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Foliar colleters in Anacardiaceae: first report for the family

Ana Paula Stechhahn Lacchia, Elisabeth E.A. Dantas Tölke, Sandra M. Carmello-Guerreiro, Lia Ascensão, and Diego Demarco

Abstract: Colleters are secretory structures widely distributed in eudicots and with taxonomic value in many families. Although glandular trichomes have been described in some Anacardiaceae species, the chemical characterization of their secretions is scarce and to date there are no reports on colleters. Light microscopy and scanning electron microscopy were used to study the distribution and structure of colleters on the vegetative buds of *Anacardium humile* A.St.-Hil., *Lithraea molleoides* (Vell.) Engl., *Spondias dulcis* Parkinson, and *Tapirira guianensis* Aubl., and to characterize their secretory products histochemically. In all of these Anacardiaceae species, colleters are multicellular and multiseriate ovoid or club-shaped glandular trichomes of protodermic origin, present on both surfaces of leaf primordia. They reach the secretory phase at early stages of leaf development, after which they gradually degenerate, become brown, and fall off. Histochemical tests indicate that the secretion within the glandular cells and outside the trichomes is a complex mixture containing mucilage, fatty acids, and phenolic compounds, which are secretory products that can play an important role in the protection of meristems against desiccation and attack by pathogens. Therefore, the distribution of these glandular trichomes, their short-life, the chemical nature of their secretions and their presumed functions support their being classified as colleters.

Key words: Anacardiaceae, colleters, glandular trichomes, histochemistry, secretion.

Résumé : Les collétères sont des structures glandulaire sécrétoires largement distribuées chez les dicotylédones vraies et d'utilité taxonomique chez plusieurs familles. Même si les trichomes glandulaires ont été décrits chez quelques espèces d'anacardiacées, la caractérisation chimique de leurs sécrétions est pauvre et jusqu'à présent, il n'existe aucun écrit portant sur leurs collétères. La microscopie optique et la microscopie électronique à balayage ont été utilisées pour étudier la distribution et la structure des collétères des bourgeons végétatifs d'*Anacardium humile* A.St.-Hil., *Lithraea molleoides* (Vell.) Engl., *Spondias dulcis* Parkinson et *Tapirira guianensis* Aubl., de même que pour caractériser leurs produits de sécrétion d'un point de vue histochimique. Chez toutes ces espèces d'anacardiacées, les collétères sont des trichomes glandulaires multicellulaires et multisériés de forme ovoïde ou en forme de massue d'origine protodermique, présents sur les deux surfaces de l'ébauche foliaire. Ils atteignent la phase sécrétoire aux stades précoces du développement foliaire, après quoi ils dégénèrent graduellement, brunissent et tombent. Les tests histochimiques indiquent que la sécrétion à l'intérieur des cellules glandulaires et à l'extérieur des trichomes consiste en un mélange complexe contenant du mucilage, des acides gras et des composés phénoliques, des produits de sécrétion qui peuvent jouer un rôle important dans la protection des méristèmes contre la dessiccation et l'attaque par des pathogènes. Ainsi, la distribution des ces trichomes glandulaires, leur courte vie, la nature chimique de leur sécrétions et leurs fonctions présumées appuient le fait qu'ils soient classifiés parmi les collétères. [Traduit par la Rédaction]

Mots-clés : anacardiacées, collétères, trichomes glandulaires, histochimie, sécrétion.

Introduction

Colleters are secretory structures, trichomes, or more complex appendages, which produce a viscous secretion containing mucilage and (or) lipophilic substances, such as fatty acids, terpenes, and phenolic compounds (Fahn 1979; Thomas 1991). They cover the vegetative and reproductive buds (Fahn 1979; Thomas 1991) and have many different functions in plants, such as (*i*) protecting the meristems against desiccation (Thomas 1991), (*ii*) facilitating the sliding of one surface over another during growth (Uphof 1962), (*iii*) promoting symbiotic association with bacteria (Lersten 1974; Machado et al. 2015), and (*iv*) acting as a

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A.P.S. Lacchia. Departamento de Biologia, Centro de Ciências Biológicas e da Saúde, Universidade Estadual da Paraíba, *campus I*, CEP 58429-600, Campina Grande, Paraíba, Brasil.
E.E.A.D. Tölke and S.M. Carmello-Guerreiro. Departamento de Biologia Vegetal, Programa de Pós Graduação em Biologia Vegetal, Instituto de Biologia, CP 6109, Universidade Estadual de Campinas. CEP 13083-970, Campinas, São Paulo, Brasil.
L. Ascensão. Centro de Estudos do Ambiente e do Mar, Faculdade de Ciências - CBV, Universidade de Lisboa, 1749 016, Lisboa, Portugal.
D. Demarco. Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo, CEP 05508-090, São Paulo, São Paulo, Brasil.
Corresponding author: Elisabeth E.A. Dantas Tölke (email: elisabeth.tolke@gmail.com).

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barrier against pathogens and herbivores (Miguel et al. 2006).

