UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"

CARACTERIZAÇÃO ESTRUTURAL E HISTOQUÍMICA DA COROLA DE REPRESENTANTES DE BIGNONIEAE E ALIANÇA TABEBUIA OCORRENTES EM CERRADO

KARISE MAMEDE MACEDO

Tese apresentada ao Instituto de Biociências, Campus de Botucatu, UNESP, para obtenção do título de Doutora no Programa de Pós-Graduação em Biologia Vegetal, Interunidades entre o Instituto de Biociências do campus de Botucatu e o Instituto de Biociências do campus de Rio Claro.

BOTUCATU - SP
- 2024 -

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FICHA CATALOGRÁFICA ELABORADA PELA SEÇÃO TÉC. AQUIS. TRATAMENTO DA INFORM.
DIVISÃO TÉCNICA DE BIBLIOTECA E DOCUMENTAÇÃO - CÂMPUS DE BOTUCATU - UNESP
BIBLIOTECÁRIA RESPONSÁVEL: ROSANGELA APARECIDA LOBO-CRB 8/7500

Macedo, Karise Mamede.
Caracterização estrutural e histoquímica da corola de representantes de Bignonieae e Aliança Tabebuia ocorrentes em Cerrado / Karise Mamede Macedo. - Botucatu, 2024

Tese (doutorado) - Universidade Estadual Paulista "Júlio de Mesquita Filho", Instituto de Biociências de Botucatu
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Capes: 20302037

1. Folhas - Anatomia. 2. Bignoniaceae. 3. Polinização. 4. Plantas dos cerrados.

Palavras-chave: Anatomia de pétalas; Bignoniaceae; Melitofilia; Microestrutura da corola; Polinização.

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Botucatu

ATA DA DEFESA PÚBLICA DA TESE DE DOUTORADO DE KARISE MAMEDE MACEDO, DISCENTE DO PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS (BOTÂNICA), DO INSTITUTO DE BIOCIÊNCIAS - CÂMPUS DE BOTUCATU.

Aos 27 dias do mês de março do ano de 2024, às 14:00 horas, por meio de Videoconferência, realizou-se a defesa de TESE DE DOUTORADO de KARISE MAMEDE MACEDO, intitulada **Caracterização estrutural e histoquímica da corola de representantes de Bignonieae e Aliança Tabebuia ocorrentes em Cerrado**. A Comissão Examinadora foi constituída pelos seguintes membros: Profa. Dra. LETÍCIA DE ALMEIDA GONÇALVES (Co-orientador(a) - Participação Virtual) do Instituto de Ciências Biológicas / Universidade Federal de Goiás, Profa. Dra. ANA PAULA FORTUNA PEREZ (Participação Virtual) do Departamento de Biodiversidade e Bioestatística / Instituto de Biociências de Botucatu UNESP, Profa. Dra. BÁRBARA DE SÁ HAIAD (Participação Virtual) do Departamento de Botânica / Museu Nacional - UFRJ / Universidade Federal do Rio de Janeiro, Prof.ª Dr.ª ROSANI DO CARMO DE OLIVEIRA ARRUDA (Participação Virtual) do Departamento de Botânica, Instituto de Biociências / Universidade Federal de Mato Grosso do Sul, Prof. Dr. JOECILDO FRANCISCO ROCHA (Participação Virtual) do Departamento de Botânica, Instituto de Ciências Biológicas e da Saúde / Universidade Federal Rural do Rio de Janeiro - UFRRJ. Após a exposição pela doutoranda e arguição pelos membros da Comissão Examinadora que participaram do ato, de forma virtual, a discente recebeu o conceito final APROVADA. Nada mais havendo, foi lavrada a presente ata, que após lida e aprovada, foi assinada pela Presidenta da Comissão Examinadora.

Profa. Dra. LETÍCIA DE ALMEIDA GONÇALVES

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À minha mãe Maria Aparecida, à minha irmã Hanna Layse, ao meu esposo Carlos Gustavo e ao meu filho Emanuel por serem minha base, fortaleza e rede de apoio.

AGRADECIMENTOS

Primeiramente agradeço a Deus por me conceder sabedoria, paciência, resiliência e perseverança, para trilhar meu caminho acadêmico ao longo dos anos.

Agradeço a CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) pela bolsa de estudos a mim concedida durante quatro anos de doutorado. A toda a equipe de professores e funcionários do Programa de Pós-Graduação em Ciências Biológicas (Botânica) – IBB UNESP Botucatu (Programa de Pós-Graduação em Biologia Vegetal – Interunidades) pela atenção, acolhida e pelo suporte para a realização do doutorado.

Ao laboratório de Pesquisa em Anatomia Vegetal (LAPAV) do Departamento de Biodiversidade e Bioestatística, IBB-UNES e ao Centro de Microscopia Eletrônica do Instituto de Biociências de Botucatu - Universidade Estadual Paulista, por disponibilizarem toda a infraestrutura necessária ao desenvolvimento deste trabalho e as análises realizadas em suas dependências.

Ao Laboratório de Anatomia Vegetal (LAV) do Instituto de Ciências Biológicas da Universidade Federal de Goiás pela disponibilização do espaço, reagentes e equipamentos indispensáveis a realização desta pesquisa.

À minha orientadora, Profª Drª Sílvia Rodrigues Machado e as minhas coorientadoras Profª Drª Elza Maria Guimarães Santos e Profª Drª Letícia de Almeida Gonçalves, pela orientação e oportunidade de crescer profissionalmente, elevando meu conhecimento e bagagem acadêmica.

À Profª. Drª. Lúcia Garcez Lohmann (Universidade de São Paulo) pela disponibilidade e auxílio na identificação das espécies coletadas para o uso nessa pesquisa.

À minha mamãe Maria Aparecida de Jesus, irmã Hanna Layse Mamede Macedo, esposo Carlos Gustavo da Silva Souza e ao meu filho Emanuel por me apoiarem incansavelmente durante essa extensa jornada e serem a minha motivação principal a cruzar essa linha de chegada. Aos meus entes queridos que já partiram desse mundo (Ana Mamede da Cunha, Claudomiro de Lima e Gilmar de Lima dos Reis), todas essas conquistas também são dedicadas a vocês.

Agradeço aos meus colegas dos laboratórios que frequentei (LAPAV e LAV) por toda a ajuda e companheirismo ao longo desses pouco mais de quatro anos de doutorado.

SUMÁRIO

RESUMO: Caracterização estrutural e histoquímica da corola de representantes de Bignonieae e Aliança Tabebuia ocorrentes em Cerrado

Bignoniaceae Juss. é composta por 112 gêneros e 840 espécies de distribuição pantropical, representada por árvores, arbustos ou lianas, muitas utilizadas na ornamentação e para fins medicinais. Os representantes desta família estão inclusos em seis tribos (Bignonieae, Catalpeae, Jacarandeae, Oroxyleae, Tecomeae e Tourrettieae) e dois clados informalmente nomeados de “Aliança Tabebuia” e “Clado Paleotropical”. A tribo Bignonieae Dumort. contém 21 gêneros e 393 espécies sendo a maioria neotropical; o clado “Aliança Tabebuia” é o segundo maior de Bignoniaceae, composto por 14 gêneros e 147 espécies. Ambos os clados estão bem representados em formações de Cerrado da região Centro-Oeste do Brasil; suas flores são zóofilas e polinizadas predominantemente por abelhas de médio a grandes portes, sendo *Pyrostegia venusta*, polinizada por beija-flores. A corola é o principal verticilo floral envolvido na atração de polinizadores, uma vez que nas pétalas ocorrem sinais visuais, táteis e olfativos que influenciam no sucesso reprodutivo das plantas. As características estruturais da corola tais como forma das células epidérmicas e rugosidade da cutícula têm forte associação com a função atrativa atuando na absorção e reflexão da luz e facilitando a aderência e locomoção do polinizador sobre a pétala. As caraterísticas do mesofilo também parecem influenciar a função atrativa, uma vez que a disposição de suas células pode aumentar a reflexão dos raios solares. Além disso, a presença de estruturas secretoras externas e internas na corola, especialmente aquelas especializadas na síntese e eliminação de compostos voláteis, exercem papel na interação com polinizadores. Apesar da comprovada relevância das características morfológicas e histoquímicas da corola em estudos de biologia reprodutiva e ecologia da polinização, a diversidade estrutural da corola e suas implicações nas interações com polinizadores em Bignoniaceae é pouco conhecida. Assim, estudos detalhados da corola com este foco se fazem necessários. Neste estudo, foram analisadas a micromorfologia da corola e a anatomia e histoquímica das pétalas de 18 espécies da tribo Bignonieae e três espécies do gênero *Handroanthus* Mattos pertencente ao clado “Aliança Tabebuia”. A presença de osmóforos foi testada em flores recém-abertas com o uso de Vermelho Neutro (VN). A seguir, as regiões coradas foram recortadas, fixadas e processadas para estudos em microscopia de luz (ML) e microscopia eletrônica de varredura (MV). O teste com VN revelou padrões distintos de distribuição de osmóforos em quatorze espécies de Bignonieae e em duas espécies de *Handroanthus*. Todas as espécies melitófilas de Bignonieae apresentaram células cônicas-papilosas na superfície adaxial da epiderme, com exceção da pétala de *Pyrostegia venusta* com células planas, polinizada por beija-flores. A maioria das espécies analisadas apresentaram cutícula rugosa, com estriações. O mesofilo variou de delgado (0,08 à 0,16 µm) a espesso (0,18 à 0,43 µm) e foi constituído de células parenquimáticas colunares, isodiamétricas ou braciformes com muitos espaços intercelulares, caracterizando parênquima aerenquimatoso. Nas regiões previamente coradas com VN, foram observados grãos de amido, gotas de lipídeos e terpenóides detectados na epiderme (com ou sem tricomas glandulares) e parênquima subepidérmico, confirmando a presença de osmóforos. Variações no padrão de distribuição e de estrutura dos osmóforos entre espécies visitadas por abelhas, incluindo variações dentro do mesmo gênero de Bignonieae, são novidades deste estudo. As variações no padrão de osmóforos, na microestrutura da superfície e nas características anatômicas do mesofilo das pétalas de Bignonieae geram um conjunto de características florais que podem orientar as visitas dos polinizadores. Epiderme composta por células papilosas foi a característica de maior importância no agrupamento das espécies visitadas por abelhas. Em *Handroanthus,* nas três espécies, a corola era composta por epiderme unisseriada composta por células cônico-papiladas, mesofilo aerenquimatoso e feixes vasculares colaterais. Superfícies rugosas, cutículas estriadas e dobradas, tricomas glandulares e não glandulares e estômatos elevados foram observados nas três espécies, o que pode ser caracteres úteis na taxonomia do grupo. Os tecidos secretores em diferentes regiões da corola das três espécies variaram em organização, com *H. coronatus* exibindo estruturas mais complexas formadas por células epidérmicas e células parenquimáticas subjacentes, sugerindo a ocorrência de osmóforo típico nesta espécie; *H. impetiginosus* apresentando apenas epiderme secretora com tricomas glandulares e não glandulares, e *H. serratifolius* exibindo epiderme secretora em paliçada ao longo de todo o comprimento do tubo da corola. Grãos de amido, gotículas lipídicas e terpenóides (exceto em *H. serratifolius*) foram encontrados nos tecidos da corola das três espécies, sendo mais abundantes nos tecidos secretores, sugerindo seu envolvimento na produção de perfume floral. Os resultados obtidos até o momento indicam um avanço considerável no conhecimento da diversidade estrutural da corola em Bignonieae. Assim, estudos detalhados sobre a corola visando este foco, são necessários para a interpretação das variações nas características da corola e relações funcionais entre flores e polinizadores, além de suporte a estudos taxonômicos.

Palavras-chave: Anatomia de pétalas**,** Bignoniaceae, Melitofilia, Microestrutura da corola, Polinização.

