Flora 275 (2021) 151760

Contents lists available at ScienceDirect

Flora

journal homepage: www.elsevier.com/locate/flora

Vegetative anatomy, morphology and histochemistry of three species of Malpighiaceae used in analogues of the Amazonian psychoactive beverage Ayahuasca

Nívea Nagamine-Pinheiro [a,*], Christopher W. Fagg [b], Sueli M. Gomes [a], Regina C. Oliveira [a], Júlia Sonsin-Oliveira [a]

[a] Universidade de Brasília (UnB), Campus Universitário Darcy Ribeiro, Departamento de Botânica - Instituto de Ciências Biológicas. Brasília, DF, 70910-900, Brazil
[b] Universidade de Brasília (UnB), Faculdade de Ceilândia. Centro Metropolitano, Ceilândia, Brasília, DF, 72220-900, Brazil

ARTICLE INFO

Editor: Alessio Papini

Keywords:
Banisteriopsis
Diplopterys
leaf anatomy
lianas
medicinal plants
wood anatomy

ABSTRACT

Ayahuasca is a traditional psychoactive beverage with pharmaceutical potential, prepared with *Banisteriopsis caapi* and *Psychotria viridis*, although the use of non-traditional plants - the so-called "Ayahuasca analogues" -, such as *Banisteriopsis* and related species, are also reported. These species are highly polymorphic and inconsistent in flowering periods, being difficult to identify when collecting to prepare the brew. To aid their separation in a vegetative state, some species used in Ayahuasca analogues (*B. laevifolia, B. muricata* and *Diplopterys pubipetala*) were characterized in their wood, outer bark and leaf morphoanatomy. We also verified whether their use is supported by histochemical data and investigated other compounds of pharmaceutical importance, in comparison with *B. caapi*. Usual techniques and methodology were used for histochemical and histological investigations, in addition to X-ray imaging for examining crystal organization and venation patterns. The wood anatomy descriptions of these species are given for the first time, and new characters were described, such as the tangential alignment of prismatic crystals in ray cells of *D. pubipetala*. Vegetative characters aid the species identification when reproductive material is unavailable, as the species differed in outer bark morphology, leaf morphology and anatomy and wood anatomy. Statistical analyses based on qualitative and quantitative anatomical features reinforce the recent distinction of *Banisteriopsis* and *Diplopterys* genera. Histochemical analyses revealed the presence of important compounds of potential pharmacological use: alkaloids, saponins, essential oils, lipids, pectin, tannins and general phenolic compounds, mostly in parenchymatous tissues in the bark and lesser in the wood. Alkaloids found mainly in the bark support the use of these plants in Ayahuasca analogues, although further studies are needed to ensure its safety and to exploit their pharmacological potential. It also raises an alternative extraction technique that could use solely strips of bark for small preparations of Ayahuasca, allowing for sustainable plant management.

1. Introduction

For the past two decades, Ayahuasca (*aya* = spirit, ancestor; *waska* = vine) has attracted much attention from both the population in general and the scientific community (Teixeira et al., 2008; Frecska et al., 2016). Also known as *daime, vegetal, hoasca, caapi, natema, yagé/yajé/iajé* or pindé, this beverage was originally used as a sacrament to elevate the state of consciousness by indigenous populations of the Amazon Basin and, nowadays, it is widespread among all continents (McKenna, 2004; Teixeira et al., 2008; Meneguetti and Meneguetti, 2014; Frecska et al., 2016; Oliveira et al., 2018). With the use of Ayahuasca, significant improvements have been reported for many neurological pathologies and even in drug addiction rehabilitation (Serrano-Dueñas et al., 2001; McKenna, 2004, Santos et al., 2007).

The globalization of Ayahuasca can threaten both native plant knowledge and environmental conservation, and this is already concerning environmental authorities (Schultes, 1994; Tupper, 2008; Teixeira et al., 2008; Ray and Lassiter, 2016). Ethnobotanical

* Corresponding author.
E-mail addresses: nivea.pinheiro@alumni.usp.br (N. Nagamine-Pinheiro), acaciafagg@gmail.com (C.W. Fagg), suelimariagomes@gmail.com (S.M. Gomes), regina711@hotmail.com (R.C. Oliveira), jsonsin@unb.br (J. Sonsin-Oliveira).

https://doi.org/10.1016/j.flora.2020.151760
Received 24 June 2020; Received in revised form 10 December 2020; Accepted 22 December 2020
Available online 24 December 2020

information can only survive with urgent investigation on related plants and recurrent field work (Schultes, 1988). Recently, fires in the Amazon basin rage at record rates, threatening many species, which could include some vines used in the tea whose biology is still unknown. Hence, studies on species related to Ayahuasca can be relevant to aid their conservation and strengthen essential cultural legacies, being even more significant under the controversial political tendencies on environmental conservation and first peoples' issues that Brazil is currently facing.

Ayahuasca is usually prepared by boiling or steeping the bark and stems of *Banisteriopsis caapi* (Spruce ex Griseb.) Morton (Malpighiaceae) in a concoction with the leaves of the Rubiaceae species *Psychotria viridis* Ruiz & Pav. (McKenna, 2004; Lunna, 2011). A synergistic interaction between alkaloids in both plants brings about the psychoactive effect (McKenna, 2004; Santos et al., 2007).

In fact, adepts of the sects discern various ethnotypes of vines used for the drink, based on different bark and trunk morphologies and their effects (Lunna, 2011). Also, many other *Banisteriopsis* C.B.Rob. species were cited for the brew composition, as well as former *Banisteriopsis* that are now classified under other genera, mainly *Diplopterys* A.Juss. (Pereda-Miranda et al., 2007 apud Garrido and Sabino, 2009). The term "Ayahuasca analogues" has been used as nontraditional combinations of plants with the same active principle of the two beforementioned (Ott, 1994; Schultes et al., 2001; Labate and Araújo, 2002).

Taxonomically, the vines used in Ayahuasca are included in Malpighiaceae family, which has its presumed diversity center in the Brazilian Cerrado (Davis and Anderson, 2010; Francener et al., 2018). As one of the largest genera in the family, with 61 accepted names in (The plant list 2013) website, *Banisteriopsis* is represented by various woody plants widespread throughout the Neotropics, particularly abundant in Brazil, with 47 accepted species, 34 of them endemic (Gates, 1982; Flora do Brasil 2020). At least half of the *Banisteriopsis* species are lianas, but subshrubs, shrubs and small trees can also occur (Gates, 1982; Davis and Anderson, 2010).

What used to be believed as a monophyletic group with three subgenera by Gates (1982) is now considered three distinct clades supported by molecular data, within the Stigmaphylloids clade (Anderson and Davis, 2007). While most *Banisteriopsis* of the homonym subgenus remained as accepted members, the subgenus *Hemiramma* (Griseb.) B. Gates is now recognized as the genus *Bronwenia* W.R.Anderson & C. Davis. Members of the subgenus *Pleiopterys* (Nied.) B. Gates, in turn, were included in an expanded *Diplopterys* genus (Anderson and Davis, 2006, 2007; Davis and Anderson, 2010).

In addition, many *Banisteriopsis* have synonyms and the same species can show great morphological variations, which makes their accurate identification somewhat challenging even in flower, as pointed by Gates (1982). The same author divided *Banisteriopsis* into several "groups" of species that share the same flower and/or fruit characteristics. Moreover, their identities are not entirely known, since collections are usually vegetative and the taxonomic understanding of the group is still under development (Wang et al., 2010).

In this context, anatomical and morphological analyses can be a special tool for distinguishing these species (Gates, 1982; Araújo et al., 2010). As *Banisteriopsis* are mainly lianas and evolutionary adjustments are expected to be found in their xylem tissue for guaranteeing efficient hydraulic conduction, analysis of their wood anatomy can aid species identification (Caballé, 1993; Figueiredo, 2011; Angyalossy et al., 2015). Leaf anatomy can provide additional data along with general morphological features for taxonomic purposes (Metcalfe and Chalk, 1983; Araújo et al., 2010). Some useful leaf characters for *Banisteriopsis* distinction were already proposed by Araújo (2014) and Gates (1982), such as leaf glands, hairs and petiole characteristics.

It is well accepted that *Banisteriopsis* species have bioactive metabolites that can be useful for human health (see Rodrigues and Carvalho, 2001; Frias et al., 2011 and Frias, 2012). Histochemistry of flower and leaf glands, seeds and fruits in many Malpighiaceae – including *Banisteriopsis* and *Diplopterys* – has been undertaken (e.g. Souto and Oliveira, 2012; Araújo, 2014; Nery et al., 2017). Stem histochemistry, however, has not been extensively analysed. Chemical studies on aerial organs of *B. caapi* have revealed that most bioactive markers are in dried bark of matured stem and branches, but not specifying in which exact cells (Wang et al., 2010).

A list of alternative Malpighiaceae species used for Ayahuasca was cited by Pereda-Miranda et al. (2007) apud Garrido and Sabino (2009). We characterized and compared the wood and leaves of three of these species: *Diplopterys pubipetala* (A.Juss.) W.R.Anderson & C.Davis (=*Banisteriopsis pubipetala* (A.Juss.) Cuatrec.), *B. laevifolia* (A.Juss.) B. Gates and *B. muricata* (Cav.) Cuatrec., with the hypothesis that we are able to separate and clearly identify the species in a vegetative state, both in the laboratory and in the field. Also, we intended to verify, through histochemical analyses, if their use in Ayahuasca analogues is supported by the presence of alkaloids in the bark and wood when compared to *B. caapi*, as well as investigate other compounds of potential pharmaceutical importance.

Table 1
Vouchers of the studied species. UB = herbarium collection; UBw = wood collection.

Species	Coordinates	Locality	Collectors	Collector n°
Banisteriopsis caapi	15°51′57.0"S 47°47′20.0"W	Cerrado, Brasília (DF)	JSO; NNP	414*
	15°44′43.1"S 47°40′26.3"W		JSO; RCO	239+
Banisteriopsis laevifolia	15°31′44.8"S 47°57′06.8"W	Cerrado, Brasília (DF)	CWF; JSO; NNP	2471
	15°31′45.5"S 47°57′06.4"W		CWF; JSO; NNP	2482
	15°31′46.2"S 47°57′06.0"W		CWF; JSO; NNP	2483
Banisteriopsis muricata	10°03′15.0"S 68°00′20.0"W	Amazônia, Rio Branco (AC)	RCO	3392
	10°03′15.0"S 68°00′20.0"W		RCO	3392
	10°03′15.0"S 68°00′20.0"W		RCO	3392
Diplopterys pubipetala	15°30′33.8"S 47°57′31.8"W	Cerrado, Brasília (DF)	CWF; JSO; NNP	2479
	15°30′33.8"S 47°57′31.8"W		CWF; JSO; NNP	2467
	15°30′32.1"S 47°57′14.9"W		CWF; JSO; NNP	2469

Notes: *B. caapi*: * young fresh sample; + mature sample previously collected.

2. Material and Methods

2.1. Botanical material

Wood and leaf samples were collected from three individuals of each species (Table 1). The vouchers were made following the taxonomical techniques found in Walter and Fagg (2015) and deposited at the University of Brasilia Herbarium (UB), including at the wood collection (UBw). One fresh sample of a young *B. caapi* and a mature one previously collected, both without inflated nodes, as described as "Tucunacá" ethnotype by Oliveira et al. (2018), were used for histochemical comparison. Plant names have been checked with http://www.theplantlist.org, which was accessed in 04/08/2019.

The stem circumference was measured and collected at 30 cm from the ground level, as it was not always possible to reach 1.3 m as proposed by Gerwing et al. (2006). Half of the wood samples were kept fresh for histochemical analyses; the remaining material was stocked in ethanol 70%. Six leaves from each specimen were collected from the fourth node (apex to the base) or, if not possible, those following below the apical meristem.

2.2. Macroscopic examination

The leaf architecture study was undertaken by diaphanization and staining for *B. muricata*. We followed the clarification and staining diaphanization method from Strittmatter (1973) in Kraus and Arduin (1997), adapted by Graciano-Ribeiro and Paiva, on which the dried leaves are rehydrated in distilled water, then brushed with coconut soap and rinsed.

For *D. pubipetala* and *B. laevifolia*, we are able to use radiographic images for evaluating the leaf architecture. They were obtained using a Faxitron MX-20 digital radiography system with a DC-12 camera (3 seconds exposure, 25 kV accelerating voltage, 0.4 mA heating current of the cathode), at Escola Superior de Agricultura Luiz de Queiroz (ESALQ). When necessary, trichomes were removed from the leaves with a razor blade before imaging. Wood samples (2.0 mm thick) were cut with IsoMet 5000 linear precision saw and kiln dried, and radiographic images were also acquired for better observation of the crystal distribution, as suggested by Matsushima et. al (2012). These images were obtained using the software Faxitron Bioptics LLC-Vision, version 2.4.1U.

For leaf morphology and venation description, we used, chiefly, Ellis et al. (2009) as a guide, in addition to Hickey (1973) and Leaf Architecture Working (Leaf Architecture Working Group, 1999), this last chosen to guide base angle descriptions, as different authors vary in methods for assessing this characteristic. The outer bark morphology analysis followed Ribeiro et al. (2002) and Sonsin et al. (2014).

2.3. Microscopic examination

Wood samples were sectioned 15–20 μm thick on their transversal and longitudinal planes by a slide microtome. The sections were bleached with 50% sodium hypochlorite, washed with distilled water and dehydrated in ethanol 30% and 50%, respectively, then stained with ethanolic Alcian blue and safranin 50% (1:4) for cellulose and lignified cell walls, respectively (Johansen, 1940; Kraus and Arduin 1997). Increasing ethanolic series were followed by butyl acetate, and permanent slides were mounted using glass varnish as mounting medium, according to Paiva et al. (2006).

Wood macerations were prepared according to Franklin (1945) modified by Kraus and Arduin (1997), using glacial acetic acid and hydrogen peroxide 1:1 at 60°C for 48 hours. They were then stained with ethanolic safranin by itself or together with Alcian blue for phloem fragments. Semi-permanent slides were mounted in aqueous glycerin 1:1.

Wood microscopy descriptors were based on "The International Association of Wood Anatomists (IAWA) Hardwood List" (IAWA Committee, 1989), together with other studies on lianas for specific terms: Angyalossy et al. (2015), Figueiredo (2011) and Pace et al. (2018).

Leaves were fixed and sectioned following Johansen (1940). Free-hand cross-sections were obtained from the middle third of the leaf blade and the base, middle and apex of the petiole. They were also bleached with sodium hypochlorite and stained with Alcian blue and safranin, and permanent slides were mounted with glass varnish (Johansen, 1940; Kraus and Arduin 1997; Paiva et al., 2006). We also mounted semi-permanent slides of leaf blade paradermal preparations, which were obtained by dissociation in Franklin's solution at 60°C (Johansen, 1940) and stained with 1% aqueous safranin (Sass, 1958).

All microscopic results were analysed and registered using an Olympus BX40 transmission light photomicroscope coupled with an Olympus SC30 digital image capture system with analySIS getIT Software. Images were digitally treated on Photoshop 5.0.

For quantitative analysis, 30 measurements of each wood anatomical feature and ten leaf measurements were taken from three samples of each species using Image-Pro Plus 6.0 software.

Principal components analysis (PCA) was performed to assess the most variant factors in the wood. For wood and leaves, a normality test was performed and, when necessary, the data were normalized. The analysis of variance (ANOVA), followed by Tukey's test or t test at 5% probability, were made to verify what was significantly different between the species. For cluster analysis, an Unweighted Pair Group Method using Arithmetic averages (UPGMA) was used. Quantitative leaf data followed Euclidean similarity index, while wood analyses was based on Jaccard coefficient for both qualitative and quantitative data, this last transformed into classes given by IAWA variables (Sneath and Sokal, 1973). All statistical analyses were performed using Past 3.20 program.

Table 2
Histochemical tests performed in wood and bark of *Banisteriopsis caapi, B. muricata, B. laevifolia* and *Diplopterys pubipetala*.

