

ARTICLE

Structural diversity of extrafloral nectaries in *Chamaecrista* sect. *Apoucouita*

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Abstract: Section *Apoucouita* (*Chamaecrista* (L.) Moench – Caesalpinioideae) is an arboreal group that is most diverse in the Amazon and Brazilian Atlantic forests. These species typically bear petiolar and (or) rachis glands called extrafloral nectaries (EFNs). However, no detailed anatomical studies or histochemical analyses have been conducted to confirm nectar secretion. We aimed at describing the structure of such EFNs, as well as determining the chemical nature of the secretion. Eighteen species (23 taxa) were studied using standard light microscopy techniques. We describe 13 types of EFNs with variable morphology. Such EFNs may be impressed, sessile, or stalked; with concave, flat or truncate, or convex secretory surfaces. Cupuliform EFNs (stalked or not) were the most common type observed and patelliform the least common. Despite the morphological variation, differences in the anatomical structure of the EFNs and the chemical composition of the secretion were not observed. EFNs with concave secretory surfaces appear to be more effective as nectar may become accumulated in the concavity, increasing the volume of available nectar. Our results show that despite the variable morphology of the EFNs, such structures share similarities on the anatomy and composition of the secretion and development of a wound-healing periderm in older EFNs. We also indicate the importance of including the morphological variation observed in the EFNs in species of sect. *Apoucouita* in future taxonomic evaluations.

Key words: anatomy, Caesalpinioideae, histochemistry, secretory structures, wound-healing periderm.

Résumé : La section *Apoucouita* (*Chamaecrista* (L.) Moench – Caesalpinioideae) constitue un groupe arboricole très varié des forêts de l'Amazone et du Brésil atlantique. Ces espèces portent typiquement des glandes sur le pétiole ou le rachis appelées nectaires extra-floraux (NEF). Toutefois, aucune étude anatomique ni analyse histochimique détaillées n'ont été réalisées afin de confirmer la sécrétion de nectar. Les auteurs avaient pour objectif de décrire la structure de tels NEF ainsi que de déterminer la nature chimique de la sécrétion. Dix-huit espèces (23 taxons) ont été étudiées par des techniques standard en microscopie optique. Les auteurs décrivent 13 types de NEF de morphologies variables. Ces NEF peuvent être rentrants, sessiles ou pédonculés, avec des surfaces sécrétrices concaves, plates ou tronquées ou convexes. Les NEF cupuliformes (pédonculés ou non) constituaient le type le plus commun, alors que les NEF patelliformes, le moins commun. Malgré les variations morphologiques, des différences de structure anatomique des NEF et de composition chimique des sécrétions n'ont pas été observées. Les NEF possédant des surfaces sécrétoires concaves semblaient plus efficaces car le nectar pouvait s'accumuler dans la concavité, augmentant le volume de nectar disponible. Les résultats des auteurs montrent que malgré la morphologie variable des NEF, de telles structures partagent des similarités sur le plan de l'anatomie et de la composition de la sécrétion, et le développement d'un périderme cicatriciel chez les NEF plus âgés. Les auteurs indiquent aussi l'importance d'inclure la variation morphologique observée chez les NEF des espèces de la section *Apoucouita* lors d'évaluations taxonomiques futures. [Traduit par la Rédaction]

Mots-clés : anatomie, Caesalpinioideae, histochimie, structures sécrétoires, périderme cicatriciel.

Introduction

Chamaecrista (L.) Moench is a legume genus of the subfamily Caesalpinioideae placed in the tribe Cassieae, subtribe Cassiinae (Irwin and Barneby 1982). This genus includes more than 330 species sorted into six sections: *Apoucouita, Absus, Caliciopsis, Chamaecrista, Grimaldia,* and *Xerocalyx. Chamaecrista* species are mainly tropical with greatest diversity in South America (Lewis 2005). The presence of extrafloral nectaries (EFNs) is remarkable in sect. *Apoucouita, Caliciopsis, Chamaecrista,* and *Xerocalyx,* along with sect. *Absus sub*sect. *Baseophyllum* (Irwin and Barneby 1982; Coutinho et al. 2012). Species of sect. *Apoucouita* are distinguished from other sections based on the following set of characteristics: woody tree habit, distichous phyllotaxy (spiral only in *Chamaecrista adiantifolia* and *Chamaecrista apoucouita*), leaves characteristically bearing petiolar and (or) rachis glands (Figs. 1A–1B), cauliflorous inflorescence with raceme glands resembling those of the leaves, and elastically dehiscent pendant fruits (Bentham 1870; Irwin and Rogers 1967).

Petiolar and (or) rachis glands of *Chamaecrista* sect. *Apoucouita* are most commonly truncate or depressed, varying from stipitate to sessile or impressed (Irwin and Rogers 1967). Although such glands in species of sect. *Apoucouita* have been called EFNs (Conceição et al. 2009; Rando 2009), no detailed anatomical studies or histochemical analyses have been performed to confirm nectar secretion. Moreover, due to morphological and topographical similarities, other secretory structures such as lipid- or resin-secreting glands, colleters, and hydathodes have been mistakenly called nectaries (Curtis and Lersten 1978; Durkee et al. 1984; McDade and Melvin 1997; Freitas et al. 2001). Fahn (1979), Mayer et al. (2011), and Radford et al. (1974) argue for the importance of histochemical analyses of the exudates to properly characterize secretory structures.

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Fig. 1. Leaf extrafloral nectaries (arrows) on the rachides of *Chamaecrista* species. (A) *C. duartei*. (B) *C. ensiformis* var. *ensiformis*. Note the secreted nectar in detail in the insets.



The following questions are addressed in our study: Do the different morphologies of the petiolar and (or) rachis glands in species of sect. *Apoucouita* necessarily reflect anatomical differences? Are the histochemical results of the secretion the same even when the gland structures are variable? How are the energy-containing compounds that will be secreted as nectar transported to such structures? The present study was undertaken to anatomically characterize the structure of the petiolar and (or) rachis glands found in species of sect. *Apoucouita* and to determine the composition of the secretion.

Materials and methods

For this study, 18 (23 taxa) of the 21 (26 taxa) species ascribed to sect. *Apoucouita* (Irwin and Barneby 1977, 1979, 1982, 1985; Barneby 1999) were sampled (Appendix A). Taxonomic authorities for all taxa mentioned in the paper (including the figures) are given in the Appendix.

Both young and fully expanded leaves collected in the field were fixed in FAA (formaldehyde, acetic acid, and 50% ethanol; 1:1:18 by volume) for 48 h and stored in 70% ethanol (Johansen 1940). Material sampled from herbarium material was microwaved in distilled water for 7 min and left to rest until the water had cooled. Rehydrated samples were then treated with 2% potassium hydroxide for 1–2 h at room temperature (Smith and Smith 1942), rinsed with tap water until the potassium hydroxide was completely removed, dehydrated in an ethanol series, and stored in 70% ethanol.

