ÍTALO ANTÔNIO COTTA COUTINHO

ANATOMIA FOLIAR COMO SUBSÍDIO PARA TAXONOMIA DE CHAMAECRISTA MOENCH COM ÊNFASE NA SEÇÃO APOUCOUITA (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.

VIÇOSA
MINAS GERAIS – BRASIL
2015

Ficha catalográfica preparada pela Biblioteca Central da Universidade Federal de Viçosa - Câmpus Viçosa

T
Coutinho, Ítalo Antônio Cotta, 1983-
C871 Anatomia foliar como subsídio para taxonomia de
2015 *Chamaecrista* Moench com ênfase na seção *Apocouita* (Leguminosae - Caesalpinioideae) / Ítalo Antônio Cotta Coutinho. – Viçosa, MG, 2015.
xi, 109f. : il. (algumas color.) ; 29 cm.

Orientador: Renata Maria Strozi Alves Meira.
Tese (doutorado) - Universidade Federal de Viçosa.
Inclui bibliografia.

1. *Caesalpinioideae*. 2. *Apocouita*. 3. *Cassieae*. 4. *Cassiineae*. 5. Fisiologia vegetal. 6. Folhas - Anatomia. 7. Coléter. 8. Nectários. I. Universidade Federal de Viçosa. Departamento de Biologia Vegetal. Programa de Pós-graduação em Botânica. II. Título.

CDD 22. ed. 583.749

ÍTALO ANTÔNIO COTTA COUTINHO

ANATOMIA FOLIAR COMO SUBSÍDIO PARA TAXONOMIA DE *CHAMAECRISTA* MOENCH COM ÊNFASE NA SEÇÃO *APOUCOUITA* (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*.

APROVADA: 26 de fevereiro de 2015.

Aristéa Alves Azevedo

Karina Lucas Barbosa Lopes Mattos

Karina Lucas Barbosa Lopes-Mattos

Flávia Cristina Pinto Garcia

Valdnéa Casagrande Dalvi

Valdnéa Casagrande Dalvi

Renata Maria Strozi Alves Meira

(Orientadora)

A ignorância gera mais frequentemente confiança do que o conhecimento: são os que sabem pouco, e não aqueles que sabem muito, que afirmam de uma forma tão categórica que este ou aquele problema nunca será resolvido pela ciência.

Charles Darwin

“Once we have sequenced all of the relevant plant genomes and have come to realize that such sequence data leaves many questions in organismal biology, unanswered, we may finally appreciate that organisms are valid and fundamental biological units worthy of our attention. Then, morphology may finally be appreciated and respected as a key to the understanding of plant organismal biology”.

Donald R. Kaplan

American Journal of Botany 88(10): 1726. 2001

E o Espírito de Deus o encheu de sabedoria, entendimento, ciência... para criar invenções... Também lhe dispôs o coração para ensinar a outros... e encheu-os de sabedoria do coração, para fazer toda a obra de mestre, até a mais engenhosa... fazendo toda a obra, e criando invenções.

Êxodo 35:31-35

Àquela que há 13 anos decidiu me orientar

Toc toc toc toc toc... sempre na passada rápida e cada vez mais alto.
Ela está chegando, e logo começa a metralhar perguntas:

Que cara é essa meu filho? O que você estava fazendo até agora?
Por que ainda não fez isso ou aquilo? Ainda temos dinheiro de projeto?
Já mandou os documentos par FUNARBE/CNPq/FAPEMIG/CAPES/SISBio?
Está tudo pronto? Tudo dominado?
E a clássica: TEM CAFÉ???
Ou mesmo atendendo ao telefone: “O que eu tenho que falar mesmo”? Ué Renata: Alô?
Sem dúvida a que mais sentirei falta é: Como você está? Com um tom sério de preocupação, de alguém que realmente se importa.

Ou pode ser ainda uma chuva de exclamações, que são mais preocupantes que as perguntas:
Você não está me convencendo disso! (Talvez mais umas 20 exclamações aqui!!!!!)
Que demora é essa meu filho! Vou cortar seu ponto, hein!
Isso está errado! Faz de novo! Não é assim, não!
Me liga! (mensagem recebida às 6h da matina)
Ítalo, passa na minha sala! (essa sim, gelava a espinha mais que Antártica Subzero!!!!)

Assim defino uma pessoa que é mais que uma orientadora, uma pessoa que durante esses anos tornou-se mais que uma amiga.
Renata, infelizmente você não é perfeita, mas absolutamente ninguém o é! No entanto, saiba que sem sombra de dúvidas, quaisquer de seus defeitos nunca irão apagar o brilhantismo de sua dedicação como orientadora, de seu potencial como pesquisadora e de sua sincera amizade.

Se fosse para recomeçar do zero, o faria tudo de novo, com muito prazer.
Não escolheria nenhuma outra pessoa para me orientar.
Que os próximos anos sejam de colheitas ainda melhores do que já tivemos.
No mais, ficará sempre a saudade de dividir a sala com você.
Meu muito obrigado.

AGRADECIMENTOS

Chego ao final de mais uma fase, e não há como não agradecê-Lo por isso. Agradeço a Deus, pelo dom da vida, pelo sustento, pela compreensão, pela paciência, pelo cuidado, pela provisão, pelo consolo, pelo renovo e pelo amor dispensado.

À Universidade Federal de Viçosa e ao Departamento de Biologia Vegetal, onde me fiz bacharel, mestre a agora doutor.

Ao CNPq, CAPES, FAPEMIG e ao projeto Floresta Escola/UNESCO-HidroEx pela concessão das bolsas e/ou auxílio financeiro à pesquisa.

A minha família, meus pais, Coutinho e Penha e meus irmãos, Marcos, Sara e Tiago pelo amor e pelas orações. Em especial a Sarinha, que nesses últimos anos tem sido benção na minha vida.

Aos meus familiares que mesmo longe, estão sempre perto. Não tenho tios ou tias, tenho pais e mães adotivos, não tenho primos, tenho irmãos. Agradeço pelo convívio e pela amizade.

Às professoras Aristéa, Luzimar Campos e Marília Ventrella, pela amizade, companheirismo e ensino.

Aos amigos do laboratório, da IPV (Igreja Presbiteriana de Viçosa) e do REC (Real English Center), os quais fizeram toda diferença durante esses anos, em especial a Pamela Torres, companheira de república.

Agradeço em especial à amizade de duas grandes amigas, Vanessa Terra e Valdnéa Dalvi, que foram um achado precioso. E também Carlota (Carla Feio) que tem sido companheira nessa fase tão complicada da vida.

Às companheiras de coleta Isabel Silva e Valéria Fernandes e ao amigo Marc Guesdon, pelas infindáveis risadas em campo.

A todos os que riram ao choraram comigo no campo.

A todos que de certa forma direta ou indiretamente contribuíram para o desenvolvimento deste trabalho.

SUMÁRIO

RESUMO

COUTINHO, Ítalo Antônio Cotta, D. Sc., Universidade Federal de Viçosa, fevereiro de 2015. **Anatomia foliar como subsídio para taxonomia de Chamaecrista Moench com ênfase na seção Apoucouita (Leguminosae - Caesalpinioideae)**. Orientadora: Renata Maria Strozi Alves Meira. Coorientadora: Adilva de Sousa Conceição.

Chamaecrista Moench (Leguminosae, Caesalpinioideae, Cassieae, Cassiineae) inclui mais de 330 espécies. A presença de estruturas secretoras é um dos parâmetros utilizados para a circunscrição das seis seções do gênero, no entanto, tal circunscrição não é corroborada pelos estudos filogenéticos. A anatomia foliar de Chamaecrista tem se mostrado uma importante ferramenta, corroborando inclusive as novas circunscrições taxonômicas baseadas em dados moleculares. Dentre as várias estruturas secretoras descritas para o gênero Chamaecrista, coléteres foram mencionados em algumas das espécies estudadas. Entretanto um estudo comparativo da diversidade e importância taxonômica dessas estruturas no gênero Chamaecrista ainda não foi realizado. Dentre as seções de Chamaecrista, poucos estudos têm investigado a seção Apoucouita que inclui espécies com glândulas no pecíolo/raque e no racemo denominadas de nectários. No entanto, tal caracterização demanda estudos complementares como análise da presença de açúcar e análises histoquímicas. Embora estudos moleculares tenham se mostrado úteis para a separação e delimitação de grupos taxonômicos, caracteres morfológicos são necessários para reconhecimento das espécies tanto em campo quanto em herbário e nestes casos a morfoanatomia foliar é uma alternativa promissora. Os materiais foram processados conforme metodologia usual de microscopia de luz e varredura utilizados em estudos anatômicos. O trabalho foi organizado em três capítulos. No capítulo I a presença e a diversidade de coléteres em folhas e flores em desenvolvimento de espécies do gênero Chamaecrista, foram avaliadas. Foram descritos seis tipos de coléteres: “club-shaped”, “racket-shaped”, “long bottle-shaped”, “short bottle-shaped”, “long digitiform” e “short digitiform”. As análises histoquímicas confirmaram a presença de proteínas totais, polissacarídeos totais, mucilagens e lipídeos na secreção dos coléteres. O tipo e posição dos coléteres corroboram as novas circunscrições com base em estudos moleculares propostas para Chamaecrista. No capítulo II procedeu-se o estudo anatômico das glândulas do pecíolo/raque para verificar se tais estruturas correspondem a nectários como descrito na literatura. Foram observados 13 tipos de nectários extraflorais (NEF) para Chamaecrista, os quais podem ser impressos, sésseis ou pedunculados, com superfície secretora côncava, plano/truncada ou convexa. Embora variações morfológicas tenham sido observadas, a estrutura anatômica e a composição química da secreção foram similares para todos os tipos de NEF. A formação de periderme de cicatrização em NEFs mais velhos é um fato inédito para Chamaecrista, uma vez que não foi relatada para as outras seções do gênero. O capítulo III aborda a morfoanatomia das folhas de espécies de C. seção Apoucouita com vistas verificar a utilidade dessas características para fins taxonomicos. O arranjo do sistema vascular no pecíolo/raque, tipo de mesofilo, idioblastos mucilaginosos na face adaxial e abaxial da epiderme, contorno das paredes periclinais das células epidérmicas (reto na adaxial e sinuoso na abaxial) e posição e tipo de estômato, (hipoestomática e paracítico-laterocíclico, respectivamente) foram caracteres comuns a todas as espécies da seção Apoucouita. A presença e posição de papilas na epiderme e a posição e tipo de NEF são caracteres promissores quanto à taxonomia de algumas das espécies do gênero.

ABSTRACT

COUTINHO, Ítalo Antônio Cotta, D. Sc., Universidade Federal de Viçosa, February 2015. **Leaf anatomy as an additional tool to the taxonomy of Chamaecrista Moench with emphasis on section Apoucouita (Leguminosae - Caesalpinioideae)**. Advisor: Renata Maria Strozi Alves Meira. Co-advisor: Adilva de Sousa Conceição.

Chamaecrista Moench (Leguminosae, Caesalpinioideae, Cassieae, Cassiineae) includes more than 330 species. The presence of secretory structures is one of the parameters used for the circumscription of the six sections of the genus. However, such circumscription is not supported by the phylogenetic studies. Leaf anatomy of Chamaecrista has proved an important tool to the new taxonomic circumscription based on molecular data. Among the secretory structures described for Chamaecrista, colleters have been mentioned in some species. However, a comparative study of the diversity and taxonomic importance of such structures for Chamaecrista has not yet been performed. Among the sections of Chamaecrista, few studies have focused on sect. Apoucouita, a section with species displaying glands on the petiole/rachis and on the raceme. Such glands have been called extrafloral nectaries (EFN), even though, no additional studies such as the analysis of the presence of sugars and histochemical tests have been performed to confirm if such glands are in fact EFNs. Although molecular studies have proved useful in the circumscription of taxonomic groups, morphological characters are necessary for recognizing species in the field and also when dried material is analyzed and in both cases, leaf anatomy is a promising alternative for the recognition of species. For this study, standard anatomical techniques for light and scanning electron microscopy were carried out. The present study is organized in three chapters. The presence and diversity of colleters on developing leaves and flowers of Chamaecrista is described in Chapter I. Six types of colleters were described: “club-shaped", "racket-shaped", "long bottle-shaped", "short bottle-shaped", "long digitiform" and "short digitiform". The histochemical analyses confirmed the presence of total proteins, total polysaccharides, mucilage and lipids in the secretion of the colleters. The type and position of colleter supported new circumscriptions based on molecular studies proposed for the Chamaecrista. The anatomical study of the petiole/rachis glands in order to verify if such structures are in fact nectaries, as described in the literature, is given in Chapter II. It was observed 13 types of ENFs for Chamaecrista. Such EFNs may be impressed, sessile or stalked, with secretory surface concave, flat/truncate or convex. Although morphological variations were observed, the anatomical structure and chemical composition of the secretion was similar to all types of EFNs. The formation of a wound-healing periderm in older EFNs is a novelty for Chamaecrista, as such was not reported for the other sections of the genus. The leaf morphoanatomy of C. sect. Apoucouita with the intent of using such characters in the taxonomy is given in Chapter III. The arrangement of the vascular system in the petiole/rachis, type of mesophyll, mucilage idioblasts in the adaxial and abaxial epidermis, outline of the anticlinal walls of the epidermal cells (straight on the adaxial side and sinuous on the abaxial side) and the position and type of stomata (hipostomatic leaves and paracytic laterocyclic, respectively) were characters common to all species from sect. Apoucouita. The presence of papillae on the epidermis as well as the position and type of extrafloral nectaries are promising characters regarding the taxonomy of some species of Chamaecrista.

1. INTRODUÇÃO GERAL

Leguminosae é uma das famílias mais importantes dentre as plantas com flores, amplamente distribuídas nas regiões tropicais, considerada a terceira maior família dentre as Angiospermas, compreendendo mais de 720 gêneros e 19.300 espécies (Lewis, 2005). Está família é tradicionalmente composta por três subfamílias: Papilionoideae, Caesalpinioideae e Mimosoideae (Polhill et al., 1981; Lewis, 1987), embora a filogenia molecular não corrobore a monofilia desses três grupos (Lewis, 2005).

O gênero Chamaecrista Moench (Leguminosae-Caesalpinioideae) está inserido na tribo Cassieae subtribo Cassiineae. A consolidação de Chamaecrista como um gênero foi feita por Irwin e Barneby (1982), quando dividiram o gênero Cassia em três: Cassia, Senna e Chamaecrista, os quais atualmente compõem a subtribo Cassiineae (Irwin e Barneby, 1982). Chamaecrista inclui mais de 330 espécies (Conceição et al., 2001, 2008; Gereau and Walters, 2003; Rando et al., 2013) com cerca de 1/3 do gênero endêmico ao Brasil, sendo 7% das espécies registradas para a região Amazônica (Lewis, 2005).

As espécies de Chamaecrista estão organizadas em seis seções: Apoucouita, Absus, Caliciopsis, Chamaecrista, Grimaldia e Xerocalyx (Irwin and Barneby, 1982). A presença de estruturas secretoras, como nectários extraflorais e tricomas, é um dos parâmetros utilizados para a circunscrição destas seções (Irwin e Barneby, 1982; Conceição et al., 2009; Francino, 2010; Coutinho et al., 2012). Tal tratamento taxonômico das seções com base apenas em caracteres morfológicos, não é corroborada pelos estudos filogenéticos baseados em dados moleculares (Conceição et al., 2009), os quais demonstram somente a sect. Apoucouita e Xerocalyx são monofiléticas.

Nos últimos dez anos, o gênero Chamaecrista tem sido foco de vários trabalhos abordando a anatomia (Francino et al., 2006; Silva, 2012; Coutinho et al., 2012, 2013, 2015; Meira et al., 2014, biologia molecular (Conceição et al., 2008, 2009), taxonomia (Camargo and Miotto, 2004; Rando, 2009; Rando and Pirani, 2011; Rando et al., 2013; Torres et al., 2011; Dantas e Silva, 2013), ecologia (Nascimento e Del-Claro, 2007, 2010) ou importância econômica (Morris, 2012).

A anatomia foliar de Chamaecrista tem se mostrado uma importante ferramenta na identificação das espécies, corroborando inclusive as novas circunscrições taxonômicas baseadas em dados moleculares (Coutinho et al. 2013; Silva, 2012; Francino et al. 2015). Dentre as várias estruturas secretoras descritas para o gênero Chamaecrista (Coutinho et al. 2012; 2013, Silva 2012; Francino et al. 2015), coléteres foram mencionados em algumas das espécies estudadas.

Coléteres são estruturas encontradas geralmente na face adaxial de estípulas e brácteas e produzem uma substância pegajosa composta de mucilagem ou uma mistura de mucilagem e resina, a qual recobre gemas foliares e florais, exercendo função protetora, principalmente contra dessecação (Foster, 1942; Fahn and Benouaiche, 1979; Paiva and Machado, 2006a; Barreiro and Machado, 2007; Sheue et al., 2012; Mayer et al., 2013). Poucos estudos em Leguminosae, assim como no gênero Chamaecrista, reportaram a presença de coléteres (Paiva and Machado, 2006a; b; De-Paula and Oliveira, 2007; Paiva, 2009; Coutinho et al., 2013), de forma que um estudo comparativo da diversidade e importância taxonômica dessas estruturas no gênero Chamaecrista ainda não foi realizado.

No que se refere à seção Apoucouita, nenhuma atenção nenhuma atenção tem sido dada desde a última revisão para a seção, feita na forma de um estudo suplementar a quase quatro décadas (Irwin e Barnerby, 1977). Para identicação das 21 espécies (26 táxons, incluindo as variedades) da seção Apoucouita, caracteres como tamanho do pecíolo/raque, número de pares de folíolos, forma do folíolo e posição dos nectários foliares são amplamente utilizados (Irwin e Rogers, 1967; Irwin e Barneby, 1977, 1982; Barneby, 1999). A identificação das variedades é uma tarefa difícil, sendo as chaves baseadas em parâmetros arbitrários e que são plásticos facilmente modificados pelo ambiente, tais como tamanho do pulvínulo, a forma do ápice do foliólulo e presença/ausência de tricomas tectores. Dessa forma, são necessários estudos complementares que dêem suporte à taxonomia clássica, isto é, aquela baseada apenas em caractéres macro morfológicos.

A seção Apoucouita difere das demais seções por compreender predominantemente espécies arbóreas das Florestas Amazônica e Atlântica (Irwin e Barneby, 1977; Conceição et al. 2009). Nestas espécies, glândulas situadas no pecíolo/raque e no racemo, foram denominadas como nectários com base apenas na morfologia e na topografia. Estruturas secretoras de compostos lipofílicos podem ser morfológica e topograficamente semelhantes aos nectários (Curtis e Lersten, 1978, 1980; Durkee et al., 1984; Jáuregui et al., 2002) e, nestes casos, a caracterização precisa demanda estudos complementares como análise da presença de açúcar e análises histoquímicas.

Nectários são estruturas secretoras especializadas na secreção de néctar, uma solução açucarada composta principalmente por glicose, frutose e sacarose (Fahn, 1979; Bentley e Elias, 1983; Roshchina e Roshchina, 1993; Nicolson et al., 2007). As glândulas presentes no pecíolo/raque das espécies da seção Baseophyllum foram descritas como NEFs por meio de estudos anatômicos minuciosos (Coutinho et al., 2012). Um estudo similar para a seção Apoucouita é desejável para a segura definição das glândulas, fornecendo dado que corroborem estudos sobre a evolução das estruturas secretoras em Chamaecrista.

Dessa forma o presente estudo se propõe (1) a investigar a presença de coléteres no gênero Chamaecrista bem como proceder o estudo morfoanatômicos dessas estruturas e sua a utilidade como caracteres que auxiliem estudos taxonômicos e filogenéticos; (2) a avaliar se as glândulas foliares presentes no pecíolo/rachis de espécies da seção Apoucouita correspondem a nectários extraflorais, procedendo sua caracterização morfoanatomica; (3) a proceder o estudo morfoanatômica das folhas de espécies da seção Apoucouita e averiguar se tais caracteres podem ser utilizados na separação de espécies e variedades.

O presente trabalho encontra-se organizado sob a forma de artigos científicos, como disposto nas normas de redação de teses da Universidade Federal de Viçosa. Cada artigo segue a formatação da revista a que será submetido ou publicado.

2. REFERÊNCIAS BIBLIOGRÁFICAS

Arambarri, A.M., S.E. Freire, M.N. Colares, N.D. Bayón, M.C. Novoa, C. Monti, and S. a Stenglein. 2006. Leaf anatomy of medicinal shrubs and trees from gallery forests of the Paranaense Province (Argentina). Part 1. Boletin de la Sociedade Argetina de Botanica 41: 233–268.

Baker, H.G. 1977. Non-sugar chemical constituents of nectar. Apidologie 8: 349–356.

Barneby, R.C. 1999. Increments to genus Chamaecrista (Caesalpiniaceae: Cassiinae) from Bolivia and from Atlantic and Planaltine Brazil. Brittonia 51: 331–339.

Barreiro, D.P., and S.R. Machado. 2007. Coléteres dendróides em Alibertia sessilis (Vell.) K. Schum., uma espécie não-nodulada de Rubiaceae. Revista Brasileira de Botânica 30: 387–399.

Bentham, G. 1870. Leguminosae II. Cassia. In C. F. P. Martius, and A. G. Eichler [eds.], Flora Brasiliensis. Enumeratio plantarum in Brasilia hactenus detectarum. XV (II), 82–176. Fried. Fleischer, Munich, Vienna, Leipzig.

Bentley, B., and T. Elias. 1983. The biology of nectaries. Columbia University Press, New York, USA.

Bentley, B.L. 1977. Extrafloral nectaries and protection by pugnacious bodyguards. Annual Review of Ecology and Systematics 8: 407–427.

Camargo, R.A., and S.T.S. Miotto. 2004. O gênero Chamaecrista Moench (Leguminosae-Caesalpinioideae) no Rio Grande do Sul. Iheringia 59: 131–148.

Carvalho, D.M.G. Anatomia foliar de Cassia ensiformis Vell. (Leguminosae - Caesalpinioideae). Arquivos do Jardim Botânico do Rio de Janeiro 27: 157–169.

Caspary, R. 1848. De nectariis. Elverfeld, Bonnae.

Conceição, A. de S. 2006. Filogenia do gênero Chamaecrista (Leguminosae-Caesalpinioideae) e taxonomia do grupo Baseophyllum. Universidade Estadual de Feira de Santana.

Conceição, A.S., L.P. Queiroz, S.M. Lambert, A.C.S. Pereira, and E.L. Borba. 2008. Biosystematics of Chamaecrista sect. Absus subsect. Baseophyllum (Leguminosae-Caesalpinioideae) based on allozyme and morphometric analyses. Plant Systematics and Evolution 270: 183–207.

Conceição, A.S., L.P. Queiroz, and G.P. Lewis. 2001. Novas espécies de Chamaecrista Moench (Leguminosae-Caesalpinioideae) da Chapada Diamantina, Bahia, Brasil. Sitientibus, Série Ciências Biológicas 1: 112–119.

Conceição, A.S., L.P. Queiroz, G.P. Lewis, M.J.G. de Andrade, P.R.M. de Almeida, A.S. Schnadelbach, and C. van den Berg. 2009. Phylogeny of Chamaecrista Moench (Leguminosae-Caesalpinioideae) based on nuclear and chloroplast DNA regions. Taxon 58: 1168–1180.

Costa, F.M.C.B., A.T. Oliveira-Filho, and P.S. Oliveira. 1992. The role of extrafloral nectaries in Qualea grandiflora (Vochysiaceae) in limiting herbivory: an experiment of ant protection in cerrado vegatation. Ecological Entomology 17: 363–365.

Coutinho, Í.A.C., D.M.T. Francino, A.A. Azevedo, and R.M.S.A. Meira. 2012. Anatomy of the extrafloral nectaries in species of Chamaecrista section Absus subsection Baseophyllum (Leguminosae, Caesalpinioideae). Flora 207: 427–435.

Coutinho, Í.A.C., D.M.T. Francino, and R.M.S.A. Meira. 2013. Leaf anatomical studies of Chamaecrista subsect. Baseophyllum (Leguminosae, Caesalpinioideae): new evidence for the up-ranking of the varieties to the species level. Plant Systematics and Evolution 299: 1709–1720.

Coutinho, Í.A.C., D.M.T. Francino, and R.M.S.A. Meira. 2015. New records of colleters in Chamaecrista (Leguminosae, Caesalpinioidae s. l.): structural diversity, secretion, functional role, and taxonomic importance. International Journal of Plant Sciences 176: 72–85.

Coutinho, Í.A.C., and R.M.S.A. Meira. 2015. Structural diversity of extrafloral nectaries in Chamaecrista sect. Apoucouita. Botanyin press.

Coutinho, Í.A.C., V.M.M. Valente, and M.R.M.S. Alves. 2010. Ontogenetic, anatomical and histochemical study of the extrafloral nectaries of Sapium biglandulosum (Euphorbiaceae). Australian Journal of Botany 58: 224–232.

Cowan, R.S. 1981. Caesalpinioideae. In Advances in Legume Systematics, Part 1., 57–64. Royal Botanical Gardens, Kew, Kew.

Curtis, J.D., and N.R. Lersten. 1978. Heterophylly in Populus grandidentata (Salicaceae) with emphasis on resin glands and extrafloral nectaries. American Journal of Botany 65: 1003–1010.

Curtis, J.D., and R.N. Lersten. 1980. Morphology and anatomy of resin glands in Salix lucida (Salicaceae). American Journal of Botany 67: 1289–1296.

Dantas, M.M., and M.J. Silva. 2013. O gênero Chamaecrista (Leguminosae, Caesalpinioideae, Cassieae) no Parque Estadual da Serra Dourada, Goiás, Brasil. Rodriguesia 64: 581–595.

Delpino, F. 1873. Ulteriori osservazioni e considerazioni sulla Dicogamia nel regno vegetale. Delle piante zooifile. Atti della Societá Italiana di Scienze Naturali 16: 151–349.

De-Paula, O., and D. Oliveira. 2007. Ocorrência de coléteres em embriões de três espécies de Chamaecrista Moench (Fabaceae: Caesalpinioideae). Revista Brasileira de Biociências 5: 348–350.

Dickson, W.C. 2000. Integrative plant anatomy. Academic Press, USA.

Durkee, L.T., C.W. Baird, and P.F. Cohen. 1984. Light and electron microscopy of the resin glands of Passiflora foetida (Passifloraceae). American Journal of Botany 71: 596–602.

Elias, T.S. 1972. Morphology and anatomy of foliar nectaries of Pithecellobium macradenium (Leguminosae). Botanical Gazette 133: 38–42.

Ellis, B., D.C. Daly, L.J. Hickey, K.R. Johnson, J.D. Mitchell, P. Wild, and S.L. Wing. 2009. Manual of leaf architecture. Cornell University Press, Ithaca, New York, USA.

Evert, R.F. 2006. Esau’s Plant Anatomy: Meristems, Cells, and Tissues of the Plant Body: Their Structure, Function, and Development. John Wiley & Sons, Inc., Hoboken, New Jersey, USA.

Fahn, A. 1979. Secretory Tissues in Plants. Academic Press, London.

Fahn, A., and P. Benouaiche. 1979. Ultrastructure, development and secretion in the nectary of banana flowers. American Journal of Botany 44: 85–93.

Fahn, A., and D.F. Cutler. 1992. Xerophytes. Gebrüder Bomtraeger, Berlin.

Fernandes, J.M. 2011. Ingea Benth. (Leguminosae. Mimosoideae) no Estado de Minas Gerais, Brasil: Taxonomia, morfoanatomia de nectários extraflorais e padrões de distribuiçao geográfica. Universidade Federal de Viçosa, Brazil.

Foster, A.S. 1942. Practical plant anatomy. D. van Nostrand Company, Inc., New York.

Francino, D.M.T. 2010. Anatomia foliar de Chamaecrista Moench (Leguminosae-Caesalpinioideae) como subsídio à taxonomia e à filogenia. Univseridade Federal de Viçosa, Brazil.

Francino, D.M.T., B.F. Sant'Anna-Santos, K.L.F. Silva, M. Thadeo, R.M.S.A. Meira, and A.A. Azevedo. 2006. Anatomia foliar e caulinar de Chamaecrista trichopoda (Caesalpinioideae) e histoquímica do nectário extrafloral. Planta Daninha 24: 695–705.

Freitas, L., G. Bernardello, L. Galetto, and A.A.S. Paoli. 2001. Nectaries and reproductive biology of Croton sarcopelatus (Euphorbiaceae). Botanical Journal of the Linnean Society 136: 267–277.

