MARINALVA DOS SANTOS SILVA

CONTRIBUIÇÕES DA ANATOMIA À TAXONOMIA E SISTEMÁTICA DE *CHAMAECRISTA* COM ÊNFASE NAS SEÇÕES *CHAMAECRISTA, CALICIOPSIS* E *XEROCALYX* (LEGUMINOSAE-CAESALPINIOIDEAE)

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do programa de Pós-Graduação em Botânica, para obtenção do título de *Doctor Scientiae*.

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APROVADA: 20 de fevereiro de 2017.

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Renata Maria Strozi Alves Meira
(Orientadora)

“Seja você quem for, seja qual for a posição social que você tenha na vida, a mais alta ou a mais baixa, tenha sempre como meta muita força, muita determinação e sempre faça tudo com muito amor e com muita fé em Deus, que um dia você chega lá. De alguma maneira você chega lá”.

(Ayrton Senna)

“Imagine uma nova história para sua vida e acredite nela”.

(Paulo Coelho)

Dedico esta tese a minha família que sempre me ensinou a valorizar o trabalho e como sempre dizia meu pai: “o justo viverá dos frutos do seu trabalho”.

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“Não há no mundo exagero mais belo que a gratidão”.
(Jean de la Bruyere)

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BIOGRAFIA

MARINALVA DOS SANTOS SILVA, filha de José Rodrigues da Silva e Anízia Ferreira dos Santos, nasceu em Guajeru, município que se localiza na região sudoeste do estado da Bahia.

Em 1997, concluiu o ensino primário na escola Estácio de Sá, na zona rural do município. No ano seguinte ingressou na 5ª série no colégio Estadual Norberto Fernandes, no município de Caculé, Bahia. Em 1999 deu sequência aos estudos na Escola Família Agrícola de Caculé, terminando assim o ensino fundamental. Em 2002, iniciou o ensino médio no Colégio Estadual Oriovaldo Santos Araújo, em Guajeru.

Em março de 2006 ingressou no curso de Licenciatura em Ciências Biológicas na Universidade do Estado da Bahia, em Caetité, graduando-se em dezembro de 2010.

Em março de 2011, ingressou no curso de Mestrado no Programa de Pós-Graduação em Biodiversidade vegetal, na Universidade do Estado da Bahia, em Paulo Afonso, defendendo a dissertação em fevereiro de 2013.

Em março de 2013, iniciou o doutorado no Programa de Pós-Graduação em Botânica, na Universidade Federal de Viçosa.

SUMÁRIO

RESUMO

SILVA, Marinalva dos Santos, D. Sc., Universidade Federal de Viçosa, fevereiro de 2017. **Contribuições da anatomia à taxonomia e sistemática de *Chamaecrista* com ênfase nas seções *Chamaecrista*, *Caliciopsis* e *Xerocalyx* (Leguminosae-Caesalpinioideae)**. Orientadora: Renata Maria Strozi Alves Meira. Colaboradores: Ítalo Antônio Cotta Coutinho e Juliana Gastaldello Rando.

Chamaecrista Moench (Leguminosae: Caesalpinioideae) possui centro de diversidade na cadeia do Espinhaço e tem ca. 330 espécies. Está organizado em seis seções, sendo *Chamaecrista* a segunda maior com ca. 75 espécies, das quais 55 são americanas, sendo distribuídas em seis séries. Por outro lado, as seções *Caliciopsis* e *Xerocalyx* têm, respectivamente, duas e três espécies. Os dados anatômicos têm se mostrado promissores para subsidiar os estudos de taxonomia em *Chamaecrista* e já foram avaliados em representantes das seções *Absus*, *Apoucouita* e *Grimaldia*. Esse estudo tem como objetivo identificar as estruturas secretoras nas espécies das seções *Chamaecrista*, *Caliciopsis* e *Xerocalyx*, caracterizando sua morfoanatomia e indicando sua localização e os compostos secretados; investigar a morfoanatomia foliar de espécies da seção *Chamaecrista*, sér. *Coriaceae* e avaliar o potencial filogenético dos caracteres morfoanatômicos no gênero *Chamaecrista* com ênfase na sér. *Coriaceae*. Foram utilizadas amostras provenientes de herbários e materiais coletados e fixados em campo. As amostras foram processadas segundo metodologia específica para observação em microscopia de luz e microscopia eletrônica de varredura. Testes histoquímicos foram realizados para identificar a natureza química da secreção produzida. Os coléteres não vascularizados são comuns às espécies estudadas e cinco tipos foram descritos, sendo que dois novos tipos foram registrados para a seção *Chamaecrista* e o tipo piriforme foi registrado pela primeira vez para o gênero. Os nectários apresentam morfoanatomia semelhante às outras espécies do gênero *Chamaecrista* e quatro formas foram descritas, sendo as mais comuns, cupuliforme e pateliforme. São comuns nas espécies estudadas as seguintes características: estômatos paracíticos, mesofilo dorsiventral, monocristais, feixes vasculares colaterais com fibras associadas na nervura mediana e no pecíolo e feixes acessórios, idioblastos fenólicos, traqueídes não alargados, nectários e coléteres. Foram observadas venação acródroma, eucamptódroma e actinódroma sendo estas características importantes para a delimitação de grupos. A distribuição dos tricomas tectores, conformação do feixe vasculares do pecíolo, hipoderme e células papilosas foram diagnósticas ao nível de espécie. Os resultados obtidos são relevantes, pois poderão contribuir para o reconhecimento das relações de proximidade entre as seções *Chamaecrista*, *Caliciopsis* e *Xerocalyx* e para a filogenia do gênero *Chamaecrista.* As espécies da sér. *Coriaceae* puderam ser delimitadas a partir dos dados de anatomia.

ABSTRACT

SILVA, Marinalva dos Santos, D. Sc., Universidade Federal de Viçosa, February, 2017. **Contribution of anatomy to taxonomy and systematic of *Chamaecrista* with emphasis on the sections *Chamaecrista*, *Caliciopsis* and *Xerocalyx* (Leguminosae-Caesalpinioideae)**. Advisor: Renata Maria Strozi Alves Meira. Co-advisor: Ítalo Antônio Cotta Coutinho and Juliana Gastaldello Rando.

Chamaecrista Moench (Leguminosae: Caesalpinioideae) has diversity center in the “Cadeia do Espinhaço” and has ca. 330 species. It is organized in six sections, with *Chamaecrista* being the second largest with ca. 75 species, of which 55 are American, being distributed in six series. On the other hand, the sections *Caliciopsis* and *Xerocalyx* have, respectively, two and three species. The anatomical data have been shown to be promising to support the taxonomic studies in *Chamaecrista* and have already been evaluated in the *Absus, Apoucouita* and *Grimaldia* sections. This study aims to identify the secretory structures in the species of the sections *Chamaecrista, Caliciopsis* and *Xerocalyx,* characterizing its morphoanatomy and indicating its location and the secreted compounds; investigates the foliar morphoanatomy of species of the *Chamaecrista* section, ser. *Coriaceae* and to evaluate the phylogenetic potential of morphoanatomic characters in the genus *Chamaecrista* with emphasis on ser. *Coriaceae.* Samples from herbariums and materials collected and fixed in the field were used. The samples were processed according to specific methodology for observation in light microscopy and scanning electron microscopy. Histochemical tests were performed to identify the chemical nature of the secretion produced. Non-vascularized colleters are common to the studied species and five types were described, two new types were recorded for the section Chamaecrista and the pyriform type was registered for the first time for the genus. The nectaries present similar morphoanatomy to the other species of the genus *Chamaecrista* and four forms were described, the most common being cupuliform and patelliform. The following characteristics are common in the studied species: Paracitic stomata, dorsiventral mesophyll, monocrystals, collateral vascularbundles with associated fibers, and accessory bundles, idioblasts with phenolic compounds, not-enlarged tracheids, nectaries and colleters. Were observed acrodromous, eucampotodromous and actinodromous venation, being these characteristics important for the delimitation of groups. The distribution of the tector trichomes, conformation of the vascular bundle of the petiole, hypodermis and papillary cells were diagnostic at the species level. The results obtained are relevant because they may contribute to the recognition of the proximity relations between the sections

Chamaecrista, *Caliciopsis* and *Xerocalyx* and for the phylogeny of the genus *Chamaecrista*, in addition the species of ser. *Coriaceae* could be delimited from the anatomy data.

1. INTRODUÇÃO GERAL

Chamaecrista Moench, com ca. 330 espécies destaca-se como um dos maiores gêneros da subfamília Caesalpinioideae, estando circunscrito na tribo Cassieae, subtribo Cassiinae (Lewis 2005). Tem distribuição ampla na América tropical e, em menor escala na África, Ásia e Austrália e com poucas espécies nas regiões temperadas (Irwin e Barneby 1982; Lewis 2005). No Brasil ocorrem 256 espécies, das quais 207 são endêmicas (Souza e Bortoluzzi 2015). O gênero possui centro de diversidade na Cadeia do Espinhaço, sobretudo nos campos rupestres (Irwin e Barneby 1978, 1982). No entanto, as espécies são largamente distribuídas, ocorrendo em todos os biomas brasileiros (Souza e Bortoluzzi 2015).

Chamaecrista difere de *Cassia* L. e *Senna* Mill., os gêneros que constituem a subtribo Cassiinae, por possuir o pedicelo com duas bractéolas situadas próximo ou acima da metade de seu comprimento, androceu actinomorfo com anteras pubescentes ao longo das suturas, fruto deiscente e nectários extraflorais côncavos no pecíolo (Irwin e Barneby 1978, 1982; Conceição et al. 2009). Os estudos moleculares sustentam o monofiletismo de *Chamaecrista* (Herendeen et al. 2003; Bruneau et al. 2008; Conceição et al. 2009), mas a taxonomia do grupo sempre foi alvo de divergências.

Segundo Irwin e Barneby (1982), *Chamaecrista* está organizado em seis seções com base no tipo de indumento, presença ou ausência de nectários extraflorais, padrão de inflorescência e padrão de venação das sépalas e folíolos. Foram circunscritas as seções *Absus* (160 spp), *Chamaecrista* (75 spp), *Apoucouita* (19 spp), *Xerocalyx* (três spp), *Caliciopsis* (duas spp) e *Grimaldia* (uma spp). Destacando-se como a segunda mais representativa, a seção *Chamaecrista* compreende 55 espécies nas Américas (Irwin e Barneby 1982; Rando e Pirani 2012; Rando et al. 2013) e é considerada um grupo parafilético (Conceição et al. 2009).

Em geral, as espécies incluídas em *Chamaecrista* sect. *Chamaecrista* são subarbustivas a arbustivas, com inflorescências em racemos reduzidos a fascículos axilares, elevado número de pares de folíolos, sementes retangulares a irregularmente rômbicas e nectários extraflorais predominantemente presentes (Irwin e Barneby 1978). Essa seção, no Novo Mundo, está organizada em seis séries: *Coriaceae* (22 spp), *Chamaecrista* (17 spp), *Prostratae* (sete spp), *Flexuosae* (cinco spp), *Bauhinianae* (duas spp) e *Greggianae* (uma sp) (Irwin e Barneby 1982; Rando e Pirani 2012; Rando et al. 2013). Ocorrem preponderantemente em ambientes abertos, contudo, nas séries *Coriaceae* e *Greggianae* foi registrado elevado grau de endemismo para determinados habitats (Irwin e Barneby 1982).

A seção *Xerocalyx* é reconhecida por seus folíolos com nervuras paralelas e sépalas multiestriadas e foram reconhecidas três espécies para a seção (Irwin e Barneby (1982). Já a seção *Caliciopsis* compreende duas espécies (três táxons) e tem caracteres morfológicos semelhantes aos representantes das seções *Xerocalyx* e *Chamaecrista*, sendo reconhecida por Irwin e Barneby (1982) como um grupo intermediário entre estas seções. Adicionalmente, nas análises moleculares, as seções *Caliciopsis*, *Chamaecrista* e *Xerocalyx* formaram um clado que apresenta como caracteres comuns as inflorescências com racemos axilares e reduzido número de cromossomos (Conceição et al. 2009).

Os caracteres anatômicos também podem ser dados adicionais para a taxonomia e para o entendimento das relações entre os grupos (Lersten e Curtis 1994; Araújo et al. 2010; Dalvi et al. 2013) e em *Chamaecrista* os trabalhos comprovam a utilidade da anatomia para a taxonomia do gênero (Coutinho et al. 2013, 2015; Francino et al. 2015). Estudos abrangentes de anatomia, incluindo um número relevante de espécies, foram conduzidos para as seções *Absus, Apoucouita* e *Grimaldia* (Coutinho et al. 2013, 2015; Francino et al. 2015). Nestes estudos, além das características anatômicas gerais como padrão de venação, ocorrência de traqueídes alargados, tipo de mesofilo, presença de hipoderme, morfologia da nervura mediana, morfoanatomia e conformação do sistema vascular do pecíolo, a morfoanatomia, localização e o tipo de estruturas secretoras se mostraram importantes caracteres diagnósticos. Tais dados reforçaram a elevação da subseção *Baseophyllum* (seção *Absus*) ao nível de seção bem como a inclusão de *C. absus*, única espécie da seção *Grimaldia*, na seção *Absus.* Quanto às seções *Chamaecrista, Caliciopsis* e *Xerocalyx*, pouco se conhece, ainda mais considerando os seus inúmeros problemas taxonômicos.

As estruturas secretoras relatadas para *Chamaecrista* foram os tricomas secretores, nectários extrarreprodutivos, coléteres e idioblastos (Irwin e Barneby 1982; Tripathi e Sahu 1991; Coutinho et al. 2012, 2013, 2015; Meira et al. 2014; Francino et al. 2015). Os tricomas secretores estão restritos às seções *Absus* (subseção *Absus* e *Adenophyllum*) e *Grimaldia* (Irwin e Barneby 1982) e segundo abordagens filogenéticas recentes, a presença de tricomas secretores é considerada como condição derivada e está correlacionada com a ausência dos nectários extraflorais (Conceição et al. 2009).

Nectários são estruturas especializadas que secretam néctar, uma solução açucarada (Elias 1983; Nicolson et al. 2007), sendo os nectários extrarreprodutivos envolvidos com a proteção das plantas contra o ataque de herbívoros e patógenos (Madureira e Sobrinho 2002; Fernandes et al. 2005; Nascimento e Del-Claro 2010). Os nectários extrarreprodutivos estão restritos às seções *Absus* (subseção *Baseophyllum* e *Otophyllum*), *Apoucouita, Chamaecrista* (exceto na ser. *Bauhinianae*) e *Xerocalyx,* grupos nos quais os tricomas secretores estão ausentes (Irwin e Barneby 1982; Conceição et al. 2009). Para a seção *Chamaecrista* foram estudados apenas os nectários de três espécies (*C. trichopoda, C. rotundata* e *C. mucronata*) (Francino et al. 2006, 2015), contudo não se comprovou a presença de açúcar nos exsudatos secretados por estas glândulas. Assim são necessários estudos com um maior número de espécies para comprovar se as glândulas foliares das espécies da seção *Chamaecrista* são, de fato, nectários, além de caracterizar a sua morfoanatomia.

Coléteres são estruturas que produzem uma secreção pegajosa constituída predominantemente por mucilagem que lubrifica e protege os meristemas vegetativos e reprodutivos contra a dessecação e ataque de patógenos (Thomas e Dave 1989; Thomas 1991; Mayer et al. 2013). Seis tipos de coléteres foram descritos para espécies de *Chamaecrista* em órgãos vegetativos e reprodutivos das seções *Absus*, *Apoucouita*, *Grimaldia*, *Chamaecrista* e *Xerocalyx.* Estas estruturas foram apontadas como marcadores morfológicos promissores para os estudos de filogenia do grupo (Coutinho et al. 2015). Entretanto, foram pouco exploradas na segunda maior seção de *Chamaecrista,* onde foram avaliadas apenas cinco espécies das 54 referidas para a América e não foram analisadas na seção *Caliciopsis*. Somente um estudo mais robusto poderá indicar se os coléteres são comuns para as seções *Chamaecrista* e *Caliciopsis* e se essas estruturas serão úteis para a taxonomia destes grupos.

Os idioblastos secretores parecem ser comuns em *Chamaecrista,* mas variam em relação à posição na lâmina foliar e ao composto secretado. Idioblastos mucilaginosos são comuns na epiderme do folíolo de espécies das seções *Apoucouita, Grimaldia* e *Absus* (subseções *Absus* e *Otophyllum*) e no mesofilo de espécies da seção *Absus,* subseção *Baseophyllum* (Coutinho et al. 2013, 2016; Francino et al. 2015). Idioblastos fenólicos foram referidos apenas para uma espécie da subseção *Absus*, série *Absoideae* (Silva et al. Dados não publicados) e outra da subseção *Baseophyllum* (Coutinho et al. 2013; Francino et al. 2015), o que pode ser um indício que sejam menos comuns que os mucilaginosos. Entretanto, somente um estudo mais abrangente poderá confirmar tal sugestão.

Diante do exposto, alguns questionamentos ainda necessitam de esclarecimentos: Coléteres são comuns nas seções *Chamaecrista* e *Caliciopsis*? Existem diferentes morfotipos de coléteres nas seções *Chamaecrista* e *Caliciopsis*? As glândulas foliares nas espécies de *Chamaecrista, Caliciopsis* e *Xerocalyx* são nectários? Há idioblastos secretores nas espécies estudadas das seções *Chamaecrista, Caliciopsis* e *Xerocalyx*? Qual a natureza química do produto secretado por eles? Existem variações morfológicas e anatômicas nas glândulas foliares? Tais variações podem ter implicações na taxonomia dos grupos? As espécies da sér. *Coriaceae* podem ser delimitadas usando os dados morfoanatômicos?

Desse modo, temos como objetivos: (1) identificar as estruturas secretoras nas espécies das seções *Chamaecrista, Caliciopsis* e *Xerocalyx*, indicando sua localização e os compostos secretados; (2) investigar a morfoanatomia foliar de espécies da seção *Chamaecrista*, sér. *Coriaceae* e (3) avaliar o potencial filogenético dos caracteres morfoanatômicos no gênero *Chamaecrista* com ênfase na sér. *Coriaceae.*

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CAPÍTULO I

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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 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 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 protoderm 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 (Serra do Cipó and Chapada Diamantina) 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 CO_2 (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 Núcleo 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 (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 characterization structural.