Samples from both the herbarium material and field-collected specimens stored in 70% ethanol were embedded in methacrylate (Historesin Leica; Leica Microsystems Nussloch GmbH, Heidelberg, Germany) according to the manufacturer's recommendation. Cross and longitudinal 4 μ m thick sections were made in an automatic rotary microtome (Leica RM2155; Leica Microsystems, Deerfield, Illinois, USA) and stained with toluidine blue at pH 4.4 (O'Brien and McCully 1981) for structural characterization. Slides were dried at room temperature and mounted in resin (Permount; Fisher Scientific, New Jersey, USA).

To study the composition of the secretion, eight *Chamaecrista* species were selected: *C. adiantifolia* var. *pteridophylla*, *C. bahiae*, *C. duartei*, *C. ensiformis* var. *ensiformis*, *C. negrensis* var. *negrensis*, *C. polystachya*, *C. scleroxylon*, and *C. xinguensis* (Appendix A). For such species, sections from field-collected material embedded in methacrylate were used in the histochemical tests carried out as follows: for total proteins, xylidine Ponceau (O'Brien and McCully 1981); for total polysaccharides, periodic acid – Schiff (PAS) (O'Brien and McCully 1981); for general phenolic compounds, ferrous chloride III (Johansen 1940); for general phenolic compounds, Sudan Black B and Sudan red (Pearse 1980).

Only Chamaecrista ensiformis var. ensiformis, C. negrensis var. negrensis, and C. polystachya were available for glucose identification in the exudates of the petiolar and (or) rachis glands using urine test strips (Alamar Tecno Científica Ltda., São Paulo, Brazil) during the field expeditions. The presence of lipids in the exudates was determined for these three species by means of Sudan stains (Sudan Black B and Sudan red). Such stains were directly applied on the secreting glands. Insect visitation to the petiolar and (or) rachis glands was also recorded.

Both observations and image capture were conducted using light microscopes. Observations were performed using a Zeiss Primo Star microscope, and images were captured with an Olympus Optical AX70TRF (Tokyo, Japan) equipped with a U-Photo system and digital camera (AxioCam HRc – Carl Zeiss – Gottingen, Germany).

Results

Structure of EFNs

The EFNs found on the petiole and (or) rachis of *Chamaecrista* sect. *Apoucouita* may be impressed, sessile, or elevated (Table 1). Impressed EFNs (Figs. 2A–2D) occurred in nine taxa (Table 1), including *C. polystachya* (Fig. 2A) and *C. bahiae* (Fig. 2B). Six of the nine taxa displayed the concave (Fig. 2D), flat or truncate (Fig. 2B), and convex (Fig. 2C) types, whereas *C. amabilis* did not present the convex type, *C. apoucouita* only flat or truncate type, and *C. polystachya* that presented both convex and flat or truncate (Table 1).

Sessile EFNs (Figs. 2E–2H) were observed in all species except *C. aspidiifolia*, *C. compitalis*, and *C. subpeltata* (Table 1). Sessile flat or truncate EFNs (Fig. 2E) were found in 14 species and sessile convex in 13 species. The sessile urceolate type (Fig. 2F) was observed in eight taxa (Table 1). Such nectaries characteristically bore two rims, one at the top, followed by a slight strangulation and then the second rim, resembling an urn (Fig. 2F). Sessile concave EFNs (Figs. 2G–2H) were observed in all but four species (Table 1).

Cupuliform EFNs (Figs. 3A–3F), were the most common type of nectary found in 17 of the 23 species studied (Table 1). The elevation of the EFNs results from the presence of a stalk. Here, "stalk" is defined as the cylindrical structure that bears the secretory apical part and is vascularized and nonsecretory. Stalked EFNs differed in the length of the stalk relative to the length of the secretory head: short-stalked EFNs, in which the stalk was shorter than the height of the apical secretory portion (Figs. 3A–3D); stalked EFNs, in which the stalk was one or two times the length of the apical secretory portion (Fig. 3E); and long-stalked EFNs, in which the stalk was more than three times the length of the apical secretory portion (Fig. 3F). Only four species (*C. aspleniifolia, C. aspidiifolia, C. compitalis,* and *C. subpeltata*) did not present sessile EFNs (Table 1) and characteristically displayed the stalked or long-stalked types. Although EFNs bearing a stalk could be observed in

	Type of EFN												
Species/Taxon	Impressed			Sessile				Cupuliform			Patelliform		
	Concave	Flat or truncate	Convex	Flat or truncate	Convex	Urceolate	Concave	Short-stalked	Stalked	Long-stalked	Short-stalked	Stalked	Long-stalked
C. adiantifolia var. adiantifolia ^a						×	×	×	×				
C. adiantifolia var. pteridophylla ^a						×	×	×	×				
C. amabilis	×	×		×	×	×	×						
C. amorimii	×	×	×	×	×		×						
C. apoucouita ^a		×		×		×	×	×	×				
C. aspidiifolia									×	×			
C. aspleniifolia ^d							×	×	×				
C. bahiae ^a	×	×	×	×	×		×	×					
C. compitalis									×	×		×	×
C. duartei	×	×	×	×	×		×						
C. eitenorum var. eitenorum				×	×		×	×					
C. eitenorum var. regana				×	×		×	×					
C. ensiformis var. ensiformis ^{bc}				×	×		×	×	×	×	×	×	×
C. ensiformis var. maranonica ^{bc}				×	×		×	×	×	×	×	×	×
C. ensiformis var. plurifoliolata ^{bc}				×	×		×	×	×	×	×	×	×
C. hymenaeifolia	×	×	×	×	×		×						
C. negrensis var. albuquerquei	×	×	×	×	×	×	×	×					
C. negrensis var. negrensis	×	×	×	×	×	×	×	×					
C. onusta							×						
C. polystachya		×	×	×	×								
C. scleroxylon						×	×	×					
C. subpeltata										×			
C. xinguensis						×	×	×	×				

Table 1. Types of extrafloral nectaries (EFN) observed on the petiole and (or) rachis of Chamaecrista.

 ${}^a\!\mathrm{Most}$ proximal EFN to the leaf base usually short-stalked while others on the rachis stalked.

^bMost proximal EFN usually short-stalked or sessile while others on the rachis stalked.

cAlthough C. ensiformis var. ensiformis and C. ensiformis var. maranonica presented long-stalked EFNs, such EFN types are common only to C. ensiformis var. plurifoliolata.

^dDiffering from other species, as the most proximal EFN is sessile concave with an oblique secretory surface in frontal view.

Fig. 2. Longitudinal (A, G and H) and cross (B–F) sections of the rachis/petiole showing sessile leaf extrafloral nectaries (EFNs) in *Chamaecrista* species. (A) *C. polystachya* showing the impressed convex type of EFN. The EFNs are composed of an epidermis (ep), a nectary parenchyma (arrows), and a subnectary parenchyma (snp). (B) *C. bahiae* showing the impressed flat/truncate type of EFN. (C) *C. negrensis* var. *albuquerquei*, sessile convex. (D) *C. ensiformis* var. *maranonica*, sessile concave. (E) *C. bahiae*, sessile concave tending to flat/truncate. (F) *C. adiantifolia* var. *adiantifolia*, sessile urceolate. (G) *C. aspleniifolia*, sessile concave with oblique secretory surface in frontal view. (H) *C. adiantifolia* var. *pteridophylla*, sessile concave. Note the vascular bundles (vb) going towards the EFN. Scale bars are 500 μm. (This figure is available in color online.)



all three varieties of *C. ensiformis*, *C. ensiformis* var. *plurifoliolata* was the variety that most commonly presented the long-stalked type of EFN (Fig. 3F).

