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Leaf glands of *Banisteriopsis muricata* (Malpighiaceae): distribution, secretion composition, anatomy and relationship to visitors

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ABSTRACT

Leaf glands are common structures in Malpighiaceae and exhibit great morphological diversity, yet information on their anatomy, secretion and type of visitors remains scarce. The aim of this study was to describe the distribution, anatomical development and chemical and functional properties of leaf glands of *Banisteriopsis muricata* (Malpighiaceae). Leaves at different stages of development were collected and processed according to standard techniques for light and scanning electron microscopy. Secretion composition was determined by histochemical tests and test-strips, while gland funciton was determined by field observation of interactions with visitors. Leaf glands were located on the petiole and on the abaxial base of the leaf blade. The gland secretion was found to be a protein-rich nectar that was foraged upon by ants (*Solenopsis*); it was found accumulated in subcuticular spaces without pores or stomata for its release. Leaf glands were found to develop from protoderm and ground meristem, and consisted of typical secretory epidermis, nectariferous parenchyma and vascularized subnectariferous parenchyma. Therefore, it can be concluded that the distribution, chemical nature of secretion and anatomy of leaf glands of *B. muricata* characterize them as EFNs, while foraging by ants indicate a mutualistic relationship that possibly protects the plant against herbivores.

Keywords: anatomy, ants, extrafloral nectaries (EFNs), histochemistry, leaf glands, nectar, ontogeny

Introduction

The presence of secretory structures in vegetative and reproductive organs is common in the family Malpighiaceae (Anderson 1979; 1990). In leaves, glands usually occur on the petiole and/or the abaxial surface of the blade, while in flowers oil-producing glands can occur on the sepals (Judd *et al.* 1999). These calyx glands are present in most Neotropical species, but are only vestigial or absent in most Paleotropical species (Anderson 1979; 1990; Judd *et al.* 1999). Within Malpighiaceae leaf glands are commonly known as extrafloral nectaries (EFNs), while calyx glands have been recognized as elaiophores (Vogel 1990). These EFNs secrete sugar solution and are generally related to the attraction of patroller insects, predominantly ants (Fahn 1979; Elias 1983; Nepi 2007). Elaiophores, on the other hand, secrete non-volatile oils (Anderson 1990; Vogel 1990) and are related to the attraction of specific bee pollinators of the tribe Centridini, which are highly specialized in oil collection (Anderson 1979; Buchmann 1987).

Extrafloral nectaries and elaiophores can be of taxonomic value in identifying genera (Anderson 1990)

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and species (Gates 1982; Machado *et al.* 2008) of the family Malpighiaceae. These glands are widely common, morphological diverse and important in ecological interactions in Neotropical and Paleotropical species of Malpighiaceae (Anderson 1990). Extrafloral nectaries, which usually occur in pairs at the base of the leaf blade and petiole, are positioned analagously to elaiophores in sepals. The morphoanatomical similarity and analogous position between EFNs and elaiophores in Malpighiaceae indicate that these two secretory structures are homologous (Anderson 1990). Although the leaf glands of Malpighiaceae have been described as EFNs because of the predominance of secretion rich in sugars (Anderson 1990; Possobom *et al.* 2010), lipids were also identified in the secretion of leaf glands of *Galphimia brasiliensis* (Castro *et al.* 2001). This evidence supports the hypothesis of homology between EFNs and elaiophores and the importance of these secretory structures to understanding aspects of the phylogenetic relationships of Malpighiaceae (Castro *et al.* 2001).

Despite the great morphological diversity and the associated taxonomic and ecological value assigned to leaf glands in Malpighiaceae (Elias 1983), anatomical studies have been restricted to certain genera such as *Banisteriopsis* (Araújo & Meira 2016), *Heteropteris*, *Peixotoa* (Machado *et al.* 2008), *Galphimia* (Castro *et al.* 2001) and *Diplopterys* (Possobom *et al.* 2010). Moreover, aspects of the ontogenetic development of these leaf glands are unknown. In most cases, only the occurrence of these leaf glands is reported, as for *Banisteriopsis muricata* (Gates 1982). For this Neotropical species, a liana with a broad distribution among all biomes of Brazil (Mamede 2012), there remains a lack of information evaluating leaf glands from structural and functional points of view. Furthermore, the integrated study of the distribution, development and anatomy of these foliar glands, as well as of their secretion profile of and interactions with visitors, are important for better understanding the ecology of the species and its interactions with other organisms.