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: 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, xylidine 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

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).

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.

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. pedicelaris* 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. simplifacta*). *C. venturiana* (ser. *Chamaecrista*) and *C. rotundifolia* var. *grandiflora* (ser. *Bauhinianae*) displayed Types I, II, III and V (Tab. 1).

Only in ser. *Chamaecrista* all five types of colleters were observed (Figs. 1C-I, 2A-H). The series *Bauhinianae, Coriaceae* and *Flexuosae* displayed Types I, II, III and V (Fig. 1B), while in ser. *Greggianae* 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 petiole and between leaflets than in stipules. The types of colleters 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 colleters 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 colleters 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 colleters, while the remaining taxa bore at least two types. Only two taxa (*C. rotundata* var. *grandistipula* and *C. gonoclada*) did not display colleters 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 colleters described. Of all the histochemical tests performed, only the secretory head of the colleters showed positive results.

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 colleters. 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*, colleters 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 (Appezzato-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 (Appezzato-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 already studied species of *Chamaecrista*, 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* by 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. simplifacta*, 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) colleters 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, colleter 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 colleters 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 colleters in *Chamaecrista*.

We showed that colleters are diverse structures in *Chamaecrista* sect. *Chamaecrista* and *Caliciopsis*. The short digitiform and short bottle-shaped colleters 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 that 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 hypothesis. In addition to the types of colleters already described for sect. *Chamaecrista*, we described two new types of colleters (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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S1 Supplementary Data

Species of *Chamaecrista* sections *Chamaecrista* and *Caliciopsis* used in the structural analysis.

Vouchers are housed in the herbaria of the Universidade Estadual de Feira de Santana (HUEFS), Universidade do Estado da Bahia (HUNEB), New York Botanical Garden (NY), Jardim Botânico do Rio de Janeiro (RB), Universidade de São Paulo (SPF) and Universidade Federal de Viçosa (VIC). Collection preceded by na asterisk (*) correspond to field-collected material fixed in FAA (formalin: acetic acid: 50% ethanol, 1:1:18 by volume) and/or FNT (neutral buffered formalin) (Johansen 1940).

C. deeringiana Small & Pennell, Small 4078, 03.IX.1912 (NY); Small 8124, 30.IV.1917 (NY); Small 8787, 15.V.1918 (NY). ***C. glandulosa*** Greene var. ***andicola*** H.S.Irwin & Barneby, Klitgaard 394, 25.II.1997 (RB). *C.* ***nictitans*** var. ***disadena*** (Steud.) H.S.Irwin & Barneby, Queiroz 4439, 30.VIII.1995 (RB); Fernandes 1, 11.IX.1998 (HUEFS). ***C. nictitans*** Moench var. ***jaliscensis*** (Greenm.) H.S.Irwin & Barneby, Calónico 4736, 10.X.1997 (HUEFS). ***C. nictitans*** Moench var. ***paraguariensis*** (Chodat & Hassl.) H.S.Irwin & Barneby, Hatschbach 34277, 16.II.1974 (RB); Silva 1262, 23.III.1993 (HUEFS); Silva 273, 03.VIII.1981 (HUEFS). ***C. obcordata*** Britton, Sastre et al. 1969, 13.VIII.1973 (NY); Galo 693, 12.II.1952 (NY). ***C. pascuorum*** (Mart. ex Benth.) H.S.Irwin & Barneby, Harley 10264, 02.II. 1977 (RB); Harley 19153, 27.II.1977 (RB); Anderson et al. 36977, 15.III.1972 (RB). ***C. pedicellaris*** Britton var. ***pedicellaris***, Ekman 7577, 25.VIII.1916 (NY); Holdridge 1126, 12.IV.1942 (NY); Leonadr & Leonard 13359, 13.II.1929 (NY). ***C. pedicellaris*** Britton var. ***adenosperma*** (Urb.) H.S.Irwin & Barneby, Almonte 8502, 10.XII.1978 (NY); Lavastre 539, XII.1962 (NY); Ekman 14498, 22.III.1930 (NY). ***C. portoricensis*** (Urb.) O.F.Cook & G.N.Collins var. ***portoricensis***, Britton & Rose 1406, 27.II.1913 (NY); Axelrod & Chavez 4707, 28.VI.1992 (NY); Britton & Boynton 288, 21.III.1925 (NY). ***C. pygmaea*** Britton var. ***pygmaea***, Shafer 10709, 05.XII.1911 (NY); Ekman 17268, 23.VIII.1923 (NY). ***C. pygmaea*** Britton var. ***savannarum*** (Britton) H.S.Irwin & Barneby,Véllez-Gavilán et al. 113, 17.VII.1996 (NY). ***C. rufa*** Britton & Rose var. ***exsul*** H.S.Irwin & Barneby, Teixeira et al. 441, 14.V.1982 (NY); Berg et al. 18501, 14.X.1973 (NY). ***C. rufa*** Britton & Rose var. ***polyphlebia*** H.S.Irwin & Barneby, Pennell 1328, 23.VIII.1917 (NY); García-Barriga 17211, 10.III.1960 (NY); Johnson 3080, 21.X.1983 (NY). ***C. venturiana*** H.S.Irwin & Barneby, Novara 2211, 26.XI.1981 (NY); Pensiero & Marino 4251, 03.XII.1992 (NY); Balcazar 21, 02.I.1994 (NY). ***C. vestita*** (Vogel) H.S.Irwin & Barneby, Krapovickas & Cristobal 40774, 12.I.1987 (NY); Hatschbach 23926, 26.II.1970 (NY); Leite 1983, 01.XI.1942 (NY). ***C. greggii*** Pollard ex A.Heller var. ***greggii***, Johnston 5459, 04.V.1960 (NY); Lavin et al. 4513, 27.VIII.1983 (NY); Worthington 8323, 02.VI.1982 (NY); Hinton et al. 24261, 04.VI.2003. *C.* ***greggii*** Pollard ex A.Heller var. ***macdougaliana*** (Rose) H.S.Irwin & Barneby, Hughes 1785, 11.XI.1993 (NY); Breckon & Kolterman 2342, 06.VII.1978 (NY); Rose & Hay 5888, 01.VIII.190 (NY). ***C. basifolia*** (Vogel) H.S.Irwin & Barneby, Van den Berg 1271, 11.II.2004 (HUEFS); Queiroz 14253, 25.IV.2009 (HUEFS). ***C. rotundifolia*** (Pers.) Greene var. ***rotundifolia***, *Silva et al.138, 139, 29.VII.2011 (HUNEB). ***C. rotundifolia*** (Pers.) Greene var. ***grandiflora*** (Benth.) H.S.Irwin & Barneby, *Silva et al. 125, 145, 25.VII.2011 (HUNEB); Conceição et al. 1498, 28.VII.2011 (HUNEB). ***C. flexuosa*** Greene var. ***flexuosa***, Carneiro-Torres 89, 29.XI.1998 (HUEFS); Conceição 361, 18.IX.1999 (HUEFS); *Coutinho & Laurenção 269, 270, 271, 18.VIII.2013 (VIC). ***C. gonoclada*** (Benth.) H.S.Irwin & Barneby, Hatschbach 45885, 11.XII.1982 (NY); Dubs 1756, 09.IX.1994 (NY). ***C. swainsonii*** (Benth.) H.S.Irwin & Barneby, Lima 6916, 27.VII.2008 (RB); Gonzaga 6, 10.VIII.1985 (RB); Conceição 702, 20.VIII.2003 (HUEFS). ***C. cordistipula*** (Mart.) H.S.Irwin & Barneby, Zardini & Guard 15477, 31.X.1989 (NY); Hatschbach 29518, 15.IV.1972 (NY); Aristeguieta 6322, VIII.1966 (NY); Saravia 2907, 20.X.1963 (NY). ***C. kunthiana*** (Schltdl. & Cham.) H.S.Irwin & Barneby, Queiroz 14171, 24.IV.2009 (HUEFS); Faria 47, 30.IV.1999 (HUEFS); Silva 1656, 18.VII.1993 (HUEFS). ***C. pilosa*** Greene var. ***pilosa***, Queiroz 369, 20.V.2005 (HUEFS); Queiroz 19, 16.VIII.2002 (HUEFS). ***C. pilosa*** Greene var. ***luxurians*** (Benth.) H.S.Irwin & Barneby, Collares 156, 02.VI.1984 (HUEFS). ***C. supplex*** (Mart.) Britton & Rose in Britton & Killip, Harley 21490, 18.IV.1980 (RB); Coradin 5932, 22.VI.1983 (RB). ***C. tenuisepala*** (Benth.) H.S.Irwin & Barneby, Queiroz 478, 19.VI.2005 (HUEFS); Delgado-Junior 45, 04.IV.2009 (HUEFS). ***C. aristata*** (Benth.) H.S.Irwin & Barneby, *Coutinho et al. 96, 14.VIII.2012 (VIC); Hatschbach 67980, 18.VII.1998 (RB); Queiroz 7528, 08.I.2003 (HUEFS). ***C. caribaea*** Britton var. ***caribaea***, Britton 3448, 28.I.1905 (NY); Small & Carter 8853, 01.II.1910 (NY); Brace 4972, 18.VIII.1906 (NY). ***C. caribaea*** Britton var. ***lucayana*** (Britton) H.S.Irwin & Barneby, Rando 963, 22.XI.2010 (NY, SPF); Brace 3985, 4.XII.1905 (NY); Wilson 7895, 28.XII.1907 (NY); Britton & Millspaugh 2841, 20.II.1905 (NY). ***C. caribaea*** Britton var. ***inaguensis*** (Britton) H.S.Irwin & Barneby, Millspaugh & Millspaugh 9303, 12.III.1911 (NY); Proctor & Gillis 34033, 11.VI.1974 (NY); Correll 43167, 26.VIII.1976 (NY). ***C. cinerascens*** (Vogel) H.S.Irwin & Barneby, Kawasaki et al. 973, 23.V.1997 (SPF); Joly et al. CFSC2198, 27.V.1972 (SPF); *Coutinho & Pereira 187, 25.IV.2013 (VIC). ***C. latifolia*** (Benth.) Rando, Souza et al. 11609, 04.VII.1996 (HUEFS); Hatschbach 68259, 25.VII.1998 (HUEFS); *Coutinho et al. 46, 47, 02.VIII.2012 (VIC). ***C. distichoclada*** (Mart. ex Benth.) H.S.Irwin & Barneby, Irwin 22733, 27.I.1969 (RB); Queiroz 2368, 04.VII.1989 (HUEFS); Azevedo 342, 26.V.2007 (HUEFS). ***C. mucronata*** (Spreng.) H.S.Irwin & Barneby, Cardoso & Conceição 597, 19.VI.2005 (HUEFS); Silva et al. 2628, 05.IX.1994 (HUEFS); Harley et al. 3784, 24.VIII.1996 (HUEFS). ***C. multinervia*** (Mart. ex Benth.) H.S.Irwin & Barneby, Hatschbach 46500, 16.V.1983 (RB); Vaillant 55, 22.VI.1978 (HUEFS). ***C. papillata*** H.S.Irwin & Barneby, Pereira 9188, 13.IX.1964 (RB); Maguire 49019, 06.VIII.1960 (RB); Souza 25025, 4.VII.2001 (RB); *Coutinho et al. 386, 387, 22.III.2014 (VIC). ***C. potentilla*** (Mart. ex Benth.) H.S.Irwin & Barneby, *Coutinho et al. 20, 27.VI.2012 (VIC); Menini-Neto 719, 25.V.2009 (RB); Lima 6366, 25.VII.2005 (RB). ***C. roraimae*** Gleason, *Silva et al. 126, 127, 128, 26.VII.2011 (HUNEB). ***C. rotundata*** (Vogel) H.S.Irwin & Barneby var. ***rotundata***, Alves 734, 21.IX.1989 (RB). ***C. rotundata*** (Vogel) H.S.Irwin & Barneby var. ***interstes*** H.S.Irwin & Barneby, Lima 1069, 13.V.1979 (RB); Martinelli 9199, 6.IV.1983 (RB). ***C. rotundata*** (Vogel) var. ***grandistipula*** (Vogel) H.S.Irwin & Barneby, *Coutinho et al. s.n., 15.VI.2011 (VIC); Romariz 112, 06.II.1947 (RB). ***C. simplifacta*** H.S.Irwin & Barneby, *Coutinho & Moura 132, 133, 19.I.2013 (VIC). ***C. tragacanthoides*** (Mart. ex Benth.) H.S.Irwin & Barneby var. ***tragacanthoides***, Stehmann 2671,21.IX.2000 (HUEFS); *Coutinho et al. 476, 26.III.2014 (VIC). ***C. tragacanthoides*** (Mart. ex Benth.) H.S.Irwin & Barneby var. ***rasa*** H.S.Irwin & Barneby, Forzza 4089, 18.VII.2005 (RB); Queiroz et al. 14932, 27.X.2010 (HUEFS). ***C. ulmea*** H.S.Irwin & Barneby, Hatschbach 41627, 21.X.1978 (RB); Pirani et al. 13034, 15.VI.1990

(HUEFS); Zappi et al. 12000, 27.V.1988 (HUEFS). ***C. venulosa*** (Benth.) H.S.Irwin & Barneby, Amorim 6202, 6239, 03.IX.2006 (RB); Gonzaga 44, 12.VIII.1995 (RB).***C. calycioides*** Greene var. ***calycioides***, Queiroz 4441, 30.VIII.1995 (HUEFS); Oliveira 719, 06.VI.2004 (HUEFS); Pesqueira et al. 86, 22.III.2008 (HUEFS). ***C. duckeana*** (P.Bezerra & Afr.Fern.) H.S.Irwin & Barneby, Lima-Verde et al. 268, 13.VI.1996 (HUEFS).

Table 1. *Chamaecrista* sections *Chamaecrista* and *Caliciopsis* Species Studied and Position of the Colleters

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

Continued

Colleter position						
Taxon	Base of stipule	Between leaflets	Insertion petiole	Base of bracteole/ bract	Base of sepal	Base of Petal
Section *Caliciopsis*						
C. calycioides var. *calycioides*	I, V	NO	I, V	I	I	I
C. duckeana	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.

[a]Field collected material.

*Analized by Coutinho *et al.* 2015.

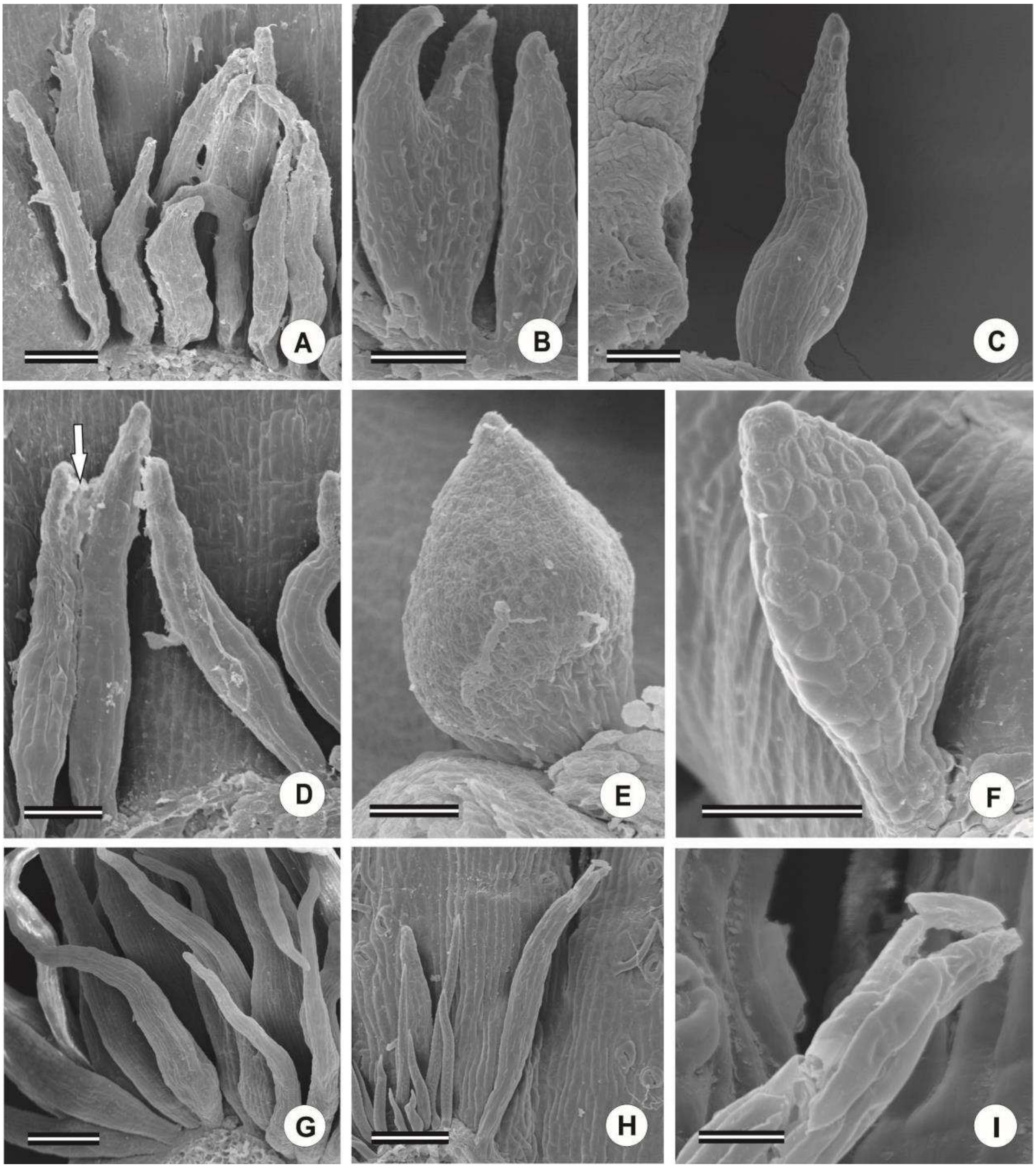

Figure 1. SEM images showing colleters on vegetative and reproductive organs of *Chamaecrista* sect. *Chamaecrista*. A. *C. basifolia:* Short digitiform Colleter on stipule. B. *C. gonoclada*: Bifurcated and short digitiform colleter on stipule. C. *C. tragacanthoides* var. *tragacanthoides*: Bottle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