Colleters are present on young vegetative and reproductive organs, such as nodes, leaves, bracts and flowers, mainly on the adaxial surface, in more than 60 dicotyledonous families (Thomas 1991; Renobales et al. 2001). In Sapindales, colleters have only been reported in Meliaceae (Fisher and Rutishauser 1990), Simaroubaceae, and Sapindaceae (Thomas 1991). Records of colleters in Anacardiaceae are lacking; however, glandular trichomes have been described in some Anacardiaceae species, on the vegetative organs, predominantly on the leaves (Metcalfe and Chalk 1979; Mitchell and Mori 1987), and on the reproductive organs, mostly on bracts, bracteoles, inflorescences axes, pedicels, ovary, pistillode, and petals (Von Teichman and Van Wyk 1994; Machado and Carmello-Guerreiro 2001). In general, the chemical composition of the secretion produced by these trichomes has not been investigated and only a few studies refer to their secretory products and putative functions (Wunnachit et al. 1992; Tomer et al. 1996). Therefore, some of the glandular trichomes reported in Anacardiaceae (Paviani 1965; Paula and Alves 1973; Naranjo and Pernia 1990; Mitchell 1992; Torres and Jáuregui 1999) could be colleters because these secretory structures are characterized not by their morphology, but by the nature of the secretory material and their functional roles.

The Anacardiaceae comprises approximately 600 species, mostly with pantropical distribution with a few occurring in temperate regions (Pell et al. 2011). In Brazil, the family is represented by many tree genera with economic importance, such as *Anacardium*, *Lithraea*, *Schinus*, *Spondias*, and *Tapirira* (Silva-Luz and Pirani 2015). Anacardiaceae species are well known for their edible fruits and seeds (mango, cashew fruit, and pistachio nut, among others), their valuable wood and production of a natural varnish and tannins that have been used to protect fine works of art and to tan leather, respectively (Pell et al. 2011). Some species are also used in folk medicine because of their bioactive compounds (Aguilar-Ortigoza and Sosa 2004).

The current study aimed to (*i*) describe the structure and distribution of glandular trichomes on the shoot apices of species including four different Anacardiaceae genera, and (*ii*) characterize the main classes of compounds present in their secretions. Herein, we present evidence to support the classification of these secretory trichomes as colleters, reporting for the first time their occurrence in Anacardiaceae.

Material and methods

Plant material

Shoot apices with leaf primordia and young leaves at different stages of development (two to three samples per individual) were collected from *Anacardium humile* A.St.-Hil., *Lithraea molleoides* (Vell.) Engl., and *Tapirira guianensis* Aubl. occurring in three distinct cerrado (Brazilian savannah) vegetation fragments in the state of São Paulo, Brazil.

Table 1. Voucher information of Anacardiaceae species examined in this study.

Species	Collection site	Voucher accession No.
Anacardium humile A.St.-Hil.	Botucatu	13 (UEC)
	Mogi Guaçu	8, 18, 19, 20, 21 (UEC)
Lithraea molleoides (Vell.) Engl.	Botucatu	11, 12 (UEC)
	Pratânia	14, 15, 16, 17 (UEC)
Spondias dulcis Parkinson	Campinas	20, 21, 22 (UEC)
Tapirira guianensis Aubl.	Mogi Guaçu	4, 7 (UEC)
	Pratânia	1, 2, 3, 10 (UEC)

Note: All collection sites were in São Paulo, Brazil; all samples (vouchers) were collected by A. Lacchia.

Similar samples were also collected from trees of *Spondias dulcis* Parkinson growing in a forest fragment (Campinas, São Paulo, Brazil). In dioecious species (*L. molleoides* and *T. guianensis*) both male and female individuals were sampled. Voucher specimens were deposited in UEC, the herbarium of the Universidade Estadual de Campinas, Brazil. The list of the four taxa studied, together with their accession numbers, collectors, and collection sites, is presented in Table 1.

Scanning electron microscopy (SEM)

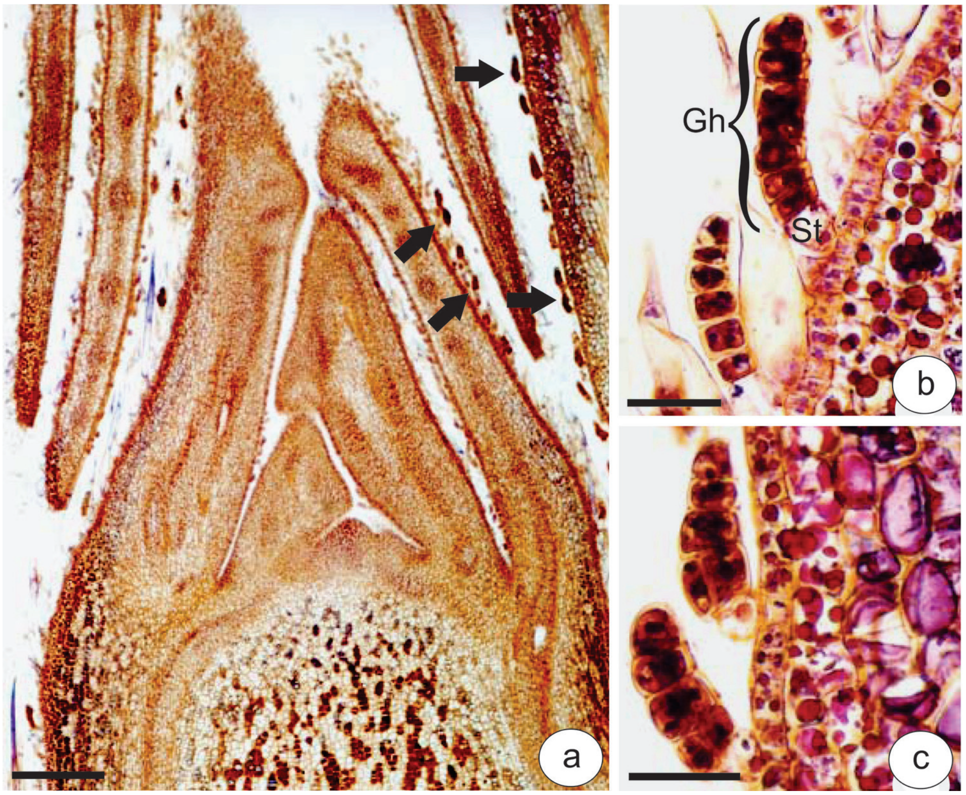
Samples were fixed in 3% glutaraldehyde in 0.1 mol/L sodium phosphate buffer at pH 7.2 for 4 h at 4 °C. After washing in the same buffer, the material was dehydrated in a graded acetone series, critical point dried with CO_2, and coated with a thin layer of gold (exposure time 200 s on a Balzers SCD-050 sputter coater). Observations were carried out on a JEOL T220 scanning electron microscope, at an accelerating voltage of 15 kV.