Considering the foregoing, the aim of this study was to characterize the leaf glands of *B. muricata* and its relationship with visitors in order to address the following questions: 1) What is the distribution, and the ontogenetic and structural patterns of these leaf glands? 2) What is the chemical profile of the secretion? 3) Who are the visitors of leaf glands and how does foraging occur?

Materials and methods

Plant material and collection area

Leaves at different developmental stages (leaf primordia to fully expanded leaves) were collected from five specimens of *Banisteriopsis muricata* (Cavanilles) Cuatrecasas in a natural population in Estação de Pesquisa, Treinamento e Educação Ambiental (EPTEA) Mata do Paraíso (20°48'08.4"S 42°51'50.9"W), a forest fragment located in Viçosa, state of Minas Gerais, Brazil. The vegetation of the area is defined as semideciduous forest (Veloso 1991), and is included within the Atlantic Forest domain (Rizzini 1997). A voucher specimen was deposited in the Herbarium of the Universidade Federal de Viçosa (VIC), under n. 36941.

Gland distribution

Ten leaves (from the 5th to 6th node) of five individuals of *B. muricata* (n=50) were collected in the field and the glands counted using a stereoscopic microscope (Zeiss, Göttinger, Germany), coupled to a digital camera (AxioCam ERc 5S, Zeiss, Göttinger, Germany) and an image capture program (AxioVision Rel. 4.8, Zeiss, Göttinger, Germany).

Light microscopy (LM)

Structural analysis

Shoot meristems and fragments of the base of petiole and leaf blade (from 1st to 5th node) were used to study anatomy and the development of leaf glands. The material was fixed in 2.5 % glutaraldehyde in phosphate buffer 0.05 M, pH 7 for 24 hours, dehydrated in an ethanol series and stored in 70 % ethanol (Johansen 1940). Subsequently, samples were dehydrated in an ascending ethanol series and embedded in methacrylate (Historesin, Leica, Heidelberg, Germany) according to Paiva *et al.* (2011). The samples were transverselly and longitudinally sectioned with a automatic rotary microtome (model RM2155, Leica Microsystems Inc., Deerfield, USA) at 5µm-thick, stained with toluidine blue, pH 4.4 (O'Brien *et al.* 1964) and mounted under cover slip with synthetic resin (Permount, Fisher Scientific, Pittsburgh, USA).

Histochemical analysis

To study the nature of the secretion, fresh or fixed leaf samples (5th node) were used and mature glands were sectioned using a table microtome (LPC, Rolemberg e Bhering Comércio e Importação Ltda, Belo Horizonte, Brazil). Methacrylate-embedded samples were also used and sectioned as described above. The following reagents were used to test the secretion: sudan black B (Pearse 1980), sudan IV (Johansen 1940), neutral red (Kirk 1970) and auramine O (Heslop-Harrison 1977) for lipids; Nile blue sulfate (Cain 1947) for acid and neutral lipids; NADI reagent (David & Carde 1964) for essential oils and oleoresins; ferric chloride (Johansen 1940) for total phenolics, Wagner reagent (Furr & Mahlberg 1981) for alkaloids, lugol (Johansen 1940) for starch, periodic acid-Schiff reagent (PAS) (McManus 1948) for neutral polysaccharides,

coriphosphine O (Ueda & Yoshioka 1976) for pectins and xylidine Ponceau (Vidal 1970) for proteins. A control was conducted simultaneously for each test, according to the specifications of each respective author.

Images were obtained with a light microscope (AX-70 TRF, Olympus Optical, Tokyo, Japan) coupled to a digital camera (Zeiss AxioCam HRc, Göttinger, Germany) and the Axion Vision image capture program. Fluorochrome analysis and autofluorescence were performed using the same equipment and a epifluorescence system with UV filter (WU: 340-380 nm), dichroic mirror (400 nm) and barrier filter (420 nm).

Scanning electron microscopy (SEM)

To observe the micromorphological characteristics of the leaf glands at different stages of development, fragments were fixed in 2.5 % glutaraldehyde as described above and dehydrated in an ethanol series, CO_2 dried to critical point (CPD 020, Bal-Tec, Balzers, Liechtenstein) and fixed on supports for metal deposition with gold (Sputter Coater equipment, FDU 010, Bal-Tec, Balzers, Liechtenstein). Observation and image capture were made using a Zeiss LEO 1430 VP scanning electron microscope (Cambridge, England).

Test-strip analysis of secretion

Branches of *B. muricata* were collected and kept in buckets with water and covered with plastic bags for 12 hours in the laboratory to prevent evaporation of secretion. Test-strips were used (Combur Test, Roche) to determine presence of glucose, nitrites and protein in the secretion.