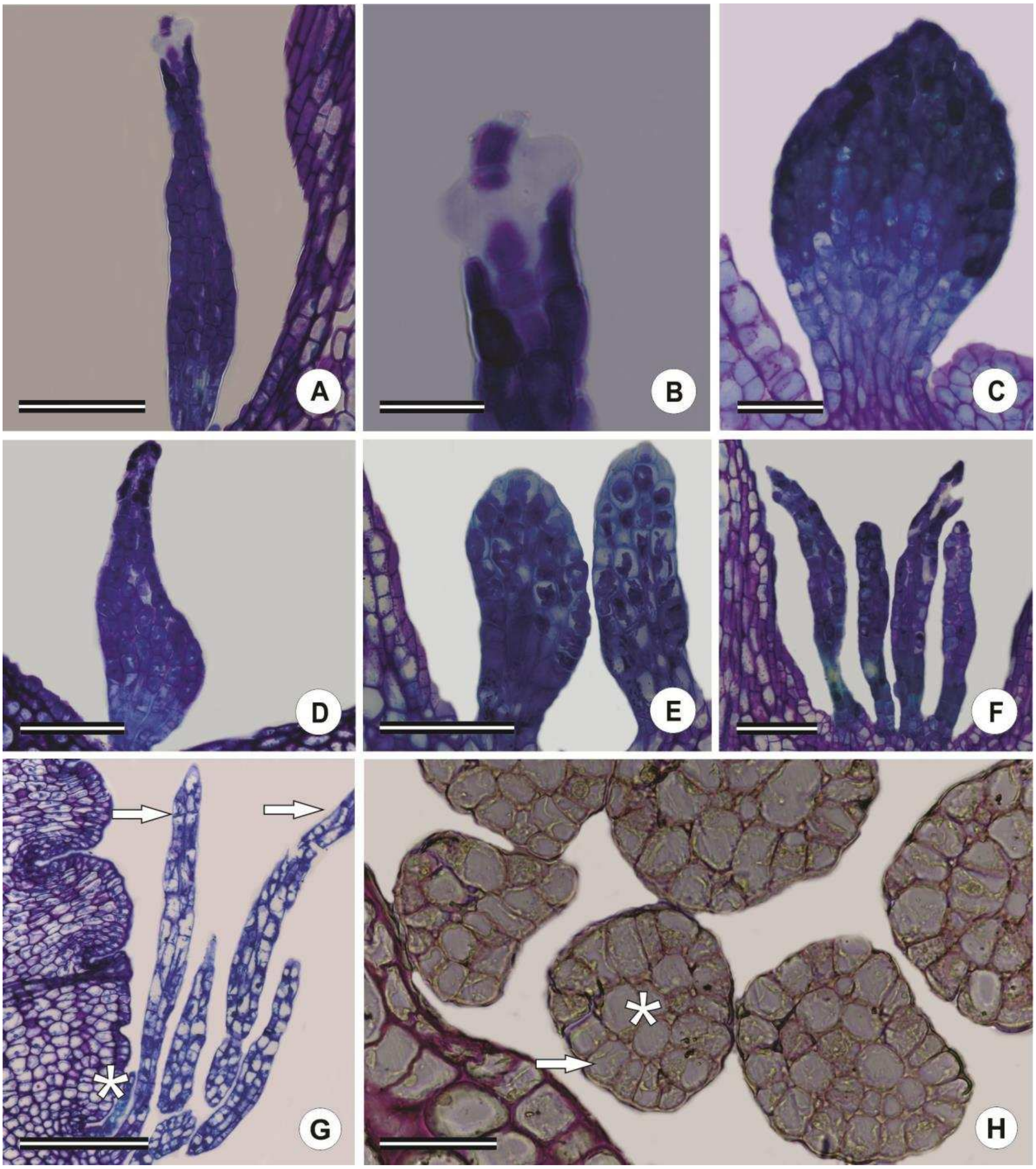

Figure 2. Anatomy of colleters of *Chamaecrista* sect. *Chamaecrista*. A, B. *C. pascuorum:* Short digitiform colleter on bracteole and apex detail colleter, respectively. C. *C. rufa* var. *exsul:* Pyriform colleter on stipule. D. *C. venturiana:* Bottle shaped colleter on bracteole. E, F. *C. mucronata:* Club shaped colleter on bracteole and short digitiform on stipule, respectively. G-H. *C. rotundata* var. *grandistipula:* Short and long digitiform colleter in the insertion of the petiole and cross-sectional view of colleter, respectively. Note the epidermis (arrow) and secretory parenchyma (asterisk). Scale bars: A, D-F: 100µm; B: 25µm; C, H: 50µm; G: 200µm.

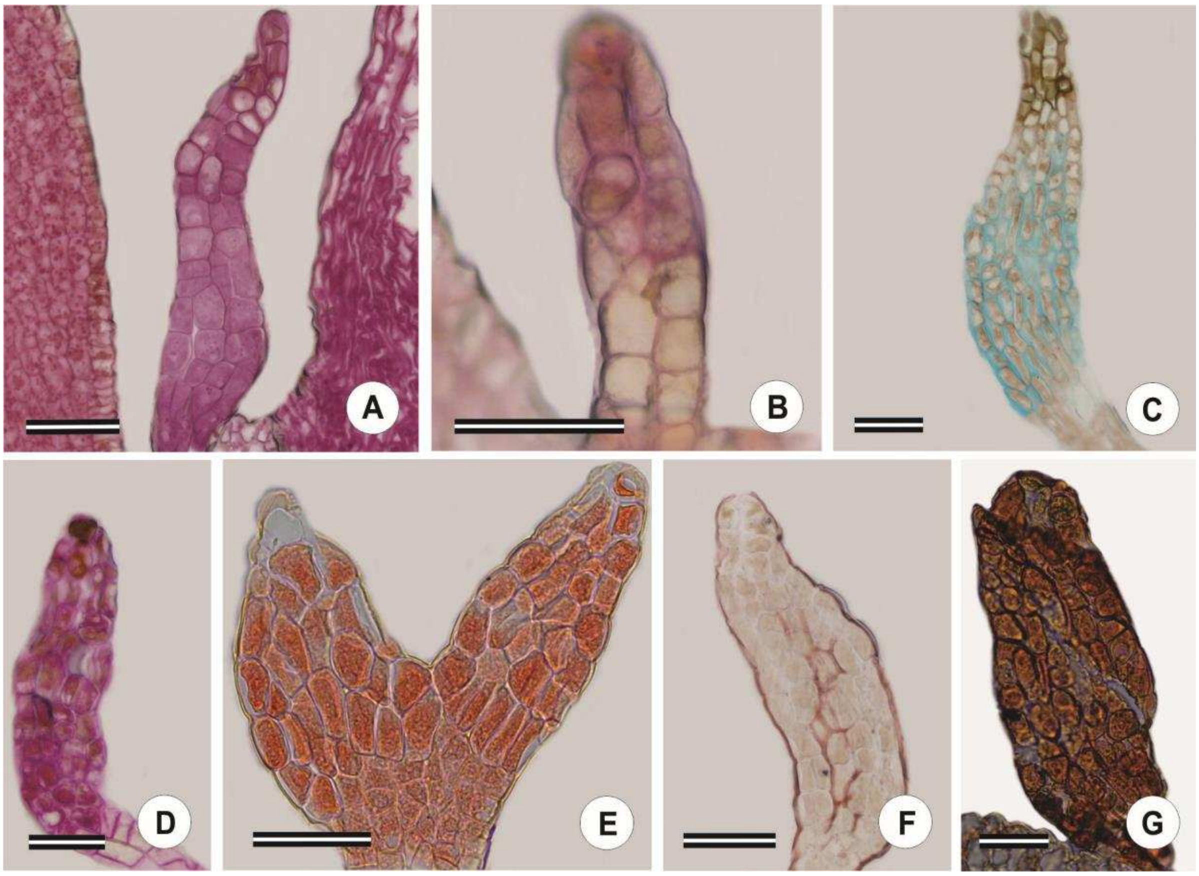

Figure 3. Histochemical tests in colleters of *Chamaecrista* sect. *Chamaecrista.* A-B. Totals polysaccharides (note the magenta coloration). A. *C. rotundifolia* var. *grandiflora:* short bottle shaped colleter on stipule. B. *C. rotundifolia* var. *rotundifolia*: club-shaped colleter on stipule. C. Acid mucopolysaccharides (Note the bluish coloration). *C. simplifacta*: short bottle shaped colleter on bracteole. D. Pectins (note the magenta coloration). *C. potentilla:* club-shaped colleter on sepal. E. Total proteins (Note the red coloration). *C. aristata:* bifurcated colleter on stipule. F, G. Total lipids (note the orange coloration). *C. aristata* and *C. mucronata:* short digitiform colleter on stipule and sepal, respectively. Scale bars: A, C, E-G: 50µm; B: 100µm; D: 40µm.

CAPÍTULO II

Este capítulo está de acordo com as normas da Acta Botanica Brasilica.

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

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Abstract

Nectaries are specialized structures that secrete nectar and are classified according to their function and/or position. These structures have been used as morphological characters for describing several families, especially Leguminosae. The sections *Chamaecrista, Caliciopsis* and *Xerocalyx* form a clade, presenting as common characters inflorescences with axillary racemes and small number of chromosomes. The section *Chamaecrista* has ca.75 species, in which 54 are American and organized in six series. The section *Xerocalyx* comprises three species and many varieties, while section *Caliciopsis* has only two species, one of them showing two varieties and has similar morphological characteristics to those representatives of sections *Xerocalyx* and *Chamaecrista.* This study sought to answer the following questions: Are the foliar glands present in species of the sections *Chamaecrista*, *Caliciopsis* and *Xerocalyx* nectaries? The glands have different morphotypes? What are the morphoanatomic characteristics of these glands? What are the secreted compounds by these glands? The morphoanatomic characteristics and secreted products have any implications for sections boundary? For that purpose, samples were obtained from herbarium and material collected in field and processed according to specific methodology for observation under light and scanning electron microscopy. Histochemical tests were performed to detect the chemical nature of the secretion produced. Nectaries occur on the petiole and rachis and four morphological types of were observed: urceolate, patelliform, verruciform and cupuliform. Anatomically, four anatomical regions were registered: epidermis, nectary parenchyma, vascularization and subnectary parenchyma. Polysaccharides, lipids, phenolic compounds and proteins were detected. The nectaries are similar to those observed in other species of *Chamaecrista*, and the data are relevant and promising to the taxonomy of the sections studied.

Keywords: Histochemical, petiole glands, morphotype, secretory structures, taxonomy

Introduction

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

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

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

In molecular phylogenetic studies, sections *Caliciopsis*, *Chamaecrista* and *Xerocalyx* are grouped in a clade which presents inflorescences with axillary racemes and reduced number of chromosome as common characters (Conceição *et al.* 2009).

There are few morphological distinctive features among these three sections. For instance, paralleled-veined leaflets, multistriate sepals, resupinate flowers and claviform seeds are observed in sect. *Xerocalyx* while subequilong sepals, short or obsolete peduncle, pinnate leaflets with high number of pairs and rectangular to irregularly rhombic seeds in sect. *Chamaecrista* (Irwin & Barneby 1982). Section *Caliciopsis* presents intermediate morphology between the sections *Xerocalyx* and *Chamaecrista,* with sepals striates similar to those of *Xerocalyx*, but differing from this because they are unequal, stipules with striate venation and many pairs of leaflets and obovate to rhombic seeds (Irwin & Barneby 1982).

Section *Chamaecrista* is the second most representative of the genera, with about 75 species (~55 in America) while *Caliciopsis* has only two species and *Xerocalyx* has three species but a high number of varieties (Irwin & Barneby 1982; Rando & Pirani 2012; Rando *et al.* 2013).

The morphoanatomy of leaf nectaries has been studied in *Chamaecrista* sect. *Apoucouita* (Coutinho & Meira 2015) and sect. *Absus* subsect. *Baseophyllum* (Coutinho *et al.* 2012) and subsect. *Otophyllum* (Francino *et al.* 2015). In sect. *Chamaecrista*, only *C. trichopoda*, *C. rotundata* and *C. mucronata* of ser. *Coriaceae* were investigated (Francino *et al.* 2006; 2015). Leaf nectaries of *Chamaecrista desvauxii* var. *langsdorfii* (sect. *Xerocalyx*) were histochemically evaluated and the presence of glucose was detected (Francino *et al.* 2015). However, although sect. *Caliciopsis* has been told to bear leaf nectaries on the petiole, as far as we are concerned, there are no morphoanatomical studies performed on the petiole nectaries present in such section.

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

Material and methods

Were studied 49 species of *Chamaecrista* sect. *Chamaecrista* including species of all series: *Coriaceae*, *Flexuosae*, *Prostratae*, *Greggiianae* and *Chamaecrista*, two species of sect. *Caliciopsis* and three species of sect. *Xerocalyx* (Tab. 1). A list with all sources of materials used and authorities for sections, subsections and species names are given in the supplementary data. Field-collected samples and voucher specimens deposited in the following herbaria were used: HUNEB, HUEFS, NY, RB, SPF an VIC(acronyms according to Thiers 2016). It were analyzed three specimens of most of species (Tab. 1).

Samples from herbarium were rehydrated (Smith & Smith 1942) and stored in 70% ethanol. Samples from species collected in field were fixed in FAA (formalin: acetic acid: 50% ethanol, 1:1:18 by volume) and NBF (neutral buffered formalin) (Johansen 1940). To detect phenolic compounds some samples were fixed in FSF (ferrous sulphate in formalin) (Johansen 1940).

Samples of petiole and rachis containing glands were dehydrated through ethanol series and embedded in methacrylate (Historesin Leica; Leica Microsystems Nussloch, Heidelberg, Germany). Cross and longitudinal sections 5 μm thick were made in an automatic rotary microtome (Leica RM2155, Deerfield, IL, USA) and subsequently stained with toluidine blue at pH 4,4 (O`Brien & Mccully 1981) for structural characterization. Some of the samples were used in histochemical tests. The slides were mounted in synthetic resin (Permount, Fisher Scientific, New Jersey, USA).

Some samples were dehydrated through *tert*-butanol series, embedded in histological paraffin enriched with dimethyl sulfoxide (Histosec®, Merck, Germany) (Johansen 1940). Cross and longitudinal sections 7 μm thick were obtained in an rotary microtome (Spencer 820 American optical Corporation, Buffalo, NY, USA). The sections were deparaffinized in xylene, rehydrated through an ethanol series, histochemical tested and the slides were mounted in synthetic resin or in water (Johansen 1940).

The presence of glucose in the gland exudates were testes by using urine test strips (Alamar Tecno Científica Ltda., São Paulo, Brazil) On the following species: *C. rotundata, C. mucronata, C. latifolia, C. potentilla, C. simplifacta, C. cinerascens, C. choriophylla, C. aristata, C. papillata* and *C. flexuosa*. The following histochemical tests were performed: neutral red (under fluorescence) and Sudan IV for total lipids (Pearse 1980); periodic acid-Schiff reagent for total polysaccharides (Maia 1979); ruthenium red for pectins/mucilage (Johansen 1940); alcian blue for acid mucopolysaccharides (Pearse 1980); xylidine Ponceau for total proteins (O'Brien & McCully 1981); phloroglucinol for lignin; and ferrous sulphate in formalin to detect phenolic compounds (Johansen 1940). 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; Carl Zeiss, Gottingen, Germany).

For scanning electron microscopy (SEM), samples of glands stored in 70% ethanol were critical-point dried with CO_2 (CPD 030, Bal-Tec, Balzers, Liechtenstein), mounted on stubs and sputter coated with gold (Modular Balzers Union FDU 010, SCA 010) (Bozzola & Russel 1991). Observations and images captures were taken using SEM (LEO, model 1430 VP, Cambridge, England) at the Centro de Microscopia e

Microanálise da Universidade Federal de Viçosa. The description of the morphology of the glands is in accordance with Radford *et al.* (1974).

Results

Due to the presence of glucose in the secretion of the petiole/rachis glands of several species of the *Chamaecrista* studied, such glands will be called hereafter nectaries. Nectaries occur in different positions on the petiole of all studied species (Fig. 1, Tab. 1). In 12 taxa (Fig. 1A), the nectaries were also present between the pairs of the leaflets (Tab. 1). Such structures are located mainly at the apex of the petiole in the ser. *Coriaceae* and in the sect. *Caliciopsis* and *Xerocalyx* (Tab. 1), while in the ser. *Prostratae* and *Greggiianae* they are predominant at the median region. In the ser. *Flexuosae* (Fig. 1G) and *Chamaecrista*, the nectaries occur at both the basal and median region (Tab. 1). Although most species have one to two nectaries, some of them have a variable number, such as *C. papillata* that has eight to nine.

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

The distribution of nectary types are as follows: urceolate nectaries occur in three taxa of sect. *Chamaecrista* ser. *Chamaecrista*; patelliform in 17 taxa of ser. *Coriaceae*, two of ser. *Flexuosae.* three of ser. *Chamaecrista* and in *C. desvauxii* var. *glauca* (sect. *Xerocalyx*), verruciform is exclusive to *C. simplifacta* (sect. *Chamaecrista*, ser. *Coriaceae*), cupuliform occur in 43 taxa (Tab. 1). Although there is no pattern of distribution of such nectaries regarding the sections, in ser. *Coriaceae*, nectaries are mostly patelliform while in the ser. *Prostratae* and *Greggiianae* the nectaries are exclusively cupuliform. The secretory surface may be concave in most of species studied, convex in *C. simplifacta* or flat in *C. vestita, C. pedicellaris* var. *pedicellaris* and *C. nictitans* var. *paraguariensis*.