Patelliform EFNs (stalked but with a convex instead of a concave secretory surface) were observed only in *C. compitalis, C. ensiformis* var. *ensiformis*, *C. ensiformis* var. *maranonica,* and *C. ensiformis* var. *plurifoliolata.* Whether sessile, impressed, or elevated, EFNs could be oblong/elliptical or round/discoid in frontal view (Table 1). As a whole, when more than one EFN was present, the most proximal to the leaf base was the largest one.

Although the morphology of the EFNs varied according to the taxon studied, all glands displayed anatomical similarities. A single-layered epidermis composed of cubical to columnar-shaped cells and devoid of stomata was observed (Figs. 2A, 2H, and 3A).

Whether convex, concave, or flat or truncate, the smallest cubical epidermal cells with the thinnest walls were observed at the center of the gland (Figs. 2H and 3A), whereas cells towards the margin were usually larger, more columnar-shaped, and possessed thicker cell walls (Figs. 2H and 3A). Similarly, the cuticle covering the epidermis was thicker towards the margin and thinner at the center of the gland. Few multicellular or unicellular, uniseriate, tector trichomes were found on the epidermis and tended to fall off in the mature nectary.

One layer (rarely two) of hypodermal cells was observed. As a whole, the hypodermal layer was similar in shape and structure to the epidermis. At the center of the nectary, between the epidermis and hypodermis, or even below the hypodermis, it was common to observe spaces filled up with secretion among the cells. **Fig. 3.** Cross sections of the rachis/petiole showing stalked leaf extrafloral nectaries (EFNs) in *Chamaecrista* species. (A) *C. ensiformis* var. *ensiformis*, short-stalked EFNs composed of a secretory apical part (ap) and a stalk (st). The EFNs is anatomically characterized for having an epidermis (ep), a nectary parenchyma (np), a subnectary parenchyma (snp), and vascular bundles (vb). Arrows indicate sclereids. (B) *C. ensiformis* var. *ensiformis* seen under polarized light. Note the presence of calcium oxalate crystals (bright spots). (C) *C. scleroxylon*, short-stalked EFN. Note that the stalk is as wide as the secretory apical part. (D) *C. aspleniifolia*, short-stalked EFN with oblique secretory surface. (E) *C. compitalis*, stalked-EFN. (F) *C. ensiformis* var. *plurifoliolata*, long-stalked EFN. Scale bars are 200 μm. (This figure is available in color online.)



The nectary parenchyma consisted of several layers of isodiametric cells (Figs. 2A, 2H, and 3A), with the smallest number of layers found in *C. subpeltata* (seven layers) and the largest in *C. onusta*. Clearly, the smaller EFNs had fewer nectary parenchyma layers. Such cells had large nuclei and dark-staining cytoplasm. Idioblasts containing prismatic crystals were not observed in the nectary parenchyma.

Despite being vascularized by both phloem and xylem (Figs. 2A, 2H, and 3A), the number of phloem cells in all EFNs was markedly higher than that of xylem cells. Additionally, 1–2 pairs of accessory bundles at each side of the petiole towards the EFNs could also be observed. The vascular tissue ends before the nectary parenchyma layers (Figs. 2A, 2H, and 3A), at an area characterized for having highly vacuolated isodiametric cells, the subnectary parenchyma. The subnectary parenchyma was composed of 1–3 layers of

cells smaller than the ground parenchyma cells (Figs. 2A, 2H, and 3A) and was frequently permeated by vascular tissue endings. As the vascular system converged from the petiole towards the nectaries, the cells surrounding the vascular tissue become sclereids instead of fibers. Such sclereids commonly contained one calcium oxalate crystal (Fig. 3B).

Composition of the secretion

The presence of glucose was confirmed in the secretion of the EFNs of *C. ensiformis* var. *ensiformis*, *C. negrensis* var. *negrensis*, and *C. polystachya* under field conditions. The presence of lipids in the secretion could not be determined by means of Sudan stains (Sudan Black B and Sudan red), as the secretion was actually rinsed by such stains.

Fig. 4. Histochemical tests of the extrafloral nectaries conducted in cross sections of the petiole/rachis of *Chamaecrista* species. Note the presence of secretion (arrowheads) among the cells of the nectary parenchyma (np). (A–C) Periodic acid – Schiff (PAS) in *C. xinguensis* (A–B) and *C. ensiformis* var. *ensiformis* (C). (D and E) Ruthenium red in *C. xinguensis* (D) and *C. polystachya* (E). Sudan red in *C. xinguensis* (F and G) and Sudan Black B in *C. ensiformis* var. *ensiformis* (H and I). ep, epidermis; snp, subnectary parenchyma; vb, vascular bundle. Scale bars are 200 μm (A, F, and H) and 30 μm (B–E, G, and I). (This figure is available in color online.)



The histochemical tests provided positive results for the following groups of metabolic compounds: total polysaccharides (Figs. 4A–4C), pectins and (or) mucilages (Figs. 4D–4E), and lipid compounds (Figs. 4F–4I). Such compounds were detected in both epidermal and nectar parenchymatic cells. Secretion was observed among the cells (in the periplasmic space and between the cell walls) (Figs. 4B–4C) and especially between the epidermal and subepidermal cell layers (Figs. 4D, 4E, 4G and 4I). The outer walls of the epidermal cells were penetrated by numerous branched microchannels oriented mainly perpendicular to the wall surface. Such microchannels were usually filled by secretions. No stomata, secretory pores or cuticle rupture were observed.

Senescence of extrafloral nectaries

During field expeditions, secretion and ant visitation occurred mostly in EFNs of developing and newly expanded leaves of *C. ensiformis* var. *ensiformis*, *C. duartei*, *C. polystachya*, and *C. negrensis* var. *negrensis*. EFNs of older leaves only rarely showed secretory activity. In addition, they turned blackish (field observations) and anatomically showed the development of a wound-healing periderm (Figs. 5). This periderm was derived from subepidermal layers (Figs. 5A–5B) or from the inner layers of the subnectary parenchyma (Figs. 5C–5F) of the EFNs. Such layers underwent periclinal cell divisions only, giving rise to several layers abundant in phenolic compounds (Figs. 5E–5F). Wound-healing and periderm formation processes were also observed in EFNs infected by molds (Fig. 5C).

