

New Records of Colleters in Chamaecrista (Leguminosae, Caesalpinioideae s.l.): Structural Diversity, Secretion, Functional Role, and Taxonomic Importance

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## NEW RECORDS OF COLLETTERS IN *CHAMAECRISTA* (LEGUMINOSAE, CAESALPINIOIDEAE S.L.): STRUCTURAL DIVERSITY, SECRETION, FUNCTIONAL ROLE, AND TAXONOMIC IMPORTANCE

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**Premise of research.** Colleters are structures that secrete a sticky product that covers and protects the shoot apex and floral buds. In *Chamaecrista*, colleters have been reported in the cotyledons of three species and on the leaves of all species belonging to sect. *Absus* subsect. *Baseophyllum*. Anatomical studies using taxonomic and phylogenetic approaches are necessary to evaluate the presence, diversity, and importance of colleters for *Chamaecrista*.

**Methodology.** We analyzed 55 species of *Chamaecrista* belonging to five of the six sections of the genus. Samples from both herbarium- and field-collected material of young vegetative and reproductive meristems were used. The material was subjected to standard anatomical study by light microscopy and SEM, and secretion was evaluated by histochemical analyses.

**Pivotal results.** Histochemical analyses for the total proteins, total polysaccharides, acid mucopolysaccharides, pectins/mucilage, and lipids generated positive results. Six types of colleters are described here: club shaped, racket shaped, long bottle shaped, short bottle shaped, long digitiform, and short digitiform. Sect. *Apoucouita* showed the short digitiform and club-shaped types and was the only section with colleters on the sepal margins. Most species of sect. *Absus* subsect. *Absus* presented the short bottle-shaped type, while all species from subsect. *Baseophyllum* presented the short digitiform and club-shaped types. Although the short bottle-shaped type was the most common among species from sect. *Chamaecrista*, the short digitiform and club-shaped types were also observed. The short bottle-shaped colleters were also found in sect. *Grimaldia*, while in sect. *Xerocalyx* only the digitiform type was found.

**Conclusions.** The topography and components identified in the secretion of the colleters suggests that such structures may be involved in the protection of developing leaves and flowers. Five of the six types described in our study are novelties for *Chamaecrista*. The distribution of colleter structural diversity provides an important source of new data that may help to clarify the taxonomy and phylogeny of *Chamaecrista*.

**Keywords:** anatomy, buds, histochemical test, secretory structures, shoots, trichomes.

### Introduction

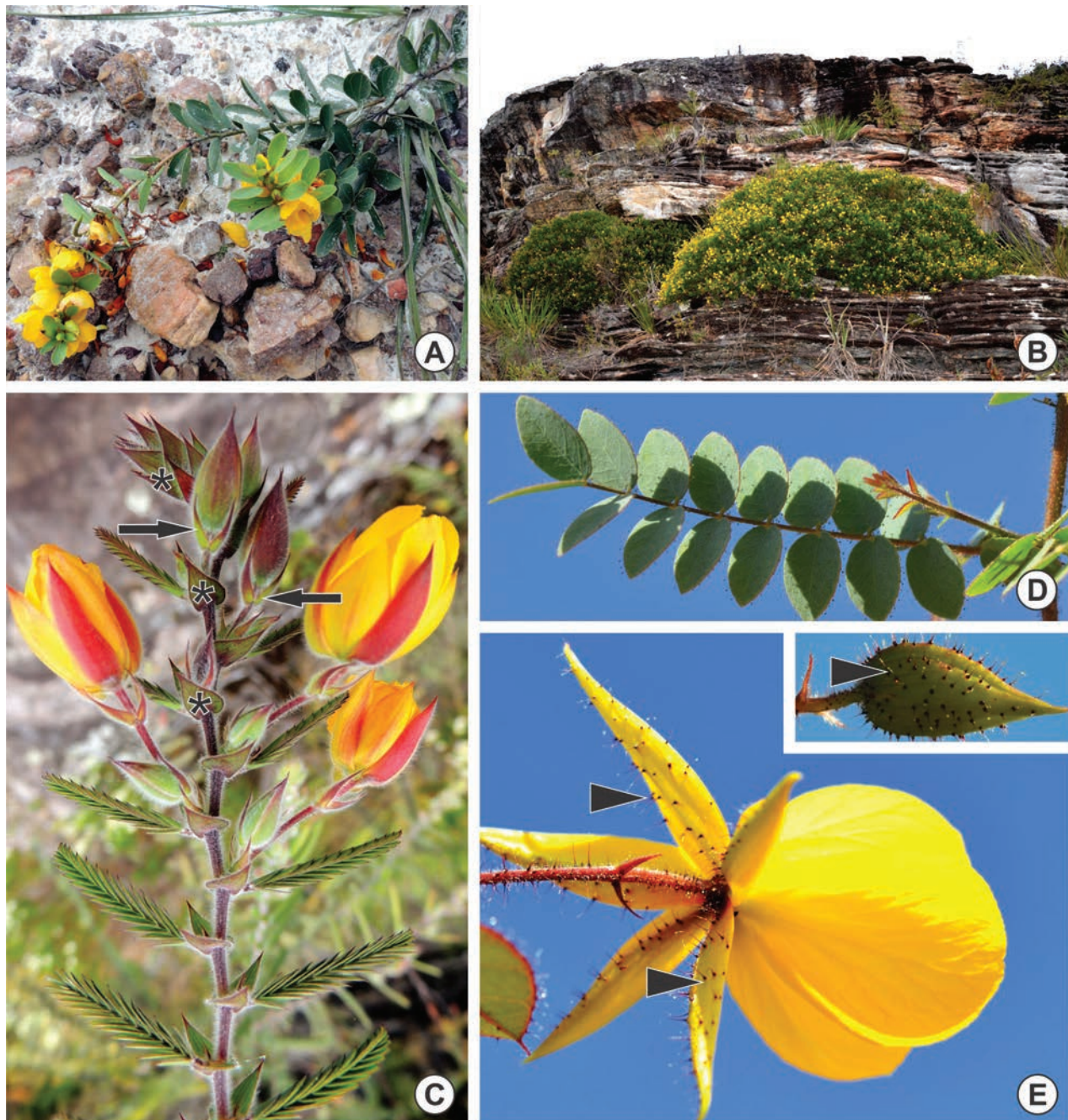
The genus *Chamaecrista* Moench (Leguminosae, Caesalpinioideae s.l.) includes more than 330 species (Lewis 2005). *Chamaecrista* species are typically herbs (fig. 1A) and shrubs (fig. 1B) with yellow flowers (fig. 1A, 1C, 1E) that are found in rocky outcrops (fig. 1A, 1B) and other open sunny areas. Woody species also appear in rain forests, especially in the Amazon and Brazilian Atlantic forests (Irwin and Barneby 1982; Lewis 2005; Conceição et al. 2009; Rando et al. 2013). Previously, *Chamaecrista* species were grouped into six sec-

tions: *Chamaecrista* sect. *Absus*, *Apoucouita*, *Caliciopsis*, *Chamaecrista*, *Grimaldia*, and *Xerocalyx* (Irwin and Barneby 1982). Molecular studies have suggested the definition of new sectional and species boundaries (Conceição et al. 2008, 2009). According to the circumscription proposed by these authors, the clades *Apoucouita* and *Xerocalyx* are recognized as sections, sect. *Absus* subsect. *Baseophyllum* should be elevated to sectional status, and sect. *Grimaldia* must now be included in sect. *Absus*. Sect. *Caliciopsis* and *Chamaecrista* were found to be paraphyletic. Although these studies demonstrate the need for taxonomic revision within the genus, taxon sampling was limited, and more data are necessary before amending taxon circumscriptions and adopting nomenclatural changes.

In the subfamily Caesalpinioideae s.l., secretory structures have been shown to be meaningful additional characters in taxonomical evaluations (Irwin and Barneby 1978, 1982; Ler-

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**Fig. 1** *Chamaecrista* species in the field. *A, B*, *Chamaecrista choriophylla* and *C. semaphora*, respectively, in rocky outcrops. *C*, Flowering branch of *C. potentilla*. Note the stipules (asterisks) and bracts (arrows). *D*, Leaf and shoot of *C. cipoana*. *E*, Flower of *C. monticola* displaying sticky glandular hairs (arrowheads) on sepals (flower bud in detail).

sten and Curtis 1993, 1994, 1996; Rudall et al. 1994; Conceição et al. 2008, 2009; Coutinho et al. 2013). In several families, colleters have stood out in taxonomical and phylogenetic interpretations (Lersten 1974; Thomas 1991; Klein et al. 2004; Silva et al. 2012; Dalvi et al. 2013, 2014). Colleters are structures that are usually found on the adaxial side of stipules and bracts and secrete a sticky product composed of mucilaginous substances or a mixture of mucilage and resin

that covers and protects the shoot apex and floral buds (Foster 1942; Thomas 1991; Paiva and Machado 2006a; Barreiro and Machado 2007; Mayer et al. 2011; Sheue et al. 2012).