Light microscopy (LM)

Shoot apices and developing leaves were fixed in formalin–acetic acid–alcohol (FAA) for 24 h (Johansen 1940) and in buffered neutral formalin (BNF) for 48 h (Lillie 1965). Afterwards, the material was dehydrated through a tertiary butyl alcohol series, embedded in Paraplast, and serial sectioned on a rotary microtome. Longisections and transections 10 μm thick were stained with either Flemming's triple stain (Johansen 1940) or Safranin and Astra blue (Gerlach 1984) for general histology.

The main classes of compounds present in the secretions were investigated in material embedded in Paraplast, using the following histochemical tests: Schiff's reagent (PAS) for total polysaccharides (Jensen 1962), tannic acid – ferric chloride for mucilage (Pizzolato and Lillie 1973), ruthenium red for acidic mucilage (Gregory and Baas 1989), Sudan black B for total lipids (Pearse 1985), Nile blue A for acidic and neutral lipids (Cain 1947), copper acetate – rubeanic acid for fatty acids (Ganter and Jollés 1969, 1970), and ferric chloride for phenolic compounds (Johansen 1940). Standard control procedures were carried out simultaneously. To confirm the presence of lipophilic substances, fresh shoot apices were maintained in a chloroform–methanol–water–HCl solution (High 1984) for 48 h, prior to being prepared

Fig. 1. Colleters on vegetative organs of *Anacardium humile*. (*a*) Longitudinal section of a shoot apex showing the distribution of colleters (arrows). (*b* and *c*) Lateral view of colleters. Note the densely stained multicellular glandular heads and the vacuolated stalk cells. Scale bars = 250 μm (*a*); 30 μm (*b* and *c*). Gh, trichome glandular head; St, trichome stalk. [Colour online.]



for histological study and used as negative controls. The results were recorded through images captured by an Olympus DP71 digital camera attached to an Olympus BX51 microscope.

Results

Distribution and structure of colleters

In the four Anacardiaceae species studied, colleters are multicellular, multiseriate glandular trichomes of protodermic origin randomly distributed on both surfaces of leaf primordia and young leaves (Figs. 1*a*, 2*a*, and 3*a*). In *A. humile* and *L. molleoides,* mature colleters are multicellular ovoid-shaped structures with uniseriate bicellular stalks and multiseriate glandular heads, where the number of cells varies in *A. humile* from 4 to 7 in each row, and the number of rows range from 4 to 8 (Figs. 1*b* and 1*c*), whereas in *L. molleoides*, colleters have 4–6 rows with 6–8 cells per row (Figs. 2*b* and 2*c*). In contrast, the mature colleters of *S. dulcis* and *T. guianensis* are club-shaped structures, composed in the former species of uniseriate stalks with 4–5 cells and elongated glandular heads with 4–7 rows of cells and 4–8 cells per row (Figs. 3*b*–3*e*). In the latter species, the uniseriate stalks have 4–6 cells, and the glandular heads have 4–5 cells in each of the 4–6 cell rows (Figs. 3*e* and 3*f*). The morphological differences in the colleters of these Anacardiaceae taxa are summarized in Table 2.