Discussion

The morphology, structural characterization, and presence of glucose and polysaccharides in secreted exudates of the leaf glands of the studied *Chamaecrista* species support the classification of such glands as nectaries (Fahn 1979; Bentley and Elias 1983;

Fig. 5. Cross (A–B, D–F) and longitudinal (C) sections of the petiole/rachis showing the development of a wound-healing periderm in petiole/ rachis extrafloral nectaries of *Chamaecrista* species. (A) *C. adiantifolia* var. *pteridophylla* and (B) *C. polystachya*. Note the periclinal division of the subepidermal layers (arrowheads) that will give rise to the wound-healing periderm (wp). (C) *C. aspleniifolia* with a fungal infection on the epidermis (arrow and magnification). Note the presence of a wound periderm formed at the transition zone, also in Figs. 4D–4F. (D) *C. ensiformis* var. *ensiformis*. (E) *C. scleroxylon*. (F) *C. apoucouita*. Note the presence of cells filled with phenolic compounds. Scale bars are 50 μ m (A–B, D, and F) and 200 μ m (C and E). (This figure is available in color online.)



Roshchina and Roshchina 1993; Nicolson et al. 2007). Based on the topography, occurrence on leaves, such glands must be classified as EFNs according to Caspary (1848) and extranuptial nectaries according to Delpino (1873), as they are visited by ants and are not near flowers.

Independently of having a stalk or being impressed or sessile, the EFNs of species belonging to *Chamaecrista* sect. *Apoucouita* are composed of three distinctive regions: nectary epidermis, nectary parenchyma, and subnectary parenchyma. This structure is consistent with descriptions in the literature for EFNs in general (Bentley and Elias 1983; Nepi 2007), as well as for EFNs in *Chamaecrista* (Francino et al. 2006; Coutinho et al. 2012; Silva 2012).

A single-layered epidermis made up of cubical or columnar cells, free of stomata, has also been observed in Chamaecrista (Francino et al. 2006; Silva 2012; Coutinho et al. 2012) and in other legumes (Elias 1972; Pascal et al. 2000; Melo et al. 2010). Accumulation of secretion below the cuticle, cuticular ruptures and (or) pores were not observed. Therefore, our understanding is that from the subepidermal layers, the pre-nectar must pass through the epidermal cells to the outside. The presence of the secretion within the microchannels of the epidermal cells indicates that nectar is secreted to the outside through such microchannels, especially at the central area of the nectary, which is composed of thin-walled cells. Such a mechanism for discharging nectar to the outside has previously been reported for other plant families (Freitas et al. 2001; Stpiczyńska et al. 2005; Koteyeva 2005), as well as for Chamaecrista sect. Absus subsect. Baseophyllum (Coutinho et al. 2012).

The subnectary parenchyma observed in the EFNs of species of *Chamaecrista* sect. *Apoucouita* is similar to what has been reported for *Chamaecrista* (Francino et al. 2006; Coutinho et al. 2012) and other genera of legumes such as *Inga* Mill. (Pascal et al. 2000; Fernandes 2011), *Senna* Mill. (Melo et al. 2010), and *Anadenanthera* Speg. (Melo et al. 2010). Furthermore, in sect. *Absus* subsect.

Baseophyllum, clusters of idioblasts containing polysaccharides and mucilage and (or) pectins were observed near the nectary parenchyma (Coutinho et al. 2012). Those authors suggested that the substances stored in the idioblasts found at the vascularization endings could act as an additional energy reservoir for nectar secretion.

Such clusters of idioblasts were not observed in species from sect. *Apoucouita*, which indicates that the energy for nectar production in sect. *Apoucouita* is supplied directly from the phloem. It appears that the nectar components are produced by the nectary parenchyma cells (including the subepidermal cell layers) and epidermis. The secretion is discharged to the periplasmic spaces, as well as spaces among the cells, and is then released to the outside via symplast or apoplast pathways, as reported in the literature (Fahn 1988; Pacini and Nepi 2007).

The presence of mucilage and (or) pectins, polysaccharides, and lipids in the nectary parenchyma and nectary epidermis, as well as in the microchannels of epidermal cells, suggests the presence of such compounds in the secreted exudates. The histochemical results were similar for all of the EFNs types, even though their morphology varied. Although fructose, glucose, and sucrose are among the components that prevail in nectar (Fahn 1979; Bentley and Elias 1983; Roshchina and Roshchina 1993; Nicolson et al. 2007), the presence of such a variety of components observed by our histochemical analysis, in addition to glucose, suggests that the nectar does not consist only of water and glucose, but also other compounds.

Although the presence of lipids has been reported in numerous flower nectars (Baker 1977; Nicolson et al. 2007), the presence of lipids is not a common feature of EFNs. Coutinho et al. (2012) hypothesized that amino acids hydrolysed from proteins found in the EFNs of species from sect. *Absus* subsect. *Baseophyllum* would make such nectar more attractive to ants. Interestingly, in species from sect. *Apoucouita*, total proteins were not found in the EFNs, but lipids instead, one of the highest-energy compounds available in nature (Nicolson et al. 2007). Several studies have shown that mutualistic interactions between EFNs and ants can reduce the damage caused by herbivores to the host plant and can even increase fruit set (Bentley 1977; Costa et al. 1992; Oliveira et al. 1999), and the same is true for *Chamaecrista* (do Nascimento and Del-Claro 2010). Ant preference for nectars with amino acids has been demonstrated (Lanza et al. 1989; Wagner and Kay 2002). However, to our knowledge, a similar study showing the response of ants to nectars rich in lipids has not been performed.

Heil and McKey (2003) reported that food bodies of obligate myrmecophyte species (symbiotic interactions) are rich in lipids and proteins, whereas those produced by myrmecophilic species (reward-based interactions) are rich in carbohydrates. Because sect. *Apoucouita* is the most basal clade of *Chamaecrista* (Conceição et al. 2009), future studies focusing on the species of ants patrolling the EFNs of species from sect. *Apoucouita* versus ants patrolling the EFNs of other groups such as sect. *Absus* subsect. *Baseophyllum* will help to evaluate evolutionary shifts in interactions between EFNs or plants and ants that may have occurred in *Chamaecrista*.

Upon analysis of the secretory surface of the EFNs in species of sect. *Apoucouita*, three types could be recognized: concave, flat or truncate, and convex. Among the studied species, concave surfaces were the most widespread among the EFNs, not observed only in *C. polystachya*. EFNs with concave secretory surfaces may be more effective than the flat or truncate and convex types because, after being secreted to the outside, the nectar may become accumulated in the concavity, possibly reducing the evaporative loss of nectar and thus increasing the volume of available nectar, as suggested by other authors (Keeler and Kaul 1979; Leitão et al. 2005; Coutinho et al. 2010).

The morphological differences in the types of EFNs in sect. Apoucouita suggest the taxonomic value of such secretory structures for the analyzed species. The types of EFNs are useful additional characters aiding in the distinction of species. Despite the morphological variation of such EFNs, the same sets of metabolic compounds are secreted. For the species of sect. Absus subsect. Baseophylllum, the chemical composition of the nectar produced was considered a phylogenetically conservative character because all species secreted the same types of compounds (Coutinho et al. 2012). The same hypothesis appears to be true for sect. Apoucouita because the EFNs found in species of this section secrete the same types of metabolic compounds. This idea is reinforced by comparisons between the secreted exudates of EFNs belonging to species of sect. Apoucouita and sect. Absus subsect. Baseophyllum and those of C. trichopoda (sect. Chamaecrista). In C. trichopoda, pectins and total polysaccharides were not found in the secretion (Francino et al. 2006). It appears that nectar compounds in Chamaecrista may be an additional pattern useful to characterize different groups.