Instead of “colleter,” other terms have been given to these structures, such as “glandular shaggy hair” (Solleder 1908; Metcalfe and Chalk 1950), “resin glands” (Curtis and Lersten 1980), “glandular trichomes” (Pascal et al. 2000), “extrafloral nectary” (Freitas et al. 2001), and “filamentous structures”

Table 1

## Chamaecrista Species Studied and Position of the Collecters

Taxon	Collector position							
	Base of stipules	Rachis between the leaflets	Base of bracts and bracteoles	Base of sepals	Margin of sepals	Base of petals	Around extrafloral nectaries	Rachis extension
Clade sect. <i>Apoucouita</i> :								
Apo:								
<i>C. adiantifolia</i> var. <i>adiantifolia</i>	I, III	I, III	I, III	I, III	III	I	NO	NO
<i>C. adiantifolia</i> var. <i>pteridophylla</i>	I, III	I, III	I, III	I, III	III	I	NO	NO
<i>C. amabilis</i>	I, III	I, III	I, III	I, III	III	I	NO	NO
<i>C. amorimii</i> <sup>a</sup>	NO	NO	I, III	I, III	III	I	NO	NO
<i>C. apoucouita</i>	I, III	I, III	I, III	I, III	III	I	NO	NO
<i>C. aspleniifolia</i>	I, III	I, III	I, III	I, III	III	I	NO	NO
<i>C. bahiae</i>	I, III	I, III	I, III	I, III	III	I	I, III	NO
<i>C. compitalis</i>	I, III	I, III	I, III	I, III	III	I	NO	NO
<i>C. duartei</i>	I, III	I, III	I, III	I, III	III	I	NO	NO
<i>C. eitenorum</i> var. <i>eitenorum</i>	I, III	I, III	I, III	I, III	III	I	NO	NO
<i>C. eitenorum</i> var. <i>regana</i>	I, III	I, III	I, III	I, III	III	I	NO	NO
<i>C. ensiformis</i> var. <i>ensiformis</i>	I, III	I, III	I, III	I, III	III	I	I, III	NO
<i>C. ensiformis</i> var. <i>maranonica</i>	I, III	I, III	I, III	I, III	III	I	NO	NO
<i>C. ensiformis</i> var. <i>plurifoliolata</i>	I, III	I, III	I, III	I, III	III	I	NO	NO
<i>C. hymenaeifolia</i>	I, III	I, III	I, III	I, III	III	I	NO	NO
<i>C. negrensis</i> var. <i>negrensis</i>	I, III	I, III	I, III	I, III	III	I	NO	NO
<i>C. polystachya</i>	I, III	I, III	I, III	I, III	III	I	I, III	NO
<i>C. scleroxylon</i>	I, III	I, III	I, III	I, III	III	I	I, III	NO
<i>C. subpeltata</i>	I, III	I, III	I, III	I, III	III	I	NO	NO
<i>C. xinguensis</i>	I, III	I, III	I, III	I, III	III	I	I, III	NO
Clade sect. <i>Absus</i> subsect. <i>Absus</i> + sect. <i>Grimaldia</i> :								
Abs:								
<i>C. barbata</i>	V	V	V	A	A	I	ENA	NO
<i>C. botryoides</i> <sup>a</sup>	V	V	V	A	A	I	ENA	NO
<i>C. cipoana</i>	V	V	V	V	A	I	ENA	NO
<i>C. clausenii</i>	V	V	V	V	A	I	ENA	NO
<i>C. conferta</i>	V	V	V	V	A	I	ENA	NO
<i>C. dentata</i>	A	A	A	A	A	A	ENA	NO
<i>C. ericifolia</i>	V	V	V	V	A	I	ENA	NO
<i>C. fagonioides</i>	NO	NO	A	A	A	A	ENA	NO
<i>C. glaucofilix</i> <sup>a</sup>	V	V	V	V	A	I	ENA	NO
<i>C. glaziovii</i>	A	A	A	A	A	A	ENA	NO
<i>C. jacobinea</i> <sup>a</sup>	V	V	V	V	A	I	ENA	NO
<i>C. monticola</i>	VI	VI	VI	VI	A	I	ENA	NO
<i>C. myrophenges</i>	V	V	V	V	A	I	ENA	NO
<i>C. ochracea</i> var. <i>purpurascens</i>	I, V	I, V	I, V	I, V	A	I	ENA	NO
<i>C. philippi</i> <sup>a</sup>	V	V	V	V	A	I	ENA	NO
<i>C. rupestrium</i>	V	V	V	V	A	I	ENA	NO
<i>C. semaphora</i>	A	A	A	A	A	A	ENA	NO
<i>C. setosa</i> <sup>a</sup>	VI	VI	VI	VI	A	I	ENA	NO
<i>C. sincorana</i>	V	V	V	V	A	I	ENA	NO
<i>C. speciosa</i> <sup>a</sup>	VI	VI	VI	VI	A	I	ENA	NO
<i>C. tephrosiifolia</i>	V	V	V	V	A	I	ENA	NO
<i>C. urophyllidia</i> <sup>a</sup>	V	V	V	A	A	I	ENA	NO
<i>C. vauthieri</i>	V	V	V	V	A	I	ENA	NO
Gri:								
<i>C. absus</i> var. <i>absus</i> <sup>a</sup>	V	V	V	V	A	I	NO	NO
<i>C. absus</i> var. <i>meonandra</i>	V	V	V	V	A	I	NO	NO
Clade sect. <i>Absus</i> subsect. <i>Baseophyllum</i> :								
Bas:								
<i>C. blanchetii</i> <sup>a</sup>	I, III	I, III	I, III	I, III	A	I	I, III	I
<i>C. brachystachya</i> <sup>a</sup>	I, III	I, III	I, III	I, III	A	I	I, III	I
<i>C. coriacea</i> <sup>a</sup>	I, III, IV	I, III, IV	I, III	I, III	A	I	I, III	I
<i>C. cytisoides</i> <sup>a</sup>	I, III	I, III	I, III	I, III	A	I	I, III	I



**Table 1**  
(Continued)

Taxon	Colleter position							
	Base of stipules	Rachis between the leaflets	Base of bracts and bracteoles	Base of sepals	Margin of sepals	Base of petals	Around extrafloral nectaries	Rachis extension
<i>C. decora</i> <sup>a</sup>	I, III	I, III	I, III	I, III	A	I	I, III	I
<i>C. depauperata</i> <sup>a</sup>	I, III	I, III	I, III	I, III	A	I	NO	I
<i>C. unijuga</i> <sup>a</sup>	I, III	I, III	I, III	I, III	A	I	I, III	I
Taxon not sampled by Conceição et al. (2009):								
Oto:								
<i>C. debilis</i>	III, IV	III, IV	III, IV	III, IV	A	I	III, IV	I
Clade sect. <i>Chamaecrista</i> + sect. <i>Xerocalyx</i> + sect. <i>Caliciopsis</i> : <sup>b</sup>								
Cha:								
<i>C. aristata</i>	I, III	I, III	I, III	I, III	A	I	I, III	NO
<i>C. caribaea</i> var. <i>caribaea</i>	I, V	I, V	I, V	I, V	A	I	NO	NO
<i>C. caribaea</i> var. <i>inaguensis</i>	I, V	I, V	I, V	I, V	A	I	NO	NO
<i>C. caribaea</i> var. <i>lucayana</i>	I, V	I, V	I, V	I, V	A	I	NO	NO
<i>C. cinerascens</i>	A	A	III, V	III, V	A	I	NO	NO
<i>C. potentilla</i> <sup>a</sup>	A	A	III, V	III, V	A	I	NO	NO
<i>C. simpliflora</i>	III, V	III, V	III, V	III, V	A	I	III, V	NO
Xer:								
<i>C. desvauxii</i> var. <i>desvauxii</i> <sup>a</sup>	I, II	I, II	I, II	I, II	A	I	A	A
<i>C. desvauxii</i> var. <i>latistipula</i>	I, II	I, II	I, II	I, II	A	I	A	A
<i>C. desvauxii</i> var. <i>graminea</i>	I	I	I	I	A	I	A	A
<i>C. ramosa</i>	I	I	I	I	A	I	A	A
<i>C. ramosa</i> var. <i>parvifoliola</i>	I	I	I	I	A	I	A	A

Note. Clades follow the molecular phylogeny performed by Conceição et al. (2009). Sections and subsections of *Chamaecrista* are as proposed by Irwin and Barneby (1982) and indicated by acronyms. Values in the following parentheses represent the number of species included in the present study and the total number of species ascribed to each taxon: Abs = sect. *Absus* subsect. *Absus* (23/169); Cha = sect. *Chamaecrista* (5/53); Bas = sect. *Absus* subsect. *Baseophyllum* (7/8); Gri = sect. *Grimaldia* (1/1); Oto = sect. *Absus* subsect. *Otophyllum* (1/1); Xer = sect. *Xerocalyx* (2/3); Apo = sect. *Apoucouita* (16/20). I = short digitiform; II = long digitiform; III = club shaped; IV = racket shaped; V = short bottle shaped; VI = long bottle shaped; A = absent; ENA = extrafloral nectaries absent; NO = not observed.