Leaf glands visitors

During field sampling, observations were made throughout the day to determine the diversity and relationships of insect visitors to the studied species. The collection of visitors was carried out during the course of two weeks, with sampling in the morning (08 to 09 h) and afternoon (16 to 17 h), time periods when there were greater frequencies of insect visits to the leaves. The collected visitors were preserved in 70 % ethanol and identified by Julio Cezar Mario Chaul (Laboratório de Ecologia de Comunidades, Departamento de Entomologia, UFV).

Results

Location

Leaf glands of *B. muricata* were found located on the abaxial surface of the base of the leaf blade and on the petiole (Fig. 1A); they are minute (≤ 0.03mm), greenish and morphologically similar to one another. The number of glands (Fig. 1B) varies from one to thirteen on the abaxial side of the leaf blade, and none, one or two opposite glands on the petiole. The glands are pedunculated protuberances on the leaf and have dilated apical regions (Fig. 1C-D). Secretion may be present and observed as a translucent drop in the central zone of the gland (Fig. 1C).

Ontogeny

The glands of the leaf blade and petiole are similar in their development and anatomy. The initial structures of the glands appear early in leaf development. The glands develop on the abaxial surface of the leaf primordia, which have protoderm with cubical cells, ground meristem with polyhedral cells (Fig. 2A) and procambial strands. Initial protodermal cells with dense cytoplasm and prominent nuclei undergo anticlinal divisions, becoming juxtaposed in a columnar fashion (Fig. 2B). Concomitantly, the cells of the ground meristem undergo divisions in different planes, which permit the identification of the site of leaf gland formation (Fig. 2B). The continued proliferation of protoderm and ground meristem cells results in the elevation of the glandular primordium above the leaf surface (Fig. 2C-D). The apical portion of the gland becomes enlarged and initiates the differentiation of the glandular tissues (Fig. 2E). Differentiation of protodermal cells results in a uniseriate secretory epidermis that is restricted to the central region of the glands, and composed of elongated and overlapping cells with thin walls, dense cytoplasm and evident nuclei (Fig. 2E-F). The other epidermal cells of the gland are cuboid, less bulky and accumulate phenolic compounds (Fig. 2F). Ground meristem cells differentiate into nectariferous and subnectariferous parenchyma. The nectariferous parenchyma is located bellow the glandular epidermis and comprises three to four layers of isodiametric, voluminous and thin-walled cells with dense cytoplasm, diminished vacuoles and conspicuous nuclei (Fig. 2F). The subnectariferous parenchyma extends to the stalk of the gland and is composed of several layers of bulkier, thick-walled and vacuolated cells, and more conspicuous intercellular spaces (Fig. 2F). Xylem and phloem cells, arising from the branch of vascular bundles of the leaf, cross the subnectariferous parenchyma and border the nectariferous parenchyma (Fig. 2F-G). The secretory epidermis is glabrous, but leaf trichomes develop at the base of the glands and partially overlying the peduncle (Fig. 2G-H). Cells containing phenolic compounds are already present in the leaf primordia as well as in the nectariferous and subnectariferous parenchyma and associated with vascular tissues in the gland peduncle (Fig. 2F-G). Calcium oxalate druses are abundant in parenchyma cells around the vascular tissue of the gland (Fig. 2F-G). The secretion accumulates in subcuticular spaces of secretory epidermis (Fig. 2G). No stomata or pores are observed in the secretory