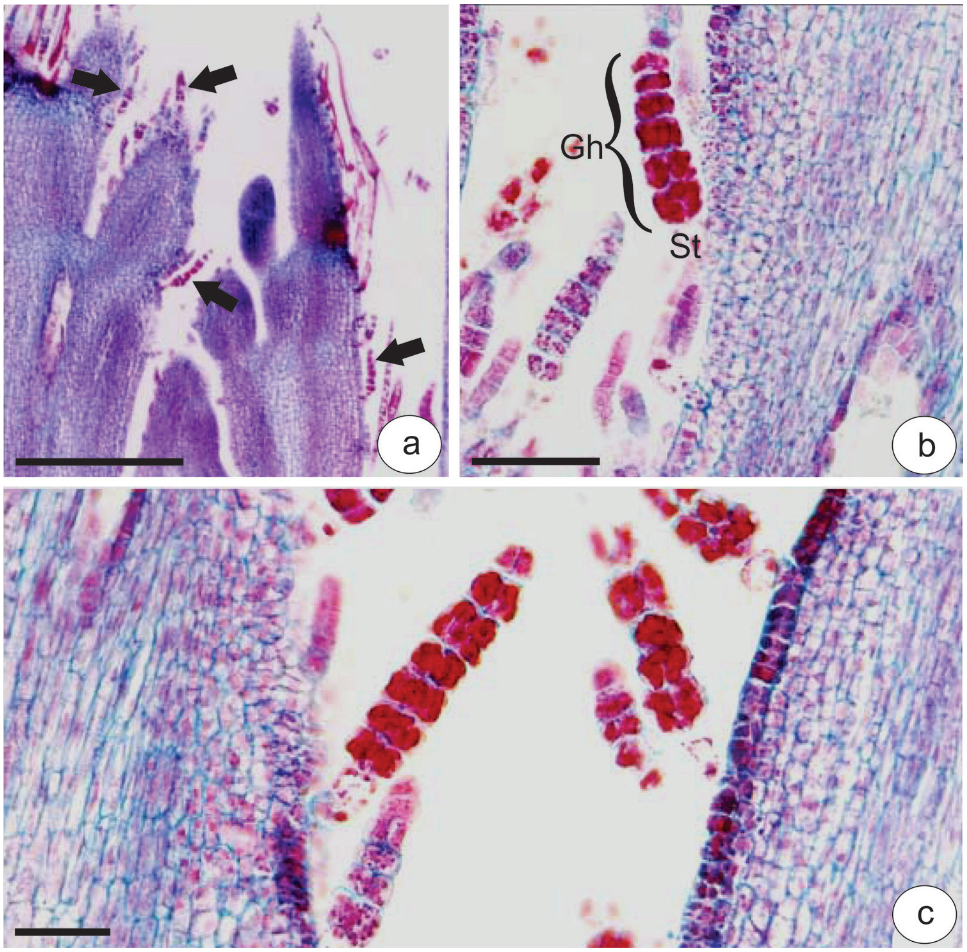
The development of these trichomatous colleters is asynchronous, and fully developed colleters are observed on very young leaves together with those in earlier stages of development (Fig. 3*d*). In mature colleters, the stalk cells are highly vacuolated, whereas those forming the glandular head have a densely stained cytoplasm, large central nucleus, and small vacuoles (Figs. 1*b*, 1*c*, 2*c*, 3*c*, and 3*d*). The secretory phase of colleters occurs early during the initial stages of leaf formation, and in general, colleters are already active in the second youngest leaf primordium of the investigated taxa.

The secretion present on the first nodes is hyaline and less viscuous than that on the older nodes, which is viscous, sticky, and quite abundant. At the level of the third or fourth node of *L. molleoides* and *S. dulcis*, a copious secretion, well-preserved in the SEM and LM samples, was found covering the colleters (Fig. 3*e*, arrowheads) and (or) filling the extracellular spaces (Figs. 4*b*, 4*h*, and 5*a*). However, no cuticle rupture or pores were observed in any of the studied species. After leaf expansion, colleters degenerate and fall off, which explains why on mature leaves only a few dark-brown colleters with a wrinkled surface were found (Fig. 3, arrows).

Histochemistry

In all of the studied species, the secreted products in the extracellular space and inside the colleter glandular cells stained positively for both hydrophilic and lipophilic compounds. The hydrophilic fraction of secretion stained deeply with PAS (Figs. 4*b*, 4*g*, 5*a*, and 5*e*), tannic

Fig. 2. Colleters on vegetative organs of *Lithraea molleoides*. (*a*) Colleters (arrows) on the shoot apex. (*b* and *c*) Developmental stages of the trichomatous colleters on leaf primordia. Scale bars = 250 μm (*a*); 50 μm (*b*); 30 μm (*c*). Gh, trichome glandular head; St, trichome stalk. [Colour online.]



acid – ferric chloride (Fig. 4*h*) and ruthenium red (Fig. 5*b*), indicating the presence of an acidic mucilage. The lipophilic fraction of the secretion was revealed with Sudan black B (Fig. 4*i*), copper acetate – rubeanic acid (Figs. 4*e*, 4*k*, 5*d*, and 5g), and Nile blue A (Figs. 4*d*, 4*j*, 5*c*, and 5*f*), showing the presence of lipids, particularly those with an acidic character. Phenolic compounds were detected with the ferric chloride test, which only gave a positive reaction in the samples fixed with BNF (Figs. 4*f* and 4*l*) because this fixative preserves lipophilic compounds (Fig. 4*c*) in contrast to FAA, which extracts lipids (Fig. 4*a*). Control sections did not react with these stains and reagents. Table 3 summarizes the results of the histochemical tests carried out to characterize the main classes of compounds present in the secreted material of the four Anacardiaceae species.

Discussion

The distribution pattern, the chemical nature of the secretions, and the structural features of the glandular trichomes present on the leaf primordia and young leaves of *Anacardium, Lithraea*, *Spondias*, and *Tapirira* allow us to infer that these trichomes are colleters. Their occurrence on differentiating young organs, their early period of secretory activity and their mucilaginous and (or) lipophilic secretion covering the meristems are characteristic features that are in agreement with the definition of colleters proposed by Fahn (1979, 1990) and Thomas (1991).