<sup>a</sup> Taxa sampled by Conceição et al. (2009).

<sup>b</sup> Sect. *Caliciopsis* is not included in this study.

(De-Paula et al. 2011), among others (Thomas 1991). Such nomenclatural diversity and/or misinterpretations are primarily based on anatomical or morphological similarities with other secretory structures. The term “colleter” is more appropriate to describe structures that secrete substances responsible for protecting developing leaves and flowers mainly against dehydration and pathogens (Thomas 1991; Mayer et al. 2011).

For Leguminosae, few studies have reported the presence of colletters (Paiva and Machado 2006b; De-Paula and Oliveira 2007, 2012; Paiva 2009; Coutinho et al. 2013). To our knowledge, there are no comparative studies of the diversity and taxonomic importance of colletters for this family. In *Chamaecrista*, colletters have been reported in the cotyledons of three species (De-Paula and Oliveira 2007, 2012) and on the leaves of all of the species of *Absus* subsect. *Baseophyllum* (Coutinho et al. 2013). However, the authors do not provide detailed information on the structural (morphological and anatomical) variation and chemical nature of the secretion. Due to the importance of colletters in taxonomy (Lersten 1974; Thomas 1991; Klein et al. 2004; Silva et al. 2012; Dalvi et al. 2013, 2014), the following questions were raised: Are colletters common secretory structures for *Chamaecrista*? Are there structural and topographical variations among the species/sections? Could such variations be used to solve taxo-

nomic problems within this group? Is there variation in the chemical nature of the exudates that are produced by the colletters? Aiming to answer such questions, this study provides the structural characterization and the histochemical analysis of the colletters found on both vegetative and reproductive meristems of *Chamaecrista*.

## Material and Methods

### Plants

A total of 55 *Chamaecrista* species (65 taxa) belonging to five of the six sections were studied (appendix). The proportion of the total taxa of the five sections of *Chamaecrista* studied is indicated in table 1. Samples of the shoots (fig. 1C, 1D) and flower buds (fig. 1C, 1E) were obtained from herbarium material and field-collected plants. For most species, at least three specimens were used as replicates.

Samples from herbarium material were microwaved in distilled water for 7 min and left to rest overnight. Samples were then treated with 2% potassium hydroxide for 1 h at room temperature (Smith and Smith 1942), rinsed with tap water three times, dehydrated in an ethanol series (10%, 30%, 50%, and 70%; 10 min each), and stored in 70% ethanol before being

subjected to standard anatomical procedures. Samples from species that were collected from the field (appendix) were fixed in FAA (formaldehyde, acetic acid, and 50% ethanol; 1:1:18 vol/vol) for 48 h and stored in 70% ethanol. Voucher specimens were deposited in the herbarium of the Universidade Federal de Viçosa (VIC), and duplicates were sent to the herbarium of the New York Botanical Garden (NY), the United States National Herbarium–Smithsonian Institution (US), and the herbarium of the Universidade de São Paulo (SPF).

#### Slide Preparation

Stipules, bracts, and bracteoles from the samples of both herbarium material and field-collected species that were stored in 70% ethanol were cleared with 10% sodium hydroxide and 20% hypochlorite solutions, stained with 50% ethanol-diluted fuchsin, and mounted in glycerinated gelatin (Johansen 1940).

The samples from both herbarium material and field-collected species that were stored in 70% ethanol were embedded in methacrylate (Historesin Leica; Leica Microsystems Nussloch, Heidelberg, Germany) in accordance with the manufacturer's recommendation. Cross and longitudinal sections that were 4  $\mu\text{m}$  thick were made in an automatic rotary microtome (Leica RM2155). For structural characterization, the sections were stained with toluidine blue at pH 4.4 (O'Brien and McCully 1981), dried at room temperature, and mounted in resin (Permout; Fisher Scientific, Fair Lawn, NJ). Some of the sections were also used in histochemical tests.

Some of the field-collected species were also embedded in histological paraffin. The material stored in 70% ethanol was dehydrated through a tert-butanol series and embedded in histological paraffin (Histosec; Merck, Darmstadt, Germany; Johansen 1940). Cross and longitudinal 7- $\mu\text{m}$ -thick serial sections were obtained from blocks using a rotary microtome (Spencer 820; American Optical, Buffalo, NY). The sections were deparaffinized in xylene and rehydrated through an ethanol series. For structural characterization, the sections were stained with safranin and astra blue, dehydrated through an ethanol/xylene series (Gerlach 1969), and mounted in resin (Permout; Fisher Scientific). Some of the sections were also used in histochemical tests.

The histochemical tests were performed on fixed samples of *C. brachystachya*, *C. cytisoides*, *C. debilis*, *C. decora*, *C. desvauxii* var. *latistipula*, *C. ensiformis* var. *ensiformis*, *C. myrophenges*, and *C. vauthiere*. The following histochemical tests were conducted on paraffin- and/or methacrylate-embedded material: for total proteins, xylydine ponceau (O'Brien and McCully 1981); for total polysaccharides, periodic acid–Schiff reagent (O'Brien and McCully 1981); for acid mucopolysaccharides, alcian blue (Pearse 1980); and for pectins/mucilage, ruthenium red (Johansen 1940). The paraffin-embedded sections of *C. debilis*, *C. desvauxii* var. *latistipula*, and *C. myrophenges* were tested for lipid compounds with Sudan IV and Sudan black B (Pearse 1980).

Paraffin- and/or resin-embedded material from six species were chosen for ontogenetic study of colleters, as follows: *C. brachystachya*, *C. cytisoides*, *C. debilis*, *C. decora*, *C. desvauxii* var. *latistipula*, and *C. myrophenges*.

Observations and photographs were obtained using a light microscope (model AX70TRF; Olympus Optical, Tokyo, Ja-

pan) equipped with a U-Photo system and digital camera (AxioCam HRC; Carl Zeiss, Göttingen, Germany).

#### SEM

Samples from herbarium and field-collected material stored in 70% ethanol were critical point dried with  $\text{CO}_2$  in a 020 CPD dryer (Bal-Tec; Balzers, Liechtenstein). The samples were mounted onto stubs and coated with gold using an FDU 010 sputter coater (Bal-Tec). Examinations and image captures were conducted using a Leo 1430VP SEM (Zeiss, Cambridge, United Kingdom) at the Centro de Microscopia e Microanálises at the Universidade Federal de Viçosa.

## Results

### Ontogeny

There is no participation of any internal meristematic tissues in the origin of the colleters. Such structures are formed from protodermal cell initials that could be easily identified due to their dense cytoplasm and large nuclei (fig. 2A–2D).

A single protodermal cell initial expands and goes through successive anticlinal divisions (fig. 2E). The cells that originated from such anticlinal divisions begin dividing on different planes (fig. 2A–2D), giving rise to a stalk and a secretory (fig. 2E, 2F). The protodermal cell divisions are not synchronous, as fully formed colleters may be observed while young ones are still going through cell divisions (fig. 2E–2G).