Gereau, R.E., and G.M. Walters. 2003. Chamaecrista mwangokae (Fabaceae, Caesalpinioideae), a new species from the Southern Highlands of Tanzania. Novon 13: 438–442.

Heil, M., and D. McKey. 2003. Protective ant-plant interactions as model systems in ecological and evolutionary research. Annual Review of Ecology, Evolution, and Systematics 34: 425–553.

Howard, R.A. 1979. The petiole. In C. R. Metcalfe, and L. Chalk [eds.], Anatomy of Dicotyledons, volume I, 88–96. Clarendon Press, Oxford.

Irwin, H.S., and R.C. Barneby. 1985. A new arborescent Chamaecrista (Caesalpiniaceae: Cassiinae). Brittonia 37: 14–16.

Irwin, H.S., and R.C. Barneby. 1977. Monographic studies in Cassia (Leguminosae: Caesalpinioideae). IV. Supplementary notes on section Apoucouita Bentham. Brittonia 29: 277–290.

Irwin, H.S., and R.C. Barneby. 1982. The American Cassiinae: A synoptical revision of Leguminosae tribe Cassieae subtribe Cassiinae in the New World. Memoirs of the New York Botanical Garden 35: 1–918.

Irwin, H.S., and R.C. Barneby. 1979a. Three new Brazilian species of Chamaecrista Moench (Leguminosae: Caesalpinioideae). Brittonia 31: 149–155.

Irwin, H.S., and R.C. Barneby. 1979b. Two new Brazilian species of Chamaecrista Moench (Leguminosae: Caesalpinioideae). Brittonia 31: 464–468.

Irwin, H.S., and D.J. Rogers. 1967. Monographic studies in Cassia (Leguminosae-Caesalpinioideae). II. A taximetric study of section Apoucouita. Memoirs of the New York Botanical Garden 16: 71–118.

Jáuregui, D., M. García, and D. Pérez. 2002. Morfoanatomia de las glándulas en cuatro especies de Passiflora L. (Passifloraceae) de Venezuela. Caldasia 24: 33–40.

Johansen, D.A. 1940. Plant microtechnique. McGraw-Hill Book, New York.

Keeler, K.H., and R.B. Kaul. 1979. Morphology and distribution of petiolar nectaries in Ipomoea (Convolvulaceae). American Journal of Botany 66: 946–952.

Koteyeva, N.K. 2005. A novel structural type of plant cuticle. Doklady Biological Sciences 403: 283–285.

Lackey, J. 1978. Leaflet anatomy of Phaseoleae (Leguminosae, Papilionoideae) and its relation to taxonomy. Botanical Gazette 139: 436–446.

Lanza, J., E.L. Vargo, S. Pulim, and Y.Z. Chang. 1989. Preferences of the fire ants Solenopsis invicta and S. geminata (Hymenbptera: Formicidae) for amino acid and sugar components of extrafloral nectars. Environmental Ecology 22: 411–417.

Leitão, C.A.E., R.M.S.A. Meira, A.A. Azevedo, J.M. de Araújo, K.L.F. Silva, and R.G. Collevatti. 2005. Anatomy of the floral, bract, and foliar nectaries of Triumfetta semitriloba (Tiliaceae). Canadian Journal of Botany 286: 279–286.

Lersten, N.R., and C.L. Brubaker. 1987. Extrafloral nectaries in Leguminosae: review and original observations in Erythrina and Mucuna (Papilionoideae; Phaseoleae). Bulletin of the Torrey Botanical Club 114: 437–447.

Lewis, G. 2005. Tribe Cassieae. In G. Lewis, B. Schrire, B. Mackinder, and M. Lock [eds.], Legumes of the World, 111–161. Royal Botanic Gardens, Kew.

Luckow, M.A. 2002. Anatomical features of the leaves in the Dichrostachys group (Leguminosae, Mimosoideae) and their utility for phylogenetic studies. Systematic Botany 27: 29–40.

Marazzi, B., P.K. Endress, L.P. de Queiroz, and E. Conti. 2006. Phylogenetic relationships within Senna (Leguminosae, Cassiinae) based on three chloroplast DNA regions: patterns in the evolution of floral symmetry and extrafloral nectaries. American Journal of Botany 93: 288–303.

Mayer, J.L.S., P. Cardoso-Gustavson, and B. Appezzato-da-Glória. 2011. Colleters in monocots: new record for Orchidaceae. Flora 206: 185–190.

Mayer, J.L.S., S.M. Carmello-Guerreiro, and P. Mazzafera. 2013. A functional role for the colleters of coffee flowers. AoB Plants 5: 1–13.

McDade, L.A., and D.T. Melvin. 1997. Structure and development of bracteal nectary glands in Aphelandra (Acanthaceae). American Journal of Botany 84: 1–15.

Meira, R.M.S.A., D.M.T. Francino, and L. Ascensão. 2014. Oleoresin trichomes of Chamaecrista dentata (Leguminosae): structure, function, and secretory products. International Journal of Plant Sciences 175: 336–345.

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

Metcalfe, C.F., and L. Chalk. 1979. Anatomy of the dicotyledons: systematic anatomy of leaf and stem with a brief history of the subject, vol I. 2nd ed. Clarendon Press, Oxford.

Metcalfe, C.R., and L. Chalk. 1950. Anatomy of the Dicotyledons: leaves, stem and wood in relation to taxonomy with notes on economic uses, vol I. Oxford Clarendon, Oxford.

Morris, J.B. 2012. Showy partridge pea [Chamaecrista fasciculata (Michx.) Greene] with potential for cultivation as a multi-functional species in the United States. Genetic Resources and Crop Evolution 59: 1577–1581.

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

Nascimento, E.A., and K. Del-Claro. 2007. Floral visitors of Chamaecrista debilis (Vogel) Irwin & Barneby (Fabaceae - Caesalpinioideae) at Cerrado of Estação Ecológica de Jataí, São Paulo State, Brazil. Neotropical Entomology 36: 619–624.

Nicolson, S.W., M. Nepi, and E. Pacini. 2007. Nectaries and nectar. Springer, Dordrecht, The Netherlands.

O'Brien, T.P., and M.E. McCully. 1981. The study of plant structure principles and selected methods. Termarcarphi Ptey. Ltd., Melbourne, Australia.

Oliveira, P.S., and H.F. Leitão-Filho. 1987. Extrafloral nectaries their taxonomic distribution and abundance in the woody flora of Cerrado vegetation in Southeast Brazil. Biotropica 19: 140–148.

Oliveira, P.S., V. Rico-Gray, C. Díaz-Castelazo, and C. Castillo-Guevara. 1999. Interaction between ants, extrafloral nectaries and insect herbivores in Neotropical coastal sand dunes: herbivore deterrence by visiting ants increases fruit set in Opuntia stricta (Cactaceae). Functional Ecology 13: 623–631.

Paiva, É.A.S. 2009. Occurrence, structure and functional aspects of the colleters of Copaifera langsdorffii Desf. (Fabaceae, Caesalpinioideae). Comptes Rendus Biologies 332: 1078–1084.

Paiva, É.A.S., and S.R. Machado. 2006a. Colleters in Caryocar brasiliense (Caryocaraceae). Ontogenesis, ultrastructure and secretion. Brazilian Journal of Biology 66: 301–308.

Paiva, É.A.S., and S.R. Machado. 2006b. Ontogênese , anatomia e ultra-estrutura dos nectários extraflorais de Hymenaea stigonocarpa Mart . ex Hayne (Fabaceae – Caesalpinioideae). Acta Botanica Brasilica 20: 471–482.

Pascal, L.M., E.F. Motte-Florac, and D.B. McKey. 2000. Secretory structures on the leaf rachis of Caesalpinieae and Mimosoideae (Leguminosae): Implications for the evolution of nectary glands. American Journal of Botany 87: 327–338.

Pearse, A.G.E. 1980. Histochemistry theoretical and applied. 4th ed. Churchill Livingston, Edinburgh.

Radford, A.E., W.C. Dickison, J.R. Massey, and C.R. Bell. 1974. Vascular Plant Systematics. Harper and Row, New York.

Rando, J.G. 2009. Chamaecrista Moench seções Apoucouita, Chamaecrista e Xerocalyx (Leguminosae - "Caesalpinioideae") na Serra do Cipó, Minas Gerais, Brasil. Instituto de Biociências da Universidade Federal de São Paulo.

Rando, J.G., B. Loeuille, and J.R. Pirani. 2013. Taxonomic novelties in Chamaecrista (Leguminosae: Caesalpinioideae) from Brazil. Phytotaxa 97: 17–25.

Rando, J.G., and J.R. Pirani. 2011. Padrões de distribuição geográfica das espécies de Chamaecrista sect. Chamaecrista ser. Coriaceae (Benth.) H. S. Irwin & Barneby, Leguminosae - Caesalpinioideae. Revista Brasileira de Botânica 34: 499–513.

Rizzini, C.T. 1976. Contribuicao ao conhecimento das floras Nordestinas. Rodriguésia 28: 173.

Roshchina, V. V, and V.D. Roshchina. 1993. The excretory function of higher plants. Springer-Verlag, Berlin, Germany.

Saheed, S.A., and H.C. Illoh. 2010. A Taxonomic study of some species in Cassiinae (Leguminosae) using leaf epidermal characters. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 38: 21–27.

Sheue, C.-R., Y.-J. Chen, and Y.-P. Yang. 2012. Stipules and colleters of the mangrove Rhizophoraceae: morphology , structure and comparative significance. Botanical Studies 53: 243–254.

Silva, M.S. 2012. Anatomia foliar como subsídio à taxonomia de espécies de Chamaecrista Moench (Leguminosae-Caesalpinioideae). Dissertação de Mestrado, Universidade do Estado da Bahia (BA), Brazil.

Smith, F.H., and E.C. Smith. 1942. Anatomy of the inferior ovary of Darbya. American Journal of Botany 29: 464–471.

So, M.L. 2004. The occurrence of extrafloral nectaries in Hong Kong plants. Botanical Bulletin of the Academia Sinica 45: 237–245.

Solereder, H. 1908. Systematic anatomy of the dicotyledons. A handbook for laboratories of pure and applied Botany. Clarendon Press, Oxford.

Stpiczyńska, M., C. Milanesi, C. Faleri, and M. Cresti. 2005. Ultrastructure of the nectary spur of Platanthera chlorantha (Custer) Rchb. (Orchidaceae) during successive stages of nectar secretion. Acta Biologica Cracoviensia 111–119.

Torres, D.C., J.P.M.S. Lima, A.G. Fernandes, E.P. Nunes, and T.B. Grangeiro. 2011. Phylogenetic relationships within Chamaecrista sect. Xerocalyx (Leguminosae, Caesalpinioideae) inferred from the cpDNA trnE-trnT intergenic spacer and nrDNA ITS sequences. Genetics and Molecular Biology 34: 244–251.

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

Weber, M.G., and K.H. Keeler. 2013. The phylogenetic distribution of extrafloral nectaries in plants. Annals of botany 111: 1251–1261.

Wilkinson, H.P. 1979. The plant surface (mainly leaf). Part I: Stomata. In C. R. Metcalfe, and L. Chalk [eds.], Anatomy of the Dicotyledons, vol I. 2nd ed, 97–117. Clarendon Press, Oxford.

CAPÍTULO I

Artigo publicado no International Journal of Plant Sciences 176 (1):72–85. 2015.
DOI: 10.1086/679016

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

Ítalo Antônio Cotta Coutinho[1], Dayana Maria Teodoro Francino[2], Renata Maria Strozi Alves Meira[1*]

[1]Universidade Federal de Viçosa – Departamento de Biologia Vegetal – Anatomia Vegetal. Minas Gerais. Viçosa, 36.570–900, Brazil.
[2]Universidade Federal dos Vales do Jequitinhonha e Mucuri, Campus JK–Minas Gerais – Departamento de Ciências Biológicas – Anatomia Vegetal. Diamantina, 39.100-000, Brazil.
[*]Author for correspondence; email: rmeira@ufv.br

Key words: anatomy; buds; histochemical test; secretory structures; shoots; trichomes.

Abstract

- Premise of the research: Colleters are structures that secrete a sticky product that covers and protects the shoot apex and floral buds. In Chamaecrista, colleters have been reported in the cotyledons of three species and on the leaves of all species belonging to sect. Absus subsect. Baseophyllum. Anatomical studies using taxonomic and phylogenetic approaches are necessary to evaluate the presence, diversity and importance of colleters for Chamaecrista.
- Methodology: We analyzed 55 species of Chamaecrista belonging to five out of the six sections of the genus. Samples from both herbarium- and field-collected material of young vegetative and reproductive meristems were used. The material was subjected to standard anatomical study by light microscopy and SEM, and the secretion was evaluated using histochemical analyses.
- Pivotal results: Histochemical analyses for the total proteins, total polysaccharides, acid mucopolysaccharides, pectins/mucilage and lipids generated positive results. Six types of colleters are described here: club-shaped, racket-shaped, long bottle-shaped, short bottle-shaped, long digitiform and short digitiform. Section Apoucouita showed the short digitiform and club-shaped types and was the only section with colleters on the sepal margins. Most species of sect. Absus subsect. Absus presented the short bottle-shaped type while all species from subsect. Baseophyllum the short digitiform and club-shaped types. Although the short bottle-shaped was the most common type among species from sect. Chamaecrista, the short digitiform and club-shaped were also observed. The short bottle-shaped colleters were also found in sect. Grimaldia while in sect. Xerocalyx only the digitiform.

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

Introduction

The genus Chamaecrista Moench (Leguminosae, Caesalpinioideae s.l.) includes more than 330 species (Lewis 2005). Chamaecrista species are typically herbs (fig. 1A) and shrubs (fig. 1B) with yellow flowers (fig. 1A, C and E) that are found in rocky outcrops (fig. 1A–B) and in other open sunny areas. Woody species also appear in rainforests, especially in the Amazon and Brazilian Atlantic forests (Irwin and Barneby 1982; Lewis 2005; Conceição et al. 2009; Rando et al. 2013). Previously, Chamaecrista species were grouped into six sections: Chamaecrista sect. Absus, Apoucouita, Caliciopsis, Chamaecrista, Grimaldia and Xerocalyx (Irwin and Barneby 1982). Molecular studies have suggested the definition of new sectional and species boundaries (Conceição et al. 2008, 2009). According to the circumscription proposed by such authors, the clades Apoucouita and Xerocalyx were recognized as sections, sect. Absus subsect. Baseophyllum should be elevated to the sectional status and sect. Grimaldia must now be included in sect. Absus. Sect. Caliciopsis and Chamaecrista were found to be paraphyletic. Although these studies demonstrate the need for taxonomic revision within the genus, taxon sampling was limited and more data are necessary before amending taxon circumscriptions and adopting nomenclatural changes.

In the subfamily Caesalpinioideae s.l., secretory structures have been shown to be meaningful additional characters in taxonomical evaluations (Irwin and Barneby 1978, 1982; Lersten and Curtis 1993, 1994, 1996; Rudall et al. 1994; Conceição et al. 2008, 2009; Coutinho et al. 2013). In several families, colleters have stood out in taxonomical and phylogenetic interpretations (Lersten 1974; Thomas 1991; Klein et al. 2004; Silva et al. 2012; Dalvi et al. 2013, 2014). Colleters are structures that are usually found on the adaxial side of stipules and bracts and secrete a sticky product composed of mucilaginous substances or a mixture of mucilage and resin that covers and protect the shoot apex and floral buds (Foster 1942; Thomas 1991; Paiva and Machado 2006b; Barreiro and Machado 2007; Mayer et al. 2011; Sheue et al. 2012).

Instead of the term colleter, other terms have been given to these structures, such as “glandular shaggy hair” (Solereder 1908; Metcalfe and Chalk 1950), “resin glands” (Curtis and Lersten 1980), “glandular trichomes” (Pascal et al. 2000), “extrafloral nectary” (Freitas et al. 2001), and “filamentous structures” (De-Paula et al. 2011), among others (Thomas 1991). Such nomenclatural diversity and/or misinterpretations are primarily based on anatomical or morphological similarities with other secretory structures. The term colleter is more appropriate to describe structures that secrete substances responsible for protecting developing leaves and flowers mainly against dehydration and pathogens (Thomas 1991; Mayer et al. 2011).

For Leguminosae, few studies have reported the presence of colleters (Paiva and Machado 2006a; De-Paula and Oliveira 2007, 2012; Paiva 2009; Coutinho et al. 2013). To our knowledge, there are no comparative studies on the diversity and taxonomic importance of colleters for this family. In Chamaecrista, colleters have been reported in the cotyledons of three species (De-Paula and Oliveira 2007, 2012) and on the leaves of all of the species of Absus subsect. Baseophyllum (Coutinho et al. 2013). However, the authors do not provide detailed information on the structural (morphological and anatomical) variation and chemical nature of the secretion. Due to the importance of colleters in taxonomy (Lersten 1974; Thomas 1991; Klein et al. 2004; Silva et al. 2012; Dalvi et al. 2013, 2014), the following questions were raised: Are colleters common secretory structures for Chamaecrista? Are there structural and topographical variations among the species/sections? Could such variations be used to solve taxonomic problems within this group? Is there variation in the chemical nature of the exudates that are produced by the colleters? Aiming to answer such questions, this study provides the structural characterization and the histochemical analysis of the colleters found on both vegetative and reproductive meristems of Chamaecrista.

Material and Methods

Plant material

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

Samples from herbarium material were microwaved in distilled water for 7 min and left to rest overnight. Samples were then treated with 2% potassium hydroxide for 1 h at room temperature (Smith and Smith 1942), rinsed with tap water three times, dehydrated in an ethanol series (10, 30, 50 and 70%, 10 min each) and stored in 70% ethanol before being subjected to standard anatomical procedures. Samples from species that were collected from the field (appendix) were fixed in FAA (formaldehyde, acetic acid and 50% ethanol; 1:1:18, v/v) for 48 h and stored in 70% ethanol. Voucher specimens were deposited at the herbarium of the Universidade Federal de Viçosa (VIC), and duplicates were sent to the herbarium of The New York Botanical Garden (NY), the United States National Herbarium – Smithsonian Institution (US) and the herbarium of the Universidade de São Paulo (SPF).

Slide preparation

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

The samples from both herbarium material and field-collected species that were stored in 70% ethanol were embedded in methacrylate (Historesin Leica, Leica Microsystems Nussloch GmbH, Heidelberg, Germany) following the manufacturer's recommendation. Cross and longitudinal sections that were 4-µm thick were made in an automatic rotary microtome (Leica RM2155, Deerfield, IL, USA). For the structural characterization, the sections were stained with toluidine blue at pH 4.4 (O'Brien and McCully 1981), dried at room temperature and mounted in resin (Permount, Fisher Scientific, NJ, USA). Some of the sections were also used in histochemical tests.

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

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

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

Observations and photographs were collected using a light microscope (model AX70TRF, Olympus Optical, Tokyo, Japan) that was equipped with a U-Photo system and digital camera (AxioCam HRc – Carl Zeiss – Gottingen, Germany).

Scanning Electron Microscopy (SEM)

Samples from herbarium and field-collected material stored in 70% ethanol were critical point-dried with CO_2 in a Bal-Tec 020 CPD dryer (Bal-Tec, Balzers, Liechtenstein). The samples were mounted onto stubs and coated with gold using an FDU 010 sputter coater (Bal-Tec). The examinations and image captures were carried out using a Leo 1430VP SEM (Zeiss, Cambridge, UK) at the Centro de Microscopia e Microanálises at the Universidade Federal de Viçosa.

Results

Ontogeny

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

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

Structure and Distribution

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

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

Six types of colleters may be described: club-shaped (figs. 3B, 4C–D), racket-shaped (figs. 3A, 4E), long bottle-shaped (whole length of the colleter > 500 µm) (fig.

4H), short bottle-shaped (< 300 µm) (figs. 3F, 4F–G), long digitiform (> 500 µm) (fig. 4B) and short digitiform (< 400 µm) (figs. 3C, 4A, D).

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

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

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

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

Histochemistry

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

Discussion

Classification, secretion and function of the colleters

The ontogenetic study of the colleters shows that such structures derive from the protodermis only and, therefore, are a kind of secretory trichomes (Fahn 1990). According to the topography, structure (micromorphology and anatomy), early secretory activity and the compounds that are present in the secreted exudates, these trichomes correspond to colleters (Foster 1942; Fahn 1990; Thomas 1991).

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

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

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

The closeness of the colleters to the developing leaf and flower, their early differentiation, the secretion period and the chemical nature of the exudates indicate a protective function of such structures in Chamaecrista.

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

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

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

Taxonomic importance of colleters

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

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

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

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

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

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

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

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

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

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

Section Xerocalyx is a monophyletic group with poorly supported branches that is placed in the clade formed by three sections (Table 1): Caliciopsis, Chamaecrista and Xerocalyx (Conceição et al. 2009; Torres et al. 2011). Interestingly, species from sect. Xerocalyx shows only the digitiform type of colleter (short and long) with the long digitiform unique for C. desvauxii var. desvauxii and C. desvauxii var. latistipula. The uniformity in the type of colleter for sect. Xerocalyx is additional evidence supporting the monophyly of this section. The variation from long to short digitiform may be related to the size of stipules/bracts. Species with larger stipules/bracts (C. desvauxii var. desvauxii and C. desvauxii var. latistipula) displayed both long and short digitiform types while species with smaller stipules/bracts (C. desvauxii var. graminea, C. ramosa and C. ramosa var. parvifoliola) displayed the short digitiform only.

Conclusion

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

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

Acknowledgments

The authors thank CNPq, CAPES, FAPEMIG and Floresta-Escola (SECTES/UNESCO/HidroEX/FAPEMIG) for financial support; CAPES and CNPq for granting a scholarship to ÍAC Coutinho and RMSA Meira; the Smithsonian Institution for granting the first author with the Cuatrecassas Award; the herbaria HUEFS, NY, RB, SPF and US for allowing sampling of voucher specimens; JG Rando along with A Nogueira for presenting us with four fixed samples of Chamaecrista sect. Apoucouita; AS Conceição for determining the species of a few collections; Instituto Chico Mendes for permission to collect specimens; and the anonymous reviewers along with PS Herendeen for helpful and critical comments that improved the manuscript.

Literature Cited

Al-Tardeh S, Sawidis T, Diannelidis B-E, Delivopoulos S 2008 Water content and reserve allocation patterns within the bulb of the perennial geophyte red squill (Liliaceae) in relation to the Mediterranean climate. Botany 86:291–299.

Ascensão L, Mota L, Castro MM 1999 Glandular trichomes on the leaves and flowers of Plectranthus ornatus: morphology, distribution and histochemistry. Ann Bot 84:437–447.

Barnes BJ, Nornan HA, Brooker NL 1997 Inhibition of three soybean fungal plant pathogens by lipid derivates and natural compounds. Pages 236–238 in JP Williams, MU Khan, NW Lem, eds. Physiol. Biochem. Mol. Biol. Plant Lipids. Springer Netherlands.

Barreiro DP, Machado SR 2007 Coléteres dendróides em Alibertia sessilis (Vell.) K. Schum., uma espécie não-nodulada de Rubiaceae. Rev Bras Botânica 30:387–399.

Chin S, Lutz S, Wen J, Potter D 2013 The bitter and the sweet: inference of homology and evolution of leaf glands in Prunus (Rosaceae) through anatomy, micromorphology, and ancestral–character state reconstruction. Int J Plant Sci 174:27–46.

Christodoulakis NS, Tsimbani H, Fasseas C 1990 Leaf structural peculiarities in Sarcopoterium spinosum, a seasonally dimorphic subshrub. Ann Bot 65:291–296.

Conceição AS, Queiroz LP, Lambert SM, Pereira CS, Borba EL 2008 Biosystematics of Chamaecrista sect. Absus subsect. Baseophyllum (Leguminosae-Caesalpinioideae) based on allozyme and morphometric analyses. Plant Syst Evol 270:183–207.

Conceição AS, Queiroz LP, Lewis GP, MJG Andrade, PRM Almeida, AS Schnadelbach, C Van den Berg 2009 Phylogeny of Chamaecrista Moench (Leguminosae-Caesalpinioideae) based on nuclear and chloroplast DNA regions. Taxon 58:1168–1180.

Coutinho ÍAC, Francino DMT, Meira RMSA 2013 Leaf anatomical studies of Chamaecrista subsect. Baseophyllum (Leguminosae, Caesalpinioideae): new evidence for the up-ranking of the varieties to the species level. Plant Syst Evol 299:1709–1720.

Curtis JD, Lersten RN 1980 Morphology and anatomy of resin glands in Salix lucida (Salicaceae). Am J Bot 67:1289–1296.

Dalvi VC, Cardinelli LS, Meira RMSA, Azevedo AA 2014 Foliar colleters in Macrocarpaea obtusifolia (Gentianaceae): anatomy, ontogeny, and secretion. Botany 92:59–67.

Dalvi VC, Meira RMSA, Francino DMT, Silva LC, Azevedo AA 2013 Anatomical characteristics as taxonomic tools for the species of Curtia and Hockinia (Saccifolieae – Gentianaceae Juss.). Plant Syst Evol 300:99–112.

De-Paula O, Oliveira D 2007 Ocorrência de coléteres em embriões de três espécies de Chamaecrista Moench (Fabaceae: Caesalpinioideae). Rev Bras Biociências 5:348–350.

De-Paula OC, Oliveira DMT 2012 Seed ontogeny of Chamaecrista and its systematic implications in Cassiinae (Leguminosae, Caesalpinioideae). Plant Syst Evol 298:1659–1669.

De-Paula OC, Sajo MG, Prenner G, Cordeiro I, Rudall PJ 2011 Morphology, development and homologies of the perianth and floral nectaries in Croton and Astraea (Euphorbiaceae-Malpighiales). Plant Syst Evol 292:1–14.

Fahn A 1979 Secretory Tissues in Plants. Academic Press, London.

Fahn A 1990 Plant Anatomy, Fourth. Pergamon Press, Oxford.

Fahn A, Cutler DF 1992 Xerophytes. Gebrüder Bomtraeger, Berlin.

Foster AS 1942 Practical plant anatomy. D. van Nostrand Company, Inc., New York.

Freitas L, Bernardello G, Galetto L, Paoli AAS 2001 Nectaries and reproductive biology of Croton sarcopelatus (Euphorbiaceae). Bot J Linn Soc 136:267–277.

Gerlarch D 1969 Botanische Mikrotechnik: Eine Einführung. Georg Thieme, Stuttgart.

Gonzalez AM, Tarragó JR 2009 Anatomical structure and secretion compounds of colleters in nine Ilex species (Aquifoliaceae) from southern South America. Bot J Linn Soc 160:197–210.

Irwin HS, Barneby RC 1978 Monographic studies in Cassia (Leguminosae-Caesalpinioideae) III. Sections Absus and Grimaldia. Mem N Y Bot Gard 30:1–300.

Irwin HS, Barneby RC 1982 The American Cassiinae, a synoptical revision of Leguminosae tribe Cassieae subtribe Cassiinae in the New World. Mem N Y Bot Gard 35:1–918.

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

Klein DS, Gomes VM, Silva-Neto SJ, Cunha M 2004 The structure of colleters in several species of Simira (Rubiaceae). Ann Bot 94:733–740.

Leitão CAE, Meira RMSA, Azevedo AA, Araújo MC, Silva KLF, Collevatti RG 2005 Anatomy of the floral, bract, and foliar nectaries of Triumfetta semitriloba (Tiliaceae). Can J Bot 286:279–286.

Lersten NR 1974 Morphology and distribution of colleters and crystals in relation to the taxonomy and bacterial leaf nodule symbiosis of Psychotria (Rubiaceae). Am J Bot 61:973–981.

Lersten NR, Curtis JD 1993 Subepidermal idioblasts in leaflets of Caesalpinia pulcherrima and Parkinsonia aculeata (Leguminosae: Caesalpinioideae). Bull Torrey Bot Club 120:319–326.

Lersten NR, Curtis JD 1994 Leaf anatomy in Caesalpinia and Hoffmannseggia (Leguminosae, Caesalpinioideae) with emphasis on secretory structures. Plant Syst Evol 192:231–255.

Lersten NR, Curtis JD 1996 Survey of leaf anatomy, especially secretory structures, of tribe Caesalpinieae (Leguminosae, Caesalpinioideae). Plant Syst Evol 200:21–39.

Lewis G 2005 Tribe Cassieae. Pages 111–161 in G Lewis, B Schrire, B Mackinder, M Lock, eds. Legumes of the World. Royal Botanic Gardens, Kew.