ABSTRACT: Structural and histochemical characterization of the corolla of representatives of Bignonieae and Tabebuia Alliance occurring in Cerrado

Bignoniaceae Juss. It is made up of 112 genera and 840 species of pantropical distribution, represented by trees, shrubs or lianas, many used for ornamentation and medicinal purposes. Representatives of this family are included in six tribes (Bignonieae, Catalpeae, Jacarandeae, Oroxyleae, Tecomeae and Tourrettieae) and two clades informally named "Tabebuia Alliance" and "Paleotropical Clade". The Bignonieae Dumort tribe. contains 21 genera and 393 species, the majority of which are neotropical; The "Tabebuia Alliance" clade is the second largest in Bignoniaceae, composed of 14 genera and 147 species. Both clades are well represented in Cerrado formations in the Central-West region of Brazil; its flowers are zoophilous and pollinated predominantly by medium to large bees, with *Pyrostegia venusta* being pollinated by hummingbirds. The corolla is the main floral whorl involved in attracting pollinators, since the petals contain visual, tactile and olfactory signals that influence the reproductive success of plants. The structural characteristics of the corolla, such as the shape of the epidermal cells and the roughness of the cuticle, have a strong association with the attractive function, acting on the absorption and reflection of light and facilitating the adherence and locomotion of the pollinator on the petal. The characteristics of the mesophyll also seem to influence the attractive function, since the arrangement of its cells can increase the reflection of solar rays. Furthermore, the presence of external and internal secretory structures in the corolla, especially those specialized in the synthesis and elimination of volatile compounds, play a role in the interaction with pollinators. Despite the proven relevance of the morphological and histochemical characteristics of the corolla in studies of reproductive biology and pollination ecology, the structural diversity of the corolla and its implications for interactions with pollinators in Bignoniaceae is little known. Therefore, detailed studies of the corolla with this focus are necessary. In this study, the micromorphology of the corolla and the anatomy and histochemistry of the petals of 18 species of the Bignonieae tribe and three species of the genus *Handroanthus* Mattos belonging to the "Tabebuia Alliance" clade were analyzed. The presence of osmophores was tested in newly opened flowers using Neutral Red (VN). Next, the stained regions were cut, fixed and processed for light microscopy (ML) and scanning electron microscopy (MV) studies. The VN test revealed distinct patterns of osmophore distribution in fourteen species of Bignonieae and two species of *Handroanthus*. All melitophilous species of Bignonieae presented conical-papillose cells on the adaxial surface of the epidermis, with the exception of the petal of *Pyrostegia venusta* with flat cells, pollinated by hummingbirds. Most of the species analyzed had rough cuticles, with striations. The mesophyll ranged from thin (0.08 to 0.16 µm) to thick (0.18 to 0.43 µm) and was made up of columnar, isodiametric or arm-shaped parenchyma cells with many intercellular spaces, characterizing aerenchymatous parenchyma. In regions previously stained with VN, starch grains, lipid drops and terpenoids were observed in the epidermis (with or without glandular trichomes) and subepidermal parenchyma, confirming the presence of osmophores. Variations in the pattern of distribution and structure of osmophores between species visited by bees, including variations within the same genus of Bignonieae, are new to this study. Variations in the pattern of osmophores, surface microstructure and anatomical characteristics of the mesophyll of Bignonieae petals generate a set of floral characteristics that can guide pollinator visits. Epidermis composed of papillose cells was the most important feature in the grouping of species visited by bees. In *Handroanthus*, in all three species, the corolla was composed of uniseriate epidermis composed of conical-papillate cells, aerenchymal mesophyll and collateral vascular bundles. Rough surfaces, striated and folded cuticles, glandular and non-glandular trichomes and elevated stomata were observed in the three species, which may be useful characters in the taxonomy of the group. The secretory tissues in different regions of the corolla of the three species varied in organization, with *H. coronatus* exhibiting more complex structures formed by epidermal cells and underlying parenchymatic cells, suggesting the occurrence of a typical osmophore in this species; *H. impetiginosus* showing only secretory epidermis with glandular and non-glandular trichomes, and *H. serratifolius* showing palisaded secretory epidermis along the entire length of the corolla tube. Starch grains, lipid droplets and terpenoids (except in *H. serratifolius*) were found in the corolla tissues of the three species, being more abundant in the secretory tissues, suggesting their involvement in the production of floral perfume. The results obtained so far indicate a considerable advance in the knowledge of the structural diversity of the corolla in Bignonieae. Therefore, detailed studies on the corolla with this focus in mind are necessary to interpret variations in corolla characteristics and functional relationships between flowers and pollinators, in addition to supporting taxonomic studies.

Keywords: Petal anatomy, Bignoniaceae, Melitophily, Corolla microstructure, Pollination.

1. INTRODUÇÃO GERAL

Bignoniaceae Juss. é composta aproximadamente por 112 gêneros e 840 espécies com distribuição pantropical e poucos representantes em regiões temperadas (Fischer et al., 2004; Lohmann e Ulloa, 2018). Inclui seis tribos (Bignonieae, Catalpeae, Jacarandeae, Oroxyleae, Tecomeae e Tourrettieae), juntamente com dois clados adicionais informalmente nomeados de “Aliança Tabebuia” e “Clado Paleotropical” (Olmstead et al., 2009). No Brasil, são estipulados cerca de 34 gêneros e 420 espécies de Bignoniaceae, sendo considerado como seu centro de diversidade (Gentry, 1980; Silva e Queiroz, 2003; Souza e Lorenzi, 2008), abrigando táxons endêmicos (o gênero *Paratecoma* Kuhlm. e 212 espécies) dos quais grande parte ocorre em florestas úmidas e secas e áreas de vegetação aberta, nos Domínios do Cerrado e Caatinga (BFG, 2015; Lohmann e Ulloa, 2016). Para o Cerrado, foram registrados 27 gêneros e 162 espécies, sendo que 26 gêneros e 96 espécies ocorrem em formações de Cerrado da região Centro-Oeste, especialmente no estado de Goiás, sendo estes táxons pertencentes às tribos Bignonieae, Crescentieae, Jacarandeae, clado “Aliança Tabebuia” e “Clado Paleotropical” (BFG, 2015). A tribo Bignonieae Dumort. contém 21 gêneros e 393 espécies sendo a maioria neotropical, representada por lianas e arbustos de folhas opostas e compostas, geralmente 3-folioladas com o folíolo terminal frequentemente substituído por gavinha simples ou multífida (Gentry, 1980; Olmstead et al., 2009; Lohmann e Taylor, 2014). Aliança Tabebuia é o segundo maior clado de Bignoniaceae, composto por 14 gêneros e 147 espécies arbóreas ou arbustivas de folhas compostas palmadas e simples, distribuídas nos neotrópicos (Bentham e Hooker, 1876; Olmstead et al., 2009).

Grande parte das espécies de Bignoniaceae é representada por árvores, arbustos ou lianas, com folhas opostas e compostas, flores conspícuas com corola zigomorfa, gamopétala e tubular, androceu composto por quatro estames e um estaminódio, gineceu bicarpelar e ovário bilocular, apresentando muitos óvulos por lóculo e frutos deiscentes do tipo cápsula ou indeiscentes (Lohmann, 2004). Diversas espécies são utilizadas como ornamentais: *Pyrostegia venusta* (Ker. Gawl.) Miers (flor-de-são-joão), *Jacaranda mimosifolia* D. Don. (jacarandá mimoso), *Crescentia cujete* L. (coité), *Tecoma stans* (L.) Juss. ex Kunth (ipê-de-jardim) e *Handroanthus albus* (Cham.) Mattos (ipê-amarelo); como madeiras nobres para construções (*Tabebuia alba* (Cham.) Sandwith e *Catalpa bignonioides* Walter) e algumas são reconhecidas por apresentarem propriedades medicinais (*Adenocalymma imperatoris-maximilianii* (Wawra) L. G. Lohmann, *Jacaranda caroba* DC e espécies do gênero *Handroanthus* Mattos), entre outras (Lohmann, 2004).

Bignoniaceae é caracterizada por apresentar grande diversidade de estruturas secretoras, tais como tricomas glandulares com morfologia diversa, nectários florais e extraflorais e osmóforos (Metcalfe e Chalk, 1950, 1979). A diversidade morfológica dessas estruturas e sua relevância em estudos farmacológicos, taxonômicos, ecológicos e evolutivos foi discutida para alguns representantes da família (Tabela 1).

Tabela 1. Estruturas secretoras florais em representantes de Bignoniaceae.

ESPÉCIES	CLADOS	ESTRUTURAS SECRETORAS	REFERÊNCIAS BIBLIOGRÁFICAS
Gênero *Campsis*	Tecomeae	Nectários florais	Elias e Gelband (1976)
Millingtonia hortensis L. Fil. *Jacaranda mimosifolia* D. Don *Tabebuia rosea* (Bertol.) Bertero ex A. DC. *Dolichandrone falcata* (Wall. ex DC.) Seem. *Stereospermum chelonoides* (L. Fil.) DC.	Oroxyleae Jacarandeae Aliança Tabebuia Tecomeae Bignonieae	Tricomas glandulares	Mehra e Kulkarni (1989) Muravnik et al. (2021)
Tabebuia serratifolia Nichols	Aliança Tabebuia	Nectários florais	Thomas e Dave (1992)
Pyrostegia venusta (Ker Gawl.) Miers	Bignonieae	Nectários florais	Galetto et al. (1994)
Argylia radiata (L.) D. Don. *Catalpa bignonioides* L. *Pandorea jasminoides* Lindl. *Podranea ricasoliana* (Tanfani) Sprague	Bignonieae Catalpeae Tecomeae	Nectários florais e tricomas glandulares	Rivera (1996)
37 táxons de Bignoniaceae	-	Nectários florais	Rivera (2000)
Lundia cordata A. DC.	Bignonieae	Tricomas glandulares	Lopes et al. (2002)
Jacaranda copaia (Aubl.) D. Don.	Jacarandeae	Tricomas glandulares, disco nectarífero e osmóforos	Maués (2006) Maués et al. (2008)
Tecoma stans (L.)	Jacarandeae	Osmóforos e nectários florais	Silva et al. (2007) Dutra e Machado (2001)
Jacaranda puberula Cham. LC. *Zeyheria montana* Mart.	Jacarandeae Tecomeae	Tricomas glandulares	Martins et al. (2008) Machado et al. (2006)
Jacaranda oxyphylla Cham.	Jacarandeae	Tricomas glandulares	Guimarães et al. (2008)
Eccremocarpus scaber R. *Anemopaegma album* Mart. ex DC. *Jacaranda oxyphylla* Cham.	Jacarandeae Bignonieae	Nectários florais	Belmonte et al. (1994) Guimarães et al. (2015,

Zeyheria montana Mart. e *Zeyheria tuberculosa* (Vell.) Bureau ex. Verl.	Tecomeae		2016, 2018) Machado et al. (2017)
Tribos Tecomeae e Bignonieae	-	Nectários florais	Galetto (2009)
Anemopaegma album Mart. ex DC. *Anemopaegma scabriusculum* Mart. ex DC.	Bignonieae	Nectários florais	Nogueira et al. (2012)
Handroanthus chrysotrichus (Mart. ex DC) mattos	Aliança Tabebuia	Osmóforos	Acra et al. (2012)
Adenocalymma magnificum Mart. ex DC. *Bignonia aequinoctialis* L.	Bignonieae	Tricomas glandulares	Gama (2013a)
Bignonia aequinoctialis L.	Bignonieae	Tricomas glandulares	Gama et al. (2013b)
Crescentia alata Kunth *Crescentia cujete* L.	Crescentieae	Tricomas glandulares e nectários florais	López et al. (2014)
Adenocalymma magnificum Mart. ex DC.	Bignonieae	Tricomas nectaríferos	Gama et al. (2016)
Jacaranda mimosifolia D. Don *Tanaecium jaroba* Sw.	Jacarandeae Bignonieae	Nectários florais e osmóforos	Alves et al. (2010) Frazão et al. (2020)
Seis espécies de *Jacaranda* Juss.	Jacarandeae	Tricomas glandulares	Oliveira (2020)
Pyrostegia venusta	Bignonieae	Osmóforos	Tunes et al. (2022)
Amphilophium mansoanum	Bignonieae	Osmóforos	Tunes et al. (em preparação)
18 espécies de Bignonieae	Bignonieae	Osmóforos	Macedo et al. (2023)

*Essa tabela foi confeccionada seguindo a mesma denominação dos autores, quanto as estruturas secretoras observadas.

A polinização das espécies da família é efetuada principalmente por abelhas de médio e grande portes, mas também atuam como vetores de polinização de algumas espécies de beija-flores, mariposas e morcegos (Gentry, 1980). Os visitantes florais, buscam recursos nas flores, entre eles, temos os visitantes esporádicos, frequentes, oportunistas, pilhadores, generalistas ou especialistas. Para ser um polinizador efetivo, o visitante precisa realizar a transferência do pólen das anteras para o estigma da flor de uma mesma espécie de planta (Alves-dos-Santos et al. 2016).

