

ISABEL REIS E SILVA

**ESTRUTURAS SECRETORAS EM LINHAGENS NEO E
PALEOTROPICAIS DE MALPIGHIACEAE: MORFOANATOMIA,
EVIDÊNCIAS FUNCIONAIS E CONTRIBUIÇÕES TAXONÔMICAS E
EVOLUTIVAS**

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
2017**

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

T

S586e
2017
Silva, Isabel Reis e, 1981-
Estruturas secretoras em linhagens Neo e Paleotropicais de
Malpighiaceae : morfoanatomia, evidências funcionais e
contribuições taxonômicas e evolutivas / Isabel Reis e Silva. –
Viçosa, MG, 2017.
v, 137f. : il. (algumas color.) ; 29 cm.

Inclui apêndices.

Orientador: Renata Maria Strozi Alves Meira.

Tese (doutorado) - Universidade Federal de Viçosa.

Inclui bibliografia.

1. Planta - Anatomia. 2. Folhas. 3. Flores. 4. *Acridocarpus*.
5. *Burdachia*. 6. *Glandonia*. 7. *Mcvaughia*. I. Universidade
Federal de Viçosa. Departamento de Biologia Vegetal. Programa
de Pós-graduação em Botânica. II. Título.


CDD 22 ed. 580

ISABEL REIS E SILVA

**ESTRUTURAS SECRETORAS EM LINHAGENS NEO E PALEOTROPICAIS
DE MALPIGHIACEAE: MORFOANATOMIA, EVIDÊNCIAS FUNCIONAIS
E CONTRIBUIÇÕES TAXONÔMICAS E EVOLUTIVAS**

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*.

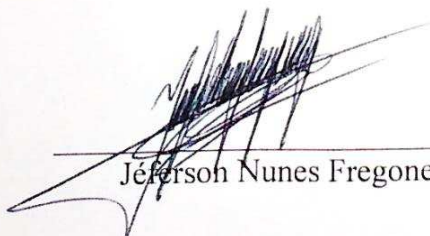
APROVADA: 17 de fevereiro de 2017



Aristéa Alves Azevedo



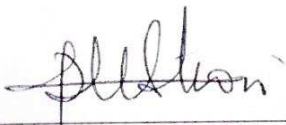
Ítalo Antônio Cotta Coutinho



Jéferson Nunes Fregonezi



Valéria Ferreira Fernandes



Renata Maria Strozi Alves Meira
(Orientadora)

AGRADECIMENTOS

Agradeço aos meus pais Marilene e Benedito pelo amor que reflete hoje nas minhas conquistas. À minha família Mari, Léo, Lucas, Marcelo, Maryse, Laurent e Anais pelo carinho mesmo na distância.

Agradeço ao meu companheiro Marco por compreender meus momentos difíceis e por sempre me apoiar. Obrigada pelo amor e pelos anos de felicidade e aventuras.

Agradeço à Universidade Federal do Amazonas-UFAM pelo incentivo à capacitação.

Agradeço à Universidade Federal de Viçosa-UFV que lapidou a profissional que acabo de me tornar, por imprimir a excelência em minha formação.

Agradeço à FAPEAM e FAPEMIG por fomentar as pesquisas e conceder a bolsa de estudo.

Agradeço aos herbários e pesquisadores colaboradores. Agradeço aos recursos humanos envolvidos na rotina laboratorial, ao precioso trabalho dos técnicos de laboratório e estagiários da Anatomia Vegetal. Agradeço a especial colaboração do especialista Dr. André Amorim.

Agradeço à Renata Maria Strozi Alves Meira, minha orientadora e amiga, por me inspirar profunda admiração por seu dinamismo, sabedoria e humanismo. Obrigada por me instigar a buscar novos desafios, sempre.

Agradeço a todos os envolvidos no desenvolvimento desse trabalho!

Agradeço aos amigos da vida e do mundo, em especial Simone, Eleonora e Karla, às amigas amazônicas Gi, Sam e Wel, meu grande amigo que Viçosa me deu, queridinho Ítalo. Obrigada as novas e fiéis amigas Aurora e Pat, às maninhas e queridinhas Val, Lú, Jú, Kat, Mari, Ana e ao meu querido amigo de café filosófico Gui e minha amiga de terapia ética Deia... Obrigada a todos os amigos especiais da Anatomia e todos amigos da Botânica com os quais convivi durante esses quatro anos.

Agradeço à música brasileira que me acompanha e me acalma em momentos difíceis.

Agradeço a inspiração em proporções infinitas que a região Norte desse Brasil me proporciona!

SUMÁRIO

RESUMO	iv
ABSTRACT	v
1. INTRODUÇÃO GERAL	1
2. CAPÍTULOS	7
CAPÍTULO I	
“To be or <i>what to be</i>”? Characterization of oilresin glands on flowers of the mcvaughiioid clade (Malpighiaceae)	7
Resumo.....	9
Introdução.....	10
Material e Métodos.....	12
Resultados.....	14
Discussão.....	19
Conclusões.....	26
Referências Bibliográficas.....	26
CAPÍTULO II	
How different are the nectaries on inflorescences and leaves of Neotropical Malpighiaceae genera?	39
Resumo.....	41
Introdução.....	42
Material e Métodos.....	44
Resultados.....	47
Discussão.....	52
Conclusões.....	58
Referências Bibliográficas.....	59
CAPÍTULO III	
Functional role and evolutionary contributions from morphoanatomy of floral glands on paleotropical genus <i>Acridocarpus</i> (MALPIGHIACEAE).....	77
Resumo.....	79
Introdução.....	80
Material e Métodos.....	82
Resultados.....	84
Discussão.....	87
Conclusões.....	95
Referências Bibliográficas.....	95
CAPÍTULO IV	
The hidrocoric Amazonian genus <i>Glandonia</i> (Malpighiaceae): new records, morphoanatomy and taxonomic contributions.....	110
Resumo.....	112
Introdução.....	113
Material e Métodos.....	114
Resultados.....	115
Discussão.....	125
Conclusões.....	128
Referências Bibliográficas.....	129
3. CONCLUSÕES GERAIS	137

RESUMO

SILVA, Isabel Reis, *D.Sc.*, Universidade Federal de Viçosa, fevereiro de 2017. **Estruturas secretoras em linhagens Neo e Paleotropicais de Malpighiaceae: morfoanatomia, evidências funcionais e contribuições taxonômicas e evolutivas.** Orientadora: Renata Maria Strozi Alves Meira.

Embora seja inegável a importância taxonômica e ecológica das estruturas secretoras em Malpighiaceae, poucos trabalhos caracterizam os tipos de glândulas em espécies de diferentes ambientes, o que dificulta a compreensão de suas funções. A biogeografia da família é peculiar e reflete diversos eventos de dispersão do Neo para o Paleotrópico. O clado neotropical mcvaughioide reúne espécies da Amazônia, Caatinga e Restinga, enquanto o paleotropical acridocarpoide, agrupa espécies de florestas e savanas da África e Ásia. Estes clados são bons modelos para estudar glândulas na folha, bractéola, cálice e corola, o que auxilia na interpretação da evolução morfológica em diferentes linhagens filogenéticas. O trabalho objetivou caracterizar a morfoanatomia das glândulas foliares e florais no clado mcvaughioide e acridocarpoide; detectar a natureza química da secreção para esclarecer sua função; comparar as estruturas nos diferentes táxons neo e paleotropicais. Para tal, o material foi coletado em campo e nas coleções herborizadas, submetido às técnicas usuais de microscopia de luz e microscopia eletrônica de varredura. Foi possível caracterizar as glândulas foliares e florais nos diferentes clados. Nas espécies do clado mcvaughioide, as glândulas foliares e bracteolares são nectários, enquanto as calicinais e petalares parecem atuar como elaióforos e osmóforos, respectivamente. No clado acridocarpoide as glândulas foliares, bracteolares e calicinais parecem atuar como nectários. Os resultados corroboram a hipótese de origem das glândulas oleíferas a partir dos nectários e contribui junto ao debate sobre a homologia entre diferentes estruturas secretoras. O trabalho registrou novas informações sobre os consumidores da secreção das glândulas foliares e florais. Os tipos e posição das estruturas secretoras foram úteis para a taxonomia. Os dados obtidos neste trabalho são promissores para o entendimento da função ecológica das glândulas foliares e florais e das relações filogenéticas e evolutivas em Malpighiaceae.

ABSTRACT

SILVA, Isabel Reis, *D.Sc.*, Universidade Federal de Viçosa, February, 2017. **Secretory structures in Neo and Paleotropical lineages of Malpighiaceae: morphoanatomy, functional evidences and taxonomical and evolutionary contributions.** Advisor: Renata Maria Strozi Alves Meira.

Despite the unquestionable importance of the secretory structures for the taxonomy and ecology of Malpighiaceae, studies characterizing the types of glands in species from different environments are rare, which makes it difficult to understand their functional role. The biogeography of Malpighiaceae is peculiar and reflects several events of dispersion from Neo to Paleotropic. The Neotropical mcvaughoid clade comprises species from the Amazon, Caatinga and Restinga while the paleotropical acridocarpoid is composed by species from both forests and savannas of the Africa and Asia. Such clades are good models to study the glands on the leaf, bracteole, calyx and corolla, since may contribute to elucidate the morphological evolution in different phylogenetic lineages. The aims of this work were to characterize the morphology of the leaf and floral glands in the mcvaughoid and acridocarpoid clade; detect the chemical nature of the secretion to clarify its function; compare the structures in the different Neo and Paleotropical taxa. For this, samples were collected in the field and herbarium material and submitted to the standard techniques for light and scanning electron microscopy. The leaf and floral glands in species belong to the different clades were described. At the mcvaughoid clade the leaf and bracteolar glands are nectary, while the calyx and petalar glands seem to act as elaiophore and osmophore, respectively. At the acridocarpoid clade the leaf, bracteolar and calyx glands seem to act as nectaries. The results corroborate the hypothesis that the oil glands are originated from the nectaries and contribute to the debate about the homology of different secretory structures. New informations about the behavior of the secretion consumers on the leaf and floral glands were registered. The secretory structures were also useful characters for the taxonomy. The data obtained in this work are promising and encourage future studies focusing on the ecological functional role of the leaf and floral glands and phylogenetics and evolutionary approaches in Malpighiaceae.

1. INTRODUÇÃO GERAL

A família Malpighiaceae Juss. é predominantemente Neotropical, com cerca de 1.300 espécies que ocupam formações florestais e savânicas (Davis e Anderson 2010). As principais classificações da família são baseadas na morfologia do fruto e reconhecem as subfamílias Byrsonimoideae, que reúne as espécies com frutos não alados, e Malpighioideae, com frutos sobretudo alados (Anderson 1978). Os gêneros *Burdachia* A. Juss., *Glandonia* Griseb. e *Mcvaughia* W. R. Anderson, com frutos não alados, foram descritos em Byrsonimoideae (Anderson 1981) e na filogenia molecular estão posicionados entre as Malpighioideae, compondo o clado mcvaughioide (Davis e Anderson 2010).

Os registros fósseis, dados paleogeográficos e moleculares indicam a América do Sul como centro de origem e diversidade da família, bem como sugerem diversos eventos de dispersão para o Paleotrópico (Hably e Manchester 2000), a partir de ancestrais compartilhados com grupos irmãos neotropicais (Davis *et al.* 2002a, 2014; Davis e Anderson 2010). O clado mcvaughioide é neotropical e vem sendo apontado como um dos grupos irmãos do clado paleotropical acridocarpoide, constituído por *Acridocarpus* Guill. & Perr. e *Brachylophon* Oliv. A filogenia molecular posiciona esses clados como grupos irmãos das demais espécies de frutos alados e apesar do monofiletismo ser altamente sustentado (Davis e Anderson 2010), apenas uma espécie de cada gênero de mcvaughioide foi amostrada.

Os clados mcvaughioide e acridocarpoide são bons modelos para interpretação evolutiva de caracteres em táxons de distribuição geográfica disjunta. As espécies de *Acridocarpus*, em geral lianas, ocorrem em florestas e savanas na África, já *Brachylophon* são arvoretas distribuídas em florestas na Ásia (Davis *et al.* 2002b). Por outro lado, *Burdachia* e *Glandonia* são árvores endêmicas de áreas alagáveis amazônicas e *Mcvaughia* é arbustivo e endêmico da Caatinga (Anderson 1979a, 1981). A origem do clado acridocarpoide teria ocorrido na África, durante a substituição de florestas tropicais úmidas por xéricas (Davis *et al.* 2002a, 2002b) e a origem de mcvaughioide teria se dado na América do Sul, durante a fragmentação e expansão das florestas tropicais em períodos secos e úmidos (Haffer e Prance 2002). Diante desta peculiar história biogeográfica, investigar variações morfológicas em linhagens neo e paleotropicais (Davis e Anderson 2010) em domínios fitogeográficos distintos, é de grande relevância para compreender a evolução de caracteres morfológicos (*id, ibid.*).

Em Malpighiaceae, as estruturas secretoras são amplamente citadas, sobretudo os nectários extraflorais (NEFs) e as glândulas calicinais, pelo seu valor diagnóstico e ecológico (Solereider 1908; Metcalfe e Chalk 1965; Anderson 1979b, 1981, 1990; Vogel 1990; Davis e Anderson 2010). Apesar das descrições taxonômicas mencionarem a ocorrência de glândulas florais (Anderson 1979b, 1981, 1990), essas são pouco compreendidas.

NEFs são estruturas secretoras de néctar (Fahn 1988; Nepi 2007), atraindo patrulhadores como formigas, que podem atuar na defesa da planta (Heil *et al.* 2004). Em Malpighiaceae, os NEFs estão distribuídos no pecíolo ou limbo foliar e em geral, quando inconspícuos são numerosos e quando conspícuos, pouco numerosos (Solereider 1908; Metcalfe e Chalk 1965). Possuem cutícula fina, epiderme secretora em paliçada, parênquima secretor vascularizado e natureza química da secreção polissacarídica (Subramanian *et al.* 1990; Vieira 2005; Machado *et al.* 2008; Araújo *et al.* 2010; Possobom *et al.* 2010).

O Neotrópico concentra uma grande diversidade de espécies, exibindo morfologia floral típica, com simetria bilateral e pares de glândulas calicinais nas cinco ou quatro sépalas (Anderson 1981; 2001; 2004). Nessas espécies, as glândulas calicinais são elaióforos, que produzem óleo fixo como principal recompensa às abelhas coletoras de óleo (Buchmann 1987; Lobreau-Callen 1989; Vogel 1990; Anderson 1979a, 1990; Simpson e Neff 1981; Endress 1996; Vinson *et al.* 1997; Sigrist e Sazima 2004).

A glândula calicinal é apontada como um caráter apomórfico na família e sua ausência no Paleotrópico, como uma condição derivada do caráter (Anderson 1979b). Uma vez que as abelhas coletoras de óleo não ocorrem no Paleotrópico, parte das espécies não sofreu pressão em reter o caráter, perdendo as glândulas calicinais (Anderson 1979b). Enquanto outras espécies paleotropicais, como as do clado acridocarpoide, possuem glândulas calicinais reduzidas que atuariam como nectários e o pólen seria o principal recurso floral (Anderson 1979b; Lobreau-Callen 1989; Vogel 1990; Ren *et al.* 2013). Portanto, a morfologia floral nas espécies neotropicais teria sido ativamente mantida por milhões de anos, como resultado da pressão seletiva exercida pelos polinizadores (Davis *et al.* 2014). Isso fica evidente ao observar que nos diferentes grupos Paleotropicais, onde as abelhas coletoras de óleo são ausentes, muitas espécies não possuem glândulas calicinais (Davis e Anderson 2010; Davis *et al.* 2014).

A associação direta das glândulas calicinais nas espécies neotropicais com a produção de óleo e nas paleotropicais com secreção de néctar é comum (Lobreau-Callen 1989). Porém, o registro de secreção lipofílica nas espécies paleotropicais (Arumugasamy *et al.* 1988, 1993; Subramanian *et al.* 1990) e mista nas neotropicais (Araújo e Meira 2016; Possobom *et al.* 2015) evidencia a necessidade de estudos adicionais em outras espécies.

O registro de secreção lipídica em supostos NEFs (Castro *et al.* 2001) demonstra que glândulas foliares e calicinais, além de se distribuírem em posições similares na folha e na sépala e compartilharem alta similaridade anatômica, podendo produzir secreção de mesma natureza (Subramanian *et al.* 1990; Vogel 1990; Castro *et al.* 2001; Possobom *et al.* 2010; 2015). Isso poderia indicar a expressão dos mesmos genes na folha e no cálice, sugerindo a homologia entre tais estruturas secretoras (Castro *et al.* 2001).

Outros tipos de glândulas florais, além das calicinais, são incomuns em Malpighiaceae. Sua presença é mencionada em poucos táxons, ocorrendo na bractéola, na margem de pétalas e no conectivo dos estames (Anderson 1979b; Lobreau-Callen 1989; Vogel 1990). Em consequência da detecção de lipídio na secreção (Lobreau-Callen 1989; Vogel 1990; Possobom *et al.* 2015), as glândulas petalares vêm sendo associadas a osmóforos e as do conectivo à múltiplas funções, como na atração e na adesão do pólen ao polinizador (Gates 1982; Possobom *et al.* 2015). No conectivo também foi detectada secreção polissacarídica (Vieira 2005) e fenólica (Cocucci *et al.* 1996; Guimarães *et al.* 2013). Assim, estudos complementares são necessários para verificar a natureza da secreção nas diferentes fases de desenvolvimento, subsidiando a determinação segura da identidade destas glândulas nas diferentes espécies.

O clado mcvaughioide é um modelo ideal para determinar os tipos de estruturas secretoras em um contexto filogenético, pois agrupa espécies com glândulas na folha, cálice, pétala e bractéola, cujas funções ecológica e da secreção permanecem desconhecidas. Ademais, as espécies de acridocarpoide também possuem glândulas foliares e florais ainda não descritas. O estudo permite comparar estruturas em posições equivalentes nas espécies dos clados mcvaughioide e acridocarpoide que apresentam distribuição em diferentes ambientes e regiões geográficas.

A partir do exposto, o desenvolvimento da proposta objetivou responder as seguintes perguntas: as glândulas foliares e florais são as mesmas já registradas na família Malpighiaceae?; a natureza química do secretado é a mesma?; tais glândulas

foliares e florais estão relacionadas às mesmas funções?; existem diferenças histoquímicas e/ou morfoanatômicas intra ou interespecíficas?; e entre os clados Neo e Paleotropicais?; a morfoanatomia das glândulas pode contribuir nas interpretações da evolução floral na família?; existem caracteres anatômicos que auxiliam na taxonomia?

Como parte dos esforços para responder as perguntas propostas, a tese foi organizada em 4 capítulos, redigidos nas normas dos seguintes periódicos, **Cap. I:** American Journal of Botany; **Cap. II:** Perspectives in Plant Ecology, Evolution and Systematics; **Cap. III:** Taxon: International journal of plant taxonomy, phylogeny and evolution e **Cap IV:** Phytotaxa.

REFERÊNCIAS BIBLIOGRÁFICAS

- Anderson, W.R. 1978 [“1977”]. Byrsonimoideae, a new subfamily of the Malpighiaceae. **Leandra** 7: 5–18.
- Anderson, W.R. 1979a. *Mcvaughia*, a new genus of Malpighiaceae from Brazil. **Taxon** 28: 157–161.
- Anderson, W.R. 1979b. Floral conservation in Neotropical Malpighiaceae. **Biotropica** 11: 219–223.
- Anderson, W.R. 1981. Malpighiaceae. The Botany of the Guayana Highland-Part XI. **Memorirs of the New York Botanical Garden** 32: 137–147.
- Anderson, W.R. 1990. The origin of the Malpighiaceae-The evidence from morphology. **Memoirs of the New York Botanical Garden** 64: 210–224.
- Anderson, W.R. 2001. Malpighiaceae. In **Flora of the Venezuelan Guayana vol 6**. Missouri: Missouri Botanical Garden Press. p. 82–139.
- Anderson, W.R. 2004. Malpighiaceae. In **Flowering Plants**. Princeton, New Jersey: The New York Botanical Garden e Princeton University Press. p. 229–232.
- Araújo, J.S.; Azevedo, A.A.; Silva, L.C.; Meira, R.M.S.A. 2010. Leaf anatomy as an additional taxonomy tool for 16 species of Malpighiaceae found in the Cerrado area (Brazil). **Plant Systematic and Evolution** 286: 117–131.
- Araújo, J.S.; Meira, R.M.S.A.. 2016. Comparative anatomy of calyx and foliar glands of *Banisteriopsis* C. B. Rob. (Malpighiaceae). **Acta Botanica Brasilica** 30: 112–123.
- Arumugasamy, K.; Inamdar, J.A.; Subramanian, R.B. 1988. Structure, ontogeny and secretion of oil secreting glands in *Hiptage acuminata* Wall. **Current Science** 58: 260–261.

- Arumugasamy, K.; Udaiyan, K.; Manian, S.; Sugavanam, V. 1993. Ultrastructure and oil secretion in *Hiptage sericeae* Hook. **Acta Societatis Botanicorum Poloniae** **62**: 17–20.
- Buchmann, S.L. 1987. The ecology of oil flowers and their bees. **Annual Review of Ecology and Systematics** **18**: 343–369.
- Castro, M.A.; Vega, A.S.; Mulgura, M.E. 2001. Structure and ultrastructure of leaf and calyx glands in *Galphimia brasiliensis* (Malpighiaceae). **American Journal of Botany** **88**: 1935–1944.
- Cocucci, A.A.; Holgado, A.M.; Anton, A.M. 1996. Estudio morfológico y anatómico de los eleoforos pedicelados de *Dinemandra ericoides*, Malpighiaceae endémica del desierto de Atacama, Chile. **Darwiniana** **34**:183–192.
- Davis, C.C.; Bell, C.D.; Mathews, S.; Donoghue, M.J. 2002a. Laurasian migration explains Gondwanan disjunctions: evidence from Malpighiaceae. **Proceedings of the National Academy of Sciences** **99**: 6833–6837.
- Davis, C.C.; Bell, C.D.; Fritsch, P.W.; Mathews, S. 2002b. Phylogeny of *Acridocarpus* - *Brachylophon* (Malpighiaceae): implications for Tertiary tropical floras and Afroasian biogeography. **Evolution** **56**:2395–2405.
- Davis, C.C.; Anderson, W.R. 2010. A complete generic phylogeny of Malpighiaceae inferred from nucleotide sequence data and morphology. **American Journal of Botany**. **97**:2031–2048.
- Davis, C.C.; Schaefer, H.; Xi, Z.; Baum, D.A.; Donoghue, M.J.; Harmon L.J. 2014. Long-term morphological stasis maintained by a plant–pollinator mutualism. **Proceedings of the National Academy of Sciences** **111**: 5914–5919.
- Endress, P.K.. 1996. **Diversity and evolutionary biology of tropical flowers**. Cambridge: University Press. 511p.
- Fahn, A. 1988. Secretory tissues in vascular plants. **New Phytologist** **108**: 229–257.
- Gates, B. 1982. *Banisteriopsis* and *Diplopterys* (Malpighiaceae). **Flora Neotropica Monograph** **30**: 1–237.
- Guimarães, A.L.; Cruz, S.M.; Vieira, A.C. 2013. Structure of floral galls of *Byrsonima sericea* (Malpighiaceae) induced by *Bruggmanniella byrsonimae* (Cecidomyiidae, Diptera) and their effects on host plants. **Plant Biology****16**:467–75.
- Hably, L.; Manchester, S.R. 2000. Fruits of *Tetrapterys* (Malpighiaceae) from the Oligocene of Hungary and Slovenia. **Review of Palaeobotany and Palynology** **111**: 93–101.
- Haffer, J.; Prance, G.T. 2002. Impulsos climáticos da evolução na Amazônia durante o Cenozóico: sobre a teoria dos Refúgios da diferenciação biótica. **Estudos Avançados** **16**: 175–206.
- Heil, M.; Hilpert, A.; Kruger, R.; Linsenmair, E. 2004. Competition among visitors to extrafloral nectaries as a source of ecological costs of an indirect defence. **Journal of Tropical Ecology** **20**: 201–208.

- Lobreau-Callen, D. 1989. Les Malpighiaceae et leurs pollinisateurs. Coadaptation ou coevolution. **Bulletin du Muséum National d'Histoire Naturelle, Adansonia** 1: 79–94.
- Machado, S.R.; Morellato, L.P.C.; Sajo, M.G.; Oliveira, P.S. 2008 Morphological patterns of extrafloral nectaries in woody plant species of the Brazilian cerrado. **Plant Biology** 10: 660–673.
- Metcalf, C.R.; Chalk, L. 1965. **Anatomy of the dicotyledons: leaves stem and wood in relation to taxonomy with notes on economic uses.** Oxford: Clarendon Press. 1500 p.
- Nepi, M. 2007. Nectary structure and ultrastructure. In Nicolson, S.W; Nepi, M.; Pacini, E.(eds.). **Nectaries and Nectar.** Dordrecht: Springer. p. 129–166.
- Possobom, C.C.F.; Guimarães, E.; Machado, S.R. 2010. Leaf glands act as nectaries in *Diplopterys pubipetala* (Malpighiaceae). **Plant Biology** 12: 863–870.
- Possobom, C.C.F.; Guimarães, E.; Machado, S.R. 2015. Structure and secretion mechanisms of floral glands in *Diplopterys pubipetala* (Malpighiaceae) and a neotropical species. **Flora** 211: 36–39.
- Ren, M.; Zhong, Y.; Song, X.. 2013. Mirror-image flowers without buzz pollination in the Asian endemic *Hiptage benghalensis* (Malpighiaceae). **Botanical Journal of the Linnean Society** 173: 764–774.
- Sigrist, M.R.; Sazima, M. 2004. Pollination and reproductive biology of twelve species of neotropical Malpighiaceae: stigma morphology and its implications for the breeding system. **Annals of Botany** 94: 33–41.
- Simpson, B.B; Neff, J.L. 1981. Floral rewards: alternatives to pollen and nectar. **Annals of the Missouri Botanical Garden** 68: 301–322.
- Solereider, H. 1908. **Systematic anatomy of the dicotyledons.** Vol. II. Oxford: Clarendon Press. 1104 p.
- Subramanian, R.B.; Arumugasamy, K.; Inamdar, J.S. 1990. Studies in secretory glands of *Hiptage sericea* (Malpighiaceae). **Nordic Journal of Botany** 10:57–62.
- Vieira, A.C.M.. 2005. **Estudos em espécies de Malpighiaceae de restingas do Rio de Janeiro: estruturas secretoras em folhas e flores e biologia floral.** Tese (Doutorado – USP), São Paulo: São Paulo [s.n.].
- Vinson, S.B; Williams, H.J; Frankie, G.W; Shrum, G. 1997. Floral lipid chemistry of *Byrsonima crassifolia* (Malpighiaceae) and a use of floral lipid by *Centris* bees (Hymenoptera: Apidae). **Biotropica** 29: 76–83.
- Vogel, S. 1990. History of the Malpighiaceae in the light of Polination Ecology. **Memoirs of the New York Botanical Garden** 55: 130–142.

1. CAPÍTULOS

CAPÍTULO I

“To be or what to be”? Characterization of oilresin glands on
flowers of the mcvaughoid clade (Malpighiaceae)

Redação de acordo com as normas de submissão do periódico: *American Journal of Botany*

**“TO BE OR *WHAT TO BE*”? CHARACTERIZATION OF OILRESIN GLANDS ON
FLOWERS OF THE MCVAUGHIOID CLADE (MALPIGHIACEAE)**

ISABEL REIS E SILVA^{a,b} AND RENATA MARIA STROZI ALVES MEIRA^{a*}

^aUniversidade Federal de Viçosa, UFV, Departamento de Biologia Vegetal, 36570-900, Viçosa, Minas Gerais, Brazil

^bUniversidade Federal do Amazonas, UFAM, Instituto de Ciências Exatas e Tecnologia, 69103-128, Itacoatiara, Amazonas, Brazil

*Corresponding author. Mailing address: ^aUniversidade Federal de Viçosa, UFV, Departamento de Biologia Vegetal, Avenida Peter Henry Rolfs, s/n, 36570-900, Viçosa, Minas Gerais, Brazil.

e-mail: rmeira@ufv.br (R. M. S. A. Meira)

ABSTRACT

Premise of the study: Historically the Malpighiaceae flowers are frequently cited by their typical calyx glands, which act as elaiophore in Neotropical species. Although this affirmative has been accepted across the years, only recently, morphoanatomical studies has helped to clarified their ultrastructure, nature of secretion, mechanisms of secretion and functional role. Besides the calyx glands, others floral glands have progressively gained attention. Since the few studies are restricted to some species from “Cerrado”, we characterized the anatomy and nature of secretion of calyx and petal glands in mcvaughoid clade, which comprises *Burdachia*, *Glandonia* and *Mcvaughia* species, endemic from Amazon, Caatinga and Atlantic Forest.

Methods: Flowers and buds of all mcvaughoid species were collected in field works and in herbarium collections. The calyx and petal glands were submitted to standard anatomical techniques to light microscopy and scanning electron microscopy

Key results: The calyx and petal glands show similar morphoanatomy, differing by the projection of the secretory surface. In *Burdachia* the calyx glands may be sessile and subsessile in *Mcvaughia* and *Glandonia*, while the petal glands are short-stalked in *Burdachia* and *Mcvaughia* and exclusively in *Glandonia* are sessile and flattened. Histochemical results of both glands showed secretion predominantly consisting by lipids of oleoresin nature, proteins, polysaccharides and phenolic compounds. The secretion produced is accumulated in the subcuticular space, only in the petal gland of *Glandonia* seems to be accumulated in intercellular spaces of secretory epidermis.

Conclusions: The morphoanatomical similarity among the floral glands in mcvaughoid species can be a signal of homology shared by such glands. Functionally, the study pointed that the petal glands act exclusively as osmophore. About the calyx glands, besides act as elaiophore they can also act secondarily as osmophore.

Keywords: calyx gland, petal gland, pollination, *Burdachia*, *Glandonia*, *Mcvaughia*.

INTRODUCTION

Malpighiaceae is a family especially known for its flowers, which bear typical calyx glands (Buchmann, 1987; Anderson, 1990; Vogel, 1990; Davis et al., 2014). Such glands are called elaiophores, structures highly specialized in fatty oil secretion (Vogel, 1990). This secretion is an important reward offered to the oil-collecting bees belonging to the tribes Centridini, Tapinotaspidini and Tetrapediini that act as pollinators (Simpson and Neff, 1981; Sazima and Sazima, 1989; Sigrist and Sazima, 2004, Alves-dos-Santos et al., 2007). Besides to the calyx gland, floral glands on petal margins and on anther connectives were mentioned for some Malpighiaceae (Anderson, 1977) and oil secretion was also reported for the petal glands from some “Amazon” species (Lobreau-Callen, 1989).

Few morphoanatomical studies describes calyx glands from neotropical species, such as *Galphimia brasiliensis* (Castro et al., 2001). The current knowledge of the ecological functional role of the petal and anther glands may be considered scarce (Lobreau-Callen, 1989; Cocucci et al., 1996). Possobom et al. (2015) described the morphoanatomy and ultrastructure of floral glands in a neotropical species from “Cerrado”. These authors reinforced the functional role of anthers glands in adherence of pollen to pollinator body, proposed by Gates (1982) and recognized the calyx and petal glands as elaiophore and osmophore (floral scent gland), respectively.

Neotropical species of Malpighiaceae show a highly stereotyped floral morphology, exhibiting symmetry zygomorphic, petals clawed, a specialized posterior petal, and indeed, calyx glands behaving as elaiophores (Anderson, 1990). Only about 10% of the family diversity occurs in Paletropic areas (Davis and Anderson, 2010),

where the most of the floral features found in Neotropical species has been lost (Davis et al., 2002), including the floral glands. Since the oil-collecting bees are restricted to America (Alves-dos-Santos et al., 2007), such typical suite of floral characters in Neotropic appears to be closely tied to their oil-bee pollinators (Vogel, 1990; Anderson, 1990). Thus, the floral calyx glands have been lost, reduced or converted to nectaries when pollination systems were changed (Davis et al., 2014).

In Malpighiaceae, calyx glands disposed in pairs represent a derived character (Anderson, 1990), being a important feature for taxonomic studies (Castro et al., 2001). Few works reported comparative morphoanatomical analyses between leaf, calyx and another floral glands (Castro et al., 2001, Possobom et al., 2010, 2015; Araújo and Meira, 2016). The similarities shared by leaf and calyx glands support the Vogel's idea (1990) that proposed the elaiophore are derivied from nectaries, as well as the similarity of secretion contitution reinforce the hypotesys of their homology (Anderson, 1990). In previous studies, the morphoanatomy of calyx glands in paleotropical species was described, behaving as elaiophore (Subramanian et al., 1990, Arumugasamy et al., 1993) or as nectaries (Ren et al., 2013; Silva et al. unpubl. data, 2017 *in* CAP III). In neotropic such glands in species from deserts (Coccuci et al., 1996) and mainly from brazilian "Cerrado" (Mamede, 1993, Possobom et al., 2010, 2015; Araújo and Meira, 2016) appear behaving exclusively as elaiophore.

Three small genera compose the mcvaughoid clade (Davis and Anderson, 2010), in which the species bear floral glands and presents peculiar biogeography. *Burdachia* Adr. Juss. and *Glandonia* Griseb. species are endemic from Amazon rainforest, while *Mcvaughia* W. R. Anderson comprises an endemic species from "Caatinga" (dryland) and another from "Restinga" in Atlantic Forest (Anderson, 1979a, 1981; Amorim and Almeida, 2015). Progress towards understanding the floral

glands in different Neotropical Malpighiaceae species from distinct environments certainly improve information about the mechanisms involved to the specific plant-pollinator mutualism. Thus, this study aimed to characterize the floral glands of the mcvaughoid species, and analyze the histochemistry of secretion and morphoanatomy of the calyx and petal glands.

MATERIAL AND METHODS

Burdachia and *Glandonia* are genera of trees from Amazonia rainforest, where the species occur in wetlands floodplain, except *G. macrocarpa* that is typical of “Terra Firme” upland forest (Anderson, 1981). On the other way, *Mcvaughia* is represented by shrubs species that occur in dry environments from different Brazilian biomes. *Mcvaughia bahiana* is endemic to dry and open habitats on sandy soils vegetation from “Caatinga” (dryland) (Anderson, 1979a), while *M. sergipana* is an endemic species from “Restinga” vegetation (sandy coastal plains) of Atlantic Forest, being restricted to a little area from Brazil (Amorim and Almeida, 2015).

Calyx and petal glands from mcvaughoid species were analyzed: *Burdachia duckei* Steyer., *B. prismatocarpa* A. Juss., *B. sphaerocarpa* A. Juss., *Glandonia macrocarpa* Griseb., *G. prancei* W.R. Anderson, *G. williamsii* Steyer., *Mcvaughia bahiana* W.R. Anderson and *M. sergipana* Amorim & Almeida. Fresh samples of buds and flowers were collected in field. Vouchers material was deposited at the herbarium VIC of the Universidade Federal de Viçosa-UFV. Samples of herbarium material from the Instituto Nacional de Pesquisas da Amazônia (INPA) were used (Appendix 1).

The samples were analysed at the Plant Anatomy Laboratory-UFV to describe the anatomical structure. The field samples were fixed in FAA (formalin, acetic acid and 50% ethanol; 1:1:18 by volume) for 48 h, NBF (neutral buffer formalin)

(Johansen, 1940) or in Karnovsky solution (Karnovsky, 1965). Some samples were fixed in FSF (ferrous sulphate formalin) to detect phenolic compounds (Johansen 1940). Both samples fixed in FAA and FSF were stored in 70% ethanol, while those fixed in NBF and Karnovsky were kept in such fixates until analysis. Samples of herbarium material were rehydrated, treated with 2% potassium hydroxide for 2 h, dehydrated in an ethanol series and stored in 70% ethanol (Smith and Smith, 1942).

Samples of both herbarium and field-collected material were embedded in methacrylate resin (Historesin Leica; Heidelberg, Germany) in accordance to the manufacturer's recommendation. Cross and longitudinal sections with 4 μ m-thickness were made with an automatic rotary microtome (Leica RM2155, Deerfield, USA). The sections were stained with toluidine blue at pH 4.7 (O'Brien et al., 1964)

The following histochemical tests were performed in section obtained in hand sectioned sections from FAA fixed samples: xyloidine ponceau (Clark, 1981) and Coomassie blue (Fisher, 1968) for total proteins; PAS (periodic acid–Schiff reagent) for total polysaccharides (McManus, 1948), ruthenium red for mucilage or pectin and lugol for starch (Johansen, 1940). Fresh sections of *Burdachia duckei* were submitted to tannin detection with acidified vanillin test (Mace and Howell, 1974). Previous tests were made *in locu*, using the entire flower (Fig. 1). Sections from NBF fixed samples were used to evidence lipid and terpenoids using Sudan black B and Sudan red for total lipid (Pearse, 1980) and NADI reagent for oil or oil-resin (David and Carde, 1964).

Posterior petal were cleared and stained, according to Vasco et al. (2014 modified), and mounted in glycerin jelly (Johansen, 1940) to describe the glands occurrence and vascularization. Photographic documentation was carried out in a light

microscope (Olympus AX70TRF) equipped with a digital camera (AxioCam HRC; Zeiss, Gottingen, Germany).

For the scanning electron microscopy analysis, samples fixed were dehydrated through an ethanol series, subjected to critical point drying with CO₂ (CPD 030, Bal-Tec, Balzers, Liechtenstein), placed on stubs with aid of carbon conductive tapes and then coated with gold (SCD 050, Bal-tec, Balzers, Liechtenstein), previously to observations. Examinations and image captures were conducted using a LEO 1430VP (Zeiss, Cambridge, UK) at the “Center for Microscopy and Microanalysis”-UFV.

The anatomical patterns were identified as **sessile**, when the secretory epidermis recovers all the projected area; **subsessile**, when the secretory epidermis is surrounded by non-secretory epidermis; and **stalked**, when a short stalk is present.

RESULTS

The morphoanatomical characters of calyx and petal glands recorded in *Burdachia*, *Glandonia* and *Mcvaughia* species are summarized at the Table 1.

Morphoanatomical characterisation of calyx glands and field observations

In mcvaughoid clade, the color of the calyx glands vary among species (Fig 1A-C). During the flowering, the glands of the *Burdachia* species are pink (Fig. 1A) or creamy-white and are yellow in the fruit. In the buds of the *Mcvaughia* species, they are greenish and at the flowers and are yellow at the fruit (Fig. 1C). The calyx gland of *Glandonia* species are white and not change (Fig. 1B). In all species the calyx glands are conspicuous structures, showing a clavate shape at frontal vision with the surface convex to flanged (Fig. 1D-F).

The calyx present a total of 10 glands, one pair in each sepal (Fig. 1D-F), medially disposed. Generally the secretory area occur at all sepalar area, only in

Glandonia the median portion of the posterior sepals is not glandular (Fig. 1E). In flower buds, the central zone of the calyx gland presents a longitudinal depression, which accumulate the secretion during the anthesis that reacted to sudan black (Fig. 1G-I) and sudan red (Fig. 1J-M). Such gland present a precocious development but the secretory activity begin only at pre-anthese stage. Although the high secretion happens in blooming stage, the activity of calyx glands was recorded until the fruiting periods.

The calyx glands occur on the connate portion of the calyx and may be distributed actinomorphyly (Fig. 2A) or zygomorphyly (Fig. 2B). The zygomorphy in *Mcvaughia* is due the lateral displacement of the glands in anterior sepal (Fig. 2B). Such glands were composed by secretory epidermis, secretory parenchyma and vascularization (Fig. 2C). In *Burdachia* the calyx glands are sessiles (fig. 2A, D) , while in *Mcvaughia* (Fig 2B, E) and *Glandonia* (Fig. 2C, F) are subsessiles (Table 1).

The secretory epidermis is one-layer composed by densely stained cytoplasm, central conspicuous nucleus and some small vacuoles with green color (Fig. 2G-I). The cuticle were thick (Fig. 2G) and a central subcuticular spaces were observed, in which generally a large amount of secretion were observed (Fig. 2I, J). The subepidermis parenchyma is constituted by cells filled with phenolic substances or druse crystals (Fig 2J, L) and the innermost of calyx gland had the most of cells with less dense cytoplasm and large vacuoles (Fig. 2M). In *Glandonia*, sclereids were frequently observed (Fig. 2N) and the secretory epidermis is less developed. The vascularisation of the calyx glands is composed by xylem and phloem tissues (Fig. M), which reached the subepidermal layers (Fig. 2C-E, J).

At the beging of the activity, the secretory epidermal cells remain juxtaposed with the cuticle typically (Fig. 2G). and due the secretory process they disconnect themselves and from cuticle (Fig. 2H-J), resulting thus a subcuticular space filled with

secretion (Fig. 2O). During field expeditions, bees visitations were observed in flowers. However, in most of the times the bees were collecting pollen (*Trigonia* sp.). Although the observation of bees with typical size and behavior of collecting oil-bees were rare. at the buds the cuticle are entirety (Fig. 2P) whereas in fertilized flowers and in fruits, exhibited a completely scraped appearance (Fig. 2Q). In the field, the aroma were felt in the bud stage inflorescence of *G. macrocarpa*.

Morphoanatomical characterization of petal glands and field observations

The corola shows different colors, being pink in *Burdachia* (Fig. 1A), white in *Glandonia* (Fig. 1B) and yellow in *Mcvaughia* (Fig. 1C). The posterior petal bear a thick erect claw (Fig. 1A-C). In *B. prismatocarpa* and *B. duckei* the claw is yellow and the petal blade is pink with yellowish margin (Fig. 3A), while in *B. sphaerocarpa* the claw and the base of petal blade is yellowish and the apex is pink (Fig. 3B). *Mcvaughia* (Fig. 3C) and *Glandonia* (Fig. 3D, E) show yellow posterior petal, with whitish margin in the *Glandonia* species. Such marginal glands presents digitiform-globoid shape (Fig. 3F-I). The margin of the petal blade has conspicuous glandular projections that can be distributed by entire blade, until the apex (Fig. 3J) or restrited to the base in *Glandonia* species with crenate apperence (Fig. 3L, M). The median portion of the posterior petal is pappilose in *Mcvaughia* and *Glandonia* species (Fig. 3N).

The test with sudan red and sudan black carried on in the fiedwork evidenced secretory areas on the petals. Positive reaction were obtained on the secretory marginal areas in *Burdachia* (Fig. 3O, P) and at the entire blade of the petal in both *Mcvaughia* (Fig. 3 R) and *Glandonia* (Fig. 3S-V). For proteins *M. sergipana* shows positive reaction (Fig. 3Q) Even though during field expeditions, the observation of bees with typical size and behavior of collecting oil-bees such as *Centris* were rare, mandible marks on the claw of the posterior petal were frequently observed (Fig. 3P). Despite

the petal glands, in some species, florivores attacks and parasites damage was frequently observed and some older flowers were clearly damaged. In the field was possible smell the sweet scent in exhaling through the flowers of *B. sphaerocarpa*.

The petal glands are vascularized (Fig. 4 A-D) and recovered by a secretory epidermis arranged in a palisade (Fig. 4D). In *Burdachia* (Fig. 4F, G, J) and *Mcvaughia* (Fig. 4N) stalked glands were observed at the proximal portion of the posterior petal, while sessile glands were observed at the distal portion. In cross section, the petal glands of *Glandonia* are dorsiventrally flattened (Fig. 4E, I) that gives the appearance of crenate margin (Fig. 4A, B). In *Glandonia*, the glands on proximal portion were identified as sessile and the secretory epidermis were less developed or absent at the abaxial surface (Fig. 4E). Only in *Glandonia*, the cells of secretory epidermis have gradually acquired a conical shape, developing intercellular spaces (Fig. 4L, M). In *G. williamsii* the secretory epidermis is less higher (Fig. 4I). The epidermical cells of median portion of posterior petal in *Glandonia* are papillose (Fig. 4O, P).