Martins FM 2012 Leaf and calycine colleters in Odontadenia lutea (Apocynaceae – Apocynoideae – Odontadenieae): their structure and histochemistry. Brazilian J Bot 35:59–69.

Mayer JLS, Cardoso-Gustavson P, Appezzato-da-Glória B 2011 Colleters in monocots: new record for Orchidaceae. Flora 206:185–190.

Mayer JLS, Carmello-Guerreiro SM, Mazzafera P 2013 A functional role for the colleters of coffee flowers. AoB Plants 5:1–13.

Meira RMSA, Francino DMT, Ascensão L 2014 Oleoresin trichomes of Chamaecrista dentata (Leguminosae): structure, function, and secretory products. Int J Plant Sci 175:336–345.

Metcalfe CR, Chalk L 1950 Anatomy of the Dicotyledons: leaves, stem and wood in relation to taxonomy with notes on economic uses. Vol I. Oxford Claredon, Oxford.

Miguel EC, Gomes VM, Oliveira MA, Cunha M 2006 Colleters in Bathysa nicholsonii K. Schum. (Rubiaceae): ultrastructure, secretion protein composition, and antifungal activity. Plant Biol 8:715–722.

O’Brien TP, McCully ME 1981 The study of plant structure principles and selected methods. Termarcarphi Ptey. Ltd., Melbourne.

Paiva EAS 2012 Colleters in Cariniana estrellensis (Lecythidaceae): structure, secretion and evidences for young leaf protection. J Torrey Bot Soc 139:1–8.

Paiva ÉAS 2009 Occurrence, structure and functional aspects of the colleters of Copaifera langsdorffii Desf. (Fabaceae, Caesalpinioideae). C R Biol 332:1078–1084.

Paiva ÉAS, Machado SR 2006a Ontogenesis, structure and ultrastructure of Hymenaea stigonocarpa (Fabaceae: Caesalpinioideae) colleters. Rev Biol Trop 54:943–950.

Paiva ÉAS, Machado SR 2006b Colleters in Caryocar brasiliense (Caryocaraceae). Ontogenesis, ultrastructure and secretion. Brazilian J Biol 66:301–308.

Pascal LM, Motte-Florac EF, McKey DB 2000 Secretory structures on the leaf rachis of Caesalpinieae and Mimosoideae (Leguminosae): Implications for the evolution of nectary glands. Am J Bot 87:327–338.

Pearse AGE 1980 Histochemistry theoretical and applied, 4th ed. Churchill Livingston, Edinburgh

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

Rudall PJ, Myers G, Lewis GP 1994 Floral secretory structures in Caesalpinia sensu lato and related genera. Pages 41–52 in IK Ferguson, S Tucker, eds. Adv. Legum. Syst. 6. Royal Botanic Gardens, Kew.

Sheue C-R, Chen Y-J, Yang Y-P 2012 Stipules and colleters of the mangrove Rhizophoraceae: morphology , structure and comparative significance. Bot Stud 53:243–254.

Silva CJ, Barbosa LCA, Marques AE, Baracat-Pereira MC, Pinheiro AL, Meira RMSA 2012 Anatomical characterisation of the foliar colleters in Myrtoideae (Myrtaceae). Aust J Bot 60:707–717.

Simões AO, Castro MM, Kinoshita LS 2006 Calycine colleters of seven species of Apocynaceae (Apocynoideae) from Brazil. Bot J Linn Soc 152:387–398.

Smith FH, Smith EC 1942 Anatomy of the inferior ovary of Darbya. Am J Bot 29:464–471.

Solereder H 1908 Systematic anatomy of the dicotyledons. A handbook for laboratories of pure and applied Botany. Clarendon Press, Oxford.

Thomas V 1991 Structural, funcional and phylogenetic aspects of the colleter. Ann Bot 68:287–305.

Torres DC, Lima JPMS, Fernandes AG, Nunes ED, Grangeiro TB 2011 Phylogenetic relationships within Chamaecrista sect. Xerocalyx (Leguminosae, Caesalpinioideae) inferred from the cpDNA trnE-trnT intergenic spacer and nrDNA ITS sequences. Genet Mol Biol 34:244–251.

Appendix. List of Chamaecrista species used in the structural analysis. Vouchers are housed in the herbaria of the Universidade Estadual de Londrina (FUEL), Universidade Estadual de Feira de Santana (HUEFS), The New York Botanical Garden (NY), Jardim Botânico do Rio de Janeiro (RB), Universidade de São Paulo (SPF), Smithsonian Institution (US) and Universidade Federal de Viçosa (VIC).

C. absus var. **meonandra** (H.S.Irwin & Barneby) H.S.Irwin - Irwin 1273, 23.VIII.1957 (NY); McVaugh et al. 19361, 22.IX.1960 (NY); McVaugh et al. 21374, 23.XI.1960-25.XI.1960 (NY); Schubert & Mario Sousa 1952, 22.IX.1963 (NY); Gentry 6536, 10.IX.1941-14.IX.1941 (NY). **C. absus** var. **absus** (L.) H.S.Irwin & Barneby - Asplund 15884, 24.III.1955 (NY); Cerrate 5209, 01.VII.1972 (NY). **C. adiantifolia** var. **adiantifolia** (Spruce ex Benth.) H. S. Irwin & Barneby - Davidse 27665, 26.VII.1984 (NY); Wurdack & Adderley 43236, 29.VI.1959 (NY). **C. adiantifolia** var. **pteridophylla** (Sandwith) H.S. Irwin & Barneby - Maguire et al. 45930, 24.VIII.1961 (NY, US); Maguire & Fanshawe 32636, 17.XI.1051 (NY, US); Silva 92, 22.IX.1942 (NY, US); Boz 548, V.1926 (NY, US); Prance et al. 22690, XVIII.IX.1974 (NY). **C. amabilis** H. S. Irwin & Barneby - Jardim & Flávia 594, 11.XI.1994 (NY); Thomas et al. 8964, 30.I.1992 (NY). **C. amorimii** Barneby - Amorim et al. 3231, 06.I.2000 (NY); Amorim et al. 4311, 23.X.2004 (RB); Amorim et al. 923, 14.XII.1992 (NY; RB). **C. apoucouita** (Aubl.) H. S. Irwin & Barneby - Forest Department of British Guiana F994, 09.I.1943 (NY). **C. aristata** (Benth.) H.S.Irwin & Barneby - Coutinho et al. 096, 14.VIII.2012 (VIC). **C. aspleniifolia** (H.S. Irwin & Barneby) H.S. Irwin & Barneby - Cardoso et al. 2407, 04.II.2009 (HUEFS); Duarte 8753, 18.I.1965 (NY, US); Pinheiro 1768, 24.I.1972 (NY); Hatschbach 47341, 18.I.1984 (NY, US); Pabst 8301 & Pereira 9410, 15.I.1965 (NY). **C. bahiae** (H.S. Irwin) H.S. Irwin & Barneby - Harley et al. 17398, 25.III.1974 (NY); Harley et al. 18071, 18.I.1977 (NY); Rando 1214, 01.III.2012 (SPF, HUEFS). **C. barbata** (Nees & Mart.) H.S. Irwin & Barneby - Souza et al. 45, 13.IV.2006 (HUEFS). **C. blanchetii** (Benth.) Conc., L.P.Queiroz & G.P.Lewis - Conceição & Leite 687, 18.VIII.2003 (HUEFS); Coutinho et al. 495, VII.2011 (VIC); Junqueira et al. 130, IX.X.2002 (HUEFS); Coutinho et al. s.n., 09.IV.2012 (VIC); Coutinho et al. s.n., 03.VII.2011 (VIC). **C. botryoides** Conc., L.P. Queiroz & G.P. Lewis - Conceição et al. 541, 29.V.2003 (HUEFS); Conceição et al. 612, 01.VI.2003 (HUEFS). **C. brachystachya** (Benth.) Conc., L.P.Queiroz & G.P.Lewis - Coutinho et al. s.n., IV.2011 Coutinho et al. s.n., 03.VII.2011 (VIC); Conceição et al. 728, 10.IX.2003 (HUEFS). **C. caribaea** var. **caribaea** (Northr.) Britton - Britton 3448, 28.I.1905 (NY); Britton & Millspaugh 5895, 16.III.1907 (NY); Small & Carter 8853, 01.III.1910 (NY). **C. caribaea** var. **inaguensis** (Britton) H.S.Irwin & Barneby - Correll 43167, 26.VIII.1976 (NY); Millspaugh &.Millspaugh 9303, 12.III.1911 (NY); Proctor & Gillis 34033, 11.VI.1974 (NY). **C. caribaea** var. **lucayana** (Britton) H.S.Irwin & Barneby - Britton & Millspaugh 2841, 20.II.1905 (NY); Wilson 7895, 28.XII.1907 (NY); Rando 963, 22.X.2010 (NY). **C. cinerascens** (Vogel) H.S. Irwin & Barneby - Coutinho & Pereira 187, 25.IV.2013 (VIC); Coutinho & Pereira 188, 25.IV.2013 (VIC); Coutinho & Pereira 189, 25.IV.2013 (VIC); Coutinho et al. 420, 24.III.2014 (VIC); Coutinho et al. 421, 24.III.2014 (VIC); Coutinho et al. 422, 24.III.2014 (VIC). **C. cipoana** (H.S. Irwin & Barneby) H.S. Irwin

& Barneby - Coutinho et al. 043, 01.VIII.2012 (VIC), Francino et al. s.n., 11.VIII.2009 (VIC); Francino & Casagrande 22, 22.VI.2009 (VIC); Francino et al. 15, 14.V.2009 (VIC). **C. claussenii** (Benth.) H.S.Irwin & Barneby - Coutinho & Lorencini 068, 08.VIII.2012 (VIC); Coutinho & Lorencini 069 08.VIII.2012 (VIC), Coutinho & Pereira 169 24.IV.2013 (VIC); Coutinho & Pereira 170, 24.IV.2013 (VIC). **C. compitalis** (H. S. Irwin & Barneby) H. S. Irwin & Barneby - Belém 3637, 24.V.1968 (NY); Belém 3643, 24.V.1968 (NY, US). **C. conferta** (Benth.) H.S.Irwin & Barneby - Coutinho et al. 047, 02.VIII.2012 (VIC); Coutinho et al. 048, 02.VIII.2012 (VIC); Coutinho & Lorencini 075, 11.VIII.2012 (VIC); Coutinho & Lorencini 076, 11.VIII.2012 (VIC); Coutinho & Moura 136, 20.I.2013 (VIC); Coutinho & Pereira 207, 27.IV.2013 (VIC); Coutinho & Pereira 208, 27.IV.2013 (VIC). **C. coriacea** (Benth.) H.S.Irwin & Barneby - Conceição et al. 869, 18.II.2005 (HUEFS, RB); Coutinho et al. 016, 27.VI.2012 (VIC). **C. cytisoides** (Collad.) H.S.Irwin & Barneby - Melo-Silva et al. 1640, 25.II.1999 (HUEFS); Coutinho et al. s.n., 11.X.2010 (VIC); Coutinho & Terra 144, 10.VII.2012. (VIC); Coutinho & Terra 323, 24.I.2014. (VIC); Coutinho & Terra 326, 24.I.2014. (VIC). **C. debilis** (Vogel) H.S.Irwin & Barneby - Coutinho & Lorencini 077, 11.VIII.2012 (VIC); Coutinho & Mora 134, 20.I.2013 (VIC); Coutinho et al. s.n., 05.VI.2011 (VIC); Coutinho et al. s.n., 05.X.2011 (VIC). **C. decora** (H.S.Irwin & Barneby) Conc., L.P.Queiroz & G.P.Lewis - Conceição et al. 716, IX.IX.2003 (HUEFS); Coutinho et al. 022, 27.VI.2012 (VIC); Coutinho et al. 093, 14.VIII.2012. (VIC); Coutinho et al. 108, 14.VIII.2012 (VIC); Coutinho & Pereira 214, 27.IV.2013 (VIC); Irwin et al. 23493, 16.III.1970 (NY); Coutinho et al. s.n., 09.X.2010 (VIC). **C. dentata** (Vogel) H.S.Irwin & Barneby - Coutinho & Moura 126, 18.I.2013 (VIC); Francino s.n., 23.III.2004 (VIC); Valente et al. 2522, 04.IV.2009 (VIC). **C. depauperata** Conc., L.P.Queiroz & G.P.Lewis - Cardoso & Conceição 433, 16.IV.2005 (HUEFS); Conceição et al. 863, II.II.2005 (HUEFS, SPF); Conceição 786, XI.I.2004 (HUEFS, SPF); Stannard et al. H51142, III.II.1992 (HUEFS, US). **C. desvauxii** var. **desvauxii** (Collad.) Killip - Coutinho et al. s.n., 03.VII.2011 (VIC). **C. desvauxii** var. **graminea** H.S. Irwin & Barneby - Coutinho et al. s.n., 03.VII.2011 (VIC); Coutinho et al. s.n., 03.VII.2011 (VIC). **C. desvauxii** var. **latistipula** (Benth.) G.P.Lewis - Coutinho & Coutinho 117, 16.I.2013 (VIC); Coutinho & Coutinho 118, 16.I.2013 (VIC); Coutinho et al. 294, 13.I.2014 (VIC); Coutinho et al. 295, 13.I.2014 (VIC). **C. duartei** (H.S.Irwin) H.S.Irwin & Barneby - Coutinho & Fernandes 303, 15.I.2014 (VIC); Duarte 8014, 19.XI.1963 (NY, US); Amorim & Lorenzi 2102, 07.XI.1997 (NY, US); Almeida & Santos 195, 31.X.1968 (NY, US); Belém & Pinheiro 3054, 12.I1967 (NY); Mori et al. 11036, 04.XI.1978 (NY). **C. eitenorum** var. **eitenorum** (H.S. Irwin) H.S. Irwin & Barneby - Drouet 2541, 01.X.1935 (US); Eiten & Eiten 10660, 18.II.1970 (US); Von Luetzelburg 446, 1970 (US). **C. eitenorum** var. **regana** (H.S. Irwin & Barneby) H.S. Irwin & Barneby - Hatschbach & Hatschbach 56931, 09.IV.1992 (US); Noblick 3034, 07.III.1984 (US); Queiroz et al. 6131, 18.II.2000 (HUEFS). **C. ensiformis** var. **ensiformis** (Vell.) H.S. Irwin & Barneby - Coutinho et al. 033, 01.VIII.2012 (VIC); Coutinho et al. 034, 01.VIII.2012 (VIC); Coutinho et al. 035, 01.VIII.2012 (VIC); Coutinho & Lorencini 070, 08.VIII.2012 (VIC); Coutinho & Moura 121, 17.I.2013 (VIC); Coutinho & Pereira 190, 25.IV.2013 (VIC); Irwin 2330, 29.XII.1958 (NY). **C. ensiformis** var. **maranonica** (H.S. Irwin) H.S. Irwin & Barneby - Daly et al. D375, 29.IX.1980 (NY, US); Hatschbach & Kummorw 38396, 26.III.1976 (NY); Prance & Silva 58692, 13.VIII.1964 (NY, US); Rosa et al. 2580, 11.VII.1978 (NY). **C. ensiformis** var. **plurifoliolata** (Hoehne) H.S. Irwin & Barneby - Belém & Magalhães 955, 25.IV.1965 (NY, US);

Santos 2952, 16.IV.1975 (NY); Kallunki et al. 523, 11.II.1994 (NY). **C. ericifolia** (Benth.) H.S. Irwin & Barneby - Coutinho & Pereira 204, 27.IV.2013 (VIC); Coutinho & Pereira 205, 27.IV.2013 (VIC); Coutinho & Pereira 206, 27.IV.2013 (VIC); Coutinho et al. 481, 27.III.2014 (VIC). **C. fagonioides** (Vogel) H.S.Irwin & Barneby - Giulietti & Harley 1622, 20.01.2000 (HUEFS); Harley & Giulietti 54031 23.VI.2000 (HUEFS), Kersten & Borgo 574, 30.VI.2002 (FUEL). **C. glaucofilix** (H.S. Irwin & Barneby) H.S. Irwin & Barneby - Conceição et al 861, 29.12.2004 (HUEFS); Coutinho et al. s.n.,03.VII.2011 (VIC). **C. glaziovii** (Harms) H.S.Irwin & Barneby - Coutinho & Moura 138, 20.I.2013 (VIC); Coutinho & Moura 139, 20.I.2013 (VIC); Coutinho & Moura 140, 20.I.2013 (VIC). **C. hymenaeifolia** (Benth.) H. S. Irwin & Barneby - Maguire et al. 36631, 27.XI.1953 (NY, US); Maguire et al. 36473, 27.XI.1953 (NY); Stevenson & Ramos 1064, 27.XI.1987 (NY, US). **C. jacobinea** (Benth.) H.S.Irwin & Barneby - Araújo 6, 17.I.2008 (HUEFS); Santos et al. 489, 12.III.2006 (HUEFS). **C. monticola** (Benth.) H.S.Irwin & Barneby - Coutinho et al. 049, 20.VIII.2012 (VIC); Coutinho et al. 052, 20.VIII.2012 (VIC); Coutinho et al. s.n., 09.X.2010 (VIC). **C. negrensis** var. **negrensis** (H.S. Irwin) H.S. Irwin & Barneby - Martins et al. 83, 04.II.1998 (NY); Nascimento et al. 1, 02.IV.1975 (NY); Prance et al. 24339, 27.XI.1976 (VIC); Pires & Silva 11304, 19.X.1967 (NY); Silva & Brazão 60789, 08.I.1966 (NY). **C. myrophenges** (H.S.Irwin & Barneby) H.S.Irwin & Barneby - Coutinho et al. 050, 02.VIII.2012 (VIC); Coutinho et al. 051, 02.VIII.2012 (VIC). **C. ochnacea** var. **purpurascens** (Benth.) H.S.Irwin & Barneby - Coutinho & Lorencini 072, 09.VIII.2012 (VIC); Coutinho & Pereira 157, 24.IV.2013 (VIC); Coutinho et al. 399, 23.III.2014 (VIC). **C. philippi** (H.S. Irwin & Barneby) H.S. Irwin & Barneby - Conceição 311, 05.V.1999 (HUEFS); Conceição & Campos 357, 13.VII.1999 (HUEFS); Queiroz et al. 7107, 15.VI.2002 (HUEFS). **C. polystachya** (Benth.) H. S. Irwin & Barneby - Steward et al. 156, I.XII.1977 (NY, US); Rando 1150, 07.II.2012 (HUEFS, SPF). **C. potentilla** (Mart. ex Benth.) Irwin & Barneby - Coutinho et al. 001, 26.VI.2012 (VIC); Coutinho et al. 004, 26.VI.2012 (VIC); Coutinho et al. 005, 26.VI.2012 (VIC); Coutinho et al. 020, 27.VI.2012 (VIC); Coutinho & Lorencini 078, 11.VIII.2012 (VIC). **C. ramosa** (Vogel) H.S. Irwin & Barneby - Coutinho et al. 423, 24.III.2014 (VIC); Coutinho et al. 449, 25.III.2014 (VIC); Coutinho et al. 450, 25.III.2014 (VIC). **C. ramosa** var. **parvifoliola** (H.S. Irwin) H.S. Irwin & Barneby - Coutinho et al. 35.639, 03.VII.2011 (VIC); Coutinho et al. 489, 03.VII.2011 (VIC). **C. rupestrium** H.S.Irwin & Barneby - Conceição et al. 588, 30.V.2003 (HUEFS); Conceição et al. 677 23.VII.2003 (HUEFS). **C. scleroxylon** (Ducke) H.S.Irwin & Barneby - G. A. Black F19A 47-1133, 31.VII. 1947 (NY, US); Rando 1212, 24.02.2012 (HUEFS, SPF). **C. semaphora** (H.S. Irwin & Barneby) H.S. Irwin & Barneby - Coutinho et al. 003, 26.VI.2012 (VIC); Coutinho et al. 019, 27.VI.2012 (VIC); Coutinho et al. 044, 01.VIII.2012 (VIC). **C. setosa** (Vogel) H.S.Irwin & Barneby - Coutinho et al. 028, 01/VIII/2012 (VIC); Francino & Dalvi 21, 22.VI.2009 (VIC). **C. simplifacta** H.S.Irwin & Barneby - Coutinho & Moura 132, 19.I.2013 (VIC); Coutinho & Moura 133 19.I.2013 (VIC). **C. sincorana** (Harms) H.S.Irwin & Barneby - Costa et al. 61, 13.X.2005 (HEUFS); Costa et al. 129, 20.I.2006 (HUEFS). **C. speciosa** Conc., L.P. Queiroz & G.P. Lewis - Coutinho et al. s.n., 03.VII.2011 (VIC). **C. subpeltata** (Rizzini) H.S. Irwin & Barneby - Lima 13469, 21.V.1976 (RB); Taylor et al. E1319, 23.IV.1983 (NY, US); Vilhena et al. 976, 10.III.1983 (NY, US). **C. tephrosiifolia** (Benth.) H.S.Irwin & Barneby - Filho 9719, 14.XII.1978 (HUEFS). **C. unijuga** (Benth.) Conc., L.P.Queiroz & G.P.Lewis -

Conceição et al. 417, 17.XI.2005 (HUEFS); Conceição & Leite 694, 19.VIII.2003 (HUEFS); Coutinho & Terra 109, 27.VIII.2012 (VIC); Coutinho & Terra 110, 27.VIII.2012 (VIC); Coutinho & Terra 111, 27.VIII.2012 (VIC); Ribeiro & Moraes 01, 05.IV.2004 (HUEFS). **C. urophyllidia** (H.S. Irwin & Barneby) H.S. Irwin & Barneby - Harley & Giulietti 54656, 06.IX.2003 (HUEFS); Coutinho et al. s.n., 03.VII.2011 (VIC); Coutinho et al. s.n., 03.VII.2001 (VIC). **C. vauthieri** (Benth.) H.S.Irwin & Barneby **-** Coutinho & Lorencini 062, 07.VIII.2012 (VIC); Coutinho & Pereira 162, 24.IV.2013 (VIC); Coutinho & Pereira 163, 24.IV.2013 (VIC). **C. xinguensis** (Ducke) H.S.Irwin & Barneby **-** Duarte 7213, 07.X.1962 (NY); Rando 1208, 23.II.2012 (HUEFS,SPF); Silva & Souza 2409, 27.VIII.1969 (NY).

Table 1

List of Chamaecrista species studied and position of the colleters

	Colleter Position							
Taxon	Base of stipules	Rachis between the leaflets	Base of bracts and bracteoles	Base of sepals	Margin of sepals	Base of petals	Around extrafloral nectaries	Rachis extension
Clade sect. Apoucouita:								
Apo:								
C. adiantifolia var. adiantifolia	I,III	I,III	I,III	I,III	III	I	NO	NO
C. adiantifolia var. pteridophylla	I,III	I,III	I,III	I,III	III	I	NO	NO
C. amabilis	I,III	I,III	I,III	I,III	III	I	NO	NO
C. amorimii[a]	NO	NO	I,III	I,III	III	I	NO	NO
C. apoucouita	I,III	I,III	I,III	I,III	III	I	NO	NO
C. aspleniifolia	I,III	I,III	I,III	I,III	III	I	NO	NO
C. bahiae	I,III	I,III	I,III	I,III	III	I	I,III	NO
C. compitalis	I,III	I,III	I,III	I,III	III	I	NO	NO
C. duartei	I,III	I,III	I,III	I,III	III	I	NO	NO
C. eitenorum var. eitenorum	I,III	I,III	I,III	I,III	III	I	NO	NO
C. eitenorum var. regana	I,III	I,III	I,III	I,III	III	I	NO	NO
C. ensiformis var. ensiformis	I,III	I,III	I,III	I,III	III	I	I,III	NO
C. ensiformis var. maranonica	I,III	I,III	I,III	I,III	III	I	NO	NO
C. ensiformis var. plurifoliolata	I,III	I,III	I,III	I,III	III	I	NO	NO
C. hymenaeifolia	I,III	I,III	I,III	I,III	III	I	NO	NO
C. negrensis var. negrensis	I,III	I,III	I,III	I,III	III	I	NO	NO
C. polystachya	I,III	I,III	I,III	I,III	III	I	I,III	NO
C. scleroxylon	I,III	I,III	I,III	I,III	III	I	I,III	NO
C. subpeltata	I,III	I,III	I,III	I,III	III	I	NO	NO
C. xinguensis	I,III	I,III	I,III	I,III	III	I	I,III	NO
Clade sect. Absus subsect. Absus + sect. Grimaldia:								
Abs:								
C. barbata	V	V	V	A	A	I	ENA	NO
C. botryoides[a]	V	V	V	A	A	I	ENA	NO
C. cipoana	V	V	V	V	A	I	ENA	NO
C. claussenii	V	V	V	V	A	I	ENA	NO
C. conferta	V	V	V	V	A	I	ENA	NO
C. dentata	A	A	A	A	A	A	ENA	NO
C. ericifolia	V	V	V	V	A	I	ENA	NO
C. fagonioides	NO	NO	A	A	A	A	ENA	NO
C. glaucofilix[a]	V	V	V	V	A	I	ENA	NO

C. glaziovii	A	A	A	A	A	A	ENA	NO
C. jacobinea[a]	V	V	V	V	A	I	ENA	NO
C. monticola	VI	VI	VI	VI	A	I	ENA	NO
C. myrophenges	V	V	V	V	A	I	ENA	NO
C. ochnacea var. purpurascens	I,V	I,V	I,V	I,V	A	I	ENA	NO
C. philippi[a]	V	V	V	V	A	I	ENA	NO
C. rupestrium	V	V	V	V	A	I	ENA	NO
C. semaphora	A	A	A	A	A	A	ENA	NO
C. setosa[a]	VI	VI	VI	VI	A	I	ENA	NO
C. sincorana	V	V	V	V	A	I	ENA	NO
C. speciosa[a]	VI	VI	VI	VI	A	I	ENA	NO
C. tephrosiifolia	V	V	V	V	A	I	ENA	NO
C. urophyllidia[a]	V	V	V	A	A	I	ENA	NO
C. vauthieri	V	V	V	V	A	I	ENA	NO
Gri:								
C. absus var. absus[a]	V	V	V	V	A	I	NO	NO
C. absus var. meonandra	V	V	V	V	A	I	NO	NO
Clade sect. Absus subsect. Baseophyllum:								
Bas:								
C. blanchetii[a]	I,III	I,III	I,III	I,III	A	I	I,III	I
C. brachystachya[a]	I,III	I,III	I,III	I,III	A	I	I,III	I
C. coriacea[a]	I,III, IV	I,III,IV	I,III	I,III	A	I	I,III	I
C. cytisoides[a]	I,III	I,III	I,III	I,III	A	I	I,III	I
C. decora[a]	I,III	I,III	I,III	I,III	A	I	I,III	I
C. depauperata[a]	I,III	I,III	I,III	I,III	A	I	NO	I
C. unijuga[a]	I,III	I,III	I,III	I,III	A	I	I,III	I
Taxon not sampled by Conceição et al. (2009):								
Oto:								
C. debilis	III,IV	III, IV	III,IV	III,IV	A	I	III,IV	I
Clade sect. Chamaecrista + sect. Xerocalyx + sect. Caliciopsis: [b]								
Cha:								
C. aristata	I,III	I,III	I,III	I,III	A	I	I,III	NO
C. caribaea var. caribaea	I,V	I,V	I,V	I,V	A	I	NO	NO
C. caribaea var. inaguensis	I,V	I,V	I,V	I,V	A	I	NO	NO
C. caribaea var. lucayana	I,V	I,V	I,V	I,V	A	I	NO	NO
C. cinerascens	A	A	III,V	III,V	A	I	NO	NO
C. potentilla[a]	A	A	III,V	III,V	A	I	NO	NO
C. simplifacta	III,V	III,V	III,V	III,V	A	I	III,V	NO
Xer:								

C. desvauxii var. desvauxii[a]	I,II	I,II	I,II	I,II	A	I	A	A
C. desvauxii var. latistipula	I,II	I,II	I,II	I,II	A	I	A	A
C. desvauxii var. graminea	I	I	I	I	A	I	A	A
C. ramosa	I	I	I	I	A	I	A	A
C. ramosa var. parvifoliola	I	I	I	I	A	I	A	A

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

[a] Taxa sampled by Conceição et al. (2009).

[b] Sect. Caliciopsis is not included in this study.