As características das pétalas ao fornecerem pistas visuais, táteis e olfativas afetam a qualidade dos sinais percebidos pelos diferentes polinizadores (Glover e Martin, 1998;

Whitney et al., 2011; Costa et al., 2016). A micromorfologia da pétala, especialmente a morfologia e as dimensões das células epidérmicas exercem forte influência na absorção de luz, na temperatura, brilho e na intensidade da cor (Kay et al., 1981; Papiorek et al., 2014; Van der Kooi et al., 2019; Wilsem et al., 2021). Geralmente, flores que são polinizadas por abelhas apresentam células epidérmicas cônicas, enquanto as que são polinizadas por pássaros, morcegos e vento possuem células epidérmicas planas (Kay et al., 1981). Algumas abelhas visitam com mais frequência flores com células cônicas do que flores que não apresentam tais formatos em suas células; tal fato se dá provavelmente devido à facilidade de pouso que as mesmas oferecem aos visitantes, sendo que a superfície epidérmica das pétalas influencia a maneira como os polinizadores interagem com a flor (Kay et al., 1981; Alcorn et al., 2012; Ojeda et al., 2012). Além disso, dimensões relacionadas a volume e comprimento das células epidérmicas cônicas alteram a absorção de luz e influenciam o brilho e a intensidade da cor, onde alguns pigmentos, como as antocianinas, quando retidos em tais células, contribuem para a atração dos polinizadores (Gorton e Vogelmann, 1996; Mol et al., 1998). Ao ocorrer a absorção de luz tais células elevam a temperatura, estimulando a atividades de células produtoras de substâncias voláteis (Kolosova et al., 2001; Whitney et al., 2011). Costa et al. (2016) descreveram a estrutura das pétalas de duas espécies de Bignoniaceae, *Tecoma stans* (L.) Juss ex. Kenth e *Spathodea campanulata* P. Beauv., sendo as mesmas compostas por células epidérmicas papilosas com variações nas estriações cuticulares da parede periclinal externa, presença de tricomas glandulares e mesofilo com células braciformes e espaços intercelulares. As células cônicas apresentaram distâncias significativas entre si e na altura, o que caracteriza uma interação tátil entre polinizador e a superfície da pétala, assim como, a presença de estrias e ceras epicuticulares associadas a essas células, que auxiliam no aumento da intensidade de cor da pétala, elevando a atratividade aos polinizadores (Costa et al., 2016). A presença de paredes celulares espessas e lignificadas nos tecidos das pétalas conferem resistência ao peso ou vibração dos polinizadores durante seu pouso (Kay et al., 1981; Alcorn et al., 2012).

O odor floral é considerado um dos principais componentes que mediam a interação entre flores e seus polinizadores (Vogel, 1990; Dudareva et al., 2013; Gervasi e Schiestl, 2017; Knudsen e Gershenzon, 2020; Dötterl e Gershenzon, 2023). Pode ser produzido e emitido por osmóforos que são estruturas complexas formadas de múltiplas camadas, incluindo células secretoras epidérmicas e várias camadas de células secretoras subepidérmicas, frequentemente apresentando gotículas lipídicas e grãos de amido (Effmert et al. 2006). As estruturas secretoras de voláteis florais diferem de outras estruturas secretoras pela composição química, local de deposição e duração da secreção (Endress, 1994; Hernández e Katinas, 2019).

A presença de osmóforos na família Bignoniaceae tem sido ressaltada em diversos estudos sobre a ecologia da polinização e biologia da reprodução (Silva et al., 2007; Guimarães et al., 2008, 2018; Alves et al., 2010; Acra et al., 2012; Frazão et al., 2020). Na maioria dos estudos (por exemplo, Zapater et al. 2009; Acra et al. 2012; Brasil & Guimarães-Brasil 2018), a presença de osmóforos tem sido descrita apenas com base no teste com Vermelho Neutro, e pouco se conhece sobre características estruturais e histoquímicas. Este estudo foi realizado utilizando a técnica tradicional de detecção de osmóforos com Vermelho Neutro no primeiro capítulo, e apenas a caracterização estrutural dessas glândulas no capítulo dois.

Apesar de apresentar certa relevância nas características morfológicas e histoquímicas da corola em estudos de biologia reprodutiva e ecologia da polinização, a revisão da literatura mostrou que estudos detalhados sobre a diversidade estrutural da corola em Bignoniaceae é ainda restrita a poucas espécies. Assim, estudos detalhados da corola com este foco se fazem necessários para inventariar as variações nas características da corola e interpretar as relações funcionais entre flores e polinizadores.

2. OBJETIVOS

Com o objetivo de investigar a diversidade estrutural da corola em Bignoniaceae e participar na ampliação da discussão sobre o papel das características da corola nas interações entre flores e polinizadores, neste trabalho analisamos a micromorfologia da superfície, a anatomia e histoquímica da corola em 21 espécies de Bignoniaceae distribuídas em oito gêneros ocorrentes em Cerrado, sendo 18 representantes da tribo Bignonieae e três do clado Aliança Tabebuia.

Com o desenvolvimento deste estudo, pretendemos responder as seguintes questões:

i) O padrão de distribuição de osmóforos é constante dentro do mesmo gênero e/ou entre espécies de gêneros distintos de Bignonieae?

ii) Existe relação entre o formato das células da epiderme das pétalas e os principais grupos de visitantes florais em Bignonieae?

iii) Existe variação na estrutura anatômica das regiões produtoras de compostos voláteis? Tais regiões são consistentes com a secreção e emissão destes compostos?

iv) As características da superfície da corola são partilhadas pelas três espécies de *Handroanthus* uma vez que são todas polinizadas por abelhas?

v) A organização dos tecidos secretores é comum às três espécies?

vi) As características anatômicas e histoquímicas da superfície da corola e das estruturas secretoras são consistentes com o esperado para espécies polinizadas por abelhas?

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APRESENTAÇÃO DE CAPÍTULOS

Conforme estabelecido pelo Programa de Pós-Graduação em Ciências Biológicas (Botânica) do IBB, UNESP, os resultados obtidos durante a execução deste projeto de doutorado foram reunidos em dois artigos científicos para publicação, os quais são apresentados de acordo com as normas de periódicos da área (Comitê de Biodiversidade da Capes).

CAPÍTULO 1: Osmophores and petal surface traits in Bignonieae species, publicado na revista The Science of Nature, Qualis A3, na área de Biodiversidade.

CAPÍTULO 2: Corolla structural and histochemical characterization in three bee-pollinated *Handroanthus* species (Bignoniaceae), submetido na revista Nordic Journal of Botany, Qualis A3, na área de Biodiversidade.

The Science of Nature (2023) 110:44
https://doi.org/10.1007/s00114-023-01873-6

ORIGINAL ARTICLE

Osmophores and petal surface traits in Bignonieae species

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Received: 19 April 2023 / Revised: 25 August 2023 / Accepted: 31 August 2023

Abstract

This study characterizes the osmophores and corolla traits in 18 species of Bignonieae Dumort., a Bignoniaceae tribe occur- ring in the Cerrado, a neotropical savanna in Brazil. To detect osmophore distribution, whole, newly opened flowers were immersed in Neutral Red Solution. Samples from the corolla tube and lobes were also fixed and analyzed micromorphologically, anatomically, and histochemically. The osmophores showed six markedly different distribution patterns that were not clearly associated with histological features. In most species, osmophores comprised papillose secretory epidermises and a few layers of subepidermal parenchyma. Starch grains, lipid droplets, and terpenes were detected in osmophores. An ornamented cuticle, cuticular folds, glandular and non-glandular trichomes, raised stomata and epicuticular wax granules are common traits in the species studied and may be useful in determining the taxonomy of the group. We found that 94% of the species visited by bees had papillose epidermises while the single hummingbird-pollinated species presented a flattened epidermis. Variations in osmophore pattern among species visited by bees, including variations within the same plant genus, are novel finding. Additionally, the Bignonieae species visited by bees presented a textured corolla surface, which has been reported as facilitating bee attachment and movement towards the floral resource. Future studies with a greater number of Bignonieae species and more detailed pollinator behavioral assays may help in the interpretation of the variations in corolla traits and functional relationships between flowers and pollinators.

Keywords Anatomy · Bee-pollination · Cuticular folds · Histochemistry · Hummingbird pollination · Osmophores · Micromorphology

Introduction

Among the Bignoniaceae tribes, Bignonieae Dumort. is an exclusively American clade, represented by lianas and shrubs, containing 21 genera and 393 species, most of which are tropical (Gentry 1980; Olmstead et al. 2009; Lohmann and Taylor 2014). The distribution of the highly diverse species of Bignonieae ranges from the humid forests of the Atlantic and Amazon forest to seasonal dry habitats, such as the savanna physiognomies of the Brazilian Cerrado, with an estimated number of 300 species in Brazil, which is its center of diversity (Lohmann 2010). Around 40% of these species, belonging to more than 85% of the genera, occur in the Cerrado, which makes it the most representative and highly diverse Bignoniaceae tribe in this phytogeographic domain (BFG 2015).

The flowers of Bignonieae species are zoophilous and are pollinated mainly by medium- and large-sized bees (Gentry 1974a; Alcantara and Lohmann 2010; Lohmann and Taylor

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Communicated by: Matthias Waltert

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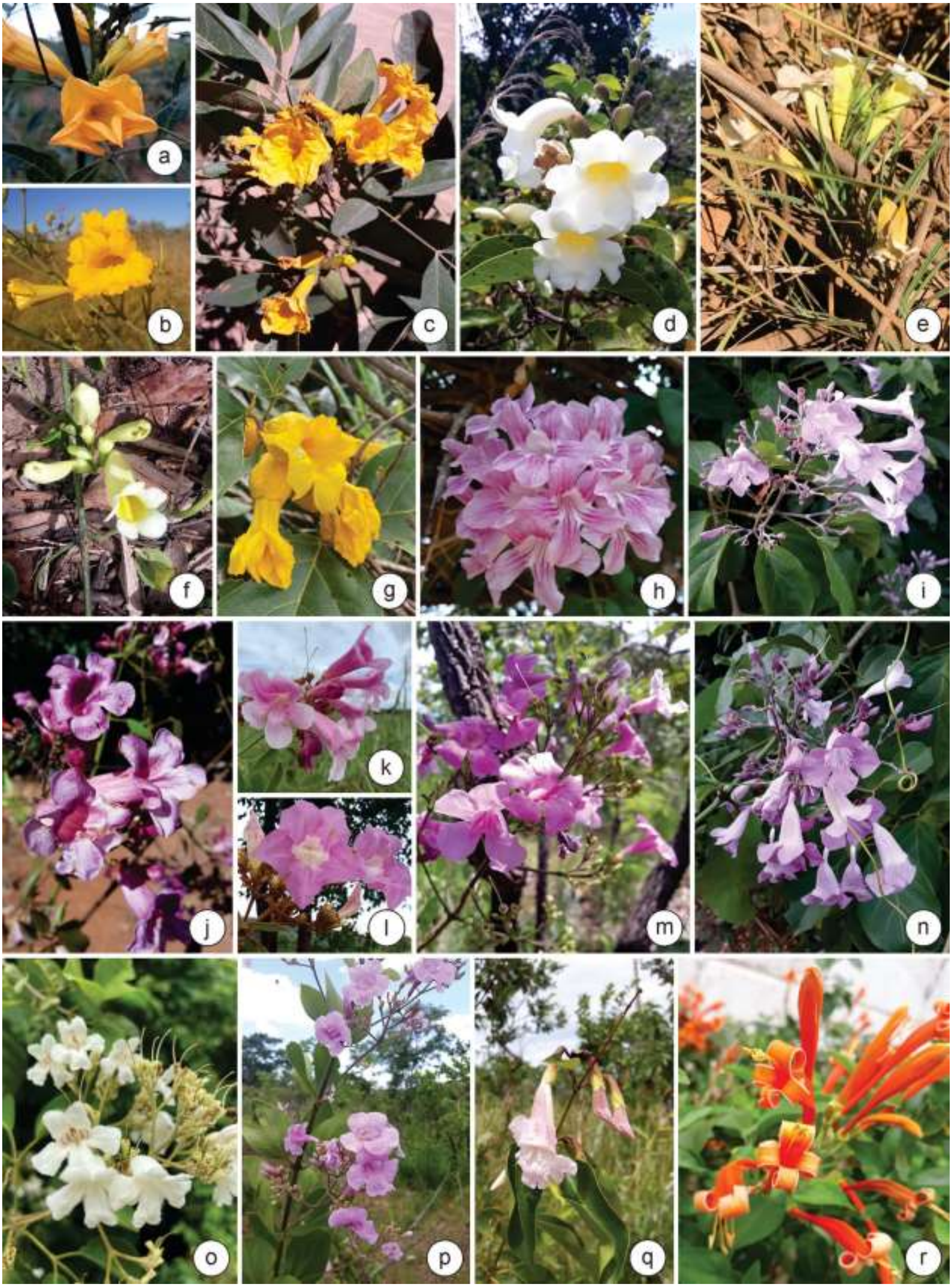
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◂**Fig. 1** Bignonieae species sampled in the Cerrado Domain. **a** *Adenocalymma nodosum*. **b** *Adenocalymma pedunculatum*. **c** *Adenocalymma peregrinum*. **d** *Amphilophium mansoanum*. **e** *Anemopaegma arvense*. **f** *Anemopaegma* sp. **g** *Callichlamys latifolia*. **h** *Cuspidaria convoluta*. **i** *Cuspidaria floribunda*. **j** *Cuspidaria pulchra*. **k** *Cuspidaria sceptrum*. **l** *Fridericia cinnamomea*. **m** *Fridericia celastroides*. **n** *Fridericia conjugata*. **o** *Fridericia florida*. **p** *Fridericia platyphylla*. **q** *Fridericia simplex*. **r** *Pyrostegia venusta*

2014). Their flowers are characterized by infundibuliform or tubular corollas, with leathery to membranous textures and variable colors, including white, yellow, pink, red, and magenta; nectar guides may be present or absent (Lohmann and Taylor 2014).