The secretory epidermis was a one-layer cell with densely stained cytoplasm, central conspicuous nucleus, generally with phenolic compounds (Fig. 4H) and covered by a thick cuticle, only in *Mcvaughia* the cuticle was thin (Fig. 4N). Generally, the subcuticular space was formed (Fig. 4J), except in *Glandonia* since as they were rarely observed. The subepidermis parenchyma was composed by cells with cytoplasm densely stained filled with phenolic compounds (Fig. 4I), while the crystals were rarely observed (Fig. 4N). In *Glandonia*, sclereids with thick wall were frequently observed (Fig. 4Q), while the crystals were observed only in *Mcvaughia* (Fig. 4N).

Histochemical tests

The results of the histochemical tests are summarized in Table 2. The histochemical tests of calyx and petal glands in all species analysed were positive for

total lipids, proteins, total polyssacharides and phenolic compounds. The results for detection of mucilage/pectine were negative.

The secretion of calyx was strongly reactive for lipids in secretory tissues and subcuticular space. In *Burdachia* the secretion drops were observed in both secretory epidermis and secretory parenchyma (Fig. 5A-G), while in *Mcvaughia* occur mainly on epidermic cells (Fig. 5H-J). In *Glandonia*, the lipids were concentrated on the epidermic cells (Fig. 5L-Q), being rarely observed in parenchyma (Fig. 5R)

Petal glands have also showed reaction for lipids. *Burdachia* (Fig. 6A-G) and *Mcvaughia* (Fig. 6H-M) species were reactive for lipids on epidermic and subepidermic cells while in *Glandonia* the reaction was concentrated at the secretory epidermic cells (Fig. 6N-S). NADI reaction detected the secretion drops as oleoresin, since resulted in an intense violet or blue-violet staining, in both calyx (Fig. 5) and petal glands (Fig. 6), while the cuticle of glands was stained of blue color.

The histochemical tests carried out in calyx (Fig. 7A-C) and petal glands detected positive results for protein on epidermic cells (Fig. 7D-F). The results for polyssacharides detection were positive in subcuticular space at the calyx (Fig. 7G-I) and petal glands (Fig. 7J-N). The PAS reaction showed the presence of starch grains in epidermic cells (Fig. 7M, N) and on parenchymal secretory cells (Fig. 7J) on the petal glands. Phenolic compounds were observed in both epidermic and subepidermic cells in calyx (Fig. 7 O-Q) and petal glands (Fig. 7R-T). The result of the test with acidified vanillin in *Burdachia* was positive indicating the presence of tannins in calyx glands.

Although the secretion was observed in the calyx and petal glands in pre-antese stages, the life of petals is ephemeral. Lipids and phenolic compounds were recorded on calyx glands at fruiting periods in *B. duckei*, *G. prancei* and *M. sergipana*.

DISCUSSION

Comparative analyses of morphoanatomical characters of calyx glands

Mcvaughoid species presented a 10-glandular calyx, and were traditionally circumscribed in the subfamily Byrsonimoideae *sensu lato* (sensu Anderson, 1977), where cases of reduction or absence of glands are rare. The loss of calyx glands in Malpighioideae *s.l.* neotropical species is frequent, presenting the absence of glands in the anterior sepal (Anderson, 2007; Araújo and Meira, 2016; Mamede, 1993; Sebastiani, 2010; Sebastiani and Mamede, 2010), anterior and lateral sepals (Coccuci et al., 1996; Anderson, 2007) and exceptionally lateral and posterior sepals one-glandular (Anderson, 2007). Cappellari et al. (2011) attributes the reduction and loss of calyx glands to changes of the pollination system.

Although *Mcvaughia*, *Burdachia* and *Glandonia* were positioned phylogenetically among the Malpighioideae *s.l.* (Davis and Anderson, 2010), they maintain the 10-glandular calyx. However, in *Mcvaughia* the anterior glands are laterally displaced, which may be a result of the pollination pressure, as suggested that the absence of anterior glands on neotropical Malpighiaceae species was related to the behavior of the oil bees (Vogel, 1990).

The anatomical structure of calyx glands described for *Mcvaughia*, *Burdachia* and *Glandonia* were reported in *Banisteriopsis* (Araújo and Meira, 2016), *Camarea* (Mamede, 1993), *Diplopterys pubipetala* (Possobom et al., 2015), *Galphimia brasiliensis* (Castro et al., 2001) and *Hiptage sericeae* (Subramanian et al., 1990; Arumugasamy et al., 1993). However the sclereids were found only in floral tissues from *Glandonia* species. According to Turner et al. (1983) and Gish et al. (2016) sclereids in drainage regions may increase the degree of mechanical defense against herbivores. In addition, *Glandonia* species are less parasitized by florivores.

Sessile calyx glands are exclusive from *Burdachia*. In the same way of *Mcvaughia* and *Glandonia* species, the calyx glands of *D. pubipetala* (Possobom et al., 2015) and *Banisteriopsis* (Araújo and Meira, 2016) are subsessile. The calyx gland in the neotropical species *Dinemandra ericoides* is exceptionally stalked (Cocucci et al., 1996). Considering the relation between the secretory epidermis and stalk, it is possible that these patterns are associated to the behavior of the pollinator (Araújo and Meira, 2016). On the other hand, species of the neotropical genus *Banisteriopsis* (Araújo and Meira, 2016), although sharing anatomical similarity of the flower glands with the genera studied, is a genus phylogenetically closest to the paleotropical species *Hiptage sericeae* (Davis & Anderson, 2010), sharing the secretory epidermis typically invaginated (Subramanian et al., 1990).

Comparative analyses of morphoanatomical characters of petal glands

Among the Byrsonimoideae *s.l.*, only the species of *Mcvaughia*, *Burdachia* and *Glandonia* have petal glands, while in other species the petal margin is generally whole (Anderson, 1977). Such findings corroborate the molecular phylogeny (Anderson and Davis, 2010), which separated *Mcvaughia*, *Burdachia* and *Glandonia* from Byrsonimoideae *s.l.* being arranged with Malpighioideae *s.l.* Take into consideration the phylogenetic positioning (Davis & Anderson, 2010) the glandular and fimbriated petal are derived only on neotropical species of Malpighioideae *s.s.*

The anatomical structure described here for the petal glands on mcvaughiid species is similar to recorded for neotropical species as *D. pubipetala* (Possobom et al., 2015) and *Dinemandra ericoides* (Cocucci et al., 1996), with typically palisade epidermis, epidermal cells with conspicuous vacuoles and vascularized parenchyma. In the species studied only the posterior petal is glandular and generally, the glands are concentrated in the proximal region.

In *D. pubipetala* all petals are glandular, but the glands are more developed and numerous in the posterior petal, mainly at the base (Possobom et al., 2015). However, the glands of the lateral petals as well as in *D. pubipetala* are not vascularized (Possobom et al., 2015). While in *Dinemandra* the five petals are glandular (Coccuci et al., 1996; Simpson, 1989). Our results recorded the subcuticular space in the petal gland, previously un notice. Calcium oxalate crystal was rarely observed in the petals, being recorded only in *M. sergipana*. Sclereids were founded only in the medial region of the posterior petal of *G. williamsii* and may be related to the exclusive erect position of the posterior petal in this species (Silva et al., Unpubl.data, 2017 in Chapter IV).

Possobom et al. (2015), associated reduction and the dorsal position of calyx glands with the posterior petal glands that are numerous and developed in the base. In *Mcvaughia*, *Burdachia* and *Glandonia*, there is not observed; on the contrary, all sepals have a pair of highly developed secretory glands and only the posterior petal are glandular. Possobom et al. (2015) relate the dorsalisation of the calyx glands and the posterior petal glands to the precise positioning of the visitor to access the reward. However, the lateral petals of *D. pubipetala* are also glandular. Thus, this relation is not confirmed. In addition, Coccuci et al. (1996) recorded in *Dinemandra ericoides* 6-glandular calyx associated with the presence of glands developed in all petals.

Histochemistry of secretion from calyx and petal glands

The calyx secretion is predominantly lipophilic. The histochemical results were similar as those recorded in the calyx glands of *Banisteriopsis campestris*, *B. laevifolia* and *B. malifolia* (Araújo and Meira, 2016), where lipids, polysaccharides and proteins were detected in the secretory epidermis. Phenolic compounds occur in the secretory epidermis and subsecretor parenchyma as reported for *D. pubipetala* (Possobom et al., 2015). The histochemical results of petal glands in mcvaughioide species also recorded

lipids, polysaccharides and proteins as in *D. pubipetala* (Possobom et al., 2015), well as phenolic compounds. The calyx and petal glands of the species studied secreted exudates of the same histochemical nature (Table 2).

In the species studied, the detection of a volatile fraction in the calyx and petal secretion, characterize them as oilresin glands according to NADI reaction. Possobom et al. (2015) recorded reaction of petal glands of *D. pubipetala* to the neutral red test, but few areas responded to NADI. However this study reported strongly the indicative of aroma production.

In the calyx and petal glands of the species studied, the cuticle is thick and the secretion is accumulated in the subcuticular space, similar to that recorded in neotropical species as *D. ericoides* (Coccuci et al., 1996), *Galphimia brasiliensis* (Castro et al., 2001) and *D. pubipetala* (Possobom et al., 2015) well as the paleotropical *H. sericeae* (Subramanian et al., 1990). In *Glandonia* species the subcuticular space was not observed in the petal glands.

The results demonstrate that the secretion of these floral glands had a chemical nature mixed. The polysaccharides could reduce the viscosity of the resinous secretion, as observed in *Anarcadium occidentale* (Miranda, 2009). Considering the demand of the female bees of the Centridini tribe (Anderson, 1979b), which are deficient at producing amino acids (Baker, 1977), the proteins reward could supply the energy demands (Nicolson and Thornburg, 2007), or may act against proliferation of microorganisms (Park and Thornburg, 2009). In the species studied the phenolic accumulation was recorded in the secretory tissues. Tannins are detected on the calyx glands of *B. duckei*, compounds known to act as deterrents against herbivores and pathogens (Swain, 1977, 1979; Raymond et al., 2011).

The presence of drops in the peripheral cytoplasm of the epidermal cells and in the subcuticular space detected in histochemical analyses of mcvaughiioid species, may corroborates the suggestion of Possobom et al. (2015) that the secretion is accumulated in the periplasmic space and is accumulated below the cuticle.

Anatomical structure x nature of secretion: homology signals and functional role evidences of floral glands

The calyx glands of the studied species show a structure similar to the leaf and bracteolar nectaries described for the same species (Silva et al. unpubl. data, 2017 *in* CAP II). The main difference between elaiophore and nectaries is the chemical nature of the secretion. Despite the precocious development, the peak of secretion of calyx glands appear occurs in the floral anthesis period, as in *D. ericoides* (Coccuci et al., 1996) and *D. pubipetala* (Possobom et al., 2015). In contrast, the activity of the calyx glands in *H. benghalensis* starts at the buds and persists in the fruit (Ren et al., 2013), what may be closely related to nectariferous secretion. It is pertinent to note that the secretory activity of calyx glands of *B. duckei* is persistent in the fruit, recorded in bracteolar nectaries of mcvaughiioid species (Silva et al. unpubl. data, 2017 *in* CAP II). The similarities between leaf and calyx glands have been reported in different studies, as in *G. brasiliensis* (Castro et al., 2001), *D. pubipetala* (Possobom et al., 2010, 2015) and *Banisteriopsis* (Araújo and Meira, 2016). Thus, this work recorded new evidences to reinforce the hypothesis of Vogel (1990) that proposed the homology between such glands and the origin of glands of lipophilics secretion from nectaries.

In addition to the morphoanatomical similarities, this work reveals that the secretory mechanisms in the calyx and petal glands could be similar. Such results could indicate homology between these floral secretory structures, as defended by

Anderson (personal com. in Castro et al., 2001), resulting from the expression of the same genes in different organs through selective pressure.

Despite the similarity shared between calyx and petal glands, they not necessarily playing the same ecological function. The oil supply of calyx gland in Malpighiaceae is an important resource for oil-bees that depend of them to complete their life cycle (Buchmann, 1987; Vinson et al., 1997). Hauman-Merk (1913) suggested that without posterior petal, the bees are disoriented and their positioning in the flower is hampered, what can explain the importance of the posterior petal.

The results confirm the recognition of calyx glands in mcvaughoid species as elaiophores (Vogel, 1990; Possobom et al., 2015). Since the gathering of secretion occurs when oil-bees target the flower, reaching the glands (Vogel, 1990; Simpson, 1982) scent production may be a important signal for such bees.

Besides calyx glands, Anderson (1977) reported that the petal glands would also be responsible for the secretion of oils. In *D. pubipetala* Possobom et al. (2015) sweet aroma was reported in the flowers, while in this studied, the aroma was rarely felt. Thus, the scent signals of the posterior petal in the studied species, besides the differentiated color, could increase the chances of visiting. According to Abrol (2012). scent and flower color are important in establishing interactions with pollinators, since the sensitivity of insects to aroma is effective at low concentrations and is a signal of available floral rewards. Thus, even though in the field activities the aroma was not perceived, it does not mean that it was not being released.

Possobom et al (2015), classified the petal glands in *D. pubipetala* as typical osmophores. These data are corroborated by the change in the shape of the epidermical cells of petal glands in *Glandonia*, which is also reported in the secretory epidermis of *Ophrys* osmophores (Orchidaceae, Ascensão et al., 2005). In addition, the presence of

starch grains on osmophores as reported in Orchidaceae, being a important source for energy costs of secretory activity (Ascensão et al., 2005).

Despite the anatomy and the histochemical nature of the calyx and petal glands, the homology shared by this structures is possible, acting as elaiophores or osmophores. This similarity is corroborated by Anderson (Castro et al., 2001), who suggested the homology between leaf and calyx glands as a clear sign of expression of the same genes in leaves and sepals of the same species. Reproductive biology studies are essential to elucidate the ecological function of such glands mainly by the presence of strong aroma in the field works to collect *G. macrocarpa* (with bud at development), which could attribute the function of scent production also to calyx glands.

This study may indicate that the number of glandular sepals may be inversely related to the number of glandular petals, as well as the relation between oil production and number of glands in the calyx may be compensatory, as reported by Carvalho et al. (2005). Although Anderson (1979b) and Zhang et al. (2010) assumed that the posterior petal, by itself, directs the positioning of the bee, more efficient signaling of the glandular petal could be advantageous in attraction, as suggested by Possobom et al. (2015) that the behavior of the bee is a result of visual and chemical perception.

The flower buds of mcvaughioide species are parasitaded by larvae of beetles (Curculionidae) that damage reproductive structures, including petals (Silva et al. unpubl. data, 2017 *in* CAP II). According to Ferreira and Torezan-Silingardi (2013) flowers of Malpighiaceae with signs of herbivory and even without the posterior petal are less visited, which affects the fruiting. Our data corroborate the ecological importance of the posterior petal, which can be a signal of health and available floral rewards. Although the observation of potential collecting bees in the study was rare, similar marks to oil-bees jaws were recorded on the claw of posterior petals.

CONCLUSIONS

The morphoanatomy of floral glands were distinguished mainly by the extension of the secretory epidermis in calyx glands and by the dorsiventral flattening in petals glands. Features such as the degree of zygomorphy of the calyx glands and the exclusive occurrence of petalar glands in Neotropical species probably carry some specific signaling in the mutualism with Malpighiaceae and oil-bees. Although the glands studied share the same nature of secretion (oilresin), they apparently play different ecological roles. Based on field observations, anatomical and histochemical data, it was possible to determine that the calyx glands act as elaiophores and the petal glands as osmophores. However, chemical and floral biology studies are imperative to elucidate if such glands performs only one function. The high morphoanatomical similarity may be a signal of homology between these glands and nectaries. Thus, the morphological and histochemical variations recorded in the studied species may be the resulted by the selective pressure from the oil-bees pollinators.

REFERENCES

- ABROL, D.P. 2012. Biochemical Basis of Plant-Pollination Interaction. *In: Pollination Biology: Biodiversity Conservation and Agricultural Production*, Springer, Berlin. pp. 413–458.
- ALVES-DOS-SANTOS, I., I.C. MACHADO, AND M.C. GAGLIANONE. 2007. História natural das abelhas coletoras de óleo. *Oecologia Brasiliensis* 11: 544–557.
- AMORIM, A.M. AND R.F. ALMEIDA. 2015. An unexpected *Mcvaughia* (Malpighiaceae) species from sandy coastal plains in northeastern Brazil. *Syst. Bot.* 40: 535–538.
- ANDERSON, C. 2007. Revision of *Galphimia* (Malpighiaceae). *Contr. Univ. Michigan Herb.* 25: 1–82.
- ANDERSON, W.R. 1978 [“1977”]. Byrsonimoideae, a new subfamily of the Malpighiaceae. *Leandra.* 7: 5–18.
- ANDERSON, W.R. 1979a. *Mcvaughia*, a new genus of Malpighiaceae from Brazil. *Taxon.* 28: 157–161.

- ANDERSON, W.R. 1979b. Floral conservatism in neotropical Malpighiaceae. *Biotropica*. 11: 219–223.
- ANDERSON, W.R. 1981. Malpighiaceae. The Botany of the Guayana Highland-Part XI. *Memorirs of the New York Botanical Garden*. 32: 137–147.
- ANDERSON, W.R. 1990. The origin of the Malpighiaceae-The evidence from morphology. *Memoirs of the New York Botanical Garden*. 64: 210–224.
- ARAÚJO, J.S. AND R.M.S.A.MEIRA. 2016. Comparative anatomy of calyx and foliar glands of *Banisteriopsis* C. B. Rob. (Malpighiaceae). *Acta Botanica Brasilica*. 30: 112–123.
- ARUMUGASAMY, K., K. UDAIYAN, S. MANIAN, AND V. SUGAVANAM. 1993. Ultrastructure and oil secretion in *Hiptage sericea* Hook. *Acta Societatis Botanicorum Poloniae*. 62: 17–20.
- ASCENSÃO, L., A. FRANCISCO, H. COTRIM, AND M. SALOMÈ PAIS. 2005. Comparative structure of the labellum in *Ophrys fusca* and *O. lutea* (Orchidaceae). *American Journal of Botany*. 92: 1059–1067.
- BAKER, H.G. 1977. Non-sugar chemical constituents of nectar. *Apidologie*. 8: 349–356.
- BUCHMANN, S.L. 1987. The ecology of oil flowers and their bees. *Annual Review of Ecology and Systematics* .18: 343–369.
- CAPPELLARI, S.C., A.M. HALEEM, A.J. MARSAIOLI, R. TIDON, AND B.B. SIMPSON. 2011. *Pterandra pyroidea*: a case of pollination shift within Neotropical Malpighiaceae. *Annals of Botany*. 107: 1323–1334.
- CARVALHO, P.D., E.L. BORBA, AND A.M. LUCCHESI. 2005. Variação no número de glândulas e produção de óleo em flores de *Stigmaphyllon paralias* A. Juss. (Malpighiaceae). *Acta Botanica Brasilica*. 19: 209–214.
- CASTRO, M.A., A.S. VEGA, AND M.E. MULGURA. 2001. Structure and ultrastructure of leaf and calyx glands in *Galphimia brasiliensis* (Malpighiaceae). *American Journal of Botany*. 88: 1935–1944.
- CLARK, S.G. 1981. *Staining procedures* Williams & Wilkins, Baltimore.
- COCUCCI, A.A., A.M. HOLGADO, AND A.M. ANTON. 1996. Estudio morfológico y anatómico de los eleoforos pedicelados de *Dinemandra ericoides*, Malpighiaceae endémica del desierto de Atacama, Chile. *Darwiniana*. 34: 183–192.
- DAVID, R. AND J.P. CARDE. 1964. Coloration différentielle des inclusions lipidique et terpeniques des pseudophylles du Pin maritime au moyen du reactif Nadi. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences*. 258: 1338–1340.
- DAVIS, C.C. AND W.R. ANDERSON. 2010. A complete generic phylogeny of Malpighiaceae inferred from nucleotide sequence data and morphology. *American Journal of Botany*. 97: 2031–2048.

- DAVIS, C.C., C.D. BELL, S.MATHEWS, AND M.J. DONOGHUE. 2002. Laurasian migration explains Gondwanan disjunctions: evidence from Malpighiaceae. *Proceedings of the National Academy of Sciences*. 99: 6833–6837.
- DAVIS, C.C., H. SCHAEFER, Z. Xi, D.A. BAUM, M.J. DONOGHUE, AND L.J. HARMON. 2014. Long-term morphological stasis maintained by a plant–pollinator mutualism. *Proceedings of the National Academy of Sciences*. 111: 5914–5919.
- FERREIRA, C.A. AND H.M. TOREZAN-SILINGARDI. 2013. Implications of the floral herbivory on Malpighiaceae plant fitness: visual aspect of the flower affects the attractiveness to pollinators *Sociobiology*. 60 (3): 323–328.
- FISHER, D.B. 1968. Protein staining of ribboned epon sections for light microscopy. *Histochemistry and Cell Biology*. 16: 92–96.
- GATES, B. 1982. *Banisteriopsis* and *Diplopterys* (Malpighiaceae). *Flora Neotropica Monograph*. 30: 1–237.
- GISH, M., M.C. MESCHERB, AND C.M. MORAESB. 2016. Mechanical defenses of plant extrafloral nectaries against herbivory. *Communicative & integrative biology*. 9(3): e1178431
- HAUMAN-MERCK, L. 1913. Observations sur la pollination d'une Malpighiacée du genre *Stigmaphyllon*. *Recueil de l'Institut Botanique* (Université Libre de Bruxelles). 9: 21–27.
- JOHANSEN, D.A. 1940. *Plant Microtechnique*. New York. Mc Graw-Hill Book Co. Inc. 523p.
- KARNOVSKY, M.J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. *Journal of Cell Biology*. 27:137.
- LOBREAU-CALLEN, D. 1989. Les Malpighiaceae et leurs pollinisateurs. Coadaptation ou coevolution. *Bulletin du Muséum National d'Histoire Naturelle. Adansonia*. 1: 79–94.
- MACE, M.E. AND C.R HOWELL. 1974. Histochemistry and identification of condensed tannin precursors in roots of cotton seedlings. *Can J Bot*. 52: 2423–2426.
- MAMEDE, M.C.H. 1993. Anatomia dos órgãos vegetativos de *Camarea* (Malpighiaceae). *Acta Botanica Brasílica*. 7: 3–19.
- MCMANUS, J.F.A. 1948. Histological and histochemical uses of periodic acid. *Stain Technology*. 23: 99–108.
- MIRANDA, R.L. 2009. Cashew tree bark secretion-perspectives for its use in rotein isolation strategies. *Open Glycoscience*. 2: 16–19.
- NICOLSON, S.W. AND R.W. THORNBURG. 2007. Nectar chemistry. *In*: S. Nicolson, M. Nepi and E. Pacini [Eds.], *Nectaries and nectar*. Springer, Dordrecht Netherlands, 215–63.
- O'BRIEN, T.P., N. FEDER, AND M.E. MCCULLY. 1964. Polycromatic staining of plant cell walls by toluidina blue O. *Protoplasma*. 59: 368–373.

- PARK, S. AND R.W. THORNBURG. 2009. Biochemistry of nectar proteins. *J. Plant Biol.* 52, 27–34. doi: 10.1007/s12374-008-9007-5
- PEARSE, A.G.E. 1980. Histochemistry: theoretical and applied, fourth ed. Churchill Livingstone, Edinburgh.
- POSSOBOM, C.C.F., E. GUIMARÃES, AND S.R. MACHADO. 2010. Leaf glands act as nectaries in *Diplopterys pubipetala* (Malpighiaceae). *Plant Biology*. 12: 863–870.
- POSSOBOM, C.C.F., E. GUIMARÃES, AND S.R. MACHADO. 2015. Structure and secretion mechanisms of floral glands in *Diplopterys pubipetala* (Malpighiaceae) and a neotropical species. *Flora*. 211: 36–39.
- RAYMOND, V., C. BARBEHENN, AND P. CONSTABEL. 2011. Tannins in Plant-Herbivore Interactions. *Phytochemistry*. 72: 1551–1565.
- REN, M., Y. ZHONG, AND X. SONG. 2013. Mirror-image flowers without buzz pollination in the Asian endemic *Hiptage benghalensis* (Malpighiaceae). *Botanical Journal of the Linnean Society*. 173: 764–774.
- SAZIMA, M. AND I. SAZIMA. 1989. Oil-gathering bees visit flowers of eglandular morphs of the oil-producing Malpighiaceae. *Botanica Acta*. 102: 106–111.
- SEBASTIANI, R. 2010. Estudos taxonomicos em *Janusia* A.Juss. (Malpighiaceae). Tese (Doutorado) — Instituto de Botanica da Secretaria de Estado do Meio Ambiente, São Paulo.
- SEBASTIANI, R. AND M.C.H. MAMEDE. 2010. Estudos taxonômicos em *Heteropterys* subsect. *Stenophyllarion* (Malpighiaceae) no Brasil. *Hoehnea*. 37: 337–366.
- SIGRIST, M.R. AND M. SAZIMA. 2004. Pollination and reproductive biology of twelve species of neotropical Malpighiaceae: Stigma morphology and its implications for the breeding system. *Annals of Botany*. 94: 33–41.
- SIMPSON, B.B. 1982. *KRAMERIA* (Krameriaceae) flowers: orientation and elaiophore morphology. *Taxon*. 31, 517–528.
- SIMPSON, B.B. 1989. Pollination Biology and Taxonomy of *Dinemandra* and *Dinemagonum* (Malpighiaceae). *Systematic Botany*. 14(3): 408–426.
- SIMPSON, B.B. AND J.L. NEFF. 1981. Floral rewards: alternatives to pollen and nectar. *Annals of the Missouri Botanical Garden*. 68: 301–322.
- SMITH, F.H. AND E.C. SMITH. 1942. Anatomy of the inferior ovary of *Darbya*. *American Journal of Botany*. 29: 464–471
- SUBRAMANIAN, R.B., K. ARUMUGASAMY, AND J.S. INAMDAR. 1990. Studies in secretory glands of *Hiptage sericea* (Malpighiaceae). *Nordic Journal of Botany*. 10: 57-62.
- SWAIN, T. 1977. Secondary compounds as protective agents. *Annual Review of Plant Physiology*. 28: 479–501.

SWAIN, T. 1979. Tannins and lignins. In G. A. Rosenthal and D. H. Janzen [Eds.], *Herbivores, their interaction with plant secondary metabolites*, 657-682. Academic Press, New York

TURNER, G.W. AND N.R. LERSTEN. 1983. Apical foliar nectary of pomegranate (*Punica granatum*: Punicaceae). *Am J Bot.* 70: 475–80.

VASCO, A., M. THADEO, M. CONOVER, AND D.C. DALY. 2014. Preparation of samples for leaf architecture studies, a method for mounting cleared leaves. *Applications in Plant Sciences.* 9: 1–4.

VINSON, S.B., H.J. WILLIAMS, G.W. FRANKIE, AND G. SHRUM. 1997. Floral lipid chemistry of *Byrsonima crassifolia* (Malpighiaceae) and a use of floral lipid by *Centris* bees (Hymenoptera: Apidae). *Biotropica.* 29: 76–83.

VOGEL, S. 1990. History of the Malpighiaceae in the light of Polination Ecology. *Memoirs of the New York Botanical Garden.* 55: 130–142.

ZHANG, W., E.M. KRAMER, AND C.C. DAVIS. 2010. Floral symmetry genes and the origin and maintenance of zygomorphy in a plant-pollinator mutualism. *Proceedings of the National Academy of Sciences, USA.* 107: 6388–6393.

APPENDIX 1. List of material used of *Burdachia*, *Glandonia* and *Mcvaughia* and voucher information of herbarium. Herbarium acronyms in parentheses follow Thiers (2016).

Species	Collector and number (Herbarium)
<i>Burdachia duckei</i> Steyerem.	Nelson 1270 (INPA), Rodrigues 10736 (INPA), Silva 301, 314 (VIC), Varejão s. n. (INPA)
<i>Burdachia prismatocarpa</i> A. Juss.	Coêlho (INPA), Guillaumet 5736 (INPA), Maas 6650 (INPA), Mota 2939 (INPA), Nelson (INPA), Silva 1164 (INPA), Silva 289; 292; 296; 297 (VIC)
<i>Burdachia sphaerocarpa</i> A. Juss.	Ferreira 4260 (INPA), Hill 12971 (INPA), Rodrigues 8886 (INPA), Silva 313 (VIC)
<i>Glandonia macrocarpa</i> Griseb.	Coêlho 3041, 3118 (INPA), Rodrigues 4945 (INPA), Silva 287; 288 (VIC)
<i>Glandonia prancei</i> W. R. Anderson	Silva 298; 299 (VIC)
<i>Glandonia williamsii</i> Steyerem.	Coêlho 484 (INPA), Silva 290 (VIC)
<i>Mcvaughia bahiana</i> W. R. Anderson	Silva 300; 301 (VIC)
<i>Mcvaughia sergipana</i> Amorim & R.F. Almeida	Silva 305; 306 (VIC)

Table 1: Distribution and general features of floral glands in *Mcvaughia*, *Burdachia* e *Glandonia* species.

	Calyx glands	Petalar glands		MEDIAN ZONE OF THE BLADE
	ABAXIAL SURFACE OF SEPAL	Proximal portion	Distal portion	
<i>M. bahiana</i>	clavate, concave-flat subsessile	digitiform	?	papillose
<i>M. sergipana</i>		stalked	sessile	
<i>B. duckei</i>	clavate, concave sessile	digitiform stalked	sessile	-
<i>B. prismatocarpa</i>				
<i>B. sphaerocarpa</i>				
<i>G. macrocarpa</i>	clavate, concave subsessile	flattened	-	papillose
<i>G. prancei</i>		immersed		
<i>G. williamsii</i>				

(-) eglandular.

Table 2: Results of histochemical tests applied on the calyx and petalar glands in *Mcvaughia*, *Burdachia* and *Glandonia* species.

	<i>Burdachia</i>						<i>Glandonia</i>						<i>Mcvaughia</i>			
	<i>Burdachia duckei</i>		<i>Burdachia prismatocarpa</i>		<i>Burdachia sphaerocarpa</i>		<i>Glandonia macrocarpa</i>		<i>Glandonia prancei</i>		<i>Glandonia williamsii</i>		<i>Mcvaughia bahiana</i>		<i>Mcvaughia sergipana</i>	
	Calyx Gland	Petal Gland	Calyx Gland	Petal Gland	Calyx Gland	Petal Gland	Calyx Gland	Petal Gland	Calyx Gland	Petal Gland	Calyx Gland	Petal Gland	Calyx Gland	Petal Gland	Calyx Gland	Petal Gland
TESTS:																
Lipids/terpenoids:																
Sudan Red	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NADI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Proteins:																
Xylidine Ponceau	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Coomassie brilliant blue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polysaccharides:																
Pas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lugol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ruthenium red	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phenolic compounds:																
Ferrous sulphate in formalin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acidified vanillin*	+															
Alkaloids:																
Wagner's reagent	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

(+) positive result; (-) negative result.

* Vanilina testes were made only at fresh samples from species available

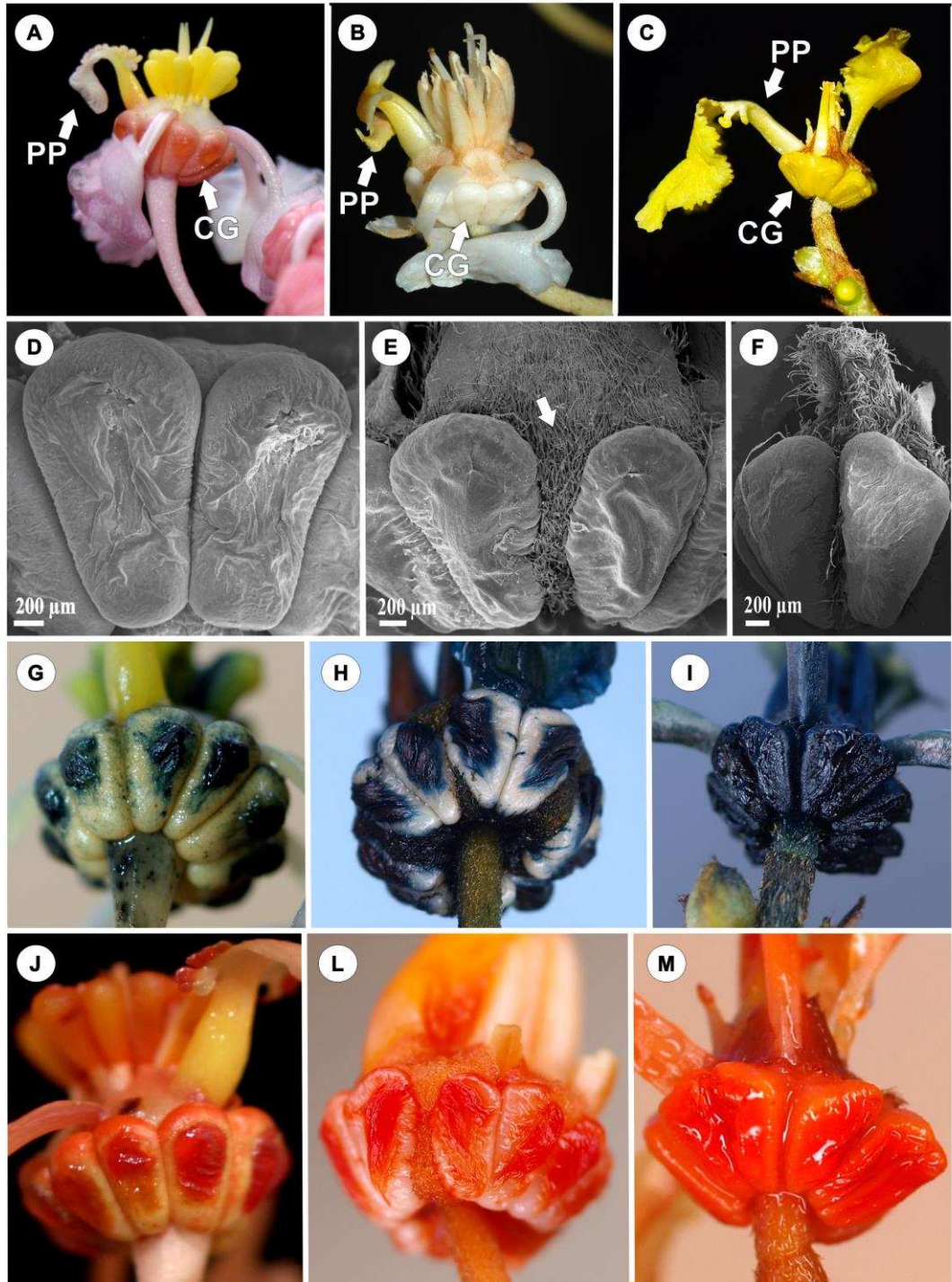


Figure 1: Floral morphological features in mcvaughiioid species. (A-C) Flower of *B. prismatocarpa* (A), *G. macrocarpa* (B) and *M. sergipana* (C). (D-F) SEM image of calyx glands in *B. prismatocarpa* (D), *G. prancei* (E) and *M. sergipana* (F), PP: posterior petal, CG: calyx glands. (G-M) Testing total lipids of calyx glands, using Sudan Black in *B. prismatocarpa* (G), *G. macrocarpa* (H) and *M. sergipana* (I); Sudan Red in *G. macrocarpa* (J), *G. macrocarpa* (L) and *M. sergipana* (M).

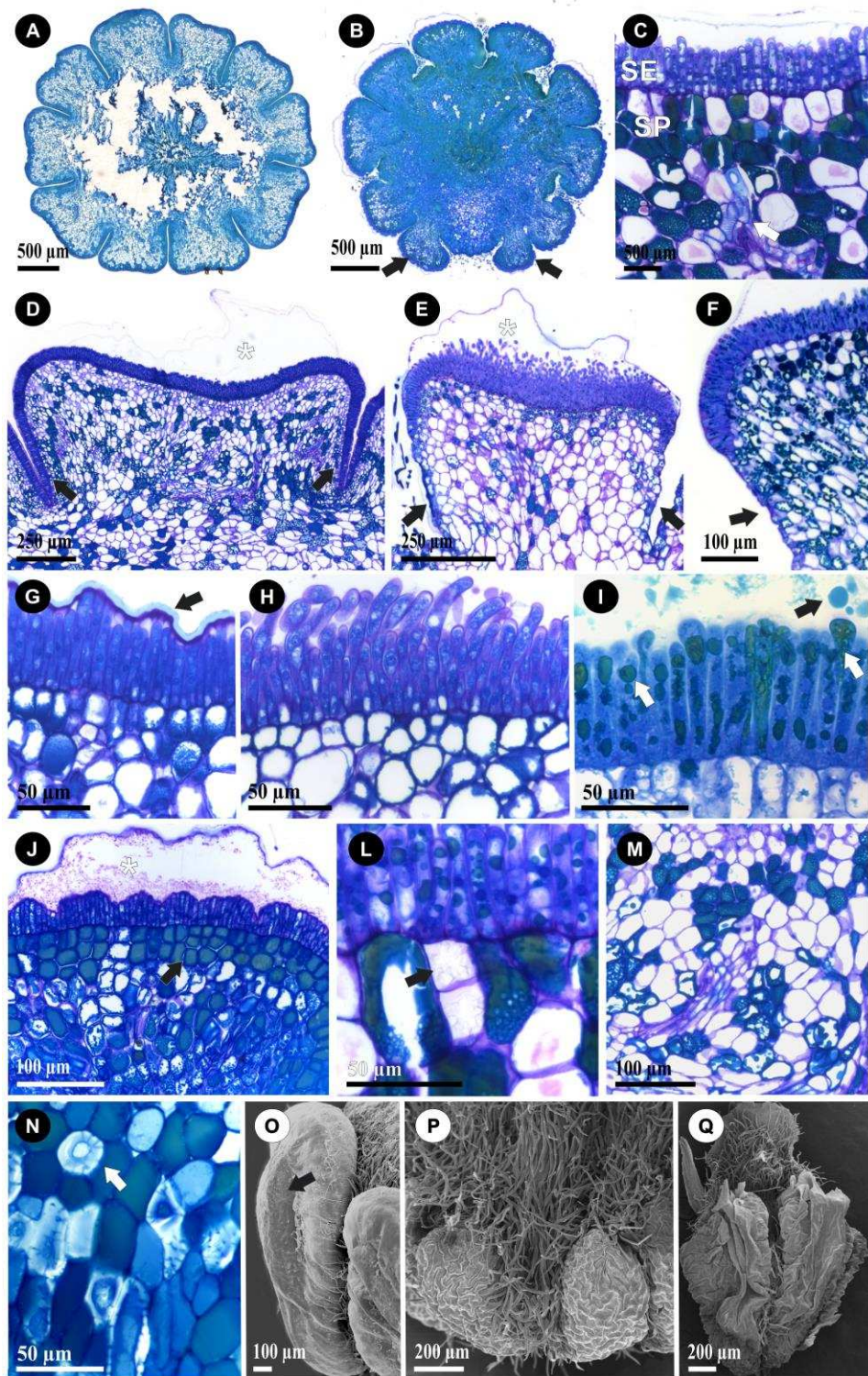


Figure 2: Anatomical characters of calyx glands. (A and B) Transversal section of calyx, showing the glands position in *B. duckei* (A) and *M. bahiana* (B). C. secretory tissues in *G. williamsii*, white arrow: vascularization. (D) Calyx gland sessile in *B. sphaerocarpa*, note the continuous secretory surface at black arrows. (E and F) subsessile calyx gland in *M. sergipana* (E) and *G. prancei* (F), black arrows: non-secretory surface. (G and H) calyx gland in *M. sergipana*, in bud (G) and flower (H) development stage. (I) secretion at black arrow in *B. prismatocarpa*, phenolic compounds in secretory cells at white arrows. (J) subepidermal layers showing cells filled with phenolic compounds in *M. bahiana* at black arrow. (L) cells with crystals at black arrow *G. williamsii*. (M) Parenchyma vascularization in *B. sphaerocarpa*. (N) sclereid (white arrow) in parenchyma secretory in *G. williamsii*. (O-Q) SEM images of calyx glands in *G. williamsii* (O), showing the space subcuticular developed and *M. sergipana* (P and Q). SE: secretory epidermis, SP: secretory parenchyma, asterisk: subcuticular space.

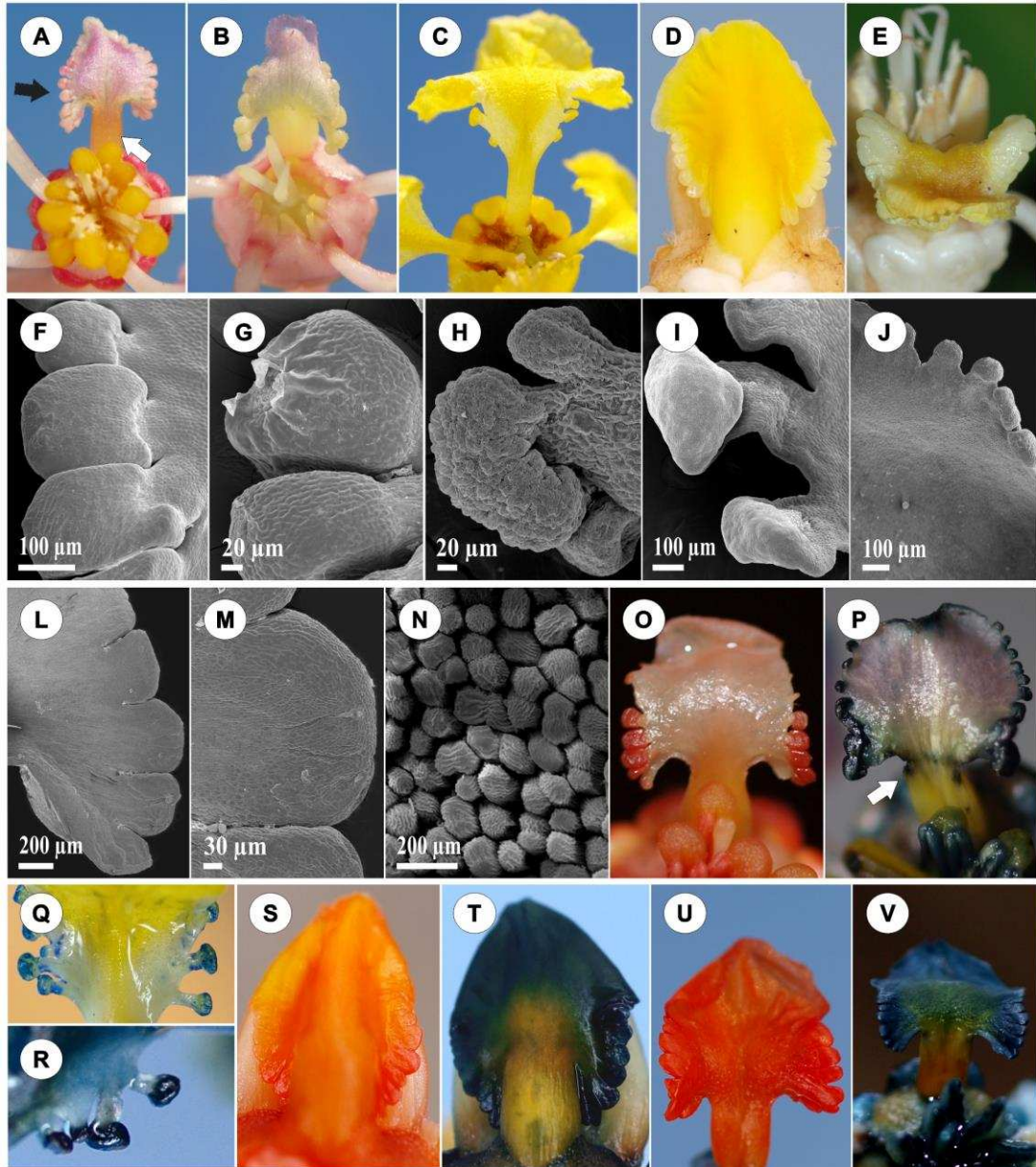


Figure 3: Morphological features of petal glands in mcvaughiioid species: (A-E) posterior petal showing a developed claw (white arrow) and marginal glands in *B. duckei* (A), *B. sphaerocarpa* (B), *M. sergipana* (C) and *G. macrocarpa* (D and E). (F-N) Posterior petal in SEM images, showing marginal glands in *B. prismatocarpa* (F and G), *M. bahiana* (H), in *M. sergipana* at the base (I) and the apex (J), flattened glands at the base petal in *G. macrocarpa* (L and M) and pappilose surface of median portion of posterior petal in *G. macrocarpa* (N). (O-V) Posterior petal submitted to Histochemical tests in field. Testing lipids in *B. prismatocarpa* using Sudan Red (O) Sudan black (P). Positive reaction for protein in *M. sergipana*; using Comassie blue (Q) and positive reaction for lipids used Sudan black (R). Testing total lipids, showing positive reaction for Sudan Red (S and U) and Sudan black (T and V) in buds of *G. marocarpa* (S and T) and in flowers of *G. macrocarpa* (U) and *G. prancei* (Q).