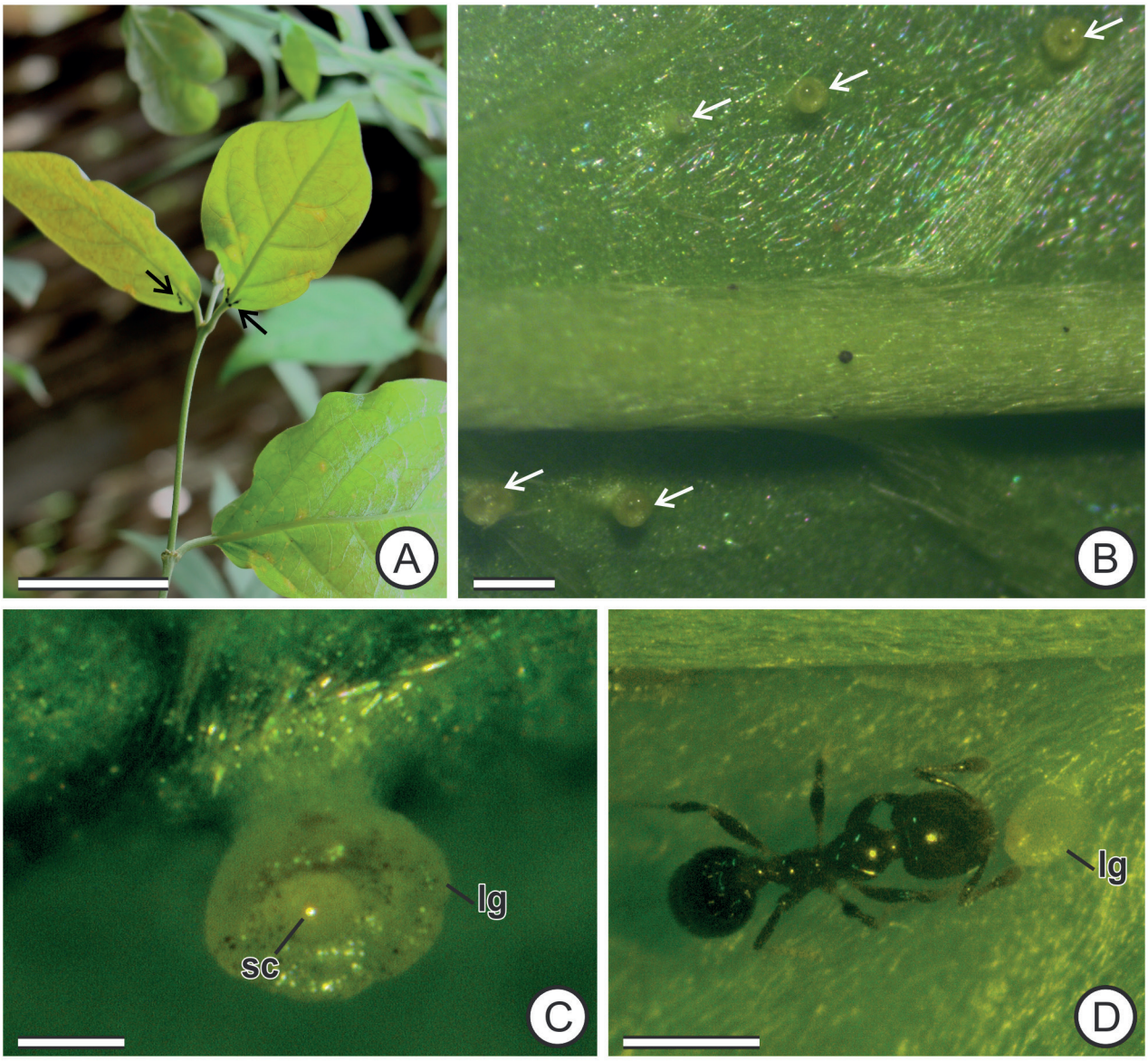

Figure 1. Distribution of leaf glands of *Banisteriopsis muricata* and its visitors. **A.** General view of a branch. Black arrows indicate the presence of ants. **B.** Detail of the basal third of the leaf blade. **C.** Gland of the petiole. **D.** Ant (*Solenopsis* sp.) foraging on a gland. White arrows indicate leaf glands. lg: leaf gland; sc: secretion. Scale bars = 100 mm (A), 0.05 mm (B, D), 0.01 mm (C).

epidermis (Fig. 2I-J), but it is possible to observe secretion deposited on the intact nectariferous surface (Fig. 2J).

Histochemistry and chemical features of secretion

The histochemical tests (Tab. 1) carried out on leaf glands confirm that the secretion accumulates in subcuticular spaces (Fig. 3). Fresh glands are green and possess chloroplasts throughout the nectariferous and subnectariferous parenchyma (not shown). Methacrylate-embedded glands not exposed to any reagent or dye exhibit translucent or slightly yellowish cells and translucent secretion (Fig. 3A). A thick cuticle is evidenced by autofluorescence (Fig. 3B), the black color of Sudan black B (Fig. 3C) and the yellow-green secondary fluorescence emitted by neutral red fluorochrome (Fig. 3D), but no lipids were identified in the subcuticular secretion. The secretory epidermal cells and subcuticular secretion possess pectins, highlighted by orange secondary fluorescence emitted by coriphosphine fluorochrome (Fig. 3E). Neutral polysaccharides were also found in the secretory epidermis and in the secretion, as shown by magenta staining with PAS (Fig. 3F). Polysaccharides in the secretory epidermis cells and secretion, as well as phenolic compounds in nectariferous parenchyma cells, were confirmed by purple and green coloration with toluidine blue, respectively (Fig. 3G). Proteins were identified by xylidine Ponceau in the secretory cells and mainly in secretion accumulated in the