Target compound	Treatment	Reference
Alkaloid	Wagner reagent	Furr and Mahlberg, 1981
Alkaloid	Dittmar reagent	Svendsen and Verpoorte, 1983
Tannins	Vanillin-hydrochloric acid	Gardner, 1975
Phenolic compounds	Ferric chloride	Johansen, 1940
Lipids	Sudan black	Pearse, 1980
Lipids	Red Sudan IV	Pearse, 1980
Essential oil	NADI reagent	David and Carde, 1964
Pectin	Ruthenium red	Gregory and Baas, 1989

Note: All protocols were taken from Figueiredo et al. (2007). The presence of saponins in bark and wood samples was also verified, following Zenid and Ceccantini (2007).

2.4. Histochemical tests

To test the major chemical classes of metabolites and cell wall nature, we performed histochemical tests, especially for alkaloids - since those are more important regarding hallucinogenic effects - and other potential phytotherapy compounds. These analyses were made with fresh stem wood and bark from the three species and *B. caapi*, following Figueiredo et al. (2007) for protocols (Table 2) and Zenid and Ceccantini (2007) specifically for saponins.

3. Results

3.1. Macroscopic vegetative characteristics

A compilation of general macroscopic vegetative characteristics of *B. laevifolia, B. muricata* and *D. pubipetala* are shown in Figs. 1–3.

All species are climbing plants when mature (Fig. 1A, 2A, 3A). Each one shows a different type of bark external morphology, considered cleft for *D. pubipetala* (with more or less straight fissures, Fig. 1B), with shallower furrows in *Banisteriopsis laevifolia* (longitudinal V-shaped fissures as seen in transverse section, Fig. 2B) and a rough bark with reddish lenticels is described for *B. muricata* (Fig. 3B, detail). These bark surface formats with grooves may correspond to their type of cambial variants. The external cylinders of secondary xylem and some of the phloem wedges in *D. pubipetala* (Fig. 1C–D) often match the bark grooves in its bark, and the cambial variant of phloem arcs in *B. laevifolia* (Fig. 2C–D) may relate to its furrows, as both the inner and outer bark bow inwards. Still, the bark is less ridged in *B. muricata*, as their external cylinders are quite continuous with the main one (Fig. 3C–D). It is relevant to mention that the wood of *B. muricata* can vary from diffuse to semi-ring-porous (Fig. 3E), which we found within the same sample.

Leaves of all studied species are simple, opposite, petiolate with marginal attachment; lamina symmetrical, with an entire margin and pinnate venation (Figs. 1E, 2E, 3F). Leaf shape and glands position can be distinctive (Figs. 1F, 2F, 3F–detail), but these and other features are further explored in the leaf section.

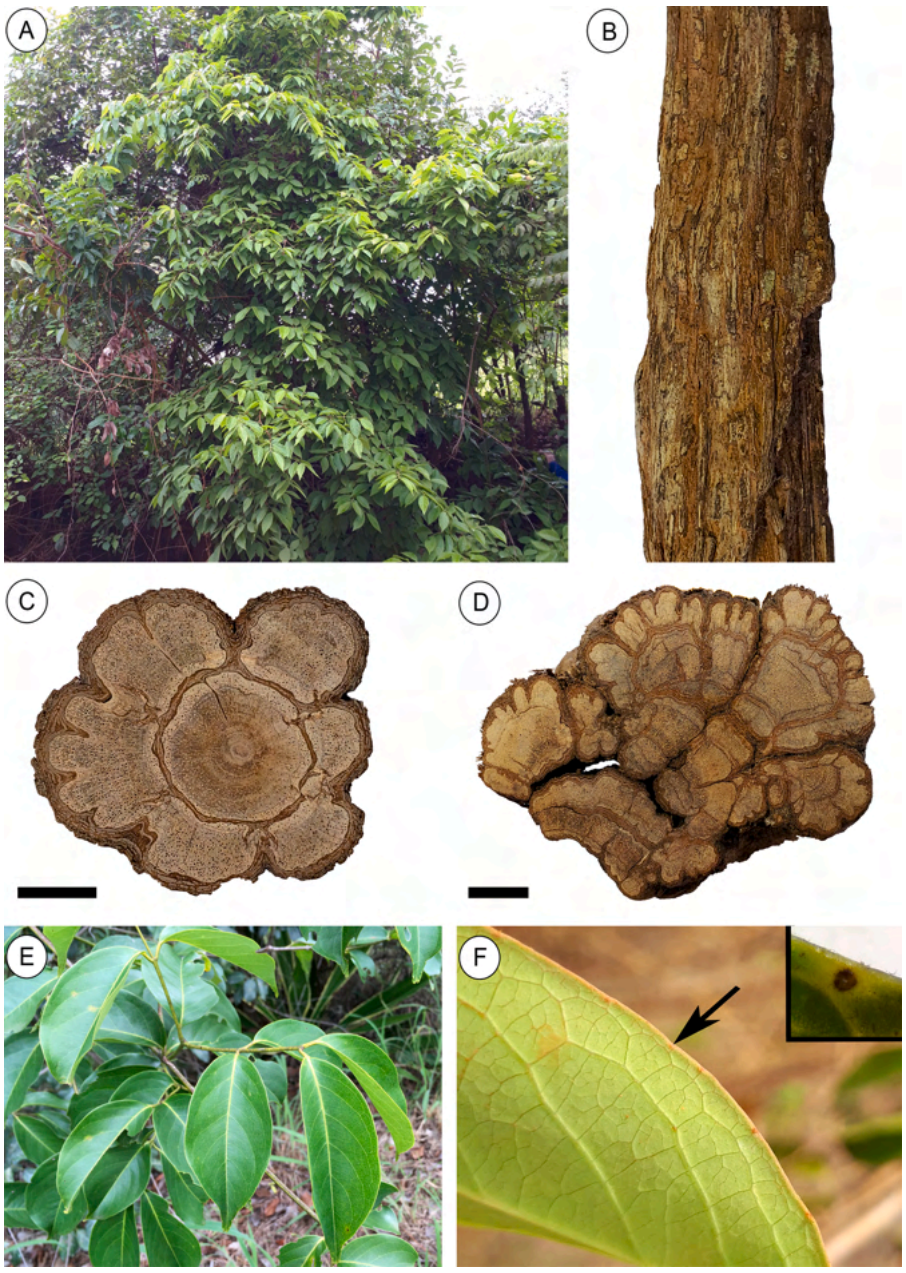

Fig. 1. *Diplopterys pubipetala* macroscopic vegetative characteristics. (A) Plant habit. (B) Cleft bark surface. (C-D) Macroscopic transverse sections with different stages of cambial variants. (E) Branch. (F) Minute marginal leaf glands (arrow and detail). Scale bars: (C-D) 1 cm.

3.2. Wood anatomy

A full description of each species is given in Supplementary Material 1, and the detailed microscopic images can be found in Figs. 4–6. In addition, the qualitative and quantitative wood anatomical features are summarized in Table 3 for better comparison.

Some qualitative characteristics are exclusive to each species. Semi-ring-porous wood (Figs. 3D-E, 4C), rays of two sizes (Fig. 4F) and ray with all cells procumbent were present uniquely in *B. muricata*. Only *D. pubipetala* had rays with procumbent cells with over 4 rows of upright and/or square marginal cells and prismatic crystals in rays. In some areas, these crystals line up in a unique tangential arrangement, viewed in transverse section, sometimes alongside the marginal band (Figs. 5A–B). We could identify these crystals in the X-ray images (Fig. 5A) by comparing them with microscopic slides and due to their shape. In Fig. 5C, we can see how the cambium produces them concomitantly. Additionally, we observed that they occur usually in pairs, in chambered enlarged upright ray cells (Fig. 5D).

Statistically significant differences between species were found for all quantitative parameters (Table 4). Although *B. laevifolia* shared most qualitative aspects with at least one of the other two species (Table 3), this was the most distinctive species regarding quantitative characters (Table 4), since it had the lowest vessel density, the smallest intervessel pits diameter, its wide vessel elements had the shortest length and its rays were more than twice as tall as for the other two species (Fig. 4E). *Diplopterys pubipetala* was distinguished due to its thicker fibre walls, thinner rays (Fig. 4D) and fewer solitary vessels. Finally, fibre length and wide vessel lumina diameter were bigger for *B. muricata*. The remaining features had significant differences between all species: narrow vessel elements length and diameter (both smaller in *B. laevifolia*); ray-vessel pits diameter (larger in *B. muricata*); and rays/mm (substantially higher for *D. pubipetala*).

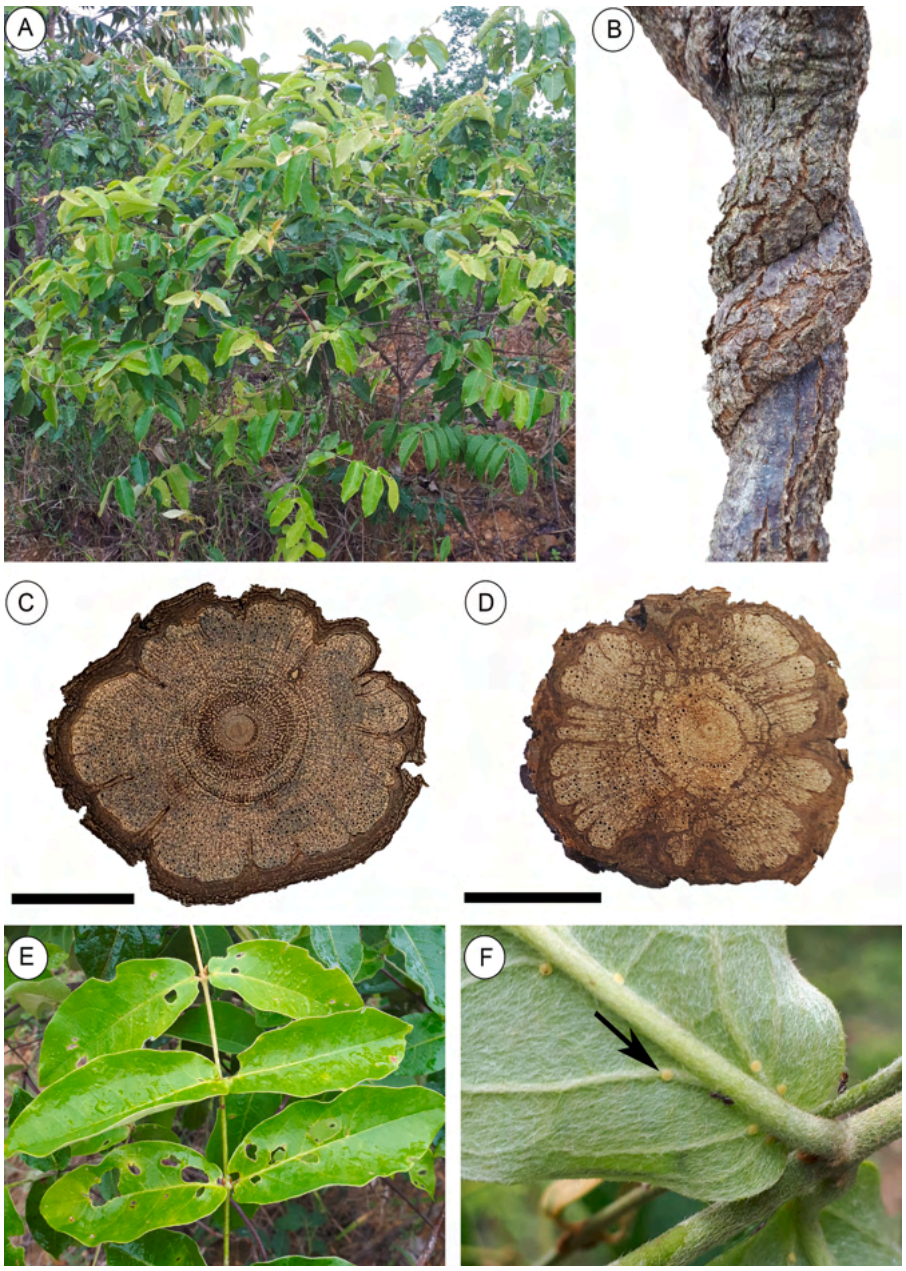

Fig. 2. *Banisteriopsis laevifolia* macroscopic vegetative characteristics. (A) Plant habit. (B) Furrowed bark surface. (C-D) Macroscopic transverse sections with different stages of cambial variants. (E) Branch. (F) Basilaminar leaf glands (arrow), usually on secondary veins and near the midrib. Scale bars: (C-D) 1 cm.

Regarding vessel groupings, multiples of 4 or more are common for all three: *D. pubipetala*-29%; *B. laevifolia*-24% and *B. muricata*-21%, reaching the maximum of 34 vessels in *B. laevifolia* and 20 in the remaining; still, multiples of 2 to 4 are more predominant (49%, 44% and 47%, respectively).

In PCA (Fig. 7), two factors explain 75% of the variance (Table 5). The first axis explains 47% of the variance and separates *B. laevifolia* from the others, being influenced by its shorter vessel elements and smaller diameter measurements for the narrow ones (Fig. 4D). The second factor, accounting for 28% of the variance, best segregated the other two, as the most influential characters were ray width and solitary vessels density, both significantly lower for *D. pubipetala*, and longer fibres for *B. muricata*.

Cluster analysis with quantitative and qualitative features separated the specimens into three expected groups (Fig. 8). In addition, *D. pubipetala* segregated into a different group from both *Banisteriopsis*.

3.3. Histochemical analysis

Histochemical tests for the stem of *B. caapi, B. laevifolia, B. muricata* and *D. pubipetala* are shown in Figs. 9, 10. In the inner bark of all species, alkaloids occur in parenchymatic cells of the secondary phloem; in the secondary xylem, they are more scarce, present in some parenchymatic cells (Figs. 9A–F, controls in 9G–H), being observed in a greater quantity of these cells in *B. muricata* (Fig. 9E). As expected, *Banisteriopsis caapi* younger wood has visibly less amounts of this compound (Fig. 9C, compare to 9A), having just a few cells stained.

The same results (i.e. presence in parenchymatic cells) were found for tannins (Fig. 10A–B), phenolic compounds (Fig. 10C–D), terpenoids of essential oils (mostly in non-lignified parenchymatic cells and near both interxylary and regular phloem; Fig. 10E) and lipids mostly in wood near the cambium area (Fig. 10H), except for the young *B. caapi*, which showed no phenols in the wood and still no tannins at all, and for

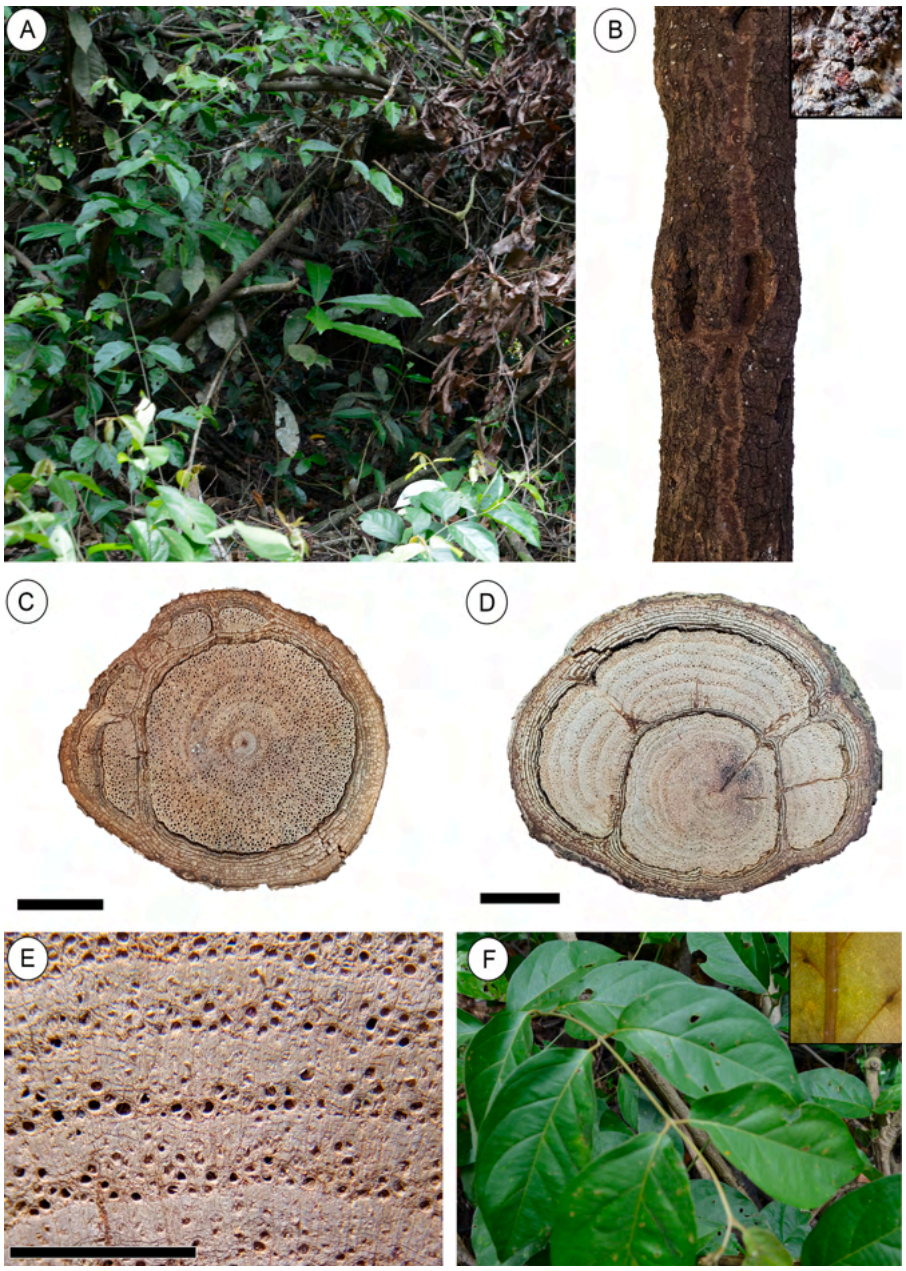

Fig. 3. *Banisteriopsis muricata* macroscopic vegetative characteristics. (A) Plant habit. (B) Rough bark surface, detail of reddish lenticels. (C-E) Macroscopic transverse sections with different stages of cambial variants. In E, detail of semi-ring-porous wood that may be found for this species. (F) Branch; detail of basilaminar leaf glands, frequently on secondary veins. Scale bars: (C-D) 1 cm; (E) 0.5 cm.