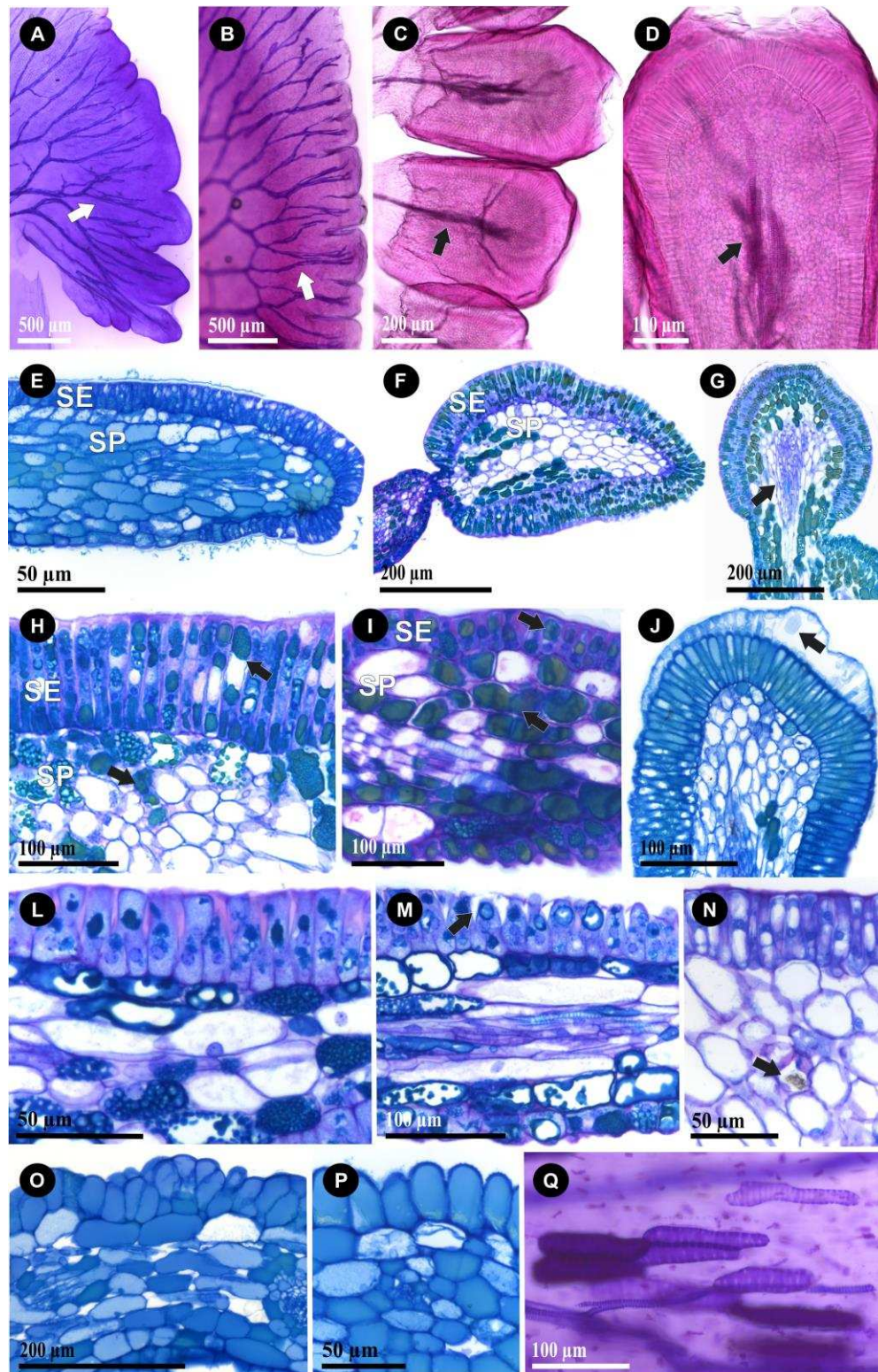


Figure 4: Anatomical characters of petal glands. (A-D) Posterior petal diaphanized of *G. prancei* (A), *G. williamsii* (B) and *B. prismatocarpa* (C and D), showing marginal glands and vascularization (arrows). (E). Sessile petal gland in *G. prancei*. (F and G). Stalked petal glands in *B. sphaerocarpa* (F) and *B. prismatocarpa* (G), showing the vascularization at the arrow (SE: secretory epidermis; SP: subepidermal parenchyma). (H and I). Secretory tissues showing cells filled with phenolic compounds (arrows) in *B. sphaerocarpa* (H) and *G. williamsii* (I). (J). subcuticular space in *B. duckei*, showing the secretion at the black arrow. (L and M). Epidermal cells in different secretory stages of development in *G. prancei*, developing intercellular space between epidermal cells (black arrow). (N). cuticle tiny in *M. sergipana* and crystal on the parenchyma (black arrow). (O and P). papillose cells on the adaxial surface in *G. macrocarpa*. (Q). Sclereids in posterior petal of *G. williamsii*.

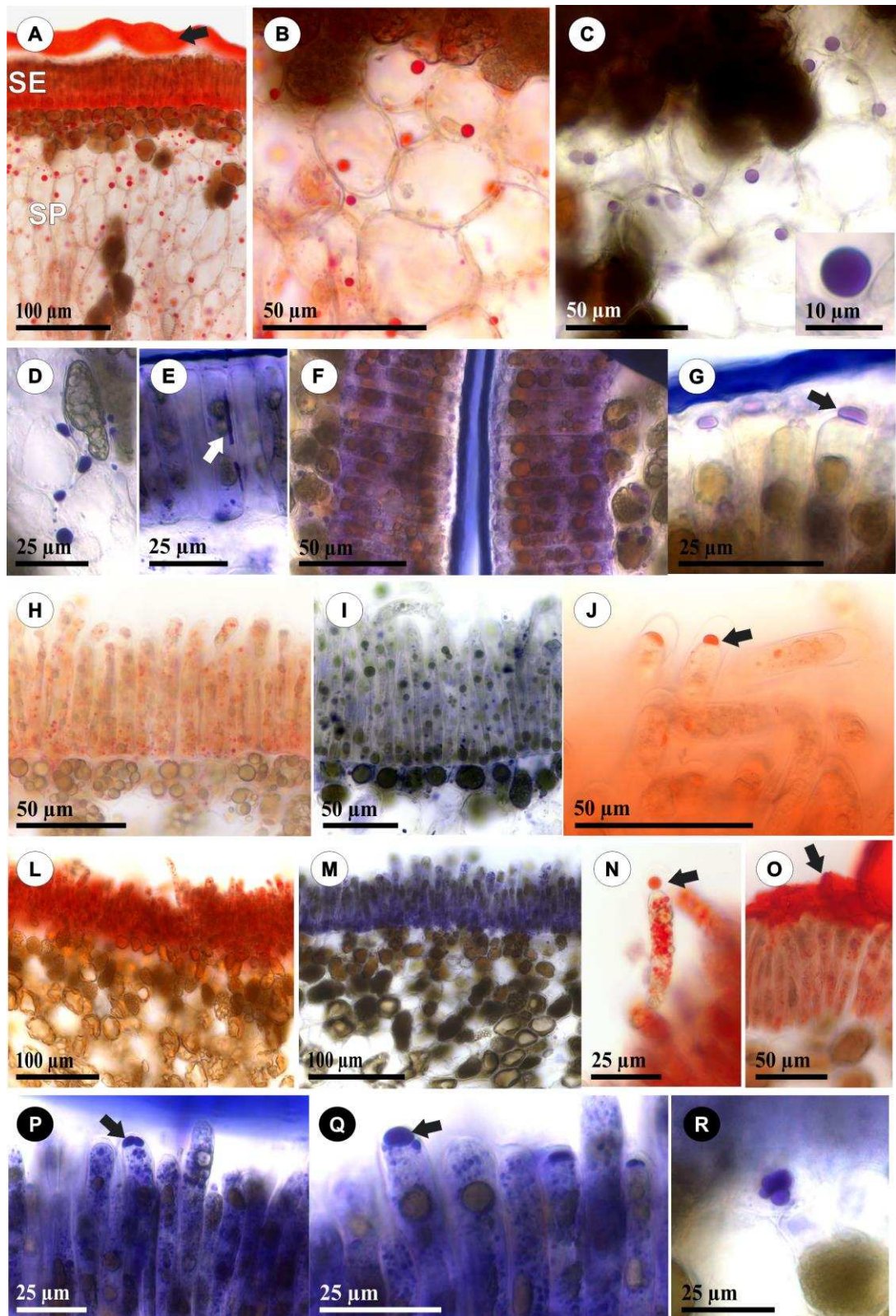


Figure 5: Histochemistry for lipid detection of calyx glands. (A-G). droplets of secretion on both secretory epidermis and parenchyma, SE and SP in *B. prismatocarpa* (A-C, F and G) in *B. sphaerocarpa* (D and E). Positive reaction to sudan red (A and B) and to NADI (C-G), detecting oilresin. (H-Q) Droplets predominantly found at the secretory epidermis in *M. sergipana* (H-J), in *G. prancei* (L-N), in *G. williamsii* (O), in *G. prancei* (P and Q). (R). Droplets at parenchyma cells in *G. prancei*. Positive reaction to sudan red (H, J, L, N and O) and to NADI (I, M, P-R), detecting oilresin. Note the secretion at black arrows.

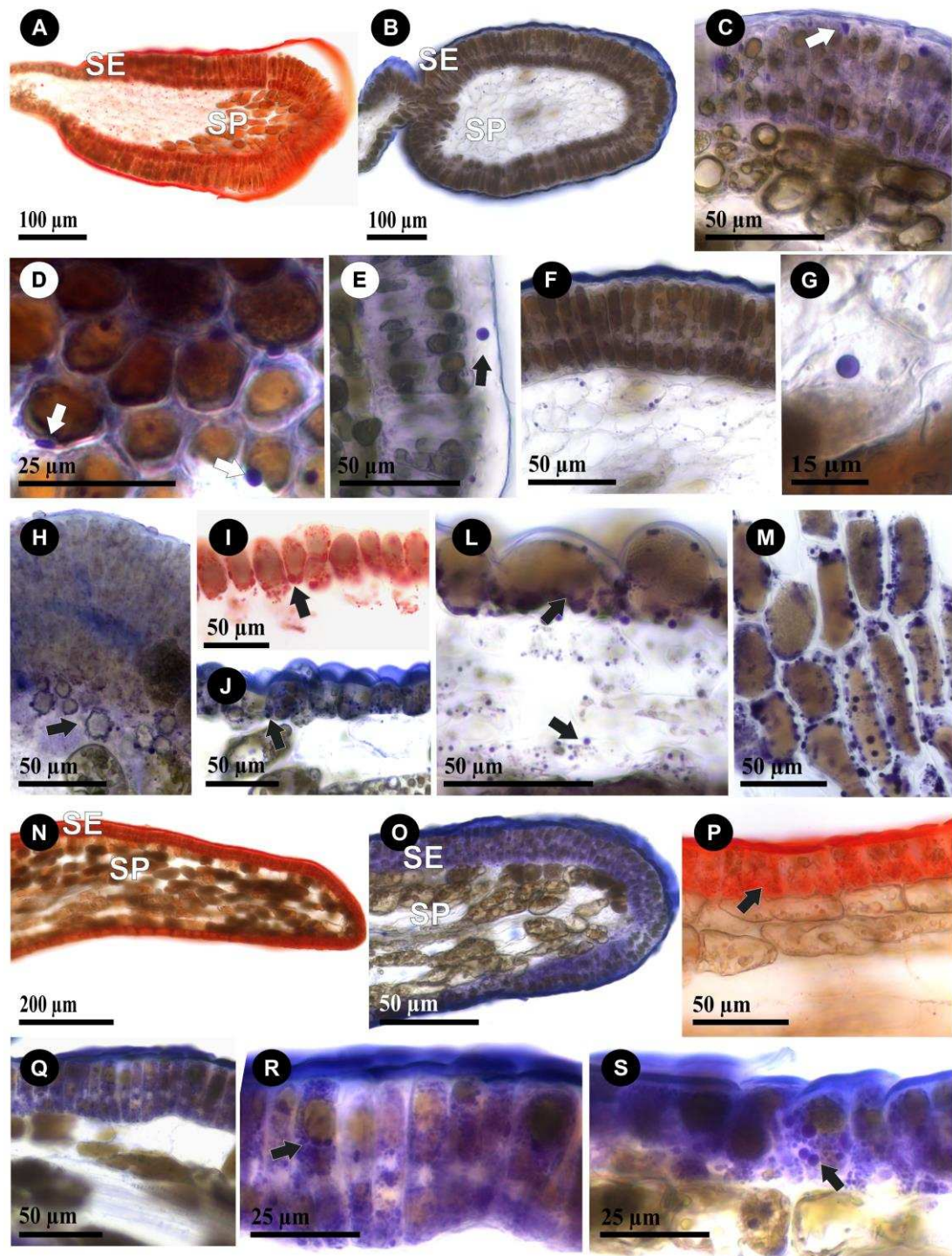


Figure 6: Histochemistry for lipid detection of petal glands. (A-M). Droplets on both secretory epidermis and parenchyma, SE and SP in *B. duckei* (A and B, F and G), in *B. sphaerocarpa* (C-G) and in *M. sergipana*, droplets at pappilose cells of the median portion of the petal (I-M), showing a parietal position; arrows: droplets of secretion. (N-S). Droplets predominantly found at secretory epidermis in *G. prancei*. Positive reaction to sudan red (A, I, N and P) and to NADI (B-H, J-M, O, Q-S), detecting oilresin. Note the secretion at black arrows.

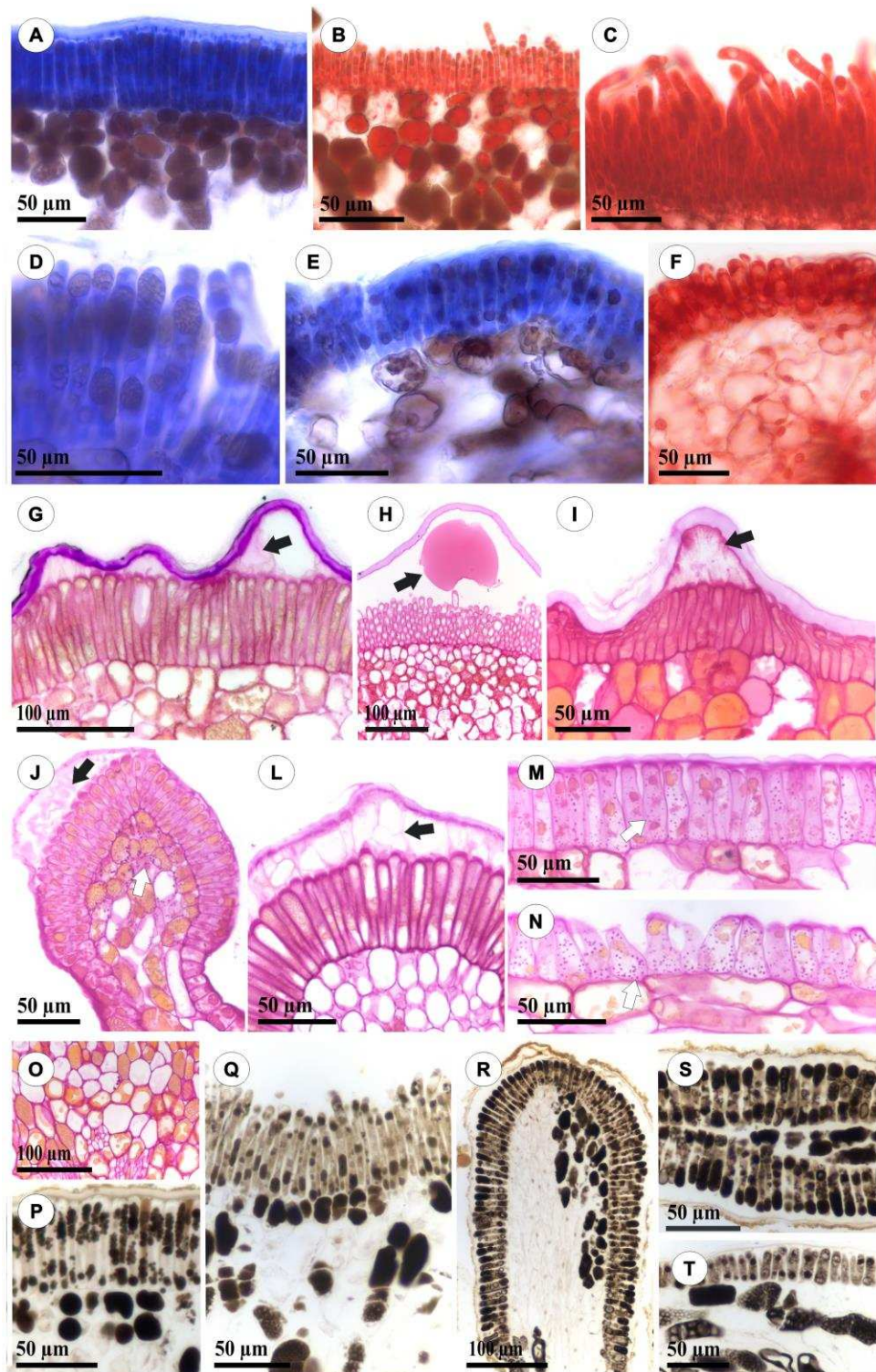


Figure 7: Histochemistry of calyx and petal glands. (A-F). Protein tests, positive reaction in epidermical cells of calyx glands in *B. duckei* (A), *G. williamsii* (B) and *M. sergipana* (C); positive reaction on the epidermical cells of petal glands in *B. duckei* (D), *G. macrocarpa* (E) and *M. sergipana* (F). (G-N) PAS, positive reaction at the subcuticular space of the calyx glands (black arrows) in *B. prismatocarpa* (G), *G. prancei* (H) and *G. williamsii* (I); positive reaction at the subcuticular space of the petal glands (black arrows) in *B. sphaerocarpa* (J), *B. duckei* (L), and in epidermical cells in different stages of development of petal gland in *G. prancei* (M and N), showing the starch grains (white arrows) (J, M and N). (O-T). Detection of phenolic compounds. Calyx glands in *B. prismatocarpa* (O). Positive reaction of calyx glands to sulphate ferreuse in *B. duckei* (P) and in *M. sergipana* (Q); and positive reaction of petal glands in *B. duckei* (R), *B. prismatocarpa* (S) and *G. macrocarpa* (T).

CAPÍTULO II

How different are the nectaries on inflorescences and leaves
of Neotropical Malpighiaceae genera?

Redação de acordo com as normas de submissão do periódico: *Perspectives in Plant Ecology, Evolution and Systematics*

How different are the nectaries on inflorescences and leaves of Neotropical Malpighiaceae genera?

Isabel Reis e Silva^{a,b}, Ítalo Antônio Cotta Coutinho^c and Renata Maria Strozi Alves Meira^{a*}

^aUniversidade Federal de Viçosa, UFV, Departamento de Biologia Vegetal, 36570-900, Viçosa, Minas Gerais, Brazil.

^bUniversidade Federal do Amazonas, UFAM, Instituto de Ciências Exatas e Tecnologia, 69103-128, Itacoatiara, Amazonas, Brazil.

^cUniversidade Federal do Ceará (UFC), Campus do Pici. Av.: Mister Hull, s/n, Departamento de Biologia, Bloco 906, CEP 60440-900, Fortaleza, CE, Brazil.

*Corresponding author. Mailing address: ^aUniversidade Federal de Viçosa, UFV, Departamento de Biologia Vegetal, Avenida Peter Henry Rolfs, s/n, 36570-900, Viçosa, Minas Gerais, Brazil.

e-mail: rmeira@ufv.br (R. M. S. A. Meira)

Abstract

Background and Aims: Although the nectaries are usually recognized as floral and extrafloral, little is known about variations between nectaries on the leaves and on inflorescences in the same species, mainly if they play similar function. The mcvaughioide clade is a good lineage in Malpighiaceae to be a model of studies, since their species has leaf and bracteolar nectaries. *Burdachia*, *Glandonia* and *Mcvaughia* genera present peculiar distribution in Amazon, “Caatinga” and “Mata Atlântica”. This study aims to compare the anatomy, the nature of secretion and record insect visitation on the leaf and bracteolar nectaries of the mcvaughioide species.

Methods: Samples of leaves and inflorescences at different stages of development were collected and submitted to standard anatomical light and scanning electron microscopy techniques. The glucose concentration of the secretion was recorded in the field and the ants with diurnal activity on such nectaries were identified.

Key Results: Leaf nectaries were classified as immersed, sessile or short-stalked, while the bracteolar as sessile or subsessile. Although the both nectaries show similar morphology and histochemistry, the bracteolar share more similarity among themselves and the concentration of sugar on the bracteolar nectar was higher. Another peculiar feature of bracteolar nectaries was the precocious development and the secretory activity persistent through frutification. Ants of eight genera were identified; being *Dolichoderus* the most common, as well the visits to consume nectar were observed generally at the inflorescences.

Conclusions: The differences between leaf and bracteolar nectaries are significant to contribute for understanding the position classification of the nectaries on leaves and inflorescences. The floral morphology evolved with pollinators, well as the occurrence of nectaries on inflorescence may be determined by nectar consumers.

Keywords: leaf gland, infloresce, floral gland, mcvaughioide clade, *Burdachia*, *Glandonia*, *Mcvaughia*.

INTRODUCTION

Malpighiaceae is a family recognized by the presence of glands on leaves and calyx, which are meaningful not only for the taxonomy (Anderson, 1990) but also for playing an important role in ecological interactions (Davis et al., 2014; Vogel, 1990). Although the calyx and leaf glands bear similar anatomical structure, such glands may produce different secretions. Leaf glands produce nectar and are therefore treated as nectaries (Araújo et al., 2010; Araújo and Meira, 2016; Castro et al., 2001; Possobom et al., 2015, 2010; Subramanian et al., 1990) while the type of exudates secreted by the calyx glands vary according to the geographic distribution of the species (Vogel, 1990). Calyx glands are said to behave as elaiophores (oil secreting structures) in the Neotropic (Anderson 1990, 1979a; Vogel, 1990) and as nectaries in the Paleotropic (Ren et al., 2013). Taxonomic treatment recorded glands at the bracteoles on inflorescences of Malpighiaceae species (Anderson 1990, 1981). However, the anatomy of such structures were not described and their functional role still needs to be unraveled.

Among the phylogenetics informal groups of Malpighiaceae, the mcvaughioide clade stands out for its peculiar biogeography as *Burdachia* and *Glandonia* are endemic trees from the Amazon while *Mcvaughia* is represented by endemic shrubs from “Caatinga” (drylands) and “Restinga” (sandy coastal plains) (Amorim and Almeida, 2015; Anderson, 1981, 1979b). Several morphological characters corroborated the recognition of the mcvaughioide clade, such as the dry, indehiscent not-winged fruit; the abaxial leaf glands; and the flowers gathered in cincinni, where

the bracteole in the pedicel bears a large gland (Anderson, 1981; Davis and Anderson, 2010). However, as far as we are concerned, the anatomy of bracteole and leaf glands have not yet been comparatively described.

Nectaries were classified as floral (FN) and extrafloral nectaries (EFN) according their position (Caspary, 1848). In other context, Delpino (1873) recognized nuptial nectary based on the involvement of nectar with pollinators and extranuptial when the nectar is not consumed by pollinators. However, the term FN have been used as a synonymous with nuptial nectaries and EFN as a synonymous with extranuptial nectaries (Elias and Gelband, 1976; Nepi et al., 2012; Nicolson and Thornburg 2007). Such functional approach is not appropriate and might be misleading, especially for nectaries that clearly occur on floral parts, e.g. axis of the inflorescence, peduncle and pedicel, bract and bracteole or perianth parts (Schimid, 1988), but are not involved in pollination. Such nectaries are obviously FN according to their position, as those occur on the inflorescences of Malpighiaceae and they may not be involved in pollination.

The functional role of EFN has been the subject of an ongoing academic debate (Del-Claro et al., 2016). Studies demonstrated that EFNs are able to attract mainly ants that feed on nectar and protect plant against herbivores and pathogens (Bentley, 1977; Elias, 1980; González-Teuber et al., 2014; Keeler and Kaul, 1984; Pacini and Nicolson, 2007). However, the mutualistic relationships between ants and plants that bear EFNs have been experimentally tested and protective, neutral or even negative effects were demonstrated (Koptur, 1992; Alves-Silva et al., 2014; Assunção et al., 2014). For shedding light on this debate, it is crucial to review the classification of nectaries based on their position and establish a pattern that allow comparing similar nectaries, since the leaf nectaries may have differences in morphology and in the

secretion (Koptur, 1994). Such differences have been ignored in ecological studies that have used the term EFN as a synonym of extranuptial nectaries.

The present study aimed to describe and compare the morphoanatomy of both leaf and bracteolar glands of *Burdachia*, *Mcvaughia* and *Glandonia* addressing the questions that follows: Are there inter and intraspecific morphoanatomical variations on such glands? Is the chemical nature of the exudates secreted the same? Does the secretion produced by such glands attract visitors?

MATERIAL AND METHODS

All species of mcvaughioide clade were analyzed: *Burdachia duckei* Steyerm., *B. prismatocarpa* A. Juss., *B. sphaerocarpa* A. Juss., *Glandonia macrocarpa* Griseb., *G. prancei* W.R. Anderson and *G. williamsii* Steyerm from wet environments; *Mcvaughia bahiana* W.R. Anderson and *M. sergipana* Amorim & Almeida from dry environments (Table 1).

Burdachia and *Glandonia* are genera from Amazonia rainforest, where the species occur in wetlands floodplain, except *G. macrocarpa* that is typical of “Floresta de Terra Firme” (Table 1). Wetlands floodplain (Fig. 1A) environments are semi aquatic areas typical from the margins of the rivers (“Várzea” and “Igapó”), which in rainy season, present extensive flooded areas (Junk et al., 2011). The upland forest or “Terra Firme” (Fig. 1B) is a complex of environments that include the lowland (called “Baixio”), which occurs closed to streams (called “Igarapés”) and is subject to temporary flooding less intense than the wetlands floodplain (Hopkins, 2005). *Mcvaughia* is typical of dry environments of different Brazilian biomes and represent an interesting geographic disjunction (Table 1). *Mcvaughia bahiana* is endemic to dry and open habitats and occurs on sandy soils vegetation of the *Caatinga* biome

(dryland) in Bahia State (Brazil). *M. sergipana* was recently described and is an endemic species to *Restinga* vegetation (sandy coastal plains) (Fig. 1C) of Atlantic Forest biome and is restricted to a little area in Sergipe State (Brazil).

Fresh samples of leaves and inflorescences at different development stages were collected in the field in different seasons (Fig. 1A-C) in the states of Amazonas, Bahia and Sergipe (Brazil). Diurnal field observations and the behavior of visitors and consumers of the secretion produced by floral and leaf glands were recorded. The natural flooding made the field observations at Wetlands floodplain easier, as the boat would float at the level of the the canopy. Glucose concentration was obtained in the field, using the urinalysis reagent strips (Insight®, Acon Laboratories, San Diego, USA). Voucher material was deposited at the herbarium VIC of the Universidade Federal de Viçosa-UFV. Julio Chaul of Communities Ecology Laboratory-UFV identified the visitor ants.

The field samples were fixed in FAA (formaldehyde, acetic acid and 50% ethanol; 1:1:18 by volume) for 48 h, NBF (neutral buffer formalin) (Johansen, 1940) or in Karnovsky solution (Karnovsky, 1965). Some samples were fixed in FSF (ferrous sulphate formalin) to detect phenolic compounds (Johansen, 1940). The samples fixed in FAA and FSF were stored in 70% ethanol, while the ones fixed in NBF and Karnovsky were kept in such fixates until analysis. Samples of herbarium material from the “Instituto Nacional de Pesquisas da Amazônia”-INPA were also used (Appendix 1). Such samples were rehydrated, treated with 2% potassium hydroxide for 2 h, dehydrated in an ethanol series and stored in 70% ethanol (Smith and Smith, 1942) for anatomical analysis at the “Plant Anatomy Laboratory”-UFV.

Samples of both herbarium and field-collected material were embedded in methacrylate resin (Histo-resin Leica; Heidelberg, Germany) in accordance to the

manufacturer's recommendation. Cross and longitudinal sections 4µm thick were made in an automatic rotary microtome (Leica RM2155, Deerfield, USA). The sections obtained were stained with toluidine blue at pH 4.7 (O'Brien et al., 1964).

Section of FAA fixed samples obtained in table microtome (LPC, Rolemberg e Bhering LTDA, Belo Horizonte, Brasil) were submitted to the following histochemical tests: Xylidine Ponceau (Clark, 1981) and Coomassie blue (Fisher, 1968) for total proteins; periodic acid–Schiff reagent (McManus, 1948) for total polysaccharides and ruthenium red (Johansen, 1940) for mucilage and pectin. Sections of NBF were submitted to histochemical tests for lipid components, using Sudan red for total lipid (Pearse, 1980) and NADI reagent for oil/resin (David and Carde, 1964).

For detecting the presence of nectaries in frontal view, samples of leaves and bracteoles from field-collected were also cleared with 10% sodium hydroxide and 20% hypochlorite solutions, stained with 50% ethanol-diluted fuchsin (Vasco et al., 2014 modified), and mounted in glycerinated gelatin (Johansen, 1940).

The analyses and photographic documentation were carried out in a light microscope (Olympus® AX70TRF) equipped with a digital camera (AxioCam HRc; Zeiss®, Göttingen, Germany). For the scanning electron microscopy analysis samples fixed were dehydrated, subjected to critical point drying with CO₂ (CPD 030, Bal-Tec®, Balzers, Liechtenstein), fixed on stubs with aid of carbon conductive tapes and sputter coated with gold (SCD 050, Bal-tec®, Balzers, Liechtenstein). Examinations and image captures were conducted using a LEO 1430VP (Zeiss®, Cambridge, UK) at the “Center for Microscopy and Microanalysis”-UFV.

We adopted the concept of (Schimid, 1988) in such a way to consider the leaf nectary as EFN and the bracteolar as FN. The anatomical patterns were recognized as **short-stalked**, when there was present a non-secretory stalk; **subsessile**, when the non-

secretory is not a typical stalk; **sessile**, when the secretory tissue are above the level of the non-secretory epidermis surrounding the gland; and **immersed** when are below.

RESULTS

The leaves and bracteoles glands are nectaries, since their secretion reacted with the urinalysis strips demonstrating the presence of glucose (Table 1). The morphoanatomical characters of both glands in all species of the mcvaughoid clade (*Mcvaughia*, *Burdachia* and *Glandonia*) are summarized at the table 2.

Morphoanatomical description of leaf nectaries

Nectaries randomly distributed throughout the leaves (Fig. 2A-D) were observed in all species (Table 2). Acropetiolar nectaries were observed only in *B. duckei* and *B. sphaerocarpa* (Fig 2M). Basilaminar nectaries were found in pairs (Fig. 2A, B, D-L) while the laminar nectaries (Fig. 2O-R) are randomly scattered through the blade and are usually smaller. In *G. macrocarpa* and *G. prancei* they are inconspicuous (Fig. 2B), in *Burdachia* they are along the middle vein closed to the branching of secondary veins (Fig. 2C) (Table 2), while those concentrated at the apex of the leaf blade only in *M. bahiana* (Fig. 2D, R).

The pairs of basilaminar and acropetiolar nectaries varied. For most species, only one pair was found (Fig. 2D-G, J, M); in *G. macrocarpa* and *G. williamsii* 1-2 pairs were observed (Fig. 2I, L) while in *M. sergipana* 1-4 pairs (Fig. 2H). Basilaminar nectaries presented convex surface in all species, except in *Glandonia williamsii* that is flat (Fig. 2L). Verrucoid-shaped nectary showed the outline elliptical (Fig. 2F) or orbicular (Fig. 2H) (Table 2), laminar nectaries were orbicular, flat to slightly convex.

The predominant color of leaf nectaries was bright yellow (Fig. 2A, B, F-I, M), except in *G. prancei* which was whitish (Fig. 2J) and *G. williamsii* was greenish (Fig.

2L). In *B. prismatocarpa* and *B. duckei*, the nectaries of older leaves showed pink coloration instead (Fig. 2E). In *Mcvaughia*, secretion drops were common in basilaminar nectaries of young and mature leaves (Fig. 2H), while the secretion in *Burdachia* was observed only in young leaves during the blooming season (Fig. 2F), but this producing was rarely observed. In addition, exudates in the leaf nectaries of *G. macrocarpa* and *G. prancei* were not observed in field work. In the blooming season, the leaf nectaries presented superficial damages at the secretory tissues.

In *M. bahiana* (Fig. 3E-G) the basilaminar nectaries are short-stalked while in *M. sergipana* (Fig. 3H-J) they are immersed, sessile in *Burdachia* the (Fig. 3L-N) and immersed in *Glandonia* (Fig. 3O-Q). The stalked and sessile nectaries were vascularized with abundant phloem, in comparison to the xylem cells, which reached the subepidermal layers of secretory parenchyma (Fig. 3J, N). The secretory epidermis is arranged in a palisade, which cells present densely stained cytoplasm, conspicuous nucleus and are covered by a thick cuticle (Fig. 3G, J, N, Q). The nectary parenchyma had about five layers of thin-walled cells with densely stained cytoplasm and conspicuous nucleus, or vacuolized cytoplasm, stored druse crystals (Fig. 3G, J) or phenolic compounds (Fig. 3G, J, N, Q). In *Glandonia* the subcuticular space were rarely observed (Fig 3G, J) due to the presence of damage in senescent stages.

The laminar nectaries were observed closed to vascularization (Fig. 3A-C) being an active structure, where drop of secretion was recorded (Fig. 2P, Q), except in *Glandonia* species. In all species they are immersed and specially in *M. sergipana* the crystals are abundant (Fig. 3C). Damaged in the secretory tissues were frequently observed at the leaf nectaries (Fig. 3D).

Morphoanatomical description of bracteole nectaries

At the base of floral pedicel in the inflorescences of *Burdachia*, *Glandonia* and *Mcvaughia* occur bracteoles, which bear only one medially positioned conspicuous nectary (Fig. 4A-D). The color of such nectaries may vary as follows: in the buds of *M. bahiana*, *M. sergipana* and *B. sphaerocarpa* showed greenish coloration (Fig. 4A, C, E-G) and yellow at flowers and fruits (Fig. 4I), while in *B. duckei* are yellow in fruiting period (Fig. 4D, L) and *B. duckei* e *B. prismatocarpa* are rose in blooming stages and yellow in fruit stages (Fig. 4I). In *Glandonia* the color of nectary did not change being white in *G. prancei* (Fig. 4B) and creamy-white in *G. macrocarpa* and *G. williamsii*. The bracteolar nectaries were active in both blooming (Fig. 4A-C, E-I) and being persistent in fruiting period (Fig. 4D, J, L). The activity of such nectaries are precocious, even in very young stages of development were active (Fig. 4F, G). In field works, variation of the volume of secretion was observed (Fig. 4M, N).

The bracteolar nectaries are hemispheric-globose in *Burdachia* and *Mcvaughia* (Fig. 5A-L) while in *Glandonia* are discoid (Fig. 5M-U). The surface is convex in all species, except in *G. prancei* that is flatted (Fig. 5O, P). In *Mcvaughia* (Fig. 5A-F) and *Glandonia* (Fig. 5M-P) are subsessiles and in *Burdachia* they are sessiles (Fig. 5G-L).

The bracteolar nectaries analyzed exhibited the same anatomical arrangement of the leaf nectaries, composed of a palisade secretory epidermis and nectary parenchyma vascularized (Fig. 5C, E, F, P, Q-S), with abundance of phloematic cells (Fig. 5S). The subcuticular spaces observed in bracteolar nectaries were most developed in relation to the leaf nectaries (Fig. 5C, J, S-U). Although the drops of secretion were easily observed, damages were rarely recorded in the cuticle surface. The secretory epidermis and the nectary parenchyma had cells filled with phenolic compounds (Fig. 5C, F, Q-S) and druse crystals (Fig. 5F, J, R) are restricted to secretory parenchyma.

Histochemical tests of leaf and bracteole nectaries

The results of the histochemical tests were similar between leaf and bracteolar nectaries, being summarized in Table 3 and illustrated in Figures 6 and 7. The PAS reaction indicated the presence of polysaccharides in the cytoplasm of nectariferous parenchyma cells and the secretory epidermis of both leaf and bracteolar nectaries, respectively (Fig. 6A-D; 7A-D). The Sudan red B test detected lipids in the secretory epidermis and nectariferous parenchyma (Fig. 6E-H, 7E-H), and the NADI reaction demonstrated the oilresin nature of the secretion (Fig. 6I-M; 7I-L). The epidermal and subepidermal layers of the bracteolar nectaries in *Mcvaughia* and *G. prancei* has thickening of the anticlinal wall of the cells, apparently forming the boundaries between the secretory parenchyma and the palisade epidermis (Fig. 7M, N). Such characters were observed only in histochemical tests.

In the secretory epidermis and in the secretory parenchyma of both nectaries, phenolic compounds (Fig. 6O, P, 7P, Q) and proteins (Fig. 6N; 7O) were detected. Phenolic compounds were detected only in the subcuticular space on the leaf and bracteolar nectaries of *B. duckei* and *M. bahiana* (Fig. 6C, D; 7D). In the cells of the fundamental parenchyma of the median vein and of the mesophyll starch grains were detected by the lugol reaction. While in the bractoles the starch grains were observed only in the cells of the fundamental parenchyma of *Mcvaughia* or in the secretory parenchyma of *G. williamsii*. In the other species, the lugol reaction was positive around the vascular bundle in the floral pedicel. Tests for the detection of alkaloids and mucilage were negative.

Chemistry of nectar, nectar consumers and ecological considerations

Although glucose has been detected in the exudate from leaf and bracteolar nectaries, the concentration was higher in the bracteolar one (Table 1). Ants

Crematogaster sp. (Fig. 2G) and *Dolichoderus quadridenticulatus* (Fig. 2N) were observed patrolling the leaves of *Burdachia* when plants are not in blooming, while *Dorymyrmex* sp. was recorded in *Mcvaughia* leaves (Fig. 2R). In *Glandonia* ants were seldom accounted (Table 4). Ants patrol all parts of leaf, including the apex of *M. bahiana*, and the feeding behavior was recorded.

In the blooming season, eight different ants species were identified visiting the axis of inflorescence (Fig. 8) (Table 4). *Crematogaster* sp. in *Glandonia* (Fig. 8A) and *Burdachia*. Others ants species founded in *Burdachia*: *Azteca* sp. (Fig. 8E, F), *Camponotus crassus* (subgênero *Myrmobrachys*), *D. bidens* (Fig. 8B), *Dolichoderus quadridenticulatus* (Fig. 8C-D, G), *D. debilis* and *D. bispinosus*. *Pseudomyrmex gracilis* and *Camponotus* sp. (Fig. 8 I-N) was recorded only in *Mcvaughia*. When the photographic apparatus or some instrument was approaching, it was common the attack positioning opening the mandibles (Fig. 8F).

The ants foraged the entire axis of the inflorescence, stooping for feeding on nectar available in the bracteolar nectaries (Fig. 8D, E, G, J). This behavior was repeated several times, following the same pattern. During the feeding of the nectar, it was observed that the ants slide the palpus on the nectary surface, collecting the secretion without damaged them (Fig. 8D). Immediately the ant visited the nectary of the neighboring bracteole, and so on, until it return to the recently visited nectaries, which usually provided a new drop of secretion (Fig. 4 I-N). The record of damages in the bracteolar nectaries was rare, only the consuming of secretion by ants were observed, and in flower whorls, ants were not observed.

Adults and larvae of phytophagous beetles of the genus *Anthonomus* (Curculionidae) (Fig. 8H) were observed on inflorescences of *Burdachia*, *Glandonia* species and *M. bahiana*. Floral buds were found parasitized by *Anthonomus* larvae,

which generally damaged reproductive structures. The most severe damage of such parasit was observed in *Burdachia*, which had many adults and larvae. In *M. sergipana* no adults or larvae were observed. Occasional visits of Vespas (Vespidae) were recorded on the inflorescences of *Burdachia*, which flew close to the flowers and eventually landed on buds intact or parasitized, no contacting the nectaries.

DISCUSSION

Morphoanatomical variations of leaf and bracteolar nectaries

The distribution of nectaries found in mcvaughioide species seems to be related to the size of leaf and bracteolar blade. In Malpighiaceae number and size of leaf nectaries can be inversely related, when numerous are small and when few are developed (Metcalf and Chalk, 1965; Solereder, 1908).

Basilaminar and laminar nectaries exhibited anatomical structure similar as reported for nectaries of Malpighiales as Euphorbiaceae (Vitarelli et al., 2015), Chrysobalanaceae and Malpighiaceae species from “Cerrado” (Machado et al., 2008) as *Diplopterys pubipetala* (Possobom et al., 2010) and *Banisteriopsis* (Araújo and Meira, 2016). The main difference among leaf nectaries were the shape and the presence of a stalk (Table 1). Although the nectaries of *Banisteriopsis* (Araújo and Meira, 2016) were considered sessile and subsessile, they should be considered as those of *M. bahiana*. The bracteolar nectaries studied herein shared more morpho-anatomical similarities among themselves, than in relation to leaf nectaries. Curiously the bracteolar nectaries are similar to the calyx glands reported at flowers of mcvaughioide species (Silva et al. unpubl. data, 2017 in CAP I)

Laminar nectaries of the *Burdachia*, *Glandonia* and *M. sergipana* were reported in Bignoniaceae (Elias and Gelband, 1976; Stephenson, 1982),

Euphorbiaceae (Coutinho et al., 2010) and Meliaceae (Morellato and Oliveira, 1994; Paiva et al., 2007). This supply of nectar in the leaves can be an efficient strategy against herbivores (Oliveira and Freitas, 2004). The restricted distribution of nectaries in *M. bahiana* that is endemic to Caatinga, may be related with leaf deciduousness. The reduction of the life time and size of the leaf can be advantageous in resource saving in dry periods typical of the Caatinga. However Nogueira et al. (2012) suggest relation of lower density of leaf nectaries in dry environments. Thus future studies are necessary to clarify the influence of environment in nectaries expression.

The reduction of the volume of secretion observed during field observations in the bracteolar nectaries (Fig 4L, M) may be a signal of reabsorption of the nectar components if accompanied by a decrease in sugar concentration (Nepi et al., 2001). Although is difficult to prove in the field, considering the high energy cost of nectar (Southwick, 1984), reabsorption would be advantageous in the species studied, since the nectaries are numerous in the inflorescences and the secretory activity is precocious and persistent. Thus, reuse the uncollected nectar would be advantageous as suggested for floral nectaries (Southwick, 1984; Stpiczyska, 2003). The mcvaughioide species show a secretory activity of the bracteolar nectaries persistent in the fruiting period, what according to Búrquez and Corbet (1991) is common in floral nectaries. Since no ruptures or pores were observed in the cuticle, it is possible that the elimination occurs through channels or cuticle permeability (Ascensão et al., 1997; Coutinho et al., 2012; Koteyeva, 2005; Miller, 1985; Stpiczynska, 2003).

In the bracteolar nectaries, a subepidermal layer of cells with Caspary's bands was a peculiarity recorded in the mcvaughioide species with upright inflorescences, being an unpublished data. Thickness of nectar-secreting trichomes were observed in Acanthaceae (McDade and Turner, 1997) and Fabaceae (Paiva, 2009); and in leaf

nectaries of Meliaceae (Lersten and Rugenstein, 1982; Paiva et al., 2007). Although Paiva and Machado (2006) classified these layer as an endoderm, they did not report Caspary band. This layer may acts to block the apoplastic transport in bracteolar nectary, since the blocking of such transport has been associated to Caspary's band (Luttge, 1971), contributing to create secretory accumulating pressure (Paiva, 2016).