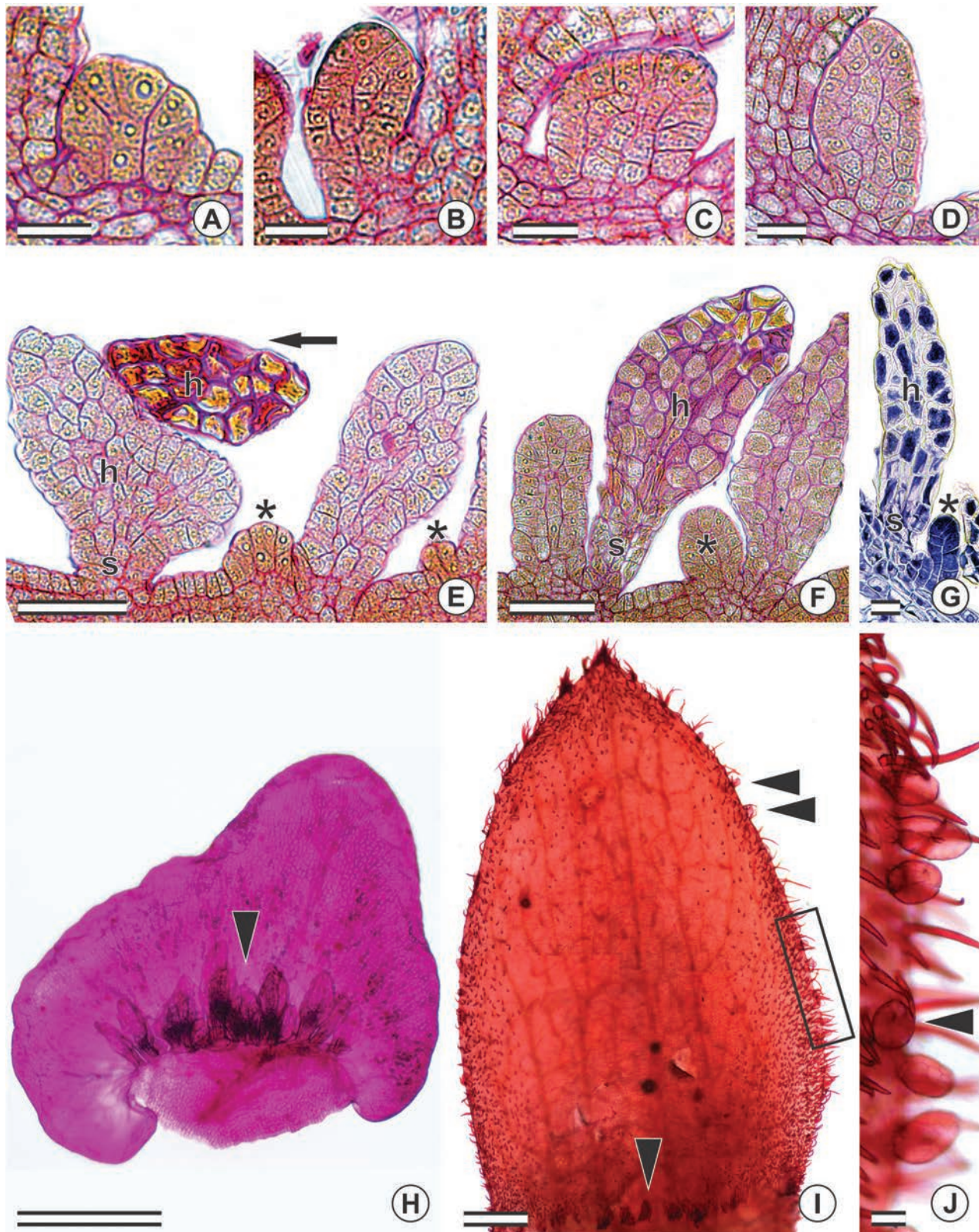
### Structure and Distribution

No vascularization was observed in the colleters of the *Chamaecrista* species studied (fig. 2F). The fully formed colleters are composed of a stalk and a secretory head (fig. 2E–2G). The stalk cells are highly vacuolated with thick outer walls, while those forming the head show a densely stained cytoplasm. Secretory pores were not observed, and both light microscopy and SEM indicated that cuticle rupture was rare. The secretory phase begins when the leaves and flowers are still being formed. When secretion ends, the colleters wither, become dark brown or black, and usually drop off.

Colleters were always found in patches (figs. 2–4) at the adaxial side of different plant structures (table 1): at the base of bracts/bracteoles (figs. 2H, 3D–3F, 4A, 4F, 4H), at the base and margins of sepals (fig. 2I, 2J), at the base of petals, on the rachides at the insertion of the leaflets (fig. 3A), and at the base of stipules (fig. 4B–4D). They were also found around extrafloral nectaries (fig. 3B, 3C) and at the extension of the rachides (fig. 3C) but not in patches. The number of colleters is not fixed for each species or plant organ/structure. All of the studied species showed at least one type of colleter except for *C. dentata*, *C. fagonioides*, *C. glaziovii*, and *C. semaphora*, in which such structures could not be found.

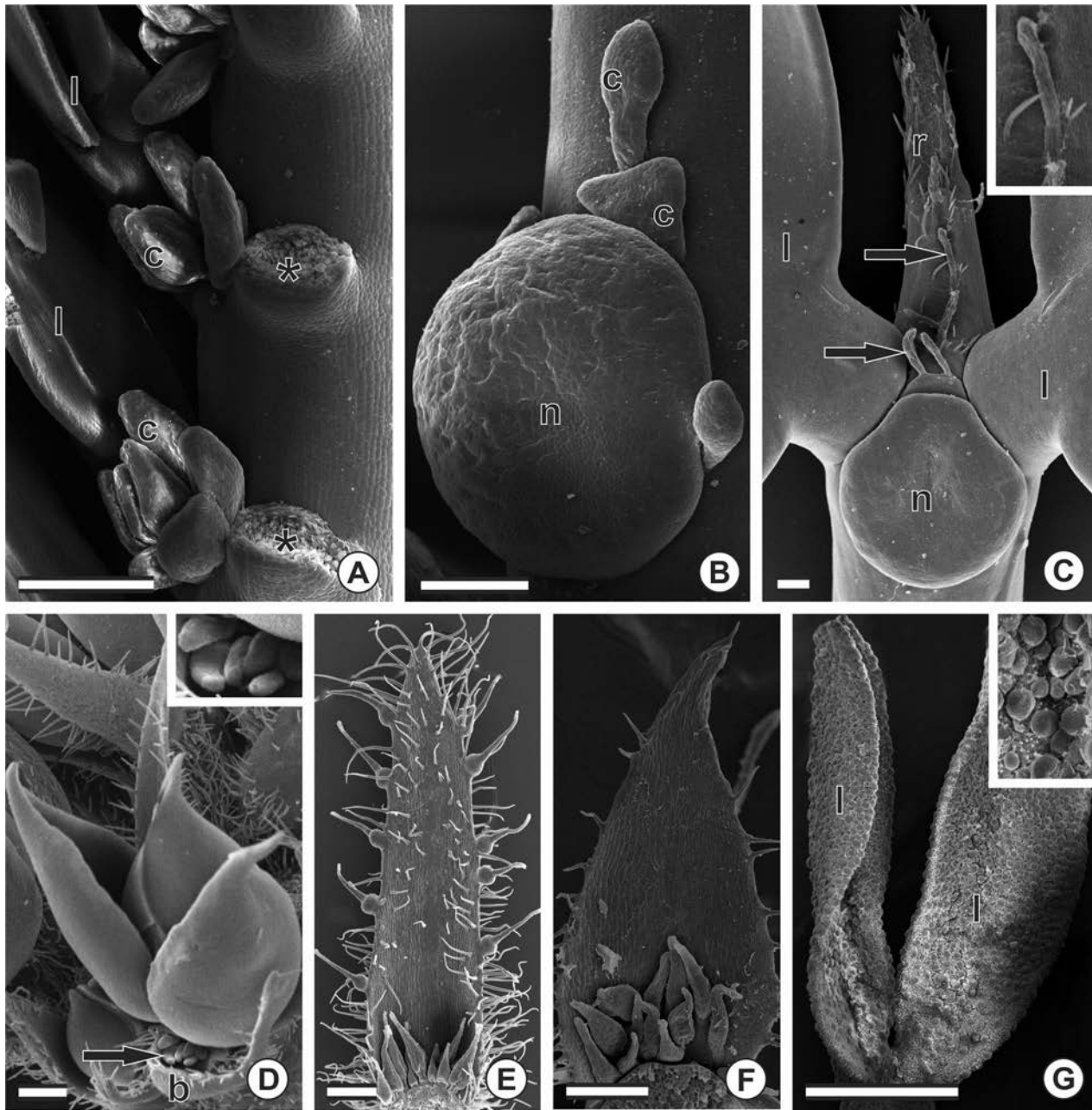
Six types of colleters may be described: club shaped (figs. 3B, 4C, 4D), racket shaped (figs. 3A, 4E), long bottle shaped (whole length of the colleter >500  $\mu\text{m}$ ; fig. 4H), short bottle shaped (<300  $\mu\text{m}$ ; figs. 3F, 4F, 4G), long digitiform (>500  $\mu\text{m}$ ; fig. 4B), and short digitiform (<400  $\mu\text{m}$ ; figs. 3C, 4A, 4D).





