



Colleters in *Chamaecrista* (L.) Moench sect. *Chamaecrista* and sect. *Caliciopsis* (Leguminosae-Caesalpinioideae): anatomy and taxonomic implications

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ABSTRACT

The genus *Chamaecrista* contains ca. 330 species organized into six sections, of which section *Chamaecrista* is the second largest (ca. 75 species) distributed among six series, while the section *Caliciopsis* contains only two species. Colleters have been described in the genus *Chamaecrista* and they show potential taxonomic and phylogenetic significance. They are associated with lubrication, desiccation prevention and protection from microbial attacks of young developing organs. Although six types of colleters have been described for the genus *Chamaecrista*, there have been no studies focusing on the diversity of colleters in the sections *Chamaecrista* and *Caliciopsis*. Samples from developing leaves and flowers of both sections were obtained from herbarium and field collections and subjected to standard methodologies for both light and scanning electron microscopy. Histochemical tests were also performed to determine the nature of the exudates. Five types of non-vascularized colleters were found: short digitiform, long digitiform, club-shaped, pyriform and short bottle-shaped. Polysaccharides, pectins, lipids and proteins were detected in the exudates of all types of colleters. Among the five types of colleters observed, pyriform is a novelty for *Chamaecrista*, reinforcing the significant morphological diversity of these secretory structures in this genus.

Keywords: colleter, flower, histochemical analysis, leaf, polysaccharides, secretory structures, taxonomy

Introduction

Chamaecrista is one of the largest genera of the subfamily Caesalpinioideae, with ca. 330 species (Lewis 2005). According to Irwin & Barneby (1982), the genus is organized into six sections on the basis of the type of indumentum, the presence or absence of extrafloral nectaries, the type of inflorescence and the type of venation of both sepals and leaflets. The six sections (and the number of species) are: *Chamaecrista* sect. *Absus* (ca. 170 spp.), *Apoucouita* (ca.

20 spp.), *Caliciopsis* (two spp.), *Chamaecrista* (ca. 75 spp.), *Grimaldia* (one sp.) and *Xerocalyx* (three spp.).

Section *Chamaecrista* is the second most speciose section, with 2/3 of its species occurring in the America (Irwin & Barneby 1982; Rando & Pirani 2012; Rando *et al.* 2013). Species grouped in sect. *Chamaecrista* are characterized as shrubs or herbs with inflorescences with racemes reduced to either a few-flowered fascicles or to a solitary flower, several pairs of pinnately-veined leaflets, seeds obovate to rhombic or trapezoid in outline and extrafloral nectaries commonly present (Irwin & Barneby 1982). Six series have

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been described for sect. *Chamaecrista*: series *Bauhinianae*, *Chamaecrista*, *Coriaceae*, *Flexuosae*, *Greggianae* and *Prostratae*. Although described by Irwin & Barneby (1982) as a separate section, a phylogenetic analysis of the genus *Chamaecrista*, based on molecular data, has brought sect. *Caliciopsis* within sect. *Chamaecrista* (Conceição *et al.* 2009).

Different secretory structures with high potential for taxonomic and phylogenetic relevance have been described in *Chamaecrista*, including: nectaries (on leaves and/or racemes), mucilage idioblasts in the mesophyll and/or epidermis, sticky glandular hairs and colleters (Irwin & Barneby 1982; Coutinho *et al.* 2012; 2013; 2015; 2016; Meira *et al.* 2014; Francino *et al.* 2015). Colleters are secretory structures that are usually found on the adaxial side of vegetative and/or reproductive structures such as stipules, bracts, sepals and petals (Fahn 1979; Thomas 1991; Mayer *et al.* 2013; Coutinho *et al.* 2015). The viscous exudates secreted by colleters are said to be involved in lubrication, desiccation prevention and protection from microbial attacks of young and developing organs (Fahn 1979; Thomas 1991; Mayer *et al.* 2013; Coutinho *et al.* 2015).

There are several types of colleters, which may originate from protodermal cells only or from all primary meristems (Lersten 1974; Thomas 1991; Appezzato-da-Glória & Estelita 2000; Rio *et al.* 2002; Silva *et al.* 2012; Coutinho *et al.* 2015). Therefore, to properly evaluate the presence of colleters in plants, their morphoanatomy, position and both time and composition of the secretion must be considered in order to avoid erroneous interpretations since such structures share morphoanatomical similarities with other secretory structures (Solereder 1908; Inamdar *et al.* 1986; Mohan & Inamdar 1986; Subramanian *et al.* 1989; Thomas 1991; Coutinho *et al.* 2015).

The presence and types of colleters are useful characters for taxonomic studies and have been reported for more than 60 families (Thomas 1991; Rio *et al.* 2005; Simões *et al.* 2006; Silva *et al.* 2012; Dalvi *et al.* 2013; Coutinho *et al.* 2015). In *Chamaecrista*, colleters have been described in vegetative and reproductive organs of species belonging to sections *Absus*, *Apoucouita*, *Grimaldia*, *Chamaecrista* and *Xerocalyx* (Coutinho *et al.* 2013; 2015). Six types of colleters were morphoanatomically described: short digitiform, long digitiform, short bottle-shaped, long bottle-shaped, club-shaped and racket-shaped (Coutinho *et al.* 2013; 2015). The type and distribution of colleters have provided new data that reinforce the sectional rearrangement indicated by the molecular phylogeny of *Chamaecrista* (Coutinho *et al.* 2015). The presence of club-shaped colleters on the margins of sepals was indicated as a synapomorphy for species of sect. *Apoucouita*. Most species of sect. *Absus* subsect. *Absus* had short bottle-shaped colleters, the same type as found in *C. absus* (sect. *Grimaldia*). Based on these data, the authors suggested that the sectional boundaries of sect. *Grimaldia* should be reconsidered in a future taxonomic revision of *Chamaecrista* to determine if this section should

be included within sect. *Absus* subsect. *Absus*. Although short bottle-shaped colleters were the most common type for sect. *Chamaecrista*, short digitiform and club-shaped types were also observed. However, only five species of sect. *Chamaecrista* were sampled by Coutinho *et al.* (2015), and none of the species of section *Caliciopsis* have been sampled in previous studies. This gap in knowledge has limited the taxonomic usefulness of colleters in sect. *Chamaecrista* and *Caliciopsis*.