A remarkable amount of information on their corolla morphology has been widely used to support taxonomic and evolutionary studies in Bignonieae (e.g., Alcantara and Lohmann 2010; Fonseca et al. 2016). On the other hand, we find that little is known of the morphoanatomical and chemical attributes of the corolla and their possible implications in the interaction between the plants and their floral visitors. Osmophores or scent glands are secretory structures directly associated with chemical attraction (Vogel 1990). These have been reported in a few studies focusing on the ecology of pollination and reproduction in representatives of other Bignoniaceae tribes (Silva et al. 2007; Guimarães et al. 2008, 2018; Alves et al. 2010; Acra et al. 2012). To date, data on chemical attractors for Bignonieae, including osmophores and floral scent features, have been reported for only three species, *Tanaecium jaroba* Sw. (Frazão et al. 2020), *Pyrostegia venusta* (Ker Gawl.) Miers (Tunes et al. 2022), and *Amphilophium mansoanum* (DC.) L.G. Lohmann (Tunes et al. in prep.).

The corolla surface displays a range of variations in the shape, cell height, and roughness of the epidermal cells as well as in optical and chemical attributes (Bräuer et al. 2017; Kraaij and van der Kooi 2020 and references therein). Several studies have assumed that the characteristics of the petal surface microstructure seem to be closely linked to the type of pollinator (Alcorn et al. 2012) and have been assumed to be a labile floral trait (Ojeda et al. 2016). Indeed, the flower surface may present adequate textures to which pollinators can easily attach at the landing site. Thus, flowers showing structured petal surfaces bearing conical and papillate epidermal cells are usually visited by bees (Bräuer et al. 2017). The structured surfaces increase the foothold of bees facilitating attachment to the flower surface during their visits (Kay et al. 1981; Papiorek et al. 2014; Ojeda et al. 2016; Bräuer et al. 2017), whereas flat epidermal cells, covered with cuticular folds or epicuticular wax crystals, impair a honey bee's attachment (Bräuer et al. 2017) and are more associated with pollinators that have more limited mechanical interaction with the flowers and that do not need to grip the flower surface, pollinators such as bats and birds (Ojeda et al. 2016). Contrary to previous studies, Kraaij and van der Kooi (2020) found no correlation between epidermal microstructure and pollination system. Thus, the functional implications of the corolla surface microstructure on interactions between the plant and its floral visitors are still open to question.

This study is a comparative investigation of the corolla surfaces and anatomies of 18 species of Bignonieae in seven genera that are visited by bees and/or hummingbirds.

Materials and methods

Plant species, collection site, and sampling

A total of 18 species of Bignonieae (Fig. 1) were sampled from the Cerrado Domain in the state of Goiás, Brazil (13° to 17°S, 47° to 50°W, Table 1). Sampling took place during the flowering periods of each species from October 2020 to February 2022. Vouchers were deposited in the Herbarium "Irina Delanova de Gemtchujnicov" (Herbarium BOTU) of the Institute of Bio- sciences, UNESP, Botucatu, São Paulo, Brazil.

The corollas in the tribe Bignonieae are gamopetalous, large (2–6 cm long), infundibuliform or less frequently tubular in form, lobes 5, varying from weakly to markedly zygomorphic, with color varying from white to bright yellow, orange, magenta, pink, or red, and often marked with nectar guides (Lohmann and Taylor 2014). The 18 species studied are distributed among seven genera. In six of these genera, the corolla is infundibuliform, except for in *Pyrostegia* that shows a narrowly tubular corolla. In five genera, the corolla is membranous, except for in *Amphilophium* and *Callichlamys* which have coriaceous corollas. In most genera, the corollas are without nectar guides, with the exception of *Anemopaegma*. The positioning and orientation of the flowers of these 18 species vary, from almost parallel to the ground to pendant to some degree. Regardless of the orientation angle relative to the ground, in frontal view towards the tube entrance, the gamopetalous corolla is organized dorsiventrally (Fig. 2) with a "floor" and a "roof" (Fig. 2a), so we will follow Guimarães et al. (2008) and use the terms tube floor and tube roof. In cross-section, we will call the inner surface of the floral tube the adaxial surface and the outer surface the abaxial surface (Fig. 2b). The reproductive structures (stamens and stigma) are located under the tube roof (Fig. 2d). From the perspective of the flower's pollinator/visitor, its body's dorsal surfaces face the tube roof (Fig. 2d), and its ventral surfaces face the

Table 1 Bignonieae species collected in the Cerrado Domain of the state of Goiás, Brazil and the respective phytophysiognomies and geographic coordinates where they were collected

Species	Phytophysiognomy	Geographic coordinates
Adenocalymma nodosum (Silva Manso) L.G. Lohmann	Cerrado *lato* sensu and anthropized cerrado	14°27.8680′S, 48°11.2040′W; 15°33.7360′S, 48°36.2620′W; 16°3.4150′S, 48°50.9600′W; 17°46.6100′S, 48°41.9060′W
Adenocalymma pedunculatum (Vell.) L.G. Lohmann	Cerrado *lato* sensu	14°7.8500′S, 47°31.4020′W; 15°58.4480′S, 47°58.3860′W
Adenocalymma peregrinum (Miers) L.G. Lohmann	Cerrado *lato* sensu and anthropized cerrado	13°42.5400′S, 47°27.4350′W
Amphilophium mansoanum (DC.) L.G. Lohmann	Savanna physiognomies of cerrado and riparian forest	14°9.3230′S, 47°38.4350′W; 16°40.3320′S, 47°28.5180′W
Anemopaegma arvense (Vell.) Stellfeld ex de Souza	Cerrado *lato* sensu and cerrado vegetation on rocky outcrops	14°24.5150′S, 48°4.6860′W
Anemopaegma sp.—cf	Cerrado *lato* sensu and riparian forest	15°58.4480′S, 47°58.3860′W
Callichlamys latifolia (Rich.) K. Schum	Open physiognomies of cerrado and anthropized cerrado	15°5.4560′S, 48°51.3430′W; 15°33.7360′S, 48°36.2620′W
Cuspidaria convoluta (Vell.) A.H. Gentry	Cerrado *lato* sensu and riparian forest	15°58.4480′S, 47°58.3860′W; 17°17.3050′S, 48°47.0410′W; 17°44.1090′S, 48°38.9720′W
Cuspidaria floribunda (DC.) A.H. Gentry		
Cuspidaria pulchra (Cham.) L.G. Lohmann	Cerrado *lato* sensu and riparian forest	
Cuspidaria sceptrum (Cham.) L.G. Lohmann	Cerrado *lato* sensu and cerrado vegetation on rocky outcrops	16°37.715′S, 49°15.026′W
Fridericia celastroides (Bureau ex K. Schum.) L.G. Lohmann	Cerrado *lato* sensu	16°5.4390′S, 50°11.0880′W
Fridericia cinnamomea (DC.) L.G. Lohmann	Cerrado *lato* sensu and cerrado vegetation on rocky outcrops	13°47.3820′S 47°20.7850′W; 14°26.4010′S, 48°9.8950′W; 15°33.7360′S, 48°36.2620′W; 16°40.3320′S, 47°28.5180′W
Fridericia conjugata (Vell.) L.G. Lohmann	Open physiognomies of cerrado, anthropized cerrado and riparian forest	15°33.7360′S, 48°36.2620′W
Fridericia florida (DC.) L.G. Lohmann	Cerrado *lato* sensu and riparian forest	15°33.7360′S, 48°36.2620′W; 15°5.4560′S, 48°51.3430′W;
Fridericia platyphylla (Cham.) L.G. Lohmann	Savanna physiognomies of cerrado	14°55.3940′S, 47°45.2820′W; 15°58.4480′S, 47°58.3860′W; 16°41.0010′S, 49°8.1370′W; 16°5.4390′S, 50°11.0880′W; 17°14.0470′S, 48°43.7020′W
Fridericia simplex (A.H. Gentry) L.G. Lohmann	Savanna physiognomies of cerrado and riparian forest	16°4.3950′S, 50°11.2840′W
Pyrostegia venusta (Ker Gawl.) Miers	Cerrado *lato* sensu, riparian forest and anthropized areas	16°36.185′S, 49°15.575′W

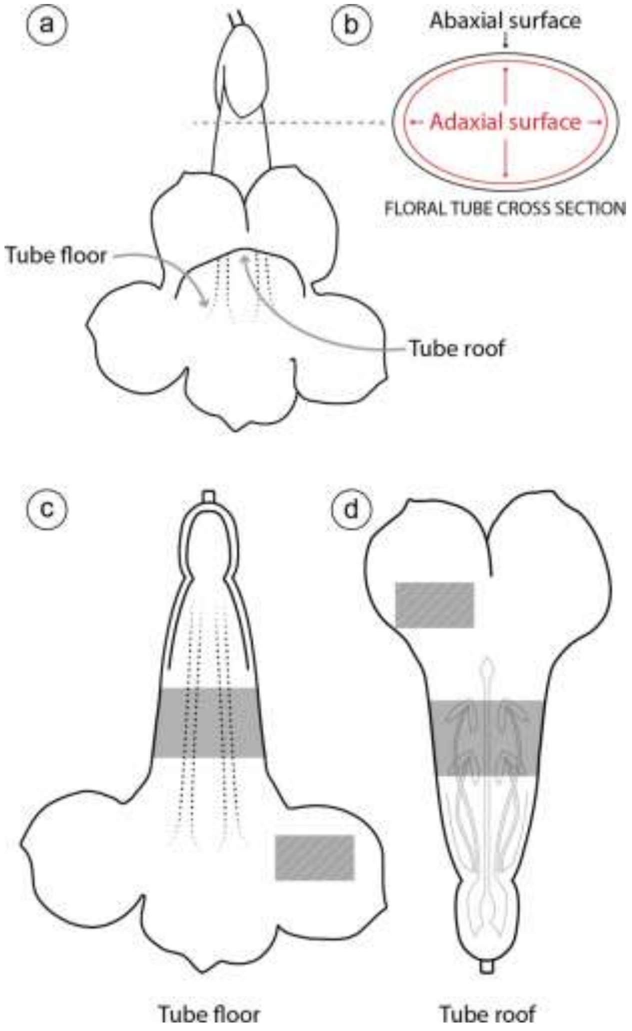


Fig. 2 Representation of a Bignonieae flower. **a** Frontal view of a flower indicating the floor and roof portions of the corolla tube. **b** Floral tube cross section showing the adaxial and abaxial surfaces. **c–d** Samples for microscopic study were taken from the median- upper portions of the corolla tube (shown in grey) and from the corolla lobes (shown in hatched grey), from the tube floor (**c**) and the tube roof (**d**)

tube floor (Fig. 2c) where the bee attaches and moves towards the flower base in search of trophic resource.

For each species, flowers from five inflorescences of at least three plants were sampled for morphological, anatomical, and histochemical study. For microscopic examinations, samples were taken from the tube floor, tube roof, and corolla lobes (Fig. 2c–d).

Detection, localization, and characterization of osmophores in situ

To locate metabolically active regions, interpreted as osmophores (Vogel 1990), 3 first-day whole flowers of at least five plants of 14 species were collected and immediately immersed in an aqueous solution of Neutral Red. This solution is used to mark regions whose metabolism is most active in the production of compounds that comprise the odor of such secretory structures (Vogel 1990). We carried out preliminary tests at different Neutral Red concentrations, 0.1%, 0.5%, and 1% (m/v), for periods ranging from 30 to 90 min for each concentration. Photographic records using a camera (Nikon) of the corolla from first-day flowers were made in the field before immersion in Neutral Red Solution. Soon after being removed from the solution, the same flowers were photographed in detail. In addition, they were sectioned longitudinally at the frontal plane (through the sagittal axis) to inspect the adaxial surface of the floor and roof tube (Fig. 2). We later identified general patterns of osmophore distribution and classified the 14 plant species accordingly.

The regions stained with Neutral Red were cut into 10 × 10 mm pieces with a scalpel after which they were washed in distilled water and then fixed for microscopic examination.

Microscopic examination

For scanning electron microscopy (SEM), the samples were fixed in 2.5% (v/v) glutaraldehyde (0.1 mol·L − 1phosphate buffer, pH 7.2), dehydrated in a graded ethanol series of 7.5, 15, 30, 50, 70, 90, and 100% (twice at each concentration for 10 min), then critical point dried (BAL-TEC AG, Balzers, Liechtenstein – Model CPD 030), using liquid CO_2. Samples of the corolla tube and corolla lobes were mounted on aluminum stubs, coated with gold (10 nm) (BAL-TEC AG, Balzers, Liechtenstein – Model SCD 050) for 160 s at a current 50 mA. The material was examined using a Quanta 200 SEM (Fei Company, FEI, Gräfelfing, Germany) at an accelerating voltage 20 kV.