**Fig. 2** Development of colleters and their distribution on bracts and sepals of *Chamaecrista*. A–F, Longitudinal sections of *C. debilis* colleters subjected to periodic acid–Schiff reaction. A–D, Development of colleters. E, F, Fully formed colleters composed of a stalk (s) and a secretory head (h). Note the young colleters (asterisks) and colleters already secreting (arrow). G, Longitudinal sections of *C. brachystachya* colleters. H, Bract clearing of *C. debilis* showing colleters (arrowhead) on the lower adaxial side. I, J, Sepal clearing of *C. hymenaeifolia* displaying colleters on the margins and lower adaxial side. Scale bars: A–D, G = 20  $\mu\text{m}$ ; E, F, J = 50  $\mu\text{m}$ ; H, I = 500  $\mu\text{m}$ .





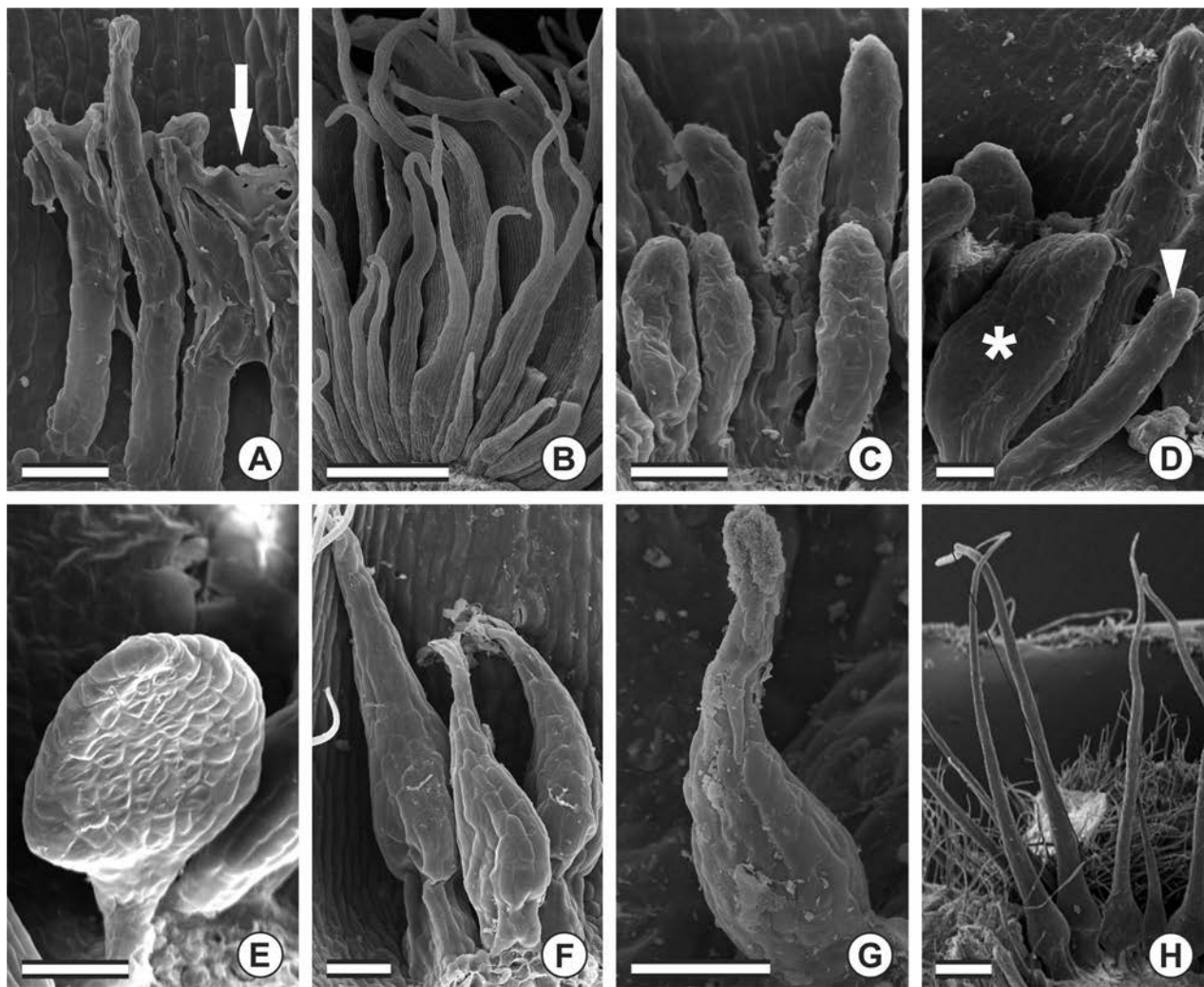
**Fig. 3** SEM images showing colleters on vegetative and reproductive organs of *Chamaecrista*. *A, B*, Leaves of *C. debilis*. *A*, Colleters (c) on the rachis between the leaflets (l). The opposite leaflet of each pair of leaflets was removed (asterisks). *B*, Colleters around the extrafloral nectary (n). *C*, Leaf of *C. unijuga* with colleters (arrows) near the extrafloral nectary and on the rachis extension (r). *D*, Flower bud of *C. aristata* displaying colleters (in detail) on the adaxial side of bracts (b). *E, F*, Bracts of *C. cipoana* and *C. conferta*, respectively, showing colleters on the lower adaxial side. Note the sticky glandular hairs on the bract margin in *E*. *G*, Leaf of *C. semaphora* with sticky glandular hairs (in detail) all over the leaflets (l). Scale bars: 200  $\mu$ m.

On petals, only the short digitiform type was found; it occurred at the base of at least one of the petals (table 1). The same types of colleters that occur on the vegetative and reproductive structures (table 1) could also be associated with the extrafloral nectaries (fig. 3B, 3C). On petals and at the base of the extrafloral nectaries, these structures were randomly found, usually less than four (table 1).

The species from sect. *Apoucouita* showed short digitiform and club-shaped types on both vegetative and floral organs (table 1). *Apoucouita* stood out for being the only section showing colleters on the sepal margins, wherein all species of that section had the club-shaped type.

Five types of colleters were observed in sect. *Absus* (table 1). Most of the species of subsect. *Absus* showed the short bottle-





**Fig. 4** SEM images showing the types of colleters in *Chamaecrista* species. A, Short digitiform type on bract of *C. unijuga*. Note the secretion (arrow) all over the colleters. B, Long and short digitiform type on stipule of *C. desvauxii* var. *latistipula*. C, Club-shaped type on stipule of *C. arisata*. D, Club-shaped (asterisk) and short digitiform (arrowhead) types on stipule of *C. cytisoides*. E, Racket-shaped type on rachis of *C. debilis*. F, G, Short bottle-shaped type on bracts of *C. cipoana* and stipule of *C. glaucofilix*, respectively. H, Long bottle-shaped type on bract of *C. monticola*. Scale bars: A, C–G = 50  $\mu\text{m}$ ; B, H = 300  $\mu\text{m}$ .

shaped type of colleters on both vegetative and reproductive organs except for *C. monticola*, *C. setosa*, and *C. speciosa*, which showed the long bottle-shaped type. Sticky glandular hairs, another type of trichome, were also present in species from subsect. *Absus* (figs. 1D, 1E, 3E, 3G). However, all of the species from subsect. *Baseophyllum* showed the short digitiform and club-shaped type, and *C. coriacea*, in addition to these, also presented the racket-shaped type. *Chamaecrista debilis*, the only species in subsect. *Otophyllum*, displayed both club-shaped and racket-shaped types.

Although the short bottle-shaped type was the most common among the species from sect. *Chamaecrista*, the short digitiform and club-shaped types were also observed (table 1). *Chamaecrista absus* var. *absus* and *C. absus* var. *meonandra*, the only two taxa from sect. *Grimaldia*, displayed the short bottle-shaped type (table 1). All of the species from sect. *Xerocalyx* showed both long and short digitiform types (table 1).