D. pubipetala, which had lipids mostly in the narrower vessels and companion cells (Fig. 10G, 10H–detail).

Pectin is present at different intensities in all cell walls, being noticeable lower in fibres, sometimes slightly marked in their inner wall (Fig. 10F). Saponins were little evident in all bark and wood samples, being only abundant in the bark of *B. muricata*.

3.4. Leaf anatomy and morphology

Leaf results for the studied species are shown in Figs. 11-13. Their main morphological features are given in Table 6. We suppressed the venation information (which can be found on Supplemental Material 2), since most of them are similar between the three species (Figs. 11A, 12A, 13A). All of them have festooned brochidodromous leaves, varying only in major secondary angle to midvein and areolation, on which *B. muricata* exhibited significantly lower frequency and development of areoles (Fig. 13B, in contrast to *D. pubipetala* - Fig. 11B and *B. laevifolia* - Fig. 12B), as well as some marginal ultimate veins incomplete. The majority of differences were found on the remaining leaf characters (Table 6).

The table on Supplementary Material 3 summarizes the leaf anatomy data for the studied species, which varied between species as follows. *Diplopterys pubipetala* could be distinguished by greater differences in thickness between adaxial and abaxial wall + cuticle layers (Fig. 11E); straight anticlinal walls in the adaxial epidermis cells (Fig. 11F, in contrast to straight to curved walls in *B. laevifolia* - Fig. 12F and *B. muricata* Fig. 13F); thicker mesophyll (mean and standard deviation of 159 μm ±1, in contrast to 111 μm ±18 for *B. laevifolia* and 79 μm ±7 for *B. muricata*, all variants according to Tukey's test at 5% in analysis of variance tests), greater layers number of spongy parenchyma; druses in the mesophyll; absence of collenchyma sheath extension on the secondary vascular bundles; prismatic crystals in spongy parenchyma (not

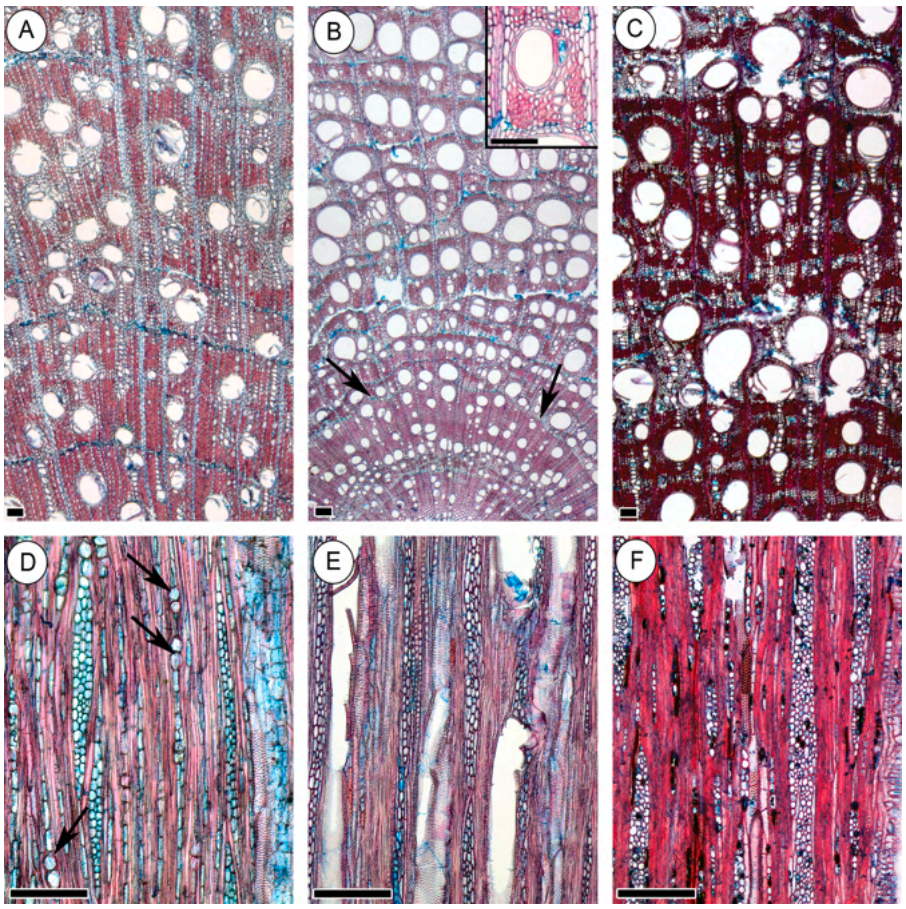

Fig. 4. General microscopic wood view. (A, D) *Diplopterys pubipetala* TS and TLS; note crystal in enlarged ray cells (arrow). (B, E) *Banisteriopsis laevifolia* TS and TLS; note self-supporting xylem (arrows) and axial parenchyma alternating with gelatinous fibres (detail). (C, F) *B. muricata* TS and TLS; note content in ray cells. TS: transverse section; TLS: tangential longitudinal section. Scale bars: 200 μm.

in the palisade parenchyma, as the others); and absence of sclerenchyma sheath in the petiole, which have lobate bundles and, sometimes, concentric amphicribal accessory bundles (Fig. 11C).

Banisteriopsis laevifolia was the only one with bilayered epidermis in some regions (Fig. 12E-detail); palisade parenchyma occupying almost half of the mesophyll; prismatic crystals in the midvein parenchyma; sclerenchyma sheath in the petiole vascular region (Fig. 12C) and radiate pedal cells (Fig. 12F).

Lastly, *B. muricata* had a smaller difference between adaxial and abaxial wall plus cuticle thickness; stomata sunken below the common cells (Fig. 13E, asterisk); lower stomata density per mm^2 (233±62 - Fig. 13G, in comparison to 464±74 - Fig. 11G for *D. pubipetala* and 504±74 - Fig. 12G for *B. laevifolia*), stomata without ledges; thinner mesophyll with less spongy parenchyma layers (Fig. 13E); plane-convex midvein, without druses in phloem (Fig. 13D, in contrast to biconvex with druses in Fig. 11D and Fig. 11D); petiole with divided vascular bundle, non-lignified sclerenchyma and, usually, less – up to 3 – accessory bundles (Fig. 13C).

The common features were the following: abaxial epidermis cells with straight to curved walls; paracytic stomata, only in abaxial epidermis; dorsiventral mesophyll, all with braciform parenchyma, idioblasts and one layer of palisade parenchyma; druses in the midvein parenchyma; collateral secondary vascular bundles, with parenchymatic sheath and fibres on major ones; midvein vascular bundle in one continuous open arc, with bicollateral sclerenchyma and collenchyma (angular to annular) and with smaller epidermis than on mesophyll; petiole with collateral accessory vascular bundles.

Cluster analysis with all leaf assessments corroborated what was found for wood analysis, with *D. pubipetala* appearing in a distinct clade from *Banisteriopsis* species. ANOVA tests (Table 6 and Supplemental Material 2) show significant differences (with $P \leq 0.001$) for all three species relatively to both palisade parenchyma and mesophyll height, petiole length and glands size. On the other hand, hair length was considered equal for *D. pubipetala* and *B. muricata*. In addition, their hairs had a significant difference between shorter and longer arms on t-test ($P = 0.002$ for *D. pubipetala* and $P = 0.0002$ for *B. muricata*), which was non-significant for *B. laevifolia* ($P = 0.92$). Hair stalks were too small to be measured in *B. muricata* and *D. pubipetala*.

4. Discussion

We identified several anatomical and morphological vegetative characters that separate the three studied species and may aid their identification, especially when fertile material is unavailable. These findings are important, since the species are hard to identify due to intrinsic difficulties of the *Banisteriopsis* species circumscription, as pointed by Gates (1982). Still, when reviewing *Banisteriopsis* Neotropical species, this author arranged several species into groups with no formal taxonomic categories. Thus, species circumscription within these groups seems unresolved.

The anatomical and morphological features achieved here constitute a contribution to the taxonomy of the studied plants and can be useful for the taxonomy of other Malpighiaceae species too. Oliveira et al. (2018) discuss the importance of stem morphology in distinguishing *B. caapi* ethnotypes, and our results agree with that. In addition, cambial variants, stem anatomy and leaf characters have discriminatory potential.

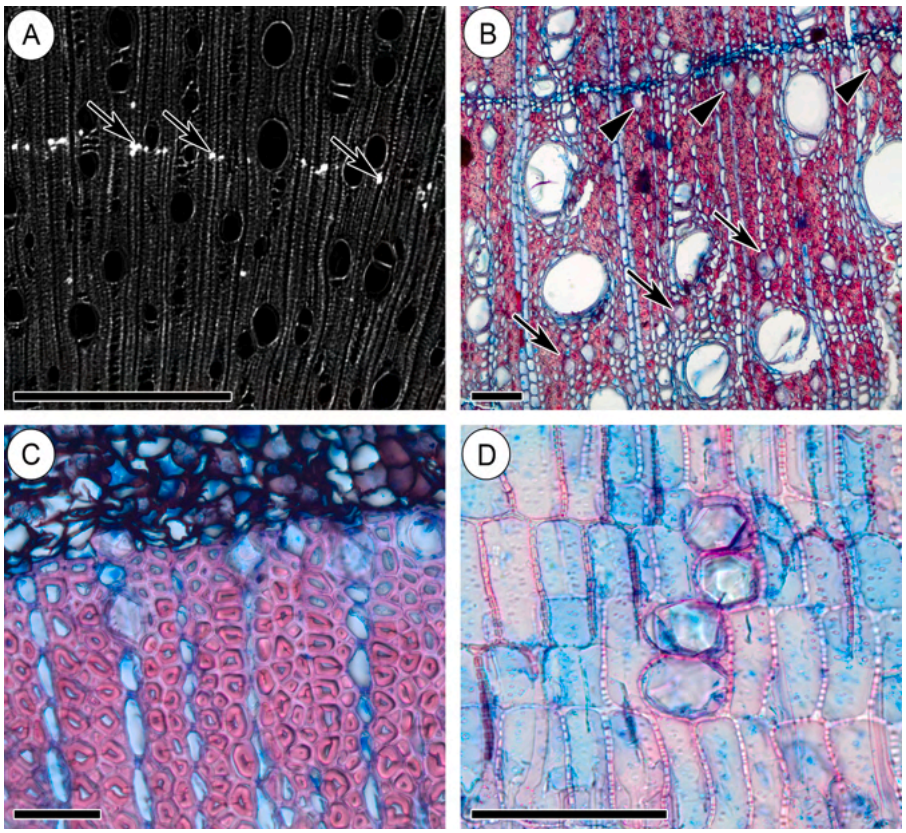

Fig. 5. Prismatic crystals in *Diplopterys pubipetala* wood. (A) Radiographic image, showing the alignment of crystals (arrows), TS. (B) Alignment following (arrowheads) or not (arrows) the growth ring, TS; (C) Concurrent crystals formation in different rays by the cambium, TS. (D) Crystals in chambered upright ray cells, RLS. TS: transverse section; RLS: radial longitudinal section. Scale bars: (A) 2,5 mm; (B-D) 100 μm.

4.1. Wood anatomy – shared, unique and novel features

Both qualitative and quantitative wood anatomical features were useful to distinguish the species, even when these data were used separately in statistical analyses. Nevertheless, some features were stable within the family.

We found anatomical features that are related to Malpighiaceae family, such as: simple perforation plate; narrow vessel in radial pattern; vestured pits; septate fibres; abundant prismatic crystals in axial parenchyma; tall heterocellular mixed rays and perforated ray cells, as mentioned by several authors (Solereder, 1908; Metcalfe and Chalk, 1950; Domingues, 2008; Amorim et al., 2017; Cabanillas et al., 2017; Pace et al., 2018).

Other attributes common for the three studied species are consistent with the lianescent vascular syndrome proposed by Angyalossy et al. (2015), such as vessels dimorphism, cambial variants, fewer fibres (mostly gelatinous), greater quantity of parenchyma cells and area of conduction (large vessels). We understand that these features are not usually reported for the whole family because lianas appear in derived groups within the Malpighiaceae (Davis & Anderson, 2010; Pace, 2015).

In this matter, vessel dimorphism, for instance, is of great importance in lianas, as narrow vessels ensure water conduction when wider vessels undergo embolism, while the latter offer lower resistance to water to flow for long distances when functional, which may compensate for the relatively small cross-sectional area of the lianas (Carlquist, 1985; Ewers, 1985). Also, variant secondary growth can benefit not only conduction in lianas but provide greater flexibility and protection for their stem, as it usually intercalates soft and stiff tissues (Pace, Lohmann, & Angyalossy, 2009; Isnard & Field, 2014; Angyalossy et al., 2015).

The high amounts of parenchymatous tissues may also reflect this search for flexibility, which we habitually expect in lianas. All nine specimens have rays with more than 1 mm high, which is not expected for the family (see Metcalfe and Chalk, 1950; Amorim et al., 2017; Pace et al., 2018), but was also found by Cabanillas et al., 2017 lianas of *Callaeum* Small genus. Also, they all have non-lignified parenchyma, which retains its meristematic capacity and allows the secondary xylem to undergo structural changes, also aiding repair of injuries to which lianas are exposed (Carlquist, 1985; Ewers & Fisher, 1991; Angyalossy et al., 2015). Additionally, it seems that bands of non-lignified parenchyma in the studied species acquire cambium activity to form secondary phloem and perhaps even secondary xylem, similar to the interxylary cambium seen in Pace et al. (2018), but further ontogenetic analyses are needed to truly understand it.

Some features have greater diagnostic value for separating species, which were mostly related to quantitative features, though qualitative ones occurred. For *D. pubipetala*, the most distinctive characteristic was perhaps the presence of prismatic crystals in upright and enlarged ray cells, usually in pairs (Fig. 5D), a few up to four. Although prismatic crystals in ray cells are not uncommon in Malpighiaceae (see Andrade, 1997), this arrangement is not usually observed in wood. In addition, their tangential alignment in ray cells is described here for the first time, being occasionally observed along with growth rings. Gourlay (1995) also observed prismatic crystals marking ring boundaries in African acacias, but it was always related to the marginal parenchyma. Also, we agree with Matsushima et al. (2012), as X-ray imaging proved to be a useful tool for crystal observation.