In this paper, we assess the occurrence, distribution and types of colleters among species of *Chamaecrista* sect. *Chamaecrista* (including samples of 32 species not previously studied) and *Caliciopsis* (including samples of all species). The composition of the exudates of the colleters, as well as the taxonomic implications for the genus *Chamaecrista*, were also evaluated.

Materials and methods

We studied 37 species (50 *taxa*) of *Chamaecrista* (L.) Moench sect. *Chamaecrista*, and two species of sect. *Caliciopsis* (Tab. 1). Taxonomic authorities for all taxa mentioned in this paper are given in S1 in supplementary material. Samples were obtained from field collections and herbarium material. Voucher specimens of field collections were deposited in the herbarium of the Universidade Federal de Viçosa (VIC) and Universidade do Estado da Bahia (HUNEB). Herbarium material was obtained from the collections of the following herbaria: Jardim Botânico do Rio de Janeiro (RB), Universidade Estadual de Feira de Santana (HUEFS), Universidade Federal de Viçosa (VIC), Universidade de São Paulo (SPF), and The New York Botanical Garden (NY).

Samples from herbaria were rehydrated (Smith & Smith 1942) and stored in 70 % ethanol. Samples from species that were collected in the field were fixed in FAA (formalin, acetic acid and 50 % ethanol; 1:1:18 by volume), NBF (neutral buffered formalin) (Johansen 1940) and FFS (formalin-ferrous sulphate) and stored in 70 % ethanol (Johansen 1940).

In order to assess the presence, position and micromorphology of the colleters we used a stereomicroscope (Zeiss Stemi 2000-C, Germany) and a scanning electron microscopy (SEM). For SEM, some samples stored in 70 % ethanol were subjected to critical-point drying using CO₂ (CPD 030, Bal-Tec, Balzers, Liechtenstein), mounted on stubs and coated with gold (Modular Balzers Union FDU 010 with a SCA 010 sputter coating attachment, Germany) (Bozzola & Russel 1991). Examination and image capture were conducted using a LEO 1430VP SEM (Zeiss, Cambridge, United Kingdom) at the Centro de Microscopia e Microanálises at the Universidade Federal de Viçosa.

Developing leaves and flowers of both dried and field-collected material stored in 70 % ethanol were dehydrated through an ethanol series and embedded in methacrylate



Table 1. *Chamaecrista* sections *Chamaecrista* and *Caliciopsis* species studied and position of the collectors.