Visitors and secretion variations of leaf and bracteolar nectaries

The most common genus recorded in this study was *Dolichoderus*. *Dolichoderus quadridenticulata*, *D. debilis*, *D. bidens* and *D. bispinosus* are commonly found in lowland rain forest and flooded forests, where they forage nectaries (Bentley, 1977; Lattke, 1986; Mackay, 1993). In Malpighiaceae ants species were documented in *Diplopterys pubipetala* (Possobom et al., 2010), *Banisteriopsis malifolia*, *Peixotoa tomentosa*, *Byrsonima intermedia* and *Heteropterys pteropetala* (Torezan-Silingardi, 2007), where *Camponotus* sp. foraging the leaf nectaries, while *Pachycondyla* sp., *Pseudomyrmex* sp., *Ectatomma* sp. and *Cephalotes* sp. patrolling the inflorescences (Torezan-Silingardi, 2007). *Camponotus* ants play an protection role of the reproductive organs in *Byrsonima crassifolia* (Fernandes et al., 2005).

Bettles from *Anthonomus* genus observed herein were also recorded as a floral parasit in *Banisteriopsis*, *Byrsonima* and *Heteropterys* from “Cerrado” (Alves-Silva et al., 2013; Torezan-Silingardi, 2007). Alves-Silva et al. (2013), proposed that wasps may protect flowers against to curculionidae, which could be happen in the species studied, since in *Burdachia*, wasps were observed visiting the inflorescence.

Some ant species observed in field works are aggressive, and nectar distribution in leaf blade could stimulate patrol throughout the organ as reported in Bignoniaceae species (Elias and Gelband, 1976; Stephenson, 1982), Meliaceae (Morellato and Oliveira, 1994; Paiva et al., 2007) and Euphorbiaceae (Coutinho et al.,

2010). In the Amazonian species studied, for long periods when plants remain partially submerged, several ants can shelter and use the nectar produced in the leaves and inflorescences as an alimentary resource. In deciduousness specie *M. bahiana*, the defense mediated by consumers of nectar is advantageous when the leaves are present. These results are in agreement with the proposition that nectar is an important resource for ants in arid environments and that the association with plants that bear nectaries is crucial for the survival of desert ant communities (Aranda-Rickert and Marazii, 2014).

As was reported above for leaf nectaries, bracteolar nectary at each pedicel seems to encourage the patrol throughout the inflorescence. According to Torezan-Silingardi (2011) the inflorescence patrol can improve plant performance by ensuring the morphofunctional integrity of the buds, attractive to florivorous insects cause the high-assimilated flow and low structural resistance. Ants visiting reproductive organs were observed in mcvaughnoid species but the damages were rare. According to the flower-distraction hypothesis (Kerner, 1878), nectaries distract the interest by flowers and when offering nectar could discourage the consumption of floral parts (Del-Claro et al., 2016). In spite of this advantage in concentrating the ant activity on the leaves (Asunção et al., 2014; Koptur, 1994; Ness, 2006), the patrol observed in the species studied was preferential in the inflorescences.

In the species studied, the higher concentration of glucose in the nectar bracteolar is a parameter that may be influencing the differential behavior of ants on the inflorescences. McKey (1979) suggest that the functional value and vulnerability of the organs justify the energy expent in their defense. Thus, the composition of nectar may vary according to the interactions between plant and animal demands (Faegri and Van der Pijl, 1976; González-Teuber and Heil, 2009; Nicolson and Thornburg, 2007). Additionally the quality and volume of the nectar in the bracteole result in performance

benefit in attracting consumers to the inflorescence, since ants prefer more concentrated solutions in sugar (Sudd and Sudd, 1985) and are attracted by nectaries that produce more nectar (Baker-Méio and Marquis, 2012). In addition, Alves-Silva and Del-Claro (2014) reported that the increase in nectar quality is associated with ants relations, where the sugar concentration is positively related to the abundance of ants in the leaf nectaries, reflecting lower rates of herbivory. Such results reinforce the significance to classify bracteolar nectaries with nectaries on flower.

The preference of ants for sugar-amino acid solutions is already know (Lanza, 1991). The proteins detected in histochemistry of leaf and bracteolar nectaries in the species studied, could enrich the nutritional value of nectar and strengthen ecological relationships with consumers as reported in other studies (Bentley, 1977; Elias, 1983; González-Teuber and Heil, 2009; Heil, 2011; Ness et al., 2009; Roshchina and Roshchina 1993). In addition, proteins of the nectar can inhibit the growth of microorganisms (Park and Thornburg, 2009). Although comparative studies in the same species are rare, (Baker et al., 1978; Blüthgen et al., 2004; Koptur, 1994) reported differences in amino acid and sugar composition between extrafloral and floral nectaries. Despite the similar histochemistry observed between the leaf and bracteolar nectaries studied, the hypothesis of different amino acids rates can not be ruled out, which would be an additional evidence that the bracteolar nectaries are floral.

The Sudan tests carried out in the leaf nectaries studied, as well as in *Galphimia brasiliensis* (Castro et al., 2001), prove the lipid portion as compound of a mixed secretion. Ultrastructural data of leaf nectary from *Diplopterys pubipetala* (Possobom et al., 2010) show mixed nature of secretion, although in histochemistry tests only hydrophilic components were detected. The same occurred in *Banisteriopsis* (Araújo and Meira, 2016), which not all histochemical tests were able to detect the lipids. Such

lipids can provide energy to nectar consumers (Real, 1983) and play an important role in the ants diet (Carroll and Janzen, 1973).

The morphology and color of nectaries have been identified as signaling characteristics for visitors (Konarska, 2011; Koptur, 1992). In addition, the oil-resin mixture detected by NADI in the leaf and bracteolar nectaries studied could act in the attraction of herbivorous predators and deterrents against herbivores and pathogens (Langenhein, 1994). Since nectar can emit chemical signals (Nicolson and Thornburg, 2007), the emission of volatiles could explain the attraction of ants in *M. bahiana*, whose leaves are smaller and the nectaries are restricted to the base and apex of blade.

The phenolic compounds in the nectaries studied, as well reported in *D. pubipetala* (Possobom et al., 2010), are frequently associated with protection against herbivores and pathogens (Nicolson and Thornburg, 2007; Subramanian et al., 1990). In some species of mcvaughioide they was observed in the subcuticular space. that can make the nectar toxic and repel some visitors (Hagler and Buchmann, 1993).

In the mcvaughioide species, leaf and bracteolar nectaries attract ants that consume their secretion. According to (Bentley, 1977) and (Koptur, 1992) the mutualism between ants and nectars acts positively on the fitness of the plant and studies have shown that the florivory increases after exclusion of the ants in species with leaf nectaries (Del-Claro 2010, Vesprini et al., 2003). In Orchidaceae, the visit of ants to nectaries of the floral pedicel decreased the herbivory, without pollination influence, increasing the productivity (Almeida and Figueiredo, 2003). Thus, ants that forage in nectaries close to the flowers can extend their protection to the reproductive structures (Del-Claro and Marquis, 2015; Rico-Gray, 1989).

Experimental studies are necessary to test the efficiency of ants against different herbivory in the mcvaughioide species. (Asunção et al., 2014) reported in

Malpighiaceae, a negative effect resulting from the presence of ants in the inflorescence, which discourages pollinators. In addition, ants may not be effective guardians against some floral herbivores (Alves-Silva et al., 2014). However, even in the face of negative effects, only further studies can explain what extent interactions with ants would be shaping different patterns in plant populations, regardless of location and environmental conditions (Del-Claro et al., 2016; Thompson 1994)

The morphoanatomy conserved of the bracteolar nectaries may be related to the standard behavior of the ants nectar consumers, since the morphoanatomy of nectaries may reflect ecological interactions (Baker and Baker, 1990; Elias, 1983; Roshchina and Roshchina, 1993). While nectaries are highly labile and arise or are lost easily throughout evolution (Heil et al., 2015), on the other hand, the calyx glands of Neotropical Malpighiaceae have been preserved for millions of years (Davis et al., 2014) due to highly specialized pollinators pressures. Thus, it is possible that the nectaries under different trophic levels pressure. However, such relationships are poorly understood in complex multitrophic systems, requiring further studies.

CONCLUSIONS

The morphoanatomical patterns of leaf shows differences in the mcvaughioide species, while the bracteolar nectaries were more conserved. Both structures presented similar histochemical results, except for the higher concentration of glucose and persistent secretory activity of the bracteolar nectaries. The ants have visited both nectaries, however, apparently the visit into the inflorescences is preferential. Thus, the morphological and histochemical results are not explained by the environment, but may be resulting from the selective pressure of the nectar consumers. The work determine differences between nectaries placed in not-reproductive floral whorls and contribute to the revision of the classification of nectaries.

Acknowledgment

This article is part of the doctoral thesis of the first author. The authors are grateful for the financial support granted by the “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq), “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior” (CAPES), “Fundação de Amparo à pesquisa do Estado de Minas Gerais” (FAPEMIG); to CNPq for the productivity grant granted to R.M.S.A. Meira. Thanks to the “Universidade Federal do Amazonas”-UFAM for the liberation of the training and the “Governo do Estado do Amazonas” through the “Fundação de Amparo à Pesquisa do Estado do Amazonas” (FAPEAM) for the scholarship (Programa RH-Interiorização) granted to I. R. e Silva. To the Brazilian herbariums INPA and VIC for the samples of the collections. We thank the “Núcleo de Microscopia e Microanálise” (NMM) and the “Laboratório de Anatomia Vegetal” of UFV. We are grateful to the “Instituto Chico Mendes de Conservação da Biodiversidade” (ICMBio), the “Centro Estadual de Unidades de Conservação” of Amazonas (CEUC), the “Instituto Piagaçú-Purus” (IPi) and the “Instituto Nacional de Pesquisas da Amazônia” (INPA) for licensing and support in field works. Thanks to Brazilian Army, the “5º Batalhão de Infantaria de Selva Batalhão Forte São Gabriel” and the “1º Pelotão Especial de Fronteira Yauareté” for the great assistance in the expeditions in remote areas of the Brazilian Amazon.

References

- Almeida, A.M., Figueiredo, R.A., 2003. Ants visit nectaries of *Epidendrum denticulatum* (orchidaceae) in a brazilian rainforest: effects on herbivory and pollination. *Braz. J. Biol.* 63(4), 551-558.
- Alves-Silva, E., Bachtold, A., Barônio, G.J., Torezan-Silingardi, H.M., Del-Claro, K., 2014. Ant–herbivore interactions in an extrafloral nectaried plant: are ants good plant guards against curculionid beetles? *Journal of Natural History.* 49, 841-851.
- Alves-Silva, E., Barônio, G.J., Torezan-Silingardi, H.M. & Del-Claro, K., 2013. Foraging behavior of *Brachygastra lecheguana* (Hymenoptera: Vespidae) on

- Banisteriopsis malifolia* (Malpighiaceae): Extrafloral nectar consumption and herbivore predation in a tending ant system. *Entomological Science*. 16, 162-169.
- Alves-Silva, E., Del-Claro, K., 2014. Fire triggers the activity of extrafloral nectaries, but ants fail to protect the plant against herbivores in a neotropical savanna. *Arthropod Plant Interactions*. 8(3), 233-240.
- Amorim, A.M., Almeida, R.F., 2015. An unexpected *Mcvaughia* (Malpighiaceae) species from sandy coastal plains in northeastern Brazil. *Syst. Bot.* 40, 535-538.
- Anderson, W.R., 1979a. Floral conservation in Neotropical Malpighiaceae. *Biotropica*. 11, 219-223.
- Anderson, W.R., 1979b. *Mcvaughia*, a new genus of Malpighiaceae from Brazil. *Taxon*. 28, 157-161.
- Anderson, W.R., 1981. Malpighiaceae. The Botany of the Guayana Highland-Part XI. *Memorirs of the New York Botanical Garden*. 32, 137-147.
- Anderson, W.R., 1990. The origin of the Malpighiaceae-The evidence from morphology. *Memoirs of the New York Botanical Garden*. 64, 210-224.
- Aranda-Rickert, A., Diez, P., Marazzi, B., 2014. Extrafloral nectar fuels ant life in deserts. *AoB*. 6 (68),10-1093
- Araújo, J.S., Azevedo, A.A., Silva, L.C., Meira, R.M.S.A., 2010. Leaf anatomy as an additional taxonomy tool for 16 species of Malpighiaceae found in the Cerrado area (Brazil). *Plant Systematic and Evolution*. 286, 117-131.
- Araújo, J.S., Meira, R.M.S.A., 2016. Comparative anatomy of calyx and foliar glands of *Banisteriopsis* C. B. Rob. (Malpighiaceae). *Acta Botanica Brasilica*. 30, 112-123.
- Ascensão, L., Marques, N., Pais, M.S., 1997. Peltate glandular trichomes of *Leonotis leonurus* leaves: Ultrastructure and histochemical characterization of secretions. *Int. J. Plant Sci.* 158(3), 249-258.
- Assunção, M.A., Torezan-Silingardi, H.M., Del-Claro, K., 2014. Do ant visitors to extrafloral nectaries of plants repel pollinators and cause an indirect cost of mutualism? *Flora*. 209, 244-249.
- Baker, D.A., Hall, H.J., Thorpe, J.R., 1978. A study of extrafloral nectaries of *Ricinus communis*. *N. Phytol.* 81,129–137.
- Baker, H.G., Baker, I, 1990. The predictive value of nectar chemistry to the recognition of pollinator type. *Israel Journal of Botany*. 39, 157-166 .
- Baker-Méio, B., Marquis, R.J., 2012. Context-dependent benefits from ant–plant mutualism in three sympatric varieties of *Chamaecrista desvauxii*. *J. Ecol.* 100, 242-252.
- Bentley, B.L., 1977. Extra-floral nectaries and protection by pugnacious bodyguards. *Annual Review of Ecology and Systematics*. 8, 407-427.

- Bluthgen, N., Stork, N.E., Fiedler, K., 2004. Bottom-up control and co-occurrence in complex communities: honeydew and nectar determine a rainforest ant mosaic. *Oikos*. 106, 344-358.
- Búrquez, A., Corbet, S.A., 1991. Do flowers reabsorb nectar? *Functional Ecology*. 5, 369-379.
- Carroll, C.R., Janzen, D.H., 1973. Ecology of foraging by ants. *Annu Rev Ecol Syst.* 4, 231-257
- Caspary, R., 1848. De nectariis. In *Botanische Zeitung*. Elverfeld, Bonnae.
- Castro, M.A., Vega, A.S., Mulgura, M.E., 2001. Structure and ultrastructure of leaf and calyx glands in *Galphimia brasiliensis* (Malpighiaceae). *American Journal of Botany*. 88, 1935-1944.
- Clark, S. G., 1981. *Staining procedures* Williams & Wilkins, Baltimore.
- Coutinho, Í.A.C., Francino, D.M.T., Azevedo, A.A., 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.
- Coutinho, I.A.C., Valente, V.M.M., Meira, R.M.S.A., 2010. Ontogenetic, anatomical and histochemical study of the extrafloral nectaries of *Sapium biglandulosum* (Euphorbiaceae). *Australian Journal of Botany*. 58, 224-232.
- David, R., Carde, J.P., 1964. Coloration différentielle des inclusions lipidique et terpéniques des pseudophylles du Pin maritime au moyen du réactif Nadi. *Comptes Rendus Hebdomadaires dès Séances de l' Academie dès Sciences*. 258, 1338-1340.
- Davis, C.C., Anderson, W.R., 2010. A complete generic phylogeny of Malpighiaceae inferred from nucleotide sequence data and morphology. *American Journal of Botany*. 97, 2031-2048
- Davis, C.C., Schaefer, H., Xi, Z., Baum, D.A., Donoghue, M.J., Harmon L.J., 2014. Long-term morphological stasis maintained by a plant–pollinator mutualism. *Proceedings of the National Academy of Sciences*. 111, 5914 - 5919.
- 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.
- Del-Claro, K., Marquis, R.J., 2015. Ant species identity has a greater effect than fire on the outcome of an ant protection system in Brazilian Cerrado. *Biotropica*. 47,459-467.
- Del-Claro, k., Rico-Gray, V., Torezan-Silingardi, H.M., Alves-Silva, E., Fagundes, R., Lange, D., Dáttilo, W., Vilela, A.A., Aguirre, A., Rodriguez-Morales, D., 2016. Loss and gains in ant–plant interactions mediated by extrafloral nectar: fidelity, cheats, and lies. *Insectes Sociaux*. 63(2), 207-221.
- 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.

- Elias, T.S., 1980. Foliar nectaries of unusual structure in *Leonardoxa africana* (Leguminosae) an African obligate myrmecophyte. *American Journal of Botany*. 67, 423–425.
- Elias, T.S., 1983. Extrafloral nectaries: their structure and distribution. In: Bentley, B. & Elias, T. (Eds.), *The Biology of Nectaries*. Columbia University Press, New York, Pp. 174--203
- Elias, T.S., Gelband, H., 1976. Morphology and anatomy of floral and extrafloral nectaries in *Campsis* (Bignoniaceae). *American Journal of Botany*. 63, 1349-1353.
- Faegri, K., Van der Pijl, L., 1976. *The principles of pollination ecology*. Pergamon Press. Oxford.
- Fernandes; G.W., Fagundes, M., Greco, M.K.B., Barbeitos, M. S., Santos, J.C., 2015. Ants and their effects on an insect herbivore community associated with the inflorescences of *Byrsonima crassifolia* (Linnaeus) H.B.K. (Malpighiaceae). *Rev. Bras. de Entomo*. 49(2), 264-269.
- Fisher, D.B., 1968. Protein staining of ribboned epon sections for light microscopy. *Histochemistry and Cell Biology*. 16, 92 - 96.
- González-Teuber, M., Heil, M., 2009. The role of extrafloral nectar amino acids for the preferences of facultative and obligate ant mutualists. *J. Chem. Ecol.* 35, 459-68.
- González-Teuber, M., Kaltenpoth, M., Boland, W., 2014. Mutualistic ants as an indirect defence against leaf pathogens. *New Phytol.* 202, 640-50.
- Hagler, J.R., Buchmann, S.L., 1993. Honey bee (Hymenoptera: Apidae) foraging responses to phenolic-rich nectars. *Journal of the Kansas Entomological Society*. 66, 223-230.
- Heil, M., 2011. Nectar: generation, regulation and ecological functions. *Trends Plant Sci.* 16,191-200.
- Heil, M., 2015. Extrafloral nectar at the plant–insect interface: a spotlight on chemical ecology, phenotypic plasticity, and food webs. *Annu Rev Entomol.* 60,213-232.
- Hopkins, M.J.G., 2005. Flora da reserva ducke, Amazonas, Brasil. *Rodriguésia*. 56 (86), 9-25.
- Johansen, D.A., 1940. *Plant Microtechnique*. New York: Mc Graw-Hill Book Co. Inc.
- Junk, W.J., Piedade, M.T.F., Schöngart, J., Cohn-haft, M.A.J., Wittmann. F.K., 2011. A Classification of Major Naturally-Occurring Amazonian Lowland Wetlands. *Wetlands*. 31, 623-640.
- Karnovsky, M.J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. *Journal of Cell Biology*. 27, 137.
- Keeler, K.H., Kaul, R.B., 1984. Distribution of defense nectaries in *Ipomoea* (Convolvulaceae). *American Journal of Botany*. 71(10), 1364-1372.
- Kerner, A., 1878. *Flowers and their Unbidden Guests*. London.

- Konarska, A., 2011. Flower nectary structure in *Cornus alba* L. *Plant Syst. Evol.* 291, 1-6.
- Koptur, S., 1992. Extrafloral nectary mediated interactions between insects and plants. In: Bernays, E., *Insect-plant interactions*, CRC Press, Boca Raton, Florida, pp. 81-129.
- Koptur, S., 1994. Floral and Extrafloral nectars of Costa Rican *Inga* trees: a comparison of their constituents and composition. *Biotropica.* 26(1), 276-284.
- Koteyeva, N.K., 2005. A novel structural type of plant cuticle. *Dokl. Biol. Sci.* 403, 283-285.
- Langenhein, J.K., 1994. Higher plant terpenoids: a phytocentric overview of their ecological roles. *J. chem. Ecol.* 20(6), 1223-1282.
- Lanza, J., 1991. Response of fire ants (Formicidae: *Solenopsis invicta* and *S. geminata*) to artificial nectars with amino acids. *Ecol. Entomol.* 16, 203-210.
- Lattke, J.E., 1987 [1986]. Notes on the ant genus *Hypoclinea* Mayr, with descriptions of three new species (Hymenoptera: Formicidae). *Rev. Biol. Trop.* 34, 259-265.
- Lersten, N.R., Rugenstein, S.R., 1982 . Foliar nectaries in mahogany (*Swietenia* Jacq.). *Annals of Botany.* 49, 397-401.
- Luttge, U., 1971. Structure and function of plant glands. *Annual Review of Plant Physiology.* 22, 23-44.
- Machado, S.R., Morellato, L.P.C., Sajo, M.G., Oliveira, P.S., 2008. Morphological patterns of extrafloral nectaries in woody plant species of the Brazilian cerrado. *Plant Biology.* 10, 660-673.
- MacKay, W.P., 1993. A review of the New World ants of the genus *Dolichoderus* (Hymenoptera: Formicidae). *Sociobiology.* 22, 1-148.
- McDade, L.A., Turner, M.D., 1997. Structure and development of bracteal nectary glands in *Aphelandra* (Acanthaceae). *American Journal of Botany.* 84, 1-15.
- McKey, D., 1979. The distribution of secondary compounds within plants. In In: Rosenthal, G.A. & Janzen, D.H. (Eds.), *Herbivores: Their Interactions with Secondary Plant Metabolites*, Academic. Press, New York, pp. 55-133.
- McManus, J.F.A. 1948. Histological and histochemical uses of periodic acid. *Stain Technology.* 23, 99-108.
- Metcalf, C.R., Chalk, L., 1965. *Anatomy of the dicotyledons: leaves stem and wood in relation to taxonomy with notes on economic uses.* Clarendon Press, Oxford.
- Miller, R.H., 1985. The prevalence of pores and canals in leaf cuticular membranes. *Annals of Botany.* 55, 459-471.
- Morellato, L.P.C., Oliveira, P.S., 1994. Extrafloral nectaries in the tropical tree *Guarea macrophylla* (Meliaceae). *Canadian Journal of Botany.* 72, 157-160.

- Nepi, M., Guarnieri, M., Pacini, E., 2001. Nectar secretion, resorption, and sugar composition in male and female flowers of *Cucurbita pepo*. *Int J Plant Sci.* 162, 353-358
- Nepi, M., Soligo, C., Nocentini, D., Abate, M., Guarnieri, M., 2012. Amino acids and protein profile in floral nectar: much more than a simple reward. *Flora.* 207, 475-81.
- Ness, J.H., 2006. A mutualism's indirect costs: the most aggressive plant bodyguards also deter pollinators. *Oikos.* 113, 506-514.
- Ness, J.H., Morris, W.F., Bronstein, J.L., 2009 For ant-protected plants, the best defense is a hungry offense. *Ecology.* 90, 2823-2831
- Nicolson, S.W., Thornburg, R.W., 2007. Nectar chemistry. In: Nicolson, S., Nepi, M., Pacini, E. (Eds.), *Nectaries and nectar.* Springer, Dordrecht Netherlands, pp. 215-63.
- Nogueira, A., Rey, P.J., Lohmann, L.G., 2012. Evolution of extrafloral nectaries: adaptive process and selective regime changes from forest to savanna. *Journal of Evolutionary Biology.* 25, 2325-2340.
- O'Brien, T.P., Feder, N., McCully, M.E., 1964. Polychromatic staining of plant cell walls by toluidine blue O. *Protoplasma* 59, 368-373.
- Oliveira, P.S., Freitas, A.V.L., 2004. Ant-plant-herbivore interactions in the neotropical cerrado savanna. *Naturwissenschaften.* 91, 557-570.
- Pacini, E., Nepi, M., 2007. Nectar production and presentation. In: Nicolson, S., Nepi, M., Pacini, E. (Eds.), *Nectaries and nectar.* Springer, Dordrecht Netherlands, pp. 167-214.
- Pacini, E., Nicolson, S.W., 2007. Introduction. In: Nicolson, S., Nepi, M., Pacini, E. (Eds.), *Nectaries and nectar.* Springer, Dordrecht Netherlands, pp. 1-18.
- Paiva, E.A.S., 2009. Ultrastructure and post-floral secretion of the pericarpial nectaries of *Erythrina speciosa* (Fabaceae). *Annals of Botany.* 104, 937-944.
- Paiva, E.A.S., 2016. How do secretory products cross the plant cell wall to be released? A new hypothesis involving cyclic mechanical actions of the protoplast. *Annals of Botany.* 117, 533-540.
- Paiva, E.A.S., Machado, S.R., 2006. Ontogênese, anatomia e ultra-estrutura dos nectários extraflorais de *Hymenaea stigonocarpa* (Fabaceae-Caesalpinioideae). *Acta Bot. Bras.* 20, 471-482.
- Paiva, E.A.S., Bueno, R.A., Delgado, M.N., 2007. Distribution and structural aspects of extrafloral nectaries in *Cedrela fissilis* (Meliaceae). *Flora.* 202, 455-461.
- Park, S., Thornburg, R.W., 2009. Biochemistry of nectar proteins. *J. Plant Biol.* 52, 27-34.
- Pearse, A.G.E., 1980. *Histochemistry: theoretical and applied*, fourth ed. Churchill Livingstone, Edinburgh.
- Possobom, C.C.F., Guimarães, E., Machado, S.R., 2010. Leaf glands act as nectaries in *Diplopterys pubipetala* (Malpighiaceae). *Plant Biology.* 12, 863-870.

- Possobom, C.C.F., Guimarães, E., Machado, S.R., 2015. Structure and secretion mechanisms of floral glands in *Diplopterys pubipetala* (Malpighiaceae) and a neotropical species. *Flora*. 211, 36 - 39.
- Real L. 1983. *Pollination Biology*. London, Academic Press.
- Ren, M., Zhong, Y., Song., X., 2013. Mirror-image flowers without buzz pollination in the Asian endemic *Hiptage benghalensis* (Malpighiaceae). *Botanical Journal of the Linnean Society*. 173, 764-774.
- Rico-Gray, V., 1989. The importance of floral and circum-floral nectar to ants inhabiting dry tropical lowlands. *Biol J Linn Soc*. 38, 173-181.
- Roshchina, V.V., Roshchina, V.D., 1993. *The excretory function of higher plants*. Springer-Verlag, Berlin, Germany.
- Schmid, R., 1988. Reproductive versus extra-reproductives nectaries – historical perspective and terminological recommendations. *Bot. Rev.* 54, 179-232.
- Smith, F.H., Smith, E.C., 1942. Anatomy of the inferior ovary of *Darbya*. *American Journal of Botany*. 29, 464-471.
- Solereeder, H., 1908. *Systematic anatomy of the dicotyledons*, vol. II. Clarendon Press, Oxford.
- South wick, E.E., 1984. Photosynthate allocation to floral nectar: a neglected energy investment. *Ecology*. 65, 1775-1779.
- Stephenson, A.G., 1982. The role of the extrafloral nectaries of *Catalpa speciosa* in limiting herbivory and increasing fruit production. *Ecology*. 63, 663-669.
- Stpiczyńska, M., 2003. Nectar resorption in the spur of *Platanthera chlorantha* Custer (Rchb.) Orchidaceae—structural and microautoradiographic study. *Plant Syst Evol*. 238,119–126.
- Subramanian, R.B., Arumugasamy, K., Inamdar, J.S., 1990. Studies in secretory glands of *Hiptage sericea* (Malpighiaceae). *Nordic Journal of Botany*. 10, 57-62.
- Sudd, J.H., Sudd, M.E., 1985. Seasonal changes in the response of wood-ants (*Formica lugubris*) to sucrose baits. *Ecol. Entomol.* 10, 89-97.
- Thompson, J.N., 1994. *The coevolutionary process*, University of Chicago Press, Chicago.
- Torezan-Silingardi, H.M., 2007. A influência dos herbívoros florais, dos polinizadores e das características fenológicas sobre a frutificação de espécies da família Malpighiaceae em um cerrado de Minas Gerais. 182p.Tese (Doutorado), Universidade de São Paulo, São Paulo.
- Torezan-Silingardi, H.M., 2011. Predatory behavior of *Pachodynerus brevithorax* (Hymenoptera: Vespidae, Eumeninae) on endophytic herbivore beetles in the Brazilian tropical savanna. *Sociobiology*. 57, 181-189.

Vasco, A., Thadeo, M., Conover, M., Daly, D.C., 2014. Preparation of samples for leaf architecture studies, a method for mounting cleared leaves. *Applications in Plant Sciences*. 9, 1-4.

Vesprini, J.L., Galetto, L., Bernardello, G., 2003. The beneficial effect of ants on the reproductive success of *Dyckia floribunda* (Bromeliaceae), an extrafloral nectary plant. *Canadian Journal of Botany*. 81,24-27.

Vitarelli, N.C., Riina, R., Caruzo, M.B.R., Cordeiro, I., Fuertes-Aguilar, J., Meira, R.M.S.A., 2015. Foliar secretory structures in Crotonaeae (Euphorbiaceae): diversity, anatomy, and evolutionary significance. *Am J Bot.* 12, 1-15.

Vogel, S., 1990. History of the Malpighiaceae in the light of Polination Ecology. *Memoirs of the New York Botanical Garden*. 55, 130-142.

Appendix 1 List of material used of *Burdachia*, *Glandonia* and *Mcvaughia* and voucher information of herbarium.

Species	Collector and number
<i>Burdachia duckei</i> Steyerm.	Nelson 1270 (INPA), Rodrigues 10736 (INPA), Silva 301, 314 (VIC), Varejão <i>s. n.</i> (INPA)
<i>Burdachia prismatocarpa</i> A. Juss.	Coêlho (INPA), Guillaumet 5736 (INPA), Maas 6650 (INPA), Mota 2939 (INPA), Nelson (INPA), Silva 1164(INPA), Silva 289; 292; 296; 297 (VIC)
<i>Burdachia sphaerocarpa</i> A. Juss.	Ferreira 4260 (INPA), Hill 12971(INPA), Rodrigues 8886 (INPA), Silva 313 (VIC)
<i>Glandonia macrocarpa</i> Griseb.	Coêlho 3041, 3118 (INPA), Rodrigues 4945(INPA), Silva 287; 288 (VIC)
<i>Glandonia prancei</i> W. R. Anderson	Silva 298; 299 (VIC)
<i>Glandonia williamsii</i> Steyerm.	Coêlho 484 (INPA), Silva 290 (VIC)
<i>Mcvaughia bahiana</i> W. R. Anderson	Silva 300; 301 (VIC)
<i>Mcvaughia sergipana</i> Amorim & R.F. Almeida	Silva 305; 306 (VIC)

Table 1: Glucose concentration in secretions of the leaf and bracteolar nectaries of macvaughiid species submitted to the urinalysis reagent strips (Insight®).

		LEAF NECTARIES	BRACTEOLAR NECTARIES
<i>Mcvaughia</i>	<i>M. bahiana</i>	250(15) mg/dL(mmol/L)	500(30) mg/dL(mmol/L)
	<i>M. sergipana</i>	1000(60) mg/dL(mmol/L)	>2000(110) mg/dL(mmol/L);
<i>Burdachia</i>	<i>B. prismatocarpa</i>	500(30) mg/dL(mmol/L)	1000(60) - 2000(110) mg/dL(mmol/L)
	<i>B. sphaerocarpa</i>	250(15) mg/dL(mmol/L)	1000(60) mg/dL(mmol/L)
<i>Glandonia</i>	<i>G. macrocarpa</i>	insufficient secretion	500(30) mg/dL(mmol/L)
	<i>G. prancei</i>	insufficient secretion	1000(60) mg/dL(mmol/L)

Table 2: Geographical informations of nectaries in *Burdachia*, *Glandonia* and *Mcvaughia* species and morphoanatomical data from the nectaries.

	<i>Mcvaughia</i>		<i>Burdachia</i>			<i>Glandonia</i>			
	<i>M. bahiana</i>	<i>M. sergipana</i>	<i>B. duckei</i>	<i>B. prismatocarpa</i>	<i>B. sphaerocarpa</i>	<i>G. macrocarpa</i>	<i>G. prancei</i>	<i>G. williamsii</i>	
Geographical distribution	Endemic to <i>Caatinga</i> (Bahia state, Brazil)	Endemic to Restinga (Sergipe state, Brazil)	Endemic to Amazon (Brazil, Venezuela, Guyana, Peru and Colombia)						
Ecosystems	Dryland seasonally arid sandy soils	Sandy Coastal Plains	“Igapó”	Wetlands floodplains “Várzea” and “Igapó”		“Terra Firme” upland forest, humid lowlands	Wetlands floodplains “Várzea” “Igapó”		
Habit	Shrubs with ca. 1,5m tall		Trees with ca. 10m tall			Trees with ca. 20m tall		Tree with ca. 15m tall	
Leaf deciduousness	deciduous	perennial							
LEAF NECTARIES	Acro/basilaminar distribution	1 pair basilaminar	1-4 pairs basilaminar	1 pair acropetiolar basilaminar acropetiolar			1-2 pairs basilaminar	1 pair basilaminar	1-2 pairs basilaminar
	Laminar distribution	few and restricted at the leaf apex	some conspicuous and scattered throughout the blade	some and conspicuous scattered throughout the blade and some along the middle vein			few and inconspicuous scattered throughout the blade (more common at the proximal portion blade)		some and conspicuous, scattered throughout the blade
	Outline of shape	orbicular		elliptical		orbicular			
	Surface	convex					convex-flat		
	Anatomical pattern	short-stalked	immersed	sessile			immersed		
BRACTEOLAR NECTARIES	Distribution	1 unit basilaminar median							
	Shape	hemispheric-globose					discoid		
	Surface	convex						flat	convex
	Anatomical pattern	subsessile		sessile			subsessile		

Table 3: Results of histochemical tests applied on the leaf (LNs) and bracteolar nectaries (BNs) in *Mcvaughia*, *Burdachia* and *Glandonia* species.

TESTS:	<i>Burdachia</i>						<i>Glandonia</i>						<i>Mcvaughia</i>			
	<i>Burdachia duckei</i>		<i>Burdachia prismatocarpa</i>		<i>Burdachia sphaerocarpa</i>		<i>Glandonia macrocarpa</i>		<i>Glandonia prancei</i>		<i>Glandonia williamsii</i>		<i>Mcvaughia bahiana</i>		<i>Mcvaughia sergipana</i>	
	LNs	BNs	LNs	BNs	LNs	BNs	LNs	BNs	LNs	BNs	LNs	BNs	LNs	BNs	LNs	BNs
Lipids/terpenoids:																
Sudan Red	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NADI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Proteins:																
Xylidine Ponceau	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Coomassie brilliant blue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polysaccharides:																
Pas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lugol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ruthenium red	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phenolic compounds:																
Ferrous sulphate in formalin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acidified vanillin*	+	+														
Alkaloids:																
Wagner's reagent	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

(+) positive result; (-) negative result.

*Vanilina testes were made only at fresh samples from species available.

Table 4: Ant species visiting nectaries of mcvaughiid species (*Burdachia*, *Glandonia* and *Mcvaughia*).

<i>Ant species:</i>	<i>Burdachia</i>			<i>Glandonia</i>		<i>Mcvaughia</i>	
	<i>B. duckei</i>	<i>B. prismatocarpa</i>	<i>B. sphaerocarpa</i>	<i>G. prancei</i>	<i>G. macrocarpa</i>	<i>M. bahiana</i>	<i>M. sergipana</i>
<i>Dolichoderus quadridenticulatus</i> Roger 1862	Bracteolar nectaries		Leaf nectaries, Bracteolar nectaries				
<i>D. debilis</i> Emery 1890			Bracteolar nectaries				
<i>D. bidens</i> Linnaeus 1758		Bracteolar nectaries	Leaf nectaries, Bracteolar nectaries				
<i>D. bispinosus</i> Olivier 1792		Bracteolar nectaries					
<i>Crematogaster sp.</i>		Leaf nectaries, Bracteolar nectaries			Bracteolar nectaries		
<i>Cephalotes atratus</i> (Linnaeus, 1758)		Leaf nectaries					
<i>Azteca sp.</i>		Leaf nectaries, Bracteolar nectaries					
<i>Pseudomyrmex gracilis</i> Fabricius 1804		Bracteolar nectaries					
<i>Dacetom armigerum</i> Latreille 1802				Leaf nectaries			
<i>Camponotus sp.</i>						Bracteolar nectaries	Bracteolar nectaries
<i>Camponotus crassus</i> (subgênero <i>Myrmobrachys</i>)		Bracteolar nectaries					
<i>Dorymyrmex sp.</i>						Leaf nectaries	



Figure 1: Specimens studied in the field. A. *Burdachia duckei* in “Igapó”; B. *Glandonia macrocarpa* in “Terra Firme” forest; C. *Mcvaughia sergipana* in Restinga

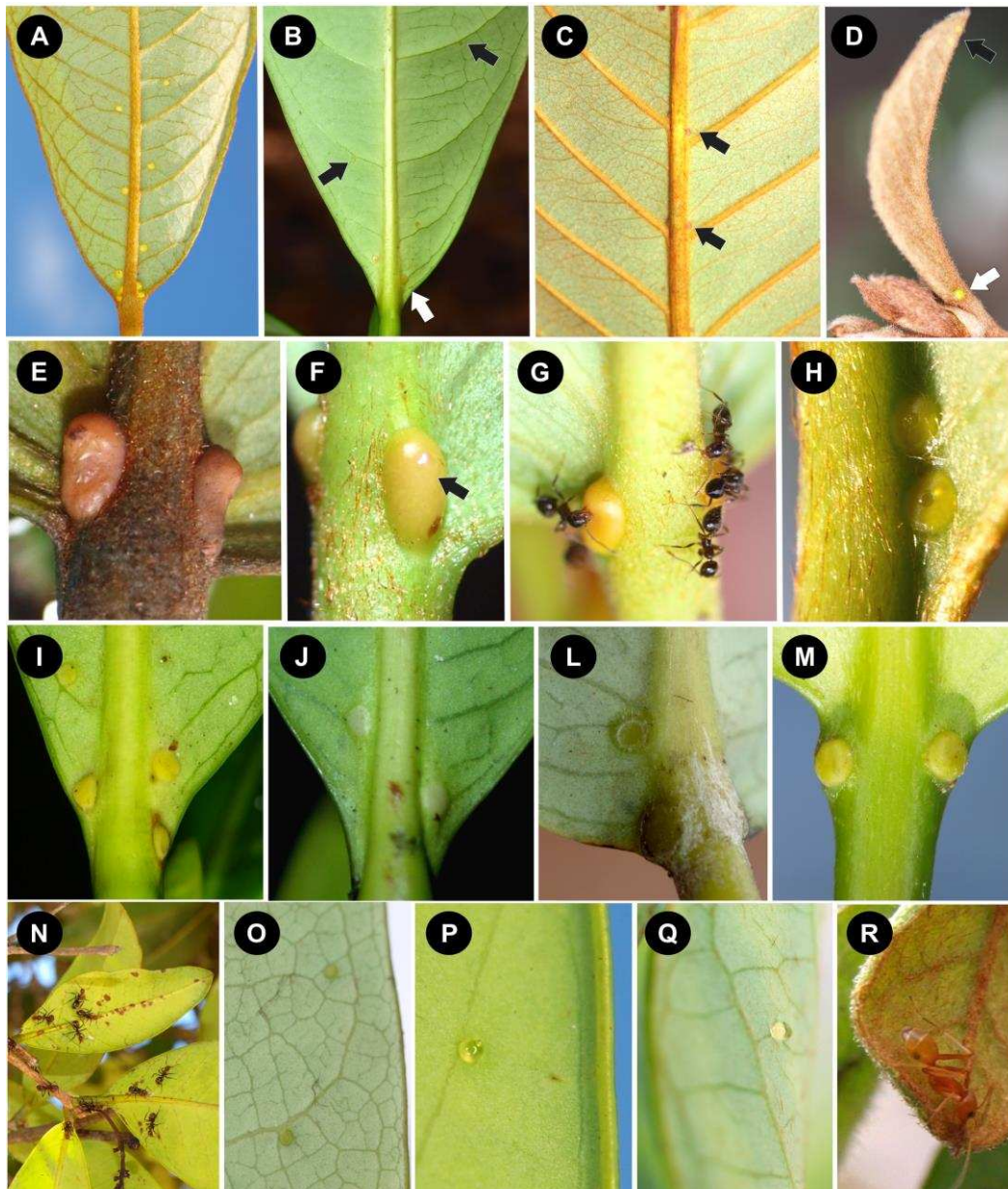


Figure 2: Morphological features of leaf nectaries. **A to D.** Patterns of distribution of nectaries through the leaf blade in *M. sergipana* (A) and *G. macrocarpa* (B); along the middle vein near the branching of secondary in *B. prismatocarpa* (C) and restricted at the base and at the apex of leaf blade in *M. bahiana* (D); white arrow: basilaminar nectaries, black arrow: laminar nectaries. **E to N.** Basilaminar nectaries

in *B. prismatocarpa* (E) showing nectar drop, arrow (F) and ants *Crematogaster* sp. collecting nectar (G); Basilar pairs of nectaries in *M. sergipana*, showing nectar drop (H); basilar pairs of nectaries in *G. macrocarpa* (I) in *G. prancei* (J) and in *G. williamsii* (L). M. Acropeciolar nectaries position in *B. sphaerocarpa*. N. *Dolichoderus quadridenticulatus* ants foraging in leaves of *B. sphaerocarpa*. O to Q. Lamina conspicuous nectaries in *G. williamsii* (O) *B. sphaerocarpa* (P) and *M. sergipana* (Q), showing nectar drop. R. *Dorymyrmex* sp. collecting nectar in lamina nectaries at the leaf apex of *M. bahiana*.

