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Morphoanatomy of nectaries of *Chamaecrista* (L.) Moench sections *Chamaecrista*, *Caliciopsis* and *Xerocalyx* (Leguminosae: Caesalpinioideae)

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ABSTRACT

Nectaries are specialized structures that secrete nectar. Several species of *Chamaecrista* possess nectaries on the petiole, which have been shown to vary widely in morphology and the chemical nature of their secretion. However, a comprehensive investigation of the nectaries of the clade formed by sect. *Chamaecrista, Caliciopsis* and *Xerocalyx* has yet to be performed. Our study aimed to confirm whether or not the leaf glands of species of this clade are in fact nectaries, determine the chemical nature of their secretion and expand the morphoanatomical database on leaf nectaries in *Chamaecrista* with the intention of contributing to the taxonomy and phylogeny of the genus. Samples from herbarium and field-collected material were subjected to standard methods for light and scanning electron microscopy. Four different forms of nectaries were observed: urceolate, patelliform, verruciform and cupuliform. The nectaries were found to comprise a single-layered epidermis, nectary parenchyma, subnectary parenchyma and vascularization. Polysaccharides, lipids, phenolic compounds and proteins were detected in secretions. Although anatomical similarities were observed among the studied species, their morphology differed. Moreover, the glands are indeed nectaries and are similar to those observed in other species of the genus *Chamaecrista*. These data hold potential taxonomic usefulness for the studied sections.

Keywords: foliar glands, histochemistry, leaf glands, secretory structures, taxonomy

Introduction

Nectaries are specialized structures for the secretion of a sugary solution called nectar, which contains sucrose, glucose and fructose as primary solutes (Fahn 1979; Nicolson *et al.* 2007). According to their location, nectaries are classified as reproductive (i.e. when found on the inflorescence axis, bracts, sepals, ovary, stamens, etc.) and extra-reproductive (i.e. when found on the petiole, rachis, leaf blade, stems, etc.) (Schmid 1988). Reproductive nectaries are usually involved in pollination strategies while extra-reproductive nectaries are related to the protection of plants from the attack of herbivores and pathogens through mutualistic interactions with ants (Schmid 1988; Madureira & Sobrinho 2002; Rutter & Rausher 2004; Fernandes *et al.* 2005; Nascimento & Del-Claro 2010; Del-Claro *et al.* 2016).

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The structural diversity and topography of nectaries are used in taxonomic and evolutionary studies (Bhattacharyya & Mareshwari 1971; Metcalfe & Chalk 1979; Coutinho *et al*. 2012; Dalvi *et al*. 2013; Coutinho & Meira 2015). In Leguminosae, leaf nectaries are most common in the subfamily Mimosoideae, followed by Caesalpinioideae and less frequently in Papilionoideae (Polhill *et al.* 1981). The association with ants is a common defense system in Mimosoideae and Caesalpinioideae, while species of Papilionoideae are more dependent on chemical defenses (Polhill *et al.* 1981).

As suggested by Conceição *et al.* (2009), the leaf nectaries of *Chamaecrista* may have a single evolutionary origin. The species of *Chamaecrista* that bear leaf nectaries are placed in the sect. *Apoucouita, Caliciopsis*, *Xerocalyx* and *Chamaecrista* (except in the ser. *Bauhinianae*) and *Absus* subsect. *Baseophyllum* and *Otophyllum* (Irwin & Barneby 1982).

Molecular phylogenetic studies group sect. *Caliciopsis*, *Chamaecrista* and *Xerocalyx* in a single clade of species possessing inflorescences with axillary racemes and a reduced number of chromosomes as common characters (Conceição *et al.* 2009). Section *Chamaecrista* is the second most representative of the genus, with about 75 species (~55 in Americas) while *Caliciopsis* has only two species and *Xerocalyx* three, but a high number of varieties (Irwin & Barneby 1982; Rando & Pirani 2012; Rando *et al.* 2013).

The morphoanatomy of leaf nectaries has been studied in *Chamaecrista* sect. *Apoucouita* (Coutinho & Meira 2015) and sect. *Absus* subsect. *Baseophyllum* (Coutinho *et al.* 2012) and subsect. *Otophyllum* (Francino *et al.* 2015). These studies have demonstrated how different extra-reprodutive nectaries can be even among species of the same genus, since the chemical nature of the secretion of extra-reproductive nectaries in *Chamaecrista* can differ and some species develop a wound-healing periderm during senescence. The extra-reproductive nectaries of the species of *Chamaecrista* may be stalked or sessile, and are anatomically composed of four distinct regions (Coutinho *et al.* 2012; Coutinho & Meira 2015; Francino *et al.* 2015).

Regarding sect. *Chamaecrista*, only *C. trichopoda, C. rotundata* and *C. mucronata* of ser. *Coriaceae* have been investigated (Francino *et al.* 2006; 2015), while in sect. *Xerocalyx* only one taxon has been evaluated, *Chamaecrista desvauxii* var. *langsdorfii*. Moreover, although sect. *Caliciopsis* has been reported to bear leaf nectaries on the petiole, as far as we are concerned, there have been no morphoanatomical studies on the petiole nectaries of this section.

This paper aims to expand the database on the morphoanatomy of leaf nectaries in the sect. *Chamaecrista, Caliciopsis* and *Xerocalyx*, with the intention of contributing to the taxonomy and phylogeny of the genus *Chamaecrista*. We address the following questions: Are the leaf glands of sect. *Chamaecrista, Caliciopsis* and *Xerocalyx* indeed nectaries? What is the chemical nature of the secreted compounds? Are there morphoanatomical differences that may indicate distinct patterns between sections or between species? Do morphoanatomical characteristics and the secreted products have any taxonomic implications at the level of section in the genus *Chamaecrista*?

Materials and methods

We studied 49 species of *Chamaecrista* (L.) Moench sect. *Chamaecrista* including species of all its series (i.e. *Coriaceae* (Benth.) H.S.Irwin & Barneby, *Flexuosae* H.S.Irwin & Barneby, *Prostratae* (Benth.) H.S.Irwin & Barneby, *Greggiianae* H.S.Irwin & Barneby and *Chamaecrista*) two species of sect. *Caliciopsis* Irwin & Barneby and three species of sect. *Xerocalyx* (Bentham) Irwin & Barneby (Tab. 1). A list of all sources of material used and authorities for sections, subsections and species names are given in the supplementary data. Field-collected samples and voucher specimens used are deposited in the following herbaria: HUNEB, HUEFS, NY, RB, SPF and VIC (acronyms according to Thiers 2016). When available, three specimens of each taxon were analyzed (List S1 in supplementary material).

Samples of fully developed leaves from herbarium material were rehydrated (Smith & Smith 1942) and stored in 70 % ethanol. Samples from species collected in the field were fixed in FAA (formalin:acetic acid:50 % ethanol, 1:1:18 by volume) and NBF (neutral buffered formalin) (Johansen 1940). Detection of phenolic compounds was performed through fixation with FSF (ferrous sulphate in formalin) (Johansen 1940).

Samples of petioles/rachises possessing glands were dehydrated through an ethanol series and embedded in methacrylate (Historesin Leica; Leica Microsystems Nussloch, Heidelberg, Germany). Cross and longitudinal sections 5 µm thick were made using an automatic rotary microtome (Leica RM2155, Deerfield, IL, USA) and subsequently stained with toluidine blue at pH 4.4 (O`Brien & McCully 1981) for structural characterization. The slides were mounted in synthetic resin (Permount, Fisher Scientific, New Jersey, USA). Some samples were dehydrated through a *tert*-butanol series, embedded in histological paraffin enriched with dimethyl sulfoxide (Histosec®, Merck, Germany) (Johansen 1940) and cross and longitudinally sectioned at 7-µm thick using a rotary microtome (Spencer 820 American optical Corporation, Buffalo, NY, USA). The sections were deparaffinized in xylene, rehydrated through a decreasing ethanol series and used in histochemical tests (Johansen 1940).