Taxa	Colleter position					
	Base of stipule	Between leaflets	Insertion petiole	Base of bracteole/bract	Base of sepal	Base of petal
Section <i>Chamaecrista</i>						
Ser. <i>Chamaecrista</i>						
<i>C. deeringiana</i>	I, II	I, II	I, II	I, II	I, II	I
<i>C. glandulosa</i> var. <i>andicola</i>	I, II	I, II	I, II	I, II, V	I, II, V	I, II
<i>C. nictitans</i> var. <i>disadena</i>	I, II	I, II	I, II	I	I	I
<i>C. nictitans</i> var. <i>jaliscensis</i>	I	I	I	I	I	I
<i>C. nictitans</i> var. <i>paraguariensis</i>	I, II	I, II	I, II	I, II	I, II	I
<i>C. obcordata</i>	I	I	I	I, II	I, II	I
<i>C. pascuorum</i>	I, II	I, II	I, II	I, II	I, II	I
<i>C. pedicellaris</i> var. <i>pedicellaris</i>	I, II	I, II	I, II	I	I	I
<i>C. pedicellaris</i> var. <i>adenosperma</i>	I, III	I, III	I, III	I	I	I
<i>C. portoricensis</i> var. <i>portoricensis</i>	I, II	I, II	I, II	I	I	I
<i>C. pygmaea</i> var. <i>pygmaea</i>	I	I	I	I, II	I, II	I
<i>C. pygmaea</i> var. <i>savannarum</i>	I, II	I, II	I, II	I, II	I, II	I, II
<i>C. rufa</i> var. <i>exsul</i>	I, VII	I, VII	I, VII	I	I	VII
<i>C. rufa</i> var. <i>polyplebia</i>	I, II	I, II	I, II	I	I	I
<i>C. venturiana</i>	I, II, III	I, II, III	I, II, III	I, II, V	I, II, V	I
<i>C. vestita</i>	I, II, V	I, II, V	I, II, V	I	I	I
Ser. <i>Greggiana</i>						
<i>C. greggii</i> var. <i>greggii</i>	I, II	I, II	I, II	I, II	I, II	I, II
<i>C. greggii</i> var. <i>macdougaliana</i>	I, II	I, II	I, II	I, II	I, II	I, II
Ser. <i>Bauhiniana</i>						
<i>C. basifolia</i>	I, II	I, II	I, II	I, II	I, II	I
^a <i>C. rotundifolia</i> var. <i>rotundifolia</i>	I, II, III	I, II, III	I, II, III	I	I	I
^a <i>C. rotundifolia</i> var. <i>grandiflora</i>	I, II, III, V	I, II, III, V	I, II, III, V	I	I	I
Ser. <i>Flexuosae</i>						
^a <i>C. flexuosa</i> var. <i>flexuosa</i>	I, II, III	I, II, III	I, II, III	I, II	I, II	I
<i>C. gonoclada</i>	I, II	I, II	I, II	I	I	NO
<i>C. swainsoni</i>	I, II	I, II	I, II	I, II, V	I, II, V	I
Ser. <i>Prostratae</i>						
<i>C. cordistipula</i>	I, II, III	I, II, III	I, II, III	I	I	I
<i>C. kunthiana</i>	I, II	I, II	I, II	I	I	I
<i>C. pilosa</i> var. <i>pilosa</i>	I, II	I, II	I, II	I, II	I, II	I
<i>C. pilosa</i> var. <i>luxurians</i>	I, II	I, II	I, II	I, II	I, II	I
<i>C. supplex</i>	I, II	I, II	I, II	I, II	I, II	I
<i>C. tenuisepala</i>	I, III	I, III	I, III	I	I	I
Ser. <i>Coriaceae</i>						
^a <i>C. aristata</i>	I, III, V	I, III, V	I, III, V	I	I	I
* <i>C. caribaea</i> var. <i>caribaea</i>	I, V	I, V	I, V	I, V	I, V	I
* <i>C. caribaea</i> var. <i>lucayana</i>	I, V	I, V	I, V	I, V	I, V	I
* <i>C. caribaea</i> var. <i>inaguensis</i>	I, V	I, V	I, V	I, V	I, V	I
^a * <i>C. cinerascens</i>	I	I	I	III, V	III, V	I
^a <i>C. latifolia</i>	I	I	I	I	I	I, III
<i>C. distichoclada</i>	I, II	I, II	I, II	I, III	I, III	I
^a <i>C. mucronata</i>	I	I	I	I, III, V	I, III, V	I, V
<i>C. multinervia</i>	I	I	I	I, II	I, II	I
^a <i>C. papillata</i>	I, II	I, II	I, II	I, III	I, III	I
^a <i>C. potentilla</i>	A	I, III	I, III	I, III	I, III	I
^a <i>C. roraimae</i>	I, III	I, III	I, III	I, III	I, III	I
<i>C. rotundata</i> var. <i>rotundata</i>	I	A	A	I	I	I
<i>C. rotundata</i> var. <i>interstes</i>	I	A	A	I	I	I
^a <i>C. rotundata</i> var. <i>grandistipula</i>	I, II	I, II	I, II	I	I	NO
^a <i>C. simpliflora</i>	I, III, V	I, III, V	I, III, V	I, III, V	I, III, V	I
^a <i>C. tragacanthoides</i> var. <i>tragacanthoides</i>	I, II, V	I, II, V	I, II, V	I, II, V	I, II, V	I



Table 1. Cont.

Taxa	Colleter position					
	Base of stipule	Between leaflets	Insertion petiole	Base of bracteole/bract	Base of sepal	Base of petal
<i>C. tragacanthoides</i> var. <i>rasa</i>	I, V	I, V	I, V	I, V	I, V	I
<i>C. ulmea</i>	I	I	I	I	I	I
<i>C. venulosa</i>	I, V	I, V	I, V	I	I	V
Section <i>Caliciopsis</i>						
<i>C. calycioides</i> var. <i>calycioides</i>	I, V	NO	I, V	I	I	I
<i>C. duckeana</i>	I, V	NO	I, V	I	I	I

Note: A: absent; NO: not observed. I: short digitiform; II: long digitiform; III: club-shaped; V: short bottle shaped and VII: pyriform.

*Field collected material.

*Analyzed by Coutinho *et al.* 2015.

(Historesin Leica; Leica Microsystems Nussloch, Heidelberg, Germany). Cross and longitudinal sections 5–7 µm thick were made with a rotary microtome (Spencer 820 American Optical Corporation, Buffalo, NY, USA). Sections were stained with toluidine blue at pH 4.4 (O'Brien & McCully 1981) and mounted in resin (Permount, Fisher Scientific, New Jersey, USA) for structural analysis.

Part of the fixed samples were dehydrated through *tert*-butanol series, embedded in histological paraffin enriched with dimethyl sulfoxide (Histosec[®], Merck, Germany), cut into 7 µm thick cross and longitudinal sections (Spencer 820 American Optical Corporation, Buffalo, New York, USA), deparaffinized in xylene, rehydrated through an ethanol series (Johansen 1940) and used in histochemical tests.

The following histochemical tests were performed on 13 taxa using material previously fixed in the field with FAA or NBF (Tab. 1): for total lipids, sudan IV (Pearse 1980); for total polysaccharides, periodic acid-Schiff reagent (Maia 1979); for pectins/mucilage, ruthenium red (Johansen 1940); for acid mucopolysaccharides, alcian blue (Pearse 1980); and for total protein, xylydine Ponceau (Clark 1981). For detection of phenolic compounds, we used samples fixed in FFS (Johansen 1940). Control samples were also tested as required for each test. All observations and image captures were obtained using a light microscope (model AX70TRF; Olympus Optical, Tokyo, Japan) equipped with a U-Photo and digital camera (AxioCam HRc; Zeiss, Gottingen, Germany). The types of colleters found are described following Coutinho *et al.* (2015), as was the data for *C. caribaea* Britton and *C. cinerascens* (Vogel) H.S.Irwin & Barneby.