Older EFNs in sect. Apoucouita species develop a wound-healing periderm. According to Evert (2006), formation of wound periderm is preceded by a sealing of the newly exposed surface by an impervious layer of cells, in which deposition of callose begins at the sites of plasmodesmata, sealing the symplastic connections at this interface. Section Apoucouita is a group of relatively large trees that is most diverse in the Amazon and Brazilian Atlantic forests (Conceição et al. 2009). The secretion of nectar by species occurring in such wet environments could favor the growth of pathogenic microorganisms at the sites of secretion. The development of a wound periderm in older EFNs seems to be unique for sect. Apoucouita, as EFNs studied of other sections (Francino et al. 2006; Coutinho et al. 2010) do not develop such tissue. It is interesting to note that species of such sections are commonly found in open sunny areas. The correlation between nectaries of species exposed to a wet environment and the development of a woundhealing periderm in such nectaries needs to be investigated to confirm whether this is a common pattern or not.

Conclusion

Although several types of EFNs in species of *Chamaecrista* sect. *Apoucouita* were observed, EFNs of all species studied share similarities in the anatomy and composition of the secretion. The development of a wound-healing periderm in older EFNs seems to be an efficient strategy against mold infections in response to the high environmental humidity where species of sect. *Apoucouita* occur. For some of the taxa studied, more than one type of EFNs was found in the same species, whereas for a few such variation was absent, which demonstrates the importance of the EFNs morphology on future taxonomic evaluations.

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References

- Baker, H.G. 1977. Non-sugar chemical constituents of nectar. Apidologie, 8: 349–356. doi:10.1051/apido:19770405.
- Barneby, R.C. 1999. Increments to genus *Chamaecrista* (Caesalpiniaceae: Cassiinae) from Bolivia and from Atlantic and Planaltine Brazil. Brittonia, **51**: 331–339. doi:10.2307/2666613.
- Bentham, G. 1870. Leguminosae II. Cassia. In Flora Brasiliensis. Enumeratio Plantarum in Brasilia Hactenus Detectarum. XV (II). Edited by C.F.P. Martius and A.G. Eichler. Fried. Fleischer, Munich, Vienna, Leipzig. pp. 82–176.
- Bentley, B.L. 1977. Extrafloral nectaries and protection by pugnacious bodyguards. Annu. Rev. Ecol. Syst. 8: 407–427. doi:10.1146/annurev.es.08.110177. 002203.
- Bentley, B., and Elias, T. 1983. The biology of nectaries. Columbia University Press, New York.
- Caspary, R. 1848. De nectariis. In Botanische Zeitung. Elverfeld, Bonnae.
- Conceição, A.S., Queiroz, L.P., Lewis, G.P., Andrade, M.J.G., de Almeida, P.R.M., de Schnadelbach, A.S., and van den Berg, C. 2009. Phylogeny of *Chamaecrista* Moench (Leguminosae-Caesalpinioideae) based on nuclear and chloroplast DNA regions. Taxon, **58**: 1168–1180.
- Costa, F.M.C.B., Oliveira-Filho, A.T., and Oliveira, P.S. 1992. The role of extrafloral nectaries in *Qualea grandiflora* (Vochysiaceae) in limiting herbivory: an experiment of ant protection in cerrado vegetation. Ecol. Entomol. 17: 363–365. doi:10.1111/j.1365-2311.1992.tb01071.x.
- Coutinho, Í.A.C., Valente, V.M.M., and Alves, M.R.M.S. 2010. Ontogenetic, anatomical and histochemical study of the extrafloral nectaries of Sapium biglandulosum (Euphorbiaceae). Aust. J. Bot. 58: 224–232. doi:10.1071/BT09200.
- Coutinho, Í.A.C., Francino, D.M.T., Azevedo, A.A., and Meira, R.M.S.A. 2012. Anatomy of the extrafloral nectaries in species of *Chamaecrista* section Absus subsection Baseophyllum (Leguminosae, Caesalpinioideae). Flora, 207: 427–435. doi:10.1016/j.flora.2012.03.007.
- Curtis, J.D., and Lersten, N.R. 1978. Heterophylly in *Populus grandidentata* (Salicaceae) with emphasis on resin glands and extrafloral nectaries. Am. J. Bot. **65**: 1003–1010. doi:10.2307/2442687.
- Delpino, F. 1873. Ulteriori osservazioni e considerazioni sulla Dicogamia nel regno vegetale. Delle piante zooifile. Atti della Soc. Ital. di Sci. Nat. **16**: 151– 349.
- Durkee, L.T., Baird, C.W., and Cohen, P.F. 1984. Light and electron microscopy of the resin glands of *Passiflora foetida* (Passifloraceae). Am. J. Bot. **71**: 596–602. doi:10.2307/2443335.
- Elias, T.S. 1972. Morphology and anatomy of foliar nectaries of *Pithecellobium* macradenium (Leguminosae). Bot. Gaz. 133: 38-42. doi:10.1086/336611.
- Evert, R.F. 2006. Esau's plant anatomy: meristems, cells, and tissues of the plant body: their structure, function, and development. John Wiley & Sons, Inc., Hoboken, New Jersey.
- Fahn, A. 1979. Secretory tissues in plants. Academic Press, London. Fahn, A. 1988. Secretory tissues in vascular plants. New Phytol. 108: 229–257. doi:10.1111/j.1469-8137.1988.tb04159.x.
- Fernandes, J.M. 2011. Ingea Benth. (Leguminosae, Mimosoideae) no Estado de Minas Gerais, Brasil: Taxonomia, morfoanatomia de nectários extraflorais e padrões de distribuiçao geográfica. D.Sc. thesis, Departamento de Botânica, Universidade Federal de Viçosa, Brazil.
- Francino, D.M.T., Sant'Anna-Santos, B.F., Silva, K.L.F., Thadeo, M., Meira, R.M.S.A., and Azevedo, A.A. 2006. Anatomia foliar e caulinar de *Chamaecrista trichopoda*

(Caesalpinioideae) e histoquímica do nectário extrafloral. Planta Daninha, 24: 695–705. doi:10.1590/s0100-83582006000400010.