#### Histochemistry

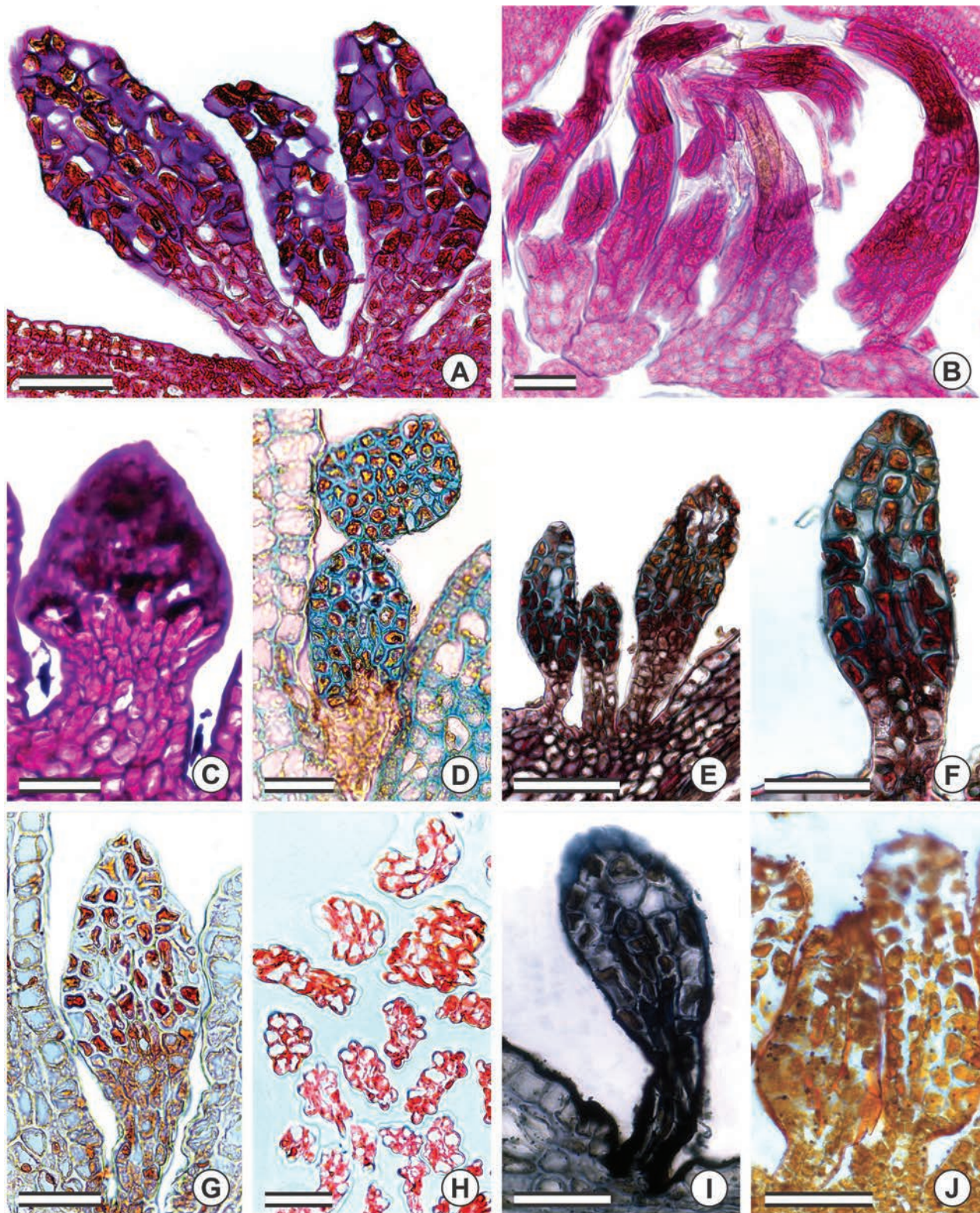
Histochemical analyses for the total polysaccharides (fig. 5A–5C), acid mucopolysaccharides (fig. 5D), pectins/mucilage (fig. 5E, 5F), total proteins (fig. 5G, 5H), and lipids (fig. 5I, 5J) generated positive results. Only the head of the colleters reacted to the histochemical analyses. The stalk cells did not secrete any of the analyzed metabolic groups. The outer of stalk cells were densely stained by Sudan black B (fig. 5I), demonstrating the presence of lipids in the cell wall composition.

#### Discussion

##### *Classification, Secretion, and Function of the Colleters*

The ontogenetic study of the colleters shows that such structures derive from the protodermis only and, therefore,





**Fig. 5** Positive histochemical results in longitudinal sections of colleters of *Chamaecrista* species. A–C, Periodic acid–Schiff reaction showing total polysaccharides in *C. debilis*, *C. desvauxii* var. *latistipula*, and *C. myrophenges*, respectively. D, Alcian blue, acid mucopolysaccharides, in *C. debilis*. E, F, Ruthenium red, pectins, in *C. debilis*. G, H, Xylidine ponceau, total proteins, in *C. debilis* (G) and *C. desvauxii* var. *latistipula* (H; cross section). I, J, Sudan black B (I) and Sudan IV (J), total lipids, in *C. debilis*. Scale bars = 50  $\mu\text{m}$ .



are a kind of secretory trichomes (Fahn 1990). According to the topography, structure (micromorphology and anatomy), early secretory activity, and compounds present in the secreted exudates, these trichomes correspond to colleters (Foster 1942; Fahn 1990; Thomas 1991).

Although colleters that are found in *Chamaecrista* display a stalk and a secretory head, a central axis and a secretory epidermis were not observed. Such colleters do not match any of the colleter descriptions of Lersten (1974). Colleters consisting of homogenous cells have been reported (Paiva and Machado 2006b; De-Paula and Oliveira 2007; Silva et al. 2012), but a classification for this type of colleter has not yet been proposed. We believe that such colleters should be called homogenous colleters on the basis of the homogeneity of the cellular composition. The homogenous colleters could then be divided into different types according to the micromorphology of such structures.

Secretory pores, which release the secretion to the exterior, were not observed, and cuticle rupture was rare. Such observations suggest an involvement of the outer cell wall in the secretion process and the releasing of the exudates via micropores or cuticle permeability (Ascensão et al. 1999; Klein et al. 2004; Paiva 2009; Mayer et al. 2011; Silva et al. 2012). The thickness and lipidic composition of the outer walls of the stalk cells may contribute to the symplastic transport of the secretion precursors toward the secretory head. Such a process is common in secretory trichomes (Fahn 1979, 1990; Leitão et al. 2005).

The histochemical tests revealed that the secreted exudates are composed of a mixture of hydrophilic (total polysaccharides, acid mucopolysaccharides, pectins, mucilages, and total proteins) and lipophilic (lipids) compounds. Polysaccharides, pectins, and mucilages may play a role in water retention due to their considerable ability to absorb water (Christodoulakis et al. 1990; Fahn and Cutler 1992; Al-Tarhah et al. 2008). This ability allows these hydrophilic compounds to lubricate both the vegetative and the reproductive meristem, thereby protecting the developing organs against dehydration (Foster 1942; Fahn 1990; Thomas 1991; Mayer et al. 2011; Martins 2012; Silva et al. 2012; Chin et al. 2013; Mayer et al. 2013). According to Paiva (2009, 2012), the hydrophilic material on the leaf surface reduces water loss to the external environment, helping to maintain adequate humidity levels for leaf development, as xylem transport is not yet efficient in developing organs. In addition to helping to lubricate the meristems, derivatives of the lipophilic fraction along with proteins present in the secretion could act as an inhibitor of fungal plant pathogens (Barnes et al. 1997; Miguel et al. 2006). In coffee flower buds, the secreted exudates produced by colleters act as an adhesive, sealing the bud until flower development is complete. As a consequence, developing flowers are protected from dehydration (Mayer et al. 2013). The closeness of the colleters to the developing leaf and flower, their early differentiation, the secretion period, and the chemical nature of the exudates indicate a protective function of such structures in *Chamaecrista*.

De-Paula and Oliveira (2007) did not observe secretion in the colleters of *C. desvauxii* var. *latistipula*. In our study, however, this species displayed secreting colleters. It is possible that De-Paula and Oliveira (2007) performed the histochemical tests when the colleters in the embryos were not yet active.

The colleters in *Chamaecrista*, especially those found on the stipules and rachides, usually fall off after completing their activity. Paiva (2012) suggests that rapid senescence may avoid possible problems with the growth of pathogens.

Of all of the studied species, *C. dentata*, *C. fagonioides*, *C. glaziovii*, and *C. semaphora* are the only ones that do not possess colleters. These species display high amounts of sticky glandular hairs all over the plant body. Meira et al. (2014) reported the presence of oleoresin secretory trichomes in *C. dentata* and correlated the presence of such secretion to protection against abiotic and biotic adverse factors. It is possible that in all of the *Chamaecrista* species that do not have colleters but instead have only sticky glandular hairs, the oily part of the trichome plays a role in lubricating the young developing structures, similar to colleters.