Results

Non-vascularized colleters composed of homogenous cells were found on both vegetative and reproductive organs (Tab. 1). Colleters were comprised of a stalk of varying length, and a secretory head of varying shape (Figs. 1, 2). The stalk cells were non-secretory and highly vacuolated while the cells from secretory head displayed densely stained cytoplasm (Fig. 2C-E). There was no differentiation of a

palisade epidermis since the epidermal cells are similar in appearance to the internal cells of the secretory head (Fig. 2H). The cuticle was thin and appeared distended or loose in some sections (Fig. 2B) or SEM images (Fig. 1I); no pores were observed in the cuticle. Older colleters exhibit a brownish color.

Colleters were located on the adaxial side of vegetative and reproductive structures including the base of stipules, bracts and bracteoles; the base of sepals and petals; on the rachis between pairs of leaflets; and on the stem at the insertion of the petiole (Tab. 1). Five types of colleters were observed (Tab. 1): Type I, short digitiform (Figs. 1A-B, D, G-H, 2A, F); Type II, long digitiform (Figs. 1G, 2G); Type III, club-shaped (Figs. 1F, 2E); type V, short bottle-shaped (Figs. 1C, 2D); and Type VII, pyriform (Figs. 1E, 2C). Pyriform colleters, described here for the first time, were about 230 µm long, and composed of a stalk that widened at the mid-height and had a short pointy apex (Figs. 1E, 2C).

Although colleters were found in all the species analyzed, their distribution among the series of section *Chamaecrista* was very distinct (Tab. 1). Type I occurred in all analyzed species and the three taxa of ser. *Coriaceae* (*C. ulmea*, *C. rotundata* var. *rotundata*, *C. rotundata* var. *interstes*); *C. nictitans* var. *jaliscensis* (ser. *Chamaecrista*) only possessed this type of colleter. Type VII was exclusive to *C. rufa* var. *exsul*. Types I and II were observed in 20 taxa, with 10 of them belonging to ser. *Chamaecrista*. Types I and III occurred in five taxa (three of ser. *Coriaceae*, *C. tenuisepala* and *C. pedicellaris* var. *adenosperma*). Five taxa from ser. *Coriaceae* (sect. *Chamaecrista*), as well as *C. calycioides* and *C. duckeana* from sect. *Caliciopsis* bore Types I and V. Colleters of the types I, II and III were found in five taxa of four different series (*C. rotundifolia* var. *rotundifolia*, *C. flexuosa* var. *flexuosa*, *C. cordistipula*, *C. distichoclada* and *C. papillata*). *C. vestita*, *C. glandulosa* var. *andicola* (ser. *Chamaecrista*), *C. swainsoni* (ser. *Flexuosae*) and *C. tragacanthoides* var. *tragacanthoides* (ser. *Coriaceae*) displayed colleters of types I, II and V. Types I, III and V occurred in four species of ser. *Coriaceae* (*C. aristata*, *C. cinerascens*, *C. mucronata* and *C. simpliflora*). *C. venturiana* (ser. *Chamaecrista*) and *C. rotundifolia* var. *grandiflora* (ser. *Bauhiniana*) displayed Types I, II, III and V (Tab. 1).