- Freitas, L., Bernardello, G., Galetto, L., and Paoli, A.A.S. 2001. Nectaries and reproductive biology of *Croton sarcopetalus* (Euphorbiaceae). Bot. J. Linn. Soc. 136: 267–277. doi:10.1111/j.1095-8339.2001.tb00572.x.
- Heil, M., and McKey, D. 2003. Protective ant-plant interactions as model systems in ecological and evolutionary research. Annu. Rev. Ecol. Evol. Syst. 34: 425– 553. doi:10.1146/annurev.ecolsys.34.011802.132410.
- Irwin, H.S., and Barneby, R.C. 1977. Monographic studies in *Cassia* (Leguminosae: Caesalpinioideae). IV. Supplementary notes on section *Apoucouita* Bentham. Brittonia, **29**: 277–290. doi:10.2307/2806200.
- Irwin, H.S., and Barneby, R.C. 1979. Two new Brazilian species of *Chamaecrista* Moench (Leguminosae: Caesalpinioideae). Brittonia, **31**: 464–468. doi:10. 2307/2805996.
- Irwin, H.S., and Barneby, R.C. 1982. The American Cassiinae: a synoptical revision of *Leguminosae* tribe *Cassieae* subtribe *Cassiinae* in the New World. Mem. N.Y. Bot. Gard. 35: 1–918. doi:10.2307/4108613.
- Irwin, H.S., and Barneby, R.C. 1985. A new arborescent *Chamaecrista* (Caesalpiniaceae: Cassiinae). Brittonia, 37: 14–16. doi:10.2307/2806236.
- Irwin, H.S., and Rogers, D.J. 1967. Monographic studies in *Cassia* (Leguminosae-Caesalpinioideae). II. A taximetric study of section *Apoucouita*. Mem. N. Y. Bot. Gard. 16: 71–118.
- Johansen, D.A. 1940. Plant microtechnique. McGraw-Hill Book, New York.
- Keeler, K.H., and Kaul, R.B. 1979. Morphology and distribution of petiolar nectaries in *Ipomoea* (Convolvulaceae). Am. J. Bot. **66**: 946–952. doi:10.2307/ 2442236.
- Koteyeva, N.K. 2005. A novel structural type of plant cuticle. Dokl. Biol. Sci. 403: 283–285. doi:10.1007/s10630-005-0109-7.
- Lanza, J., Vargo, E.L., Pulim, S., and Chang, Y.Z. 1989. Preferences of the fire ants Solenopsis invicta and S. geminata (Hymenoptera: Formicidae) for amino acid and sugar components of extrafloral nectars. Environ. Ecol. 22: 411–417. doi: 10.1093/ee/22.2.411.
- Leitão, C.A.E., Meira, R.M.S.A., Azevedo, A.A., de Araújo, J.M., Silva, K.L.F., and Collevatti, R.G. 2005. Anatomy of the floral, bract, and foliar nectaries of *Triumfetta semitriloba* (Tiliaceae). Can. J. Bot. 83(3): 279–286. doi:10.1139/b05-001.
- Lewis, G. 2005. Tribe Cassieae. In Legumes of the world. Edited by G. Lewis, B. Schrire, B. Mackinder, and M. Lock. Royal Botanic Gardens, Kew. pp. 111–161.
- Mayer, J.L.S., Cardoso-Gustavson, P., Appezzato-da-Glória, B. 2011. Colleters in monocots: new record for Orchidaceae. Flora, 206: 185–190. doi:10.1016/j.flora. 2010.09.003.
- McDade, L.A., and Melvin, D.T. 1997. Structure and development of bracteal nectary glands in *Aphelandra* (Acanthaceae). Am. J. Bot. 84: 1–15. doi:10.2307/ 2445877.
- Melo, Y., Machado, S.R., and Alves, M. 2010. Anatomy of extrafloral nectaries in Fabaceae from dry-seasonal forest in Brazil. Bot. J. Linn. Soc. **163**: 87–98. doi:10.1111/j.1095-8339.2010.01047.x.
- do Nascimento, E.A., and 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. doi:10.1016/j.flora. 2009.12.040.
- Nepi, M. 2007. Nectary structure and ultrastructure. In Nectaries and nectar. Edited by S.W. Nicolson, M. Nepi, and E. Pacini. Springer, Dordrecht, the Netherlands. pp. 129–166. doi:10.1007/978-1-4020-5937-7.
- Nicolson, S.W., Nepi, M., and Pacini, E. 2007. Nectaries and nectar. Springer, Dordrecht, the Netherlands. doi:10.1007/978-1-4020-5937-7.
- O'Brien, T.P., and McCully, M.E. 1981. The study of plant structure principles and selected methods. Termarcarphi Ptey. Ltd., Melbourne, Australia.
- Oliveira, P.S., Rico-Gray, V., Díaz-Castelazo, C., and Castillo-Guevara, C. 1999. Interaction between ants, extrafloral nectaries and insect herbivores in Neotropical coastal sand dunes: herbivore deterrence by visiting ants increases fruit set in *Opuntia stricta* (Cactaceae). Funct. Ecol. **13**: 623–631. doi:10.1046/j. 1365-2435.1999.00360.x.
- Pacini, E., and Nepi, M. 2007. Nectar production and presentation. In Nectaries and nectar. Edited by S.W. Nicolson, M. Nepi, and E. Pacini. Springer, Dordrecht, the Netherlands. pp. 167–214. doi:10.1007/978-1-4020-5937-7.
- Pascal, L.M., Motte-Florac, E.F., and McKey, D.B. 2000. Secretory structures on the leaf rachis of Caesalpinieae and Mimosoideae (Leguminosae): implications for the evolution of nectary glands. Am. J. Bot. 87: 327–338. doi:10.2307/ 2656628. PMID:10718993.
- Pearse, A.G.E. 1980. Histochemistry: theoretical and applied. 4th ed. Churchill Livingston, Edinburgh.
- Radford, A.E., Dickison, W.C., Massey, J.R., and Bell, C.R. 1974. Vascular plant systematics. Harper and Row, New York.
- Rando, J.G. 2009. Chamaecrista Moench seções Apoucouita, Chamaecrista e Xerocalyx (Leguminosae - "Caesalpinioideae") na Serra do Cipó, Minas Gerais, Brasil. Instituto de Biociências da Universidade Federal de São Paulo.
- Roshchina, V.V., and Roshchina, V.D. 1993. The excretory function of higher plants. Springer-Verlag, Berlin, Germany. doi:10.1007/978-3-642-78130-8.
- Silva, M.S. 2012. Anatomia foliar como subsídio à taxonomia de espécies de Chamaecrista Moench (Leguminosae-Caesalpinioideae). M.Sc. dissertation, Departamento de Educação, Universidade do Estado da Bahia (BA), Brazil.

- Smith, F.H., and Smith, E.C. 1942. Anatomy of the inferior ovary of *Darbya*. Am. J. Bot. 29: 464–471. doi:10.2307/2437312.
- Stpiczyńska, M., Milanesi, C., Faleri, C., and Cresti, M. 2005. Ultrastructure of the nectary spur of *Platanthera chlorantha* (Custer) Rchb. (Orchidaceae) during successive stages of nectar secretion. Acta Biol. Cracoviensia, 47: 111–119.
- Wagner, D., and Kay, A. 2002. Do extrafloral nectaries distract ants from visiting flowers? An experimental test of an overlooked hypothesis. Evol. Ecol. Res. 4: 293–305.

Appendix A

List of *Chamaecrista* species used in the anatomical study and vouchers. Vouchers are housed in the herbaria of the Universidade Estadual de Feira de Santana (HUEFS), The New York Botanical Garden (NY), Jardim Botânico do Rio de Janeiro (RB), Universidade de São Paulo (SPF), Smithsonian Institution (US), and Universidade Federal de Viçosa (VIC). Collections fixed in FAA (formaldehyde, acetic acid, and 50% ethanol) and used in histochemical tests are indicated by an asterisk (*).