We also observed a horizontal alignment of vessel elements as viewed on the radial section, reported here for the three species (Fig. 6D), which has not been acknowledged in previous works (since storied structures are determined from the tangential section only, IAWA Committee, 1989). Even so, this aspect can be observed in different degrees (for all or some narrow vessel elements) in many

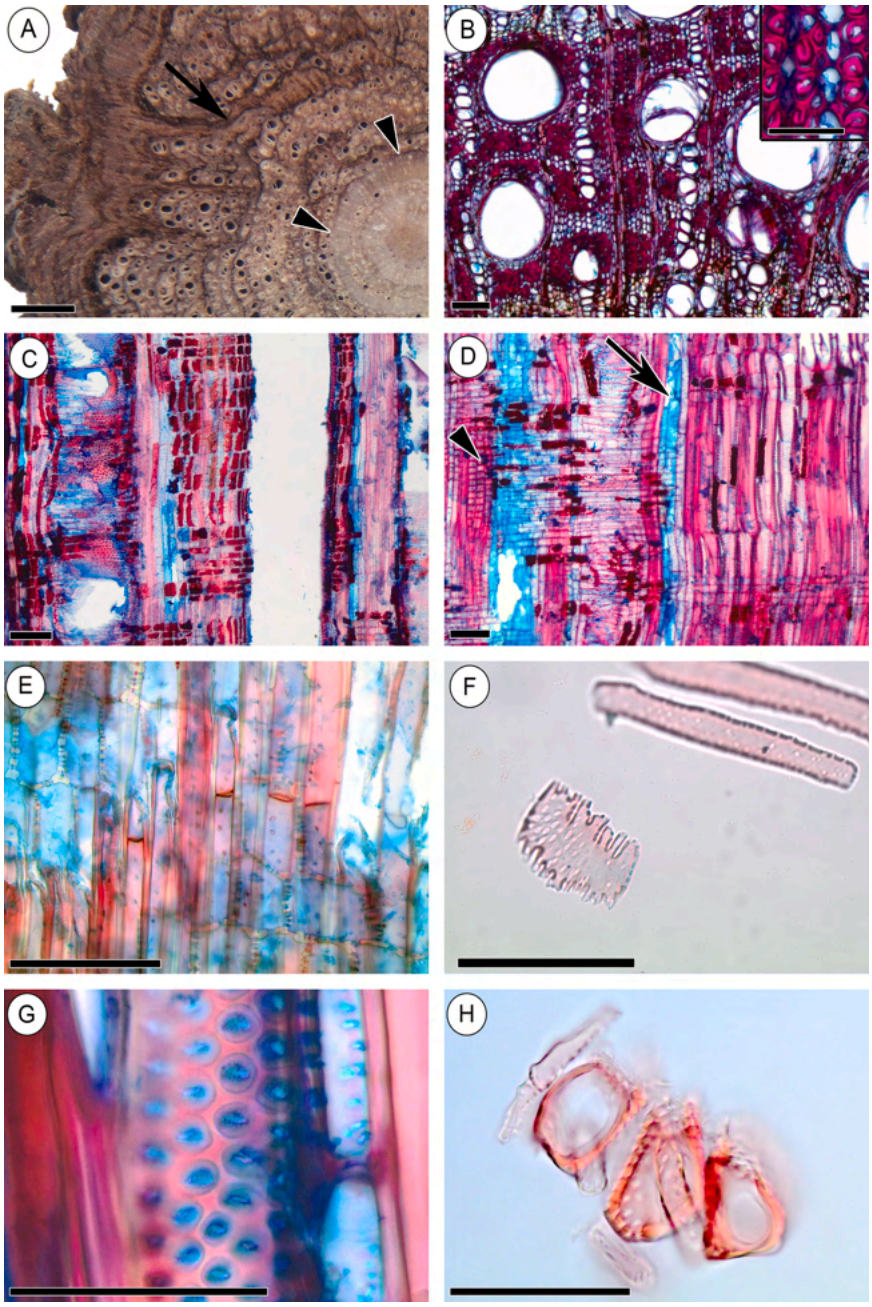

Fig. 6. Common wood anatomical features for the studied species. (A) *Banisteriopsis laevifolia*, TS: Cambial variation with interxylary phloem (arrow); note self-supporting secondary xylem (arrowhead). (B) *B. muricata*, TS: Vessel dimorphism, with narrow vessels in radial pattern; note different types of axial parenchyma; detail: gelatinous fibres. (C-D) *B. muricata*, RLS: (C) Heterocellular rays; parenchymatic cells with content; (D) Non-lignified axial parenchyma (arrow); prismatic crystals in chambered axial parenchyma (arrowhead); simple perforation plate; note the alignment of narrow vessel elements. (E) *Diplopterys pubipetala*, RLS: Septate fibres; (F) *B. laevifolia*, macerate: Disjunctive ray parenchyma cell walls. (G) *Diplopterys pubipetala*, TLS: vestured pits. (H) *B. muricata*, macerate: perforated ray cells. TS: transverse section; TLS: tangential longitudinal section; RLS: radial longitudinal section. Scale bars: (A) 1 cm; (B-F), (H) 100 μm; (B-detail), (G) 50 μm.

species which have a radial pattern, both within Malpighiaceae and in other families (see, for instance, *Byrsonima basiloba* A.Juss., *Baccharis trimera* (Less.) DC. and *Styrax ferrugineus* Nees & Mart. in Sonsin et al., 2014). Ontogenetic studies may provide a better understanding of this characteristic, on which radially adjacent vessel elements seem to have a synchronized development from radially aligned fusiform initials. As this feature may be relevant in descriptions, we here suggest reporting it as "radially storied vessel elements". Further analyses should be done, but we expect that this trait could aid conduction, as the occurrence of perforation plates at the same level may cause adjacent narrow vessels elements to function as one, but avoiding the risks of being susceptible to cavitation such as for wide vessels.

Despite being described for the family in Metcalfe and Chalk (1950), vessels in radial pattern occur only for narrow ones, predominantly in *B. muricata* and *D. pubipetala*. In these cases, we found at least 60% of multiple vessels, sometimes reaching more than 20 vessels in a single cluster.

The most dominant types of axial parenchyma in the studied species are scanty, vasicentric and diffuse, and when occurring in bands they are sometimes non-lignified, which was also found for two variants of *B. caapi* used for Ayahuasca studied by Sonsin-Oliveira and colleagues (unpubl. data).

Regarding gelatinous fibres, they appear as irregular bands in *B. muricata* and *D. pubipetala*. However, *B. laevifolia* seems to have developed a specialized organization, on which each axial parenchyma lines (lignified or not) are commonly followed by an external area of gelatinous fibres, which in turn is adjacent to an area with no gelatinous fibres just before the next line (Fig. 6B-detail). We speculate that this arrangement – found similarly in *B. caapi* by Sonsin-Oliveira and colleagues (unpubl. data) – guarantees more flexibility, which is so indispensable in the habit.

In addition, *D. pubipetala* was separated by its thinner rays, most with procumbent cells with over 4 rows of upright and/or square marginal cells, thicker fibre walls and lower frequency of solitary vessels. In turn,

Table 3
Summarized description of wood anatomical features of studied species.

Anatomical features	Species		
	Bl	Bm	Dp
Growth rings well defined (Fig. 4A, C)		*	+
Growth rings poorly defined	*	*	*
Growth rings absent (Fig. 4B)	+	*	
Wood diffuse-porous (Fig. 4A-B)	X	*	X
Semi-ring-porous (Figs. 3E, 4C)		+	
Small vessels in radial pattern (Fig. 4A-C)	X	X	X
Multiples and solitary vessels	X	X	X
Simple perforation plate	X	X	X
Intervessel pits alternate, circular (Fig. 6G)	X	X	X
Vestured pits (Fig. 6G)	X	X	X
Vessel-ray pits with distinct border, similar to intervessel pits in size and shape	X	X	X
Vessel dimorphism (Figs. 4A-C, 6B)	X	X	X
Tyloses in vessels	+	+	
Deposits in vessels	X	X	X
Tracheids	*	+	
Fibres with simple to minutely bordered pits	X	X	X
Septate fibres (Fig. 6E)	X	*	X
Fibres thin- to thick-walled	X	X	X
Gelatinous fibres (Fig. 6B, detail)	X	X	X
Apotracheal axial parenchyma diffuse	X	X	X
Apotracheal axial parenchyma diffuse-in-aggregate	X	X	+
Axial parenchyma scanty	X	X	X
Axial parenchyma vasicentric	X	X	X
Axial parenchyma confluent	X	X	+
Axial parenchyma in narrow bands or lines up to three cells wide (irregular)	X	X	
Axial parenchyma in marginal bands	*	X	X
2 cells per parenchyma strand	+	X	+
3-4 cells per parenchyma strand	X	X	X
5-8 cells per parenchyma strand	+		+
Non-lignified parenchyma (Figs. 4A-C, 6C-D)	X	X	X
Ray width 1-3 cells (Fig. 4D-F)	X	X	X
Larger rays commonly 4- to 10-seriate (Fig. 4F)	*	X	*
Ray height >1 mm (Fig. 4D-F)	X	X	X
Rays of two distinct sizes (Fig. 4F)		X	
All ray cells procumbent		+	
All ray cells upright and/or squares	X	X	X
Body ray cells procumbent with 1 rows of upright/squares marginal cells		X	X
Body ray cells procumbent with 2-4 rows of upright/squares marginal cells		X	X
Body ray cells procumbent with over 4 rows of upright/squares marginal cells			X
Ray with procumbent, upright and squares mixed throughout the ray (Fig. 6C)	X	X	X
Sheath cells	X	X	X
Perforated ray cells (Fig. 6H)	X	X	X
Disjunctive ray parenchyma cell walls (Fig. 6F)	X	X	X
Parenchyma cells with content (Fig. 4F, 6C-D)	+	X	X
Prismatic crystals in chambered upright/square ray cells (Figs. 4D, 5)			X
Prismatic crystals in chambered axial parenchyma cells (Fig. 6D)	X	X	X
Prismatic crystals in non-lignified axial parenchyma cells	X	X	X
Cambial variants (Figs. 1C-D, 2, 3C-D, 6A)	+	X	X

Notes: X = presence in all individuals; + = presence in two individuals; * = presence in only one individual; blank = not present; Bl = *Banisteriopsis laevifolia*; Bm = *B. muricata*; Dp = *Diplopterys pubipetala*.

Table 4
Statistical data with analysis of variance (ANOVA) of quantitative measurements of *Banisteriopsis laevifolia, B. muricata* and *Diplopterys pubipetala*.

Character	Species	A ± SD	Tukey	F_{df}
Fibre length (μm)	Bl	557 ± 153	a	28.53
	Bm	796 ± 279	**b**	
	Dp	555 ± 147	a	
Wide vessel element length (μm)	Bl	213 ± 44.2	**b**	37.32
	Bm	275 ± 43.5	a	
	Dp	275 ± 74.9	a	
Narrow vessel element length (μm)	Bl	233 ± 53.0	**a**	50.67
	Bm	292 ± 50.3	**b**	
	Dp	320 ± 56.7	**c**	
Intervessel pits diameter (μm)	Bl	5.6 ± 1.1	**b**	161.2
	Bm	8.2 ± 0.9	a	
	Dp	7.9 ± 1.1	a	
Ray-vessel pits diameter (μm)	Bl	3.9 ± 0.8	**a**	339.4
	Bm	6.7 ± 0.9	**b**	
	Dp	4.5 ± 0.6	**c**	
Wide vessel lumina diameter (μm)	Bl	128 ± 43.7	a	43.57
	Bm	201 ± 50.0	**b**	
	Dp	144 ± 39.9	a	
Narrow vessel lumina diameter (μm)	Bl	15.9 ± 5.0	**a**	35.85
	Bm	23.7 ± 6.6	**b**	
	Dp	20.2 ± 7.9	**c**	
Fibre wall thickness (μm)	Bl	5.1 ± 1.3	a	9.82
	Bm	5.8 ± 2.0	a	
	Dp	7.1 ± 3.2	**b**	
Rays/mm	Bl	6.4 ± 1.9	**a**	271.6
	Bm	9.6 ± 2.5	**b**	
	Dp	16.7 ± 4.1	**c**	
Vessels density (mm^2)	Bl	66.0 ± 26.9	**b**	24.14
	Bm	94.5 ± 35.2	a	
	Dp	88.4 ± 31.0	a	
Ray height (μm)	Bl	955 ± 937	**b**	21.48
	Bm	408 ± 326	a	
	Dp	472 ± 318	a	
Ray width (μm)	Bl	24.2 ± 11.4	a	8.03
	Bm	27.9 ± 16.5	a	
	Dp	18.6 ± 10.9	**b**	
Solitary vessels (%)	Bl	40.2 ± 10.2	a	15.15
	Bm	40.0 ± 10.8	a	
	Dp	32.0 ± 13.4	**b**	

Notes: A = Average; SD = Standard Deviation; Fdf value refer to the ANOVA; P value was < 0.001 for all features. Equal letters indicate statistical similarities among the species to each variable according to Tukey's test at 5%; variants in bold; Bl = *Banisteriopsis laevifolia*; Bm = *B. muricata*; Dp = *Diplopterys pubipetala*.

both *Banisteriopsis* species had tracheids, tyloses in vessels and irregular lines of axial parenchyma, characteristics not found on *D. pubipetala*. These differences, which were explored in PCA, summed with our cluster analysis corroborate the placement of this species in a clade distinct from *Banisteriopsis*, as proposed by Anderson and Davis (2006).

One particular cambial variant found on *B. laevifolia* seems to derive from a close variation of the interxylary cambia cited by Pace et al. (2018), on which cambial tissue appears within the secondary xylem, but not in a scale-like pattern as the authors described. As they found on *B. nummifera* group, *B. laevifolia* of this study seems to exhibit a centripetal differentiation from non-lignified axial parenchyma bands near the regular cambium to the center, but ontogenetic investigations should be done to elucidate this matter.

As for *B. muricata*, the rays were of distinct sizes, the wider commonly having more than 4 cells in width. Only in this species we found ray composition with all cells procumbent, besides the other types. Additionally, analysis of variance isolated this species for its

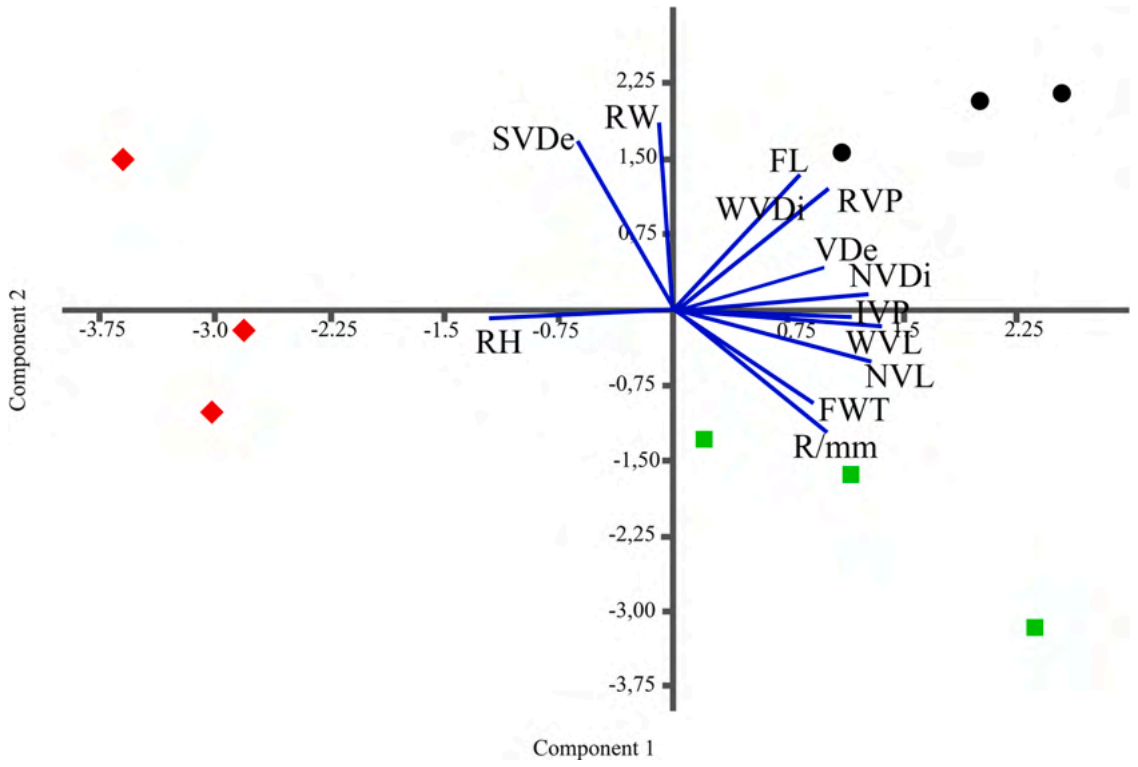

Fig. 7. Principal component analysis. ■ *Diplopterys pubipetala*; ◆ *Banisteriopsis laevifolia*; ● *B. muricata*; VDe: vessels density; SVDe: solitary vessels density; WVL: wide vessel element length; NVL: narrow vessel element length; WVDi: Wide vessel lumina diameter; NVDi: narrow vessel lumina diameter; FL: fibre length; FWT: fibre wall thickness; R/mm: rays/mm; IVP: intervessel pits diameter; RVP: ray-vessel pit diameter; RH: ray height; RW: ray width.