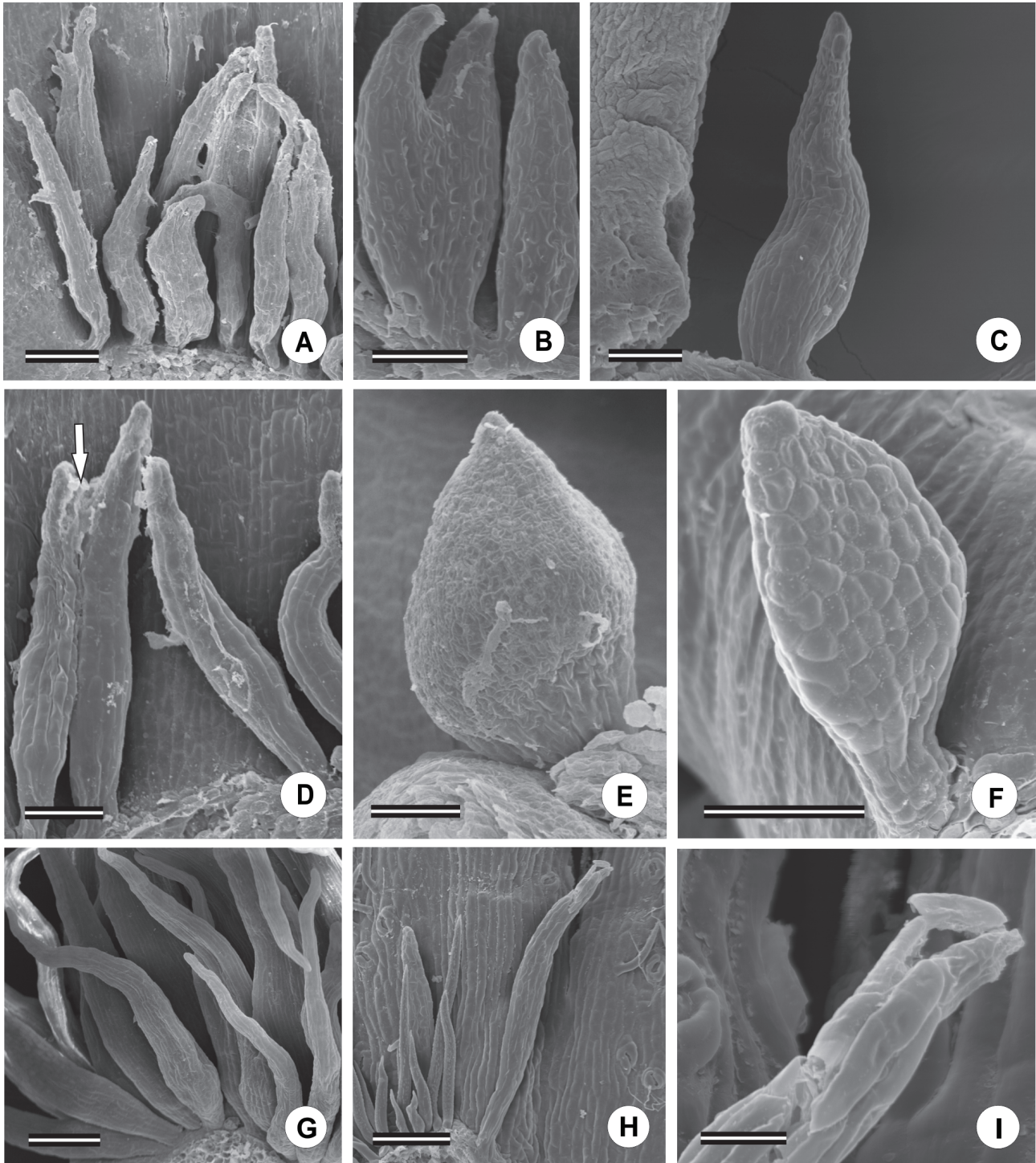


Figure 1. SEM images showing colleters on vegetative and reproductive organs of *Chamaecrista* sect. *Chamaecrista*. A. *C. basifolia*: Colleter short digitiform on stipule. B. *C. gonoclada*: Colleter bifurcated and short digitiform on stipule. C. *C. tragacanthoides* var. *tragacanthoides*: Colleter bottle shaped on sepal. D. *C. nictitans* var. *paraguayensis*: Colleter short digitiform on bracteole. Note the secretion (arrow). E. *C. rufa* var. *exsul*: Colleter pyriform on petal. F. *C. potentilla*: Colleter club shaped on sepal. G. *C. rotundata* var. *grandistipula*: Colleter short and long digitiform on stipule. H, I. *C. tragacanthoides* var. *tragacanthoides*: Colleter short digitiform on stipule and apex detail colletel, respectively. Scale bars: A, I: 100µm; B-F: 60µm; G: 200µm; H: 20µm.

Only in ser. *Chamaecrista* were all five types of colleters observed (Figs. 1C-I, 2A-H). The series *Bauhinianae*, *Coriaceae* and *Flexuosae* displayed Types I, II, III and V (Fig. 1B), while

in ser. *Greggiana* only Types I and II were observed. Species belonging to ser. *Prostratae* bore Types I, II and III. There were fewer colleters on the stem at the insertion of the

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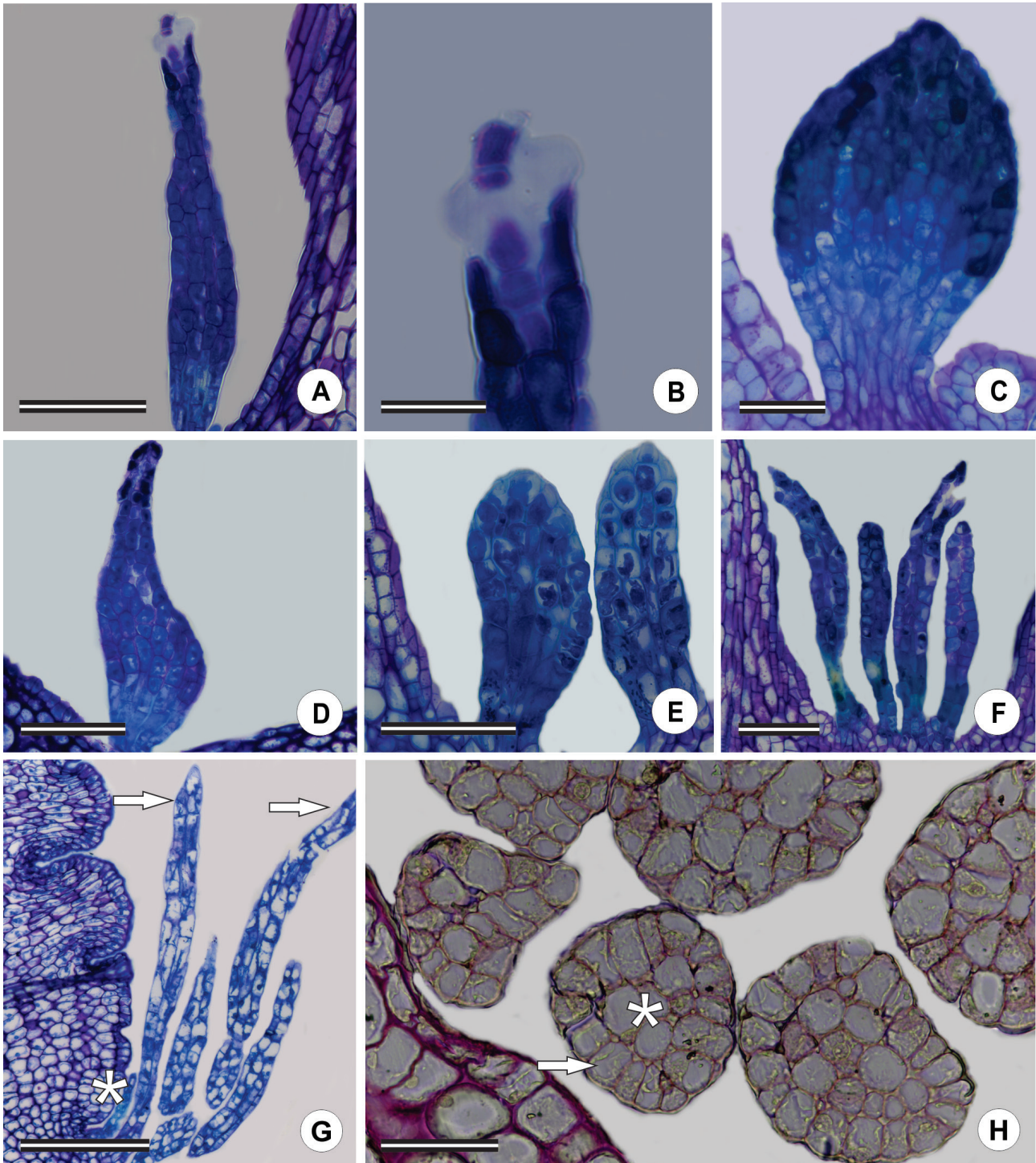