Glandular trichomes have been observed in several Anacardiaceae species, but considering the number of species included in this family, the number of the taxa studied is still very small. In some *Anacardium* species, multicellular glandular trichomes were found in the secondary vein axils of the abaxial leaf surface, at the bottom of pit-like cavities, which were previously called laminar crypts and are nowadays classified as mite domatia (Mitchell and Mori 1987; Mitchell 1992). Recently, in *Anacardium humile*, these foliar pit-like cavities were classified as extrafloral nectaries according to the histochemical characterization of the trichome secreted products (Lacchia et al. 2016). In general, the glandular trichomes in *Anacardium* species are multicellular, but on the leaves and stems of *A. spruceanum*, only secretory unicellular trichomes were observed (Paula and Alves 1973).

Despite the several studies related to the presence of trichomes in Anacardiaceae, only a few records concerning

Fig. 3. Light (*a–d* and *f*) and SEM (*e* and *g*) micrographs of colleters on the vegetative organs of *Spondias dulcis* (*a–e*) and *Tapirira guianensis* (*f* and *g*). (*a*) Colleters (arrows) on the first nodes of shoot apex. (*b–g*) Details of colleters. Note the abundant secretion surrounding the colleters (arrowheads) and the wrinkly surface of the colleter in the post-secretory phase (*e*). Scale bars = 300 μm (*a*); 30 μm (*b–d*); 40 μm (*f*); 10 μm (*e* and *g*). Gh, trichome glandular head; St, trichome stalk. [Colour online.]