#### Taxonomic Importance of Colleters

In *Chamaecrista*, colleters have been reported for a few species (De-Paula and Oliveira 2007, 2012; Coutinho et al. 2013). However, 51 of the 55 studied species displayed colleters, demonstrating how common these structures are for this genus. As the colleters on the fully expanded leaves and flowers may have withered or dropped off, such structures may go unnoticed.

Colleters of exclusively protodermal origin were observed in the five species of *Chamaecrista* that were ontogenetically studied and in *Hymenaea stigomocarpa* (Paiva and Machado 2006b). A protodermal origin for colleters seems to be a rare pattern of development, as colleters are commonly formed from both protodermal and ground meristem and sometimes even from the procambial activity (Thomas 1991; Klein et al. 2004; Gonzalez and Tarragó 2009).

In *Chamaecrista*, six types of homogenous colleters could be distinguished. The anatomical and morphological variations observed in colleters have been used as taxonomic characteristics for several families (Thomas 1991)—Apocynaceae (Simões et al. 2006), Aquifoliaceae (Gonzalez and Tarragó 2009), Myrtaceae (Silva et al. 2012), Rubiaceae (Lersten 1974; Klein et al. 2004), Salicaceae (Curtis and Lersten 1980), and Rhizophoraceae (Sheue et al. 2012)—but have not yet been used in Leguminosae.

Colleters associated with the extrafloral nectaries, the adaxial base of petals, and the rachis extension varied among the taxa (species and sections). However, we could not demonstrate a useful pattern to delimitate sections or species groups.

Although colleters in *Chamaecrista* are found in groups in both vegetative and reproductive organs, the number of colleters is not fixed. On the other hand, the variation in their position is noteworthy. For instance, species from sect. *Apoucouita* are the only studied species to display colleters on the margin of the sepals. Conceição et al. (2009) noted that sect. *Apoucouita* actually forms a monophyletic group. The presence of club-shaped colleters on the margins of the sepals of species belonging to sect. *Apoucouita* is a synapomorphy for this section.

Sect. *Absus* subsect. *Baseophyllum* stood out for showing the short digitiform and club-shaped types of colleter. The same types of colleters were found in sect. *Apoucouita* (table 1), the sister clade to the remaining sections of *Cham-*



*aecrista* (Conceição et al. 2009). The absence of the short bottle-shaped type of colleter, characteristic of sect. *Absus* subsect. *Absus*, in all species of subsect. *Baseophyllum* supports the elevation of subsect. *Baseophyllum* as a section distinct from sect. *Absus*, as shown by molecular and anatomical studies (Conceição et al. 2009; Coutinho et al. 2013).

*Chamaecrista debilis* is the only species in sect. *Absus* subsect. *Otophyllum*. This species displayed both the club-shaped and the racket-shaped type of colletes. The racket-shaped type appeared only in *C. debilis* and *C. coriacea* (subsect. *Baseophyllum*), bringing *C. debilis* close to the species of subsect. *Baseophyllum*. The inclusion of *C. debilis* in future molecular studies is necessary to confirm this possible affinity.

Most of the species of sect. *Absus* subsect. *Absus* had the short bottle-shaped type of colleter on both vegetative and reproductive organs. The only two taxa that belong to sect. *Grimaldia*, *C. absus* var. *absus* and *C. absus* var. *meonandra*, also display the same type of colleter (short bottle shaped). The similarity in the type of colletes of these species reinforces the affinity between these two groups, supporting the recent molecular data that included sect. *Grimaldia* and sect. *Absus* subsect. *Absus* in a single clade (table 1). Therefore, sect. *Grimaldia* should be treated as a synonym of sect. *Absus* subsect. *Absus* in a future taxonomic revision of *Chamaecrista*.

The most common type of colleter in sect. *Chamaecrista* was the short bottle-shaped type. However, other types of colletes (club shaped and short digitiform) also appeared. Sect. *Chamaecrista* is paraphyletic (Conceição et al. 2009; table 1), and it is possible that the diversity of colletes in this section reflects such paraphyletism. *Chamaecrista calycioides* of sect. *Caliciopsis* is well supported within sect. *Chamaecrista*, while the position of *C. supplex* is poorly supported. Unfortunately, our study did not include either of these species. Additional anatomical studies including these two species are necessary to evaluate the importance of colletes in providing better resolution to phylogenetic analyses. Future anatomical and phylogenetic studies need to include broader taxon sampling of sect. *Chamaecrista* before any taxonomical considerations are taken into account.

Sect. *Xerocalyx* is a monophyletic group with poorly supported branches that is placed in the clade formed by three sections (table 1): *Caliciopsis*, *Chamaecrista*, and *Xerocalyx* (Conceição et al. 2009; Torres et al. 2011). Interestingly, species from sect. *Xerocalyx* show only the digitiform type of colleter (short and long), with the long digitiform unique for *C. desvauxii* var. *desvauxii* and *C. desvauxii* var. *latistipula*.

The uniformity in the type of colleter for sect. *Xerocalyx* is additional evidence supporting the monophyly of this section. The variation from long to short digitiform may be related to the size of stipules/bracts. Species with larger stipules/bracts (*C. desvauxii* var. *desvauxii* and *C. desvauxii* var. *latistipula*) displayed both long and short digitiform types, while species with smaller stipules/bracts (*C. desvauxii* var. *graminea*, *C. ramosa*, and *C. ramosa* var. *parvifoliola*) displayed the short digitiform type only.

## Conclusion

On the basis of the topography, structural characters, ontogeny, and components that were identified in the secretion, the secretory structures found in young leaves and flowers of *Chamaecrista* species are colletes. Such structures may be involved in the protection of developing leaves and flowers. Report of the presence of colletes on leaves and flowers is new for *Chamaecrista*. Five of the six types of colletes described in our study are novelties in the genus.

The structural diversity of colletes in *Chamaecrista* provides characters that have proved useful to taxonomy, as they support many of the clades recognized in a recent phylogenetic study (Conceição et al. 2009). However, taxon sampling for both phylogeny and anatomical studies is inadequate to justify a revised classification. Future research will focus on broadening the taxon sampling. In addition, future taxon sampling should give high priority to sampling the same species in phylogenetic and anatomical analyses.

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## Appendix

### *Chamaecrista* Species Used in the Structural Analysis

Vouchers are housed in the herbaria of the Universidade Estadual de Londrina (FUEL), Universidade Estadual de Feira de Santana (HUEFS), New York Botanical Garden (NY), Jardim Botânico do Rio de Janeiro (RB), Universidade de São Paulo (SPF), the United States National Herbarium–Smithsonian Institution (US), and Universidade Federal de Viçosa (VIC).

*C. absus* var. *meonandra* (H.S. Irwin & Barneby) H.S. Irwin—Irwin 1273, 23.VIII.1957 (NY); McVaugh et al. 19361, 22.IX.1960 (NY); McVaugh et al. 21374, 23.XI.1960–25.XI.1960 (NY); Schubert & Mario Sousa 1952, 22.IX.1963 (NY); Gentry 6536, 10.IX.1941–14.IX.1941 (NY). *C. absus* var. *absus* (L.) H.S. Irwin & Barneby—Asplund 15884, 24.III.1955 (NY); Cerrate 5209, 01.VII.1972 (NY). *C. adiantifolia* var. *adiantifolia* (Spruce ex Benth.) H.S. Irwin & Barneby—Davidse 27665, 26.VII.1984 (NY); Wurdack & Adderley 43236, 29.VI.1959 (NY). *C. adiantifolia* var. *pteridophylla* (Sandwith) H.S. Irwin & Barneby—Maguire et al. 45930, 24.VIII.1961 (NY, US); Maguire & Fanshawe 32636, 17.XI.1051 (NY, US); Silva 92, 22.IX.1942