The presence of glucose in gland exudates was tested using urinetest strips (Alamar Tecno Científica Ltda., São Paulo, Brazil) for the following species: *C. rotundata* (Vogel) H.S.Irwin & Barneby, *C. mucronata* (Spreng.) H.S.Irwin & Barneby, *C. latifolia* (Benth.) Rando, *C. potentilla* (Mart. ex Benth.) H.S.Irwin & Barneby, *C. simplifacta* H.S.Irwin & Barneby, *C. cinerascens* (Vogel) H.S.Irwin & Barneby, *C.*

choriophylla (Vogel) Irwin & Barneby, *C. aristata* (Benth.) H.S.Irwin & Barneby, *C. papillata* H.S.Irwin & Barneby and *C. flexuosa* Greene. The following histochemical tests were performed: neutral red (under fluorescence) and Sudan IV for total lipids (Pearse 1980); periodic acid-Schiff reagent for total polysaccharides (Maia 1979); ruthenium red for pectins/mucilage (Johansen 1940); alcian blue for acid mucopolysaccharides (Pearse 1980); xylidine Ponceau for total proteins (O'Brien & McCully 1981); phloroglucinol for lignin; and ferrous sulphate in formalin for phenolic compounds (Johansen 1940). All observations and image captures were obtained using a light microscope (model AX70TRF; Olympus Optical, Tokyo, Japan) equipped with U-Photo and a digital camera (AxioCam HRc; Carl Zeiss, Gottingen, Germany).

For scanning electron microscopy (SEM) analysis, samples of glands stored in 70 % ethanol were critical-point dried with CO_2 (CPD 030, Bal-Tec, Balzers, Liechtenstein), mounted on stubs and sputter coated with gold (Modular Balzers Union FDU 010, SCA 010) (Bozzola & Russel 1991). Observations and image captures were obtained using a LEO model 1430 VP SEM (Cambridge, England). Morphological descriptions of glands are in accordance with Radford *et al.* (1974).

Results

Due to the presence of glucose in the secretion of the petiole/rachis glands of all the species tested with urinetest strips (i.e. *C. rotundata*, *C. mucronata*, *C. latifolia*, *C. potentilla*, *C. simplifacta*, *C. cinerascens*, *C. choriophylla*, *C. aristata*, *C. papillata* and *C. flexuosa*), the glands are hereafter considered nectaries. Nectaries occured in a variety of positions on the petioles of the studied species (Fig. 1, Tab. 1). In 11 taxa (Tab. 1), nectaries were also present between the pairs of leaflets (Fig. 1A). These nectaries were located mainly at the apex of the petiole in ser. *Coriaceae* and sect. *Caliciopsis* and *Xerocalyx* (Tab. 1), while in the ser. *Prostratae* and *Greggiianae* they were located predominantly in the median region. In ser. *Flexuosae* (Fig. 1G) and *Chamaecrista*, the nectaries were found in both the basal and median regions (Tab. 1). Although most species had one to two nectaries, some had a variable number, such as *C. aristata* (Fig. 1A).

Four forms of nectaries were recorded: urceolate (Fig. 2A, B), patelliform (Fig. 2C, D), verruciform (Fig. 2E, F) and cupuliform (Figs. 2G, H, 3A-E). Secretory surfaces were found to be concave (Fig. 2D, H, 3B), convex (Fig. 2F) or flat (Fig. 3D). Some nectaries were stalked (i.e. a cylindrical structure that is vascularized and nonsecretory, but which bears a secretory portion at the top) while others were sessile (Tab. 1). Urceolate nectaries were sessile; patelliform were sessile (Fig. 2D) or short stalked; verruciform were short-stalked (2F) and cupuliform were short-stalked, stalked (Fig. 3B) or long-stalked (Fig. 3C-E).

The distribution of nectary forms is as follows: urceolate nectaries occured in three taxa of sect. *Chamaecrista* ser. *Chamaecrista*; patelliform nectaries occurred in 17 taxa of ser. *Coriaceae*, two of ser. *Flexuosae*, three of ser. *Chamaecrista* and in *C. desvauxii* var. *glauca* (sect. *Xerocalyx*); verruciform nectaties were exclusive to *C. simplifacta* (sect. *Chamaecrista* ser. *Coriaceae*); and cupuliform nectaries occured in 43 taxa (Tab. 1). Although there was no pattern to the distribution of nectary forms with regard to sections, in ser. *Coriaceae*, nectaries were mostly patelliform, while in the ser. *Prostratae* and *Greggiianae* the nectaries were exclusively cupuliform. The secretory surface was concave in most of species studied, but convex in *C. simplifacta* and flat in *C. vestita*, *C. pedicellaris* var. *pedicellaris* and *C. nictitans* var. *paraguariensis*.

The nectaries were mostly short-stalked in ser. *Coriaceae* (Fig. 2F; Tab. 1). In ser. *Flexuosae* and in the sect. *Xerocalyx* the nectaries were short-stalked or stalked. On the other hand, the species of the ser. *Prostratae* and those of sect. *Caliciopsis* were found to have long-stalked nectaries. In the ser. *Greggiianae*, nectaries were stalked or long-stalked. As for ser. *Chamaecrista*, nectaries were found to be sessile, short-stalked, stalked or long-stalked (Fig. 3D).

In all species studied, the nectaries were characterized by having four distinct regions: a single-layered epidermis, a nectary parenchyma, a subnectary parenchyma and vascularization (Figs. 2B, D, F, H, 3B, D, F). The epidermis was uniseriate, deprived of stomata, and with more columnar shaped cells at the edges of the nectary and smaller and more cuboidal, sometimes papillary, cells in the center (Figs. 2B, D, 4). Throughout the nectary, the cuticle was thick except at the center of the secretory epidermis (Fig. 4I). In most samples studied, a distended cuticle was observed in the central area (Figs. 2A, H, 3E). Very prominent intercellular spaces were oobserved at the edges of the nectary (Fig. 2B, H, 3B). Non-glandular trichomes were observed, especially in the epidermal cells of the stalk (Fig. 3A).

The nectary parenchyma was typically formed of small polyhedral cells with dense cytoplasm (Fig. 4E). The number of cell layers in the secretory parenchyma was variable, from five, as observed in *C. kunthiana*, to more than 20, as was the case for *C. repens* (Fig. 2B). The subnectary parenchyma possessed highly vacuolated cells, which are larger than those of the secretory parenchyma (Fig. 3B). The number of cell layers in the subnectary parenchyma was also found to vary (Fig. 3F).

The nectaries of all species studied were vascularized predominantly by phloem (Figs. 3F). Vascularization originates from the main vascular bundles in most taxa (Figs. 2F, 4D; Tab. 1). In nine taxa, only the accessory bundles contributed to nectary vascularization (Tab. 1), while in nine other taxa both the main and accessory vascular bundles contributed to nectary vascularization (Tab. 1).