For light microscopy (LM), samples from of the corolla tube and corolla lobes (Fig. 2) of the 18 Bignonieae species were fixed in neutral buffered formalin (TNF) for 48 h (Lillie 1965), stored in 70% ethanol, dehydrated in ethanol series (90% ethanol and P.A.), and embedded in glycol methacrylate historesin (Leica Microsystems Inc., Heidelberger, Germany). Transverse sections of the corolla pieces were cut to 5 µm thickness using a rotary microtome 2245 (Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany). The sections were stained with 0.05% (w/v) aqueous toluidine blue O (TBO) for 1 min at 60 °C on a hot plate (Feder and O'Brien 1969). The sections were mounted on glass slides with synthetic resin (Entellan New, Merck, Darmstadt, Germany).

To determine the chemical composition of the cellular inclusions and osmophore secretions, samples were subjected to histochemical tests. A Lugol solution (5% iodine solution added with 1% potassium iodide) was used to detect starch grains (Johansen 1940). The NADI reagent (N-dimethyl-p-phenylenediamine) was used to detect terpenes (David and Carde 1964). A 70% (w/v) ethanolic solution of Sudan IV was used to localize lipid (Johansen 1940). A 10% (w/v) aqueous solution of $FeCl_3$ was used to detect phenolic compounds (Johansen 1940). For each histochemical test, we carried out the standard control procedures suggested by authors of the respective techniques.

The sections were observed using a Leica DM 500 (Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany) optical microscope equipped with a digital camera (Leica ICC 50®).

The cuticle thickness was determined by morphometric analysis using ImageJ software (Schindelin et al. 2012). Values are reported as the minimum and maximum measurements (*n* = 3 cross-sections per species) using a light microscope with a × 400 objective.

Results

Osmophore distribution pattern

We found that 0.5% Neutral Red for 30 to 60 min gave the most satisfactory results in terms of clearly marking the regions bearing osmophores. A positive reaction to Neutral Red confirmed the presence of osmophores in 13 of the 14 species of Bignonieae examined; *Pyrostegia venusta* did not show any osmophores. We identified six patterns of osmophore distribution on the adaxial surfaces of the corollas (Fig. 3, Table 2): pattern [A], tiny spots distributed along the tube floor, ranging from the fauces to the constriction at the bottom of the floral tube, and scarcely present on the petal lobes (Fig. 3a); pattern [B], irregular and elongated spots on the floor and lateral areas of the floral tube but not including on the central region of the roof where the anthers and stigmas are placed (Fig. 3b); pattern [C], dense continuous lines along the entire length of the floral tube, on the floor and lateral areas, but not including on the central roof region area where the anthers and stigmas are placed (Fig. 3c); pattern [D], a large dense spot on the roof of the floral tube, corresponding to the area where the anthers and stigma are placed, and tiny spots distributed all over the floor of the floral tube (Fig. 3d); pattern [E], a continuous thick ring-shaped band in the upper third of the floral tube (Fig. 3e); and pattern [F], distributed over entire floral tube, from the fauces to the constriction at the bottom of the floral tube (Fig. 3f).

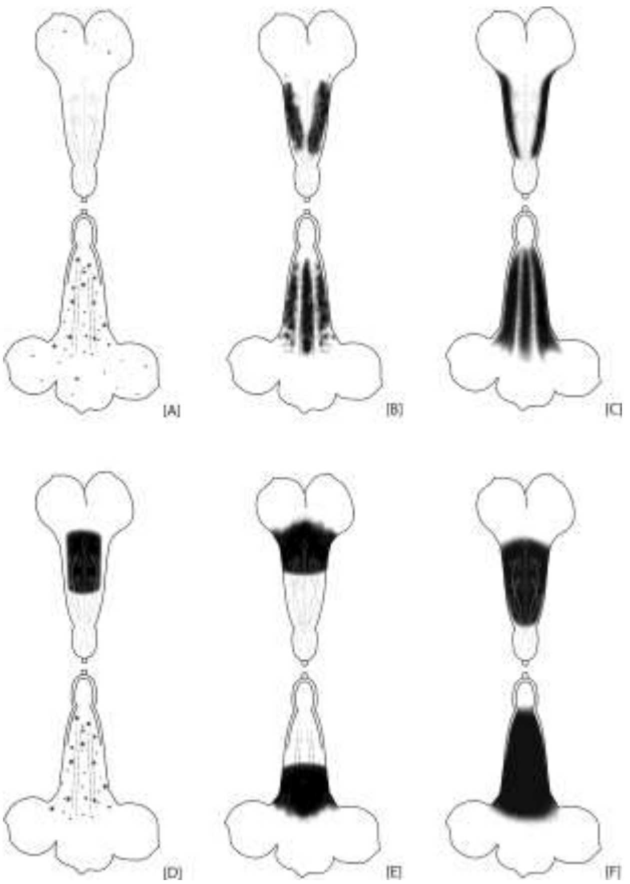


Fig. 3 Representation of the six patterns (A–F) of osmophore distribution (shown in dark), revealed by Neutral Red. The tube floor is represented below and the tube roof above in each figure. **A** Tiny spots distributed along the tube floor, ranging from the fauces to the constriction at the base of the floral tube, and sparsely distributed on the petal lobes. **B** Irregular and elongated spots in the floor and lateral portion of the floral tube, not encompassing the central region of the roof where the anthers and stigmas are located. **C** Dense continuous lines along the entire length of the floral tube, on the floor and lateral portion, without encompassing the central roof portion where the anthers and stigmas are located. **D** Dense large spot on the roof of the floral tube, corresponding to the area where anthers and stigma are located, and tiny spots distributed throughout the floor of the floral tube. **E** Continuous thick, ring-shaped band in the upper third of the floral tube. **F** Entire floral tube, ranging from the fauces to the con- striction at the base of the floral tube

Table 2 shows the different patterns of osmophore distribution observed in the 14 species Bignonieae species examined here and also provides information on the groups of pollinators/floral visitors recorded in the literature for these species (Fig. 4).

Table 2 Species of the Bignonieae tribe, osmophore distribution pattern based on positive reaction to the treatment with Neutral Red, as proposed by Vogel (1990), and their respective pollinators/floral visitors according to the literature.

Species	Osmophore distribution pattern	Pollinators/floral visitors	References for pollinator determination
Adenocalymma nodosum	[E]	Medium- to large-sized bees (Centridini)	Sampaio et al. (2016)
Adenocalymma pedunculatum	[A]	Medium- to large-sized bees (Centridini and Euglossini) and Hummingbirds	Lima et al. (2014)
Adenocalymma peregrinum	[C]	Medium- to large-sized bees (Centridini)	Sampaio et al. (2016)
Amphilophium mansoanum	[F]	Medium- to large-sized bees (Centridini)	Balduino et al. (2023)
Anemopaegma arvense	[B]	Medium- to large-sized bees (Centridini and Euglossini)	Firetti-Leggieri et al. (2013)
Anemopaegma sp.	-	Medium- to large-sized bees	Gentry (1974a)
Callichlamys latifolia	[F]	Medium- to large-sized bees (Euglossini)	Gentry (1974a)
Cuspidaria convoluta	[A]	Large-sized bees	Polatto (2020)
Cuspidaria floribunda	[A]	Medium- to large-sized bees	Genini et al. (2010)
Cuspidaria pulchra	[A]	Medium- to large-sized bees	Gentry (1974a)
Cuspidaria sceptrum	[A]	Medium- to large-sized bees	Gentry (1974a)
Fridericia celastroides	-	Bees	Gentry (1974a)
Fridericia cinnamomea	[D]	Medium- to large-sized bees	Gentry (1974a); Alcantara and Lohmann (2010)*
Fridericia conjugata	-	Medium- to large-sized bees (Centreline)	Gentry (1974a, b); Correia et al. (2005)
Fridericia florida	[A]	Small-sized bees	Gentry (1974a, b) *Fridericia*
platyphylla	[A]	Medium- to large-sized bees (Centridini)	da Rocha-Filho et al. (2018)
Fridericia simplex	-	Bees	Gentry (1974a)
Pyrostegia venusta	n.o	Hummingbirds	Gobatto-Rodrigues and Stort (1992); Tunes et al. (2022)

*Pollinator inferred based on the classification of this species as *Anemopaegma*-type

[A]: tiny spots distributed along the floor tube, ranging from the fauces to the constriction at the bottom of the floral tube, and scarcely distributed on the petal lobes. [B]: irregular and elongated spots in the floor and lateral portion of the floral tube, not encompassing the central region of the roof where the anthers and stigmas are placed. [C]: dense continuous lines along the entire length of the floral tube, on the floor and lateral portion, without encompassing the central roof portion where the anthers and stigmas are placed. [D]: dense large spot on the roof of the floral tube, corresponding to the area where anthers and stigma are placed, and tiny spots distributed throughout the floor of the floral tube. [E]: continuous thick ring-shaped band in the upper third of the floral tube. [F]: entire floral tube, ranging from the fauces to the constriction at the bottom of the floral tube. *n.o.*, not observed; the species not tested with Neutral Red are indicated by a small dash (-)

Anatomy and histochemistry of osmophores

Based on the above described classification of osmophore distribution, i.e., the patterns indicated by the Neutral Red test (Fig. 3, Table 2), the stained regions were isolated for anatomical and histochemical characterization. In cross sections, the osmophores comprised a single-layered papillose epidermis only, or an epidermis together with distinct subepidermal parenchyma cells, of variable shape from ovoid to tangentially elongated, arranged in from one to several layers (Fig. 5). When compared to the adjacent normal epidermal cells, the osmophore cells exhibited relatively large nuclei, higher densities of cytoplasm containing granulations and more developed vacuoles (Fig. 5b–d, f–g, h).

Among the species classified in pattern [A], the osmophores were variable. They comprised a single-layered epidermis in *Cuspidaria pulchra* (Fig. 5a) and *Fridericia florida* (Fig. 5b); or a single-secretory epidermis together with subepidermal parenchyma cells of ovoid to tangentially-elongated shape, arranged in one layer, as in *Fridericia platyphylla* (Fig. 5cd); or in two to three layers, as observed in most of the other species (Fig. 5e–h), including *Cuspidaria convoluta* (Fig 5e), *C. floribunda* (Fig. 5f), *C. sceptrum* (Fig. 5g), and *Adenocalymma pedunculata* (Fig. 5h). Tall, narrow cells with voluminous nuclei featuring a palisade secretory epidermis were observed in *Cuspidaria convoluta* (Fig. 5e), *C. floribunda* (Fig. 5f), and *C. sceptrum* (Fig. 5g). Raised osmophores with conspicuous secretory cells with

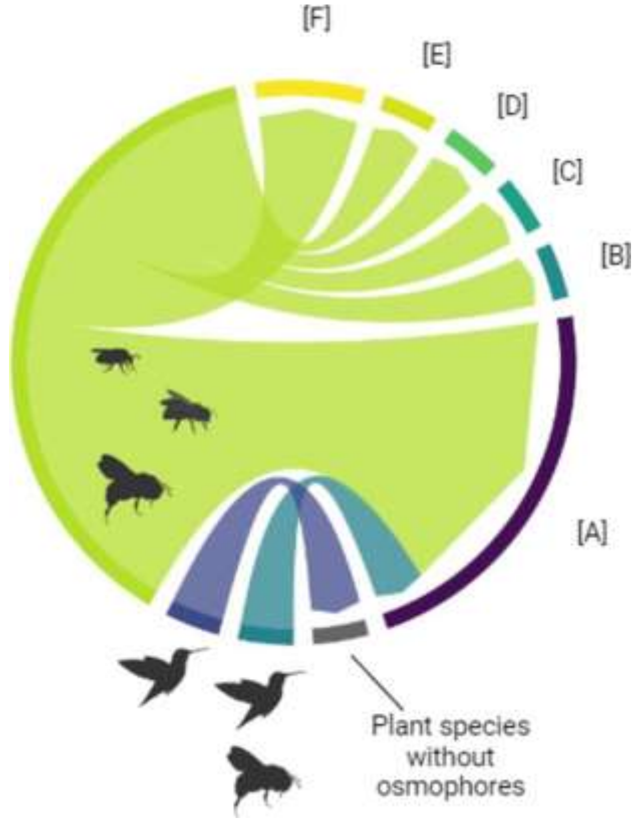


Fig. 4 Chord diagram showing the relationship between the absence/ presence pattern of osmophore distribution and the main groups of floral visitors of 14 Bignonieae species. The chords are unidirectional, and their thicknesses correspond to the number of species belonging to each category that are related to a given group of floral visitors. Built with BioRender.com

dense contents stained with toluidine blue occurred in *Adenocalymma pedunculata* (Fig. 5h).