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

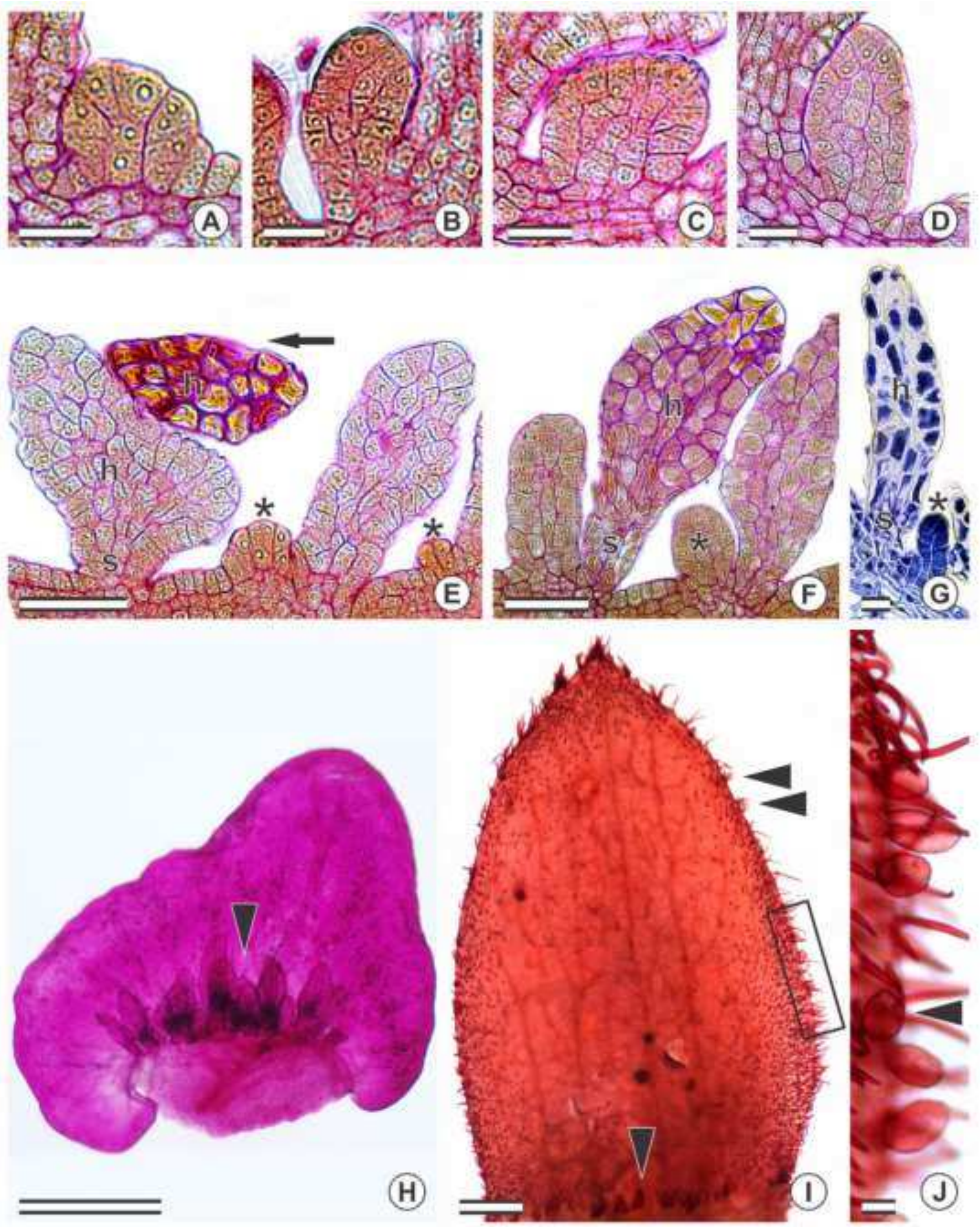

Figure 2. Development of colleters and their distribution on bracts and sepals of Chamaecrista. **A–F.** Longitudinal sections of C. debilis colleters subjected to PAS reaction. **A–D.** Development of colleters. **E–F.** Fully-formed colleters composed of a stalk (s) and a secretory head (h). Note young colleters (asterisk) and colleters already secreting (arrow). **G.** Longitudinal sections of C. brachystachya colleters. **H.** Bract clearing of C. debilis showing colleters (arrowhead) on the lower adaxial side. **I–J.** Sepal clearing of C. hymenaeifolia displaying colleters on the margins and lower adaxial side. Bars = 20 µm, A–D, G; 50 µm, E–F, J; 500 µm, H–I.

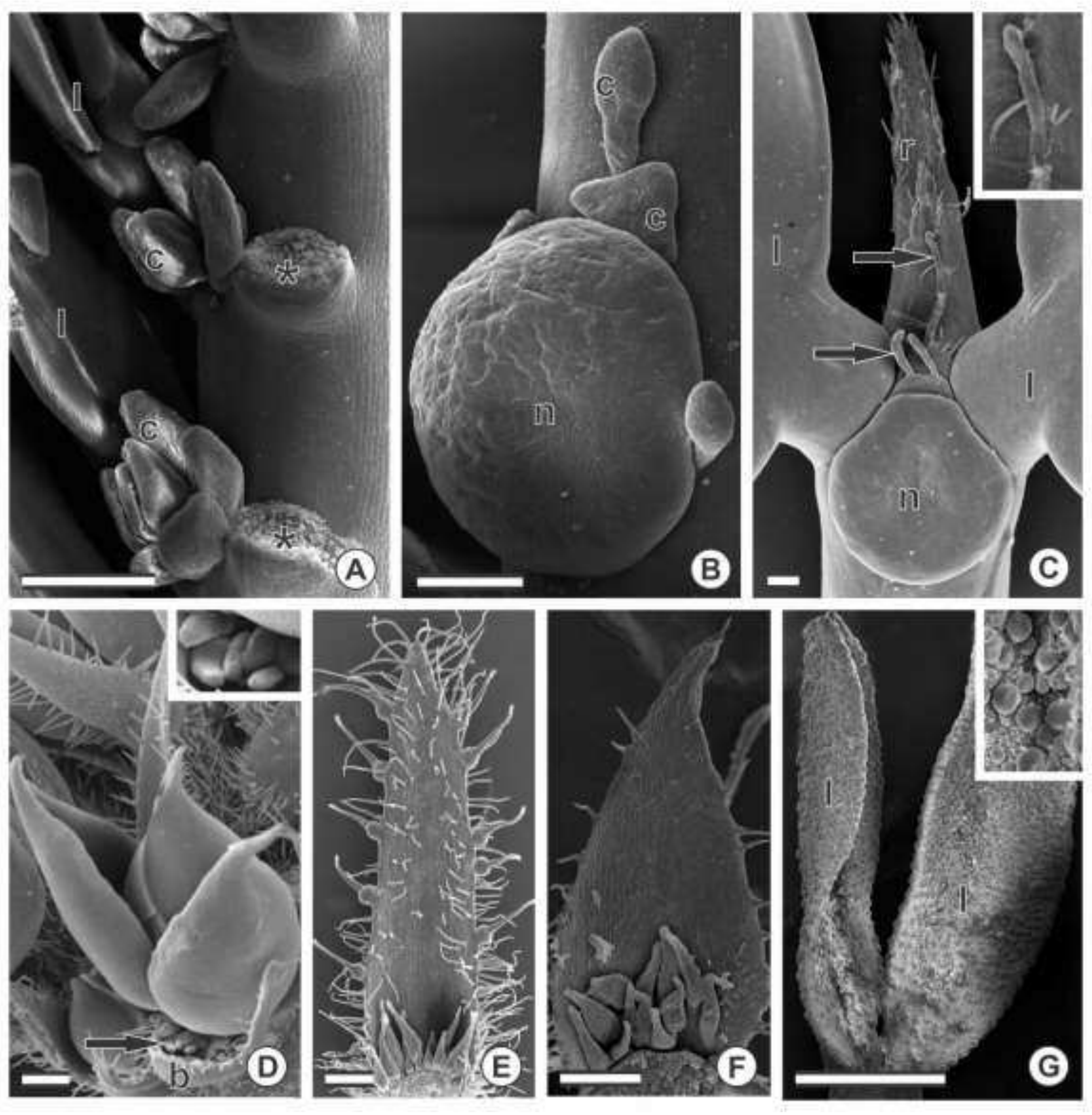

Figure 3. SEM showing colleters on vegetative and reproductive organs of Chamaecrista. **A–B.** Leaves of C. debilis. **A.** Colleters (c) on the rachis between the leaflets (l). The opposite leaflet of each pair of leaflets was removed (asterisk). **B.** Colleters around the extrafloral nectary (n). **C.** Leaf of C. unijuga with colleters (arrow) near the extrafloral nectary and on the rachis extension (r). **D.** Flower bud of C. aristata displaying colleters (in detail) on the adaxial side of bracts (b). **E–F.** Bracts of C. cipoana and C. conferta, respectively, showing colleters on the lower adaxial side. **E.** Note the sticky glandular hairs (SGH) on the bract margin. **G.** Leaf of C. semaphora with sticky glandular hairs (in detail) all over the leaflets (l). Bars = 200 µm

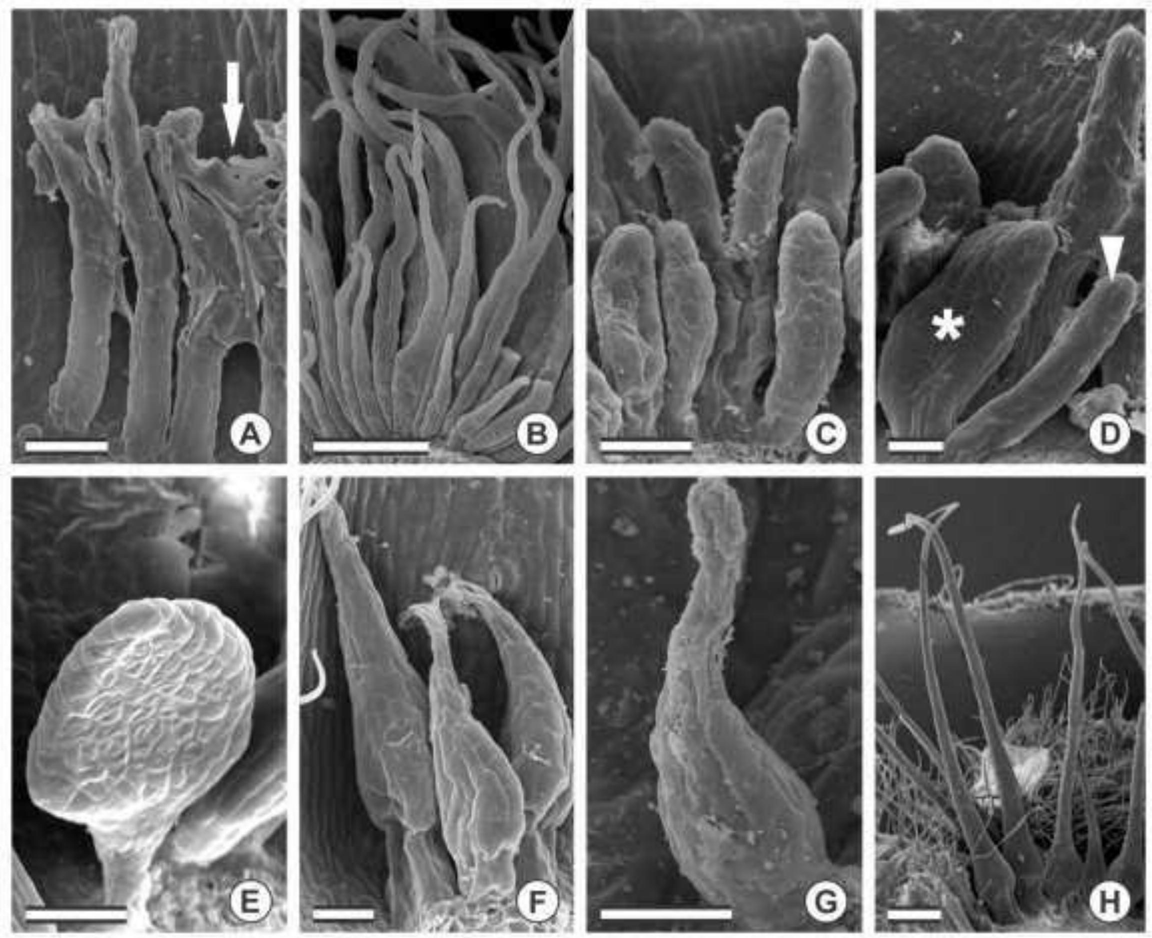

Figure 4. SEM showing the types of colleters in Chamaecrista species. **A.** Short digitiform type on bract of C. unijuga. Note the secretion (arrow) all over the colleters. **B.** Long and short digitiform type on stipule of C. desvauxii var. latistipula. **C.** Club-shaped type on stipule of C. aristata. **D.** Club-shaped (asterisk) and short digitiform (arrowhead) types on stipule of C. cytisoides. **E.** Racket-shaped type on rachis of C. debilis. **F–G.** Short bottle-shaped type on bracts of C. cipoana and stipule of C. glaucofilix, respectively. **H.** Long bottle-shaped type on bract of C. monticola. Bars = 50 µm, except B and H = 300 µm.

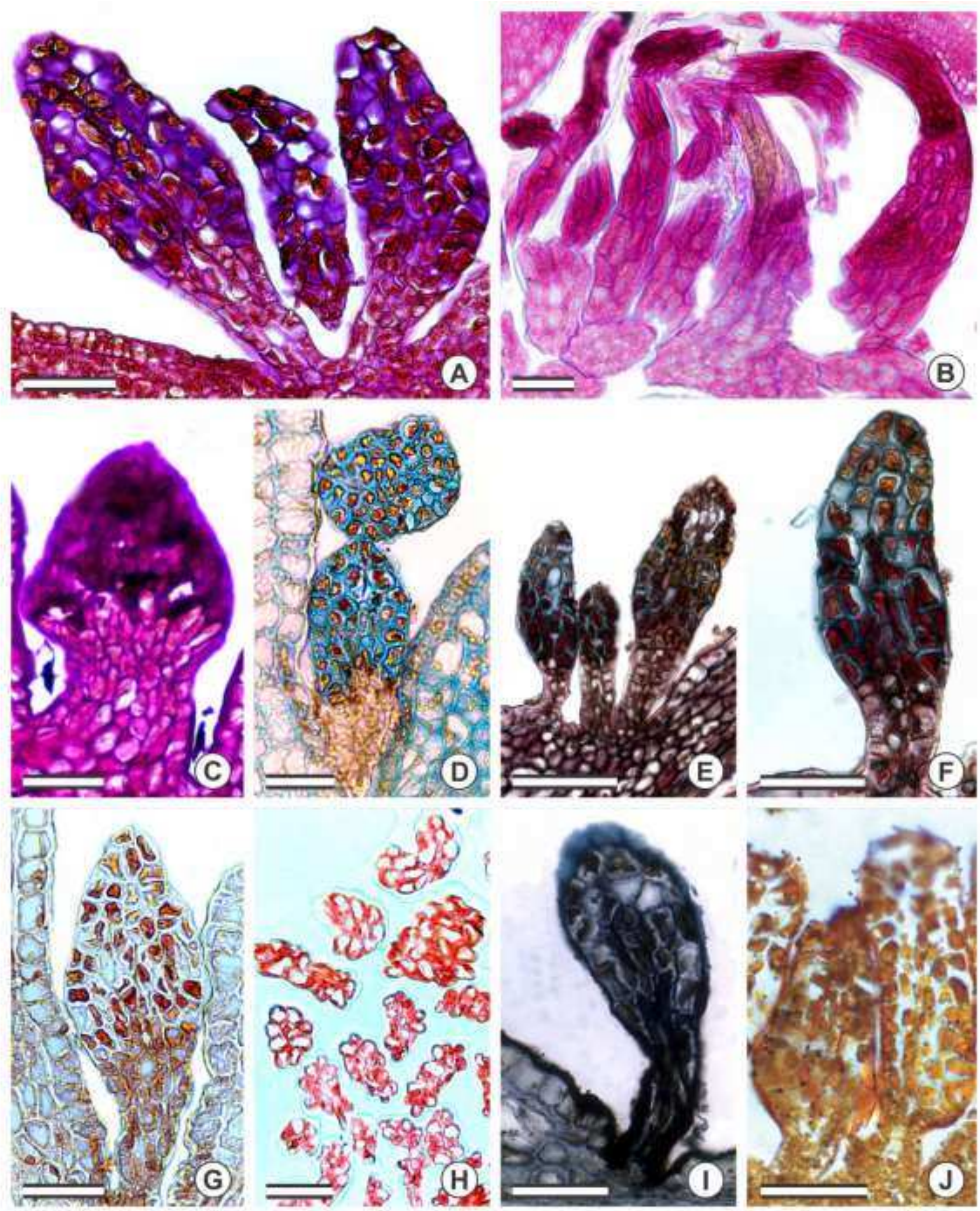

Figure 5. Positive histochemical results in longitudinal sections of colleters of Chamaecrista species. **A–C.** PAS reaction showing total polysaccharides in C. debilis, C. desvauxii var. latistipula and C. myrophenges, respectively. **D.** Alcian blue, acid mucopolysaccharides, in C. debilis. **E–F.** Ruthenium red, pectins, in C. debilis. **G–H.** Xylidine Pounceau, total proteins, in C. debilis (**G**) and C. desvauxii var. latistipula (**H**, cross section). **I–J.** Sudan black B (**I**) and Sudan IV (**J**), total lipids, in C. debilis. Bars = 50 µm.

CAPÍTULO II

Artigo aceito na Botany (Canadian Journal of Botany)

Structural diversity of extrafloral nectaries in Chamaecrista sect. Apoucouita

Ítalo Antônio Cotta Coutinho[1], Renata Maria Strozi Alves Meira[2*]

[1]Universidade Federal de Viçosa – Departamento de Biologia Vegetal – Anatomia Vegetal. Av. P. H. Rolfs, s/n, Campus Universitário - Viçosa, MG, Brasil. CEP 36570-900 (italocoutinho@hotmail.com).

[2]Universidade Federal de Viçosa – Departamento de Biologia Vegetal – Anatomia Vegetal. Av. P. H. Rolfs, s/n, Campus Universitário - Viçosa, MG, Brasil. CEP 36570-900 (rmeira@ufv.br).
[*]corresponding author

Abstract

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

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

Introduction

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

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

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

Materials and Methods

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

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

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

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

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

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

Results

Structure of extrafloral nectaries

The extrafloral nectaries (EFNs) found on the petiole/rachis of Chamaecrista sect. Apoucouita may be impressed (Fig.2), sessile (Fig. 2) or elevated (Fig. 3) (Table 1). Impressed EFNs (Fig. 2A–D) occurred in nine taxa (Table 1), including C. polystachya (Fig. 2A) and C. bahiae (Fig. 2B). Six of the nine taxa displayed the concave (Fig. 2D), flat/truncate (Fig. 2B) and convex (Fig. 2C) types, whereas C. amabilis did not present the convex, C. apoucouita only flat/truncate and C. polystachya that presented both convex and flat/truncate (Table 1).

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

Cupuliform EFNs (Fig. 2F–G and Fig. 3),were the most common type of nectary found in 17 of the 23 species studied (Table 1). The elevation of the EFNs results from the presence of a stalk. Here, ‘stalk’ is defined as the cylindrical structure that bears the secretory apical part and is vascularized and non-secretory. Stalked EFNs differed in the length of the stalk relative to the length of the secretory head: short-stalked EFNs, in which the stalk was shorter than the height of the apical secretory portion (Fig. 3A–D);

stalked EFNs, in which the stalk was one or two times the length of the apical secretory portion (Fig. 3E); and long-stalked EFNs, in which the stalk was more than three times the length of the apical secretory portion (Fig. 3F). Only four species (C. aspleniifolia, C. aspidiifolia, C. compitalis and C. subpeltata) did not present sessile EFNs (Table 1) and characteristically displayed the stalked or long-stalked types. Although EFNs bearing a stalk could be observed in all three varieties of C. ensiformis, C. ensiformis var. plurifoliolata was the variety that most commonly presented the long-stalked type of EFN (Fig.3F).

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

Although the morphology of the EFNs varied according to the taxon studied, all glands displayed anatomical similarities. A single-layered epidermis composed of cubical to columnar-shaped cells and devoid of stomata was observed (Fig. 2A, H and 3A). Whether convex, concave or flat/truncate, the smallest cubical epidermal cells with the thinnest walls were observed at the center of the gland (Fig. 2H and 3A), whereas cells towards the margin were usually larger, more columnar-shaped and possessed thicker cell walls (Fig. 2H and 3A). Similarly, the cuticle covering the epidermis was thicker towards the margin and thinner at the center of the gland. Few multicellular or unicellular, uniseriate, tector trichomes were found on the epidermis and tended to fall off in the mature nectary.

One layer (rarely two) of hypodermal cells was observed. As a whole, the hypodermal layer was similar in shape and structure to the epidermis. At the center of the nectary, between the epidermis and hypodermis, or even below the hypodermis, it was common to observe spaces filled up with secretion among the cells.

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

Despite being vascularized by both phloem and xylem (Fig. 2A, H and 3A), the number of phloem cells in all EFNs was markedly higher than that of xylem cells. Additionally, 1-2 pairs of accessory bundles at each side of the petiole towards the EFNs could also be observed. The vascular tissue ends before the nectary parenchyma layers (Fig. 2A, H and 3A), at an area characterized for having highly vacuolated isodiametric cells, the subnectary parenchyma. The subnectary parenchyma was composed of 1-3 layers of cells smaller than the ground parenchyma cells (Fig. 2A, H and 3A) and was frequently permeated by vascular tissue endings. As the vascular system converged from the petiole towards the nectaries, the cells surrounding the vascular tissue become sclereids instead of fibers. Such sclereids commonly contained one calcium oxalate crystal (Fig. 3B).

Composition of the secretion

The presence of glucose was confirmed in the secretion of the EFNs of C. ensiformis var. ensiformis, C. negrensis var. negrensis and C. polystachya under field conditions. The presence of lipids in the secretion could not be determined by means of

Sudan stains (Sudan Black B and Sudan red), as the secretion was actually rinsed by such stains.

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

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

Discussion

The morphology, structural characterization and presence of glucose and polysaccharides in secreted exudates of the leaf glands of the studied Chamaecrista species support the classification of such glands as nectaries (Fahn 1979; Bentley and Elias 1983; Roshchina and Roshchina 1993; Nicolson et al. 2007). Based on the topography, occurrence on leaves, such glands must be classified as extrafloral nectaries (EFNs) accordingly to Caspary (1848) and extranuptial nectaries accordingly to Delpino (1873), as they are visited by ants and are not near flowers.

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

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

The subnectary parenchyma observed in the EFNs of species of Chamaecrista sect. Apoucouita is similar to what has been reported for Chamaecrista (Francino et al.

2006; Coutinho et al. 2012) and other genera of legumes such as Inga Mill. (Pascal et al. 2000; Fernandes 2011), Senna Mill. (Melo et al. 2010) and Anadenanthera Speg. (Melo et al. 2010). Furthermore, in sect. Absus subsect. Baseophyllum, clusters of idioblasts containing polysaccharides and mucilage/pectins were observed near the nectary parenchyma (Coutinho et al. 2012). Those authors suggested that the substances stored in the idioblasts found at the vascularization endings could act providing an additional energy reservoir for nectar secretion.

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

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

Although the presence of lipids has been reported in numerous flower nectars (Baker 1977; Nicolson et al. 2007), the presence of lipids is not a common feature of

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

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

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

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

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

Conclusion

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

Acknowledgments

The authors thank CNPq, CAPES, FAPEMIG and Floresta Escola (SECTES/UNESCO/HidroEX/FAPEMIG) for financial support; CAPES and CNPq for granting a scholarship to ÍAC Coutinho and RMSA Meira; the herbaria HUEFS, NY, RB, SPF and US for allowing sampling of voucher specimens and especially the Smithsonian Institution for granting the first author the Cuatrecasas Award; and JG Rando and A Nogueira for providing five fixed samples of Chamaecrista sect. Apoucouita.

References

Baker, H.G. 1977. Non-sugar chemical constituents of nectar. Apidologie **8**: 349–356. doi: 10.1051/apido:19770405.

Barneby, R.C. 1999. Increments to genus Chamaecrista (Caesalpiniaceae: Cassiinae) from Bolivia and from Atlantic and Planaltine Brazil. Brittonia **51**: 331–339. doi:10.2307/2666613.

Bentham, G. 1870. Leguminosae II. Cassia. In Flora Brasiliensis. Enumeratio Plantarum in Brasilia Hactenus Detectarum. XV (II). Edited by C.F.P. Martius and A.G. Eichler. Fried. Fleischer, Munich, Vienna, Leipzig. pp. 82–176.

Bentley, B., and Elias, T. 1983. The Biology of Nectaries. Columbia University Press, New York, USA.

Bentley, B.L. 1977. Extrafloral nectaries and protection by pugnacious bodyguards. Annu. Rev. Ecol. Syst. **8**: 407–427. doi: 10.1146/annurev.es.08.110177.002203.

Caspary, R. 1848. De nectariis. In Botanische Zeitung. Elverfeld, Bonnae.

Conceição, A.S., Queiroz, L.P., Lewis, G.P., Andrade, M.J.G. de, Almeida, P.R.M. de, Schnadelbach, A.S., and van den Berg, C. 2009. Phylogeny of Chamaecrista Moench (Leguminosae-Caesalpinioideae) based on nuclear and chloroplast DNA regions. Taxon **58**: 1168–1180.

Costa, F.M.C.B., Oliveira-Filho, A.T., and Oliveira, P.S. 1992. The role of extrafloral nectaries in Qualea grandiflora (Vochysiaceae) in limiting herbivory: an experiment of ant protection in cerrado vegatation. Ecol. Entomol. **17**: 363–365. doi.: 10.1111/j.1365-2311.1992.tb01071.x.

Coutinho, Í.A.C., Francino, D.M.T., Azevedo, A.A., and Meira, R.M.S.A. 2012. Anatomy of the extrafloral nectaries in species of Chamaecrista section Absus subsection Baseophyllum (Leguminosae, Caesalpinioideae). Flora **207**: 427–435. Elsevier GmbH. doi: 10.1016/j.flora.2012.03.007.

Coutinho, Í.A.C., Valente, V.M.M., and Alves, M.R.M.S. 2010. Ontogenetic, anatomical and histochemical study of the extrafloral nectaries of Sapium biglandulosum (Euphorbiaceae). Aust. J. Bot. **58**: 224–232. doi: 10.1071/BT09200.

Curtis, J.D., and Lersten, N.R. 1978. Heterophylly in Populus grandidentata (Salicaceae) with emphasis on resin glands and extrafloral nectaries. Am. J. Bot. **65**: 1003–1010. doi: 10.2307/2442687.

Delpino, F. 1873. Ulteriori osservazioni e considerazioni sulla Dicogamia nel regno vegetale. Delle piante zooifile. Atti della Soc. Ital. di Sci. Nat. **16**: 151–349.

Durkee, L.T., Baird, C.W., and Cohen, P.F. 1984. Light and electron microscopy of the resin glands of Passiflora foetida (Passifloraceae). Am. J. Bot. **71**: 596–602. doi: 10.2307/2443335.

Elias, T.S. 1972. Morphology and anatomy of foliar nectaries of Pithecellobium macradenium (Leguminosae). Bot. Gaz. **133**: 38–42. doi: 10.1086/336611.

Elias, T. 1983. Extrafloral nectaries: their structure and distribution. In The Biology of Nectaries. Edited by B. Bentley, and T. Elias. Columbia University Press, New York, USA. pp. 174-203.

Evert, R.F. 2006. Esau's Plant Anatomy: Meristems, Cells, and Tissues of the Plant Body: Their Structure, Function, and Development. John Wiley & Sons, Inc., Hoboken, New Jersey, USA.

Fahn, A. 1979. Secretory Tissues in Plants. Academic Press, London.

Fahn, A. 1988. Secretory tissues in vascular plants. New Phytol. **108**: 229-257. doi: 10.1111/j.1469-8137.1988.tb04159.x.

Fernandes, J.M. 2011. Ingea Benth. (Leguminosae. Mimosoideae) no Estado de Minas Gerais, Brasil: Taxonomia, morfoanatomia de nectários extraflorais e padrões de distribuiçao geográfica. D. Sc. thesis, Departamento de Botânica, Universidade Federal de Viçosa, Brazil.

Francino, D. M. T. 2010. Anatomia foliar de Chamaecrista Moench. (Leguminosae-Caesalpinioideae) como subsídio à taxonomia e à filogenia. D. Sc. thesis, Departamento de Botânica, Universidade Federal de Viçosa, Brazil.