Fibers adjacent to the vascularization that reaches the nectary were noticed in 56 taxa (Figs. 2F, 3D, Tab. 1). A layer of sclereids was found to clearly separate the secretory

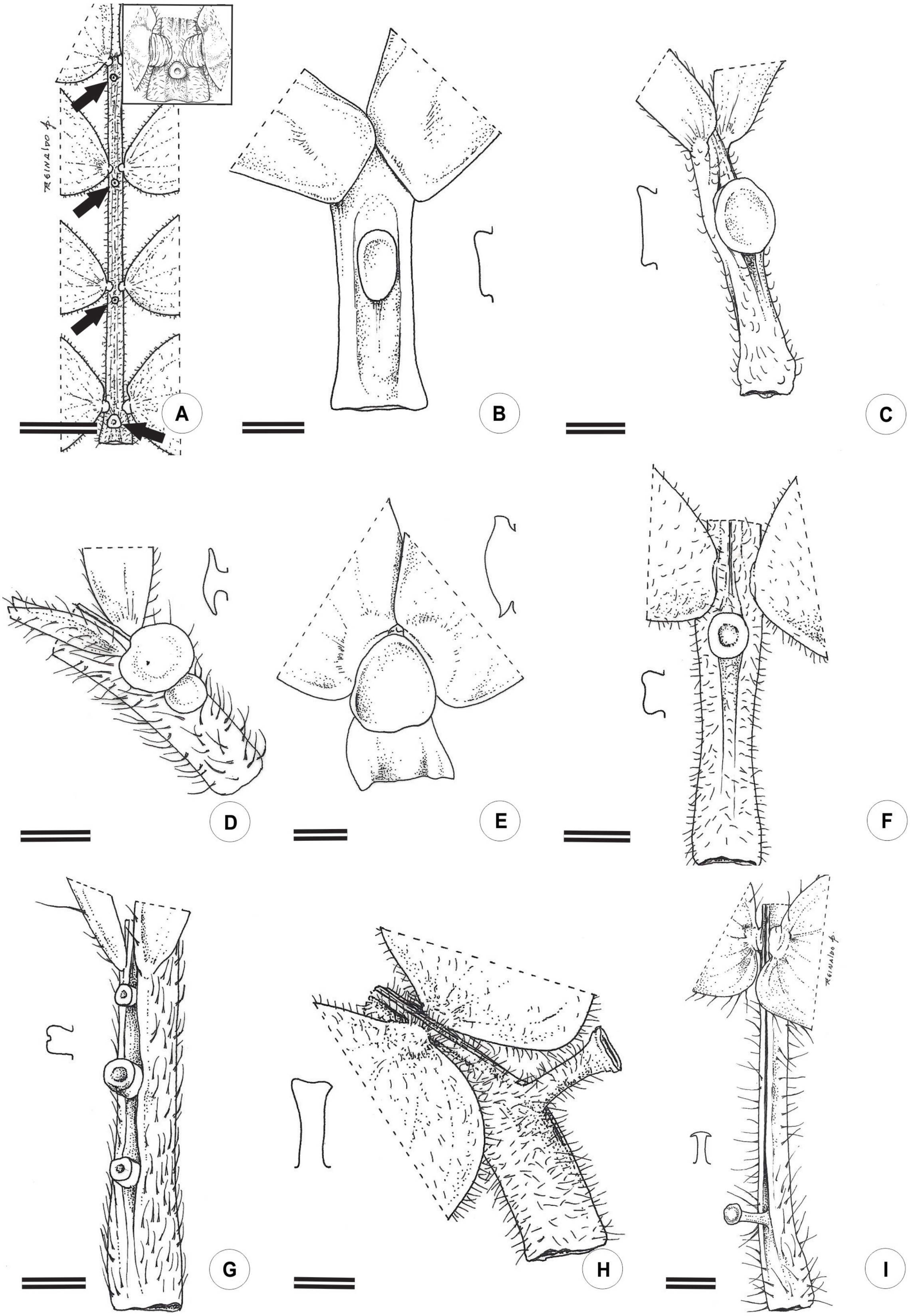

Figure 1. Nectaries on leaves of species of *Chamaecrista* sect. *Chamaecrista* and *Xerocalyx*. **A.** *C. aristata:* nectary on petiole and raquis. **B.** *C. lagotois*: sessile patelliform. **C-D.** *C. venturiana* and *C. nictitans* var. *paraguariensis*: sessile and short-stalked patelliform respectivelly. **E.** *C. simplifacta:* verruciform. **F.** *C. desvauxii* var. *desvauxii:* short-stalked cupuliform. **G.** *C. flexuosa* var. *flexuosa:* stalked cupuliform. **H.** *C. pascuorum:* long-stalked cupuliform. **I.** *C. vestita:* long-stalked cupuliform. Scale bars: A: 5000 μm; B: 2000 μm; C-I: 1000 μm.

Table 1. Nectaries in *Chamaecrista* sections *Chamaecrista*, *Caliciopsis* and *Xerocalyx*.

Taxa	Form							N°	Position				Origin vascularization			Fibers adjacent to the vascularization
	Urceolate	Patelliform		Verruciform	Cupuliform				Petiole			Rachis				
	Sessile	Sessile	Short stalked	Short stalked	Short stalked	Stalked	Long stalked		A	M	B		1	2	3	
Sect. *Chamaecrista*																
Ser. *Coriaceae*																
C. anceps		X						1-3	X				X			X
C. aristata			X					+1	X			X			X	X
C. burchelli		X						1-2		X			X			X
C. cardiostegia		X						1	X				X			X
C. caribaea var. *caribaea*							X	+1		X		X	X			X
C. caribea var. *lucayana*							X	+1		X		X			X	X
C. caribea var. *inaguensis*							X	+1		X		X	X			X
C. cinerascens			X					1	X				X			X
C. choriophylla		X						1	X				X			X
C. rossicorum			X					1	X				X			X
C. latifolia			X					1	X				X			X
C. distichoclada			X					1	X					X		
C. lagotois		X						1	X				X			X
C. mucronata			X					1-4	X			X	X			X
C. multinervia						X		+1	X				X			X
C. olesiphylla			X					+1	X				X			X
C. papillata			X					8-9	X			X	X			
C. potentilla					X			1	X				X			X
C. roraimae						X		+1	X			X	X			
C. rotundata			X					1	X				X			
C. rotundata var. *interstes*			X					1	X				X			
C. rotundata var. *grandistipula*						X		1	X				X			
C. simplifacta				X				1	X				X			X
C. tragacanthoides						X		1	X				X			
C. tragacanthoides var. *rasa*			X					1	X				X			
C. ulmea			X					1	X				X			X
C. venulosa							X	1	X	X					X	X
Ser. *Flexuosae*																
C. flexuosa var. *flexuosa*						X		1-4	X	X			X			
C. flexuosa var. *texana*						X		1-2	X	X				X		
C. gonoclada			X					1			X		X			
C. gonoclada			X					1			X		X			
C. parvistipula			X					1		X					X	
C. swainsoni						X		1			X		X			

Table 1. Cont.