In the species showing pattern [B], *Anemopaegma arvense*, the osmophore region comprised papillose secretory cells alternating with peltate glandular trichomes, and voluminous, juxtaposed subepidermal parenchyma cells arranged in one layer (Fig. 5i). In the species showing pattern [C], *Adenocalymma peregrinum*, the osmophores comprised a single-layered secretory epidermis with prominent papillose cells featuring rounded apices and inner wall projections and one to three layers of voluminous subepidermal parenchyma cells (Fig. 5j). In *Fridericia cinnamomea*, pattern [D], the secretory tissue in the dorsal portion of the corolla comprised a single-layered epidermis and tangentially expanded sub-epidermal parenchyma cells arranged in three to six layers (Fig. 5k); in this area, the secretory tissue was extensive and vascularized with xylem and phloem elements from the petal vascular bundles. In the ventral portion of the corolla, the osmophores comprised a single-layered secretory epidermis and subepidermal clusters formed by two to three voluminous parenchyma cells (Fig. 5l). In the species that displayed osmophores in pattern [E], *Adenocalymma nodosum* (Fig. 5m), the secretory tissue comprised papillose cells and parenchyma cells arranged in two to three subepidermal layers. In the species classified as pattern [F], *Amphilophium mansoanum* and *Callichlamys latifolia*, the secretory tissue was very distinct. In *A. mansoanum*, the secretory epidermis comprised irregularly shaped cells (somewhat flattened) alternating with non-secretory and secretory trichomes and about six layers of tangentially elongated subepidermal parenchyma cells; xylem and phloem elements from the petal vascular bundles were linking them with the parenchyma (Fig. 5n–o); papillose cells were characterized by irregular dimension and thickened walls (Fig. 5o). In *Callichlamys latifolia*, the secretory epidermis comprised papillose cells alternating with flattened cells presenting thin walls and subepidermal parenchyma cells arranged in two layers (Fig. 5p).

Although lipids were commonly present in all petal tissues, they were especially abundant in the epidermis and parenchyma cells of the osmophores (Fig. 6a–c, Table 3). Oil bodies were commonly larger in the parenchyma cells (Fig. 6b, c) and were seen as small droplets in the protoplast and walls of the epidermal cells (Fig. 6a–c). Starch grains, detected with Lugol solution, were uniformly distributed at the basal pole of the secretory epidermal cells (Fig. 6d–g) forming a continuous layer of small granules. Terpenes were detected as cell inclusions stained violet and small droplets stained blue in the epidermal and parenchyma secretory cells (Fig. 6h–j).

Corolla microstructure

Under the SEM, the corolla of the 18 species showed common epidermal cells of variable dimensions and shapes, with different degrees of undulations of the anticlinal walls and projections of the outer periclinal walls (Fig. 7), cuticular ornamentation (Fig. 8), stomata (Fig. 9), and trichomes with variable morphologies (Fig. 10). Detailed results of the surface micromorphology are shown in Table 4.

The undulation of the anticlinal walls varied from straight (Fig. 7a, c, i), to curved (Fig. 7b, f, k), to slightly undulate (Fig. 7d–e, g) to undulate (Fig. 7h, j). The curvature of the outer wall ranged from flat (Fig. 7a), to slightly convex (Fig. 7b–e) to conical (Fig. 7f–h), to rounded, to dome-shaped papillae (Fig. 7i–j) and half-spheres (Fig. 7k–l).

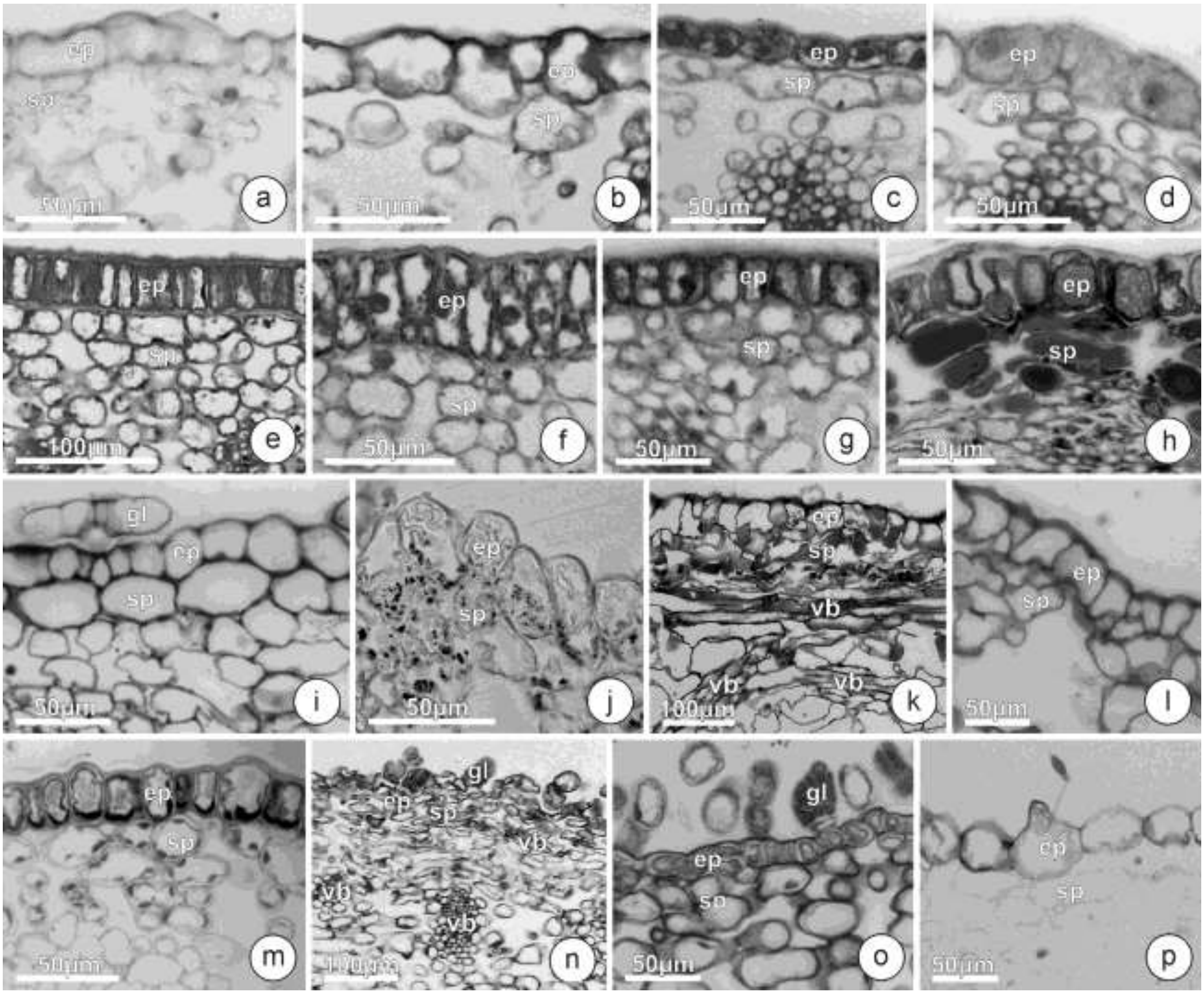


Fig. 5 Anatomical features of the osmophores in cross-sections of the adaxial surface of corolla of Bignonieae species. **a–b** Osmophores consisted of a single-layered epidermis in *Cuspidaria pulchra* (**a**) and *Fridericia florida* (**b**). **c–d** *Fridericia platyphylla* osmophores composed of a single-layered secretory epidermis and subepidermal parenchyma cells arranged in one layer. **e–h** Osmophores composed of a single-layered secretory epidermis and subepidermal parenchyma cells arranged in two to three layers in *Cuspidaria convoluta* (**e**), *C. floribunda* (**f**), *C. sceptrum* (**g**), and *Adenocalymma pedunculata* (**h**). **i** *Anemopaegma arvense* osmophore composed of papillose secretory cells alternating with glandular trichomes and subepidermal parenchyma cells arranged in one layer. **j** *Adenocalymma peregrinum* osmophores composed of prominent papillose cells with inner wall projections and one to three layers of subepidermal parenchyma cells. **k–l** *Fridericia cinnamomea* osmophores consisted of single-layered epidermis and several layers of tangentially expanded subepidermal parenchyma cells in the roof of the corolla tube (**k**), and clusters of subepidermal parenchyma cells in the floor of the corolla tube (**l**). Note the vascular bundle bordering the secretory parenchyma (**k**). **m** *Adenocalymma nodosum* osmophores were composed of papillose cells with dense parietal cytoplasm and parenchyma cells arranged in two to three subepidermal layers. **n–o** *Amphilophium mansoanum* osmophores consisted of irregularly shaped cells alternating with non-glandular and glandular trichomes, and several layers of tangentially elongated subepidermal parenchyma cells. Note the vascular bundles bordering the parenchyma (**n**). **p** *Callichlamys latifolia* osmophores were composed of papillose cells alternating with flattened thin-walled cells and subepidermal parenchyma cells arranged in two layers. Symbols: ep, epidermis; gl, glandular trichomes; sp, secretory parenchyma; vb, vascular bundles.

Based on the curvatures and projections of the outer periclinal wall, the corolla surface was classified in two patterns: flattened and papillose (Table 4). Among the bee-visited species, 94% had papillose surfaces, and only the hummingbird-pollinated species had flattened surfaces (Fig. 8).

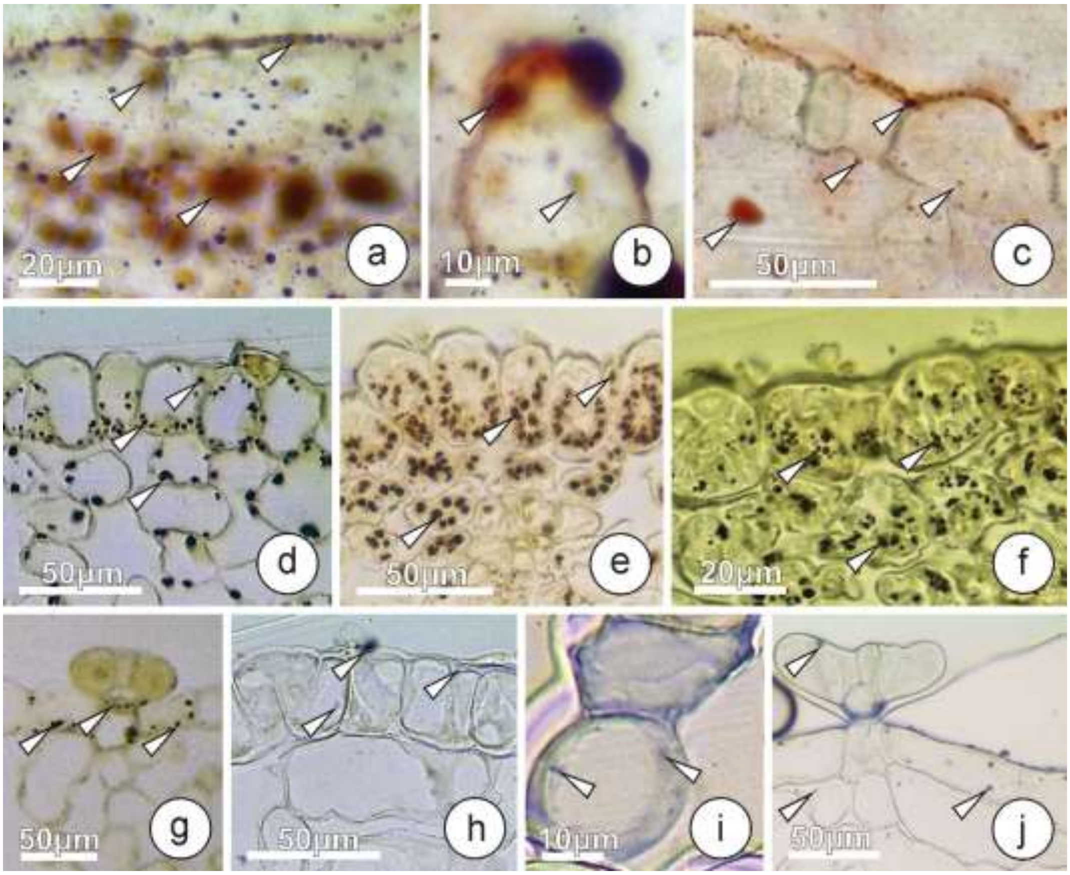


Fig. 6 Histochemical features of the osmophores in Bignonieae species. Cross sections of the corolla showing the adaxial surface of *Adenocalymma pedunculatum* (**a**, **b**, **f**); *Amphilophium mansoanum* (**c**, **i**), *Adenocalymma peregrinum* (**d**), *Adenocalymma nodosum* (**e**) and *Anemopaegma arvense* (**g**, **j**). **a**–**c** Detection of lipid bodies (red color) in the parenchyma and epidermal cells with Sudan IV. **d**–**g** Detection of starch grains (brown to black colors) with Lugol solution. **h**–**j** Detection of terpenes (cell inclusion stained blue or violet colors) with NADI reagent. Symbols: (arrowheads) positive reactions

In most of the species studied, the corolla had an ornamented cuticle (Figs. 7, 9). Cuticular folds varied from thin to thick and also occurred as short curved bands confined to each cell (Fig. 9a–c) to long straight, or curved, and continuous parallel folds distributed longitudinally over the whole corolla surface (Fig. 7b, Fig. 9d–e, g). Various cuticular folds were observed in the petals of the different species, such as the following: continuous and parallel running curved ones (Fig. 9d); strongly curved ones on the outer wall with radiating ones across the anticlinal wall boundaries (Fig. 9e); folds forming a dense reticulum on buttressed ridges (Fig. 9f–g); dense, intricate networks of short and long curved folds on buttressed ridges with deep sulci at its sides (Fig. 9h); and a coarse reticulum of tall folds (Fig. 9i) and folded cuticle around the papillae (Fig. 9j). Epicuticular wax granules were observed on the cuticles of *Adenocalymma nodosum* (Fig. 9j) and *A. peregrinum* (Fig. 9i).