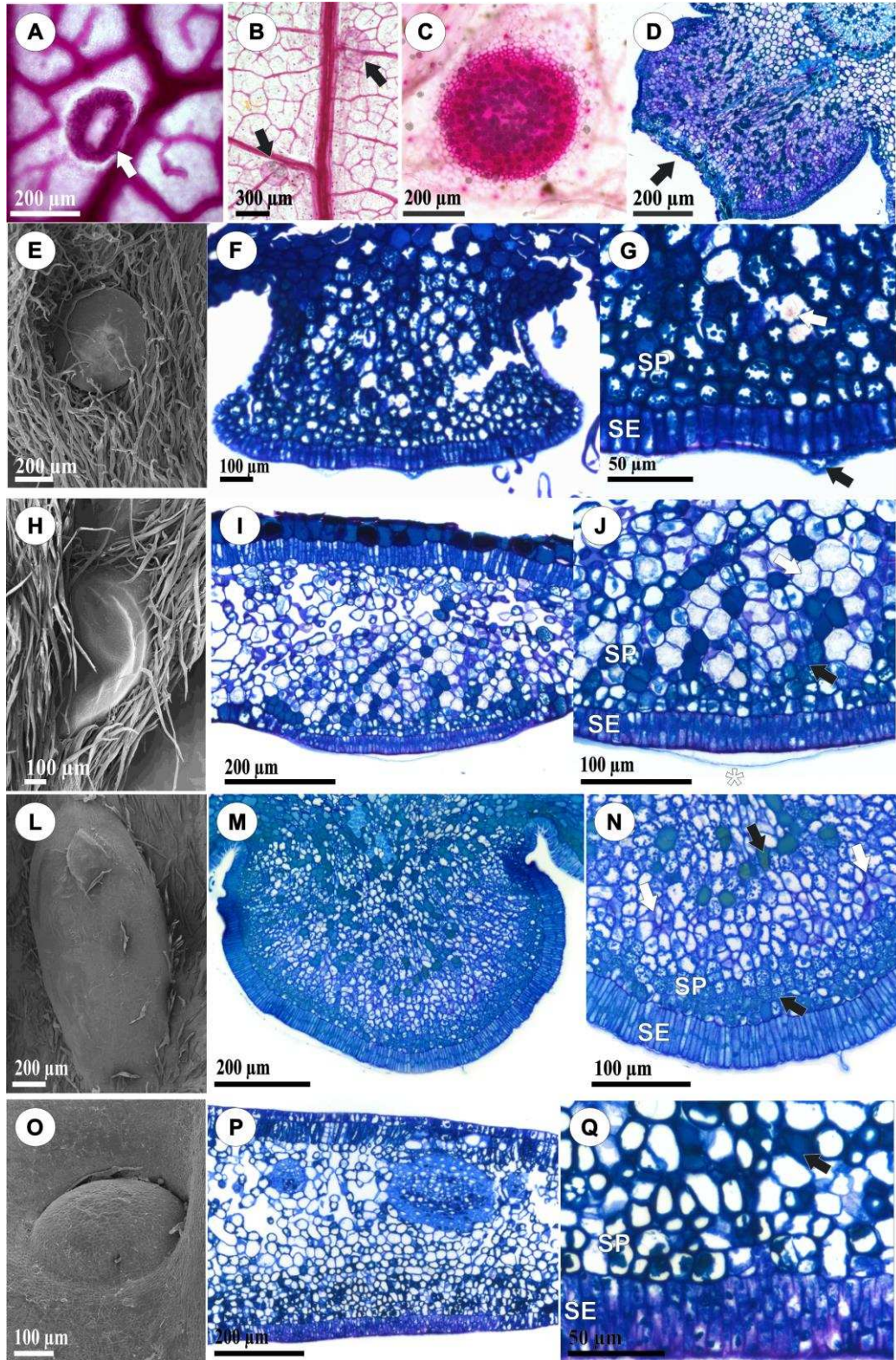


Figure 3: Mophoanatomical features of leaf nectaries. A to C. Diafanized leaves. Lamina nectary in *B. prismatocarpa* (A), at the apex leaf blade in *M. bahiana* (B) and in *M. sergipana* (C). D. Damage on

the basilamar nectary tissues in *B. prismatocarpa*. **E to G.** Basilaminar nectary in *M. bahiana*; SEM image (E); transversal view of short-stalked nectary (F); detail of secretory epidermis (SE), secretory parenchyma (SP), black arrow: subcuticular space and white arrow: crystals. (G). **H to J.** Basilaminar nectary in *M. sergipana*. SEM image (H) transversal view of immersed nectary (I); detail of secretory epidermis (SE), secretory parenchyma (SP) (J), black arrow: cell filled with phenolic compounds and white arrow: crystals, asterisk: cuticle. **L to N.** Basilaminar nectary in *B. prismatocarpa*. SEM image (L); transversal view of sessile nectary (M); detail of secretory epidermis (SE), secretory parenchyma (SP) (N), black arrow: cell filled with phenolic compounds and white arrow: phloematic cells. **O to Q.** Basilaminar nectary in *G. macrocarpa*. SEM image (O); transversal view of immersed nectar (P); detail of secretory epidermis (SE), secretory parenchyma (SP) (Q), black arrow: cell filled with phenolic compounds.

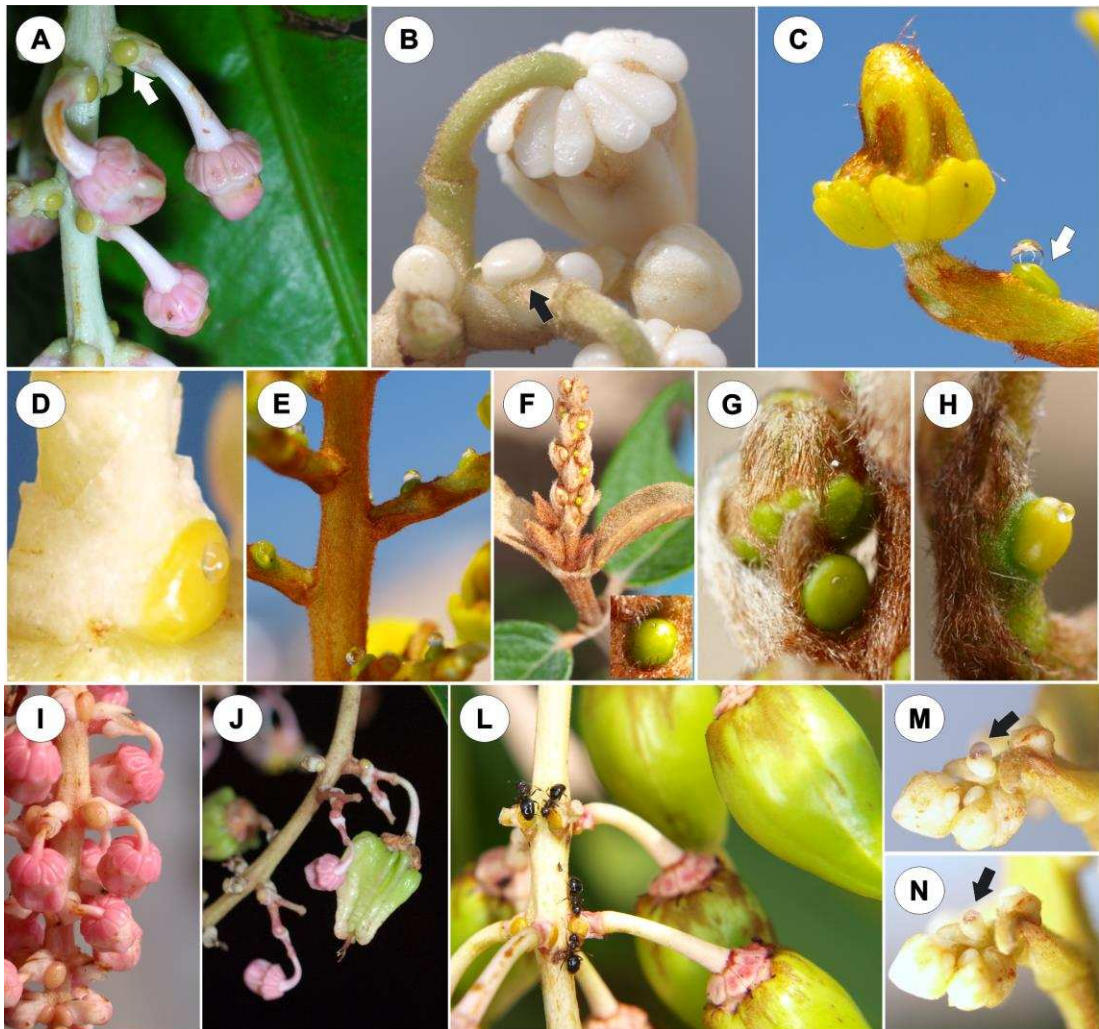


Figure 4: Morphological features of bracteolar nectarines. **A to C.** Bracteolar nectarines (arrows) in *B. sphaerocarpa* (A), in *G. prancei* (B) and in *M. sergipana* (C). **D.** Detail of drop nectar in *B. sphaerocarpa*. **E.** Nectar available along the inflorescence in *M. sergipana*. **F to H.** Inflorescence of *M. bahiana* in development, note bracteolar nectarines active in blooming period (G) and fruiting period (H). **I.** Bracteolar nectar drop in *B. prismatocarpa*. **J and L.** Nectarines active in frutification period in *B. prismatocarpa* (J) and *B. sphaerocarpa* (L). **M and N.** Nectar volume reduction in *G. macrocarpa*.

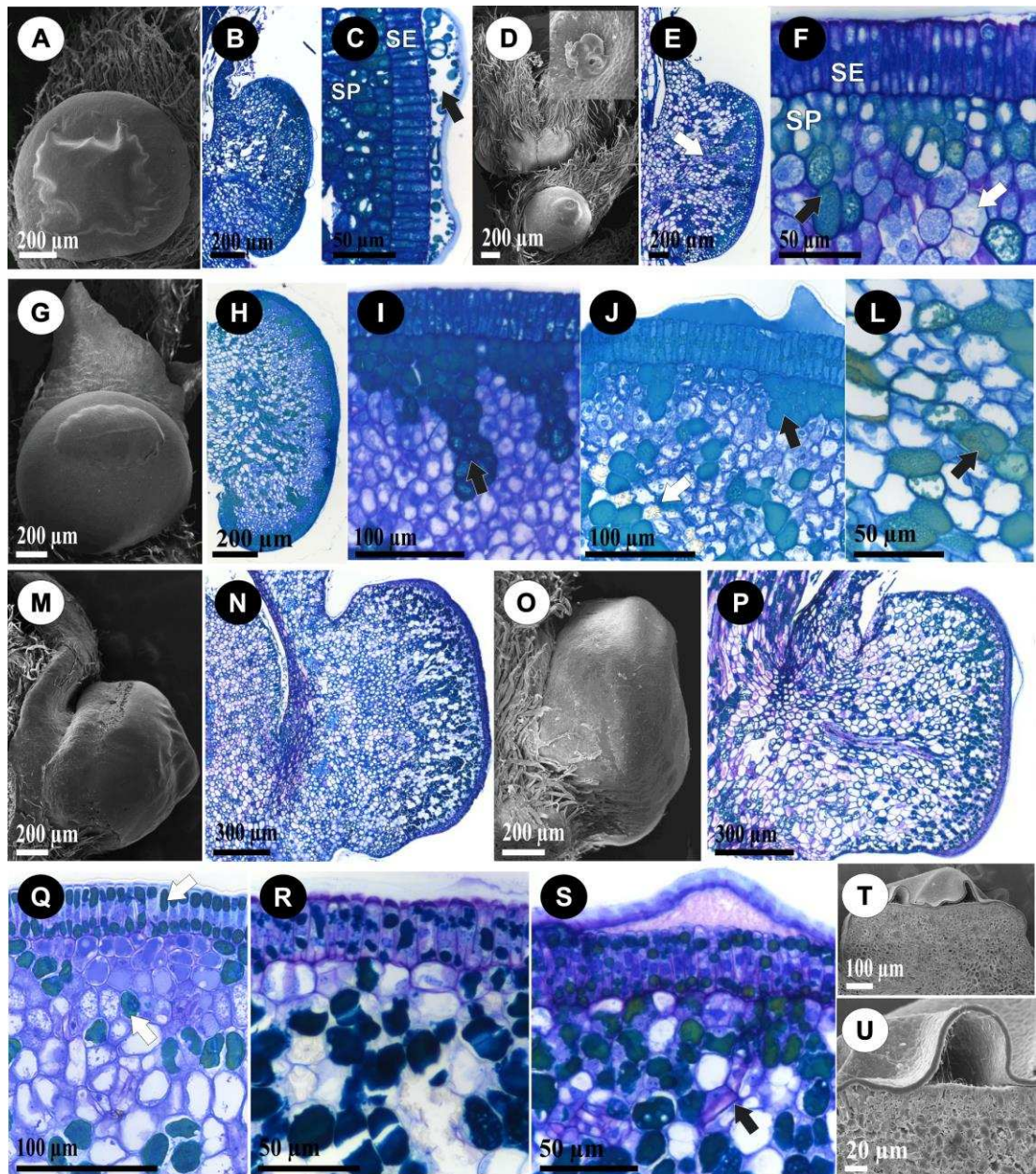


Figure 5: Mophoanatomical features of bracteolar nectaries. **A to C.** Bracteolar nectary in *M. bahiana*; SEM image (A); longitudinal view of sessile nectary (B); Detail of secretory epidermis (SE) and secretory parenchyma (SP), black arrow: subcuticular space (C). **D to F:** Bracteolar nectary in *M. sergipana*; SEM image (D), detail of secretion; longitudinal view of sessile nectary (E), arrow: vascularization; detail of secretory epidermis (SE), secretory parenchyma (SP), black arrow: cells filled with phenolic compounds and white arrows: crystals (F). **G and H:** Bracteolar nectary in *B. duckei*, SEM image (G), transversal section, showing cells filled with phenolic compounds (H). **I to L.** longitudinal view of sessile nectary in *B. prismatocarpa*, white arrow: crystals and black arrow: cells filled with phenolic compounds. **M to P.** Sessile nectary in *G. macrocarpa* in SEM image (M) and transversal view (N) and *G. prancei* in SEM image (O) and transversal view (P). **Q to S.** Detail in *B. prismatocarpa* (Q), *G. williamsii* (R) and *G. prancei* (S), white arrows: cells filled with phenolic compounds in secretory epidermis and secretory parenchyma, black arrows: phloematic cells and subcuticular space with secretion accumulated. **T and U.** SEM image of subcuticular space in *G. prancei*.

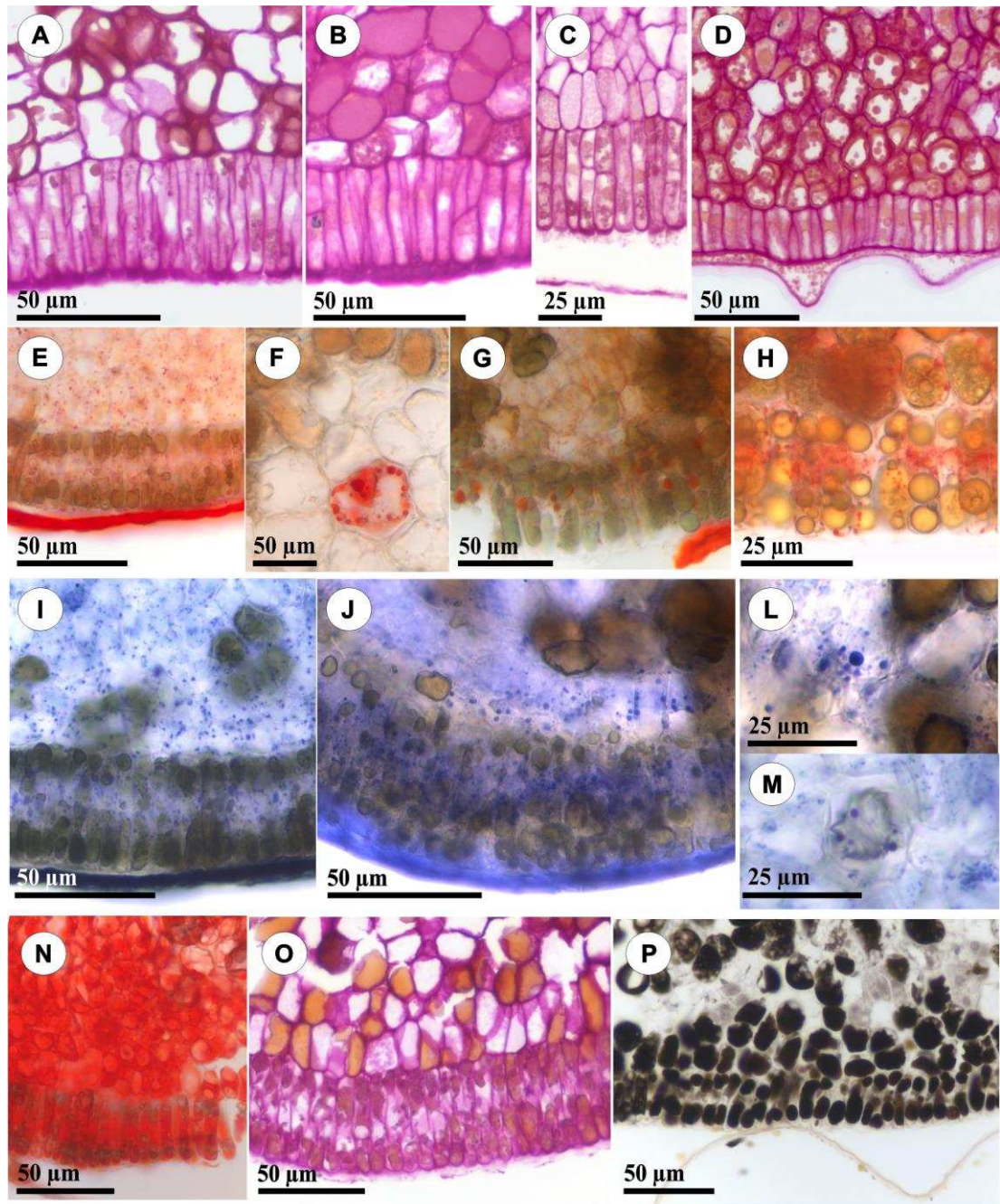


Figure 6: Histochemistry of leaf nectaries. **A to D.** Polysaccharides test, note positive PAS reaction in the subcuticular space in *G. macrocarpa* (A), *G. prancei* (B), *B. duckei* (C) and *M. bahiana* (D). **E to H.** Testing total lipids, note positive reaction to Sudan Red in the drops at *B. duckei* (E), *B. prismatocarpa* (F), *B. sphaerocarpa* (G) and *M. bahiana* (H). **I and J:** NADI test, showing positive reaction to oilresin, in *B. duckei* (I) and *B. sphaerocarpa* (J). **L and M.** droplets and crystals. **N.** Testing proteins using Xylidine Ponceau, note the positive reaction in secretory cells in *B. prismatocarpa*. **O and P.** Phenolic compounds in yellow coloration in PAS test and black color in SFF test in *B. sphaerocarpa* (O) and *M. bahiana* (P).

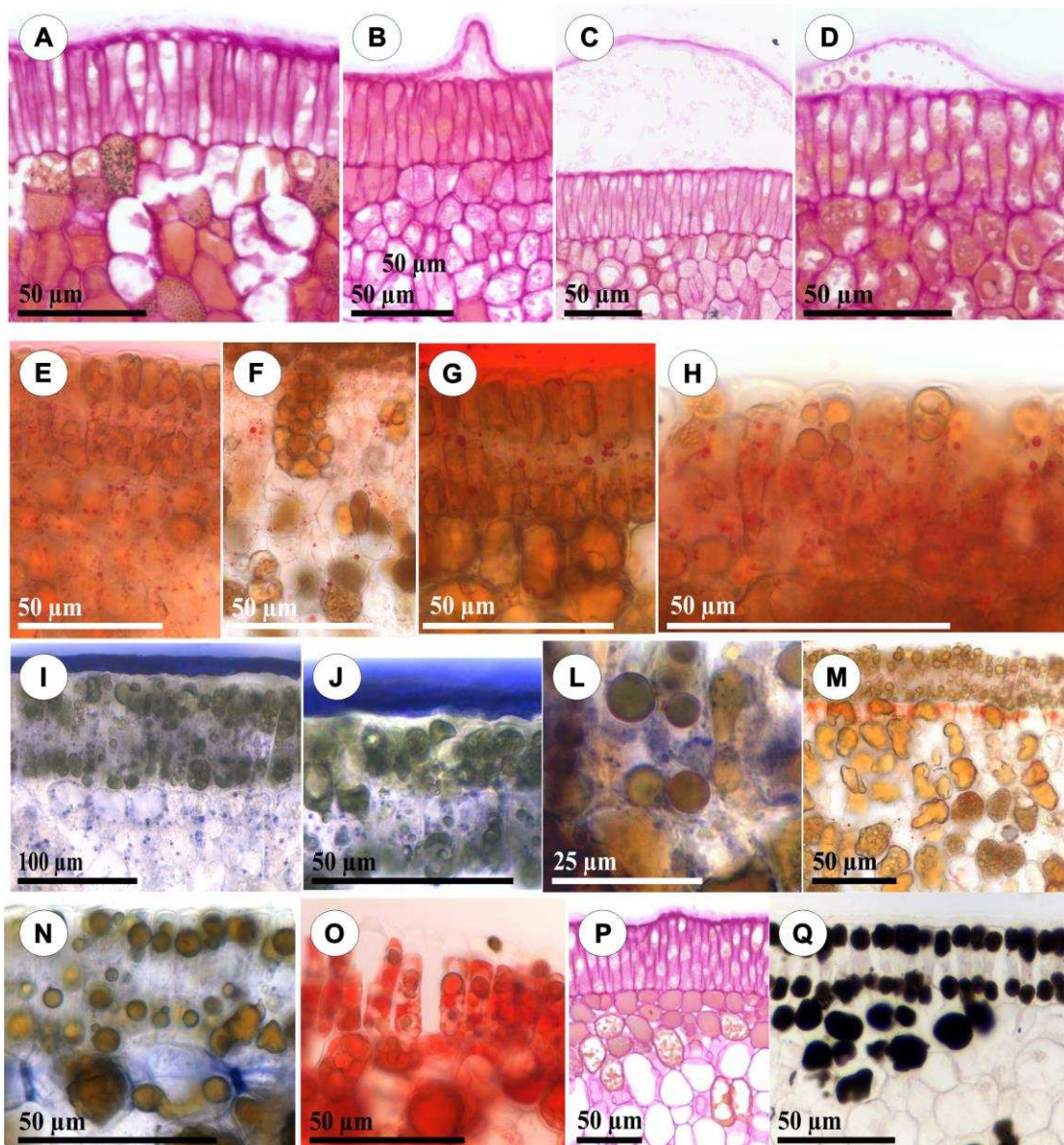


Figure 7: Histochemistry of bracteolar nectaries. **A to D.** Testing polysaccharides, note positive PAS reaction in the subcuticular space in *G. williamsii* (A), *B. prismatocarpa* (B), *M. sergipana* (C) and *M. bahiana* (D). **E to H.** Total lipids test, using Sudan Red, note drops with positive reaction in *B. prismatocarpa* (E and F) *G. macrocarpa* (G) and *M. bahiana* (H). **I to L:** NADI test, showing positive reaction to oilresin, in *B. sphaerocarpa* (I and J) and *M. bahiana* (L). **M and N.** Casparian-band-like thickenings of the wall in *G. prancei*. **O.** Testing proteins using Xylidine Ponceau, note the positive reaction in secretory cells in *G. williamsii*. **P and Q.** Phenolic compounds in yellow color in PAS reaction and in SFF test in *B. duckei* (P) and *B. prismatocarpa* (Q).

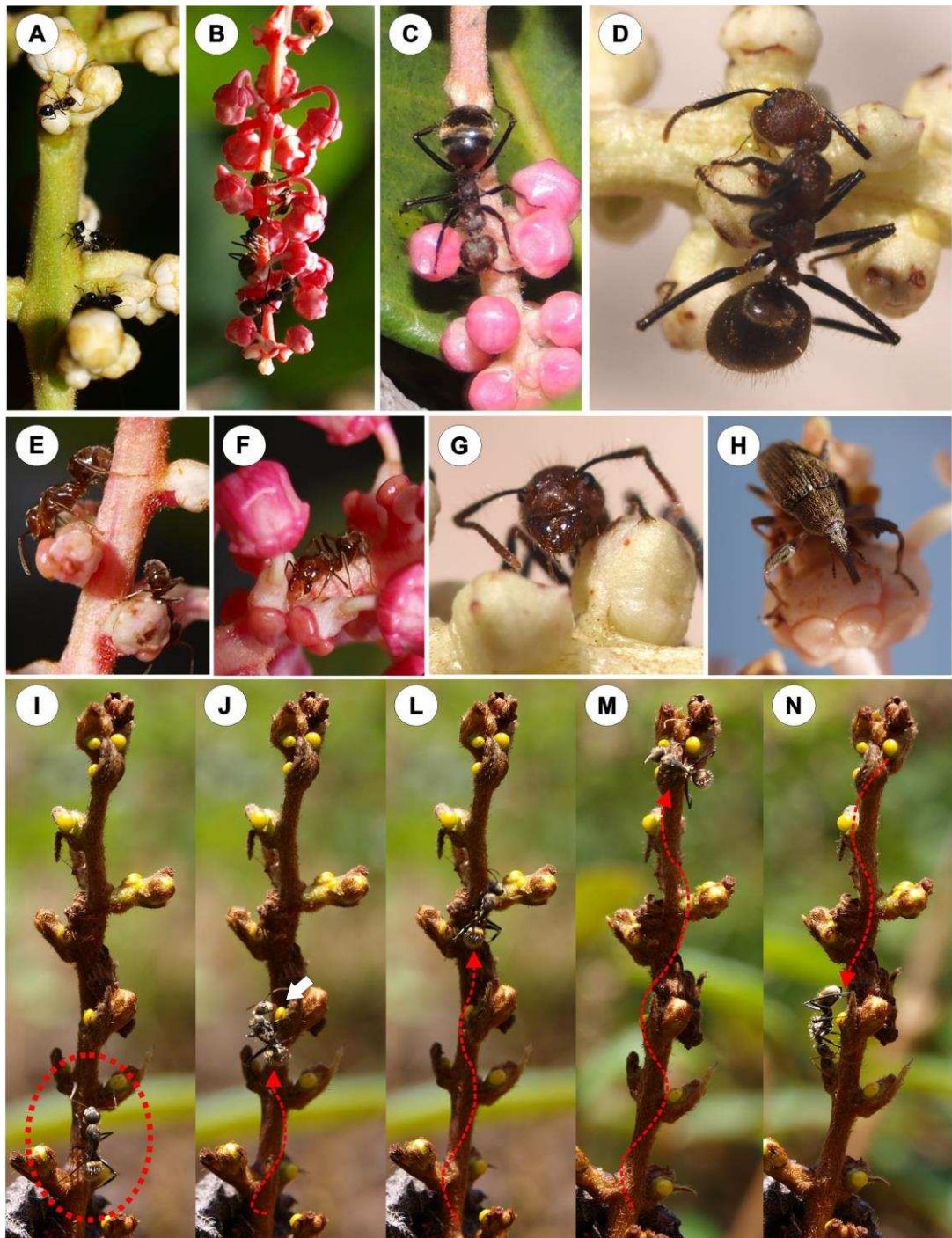


Figure 8: Ants foraging the bracteolar nectaries. **A.** *Crematogaster* sp. in *G. macrocarpa*. **B.** *Dolichoderus bidens* in *B. prismatocarpa*. **C and D.** *D. quadridenticulatus*, in *B. duckei* (C) and in *B. sphaerocarpa* (D), using the palpus to collect the nectar. **E and F.** *Azteca* sp. in *B. prismatocarpa*, collecting nectar (E) and in attack position (F). **G.** *D. quadridenticulatus* in *B. sphaerocarpa* showing mandibles filled of nectar. **H.** Curliionideae *Anthomonus* sp. in *B. sphaerocarpa*. **I to N.** Foraging route of *Camponotus* sp. along the axis of inflorescence in *M. bahiana*, collecting nectar drop (arrow).

CAPÍTULO III

Functional role and evolutionary contributions from
morphoanatomy of floral glands on paleotropical genus

Acridocarpus (Malpighiaceae)

Redação de acordo com as normas de submissão do periódico: *Taxon, International journal of plant taxonomy, phylogeny and evolution*

**FUNCTIONAL ROLE AND EVOLUTIONARY CONTRIBUTIONS FROM
MORPHOANATOMY OF FLORAL GLANDS ON PALEOTROPICAL
GENUS *ACRIDOCARPUS* (MALPIGHIACEAE)**

Isabel Reis e Silva^{a,b}, Jeferson Nunes Fregonezi^a, André Márcio Amorim^{c,d} and
Renata Maria Strozi Alves Meira^{a*}

^aUniversidade Federal de Viçosa, UFV, Departamento de Biologia Vegetal, 36570-900, Viçosa, Minas Gerais, Brazil

^bUniversidade Federal do Amazonas, UFAM, Instituto de Ciências Exatas e Tecnologia, 69103-128, Itacoatiara, Amazonas, Brazil

^c*Universidade Estadual de Santa Cruz, UESC, Departamento de Ciências Biológicas, 45662-900, Ilhéus, Bahia, Brazil*

^d*Centro de Pesquisas do Cacau, Herbário CEPEC, 45650-970, Itabuna, Bahia, Brazil*

*Corresponding author. Mailing address: ^aUniversidade Federal de Viçosa, UFV, Departamento de Biologia Vegetal, Avenida Peter Henry Rolfs, s/n, 36570-900, Viçosa, Minas Gerais, Brazil.

e-mail: rmeira@ufv.br (R. M. S. A. Meira)

Abstract

The Paleotropical genus *Acridocarpus* comprises species from Africa, Madagascar, Arabian Peninsula and New Caledonia. Several events of dispersion from Neo to Paleotropic were identified in the phylogeny of Malpighiaceae and a typical change in the floral morphology traits is the lost of the floral glands. Among the paleotropical lineages, *Acridocarpus* is an exception by the conservation of such glands. To analyse the morphoanatomy of bracteolar and calyx glands in the genus, flowers of 26 species were studied using standard anatomical light microscopy techniques. Histochemical tests and glucose test strips were made in fresh samples to clarify the nature of the exudate. Bracteal and calyx secretion were positive strips results for glucose and positive histochemical results for protein and phenolic compounds. The anatomy of bracteolar and calyx glands was similar in the species studied, showing a typical structure of nectaries with a palisade epidermis, parenchyma secretory vascularized. The bracteolar nectaries were classified as sessile or short-stalked and vary in number of one, two-glandular or eglandular bracteoles. Ten different patterns of distribution of nectaries were observed in the calyx and about the anatomical arrange, they were recognized as immersed or sessile. This study revealed new records about the occurrence of floral glands in paleotropical species of Malpighiaceae. The *Acridocarpus* species studied show a high diversity on the patterns of distribution, mainly in the calyx. This records reinforced the importance of polinators in morphological floral issues and corroborat the role of oil-bees mutualism in the stereotyped Neotropical morphology, since in their absence in the Paleotropic the morphology vary. Our results are promising for investigations of floral traits evolution in differents lineages of Malpighiaceae and the relation with the pollinator syndrome.

Keywords: floral traits, nectaries, inflorescence.

Introduction

Malpighiaceae is a family composed of about 1.300 species, which occur in savannas, tropical and subtropical forests of the Neo and Paleotropic. However 90% of the species are presented in the Neotropics (Davis & al., 2002a; Davis & Anderson, 2010). The vicariancy of Gondwanan biota is the main explanation for the disjunct distribution patterns between Neo and Paleotropic species (Raven & Axelrod, 1974; Vogel, 1990). However, based on fossil evidence (Taylor & Crepet, 1987; Hably & Manchester, 2000) and phylogenetics analyses, Davis & al. (2002b) proposed that the South America was the center of origin of Malpighiaceae in a post Gondwananian time, being in agreement with Anderson's hypothesis (1979). The divergence-time estimatives and the paleogeographical scenario of eoceno period allowed sugesttins that the actual biogeography patterns was resulted of dispersions from Neo for Paleotropic (Davis & al., 2002b). A total of nine dispersions events were recognised at the last complety generic phylogeny of Malpighiaceae (Davis & Anderson, 2010), which linked paleo and neotropical lineages as sister groups (Davis & al., 2014).

The floral morphology of the Neotropical Malpighiaceae species is highly conserved, typically bear pentamerous zygomorphic flowers with glandular calyx, clawed petals, trimerous gynoecium and one ovule per locule (Anderson, 1981; Vogel, 1990; Anderson, 1990, 2001, 2004). The posterior petal upright positioned and differentiated from the others (Vogel, 1990; Anderson 1979, 1990) is strongly correlated with the calyx glands that produce fatty oil as pollinators reward (Buchmann, 1987; Lobreau-Callen, 1989; Vogel, 1990; Anderson, 1979, 1990; Simpson & Neff , 1981; Endress, 1996; Vinson & al., 1997; Sigrist & Sazima, 2004; Zahng & al., 2010; Davis & al., 2014). The oil exudates is exclusively used by females oil-collecting bees as food resource and in nest construction (Neff & Simpson, 1981;

Vogel, 1990). The bees cling to the posterior petal and use their specialized legs to scratch the glands (Anderson, 1979; Vogel, 1990; Sigrist & Sazima, 2004). The high variability of others morphological characters suggests that the specialized system of pollination has driven the floral evolution in Neotropics (Anderson, 1979; Davis & al., 2014). Thus, such floral features were maintained for millions of years (Davis & al., 2014) by specialist oil-bees pollinators of the tribes Centridini, Tapinotaspidini and Tetrapediini (Vogel, 1990).

Specialist oil-collecting bees are absence at Paleotropic region (Vogel, 1990), resulting in lost of the selective pressure for maintenance of the Neotropical typically floral morphology (Anderson, 1979). The oil-bee pollination syndrome was lost by the Paleotropical clades and by few Neotropical species that bear actinomorphic eglandular flowers (Zhang & al., 2013) and present modified petals, anthers and stigma (Vogel, 1990; Davis & Anderson, 2010; Davis & al., 2014). The Paleotropical genera *Acridocarpus*, *Hiptage* and *Tristellateia* are considered exceptions due maintenance of the floral zygomorphy and of the calyx glands (Vogel, 1990) that seem to behave as nectaries, although the pollen is considered the only reward to pollinators (Anderson, 1979; Lobreau-Callen, 1989; Vogel, 1990; Ren & al., 2013).

Acridocarpus comprises about 30 species distributed in Africa, Madagascar, Arabian Peninsula, and one species of New Caledonia (Niendenzu, 1928; Davis & al., 2014), covering rainforest to savannas (Davis & al., 2002a). Besides the leaf glands frequently mentioned (Anderson, 1990; Vogel, 1990), *Acridocarpus* species may present bracteal, bracteolar and calyx glands (Niendenzu, 1921,1928; Launert 1995), which the anatomy and ecological function have not been elucidated yet.

Because the peculiar biogeographic history, Malpighiaceae is a special group to test hypothesis about morfological evolution, since there are lineages neo and

paleotropical with distinct pollinator syndrome (Vogel, 1990; Michener, 2000; Davis & Anderson, 2010). Thus, evaluate the occurrence and types of floral glands in the paleotropical species of *Acridocarpus* would answer questions such as: are there morphoanatomical patterns between bracteole and calyx glands in different species? These glands can be classified as nectaries? The biogeography of species can be related with morphoanatomical patterns of the calyx and bracteole glands?

Material and Methods

Acridocarpus is characterized by erect or climber shrubs and rarely small trees, with simple leaves exceptionally alternated. The flowers are clustered in racemes or in terminal panicles with bracts and two bracteoles at the pedicel base (Fig. 1A). Generally the calyx has sepal glands, the blade of petals are entire or lacerate (Launert, 1995) and the claw is reduced (Vogel, 1990). The anthers are poricidal, attractants for pollen-gathering insects such as buzzing bees (Vogel, 1990). The corolla is constituted by two posterior petals, two lateral petals and only one median anterior petal, which serves as attractive and landing zone to pollinators. The calyx is also reoriented (Fig. 1B), presenting only one median posterior sepal, two lateral sepals and two anterior sepals (Vogel, 1990; Zhang & al., 2010, 2013).

Floral samples were obtained from herbarium material of 26 *Acridocarpus* species, at collection of the “Muséum National d’Histoire Naturelle”-P, Paris, France (Appendix 1). The bracteoles and calyx glands were analyzed and pictures were taken in stereomicroscopy (Stemi 2000-C Zeiss, Gottingen, Germany) equipped with a digital camera (AxioCam ERc; Zeiss, Gottingen, Germany).

The glands present at the bracts on the base of inflorescence, on the bracteoles and on the calyx (Fig. 1.A) of *A. longifolius* were observed and collected in plant

nursery from collections of “Jardin Botanique de Meise”-BR, Belgium (Appendix 1). The nature of secretion in the floral glands of *A. longifolius* was investigated as a model for the genus since this species bears inflorescences with bracteal, bracteolar and calyx glands. The secretion exsudated by these glands was submitted *in locu* to tests of glucose and protein concentration, using the urinalysis reagent strips (Insight, Acon Laboratories, San Diego, USA). Samples were fixed in FAA (formalin, acetic acid and 50% ethanol; 1:1:18 by volume) for 48 h and then stored in 70% ethanol.

The procedures of light microscopy were made at the Laboratory of Plant Anatomy of the Federal University of Viçosa-UFV. Samples acquired from herbarium specimens were subjected to the herborization reversion process (Smith & Smith, 1942), dehydrated in an ethanol series and stored in 70% ethanol. Samples of both herbarium and field-collected material were embedded in methacrylate (Historesin Leica; Heidelberg, Germany) prepared in accordance with the manufacturer’s recommendation. Cross and longitudinal sections with 4µm thick were made in an automatic rotary microtome (Leica RM2155, Deerfield, USA). The sections obtained were stained with toluidine blue at pH 4.7 (O’Brien & al., 1964) and the slides were mounted in Permount (Fisher Scientific, NJ, USA).

The following histochemical tests were performed in the fixated samples of *A. longifolius*: for total proteins, xyloidine ponceau (Clark, 1981) and Coomassie blue (Fisher, 1968); for total polysaccharides, periodic acid–Schiff reagent (McManus, 1948); for mucilage and pectin, ruthenium red (Johansen, 1940); for phenolics compounds, ferric chloride and for starch, lugol (Johansen, 1940); for total lipid Sudan red (Pearse, 1980).

Images were captured using a light microscope (Olympus AX70TRF) equipped with a digital camera (AxioCam HRc; Zeiss, Gottingen, Germany). Examinations and

image captures on scanning electron microscopy (SEM) were conducted at the “Núcleo de Microscopia e Microanálise-UFV”, using a LEO 1430VP (Zeiss, Cambridge, UK). For SEM studies, the samples from herbarium material were dehydrated, subjected to critical point drying with CO₂ (CPD 030, Bal-Tec, Balzers, Liechtenstein), placed on stubs with carbon conductive tapes and then coated with gold (SCD 050, Bal-tec, Balzers, Liechtenstein).

The anatomical patterns were classified as **stalked**, when a short stalk is present; **sessile**, when the secretory epidermis is above the level of the non-secretory epidermis around; and **immersed** when the secretory tissues are distributed below the level of the non-secretory epidermis surrounding the gland.

Results

Morphoanatomy of bracteolar glands:

Considering the presence and position of bracteolar glands at the inflorescence of *Acridocarpus*, three groups were recognized: **Group I:** eglandular, in *A. alternifolius*, *A. camerunensis*, *A. chevalieri*, *A. chloropterus*, *A. congolensis*, *A. macrocalyx*, *A. monodii*, *A. natalitius*, *A. obovatus*, *A. orientalis*, *A. plagiopterus*, *A. socrotanus*, *A. spectabilis*, *A. vanderystii* and *A. zanzibaricus*; **Group II:** one basilaminar gland (Fig. 2A-D), in *A. adenophorus*, *A. austrocaledonicus*, *A. humbertii*, *A. katangensis*, *A. longifolius*, *A. prasinus* and *A. smeathmanni*; **Group III:** two glands medially positioned (Fig. 2E-I), in *A. alopecurus*, *A. excelsus*, *A. perrieri* and *A. vivy*. The shapes of such glands were hemispheric-globoid with outlining orbicular (Fig. 2E).

The bracteolar glands are composed of a secretory epidermis and secretory parenchyma vascularized with abundant phloem cells (Fig. 2D). The secretory epidermis cells are typically arranged in a palisade and bear densely stained cytoplasm

with conspicuous nucleus and a thick cuticle (Fig. 2L), which develop a subcuticular space (Fig. 2L-N). No secretory pores or rupture cuticles were observed, even though at SEM images (Fig. 2A, E). The secretory parenchyma is consisted of some layers of cells (Fig. 2D, L), which the cells may filled with phenolic compounds (Fig. 2M). Idioblasts containing druse crystals were frequently observed scattered among the secretory parenchyma (Fig. 2F, M).

Only in *A. adenophorus*, *A. excelsus*, *A. humbertii* and *A. perrieri* the glands were stalked (Fig. 2F, H-J, N). and remain species bear sessile glands. The secretory surface may be convex (Fig. 2C) or plane (Fig. 2F).

Morphoanatomy of calyx glands:

The mostly of species analyzed showed sessile calyx glands (Fig. 3B, D, F-N) in *A. longifolius* and *A. zanzibaricus* are immersed (Fig. 3A, C, E). The calyx comprised by a secretory epidermis and secretory parenchyma vascularized with abundant phloem (Fig. 3C-G). The subcuticular space was frequently observed (Fig. 3F, G) well as crystalliferous (Fig. 3F) and phenolics (Fig. 3E, G) idioblasts.

The position of calyx glands may be intersepal (Fig. 3A) or marginal (Fig. 3B, H). The distribution of glands at the calyx cover the posterior (Fig. 3I, J), ventral (Fig. 3L) or both dorsal-ventral plane (Fig. 3 M, N). Morphoanatomical analysis allowed establishing ten types of distribution patterns of glands in the calyx (Fig. 4).

The calyx glands were distributed only on posterior plane (Types II and III) in *A. congolensis*, *A. obovatus*, *A. plagiopterus*, *A. socotranus* and *A. prasinus* or only at the ventral plane (Types IV-VII) in *A. chevalieri*, *A. chloropterus*, *A. longifolius*, *A. monodii*, *A. natalitius*, *A. smeathmanni*, *A. spectabilis* and *A. zanzibaricus*. While *A. alternifolius*, *A. orientalis* (Type VIII), *A. camerunensis* (Type IX) and *A. vanderystii* (Type X) exhibited glands in both planes. The occurrence of different types in the same

specimen has observed in *A. macrocalyx* and *A. katangensis* (Type II and III), and *A. alopecurus* (Type VII and VIII). *A. vanderystii* was the only species that bears exclusively intersepal glands in the whole calyx (Type X).

The morphology of calyx glands appear to be individualized (Fig. 5A-D). However, in *A. spectabilis* and *A. monodii* the pair of neighbors glands were partially fused (Fig. 5E, F), in less or higher degree respectively, where the secretory epidermis is continued without non-secretory epidermis either subepidermis tissues between pair of glands. *A. spectabilis* showed independent vascularization of glands and secretory epidermis continuous in the limit of the neighbor's pair glands (Fig. 5H). A higher degree of fusion by the secretory epidermis in *A. monodii* due to the part of secretory parenchyma shared (Fig. 5I). Whereas in *A. longifolius* (Fig. 5C, 3C) and *A. zanzibaricus* (Fig. 5D) the median anterior gland is intersepal (Fig. 5D), showing completely sharing of the secretory parenchyma and a regular surface of the secretory epidermis due to completely fused (Fig. 5G, J).

The calyx glands are impressed with the outlining sagittate at the basal portion and acute in the apical portion (Fig. 5C, D) in *A. longifolius* and *A. zanzibaricus*. The remain species exhibit may be hemispheric-globose calyx glands with orbicular or elliptical outlining (Fig. 3B, H; 5A, B).

Secretion of floral glands in Acridocarpus longifolius

A. longifolius bears inflorescences with bracteal, bracteolar and calyx glands (Fig. 6A-G). The coloration of such glands varies: it is yellow in the calyx (Fig. 6B-D), greenish in the bracts (Fig. 6E) and red in the bracteoles (Fig. 6F, G).

During the observation at the plant nursery, the secretion of bracteolar gland was very scarce, while in the calyx and the bracteal glands were copiose (Fig. 6E). It was possible to test only calyx and bracteal exsudate that reacted with the urinalysis

strips, demonstrating the presence of glucose and proteins, the mains constitutive of the nectar. The concentration of glucose was higher in the calyx gland [1000(60) mg/dL (mmol/L)] than of the bracteal glands [250(15) mg/dL (mmol/L)]. On the other hand the concentration of proteins was not varied since both glands showed the same value: 30(0,3) mg/dL(g/L). The secretory activity starts in blooming periods and kept active until fruiting periods (Fig. 6D) and we could document visitation of ants consuming the exsudate of calyx glands (fig. 6C).

The anatomical analyses in *A. longifolius* showed that the bracteal and calyx glands are actually secretory (Fig. 3C, E; Fig. 6H, I), while no secretory features were observed at the bracteolar tissues (Fig. 6J, L). The histochemical tests of calyx glands provided positive results for total polysaccharides (Fig. 7 A-C), protein (Fig. 7 D-F) and phenolic compounds. Proteins and phenolic compounds were distributed on epidermis and secretory parenchyma. Starch grains were observed in the parenchyma not secretory and around the vascular tissues. Positive reaction with Sudan allowed detecting lipids compounds only at the cuticle (Fig. 7G-I).

Discussion

Bracteolar and calyx glands: insights on taxonomy and functional evidences

Although in the taxonomic descriptions the floral glands are mentioned for *Acridocarpus*, in the present study the presence of these glands was checked for 26 species (Table 1). In *A. autrocaledonicus*, *A. congolensis*, *A. katangensis*. *A. longifolius* bracteolar glands were not reported (Niedenzu, 1921, 1928; Hutchinson & Dalziel, 1954; Launert, 1995) and in the present work a basilaminar medium gland was documented in these species. Bracteole glandular was reported for *A. adenophorus*, *A. excelsus*, *A. humbertii*, *A. perrieri* and *A. vivy* (Niedenzu, 1921, 1928;

Arènes, 1946, 1950, 1955), being in the present work detected two glands in each bracteole. On the other hand, the bracteolar glands of *A. vanderystii* reported by Wilczek (1955) were not observed in the samples analyzed. Such results reinforce the importance of the anatomical studies to be safe in the characterization of floral secretory structures in Malpighiaceae.