Taxa	Form							Nº	Position				Origin vascularization			Fibers adjacent to the vascularization
	Urceolate	Patelliform		Verruciform	Cupuliform				Petiole			Rachis				
	Sessile	Sessile	Short stalked	Short stalked	Short stalked	Stalked	Long stalked		A	M	B		1	2	3	
Ser. *Prostratae*																
C. cordistipula							X	1	X					X		X
C. kunthiana							X	1		X				X		X
C. pilosa var. *pilosa*							X	1		X			X			X
C. pilosa var. *luxurians*							X	1-2		X					X	X
C. serpens var. *serpens*							X	1		X				X		X
C. supplex							X	1		X				X		X
C. tenuisepala							X	1		X					X	X
C. trichopoda							X	1-2	X						X	X
Ser. *Greggianae*																
C. greggii var. *greggii*							X	1		X			X			X
C. greggii var. *macdougaliana*							X	1		X				X		X
C. greggii var. *potosini*						X		1		X					X	X
Ser. *Chamaecrista*																
C. cuprea							X	1		X			X			X
C. deeringiana			X					1		X			X			X
C. fasciculata						X		1-2		X			X			X
C. glandulosa						X		1		X			X			X
C. lineata							X	+1		X		X	X			X
C. nictitans var. *paraguariensis*			X					1-2		X			X			X
C. nictitans var. *disadena*	X							+1		X	X	X	X			X
C. nictitans var. *jaliscensis*							X	1-2		X			X			X
C. obcordata							X	1-9			X		X			X
C. pascuorum							X	1-2			X		X			X
C. pedicellaris var. *pedicellaris*							X	1-2			X		X			X
C. pedicellaris var. *adenosperma*							X	1-2			X		X			X
C. portoricensis var. *portoricensis*						X		1-4		X			X			X
C. pygmaea var. *pygmaea*						X		1-2		X			X			X
C. pygmaea var. *savannarum*						X		1-2		X			X			X
C. repens var. *repens*	X							+1		X		X	X			X
C. repens var. *multijuga*	X							1		X			X			X
C. rufa var. *exsul*							X	1-2		X		X	X			X
C. rufa var. *polyplebia*						X		1-2		X			X			X
C. venturiana		X						1-2					X			X
C. vestita							X	1-2			X				X	X

Table 1. Cont.

Taxa	Form							N°	Position				Origin vascularization			Fibers adjacent to the vascularization
	Urceolate	Patelliform		Verruciform	Cupuliform				Petiole			Rachis				
	Sessile	Sessile	Short stalked	Short stalked	Short stalked	Stalked	Long stalked		A	M	B		1	2	3	
Sect. *Caliciopsis*										X						
C. calycioides var. *calycioides*							X		X					X		X
C. duckeana							X		X				X			
Sect. *Xerocalyx*																
C. desvauxii var. *desvauxii*					X				X				X			X
C. desvauxii var. *glauca*			X			X			X				X			
C. desvauxii var. *graminea*						X			X				X			X
C. diphylla						X			X				X			X
C. ramosa var. *parvifoliola*						X			X					X		X

Note: A: Apex; M: Middle; B: Base. 1: vascularization originates from the main vascular bundles; 2: vascularization originates from the accessory bundles; 3: Vascularization originates from both vascular accessory bundles and main vascular bundles.

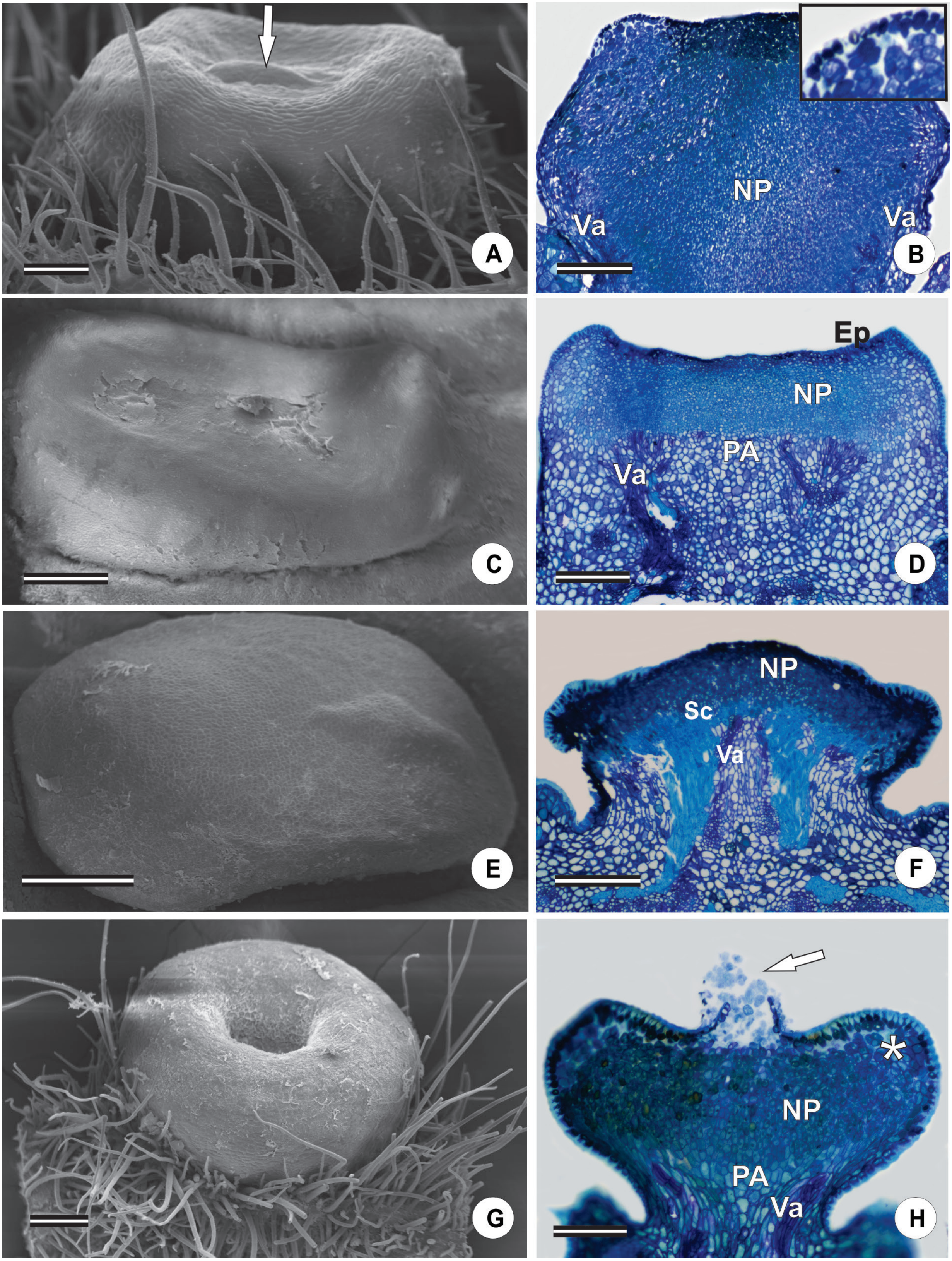

Figure 2. SEM images and anatomical sections (stained with toluidine blue) of nectaries of *Chamaecrista* sect. *Chamaecrista*. **A-B.** *C. repens* var. *repens*: sessile urceolate (note detail of intercellular spaces). **C-D.** *C. lagotois*: sessile patelliform. **E-F.** *C. simplifacta*: short-stalked verruciform. **G-H.** *C. potentilla:* short-stalked cupuliform. Note the cuticle distended (arrow) and intercellular spaces (asterisk). (Ep: epidermis; Sc: sclereids; NP: nectary parenchyma; PA: subnectary parenchyma; Va: vascular tissue). Scale bars: A: 200 µm; B, G: 100 µm; C, D: 400 µm; H: 50 µm; E, F: 300 µm.