Francino, D.M.T., Sant'Anna-Santos, B.F., Silva, K.L.F., Thadeo, M., Meira, R.M.S.A., and Azevedo, A.A. 2006. Anatomia foliar e caulinar de Chamaecrista trichopoda (Caesalpinioideae) e histoquímica do nectário extrafloral. Planta Daninha **24**: 695–705. doi: 10.1590/s0100-83582006000400010.

Freitas, L., Bernardello, G., Galetto, L., and Paoli, A.A.S. 2001. Nectaries and reproductive biology of Croton sarcopelatus (Euphorbiaceae). Bot. J. Linn. Soc. **136**: 267–277. doi: 10.1006/bojl.2000.0437.

Heil, M., and McKey, D. 2003. Protective ant-plant interactions as model systems in ecological and evolutionary research. Annu. Rev. Ecol. Evol. Syst. **34**: 425–553. doi: 10.1146/annurev.ecolsys.34.011802.132410.

Irwin, H.S., and Barneby, R.C. 1977. Monographic studies in Cassia (Leguminosae: Caesalpinioideae). IV. Supplementary notes on section Apoucouita Bentham. Brittonia **29**: 277–290. doi: 10.2307/2806200.

Irwin, H.S., and Barneby, R.C. 1979. Two new Brazilian species of Chamaecrista Moench (Leguminosae: Caesalpinioideae). Brittonia **31**: 464–468.

Irwin, H.S., and Barneby, R.C. 1982. The American Cassiinae: A synoptical revision of Leguminosae tribe Cassieae subtribe Cassiinae in the New World. Mem. N. Y. Bot. Gard. **35**: 1–918. 10.2307/4108613.

Irwin, H.S., and Barneby, R.C. 1985. A new arborescent Chamaecrista (Caesalpiniaceae: Cassiinae). Brittonia **37**: 14–16. doi: 10.2307/2806236.

Irwin, H.S., and Rogers, D.J. 1967. Monographic studies in Cassia (Leguminosae-Caesalpinioideae). II. A taximetric study of section Apoucouita. Mem. N. Y. Bot. Gard. **16**: 71–118.

Johansen, D.A. 1940. Plant Microtechnique. McGraw-Hill Book, New York, USA.

Keeler, K.H., and Kaul, R.B. 1979. Morphology and distribution of petiolar nectaries in Ipomoea (Convolvulaceae). Am. J. Bot. **66**: 946–952. doi: 10.2307/2442236.

Koteyeva, N.K. 2005. A novel structural type of plant cuticle. Dokl. Biol. Sci. **403**: 283–285. doi: 10.1007/s10630-005-0109-7.

Lanza, J., Vargo, E.L., Pulim, S., and Chang, Y.Z. 1989. Preferences of the fire ants Solenopsis invicta and S. geminata (Hymenoptera: Formicidae) for amino acid and sugar components of extrafloral nectars. Environ. Ecol. **22**: 411–417. doi: 10.1093/ee/22.2.411.

Leitão, C.A.E., Meira, R.M.S.A., Azevedo, A.A., Araújo, J.M. de, Silva, K.L.F., and Collevatti, R.G. 2005. Anatomy of the floral, bract, and foliar nectaries of Triumfetta semitriloba (Tiliaceae). Can. J. Bot. **286**: 279–286. doi: 10.1139/b05-001.

Lewis, G. 2005. Tribe Cassieae. In Legumes of the World. Edited by G. Lewis, B. Schrire, B. Mackinder, and M. Lock. Royal Botanic Gardens, Kew. pp. 111–161.

Mayer, J.L.S., Cardoso-Gustavson, P., and Appezzato-da-Glória, B. 2011. Colleters in monocots: new record for Orchidaceae. Flora **206**: 185–190. Elsevier GmbH. doi: 10.1016/j.flora.2010.09.003.

McDade, L.A., and Melvin, D.T. 1997. Structure and development of bracteal nectary glands in Aphelandra (Acanthaceae). Am. Fern Soc. **84**: 1–15. doi: 10.2307/2445877.

Melo, Y., Machado, S.R., and Alves, M. 2010. Anatomy of extrafloral nectaries in Fabaceae from dry seasonal forest in Brazil. Bot. J. Linn. Soc. **163**: 87–98. doi: 10.1111/j.1095-8339.2010.01047.x.

Nascimento, E.A. do, and Del-Claro, K. 2010. Ant visitation to extrafloral nectaries decreases herbivory and increases fruit set in Chamaecrista debilis (Fabaceae) in a Neotropical savanna. Flora **205**: 754–756. Elsevier GmbH. doi: 10.1016/j.flora.2009.12.040.

Nepi, M. 2007. Nectary structure and ultrastructure. In Nectaries and Nectar. Edited by S.W. Nicolson, M. Nepi, and E. Pacini. Springer, Dordrecht, The Netherlands. pp. 129-166. doi: 10.1007/978-1-4020-5937-7.

Nicolson, S.W., Nepi, M., and Pacini, E. 2007. Nectaries and Nectar. Springer, Dordrecht, The Netherlands. doi: 10.1007/978-1-4020-5937-7.

O'Brien, T.P., and McCully, M.E. 1981. The study of plant structure principles and selected methods. Termarcarphi Ptey. Ltd., Melbourne, Australia.

Oliveira, P.S., Rico-Gray, V., Díaz-Castelazo, C., and Castillo-Guevara, C. 1999. Interaction between ants, extrafloral nectaries and insect herbivores in Neotropical coastal sand dunes: herbivore deterrence by visiting ants increases fruit set in Opuntia stricta (Cactaceae). Funct. Ecol. **13**: 623–631. doi: 10.1046/j.1365-2435.1999.00360.x.

Pacini, E., and Nepi, M. 2007. Nectar production and presentation. In Nectaries and Nectar. Edited by S.W. Nicolson, M. Nepi, and E. Pacini. Springer, Dordrecht, The Netherlands. pp. 167-214. doi: 10.1007/978-1-4020-5937-7.

Pascal, L.M., Motte-Florac, E.F., and McKey, D.B. 2000. Secretory structures on the leaf rachis of Caesalpinieae and Mimosoideae (Leguminosae): Implications for the evolution of nectary glands. Am. J. Bot. **87**: 327–338. doi: 10.2307/2656628.

Pearse, A.G.E. 1980. Histochemistry Theoretical and Applied. 4th edition. Churchill Livingston, Edinburgh.

Radford, A.E., Dickison, W.C., Massey, J.R., and Bell, C.R. 1974. Vascular Plant Systematics. Harper and Row, New York.

Rando, J.G. 2009. Chamaecrista Moench seções Apoucouita, Chamaecrista e Xerocalyx (Leguminosae - "Caesalpinioideae") na Serra do Cipó, Minas Gerais, Brasil. Instituto de Biociências da Universidade Federal de São Paulo.

Roshchina, V. V, and Roshchina, V.D. 1993. The Excretory Function of Higher Plants. Springer-Verlag, Berlin, Germany. doi: 10.1007/978-3-642-78130-8.

Silva, M.S. 2012. Anatomia foliar como subsídio à taxonomia de espécies de Chamaecrista Moench (Leguminosae-Caesalpinioideae). M. Sc. dissertation, Departamento de Educação, Universidade do Estado da Bahia (BA), Brazil.

Smith, F.H., and Smith, E.C. 1942. Anatomy of the inferior ovary of Darbya. Am. J. Bot. **29**: 464–471. doi: 10.2307/2437312.

Stpiczyńska, M., Milanesi, C., Faleri, C., and Cresti, M. 2005. Ultrastructure of the nectary spur of Platanthera chlorantha (Custer) Rchb. (Orchidaceae) during successive stages of nectar secretion. Acta Biol. Cracoviensia **47**: 111–119.

Wagner, D., and Kay, A. 2002. Do extrafloral nectaries distract ants from visiting flowers? An experimental test of an overlooked hypothesis. Evol. Ecology Res. **4**: 293–305.

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

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

(NY, US); Maciel & Cordeiro 202, 07.XII.1978 (NY); Bastos et al. 126, 12.XII.1978 (NY); Plowman et al. 9863, 21.III.1980 (US); Plowman et al. 9885, 22.III.1980 (US); Rabelo & Jonas 2201, 14.V.1983 (NY); Daly & Cardoso 3826, 19.XII.1984 (US); Pirani et al. 3481, 08.XII.1994 (NY); Amorim et al. 2111, 21.I.1998 (NY); Hatschbach et al. 68571, 17.X.1998 (NY); *Rando 1214, 01.III.2012 (SPF, HUEFS). **C. compitalis** (H.S. Irwin & Barneby) H.S. Irwin & Barneby - Belém 3637, 24.V.1968 (NY); Belém 3643, 24.V.1968 (NY, US). **C. duartei** (H.S.Irwin) H.S.Irwin & Barneby – Duarte 8014, 19.XI.1963 (NY, US); Belém & Pinheiro 2946, 30.XI.1966 (NY); Soares 2191, 06.XII.1966 (US); Pinheiro 248, 26.IX.1967 (US); Belém & Pinheiro 3054, 12.I.1967 (NY); Almeida & Santos 195, 31.X.1968 (NY, US); Santos 2227, 27.XI.1971 (NY); Mori et al. 11036, 04.XI.1978 (NY); Santos 3422, 05.XII.1978 (US); Santos et al. 4565, 24.V.1990 (NY); Thomas et al. 8915, 29.I.1992 (NY); Amorim et al. 1290, 26.V.1993 (NY, US); Amorim et al. 1335, 13.IX.1993 (US); Silva et al. 3624, 30.X.1997 (MY); Amorim & Lorenzi 2102, 07.XI.1997 (NY, US); *Coutinho & Fernandes 303, 15.I.2014 (VIC). **C. eitenorum** var. **eitenorum** (H.S. Irwin) H.S. Irwin & Barneby - Drouet 2541, 01.X.1935 (US); Eiten & Eiten 10660, 18.II.1970 (US); Von Luetzelburg 446, 1970 (US); Santos 1321, 14.I.1971 (US). C. eitenorum var. regana (H.S. Irwin & Barneby) H.S. Irwin & Barneby - Noblick 3034, 07.III.1984 (US); Hatschbach & Hatschbach 56931, 09.IV.1992 (US); Queiroz et al. 6131, 18.II.2000 (HUEFS). **C. ensiformis** var. **ensiformis** (Vell.) H.S. Irwin & Barneby - Riedel 1201, X.1832 (NY); Guillemin 145, 1838 (NY); Rosa , 30.VII. 1936 (NY); Irwin 2330, 29.XII.1958 (NY); Hoehne 5642, 24.III.1964 (NY); Sucre 5091 & Plowmann, 27.V.1969 (NY); Santos 2120, 11.XI.1971 (NY); Euponino 178, 24.I.1972 (NY); Santos 2272, 20.IV.1972 (NY); Pinheiro 1850, 03.VII.1972 (NY); Anderson et al. 11192, 01.II.1975 (NY); Thomas 6169, 26.II.1988 (NY); Amorim et al. 1433, 10.XI.1993 (NY); Amorim et al. 2145, 25.I. 1998 (NY) *Coutinho et al. 033, 01.VIII.2012 (VIC); *Coutinho et al. 034, 01.VIII.2012 (VIC); *Coutinho et al. 035, 01.VIII.2012 (VIC); *Coutinho & Lorencini 070, 08.VIII.2012 (VIC); *Coutinho & Moura 121, 17.I.2013 (VIC); *Coutinho & Pereira 190, 25.IV.2013 (VIC). **C. ensiformis** var. **maranonica** (H.S. Irwin) H.S. Irwin & Barneby – Froés 28501, 20.VIII.1952 (US); Silva 57782, 12.XI.1963 (US); Maguire et al. 56079, 09.VIII.1963 (NY); Prance & Silva 58634, 09.VIII.1964 (US); Prance & Silva 58692, 13.VIII.1964 (NY, US); Prance & Silva 58956, 30.VIII.1964 (NY, US); Prance & Silva 58976, 31.VIII.1964 (NY, US); Silva 378, 15 Jan 1966 (NY); Hatschbach & Kummorw 38396, 26.III.1976 (NY); Silva et al. 2747, 03.IX.1976 (NY); Silva & Bahia 3143, 25.V.1977 (NY, US); Rosa et al. 2580, 11.VII.1978 (NY); Maciel et al. 410, 03.X.1979 (NY); Daly et al. D375, 29.IX.1980 (NY, US); Daly et al. D571, 10.X.1980 (NY, US); Taylor et al. E1180, 10.IV.1983 (NY); Taylor et al. E1303, 22.IV.1983 (NY); Pereira 2555, 07.IX.1993 (NY). **C. ensiformis** var. **plurifoliolata** (Hoehne) H.S. Irwin & Barneby - Warming s.n., 18.VIII.1863 (US); Curran 4675, 29.III.1921 (NY); Nunes s.n., 1930 (NY); Ser. refl. Est. Ferro No. 18, 30.VIII.1930 (NY); Pereira 9551 & Pabst 8440, 19.I.1965 (NY); Belém & Magalhães 955, 25.IV.1965 (NY, US); Santos 2952, 16.IV.1975 (NY); Kallunki et al. 523, 11.II.1994 (NY). **C. hymenaeifolia** (Benth.) H. S. Irwin & Barneby - Maguire et al. 36631, 27.XI.1953 (NY, US); Maguire et al. 36473, 27.XI.1953 (NY); Maguire et al. 42612, 02.I.1958 (NY, US); Lima et al. 3217, 17.XI.1987 (NY); Stevenson & Ramos 1064, 27.XI.1987 (NY, US). **C. negrensis** var. **albuquerquei** H.S. Irwin & Barneby - Coêlho & Coêlho s.n., 06.II.1968 (NY); Santos 144, 04.III.1977 (NY); Henderson et al. 396, 09.V.1985 (NY). **C. negrensis** var. **negrensis** (H.S. Irwin) H.S. Irwin & Barneby - Silva &

Brazão 60789, 08.I.1966 (NY); Pires & Silva 11304, 19.X.1967 (NY); Nascimento et al. 1, 02.IV.1975 (NY); Irwin 37202, 28.VI.1976 (NY); Prance et al. 24339, 27.XI.1976 (VIC); Alencar 596, 12.VII.1979 (NY); Silva 165, 28.VI.1985 (NY); Silva 288, 05.VII.1985 (NY); Ferreira et al. 6846, 19.III.1986 (NY); Belém 2056, 29.III.1986 (NY); Baleé 2447, 12.VI.1986 (NY); Baleé 2480, 13.VI.1986 (NY); Martins et al. 83, 04.II.1998 (NY); *Coutinho et al. 537, 01.I.2015. **C. onusta** Irwin & Barneby - Carvalho et al. 179, 26.II.1980 (NY); Lewis & Carvalho 1024, 07.I.1982 (NY); Carvalho & Chautems 1669, 08.III.1983 (NY); Carvalho et al. 6800, 06.IX.1999 (NY); Amorim et al. 4311, 23.X.2004 (NY). **C. polystachya** (Benth.) H.S. Irwin & Barneby - Schomburgk 1842-3, 1842-1843 (NY); Coradin et al. 558, 08.X.1977 (NY); Steward et al. 156, I.XII.1977 (NY, US); Steward et al. 226, 29.XI.1977 (NY, US); *Rando 1150, 07.II.2012 (HUEFS, SPF); *Coutinho et al. 530, 31.XII.2014;* Coutinho et al. 531, 31.XII.2014. **C. scleroxylon** (Ducke) H.S.Irwin & Barneby – Ducke 16605, 13.XII.1916 (US); Black 47-1031 F19 A, 17.VII.1947 (NY); Black F19A 47-1133, 31.VII. 1947 (NY, US); Fróes 31813, V.1955 (NY); Fróes 32171, 10.X.1955 (US); Fróes 33833, VI.1957 (NY); Fróes 33881, VI.1957 (NY); Duarte 7213, 07.X.1962 (US); Silva & Souza 2557, 11.IX.1969 (NY); *Rando 1212, 24.02.2012 (HUEFS, SPF). **C. subpeltata** (Rizzini) H.S. Irwin & Barneby - Lima 13469, 21.V.1976 (RB); Vilhena et al. 976, 10.III.1983 (NY, US); Taylor et al. E1319, 23.IV.1983 (NY, US). **C. xinguensis** (Ducke) H.S.Irwin & Barneby - Fróes 31033, 20.XIII.1954 (NY); Duarte 7213, 07.X.1962 (NY); Silva & Souza 2259, 14.VIII.1969 (NY); Silva & Souza 2409, 27.VIII.1969 (NY); Silva et al. 3421, 21.X.1977 (NY); Bahia 13, 12.VIII.1978 (NY); Baleé 2424, 12.VI.1986 (NY); Vasconcelos et al. 250, 12.X.1986 (NY); Souza et al. 376, 18.X.1986 (NY); *Rando 1208, 23.II.2012 (HUEFS, SPF).

Table 1. Types of extrafloral nectaries observed on the petiole/rachis of Chamaecrista.

Species/Taxon	**Type of EFN**												
	Impressed			Sessile				Cupuliform			Patelliform		
	Concave	Flat/ truncate	Convex	Flat/ truncate	Convex	Urceolate	Concave	Short-stalked	Stalked	Long-stalked	Short-stalked	Stalked	Long-stalked
C. adiantifolia var. adiantifolia [a]						x	x	x	x				
C. adiantifolia var. pteridophylla [a]						x	x	x	x				
C. amabilis	x	x		x	x	x	x						
C. amorimii	x	x	x	x	x		x						
C. apoucouita [a]		x		x		x	x	x	x				
C. aspleniifolia [d]								x	x				
C. aspidiifolia									x	x			
C. bahiae [a]	x	x	x	x	x		x	x					
C. compitalis									x	x		x	x
C. duartei	x	x	x	x	x		x						
C. eitenorum var. eitenorum				x	x		x	x					
C. eitenorum var. regana				x	x		x	x					
C. ensiformis var. ensiformis [b,c]				x	x		x	x	x	x	x	x	x
C. ensiformis var. maranonica [b,c]				x	x		x	x	x	x	x	x	x
C. ensiformis var. plurifoliolata [b,c]				x	x		x	x	x	x	x	x	x
C. hymenaeifolia	x	x	x	x	x		x						

C. negrensis var. albuquerquei	x	x	x	x	x	x	x	x		
C. negrensis var. negrensis	x	x	x	x	x	x	x	x		
C. onusta							x			
C. polystachya		x	x	x	x					
C. scleroxylon						x	x	x		
C. subpeltata										x
C. xinguensis						x	x	x	x	

[a] Most proximal EFN usually short-stalked while others on the rachis stalked; [b] Most proximal EFN usually short-stalked or sessile while others on the rachis stalked; [c] Although C. ensiformis var. ensiformis and C. ensiformis var. maranonica presented long-stalked EFNs, these are common only to C. ensiformis var. plurifoliolata; [d] Differing from other EFNs due to the common presence of an oblique secretory surface in frontal view.

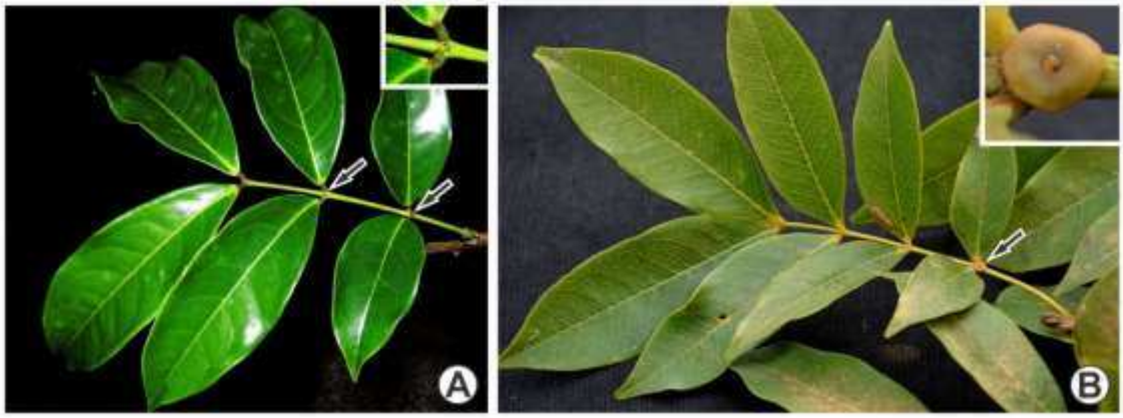

Figure 1. Leaf extrafloral nectaries on the rachides of Chamaecrista species. A, C. duartei. B. C. ensiformis var. ensiformis. Note the secreted nectar in detail.

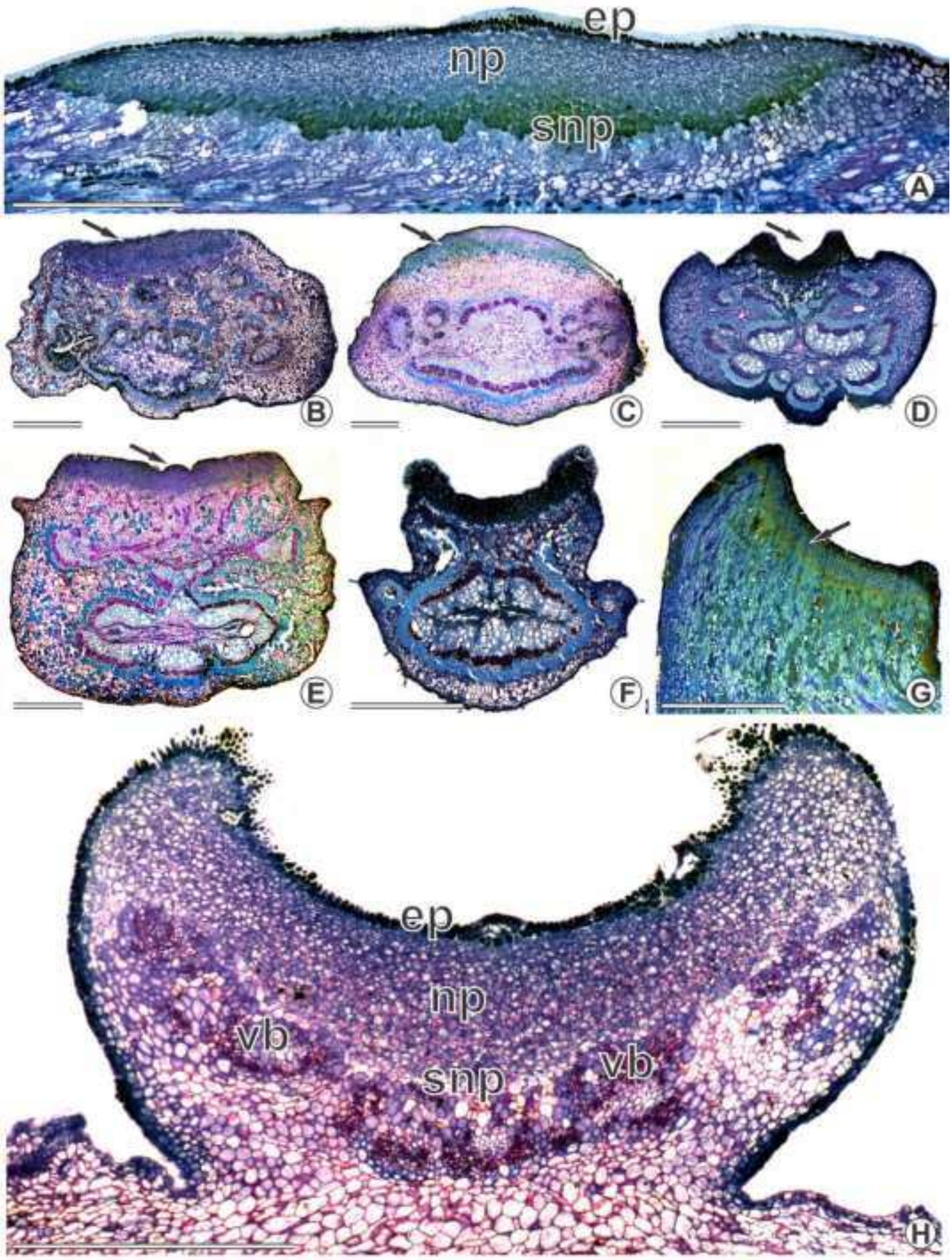

Figure 2. Longitudinal (A, G and H) and cross (B-F) sections of the rachis/petiole showing sessile leaf extrafloral nectaries (EFNs) in Chamaecrista species. A, C. polystachya showing the impressed convex type of EFN. The EFNs are composed of an epidermis (ep), a nectary parenchyma (np) and a subnectary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

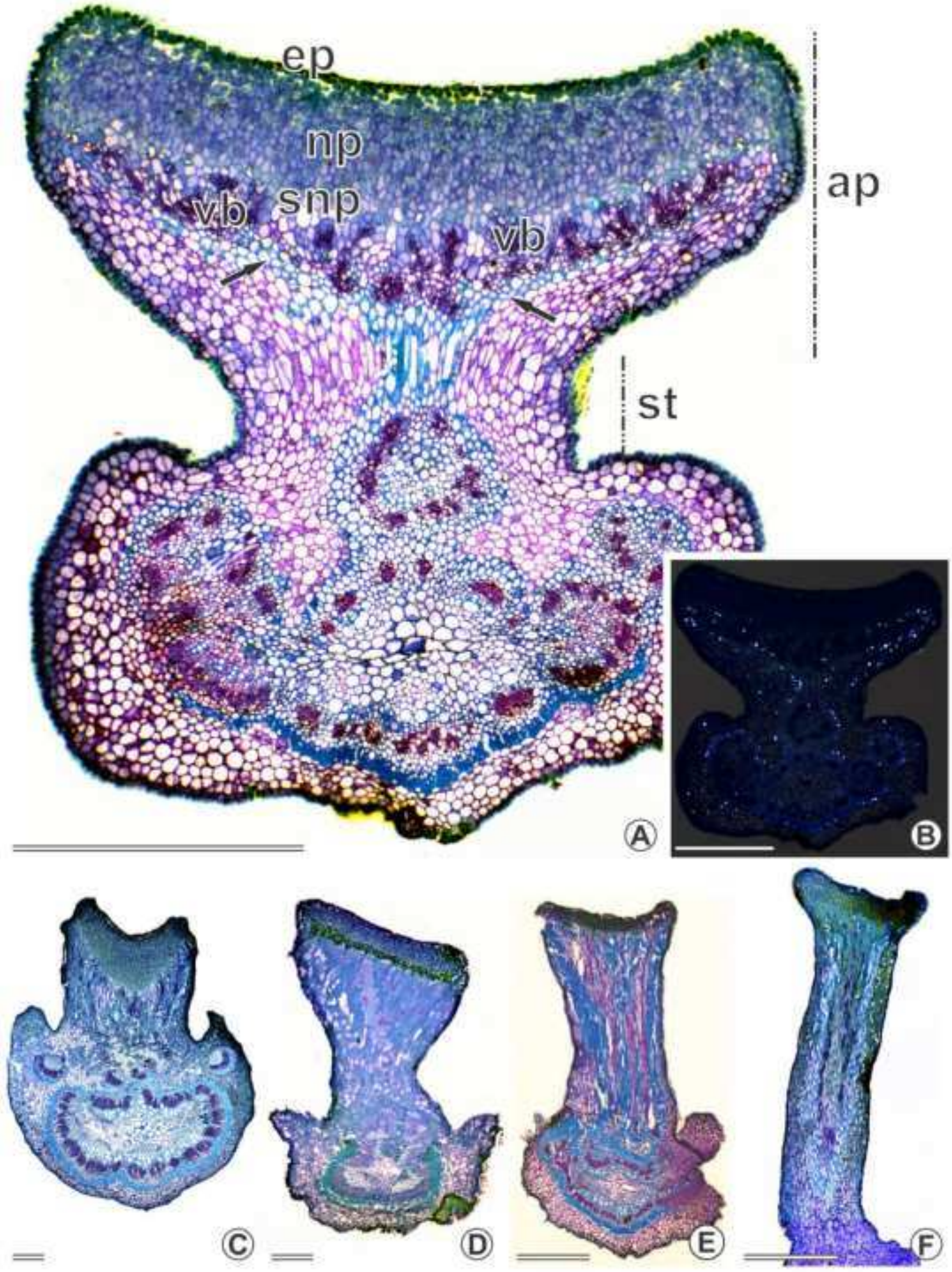

Figure 3. Cross sections of the rachis/petiole showing stalked leaf extrafloral nectaries (EFNs) in Chamaecrista species. Stalked leaf extrafloral nectaries (EFNs) in species of Chamaecrista species. A, C. ensiformis var. ensiformis, short-staked EFNs composed of a secretory apical part (ap) and a stalk (s). The EFNs is anatomically characterized for having an epidermis (ep), a nectary parenchyma (np), a subnectary parenchyma (snp) and vascular bundles (vb). Arrows indicate sclereids. B, C. ensiformis var. ensiformis seen under polarised light. Note the presence of calcium oxalate crystals (bright spots). C, C. scleroxylon, short-staked EFN. Note that the stalk is as wide as the secretory apical part. D, C. aspleniifolia, short-stalked EFN with oblique secretory surface. E, C. compitalis, stalked-EFN. F, C. ensiformis var. plurifoliolata, long-stalked EFN. Scale bars, 200 µm.