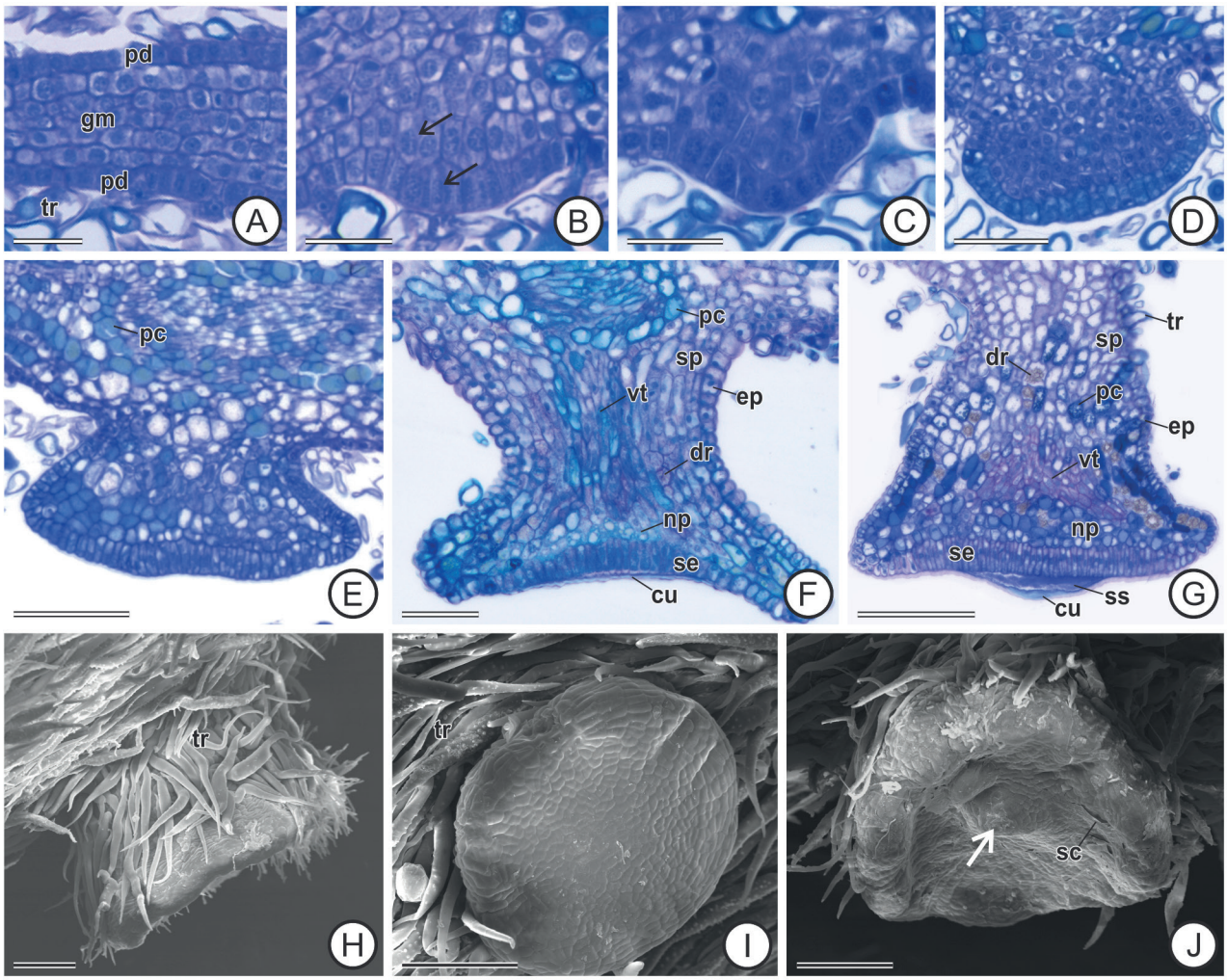

Figure 2. Ontogeny of the leaf glands of *Banisteriopsis muricata*. Photomicrographs of cross sections of leaf primordia and leaves stained with toluidine blue **(A-G)** and scanning electromicrographs **(H-J)**. **A-G, I-J**. Leaf blade. **H.** Petiole. **A.** Leaf primordium before the emergence of the first gland cells. **B.** Leaf primordium with the first cellular divisions in the protoderm and ground meristem (black arrows). **C-E.** Developing leaf glands with progressive increase in number and size of cells. **F-J.** Differentiated leaf glands. **G, J.** Differentiated leaf glands with the cuticle dilated by the accumulation of secretion (white arrow). **H-J.** Note that there are no stomata or pores in the secretory epidermis and trichomes only surround the glands. cu: cuticle; dr: druse; ep: epidermis; gm: ground meristem; np: nectariferous parenchyma; pc: phenolic cell; pd: protoderm; sc: secretion; se: secretory epidermis; sp: subnectariferous parenchyma; ss: subcuticular space; tr: trichome; vt: vascular tissue. Scale bars = 25 μm (A-D), 100 μm (E-J).