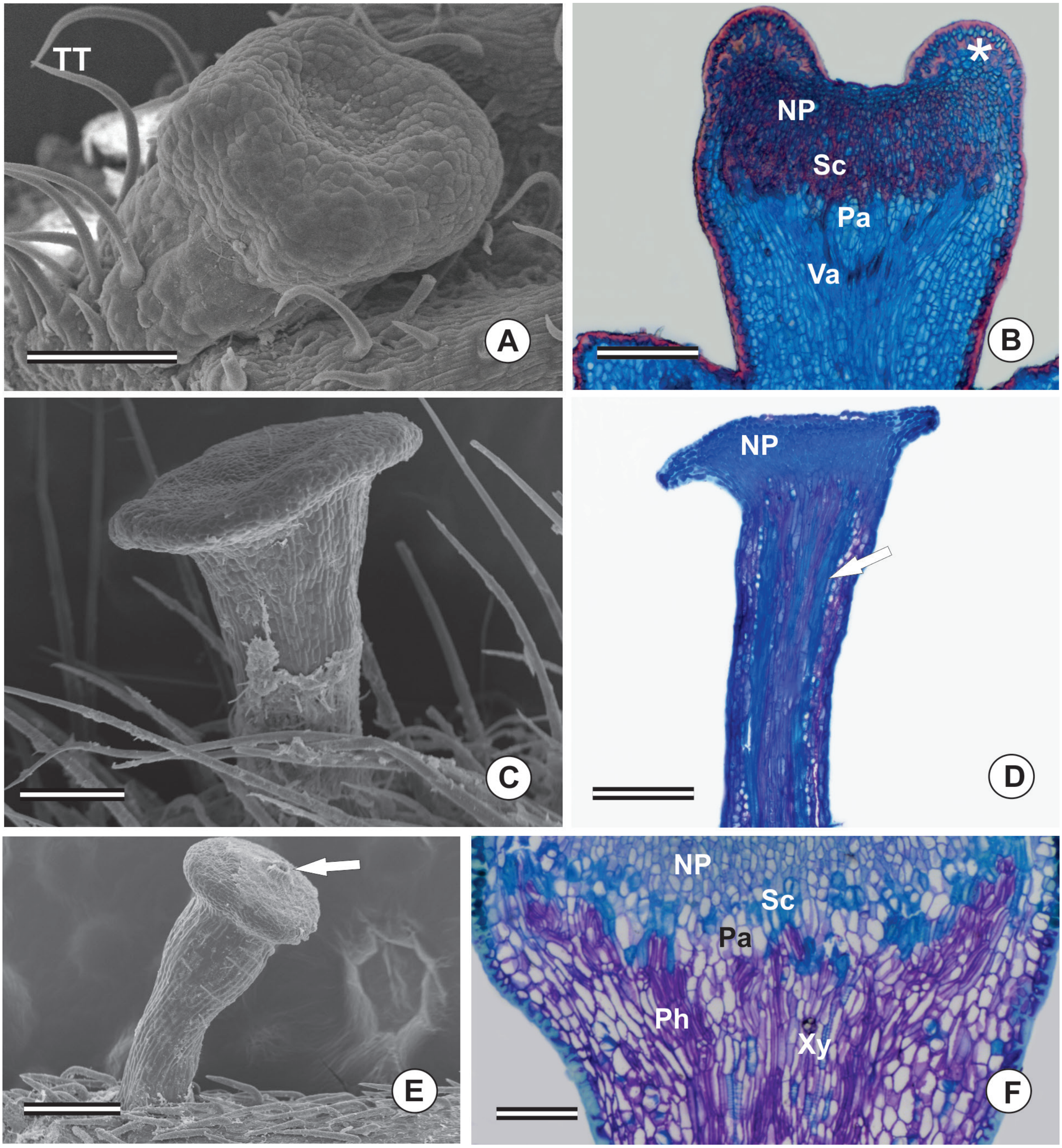

Figure 3. SEM images and anatomicals sections (stained with toluidine blue) of nectaries of *Chamaecrista* sect. *Chamaecrista*. **A-B.** *C. roraimae*: stalked cupuliform. **C-E.** *C. vestita* and *C. pedicellaris* var. *pedicellaris*, respectivelly: long-stalked cupuliform (Note the fibers: arrow). **F.** *C. mucronata*. Note the cuticle distended (arrow) and intercellular spaces (asterisk). (NP: nectary parenchyma; Va: vascular tissue; Pa: subnectary parenchyma; Ph: phloem; Xy: xylem; Sc: esclereides; TT: non-glandular trichomes). Scale bars: A, C, E, F: 200 µm; B, D: 300 µm.

parenchyma of the vascular region (Figs. 2F, 3B, F, 4F) in *C. anceps*, *C. aristata*, *C. cardiostegia*, *C. cinerascens*, *C. multinervia*, *C. rotundata* var. *rotundata*, *C. ulmea*, *C. roraimae*, *C. mucronata*, *C. latifolia*, *C. potentilla* and *C. simplifacta* (series *Coriaceae*).

The histochenical results are summarizated in table 2. Total polysaccharides (Fig. 4A, B), acid muco-polysaccharides (Fig. 4C) and pectin (4D) were detected in the secretory parenchyma as well as in the intercellular spaces; proteins (Fig.4E) were detected only in the protoplast of cells of the secretory parenchyma; general phenolic compounds (Fig. 4G) were detected in secretory parenchyma, subnectary parenchyma and epidermis; and lipids (Fig. 4H-J) were detected in the intercellular spaces and the cuticle.