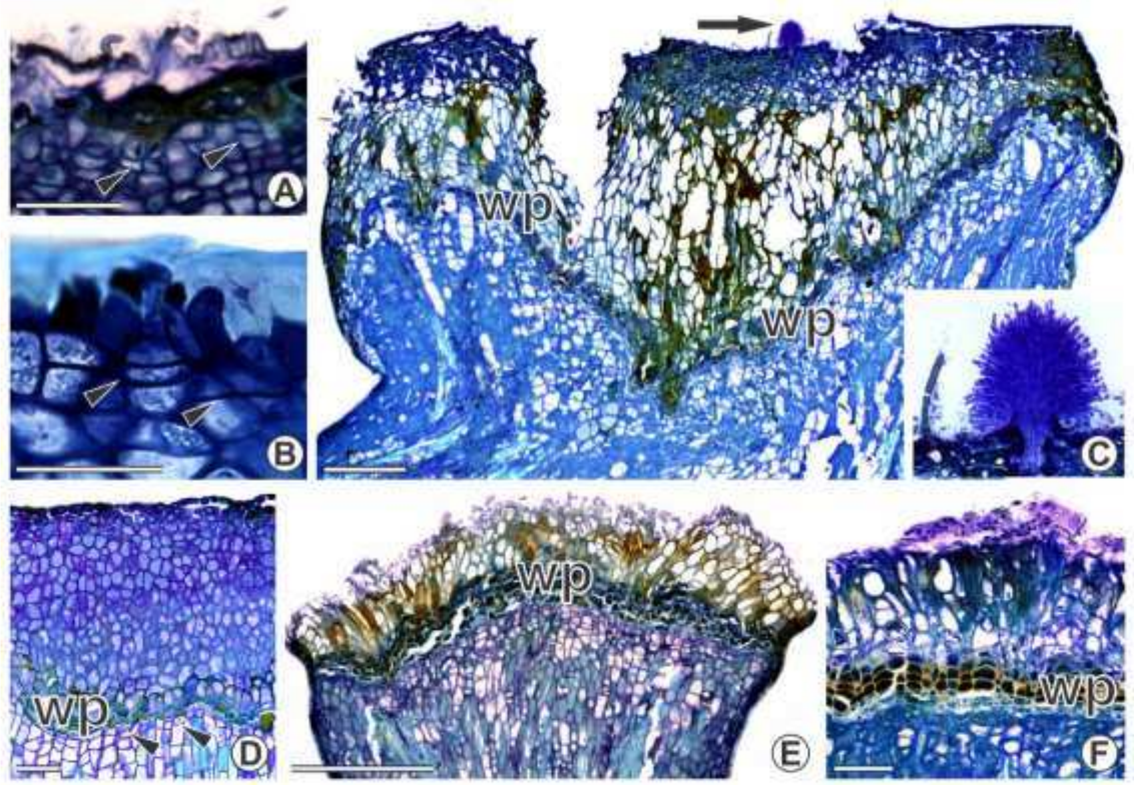

Figure 4. Cross (A-B, D-F) and longitudinal (C) section of the petiole/rachis showing the development of a wound-healing periderm in petiole/rachis extrafloral nectaries Chamaecrista species. A, C. adiantifolia var. pteridophylla and B, C. polystachya. Note the periclinal division of the subepidermal layers that will give rise to the wound-healing periderm (wp). C, C. aspleniifolia with a fungal infection on the epidermis (arrow and magnification). Note the presence of a wound periderm formed at the transition zone, also in D-F. D, C. ensiformis var. ensiformis. E, C. scleroxylon. F, C. apoucouita . Note the presence of cells filled with phenolic compounds. Scale bars, 50 µm (A-B, D and F); 200 µm (C and E).

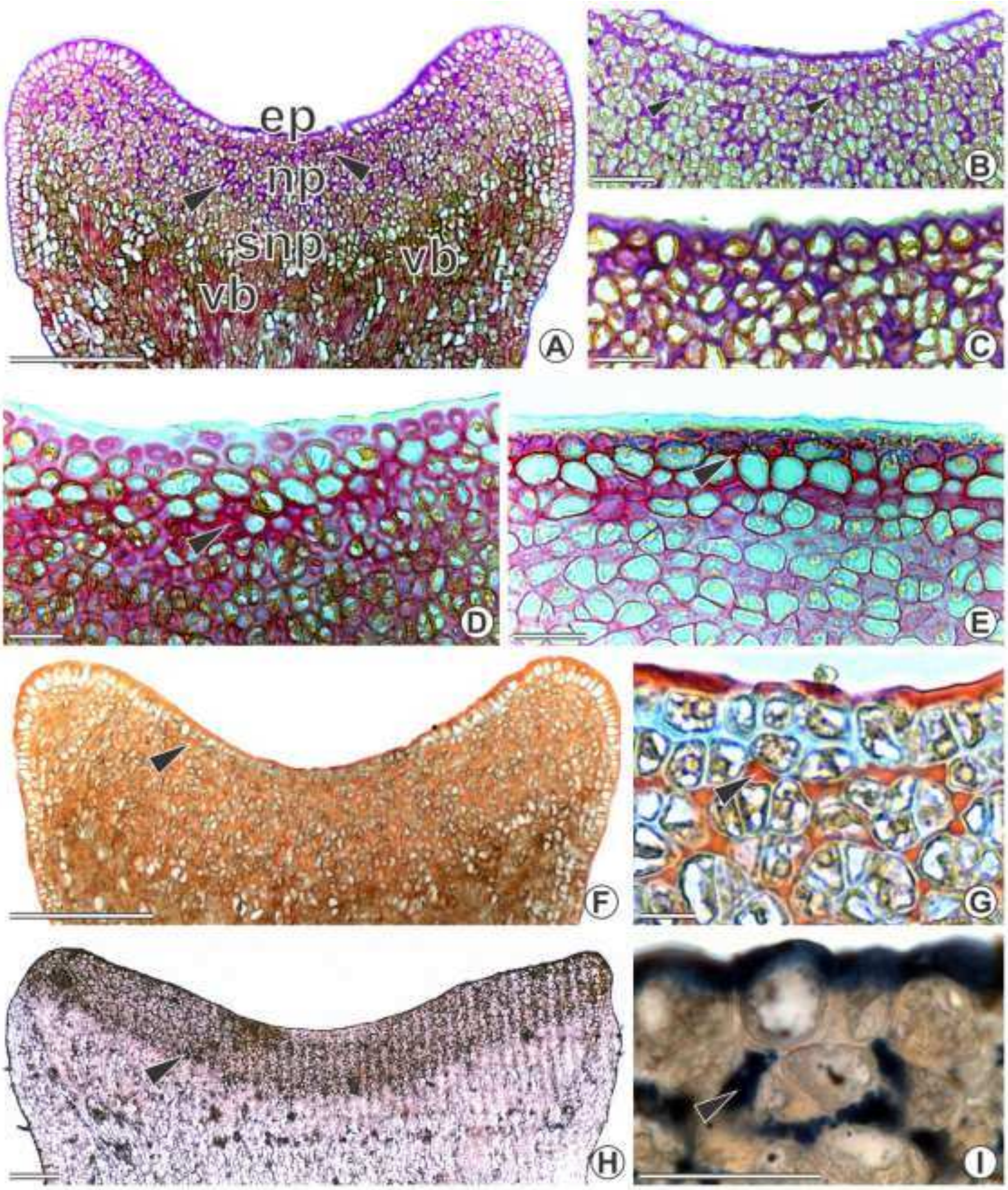

Figure 5. Histochemical tests of the EFNs conducted in cross sections of the petiole/rachis of Chamaecrista species. A-C, periodic acid Schiff (PAS) in C. xinguensis (A-B) and C. ensiformis var. ensiformis. Note the presence of secretion (arrow head) among the cells of the nectary parenchyma (np). D and E, ruthenium red in C. xinguensis (D) and C. polystachya (E). Sudan red in C. xinguensis (F and G) and sudan black B in C. ensiformis var. ensiformis (H and I). ep, epidermis; snp, subnectary parenchyma; vb, vascular bundle. Scale bars, 200 µm (A, F and H); 30 µm (B-E, G and I).

CAPÍTULO III

Artigo nas normas da South African Journal of Botany

A study of the leaf morphoanatomical characters in Chamaecrista Moench sect. Apoucouita

Ítalo Antônio Cotta Coutinho[1]; Adilva de Souza Conceição[2], Renata Maria Strozi Alves Meira[1*]

[1]Universidade Federal de Viçosa (UFV) – Departamento de Biologia Vegetal – Anatomia Vegetal. Av. P. H. Rolfs, s/n, Campus Universitário - Viçosa, MG, Brasil. CEP 36570-900.

[2]Universidade do Estado da Bahia – Departamento de Educação, Herbário HUNEB (Coleção Paulo Afonso), Campus VIII. R. do Gangorra 503, CHESF, Alves de Sousa, 48608-240 - Paulo Afonso, BA, Brasil.

[*]corresponding author.

Abstract

Chamaecrista Moench is one of the most diverse genera of Leguminosae-Caesalpinioideae. Although Chamaecrista has received much attention in the last ten years, little attention has been paid to species of C. sect. Apoucouita, especially regarding anatomical studies. Additionally, the recognition of the varieties within species of C. sect. Apoucouita seems to be whether arbitrary and non-reproductive specimens of such section may be quite difficult to have their species determined as vegetative material may resemble species of the genus Inga. The present study has focused on recognizing leaf morphoanatomical characters that support the taxonomy of the species and varieties of C. sect. Apoucouita by employing standard anatomical techniques. The arrangement of the vascular system in the petiole/rachis and leaflets (similar to siphonostele), dorsiventral mesophyll, mucilage idioblasts in the adaxial and abaxial epidermis, outline of the anticlinal walls of the epidermal cells (straight or lightly sinuous on the adaxial side and sinuous on the abaxial side) and position and type of stomata (hipostomatic leaves and paracytic laterocyclic, respectively) were characters shared by all species of C. sect. Apoucouita and together with morphological data contribute to the consolidation of this group of species as a monophyletic section. The presence of papillae on the epidermis as well as the position and type of extrafloral nectaries are characters that may aid the taxonomy of some species. Moreover, our anatomical data provide no evidence for the recognition the varieties ascribed to some of the species of C. sect. Apoucouita.

Introduction

Chamaecrista Moench is one of the most diverse genera of Leguminosae-Caesalpinioideae, including about 330 species (Lewis, 2005). Chamaecrista species can be readily recognized due to the presence of pinnate leaves (Fig. 1A–C), yellow flowers (Fig. 1D–F) strongly asymmetric, 2 cycles of stamens often of different lengths, androecium (erratically) actinomorphic, a pair of bracteoles at the flower pedicel, pod elastically dehiscent with coiling valves (Fig. 1 G) and either the presence of extrafloral nectaries (Fig. 1A–C) on the leaves or sticky glandular hairs usually all over the plant body (Irwin and Barneby, 1982). Species of Chamaecrista are organized into six sections: C. sect. Apoucouita, Absus, Caliciopsis, Chamaecrista, Grimaldia and Xerocalyx.

Chamaecrista sect. Apoucouita, composed of 21 species (26 taxa), groups most of the woody species of the genus. Besides including arborescent species (Fig. 1D), C. sect. Apoucouita may be characterized for having cauliflorous inflorescences (Fig. 1E) with minute truncate or depressive inflorescence glands (Fig. 1F) and pendant elastically dehiscent pods (Fig. 1G). Such woody species are usually found in neotropical rain forests, most diverse in the Amazon and Brazilian Atlantic forests (Irwin and Rogers, 1967; Irwin and Barneby, 1977; Conceição et al., 2009).

It is true that Chamaecrista has received much attention in the last 10 years as anatomical (Francino et al., 2006; Coutinho et al., 2012, 2013, 2015; Silva, 2012; Meira et al., 2014), molecular (Conceição et al., 2008, 2009; Torres et al., 2011), taxonomic (Camargo and Miotto, 2004; Rando, 2009; Dantas and Silva, 2013; Rando et al., 2013), ecological, geographical (Rando and Pirani, 2011), ecological and economic importance (Morris, 2012) studies have focused on such genus and yet little attention has been paid to species of C. sect. Apoucouita, especially regarding anatomical studies.

Additionally, non-reproductive individuals may be quite difficult to have their species determined as vegetative material may resemble species of the genus Inga Mill., especially due to the winged petiole and presence of leaf extrafloral nectaries. Morphoanatomical characters have proved to be useful as an additional tool to taxonomy and phylogeny not only of Leguminosae (Solereder, 1908; Metcalfe and Chalk, 1950, 1979; Lackey, 1978; Luckow, 2002; Arambarri et al., 2006) but also Chamaecrista (Francino, 2010; Coutinho et al., 2013, 2015).

Moreover, the recognition of the varieties within species of C. sect. Apoucouita seems to be whether arbitrary. For instance, varieties of C. adiantifolia were once defined based on the pubescence and flower size (Irwin and Rogers, 1967) and later on length of pulvinule and shape of leaflet apex (Irwin and Barneby, 1977). The authors themselves stated that ‘the varietal key might be emended as follows, but requires testing against the full range of material available to the taximetric study’ (Irwin and Barneby, 1977). Morphoanatomy has proved used in supporting molecular studies in Chamaecrista which up-ranked varieties to the species level (Conceição, 2006; Conceição et al., 2008; Coutinho et al., 2013).

Owing to the importance of morphoanatomical characters as an additional tool to taxonomic and phylogenetical studies, the following questions are here addressed: Are there recognizable leaf morphoanatomical characters that support the taxonomy of the species of C. sect. Apoucouita? Could such characters be used to build an identification key? Can morphoanatomy support the species varieties ascribed to C. sect. Apoucouita?

Material and Methods

We sampled 23 (18 spp.) of the 26 taxa (21 spp.) which belong to C. sect. Apoucouita (Irwin and Barneby, 1982, 1985; Barneby, 1999) (Appendix). Taxonomic authorities for all taxa used in the paper are given in the appendix. Both field-collected and herbarium material were used. Voucher specimens were deposited at the herbarium of the Universidade Federal de Viçosa (VIC).

Fully expanded leaves were collected in the field and fixed in FAA (formaldehyde, acetic acid and 50% ethanol; 1:1:18 by volume) for 48 h and stored in 70% ethanol (Johansen, 1940). Material sampled from herbarium specimens was rehydrated by microwaving in distilled water for 7 min and left to rest overnight. Rehydrated samples were then treated with 2% potassium hydroxide for 1-2 h and dehydrated in an ethanol series and stored in 70% ethanol (Smith and Smith, 1942). For identification of the venation pattern and stomata types, whole and/or fragments of mature leaflets stored in 70% ethanol of both field-collected and herbarium materials were cleared with 10% sodium hydroxide and 20% hypochlorite solutions, stained with diluted fuchsin and mounted in glycerinated gelatin (Johansen 1940).

For structural characterization, fragments from the middle portion of both the leaflet (including the middle vein, margin and area between the margin and the middle vein) and petiole/rachis (base of the petiole right after the pulvinus and rachis right after the insertion of the first pair of leaflets) were taken. The fragments from both the herbarium and field-collected specimens stored in 70% ethanol were embedded in methacrylate (Historesin Leica, Leica Microsystems Nussloch GmbH, Heidelberg, Germany) as recommended the manufacturer. Cross and longitudinal 4-µm-thick sections were made in an automatic rotary microtome (Leica RM2155, Deerfield, IL, USA), placed in glass slides, stained with toluidine blue at pH 4.4 (O'Brien and

McCully, 1981), dried at room temperature and mounted in resin (Permount, Fisher Scientific, NJ, USA).

Both observations and image captures were conducted using an Olympus Optical AX70TRF (Tokyo, Japan) equipped with a U-Photo system and digital camera (AxioCam HRc – Carl Zeiss – Gottingen, Germany). The classification of the venation types, stomata types, and vascular arrangements of the petiole/rachis followed Ellis et al. (2009), Wilkinson (1979) and Howard (1979), respectively. The types of leaf extrafloral nectaries, their position as well as data on the morphology of leaves/leaflets (petiole and blade) were taken from the literature (Irwin and Rogers, 1967; Rizzini, 1976; Irwin and Barneby, 1977, 1979a; b, 1982, 1985; Barneby, 1999; Coutinho and Meira, 2015).

Results

Petiole/Rachis

Petiole shorter than 15 mm was observed in four taxa (C. adiantifolia var. adiantifolia, C. adiantifolia var. pteridophylla, C. aspleniifolia and C. aspidiifolia), longer than 50 mm in C. amorimii only the remaining 18 taxa longer than 15 mm and shorter than 50 mm (Table 1). Winged petiole/rachis (Fig. 2A–F) was present in 16 taxa, from which all varieties of C. ensiformis and C. eitenorum as well as C. amabilis and C. hymenaeifolia may or not present it. Non-secretory trichomes on the petiole/rachis were found in 15 taxa (Table 1), from which five may or not present them (C. duartei, all varieties of C. ensiformis and C. scleroxylon).

Petiole/rachis showed that all species studied had the vascular system forming a siphonostele (Fig. 2 A–D and G–I) made up by a central pith of parenchymatic cells surrounded by layers (three or more) of phloem and xylem, respectively, and then by layers of fibers (Fig. 2I). Sometimes the vascular system did not form concentric layers around the central pith and interruptions were observed as if the siphonostele were incomplete (Fig. 2E, F). The siphonostele along with the surrounding layers of fibers usually displayed a circular shape (Fig. 2B, I). The vascular system occasionally was more like three semi-circles interconnected. Such variations on the vascular system arrangement were observed spread throughout the species and within a species.

Two-four accessory bundles on the adaxial side of the petiole were commonly counted (Fig. 2), but in rare occasion this number could be up to eight. When petiole/rachis was winged, the accessory bundles were usually placed in the wings (Fig. 2A–F).

Sclereids were accounted in the cortex of the petiole/rachis of all studied species, especially in the petiole (Fig. 2G, H–I). Larger leaves presented higher amount of sclereids in the cortex of the petiole. Druses were found inside the sclereids and a single-layered sheath of druses was also present encircling the outermost layer of fiber.

Leaflet blade

The smallest leaflets were found in C. adiantifolia, C. aspidiifolia, C. aspleniifolia and C. subpeltata while the largest in C. amorimii, C. duartei and C. negrensis var. negrensis (Table 1). The highest number of pair of leaflets was found in C. adiantifolia, C. aspidiifolia and C. aspleniifolia while the other species varied from 2–10 pairs (Table 1). All species presented entire pinnate leaves with brochidodromous venation (Fig. 3 A, B), except C. hymenaeifolia that presented craspedodromous-brochidodromous with perimarginal veins of marginal secondary type (Fig. 3C). Marginal ultimate venation was commonly looped (Fig. 3D) for all species but incomplete marginal ultimate venation (Fig. 3D) was also observed in the same leaflet.

Enlarged tracheids (tracheoids) at the vein endings were common among all species (Fig. 3E).

Non –secretory trichomes (Fig. 3F–H) on the leaflets were observed in 14 taxa (Table 1), from which 10 may or not present trichomes on leaflets and when present found mainly on the midrib (Fig. 3F and 4A) and margins (Fig. 3G–H and 4B). Only four taxa presented all collection with trichomes: C. aspidiifolia, C. ensiformis var. maranonica, C. scleroxylon and C. subpeltata.

All species displayed paracytic laterocyclic stomata (Fig. 3I–J) and hipostomatic leaflets (Fig. 3I–M). However, we could rarely observe few stomata scattered along the midrib on the adaxial side. As a whole, the anticlinal cell walls of epidermal cells on the adaxial were straight or lightly sinuous (Fig. 3J–L and Fig. 4C, D) and sinuous on the abaxial side (Fig. 3I–M). However, few exceptions could be observed in collections of the two varieties of C. regana and C. xinguensis and C. onusta, as sinuosity on the adaxial side could also be observed. Exceptions were also displayed by a few collections of the C. amabilis, C. amorimii, C. bahiae, C. compitalis, C. ensiformis, C. hymenaeifolia, C. negrensis, C. onusta and C. polystachya as straight outline of the anticlinal cell walls of epidermal cells on the abaxial was observed. Leaflets showed a single-layered adaxial and abaxial epidermis for all species (Fig. 5).

Papillae (Fig. 4) were observed in five taxa only (Table 1). Two different types of papillae were found: several domes per cell (Fig. 4A–C and Fig. 5B, C) and one dome per cell (Fig. 4D–E and Fig. 5A, D, F). One dome papillae were found in C. aspleniifolia (Fig. 5A, F), C. polystachya (Fig. 4D) and C. hymenaeifolia (Fig. 4E, 5D), the first species displaying such papillae on both adaxial and abaxial sides (Fig. 5A, F) while the other two only on the abaxial side (Fig. 5D). Several domes papillae were found only in the two varieties of C. adiantifolia and were always found on the abaxial side (Fig. 5B, C).

Secretory idioblasts (Fig. 5A, B) were observed on the adaxial and abaxial epidermis but were less frequent on the abaxial epidermis, especially when such epidermis was composed of papillae (Fig. 5B). Sometimes the secretory idioblasts displayed a thinner periclinal cell wall, dividing the cell into two, giving to the epidermis and aspect of double layer (Fig. 5A, B).

Dorsiventral mesophyll (Fig. 5A, B, E–G) was found in all species. Adaxial palisade parenchyma was composed of 1–3 layers of cells while abaxial spongy parenchyma 3–12 layers. The number of layers of both palisade and spongy parenchyma were not fixed accordingly to the species. Sclereids scattered among the mesophyll were exclusive to C. amorimii (Fig. 5E).

Midrib showed the vascular system arranged as collateral vascular bundles (Fig. 5A, G–H). Additionally, we could also observe that some of the samples of all species presented collateral vascular bundles with 3–5 accessory bundles on the adaxial side (Fig. 5H). Similarly to the vascular system in the petiole, layers of fibers surrounded vascular system. At the midrib area, secretory idioblasts in the epidermis were rarely observed (Fig. 5G, H). Layers of collenchyma below the epidermis followed layers of chlorenchyma were found in all species except the two varieties of C. adiantifolia and C. aspleniifolia. However, such character was not fixed as sometimes collenchyma was not found but just a few layers of chlorenchyma or parenchyma cells instead. The chlorenchyma on both adaxial and abaxial side at the midrib could not be classified into palisade or spongy parenchyma as only compact isodiametric cells were found. Only the two varieties of C. adiantifolia and C. aspleniifolia truly presented palisade parenchyma on the adaxial side at the midrib area (Table 1).

Extrafloral Nectaries

Six taxa presented the most basal extrafloral nectary (EFN) exclusively on the petiole proper, as C. hymenaeifolia (Fig. 1B) and C. polystachya (Fig. 1C) (Table 1), from which three may uncommonly present it below the basal pair of leaflets instead (the two varieties of C. adiantifolia and C. xinguensis). Seventeen had the most basal EFN between/immediately below the basal pair of leaflets (Table 1), as in C. eitenorum var. regana (Fig. 1A). The others EFNs found on the leaves of all species were placed between/immediately below other pairs of leaflets, except in C. hymenaeifolia and C. polystachya found only below other pairs of leaflets.

EFNs impressed were observed in eight species, sessile (concave, flat/truncate, convex and/or urceolate) in nineteen, cupuliform (short-stalked, stalked and/or long-stalked) in seventeen and patelliform (short-stalked, stalked and/or long-stalked) only in four (Table 1). The following species may be characterized for having the following types of EFNs: C. aspleniifolia and C. aspidiifolia, cupuliform; C. polystachya, impressed and sessile flat/truncate and/or convex; C. adiantifolia, C. scleroxylon and C. xinguensis, sessile concave and urceolate as well as cupuliform; C. compitalis, cupuliform and patelliform, both stalked and long-stalked; C. amabilis, C. amorimii, C. duartei and C. hymenaeifolia, impressed or sessile; C. eitenorum, sessile or cupuliform; C. subpetalta showed only the long-stalked cupuliform type; C. onusta, sessile concave. C. apoucouita, C. bahiae, C. ensiformis and C. negrensis were the species with the most variable EFNs.

Discussion

The position of the extrafloral nectary (EFN) as well as the type of EFN provided an important set of data that allow the recognition of C. adiantifolia, C. aspleniifolia, C. aspidiifolia, C. hymenaeifolia, C. onusta, C. polystachya and C. subpeltata as little variation was observed in such EFNs. For the other species, the types of EFNs are quite variable which limits their use on taxonomy while their position seems to be a promising character. Position of EFNs has already been successfully used in species of C. sect. Absus subsect. Baseophyllum (Conceição, 2006; Conceição et al., 2008) as well as in Leguminosae (Lersten and Brubaker, 1987; Pascal et al., 2000; Marazzi et al., 2006; Melo et al., 2010) and other plant families (Keeler and Kaul, 1979; Oliveira and Leitão-Filho, 1987; So, 2004; Weber and Keeler, 2013).

The vascular arrangement of the petiole similar to a siphonostele was common for all C. sect. Apoucouita species, even though when the vascular system was formed by three semi-circles interconnected. Such pattern of arrangement as well as the presence of accessory bundles (with accessory bundles in the wings, when such wings are present) have already been reported in the literature for Chamaecrista belonging to different sections (Metcalfe and Chalk, 1950; Francino, 2010; Coutinho et al., 2013). Although the amount of sclereids varied according the specimen analyzed, such character could not be associated with any particular species but could be associated with the size of leaves. The presence of higher amount of sclereids in the cortex of the petiole of larger leaves present indicates that such cells may be involved in the mechanical support of such leaves (Evert, 2006). The arrangement of the vascular tissue in the petiole/rachis as well as the presence of sclereids scattered among the cortex could be useful to distinguish vegetative branches of species of Chamaecrista that resemble Inga species when they are non-flowering. For Inga punctata Willd. and Inga verna subsp. affinis (DC.) T.D. Penn, petiole/rachis in transverse sections also exhibits a continuous or interrupted ring of bundles, accompanied by accessory bundles in the wings. However, the main difference between species of Chamaecrista and species of Inga is the presence of one/two large accessory, centric, medullary bundle with central phloem and the lack of sclereids in Inga (Metcalfe and Chalk, 1950; Arambarri et al., 2006). Although more species of Inga should be checked to confirm if such pattern of arrangement of the vascular system is common for all species of Inga, the arrangement of the vascular tissue in the petiole/rachis is a promising character in the separation of Chamaecrista and Inga. Such difference can be easily observed in the field upon staining cross sections of the petiole/rachis with phloroglucinol, a die that stains lignified cell walls such as fibers and xylem cells (Johansen 1940).

Brochidodromous venation was found in all species but C. hymenaeifolia. The brochidodromous venation agrees with the pinnate venation described for the C. sect. Apoucouita (Irwin and Rogers, 1967; Irwin and Barneby, 1977, 1982). However, the craspedodromous-brochidodromous venation with perimarginal veins of marginal secondary type is a novelty for C. sect. Apoucouita and is a unique to C. hymenaeifolia. Druses associated with the vascular bundles and enlarged tracheids at the vein endings were also observed in all species of C. sect. Apoucouita and has already been reported for other species of Chamaecrista belonging to other sections (Francino, 2010; Silva, 2012; Coutinho et al., 2013) in a way that such characters seem to be common for such genus.

All studied species have hipostomatic leaves, a character that is not widely spread in the genus, as other species from other sections (sect. Absus, Chamaecrista, Grimaldia and Xerocalyx) usually display amphistomatic leaves, except C. sect. Absus subsect. Baseophyllum with species having epistomatic leaves (Francino, 2010; Silva, 2012; Coutinho et al., 2013). In Chamaecrista, hipostomatic leaves are common in species found in rain forests (C. sect. Apoucouita) while amphistomatic/epistomatic leaves are usually found in species from open sunny areas (sect. Absus, Chamaecrista, Grimaldia and Xerocalyx) which demonstrates that Chamaecrista display the typical ecological pattern of stomatal position described in the literature (Fahn and Cutler, 1992; Dickson, 2000).