Stomata occurred in 16 of the 18 species examined: in four species, they were found on both surfaces of the petals (amphistomatic), in four species on the adaxial surface (epistomatic), and in six species on the abaxial surface (hypostomatic). The details of stomatal distribution in each corolla portion and petal surface are summarized in Table 4. Morphologically, various patterns of stomata were observed (Fig. 10). Ordinary stomata presenting a long, narrow aperture, with outer stomatal rim were observed in the abaxial surfaces of *Adenocalymma nodosum* (Fig. 10a); prominent stomata with narrow, ellipsoidal aperture surrounded with outer stomatal rim, and smooth subsidiary cells were observed in *Pyrostegia venusta* (Fig. 10b). Stomata with subsidiary cells radially arranged, presenting epicuticular wax granules, occurred in *Callichlamys latifolia* (Fig. 10c). Slightly sunken stomata with long, wide aperture and a distinct

Table 3 Compounds detected in the osmophores of 14 species of Bignonieae and portions of the corolla in which they were detected.

Species	Starch grains	Lipid drops	Terpenes
Adenocalymma nodosum	Roof: P Lobes: E, P	Roof: E, P Lobes: E, P Floor: E, P	n.r
Adenocalymma pedunculatum	Roof: E, P Lobes: E, P Floor: E, P	Roof: E, P Lobes: E, P Floor: E, P	n.r
Adenocalymma peregrinum	Roof: E, P Lobes: E, P Floor: E, P	Roof: E, P Lobes: E, P Floor: E, P	n.r
Amphilophium mansoanum	Roof: E Lobes: E	Roof: E, P Lobes: E, P	n.r
Anemopaegma arvense	-	Roof: E, P Lobes: E, P Floor: E, P	n.r
Callichlamys latifolia	-	Roof: E, P Lobes: E, P Floor: E, P	n.r
Cuspidaria convoluta	Roof: E, P Lobes: E, P	Roof: E, P Lobes: E, P Floor: E, P	n.r
Cuspidaria floribunda	Roof: E Lobes: P	Roof: E, P Lobes: E, P Floor: E, P	n.r
Cuspidaria pulchra	Roof: E, P Floor: E, P	Roof: E, P Lobes: E, P Floor: E, P	n.r
Cuspidaria sceptrum	Roof: E, P	Roof: E, P Lobes: E, P Floor: E, P	n.r
Fridericia cinnamomea	Roof: E	Roof: E, P Lobes: E, P	n.r
Fridericia florida	Roof: E, P Lobes: E, P	Roof: E, P Lobes: E, P	n.r
Fridericia platyphylla	Roof: E, P Lobes: E, P Floor: E, P	Roof: E, P Lobes: E, P Floor: E, P	Floor: E
Pyrostegia venusta	-	Roof: E, P Lobes: E, P Floor: E, P	Floor: E

Roof tube roof, *Lobes* corolla lobes, *Floor* tube floor, *E* epidermis, *P* parenchyma, *n.r.* negative result/no detection. The species not tested for starch grains are indicated by a small dash (-)

outer stomatal rim with short laterally extended cuticular folds occurred in *Cuspidaria sceptrum* (Fig. 10d). Raised stomata surrounded by curved folds of cuticle on the subsidiary cells were found in different species, such as *Anemopaegma arvense* (Fig. 10e), *Adenocalymma pedunculatum* (Fig. 10f), and *Cuspidaria sceptrum* (Fig. 10g). Giant stomata, with prominent aperture and stomatal rim, raised by their subsidiary cells occurred in *Amphilophium mansoanum* (Fig. 10h). Giant raised stomata with concentric rings of striae occurred in *Fridericia simplex* (Fig. 10i).

There were both non-glandular and glandular trichomes all over the corolla surfaces in 16 of the 18 species studied, while in two species, the surfaces were glabrous (Table 4).

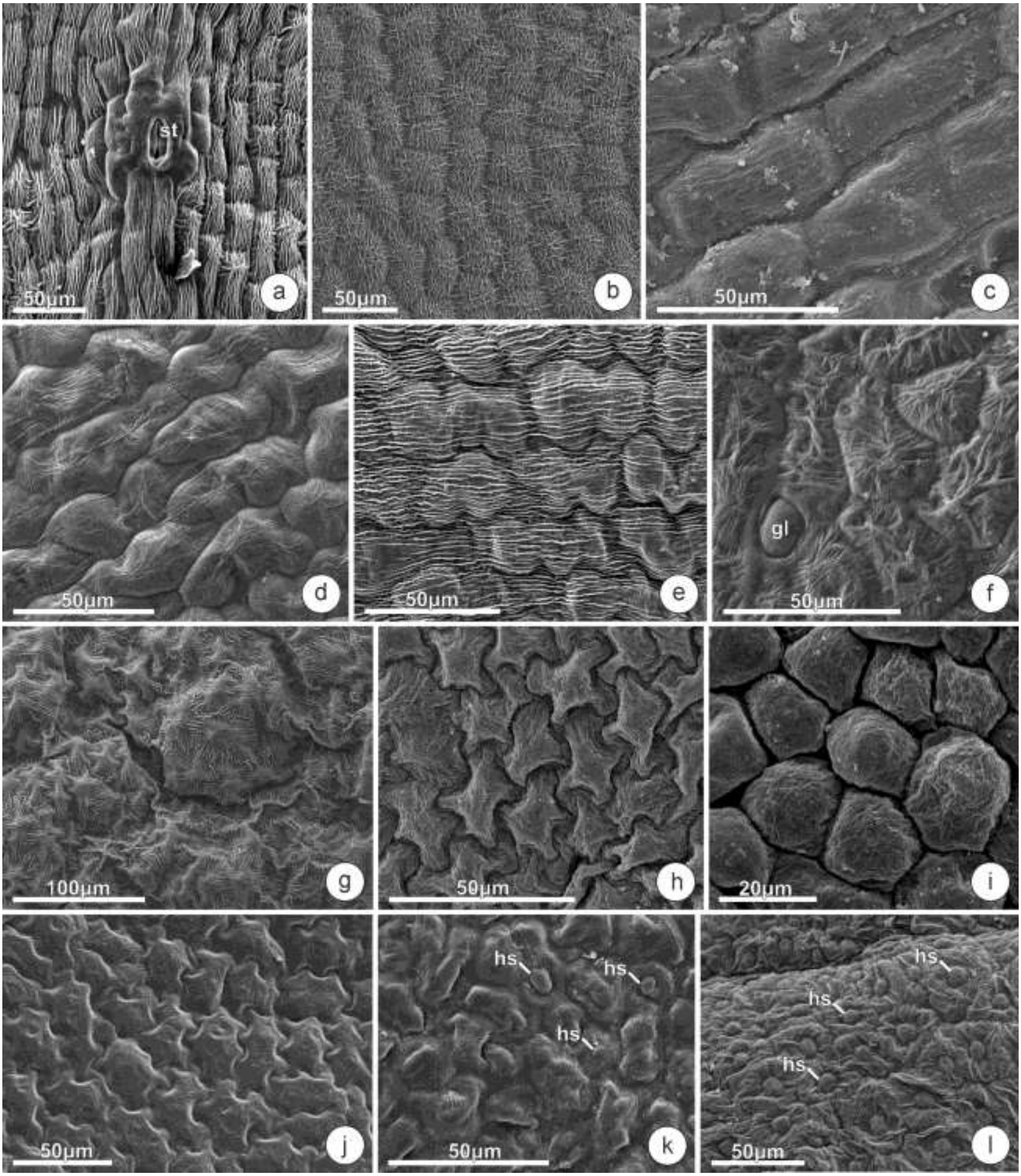
The non-glandular trichomes were of variable size in one or both the corolla surfaces in several species (Table 4). These non-glandular trichomes were of the following types: Simple, uniseriate, and multicellular, slightly curved, parallel to the epidermal surface (Fig. 11a), with thin (Fig. 11b) or globular bases (Fig. 11c), with pointed apical cells (Fig. 11b) or broader apices (Fig. 11d) were commonly observed in the species studied. The majority of non-glandular trichomes exhibited linear warty ornamentations (Fig. 11a–d). Long non-glandular trichomes, adpressed to the corolla surface, with smooth surfaces were observed in a few species (Fig. 11e–f).

Multicelular stalked, two-to-five-armed trichomes occurred only on the corolla abaxial surfaces of a few species, such as *Callichlamys latifolia* (Fig. 11h).

Two types of glandular trichomes occurred: peltate and capitate (Table 4). Both types were variable in gross morphology. Sessile peltate trichomes with flattened multicellular secretory heads covered with smooth cuticle (Fig. 11i) and peltate trichomes with unicellular heads usually sunken into the adaxial surface (Fig. 11f) were common and widely distributed throughout the corolla of *Anemopaegma* species. Capitate glandular trichomes were variable in dimension and thickness of the stalk and in the shape of the secretory head (Fig. 11j–p). Short-stalked capitate trichomes with multicellular and biseriate bulbous heads were observed in the adaxial surface of the tube floor in *Anemopaegma arvense* (Fig. 11j), and short-stalked capitate trichomes with multicellular and uniseriate ovoid heads occurred in several species, as in *Fridericia simplex* (Fig. 11k). Capitate glandular trichomes slightly raised and formed by an inflated short stalk and flattened head occurred in *Adenocalymma peregrinum* (Fig. 11l) and with a bulbous head in *Cuspidaria convoluta* (Fig. 11m) and *Fridericia conjugata* (Fig. 11n). Trichomes with thickened long stalks with a folded cuticle surface and raised by a pedestal formed with radially arranged common epidermal cells and a small multicellular, uniseriate flattened head occurred in *Cuspidaria* species (Fig. 11o–p).

Corolla anatomical features

The corolla tube, in cross section, in all species studied exhibits a single-layered epidermis composed of flattened to papillose cells of irregular dimension, often alternating



with trichomes, mesophyll variable in structure and thickness, and vascular bundles of the collateral type (Fig. 12).

An epidermis composed of conical to rounded cells was observed on the adaxial and abaxial surfaces in *Adenocalymma nodosum* (Fig. 12a). Slightly convex epidermal cells on both surfaces alternating with glandular trichome on the adaxial surface were observed in *A. pedunculatum* (Fig. 12b). Papillose cells alternating with

◂ **Fig. 7** SEM images of the corolla surface of Bignonieae species illustrating undulations of the anticlinal walls and projections of the outer periclinal wall. **a** *Pyrostegia venusta*, adaxial surface of the tube floor covered by long, flat cells with straight walls and folded cuticle. **b** *Pyrostegia venusta*, adaxial surface of the lobe floor covered by slightly convex square cells with curved walls and folded cuticle. **c** *Callichlamys latifolia*, adaxial surface of the tube roof covered by long, slightly convex cells featured with straight walls. **d** *Anemopaegma arvense*, adaxial surface of tube roof covered by slightly convex cells with slightly undulate walls. **e** *Anemopaegma arvense*, adaxial surface of the tube floor covered by slightly convex cells with slightly undulate cell walls. **f** *Anemopaegma* sp., adaxial surface of the tube floor covered by conical cells with curved cell walls. Note the glandular trichome sunken into the surface. **g** *Fridericia platyphylla,* abaxial surface of the tube roof covered by conical cells with slightly undulate cell walls. **h** *Adenocalymma pedunculatum*, abaxial surface of the tube roof covered by conical cells with undulate cell walls. **i** *Adenocalymma pedunculatum*, adaxial surface of the tube floor covered by rounded, dome-shaped papillae with straight cell walls. **j** *Adenocalymma nodosum,* abaxial surface of the tube roof covered by dome- shaped papillae with undulate cell walls. **k** *Adenocalymma nodosum*, adaxial surface of the tube floor covered by half-sphere papillae with curved cell walls. **l** *Adenocalymma nodosum*, adaxial surface of the lobe floor covered by half-sphere papillae. Note the slightly collapsed cells by the preparation method in d, f, g, i, l. Symbols: st, stomata; gl, glandular trichome; hs, half-sphere papillae

globose base of non-glandular trichomes on both surfaces were observed in *A. peregrinum* (Fig. 12c). Epidermis with abundant trichomes on both surfaces was observed in *Amphilophium mansoanum* (Fig. 12 d). Papillose epidermis alternating with peltate glandular trichomes in was observed *Anemopaegma arvense* (Fig. 12e) and *Anemopaegma* sp. (Fig. 12f). Papillose epidermis on the adaxial surface and flattened rectangular cells was observed on the abaxial surface in *Callichlamys latifolia* (Fig. 12g). Papillose cells on both surfaces, alternating with non-glandular trichomes on the abaxial surface were observed in *Cuspidaria convoluta* (Fig. 12h). Palisade epidermis on both surfaces and glandular trichomes on the abaxial surface was observed in *Cuspidaria floribunda* (Fig. 12i). Papillose epidermis on both surfaces, alternating with conspicuous trichomes on the abaxial surface was observed in *Cuspidaria pulchra* (Fig. 12j). Palisade epidermis on both surfaces was observed in *Cuspidaria sceptrum* (Fig. 12k). Papillose epidermis alternating with non-glandular and glandular trichomes on both surfaces was observed in *Fridericia celastroides* (Fig. 12l), *F. cinnamomea* (Fig. 12m), and *F. florida* (Fig. 12o), while trichomes only on the abaxial surface were observed in *F. conjugata* (Fig. 12n), *F. platyphylla* (Fig. 12p), and *F. simplex* (Fig. 12q). In *F. platyphylla*, rectangular epidermal cells on the adaxial surface and tall cells on the abaxial surface were observed (Fig. 12p). Epidermis composed of conspicuous conical cells with flattened outer periclinal walls was observed on both surfaces in *Pyrostegia venusta* (Fig. 12r).