Table 5
High value data of principal component analysis.

Features	C1	C2
Fibre length (μm)	0.22	**0.36**
Wide vessel element length (μm)	**0.36**	-0.04
Narrow vessel element length (μm)	**0.35**	-0.14
Intervessel pits diameter (μm)	0.31	-0.02
Vessel-ray pit diameter (μm)	0.27	0.33
Wide vessel lumina diameter (μm)	0.27	0.31
Narrow vessel lumina diameter (μm)	**0.34**	0.05
Fibre wall thickness (μm)	0.25	-0.25
Rays/mm	0.27	-0.33
Vessels/mm^2	0.26	0.11
Ray height (μm)	-0.32	-0.02
Ray width (μm)	-0.03	**0.50**
Solitary vessels/mm^2	-0.17	**0.45**

Notes: C1 = Component 1; C2 = Component 2. Features that contributed most for axes are in bold.

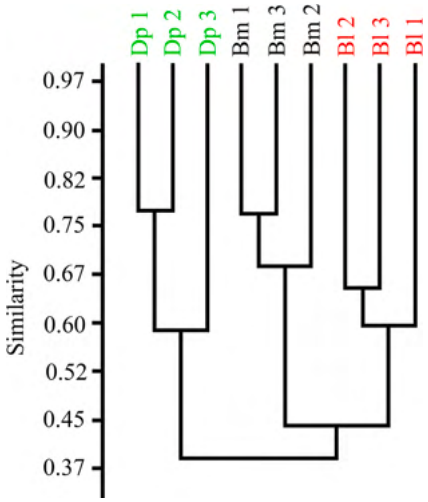

Fig. 8. Dendrogram of cluster analysis for wood features (Jaccard similarity. UPGMA method). Bl = *Banisteriopsis laevifolia*; Bm = *B. muricata*; Dp = *Diplopterys pubipetala*; numbers refer to each collected individual.

longer fibres and wider vessels, which led them to the last class of IAWA Committee (1989) for vessel lumina diameter (greater than 200 μm). *Banisteriopsis muricata* specimens had greater development, visibly reaching higher heights when compared to the other two species, which were smaller and sometimes still seeking for better subtracts to climb on. Possibly, some of their distinctive features (as wider rays and vessels) are related to a better adaption for this habit.

Semi-ring-porous woods, found in some areas of *B. muricata*, have also been reported for *B. oxyclada* (Andrade, 1997). They are also present in at least one development stage in most Bignoniaceae lianas (Lima et al., 2010). Indeed, IAWA Committee (1989) states that it is not unusual for many plants to range from semi-ring-porous to diffuse-porous.

Additionally, the inconsistence in growth ring distinction between individuals of the same species, as found here, is not rare in tropical species (see Alves & Angyalossy-Alfonso, 2000; Gasson et al., 2010). It is known that growth rings are influenced both by genetic factors and the environment (Schweingruber et al., 2008). Silva et al. (2019) discuss that tropical species indeed have great variations and complexity in growth rings, being far less accurate than in the ones from temperate regions, which are commonly continuous, strongly demarcated and annual.

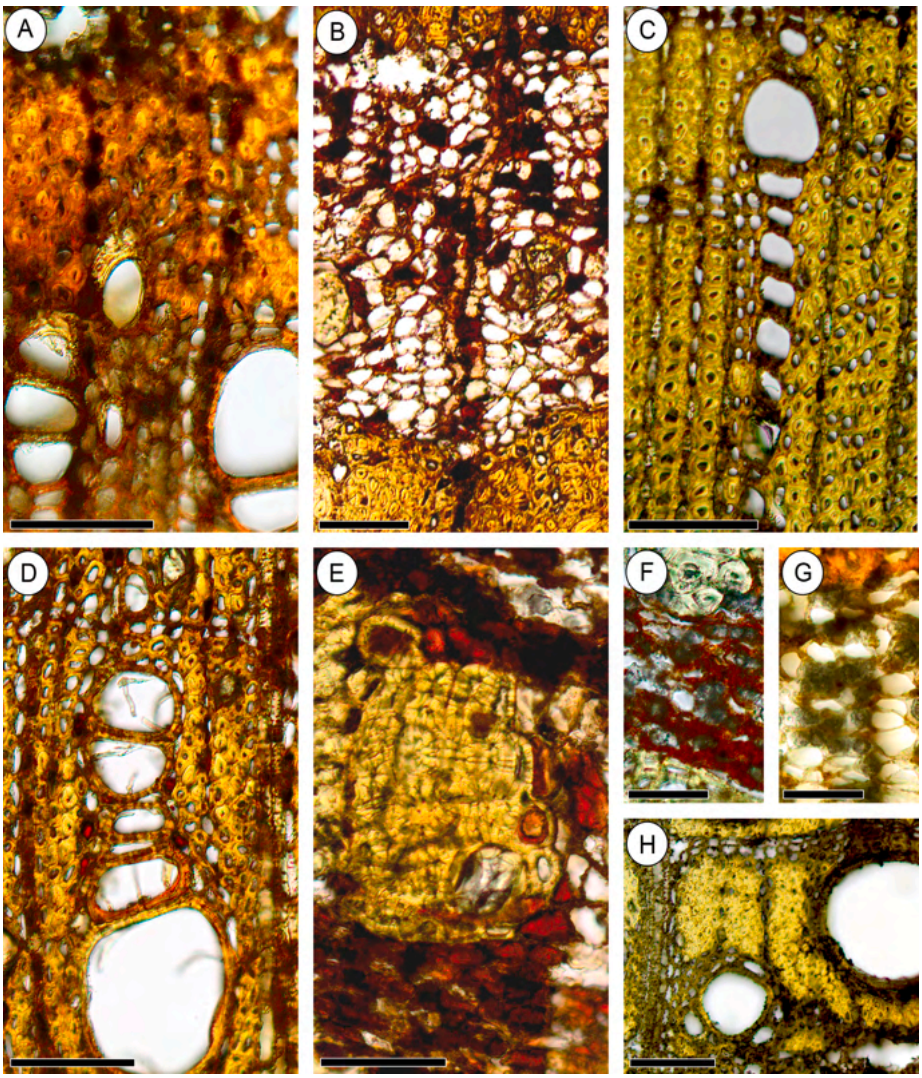

Fig. 9. Alkaloid histochemical tests in transverse sections, alkaloids are colored in reddish-brown. (A, C-D, H) Secondary xylem; (B, E-G) Secondary phloem. (A-D, F) Wagner reagent; (E) Dittmar reagent; (G-H) controls. (A-C) Banisteriopsis caapi, C in young stage. (D, F-G) Diplopterys pubipetala. (E) B. muricata. (H) B. laevifolia. Scale bars (A-E, H) 100 μm; (F-G) 50 μm.

4.2. Alkaloids and beyond

The active principles in *B. caapi* are the β-carboline alkaloids harmine, harmaline and tetrahydroharmine (Callaway et al., 2005). Alkaloids were found in a greater quantity of parenchymatic cells in *B. laevifolia, B. muricata* and *D. pubipetala* as expected, since they are used in Ayahuasca analogs, and this result preliminarily supports this alternative use. The findings for *B. muricata* are consistent with Davis and Yost (1983), which report that Peruvian Witotos of the Ampiyacu river call this species *sacha Ayahuasca* ("wild Ayahuasca"), claiming it can be used in the same way as *B. caapi*, even though it induces weaker effects. Costa et al. (2020) also detected different alkaloids in leaf and stem partitions of *D. pubipetala*.

Wang et al. (2010) found higher concentrations of alkaloids in *B. caapi* in dried bark and, secondly, in whole dried stems, as we also found in the three studied species. This discrepancy could be used to improve management plans of extraction, on which the plant could be spared in the beverage's preparation with techniques that only removes parts of the bark, especially when prepared in a low scale. Davis and Yost (1983) reported that the Waorani Indians from Ecuador scrape the bark of *B. muricata* to make a hallucinogenic brew (*mii*), and this could likewise be extended to other species.

However, the findings by Wang et al. (2010) are applied to matured stems only. Indeed, young *B. caapi* stems exhibited a weak reaction for alkaloids detection in our work, both in bark and wood. Still, Callaway et al. (2005) found that older plants (8 years old) also had low levels of harmala alkaloids. Thus, there might be an optimum stage when these plants concentrate highest amounts of alkaloids, indicating the best age for an optimal yield.

Alkaloid presence is not constant throughout the Malpighiaceae family, being more restricted to liana groups such as *Banisteriopsis, Diplopterys, Tetrapterys* and *Stigmaphyllon* (see Ghosal and Mazumder, 1971; Frias et al., 2012; Guilhon-Simplicio et al., 2013; Queiroz et al., 2015; Guimarães et al., 2016). The broad prevalence of alkaloids in *Banisteriopsis* species and close relatives might explain why there is such a variety of alternative species used for Ayahuasca. We believe that the common presence of such alkaloids in these genera summed with the difficulties of discrimination between species within them entail people to collect different plants that look alike but bring on similar effects, with little concern to precisely identify and use solely *B. caapi*. Yet, supplementary analysis is needed and caution should be taken to avoid intoxications, as some alternative species may contain additional toxic alkaloids such as bufotenine (*e.g. Tetrapterys mucronata* Cav., according to Queiroz et al., 2015). Colleagues are now undertaking further

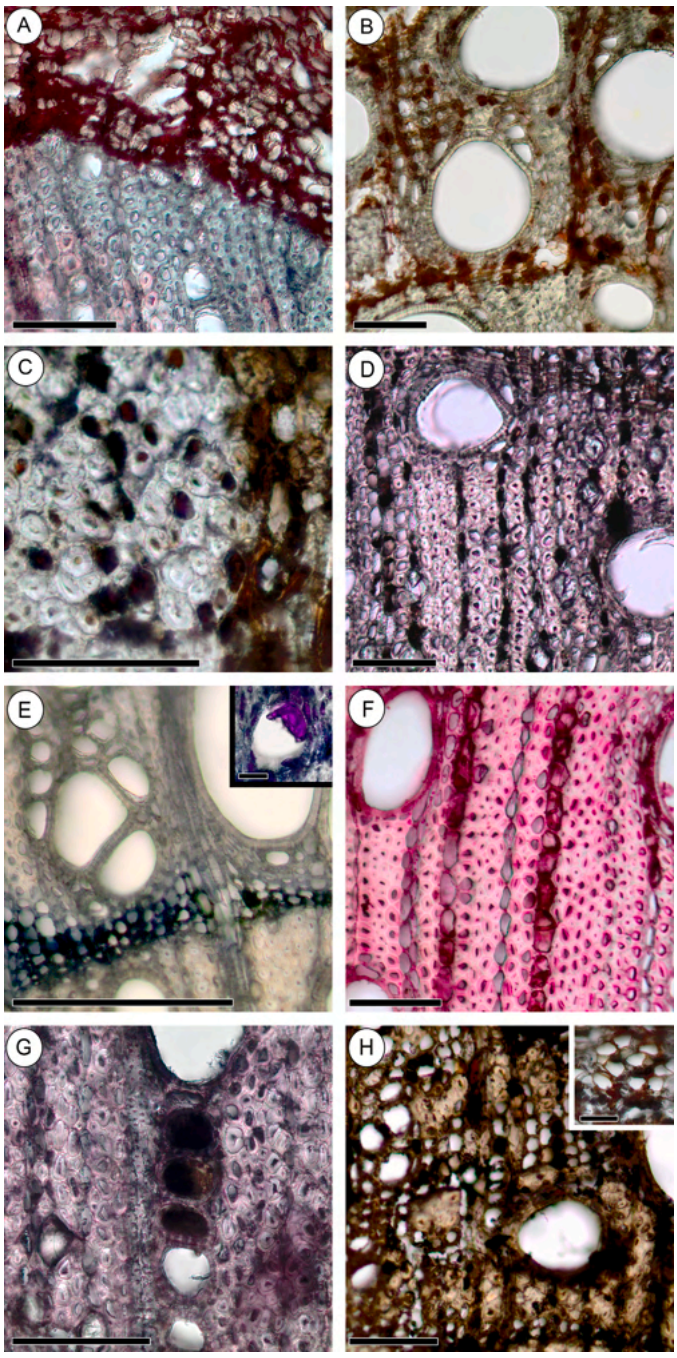

Fig. 10. Histochemical tests in transverse sections. (A, C, H-detail) Inner bark; (B, D-H) Stem. (A-B) Vanillin-hydrochloric acid test for tannins, in red. (C-D) Ferric chloride test for phenolic compounds, in dark. (E) NADI test for essential oil, in dark blue. Detail of vessel content, with both essential oil and resin acid, in purple. (F) Ruthenium red test for pectin, in intense pink. (G-H) Sudan Black test for lipids, in black; detail of stained companion cells; note tannins in orange brown, also stained by Sudan Black. (A, D, F-G, H-detail) *Diplopterys pubipetala*; (B-C, E) *Banisteriopsis laevifolia*; (E-detail) *B. caapi*; (H) *B. muricata*. Scale bars: (A-H) 100 μm; (E, H details) 50 μm.

phytochemical analyses of these samples and samples of *B. caapi* from different regions of Brazil.

At a cellular level, vessel contents had positive reactions only for lipids in *D. pubipetala* and for essential oil and resin acid in *B. caapi*. Also, Andrade (1997) verified a dense cytoplasm in companion cells of *B. oxyclada* and other Malpighiaceae species. This could partly refer to lipids, which were located in these cells of *D. pubipetala* in the present work. Yet in relation to *D. pubipetala*, our findings for tannins, phenols and saponins conform to what was found by Santos et al. (2015) in the bark of this species. These metabolites, also present in the other studied species, can be of great value for human use.

Positive tests of saponins in the studied species may be promising, as they are also important for having bactericide and fungicide properties (Santos et al., 2015). Their presence, in particular at relative high levels in *B. muricata*, raises the question on how would they be interacting with harmine alkaloids. Milugo et al. (2013) showed that alkaloids and saponins appeared to have an antagonist effect, exhibiting lower antioxidant activity when combined. If this is the case for the species we studied, effects of the Ayahuasca beverage may even be affected at some level.