Paracytic laterocyclic stomata were present in all species of C. sect. Apoucouita and agrees with previous reports in Caesalpinioideae (Solereder, 1908; Metcalfe and Chalk, 1950; Cowan, 1981; Saheed and Illoh, 2010) and Chamaecrista (Francino, 2010; Silva, 2012).

Even though a few exceptions were observed, straight or lightly sinuous outline of the anticlinal cell wall of the epidermal cells on the adaxial side and sinuous outline on the abaxial side was common for all species of C. sect. Apoucouita. Such character is not very common in Chamaecrista as species tend to display straight contour on both sides (Francino, 2010). The few exceptions regarding the outline of the epidermal cells observed may have been caused as a result of the exposure of leaves to sun/shade. As most of our material came from herbarium specimens we do not know if the collected branches were exposed to sun or shade. Leaves exposed to sun usually have a straight outline while leaves exposed to shade display a sinuous outline (Wilkinson, 1979). Epidermal papillae were one of the most distinctive characters for species of C. sect. Apoucouita as only five species displayed them. Papillae on the adaxial and/or abaxial epidermal cells is not a common trait for Chamaecrista as they are usually restricted to the midvein only (Francino, 2010).

The secretory epidermal cells are most probably mucilage idioblasts as the reddish purple staining of cells indicate the presence of pectins (O’Brien and McCully, 1981). The secretory idioblasts found in the mesophyll of species of C. sect. Absus subsect. Baseophyllum revealed to be mucilage idioblasts as proved by histochemical analyses (Coutinho et al., 2013). Such idioblasts also displayed a reddish purple color when stained with toluidine blue (Coutinho et al., 2013) similarly to the present our results. Although Chamaecrista ensiformis var. ensiformis had already been anatomically studied Carvalho (1983-1985), the author failed to describe the presence of secretory idioblasts in the epidermis, even though a histochemical study was carried out. Epidermal mucilage idioblasts were also observed in other species of C. sect. Absus as well as C. sect. Grimaldia (Francino, 2010). Coutinho et al. 2013 suggests that mucilage idioblasts may represent an adaptation to aridity as Chamaecrista is a genus typically found in open sunny areas. However, species of C. sect. Apoucouita are mainly found in the Amazon and Brazilian Atlantic forests, a habitat that is well supplied by water. As C. sect. Apoucouita is the most basal clade of Chamaecrista (Conceição et al., 2009), mucilage idioblasts is probably a character that was already present in the ancestor of the genus.

Dorsiventral mesophyll and open arch collateral vascular bundles which may present 3–5 accessory bundles on the adaxial side were common for all species studied and had already been reported for other species of Chamaecrista (Metcalfe and Chalk, 1950; Francino, 2010; Coutinho et al., 2012). Considering C. sect. Apoucouita only, the presence of sclereids scattered in the mesophyll is a unique character for C. amorimii. Interestingly, C. amorimii is the species with the largest leaf in Chamaecrista, indicating that the presence of sclereids in the mesophyll is an important evolutionary character for such species, aiding on the mechanical support of large leaves (Evert, 2006).

Our morphoanatomical data were not enough to build an identification key for the species of C. sect. Apoucouita as most characters are shared by all species such as the arrangement of the vascular system in the petiole/rachis and leaflets, dorsiventral mesophyll, mucilage idioblasts in the adaxial and abaxial epidermis, outline of the anticlinal walls of the epidermal cells and position and type of stomata. Although such characters may be found in species of other sections of Chamaecrista (Francino, 2010; Coutinho et al., 2012; Silva, 2012), their presence in all species of C. sect. Apoucouita together with morphological characters contribute to the consolidation of this of group of species as a monophyletic section as demonstrated by Conceição et al. (2009).

No anatomical differences have been observed in the varieties ascribed to C. adiantifolia, C. ensiformis and C. negrensis. The recognition of such varieties are still based on the characters pointed out by previous authors (Irwin and Rogers, 1967; Irwin and Barneby, 1977). The only character that could be used for separating C. ensiformis var. maranonica from the other two varieties of C. ensiformis is the presence of non-secretory trichomes on both adaxial and abaxial sides of the leaflets. However, the other two varieties may also bear such trichomes which restricts the use of such character. Moreover, the type EFN is one of the characters used to distinguish the two varieties of C. negrensis yet based on our present data and Coutinho and Meira (2015), such character should no longer be considered as both varieties may present the same types of EFNs.

Conclusion

When the position on the petiole/rachis of EFNs and their types are analyzed together, such characters provide important data for the identification of some species of C. sect. Apoucouita. Paracytic laterocyclic stomata, vascular system with accessory bundles in petiole/rachis and mucilage idioblasts in the epidermis are importat characters present in all species of C. sect. Apoucouita, the most basal clade of Chamaecrista, that must be taken into account in evolutionary studies of the genus. Our data provide evidence for the taxonomic re-evaluation of the varieties of some of the species of Chamaecrista as no anatomical differences were observed in varieties of the same species.

References

Arambarri, A.M., S.E. Freire, M.N. Colares, N.D. Bayón, M.C. Novoa, C. Monti, and S. a Stenglein. 2006. Leaf anatomy of medicinal shrubs and trees from gallery forests of the Paranaense Province (Argentina). Part 1. Boletin de la Sociedade Argetina de Botanica 41: 233–268.

Barneby, R.C. 1999. Increments to genus Chamaecrista (Caesalpiniaceae: Cassiinae) from Bolivia and from Atlantic and Planaltine Brazil. Brittonia 51: 331–339. Available at: http://link.springer.com/10.2307/2666613 [Accessed January 21, 2015].

Camargo, R.A., and S.T.S. Miotto. 2004. O gênero Chamaecrista Moench (Leguminosae-Caesalpinioideae) no Rio Grande do Sul. Iheringia 59: 131–148.

Carvalho, D.M.G. Anatomia foliar de Cassia ensiformis Vell. (Leguminosae - Caesalpinioideae). Arquivos do Jardim Botânico do Rio de Janeiro 27: 157–169.

Conceição, A. de S. 2006. Filogenia do gênero Chamaecrista (Leguminosae-Caesalpinioideae) e taxonomia do grupo Baseophyllum. D. Sc., thesis, Universidade Estadual de Feira de Santana.

Conceição, A.S., L.P. Queiroz, S.M. Lambert, A.C.S. Pereira, and E.L. Borba. 2008. Biosystematics of Chamaecrista sect. Absus subsect. Baseophyllum (Leguminosae-Caesalpinioideae) based on allozyme and morphometric analyses. Plant Systematics and Evolution 270: 183–207.

Conceição, A.S., L.P. Queiroz, G.P. Lewis, M.J.G. de Andrade, P.R.M. de Almeida, A.S. Schnadelbach, and C. van den Berg. 2009. Phylogeny of Chamaecrista Moench (Leguminosae-Caesalpinioideae) based on nuclear and chloroplast DNA regions. Taxon 58: 1168–1180.

Coutinho, Í.A.C., D.M.T. Francino, A.A. Azevedo, and R.M.S.A. Meira. 2012. Anatomy of the extrafloral nectaries in species of Chamaecrista section Absus subsection Baseophyllum (Leguminosae, Caesalpinioideae). Flora 207: 427–435.

Coutinho, Í.A.C., D.M.T. Francino, and R.M.S.A. Meira. 2013. Leaf anatomical studies of Chamaecrista subsect. Baseophyllum (Leguminosae, Caesalpinioideae): new evidence for the up-ranking of the varieties to the species level. Plant Systematics and Evolution 299: 1709–1720.

Coutinho, Í.A.C., D.M.T. Francino, and R.M.S.A. Meira. 2015. New records of colleters in Chamaecrista (Leguminosae, Caesalpinioidae s. l.): structural diversity, secretion, functional role, and taxonomic importance. International Journal of Plant Sciences 176: 72–85.

Coutinho, Í.A.C., and R.M.S.A. Meira. 2015. Structural diversity of extrafloral nectaries in Chamaecrista sect. Apoucouita. Botany (in press).

Cowan, R.S. 1981. Caesalpinioideae. In Advances in Legume Systematics, Part 1., 57–64. Royal Botanical Gardens, Kew, Kew.

Dantas, M.M., and M.J. Silva. 2013. O gênero Chamaecrista (Leguminosae, Caesalpinioideae, Cassieae) no Parque Estadual da Serra Dourada, Goiás, Brasil. Rodriguesia 64: 581–595.

Dickson, W.C. 2000. Integrative plant anatomy. Academic Press, USA.

Ellis, B., D.C. Daly, L.J. Hickey, K.R. Johnson, J.D. Mitchell, P. Wild, and S.L. Wing. 2009. Manual of leaf architecture. Cornell University Press, Ithaca, New York, USA.

Evert, R.F. 2006. Esau's Plant Anatomy: Meristems, Cells, and Tissues of the Plant Body: Their Structure, Function, and Development. John Wiley & Sons, Inc., Hoboken, New Jersey, USA.

Fahn, A., and D.F. Cutler. 1992. Xerophytes. Gebrüder Bomtraeger, Berlin.

Francino, D.M.T. 2010. Anatomia foliar de Chamaecrista Moench (Leguminosae-Caesalpinioideae) como subsídio à taxonomia e à filogenia. Univseridade Federal de Viçosa, Brazil.

Francino, D.M.T., B.F. Sant'Anna-Santos, K.L.F. Silva, M. Thadeo, R.M.S.A. Meira, and A.A. Azevedo. 2006. Anatomia foliar e caulinar de Chamaecrista trichopoda (Caesalpinioideae) e histoquímica do nectário extrafloral. Planta Daninha 24: 695–705.

Howard, R.A. 1979. The petiole. In C. R. Metcalfe, and C. L [eds.], Anatomy of Dicotyledons, volume I, 88–96. Clarendon Press, Oxford.

Irwin, H.S., and R.C. Barneby. 1985. A new arborescent Chamaecrista (Caesalpiniaceae: Cassiinae). Brittonia 37: 14–16.

Irwin, H.S., and R.C. Barneby. 1977. Monographic studies in Cassia (Leguminosae: Caesalpinioideae). IV. Supplementary notes on section Apoucouita Bentham. Brittonia 29: 277–290.

Irwin, H.S., and R.C. Barneby. 1982. The American Cassiinae: A synoptical revision of Leguminosae tribe Cassieae subtribe Cassiinae in the New World. Memoirs of the New York Botanical Garden 35: 1–918.

Irwin, H.S., and R.C. Barneby. 1979a. Three new Brazilian species of Chamaecrista Moench (Leguminosae: Caesalpinioideae). Brittonia 31: 149–155.

Irwin, H.S., and R.C. Barneby. 1979b. Two new Brazilian species of Chamaecrista Moench (Leguminosae: Caesalpinioideae). Brittonia 31: 464–468.

Irwin, H.S., and D.J. Rogers. 1967. Monographic studies in Cassia (Leguminosae-Caesalpinioideae). II. A taximetric study of section Apoucouita. Memoirs of the New York Botanical Garden 16: 71–118.

Johansen, D.A. 1940. Plant microtechnique. McGraw-Hill Book, New York.

Keeler, K.H., and R.B. Kaul. 1979. Morphology and distribution of petiolar nectaries in Ipomoea (Convolvulaceae). American Journal of Botany 66: 946–952.

Lackey, J. 1978. Leaflet anatomy of Phaseoleae (Leguminosae, Papilionoideae) and its relation to taxonomy. Botanical Gazette 139: 436–446.

Lersten, N.R., and C.L. Brubaker. 1987. Extrafloral nectaries in Leguminosae: review and original observations in Erythrina and Mucuna (Papilionoideae; Phaseoleae). Bulletin of the Torrey Botanical Club 114: 437–447.

Lewis, G. 2005. Tribe Cassieae. In G. Lewis, B. Schrire, B. Mackinder, and M. Lock [eds.], Legumes of the World, 111–161. Royal Botanic Gardens, Kew.

Luckow, M.A. 2002. Anatomical features of the leaves in the Dichrostachys group (Leguminosae, Mimosoideae) and their utility for phylogenetic studies. Systematic Botany 27: 29–40.

Marazzi, B., P.K. Endress, L.P. de Queiroz, and E. Conti. 2006. Phylogenetic relationships within Senna (Leguminosae, Cassiinae) based on three chloroplast DNA regions: patterns in the evolution of floral symmetry and extrafloral nectaries. American Journal of Botany 93: 288–303.

Meira, R.M.S.A., D.M.T. Francino, and L. Ascensão. 2014. Oleoresin trichomes of Chamaecrista dentata (Leguminosae): structure, function, and secretory products. International Journal of Plant Sciences 175: 336–345.

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

Metcalfe, C.F., and L. Chalk. 1979. Anatomy of the dicotyledons: systematic anatomy of leaf and stem with a brief history of the subject, vol I. 2nd ed. Clarendon Press, Oxford.

Metcalfe, C.R., and L. Chalk. 1950. Anatomy of the Dicotyledons: leaves, stem and wood in relation to taxonomy with notes on economic uses, vol I. Oxford Clarendon, Oxford.

Morris, J.B. 2012. Showy partridge pea [Chamaecrista fasciculata (Michx.) Greene] with potential for cultivation as a multi-functional species in the United States. Genetic Resources and Crop Evolution 59: 1577–1581.

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

Nascimento, E.A. do, and K. Del-Claro. 2007. Floral visitors of Chamaecrista debilis (Vogel) Irwin & Barneby (Fabaceae - Caesalpinioideae) at Cerrado of Estação Ecológica de Jataí, São Paulo State, Brazil. Neotropical Entomology 36: 619–624.

O'Brien, T.P., and M.E. McCully. 1981. The study of plant structure principles and selected methods. Termarcarphi Ptey. Ltd., Melbourne, Australia.

Oliveira, P.S., and H.F. Leitão-Filho. 1987. Extrafloral nectaries their taxonomic distribution and abundance in the woody flora of Cerrado vegetation in Southeast Brazil. Biotropica 19: 140–148.

Pascal, L.M., E.F. Motte-Florac, and D.B. McKey. 2000. Secretory structures on the leaf rachis of Caesalpinieae and Mimosoideae (Leguminosae): Implications for the evolution of nectary glands. American Journal of Botany 87: 327–338.

Rando, J.G. 2009. Chamaecrista Moench seções Apoucouita, Chamaecrista e Xerocalyx (Leguminosae - "Caesalpinioideae") na Serra do Cipó, Minas Gerais, Brasil. Instituto de Biociências da Universidade Federal de São Paulo.

Rando, J.G., B. Loeuille, and J.R. Pirani. 2013. Taxonomic novelties in Chamaecrista (Leguminosae: Caesalpinioideae) from Brazil. Phytotaxa 97: 17–25.

Rando, J.G., and J.R. Pirani. 2011. Padrões de distribuição geográfica das espécies de Chamaecrista sect. Chamaecrista ser. Coriaceae (Benth.) H. S. Irwin & Barneby, Leguminosae - Caesalpinioideae. Revista Brasileira de Botânica 34: 499–513.

Rizzini, C.T. 1976. Contribuicao ao conhecimento das floras Nordestinas. Rodriguésia 28: 173.

Saheed, S.A., and H.C. Illoh. 2010. A Taxonomic study of some species in Cassiinae (Leguminosae) using leaf epidermal characters. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 38: 21–27.

Silva, M.S. 2012. Anatomia foliar como subsídio à taxonomia de espécies de Chamaecrista Moench (Leguminosae-Caesalpinioideae). M. Sc., dissertation, Universidade do Estado da Bahia (BA), Brazil.

Smith, F.H., and E.C. Smith. 1942. Anatomy of the inferior ovary of Darbya. American Journal of Botany 29: 464–471.

So, M.L. 2004. The occurrence of extrafloral nectaries in Hong Kong plants. Botanical Bulletin of the Academia Sinica 45: 237–245.

Solereder, H. 1908. Systematic anatomy of the dicotyledons. A handbook for laboratories of pure and applied Botany. Clarendon Press, Oxford.

Torres, D.C., J.P.M.S. Lima, A.G. Fernandes, E.P. Nunes, and T.B. Grangeiro. 2011. Phylogenetic relationships within Chamaecrista sect. Xerocalyx (Leguminosae, Caesalpinioideae) inferred from the cpDNA trnE-trnT intergenic spacer and nrDNA ITS sequences. Genetics and Molecular Biology 34: 244–251.

Weber, M.G., and K.H. Keeler. 2013. The phylogenetic distribution of extrafloral nectaries in plants. Annals of botany 111: 1251–1261. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23087129 [Accessed April 4, 2014].

Wilkinson, H.P. 1979. The plant surface (mainly leaf). Part I: Stomata. In C. R. Metcalfe, and L. Chalk [eds.], Anatomy of the Dicotyledons, vol I. 2nd ed, 97–117. Clarendon Press, Oxford.

Table 1. List of morphoanatomical characters and species of the Chamaecrista sect. Apoucouita used in the study. 0 = absent; 1 = present; 0/1 = not always present; [a] usually on the petiole proper but may be placed below the first pair of leaflets; [b] concave and urceolate only; [c] cupuliform stalked and long-stalked only; [d] mainly on the midrib and/or margins; [e] sessile concave only; [f] flat/truncate and/or convex only; [g] cupuliform long-stalked only.

Nº	**Morphoanatomical character**	adia adia	adia pter	amab	amor	apou	aspl	aspi	bahi	comp
01	ENF on the petiole proper	1[a]	1[a]	0	0	0	1	0	0	0
02	EFN between/immediately below the basal pair of leaflets	0	0	1	1	1	0	1	1	1
03	EFN between/immediately below other pairs of leaflets	1	1	1	1	1	1	1	1	1
04	EFN below other pairs of leaflets	0	0	0	0	0	0	0	0	0
05	EFN impressed	0	0	1	1	0	0	0	1	0
06	EFN sessile (flat/truncate, convex, concave and/or urceolate)	1[b]	1[b]	1	1	0	0	1	0	0
07	EFN cupuliform (short-stalked, stalked and/or long-stalked)	1	1	0	0	1	1	1	1	1[c]
08	ENF patelliform (short-stalked, stalked and/or long-stalked)	0	0	0	0	0	0	0	0	1
09	Petiole length in millimeters	<15	<15	>15;<50	>50	>15;<50	<15	<15	>15;<50	>15;<50
10	Non-secretory trichomes on the petiole	1	1	0	0	1	1	1	1	1
11	Petiole winged	1	1	0/1	0	1	1	1	0	1
12	Brochidodromous venation	1	1	1	1	1	1	1	1	1
13	Brochidodromous-craspedodromous	0	0	0	0	0	0	0	0	0
14	Perimarginal veins	0	0	0	0	0	0	0	0	0
15	Number of pairs of leaflets	(15-)20-35	(15-)20-35	2	5	2-4	(10)13-19	9-13	2	(3-)4(-5)
16	Length of leaflets in millimeters	10-20	10-20	40-120	90-200	40-120	10-20	(5)6-25	80-120	10-70
17	Non-secretory trichomes on the adaxial epidermis	0/1[d]	0/1[d]	0	0	0/1[d]	0/1[d]	1[d]	0	0/1[d]
18	Non-secretory trichomes on the abaxial epidermis	0/1[d]	0/1[d]	0	0	0/1[d]	0/1[d]	1[d]	0	0/1[d]
19	Several-dome papillae on the abaxial epidermis	1	1	0	0	0	0	0	0	0
20	One-dome papillae on the adaxial epidermis	0	0	0	0	0	1	0	0	0
21	One-dome papillae on the abaxial epidermis	0	0	0	0	0	1	0	0	0
22	Palisade parenchyma on the adaxial side at the midvein	1	1	0	0	0	1	0	0	0
23	Sclereids in the mesophyll	0	0	0	1	0	0	0	0	0

Table 1 continued

Nº	Morphoanatomical character	duar	eite eite	eite rega	ensi ensi	ensi mara	ensi plur	hyme
01	ENF on the petiole proper	0	0	0	0	0	0	1
02	EFN between/immediately below the basal pair of leaflets	1	1	1	1	1	1	0
03	EFN between/immediately below other pairs of leaflets	1	1	1	1	1	1	0
04	EFN below other pairs of leaflets	0	0	0	0	0	0	1
05	EFN impressed	1	0	0	0	0	0	1
06	EFN sessile (flat/truncate, convex, concave and/or urceolate)	1	1	1	1	1	1	1
07	EFN cupuliform (short-stalked, stalked and/or long-stalked)	0	1	1	1	1	1	0
08	ENF patelliform (short-stalked, stalked and/or long-stalked)	0	0	0	1	1	1	0
09	Petiole length in millimeters	>15;<50	>15;<50	>15;<50	>15;<50	>15;<50	>15;<50	>15;<50
10	Non-secretory trichomes on the petiole	0/1	0	0	0/1	0/1	0/1	1
11	Winged petiole	0	0/1	0/1	0/1	0/1	0/1	0/1
12	Brochidodromous venation	1	1	1	1	1	1	0
13	Brochidodromous-craspedodromous	0	0	0	0	0	0	0
14	Perimarginal veins	0	0	0	0	0	0	0
15	Number of pairs of leaflets	2-4	(1)2-4	(1)2-4	(2)3-5	(2)3-5	5-8	2-3
16	Length of leaflets in millimeters	120-160	(30-)40-100	(30-)40-100	20-100	20-100	20-100	30-50(-75)
17	Non-secretory trichomes on the adaxial epidermis	0	0	0	0/1[d]	1	0/1[d]	0/1[d]
18	Non-secretory trichomes on the abaxial epidermis	0	0	0	0/1[d]	1	0/1[d]	0/1[d]
19	Several-dome papillae on the abaxial epidermis	0	0	0	0	0	0	0
20	One-dome papillae on the adaxial epidermis	0	0	0	0	0	0	0
21	One-dome papillae on the abaxial epidermis	0	0	0	0	0	0	1
22	Palisade parenchyma on the adaxial side at the midvein	0	0	0	0	0	0	0
23	Sclereids in the mesophyll	0	0	0	0	0	0	0

Table 1 continued

Nº	**Morphoanatomical character**	negr albu	negr negr	onus	poly	scle	subp	xing
01	ENF on the petiole proper	0	0	0	1	0	0	1[a]
02	EFN between/immediately below the basal pair of leaflets	1	1	1	0	1	1	0
03	EFN between/immediately below other pairs of leaflets	1	1	1	0	1	1	1
04	EFN below other pairs of leaflets	0	0	0	1	0	0	0
05	EFN impressed	1	1	0	1	0	0	0
06	EFN sessile (flat/truncate, convex, concave and/or urceolate)	1	1	1[e]	1[f]	1[b]	0	1[b]
07	EFN cupuliform (short-stalked, stalked and/or long-stalked)	1	1	0	0	1	1[g]	1
08	ENF patelliform (short-stalked, stalked and/or long-stalked)	0	0	0	0	0	0	0
09	Petiole length in millimeters	>15;<50	>15;<50	>15;<50	>15;<50	>15;<50	>15;<50	>15;<50
10	Non-secretory trichomes on the petiole	0	0	0	0	0/1	1	1
11	Winged petiole	0	0	0	0	1	1	1
12	Brochidodromous venation	1	1	1	1	1	1	1
13	Brochidodromous-craspedodromous	1	0	0	0	0	0	0
14	Perimarginal veins	1	0	0	0	0	0	0
15	Number of pairs of leaflets	3-4	2-3	(2)3	2-4	(2-)3(-5)	7-9	6-10
16	Length of leaflets in millimeters	50-100	100-200	40-200	50-60	60-100	10-40	50-90
17	Non-secretory trichomes on the adaxial epidermis	0	0	0	0/1	0/1	1	1[d]
18	Non-secretory trichomes on the abaxial epidermis	0	0	0	0/1	0/1	1	1[d]
19	Several-dome papillae on the abaxial epidermis	0	0	0	0	0	0	0
20	One-dome papillae on the adaxial epidermis	0	0	0	0	0	0	0
21	One-dome papillae on the abaxial epidermis	0	0	0	1	0	0	0
22	Palisade parenchyma on the adaxial side at the midvein	0	0	0	0	0	0	0
23	Sclereids in the mesophyll	0	0	0	0	0	0	0

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

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

(NY, US); Maciel & Cordeiro 202, 07.XII.1978 (NY); Bastos et al. 126, 12.XII.1978 (NY); Plowman et al. 9863, 21.III.1980 (US); Plowman et al. 9885, 22.III.1980 (US); Rabelo & Jonas 2201, 14.V.1983 (NY); Daly & Cardoso 3826, 19.XII.1984 (US); Pirani et al. 3481, 08.XII.1994 (NY); Amorim et al. 2111, 21.I.1998 (NY); Hatschbach et al. 68571, 17.X.1998 (NY); *Rando 1214, 01.III.2012 (SPF, HUEFS). **C. compitalis** (H.S. Irwin & Barneby) H.S. Irwin & Barneby - Belém 3637, 24.V.1968 (NY); Belém 3643, 24.V.1968 (NY, US). **C. duartei** (H.S.Irwin) H.S.Irwin & Barneby – Duarte 8014, 19.XI.1963 (NY, US); Belém & Pinheiro 2946, 30.XI.1966 (NY); Soares 2191, 06.XII.1966 (US); Pinheiro 248, 26.IX.1967 (US); Belém & Pinheiro 3054, 12.I.1967 (NY); Almeida & Santos 195, 31.X.1968 (NY, US); Santos 2227, 27.XI.1971 (NY); Mori et al. 11036, 04.XI.1978 (NY); Santos 3422, 05.XII.1978 (US); Santos et al. 4565, 24.V.1990 (NY); Thomas et al. 8915, 29.I.1992 (NY); Amorim et al. 1290, 26.V.1993 (NY, US); Amorim et al. 1335, 13.IX.1993 (US); Silva et al. 3624, 30.X.1997 (MY); Amorim & Lorenzi 2102, 07.XI.1997 (NY, US); *Coutinho & Fernandes 303, 15.I.2014 (VIC). **C. eitenorum** var. **eitenorum** (H.S. Irwin) H.S. Irwin & Barneby - Drouet 2541, 01.X.1935 (US); Eiten & Eiten 10660, 18.II.1970 (US); Von Luetzelburg 446, 1970 (US); Santos 1321, 14.I.1971 (US). C. eitenorum var. regana (H.S. Irwin & Barneby) H.S. Irwin & Barneby - Noblick 3034, 07.III.1984 (US); Hatschbach & Hatschbach 56931, 09.IV.1992 (US); Queiroz et al. 6131, 18.II.2000 (HUEFS). **C. ensiformis** var. **ensiformis** (Vell.) H.S. Irwin & Barneby - Riedel 1201, X.1832 (NY); Guillemin 145, 1838 (NY); Rosa , 30.VII. 1936 (NY); Irwin 2330, 29.XII.1958 (NY); Hoehne 5642, 24.III.1964 (NY); Sucre 5091 & Plowmann, 27.V.1969 (NY); Santos 2120, 11.XI.1971 (NY); Euponino 178, 24.I.1972 (NY); Santos 2272, 20.IV.1972 (NY); Pinheiro 1850, 03.VII.1972 (NY); Anderson et al. 11192, 01.II.1975 (NY); Thomas 6169, 26.II.1988 (NY); Amorim et al. 1433, 10.XI.1993 (NY); Amorim et al. 2145, 25.I. 1998 (NY) *Coutinho et al. 033, 01.VIII.2012 (VIC); *Coutinho et al. 034, 01.VIII.2012 (VIC); *Coutinho et al. 035, 01.VIII.2012 (VIC); *Coutinho & Lorencini 070, 08.VIII.2012 (VIC); *Coutinho & Moura 121, 17.I.2013 (VIC); *Coutinho & Pereira 190, 25.IV.2013 (VIC). **C. ensiformis** var. **maranonica** (H.S. Irwin) H.S. Irwin & Barneby – Froés 28501, 20.VIII.1952 (US); Silva 57782, 12.XI.1963 (US); Maguire et al. 56079, 09.VIII.1963 (NY); Prance & Silva 58634, 09.VIII.1964 (US); Prance & Silva 58692, 13.VIII.1964 (NY, US); Prance & Silva 58956, 30.VIII.1964 (NY, US); Prance & Silva 58976, 31.VIII.1964 (NY, US); Silva 378, 15 Jan 1966 (NY); Hatschbach & Kummorw 38396, 26.III.1976 (NY); Silva et al. 2747, 03.IX.1976 (NY); Silva & Bahia 3143, 25.V.1977 (NY, US); Rosa et al. 2580, 11.VII.1978 (NY); Maciel et al. 410, 03.X.1979 (NY); Daly et al. D375, 29.IX.1980 (NY, US); Daly et al. D571, 10.X.1980 (NY, US); Taylor et al. E1180, 10.IV.1983 (NY); Taylor et al. E1303, 22.IV.1983 (NY); Pereira 2555, 07.IX.1993 (NY). **C. ensiformis** var. **plurifoliolata** (Hoehne) H.S. Irwin & Barneby - Warming s.n., 18.VIII.1863 (US); Curran 4675, 29.III.1921 (NY); Nunes s.n., 1930 (NY); Ser. refl. Est. Ferro No. 18, 30.VIII.1930 (NY); Pereira 9551 & Pabst 8440, 19.I.1965 (NY); Belém & Magalhães 955, 25.IV.1965 (NY, US); Santos 2952, 16.IV.1975 (NY); Kallunki et al. 523, 11.II.1994 (NY). **C. hymenaeifolia** (Benth.) H. S. Irwin & Barneby - Maguire et al. 36631, 27.XI.1953 (NY, US); Maguire et al. 36473, 27.XI.1953 (NY); Maguire et al. 42612, 02.I.1958 (NY, US); Lima et al. 3217, 17.XI.1987 (NY); Stevenson & Ramos 1064, 27.XI.1987 (NY, US). **C. negrensis** var. **albuquerquei** H.S. Irwin & Barneby - Coêlho & Coêlho s.n., 06.II.1968 (NY); Santos 144, 04.III.1977 (NY); Henderson et al. 396, 09.V.1985 (NY). **C. negrensis** var. **negrensis** (H.S. Irwin) H.S. Irwin & Barneby - Silva &