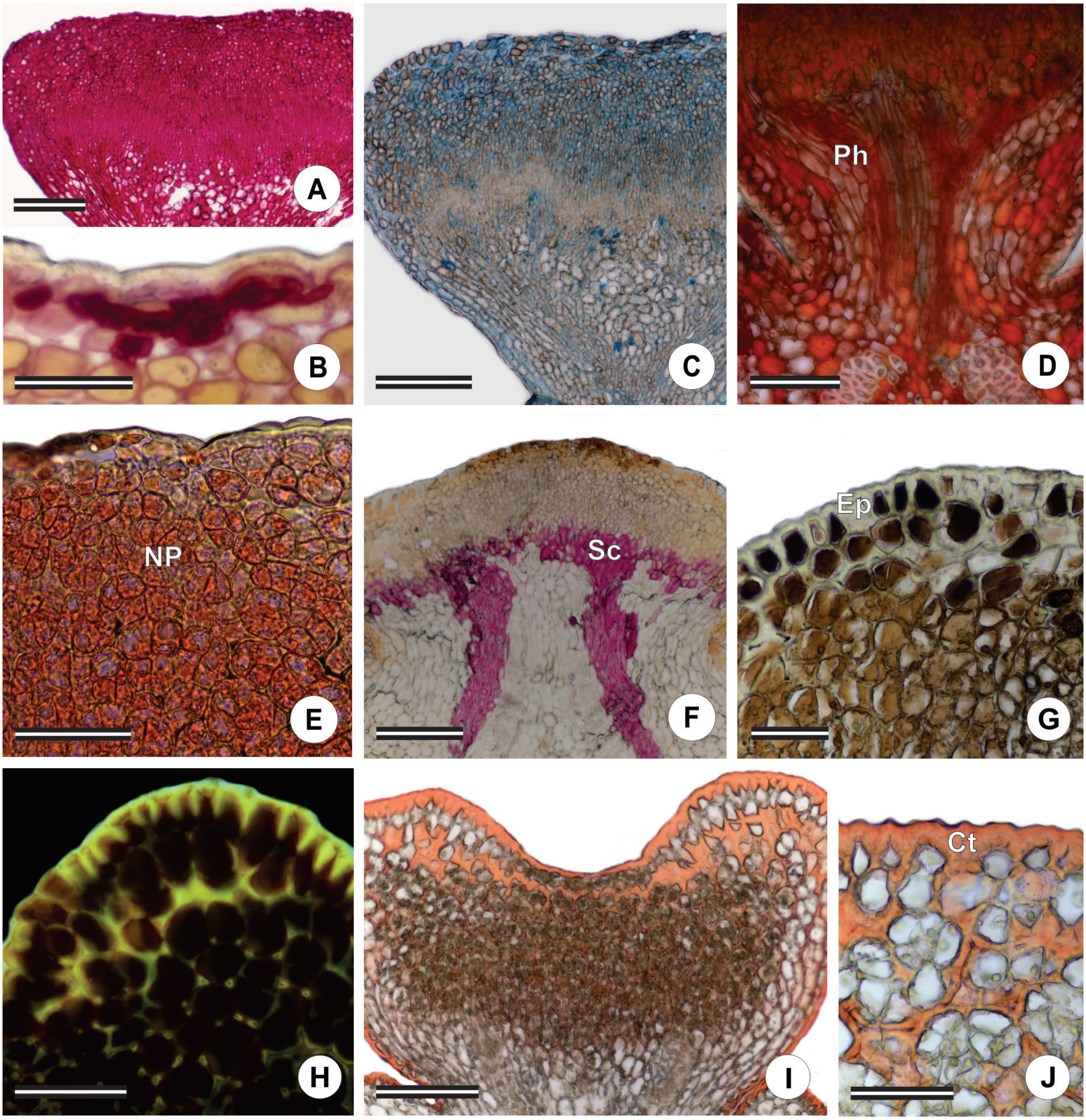

Figure 4. Histochemical tests in nectaries of *Chamaecrista* sect. *Chamaecrista*. **A-B.** Totals polysaccharides (periodic acid-Schiff reagent). **A.** *C. desvauxii* var. *glauca*. **B.** *C. tragacanthoides* var. *tragacanthoides*. **C.** Acid mucopolysaccharides (alcian blue). *C.desvauxii* var. *glauca*. **D.** Pectins (ruthenium red). *C. roraimae*. **E.** Totals proteins (xylidine Ponceau). *C. mucronata*. **F.** Lignin (phloroglucinol). *C. simplifacta*. **G.** General phenolic compounds (ferrous sulfate in formalin). *C. simplifacta*. **H-J.** Totals lipids (Sudan IV/ neutral red). **H.** *C. roraimae* (fluorescence). **I.** *C. potentilla*. **J.** *C. lagotois*. (Ep: epidermis; Ct: cuticle; Ph: phloem; Sc: sclereids; NP: nectary parenchyma) Scale bars: A, C: 300 μm; B: 25 μm; D, I: 100 μm; E, G, H, J: 50 μm; F: 200 μm.

Table 2. Histochemical tests in *Chamaecrista* studied.

Taxa	Histochemical tests								
	Total lipids		Total polysaccharides	Pectins/ mucilage	Acid mucopolysaccharides	Total proteins	Lignin	Phenolic compounds	Urinetest strips
	Neutral red	Sudan IV	Periodic acid-Schiff reagent	Ruthenium red	Alcian blue	Xylidine Ponceau	Phloroglucinol	Ferrous sulphate in formalin/	
C. aristata		+ (1, 3)	+ (3, 4)	+ (6)		+ (4)			+
C. choriophylla			+ (3, 4)	+ (6)					+
C. cinerascens			+ (3, 4)	+ (6)			+ (7)		+
C. desvauxii		+ (1, 3)	+ (3, 4)	+ (3, 4, 6)					
C. desvauxii var. *glauca*		+ (1, 3)	+ (3, 4)	+ (3, 4, 6)	+ (3, 4)				
C. desvauxii var. *graminea*			+ (3, 4)	+ (6)					
C. diphylla			+ (3, 4)	+ (6)					
C. flexuosa		+ (1, 3)	+ (3, 4)	+ (6)				+ (2)	+
C. lagotois		+ (1, 3)	+ (3, 4)	+ (6)		+ (4)		+ (2)	
C. latifólia			+ (3, 4)	+ (6)					+
C. mucronata		+ (1, 3)	+ (3, 4)	+ (6)		+ (4)	+ (7)	+ (2)	+
C. papillata			+ (3, 4)	+ (6)		+ (4)		+ (2)	+
C. potentilla		+ (1, 3)	+ (3, 4)	+ (6)		+ (4)			+
C. ramosa var. *parvifoliola*			+ (3, 4)						
C. rotundata		+ (1, 3)	+ (3, 4)	+ (6)				+ (2)	+
C. roraimae	+ (1, 3)		+ (3, 4)				+ (7)	+ (2, 4, 5)	
C. simplifacta		+ (1, 3)	+ (3, 4)	+ (6)		+ (4)	+ (7)	+ (2, 4, 5)	+
C. tragacanthoides		+ (1, 3)	+ (3, 4)	+ (6)		+ (4)		+ (2, 4, 5)	

1: Cuticle; 2: Epidermis; 3: Intercellular spaces; 4: Secretory parenchyma; 5: Subnectary parenchyma; 6: Phloem; 7: Fibers and /or sclereids.

Discussion

The glandular structures present on the petioles/rachises of the studied species are classified as extra-reproductive nectaries based on their topography, morphology, anatomical structure and presence of glucose. The structure of the nectaries studied here is similar to that of nectaries described for other species of *Chamaecrista* and for other genera of the subfamily Caesalpinioideae (Bhattacharyya & Maheshwari 1971; Elias 1983; Francino *et al.* 2006; 2015; Paiva & Machado 2006; Melo *et al.* 2010; Coutinho *et al.* 2012; Coutinho & Meira 2015).

Cupuliform nectaries were the most common form recorded for the three sections studied. Patelliform nectaries are almost exclusive to species of the sect. *Chamaecrista* ser. *Coriaceae*. Our observations confirmed the occurrence of patelliform nectaries in three previously studied species of ser. *Coriaceae* and in *C. desvauxii* var. *langsdorfii* (section *Xerocalyx*) (Francino *et al.* 2015). Verruciform and urceolate nectaries were uncommon. The presence of cupuliform nectaries in *C. flexuosa* and *C. swainsoni* (ser. *Flexuosae*) and in the eight taxa of ser. *Coriaceae* studied is a morphological similarity that reinforces the hypothesis made by Conceição *et al.* (2009) and Rando *et al.* (2016), that ser. *Flexuosae* is the sister group to ser. *Coriaceae*. Additionally, *C. caribaea, C. venulosa* and *C. roraimae*, which all have the same type of nectary, were separated from the other species of ser. *Coriaceae* and considered related to species of ser. *Chamaecrista,* ser. *Prostratae* and sect. *Caliciopsis,* respectively (Rando *et al.* 2016). The species of sect. *Caliciopsis*, and most of the species of the sect. *Xerocalyx* studied, exhibited cupuliform nectaries, the same as observed in 37 taxa of sect. *Chamaecrista*. These data demonstrate affinities between the three sections, a relationship that had already been proposed in studies of molecular phylogeny (Conceição *et al.* 2009; Rando *et al.* 2016).

Nectaries are an effective tool for taxonomy because of variation in their shape and position on the plant body (Keeler & Kaul 1979; Bentley & Elias 1983). In species of *Chamaecrista* sect. *Apoucouita,* Coutinho & Meira (2015) and Coutinho *et al.* (2016) observed 13 different types of extra-reproductive nectaries and demonstrated their important role in the taxonomy of the genus. On the other hand, morphoanatomical similarities among the extra-reproductive nectaries of sect. *Absus* subsect. *Baseophyllum* seems to support the elevation of this subsection to sectional level (Coutinho *et al.* 2012), as proposed by Conceição *et al.* (2009) with studies based on molecular data. Although morphologically different, our study found anatomical similarities among the extra-reproductive nectaries of *Chamaecrista* in that all of them comprise a single layered epidermis, several layers of nectary parenchyma with underlying layers of subnectary parenchyma and vascularization. Such anatomical similarity is also shared with other species of *Chamaecrista* that bear extra-reproductive nectaries (Coutinho *et al*. 2012; Coutinho & Meira 2015; Francino *et al*. 2015).