Essential oils were found in the bark and in the wood near the cambium, frequently in the axial parenchyma cells. They also can have

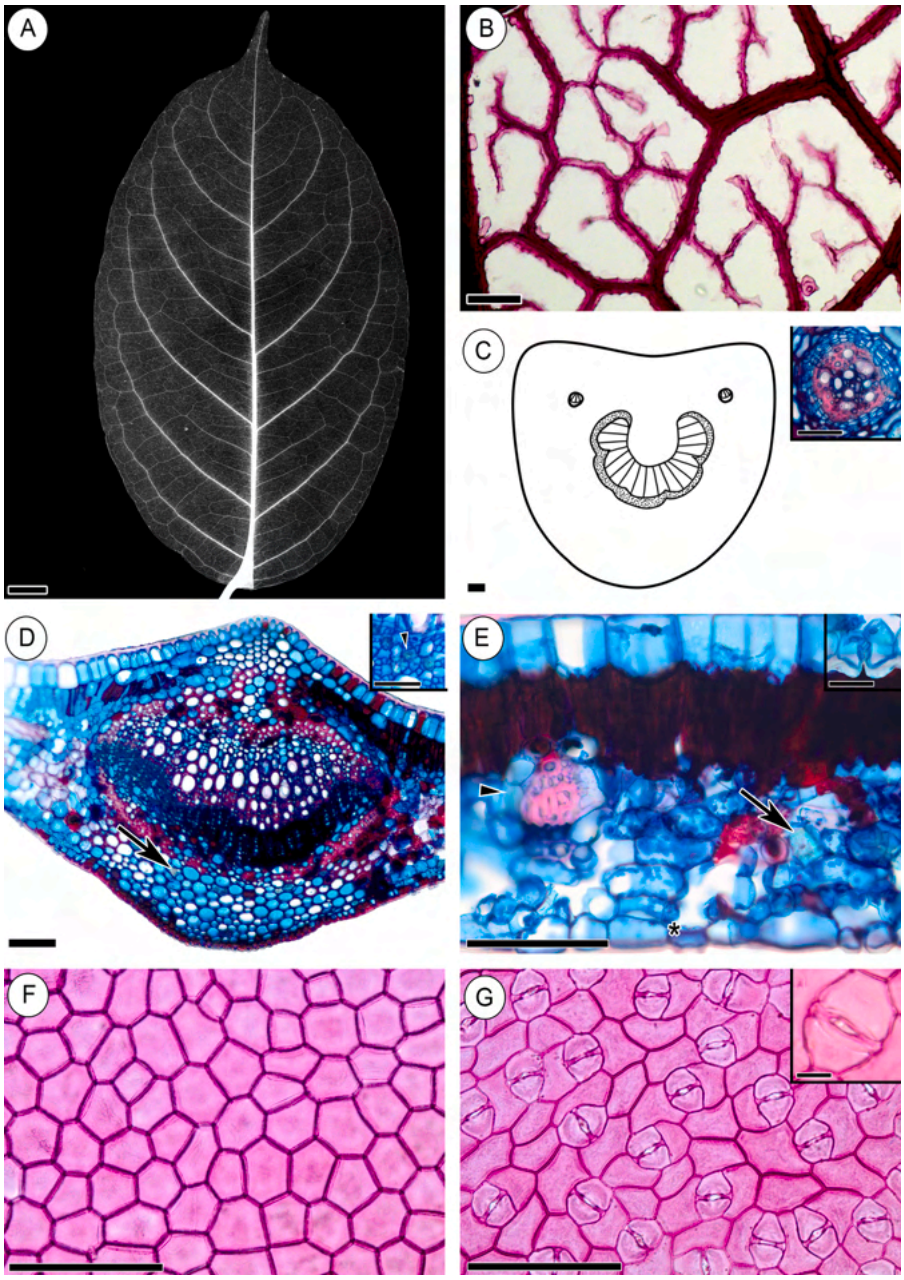

Fig. 11. *Diplopterys pubipetala* leaf. (A) Radiographic image: leaf shape and general venation pattern. (B) Vein detail: well-developed areoles. (C) Diagram of the petiole cross-section and detail of the concentric amphicribal accessory bundle. (D) Midrib, TS: druses in the midvein cortex (arrows) and phloem (detail, arrowhead); (E) Leaf blade, TS: adaxial epidermis 2-3 times taller than abaxial; abundant idioblasts; prismatic crystal in the spongy parenchyma (arrow); well-developed sheath in the minor vein (arrowhead); stomata located in the abaxial epidermis, with subsidiary cells with ledges (* and detail). (F-G) Paradermal preparations: (F) Epidermal cells in the adaxial face, with straight anticlinal walls; (G) Epidermal cells in the abaxial face, with curved walls; detail of paracytic stomata. Dot-filled areas = phloem; stripped areas = xylem. TS: transverse sections. Scale bars: (A) 1cm; (B-G) 100 μm; (C-detail) 50 μm; (D-E, details) 50 μm; (G-detail) 10 μm.

many useful effects, ranging from antibacterial, antifungal and insecticidal to cosmetic applications (Bakkali et al., 2008; Miller et al., 2015). Few tests have been performed to investigate essential oils in different organs (such as flowers by Rocha et al. (2018) and leaves by Araújo and Meira (2016)) of *Banisteriopsis* and related species. We here suggest that some focus should also be given to the bark when studying essential oils in this genus.

Gelatinous fibres had almost no reaction or staining. Mellerowicz and Gorshkova (2012) explain that the crystalline cellulose in the G-layer of gelatinous fibres hardly ever gets stained. In our ruthenium red test, the slight pink staining of this layer corroborated their speculation that spaces between these cellulose macrofibrils were occupied by pectins and proteins. In addition, the abundance of gelatinous fibres in these lianas could be of great value if we acknowledge the novel technologies that may use them, such as in biofuel making, saccharification processes and development of biomimetic materials (Mellerowicz and Gorshkova, 2012).

4.3. Leaf analysis – an ally to species distinction

Regarding leaf features, the most distinctive characters were its general shape as well as aspects and distribution of trichomes and glands. These latter have been mentioned in the Malpighiaceae family and are important for taxonomical identification of genus and species within it (Metcalfe and Chalk, 1950; Gates, 1982; Elias, 1983; Cronquist, 1988).

While *D. pubipetala* and *B. laevifolia* gland shapes corroborate previous works, there seems to be no accordance in relation to *B. muricata* (Araújo, 2014; Possobom et al., 2010). Araújo (2014) claims that the glands are sessile in *B. muricata*, as we found; nevertheless, Nery et al. (2017) describe them as pedunculated. In the collection of UB Herbarium, both morphotypes can indeed be found, raising the query of whether this would be just an expected variation, as it has a wide distribution among different biomes, or if two distinct plants are being called by the same name.

Gates (1982) values the gland position as a taxonomic character. This

Table 6
Leaf morphological features of studied species.

Character			Species		
			Bl	Bm	Dp
Petiole	Cross section	Plane-convex	X		
		Concave-convex	X	X	X
	Length (cm)		**0.24b**±0.1	**1.17c**±0.4	**0.71a**±0.1
Lamina	Size	Area (cm^2)	57b±3.3	54b±4.5	**78a**±13
	Length/width ratio	2:1	X	X	X
		3:1	X		
	Shape	Elliptic to narrow elliptic	X		
		Elliptic to large elliptic			X
		Narrow ovate to lanceolate	X		
		Ovate to wide ovate		X	
	Color	Discolor	X	X	X
	Midrib	Prominent on both faces	X	X	X
Apex	Angle	Acute	X	X	X
		Obtuse			X
	Shape	Strongly acuminate with tip drip			X
		Straight to slightly acuminate	X	X	
Base	Angle	Acute	X	X	X
		Obtuse		X	X
	Shape	Convex to rounded		X	X
		Cordate	X		
Glands	Position	Basilaminar	X	X	
		Marginal			X
		Abaxial	X	X	X
	Size	Length (μm)	**816b**±82	**499c**±45	**247a**±64
		Width (μm)	**624b**±88	**409c**±29	**203a**±60
Indumentum	Adaxial	Sparse pubescent		X	
		Glabrous			X
		Glabrescent	X		
	Abaxial	Dense pubescent	X		
		Sparse pubescent		X	
		Glabrescent			X
	Malpighiaceous hair	Size: arms (μm)	**1482b**±257	296a±22	300a±41
		Size: stalk (μm)	53±25	-	-
		Arms with same size	X		
		Arms shape straight		X	X
		Arms shape twisted	X		

Notes: X = present; blank = absent; - = immensurable; mean ± standard deviation; Bl = *Banisteriopsis laevifolia*; Bm = *B. muricata*; Dp = *Diplopterys pubipetala*; bold = variants according to Tukey's test (5%) in analysis of variance tests, equal letters indicate statistical similarities.

feature has been shown to be useful for distinguishing *D. pubipetala* (located all along the leaf border, Fig. 1D) from the other studied species. Also, its glands are significantly smaller than other *Banisteriopsis* species. Elias and Gelband (1976) suggested that minute glands can be compensated for their abundance, conferring an advantage as they increase the area visited and protected by ants, which could be the case for *D. pubipetala*. In addition, Levin (1973) concluded that pubescence in leaves could hamper the passage of insects; this could explain in some extension the position of glands near the margin and near the base of leaves on the studied species and other Malpighiaceae, as these areas would be more easily accessible.

In the matter of trichomes, Gates (1982) defines "malpighiaceous (T shaped)" hairs as unicellular and medifixed, often slightly eccentrically attached in *Banisteriopsis*. In the present work, in *B. laevifolia*, the trichomes are strictly T-shaped with twisted arms and very long and centrally positioned stalks with a peculiar radiate arrangement of pedal cells that might be of taxonomic value. In the other species, trichomes were significantly eccentric, with almost no stalk and without pedal cells. This morphological variance is expected within *Banisteriopsis* genus, which used to include *Diplopterys pubipetala* as well (Metcalfe and Chalk, 1950; Gates, 1982; Cronquist, 1988).

The difference in overall pubescence can further be influenced by the environment in which the specimens were collected. While *B. muricata*, collected in the shade of the Amazon forest, has sparse pubescence, *B. laevifolia* from an open Cerrado has abundant trichomes on its abaxial surface, appearing discolor. It is well known that in drier environments, these structures are more prevalent and can reduce air movement on the leaf surface, thereby decreasing transpiration rates, besides reflecting radiation to prevent overheating and reducing insect herbivory (Ehleringer and Mooney, 1978; Fahn and Cutler, 1992).

Brochidodromous frameworks are common in several genera of Malpighiaceae, particularly in those of Cerrado (Mamede, 1993), and it was confirmed here. The remaining venation patterns are also quite stable among specimens, but the variation of the major secondary angle to midvein and areoles development were not the same for the studied specimens (Table 6), which could be helpful in other investigative works on the family.

Araújo (2014) and Metcalfe and Chalk (1979) agree that petiole characteristics are reliable for differentiating Malpighiaceae taxa, and our results supports them. Although the vascular system is divided at petiole base in *D. pubipetala* and *B. laevifolia*, it remains in separated bundles only in *B. muricata*, which is a diagnostic feature (Fig. 14C). In addition to the description made by Araújo (2014), we found that *B. laevifolia* can have both plane-convex and concave-convex outlines. Petiole length can also be quite distinctive, as claimed by Gates (1982), such as the short petioles we found in *B. laevifolia*. Also, the petiole of *D. pubipetala* showed more lignified cells, what would be predictable, as their leaves were thicker and broader, requiring more rigid tissues. The number of petiolar accessory bundles varied within the species of *Banisteriopsis* and *Diplopterys*, indicating that it is not a reliable taxonomic tool for distinguishing between these two genera, as also stated by Araújo (2014).

Anatomical characteristics have been evidenced as more distinctive than morphological ones. Cell wall plus cuticle are more than 1.5x thicker in the adaxial than abaxial epidermis in *B. laevifolia* and more than 2x in *D. pubipetala*, both collected in Cerrado (Figs. 12E, 13E).

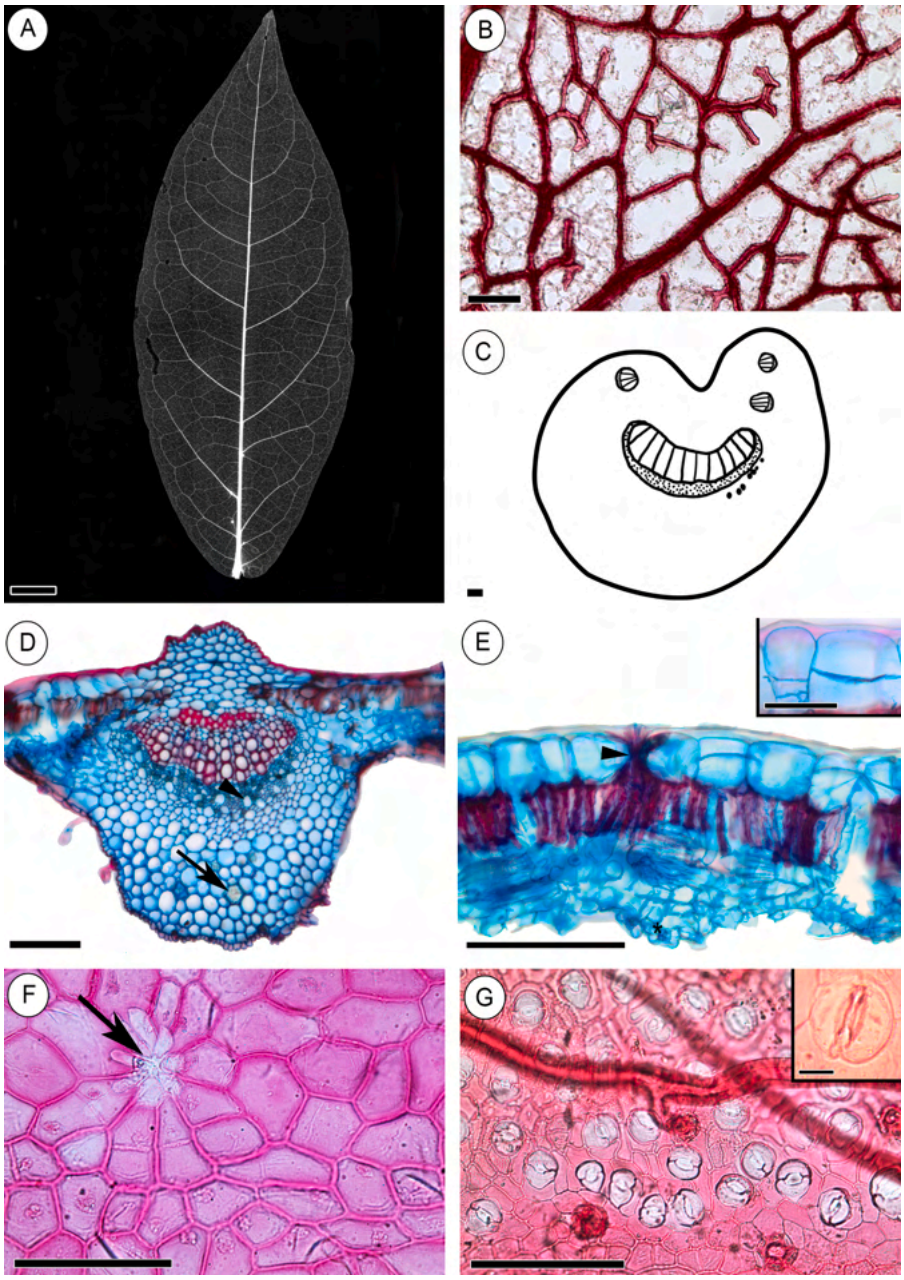

Fig. 12. *Banisteriopsis laevifolia* leaf. (A) Radiographic image: leaf shape and general venation pattern. (B) Vein detail: well-developed areoles. (C) Diagram of the petiole cross-section. (D) Midrib, TS: druses in the cortex (arrow) and phloem, idioblasts in the cortex (arrowhead). (E) Leaf blade, TS: stomata in adaxial epidermis (*) and lignified hair attachment (arrowhead); note abundant idioblasts; region of bistratified epidermis (detail). (F-G) Paradermal preparations: (F) Epidermal cells in the adaxial face, with straight to curved walls and trichome insertion region with radiate pedal cells (arrow); (G) Epidermal cells in the abaxial face, with straight to curved walls; paracytic stomata (detail). Dot-filled areas = phloem; stripped areas = xylem; dark areas = fibres. TS: transverse sections. Scale bars: (A) 1cm; (B-G) 100 μm; (D, detail) 50 μm; (G, detail) 10 μm.