The bracteolar glands are similar among the studied species of *Acridocarpus*, varying only in the presence of the stalk. The anatomical structure is the same as in the foliar and bracteolar glands in the closely related genera of the Neotropical mcvaughoiide clade (Silva et al., Unpubl.data, 2017 in Chapter II).

As for calyx glands (Table 1), this is the first record for *A. congolensis* that was unnoticed in the description (Nieden zu, 1921, 1928). In *A. camerunensis*, *A. chevalieri*, *A. chloropterus*, *A. natalitius*, *A. obovatus*, *A. Orientalis*, *A. smeathmanni*, *A. Spectabilis*, *A. zanzibaricus* the number of calyx glands observed in this study corresponds to the range of previously recorded (Morot, 1909; Hutchinson & Dalziel, 1954; Oliver, 1868; Niedenzu 1921, 1928; Launert, 1995; Thulin, 1993). On the other hand, in this work high numbers of calyx glands were recorded in *A. alternifolius*, *A. macrocalyx*, *A. monodii*, *A. prasinus*, and *A. vandersystii* (Nieden zu, 1921, 1928; Chipp & Dawe, 1922; Arènes, 1950; Wilczek 1955; Hutchinson & Dalziel, 1954; Wilczek, 1958; Launert, 1995; Oliver, 1868; Doorn-Hoekman, 1975; Birnbaum & Florence, 2005). Only in *A. adenophorus*, *A. austrocaledonicus*, *A. excelsus*, *A. plagiopterus*, *A. socotranus* and *A. vivy*, the number of calyx glands observed here was identical to that reported by Niedenzu (1921, 1928), Arènes (1950) Guillemin & al. (1831) and Oliver (1868).

Most of the analyzed species of *Acridocarpus* have more than one calyx gland, sessile and subcircular and these results are in agreement with the observations

recorded in the taxonomic descriptions (Launert, 1995). Although calyx gland stands out for its taxonomic, ecological and evolutionary importance (Anderson, 1979, 1990; Vogel, 1990; Davis & Anderson, 2010; Davis & al., 2014), in some taxonomic descriptions these structures were not observed, either cited. The results obtained in the present work demonstrate the importance of the morphoanatomical evaluation to assure the usefulness of these characteristics for taxonomic purposes in *Acridocarpus*.

The calyx glands distribution patterns of *Acridocarpus* showed labile character in some studied species, whereas in *A. katangensis*, *A. macrocalyx* and *A. alopecururs* more than one type of distribution has been observed, for example, related types II and III; VII and VIII). In contrast, in neotropical species, calyx glands stand in pairs in all sepals or are absent in the previous pair (Vogel, 1990). It is interesting to note that in the paleotropical species *Hiptage sericeae* (Subramanian & al., 1990; Arumugasamy & al., 1993) and *H. acuminata* (Arumugasamy & al., 1989) the calyx glands follow the same distribution pattern of neotropical species, besides being similar morphoanatomical features and acting as elaiophores. By the other side, in *H. bengalensis* only a calyx gland occurs that was characterized as nectary (Ren & al., 2013). Different reduction patterns were also detected in the species studied.

The studied *Acridocarpus* calyx glands are similar to leaf nectaries of different Neotropical species of *Banisteriopsis*, *Burdachia*, *Diplopterys*, *Galphimia* and *Glandonia*, *Mcvaughia* (Castro & al., 2001;; Possobom & al., 2015; Araújo & Meira, 2016; Silva & al., unpubl. data) as well as the paleotropical species of *Hiptage* (Arumugasamy & al., 1989).

Immersed calyx glands were recorded only in *A. longifolius* and *A. zanzibaricus*. This anatomical pattern was reported only in leaf nectaries (Elias, 1980; Silva & al., unpubl. data) suggesting the the function of calyx glands in *Acridocarpus*.

The function of the floral glands of Paleotropical species needs to be clarified, since Vogel (1990) makes inferences of their performance as nectaries, mainly based on the absence of Paleotropic oil-bees. However, there is a lack of morpho-anatomical and ecological data to support such assumptions. Some evidences of the studied anatomy glands reinforce the idea of its nectariferous function, such as: the epidermis palisade, the developed subcuticular space, layer between the epidermis and the secretory parenchyma, phenolic and crystal idioblasts in the parenchyma and persistent secretory activity on the fruit, characteristics commonly reported in nectaries (Possobom & al., 2010; Ren & al., 2013; Araújo & Meira, 2016). These anatomical characteres may suggest the related origin of elaiophores from nectaries, proposed by Vogel (1990).

Chemical features of secretion and ecological role of floral glands from A. longifolius

The results obtained in the histochemical analysis and concentration tests of exudate components of *A. longifolius* floral glands allowed proving their performance as nectaries. Throughout *A. longifolius* inflorescence, the distribution of bracteal, bracteolar and calyx glands favors the visitation of nectar consumers, who act against herbivory, as occurs in leaves (Elias & Gelband, 1976; Stephenson, 1982; Morellato & Oliveira, 1994; Paiva & al., 2007; Coutinho & al., 2010) and inflorescences of neotropical species (Silva & al., unpubl. data). The ants foraging observed in secretory structures of *A. longifolius*, supports recognition of bracteolar and leaf glands nectaries as reported by Vogel (1990).

Components detected in the bracteal and calyx glands nectar suggest mutualism with ants, since they prefer sugary solutions and with amino acids (Lanza, 1991). Proteins found in the nectar of different nectaries of *A. longifolius* inflorescence, could enrich their nutritional value and strengthen ecological relations

with consumers as cited in other studies (Bentley, 1977; Fahn, 1979; Elias, 1983; Roshchina & Roshchina, 1993; Ness & al., 2009; González-Teuber & Heil, 2009; Heil, 2011). Another possibility is the proteins action inhibiting the growth of microorganisms (Park & Thornburg, 2009).

Differences in the secretion of the glands studied in *A. longifolius* can be used as advantages for the different regions of the inflorescence. The composition of nectar may also vary according the interactions between plant and animal demands (Faegri & Van der Pijl, 1976; Nicolson & Thornburg, 2007; González-Teuber & Heil, 2009; Nepi & al., 2009, 2012; Heil, 2011, 2015). The biggest size of the secretion drops and the higher concentration of glucose in calyx nectar from *A. longifolius*, may be related to the functional value and vulnerability of the reproductive organs, which according to McKey (1979) justify the investment in the flowers protection. Studies have shown that ants are attracted by larger nectaries, which produce nectar in bulk and with higher sugar content (Sudd & Sudd, 1985, Baker-Méio & Marquis, 2012).

Bracteolar and leaf glands are cited for establishing mutualism with ants (Vogel, 1990). The calyx glands, although acting mainly as elaiophores in the Neotropic, in *Acridocarpus* are associated to the mutualism with ants (Davis & al., 2002a). The attraction of ants observed in bracteolar and calyx glands in *A. longifolius* is an indication of this mutualism, which according to Bentley (1977) and Koptur (1992) in exchange for food resources, ants act as defense agents. Lobreau-Callen (1989) and Ren & al. (2013) observed as main consumers of calyx nectar in paleotropical species, carnivorous species of ants, whose predation behavior suggests its important role against herbivory in inflorescences, without interference in pollination (Ren & al., 2013).

Phenolic compounds were detected in different glands of *Acridocarpus* analyzed. Such compounds are used to protect against herbivores and plants, especially in leaves (Subramanian & al., 1990; Nicolson & Thornburg, 2007). The presence of these compounds was reported in foliar nectaries, floral and in the elaiophores of Neotropical Malpighiaceae (*D. pubipetala* Possobom & al., 2010; *Burdachia*, *Glandonia* and *Mcvaughia* Silva & al., unpubl. data).

The sudan tests did not detect lipids in the glands of the inflorescence in *A. longifolius*, unlike the Neotropical species *Galphimia brasiliensis* (Castro & al., 2001), *Diplopterys pubipetala* (Possobom & al., 2010) *Banisteriopsis* (Araújo & Meira, 2016), *Mcvughia*, *Burdachia* and *Glandonia* (Silva & al., unpubl. data). The rare studies in paleotropical genera shows up that the calicinal secretion in *H. benghalensis* is hydrophilic, composed mainly of sucrose, fructose and glucose and without lipophilic components (Ren & al., 2013) which characterizes these glands as nectaries. Thus, as reported for the calyx nectaries in *H. benghalensis* (Ren & al., 2013), in *A. longifolius* the secretory activity of bracteolar and calyx glands is early and persistent in the fruits, with no cuticle rupture occurring.

According to Vogel (1990) the calyx glands are the same of leaf and bracts, having a similar arrangement in the neo and paleotropical species. The homology between nectaries and elaiophores is quite discussed (Vogel, 1990; Castro & al., 2001; Araújo & Meira, 2016) and due to the nectaries frequency in the family, Vogel (1990) considers them to be plesiomorphics, from which elaiophores derived. Before long, in *Acridocarpus* the character would have undergone reversal, modifying the secretion to nectar (Vogel, 1990), an expected convergence to the lost of pollination syndrome from oil-bees pollination. Thus, the character maintenance and the secretion change would be a reflection of the selective pressure exerted by nectar consumers.

Morphoanomical data: evolutionary trends and phylogeographycal contributions

Vogel (1990) points out that calyx glands in the Paleotropic show marked differences, being smaller than elaiophores and positioned in the intersepal region, which would be the result of the fusion of neighboring glands. However, the variations observed herein indicate that in *Acridocarpus* calyx glands are mostly marginal. Exceptionally in *A. longifolius* and *A. zanzibaricus*, the median gland is intersepal and the sagittate-acute outline may be a residue of fusion. However, in *A. vanderystii*, although intersepal position of the glands suggest fusion, the outline is circular.

Only in this study, it was noted that the juxtaposed calyx glands of *A. monodii* and *A. spectabilis* are partially fused. In this way, the anatomy of the calyx gland of *A. spectabilis*, *A. monodii*, *A. longifolius* and *A. zanzibaricus* suggests an evolutionary sequence of related character states (Fig. 4). *Acridocarpus spectabilis*, *A. longifolius* and *A. zanzibaricus* were sampled in phylogeny (Davis & al., 2002a; Davis & Anderson, 2010), where the first two are placed at more basal nodes and *A. zanzibaricus* at derived nodes. Therefore, juxtaposed glands would be a plesiomorphic condition in relation to the medial glands fusion of the ventral sepals. On the other hand, the fusion of calyx glands in the Neotropics should be investigated since only in *Lophopterys* the calyx exhibits one gland by sepal (Anderson & Davis, 2001).

In addition , according to the phylogeny (Davis & al., 2002a; Davis & Anderson, 2010), in *Acridocarpus* there is a ventralisation of the position of glands in the calyx being a derived condition. According to Vogel (1990), among glandular paleotropical taxa, only in *Hiptage* there is a medial, intersepal and posterior gland, with a clear dorsalisation and reorientation of zygomorphy in relation to *Acridocarpus* and *Tristellateia*. This could indicate different selective pressures exerted by floral visitors. In the Neotropic, 10-glandular calyx is plesiomorphic and the restriction of

glands in the dorsal plane of the calyx is derived (Vogel, 1990), and the absence of glands in the ventral plane is a consequence of the positioning of the oil-bees in the flowers during the visitation (Vogel, 1990).

Although reproductive biology data in *Acridocarpus* are non-existent, the actuation of the calyx glands as nectaries and the poricidal anthers may be indicative of the change in the pollination syndrome. In the Neotropics, the reduction or loss of calyx glands is associated with secretion changes, the symmetry and the morphology of the anthers, which indicate a change in pollination syndrome (Anderson, 1979; Teixeira & Machado, 2000; Anderson, 2007; Anderson & Corso, 2007).

The data obtained reinforce the idea of calyx glands tend to disappear (Davis & al., 2002a; Zhang & al., 2010), since the Madagascar species studied have eglandular calyx. However, in these species, bracteolar glands were recorded, suggesting a correlation between glandular bracteole and glandular calyx. A similar relationship was detected in *A. austrocaledonicus*, occurring in New Caledonia, corroborating the phylogenetic proximity with Madagascar species indicated by Davis and Anderson (2010).

Although eglandular bracteole is common in *Acridocarpus* species with a higher number of calyx glands, no clear correlation was observed of floral glands in relation to the diversification of the genus from the aridification (Davis & al., 2002a). *Acridocarpus* adapted to the retraction of the tropical forests and its diversification occurred from West Africa towards the East, coinciding with the formation of the first savannas (Davis & al., 2002a). Phylogeny and the distribution of *Acridocarpus* suggest that this strain has diversified in response to aridification (Davis & al., 2002a). Species of East Africa and Arabian Peninsula have an eglandular bracteole, regardless the

calyx glands number. Thus, selective pressures maintained calyx glands, but the number does not seem to be a factor that offers some adaptive advantage.

CONCLUSIONS

In addition to the typical floral morphology of *Acridocarpus*, this work showed that the floral glands are nectaries, which probably act against herbivore attack. The bracteolar and calyx gland showed different patterns of distribution. Morphoanatomy of bracteole and calyx glands are typical in species from Madagascar and New Caledonia. In African species, the morphoanatomy indicates that the fusion of calyx glands would be a derived condition. Morphoanatomical patterns in Africa and Arabian Peninsula species does not seem to be associated with the history of diversification of the genus from the environment aridification. It is possible that the maintenance of the character has undergone selective pressure of the consumers, but the structures distribution may be variable. In view of the loss of the relation with the oil-bees due to the dispersion to the Palearctic, the floral glands, especially from calyx, are significantly more labile in the absence of this mutualism. Our results corroborate the importance of oil-bees mutualism in floral stereotyped morphology in the Neotropics and contribute to understanding of floral evolution in Malpighiaceae.

References

- Anderson, W.R.** 1979. Floral conservatism in Neotropical Malpighiaceae. *Biotropica* 11: 219--223.
- Anderson, W.R.** 1981. Malpighiaceae. The Botany of the Guayana Highland-Part XI. *Memorirs of the New York Botanical Garden* 32: 137--147.
- Anderson, W.R.** 1990. The origin of the Malpighiaceae-The evidence from morphology. *Memoirs of the New York Botanical Garden* 64: 210--224.
- Anderson, W. R.** 2001. Malpighiaceae. Pp. 82--185. *in*: Berry, P. E., Yatskievych, K. & B. K. (ed.), *Flora of the Venezuelan Guayana*. Holst, vol. 6.

- Anderson, W.R.** 2004. Malpighiaceae. Pp. 229--232 in: Smith, N. & al. (ed.), *Flowering Plants of the Neotropics*.
- Anderson, C.** 2007. Revision of *Galphimia* (Malpighiaceae). *Contr. Univ. Michigan Herb.* 25: 1--82.
- Anderson, W.R. & S. Corso.** 2007. *Psychopterys*, a new genus of Malpighiaceae from Mexico and Central America. *Contr. Univ. Michigan Herb.* 25: 113--135.
- Anderson, W.R. & C.C. Davis.** 2001. Monograph of *Lophopterys* (Malpighiaceae). *Contr. Univ. Michigan Herb.* 23: 83--105.
- Arènes, J.** 1946. Pp. 185. in: *Not. Syst.*, Humbert, E. (ed.) *Not. Syst.*, ed. Humbert.
- Arènes, J.** 1950. Famille 108—Malpighiacées. in: Humbert, H. (ed.) *Flore de Madagascar et des Comores*. Catalogue of Vascular Plants of Madagascar, Tropicos.org, Missouri Botanical Garden.
- Arènes, J.** 1955 ["1954"]. Un *Acridocarpus* nouveau de Madagascar. *Notul. Syst.* (Paris) 15: 4--5.
- Araújo, J.S. & Meira, R.M.S.A.** 2016. Comparative anatomy of calyx and foliar glands of *Banisteriopsis* C. B. Rob. (Malpighiaceae). *Acta Botanica Brasilica* 30: 112--123.
- Arumugasamy, K., Inamdar, J.A. & Subramanian, R.B.** 1989. Structure, ontogeny and secretion of oil secreting glands in *Hiptage acuminata*. *Wall. Current Science* 58: 260--261.
- Arumugasamy, K., Udaiyan, K., Manian, S. & Sugavanam, V.** 1993. Ultrastructure and oil secretion in *Hiptage sericeae* Hook. *Acta Societatis Botanicorum Poloniae* 62: 17--20.
- Baker-Méio, B. & Marquis, R.J.** 2012. Context-dependent benefits from ant-plant mutualism in three sympatric varieties of *Chamaecrista desvauxii*. *J Ecol* 100:242--252.
- Bentley, B.L.** 1977. Extra-floral nectaries and protection by pugnacious bodyguards. *Annual Review of Ecology and Systematics* 8: 407--427.
- Birnbaum, P. & J. Florence.** 2005. Validation d'*Acridocarpus monodii* Arènes & Jaeger ex Birnbaum & J. Florence, sp. nov. (Malpighiaceae). *Notes sur biologie. Adansonia* 27: 235--241.
- Buchmann, S.L.** 1987. The ecology of oil flowers and their bees. *Annual Review of Ecology and Systematics* 18: 343--369.
- Castro, M.A, Vega, A.S. & Mulgura, M.E.** 2001. Structure and ultrastructure of leaf and calyx glands in *Galphimia brasiliensis* (Malpighiaceae). *American Journal of Botany* 88: 1935--1944.
- Clark, S. G.** 1981. *Staining procedures* Williams & Wilkins, Baltimore.

- Coutinho, Í.A.C., Valente, V.M.M. & Meira, R.M.S.A.** 2010. Ontogenetic, anatomical and histochemical study of the extrafloral nectaries of *Sapium biglandulosum* (Euphorbiaceae). *Australian Journal of Botany* 58: 224--232.
- Davis, C.C., Bell, C.D., Fritsch, P.W. & Mathews, S.** 2002a. Phylogeny of *Acridocarpus* - *Brachylophon* (Malpighiaceae): implications for Tertiary tropical floras and Afroasian biogeography. *Evolution* 56:2395--2405.
- Davis, C.C., Bell, C.D., Mathews, S. & Donoghue, M.J.** 2002b. Laurasian migration explains Gondwanan disjunctions: evidence from Malpighiaceae. *Proceedings of the National Academy of Sciences* 99: 6833--6837.
- Davis, C.C. & Anderson, W.R.** 2010. A complete generic phylogeny of Malpighiaceae inferred from nucleotide sequence data and morphology. *American Journal of Botany* 97:2031--2048
- Davis, C.C., Schaefer, H., Xi, Z., Baum, D.A., Donoghue, M.J. & Harmon L.J.** 2014. Long-term morphological stasis maintained by a plant-pollinator mutualism *Proceedings of the National Academy of Sciences*. 111: 5914--5919.
- Doorn-Hoekman, H. van.** 1975. *Rhinopteryx* Niedenzu and *Acridocarpus* (G.Don) Guill. et. Perr. (Malpighiaceae) united. *Acta Bot. Neerl.* 24: 69--82.
- Elias, T.S.** 1980. Foliar nectaries of unusual structure in *Leonardoxa africana* (Leguminosae) an African obligate myrmecophyte. *American Journal of Botany* 67: 423--425.
- Elias, T.S.** 1983. Extrafloral nectaries: their structure and distribution. Pp. 174--203 in: Bentley, B. & Elias, T. (eds.), *The Biology of Nectaries*. New York: Columbia University Press.
- Elias, T.S. & Gelband, H.** 1976. Morphology and anatomy of floral and extrafloral nectaries in *Campsis* (Bignoniaceae). *American Journal of Botany* 63: 1349--1353.
- Endress, P.K.** 1996. *Diversity and evolutionary biology of tropical flowers*. Cambridge: University Press. 511p.
- Fahn, A.** 1979. *Secretory Tissues in Plants*. Academic Press, London.
- Faegri, K. & Van der Pijl, L.** 1976. *The principles of pollination ecology*. Pergamon Press, Oxford
- Fisher, D.B.** 1968. Protein staining of ribboned epon sections for light microscopy. *Histochemistry and Cell Biology* 16: 92--96.
- Guillemin, J.A., Perrottet, S. & Richard, A.** 1831. Florae Senegambiae tentamen. Malpighiaceae. 29: 123--124.
- González-Teuber, M & Heil, M.** 2009. The role of extrafloral nectar amino acids for the preferences of facultative and obligate ant mutualists. *J. Chem. Ecol.* 35:459--68
- Hably, L. & Manchester, S.R.** 2000. Fruits of *Tetrapterys* (Malpighiaceae) from the Oligocene of Hungary and Slovenia. *Review of Palaeobotany and Palynology* 111: 93-101.

- Heil, M.** 2015. Extrafloral nectar at the plant–insect interface: a spotlight on chemical ecology, phenotypic plasticity, and food webs. *Annu Rev Entomol* 60:213--232.
- Heil, M.** 2011. Nectar: generation, regulation and ecological functions. *Trends Plant Sci.* 16:191--200
- Hutchinson, J. & Dalziel, J. M.** 1954. Pp. 350--354 in: R. W. J. Keay and F. N. Hepper Flora of West tropical Africa. Malpighiaceae 1958.
- Johansen, D.A.** 1940. *Plant Microtechnique*. New York: Mc Graw-Hill Book Co. Inc.
- Koptur, S.** 1992. Extrafloral nectary mediated interactions between insects and plants. Pp. 81--129 in: Bernays, E. (ed.) *Insect–plant interactions*. Boca Raton, Florida: CRC Press.
- Lanza, J.** 1991. Response of fire ants (Formicidae: *Solenopsis invicta* and *S. geminata*) to artificial nectars with amino acids. *Ecol. Entomol.* 16, 203--210.
- Launert, E.** 1995. Malpighiaceae. Pp. 257--263. in: Edwards, S. & al. (eds.), *Flora of Ethiopia and Eritrea*.
- Lobreau-Callen, D.** 1989. Les Malpighiaceae et leurs pollinisateurs. Coadaptation ou coevolution. *Bulletin du Muséum National d'Histoire Naturelle, Adansonia* 1: 79--94.
- McKey, D.** 1979. The distribution of secondary compounds within plants. Pp. 55--133 in: Rosenthal, G.A. & Janzen, D.H. (eds.), *Herbivores: Their Interactions with Secondary Plant Metabolites*. New York: Academic Press.
- McManus, J.F.A.** 1948. Histological and histochemical uses of periodic acid. *Stain Technology* 23: 99--108.
- Michener, C.D.** 2000. *The bees of the world.*, Baltimore, London: The John Hopkins University Press.
- Morellato, L.P.C. & Oliveira, P.S.** 1994. Extrafloral nectaries in the tropical tree *Guarea macrophylla* (Meliaceae). *Canadian Journal of Botany* 72: 157--160.
- Neff, J.L. & Simpson, B.B.** 1981. Oil-collecting structures in the Anthoporidae (Hymenoptera): morphology, function and use in systematics. *Journal of the Kansas entomological society* 54(1): 95--123.
- Nepi, M., Soligo, C., Nocentini, D., Abate, M. & Guarnieri, M.** 2012. Amino acids and protein profile in floral nectar: much more than a simple reward. *Flora* 207:475--81
- Nepi, M., von Aderkas, P., Wagner, R., Mugnaini, S., Coulter, A. & Pacini, E.** 2009. Nectar and pollination drops: How different are they? *Ann. Bot.* 104:205--19
- Ness, J.H., Morris, W.F. & Bronstein, J.L.** 2009 For ant-protected plants, the best defense is a hungry offense. *Ecology* 90:2823--2831
- Nicolson, S.W & Thornburg, R.W.** 2007. Nectar chemistry. Pp. 215--63 in: Nicolson, S.W, Nepi, M. & Pacini, E. (eds.), *Nectaries and Nectar*. Dordrecht: Springer.

- Niedenzu, F.** 1921. De genere Acridocarpo. Arbeiten aus dem botanischen Institut der Staatlichen Akademie (vorm. Kgl. Lyceum hosianum). Braunsberg 1--20.
- Niedenzu, F.** 1928. Malpighiaceae. In: Das Pflanzenreich, A. Engler, IV. 141: 1--870. Part 1 (Heft 91; pp.1--246); part 2 (Heft 93; pp. 247--572); part 3 (Heft 94; pp. 573--870).
- Oliver, D.** 1868. Malpighiaceae. Pp. 276--282 in: Oliver, D. (ed.) *Flora of tropical Africa*.
- O'Brien, T.P., Feder, N. & McCully, M.E.** 1964. Polychromatic staining of plant cell walls by toluidine blue O. *Protoplasma* 59: 368--373.
- Paiva, E.A.S., Buono, R.A. & Delgado, M.N.** 2007. Distribution and structural aspects of extrafloral nectaries in *Cedrela fissilis* (Meliaceae). *Flora* 202: 455--461.
- Park, S. & Thornburg, R. W.** (2009). Biochemistry of nectar proteins. *J. Plant Biol.* 52: 27--34. doi: 10.1007/s12374-008-9007-5
- Pearse, A.G.E.** 1980. *Histochemistry theoretical and applied* (4th ed.) Churchill Livingston, Edinburgh, UK.
- Possobom, C.C.F., Guimarães, E. & Machado, S.R.** 2010. Leaf glands act as nectaries in *Diplopterys pubipetala* (Malpighiaceae). *Plant Biology* 12: 863--870.
- Possobom, C.C.F., Guimarães, E. & Machado, S.R.** 2015. Structure and secretion mechanisms of floral glands in *Diplopterys pubipetala* (Malpighiaceae) and a neotropical species. *Flora* 211: 36--39.
- Raven, P. H. & Axelrod, D. I.** 1974. Angiosperm biogeography and past continental movements. *Ann. Mo. Bot. Gard.* 61: 39--637.
- Ren, M., Zhong, Y. & Song, X.** 2013. Mirror-image flowers without buzz pollination in the Asian endemic *Hiptage benghalensis* (Malpighiaceae). *Botanical Journal of the Linnean Society* 173: 764--774.
- Roshchina, V.V. & Roshchina, V.D.** 1993. *The excretory function of higher plants*. Berlin, Germany: Springer-Verlag.
- Sigrist, M.R. & Sazima, M.** 2004. Pollination and reproductive biology of twelve species of neotropical Malpighiaceae: stigma morphology and its implications for the breeding system. *Annals of Botany* 94: 33--41.
- Simpson, B.B. & Neff, J.L.** 1981. Floral rewards: alternatives to pollen and nectar. *Annals of the Missouri Botanical Garden* 68: 301--322.
- Smith, F.H. & Smith, E.C.** 1942. Anatomy of the inferior ovary of *Darbya*. *American Journal of Botany* 29: 464--471
- Stephenson, A.G.** 1982. The role of the extrafloral nectaries of *Catalpa speciosa* in limiting herbivory and increasing fruit production. *Ecology* 63: 663--669.

- Subramanian, R.B., Arumugasamy, K. & Inamdar, J.S.** 1990. Studies in secretory glands of *Hiptage sericea* (Malpighiaceae). *Nordic Journal of Botany* 10:57--62.
- Sudd, J.H. & Sudd, M.E.** 1985. Seasonal changes in the response of wood-ants (*Formica lugubris*) to sucrose baits. *Ecol. Entomol.* 10: 89--97.
- Taylor, D.W., & Crepet, W.L.** 1987. Fossil floral evidence of Malpighiaceae and an early plant-pollinator relationship. *Amer. J. Bot.* 74: 274--286.
- Teixeira, L.A.G. & Machado, I.C.** 2000. Sistemas de polinização e reprodução de *Byrsonima sericea* DC (Malpighiaceae). *Acta Botanica Brasilica* 14:347--357.
- Thulin, M.** 1993. Malpighiaceae. Pp. 260--264 in: Thulin, M. (ed.), *Flora of Somalia*.
- Vinson, S.B, Williams, H.J, Frankie, G.W & Shrum, G.** 1997. Floral lipid chemistry of *Byrsonima crassifolia* (Malpighiaceae) and a use of floral lipid by *Centris* bees (Hymenoptera: Apidae). *Biotropica* 29: 76--83.
- Vogel, S.** 1990. History of the Malpighiaceae in the light of Polination Ecology. *Memoirs of the New York Botanical Garden* 55: 130--142.
- Wilczek, R.** 1955. Novitates africanæ I (Malpighiaceae et Linaceae). Bull. Jard. Bot. État. Bruxelles 25: 303--313.
- Wilczek, R.** 1958. Malpighiaceae. Pp. 214--234. in: *Flore du Congo-Belge et du Ruanda-Urundi*.
- Zhang, W., Kramer, E.M. & Davis, C.C.** 2010 Floral symmetry genes and the origin and maintenance of zygomorphy in a plant-pollinator mutualism. *Proceedings of the National Academy of Sciences* 107:6388--6393.
- Zhang, W., Steinmann, V.W., Nikolov, L., Kramer, E.M. & Davis, C.** 2013 Divergent genetic mechanisms underlie reversals to radial floral symmetry from diverse zygomorphic flowered ancestors. *Front Plant Sci.* 4:302.

Table 1: Distribution of glands on inflorescences in *Acridocarpus*, occurrence and number of glands in the species analyzed.

Species	Bracteole gland	Calyx gland	Literature premise		Geographical distribution	
			Bracteolar gland	Calyx gland		
<i>denophorus</i>	2	-	1	-	Niedenzu 1921, 1928; Arènes 1950	Madagascar
<i>A. excelsus</i>	2	-	-; 1	-	Niedenzu 1921,1928; Arènes 1950	Madagascar
<i>A. humbertii</i>	2	-	1	-	Arènes 1955	Madagascar
<i>A. perrieri</i>	2	-	1	-	Humberti 1945; Arènes 1950	Madagascar
<i>A. vivy</i> var. <i>glandulifera</i>	2	-	1	-	Arènes 1950	Madagascar
<i>A. austrocaledonicus</i>	1	-	-	-	Niedenzu 1921, 1928	New Caledonia
<i>A. alternifolius</i>	-	5	-	1-4; 2	Oliver 1868; Niedenzu 1921,1928; Hutchinson & Dalziel 1954	West of Africa
<i>A. camerunensis</i>	-	4	-	3-4	Niedenzu 1921,1928	West of Africa
<i>A. chevalieri</i>	-	3	-	2-3	Morot 1909; Hutchinson & Dalziel 1954	West of Africa
<i>A. chloropterus</i>	-	3	-	2-3	Oliver1868; Niedenzu 1921,1928; Launert 1963, 1968	West of Africa
<i>A. congolensis</i>	1	2	-	-	Niedenzu 1921,1928	West of Africa
<i>A. katangensis</i>	1	2(3)	-; 1	2; 2-3	Niedenzu 1921, 1928; Launert 1963	West of Africa
<i>A. longifolius</i>	Irrelictual	1	-	1	Niedenzu 1921,1928; Hutchinson & Dalziel 1954	West of Africa
<i>A. macrocalyx</i>	-	2(4)	-	2; 2-3	Niedenzu 1921,1928; R. Wilczek (1958)	West of Africa
<i>A. monodii</i>	-	4	-	2	Birnbaum & J.Florence 2005	West of Africa
<i>A. obovatus</i>	-	2	-	2	C. Davis 2002	West of Africa
<i>A. plagiopterus</i>	-	2	-	2; 3	Guillemin & Perrottet 1831; Oliver1868; Niedenzu 1921,1928;	West of Africa
<i>A. prasinus</i>	1	4	-; 1	-; 2-3	Niedenzu 1921,1928; Launert 1968	West of Africa
<i>A. smeathmanni</i>	1	3	1	3; 2 (3-4); 2-3 (4)	Oliver1868; Niedenzu 1921, 1928; Launert 1968,	West of Africa
<i>A. spectabilis</i>	-	4	-	3; 2-4	Niedenzu 1921, 1928; Chipp & Dawe 1922; Doorn-Hoekman 1975	West of Africa
<i>A. vanderystii</i>	-	5	+	2-3	Wilczek 1955	West of Africa
<i>A. alopecurus</i>	1	3(5)	-; 1	3(5)	Niedenzu 1921,1928; Launert 1968	East of Africa
<i>A. natalitius</i>	-	3	-	3; 4	Niedenzu 1921, 1928; Launert 1963,	East of Africa
<i>A. zanzibaricus</i>	-	3	-	2; 3; 2-3	Oliver1868; Niedenzu 1921, 1928; Launert 1968	East of Africa
<i>A. socotranus</i>	-	2	-	2	Niedenzu 1921, 1928	Arabian peninsula
<i>A. orientalis</i>	-	5	-	3-4; 5	Niedenzu 1921,1928; Thulin 1993	Arabian peninsula

(-) eglandular.

Appendix 1

List of material used of *Acridocarpus* and voucher information of MNHN herbarium (P).

Species	Collector and number
<i>Acridocarpus adenophorus</i> A. Juss.	Capuron 8883 (P)
<i>Acridocarpus alopecurus</i> Sprague	Haerdi 447 (P), Sacleux 779 (P)
<i>Acridocarpus alternifolius</i> (Schum. & Thonn.) Nied.	Meikle 845 (P), Breteler 5322 (P)
<i>Acridocarpus austrocaledonicus</i> Baill.	McPherson 3306 (P)
<i>Acridocarpus camerunensis</i> Nied.	Le Testu 7800 (P)
<i>Acridocarpus chevalieri</i> Sprague	Jaeger 3461 (P)
<i>Acridocarpus chloropterus</i> Oliv.	Schlieben 2427 (P)
<i>Acridocarpus congolensis</i> Sprague	Chevalier 28381 (P)
<i>Acridocarpus excelsus</i> A. Juss.	Schatz 2984 (P)
<i>Acridocarpus humbertii</i> Arènes	Phillipson 5989 (P)
<i>Acridocarpus katangensis</i> De Wild.	Gathy 1950 (P)
<i>Acridocarpus longifolius</i> (G. Don) Hook. F.	Bos 4924 (P), Chevalier 26184 (P)
<i>Acridocarpus macrocalyx</i> Engl.	Letouzey 11775 (P), Carvalho 3455 (P)
<i>Acridocarpus monodii</i> Arènes & Jaeger ex Birnbaum & J.Florence	Griaule 60 (P), Birnbaum 615 (P)
<i>Acridocarpus natalitius</i> A. Juss.	Phillipson 3807 (P)
<i>Acridocarpus obovatus</i> (G.Don) C.Cav.	Chevalier 20357 (P)
<i>Acridocarpus orientalis</i> A. Juss.	Popov 706 (P)
<i>Acridocarpus perrieri</i> Arènes	Rakotondrajaona 397 (P)
<i>Acridocarpus plagiopterus</i> Guill. & Perr.	Chevalier 14767 (P)
<i>Acridocarpus prasinus</i> Exell	Sita 3148 (P)
<i>Acridocarpus smeathmannii</i> (DC.) Guill. & Perr.	Leeuwenberg 2409 (P)
<i>Acridocarpus socrotanus</i> Oliv.	Smith 204 (P)
<i>Acridocarpus spectabilis</i> Doorn-Hoekm.	Valenza 420 (P), Birnbaum 751 (P)
<i>Acridocarpus vanderystii</i> R.Wilczek	Koechlin 6027 (P), Chevalier 11097 (P)
<i>Acridocarpus vivy</i> Arènes	Schatz 4165 (P)
<i>Acridocarpus zanzibaricus</i> A. Juss.	Zhang 154 (P)

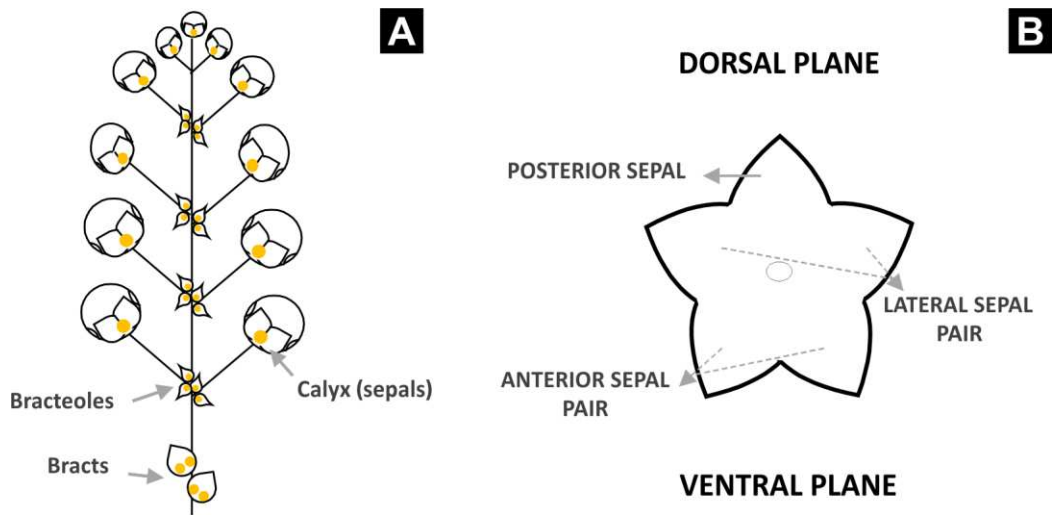


Figure 1: Diagram from hypothetical *Acridocarpus*. **A.** Inflorescence type representing a raceme of cincinnis (1-flowered) with bracteal, bracteolar and calyx glands (yellow circles). **B.** Orientation of calyx whorl units in frontal view.

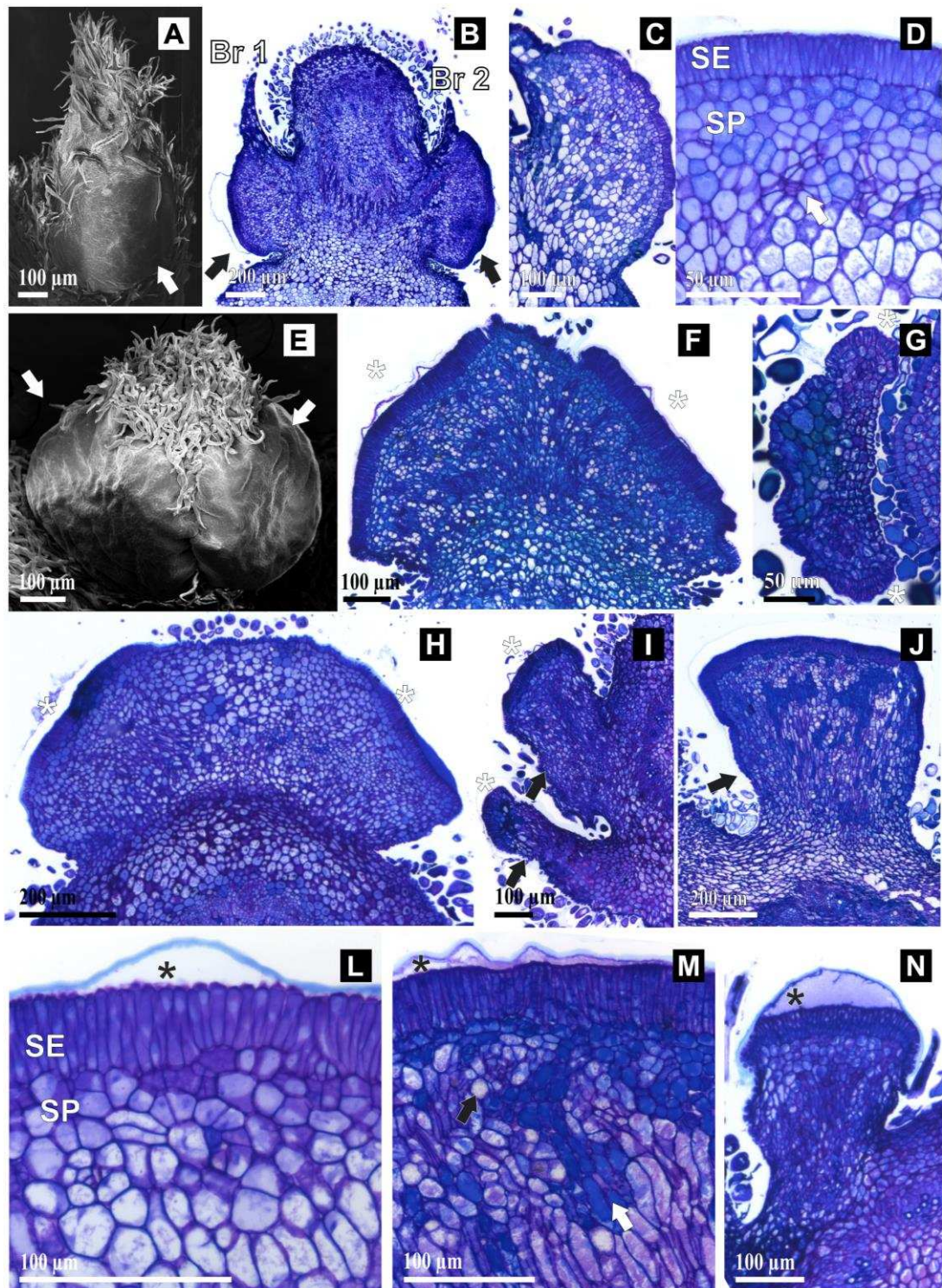


Figure 2: Morphoanatomy of bracteolar glands. **A.** SEM image of one-glandular bracteole in *A. smeathmanni*, white arrow: gland. **B.** bracteole pair of floral pedicel in *A. smeathmanni*, showing the basilaminar position of one gland in each bracteole of the floral pedicel, Br: bracteole, black arrows: glands. **C.** Transversal view of bracteolar gland in *A. alopecurus*. **D.** Secretory epidermis (SE) and secretory parenchyma (SP) of bracteolar gland in *A. katangensis*, white arrow: vascularization. **E.** SEM image of two-glandular bracteole in *A. perrieri*, white arrows: glands. **F-H.** transversal view of two-glandular bracteole, showing medially position of glands at the bracteole in *A. perrieri*, *A. vivy* and *A. excelsus*, respectively, white asterisks: glands. **I., J.** Short-stalked glands in *A. humbertii* and *A. adenophorus* respectively, black arrows: stalk. **L.** Transversal view of bracteole gland in *A. prasinus*, *A. adenophorus* and *A. humbertii*, respectively, black asterisks: subcuticular space, white arrow: cristals, black arrow: cells filled with phenolic compounds.

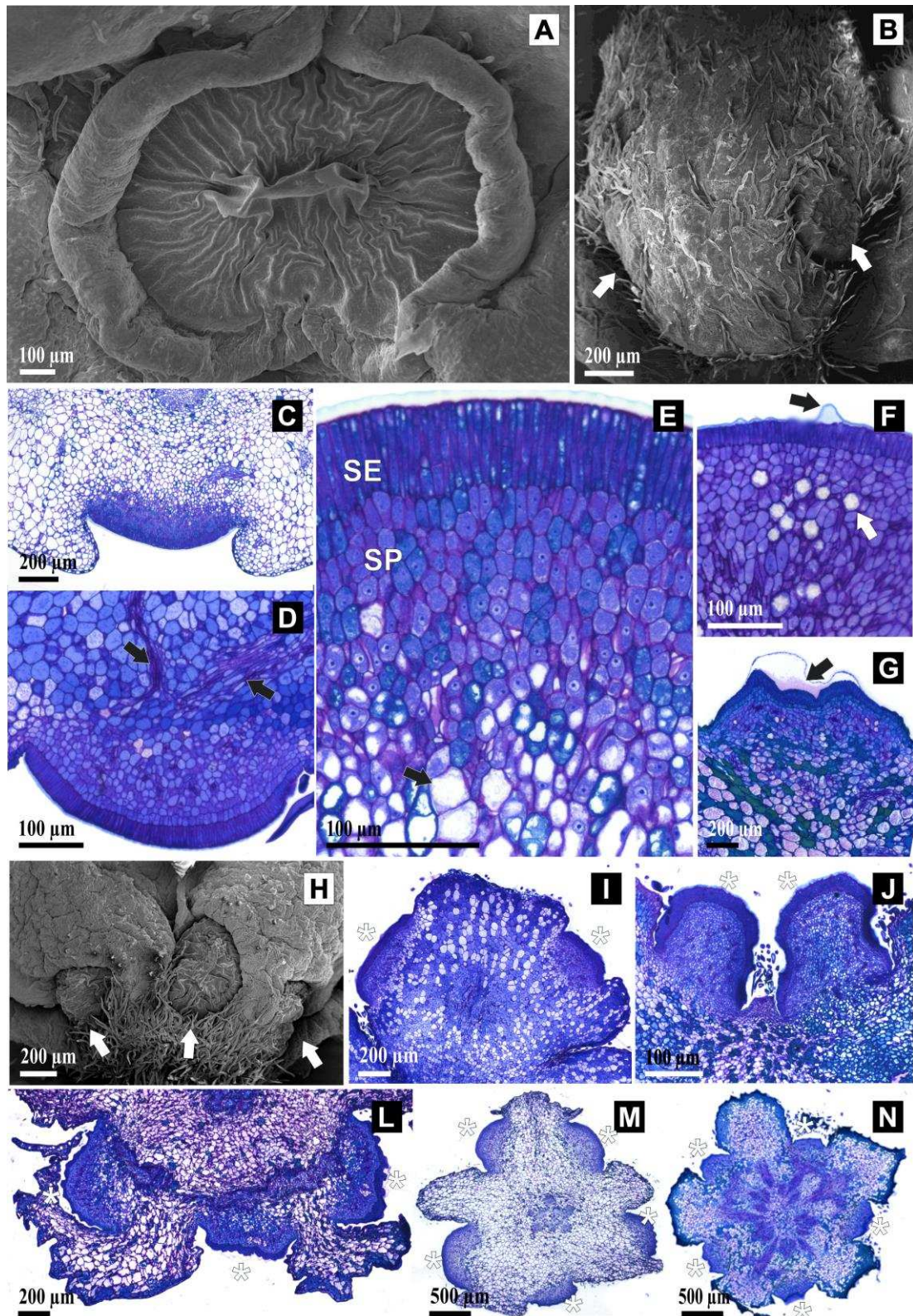


Figure 3: Morphoanatomy of calyx glands. **A, B.** Calyx glands at SEM images, immersed and sessile in *A. longifolius* and *A. smeathmanni*, respectively; white arrows: glands. **C.** and **D.** Transversal view of immersed and sessile glands, in *A. longifolius* and *A. smeathmanni*, respectively; black arrows: vascularization. **E.** Secretory epidermis (SE) and secretory parenchyma (SP) of calyx gland in *A. longifolius* black arrow: crystal. **F, G.** subcuticular space in *A. spectabilis* and *A. vanderystii*, respectively; black arrow: subcuticular space and white arrow: crystal. **H.** SEM image of *A. alopecurus*, showing marginal calyx glands, white arrow: glands. **I-N:** different patterns of calyx glands distribution in *A. socrotanus*, *A. prasinus*, *A. alopecurus*, *A. alternifolius* and *A. vanderystii*, respectively; white asterisks: glands.