Figure 2. Anatomical sections of collaters of *Chamaecrista* sect. *Chamaecrista*. A, B. *C. pascuorum*: Collater short digitiform on bracteole and apex detail collater, respectively. C. *C. rufa* var. *exsul*: Collater pyriform on stipule. D. *C. venturiana*: Collater bottle shaped on bracteole. E, F. *C. mucronata*: Collater club shaped on bracteole and short digitiform on stipule, respectively. G-H. *C. rotundata* var. *grandistipula*: Collater short and long digitiform the insertion of the petiole and cross-sectional view of collater the insertion of the petiole, respectively. Note the epidermis (arrow) and secretory parenchyma (asterisk). Scale bars: A, D-F: 100µm; B: 25µm; C, H: 50µm; G: 200µm.

petiole and between leaflets than in stipules. The types of collaters varied among sepals, petals, bracteoles and bracts. Type I was present on petals of all species (Tab. 1) except in *C. venulosa* and *C. rufa* var. *exsul*, which only displayed

Types V and VII, respectively.

Types I, II and III were the most common types of collaters observed among the studied species (Tab. 1), with Type I being found in all taxa, while Type II occurred

in all series studied (Tab. 1). Type VII was observed at the base of stipules and petals, between leaflets and on the stem at the insertion of the petiole of *C. rufa* var. *exsul* (ser. *Chamaecrista*). Bifurcated colleter were presented in seven taxa (Tab. 1) and were not considered a different type of colleter. *Chamaecrista rotundata* var. *rotundata*, *C. rotundata* var. *interstes*, *C. ulmea* and *C. nictitans* var. *jaliscensis* possessed only Type I collectors, while the remaining taxa

bore at least two types. Only two taxa (*C. rotundata* var. *grandistipula* and *C. gonoclada*) did not display collectors on petals (Tab. 1).

The presence of total polysaccharides (Fig. 3A, B), mucopolysaccharides (Fig. 3C), pectins (Fig. 3D), proteins (Fig. 3E) and total lipids (Fig. 3F, G) was confirmed by the histochemical tests for all types of collectors described. Of all the histochemical tests performed, only the secretory head of the collectors showed positive results.