However, it hardly exceeded 1.5x in *B. muricata* (Fig. 13E), collected in a much more humid environment (Amazon biome). The thickness of the epidermal cell wall and cuticle is inversely proportional to epidermal water loss (Ristic and Jenks, 2002); thus, this can explain the variation of this feature between humid and drier environments.

Large adaxial epidermal cells, as observed here, are not uncommon in Malpighiaceae family (Metcalfe and Chalk, 1950). Several other leaf features that we found followed descriptions for Malpighiaceae, such as paracytic stomata in the abaxial epidermis, dorsiventral mesophyll, druses or solitary-prismatic crystals and idioblasts in the mesophyll and arc-shaped vascular strands with accessory bundles in the petiole (Watson and Dallwitz, 1992; Metcalfe and Chalk, 1950). Druses were also found by Andrade (1997) in palisade parenchyma of *B. oxyclada*, which seem to be quite common in the genus.

Ledges formed by the cuticle on the guard cells were described in *Banisteriopsis* by Araújo (2014). They may aid ostiole closure as an additional sealing, possibly retaining water (Mauseth, 1988; Fricker and Willmer, 1996). We also found ledges in *B. laevifolia* (Fig. 12E) and *D. pubipetala* (Fig. 11E-detail), but in the subsidiary cells, which may also reduce water loss in their seasonally dry environment.

Two cell layers can occur in some leaf epidermal regions of *B. laevifolia*. This feature was also found in *Tetrapteris, Janusia* and other *Banisteriopsis* species, sometimes related to mucilaginous secretion (Metcalfe and Chalk, 1950; Andrade, 1997; Araújo, 2014).

Almost half of the parenchyma in *B. laevifolia* was palisade, while it represented 1/3 of the mesophyll for the other species. Fahn and Cutler (1992) affirm that it can conduct water to the epidermis more efficiently than spongy parenchyma. A greater area of this parenchymatous type could be an adaptative advantage in biomes like Cerrado, increasing photosynthetic area. This parenchyma almost invades the midvein for all three species, indicating a putative tendency to increase this area.

5. Conclusions

We were able to separate *Banisteriopsis laevifolia, B. muricata* and *Diplopterys pubipetala* both by wood and leaf anatomy and morphology.

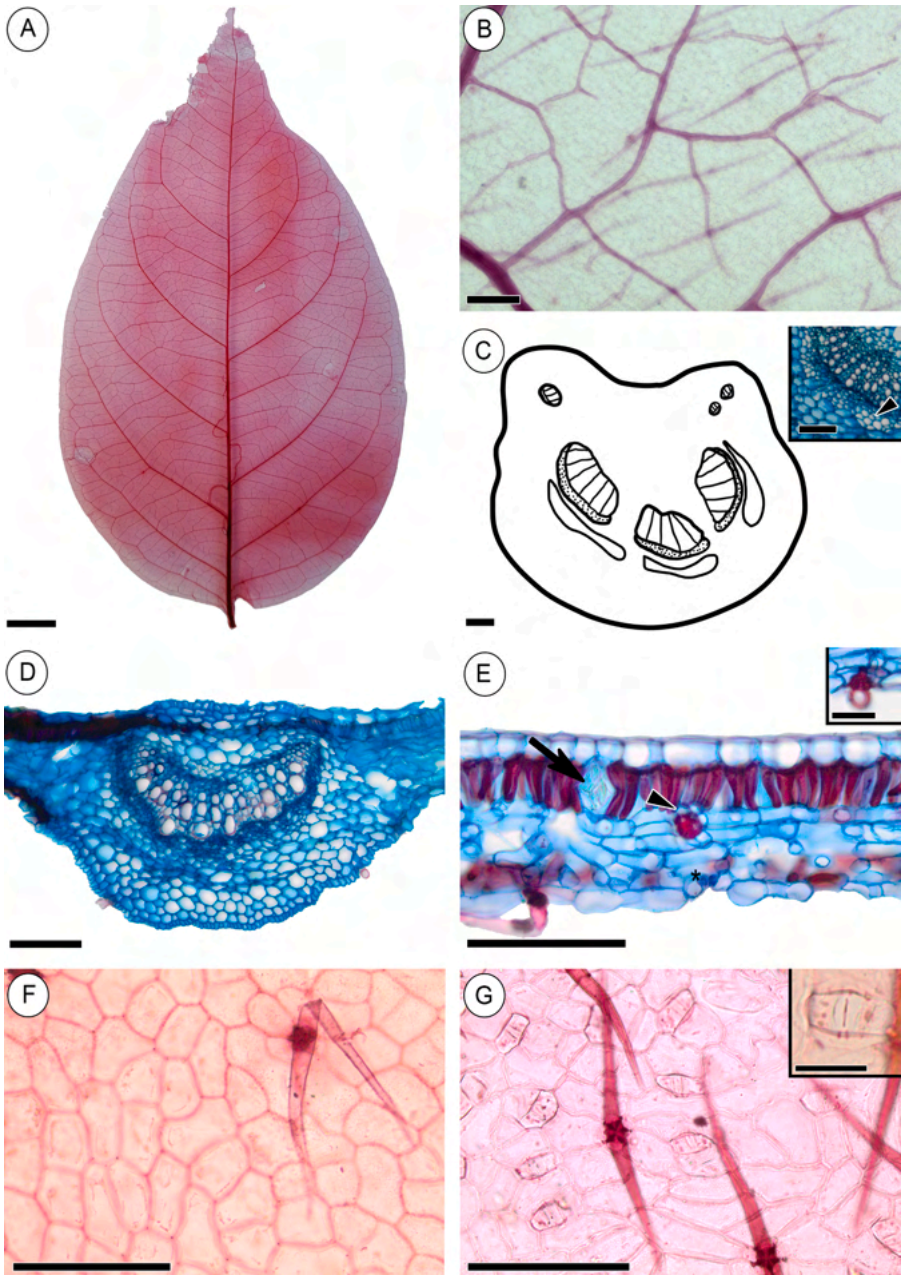

Fig. 13. *Banisteriopsis muricata* leaf. (A-B) Diaphanized leaf: (A) Leaf shape and general venation pattern; (B) Detail of moderately-developed areoles; note parallel pattern in trichomes. (C) Diagram of the petiole cross-section. (D) Midrib, TS. (E) Leaf blade, TS: mesophyll with prismatic crystals (arrow), vascular bundles with parenchymatic sheath (arrowhead), sunken stomata in adaxial epidermis (*); lignified insertion of hair with virtually no stalk (detail); note idioblasts. (F-G) Paradermal preparations: (F) Epidermal cells in the adaxial face, with straight to curved walls; (G) Epidermal cells in the abaxial face, with straight to curved walls; paracytic stomata (detail). TS: transverse sections. Dot-filled areas = phloem; stripped areas = xylem; blank area = non-lignified sclerenchyma. TS: transverse sections. Scale bars: (A) 1cm; (B-G) 100 μm; (D, detail) 50 μm; (G, detail) 10 μm.

Novel descriptions in wood anatomy are presented: the unique tangential alignment of prismatic crystals in ray cells in *D. pubipetala* and the horizontal alignment of vessel elements as viewed on radial section. The presence of alkaloids, mostly in parenchymatous tissues in the bark and, to a minor extent, in the wood of the studied species, may justify their use as alternatives to *B. caapi* in the Ayahuasca beverage preparation. Still, since there are many different types of alkaloids, further studies are needed to specify which alkaloids occur in each species and to ensure its safety of use.

CRediT authorship contribution statement

Nívea Nagamine-Pinheiro: Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing. **Christopher W. Fagg:** Investigation, Supervision, Writing - review & editing, Funding acquisition. **Sueli M. Gomes:** Conceptualization, Methodology, Writing - review & editing. **Regina C. Oliveira:** Conceptualization, Investigation, Writing - review & editing. **Júlia Sonsin-Oliveira:** Conceptualization, Methodology, Formal analysis, Investigation, Writing - review & editing, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank M.C.H. Mamede and R.F. Sebastiani for aid with identification, M. Tommasiello Filho and ESALQ for help with X-ray imaging. This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq (0193.000881/2015 – CWF and 130978/2017-5 – NNP).

References

Alves, E.S., Angyalossy-Alfonso, V., 2000. Ecological trends in the wood anatomy of some Brazilian species. 1. Growth rings and vessels. IAWA J 21, 3–30. https://doi.org/10.1163/22941932-90000233.

Amorim, A.M., Marinho, L.C., Pessoa, C., Pace, M.R., 2017. A new *Heteropterys* (Malpighiaceae) from semideciduous forest, with notes on wood anatomy. Plant Syst. Evol. 303, 177–185. https://doi.org/10.1007/s00606-016-1360-0.

Anderson, W.R., Davis, C.C., 2007. Generic adjustments in Neotropical Malpighiaceae. Contrib. from Univ. Michigan Herb. 25, 137–166.

Anderson, W.R., Davis, C.C., 2006. Expansion of *Diplopterys* at the expense of *Banisteriopsis* (Malpighiaceae). Harvard Pap. Bot. 11, 1–16. 10.3100/1043-4534(2006)95[1:EODATE]2.0.CO;2.

Andrade, R.M.C., 1997. Anatomia foliar e caulinar de 3 espécies de lianas pertencentes a família Malpighiaceae. MSc Dissertation, Universidade Estadual de São Paulo.

Angyalossy, V., Pace, M.R., Lima, A.C., 2015. Liana anatomy: a broad perspective on structural evolution of the vascular system. In: Schnitzer, S.A., Bongers, F., Burnham, R.J., Putz, F.E. (Eds.), Ecology of Lianas, Eds. John Wiley & Sons, Ltd, pp. 253–287.

Araújo, J.S., 2014. Anatomia de espécies de *Banisteriopsis* C. B. Rob. (Malpighiaceae) ocorrentes no Brasil.. Thesis, Universidade Federal de Viçosa.

Araújo, J.S., Azevedo, A.A., Silva, L.C., Meira, R.M.S.A., 2010. Leaf anatomy as an additional taxonomy tool for 16 species of Malpighiaceae found in the Cerrado area (Brazil). Plant Syst. Evol. 286, 117–131. https://doi.org/10.1007/s00606-010-0268-3.

Araújo, J.S., Meira, R.M.S.A., 2016. Comparative anatomy of calyx and foliar glands of *Banisteriopsis* C. B. Rob. (Malpighiaceae). Acta Bot. Brasilica 30, 112–123. https://doi.org/10.1590/0102-33062015abb0248.

Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M., 2008. Biological effects of essential oils – A review. Food Chem. Toxicol. 46, 446–475. https://doi.org/10.1016/j.fct.2007.09.106.

Caballé, G., 1993. Liana structure, function and selection: a comparative study of xylem cylinders of tropical rainforest species in Africa and America. Bot. J. Linn. Soc. 113, 41–60. https://doi.org/10.1111/j.1095-8339.1993.tb00328.x.

Cabanillas, P.A., Pace, M.R., Cabanillas, P.A., Angyalossy, V., 2017. Structure and ontogeny of the fissured stems of *Callaeum* (Malpighiaceae). IAWA J 38, 49–66. https://doi.org/10.1163/22941932-20170156.

Callaway, J.C., Brito, G.S., Neves, E.S., 2005. Phytochemical Analyses of *Banisteriopsis caapi* and *Psychotria viridis*. J. Psychoactive Drugs 37, 145–150. https://doi.org/10.1080/02791072.2005.10399795.

Carlquist, S., 1985. Observations on Functional Wood Histology of Vines and Lianas. Aliso 11, 139–157.

Costa, M.O.N., Santos, K.T., Almeida, C.A., Fonseca, F.S.A., Angolini, C.F.F., Oliveira, D. A., Júnior, A.F.M., Menezes, E.V., Royo, V.A., 2020. Chemical Composition of Diplopterys pubipetala (Malpighiaceae). European J. Med. Plants 31, 43–48. https://doi.org/10.9734/ejmp/2020/v31i1530323.

Cronquist, A., 1988. The Evolution and Classification of Flowering Plants, 2nd ed. New York Botanical Garden, Bronx.

Davis, C.C., Anderson, W.R., 2010. A complete generic phylogeny of Malpighiaceae inferred from nucleotide sequence data and morphology. Am. J. Bot. 97, 2031–2048. https://doi.org/10.3732/ajb.1000146.

Davis, E.W., Yost, J.A., 1983. Novel hallucinogens from eastern Ecuador. Bot. Museum Leafl. 97, 291–295.

Domingues, D.F., 2008. Anatomia foliar e caulinar do gênero *Heteropterys* Kunth (Malpighiaceae).. Thesis, Universidade Federal do Rio de Janeiro.

Ehleringer, J.R., Mooney, H.A., 1978. Leaf Hairs: Effects on Physiological Activity and Adaptive Value to a Desert Shrub. Oecologia 37, 183–200.

Elias, T.S., 1983. Extrafloral nectaries: their structure and distribution. In: Bentley, B., Elias, T. (Eds.), The Biology of Nectaries, Eds. Columbia University Press, New York, pp. 174–203.

Elias, T.S., Gelband, H., 1976. Morphology and anatomy of floral and extrafloral nectaries in *Campsis* (Bignoniaceae). Am. J. Bot. 63, 1349–1353. https://doi.org/10.1002/j.1537-2197.1976.tb13220.x.

Ellis, B., Daly, D.C., Hickey, L.J., Mitchell, J.V., Johnson, K.R., Wilf, P., Wing, S.L., 2009. Manual of leaf architecture. Cornell University Pres, New York.

Ewers, F.W., 1985. Xylem structure and water conduction in conifer trees, dicot trees and lianas. IAWA J 6, 309–317. https://doi.org/10.1163/22941932-90000959.

Ewers, F.W., Fisher, J.B., 1991. Why vines have narrow stems: Histological trends in *Bauhinia* (Fabaceae). Oecologia 88, 233–237. https://doi.org/10.1007/BF00320816.

Fahn, A., Cutler, D.F., 1992. Xerophytes, Encyclopedia of Plant Anatomy. Gebrüder Borntraeger, Berlin.

Figueiredo, A.C.S., Barroso, J.M.G., Ascensão, L.M.G.L., 2007. Histoquímica e citoquímica em plantas: princípios e protocolo, 1st ed. Faculdade de Ciências da Universidade de Lisboa, Centro de Biotecnologia Vegetal, Lisboa.

Figueiredo, C.F., 2011. Anatomia e identificação macroscópica das lianas da Reserva Florestal do Instituto de Biociências da Universidade de São Paulo, São Paulo-SP, Brasil. MSc Dissertation, Universidade de São Paulo.

Flora do Brasil, under construction [WWW Document], 2020. Jard. Botânico do Rio Janeiro. URL http://reflora.jbrj.gov.br (accessed 1.24.18).

Francener, A., Almeida, R.F., Sebastiani, R., 2018. Check-List de Malpighiaceae do Estado de Mato Grosso do Sul. Iheringia, Série Botânica 73, 264–272. https://doi.org/10.21826/2446-8231201873s264.

Franklin, G.L., 1945. Preparation of thin sections of synthetic resins and wood-resin Composites, and a new macerating method for Wood. Nature 155, 51. https://doi.org/10.1038/155051a0.

Frecska, E., Bokor, P., Winkelman, M., 2016. The therapeutic potentials of Ayahuasca: Possible effects against various diseases of civilization. Front. Pharmacol. 7, 35. https://doi.org/10.3389/fphar.2016.00035.

Frias, U.A., Costa, M.C.M., Takahashi, J.A., 2011. Caracterização fitoquímica e avaliação das atividades antibacteriana e anticolinesterásica de extratos de *Banisteriopsis anisandra* A. Juss. (Malpighiaceae). Rev. Cuba. Plantas Med. 16, 60–71.

Frias, U.A., 2012. *Banisteriopsis* species: a source of bioactive of potential medical application. Int. J. Biotechnol. Wellness Ind. 1, 163–171. https://doi.org/10.6000/1927-3037/2012.01.03.02.

Fricker, M., Willmer, C., 1996. Stomata, 2nd ed. Springer Science & Business Media, London.

Garrido, R.G., Sabino, B.D., 2009. Ayahuasca: entre o legal e o cultural. Saúde, Ética & Justiça 14, 44–53. https://doi.org/10.11606/issn.2317-2770.v14i2p44-53.