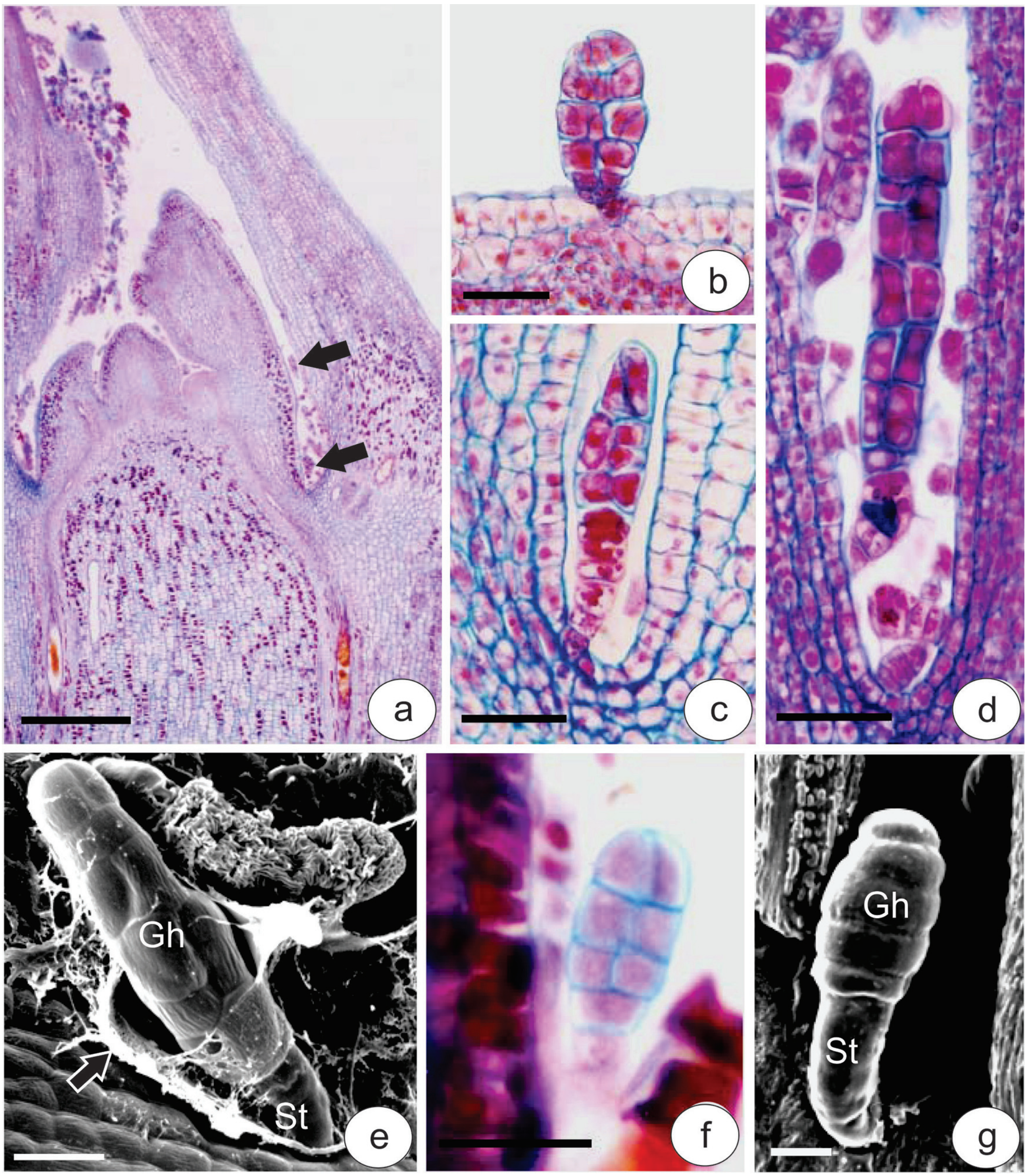
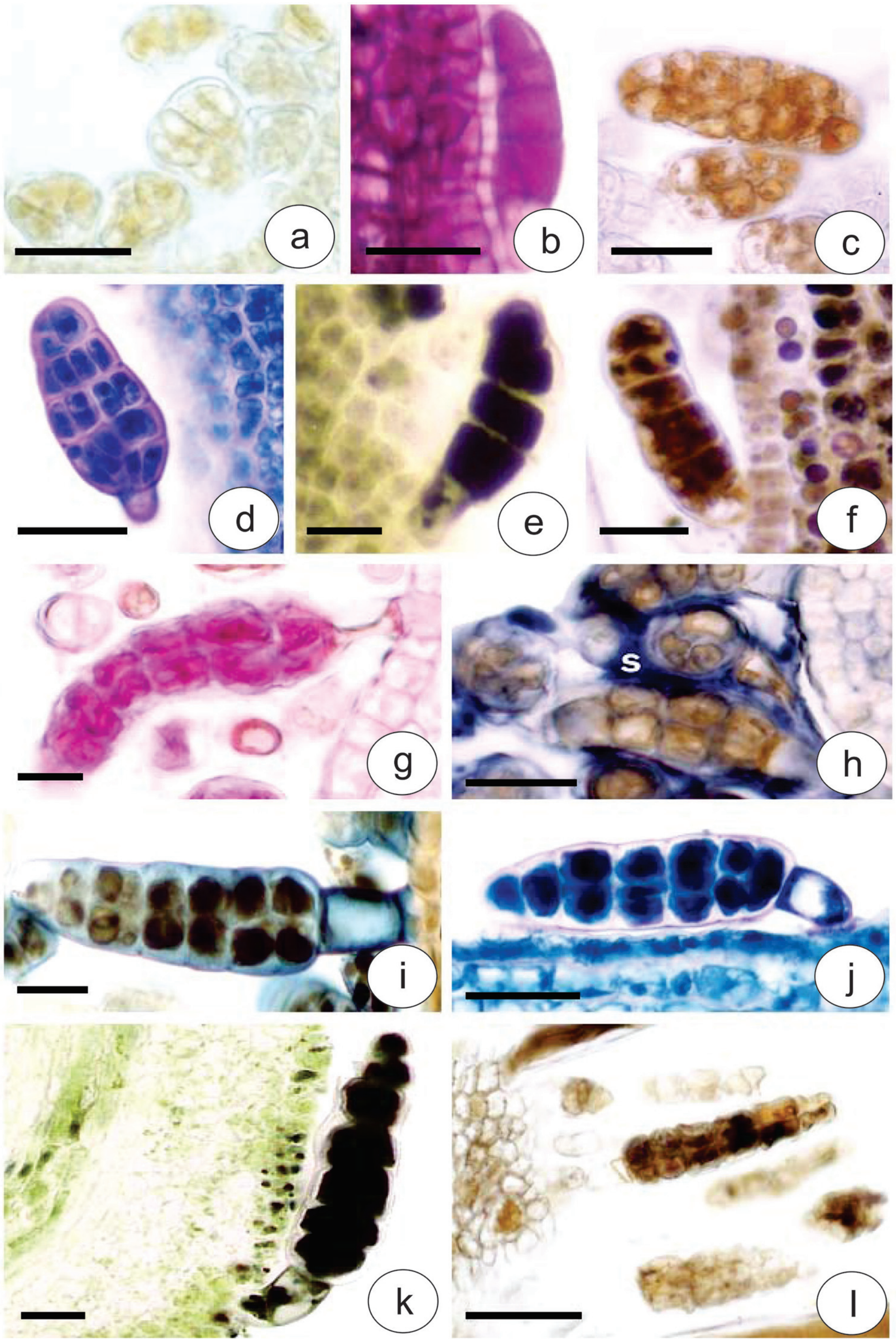


Table 2. General characteristics of the colleters present in the four Anacardiaceae species examined in this study.

Taxon	Shape	Glandular head	Stalk
Anacardium humile A.St.-Hil.	Ovoid	Multicellular (4–7 cells/row) multiseriate (4–8 rows)	Uniseriate and bicellular
Lithraea molleoides (Vell.) Engl.	Ovoid	Multicellular (6–8 cells/row) multiseriate (4–6 rows)	Uniseriate and bicellular
Spondias dulcis Parkinson	Club	Multicellular (4–8 cells/row) multiseriate (4–7 rows)	Uniseriate (4–5 cells)
Tapirira guianensis Aubl.	Club	Multicellular (4–5 cells/row) multiseriate (4–6 rows)	Uniseriate (4–6 cells)

the composition of their secretory products are available. A resiniferous secretion was recognized in the leaf glandular trichomes of *Rhus* (Metcalfe and Chalk 1979), tannin-like substances were observed inside the glandular cells of the peltate trichomes of *Mangifera indica* (Tomer et al. 1996), and a nectar rich in lipids and phenolic compounds was characterized in the leaf glandular trichomes of *Anacardium humile* (Lacchia et al. 2016). Records of colleters in Anacardiaceae