(NY, US); Boz 548, V.1926 (NY, US); Prance et al. 22690, XVIII.IX.1974 (NY). *C. amabilis* H.S. Irwin & Barneby—Jardim & Flávia 594, 11.XI.1994 (NY); Thomas et al. 8964, 30.I.1992 (NY). *C. amorimii* Barneby—Amorim et al. 3231, 06.I.2000 (NY); Amorim et al. 4311, 23.X.2004 (RB); Amorim et al. 923, 14.XII.1992 (NY; RB). *C. apoucouita* (Aubl.) H.S. Irwin & Barneby—Forest Department of British Guiana F994, 09.I.1943 (NY). *C. aristata* (Benth.) H.S. Irwin & Barneby—Coutinho et al. 096, 14.VIII.2012 (VIC). *C. aspleniifolia* (H.S. Irwin & Barneby) H.S. Irwin & Barneby—Cardoso et al. 2407, 14.II.2009 (HUEFS); Duarte 8753, 18.I.1965 (NY, US); Pinheiro 1768, 24.I.1972 (NY); Hatschbach 47341, 18.I.1984 (NY, US); Pabst 8301 & Pereira 9410, 15.I.1965 (NY). *C. bahiae* (H.S. Irwin) H.S. Irwin & Barneby—Harley et al. 17398, 25.III.1974 (NY); Harley et al. 18071, 18.I.1977 (NY); Rando 1214, 01.III.2012 (SPF, HUEFS). *C. barbata* (Nees & Mart.) H.S. Irwin & Barneby—Souza et al. 45, 13.IV.2006 (HUEFS). *C. blanchetii* (Benth.) Conc., L.P. Queiroz & G.P. Lewis—Conceição & Leite 687, XVIII.VIII.2003 (HUEFS); Coutinho et al. 495, VII.2011 (VIC); Junqueira et al. 130, IX.X.2002 (HUEFS); Coutinho et al. s.n., 09.IV.2012 (VIC); Coutinho et al. s.n., 03.VII.2011 (VIC). *C. botryooides* Conc., L.P. Queiroz & G.P. Lewis—Conceição et al. 541, 29.V.2003 (HUEFS); Conceição et al. 612, 01.VI.2003 (HUEFS). *C. brachystachya* (Benth.) Conc., L.P. Queiroz & G.P. Lewis—Coutinho et al. s.n., IV.2011 Coutinho et al. s.n., 03.VII.2011 (VIC); Conceição et al. 728, 10.IX.2003 (HUEFS). *C. caribaea* var. *caribaea* (Northr.) Britton—Britton 3448, 28.I.1905 (NY); Britton & Millspaugh 5895, 16.III.1907 (NY); Small & Carter 8853, 01.III.1910 (NY). *C. caribaea* var. *inaguensis* (Britton) H.S. Irwin & Barneby—Correll 43167, 26.VIII.1976 (NY); Millspaugh & Millspaugh 9303, 12.III.1911 (NY); Proctor & Gillis 34033, 11.VI.1974 (NY). *C. caribaea* var. *lucayana* (Britton) H.S. Irwin & Barneby—Britton & Millspaugh 2841, 20.II.1905 (NY); Wilson 7895, 28.XII.1907 (NY); Rando 963, 22.X.2010 (NY). *C. cinerascens* (Vogel) H.S. Irwin & Barneby—Coutinho & Pereira 187, 25.IV.2013 (VIC); Coutinho & Pereira 188, 25.IV.2013 (VIC); Coutinho & Pereira 189, 25.IV.2013 (VIC); Coutinho et al. 420, 24.III.2014 (VIC); Coutinho et al. 421, 24.III.2014 (VIC); Coutinho et al. 422, 24.III.2014 (VIC). *C. cipoana* (H.S. Irwin & Barneby) H.S. Irwin & Barneby—Coutinho et al. 043, 01.VIII.2012 (VIC), Francino et al. s.n., 11.VIII.2009 (VIC); Francino & Casagrande 22, 22.VI.2009 (VIC); Francino et al. 15, 14.V.2009 (VIC). *C. clausenii* (Benth.) H.S. Irwin & Barneby—Coutinho & Lorencini 068, 08.VIII.2012 (VIC); Coutinho & Lorencini 069 08.VIII.2012 (VIC), Coutinho & Pereira 169 24.IV.2013 (VIC); Coutinho & Pereira 170, 24.IV.2013 (VIC). *C. compitalis* (H.S. Irwin & Barneby) H.S. Irwin & Barneby—Belém 3637, 24.V.1968 (NY); Belém 3643, 24.V.1968 (NY, US). *C. conferta* (Benth.) H.S. Irwin & Barneby—Coutinho et al. 047, 02.VIII.2012 (VIC); Coutinho et al. 048, 02.VIII.2012 (VIC); Coutinho & Lorencini 075, 11.VIII.2012 (VIC); Coutinho & Lorencini 076, 11.VIII.2012 (VIC); Coutinho & Moura 136, 20.I.2013 (VIC); Coutinho & Pereira 207, 27.IV.2013 (VIC); Coutinho & Pereira 208, 27.IV.2013 (VIC). *C. coriacea* (Benth.) H.S. Irwin & Barneby—Conceição et al. 869, 18.II.2005 (HUEFS, RB); Coutinho et al. 016, 27.VI.2012 (VIC). *C. cytisoides* (Collad.) H.S. Irwin & Barneby—Melo-Silva et al. 1640, 25.II.1999 (HUEFS); Coutinho et al. s.n., 11.X.2010 (VIC); Coutinho & Terra 144, 10.VII.2012. (VIC); Coutinho & Terra 323, 24.I.2014. (VIC); Coutinho & Terra 326, 24.I.2014. (VIC). *C. debilis* (Vogel) H.S. Irwin & Barneby—Coutinho & Lorencini 077, 11.VIII.2012 (VIC); Coutinho & Mora 134, 20.I.2013 (VIC); Coutinho et al. s.n., 05.VI.2011 (VIC); Coutinho et al. s.n., 05.X.2011 (VIC). *C. decora* (H.S. Irwin & Barneby) Conc., L.P. Queiroz & G.P. Lewis—Conceição et al. 716, IX.IX.2003 (HUEFS); Coutinho et al. 022, 27.VI.2012 (VIC); Coutinho et al. 093, 14.VIII.2012. (VIC); Coutinho et al. 108, 14.VIII.2012 (VIC); Coutinho & Pereira 214, 27.IV.2013 (VIC); Irwin et al. 23493, 16.III.1970 (NY); Coutinho et al. s.n., 09.X.2010 (VIC). *C. dentata* (Vogel) H.S. Irwin & Barneby—Coutinho & Moura 126, 18.I.2013 (VIC); Francino s.n., 23.III.2004 (VIC); Valente et al. 2522, 04.IV.2009 (VIC). *C. depauperata* Conc., L.P. Queiroz & G.P. Lewis—Cardoso & Conceição 433, 16.IV.2005 (HUEFS); Conceição et al. 863, II.II.2005 (HUEFS, SPF); Conceição 786, XI.I.2004 (HUEFS, SPF); Stannard et al. H51142, III.II.1992 (HUEFS, US). *C. desvauxii* var. *desvauxii* (Collad.) Killip—Coutinho et al. s.n., 03.VII.2011 (VIC). *C. desvauxii* var. *graminea* H.S. Irwin & Barneby—Coutinho et al. s.n., 03.VII.2011 (VIC); Coutinho et al. s.n., 03.VII.2011 (VIC). *C. desvauxii* var. *latistipula* (Benth.) G.P. Lewis—Coutinho & Coutinho 117, 16.I.2013 (VIC); Coutinho & Coutinho 118, 16.I.2013 (VIC); Coutinho et al. 294, 13.I.2014 (VIC); Coutinho et al. 295, 13.I.2014 (VIC). *C. duartei* (H.S. Irwin) H.S. Irwin & Barneby—Coutinho & Fernandes 303, 15.I.2014 (VIC); Duarte 8014, 19.XI.1963 (NY, US); Amorim & Lorenzi 2102, 07.XI.1997 (NY, US); Almeida & Santos 195, 31.X.1968 (NY, US); Belém & Pinheiro 3054, 12.I.1967 (NY); Mori et al. 11036, 04.XI.1978 (NY). *C. eitenorum* var. *eitenorum* (H.S. Irwin) H.S. Irwin & Barneby—Drouet 2541, 01.X.1935 (US); Eiten & Eiten 10660, 18.II.1970 (US); Von Luetzelburg 446, 1970 (US). *C. eitenorum* var. *regana* (H.S. Irwin & Barneby) H.S. Irwin & Barneby—Hatschbach & Hatschbach 56931, 09.IV.1992 (US); Noblick 3034, 07.III.1984 (US); Queiroz et al. 6131, 18.II.2000 (HUEFS). *C. ensiformis* var. *ensiformis* (Vell.) H.S. Irwin & Barneby—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); Irwin 2330, 29.XII.1958 (NY). *C. ensiformis* var. *maranonica* (H.S. Irwin) H.S. Irwin & Barneby—Daly et al. D375, 29.IX.1980 (NY, US); Hatschbach & Kummorw 38396, 26.III.1976 (NY); Prance & Silva 58692, 13.VIII.1964 (NY, US); Rosa et al. 2580, 11.VII.1978 (NY). *C. ensiformis* var. *plurifoliolata* (Hoehne) H.S. Irwin & Barneby—Belém & Magalhães 955, 25.IV.1965 (NY, US); Santos 2952, 16.IV.1975 (NY); Kallunki et al. 523, 11.II.1994 (NY). *C. ericifolia* (Benth.) H.S. 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