subcuticular space (Figure 3H). The test-strip analysis of the secretion confirmed the presence of proteins, as identified in the histochemical tests, and also indicated the presence of glucose. Therefore, the secretion of leaf glands is a mixture of glucose, proteins and pectins. The secretion produced in each EFN was so minimal that it was not possible to measure its volume.

Visitors

Ants of the genera *Solenopsis*, *Pheidole* and *Camponotus* were observed foraging leaves and branches of *B. muricata*. However, only individuals of *Solenopsis* were observed making direct contact with the glands (Fig. 1D). The behavior of these ants involves approaching the glands and projecting the front legs and antennae onto them, and then passing the appendages rapidly on the surface of the glands. Bristles were observed in the forepaws and the antenna, indicating that these structures are used to "scan" the surface of glands and collect secretion. After foraging, the legs and antennae touch, as if the ants are cleaning them, and then borugh to touch the mouthparts, which seems to be a behavior of deposition of the glandular secretion.

Discussion

The distribution of the leaf glands of *Banisteriopsis muricata* varies among different leaves, similar to that

Table 1. Histochemical characterization of the leaf glands of *Banisteriopsis muricata*.

Chemical compounds		Reagent	Reaction			
			Cuticle	Secretion	Secretory epidermis	Nectariferous parenchyma
Lipids		Sudan IV	+	-	-	-
		Sudan black B	+	-	-	-
		Neutral red	+	-	-	-
		Auramine O	+	-	-	-
Terpenoids		NADI reagent	+	-	-	-
Phenolic compounds		Ferric chloride	-	-	+	+
Alkaloids		Wagner reagent	-	-	-	-
Carbohydrates	Polysaccharides	PAS	-	++	+	-
	Starch	Lugol	-	-	-	-
	Pectins	Ruthenium red	-	+	+	-
		Coriphosphine O	-	++	+	+
Proteins		Xylidine Ponceau	-	++	+	+

+ positive reaction; - negative reaction.