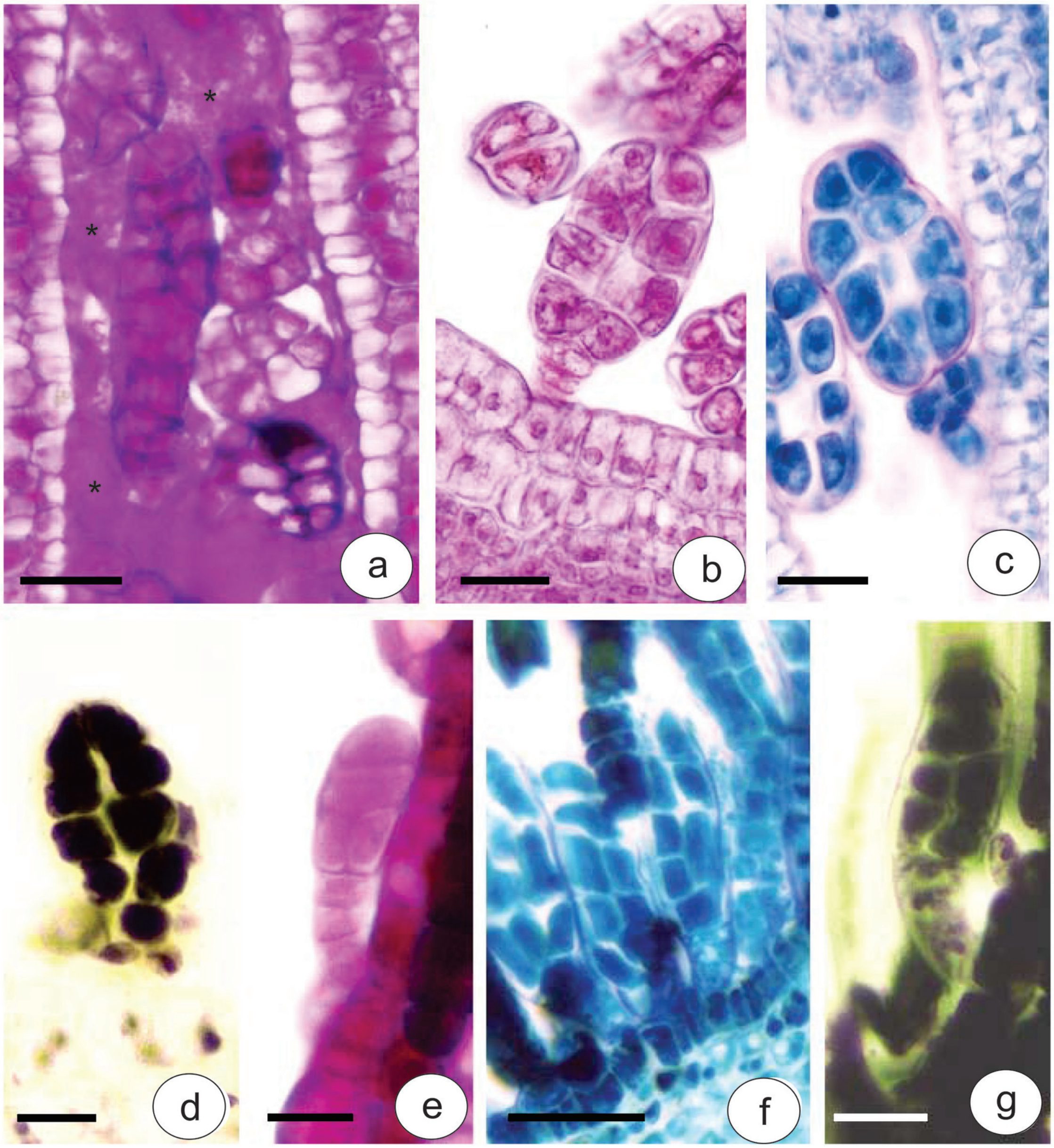
Fig. 4. Histochemical characterization of colleter secretions of *Anacardium humile* (*a*–*f*) and *Lithraea molleoides* (*g*–*l*) colleters. Material fixed in FAA (*a*) and BNF (*c*). (*b* and *g*) PAS reagent for total polysaccharides. (*d* and *j*) Nile blue A for acidic and neutral lipids. (*e* and *k*) Copper acetate – rubeanic acid for fatty acids. (*f* and *l*) Ferric chloride for phenolic compounds. (*h*) Tannic acid – ferric chloride for mucilage. Note the profuse secretions around the trichomes. (*i*) Sudan black B for total lipids. Scale bars = 30 μm (*a*–*d*, *h*, *j*, and *l*); 15 μm (*e*, *g*, *i*, and *k*); 25 μm (*f*). [Colour online.]



are lacking. Although deciduous uniseriate glandular trichomes have been described on the leaf primordia of *Pachycormus discolor*, no reference has been made to the chemistry of their secretions (Gibson 1981).