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

The nectaries are characterized by having four distinct regions: a single-layered epidermis, a nectary parenchyma, the vascular bundles and a subnectary parenchyma (Figs. 2B, D, F, H, 3B, D, F). The epidermis is uniseriate, deprived of stomata, with more columnar shaped cells at the nectary edges but smaller and more cuboids in the center, sometimes slightly papillary (Figs. 2B, D, 4G, H). Throughout the nectary the cuticle is thick, except at the central area of the secretory epidermis. In most samples studied, a distended cuticle at central area was observed (Figs. 2A, H, 3E). At the edges of the nectary, there are very prominent intercellular spaces (Fig. 2B, H, 3B). Unicellular or multicellular tector trichomes were observed, especially in the epidermal cells of the stalk (Fig. 3A).

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

All species have nectaries vascularized predominantly by phloem (Figs. 3F). The vascularization originates from the main vascular bundles in most taxa (Figs. 2F, 4D; Tab. 1). In nine taxa only the accessory bundles contribute to the nectary vascularization (Tab. 1). Vascularization originates from both vascular accessory bundles and main vascular bundles also in nine taxa (Tab. 1).

Fibers adjacent to the vascularization that reaches the nectary were noticed in 56 taxa (Fig. 2F, 3D; tab. 1). A layer of sclereids, clearly separates the secretory parenchyma of the vascular region (Figs. 2F, 3B, F, 4F) in *C. anceps, C. aristata, C. cardiostegia, C. cinerascens, C. multinervia, C. rotundata* var. *rotundata, C. ulmea, C. roraimae, C. mucronata, C. latifolia, C. potentilla* and *C. simplifacta* (series *Coriaceae*).

Total polysaccharides (Fig. 4A, B), acid muco-polysaccharides (Fig. 4C), pectin (4D), proteins (Fig.4E), general phenolic compounds (Fig. 4G), lignin (4F), and lipids (Fig. 4H-J) were detected in the secretory parenchyma and epidermis. The presence of cuticle was also shown (Fig. 4H-J).

Discussion

The glandular structures present on the petiole/rachis of the studied species are classified as extrareproductive nectaries based on their topography, morphology, anatomical structure and presence of sugar (i.e. glucose). The nectaries here studied have similar structure to the nectaries described for other species of *Chamaecrista* and for other genera of subfamily Caesalpinioideae (Bhattachryya & Maheshwari 1971; Elias 1983; Francino *et al.* 2006; 2015 Paiva & Machado 2006; Melo *et al.* 2010; Coutinho *et al.* 2012; Coutinho & Meira 2015).

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

Nectaries are an effective tool for taxonomy, because they vary in type, shape and location (Keeler & Kaul 1979; Bentley & Elias 1983). In species of *Chamaecrista* sect. *Apoucouita,* Coutinho & Meira (2015) and Coutinho *et al*. (2016) observed 13 different types of extrareproductive nectaries and demonstrated their important role in the taxonomy of the genus. On the other hand, the similarity on the morphoanatomical characteristics among the extrareproductive nectaries of sect. *Absus* subsect. *Baseophyllum* seems to support the elevation of such a subsection to the sectional level (Coutinho *et al.* 2012), as had already been proposed in molecular studies (Conceição *et al*. 2009).

Although morphologically different, the anatomical similarities among the extrareproductive nectaries of *Chamaecrista* is evident as all of them are made up by a single layered epidermis, several layers of nectary parenchyma with underlying layers of subnectary parenchyma and vascularization. Such anatomical similarity is also shared by other species of *Chamaecrista* that bear extrareproductive nectaries (Coutinho el al. 2012; Coutinho & Meira 2015; Francino et al. 2015).

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

The distended cuticle in the central area of the secreting portion of the nectaries leads us to conclude that the nectar is accumulated below the cuticle and that later it is secreted to the outer side through cuticle bursting. Such way to release the secretion is in accordance with several authors (Fahn 1979; Elias 1983; Paiva & Machado 2006; Nepi 2007; Thadeo *et al.* 2008; Rocha *et al.* 2009).

Conceição *et al.* (2009) support the idea of a single origin for the extrareproductive nectaries in *Chamaecrista* and so far the anatomical similarity among such structures may reflect such idea. However, despite their unique origin, the extrareproductive nectaries in *Chamaecrista* may have followed different evolutionary lines and the type of secretion released may reinforce such hypothesis. The extrareproductive nectaries of the species of sect. *Apoucouita* secrete lipids in addition to carbohydrates and sugars (Coutinho & Meira 2015). In species of sect. *Absus* subsect. *Baseophyllum*, lipids were not detected but phenolic compounds instead (Coutinho *et al.* 2012) while in the taxa of sect. *Chamaecrista, Caliciopsis* and *Xerocalyx* studied herein we demonstrated that the nectaries secrete both lipids, phenolic compounds and proteins. We may correlate the differences in the nature of the secretion produced by the extrareproductive nectaries in *Chamaecrista* to their ecological function as different visitors may favor one particular type of nectar what would contribute to the evolution of mutualistic strategies. Such hypothesis have already been reported by Davis *et al.* (2014) for the family Malpighiaceae in which the highly specialized mutualism with oilseed bees would have allowed the conservation of floral characteristics in the species of the New World species and influenced in their diversification. Comprehensive evolutionary and ecological studies are needed with the species of the genus *Chamaecrista* in an attempt to understand the role of the secretion in mediating interactions with visitors and promoting the evolution and diversification of the group.

The lipid content found in the intercellular spaces of the species studied here is in accordance with reports for other genera, including *Chamaecrista* (Baker *et al.* 1978; Fahn 1979; 1988; 2000; Coutinho & Meira 2015). As stated by Paiva & Machado

(2006), the presence of lipids in the intercellular spaces of the secretory parenchyma indicates that these compounds are part of the secretion and that the plant offers reward in a more energetic form. Additionally, extreme environmental conditions may require a high-energy food resource for visitors, an event mentioned by Forcone *et al.* (1997) and Bernardello *et al.* (1999). The phenolic compounds within idioblasts may act in defense against herbivores as their content renders unpalatability to plant organs/structures, besides protecting them against pathogens (Nicolson & Thornburg 2007). Some studies suggest that plants with high levels of amino acids in their nectars attract more ants and therefore may suffer less herbivory (Lanza 1991; Wagner & Kay 2002; Wilder & Eubanks 2009).

The secretory structures present in the petiole/rachis of the species from sect. *Chamaecrista, Caliciopsis* and *Xerocalyx* correspond to extrareproductive nectaries. Although anatomical similarities were observed, the morphology differ. The nectar released is complex, made up by a mixture of compounds which may be important in plant-animal interaction. The nectary form of the series *Prostratae* and *Greggiianae* are well defined and this is an important data for the taxonomy of these groups. The data obtained here showed that the nectaries of the sections *Caliciopsis* and *Xerocalyx* are to a large extent similar to those of the section *Chamaecrista*. Our study also presents unprecedented data on the anatomy of the extrareproductive nectaries of the species of the sect. *Caliciopsis*. The database provided by us is vital for future studies with a taxonomic and phylogenetic approach.

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Supplementary Data

Species of *Chamaecrista* studied.

Vouchers are housed in the herbaria of the Universidade Estadual de Feira de Santana (HUEFS), Universidade do Estado da Bahia (HUNEB), New York Botanical Garden (NY), Jardim Botânico do Rio de Janeiro (RB), Universidade de São Paulo (SPF) and Universidade Federal de Viçosa (VIC). Collection preceded by an asterisk (*) correspond to field-collected material fixed in FAA (formalin: acetic acid: 50% ethanol, 1:1:18 by volume) and/or FNT (neutral buffered formalin) (Johansen 1940).

C. cuprea H.S.Irwin & Barneby, Carvalho 1936, 12.X.1983 (RB). ***C. deeringiana*** Small & Pennell, Small 4078, 03.IX.1912 (NY); Small 8124, 30.IV.1917 (NY); Small 8787, 15.V.1918 (NY). ***C. fasciculata*** (Michx.) Greene, Miranda et al. 1747, 18.VI.1994 (HUEFS); Oliveira et al. 42 02.VIII.1996 (HUEFS). ***C. glandulosa*** Greene var. ***andicola*** H.S.Irwin & Barneby, Klitgaard 394, 25.II.1997 (RB). ***C. lineata*** Greene, Rando 964, 960, 20.XI.2010 (SPF). ***C. nictitans*** var. ***disadena*** (Steud.) H.S.Irwin & Barneby, Queiroz 4439, 30.VIII.1995 (RB); Fernandes 1, 11.IX.1998 (HUEFS). ***C. nictitans*** Moench var. ***jaliscensis*** (Greenm.) H.S.Irwin & Barneby, Calónico 4736, 10.X.1997 (HUEFS). ***C. nictitans*** Moench var. ***paraguariensis*** (Chodat & Hassl.) H.S.Irwin & Barneby, Hatschbach 34277, 16.II.1974 (RB); Silva 1262, 23.III.1993 (HUEFS); Silva 273, 03.VIII.1981 (HUEFS). ***C. obcordata*** Britton, Sastre et al. 1969, 13.VIII.1973 (NY); Galo 693, 12.II.1952 (NY). ***C. pascuorum*** (Mart. ex Benth.) H.S.Irwin & Barneby, Harley 10264, 02.II. 1977 (RB); Harley 19153, 27.II.1977 (RB); Anderson et al. 36977, 15.III.1972 (RB). ***C. pedicellaris*** Britton var. ***pedicellaris***, Ekman 7577, 25.VIII.1916 (NY); Holdridge 1126, 12.IV.1942 (NY); Leonadr & Leonard 13359, 13.II.1929 (NY). ***C. pedicellaris*** Britton var. ***adenosperma*** (Urb.) H.S.Irwin & Barneby, Almonte 8502, 10.XII.1978 (NY); Lavastre 539, XII.1962 (NY); Ekman 14498, 22.III.1930 (NY). ***C. portoricensis*** (Urb.) O.F.Cook & G.N.Collins var. ***portoricensis***, Britton & Rose 1406, 27.II.1913 (NY); Axelrod & Chavez 4707, 28.VI.1992 (NY); Britton & Boynton 288, 21.III.1925 (NY). ***C. pygmaea*** Britton var. ***pygmaea***, Shafer 10709, 05.XII.1911 (NY); Ekman 17268, 23.VIII.1923 (NY). ***C. pygmaea*** Britton var. ***savannarum*** (Britton) H.S.Irwin & Barneby,Véllez-Gavilán et al. 113, 17.VII.1996 (NY). ***C. repens*** (Vogel) H.S.Irwin & Barneby var. ***repens,*** Fernandes s/n, 19.VII.1977 (HUEFS); Queiroz & Machado 12604, 29.II.2006 (HUEFS). ***C. repens*** (Vogel) H.S.Irwin & Barneby var. ***multijuga*** (Benth.) H.S.Irwin & Barneby, Harley 21111, 09.IV.1980 (RB); Silva 2497, 01.IX.1994 (RB); Carvalho 2941, 20.III.1990 (RB). ***C. rufa*** Britton & Rose var. ***exsul*** H.S.Irwin & Barneby, Teixeira et al. 441, 14.V.1982 (NY); Berg et al. 18501, 14.X.1973 (NY). ***C. rufa*** Britton & Rose var. ***polyphlebia*** H.S.Irwin & Barneby, Pennell 1328, 23.VIII.1917 (NY); García-Barriga 17211, 10.III.1960 (NY); Johnson 3080, 21.X.1983 (NY). ***C. venturiana*** H.S.Irwin & Barneby, Novara 2211, 26.XI.1981 (NY); Pensiero & Marino 4251, 03.XII.1992 (NY); Balcazar 21, 02.I.1994 (NY). ***C. vestita*** (Vogel) H.S.Irwin & Barneby, Krapovickas & Cristobal 40774, 12.I.1987

(NY); Hatschbach 23926, 26.II.1970 (NY); Leite 1983, 01.XI.1942 (NY). ***C. greggii*** Pollard ex A.Heller var. ***greggii***, Johnston 5459, 04.V.1960 (NY); Lavin et al. 4513, 27.VIII.1983 (NY); Worthington 8323, 02.VI.1982 (NY); Hinton et al. 24261, 04.VI.2003. *C.* ***greggii*** Pollard ex A.Heller var. ***macdougaliana*** (Rose) H.S.Irwin & Barneby, Hughes 1785, 11.XI.1993 (NY); Breckon & Kolterman 2342, 06.VII.1978 (NY); Rose & Hay 5888, 01.VIII.190 (NY). ***C. greggii*** Pollard ex A.Heller var. ***potosini***, Crutchfield 5383B, 20.IV.1960 (NY); Stanford 871, 12. VIII.1941 (NY). ***C. flexuosa*** Greene var. ***flexuosa***, Carneiro-Torres 89, 29.XI.1998 (HUEFS); Conceição 361, 18.IX.1999 (HUEFS); *Coutinho & Laurenção 269, 270, 271, 18.VIII.2013 (VIC). *C.* ***flexuosa*** Greene var. ***texana*** (Buckley) H.S.Irwin & Barneby, MacDougall s/n, 30.IV.1970 (NY); Sánchez 5872, 20.VIII.1976 (NY). ***C. gonoclada*** (Benth.) H.S.Irwin & Barneby, Hatschbach 45885, 11.XII.1982 (NY); Dubs 1756, 09.IX.1994 (NY) ***C. parvistipula*** (Benth.) H.S.Irwin & Barneby, Kirkbride 3807, 12.II.1981 (RB); Irwin 11610, 12.I.1966 (RB); Irwin 25344, 25.I.1970 (RB).***C. swainsonii*** (Benth.) H.S.Irwin & Barneby, Lima 6916, 27.VII.2008 (RB); Gonzaga 6, 10.VIII.1985 (RB); Conceição 702, 20.VIII.2003 (HUEFS). ***C. cordistipula*** (Mart.) H.S.Irwin & Barneby, Zardini & Guard 15477, 31.X.1989 (NY); Hatschbach 29518, 15.IV.1972 (NY); Aristeguieta 6322, VIII.1966 (NY); Saravia 2907, 20.X.1963 (NY). ***C. kunthiana*** (Schltdl. & Cham.) H.S.Irwin & Barneby, Queiroz 14171, 24.IV.2009 (HUEFS); Faria 47, 30.IV.1999 (HUEFS); Silva 1656, 18.VII.1993 (HUEFS). ***C. pilosa*** Greene var. ***pilosa***, Queiroz 369, 20.V.2005 (HUEFS); Queiroz 19, 16.VIII.2002 (HUEFS). ***C. pilosa*** Greene var. ***luxurians*** (Benth.) H.S.Irwin & Barneby, Collares 156, 02.VI.1984 (HUEFS). ***C. serpens*** Greene var. ***serpens***, Queiroz 2894, 26.XI.1992 (HUEFS, RB); Dutra 03, 12.V.1994 (HUEFS); Queiroz 2904, 29.XI.1992 (HUEFS, RB). ***C. supplex*** (Mart.) Britton & Rose in Britton & Killip, Harley 21490, 18.IV.1980 (RB); Coradin 5932, 22.VI.1983 (RB). ***C. tenuisepala*** (Benth.) H.S.Irwin & Barneby, Queiroz 478, 19.VI.2005 (HUEFS); Delgado-Junior 45, 04.IV.2009 (HUEFS ***C. trichopoda*** Britton & Rose ex Britton & Killip, Oliveira et al. 446, 18.IV.2000 (HUEFS); Silva et al. 3361, 27.II.1996 (HUEFS, NY). ***C. anceps*** (Benth.) H.S.Irwin & Barneby, Damazio 2014, 1908 (RB). ***C. aristata*** (Benth.) H.S.Irwin & Barneby, *Coutinho et al. 96, 14.VIII.2012 (VIC); Hatschbach 67980, 18.VII.1998 (RB); Queiroz 7528, 08.I.2003 (HUEFS). ***C. burchelli*** (Benth.) H.S.Irwin & Barneby, Irwin 34703, 21.I.1972 (RB); Silva 2244, 1.XII.2003 (RB). ***C. cardiostegia*** H.S.Irwin & Barneby, Irwin 25482, 20.I.1970 (RB, NY); Magalhães 5434; Duarte 2802. ***C. caribaea*** Britton var. ***caribaea***, Britton 3448, 28.I.1905 (NY); Small & Carter 8853, 01.II.1910 (NY); Brace 4972, 18.VIII.1906 (NY). ***C. caribaea*** Britton var. ***lucayana*** (Britton) H.S.Irwin & Barneby, Rando 963, 22.XI.2010 (NY, SPF); Brace 3985, 4.XII.1905 (NY); Wilson 7895, 28.XII.1907 (NY); Britton & Millspaugh 2841, 20.II.1905 (NY). ***C. caribaea*** Britton var. ***inaguensis*** (Britton) H.S.Irwin & Barneby, Millspaugh & Millspaugh 9303, 12.III.1911 (NY); Proctor & Gillis 34033, 11.VI.1974 (NY); Correll 43167, 26.VIII.1976 (NY). ***C. cinerascens*** (Vogel) H.S.Irwin & Barneby, Kawasaki et al. 973, 23.V.1997 (SPF); Joly et al. CFSC2198, 27.V.1972 (SPF); *Coutinho & Pereira 187, 25.IV.2013 (VIC). ***C. choriophylla*** (Vogel) Irwin & Barneby, *Coutinho et al. 11, 21.VI.2012 (VIC). ***C. lagotois*** H.S.Irwin & Barneby, *Coutinho et al. 407, 408, 409, 24.III.2014 (VIC). ***C. latifolia*** (Benth.) Rando, Souza et al. 11609, 04.VII.1996 (HUEFS); Hatschbach 68259, 25.VII.1998 (HUEFS); *Coutinho et al. 46, 47, 02.VIII.2012 (VIC). ***C. distichoclada*** (Mart. ex Benth.) H.S.Irwin & Barneby, Irwin 22733, 27.I.1969 (RB); Queiroz 2368, 04.VII.1989 (HUEFS); Azevedo 342, 26.V.2007 (HUEFS). ***C. mucronata*** (Spreng.) H.S.Irwin & Barneby, Cardoso & Conceição 597, 19.VI.2005 (HUEFS); Silva et al. 2628, 05.IX.1994 (HUEFS); Harley et al. 3784, 24.VIII.1996 (HUEFS). ***C. multinervia*** (Mart. ex Benth.) H.S.Irwin &