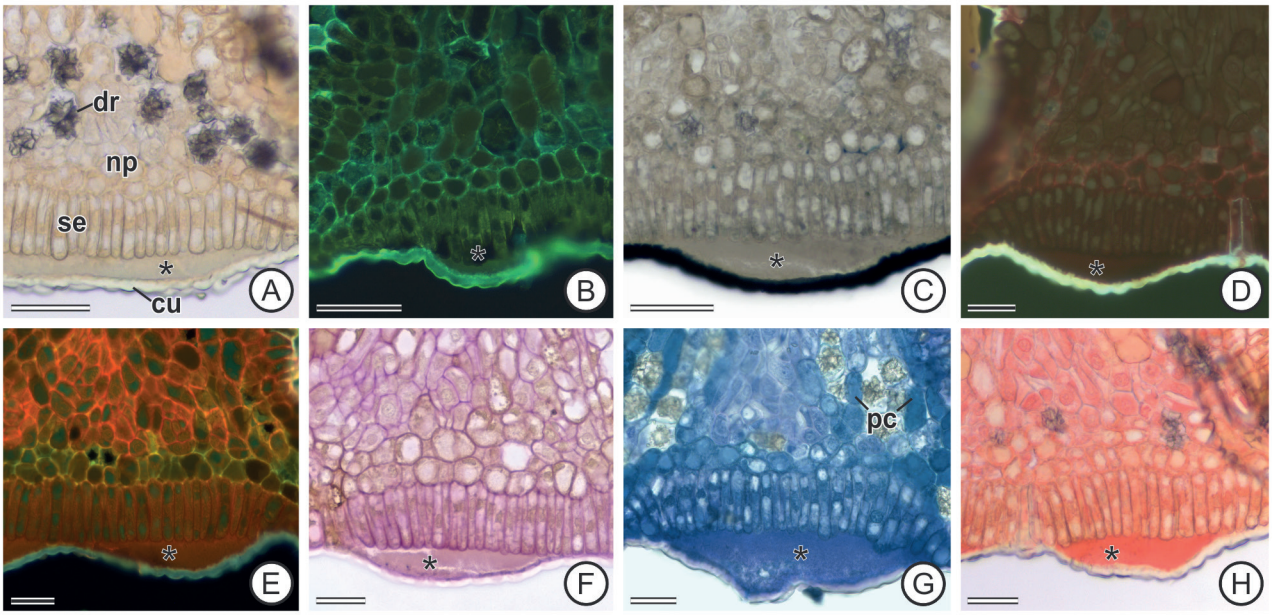

Figure 3. Leaf glands of *Banisteriopsis muricata* submitted to different histochemical tests. **(A-H)** Photomicrographs of methacrylate-embedded material. **A.** No application of dyes or reagents. **B.** Autofluorescence. **C.** Sudan black B; black color indicates lipids. **D.** Neutral red; yellow-green secondary fluorescence indicates lipids. **E.** Coriphosphine; orange secondary fluorescence indicates pectins. **F.** Periodic acid/Schiff reagent (PAS); magenta staining indicates neutral polysaccharides. **G.** Toluidine blue; purple color indicates polysaccharides. **H.** Xylidine Ponceau; reddish color indicates proteins. cu: cuticle; dr: druse; np: nectariferous parenchyma; pc: phenolic cell; se: secretory epidermis; * secretion. Scale bars = 25 μm. Please see the PDF version for color reference.

observed in other species of Malpighiaceae (Castro *et al.* 2001; Machado *et al.* 2008). The occurrence of eglandulated leaves and leaves with two to four pairs of glands in the basal third of leaf blade and petiole have been previously described for *B. muricata* (Gates 1982), but this was not exactly the pattern found in the present study. In addition to eglandulated leaves, the present study found *B. muricata* to also possess leaves with a highly variable number of glands (1-13), as in other Malpighiaceae (Anderson 1990). The difference between these accounts can be justified by the small size of the glands and the difficulty in observing them with the naked eye, as was done by Gates (1982).

The presence of numerous small glands, as in *B. muricata*, may represent an advantageous strategy compared to other species that have leaves with a small number of large glands. Some of these glands can be injured and lose functionality, and having a greater number can act as a compensatory mechanism (Subramanian & Inamdar 1985). Variaiton in

location and abundance of glands on the leaf blade favor ant patrolling across the entire leaf in search of nectar, and favoring organ protection against attack by herbivorous insects (Bentley 1977; Paiva & Machado 2006). In addition, the small volume of secretion of each leaf gland in *B. muricata* would also be compensated by the abundance of glands on the leaf, favoring the continued the production of secretion necessary to attract and reward ants (Paiva *et al*. 2007), and thus ensuring the protection of young leaves and buds.

These glands are cup-shaped with a short peduncle, a discoidal apical portion and a slightly concave secretory surface, which characterize them as "high type" according to the classification of Elias (1983). Similar morphology has been observed in the leaf glands of other species of *Banisteriopsis* (Machado *et al*. 2008; Araújo & Meira 2016), but in yet others the leaf glands are sessile (Machado *et al*. 2008). The leaf glands of *Banisteriopsis muricata* possess a secretory surface that is restricted to the central region of the gland, which is common in some species of *Banisteriopsis* (Araújo & Meira 2016) and *Galphimia brasiliensis* (Castro *et al*. 2001), but not in other Malpighiaceae, such as *Peixotoa reticulata* (Machado *et al*. 2008).

The secretion of leaf glands of *B. muricata* is a mixture of water, glucose, pectins and proteins. The presence of glucose in the secretion confirms the nectariferous nature of these glands (Bentley 1977; Fahn 1979). Thus, the leaf glands of *B. muricata* can be considered extrafloral nectaries (EFNs) and the secretion as nectar. Moreover, the absence of lipids in the secretion dismisses the possibility of these leaf glands acting as elaiophores, as suggested by Castro *et al*. (2001). These authors identified lipids in the secretion of the leaf glands of *Galphimia brasiliensis* by histochemical tests, but they did not provide images and so these results can not be verified.

Pectins and water form a mucilaginous phase and, consequently, increase the viscosity of nectar (Nepi 2007), which may be a mechanism for regulating secretion release (Paiva 2016). The proteins found in the nectar of EFNs of *B. muricata* may be crucial to the establishment of mutualistic relationships between individual plants and various animals (Fahn 1979; Roshchina & Roshchina 1993; Heil 2011).