Glandular trichomes are rarely considered colleters; commonly, colleters are complex appendages (emergences) that occur on vegetative and floral buds, usually on the adaxial surface of developing organs (Thomas 1991). However, colleters of protodermic origin, like those described here for the first time in Anacardiaceae species, have also been reported in two species of *Hibiscus* (Malvaceae) (Rocha et al. 2002), in species of *Hymenaea, Copaifera*,

Fig. 5. Histochemical characterization of colleter secretions of *Spondias dulcis* (*a–d*) and *Tapirira guianensis* (*e–g*). (*a* and *e*) Colleters; PAS reagent for total polysaccharides. Note the abundant secretion filling the extracellular spaces among the trichomes (*) (*a*). (*b*) Ruthenium red for acidic mucilage. (*c* and *f*) Nile blue A for acidic and neutral lipids. (*d* and *g*) Copper acetate – rubeanic acid for fatty acids. Scale bars = 25 μm (*a*); 15 μm (*b*, *d*, *e*, and *g*); 30 μm (*c* and *f*). [Colour online.]



and *Chamaecrista* (Leguminosae) (Paiva and Machado 2006*a*; Paiva 2009; Coutinho et al. 2015), and in several Orchidaceae species (Leitão and Cortelazzo 2008; Mayer et al. 2011; Cardoso-Gustavson et al. 2014). The colleters of *Anacardium*, *Lithraea*, *Spondias*, and *Tapirira* are present on both leaf primordium surfaces as those found in *Hymenaea stigonocarpa* stipules (Paiva 2009), suggesting that their main role is to protect the shoot apex.

The histochemical characterization of the secretions produced by the trichomatous colleters of the four Anacardiaceae species studied indicates the presence of a complex mixture containing mucilage, fatty acids, and phenolic compounds. Exudates with a similar composition were reported to occur in other families of eudicots, such as Apocynaceae (Thomas and Dave 1989), Caryocaraceae (Paiva and Machado 2006*b*), Orchidaceae (Mayer et al. 2011; Cardoso-Gustavson et al. 2014), Euphorbiaceae (Machado et al. 2015), Rubiaceae (Muravnik et al. 2014), and Leguminosae (Paiva and Machado 2006*a*; Paiva 2009). The mucilage may protect the developing meristem and young differentiating leaves against desiccation, owing to its notable water absorption capacity and water-retaining ability (Fahn 1979; Clifford et al. 2002), whereas phenolic compounds may act as chemical barriers against herbivores and microorganisms, and may protect young organs against attack by a wide range of potential pests (Lattanzio et al. 2006; Castro

Table 3. Results of the histochemical characterization of the main secretory products of colleters of *Anacardium humile*, *Lithraea molleoides*, *Spondias dulcis*, and *Tapirira guianensis*.

Histochemical test	Target class of compound	Reaction color	*A. humile* (Fig.)	*L. molleoides* (Fig.)	*S. dulcis* (Fig.)	*T. guianensis* (Fig.)
PAS reaction	Total polysaccharides	Purplish red	+ (4*b*)	+ (4*g*)	+ (5*a*)	+ (5*e*)
Tannic acid – ferric chloride	Mucilage	Black	+	+ (4*h*)	+	+
Ruthenium red	Acid mucilage	Pink to bright red	+	+	+ (5*b*)	+
Sudan black B	Total lipids	Black	+	+ (4*i*)	+	+
Nile blue A	Acidic lipids	Blue	+ (4*d*)	+ (4*j*)	+ (5*c*)	+ (5*f*)
Nile blue A	Neutral lipids	Pink to read	–	–	–	–
Cooper acetate – rubeanic acid	Fatty acids	Dark-green	+ (4*e*)	+ (4*k*)	+ (5*d*)	+ (5g)
Ferric chloride[a]	Phenolic compounds	Black; brown	–	–	–	–
Ferric chloride[b]	Phenolic compounds	Black; brown	+ (4*f*)	+ (4*l*)	+	+

Note: +, positive reaction; –, negative reaction; PAS, •••.
[a]Material fixed in FAA.
[b]bMaterial fixed in BNF.

and Demarco 2008). Mucilage and lipids together may act as a lubricant, facilitating sliding of young leaf surfaces against each other during their expansion and growth in the shoot apex (Uphof 1962; Modenesi et al. 1984).

In different types of secretory structures that produce a polysaccharide secretion, several authors have assumed that the exudate is released through micropores in the cuticle covering the head cells (Ascensão et al. 1999; Mayer et al. 2011; Naidoo et al. 2014; Canaveze and Machado 2015); a process that may also occur in the colleters described here, since no ruptures in the cuticles in any of the studied taxa were observed. However, in some nectariferous trichomes, the cuticle is not interrupted, owing to the presence of cell wall projections crossing it; therefore, the nectar flows and is released to the outside, avoiding the disruption of the cuticle, which is a hydrophobic layer, by passage of the nectar, which is a hydrophilic secretion (Gama et al. 2016).

In the last decade, the relevance of colleters for taxonomy has been demonstrated and their structure and distribution patterns have been used as taxonomic characters for many eudicot families, such as the Apocynaceae (Simões et al. 2006), Celastraceae (Simões-Mercadante and Paiva 2013), Leguminosae (Coutinho et al. 2015), Myrtaceae (Silva et al. 2012), Rhizophoraceae (Sheue et al. 2012), Rubiaceae (Miguel et al. 2006), and Turneraceae (González 1998).

In conclusion, this study demonstrates for the first time that the multicellular, multiseriate glandular trichomes that occur on the leaf primordia and young leaves of *Anacardium*, *Lithraea*, *Spondias*, and *Tapirira* are colleters. They synthesize a heterogeneous secretion mainly composed of mucilage, fatty acids, and phenolic compounds, which are secreted products that may be involved in bud and shoot protection. As this is the first report of the occurrence of colleters in Anacardiaceae, further studies are needed to evaluate their taxonomic importance in this family.

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