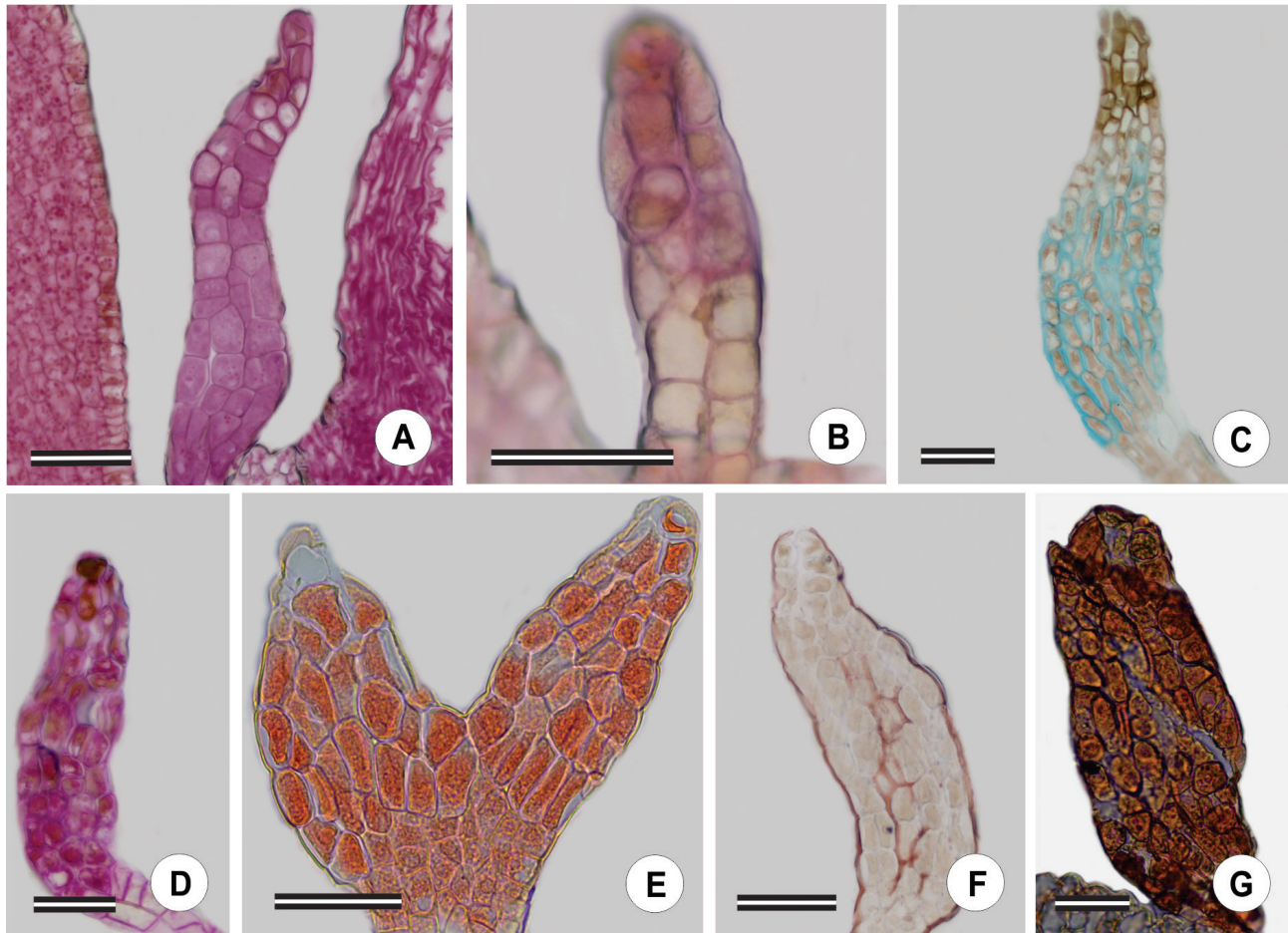


Figure 3. Histochemical tests in collectors of *Chamaecrista* sect. *Chamaecrista*. A-B. Totals polysaccharides. A. *C. rotundifolia* var. *grandiflora*: colleter short bottle shaped on stipule. B. *C. rotundifolia* var. *rotundifolia*: colleter club-shaped on stipule. C. Acid mucopolysaccharides, *C. simplifecta*: colleter short bottle shaped on bracteole. D. Pectins, *C. potentilla*: colleter club-shaped on sepal. E. Totals proteins, *C. aristata*: colleter bifurcated on stipule. F, G. Totals lipids, *C. aristata* and *C. mucronata*: colleter short digitiform on stipule and sepal, respectively. Scale bars: A, C, E-G: 50µm; B: 100µm; D: 40µm.

Discussion

According to the position, morphology, anatomy and the composition of the exudates, the secretory structures present on the base of stipules, between leaflets, at the petiole insertion, and among sepals, petals and bracts/bracteoles of the studied species of *Chamaecrista* sect. *Chamaecrista* and *Caliciopsis* are indeed collectors. Such

structures are typically related to the protection of young leaves and flowers from desiccation and attack from microorganisms since the secretion present is made of mucilage or a mixture of mucilage, resin and protein (Thomas & Dave 1989; Thomas 1991; Rocha *et al.* 2009).

In *Chamaecrista* sect. *Chamaecrista*, collectors produce a mixture of hydrophilic (polysaccharides and proteins) and lipophilic (lipids) compounds. Polysaccharides are able to retain water and hence their presence in colleter secretion

may indicate that they play an important functional role in keeping young structures hydrated (Fahn & Cutler 1992). Lipids have been detected in the colleter secretion of species belong Apocynaceae (Appezato-da-Glória & Estelita 2000), Leguminosae (Paiva 2009), Gentianaceae (Dalvi *et al.* 2013), Orchidaceae (Mayer *et al.* 2011) and Rubiaceae (Machado *et al.* 2012; Tullii *et al.* 2013). As claimed for polysaccharides, lipid compounds may help avoid water loss, in addition to prohibiting fungal and microorganism attack, since this hydrophobic substance lubricates the surface of young leaves and floral buds, (Fahn 1979; Thomas & Dave 1989; Thomas 1991; Evert 2006; Paiva 2009; Mayer *et al.* 2013). Proteins have also been reported as a component of the secretion produced by colleters from a variety of different species (Thomas & Dave 1990; Klein *et al.* 2004; Miguel *et al.* 2006; González & Tarragó 2009; Mayer *et al.* 2011; Dalvi *et al.* 2013). Some authors have suggested that such proteins may provide additional protection from fungi and parasites (Miguel *et al.* 2006; Vieira *et al.* 2006; Mayer *et al.* 2011). However, research aiming to unravel how exudates produced by colleters may contribute to the success of species of *Chamaecrista* in stressful environments is still needed.

Although cuticular pores were not observed among the colleters of the studied species, a few samples had loosely packed secretory cells at the colleter apex and a distended cuticle. This arrangement of cells and cuticle is an indication that the secretion may be released to the outside by cuticle rupture. Releasing of secretion by cuticle rupture has been suggested for colleters of *Hymenaea stigonocarpa* (Paiva & Machado 2006b) and demonstrated in colleters of *Senna macranthera* (Souza 2014), both belonging to Caesalpinioideae, as well as other families (Paiva & Machado 2006a; Tullii *et al.* 2013).

As observed in the species of the sect. *Chamaecrista* and *Caliciopsis* studied, non-vascularized colleters have been previously reported for other species of *Chamaecrista* belonging to other sections (De-Paula & Oliveira 2007; Coutinho *et al.* 2013; 2015), other species of Caesalpinioideae (Paiva & Machado 2006b; Paiva 2009; Souza 2014), and even species of other families (Paiva & Machado 2006a; Paiva 2009; Vitarelli & Santos 2009; Silva *et al.* 2012; Dalvi *et al.* 2013). Anatomically, the colleters described for the species of sect. *Chamaecrista* and *Caliciopsis* studied exhibit the same homogeneous pattern of cells as reported for other *Chamaecrista* species (Coutinho *et al.* 2015).