Extra-reproductive nectaries vascularized by xylem and phloem are common in species of the genus *Chamaecrista* (Coutinho *et al.* 2012; Coutinho & Meira 2015), and the vascularization often originates from the main vascular system of the petiole and/or rachis (Francino *et al.* 2006; Coutinho *et al.* 2012), as observed for most species in our study. The layer of sclereids that has been observed only in species of the ser. *Coriaceae* is similar to that found by Coutinho *et al.* (2012) in species of *Chamaecrista* sect. *Absus* subsect. *Baseophyllum* and may serve to provide mechanical support for nectaries. Paiva & Machado (2006) reported the presence of an endoderm with lignified and suberized cells in the nectary of *Hymenaea stigonocarpa*, and according to them the endoderm may prevent the reflux of nectar and direct its release extenally. A similar function can be attributed to the boundary layer of sclereids observed in the nectaries studied herein. The non-secreting parenchyma has also been considered a barrier to apoplastic transport, preventing the reflux of nectar to internal tissues (Contreras & Lersten 1984; Francino *et al.* 2006; Paiva & Machado 2006; Melo *et al.* 2010). The conspicuous intercellular spaces present in the secretory tissue of the nectaries of the species studied here have already been observed in other species of *Chamaecrista* (Coutinho *et al.* 2012; Coutinho & Meira 2015), and is considered the likely location for the accumulation of nectar prior to its being released to the exterior, as well as contributing to apoplastic transport of nectar (Vassilyev 2010).

The distended cuticle in the center of the secreting portion of the nectaries leads us to conclude that nectar is accumulated below the cuticle and that it is later secreted to the outside through cuticular burst. This manner of secretion release is in accordance with several authors (Fahn 1979; Elias 1983; Paiva & Machado 2006; Nepi 2007; Thadeo *et al.* 2008; Rocha *et al.* 2009; Paiva 2016).

Conceição *et al.* (2009) hypothesized a single origin for extra-reproductive nectaries in *Chamaecrista*, which is supported, thus far, by the anatomical similarity among these structures. However, despite their singular origin, the extra-reproductive nectaries of *Chamaecrista* may have followed different evolutionary trends, which may be supported by the type of secretion released. The extra-reproductive nectaries of the species of sect. *Apoucouita* secrete lipids in addition to carbohydrates and sugars (Coutinho & Meira 2015). In species of sect. *Absus* subsect. *Baseophyllum*, lipids were not detected, but phenolic compounds were (Coutinho *et al.* 2012). Similar to other species of *Chamaecrista* already studied, the nectar released by species of sect. *Chamaecrista, Caliciopsis* and *Xerocalyx* studied herein are complex. This nectar is made up of a mixture of lipids, phenolic compounds and proteins, which may be important in plant-animal interactions. Lanza *et al.* (1993) showed that *Solenopsis geminata* and *S. invicta* exhibited different preferences regarding nectar composition; *S. gemitala* workers preferred extra-reproductive nectars that were richer in aminoacids, while *S. invicta* did not discrinnate between the two types of nectar offered. Nectar richer in aminoacids is produced by *Impatiens sultani* when plants are subjected to simulated herbivory (Lanza *et al*. 1993). Therefore, if a mutualistic ant, such as *S. geminata*, which prefers aminoacid-rich nectars, was involved, such behavior could favor the survival of species with aminoacid-rich extra-reproductive nectars. Such preferential behavior may also exist for other substances, such as the presence/absence of lipids, cardohydrates and so on. Comprehensive evolutionary and ecological studies are needed for the species of the genus *Chamaecrista* in order to better understand the role of secretions in mediating interactions with visitors and promoting the evolution and diversification of the group.

The lipid content found in the intercellular spaces of the species studied here is in accordance with reports for other genera, including *Chamaecrista* (Baker *et al.* 1978; Fahn 1979; 1988; 2000; Coutinho & Meira 2015). As stated by Paiva & Machado (2006), the presence of lipids in the intercellular spaces of the secretory parenchyma indicates that these compounds are part of the secretion and that the plant offers a reward in a more energetic form. Additionally, extreme environmental conditions may require high-energy food resource for visitors, a hypothesis proposed by Forcone *et al.* (1997) and Bernardello *et al.* (1999). The phenolic compounds found within idioblasts may act in defense against herbivores, as their content renders a plants organs/structures unpalatable, in adidiiton to providing protection from pathogens (Nicolson & Thornburg 2007). Some studies have suggested that plants with high levels of amino acids in their nectar attract more ants, and therefore may suffer less herbivory (Lanza 1991; Wagner & Kay 2002; Wilder & Eubanks 2009).

Although anatomical similarities were observed among the species studied, the morphology differed. The form of the nectaries of ser. *Prostratae* and *Greggiianae* were found to be well-defined, representing important data for the taxonomy of these series. The similarity among the nectaries of the three studied sections (i.e. *Chamaecrista*, *Caliciopsis* and *Xerocalyx*) may also be of taxonomic value in justifying the grouping of these three sections into a single clade.

Our study also provides unprecedented data regarding the anatomy of the extra-reproductive nectaries of the species of the sect. *Caliciopsis*. The database provided by this work will likely be important for future studies into the taxonomy and phylogeny of these plants.

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References

Baker DA, Hall JL, Thorpe JR A. 1978. Study of the extrafloral nectaries of *Ricinus communis*. New Phytologist 81: 129-137.

Bentley B, Elias TS. 1983. The biology of nectaries. New York, Columbia University Press.

Bernardello L, Galetto L, Forcone A. 1999. Floral nectar chemical composition of some species from Patagonia. II. Biochemical Systematics and Ecology 27: 779-790.

Bhattacharyya B, Maheshwari JK. 1971. Studies on extrafloral nectaries of the Leguminales – II. The genus *Cassia* Linn. (Caesalpiniaceae). Proceedings of the Indian National Science Academy 37: 74-90.

Bozzola JJ, Russel LD. 1991. Electron microscopy: principles and techniques for biologists. New York, Jones and Bartlett Publishers.

Conceição AS, Queiroz LP, Lewis GP, *et al*. 2009. Phylogeny of *Chamaecrista* Moench (Leguminosae-Caesalpinioideae) based on nuclear and chloroplast DNA regions. Taxon 58: 1168-1180.

Contreras LS, Lersten NR. 1984. Extrafloral nectaries in Ebenaceae: anatomy, morphology and distribution. American Journal of Botany 71: 865-872.

Coutinho IAC, Francino DMT, Azevedo AA, Meira RMSA. 2012. Anatomy of the extrafloral nectaries in species of *Chamaecrista* section *Absus* subsection *Baseophyllum* (Leguminosae, Caesalpinioideae). Flora 207: 427-435.

Coutinho IAC, Meira RMSA. 2015. Structural diversity of extrafloral nectaries in *Chamaecrista* sect. *Apoucouita*. Botany 93: 379-388.

Coutinho IAC, Rando JG, Conceição AS, Meira RMSA. 2016. A study of the morphoanatomical characters of *Chamaecrista* sect. *Apoucouita*. Acta Botanica Brasilica 30: 205-221.

Dalvi VC, Meira RMSA, Azevedo AA. 2013. Extrafloral nectaries in neotropical Gentianaceae: Occurrence, distribution patterns, and Anatomical characterization. American Journal of Botany 100: 1-11.