Chamaecrista adiantifolia var. adiantifolia (Benth.) H.S.Irwin & Barneby: Ducke 333, 18.XI.1936 (NY); Wurdack & Adderley 43236, 29.VI.1959 (NY, US); Zarucchi 1723 & Balick, 24.VI. 1976 (NY); Zarucchi 1935, 02.IX.1976 (NY, US); Farney et al. 1748 16.X.1987 (NY, US); Davidse 27665, 23.VII.1984 (NY); Davidse 27741, 23-25.VII.1984 (US); Davidse 27998, 26.VII.1984 (NY); Lima 3274, 22.XI.1987 (NY). Chamaecrista adiantifolia var. pteridophylla (Sandwith) H.S.Irwin & Barneby: Boz 548, V.1926 (NY, US); Pinkus 222, 14.II.1939 (NY); Ducke 601, 10.X.1940 (US); Silva 92, 22.IX.1942 (NY, US); Ducke 2010, 10.X.1946 (NY); Black 48-3268, 27.IX.1948; Maguire & Fanshawe 32636, 17.XI.1951 (NY, US); Maguire et al. 41983, 02.XI.1957 (US); Maguire et al. 45930, 24.VIII.1961 (NY, US); Prance et al. 22690, 18.IX.1974 (NY, US); Zarucchi & Balick 1723, 24.VI.1976 (US); Silva 4455, 22.IX.1976 (NY, US); Daly et al. 1001, 28.X.1981 (US); Silva 239, 03.VII.1985; Ferreira et al. 8220 20.IX.1986 (NY, US); Ferreira et al. 9329 (NY), 10.X.1987 (NY); *Rando & Nogueira 1197, 17.II.2012 (SPF). Chamaecrista amabilis H.S.Irwin & Barneby: Lewis & Carvalho 1068, 10.I.1982 (NY); Martinelli et al. 8901, 15.XII.1982 (RB); Santos et al. 4551, 24.V.1990 (RB); Santos et al. 4565, 24.V.1990 (RB); Thomas et al. 8964, 30.I.1992 (NY); Jardim & Flávia 594, 11.XI.1994 (NY). Chamaecrista amorimii Barneby: Mori et al. 13756, 27.IV.1981 (NY); Amorim et al. 923, 14.XII.1992 (NY; RB); Amorim et al. 3231, 06.I.2000 (NY); Amorim et al. 4311, 23.X.2004 (RB). Chamaecrista apoucouita (Aubl.) H.S.Irwin & Barneby: Riedel 1240, 1827; Samuels 545, 03.VII.1916 (NY); Forest Department of British Guiana F994, 09.I.1943 (NY); Maguire 26170 & Fanshawe, 28.IV.1944 (NY); Schultes & Cabrera 16995, 22.VIII.1952 (US); Zarucchi 1563, 15.V.1976 (NY, US); Pipoly & Boayn 8610, 17.IX.1986 (NY, US); Pipoly & Boayn 8954, 20.XI.1986 (NY, US); Pipoly & Boayn 8972, 20.XI.1986 (NY); Pipoly & Boayn 10055, 27.I. 1987 (NY); Prévost & Sabatier 4630, 21.X.2002 (NY). Chamaecrista aspidiifolia H.S.Irwin & Barneby: Castellanos 27069, 19.XII.1967 (NY); Santos 406, 02.X.1969 (NY); Guedes et al. 5220, 08.II.1997 (HUEFS). Chamaecrista aspleniifolia (H.S.Irwin & Barneby) H.S.Irwin & Barneby: Pabst 8301 & Pereira 9410, 15.I.1965 (NY); Duarte 8753, 18.I.1965 (NY, US); Belém 1562, 12.VIII.1965 (NY); Lindeman & Haas 4563, 01.II.1967 (NY); Pinheiro 1768, 24.I.1972 (NY); Lino 40, 10.III.1972 (NY); Folli 70, 27.I.1979 (NY); Hatschbach 47341, 18.I.1984 (NY, US); Cardoso et al. 2407, 04.II.2009 (HUEFS). Chamaecrista bahiae (H.S.Irwin) H.S.Irwin & Barneby: Kuhlmann 6685, 16.XII.1943 (NY); Belém & Pinheiro 3199, Fróes 27032, 01.V.1951 (US); Fróes 27076, 14.V.1951 (NY); Fróes 27259, 05.VII.1951 (US); 30.I.1967 (NY); Oliveira 4297, 22.IV.1968 (NY); Oliveira 4557, 17.VI.1968 (NY); Silva 1761, 03.III.1969 (NY); Silva 1954, 30.IV.1969 (NY); Pinheiro 1725, 18.I.1972 (NY); Santos 2235, 10.II.1972 (NY); Harley et al. 17398, 25.III.1974 (NY): Santos 2880, 20.II.1975 (NY): Harley et al. 18071. 18.I.1977 (NY, US); Oliveira 6562, 08.II.1977 (NY); Silva & Bahia 3054, 4.VI.1977 (NY); Mori et al. 10321, 26.VII. 1978 (NY, US); Maciel & Cordeiro 202, 07.XII.1978 (NY); Bastos et al. 126, 12.XII.1978 (NY); Plowman et al. 9863, 21.III.1980 (US); Plowman et al. 9885, 22.III.1980 (US); Rabelo & Jonas 2201, 14.V.1983 (NY); Daly & Cardoso 3826, 19.XII.1984 (US); Pirani et al. 3481, 08.XII.1994 (NY); Amorim et al. 2111, 21.I.1998