Barneby, Hatschbach 46500, 16.V.1983 (RB); Vaillant 55, 22.VI.1978 (HUEFS). ***C. olesiphylla*** (Vogel) H.S.Irwin & Barneby, Giulietti s/n, 30.VIII.1981 (RB); Forzza 4844, 14.I.2007 (RB). ***C. papillata*** H.S.Irwin & Barneby, Pereira 9188, 13.IX.1964 (RB); Maguire 49019, 06.VIII.1960 (RB); Souza 25025, 4.VII.2001 (RB); *Coutinho et al. 386, 387, 22.III.2014 (VIC). ***C. potentilla*** (Mart. ex Benth.) H.S.Irwin & Barneby, *Coutinho et al. 20, 27.VI.2012 (VIC); Menini-Neto 719, 25.V.2009 (RB); Lima 6366, 25.VII.2005 (RB). *****C. roraimae*** Gleason, Silva et al. 126, 127, 128, 26.VII.2011 (HUNEB). ***C. rossicorum*** (H.S. Irwin & Barneby) Rando, Conceição et al. 739, 12.IX.2003 (HUEFS); Silva et al. 2888, 14.IX.1994 (HUEFS); Silva 2891,15.IX.1994 (HUEFS). ***C. rotundata*** (Vogel) H.S.Irwin & Barneby var. ***rotundata***, Alves 734, 21.IX.1989 (RB). ***C. rotundata*** (Vogel) H.S.Irwin & Barneby var. ***interstes*** H.S.Irwin & Barneby, Lima 1069, 13.V.1979 (RB); Martinelli 9199, 6.IV.1983 (RB). ***C. rotundata*** (Vogel) var. ***grandistipula*** (Vogel) H.S.Irwin & Barneby, *Coutinho et al. s.n., 15.VI.2011 (VIC); Romariz 112, 06.II.1947 (RB). ***C. simplifacta*** H.S.Irwin & Barneby, *Coutinho & Moura 132, 133, 19.I.2013 (VIC). ***C. tragacanthoides*** (Mart. ex Benth.) H.S.Irwin & Barneby var. ***tragacanthoides***, Stehmann 2671,21.IX.2000 (HUEFS); *Coutinho et al. 476, 26.III.2014 (VIC). ***C. tragacanthoides*** (Mart. ex Benth.) H.S.Irwin & Barneby var. ***rasa*** H.S.Irwin & Barneby, Forzza 4089, 18.VII.2005 (RB); Queiroz et al. 14932, 27.X.2010 (HUEFS). ***C. ulmea*** H.S.Irwin & Barneby, Hatschbach 41627, 21.X.1978 (RB); Pirani et al. 13034, 15.VI.1990 (HUEFS); Zappi et al. 12000, 27.V.1988 (HUEFS). ***C. venulosa*** (Benth.) H.S.Irwin & Barneby, Amorim 6202, 6239, 03.IX.2006 (RB); Gonzaga 44, 12.VIII.1995 (RB).***C. calycioides*** Greene var. ***calycioides***, Queiroz 4441, 30.VIII.1995 (HUEFS); Oliveira 719, 06.VI.2004 (HUEFS); Pesqueira et al. 86, 22.III.2008 (HUEFS). ***C. duckeana*** (P.Bezerra & Afr.Fern.) H.S.Irwin & Barneby, Lima-Verde et al. 268, 13.VI.1996 (HUEFS). ***C. desvauxii*** (Collad.) Killip var. ***desvauxii***, *Coutinho et al. s/n, 03.VII.2011 (VIC). ***C. desvauxii*** (Collad.) Killip var. ***glauca*** (Hassl.) H.S.Irwin & Barneby, *Coutinho 125, 18.I.2003 (VIC); Coutinho 118, 16.I.2003 (VIC) *Coutinho et al. 294, 295 13.I.2014 (VIC). ***C. desvauxii*** (Collad.) Killip var. ***graminea*** H.S.Irwin & Barneby, *Coutinho et al. s/n, 03.VII.2011 (VIC). ***C. diphylla*** (L.) Greene, *Coutinho 533, 534 (VIC). ***C. ramosa*** (Vogel) H.S.Irwin & Barneby var. ***parvifoliola*** H.S.Irwin & Barneby, *Coutinho et al. s/n, 03.VII.2011 (VIC).

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

Taxa	**Form**							**Nº**	**Position**				**Origin vascularization**			**Fibers adjacent to the vascularization**
	Urceolate	**Patelliform**		**Verruciform**	**Cupuliform**				**Petiole**			**Rachis**				
	Sessile	**Sessile**	**Short stalked**	**Short stalked**	**Short stalked**	**Stalked**	**Long stalked**		**A**	**M**	**B**		**1**	**2**	**3**	
Sect. *Chamaecrista*																
Ser. *Coriaceae*																
C. anceps		X						1-3	X				X			X
C. aristata			X					+1	X			X			X	X
C. burchelli		X						1-2		X			X			X
C. cardiostegia		X						1	X				X			X
C. caribaea var. *caribaea*							X	+1		X		X	X			X
C. caribea var. *lucayana*							X	+1		X		X			X	X
C. caribea var. *inaguensis*							X	+1		X		X	X			X
C. cinerascens			X					1	X				X			X
C. choriophylla		X						1	X				X			X
C. rossicorum			X					1	X				X			X
C. latifólia			X					1	X				X			X
C. distichoclada			X					1	X					X		
C. lagotois		X						1	X				X			X
C. mucronata			X					1-4	X			X	X			X
C. multinervia						X		+1	X				X			X
C. olesiphylla			X					+1	X				X			X
C. papillata			X					8-9	X			X	X			
C. potentilla					X			1	X				X			X
C. roraimae						X		+1	X			X	X			
C. rotundata			X					1	X				X			
C. rotundata var. *interstes*			X					1	X				X			
C. rotundata var. *grandistipula*						X		1	X				X			
C. simplifacta				X				1	X				X			X
C. tragacanthoides						X		1	X				X			
C. tragacanthoides var. *rasa*			X					1	X				X			
C. ulmea			X					1	X				X			X
C. venulosa							X	1	X	X					X	X
Ser. *Flexuosae*																

Taxa	Form							Nº	Position				Origin vascularization			Fibers adjacent to the vascularization
	Urceolate	**Patelliform**		**Verruciform**	**Cupuliform**				**Petiole**			**Rachis**				
	Sessile	**Sessile**	**Short stalked**	**Short stalked**	**Short stalked**	**Stalked**	**Long stalked**		**A**	**M**	**B**		**1**	**2**	**3**	
C. flexuosa var. *flexuosa*						X		1-4	X	X			X			
C. flexuosa var. *texana*						X		1-2	X	X				X		
C. gonoclada			X					1			X		X			
C. parvistipula			X					1		X					X	
C. swainsoni						X		1			X		X			
Ser. *Prostratae*																
C. cordistipula							X	1	X					X		X
C. kunthiana							X	1		X				X		X
C. pilosa var. *pilosa*							X	1		X			X			X
C. pilosa var. *luxurians*							X	1-2		X					X	X
C. serpens var. *serpens*							X	1		X				X		X
C. supplex							X	1		X				X		X
C. tenuisepala							X	1		X					X	X
C. trichopoda							X	1-2	X						X	X
Ser. *Greggianae*																
C. greggii var. *greggii*							X	1		X			X			X
C. greggii var. *macdougaliana*							X	1		X				X		X
C. greggii var. *potosini*						X		1		X					X	X
Ser. *Chamaecrista*																
C. cuprea							X	1		X			X			X
C. deeringiana			X					1		X			X			X
C. fasciculata						X		1-2		X			X			X
C. glandulosa						X		1		X			X			X
C. lineata							X	+1		X		X	X			X
C. nictitans var. *paraguariensis*			X					1-2		X			X			X
C. nictitans var. *disadena*	X							+1		X	X	X	X			X
C. nictitans var. *jaliscensis*							X	1-2		X			X			X
C. obcordata							X	1-9			X		X			X

Taxa	Form							Nº	Position				Origin vascularization			Fibers adjacent to the vascularization
	Urceolate	Patelliform		Verruciform	Cupuliform				Petiole			Rachis				
	Sessile	Sessile	Short stalked	Short stalked	Short stalked	Stalked	Long stalked		A	M	B		1	2	3	
C. pascuorum							X	1-2			X		X			X
C. pedicellaris var. *pedicellaris*							X	1-2			X		X			X
C. pedicellaris var. *adenosperma*							X	1-2			X		X			X
C. portoricensis var. *portoricensis*						X		1-4		X			X			X
C. pygmaea var. *pygmaea*						X		1-2		X			X			X
C. pygmaea var. *savannarum*						X		1-2		X			X			X
C. repens var. *repens*	X							+1		X		X	X			X
C. repens var. multijuga	X							1		X			X			X
C. rufa var. *exsul*							X	1-2		X		X	X			X
C. rufa var. *polyplebia*						X		1-2		X			X			X
C. venturiana		X						1-2					X			X
C. vestita							X	1-2			X				X	X
										X						
Sect. *Caliciopsis*																
C. calycioides var. *calycioides*							X							X		X
C. duckeana							X						X			
Sect. *Xerocalyx*																
C. desvauxii var. *desvauxii*					X								X			X
C. desvauxii var. *glauca*						X							X			
C. desvauxii var. *graminea*						X							X			X
C. diphylla						X							X			X
C. ramosa var. *parvifoliola*						X								X		X

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

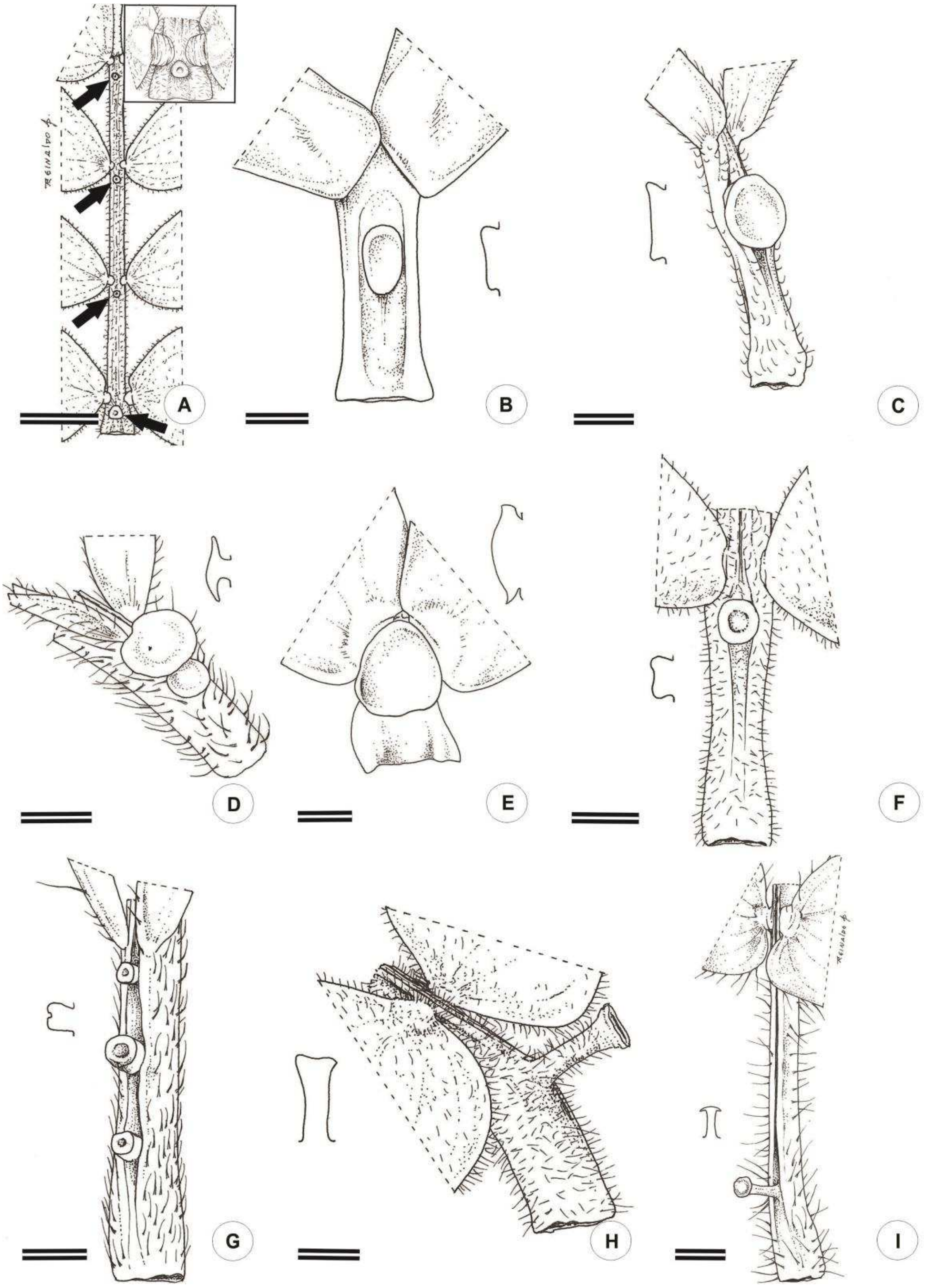

Figure 1. Nectaries on leaves of species of *Chamaecrista* sect. *Chamaecrista* and *Xerocalyx*. A. *C. aristata:* nectary on petiole and raquis. B. *C. lagotois*: patelliform sessile. C, D. *C. venturiana* and *C. nictitans* var. *paraguariensis*: patelliform sessile and short-stalked respectivelly.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

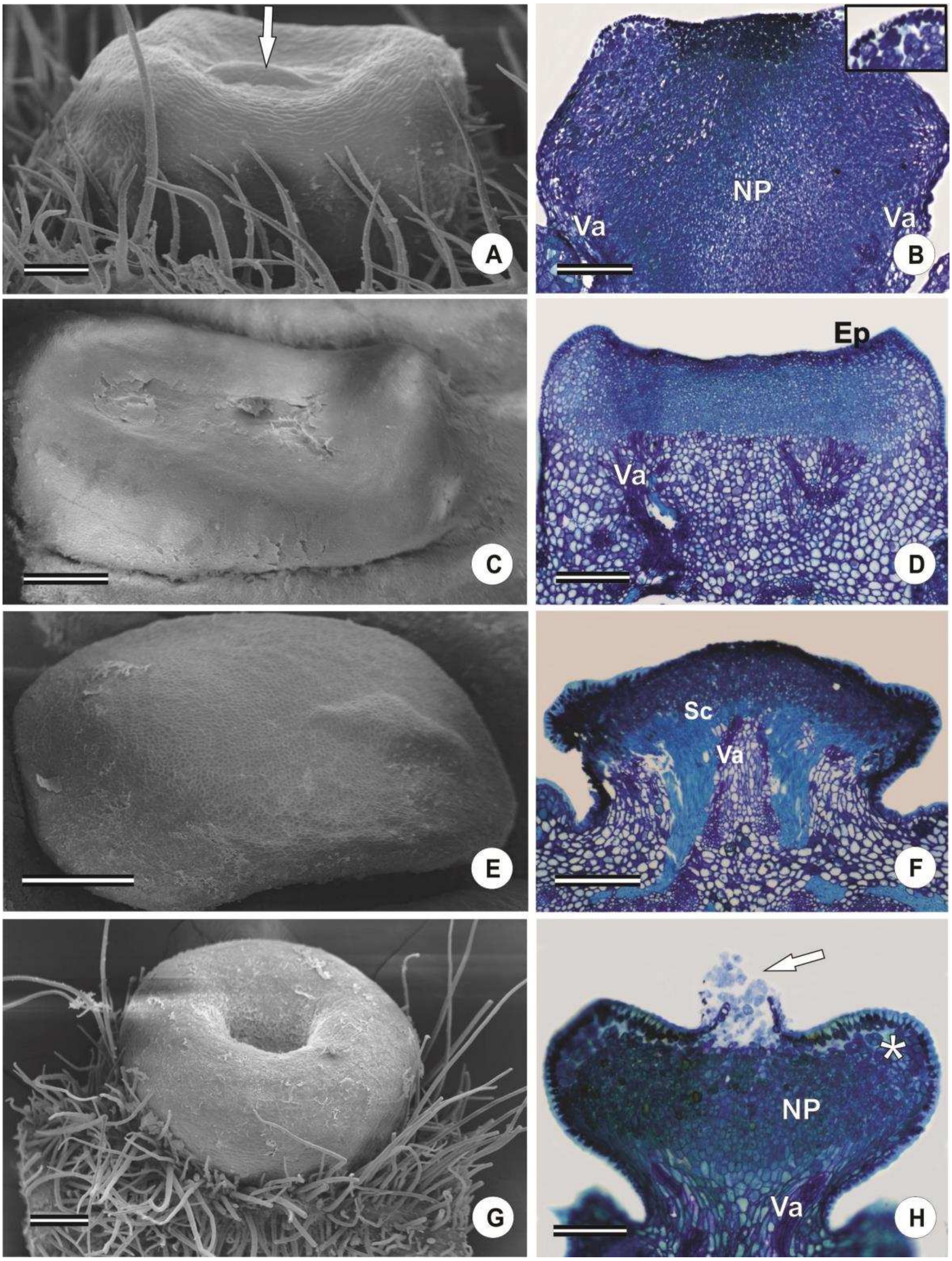

Figure 2. SEM images and anatomical sections of nectaries of *Chamaecrista* sect. *Chamaecrista*. A, B. *C. repens* var. *repens*: sessile urceolate (note detail of intercellular spaces). C, D. *C. lagotois*: sessile patelliform. E, F. *C. simplifacta*: short-stalked verruciform. G, H. *C. potentilla:*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