Del-Claro K, Rico-Gray V, Torezan-Silingardi, *et al*. 2016. Loss and gains in ant–plant interactions mediated by extrafloral nectar: fidelity, cheats, and lies. Insectes Sociaux 63: 207-221.

Elias TS. 1983. Extrafloral nectaries: their structure and distribution. In: Bentley BL, Elias TS. (eds.) The Biology of Nectaries. New York, Columbia University Press. p. 174-203.

Fahn A. 1979. Secretory tissues in plants. London, Academic Press.

Fahn A. 1988. Secretory tissues in vascular plants. New phytologist 108: 229-257.

Fahn A. 2000. Structure and function of secretory cells. Advances in Botanical Research 31: 37-75.

Fernandes GW, Fagundes M, Greco MKB, Barbeitos MS, Santos JC. 2005. Ants and their effects on an insect herbivore community associated with the inflorescences of *Byrsonima crassifolia* (Linneaus) H.B.K. (Malpighiaceae). Revista Brasileira de Entomologia 49: 264-269.

Forcone A, Galetto L, Bernardello L. 1997. Floral nectar chemical composition of some species from Patagonia. Biochemical Systematics and Ecology 25: 395-402.

Francino DMT, Coutinho IAC, Dalvi VC, Azevedo AA, Conceição, AS, Meira RMSA. 2015. Anatomical interpretations of the taxonomy of *Chamaecrista* section *Absus* (Leguminosae - Caesalpinioideae s.l.). Plant Systematics and Evolution 301: 2087-2103.

Francino DMT, Sant'Anna-Santos BF, Silva KLF. 2006. Anatomia foliar e caulinar de *Chamaecrista trichopoda* (Caesalpinioideae) e histoquímica do nectário extrafloral. Planta Daninha 2: 695-705.

Irwin HS, Barneby RC. 1982. The American Cassiinae, a synoptical revision of Leguminosae tribe Cassieae subtribe Cassiinae in the New World. Memoirs of the New York Botanical Garden 35: 1-918.

Johansen DA. 1940. Plant microtechnique. New York, McGraw-Hill.

Keeler KH, Kaul RB. 1979. Morphology and distribution of petiolar nectarines in *Ipomoea* (Convolvulaceae). American Journal of Botany 66: 946-952.

Lanza J. 1991. Response of fire ants (Formicidae: *Solenopsis invicta* and *S. geminata*) to artificial nectars with amino acids. Ecological Entomology 16: 203-210.

Lanza J, Vargo E, Pulim S, Chang Yu Z. 1993. Preferences of the fire ants *Solenopsis invicta* and *S. geminata* (Hymenoptera: Formicidae) for amino acid and sugar components of extrafloral nectars. Environmental Entomology 22: 411-417.

Madureira M, Sobrinho TG. 2002. Evidência de mutualismo entre *Qualea cordata* (Vochysiaceae) e *Cephalotes* sp. (Hymenoptera: Formicidae). Academia Insecta 2: 1-4.

Maia V. 1979. Técnica histológica. São Paulo, Atheneu.

Melo Y, Machado SR, Alves M. 2010. Anatomy of extrafloral nectaries in Fabaceae from dry-seasonal forest in Brazil. Botanical Journal of the Linnean Society 163: 87-98.

Metcalfe CR, Chalk L. 1979. Anatomy of the Dicotyledons. Vol. 1. Oxford, Clarendon Press.

Nascimento EA, Del-Claro K. 2010. Ant visitation to extrafloral nectaries decreases herbivory and increases fruit set in *Chamaecrista debilis* (Fabaceae) in a Neotropical savanna. Flora 205: 754-756.

Nepi M. 2007. Nectary structure and ultrastructure. In: Nicolson SW, Nepi M, Pacini E. (eds.) Nectaries and nectar. Dordrecht, Springer. p. 129-166.

Nicolson SW, Thornburg RW. 2007. Nectar chemistry. In: Nicolson SW, Nepi M, Pacini E. (eds.) Nectaries and nectar. Dordrecht, Springer. p. 215-264.

Nicolson SW, Nepi M, Pacini E. 2007. Nectaries and nectar. Dordrecht, Springer.

O`Brien TPE, Mccully ME. 1981. The study of plant structure principles and select methods. Melbourne, Termarcarphi Pty.

Paiva EAS. 2016. How do secretory products cross the plant cell wall to be released? A new hypothesis involving cyclic mechanical actions of the protoplast. Annals of Botany 117: 533-540.

Paiva EAS, Machado RS. 2006. Ontogênese, anatomia e ultra-estrutura dos nectários extraflorais de *Hymenaea stigonocarpa* Mart. ex Hayne (Fabaceae-Caesalpinioideae). Acta Botanica Brasilica 20: 471-482.

Pearse AGE. 1980. Histochemistry theoretical and applied. Vol 2. Edinburgh, Churchill Livingston.

Polhill RM, Raven PH, Stirton CH. 1981. Evolution and systematics of the Leguminosae. In: Polhill RM, Raven PH. (eds.) Advances in Legumes systematic Part 1. Kew, Royal Botanic Gardens. p 1-26.

Radford AE, Dickison WC, Massey JR, Bell CR. 1974. Vascular plant systematics. New York, Harper & Row.

Rando JG, Pirani JR. 2012. A new species of *Chamaecrista* sect. *Chamaecrista* ser. *Flexuosae* (Leguminosae, Caesalpinioideae) from Serra do Cipó, Minas Gerais, Brazil. Brittonia 64: 241-245.

Rando JG, Loeuille B, Pirani JR. 2013. Taxonomic novelties in *Chamaecrista* (Leguminosae: Caesalpinioideae) from Brazil. Phytotaxa 97: 17-25.

Rando JG, Zuntini AR, Conceição AS, Berg C, Pirani JR, Queiroz LP. 2016. Phylogeny of *Chamaecrista* ser. *Coriaceae* (Leguminosae) unveils a lineage recently diversified in Brazilian Campo Rupestre vegetation. International Journal of Plant Sciences 177: 3-17.

Rocha DI, Silva LC, Valente VMM, Francino DMT, Meira RMSA. 2009. Morphoanatomy and development of leaf secretory structures in *Passiflora amethystina* Mikan (Passifloraceae). Australian Journal of Botany 57: 619-626.

Rutter MT, Rausher MD. 2004. Natural selection on extrafloral nectar production in *Chamaecrista fasciculata*: the costs and benefits of a mutualism trait. Evolution 58: 2657-2668.

Schmid R. 1988. Reproductive versus extra-reproductive nectarines – historical perspective and terminological recommendations. Botanical Review 54: 179-227.

Smith FH, Smith EC. 1942. Anatomy of the inferior ovary of *Darbya*. American Journal of Botany 29: 464-471.

Thadeo M, Cassino MF, Vitarelli NC, *et al*. 2008. Anatomical and histochemical characterization of extrafloral nectaries of *Prockia. crucis* (Salicaceae). American Journal of Botany 95: 1515-1522.

Thiers B. 2016. Index Herbariorum: A global directory of public herbaria and associated staff. New York, New York Botanical Garden's Virtual Herbarium.

Vassilyev AE. 2010. On the mechanisms of nectar secretion: revisited. Annals of Botanany 105: 349-54.

Wagner D, Kay A. 2002. Do extrafloral nectaries distract ants from visiting flowers? An experimental test of an overlooked hypothesis. Evolutionary Ecology Research 4: 293-305.

Wilder SM, Eubanks MD. 2009. Extrafloral nectar content alters foraging preferences of a predatory ant. Biology Letters. 6: 177-179.