The EFNs of *B. muricata* differentiate early in the leaf primordia and remain active in expanded leaves. This pattern of development seems to guarantee indirect protection against herbivores for an extended period of time, since the glands do not show damage after foraging by ants. The ants found on the EFNs of *B. muricata* forage the gland in search of sugars for adult nutrition and protein for the nutrition of the larvae (Bentley 1977). In *B. muricata*, only ants of the genus *Solenopsis*, attracted by nectars rich in sugars and amino acids (Lanza *et al.* 1993; Ness *et al.* 2010; Byk & Del-Claro 2011), were observed foraging on the EFNs. However, patrolling by ants of other genera that did not forage on the EFNs, such as *Pheidole* and *Camponotus*, could also act to protect the plant by increasing the period and frequency of patrolling (Bentley 1977).

The EFNs of *B. muricata* consist of secretory epidermis, nectariferous parenchyma and vascularized subnectariferous parenchyma, as is typical for EFNs of species of Malpighiaceae (Machado *et al.* 2008; Possobom *et al*. 2010; Araújo & Meira 2016). The accumulation of secretion in the subcuticular spaces and the absence of stomata, or any other type of opening to release the secretion of EFNs of *B. muricata*, suggest that the elimination of nectar occurs gradually via permeability of the cuticle or by its rupture after foraging by ants. Curiously, no EFNs were observed with ruptured cuticles in *B. muricata*, which favors the former release mechanism over the latter. There is also the possibility that hydrophilic microchannels occur in the cuticle and favor the release of predominantly hydrophilic secretions by the apoplastic pathway (Fahn 1988; Paiva 2016).

The nectariferous parenchyma of the EFNs of *B. muricata* is similar to that observed in other species of the genus *Banisteriopsis* (Araújo & Meira 2016) and other genera of the family Malpighiaceae, such as *Peixotoa* (Machado *et al*. 2008) and *Diplopterys* (Possobom *et al.* 2010). Dense cytoplasm and conspicuous nuclei are cytological features of the nectariferous parenchyma cells of *B. muricata*, and secretory cells in general, indicating high tissue metabolic activity (Fahn 1979). This suggests the participation of nectariferous parenchyma in the transformation of the solutions received from the vascular system that runs through the subnectariferous parenchyma to the final composition of the nectar (Fahn 1979; Nepi 2007). Both the nectariferous parenchyma and the subnectariferous parenchyma of *B. muricata* EFNs are green, have chloroplasts and, consequently, photosynthetic activity, which corroborates the possibility of the local incorporation of photoassimilated products into the nectariferous secretion (Vassilyev 2010). Highlighted in the subnectariferous parenchyma of the EFNs of *B. muricata*, are cells with phenolic compounds and cells containing calcium oxalate crystals, which are related to chemical (Mandal *et al*. 2010) and mechanical (Franceschi & Nakata 2005) protection, respectively, of this secretory structure. However, the presence of calcium oxalate crystals in nectaries, although common, has been interpreted differently. Fixation of calcium in crystals could represent a mechanism of physiological adaptation to control cellular calcium levels (Franceschi & Nakata 2005; Paiva & Machado 2005), since at high concentrations, calcium ions are toxic to plants (Franceschi & Nakata 2005).

Despite the presence of chloroplasts in the nectariferous parenchyma, there are no stomata throughout the epidermis of EFNs of *B. muricata* to maintain gas exchange and supply the photosynthetic process. However, the presence and abundance of calcium oxalate crystals could represent a source of carbon dioxide for the maintenance of the photosynthetic process under these conditions (Tooulakou *et al*. 2016). These authors showed a new photosynthetic pathway that uses mesophyll calcium oxalate crystals as

a CO_2 source when stomata are closed, which provides adaptive advantages under drought conditions. This photosynthetic pathway could also occur in nectaries in general, where such crystals are so abundant.

Extrafloral nectaries may be vascularized by xylem and phloem (Fahn 1979; Elias 1983), as in *B. muricata* and other species of Malpighiaceae (Machado *et al*. 2008; Possobom *et al*. 2010; Araújo & Meira 2016), but they may also possess only one type of vascular tissue or no vascular tissues at all (Fahn 1979; Paiva *et al*. 2007). The amount of vascular tissue in nectaries is considered proportional to their size (Carlquist 1969) and to the volume of secretion produced (Paiva *et al*. 2007). In the case of *B. muricata*, the reduced size of the EFNs may be the only factor explaination the small volume of secretion produced, since the proportion of vascular tissue in relation to the size of the EFNs is large.

Therefore, it can be concluded that the distribution, chemical composition of the secretion and anatomy of leaf glands of *B. muricata* characterize them as EFNs, while foraging by ants of the genus *Solenopsis* indicate a mutualistic relationship that possibly protects the plant against herbivores.

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