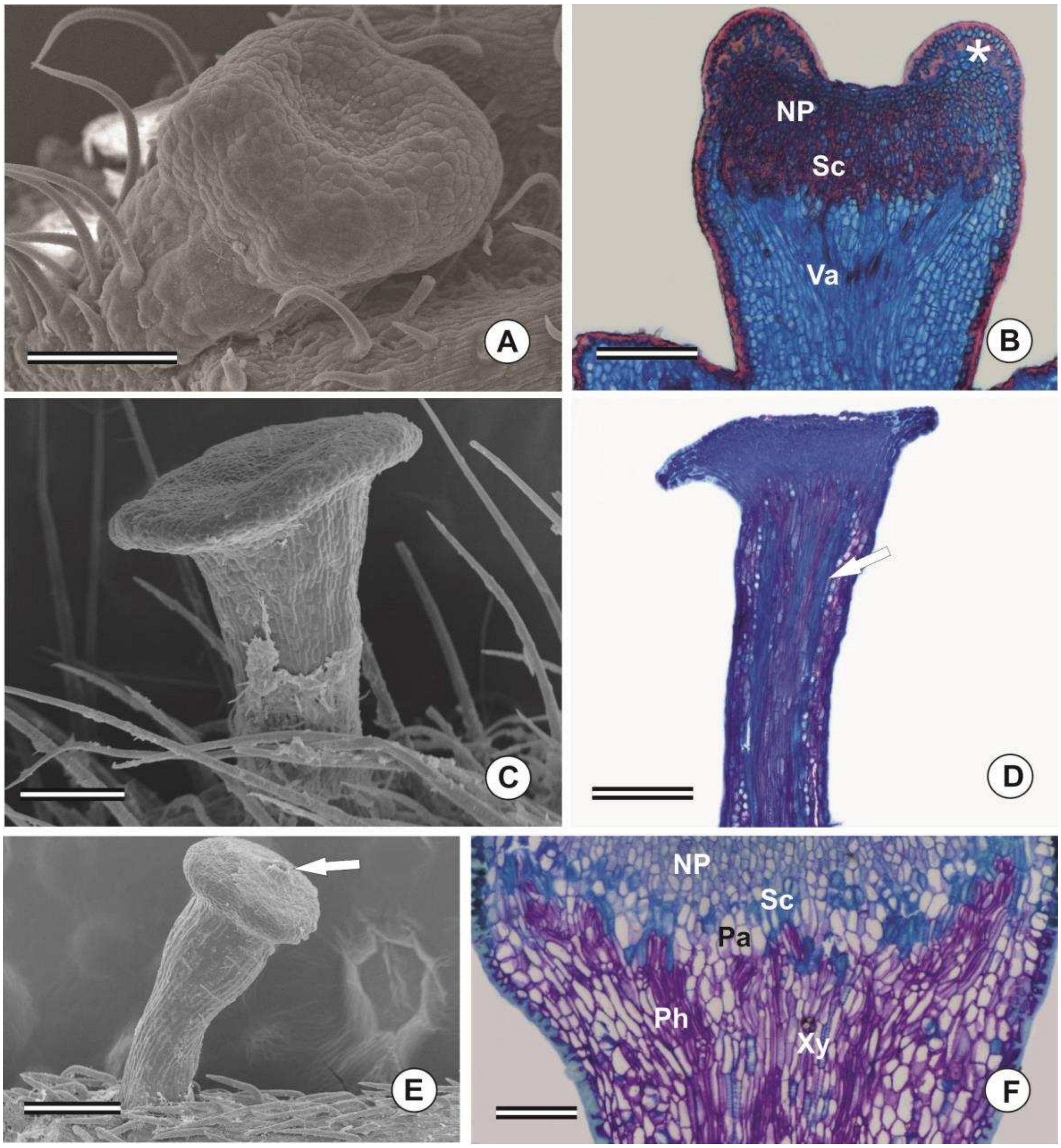

Figure 3. SEM images and anatomicals sections of nectaries of *Chamaecrista* sect. *Chamaecrista*. A, B. *C. roraimae*: stalked cupuliform. C, D, E. *C. vestita* and *C. pedicellaris* var. *pedicellaris*, respectivelly: long-stalked cupuliform (Note the fibers: arrow). F. *C. mucronata*. Note the cuticle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

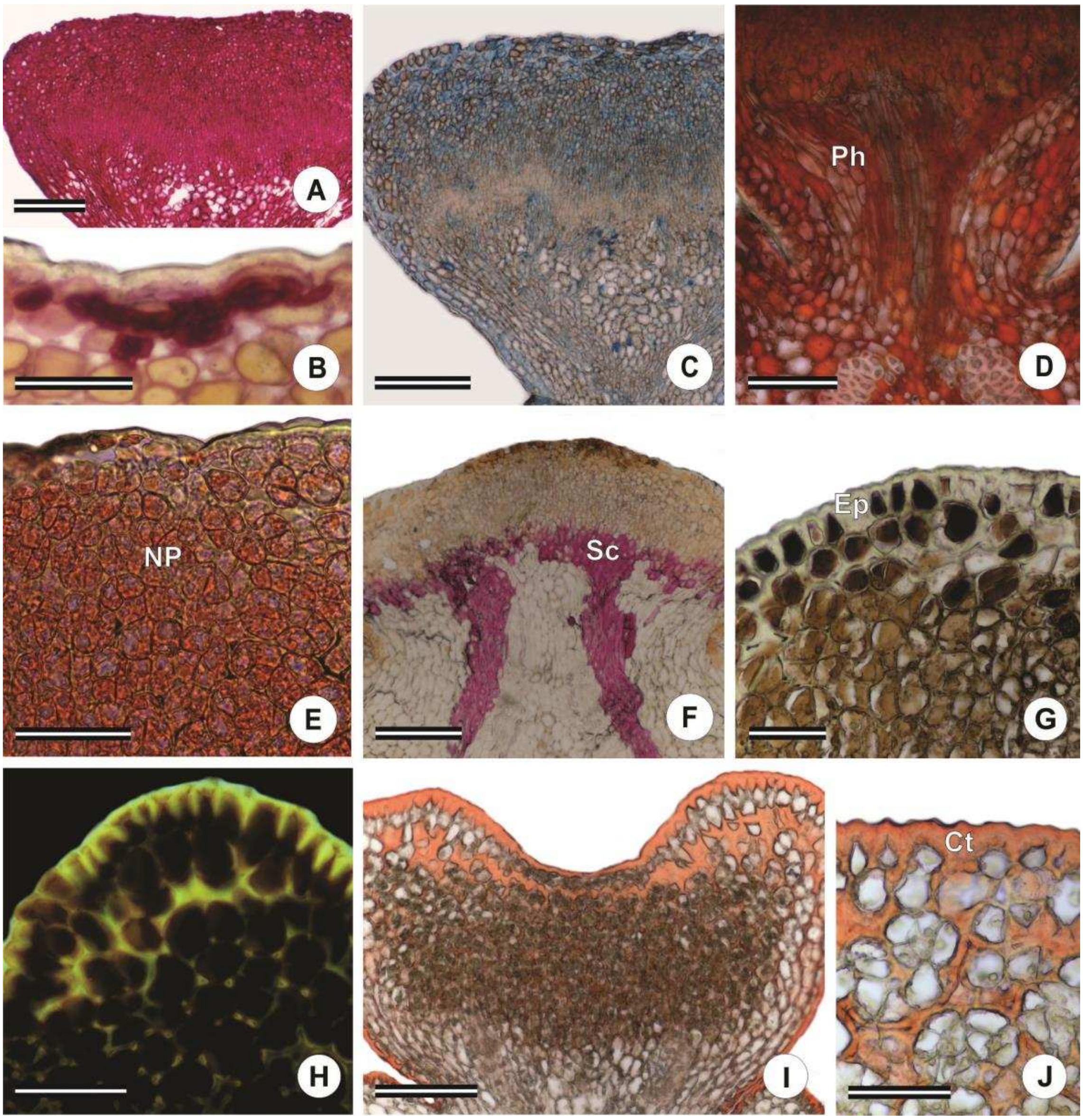

Figure 4. Histochemical tests in nectaries of *Chamaecrista* sect. *Chamaecrista*. A-B: Total polysaccharides (magenta coloration). A. *C. desvauxii* var. *glauca*. B. *C. tragacanthoides* var. *tragacanthoides*. C. Acid mucopolysaccharides (bluish coloration). *C. desvauxii* var. *glauca*. D. Pectins (magenta coloration). *C. roraimae*. E. Total proteins (red color). *C. mucronata*. F. Lignin (magenta coloration). *C. simplifacta*. G. General phenolic compounds (black coloration). *C. simplifacta*. H (yellow coloration)-J (orange coloration) Total lipids. H. *C. roraimae* (fluorescence). I. *C. potentilla*. J. *C. lagotois*. (Ep: epidermis; Ct: cuticle; Ph: phloem; Sc: sclereids; NP: nectary parenchyma) Scale bars: A, C: 300µm; B: 25µm; D, I: 100µm; E, G, H, J: 50µm; F: 200µm.

CAPÍTULO III

Este capítulo está de acordo com as normas do periódico Plant Systematics and Evolution.

Leaf morphoanatomy of section *Chamaecrista* series *Coriaceae* (Leguminosae: Caesalpinioideae)

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Abstract

Chamaecrista (L.) Moench (Leguminosae: Caesalpinioideae) is a monophyletic genus with ca. 330 species organized in six sections. The species belong to sect. *Chamaecrista*, the second largest section, are distributed into six series. The *Coriaceae* series were found poliphyletc and the “campo rupestre” is the mail enviroment where the species from this series occurs. Taking into consideration the importance of morphoanatomy for taxonomy of the genus and the studies available for the genus *Chamaecrista*, our work aims to describe the morphoanatomy of species from section *Chamaecrista* ser. *Coriaceae* and evaluate data that may be useful for the systematic approaches. Samples from herbarium and field were processed according specific methodology for light microscopy. Paracitic stomata, dorsiventral mesophyll, monocrystals, collateral bundles with associate fibers, accessory bundles on the petiole, idioblasts with compounds phenolic, not-enlarged tracheids, nectaries and colleters are common in the species studied. Were observed acrodromous, eucampotodromous and actinodromous venation. The distribution of the tector trichomes, conformation of the vascular bundle of the petiole, hypodermis and papillary cells were diagnostic at the species level.

Keywords: “Campo rupestre”, colleter, leaf anatomy, nectaries, secretory structures.

Introduction

Chamaecrista Moench is one of the largest genera of the subfamily Caesalpinioideae (Cassieae: Cassiinae) with ca. 330 species distributed mainly in tropical America (Irwin and Barneby 1982; Lewis 2005). There are about 210 endemic species to Brazil (Flora do Brasil 2017).

The genus is organized into six sections based on the type of indument, presence or absence of extrafloral nectaries, inflorescence pattern and venation of the sepals and leaflets (Irwin and Barneby 1982). In this context, six sections were recognized: *Absus*, *Apoucouita*, *Caliciopsis*, *Chamaecrista*, *Grimaldia* and *Xerocalyx* (Irwin and Barneby 1982). Sections *Absus* and *Grimaldia* are differentiated by the presence of secretory trichomes, while the other sections have extrafloral nectaries instead (Irwin and Barneby 1982). Aditionally, in molecular phylogenie the genus emerged as a monophyletic group (Conceição 2006; Rando et al. 2016).

Chamaecrista sect. *Chamaecrista* is the second most representative section, with 76 species, being 52 from new world (Rando and Pirani 2012). Based on the position of inflorescences, number of stamens, leaflet venation pattern and presence of xylopodium sect. *Chamaecrista* is divided in six series: *Bauhinianae*, *Chamaecrista*, *Coriaceae*, *Flexuosae*, *Greggianae* and *Prostratae* (Irwin and Barneby 1982). *Chamaecrista* sect. *Chamaecrista* ser. *Coriaceae* with 22 species presents shrubby spceis with xylopodium, nectaries on the petiole, thick-textured leaflets and axillary peduncles (Irwin and Barneby, 1982; Rando et al. 2013, 2016). Within ser. *Coriaceae*, *C. caribaea* is the only extra-Brazilian species, being registered in Bahamas (Irwin and Barneby 1982) and most of the species are endemic to the “Campos rupestres” (Rando and Pirani, 2011).

According phylogenetic studies based on molecular and morphological data, ser. *Coriaceae* is polyphyletic (Rando et al. 2016). Such authors have proposed the exclusion of *C. roraimae, C. venulosa* and *C. caribaea* in a way that the clade Coriaceae would then comprise only 18 species.

Leaf anatomical characters have been successfuly used in distinct plant families as an addidional tool for the taxonomy (Dianne et al. 2003; Rio et al. 2005; Silva and Potiguara 2008; Martinez-Cabrera et al. 2009; Araújo et al. 2010; Oliveira et al. 2011; Dalvi et al. 2013), including Leguminosae (Lackey 1978; Lersten and Curtis 1994; Crow et al. 1997; Flores-Cruz et al. 2004; Coutinho et al. 2013, 2016; Francino et al. 2015). In the same way, anatomy has proved useful in taxonomical studies in *Chamaecrista* for sect. *Absus, Apoucouita* and *Grimaldia* (Coutinho et al. 2013, 2016; Francino et al. 2015). Among the anatomical characters used in studies in *Chamaecrista,* secretory structures have stood out as a rich source of data for taxonomy. Little is known on the anatomy of set. *Chamaecrista, Caliciopsis* and *Xerocalyx* as only four from sect. *Chamaecrista* and one species from sect. *Xerocalyx* were studied (Francino et al. 2015) while sect. *Caliciopsis* remains unkown.

Taking into consideration the importance of the morphoanatomy for the taxonomy of the *Chamaecrista* and the studies available for the genus, our aim was to performe an anatomical description of species from sect. *Chamaecrista* ser. *Coriaceae*, and to identify data useful to the systematic.

Material and methods

Were studied all taxa of section *Chamaecrista* ser. *Coriaceae* (27 taxa). Thirteentaxa were obtained and fixed in field and the voucher specimens of field collections were deposited in the herbarium of the Universidade do Estado da Bahia (HUNEB-Coleção Paulo Afonso) and herbarium of the Universidade Federal de Viçosa (VIC). Herbarium material were obtained from collections of the following herbaria: HUEFS, VIC, RB, SPF and NYBG. Samples from herbarium were rehydrated (Smith and 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).

Samples petiole (apex, middle or base) and leaf blade were used. The fragments were dehydrated through an ethanol series and embedded in methacrylate (Historesin, Leica Instruments, Heidelberg, Germany). Cross sections 5-7 µm thick were made in a rotary microtome (Spencer 820 American Optical Corporation, Buffalo, NY, USA). Sections were stained with toluidine blue at pH 4.4 (O'Brien and McCully 1981) and mounted in resin (Permount, Fisher Scientific, New Jersey, USA) for structural characterization. Some of the sections were also used in histochemical tests.

Whole leaflets were cleared with 10% sodium hydroxide followed by 20% sodium hypochlorite, rinsed with distilled water, stained with 0.1% basic fuchsin solution in 50% ethanol or 1% aqueous safranin solution, and mounted in gelatin-glycerine (Johansen 1940) for observation of the venom pattern, stomata and trichomes.

The stomata were classified according to Howard (1979); the venation pattern, Hickey (1979) and Ellis et al.(2009); the arrangement of the vascular system of the petiole, Francino et al. (2015); trichomes, Theobald et al. (1979). The description of colleters was obtained of Silva et al. (2017-chapter I) and nectaries were obtained of Silva et al. (2017 chapter II).

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

The histochemical tests were performed using sudan IV for total lipids (Pearse 1980); periodic acid-Schiff reagente, for total polysaccharides (Maia 1979); ruthenium red, for pectins/mucilage, (Johansen 1940); alcian blue, for acid mucopolysaccharides (Pearse 1980); for detect lignin, phloroglucinol, and for detect phenolic compounds, fixation in ferrous sulphate in formalin (Johansen 1940). The slides were mounted in water and/or jelly glycerin-gelatine (Kaiser 1880). 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; Carl Zeiss, Gottingen, Germany).

Results

The morphoanatomic characters analyzed are listed in Table 1. The following venation types were observed in the species (Tab. 1, 2): acrodromous (Fig. 1a), eucamptodromous (Fig. 1b) and actinodromous (Fig. 1c). Acrodromous pattern was observed in eight species, as in *C. lagotois.* Eucamptodromous venation was only reported for *C. caribaea*. All remaining taxa bear actinodromous venation. Vascular endings with tracheids not enlarged were observed in all taxa (Fig. 1d).

All remaining species have amphistomatic leaflets (Fig. 1e, f). Paracytic stomata were the most commun type as they occurred in all species (Fig. 1e, f; Tab. 1, 2). In frontal view, the contour of the epidermal cell wall was straight on the surfaces adaxial and abaxial in 16 studied taxa, as in *C. roraimae* (Fig. 1f). In seven taxa such contour was sinuous on both sides of the leaflets (Fig. 1e) and in four taxa it was straight on the adaxial side of the leaflets and sinuous on the abaxial side (Tab. 1, 2).

Tector trichomes were observed in all species (Fig. 1g), except in *C. aristata, C. burchelli, C. lagotois, C. choriophylla, C. latifolia* and *C. simplifacta*. Trichomes were present in both sides of the leaflet in six taxa or only at the margin of the leaflet and in all its extension in seven taxa (Tab. 2). Trichomes only at the basal region of the margin of the leaflet were noticed in eight taxa (Tab. 2).

The external periclinal wall of the epidermal cells was thick in most species (Fig. 2a-d). All species have uniseriate epidermis on both sides of the leaflet. However, the epidermal cells of the adaxial side were higher than those of the abaxial side in seven taxa, while in the remainining species the epidermal cells on both of the leaflets sides were similar. Papillary epidermis was observed in six taxa (Fig. 2c); idioblasts with phenolic compounds in *C. roraimae, C. cinerascens* and *C. distichoclada* (Fig. 2d).