(NY): Hatschbach et al. 68571, 17.X.1998 (NY): *Rando 1214, 01.III.2012 (SPF, HUEFS). Chamaecrista compitalis (H.S.Irwin & Barneby) H.S. Irwin & Barneby: Belém 3637, 24.V.1968 (NY); Belém 3643, 24.V.1968 (NY, US). Chamaecrista duartei (H.S.Irwin) H.S.Irwin & Barneby: Duarte 8014, 19.XI.1963 (NY, US); Belém & Pinheiro 2946, 30.XI.1966 (NY); Soares 2191, 06.XII.1966 (US); Pinheiro 248, 26.IX.1967 (US); Belém & Pinheiro 3054, 12.I.1967 (NY); Almeida & Santos 195, 31.X.1968 (NY, US); Santos 2227, 27.XI.1971 (NY); Mori et al. 11036, 04.XI.1978 (NY); Santos 3422, 05.XII.1978 (US); Santos et al. 4565, 24.V.1990 (NY); Thomas et al. 8915, 29.I.1992 (NY); Amorim et al. 1290, 26.V.1993 (NY, US); Amorim et al. 1335, 13.IX.1993 (US); Silva et al. 3624, 30.X.1997 (MY); Amorim & Lorenzi 2102, 07.XI.1997 (NY, US); *Coutinho & Fernandes 303, 15.I.2014 (VIC). Chamaecrista eitenorum var. eitenorum (H.S.Irwin & Barneby) H.S.Irwin & Barneby: Drouet 2541, 01.X.1935 (US); Eiten & Eiten 10660, 18.II.1970 (US); Von Luetzelburg 446, 1970 (US); Santos 1321, 14.I.1971 (US). Chamaecrista eitenorum var. regana (H.S.Irwin & Barneby) H.S.Irwin & Barneby: Noblick 3034, 07.III.1984 (US); Hatschbach & Hatschbach 56931, 09.IV.1992 (US); Queiroz et al. 6131, 18.II.2000 (HUEFS). Chamaecrista ensiformis var. ensiformis (Vell.) H.S.Irwin & Barneby: Riedel 1201, X.1832 (NY); Guillemin 145, 1838 (NY); Rosa, 30.VII. 1936 (NY); Irwin 2330, 29.XII.1958 (NY); Hoehne 5642, 24.III.1964 (NY); Sucre 5091 & Plowmann, 27.V.1969 (NY); Santos 2120, 11.XI.1971 (NY); Euponino 178, 24.I.1972 (NY); Santos 2272, 20.IV.1972 (NY); Pinheiro 1850, 03.VII.1972 (NY); Anderson et al. 11192, 01.II.1975 (NY); Thomas 6169, 26.II.1988 (NY); Amorim et al. 1433, 10.XI.1993 (NY); Amorim et al. 2145, 25.I. 1998 (NY) *Coutinho et al. 033, 01.VIII.2012 (VIC); *Coutinho et al. 034, 01.VIII.2012 (VIC); *Coutinho et al. 035, 01.VIII.2012 (VIC); *Coutinho & Lorencini 070, 08.VIII.2012 (VIC); *Coutinho & Moura 121, 17.I.2013 (VIC); *Coutinho & Pereira 190, 25.IV.2013 (VIC). Chamaecrista ensiformis var. maranonica (H.S.Irwin) H.S.Irwin & Barneby: Froés 28501, 20.VIII.1952 (US); Silva 57782, 12.XI.1963 (US); Maguire et al. 56079, 09.VIII.1963 (NY); Prance & Silva 58634, 09.VIII.1964 (US); Prance & Silva 58692, 13.VIII.1964 (NY, US); Prance & Silva 58956, 30.VIII.1964 (NY, US); Prance & Silva 58976, 31.VIII.1964 (NY, US); Silva 378, 15 Jan 1966 (NY); Hatschbach & Kummorw 38396, 26.III.1976 (NY); Silva et al. 2747, 03.IX.1976 (NY); Silva & Bahia 3143, 25.V.1977 (NY, US); Rosa et al. 2580, 11.VII.1978 (NY); Maciel et al. 410, 03.X.1979 (NY); Daly et al. D375, 29.IX.1980 (NY, US); Daly et al. D571, 10.X.1980 (NY, US); Taylor et al. E1180, 10.IV.1983 (NY); Taylor et al. E1303, 22.IV.1983 (NY); Pereira 2555, 07.IX.1993 (NY). Chamaecrista ensiformis var. plurifoliolata (Hoehne) H.S.Irwin & Barneby: Warming s.n.,

18.VIII.1863 (US): Curran 4675, 29.III.1921 (NY): Nunes s.n., 1930 (NY); Ser. refl. Est. Ferro No. 18, 30.VIII.1930 (NY); Pereira 9551 & Pabst 8440, 19.I.1965 (NY); Belém & Magalhães 955, 25.IV.1965 (NY, US); Santos 2952, 16.IV.1975 (NY); Kallunki et al. 523, 11.II.1994 (NY). Chamaecrista hymenaeifolia (Benth.) H. S.Irwin & Barneby: Maguire et al. 36631, 27.XI.1953 (NY, US); Maguire et al. 36473, 27.XI.1953 (NY); Maguire et al. 42612, 02.I.1958 (NY, US); Lima et al. 3217, 17.XI.1987 (NY); Stevenson & Ramos 1064, 27.XI.1987 (NY, US). Chamaecrista negrensis var. albuquerquei (H.S.Irwin & Barneby) H.S.Irwin & Barneby: Coêlho & Coêlho s.n., 06.II.1968 (NY); Santos 144, 04.III.1977 (NY); Henderson et al. 396, 09.V.1985 (NY). Chamaecrista negrensis var. negrensis (H.S.Irwin) H.S.Irwin & Barneby: Silva & Brazão 60789, 08.I.1966 (NY); Pires & Silva 11304, 19.X.1967 (NY); Nascimento et al. 1, 02.IV.1975 (NY); Irwin 37202, 28.VI.1976 (NY); Prance et al. 24339, 27.XI.1976 (VIC); Alencar 596, 12.VII.1979 (NY); Silva 165, 28.VI.1985 (NY); Silva 288, 05.VII.1985 (NY); Ferreira et al. 6846, 19.III.1986 (NY); Belém 2056, 29.III.1986 (NY); Baleé 2447, 12.VI.1986 (NY); Baleé 2480, 13.VI.1986 (NY); Martins et al. 83, 04.II.1998 (NY); *Coutinho et al. 537, 01.I.2015. Chamaecrista onusta H.S.Irwin & Barneby: Carvalho et al. 179, 26.II.1980 (NY); Lewis & Carvalho 1024, 07.I.1982 (NY); Carvalho & Chautems 1669, 08.III.1983 (NY); Carvalho et al. 6800, 06.IX.1999 (NY); Amorim et al. 4311, 23.X.2004 (NY). Chamaecrista polystachya (Benth.) H.S.Irwin & Barneby: Schomburgk 1842-3, 1842-1843 (NY); Coradin et al. 558, 08.X.1977 (NY); Steward et al. 156, I.XII.1977 (NY, US); Steward et al. 226, 29.XI.1977 (NY, US); *Rando 1150, 07.II.2012 (HUEFS, SPF); *Coutinho et al. 530, 31.XII.2014; *Coutinho et al. 531, 31.XII.2014. Chamaecrista scleroxylon (Ducke) H.S.Irwin & Barneby: Ducke 16605, 13.XII.1916 (US); Black 47-1031 F19 A, 17.VII.1947 (NY); Black F19A 47-1133, 31.VII. 1947 (NY, US); Fróes 31813, V.1955 (NY); Fróes 32171, 10.X.1955 (US); Fróes 33833, VI.1957 (NY); Fróes 33881, VI.1957 (NY); Duarte 7213, 07.X.1962 (US); Silva & Souza 2557, 11.IX.1969 (NY); *Rando 1212, 24.02.2012 (HUEFS, SPF). Chamaecrista subpeltata (Rizzini) H.S.Irwin & Barneby: Lima 13469, 21.V.1976 (RB); Vilhena et al. 976, 10.III.1983 (NY, US); Taylor et al. E1319, 23.IV.1983 (NY, US). Chamaecrista xinguensis (Ducke) H.S.Irwin & Barneby: Fróes 31033, 20.XIII.1954 (NY); Duarte 7213, 07.X.1962 (NY); Silva & Souza 2259, 14.VIII.1969 (NY); Silva & Souza 2409, 27.VIII.1969 (NY); Silva et al. 3421, 21.X.1977 (NY); Bahia 13, 12.VIII.1978 (NY); Baleé 2424, 12.VI.1986 (NY); Vasconcelos et al. 250, 12.X.1986 (NY); Souza et al. 376, 18.X.1986 (NY); *Rando 1208, 23.II.2012 (HUEFS, SPF).

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