Gasson, P., Miller, R., Stekel, D.J., Whinder, F., Zieminska, K., 2010. Wood identification of *Dalbergia nigra* (CITES Appendix I) using quantitative wood anatomy, principal components analysis and naïve Bayes classification. Ann. Bot. 105, 45–56. https://doi.org/10.1093/aob/mcp270.

Gates, B., 1982. *Banisteriopsis, Diplopterys* (Malpighiaceae). Flora Neotrop. Monogr. 30, 1–236.

Gerwing, J.J., Schnitzer, S.A., Burnham, R.J., Bongers, F., Chave, J., DeWalt, S.J., Ewango, C.E.N., Foster, R., Kenfack, D., Martínez-Ramos, M., Parren, M., Parthasarathy, N., Pérez-Salicrup, D.R., Putz, F.E., Thomas, D.W., 2006. A Standard Protocol for Liana Censuses 1. Biotropica 38, 256–261. https://doi.org/10.1111/j.1744-7429.2006.00134.x.

Ghosal, S., Mazumder, U.K., 1971. Alkaloids of the leaves of *Banisteriopsis argentea*. Phytochemistry 10, 2840–2841.

Gourlay, I.D., 1995. The definition of seasonal growth zones in some African Acacia species - A Review. IAWA J 16, 353–359. https://doi.org/10.1163/22941932-90001425.

Guilhon-Simplicio, F., Souza, T.P., Almeida, P.D.O., Alex, P., Ohana, D.T., Lima, E.S., De Meneses Pereira, M., 2013. Antioxidant activity of a standardized extract of *Byrsonima japurensis* A. Juss. (Malpighiaceae) stem bark. J. Med. Plants Res. 7, 1926–1930. https://doi.org/10.5897/JMPR12.1133.

Guimarães, A.L.A., Costa, R.P.C., Cabral, L.M., Vieira, A.C.M., 2016. Comparative anatomy and chemical analysis of the vegetative organs of three species of *Stigmaphyllon* (Malpighiaceae). Flora 224, 30–41. https://doi.org/10.1016/j.flora.2016.07.001.

Hickey, L.J., 1973. Classification of the Architecture of Dicotyledonous Leaves. Am. J. Bot. 60, 17. https://doi.org/10.2307/2441319.

IAWA Committee, 1989. IAWA list of microscopic features for hardwood identification. IAWA Bull. n.s 10, 219–332. https://doi.org/10.2307/4110625.

Isnard, S., Feild, T.S., 2014. The evolution of angiosperm lianescence: a perspective from xylem structure-function. In: Schnitzer, S.A., Bongers, F., Burnham, R.J., Putz, F.E. (Eds.), Ecology of Lianas, Eds. John Wiley & Sons, Ltd, Chichester, pp. 221–238. https://doi.org/10.1002/9781118392409.ch17.

Johansen, S.A., 1940. Plant microtechnique. Mcgraw Hill Book Co, New York.

Kraus, J.E., Arduin, M., 1997. Manual básico de métodos em morfologia vegetal. EDUR, Seropédica.

Labate, B.C., Araújo, W.S., 2002. O Uso Ritual da Ayahuasca, 1st ed. Mercado das Letras, Campinas.

Leaf Architecture Working Group, 1999. Manual of Leaf Architecture: Morphological Description and Categorization of Dicotyledonous and Net-Veined Monocotyledonous Angiosperms, 1st ed. Smithsonian, Washington.

Levin, D.A., 1973. The Role of Trichomes in Plant Defense. Q. Rev. Biol. 48, 3–15. https://doi.org/10.1086/407484.

Lima, A.C., Pace, M.R., Angyalossy, V., 2010. Seasonality and growth rings in lianas of Bignoniaceae. Trees 24, 1045–1060. https://doi.org/10.1007/s00468-010-0476-z.

Lunna, L.E., 2011. Indigenous and mestizo use of ayahuasca: an overview. In: Santos, R. G. (Ed.), The Ethnopharmacology of Ayahuasca, Ed. Transworld Research Network, Kerala, pp. 1–21.

Mamede, M.C.H., 1993. Anatomia dos órgáos vegetativos de *Camarea* (Malpichiaceae). Acta Bot. Brasilica 7, 3–19. https://doi.org/10.1590/S0102-33061993000100001.

Matsushima, U., Hilger, A., Graf, W., Zabler, S., Manke, I., Dawson, M., Choinka, G., Herppich, W.B., 2012. Calcium oxalate crystal distribution in rose peduncles: Non-invasive analysis by synchrotron X-ray micro-tomography. Postharvest Biol. Technol. 72, 27–34. https://doi.org/10.1016/j.postharvbio.2012.04.013.

Mauseth, J.D., 1988. Plant Anatomy, 1st ed. The Blackburn Press, California.

McKenna, D.J., 2004. Clinical investigations of the therapeutic potential of ayahuasca: rationale and regulatory challenges. Pharmacol. Ther. 102, 111–129. https://doi.org/10.1016/j.pharmthera.2004.03.002.

Mellerowicz, E.J., Gorshkova, T.A., 2012. Tensional stress generation in gelatinous fibres: a review and possible mechanism based on cell-wall structure and composition. J. Exp. Bot. 63, 551–565. https://doi.org/10.1093/jxb/err339.

Meneguetti, D.U.O., Meneguetti, N.F.S., 2014. Benefícios à saúde ocasionados pela ingestão da ayahuasca: contexto social e ação neuropsicológica, fisioimunológica, microbiológica e parasitária. Cad. Bras. Saúde Ment. 6, 104–121.

Metcalfe, C.R., Chalk, L., 1983. Anatomy of the Dicotyledons: Wood Structure and Conclusion of the General Introduction, 2nd ed. Clarendon Press, Oxford.

Metcalfe, C.R., Chalk, L., 1979. Anatomy of the Dicotyledons. Clarendon Press, Oxford.

Metcalfe, C.R., Chalk, L., 1950. Anatomy of the Dicotyledons: Leaves, Stems, and Wood in Relation to Taxonomy with Notes on Economic Uses. Clarendon Press, Oxford.

Miller, A.B., Cates, R.G., Lawrence, M., Soria, J.A.F., Espinoza, L.V., Martinez, J.V., Arbizú, D.A., 2015. The antibacterial and antifungal activity of essential oils extracted from Guatemalan medicinal plants. Pharm. Biol. 53, 548–554. https://doi.org/10.3109/13880209.2014.932391.

Milugo, T.K., Omosa, L.K., Ochanda, J.O., Owuor, B.O., Wamunyokoli, F.A., Oyugi, J.O., Ochieng, J.W., 2013. Antagonistic effect of alkaloids and saponins on bioactivity in the quinine tree (*Rauvolfia caffra* sond.): further evidence to support biotechnology in traditional medicinal plants. BMC Complement. Altern. Med. 13, 285. https://doi.org/10.1186/1472-6882-13-285.

Nery, L.A., Vieira, M.F., Ventrella, M.C., 2017. Leaf glands of *Banisteriopsis muricata* (Malpighiaceae): distribution, secretion composition, anatomy and relationship to visitors. Acta Bot. Brasilica 31, 459–467. https://doi.org/10.1590/0102-33062017abb0108.

Oliveira, R.C., Fagg, C.W., Labate, B.C., Sonsin-Oliveira, J., 2018. The urgent need to review the botanical classification of the Ayahuasca vine [WWW Document]. URL https://chacruna.net/urgent-botanical-classification-ayahuasca/ (accessed 11.30.18).

Ott, J., 1994. Ayahuasca analogues: Pangæan entheogens, 1st ed. Natural Products Company, Kennewick.

Pace, M.R., Acevedo-Rodríguez, P., Amorim, A.M., Angyalossy, V., 2018. Ontogeny, structure and occurrence of interxylary cambia in Malpighiaceae. Flora 241, 46–60. https://doi.org/10.1016/j.flora.2018.02.004.

Pace, M.R., Lohmann, L.G., Angyalossy, V., 2009. The rise and evolution of the cambial variant in Bignonieae (Bignoniaceae). Evol. Dev. 11, 465–479. https://doi.org/10.1111/j.1525-142X.2009.00355.x.

Pace, M.R., 2015. Evolution of the vascular system in lineages that contain lianas. Thesis, Universidade de São Paulo.

Paiva, J.G.A., Fank-de-Carvalho, S.M., Magalhães, M.P., Graciano-Ribeiro, D., 2006. Verniz vitral incolor 500®: uma alternativa de meio de montagem economicamente viável. Acta Bot. Brasilica 20, 257–264. https://doi.org/10.1590/S0102-33062006000200002.

Pereda-Miranda, R., Taketa, A.T.C., Villatoro-Vera, R.A., 2007. Alucinógenos naturais: etnobotânica e psicofarmacologia. In: Simões, C.M.O., Schechenkel, E.P., Gosmann, G., Mello, J.C.P., Mentz, L.A., Petrovick, P.R. (Eds.), Farmacognosia: Da Planta Ao Medicamento, Eds. Editora da UFSC, Florianópolis, pp. 919–958.

Possobom, C.C.F., Guimarães, E., Machado, S.R., 2010. Leaf glands act as nectaries in *Diplopterys pubipetala* (Malpighiaceae). Plant Biol 12, 863–870. https://doi.org/10.1111/j.1438-8677.2009.00304.x.

Queiroz, M.M.F., Marti, G., Queiroz, E.F., Marcourt, L., Castro-Gamboa, I., Bolzani, V.S., Wolfender, J.-L., 2015. LC-MS/MS Quantitative determination of *Tetrapterys mucronata* alkaloids, a plant occasionally used in Ayahuasca Preparation. Phytochem. Anal. 26, 183–188. https://doi.org/10.1002/pca.2548.

Ray, R.R., Lassiter, K.S., 2016. Ayahuasca treatment center safety for the Western seeker. Anthropol. Conscious. 27, 121–150. https://doi.org/10.1111/anoc.12060.

Ribeiro, J.E.L.S., Hopkins, M.J.G., Vicentini, A., Sothers, C.A., S. Costa, M.A., Brito, J.M., Souza, M.A.D., Martins, L.H.P., Lohmann, L.G., Assuncao, P.A.C.L., Pereira, E.C., Silva, C.F., Mesquita, M.R., Procopio, L.C., 2002. Flora da Reserva Ducke. Guia de identificação das plantas vasculares de uma floresta de terra-firme na Amazonia Central. Kew Bull 57, 238. https://doi.org/10.2307/4110841.

Ristic, Z., Jenks, M.A., 2002. Leaf cuticle and water loss in maize lines differing in dehydration avoidance. J. Plant Physiol. 159, 645–651. https://doi.org/10.1078/0176-1617-0743.

Rocha, E.O., Cunha, L.C.S., Silva, M.V.S.G., Freitas, T.R., Nascimento, E.A., Silva, L.O., Martins, C.H.G., Aquino, F.J.T., Chang, R., Morais, S.A.L., 2018. Composição química e atividade antimicrobiana do óleo essencial das flores de *Banisteriopsis campestris* (A. Juss.) Little. Rev. Virtual Quim. 10, 1562–1577.

Rodrigues, V.E.G., Carvalho, D.A., 2001. Levantamento etnobotânico de plantas medicinais no domínio cerrado na região do Alto Rio Grande - Minas Gerais. Ciência e Agrotecnologia 25, 102–123.

Santos, K.T., Sacramento, V.D.M., Royo, V.A., Oliveira, D.A., Menezes, E.V., 2015. Perfil fitoquímico das folhas e casca de *Banisteriopsis pubipetala* com potencial para uso fitoterápico. 9°Fórum de Ensino Pesquisa e Extensão da Universidade Estadual de Montes Claros, Montes Claros.

Santos, R.G., Landeira-Fernandez, J., Strassman, R.J., Motta, V., Cruz, A.P.M., 2007. Effects of ayahuasca on psychometric measures of anxiety, panic-like and hopelessness in Santo Daime members. J. Ethnopharmacol. 112, 507–513. https://doi.org/10.1016/j.jep.2007.04.012.

Sass, J.E., 1958. Elements of botanical microtechnique, 3o. ed. McGraw-Hill Book Company Inc., New York-London.

Schultes, R.E., 1994. The Importance of ethnobotany in environmental conservation. Am. J. Econ. Sociol. 53, 202–206. https://doi.org/10.1111/j.1536-7150.1994.tb02586.x.

Schultes, R.E., 1988. Ethnopharmacological conservation: a key to progress in medicine. Acta Amaz 18, 393–406. https://doi.org/10.1590/1809-43921988185406.

Schultes, R.E., Hofmann, A., Rätsch, C., 2001. Plants of the Gods: their sacred, healing, and hallucinogenic powers, 2nd ed. Healing Arts Press, Rochester.

Schweingruber, F.H., Börner, A., Schulze, E.-D., 2008. Atlas of Woody Plant Stems: Evolution, Structure, and Environmental Modifications, 2o. ed. Springer-Verlag, Berlin-Heidelberg. 1010079783540325253.

Serrano-Dueñas, M., Cardozo-Pelaez, F., Sánchez-Ramos, J.R., 2001. Effects of *Banisteriopsis caapi* extract on Parkinson's disease. Sci. Rev. Altern. Med. 5, 127–132.

Silva, M.S., Funch, L.S., Silva, L.B., 2019. The growth ring concept: seeking a broader and unambiguous approach covering tropical species. Biological Reviews 94, 161–178. https://doi.org/10.1111/brv.12495.

Sneath, P.H.A., Sokal, R.R., 1973. Numerical taxonomy: the principles and practice of numerical classification. W H Freeman & Co., San Francisco.

Solereder, H., 1908. Systematic Anatomy of the Dicotyledons. Clarendon Press, Oxford.

Sonsin, J.O., Gasson, P.E., Machado, S.R., Caum, C., Marcati, C.R., 2014. Atlas da diversidade de madeiras do cerrado paulista, first, ed. Fundação de Estudos e Pesquisas Agrícolas e Florestais, Botucatu.

Souto, L.S., Oliveira, D.M.T., 2012. Pericarp structure in *Banisteriopsis* C.B.Rob. and *Diplopterys* A.Juss. (Malpighiaceae): new data supporting generic segregation. Acta Bot. Brasilica 26, 527–536. https://doi.org/10.1590/S0102-33062012000300003.

Strittmatter, C.G.D., 1973. Nueva Técnica de diafanizacion, 15. Boletín da Soc. Argentina Botánica, pp. 126–129.

Teixeira, D.C., Quinteiro, M.M.C., Baptista, A.A., Silva, J.G., 2008. Uso e manejo de plantas ritualísticas na comunidade de Santo Daime em Galdinópolis, Nova Friburgo/RJ. Brasil. Rev Ciên Vida 28, 63–74.

The plant list [WWW Document], 2013. URL http://www.theplantlist.org (accessed 8.4.20).

Tupper, K.W., 2008. The globalization of ayahuasca: Harm reduction or benefit maximization? Int. J. Drug Policy 19, 297–303. https://doi.org/10.1016/j.drugpo.2006.11.001.

Walter, B.M., Fagg, C.W., 2015. In: Eisenlohr, P.V, Felfili, J.M., Fiuza-de-Melo, M., Andrade, L., Meira-Neto, J. (Eds.), Eds. Fitossociologia No Brasil Métodos e Estudos de Casos. Universidade Federal de Viçosa, Viçosa, pp. 13–30.

Wang, Y.-H., Samoylenko, V., Tekwani, B.L., Khan, I.A., Miller, L.S., Chaurasiya, N.D., Rahman, M.M., Tripathi, L.M., Khan, S.I., Joshi, V.C., Wigger, F.T., Muhammad, I., 2010. Composition, standardization and chemical profiling of *Banisteriopsis caapi*, a plant for the treatment of neurodegenerative disorders relevant to Parkinson's disease. J. Ethnopharmacol. 128, 662–671. https://doi.org/10.1016/j.jep.2010.02.013.

Watson, L., Dallwitz, M.J., n.d. The families of Flowering Plants: descriptions, illustrations, identification, and information retrieval. [WWW Document], 1992 onwards. URL http://biodiversity.uno.edu/delta/ (accessed 10.14.2018).

Zenid, G.J., Ceccantini, G.C.T., 2007. Identificação macroscópica de madeiras. IPT, São Paulo.