Chamaecrista roraimae have hypodermis at the abaxial side (Fig. 2a). In 14 taxa idioblasts with phenolic compounds were observed in the mesophyll (Fig. 2a; Tab. 1, 2). Mesophyll dorsiventral cell 1-3 layered is presented in all species (Fig. 2a, b), except *C. simplifacta* that was isobilateral (Fig. 2c).

Nine taxa showed prominent midvein (Fig. 2e; Tab. 1, 2) with a single colateral arc-shaped vascular bundle (Fig. 2a, e). A sheath with monocrystals and fibers associated to the vascular bundles were common to all species. The fibers form a cap on both sides of the leaflets in all species (Fig. 2a, e).

The epidermis is unisseriate in the petiole (Fig. 3a-d) with thick cuticle in almost all species. Tector trichomes and scattered stomata were also observed. The number of the cortical parenchyma layers was variable and monocrystals were common. The vascular system was composed by the main vascularization and accessory bundles on the adaxial side (Fig. 3a-d). Sclerenchymatics fibers are associated to the vascular bundles.

Seven conformation types of the vascular bundles on the petiole were reported: type-II, IV, V, XV, XVII, XVIII and XIX. Types XVII, XVIII and XIX are described herein (Fig. 3a-d; Tab. 1, 2). *Chamaecrista aristata* have the type II. *Chamaecrista mucronata* type IV (Fig. 3a). Type V was observed in nine taxa, as *C. tragacanthoides*

(Fig. 3b). Type XV was reported in *C. venulosa*. Type XVII was observed in nine taxa (Fig. 3c) and consists of a bundle in arc partially surrounded by fibers. *Chamaecrista distichoclada* and *C. rotundata* had type XVIII (Fig. 3d) that is characterized by four vascular bundles fully surrounded by fibers and 2-4 accessory bundles surrounded or not by fibers. *Chamaecrista latifolia* and *C. ulmea* displayed type XIX, which had five main vascular bundles partially surrounded by fibers and two accessory bundles.

Nectaries were present only at the petiole in 20 taxa while on both petiole and rachis, between or above the pairs of leaflets, in seven taxa (Tab. 2). Three forms of nectaries (Tab.1, 2): patelliform in 17; verruciform short-stalked only in *C. simplifacta;* and cupuliform in nine taxa. Nectaries were anatomically similar: vascularized by xylem and phloem and made up by subnectary parenchyma and nectary parenchyma. In many species fibers were associate with vascular tissue and, in others, there was sheath of sclereids.

Non-vascularized colleters were found in 20 studied taxa. Such structures occur at the insertion of the petiole, base of the stipules, bracts/bracteoles, petals and sepals, on the rachis. Colleters are composed by a short-peduncle and a secretory portion. The following types were identified: type I-short-digitiform, type II-long-digitiform, type III-club-shaped and type V-short bottle shaped. Short digitiform colleters were recorded in all taxa; the long-digitiform in five, club-shaped in nine, and short bottle shaped in ten (Tab. 2).

Identification key for species of *Chamaecrista* ser.*Coriaceae*.

1. Nectary on the petiole and rachis..2
1'. Nectary only on petiole...7
2. Acrodromous venation...*C. aristata*
2'. Eucamptodromous or actinodromous venation..3

3. Eucamptodromous venation...4

3’. Actinodromous venation...5

4. Tector trichomes at the basal region of the margin of the leaflet only……..*C. caribaea* var. *caribaea*

4’. Tector trichomes at the margin of the leaflet and in all its extension only...*C. caribaea* var. *inaguensis* and*C. caribaea* var. *lucayana*

5. Cupuliform nectary and hypodermis at the abaxial side of leaflets............*C. roraimae*

5’. Patelliform nectary and absence of hypodermis...6

6. Vascular bundles of the petiole type IV...*C. mucronata*

6’. Vascular bundles of the petiole type V...*C. papillata*

7. Verruciform nectary and isobilateral mesophyll......................................*C. simplifacta*

7’. Cupuliform or patelliform nectary and dorsiventral mesophyll..................................8

8. Cupuliform nectary...9

8’. Patelliform nectary..13

9. Vascular bundles of the petiole type XVII...*C. multinervia*

9’. Vascular bundles of the petiole type V, XV or XVIII..10

10. Vascular bundles of the petiole type XV...*C. venulosa*

10’. Vascular bundles of the petiole type V or XVIII..11

11. Vascular bundles of the petiole type V...12

11’. Vascular bundles of the petiole type XVIII……....*C. rotundata* var. *grandistipula*

12. Epidermal papillae on the abaxial side of the leaflet……………………………………………*C. tragacanthoides* var. *tragacanthoides*

12’. Absence ofepidermal papillae on the abaxial side of the leaflets...........*C. potentilla*

13. Acrodromous venation...14

13’. Actinodromous venation...18

14. Tector trichomes absent..15

14’. Tector trichomes only at the margin of the leaflet and in all its extension………………………………………………………………...*C. rossicorum*

15. Vascular bundles of the petiole type XVII..16

15’. Vascular bundles of the petiole type V ou XIX...17

16. Contour of the epidermal cell wall straight on both sides of the leaflet……………………………………………………………….*C. choriophylla*

16’. Contour of the epidermal cell wall sinuous on both sides of the leaflets...*C. lagotois*

17. Conformation of vascular bundles of the petiole type V...........................*C. burchelli*

17'. Conformation of vascular bundles of the petiole type XIX........................*C. latifolia*

18. Idioblasts with phenolic compounds in the epidermis of the leaflet.........................19

18'. Absence ofIdioblasts with phenolic compounds in the epidermis of the leaflet......20

19. Conformation of vascular bundles of the petiole type V.......................*C. cinerascens*

19'. Conformation of vascular bundles of the petiole type XVIII............*C. distichoclada*

20. Idioblasts with phenolic compounds in the mesophyll...21

20'. Absence ofIdioblasts with phenolic compounds in the mesophyll................*C. ulmea*

21. Conformation of vascular bundles of the petiole type XVII.........................*C. anceps*

21'. Conformation of vascular bundles of the petiole type V ou XVIII..........................22

22. Conformation of vascular bundles of the petiole type V..23

22'. Conformation of vascular bundles of the petiole type XVIII..*C. rotundata* var. *rotundata* e *C. rotundata* var. *interstes*

23. Epidermal papillae on the abaxial side of the leaflets.............................*C. olesiphylla*

23'. Absence ofEpidermal papillae on the abaxial side of the leaflets............................24

24. Tector trichomes only at the margin of the leaflet and in all its extension...*C. tragacanthoides* var. *rasa*

24'. Tector trichomes only in the basal region of the margin of the leaflet…………………………………………………………………...*C. cardiostegia*

Discussion

Some of the morphoanatomical characters described here have proved useful to the taxonomy. The pattern of venation allowed grouping few species and seems to be promissor for future taxonomical studies. The actinodromous venation was observed in the majority of the studied taxa. Only *C. caribaea* (series *Coriacea*) that is endemic from Caribe has eucamptodromous venation. In the molecular phylogeny *C. caribaea, C. roraimae* and *C. venulosa* have been emerged in a distinct lineage from the other species of the series *Coriaceae* and, they must be excluded from this group (Rando et al. 2016), thus the venation type reinforce such interpretation for *C. caribaea*. *Chamaecrista lagotois, C. simplifacta, C. choriophylla, C. latifolia* and *C. rossicorum* exhibit acrodromous venation and have been considered as closely related by Irwin and

Barneby (1982) and Rando et al. (2013, 2016), these can be distinguished by the size and form of the leaflets, stipules, and pedicels (Rando et al. 2013).

Tracheids not-enlarged were observed in all species, instead of the typical enlarged tracheids observed in species from sections *Absus, Grimaldia* and *Apoucouita* (Coutinho et al. 2012, 2016; Francino et al. 2015).

The "campos rupestres” is the environment where most of the studied species occur. Thus, some of anatomical characters observed, such as thick cuticle and outer epidermal cell wall, high density of the tector trichomes, and both vascularization and mechanical tissues highly developed may be related to the environmental conditions (Fahn 1986; Dickison 2000; Evert 2006).

Amphistomatic leaflets and paracytic stomata are common in the species studied and also in other species of *Chamaecrista,* which were reported for species from sect. *Absus, Chamaecrista, Xerocalyx* and *Grimaldia* (Coutinho et al. 2013; Francino et al. 2015; Silva et al. Unpublished data). Amphistomatic leaflets have been argued as an adaptation to xeric environments (i.e. such as the "campos rupestres”) as they may contribute to the photosynthetic performance (Fahn 1990).

In *Chamaecrista*, the secretory idioblasts vary in relation to the secreted compounds and the position they occupy in leaf blade. Phenolic compounds may act by inhibiting the action of ultraviolet rays as well as protection of the leaves against herbivors. The pattern of idioblast distribution, as well the composition of secretion was useful for taxonomic approache in Caesalpinioideae (Lersten and Curtis1993). As not all species presented idioblasts with phenolic compounds, such character was useful to distinguish species.

Papillose cells were reported for only six taxa studied which reinforces that such character is incommon in *Chamaecrista* (Francino et al.2015) and may be used for distinguishing species (Coutinho et al. 2016). Likewise, hypodermis is not frequent in the studied species, being only observed in *C. roraimae* and species of the *Absus*, subsect. *Baseophyllum* and *Otophyllum* (Coutinho et al. 2013; Francino et al. 2015). Hypodermis may protect the leaf photosynthetic tissue especially when there are nutrient deficiency and excessive light, which can limit the metabolism (Feller 1996).

Dorsiventral mesophyll was observed in most species of *Chamaecrista* studied as well as in other species of the genus and Caesalpinioideae species (Metcalfe and Chalk 1950; Coutinho et al. 2013; Francino et al. 2015). On the other hand, isobilateral mesophyll was exclusive to *C. simplifacta*, a character also reported for the sect. *Absus* (Coutinho et al. 2013; Francino et al. 2015).

The arrangement of the vascular bundles on the petiole was useful for taxonomy and reported for other families (Crow et al.1997; Gomes et al. 2005; Araújo et al. 2010; Oliveira et al. 2011). Although such pattern was not usefull to group species, it allowed distinguishing species.

In *Chamaecrista*, the prismatic crystals are very common and they form a sheath lining the inner layer around the vascular tissues in the petiole or around the midvein (Coutinho et al. 2013; Francino et al. 2015). Such crystals may protect the cells against herbivorous or keep excess calcium ions out of the cytosol (Cutter 1986; Zindler-Frank 1987). The “campos rupestres” commonly have soils with high aluminium and iron oxides concentration (Benites et al. 2007; Vincente and Meguro 2008).

Colleters are common in *Chamaecrista*, however recently described for the genus (Coutinho et al. 2015; Francino et al. 2015; Silva et al. 2017) and even varying the morphoanatomy its function is probably the same. Colleteres were related to protection meristems and young leaves and flowers against both dehydration and pathogens attack (Thomas and Dave 1989; Rocha et al. 2009).

The nectaries are morphoanatomically similar to others reported for *Chamaecrista* as well as other genera from Caesalpinioideae (Bhattachryya and

Maheshwari 1971; Elias 1983; Francino et al. 2006, 2015; Paiva and Machado 2006; Coutinho et al. 2012), and they may be useful for phylogenetic purposes. Additionally, it was suggested that in *Chamaecrista* the nectaries were present in the ancestral of the genus with a posterior losses in the sect. *Grimaldia*, *Absus*, subsect. *Absus* and sect. *Chamaecrista* ser. *Bauhinianae* (Conceição et al. 2009). In the sect. *Grimaldia* and *Absus* the secretory trichomes were interpreted as a new strategy of defense, which arised as a substitution to the nectaries(Conceição et al. 2009).

The position and type of nectaries were useful for distinguishing the studied species and were used at the identification key. The patelliform was the most common type of nectary in the series *Coriaceae*. The simarity between the types of nectaries in *C. caribaea, C. roraimae* and *C. venulosa* is an additonal charater that could be used to justify the exclusion of these species from the ser. *Coriaceae*, according the molecular phylogeny (Rando et al. 2009). The verruciforme type obseved in the presente study in *C. simplifacta* is an important data as it was also reported for *C. debilis* from the monospecific sect. *Absus* subsect. *Otophyllum* (Francino et al. 2015).

Thickening of outer epimdermal cell wall and cuticle, high density of tector trichomes, vascularization and sclerenchyma highligly developed were pointed out as xeromorphic characters for the studied species. Uniseriate epidermis, paracytic stomata, amphistomatic leaflets, tracheids not-enlarged and petiole with accessory bundles and associated fibers are common characteristics in species of ser. *Coriaceae*. The arrangement of the vascular tissue in the petiole, presence of hypodermis, papillose cells and distribution of tector trichomes and the phenolic idioblasts were useful to distinguish species. The anatomical differences observed in *C. caribaea*, *C. roraimae* and *C. venulosa* may be useful to strengthen the proposal of the exclusion of such species from the ser. *Coriaceae* in order to make such a series a monophyletic group.

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Supplementary Material

Species of *Chamaecrista* studied.

Vouchers are housed in the herbaria of the Universidade Estadual de Feira de Santana (HUEFS), Universidade do Estado da Bahia (HUNEB), New York Botanical Garden (NY), Jardim Botânico do Rio de Janeiro (RB), Universidade de São Paulo (SPF) and Universidade Federal de Viçosa (VIC).

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Table 1. Anatomical characters of the species of ser. *Coriaceae*

Nº	Anatomical characters
1.	Venation acrodromous: absent (0)/ present (1)
2.	Venation actinodromous: absent (0)/ present (1)
3.	Venation eucamptodromous: absent (0)/ present (1)
4.	Contour of the epidermal cell wall straight on the adaxial side of the leaflets and sinuous on the abaxial side: absent (0)/ present (1)
5.	Contour of the epidermal cell wall sinuous on both sides of the leaflets: absent (0)/ present (1)
6.	Contour of the epidermal cell wall straight on both sides of the leaflets: absent (0)/ present (1)
7.	Tector trichomes on both sides of the leaflet: absent (0)/ present (1)
8.	Tector trichomes only at the margin of the leaflet and in all its extension: absent (0)/ present (1)
9.	Tector trichomes only in the basal region of the margin of the leaflet: absent (0)/ present (1)
10.	Epidermal cells of the adaxial side higher than those of the abaxial side: absent (0)/ present (1)
11.	Epidermal cells of similar height on both sides: absent (0)/ present (1)
12.	Epidermal papillae on the abaxial side of the leaflets: absent (0)/ present (1)
13.	Idioblasts with phenolic compounds in the epidermis of the leaflet: absent (0)/ present (1)
14.	Idioblasts with phenolic compounds in the mesophyll: absent (0)/ present (1)
15.	Mesophyll type: isobilateral (0)/ dorsiventral (1)
16.	Hypodermis at the abaxial side of leaflets: absent (0)/ present (1)
17.	Prominent midvein: absent (0)/ present (1)
18.	Conformation of vascular bundles of the petiole type II: absent (0)/ present (1)
19.	Conformation of vascular bundles of the petiole type IV absent (0)/ present (1)
20.	Conformation of vascular bundles of the petiole type V: absent (0)/ present (1)
21.	Conformation of vascular bundles of the petiole type XV: absent (0)/ present (1)
22.	Conformation of vascular bundles of the petiole type XVII: absent (0)/ present (1)
23.	Conformation of vascular bundles of the petiole type XVIII: absent (0)/ present (1)
24.	Conformation of vascular bundles of the petiole type XIX: absent (0)/ present (1)
25.	Nectary only on petiole: absent (0)/ present (1)
26.	Nectary on the petiole and rachis: absent (0)/ present (1)
27.	Nectary patelliform: absent (0)/ present (1)
28.	Nectary verruciform short-stalked: absent (0)/ present (1)
29.	Nectary cupuliform: absent (0)/ present (1)
30.	Colleter type I: absent (0)/ present (1)
31.	Colleter type II: absent (0)/ present (1)
32.	Colleter type III: absent (0)/ present (1)
33.	Colleter type V: absent (0)/ present (1)

Table 2. Matrix the characters morphoanatomical analized is species of ser. *Coriaceae*

Taxa	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**
ari	1	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	1	0	0	1	0	1	1
anc	0	1	0	0	0	1	0	0	1	1	0	0	0	1	1	0	1	0	0	0	0	1	0	0	1	0	1	0	0	0	0	0	0
bur	1	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0
car	0	1	0	1	0	0	0	0	1	0	1	0	0	1	1	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0
carc	0	0	1	0	0	1	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0	1	1	0	0	1
cari	0	0	1	0	1	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0	1	1	0	0	1
carl	0	0	1	0	1	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0	1	1	0	0	1
cin	0	1	0	0	0	1	0	0	1	1	0	1	1	1	1	0	0	0	0	1	0	0	0	0	1	0	1	0	0	1	0	1	1
cho	1	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	1	0	0	1	0	1	0	0	0	0	0	0
ros	1	0	0	0	0	1	0	1	0	0	1	0	0	0	1	0	1	0	0	0	0	1	0	0	1	0	1	0	0	0	0	0	0
lat	1	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	1	1	0	1	0	0	1	0	1	0
dis	0	1	0	0	0	1	0	1	0	0	1	0	1	1	1	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0
lag	1	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	1	0	0	1	0	1	0	0	0	0	0	0
muc	0	1	0	0	1	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	1	0	0	1	0	1	1
mul	0	1	0	0	0	1	1	0	0	1	0	1	0	1	1	0	0	0	0	0	0	1	0	0	1	0	0	0	1	1	1	0	0
ole	0	1	0	0	0	1	1	0	0	0	1	1	0	1	1	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0
pap	0	1	0	0	0	1	1	0	0	0	1	1	0	1	1	0	1	0	0	1	0	0	0	0	0	1	1	0	0	1	1	1	0
pot	0	1	0	0	0	1	0	1	0	0	1	0	0	1	1	0	0	0	0	1	0	0	0	0	1	0	0	0	1	1	0	1	0
ror	0	1	0	0	0	1	0	1	0	0	1	0	1	1	1	1	0	0	0	1	0	0	0	0	0	1	0	0	1	1	0	1	0
rotr	0	1	0	0	0	1	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	0	0	0
roti	0	1	0	0	0	1	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	0	0	0
rotg	0	1	0	0	1	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0	0	1	0	1	0	0	0	1	1	1	0	0
sim	1	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	1	0	1	0	1	1
trat	0	1	0	0	0	1	1	0	0	0	1	1	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	1	1	1	0	1
trar	0	1	0	1	0	0	0	1	0	0	1	0	0	1	1	0	0	0	0	1	0	0	0	0	1	0	1	0	0	1	0	0	1
ulm	0	1	0	0	1	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	1	0	0	1	0	0	0
ven	0	1	0	0	1	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	0	1	0	0	0	1	1	0	0	1

ari: *C. aristata;* anc: *C. anceps;* bur:*C. burchelli;* car: *C. cardiostegia;* carc: *C. caribaea* var. *caribaea;* cari: *C. caribaea* var. *inaguensis;* carl: *C. caribaea* var. *lucayana;* cin: *C. cinerascens;* cho: *C. choriophylla;* ros: *C. rossicorum;* lat: *C. latifolia;* dis: *C. distichoclada;* lag: *C. lagotois;* muc: *C. mucronata;* *mul: *C. multinervia;* ole: *C. olesiphylla;* pap: *C. papillata;* pot: *C. potentilla;* ror: *C. roraimae;* rotr: *C. rotundata;* roti: *C. rotundata* var. *interstes;* rotg: *C. rotundata* var. *grandistipula;* sim: *C. simplifacta;* trat: *C. tragacanthoides;* trar: *C. tragacanthoides* var. *rasa;* ulm: *C. ulmea;* ven: *C. venulosa*..