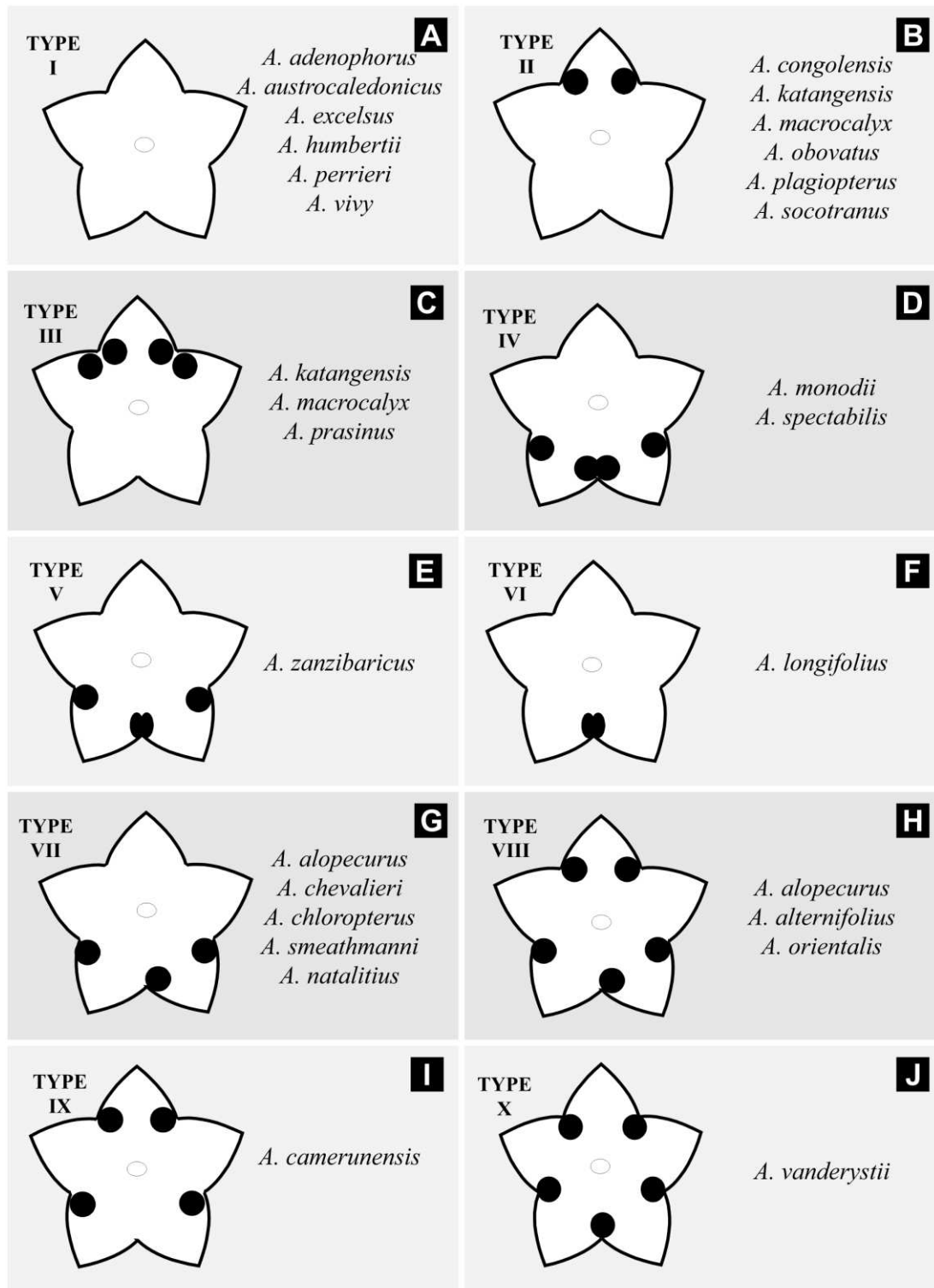


Figure 4: Diagram in frontal view from the morphoanatomical patterns of calyx glands distribution. **A:** Type I, eglandular; **B:** Type II, two marginal glands in posterior sepal; **C:** Type III, two marginal glands in posterior sepal and one marginal gland at posterior side in each one of lateral sepals; **D:** Type IV, each anterior sepal bear two glands; **E:** Type V, is distinct of type IV due to have one anterior gland in a intersepal position; **F:** Type VI, one gland in a intersepal portion of the anterior sepal pair; **G:** Type VII, two marginal glands in one of anterior sepals, add one marginal gland in the other anterior sepal; **H:** Type VIII, two marginal glands in posterior sepal, two marginal glands in one of anterior sepals, one marginal gland in the other anterior sepal; **I:** Type IX, two marginal glands in posterior sepal and one marginal gland at posterior side of each anterior sepal; **J:** Type X, one gland in each intersepal portions.

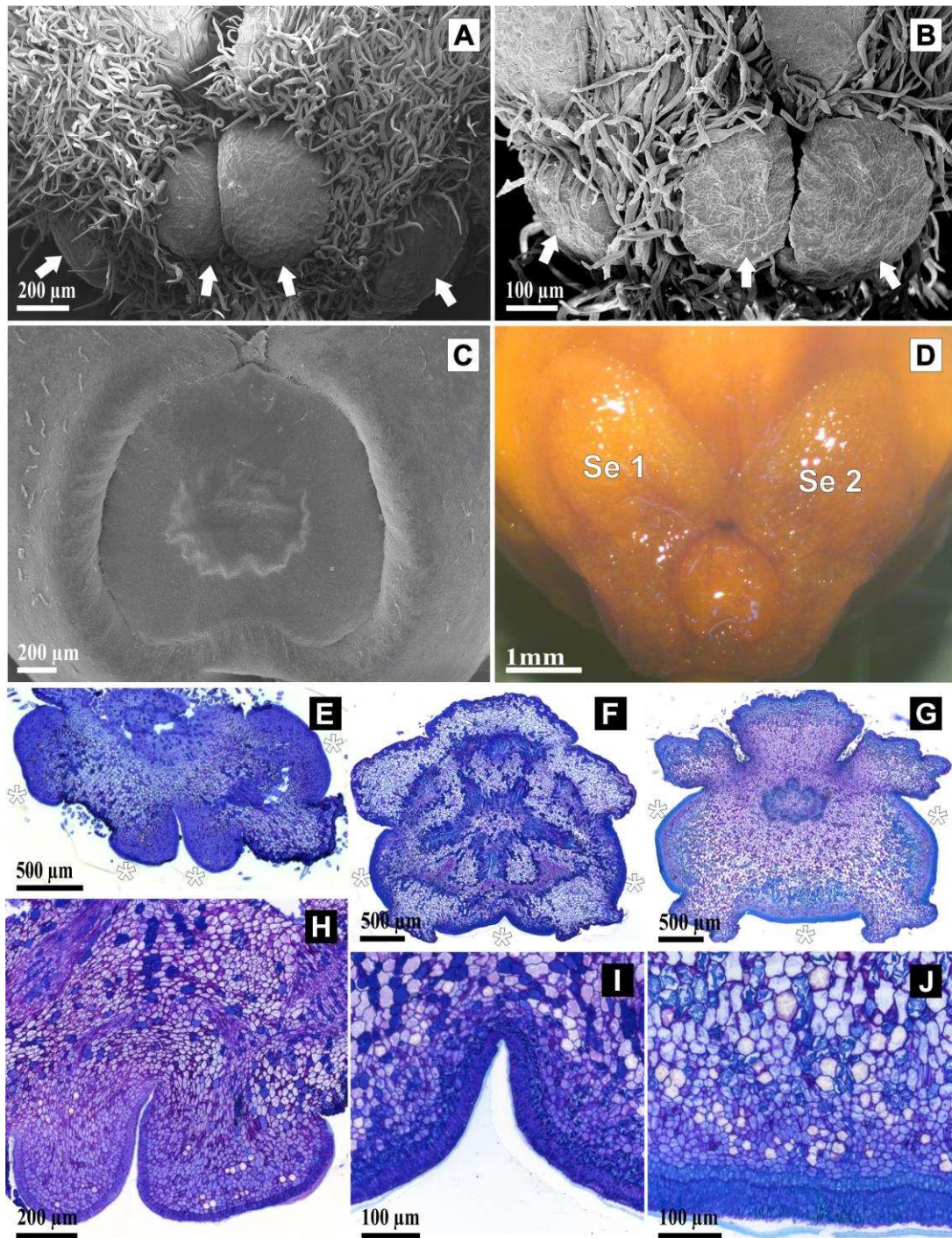


Figure 5: A-D. Morphological appearance of individualized glands in SEM images and stereomicroscopy image in *A. spectabilis*, *A. monodii*, *A. longifolius* and *A. zanzibaricus* respectively; black arrows: calyx glands; Se: sepal. C-D. sagittate-acute outline of calyx gland. E-G. distribution of calyx glands in *A. spectabilis*, *A. monodii* and *A. zanzibaricus* respectively, white asterisks: glands. H-J. different degrees of glandular fusion: H. secretory epidermis continuous in neighbors glands in *A. spectabilis*. I. Epidermis and secretory parenchyma shared in *A. monodii*. J. Completely sharing of secretory tissues in *A. zanzibaricus*.

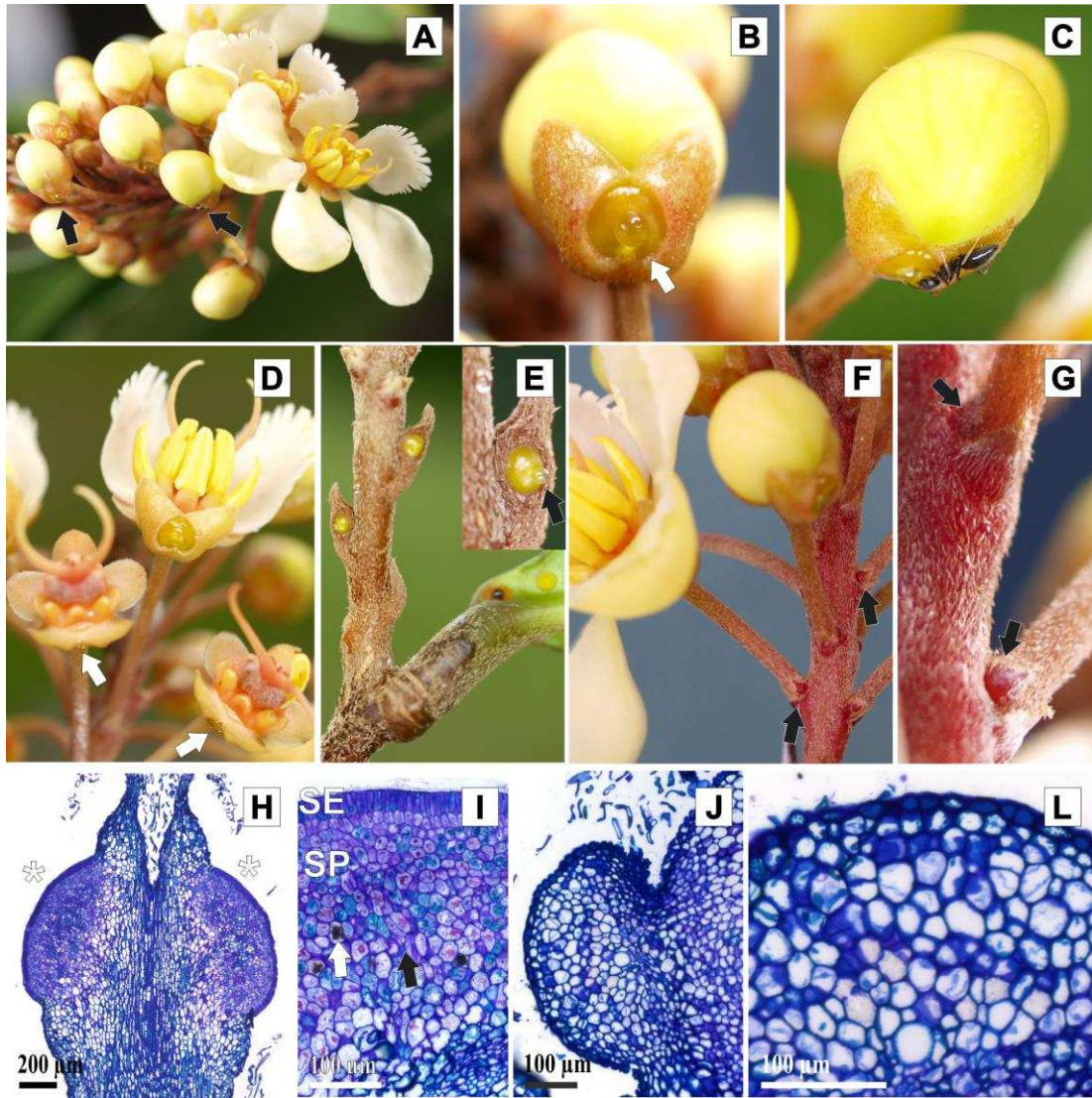


Figure 6: Floral glands in *Acridocarpus longifolius*. **A, B.** General view of inflorescence and calyx gland, black arrows: nectar drop. **C.** Consuming of nectar by an ant visitor. **D.** Nectar available in calyx glands at fruiting period. **E.** General view of the base of inflorescence, showing glandular bracts, bracteal gland detail, black arrow: nectar drop. **F.** View of the axis of inflorescence, showing bracteoles at the floral pedicel, black arrows: glands. **G.** Bracteole glands detail. **H.** Anatomical cross of bract, white asterisks: glands. **I.** secretory tissues of bracteal gland, SE and SP: secretory epidermis and secretory parenchyma respectively; white arrow: crystal, black arrow: vascularization. **J.** Bracteole gland. **L.** Detail of bracteole gland, showing epidermis and parenchyma not secretory.

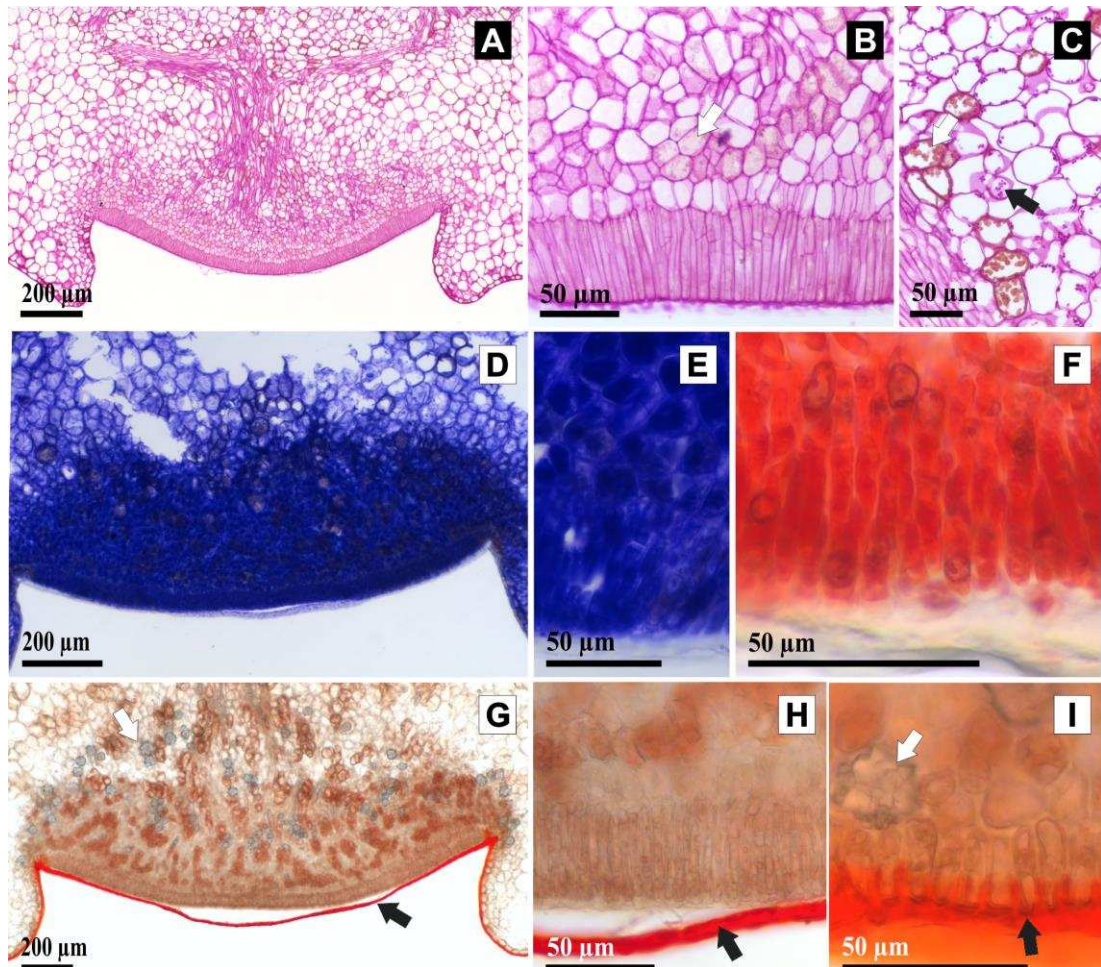


Figure 7: Histochemistry of calyx gland in *A. longifolius*. **A-C.** Testing polyssacharides, **A.** positive reaction to PAS, white arrow: cell filled with phenolic compounds; black arrow: starch grains. **D, E.** Test for protein, **D and E,** positive reaction to Blue Comassie test, **F.** positive reaction to Xylidine ponceae test. **G-I.** Testing total lipids, note the negative reaction to Sudan red test. white arrows: crystals; black arrows: cuticle (G and H) and thickness of epidermical wall (I).

CAPÍTULO IV

The hidrocoric Amazonian genus *Glandonia* (Malpighiaceae): new records, morphoanatomy and taxonomic contributions

Redação de acordo com as normas de submissão do periódico: *Phytotaxa, a rapid international journal for accelerating the publication of botanical taxonomy*

The hidrocoric Amazonian genus *Glandonia* (Malpighiaceae): new records, morphoanatomy and taxonomic contributions

ISABEL REIS e SILVA^{a, b*}, ANDRÉ MÁRCIO AMORIM^{c, d} & RENATA MARIA STROZI ALVES MEIRA^a

^a*Universidade Federal de Viçosa, UFV, Departamento de Biologia Vegetal, 36570-900, Viçosa, Minas Gerais, Brazil*

^b*Universidade Federal do Amazonas, UFAM, Instituto de Ciências Exatas e Tecnologia, 69103-128, Itacoatiara, Amazonas, Brazil*

^c*Universidade Estadual de Santa Cruz, UESC, Departamento de Ciências Biológicas, 45662-900, Ilhéus, Bahia, Brazil*

^d*Centro de Pesquisas do Cacau, Herbário CEPEC, 45650-970, Itabuna, Bahia, Brazil*

*Corresponding author. Mailing address: ireis@ufam.edu.br

Abstract

Glandonia is a scarce and small genus with only three species confined to the Amazonian rainforest. The herbarium register of *G. prancei* has restricted to small collection of the '70s, when this species was described. Such species was recently rediscovered in the collection of *G. macrocarpa*, which has been recorded in some locations from Municipality Manaus, Brazil. *Glandonia williamsii* was not collected in Brazil at the last three decades. The lack of understanding about such species resulted in some identification mistakes over the years. The taxonomy of the genus was reexamined in this work, which showing notes on morphoanatomy, distribution and phenology, based on exhaustive field survey and examination of herbarium specimens. In front of the similarity of the fruits, new morphoanatomical characters such as from leaf and floral glands are provided to better diagnose of these species.

Keywords: Malpighiales, *mcvaughioide* clade, taxonomy

Resumo

Glandonia é um gênero raro e pequeno com três espécies, típico da Amazônia. Os registros de *G. prancei* em herbários estava restrito a uma pequena coleção dos anos 70, quando a espécie foi descrita. Esta espécie foi recentemente redescoberta na coleção de *G. macrocarpa*, que foi coletada em algumas localidades do município de Manaus, Brasil. *Glandonia williamsii* não foi coletada no Brasil nas últimas três décadas. A falta de conhecimento sobre as espécies de *Glandonia* resultou em alguns erros de identificação ao longo dos anos. Neste trabalho, a taxonomia do gênero foi revisitada, trazendo notas da morfoanatomia, distribuição geográfica e fenologia, baseadas em exaustivo trabalho de campo e análise de espécimes herborizados. Diante da similaridade dos frutos, novos caracteres morfoanatômicos como das glândulas foliares e florais proporcionaram melhorias na diagnose das espécies.

Palavras-chave: Malpighiales, clado *mcvaughioide*, taxonomia

Introduction

Although the occurrence of Malpighiaceae is concentrated in open environments, such as Brazilian "Cerrado" (Mamede *et al.* 2013), some species are distributed in different environments and in higher elevations (Anderson 1981). *Glandonia* Griseb. (Grisebach 1858: 23) is an endemic genus from Amazonian rainforest (Anderson 1981) where occur in wetlands subjected to oscillating water levels "várzea" and "igapó" and in upland forest "terra firme". Wetlands floodplains are environments semi aquatic and typical from the margins of the white and black rivers, which in rainy season, present extensive flooded areas (Junk *et al.* 2011). The "terra firme" forest is a complex patchwork of environments, including lowlands called "baixio" that occur closed to streams ("igarapés") and is subject to temporary shallow flood during the rainy season (Hopkins 2005).

Glandonia is comprised of only three species: *G. macrocarpa* Griseb. (Grisebach 1858: 24), *G. prancei* W.R. Anderson (Anderson 1981: 139) and *G. williamsii* Steyermark. (Steyermark 1952: 288). Since the *Glandonia* species presents a restricted occurrence at different rivers of Amazon basin, Anderson (1981) proposed a geographical disjunct distribution.

In Neotropical species of Malpighiaceae the floral morphology is highly stereotyped (Anderson 1990) and the diversity of fruit has been used by a long time to the taxonomy (Davis & Anderson 2010). However, according Davis & Anderson (2010), the evidence taxonomic provides by fruits, seems to be the reason for taxonomic problems in traditional systems, for example, Niedenzu (1928) and Hutchinson (1967) treatments (Davis & Anderson 2010). The most of species of Malpighiaceae is wing-fruited, which are wind-dispersed (Anderson 1977; Davis &

Anderson 2010). *Glandonia* is one of the exceptions since the species bear not winged and not schizocarpic fruits, probably adapted for water dispersion (Anderson 1981).

In *Glandonia*, the three species exhibit morphological similarities, mainly between *Glandonia macrocarpa* and *G. prancei* (Anderson 1981). Thus, in this study the taxonomy of *Glandonia* were revisited and morphoanatomical data were described in order to improve informations about this genus, providing more accurate diagnosis to avoid identification mistakes, as has been notice. The fieldwork efforts allowed finding unnotice records and reevaluating the geographic distribution pattern of all *Glandonia* species.

Material and Methods

Taxonomic studies

This study was based on the analysis of collections deposited in the following herbaria: IAN, INPA, MG and VIC in Brazil; COL in Colombia; GUYN, PORT and VEN in Venezuela; P in France (acronyms according to Thiers 2016). All specimens and type collections were analyzed in stereomicroscope or through images available at the JSTOR Global Plants website (<http://plants.jstor.org/>) and Reflora website (<http://herbariovirtualreflora.jbrj.gov.br/>).

Fieldwork was carried out at different environments in Brazil (Fig. 1A and B) where fresh samples were collected and field observations were recorded during the vegetative, flowering and fruiting periods (Fig. 1C). Vouchers materials were deposited at the herbarium VIC of the Universidade Federal de Viçosa-UFV. Morphological characters were observed using a stereomicroscopy (Stemi 2000-C Zeiss, Gottingen, Germany) equipped with a digital camera (AxioCam ERc; Zeiss, Gottingen, Germany). The terminology adopted was based on Anderson (1981, 1990).

Map was elaborated using ArcGIS (ESRI 2010), according to geographical coordinates from herbarium, from Google Earth (Online version) and from GPS (Global Positioning System) coordinates taken in fieldworks. The conservation assessment of the species was based on IUCN Red List categories and criteria (IUCN 2012) and the Extent of Occurrence (EOO) and Area of Occupancy (AOO) values (Bachman *et al.* 2011) were found using the GeoCAT tool (<http://geocat.kew.org/>).

Leaf and floral anatomy studies

Leaf and floral samples of *Glandonia macrocarpa* (Silva *et al.* 287, 288), *G. prancei* (Silva *et al.* 298, 299) and *G. williamsii* (Silva *et al.* 290) were prepared for light microscopy and scanning electron microscopy following the standard methods. Fragments of leaf and whole posterior petals were cleared according to Vasco *et al.* (2014) while dissociation of leaf epidermis followed Franklin (1945). Such analysis were conducted at the “Plant Anatomy Laboratory” and the “Center for Microscopy and Microanalysis” of UFV. Observations and photographs were obtained using a light microscope (AX70TRF; Olympus Optical, Tokyo, Japan) equipped with a digital camera (AxioCam HRc; Zeiss, Gottingen, Germany). Scanning electron microscopy followed Bozzola & Russel (1992), submitting the samples to dehydration in a graded ethanol series, critical-point dryinf of samples, sputter coating with gold and observed using a Leo 1430VP SEM (Zeiss, Cambridge, United Kingdom).

Results

Detailed descriptions of the *Glandonia* species were provided herein, incorporating atualizations and new morphoanatomical features (Fig. 2-5) not previously reported by Anderson (1981) early.

Glandonia macrocarpa Griseb. in Mart., Fl. bras. 12 (1): 24. 1858.

Type—BRAZIL. Amazonas. Manaus, Rio Negro, *Spruce 1090* (holotype GOET; isotypes GH, M, NY!, P!).

(Figures 1B; 2A; 3D, H, I, L, M; 4A, I; 5A-C)

Description—Trees 12–20 m; branches glabrous. Leaf entire; lamina (10–)12–26 × (3–) 5–11 cm, strongly obovate, base cuneate, apex acuminate, margin flat or slightly revolute, papyraceous, adaxial and abaxial surface glabrescent, 1–2 pairs of basilaminar glands, with yellow color, laterally positioned at the median vein in abaxial surface and at the same surface smaller glands randomly distributed throughout the leaf blade on the proximal portion; petiole (7–)8–20(–2.6) mm, glabrous; stipules (8–)10–20 mm long. Raceme of cincinni inflorescence (13–)17–28 cm long, adpressed-tomentose, cincinni with 2–5 flowers; bracts and bracteoles widely ovate, concave, abaxial surface sericeous, bracts 3.5–6 mm long, bracteoles 1.5–4 mm long, bracteolar gland with creamy-white color and discoid-convex secretory surface; floriferous peduncle 3–3.5 cm long. Pedicel articulated 10–15 mm long, adpressed-tomentose. Sepals 1.5–2 mm long beyond calyx glands, 2–2.5 mm wide, rounded to ovate, abaxial surface sericeous, calyx glands 2–3.5 mm long and white color. Petals glabrous, outermost petal helmet shaped 5–6.5 × ca. 5.5 mm, denticulate, claw 4–6 mm long, lateral petals white, limb 5–6.5 × 5–6.5 mm, sagittate, eglandular, margin entire, claw 4.5–6 mm long, posterior petal yellow, limb ca. 6 × 5 mm, base sagittate, 5–9 pairs of large marginal glands at the proximal portion of the reflexed posterior petal, claw 4–5 mm long. Filaments 1–1.5 mm long, densely hirsute, tuft of straight hairs just above of the insertion of anther; anthers 4.3–5.5 mm long. Ovary ca. 1.3 mm long, pyramidal, glabrous; three styles 5–5.5 mm long, subequal, glabrous, 0.5–0.7 mm ends at the apex bent, small apical stigma. Fruit (1.8–) 1.9–2.8 × 0.7–1.8 cm, cylindrical becoming

truncate-conoid, 17–20 longitudinal grooves, forming rounded projections at the base, styles apex permanent.

Specimens examined—BRAZIL. AMAZONAS: Manaus, Reserva Adolpho Ducke, December 1901 (fr), *s.c.* 5956 (MG); *ibid.*, Igapó dos riachos, 2 November 1929 (bud), *A. Ducke s.n.* (INPA 16283); *ibid.*, Estrada do Aleixo, 2 December 1942 (fl), *A. Ducke 63* (IAN); *ibid.*, 2 December 1942 (fl), *A. Ducke 63* (MG); *ibid.*, Igarapé do Crespo, 13 February 1943 (fr), *A. Ducke 1182* (IAN); *ibid.*, Cachoeira baixa do Tarumã, 18 November 1955 (bud), *W. Rodrigues s.n.* (2934 INPA); *ibid.*, Estrada dos Franceses, 28 November 1955 (bud), *W. Rodrigues s.n.* (INPA 2979); *ibid.*, Cachoeira baixa do Tarumã, 6 December 1955 (bud, fl), *D. Coelho s.n.* (IAN 92214; INPA 3041); *ibid.*, BR 17 Km 17, 14 December 1955 (bud, fl), *D. Coelho s.n.* (INPA 3118); *ibid.*, 28 December 1955 (fl), *Luis e Francisco s.n.* (IAN 110940); *ibid.*, 28 December 1955 (bud, fl), *L. Coelho & F. Mello s.n.* (INPA 3230); *ibid.*, Igarapé Santa Maria, 24 February 1956 (fr), *J. Chagas & D. Coelho s.n.* (IAN 110956; INPA 3505); *ibid.*, Rio Negro, Igarapé do Goiabinha, 15 March 1958 (fr), *s.n.* (IAN 98780); *ibid.*, 19 March 1958 (fr), *s.c.*, *s.n.* (INPA 622); *ibid.*, Cachoeira baixa do Tarumã, 07 November 1958 (bud, fl), *s.n.* (INPA 6047); *ibid.*, Rio Preto, 30 January 1962 (fr), *W. Rodrigues & J. Chagas 4168* (INPA); *ibid.*, Rio Cuieiras, 19 December 1961 (bud, fl), *W. Rodrigues & B. Willson 3986* (INPA); *ibid.*, Cachoeira baixa do Tarumã, 26 December 1962 (bud, fl), *W. Rodrigues et al. 4945* (INPA); *ibid.*, Estrada Torquato Tapajós, 1° April 1975 (fr), *A. Loureiro et al. s.n.* (INPA 48328); *ibid.*, Ponta Negra, 11 February 1977 (fr), *M. F. Silva et al. 2065* (INPA); *ibid.*, BR 17 Km 21, 28 December 1955 (bud, fl), *Coelho & F. Mello s.n.* (INPA 3230); São Gabriel da Cachoeira, Rio Curicuriari, 12. July 1979 (fr), *L. A. Maia et al 571* (INPA); *ibid.*, Reserva Adolpho Ducke, Igarapé do Acará, 7 April 1994 (fr), *Ribeiro, J. E. L. S. 1262 et al.* (INPA); *ibid.*, 27 November

1997 (bud, fl), *P. A. C. L. Assunção 725 et al.* (IAN; INPA); *ibid.*, 11 January 2007 (fl), *I. R. Silva 15* (INPA), 22 December 2014 (bud, fl), *I. R. Silva et al. 287; 288* (VIC).

Notes on morphology— The above description of *Glandonia macrocarpa* expands the original one by Anderson (1981) with the following additions: quantitative atualizations were made, about vegetative and reproductive characters; leaf, petiole and stipules dimensions; pedicel, sepal, claw, filament and anther longitude, even as fruit dimensions. New characters were observed in fieldwork, such as the yellow color of the leaf glands (Fig. 3D) and of the posterior petal (Fig. 3L), creamy-white color of bracteolar gland (Fig. 3I) and withe color of calyx glands (Fig. 3L, M).

Notes on anatomy— The sinuoses outline of anticlinal wall and scars of indument at the adaxial (Fig. 4A) and abaxial surface of leaf was perfomed. Anatomical analyses were crucial to define the presence and distribution of both basilaminar and laminar glands on the leaf. The anatomical procedures allow observing the smaller glands (Fig. 3H) that are not well visualized in a naked through the blade (as in *G. prancei* Fig. 4H). The number of glands at the base of petal limb was also evalueted (Fig. 5B, C). In addction the cross section of bracteolar glands show secretory surface convex (Fig 4I).

Common names— “riteira”.

Distribution, habitat and conservation— The species is restricted to lowlands of “Terra Firme” in the vicinity of Manaus, Brazil (Fig. 1A). The following specimens NY01856587, NY01856583, NY01856584, NY01856586, NY01856587 and RB00213108 (that were determined as *Glandonia macrocarpa*) were revised herein and recognized as *G. williamsii*. Such dubious previous

identifications were records in the Upper Rio Negro but this collection has many vegetative atypical charateres. As these specimens were collected in frutification period, the presence of conspicuous nectaries through the leaf blade was the main feature for recognized them as *G. williamsii*.

According to IUCN (2012) criterias, *G. macrocarpa* is Endangered (EN): EOO (extent of occurrence) less than 2,000 km² and AOO (area of occupancy) is equal a 16 km². Furthermore, such species was collected only in Manaus vicinity.

Phenology— *Glandonia macrocarpa* has been collected with flowers from November to February, and with fruits from January to May.

Glandonia prancei W.R. Anderson, Mem. New York Bot. Gard. 32: 139. 1981.

Type:—BRAZIL. Amazonas: Humaitá, Rio Madeira, Rio Ipixuna, *Prance, Pena & Ramos* 3363 (holotype INPA!, isotypes K, NY!, MO, S).

(Figures 1C; 2C; 3B, C, E, J; 4B, H, J; 5D-F)

Description—Trees ca. 18m. Leaf entire, lamina 9–21 × 4–6 cm long, elliptical, 0–1 pair of basilaminar white glands, lateraly positioned at the median vein in abaxial surface and at the same surface smaller glands randomly distributed throughout the blade on the proximal portion, stipules 12–24 mm long. Raceme of cincinni inflorescence 5–11 cm long, cincinnis ca. 1 cm apart; bracts 2–4 mm long, bracteoles 1–3 mm long, ovate, bracteolar gland with white color and discoid-flat secretory surface; floriferous peduncle 2–3 mm long. Pedicel articulated 6–8 mm long, pedicel foot 3–5 mm besides bracteole apex, calyx glands ca. 3 mm long and white color. Helmet shaped petal ca. 6 × 4.5 mm, claw ca. 5 mm; lateral petals ca. 6 × 6 mm, claw

ca. 3.5 mm; posterior petal ca. 6×5 mm reflexed, bear about 12 pairs of small marginal glands at the proximal portion, claw 3.5–4 mm long; filament ca. 1 mm long, anthers 3–4.5 mm long. Fruit 1.6–3.3 \times 0.6–1.9 cm, cylindrical becoming truncate-conoid, 16–18 longitudinal grooves, forming rounded projections at the base.

Specimens examined—BRAZIL. AMAZONAS: Igapó-Açú, BR 319, 15 March 1974 (fr), *G. T. Prance et al.* 20557 (INPA, MG, NY); Humaitá, Banks of Rio Ipixuna, 26 November 1966 (fl), *G. T. Prance et al.* 8058 (INPA, MG, NY). Lago Preto, norte de Lábrea, 29 October 1968 (bud), *Prance P04870630* (P, NY); Beruri, RDS Piagaçu-Purus, 22 January 2011 (bud), *B.G. Luizze 304* (INPA); *ibid.*, 28 February 2015 (bud, fl), *Silva et al.* 298, 299 (VIC).

Notes on morphology— The additions that expand the original description are about quantitative atualizations in vegetative and reproductive characters that differ to *G. macrocarpa*: leaf, stipule, petals dimensions, and fruit dimensions. The pedicel in *G. prancei* is distinct to *G. macrocarpa* by an articulated portion beside the bracteole (Fig. 3I, J respectively). Morphological characters were observed in field as the same color of the posterior petal (Fig. 5D) observed in *G. macrocarpa*, but besides the calyx glands, the bracteolar glands (Fig. 3J) and basilaminar leaf glands are white (Fig. 3E).

Notes on anatomy— The distribution of inconspicuous laminar leaf glands (Fig. 4H), and the sinuoses outline of anticlinal wall at the epidermal cells on the abaxial surface of the leaf (Fig. 4B) are similar to *G. macrocapa*. The main distinguish features from *G. prancei* to *G. macrocarpa* are flat surface of the bracteole glands (Fig. 4J) and amount and size from the petal glands (Fig. 5E, F).

Etymology— The specific epithet honours the botanic Ghillean Tolmie Prance.

Distribution, habitat and conservation— In the genus, such species is the most scarced, being colleted in only four areas from of Solimões River Basin (Fig. 1A).

Glandonia prancei was recently rediscovered in the *Glandonia macrocarpa* collection. This specimen was collected in the “Reserva do Desenvolvimento Sustentável Piagaçu-Purus” (Luize 304) in 2011 and it is the first record of *Glandonia prancei* since 1974 (Prance et al. 20557). Therefore, the new record in the Lower “Rio Purus” is a strong evidence of the sub-sampling of Amazonian flora and reinforce the diagnose problems of such species.

Based on EOO (49,090.842 km²) and AOO (16.000 km²) values, the analyses suggests the status of *G. prancei* according to IUCN (2012), to be Least Concern (LC) and Endangered (EN), respectively. The sampling efforts made in this work in nearby from the type location have failed to find any populations. On the other hand, fieldworks in Lower Purus have recorded this species in a new location. Although the EOO of *G. prancei* is higher, the species was recently collected only in a Conservation Unit area.

Phenology— *Glandonia prancei* has been collected with flowers from November to February, and with fruits from March to April.

Glandonia williamsii Steyerl., Fieldiana Bot. 28(2): 288. 1952.

Type:—VENEZUELA. Amazonas: Alto Orinoco, Ll. Williams 14154 (holotype F; isotypes F, MICH, VEN!).

(Figures 1C; 2B; 3A, F, G; 4C-G, L-N; 5G-I)

Description—Small trees 8(–15) m, branches glabrous. Leaf entire, lamina 9–26 × 3–10.8 cm, elliptical, base rounded, margin revolute, apex acuminate, coriaceous, adaxial and abaxial surface glabrescent, abaxial surface papillose, (0)1–2 pairs of basilaminar glands, with green-yellowish color, laterally positioned at the medivein in abaxial surface and at the same surface smaller glands randomly distributed

throughout all the leaf blade; veins abaxially prominent; the median portion of the keel shaped leaves; petiole 10–24(–29) mm long, glabrous; stipules 9–21 mm long, abaxially glabrescent. Raceme of cincinni inflorescence 5–18 cm long, adpressed-tomentose or sericeous, cincinnis mostly less than 1 cm apart, with 3–4(–5) flowers; bracts and bracteoles ovate, abaxial surface, bracts 3–5 mm long, bracteoles 2–3 mm long, bracteolar gland with creamy-white color and discoid-convex secretory surface; floriferous peduncle 1–2 cm long; Pedicel articulated 8–10 mm long, pedicel foot 2–4 mm besides bracteole apex, tomentose to sericeous. Sepals 1.5–2 mm long beyond the calyx glands, 2–2.5 mm wide, rounded apex, abaxial surface sericeous, calyx glands 2–3.2 mm long and white color. Petals glabrous, outermost petal helmet shaped 4–6 × 4–4.5 mm, denticulate, claw 3–4 mm long, lateral petals white, limb 4–6 × 4–5 mm, obtusely sagittate, eglandular, claw 3.5–4.5 mm long, posterior petal yellow, limb 4–5.5 × 3–4 mm, base sagittate, ca. 18 pairs of small marginal glands at the proximal portion of the erect posterior petal, claw ca. 3.5 mm long Filaments 1–1.3 mm long, densely hirsute; anthers 3.5–5 mm long, tuft of straight hairs just above of the insertion of anther. Ovary 1–1.5 mm long, pyramidal, glabrous; styles 4–5.5 mm long, subequal, glabrous, ca. 0.5 mm ends at the apex bent. Fruit (1.3–)1.4–2.4 × 0.8–1.6 cm, cylindrical becoming slightly acute-conoid, 7–15 longitudinal grooves, forming rounded projections at the base, styles apex permanent.