Brazão 60789, 08.I.1966 (NY); Pires & Silva 11304, 19.X.1967 (NY); Nascimento et al. 1, 02.IV.1975 (NY); Irwin 37202, 28.VI.1976 (NY); Prance et al. 24339, 27.XI.1976 (VIC); Alencar 596, 12.VII.1979 (NY); Silva 165, 28.VI.1985 (NY); Silva 288, 05.VII.1985 (NY); Ferreira et al. 6846, 19.III.1986 (NY); Belém 2056, 29.III.1986 (NY); Baleé 2447, 12.VI.1986 (NY); Baleé 2480, 13.VI.1986 (NY); Martins et al. 83, 04.II.1998 (NY); *Coutinho et al. 537, 01.I.2015. **C. onusta** Irwin & Barneby - Carvalho et al. 179, 26.II.1980 (NY); Lewis & Carvalho 1024, 07.I.1982 (NY); Carvalho & Chautems 1669, 08.III.1983 (NY); Carvalho et al. 6800, 06.IX.1999 (NY); Amorim et al. 4311, 23.X.2004 (NY). **C. polystachya** (Benth.) H.S. Irwin & Barneby - Schomburgk 1842-3, 1842-1843 (NY); Coradin et al. 558, 08.X.1977 (NY); Steward et al. 156, I.XII.1977 (NY, US); Steward et al. 226, 29.XI.1977 (NY, US); *Rando 1150, 07.II.2012 (HUEFS, SPF); *Coutinho et al. 530, 31.XII.2014;* Coutinho et al. 531, 31.XII.2014. **C. scleroxylon** (Ducke) H.S.Irwin & Barneby – Ducke 16605, 13.XII.1916 (US); Black 47-1031 F19 A, 17.VII.1947 (NY); Black F19A 47-1133, 31.VII. 1947 (NY, US); Fróes 31813, V.1955 (NY); Fróes 32171, 10.X.1955 (US); Fróes 33833, VI.1957 (NY); Fróes 33881, VI.1957 (NY); Duarte 7213, 07.X.1962 (US); Silva & Souza 2557, 11.IX.1969 (NY); *Rando 1212, 24.02.2012 (HUEFS, SPF). **C. subpeltata** (Rizzini) H.S. Irwin & Barneby - Lima 13469, 21.V.1976 (RB); Vilhena et al. 976, 10.III.1983 (NY, US); Taylor et al. E1319, 23.IV.1983 (NY, US). **C. xinguensis** (Ducke) H.S.Irwin & Barneby - Fróes 31033, 20.XIII.1954 (NY); Duarte 7213, 07.X.1962 (NY); Silva & Souza 2259, 14.VIII.1969 (NY); Silva & Souza 2409, 27.VIII.1969 (NY); Silva et al. 3421, 21.X.1977 (NY); Bahia 13, 12.VIII.1978 (NY); Baleé 2424, 12.VI.1986 (NY); Vasconcelos et al. 250, 12.X.1986 (NY); Souza et al. 376, 18.X.1986 (NY); *Rando 1208, 23.II.2012 (HUEFS, SPF).

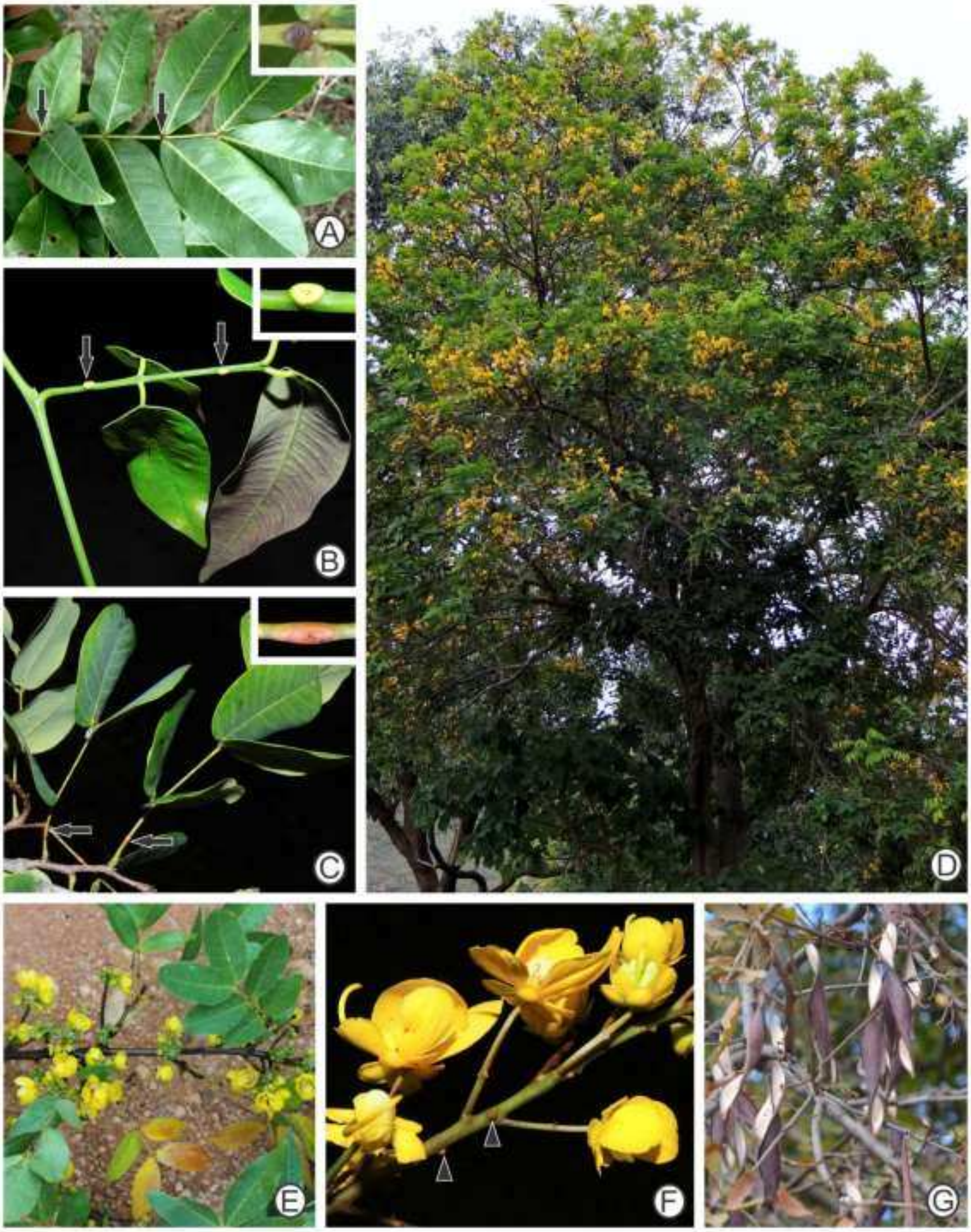

Figure 1. Species of Chamaecrista: Extrafloral nectaries (EFN, arrows) on the petiole/rachis of (A) C. eitenorum var. regana, (B) C. hymenaeifolia and C. polystachya. Note the detail of EFN in A-C. (D, E) C. ensiformis var. ensiformis. Note the cauliflorous inflorescences in (E); (F) Raceme of C. polystachya charged with glands (arrowheads); (G) Pendant elastically dehiscent pods of C. ensiformis var. ensiformis.

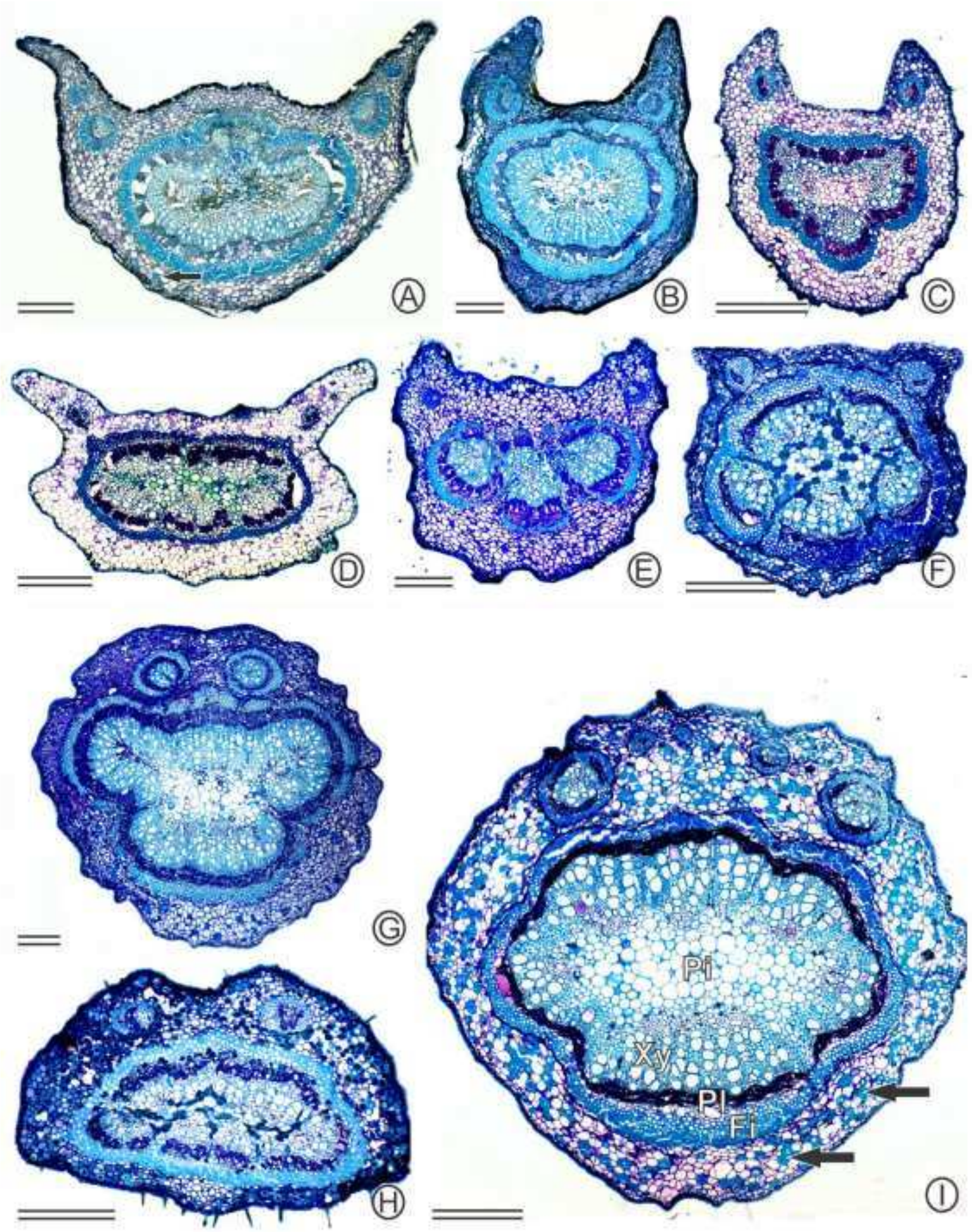

Figure 2. Arrangement of the vascular system in cross sections of the petiole/rachis of Chamaecrista species. (A-D, G-I) Vascular system forming a siphonostele in C. apoucouita (A, B), C. subpeltata (C), C. adiantifolia var. pteridophylla (D), C. bahiae (G), C. hymenaeifolia (H) and C. negrensis var. negrensis (I). Note petiole winged in A-F. Vascular system formed by three semi-circles interconnected in (E) and resembling an incomplete siphonostele in C. ensiformis var. ensiformis (F). Note the presence of non-secretory trichomes in (H). Central pith (Pi); Xylem (Xy); Phloem (Pl); Sclereids (arrow). Bars = 300µm.

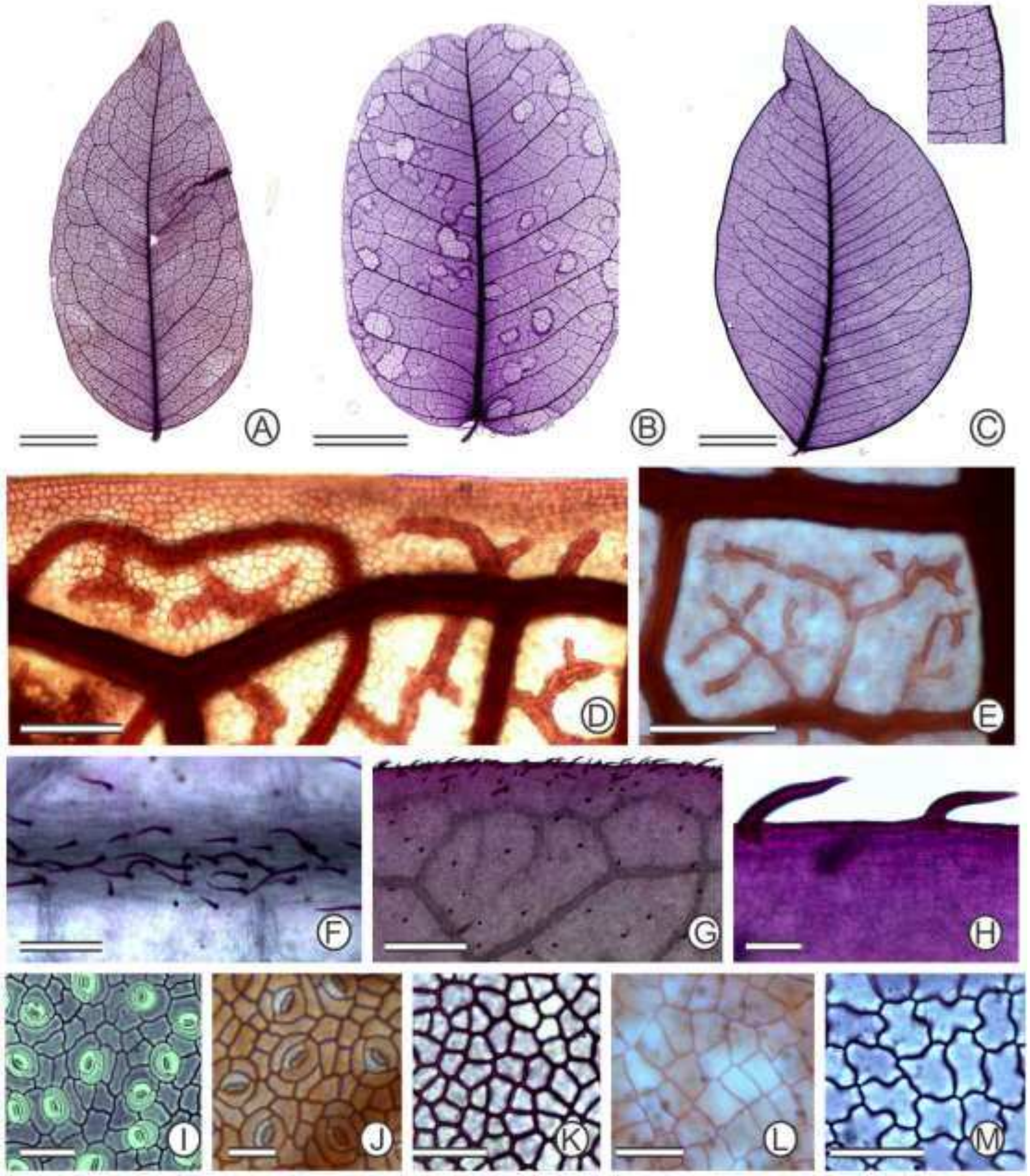

Figure 3. Leaflet clearings of Chamaecrista species. (A) C. ensiformis var. ensiformis and (B) C. polystachya with brochidodromous venation. (C) C. hymenaeifolia with craspedodromous-brochidodromous with detail of the perimarginal veins of marginal secondary type. (D) C. polystachya showing marginal ultimate venation looped and incomplete. (E) C. eitenorum var. eiteronorum with enlarged tracheids (tracheoids) at the vein endings. Non-secretory trichomes on the midvein in C. ensiformis var. maranonica (F) and mostly on the leaflet margin in C. subpeltata (G, H). Paracytic laterocyclic stomata on the abaxial side of the epidermis in C. apoucouita (I) and C. amorimii (J). Note the sinuous outline of anticlinal walls of the epidermal cells on the abaxial side in (I) and straight in (J). Outline of the anticlinal walls of the epidermal cells straight on the adaxial side in C. amorimii (K) and C. eitenorum var. eitenorum and sinuous in C. xinguensis (M). Bars = A–C, 20 mm; D–F, 200 µm; G, 500µm, H–M, 50 µm.

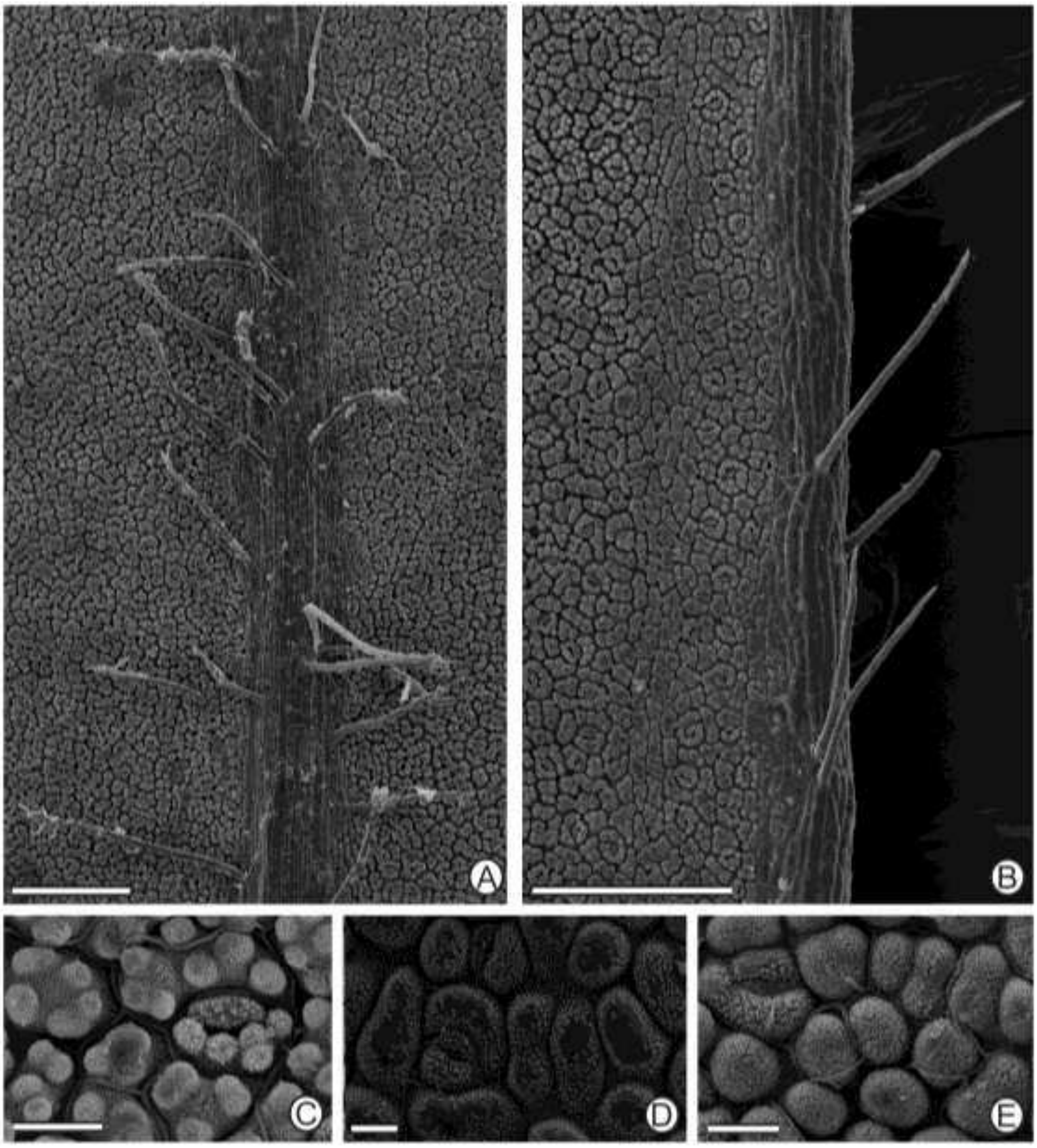

Figure 4. Scanning electron microscopy of the abaxial side of leaflets of Chamaecrista species. (A–C) C. adiantifolia var. pteridophylla. Note the non-secretory trichomes on the midvein (A) and (B) margins of the leaflets and the several domes per cell papillae (C). (D) C. polystachya and (E) C. hymenaeifolia displaying one dome per cell papillae. Bars = A–B, 200 µm; C–E, 20 µm.

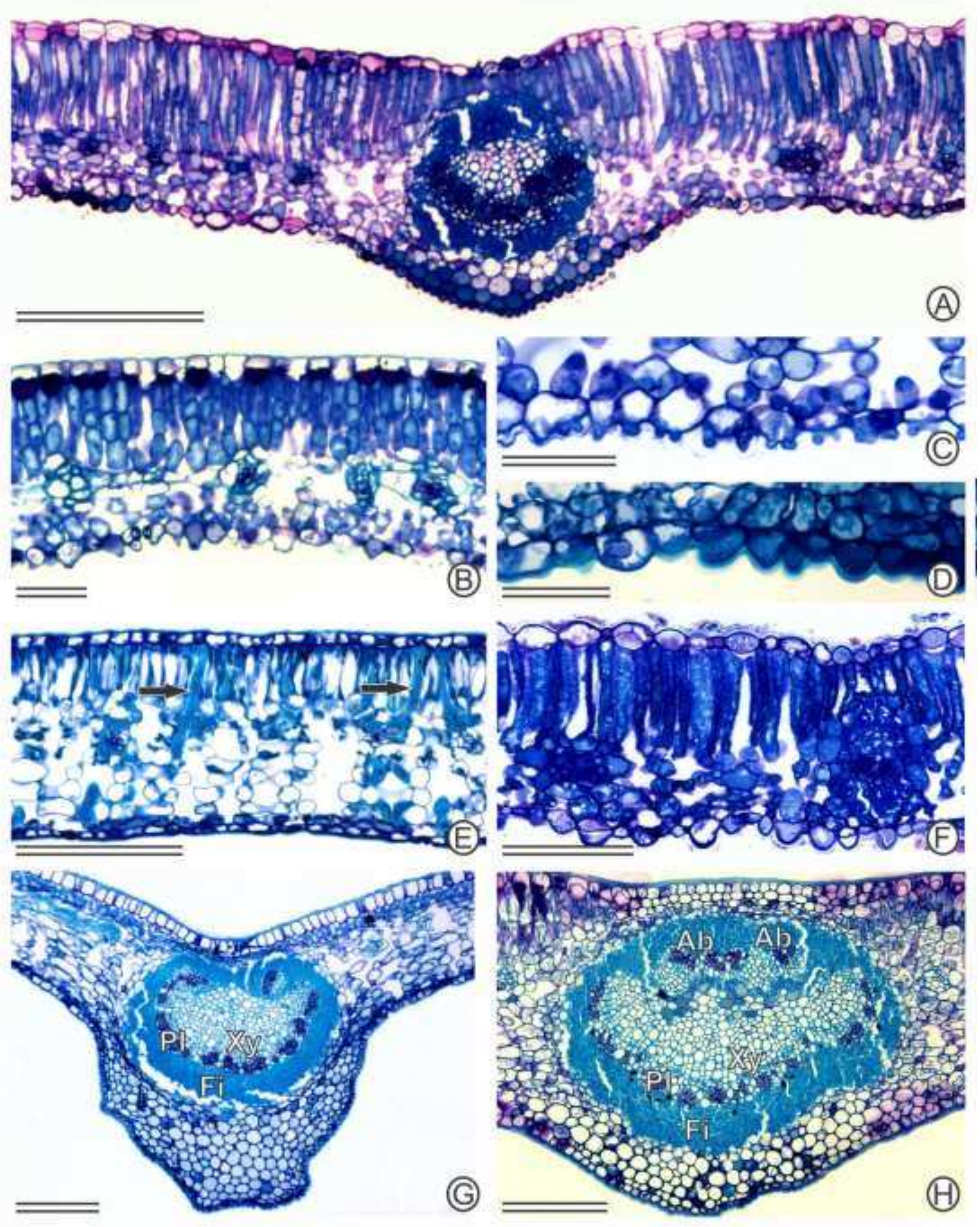

Figure 5. Cross sections of leaflets in Chamaecrista species. (A) C. aspleniifolia with one-dome papillae on both sides of the epidermis and collateral arrangement of the vascular bundles. (B–C) C. adiantifolia var. pteridophylla with several-dome papillae on the adaxial side of the leaflet. Note mucilage idioblasts in the epidermis.and a thin periclinal cell wall dividing some of the cells into two (B). (D) C. hymenaeifolia with one-dome papillae on the abaxial side. (E) C. amorimii with sclereids (arrows) in the mesophyll. (F) C. aspleniifolia, note epidermis with one-dome papillae on both sides. Cross section of the midvein showing collateral vascular bundles in C. onusta (G) and collateral vascular bundles with accessory bundles in C. amabilis (H). Bars = A, E–H, 200 µm; B–D, 50 µm.

4. CONCLUSÕES GERAIS

Com base na topografia, na estrutura, na ontogenia e nos compostos detectados na secreção, as estruturas secretoras encontradas nos ápices foliares e botões florais de espécies de Chamaecrista são coléteres, estruturas envolvidas na proteção contra dessecação dos órgãos em desenvolvimento. Cinco dos seis tipos de coléteres descritos neste trabalho são inéditos para o gênero. A diversidade estrutural dos coléteres em Chamaecrista forneceu caracteres úteis para a taxonomia, pois suportam os clados resultantes dos estudos de filogenia molecular.

Embora vários tipos de nectários extraflorais (NEF) em espécies de C. seção Apoucouita tenham sido observados, todas as espécies estudadas compartilham similaridades anatômicas e secretam o mesmo tipo de exsudado. O desenvolvimento de uma periderme de cicatrização em NEFs mais velhos parece ser uma estratégia eficiente contra infecções fúngicas em resposta a alta umidade ambiental onde as espécies de C. seção Apoucouita ocorrem. A periderme de cicatrização não foi reportada para os NEFs de espécies pertencentes a outras seções de Chamacrista, as quais ocorrem predominantemente em ambientes abertos e ensolarados.

Quando ao tipo e a posição dos NEFs (pecíolo/raque) são analisados em conjunto, tais caracteres fornecem dados que podem ser úteis para a identificação das espécies de algumas das espécies de C. sect. Apoucouita. Entretanto, para alguns táxons, este parâmetro, foi de pouca utilidade para taxonomia, uma vez que algumas espécies compartilham mais de um tipo de NEF.

Chamaecrista seção Apoucouita é o clado mais basal na filogenia do gênero, o que indica que alguns dos caracteres aqui descritos, tais como estômatos paracíticos-laterocíclicos, folhas hipoestomáticas, arranjo do sistema vascular do pecíolo/raque similar a um sifonostelo e idioblastos mucilaginosos na epiderme, poderiam estar presentes no ancestral.