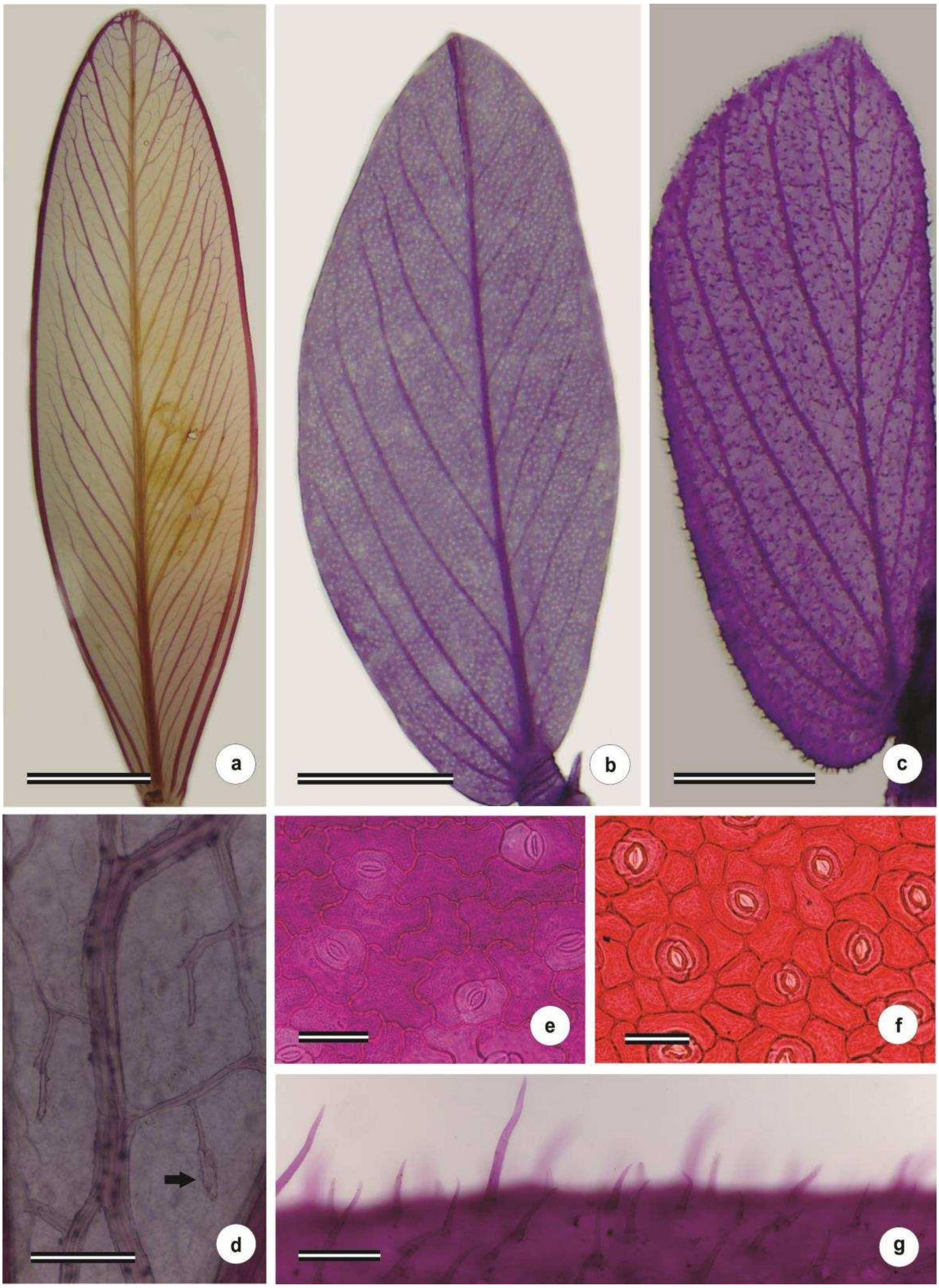

Fig 1 Venation and epidermis in the species of *Chamaecrista* studied. **a** *C. lagotois:* venation acrodromous. **b** *C. caribaea* var. *caribaea:* venation eucamptodromous. **c** *C. venulosa:* venation actinodromous. **d** *C. venulosa:* vascular endings (arrow). **e** *C. venulosa:* epidermal cells with anticlinal wall of sinuous contour in the adaxial side and stomata paracytic. **f** *C. roraimae*: epidermal cells with anticlinal wall of straight contour in the abaxial side and stomata paracytic. **g** *C. olesiphylla*: tector trichomes. *Scale bars* **a**1cm, **b, c** 0,25cm, **d, g** 100µm, **e**, **f** 50µm

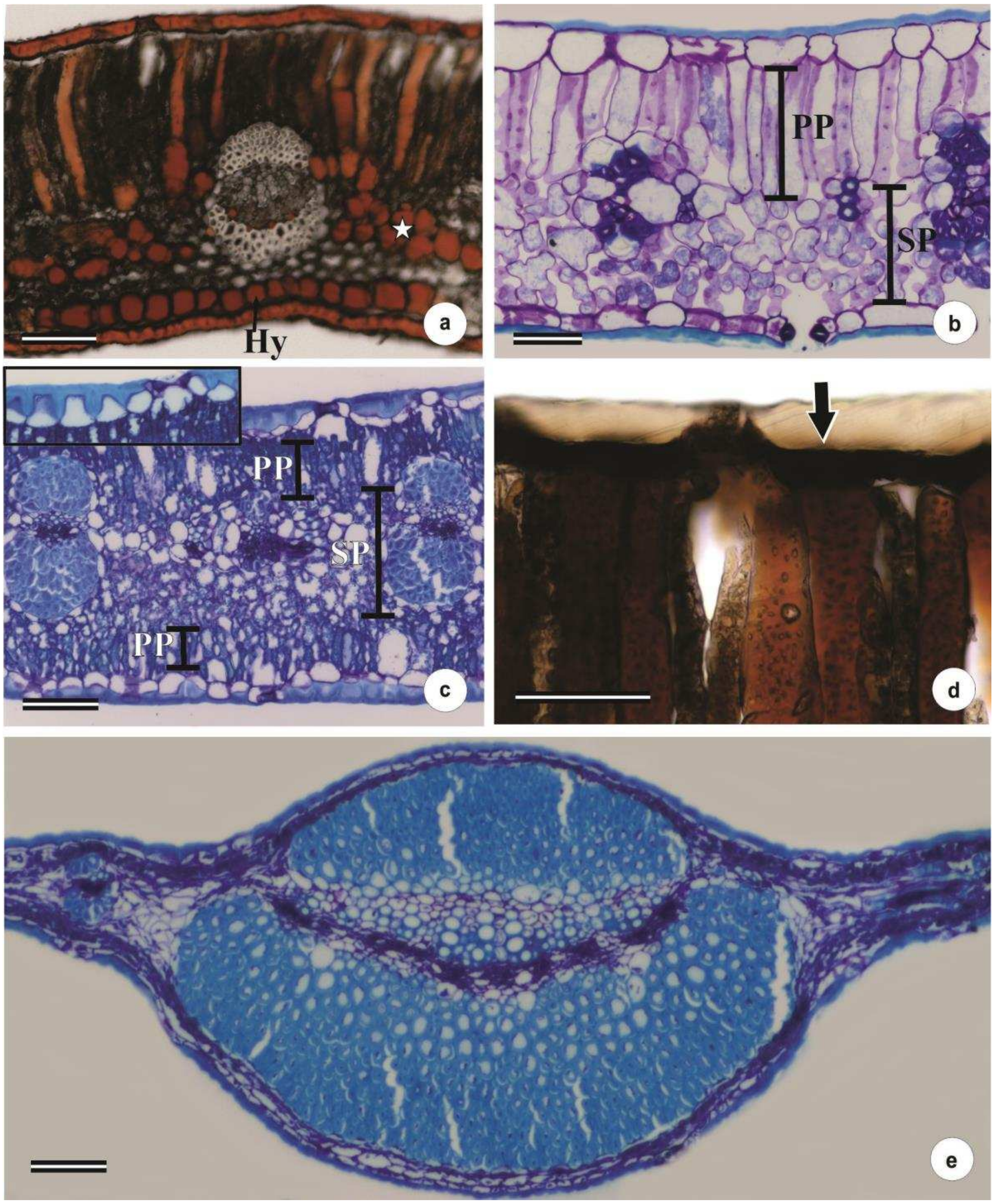

Fig. 2 Leaf blade of species of *Chamaecrista* studied. **a** *C. roraimae:* hypodermis in the abaxial side and idioblasts in the chlorenchyme with phenolic compounds. **b** *C. caribaea* var. *lucayana:* dorsiventral mesophyll and epidermal cells of the adaxial side higher than the cells from the abaxial side. **c** *C. simplifacta:* isobilateral mesophyll and detailof the papillose cells. **d** *C. roraimae:* idioblasts with phenolic compounds in the epidermis (arrow). **e** *C. rossicorum:* prominent midvein. (Hy, hypodermis, PP, palisade parenchyma; SP, spongy parenchyma; Fi, fibers). *Scale bars* **a**-**c**-**e** 100µm, **b**-**d** 50µm

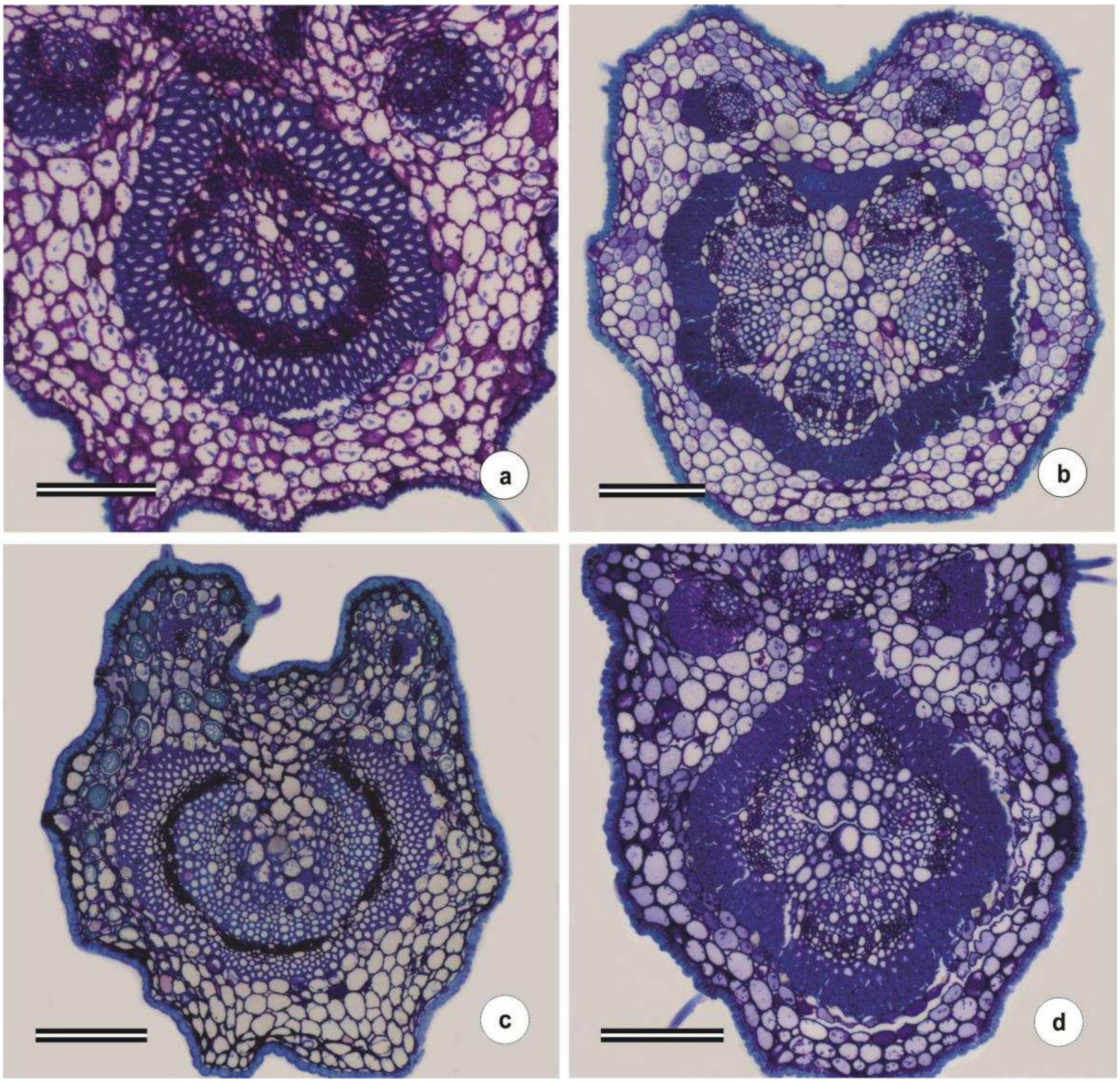

Fig. 3 Type of conformation of vascular bundles of the petiole in the species of *Chamaecrista* studied. **a** *C. mucronata:* type IV. **b** *C. tragacanthoides* var. *rasa:* type V. **c** *C. caribaea* var. *lucayana:* type XVII. **d** *C. rotundata* var. *interstes*: type XVIII. *Scale bars* **a**-**d** 200µm, **b**-**c** 300µm

3. CONCLUSÕES GERAIS

De acordo com a posição, morfologia, anatomia e composição dos exsudatos, as estruturas secretoras presentes na base das estipulas, brácteas/bractéolas, sépalas e pétalas, na raque entre os pares de folíolos e na inserção do pecíolo com o caule nas espécies estudadas das seções *Chamaecrista* e *Caliciopsis* são coléteres.

Estas estruturas secretoras são comuns e diversas nos representantes dessas seções e cinco tipos de coléteres não vascularizados foram encontrados, sendo o curto e longo digitiforme os mais comuns. Registramos dois novos tipos de coléteres para a seção *Chamaecrista*, além disso, o tipo piriforme é inédito para o gênero *Chamaecrista*. Adicionalmente, o registro de coléteres para a seção *Caliciopsis* está sendo feito pela primeira vez nesse estudo. Polissacarídeos, pectinas, lipídeos, compostos fenólicos e proteínas foram detectados na secreção dos coléteres.

As estruturas secretoras presentes no pecíolo e raque das espécies das seções *Chamaecrista*, *Caliciopsis* e *Xerocalyx* são nectários de acordo com a morfologia, estrutura anatômica e reação positiva para o açúcar. Quatro formas foram registradas, sendo cupuliforme e pateliforme as mais comuns. Em *Caliciopsis* há apenas o morfotipo cupuliforme; em *Xerocalyx*, cupuliforme e pateliforme, por outro lado na seção *Chamaecrista* todas as formas foram registradas. Os nectários das espécies da seção *Caliciopsis* nunca haviam sido estudados, assim nossos dados são inéditos para o grupo.

Anatomicamente, os nectários são semelhantes aos observados em outras espécies do gênero *Chamaecrista* e são divididos em quatro regiões distintas: epiderme, parênquima nectarífero, parênquima subnectarífero e região vascular. Quanto à secreção, além de polissacarídeos foram detectados lipídios, compostos fenólicos e proteínas.

Observamos similaridades entre as formas dos nectários das três seções estudadas, dando suporte aos estudos de filogenia molecular que mostraram que as seções *Chamaecrista, Caliciopsis* e *Xerocalyx* formam um único clado. Assim, tais dados poderão servir para o entendimento das relações entre as três seções estudadas e amplia a base de dados para trabalhos de cunho evolutivo e filogenético.

Algumas características anatômicas que foram encontradas na maior parte das espécies estudadas são típicas do gênero *Chamaecrista*: estômato paracítico, folíolos anfiestomáticos, mesofilo dorsiventral, traqueídes não alargados, feixes vasculares com fibras associadas e feixes acessórios no pecíolo, além de estruturas secretoras como idioblastos, nectários e coléteres. Quanto aos idioblastos, foram registados somente os fenólicos os quais são incomuns na epiderme e mais abundantes no mesofilo.

Características como parede celular e cutícula espessa, alta densidade de tricomas tectores, vascularização e esclerênquima foram registradas como adaptações ao ambiente em que as espécies vivem. O tipo de conformação dos feixes vasculares do pecíolo, hipoderme, células papilosas e distribuição dos tricomas tectores foram diagnósticos ao nível de espécie.

As diferenças anatômicas observadas em *C. caribaea, C. roraimae* e *C. venulosa* podem ser úteis para fortalecer a proposta de exclusão dessas espécies de *Coriaceae*, a fim de tornar essa série um grupo monofilético.