Specimens examined—BRAZIL. AMAZONAS: São Gabriel da Cachoeira, Rio Negro, 1947 (fr), *R. E. Schultes* 9565 (IAN); *ibid.*, Içana, Rio Negro, 05 May 1947 (fl), *R. L. Fróes* 22280 (MG); *ibid.*, Cachoeira Maçarico, 26 April 1947 (fr), *R. L. Fróes* 22233 (IAN); *ibid.*, Rio Negro, Igarapé Toury, 18 March 1952 (bud, fl), *R. L. Fróes* 27909 (IAN); *ibid.*, Taraquá, Igarapé da Chuva, Uaupés, 7 June 1962 (fr), *O. C. Nascimento* 575 (IAN); *ibid.*, Rio Uneiuxi, 22 October 1971 (bud), *G. T. Prance et al.*

s.n. (INPA 33733); Rio Urubaxi, 4 June 1976 (fr), *L. R. Marinho* 420 (IAN); *ibid.*, Rio Negro, Rio Canaburi, 16 June 1976 (fr), *L. F. Coelho* 984 (INPA; MG); *ibid.*, Rio Curicuriari, 12 July 1979 (fr), *J. M. Pires & N. T. Silva* 7970 (MG); *ibid.*, 13 July 1979 (fr), *J. M. Poole* 1982 (MG); *ibid.*, Rio Uapés, Taraquá, January 2015 (bud, fl), *I. R. Silva et al.* 290 (VIC); COLOMBIA. VAUPÉS: Mitú, Rio Vaupés, 19 November 1976 (bud, fl), *J. L. Zarucchi s.n.* (COL 172039; INPA 76785); GUAIANIA: Caño Minas, abajo de Sejalito, 05 March 1995 (ste), *M. P. Córdoba et al.* 979 (COL). VENEZUELA. AMAZONAS: São José Casiquiare, 12 December 1945 (fl), *R. L. Fróes* 21509 (IAN); Along Yapacana, 20 March 1953 (fr), *B. Maguire & J. J. Wurdack s.n.* (VEN 178462); Rio Negro, cerca Rio Pasimoni, 9 February 1981 (bud, fl, fr), *O. Huber & E. Medina s.n.* (COL 259369; MG 107371); *s.l.*, 18 February 1986 (bud, fl), *B. Stergios & G. Aymard s.n.* (PORT 31685); Rio Esoni, 16-27 January 1987 (fr), *B. Stergios, G. Aymard & R. Estévez s.n.* (PORT 32975); Río Casiquiare, Caño Kuka, 10-22 February 1989 (fl, fr), *B. Stergios et al. s.n.* (PORT 42084); Rio Guayapo, May 1989 (fr), *E. Foldats & J. Velazco s.n.* (PORT 49490); Entere Caño Cotúa y Cerro Yapacana, November 1989 (bud, fl), *E. Marin s.n.* (PORT 60136); Caño Yagua, November 1989 (fl), *E. Marin s.n.* (PORT 52001); San Entre Caño Cutúa y Cerro Yapacana, November 1989 (bud), *E. Marin s.n.* (GUYN 7507); Laja Cúcuta, Rio Atacavi, November 1989 (bud, fl), *J. Velazco s.n.* (PORT 41135); Atabapo, confluencia del Caño Yagua em el Orinoco, May 1990 (fr), *E. Marin s.n.* (PORT 62675); Varía, April 1991 (fl), *J. Velazco s.n.* (PORT 51484); Rio Varía, April 1991 (ste), *J. Velazco s.n.* (PORT 56484); Casiquiare, Caño San Miguel, 18 April 1991 (fr), *G. Aymard s.n.* (PORT 58955); Casiquiare, Rio Negro, 3 February 1992 (bud, fl), *B. Stergios et al. s.n.* (PORT 67208); Rio Baría, 7 November 1994 (bud, fl), *B. Stergios et al. s.n.* (PORT 61007; VEN 288790); Autana, Rio Cuao, entre Piedra Picure y Santa

Elena, 14 August 1997 (fr), *A. Castillo 5079* (VEN); Autana, Rio Cuao, entre Raudal del Danto e Isla Picurela, 29 January 1997 (fr), *A. Castillo 4488* (VEN); Casiquiare, April 2000 (ste), *B. Stergios et al. s.n.* (GUYN 12943); *ibid.*, April 2000 (ste), *B. Stergios, & W. Schargel s.n.* (PORT72216); Rio Sipapo, cerca de Cerro Pelota, 22 February 2001 (fl), *A. Castillo & B. Camaripano. 8995* (VEN); Rio Sipapo, entre Cano Veneno y Pendare, margen izquierda, 19 February 2001 (bud, fl), *A. Castillo & B. Camaripano. 8242* (VEN); Médio Ventuari, March 2002 (ste), *B. Stergios, F. Molina & A. Vicentini s.n.* (PORT 82468); Río Yatuá, 30 January 2005 (bud), *K. M. Redden 3408* (GUYN); Río Baría, Caño Eruvichi, 14 April 2005 (bud, fr), *W. Diaz 7477* (GUYN).

Notes on morphology— Concerning the original description, the following additions were made: keel shaped leaves (Fig. 3A), the slightly acute apex of the fruit (Fig. 2C) and an update about vegetative and reproductive quantitative characters was made for leaf and fruit dimensions. The pedicel in *G. williamsii* is also articulated as in *G. prancei*. Field observations able to confirm the erect position and yellow color of the posterior petal (Fig. 5G), besides the green-yellowish color of leaf glands (Fig. 3F) and creamy-white color of bracteolar glands (Fig. 5G).

Notes on anatomy— Although the anatomical characters are similar to the *G. macrocarpa* and *G. prancei*, the size of laminar glands is bigger (Fig. 4G) and visualized on the naked eye (Fig. 3G). In additional, the abaxial epidermis is papillose (Fig. 4C-E) and the numerous and small petal glands (Fig. 5H, I) were recorded. One feature exclusively found on the posterior petal of *G. williamsii* is the presence of brachysclereids and macrosclereids in the adaxial subepidermis layer (Fig. 4L-N).

Etymology— The specific epithet pays homage to the type collector, Llewelyn Williams.

Distribution, habitat and conservation— *Glandonia williamsii* is distributed in Lower Uaupés, Upper Rio Negro and Orinoco, covering “Igapó” areas in Colombia, Venezuela and Brazil (Fig. 1A). According to IUCN criterias (2012), *G. williamsii* is Least Concern (LC) and Endangered (EN), respectively: EOO (extent of occurrence) is more than 200,000.000 km², while the AOO (area of occupancy) is equal a 108.000 km². Therefore, such species was collected only in Upper Rio Negro areas.

Phenology— *Glandonia williamsii* has been collected with flowers from Outubro to May, and with fruits from February to June.

DISCUSSION

Taxonomic comments— *Glandonia* species differs on the shape of leaves, cuneate-obovate in *G. macrocarpa*, cuneate-elliptical in *G. prancei* and rounded-elliptical in *G. williamsii*. In this work were recorded informations about the color of the glands, unnotice in original description of these species (Anderson 1981). The leaf glands are yellow in *G. macrocarpa*, white in *G. prancei* and green-yellowish in *G. williamsii*, while the bracteolar glands are creamy-white in *G. macrocarpa* and *G. williamsii*, and white only in *G. prancei*. All three species present calyx glands white and about the corolla color, only the posterior petal is yellow while the other petals are white (helmet shaped and lateral petals). The smallest petals were observed in *G. prancei* and only in *G. williamsii* the reflex position of the posterior petal limb was not observed. Another difference among the species is the fruit shape: truncate-conoid in *G. macrocarpa* and *G. prancei* or acute-conoid in *G. williamsii*. Table 1 shows a comparasion among the species.

Notes on leaf and floral anatomy— The three species of *Glandonia* showed morphoanatomical differences in their leaves, bracteoles and flowers. The anatomy studies help to elucidate the presence of the leaf glands: general two pairs of basilaminar leaf glands in *G. macrocarpa* and *G. williamsii*, absent or only one pair in *G. prancei*; and laminar leaf glands is conspicuous in *G. williamsii*, while in *G. macrocarpa* and *G. prancei* they are inconspicuous. In the original description of the three species (Anderson 1981) the leaf glands were not mentioned or were reported without detail about position and size. Differences of leaf anatomical characters were observed among *Glandonia* species, as the presence and form of the papillose cells in abaxial surface, which recovered the stomata. Papillae were one of the most distinctive anatomical leaf characters since only the epidermal abaxial surface of *G. williamsii* displayed them. Such character can play an important functional role in taxonomy as reported by others works (Coutinho *et al.* 2016).

The glands of the pedicel bracteole of flowers, showed a convex surface in *G. macrocarpa* and *G. williamsii*, while only in *G. prancei* is flat. The leaf and bracteolar glands are nectaries and attract insects, mainly ants (Silva *et al.* unpublished data), which probably act against herbivorous agents (Fernandes *et al.* 2005). The presence of sclereids on the posterior petal of *G. williamsii* promotes the sustentation to their position erect. Another peculiar distinctive character observed at the corolla are the number and size of petal glands, being large in *G. macrocarpa*, few smaller glands in *G. prancei* and numerous and small in *G. williamsii*. Although the secretion produced by such glands are actually similar to reported for the calyx gland that act as elaiophore (Vogel 1990), they can also act as osmophore (Possobom *et al.* 2015, Silva *et al.* unpublished data). All anatomical data are promising for future taxonomic, phylogenetic approaches.

Key to the species of *Glandonia* (Anderson 1981, modified)

1. Leaf elliptical, rounded at the base, coriaceous, abaxial surface papillose, conspicuous basilaminar and laminar leaf glands; posterior petal with about 18 pairs of small marginal glands at the proximal portion of the erect petalar limb, posterior petal bear tracheids; fruit apex slithly acute.....*Glandonia williamsii*

- Leaf obovate to elliptical, cuneate at the base, papyraceous, abaxial surface not papillose, conspicuous basilaminar leaf glands and inconspicuous laminar leaf glands; about 12 pair of marginal glands at the posterior petal, which not present tracheids; fruit apex truncate.....2.

2. Leaf strongly obovate, 1-2 pairs of basilaminar leaf glands (yellow); convex secretory surface of bracteolar glands (creamy-white); posterior petal with 6-9 pairs of large marginal glands at the proximal portion of the limb.....*Glandonia macrocarpa*

- Leaf ellipical to slithly obovate, 0-2 pairs of basilaminar leaf glands (white); flat secretory surface of bracteolar glands (white); posterior petal with about 12 pairs of smallers marginal glands at the proximal portion of the limb.....*Glandonia prancei*

Biogeographical comments— According to the phylogeny presented by Davis and Anderson (2010), *Glandonia* is positioned in the “mcvaughoid clade”, as a sister-group of *Burdachia* and *Mcvaughia*. As *Glandonia*, *Burdachia* is a little endemic genus of trees from Amazonia wetlands floodplain (Anderson 1981). On the other side, *Mcvaughia* is shrubs restricted to Caatinga (Anderson 1979) and sandy coastal plains from Atlantic forest in Brazil (Amorim & Almeida 2015). The endemism of *Glandonia* was reforced by the records added herein (Fig. 1), since the disjunct distribution of the three species of *Glandonia* was mainted as described by Anderson (1981). About 30% of the seven million square kilometers that make up the Amazon basin is covered by periodically flooded forests (Junk *et al.* 2011). Hidrocorical dispersion is a commun adaptation of trees in inundated forest reported by Ducke (1949) and Parolin *et al.* (2013), the fruits of *Glandonia* species is an evidence of such syndrome. In general, high degree of endemism of trees species has been point for the Amazonian flooded habitats, mainly in the Upper Rio Negro (Kubtzki 1989). One of the hypotheses to explain the high endemins in flooded areas is related to climatic oscillations of the

Pleistocene, which were postulated that the speciation was resulted of biota isolation in refuges during arid periods (Prance 1982).

In additional, such species occur in a place that is very difficult to access, what may explain the collection to be restricted to some specimens. Hopkins (2007) recognized that the botanical knowledge about the Amazon is concentrated in certain areas, which reflects in underestimated information about the diversity and geographic distribution of the amazonian flora. In this way, the occurrence areas of *Glandonia* species may encompass part of this knowledge gap. The recent collection of *G. prancei* in the Lower Purus River corroborates this idea, since this species was considered by Anderson (1981) endemic to the Upper Rio Purus and Rio Madeira. Apparently, the lack of new records is a consequence of the low effort of fieldwork in hard-to-access areas. Therefore, future expeditions along the Negro River Basin and Rio Solimões Basin may reveal new records for the genus, clarifying the controversial about the disjunct distribution of the three species.

Acknowledgements

We acknowledge the “Fundação de Amparo à Pesquisa do Estado do Amazonas” (FAPEAM) for granting the first author’s Doctoral scholarship “PROGRAMA RH-INTERIORIZAÇÃO”; the “Programa de Pós Graduação em Botânica” of the “Universidade Federal de Viçosa” (UFV) for providing logistic structure; the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for awarding a Research Productivity Fellowship to AMA and RMSAM (grant # 310717/2015-9 and grant # 308389/2013-1, respectively), and all herbaria for kindly loaning specimens of the *Glandonia* species. We are also grateful to “Centro Estadual de Unidades de Conservação” (CEUC), to the “Instituto Piagaçu-Purus” and “RDS

Piagaçu-Purus”, to the “Instituto Nacional de Pesquisas da Amazonia” (INPA) and the “Exército Brasileiro” for field support in the conservation units; and the “Fundação de Amparo à Pesquisa do Estado de Minas Gerais” (FAPEMIG) for the financial resources.

References

- Amorim, A.M. & Almeida, R.F. (2015) An unexpected *Mcvaughia* (Malpighiaceae) species from sandy coastal plains in northeastern Brazil. *Systematic Botany* 40: 535–538.
- Anderson, W.R. (1977) Byrsonimoideae, a new subfamily of the Malpighiaceae. *Leandra* 7: 5–18.
- Anderson, W.R. (1979) *Mcvaughia*, a new genus of Malpighiaceae from Brazil. *Taxon* 28: 157–161.
- Anderson, W.R. (1981) Malpighiaceae. The Botany of the Guayana Highland-Part XI. *Memorirs of the New York Botanical Garden* 32: 137–147.
- Anderson, W.R. (1990) The origin of the Malpighiaceae-The evidence from morphology. *Memoirs of the New York Botanical Garden* 64: 210–224.
- Bachman, S., Moat, J., Hill, A., de la Torre, J. & Scott, B. (2011) Supporting Red List threat assessments with GeoCAT: geospatial conservation assessment tool. *In*: Smith, V. & Penev, L. (Eds.) e-Infrastructures for data publishing in biodiversity science. *ZooKeys* 150: 117–126. (Online version). GeoCAT tool available from: <http://geocat.kew.org/> (accessed 1 February 2017)
- Bozzola, J.J. & Russel, L.D. (1992) *Electron microscopy*. Jones and Bartlett Publishers, Boston, Massachusetts, USA. 115 pp.
- Coutinho, Í.A.C., Rando, J.G., Conceição, A.S. & R.M.S.A., Meira (2016) A study of the morphoanatomical characters of the leaves of *Chamaecrista* (L.) Moench sect. Apoucouita (Leguminosae-Caesalpinioideae). *Acta Botanica Brasilica* 30(2): 205–221.
- Davis, C.C. & Anderson, W.R. (2010) A complete generic phylogeny of Malpighiaceae inferred from nucleotide sequence data and morphology. *American Journal of Botany*. 97: 2031–2048.
- Ducke, A. (1948) Árvores amazônicas e sua propagação. *Boletim do Museu Paraense Emílio Goeldi* 10:81–92.
- Environmental Systems Research Institute-ESRI (2010) ArcGIS, version 9.3.1. *Environmental Systems Research Institute*. United States of America.

- Fernandes, G.W., Fagundes, M., Greco, M.K.B., Barbeitos, M.S. & Santos, J.C. (2005) Ants and their effects on an insect herbivore community associated with the inflorescences of *Byrsonima crassifolia* (Linnaeus) H.B.K. (Malpighiaceae). *Revista Brasileira de Entomologia* 49(2): 264–269.
- Franklin, G.L. (1945) Preparation of thin sections of synthetic resins and wood-resin composites, and a new macerating method for wood. *Nature* 155:51.
- Grisebach, A. (1858) Malpighiaceae. *In*: Martius, K.F.P. (Ed.) *Flora brasiliensis*. F. Fleischer, Lipsiae, pp. 1–124.
- Hopkins, M.J.G. (2005) Flora da Reserva Ducke, Amazonas, Brasil. *Rodriguésia* 56 (86): 9–25.
- Hopkins, M.J.G. (2007) Modelling the known and unknown plant biodiversity of the Amazon Basin. *Journal of Biogeography* 34: 1400–1411.
- Hutchinson, J. (1967) The genera of flowering plants. Clarendon Press, Oxford, UK.
- IUCN (2012) *IUCN Red List Categories and Criteria: Version 3.1*. Second edition. Gland, Switzerland and Cambridge, UK: IUCN. Available at: www.iucnredlist.org/technical-documents/categories-and-criteria. (Accessed: 16 Jan 2017).
- JSTOR Global Plants website (<http://plants.jstor.org/>) (Accessed: 16 Jan 2017)
- Junk, W.J., Piedade, M.T.F., Scheongart, J., Cohn-Haft, M.A.J., & Wittmann, F.K. (2011) A Classification of Major Naturally-Occurring Amazonian Lowland Wetlands. *Wetlands* 31: 623–640.
- Kubtzki, K. (1989) The ecogeographical differentiation of Amazonian inundation forests. *Plant Systematics and Evolution* 162: 285–304
- Mamede, M.C.H., Sebastiani, R. & Amorim, A.M. (2013) Malpighiaceae. *In*: *Lista de espécies da flora do Brasil*. Rio de Janeiro: Jardim Botânico. Disponível em: <http://floradobrasil.jbrj.gov.br/2012/FB000102/> (acesso em 16 Jan 2017).
- Niedenzu, F. (1928) Malpighiaceae. *In*: Engler, A. (Ed.) *Das Pflanzenreich* IV 141. W. Engelmann, Leipzig, pp.1–870.
- Parolin, P., Wittmann, F. & Ferreira, L.V. (2013) Fruit and seed dispersal in Amazonian floodplain trees: a review. *Ecotropica* 19: 19–36.
- Possobom, C.C.F., Guimarães, E. & Machado, S.R. (2015) Structure and secretion mechanisms of floral glands in *Diplopterys pubipetala* (Malpighiaceae) and a neotropical species. *Flora* 211: 36–39.
- Prance, G.T. (1982) Biological diversification in the tropics. Columbia University Press, New York, USA.
- REFLORA website (<http://herbariovirtualreflora.jbrj.gov.br/>) (Accessed: 16 Jan 2017)

Steyermark, J.A. (1952) Contribution to the flora of Venezuela. *Fieldiana. Botany* 28 (2): 280–288.

Thiers, B. (2016) (and continuously updated). Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. Available at: <http://sweetgum.nybg.org/ih/> (Accessed: 16 Jan 2017)

Vasco, A., Thadeo, M., Conover, M. & Daly, D.C. (2014) Preparation of samples for leaf architecture studies, a method for mounting cleared leaves. *Applications in Plant Sciences* 9: 1–4.

Vogel, S. (1990) History of the Malpighiaceae in the light of Polination Ecology. *Memoirs of the New York Botanical Garden* 55: 130–142.

TABLE 1. Comparison of diagnostic morphoanatomical characters among *Glandonia* species.

Morphoanatomical characters	<i>G. macrocarpa</i>	<i>G. prancei</i>	<i>G. williamsii</i>
Vegetative:			
Leaf shape	obovate	elliptical	elliptical
Leaf base shape	cuneate	cuneate	rounded
Number of basilaminar leaf glands	2 (4)	(0) 2	(0) 2-4 (6)
Color of basilaminar leaf glands	yellow	white	green-yellowish
Distribution of laminar leaf glands	more visible at the proximal portion	more visible at the proximal portion	entire leaf blade
Aspect of laminar leaf glands	inconspicuous	inconspicuous	conspicuous
Anticlinal outline of abaxial surface	sinuous	slightly sinuous	curve
Periclinal outline of abaxial surface	flat	flat	papillose
Reproductive:			
Pedicel articulated beside bracteole	absent	present	present
Bracteolar gland shape	discoïd-convex	discoïd-flat	discoïd-convex
Color of bracteolar gland	creamy-white	white	creamy-white
Number of posterior petal glands	6-9 glands	±12 glands	± 18 glands
Size of posterior petal glands	large	small	small
Posterior petal position	reflexed	reflexed	erect
Sclereids on the posterior petal	absent	absent	present
Shape of mature fruit	truncate-conoid	truncate-conoid	slightly acute-conoid

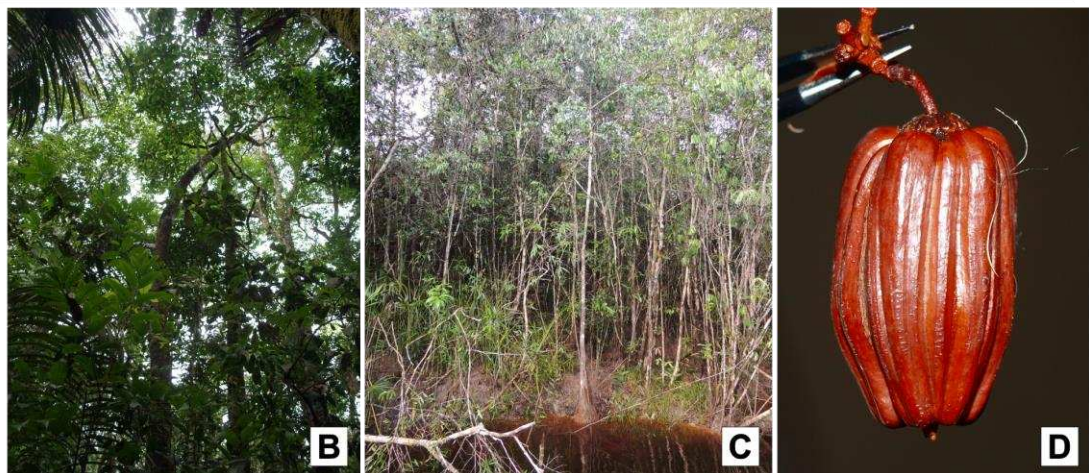
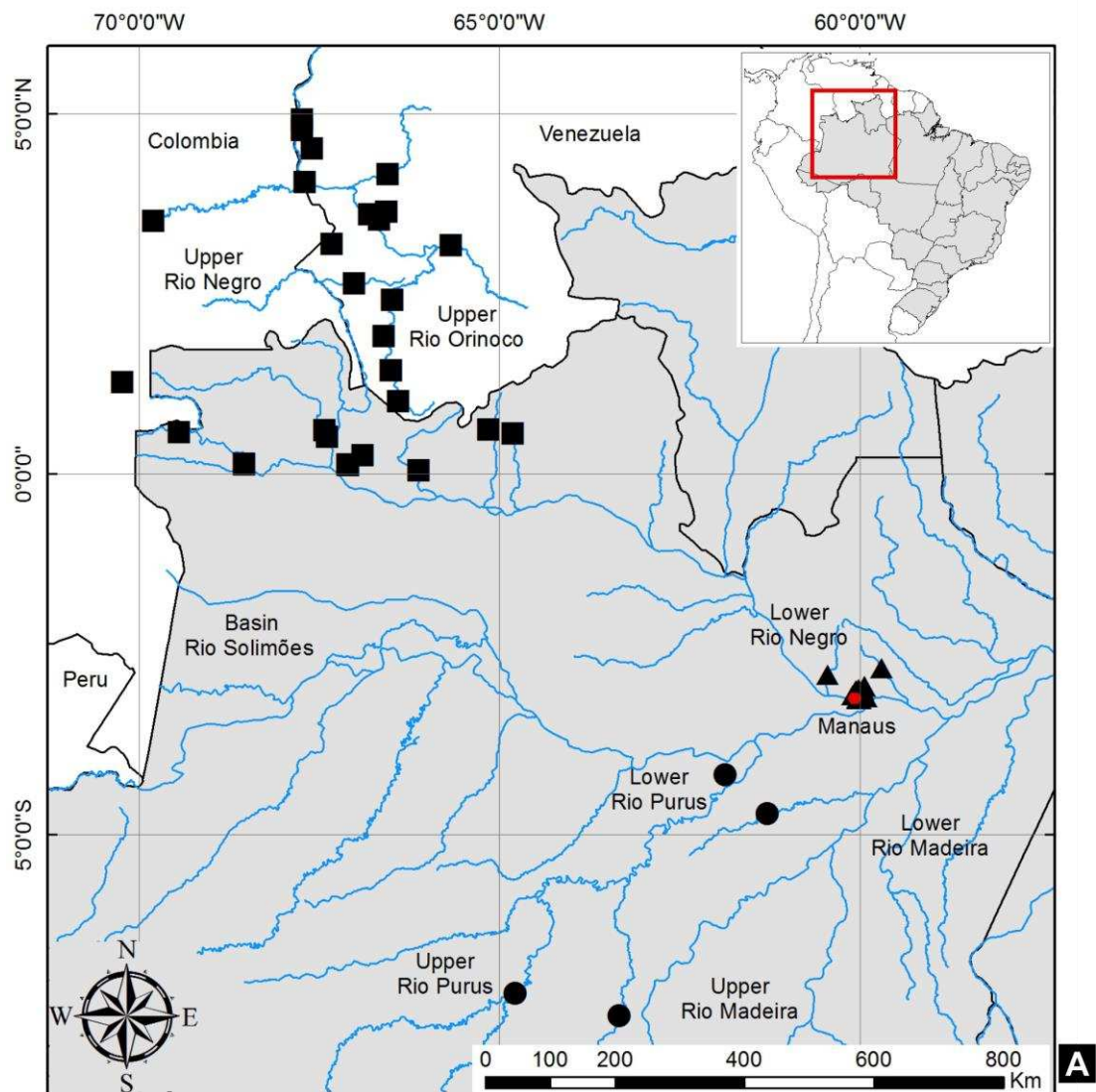


FIGURE 1. **A.** Map showing the distribution of *Glandonia macrocarpa* (black triangles), *G. prancei* (black circles) and *G. williamsii* (black squares). **B.** *Glandonia macrocarpa* specimen in “Terra Firme” forest. **C.** *Glandonia williamsii* specimen “Igapó”. **D.** Hidrocorical fruit of *G. prancei*. Photos B-D by I. R. Guesdon.

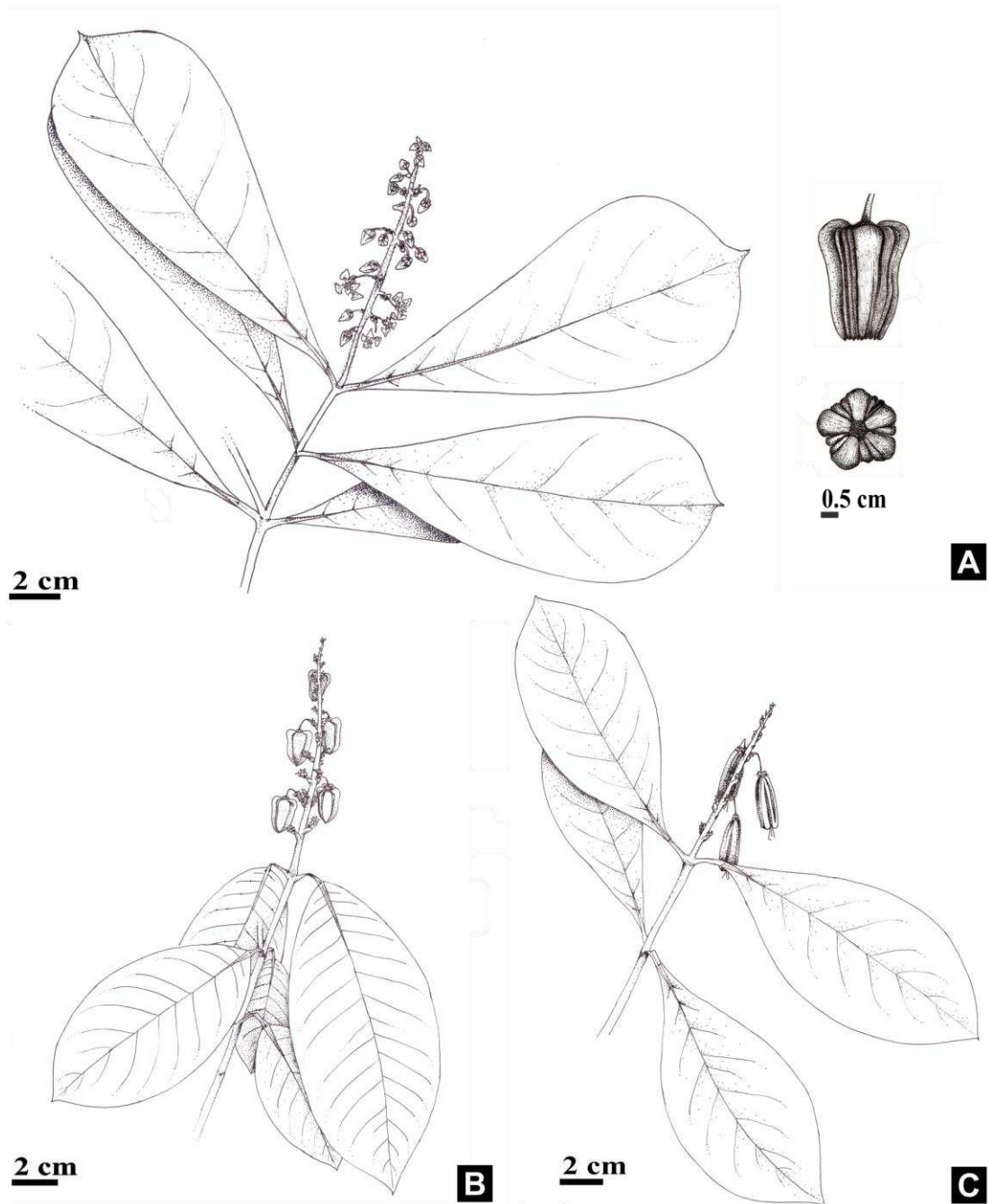


FIGURE 2. General view of *Glandonia* species: **A.** Floriferous branch in *G. macrocarpa* and lateral and frontal view of fruit. **B.** Frutiferous branch in *G. williamsii*. *Glandonia macrocarpa*: **C.** Frutiferous branch in *G. prancei*. Draw A from Silva 14; Ducke 793; B from Fróes 28782 and C from Prance et al. 20557

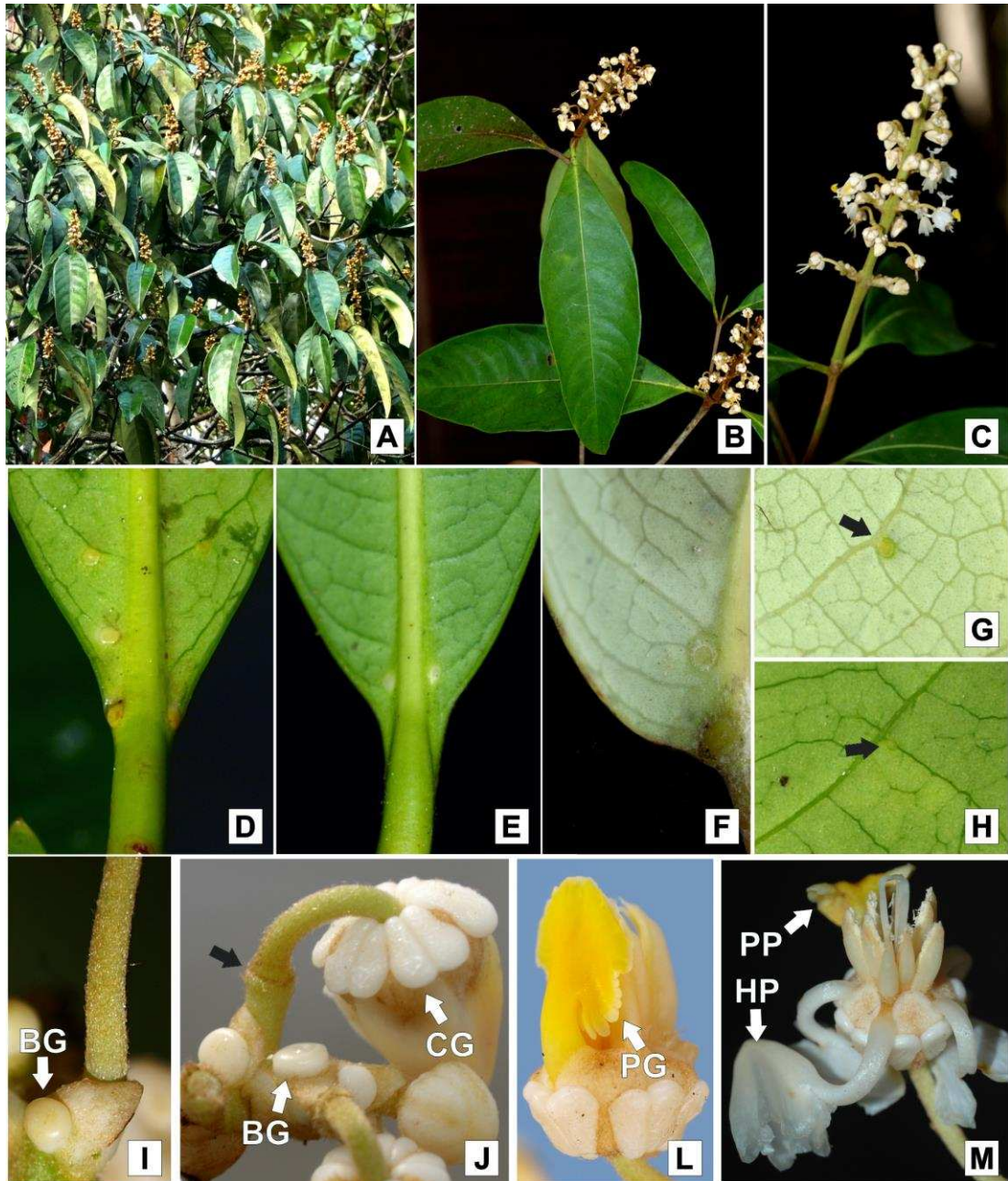


FIGURE 3. Vegetative and reproductive characters in *Glandonia* species: **A.** keel shaped leaves in *G. williamsii*, **B.** and **C.** general view of leaves and inflorescence of *G. prancei*. Basilaminars leaf nectaries: **D.** *Glandonia macrocarpa*, **E.** *Glandonia. prancei* and **F.** *Glandonia williamsii*. Laminars leaf glands: **G.** *Glandonia williamsii* and **H.** *Glandonia macrocarpa*. Floral glands: **I.** bracteolar gland (BG) in *G. macrocarpa*, **J.** bracteolar and calyx glands in *G. prancei* (BG and CC respectively), black arrow: articulated pedicel. **L.** Posterior petal in bud of *G. macrocarpa*, note the petal glands (arrow) **M.** Helmet shaped petal and posterior petal reflex in *G. macrocarpa* (HP and PP respectively).

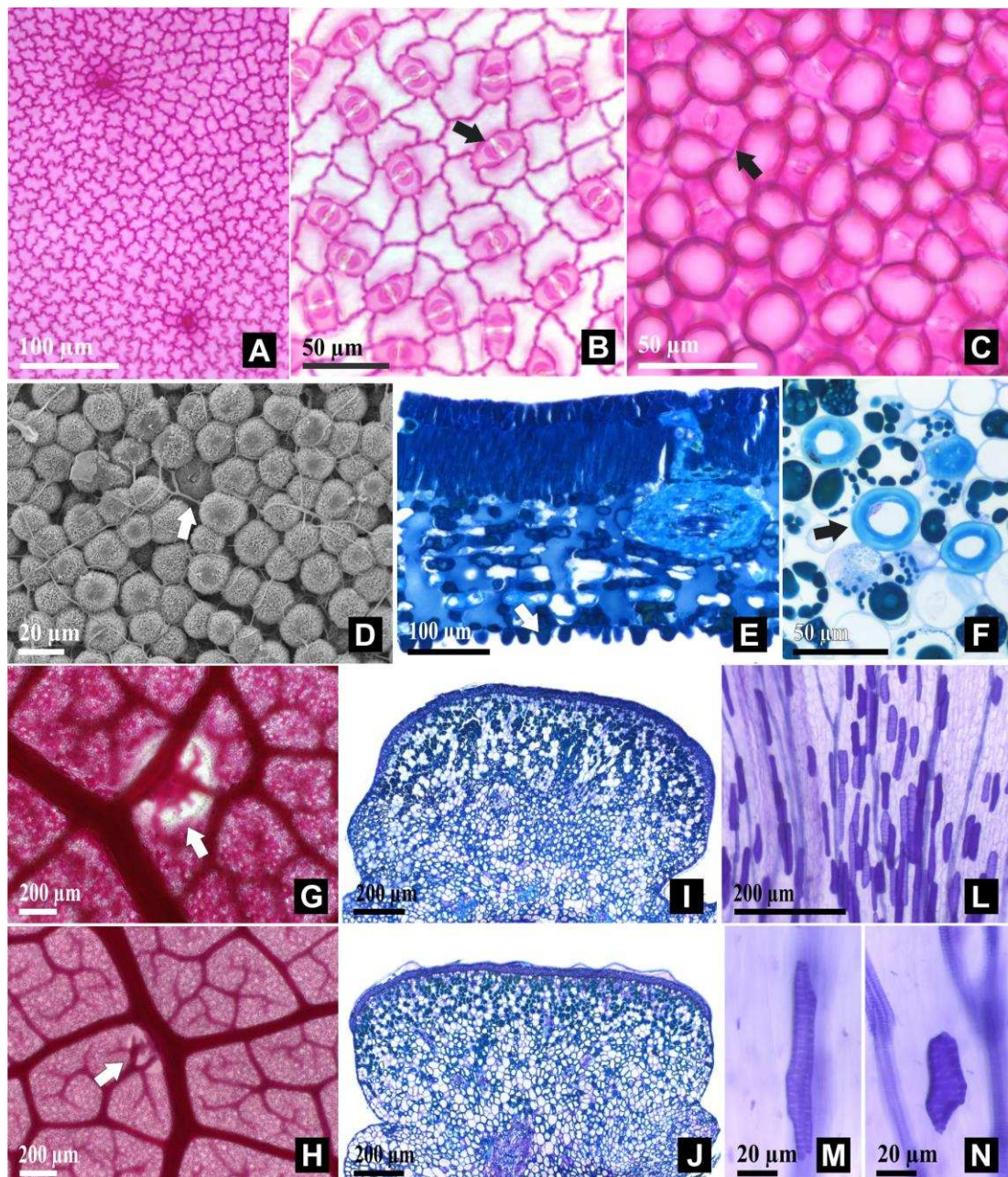


FIGURE 4. Anatomical characters in *Glandonia* species. **A.** Adaxial surface in *G. macrocarpa*, showing scars of indumentums, **B.** Abaxial surface in *G. prancei*, showing a stoma (black arrow) and outline sinuoses of the anticlinal epidermal wall, **C.** Abaxial surface in *G. williamsii*, showing papillae that recurved the stomata (black arrow), **D.** Papillae of *G. williamsii* at SEM image, a stoma (white arrow). **E.** Transversal section of leaf *G. williamsii*, papillae (white arrow), **F.** Sclereids in median vein of the leaf blade in *G. williamsii* (black arrow). **G.** Diafanized leaf blade of *G. williamsii* and **H.** *G. prancei*, note the laminar nectar (arrow). **I.** Bracteolar glands in *G. macrocarpa*, showing convex surface at transversal view, **J.** bracteolar gland in *G. prancei* with flat surface at transversal view. **L-N.** Sclereids on subepidermal layers at the median portion of posterior petal of the posterior petal in *G. williamsii*, **M.** Macrosclereids, **N.** Brachysclereids.

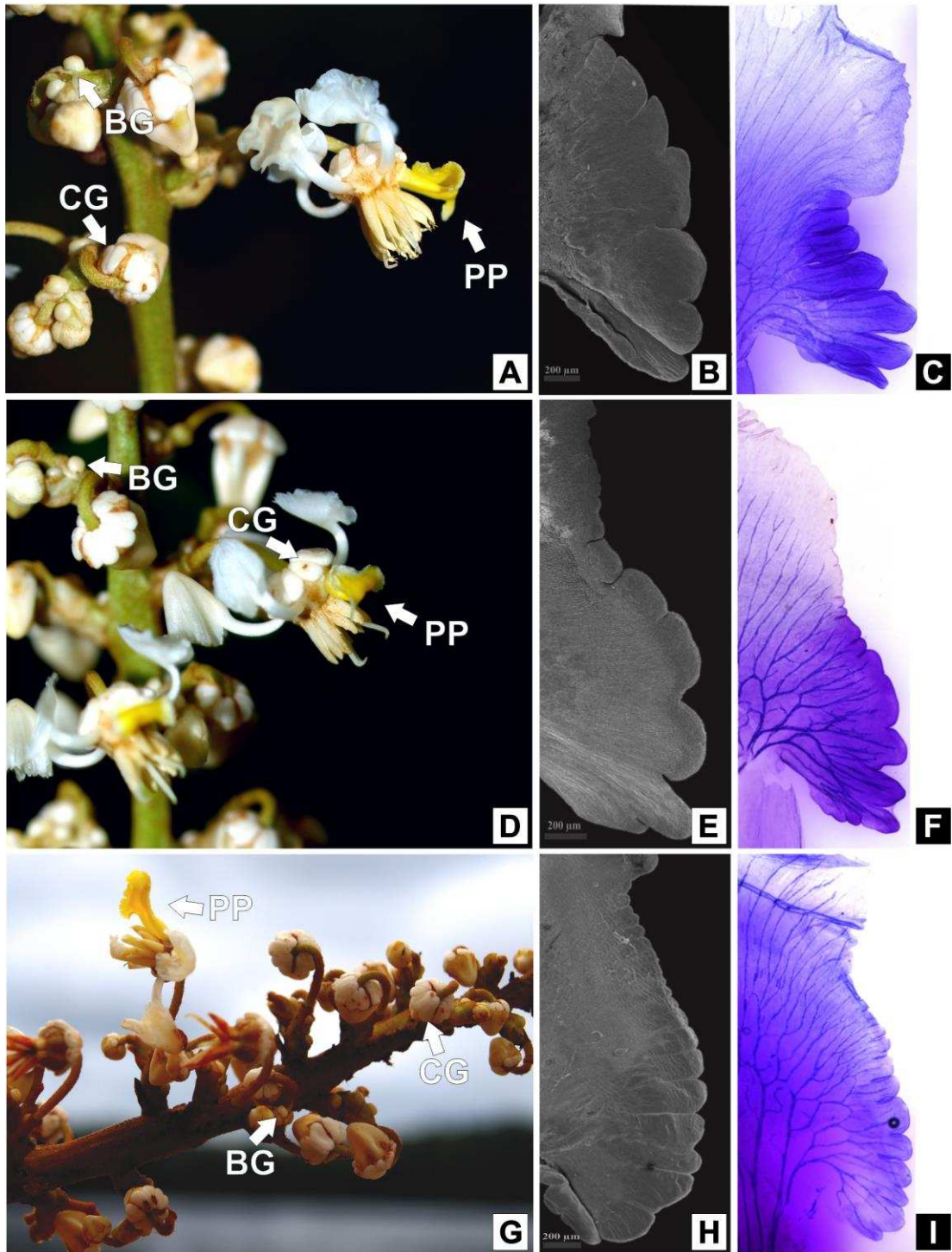


FIGURE 5. Morphoanatomical floral characters in *Glandonia* species. **A-C:** *Glandonia macrocarpa*: **A.** General view of the flowers, **B.** Posterior petal margin in SEM image and **C.** Posterior petal diafanized; **D-F:** *Glandonia prancei*: **D.** General view of the flowers, **E.** Posterior petal margin in SEM image and **F.** Posterior petal diafanized; **G-I:** *Glandonia williamsii*: **G.** General view of the flowers **H.** Posterior petal margin in SEM image and **I.** Posterior petal diafanized. **PP:** Posterior petal; **CG:** Calyx glands, **BG:** Bracteolar gland.

3. CONCLUSÕES GERAIS

Os padrões morfológicos e anatômicos das glândulas estudadas revelaram diferenças intra e interespecíficas. Dentre os nectários do clado mcvaughioide, os bracteolares são os mais conservados enquanto os foliares variam morfo e anatomicamente. Porém não foi detectada variações de distribuição relacionadas aos diferentes ambientes. Os resultados dos testes histoquímicos dos nectários foliares são similares aos bracteolares, mas diferem na concentração de glicose da secreção. Além disso, a visita das formigas foi observada sobretudo nos nectários bracteolares, onde concentração de glucose do néctar é maior.

Nas glândulas florais das espécies do clado mcvaughioide também foram observados diferentes padrões morfoanatômicos no cálice e pétala. No entanto, a composição anatômica e a natureza histoquímica da secreção são idênticas. Tal similaridade pode ser um sinal de homologia, apesar de suas respectivas funções ecológicas serem possivelmente diferentes. As glândulas do cálice parecem atuar como elaióforos e as petalares como osmóforos.

As glândulas florais no gênero paleotropical *Acridocarpus* apresentam composição anatômica típica de nectários. Diante dos testes histoquímicos realizados em *A. longifolius* tais glândulas foram consideradas como nectários nas bracteolas e no cálice. Nas bracteolas e no cálice a distribuição foi variável, onde as glândulas podem ser ausentes e variar em número e grau de fusão. Alguns padrões parecem ser específicos de determinadas regiões geográficas como por exemplo o cálice eglandular nas espécies de Madagaskar. A alta variabilidade desses caracteres florais parecem refletir a ausência do mutualismo com as abelhas coletoras de óleo.

Adicionalmente, a caracterização morfoanatomica das glândulas foliares e florais apresentam importância taxonômica na distinção das espécies de *Glandonia* (clado mcvaughioide). Assim, foi apresentada nova chave de identificação e atualizações nas descrições das espécie.

O presente trabalho registrou importantes dados sobre as glândulas foliares e florais de espécies Neo e Paleotropicais da família Malpighiaceae, contribuindo com informações da flora de áreas de difícil acesso e pouco conhecidas. Os resultados são promissores para estudos de evolução de caracteres foliares e da evolução floral na família, além de estudos da relação entre as Malpighiaceae com seus consumidores de néctar e polinizadores.