Although Lersten (1974) pointed out that the standard type of colleter is the most widespread, variation in the morphoanatomy of these structures has since been observed such that now several different types of colleters are recognized (Mayer *et al.* 2011; Silva *et al.* 2012; Coutinho *et al.* 2015). Standard type colleters are comprised of a secretory palisade epidermis covering a non-secretory central axis, which may or may not be vascularized with xylem and phloem (Lersten 1974). In contrast to this

standard type of colleter, which has been described for other botanical families (Appezato-da-Glória & Estelita 2000; Paiva & Machado 2006a; Vitarelli & Santos 2009), the most common type of colleter for the genus *Chamaecrista*, as shown by our data along with data provided by Coutinho *et al.* (2015), are comprised of homogenous cells.

As the colleters of the studied species of *Chamaecrista* age, they exhibit a brownish color and usually fall off when they stop secreting. Similar observations were reported for other species of *Chamaecrista* (Coutinho *et al.* 2015). This change in color may be the result of the oxidation of phenolic compounds accumulated within the cells, which is followed by the shrinking of the cytoplasm of the apical cells of colleters (Souza 2014). Similar descriptions were reported for colleters from a variety of botanical families (Thomas 1991; Paiva 2009; Souza 2014).

Colleters were observed in all of the analyzed taxa from both sect. *Chamaecrista* and *Caliciopsis*. Within subfamily Caesalpinioideae, the presence of colleters stands out when we take species from subtribe Cassinae (tribe Cassieae) into account, since five species of *Senna* (Souza 2014) and 55 species of *Chamaecrista* (De-Paula & Oliveira 2007; Coutinho *et al.* 2015) display these secretory structures. Bifurcated colleters, like those observed in seven of the taxa studied here, were also reported for *Temnadenia violacea* (Martins *et al.* 2010), *Prestonia coalita* (Rio 2001), *Forsteronia* (Rio 2006), *Mandevilla pycnantha* and *M. tenuifolia* (Simões *et al.* 2006), all species that belong to Apocynaceae, as well as in *Curtia* and *Hockinia* (Dalvi *et al.* 2013), species of Gentianaceae. We suspect that this bifurcation is the result of abnormalities during colleter development, and so do not correspond to a distinct type of colleter.

Although six distinct types of colleters have been described for species of *Chamaecrista* (Coutinho *et al.* 2015), only five species of ser. *Coriaceae* (sect. *Chamaecrista*) were sampled and nothing about sect. *Caliciopsis* was provided. The same authors reported short digitiform (I), club-shaped (III) and short bottle shaped (V) colleters for sect. *Chamaecrista*. Our more comprehensive sample of sect. *Chamaecrista* enabled us to observe two additional types of colleters for this section: long digitiform and pyriform. New information on the occurrence of colleters at the insertion of the petiole of *C. aristata*, *C. caribaea*, *C. cinerascens*, *C. potentilla* and *C. simpliflora*, which was overlooked by previous authors, is also provided here. The pyriform (VII) type of colleter is a novelty for *Chamaecrista*, and is exclusive to *C. rufa* var. *exsul* (ser. *Chamaecrista*), while racket-shaped (IV) and long bottle-shaped (VI) colleters were not detected in the sections studied.

Coutinho *et al.* (2015) suggested that the length (short and long) of colleters Type I and II is related to the size of the structure on which they are located such that larger stipules and bracts may bear both colleter types, while in smaller ones only the short digitiform type of colleter (Type I) would develop. However, among the species studied herein, we were able to observe that both types of colleters



are independent of the size of the bearing structure.

Short digitiform (I) and short bottle shaped (V) collectors have been observed only in *C. tragacanthoides* var. *rasa*, *C. venulosa*, the three varieties of *C. caribaea*, species that belong to ser. *Coriaceae*, and two taxa of sect. *Caliciopsis*. Considering that the infrageneric relationships of *Chamaecrista* remain unclear, collector type is emerging as a relevant and promising character for understanding the relationships among the species of *Chamaecrista*. According to both molecular and morphological data, *Chamaecrista* ser. *Coriaceae* was recognized as a monophyletic group, excluding *C. caribaea*, *C. roraimae*, and *C. venulosa* (Rando *et al.* 2016). The diversity of types and different positions reported for collectors in *Chamaecrista*, both by us and other authors, emphasizes how important and poorly understood this structure is. Such data promise to be useful for both taxonomic and phylogenetic studies, as has been the case for other taxa (Lersten 1975; Curtis & Lersten 1980; Thomas 1991; González 1998; Simões *et al.* 2006; González & Tarragó 2009; Sheue *et al.* 2012; Silva *et al.* 2012; Vitarelli *et al.* 2015; Fernandes *et al.* 2016). However, further studies are necessary to thoroughly evaluate the evolutionary history of collectors in *Chamaecrista*.

We showed that collectors are diverse structures in *Chamaecrista* sect. *Chamaecrista* and *Caliciopsis*. The short digitiform and short bottle-shaped collectors found in the two species of sect. *Caliciopsis* were also observed in two (*C. caribaea* and *C. venulosa*) of the three species that should be excluded from ser. *Coriaceae* (sect. *Chamaecrista*), as suggested by a recent molecular phylogenetic study. Such similar characters may suggest species of sect. *Caliciopsis* are closer to species of ser. *Coriaceae*, therefore, the position of the such species of ser. *Coriaceae* should be revised. However, only a comprehensive study of species of sect. *Chamaecrista*, including representatives of sect. *Caliciopsis*, will be able to confirm such a hypothesis. In addition to the types of collectors already described for sect. *Chamaecrista*, we added two new types of collectors (long digitiform and pyriform). Besides, the pyriform type is a novelty for the genus *Chamaecrista*. Our results are promising as they may be useful to future analyses combining phylogenetic and evolutionary approaches, and encourage further research on other members of Caesalpinioideae.

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