The cuticle, in cross section, was classified as thick (between 0.18 and 0.43 µm) in 12 species (*Adenocalymma nodosum*, *A. pedunculatum*, *Amphilophium mansoanum*, *Anemopaegma* sp., *Callichlamys latifolia*, *Cuspidaria convoluta*, *C. sceptrum*, *Fridericia cinnamomea*, *F. conjugata*, *F. florida*, *F. platyphylla*, and *Pyrostegia venusta*) or thin (between 0.08 and 0.16 µm) in six species (*Adenocalymma peregrinum*, *Anemopaegma arvense*, *Cuspidaria floribunda*, *C. pulchra, Fridericia celastroides*, and *F. simplex*).

In cross section, the mesophyll of the 18 species was composed of a variable number of thin-walled parenchyma cells of various shapes and sizes. The mesophyll structure ranged from relatively compact and homogeneous, com- posed of isodiametric cells and small intercellular spaces (Fig. 12d, i, k, r) to a loose structure, with branched cells and large intercellular spaces characterizing a typical aerenchymatous mesophyll (Fig. 12a, j, l–q).

In all species, the corollas were vascularized by collateral-type bundles (Fig. 12) of different diameters and distributed at different levels in the mesophyll.

Discussion

This study presents a characterization of the corollas in several species of the Bignonieae tribe. We found six patterns of osmophore distribution in the 14 species analyzed. The osmophore distribution varied from over the whole floral tube to occurring in small, scattered spots only on the floral tube floor, the latter being present in 50% of these species. More than 90% of species were bee-visited and presented osmophores. Only one species was exclusively hummingbird pollinated and showed no evidence of osmophores. In most species, osmophores comprised a papillose secretory epidermis and a few layers of subepidermal parenchyma. An ornamented (folded) cuticle, trichomes, and raised stomata were common features of the petal surface in the species studied. We found that 94% of the bee-visited species had papillose epidermises, and the single hummingbird-pollinated species had an irregular epidermis (somewhat flattened). Our results contribute to a broadening discussion of the morphological and histochemical characteristics of petals and their potential role in plant-pollinator interactions, as discussed here.

The presence of osmophores with different patterns of distribution on the corolla surface is a notable feature, being present in more than 90% of the members of Bignonieae studied. In general, Bignoniaceae flowers

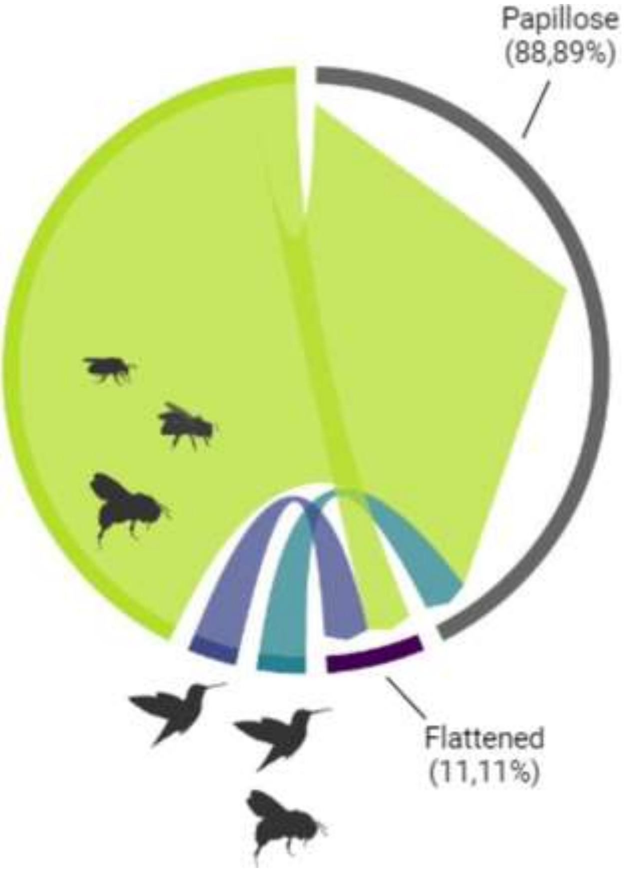


Fig. 8 Chord diagram showing the relationships between the types of corolla adaxial surface of the tube roof and the main groups of floral visitors reported for the 18 Bignonieae plant species. The chords are unidirectional, and their thicknesses correspond to the number of species that present each type of corolla adaxial surface and are related to a given group of floral visitors. Built with BioRender.com

are visually conspicuous; however, they are usually not strongly scented (see Guimarães et al. 2018; Souza et al. 2019; Tunes et al. 2022). In Bignonieae, this can be related to the observation that the most common osmophore distribution is pattern [A], and this is present in 50% of the species studied. In pattern [A], the secretory area is smaller than that in the other five patterns. Osmophores are responsible for the production and release of volatile substances commonly associated with pollinator attraction during anthesis (Vogel 1990; Knudsen and Gershenzon 2020). The occurrence of osmophores has been reported in two species of Jacarandeae (Guimarães et al. 2008; Alves et al. 2010) and in only one species of Bignonieae, *Tanaecium jaroba* (Frazão et al. 2020). In the Bignonieae species studied here, the secretory tissue was located mainly on the adaxial corolla tube surface and on the lobes, and it is composed of a secretory epidermis and underlying parenchyma cells, in a variable number of layers. The high cytoplasmic density, relatively large nuclei, and the abundance of starch in the epidermis and subepidermal layers in the species investigated are typical of scent-producing cells (Vogel 1990). In addition, positive reactions for lipids and terpenes confirmed the presence of osmophores, as usually reported for scent glands (Marinho et al. 2014). A small amount of lipid material was present on the surface of the epidermal cells, with small accumulations of lipids and terpenes inside the cells. This confirms the osmophore function (Stern et al. 1987; Vogel 1990; Kowalkowska et al. 2012, 2015). The accumulation of starch grains in the pre-secretory stage and its hydrolyzation at anthesis is a common feature of osmophore tissue and represents a source of energy for scent production (Vogel 1990). Scent may be composed of mixtures of many constituents and usually is produced and released periodically (Vogel 1990; Lynch et al. 2020) without accumulation on the organ surface. Scent volatilization can occur via cuticular diffusion (Vogel 1990; Stern et al. 1987; Curry et al. 1988; Stpiczynska 1993, 2001) or through cuticular pores (Melo et al. 2010). There were no pores or cracks observed on the epidermal epithelial cells of the osmophores in the species studied. On the other hand, the presence of raised stomata was noticeable on the adaxial surface of the corollas in Bignonieae, often associated with the osmophore regions. Our findings suggest that large, raised, stomata close to osmophores may play a role in floral scent volatilization.

Floral scent is considered one of the major components mediating the interaction between flowers and their pollinators (Gervasi and Schiestl 2017). Indeed, the presence of osmophores in bee-pollinated species and their absence in hummingbird-pollinated species are consistent with the idea that bees are known to use floral scent as a guide to locating flowers (Buchmann 2023), while humming-birds do not (Knudsen and Gershenzon 2020; Tunes et al. 2022).

Floral volatiles act as indicators of the presence of the flower itself and thus floral resources, optimizing the contact of pollinators with the reproductive structures of flowers (Lynch et al. 2020). Therefore, the osmophores distributed on the tube floor and along the grooves throughout the floral tube may act as a floral guide, sensu Dafni and Giurfa (1996), for bee pollinators, especially in species that have the floral resource hidden in a nectar chamber at the base of the floral tube, as Bignonieae species do (Galetto 2009; Guimarães et al. 2016). In several of the bee-visited species, *Adenocalymma peduncuIatum*, all *Cuspidaria* species, *Fridericia florida*, *F. platyphylla*, and *Anemopaegma arvense*, we observed a combination of osmophores as spots

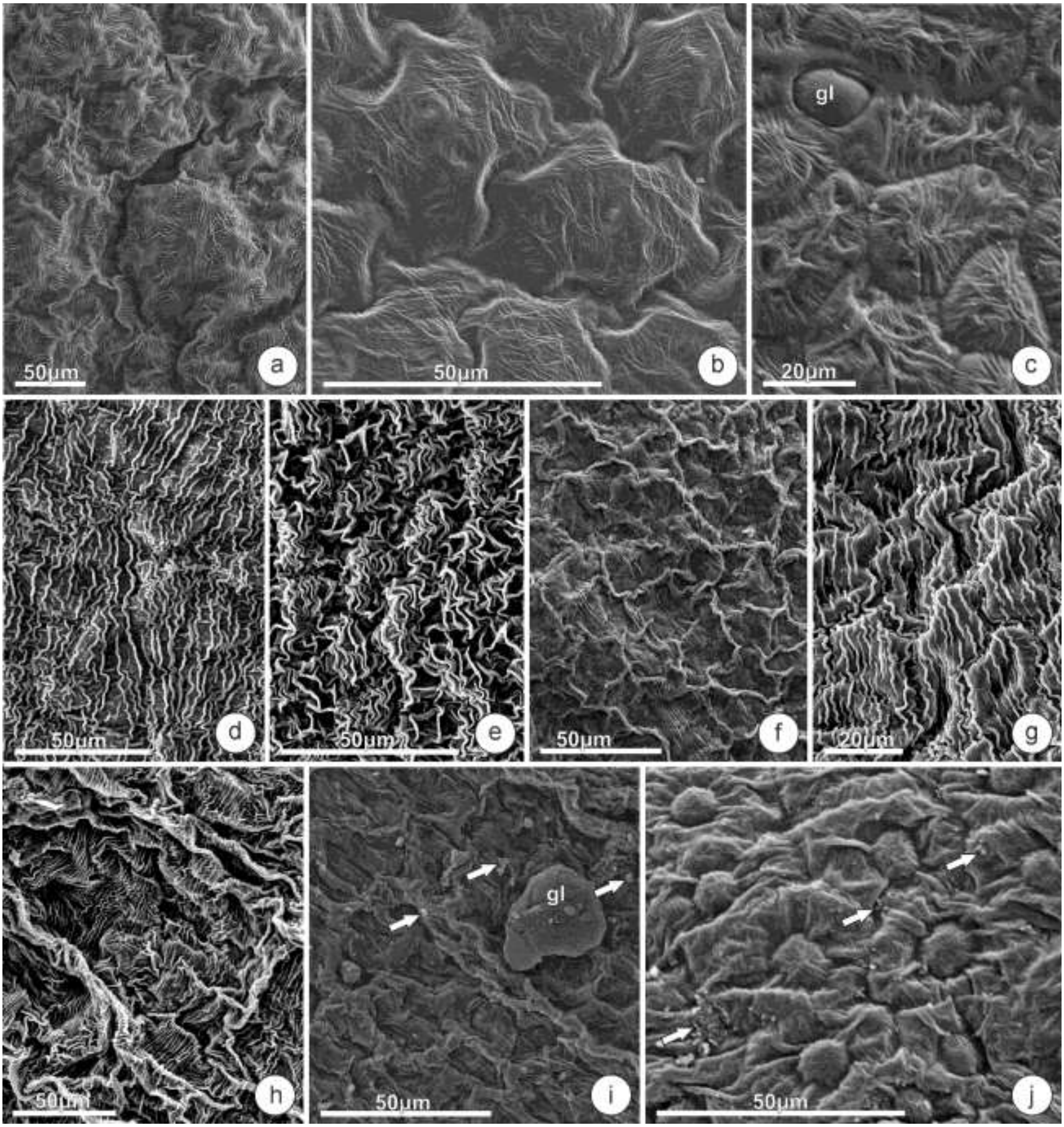
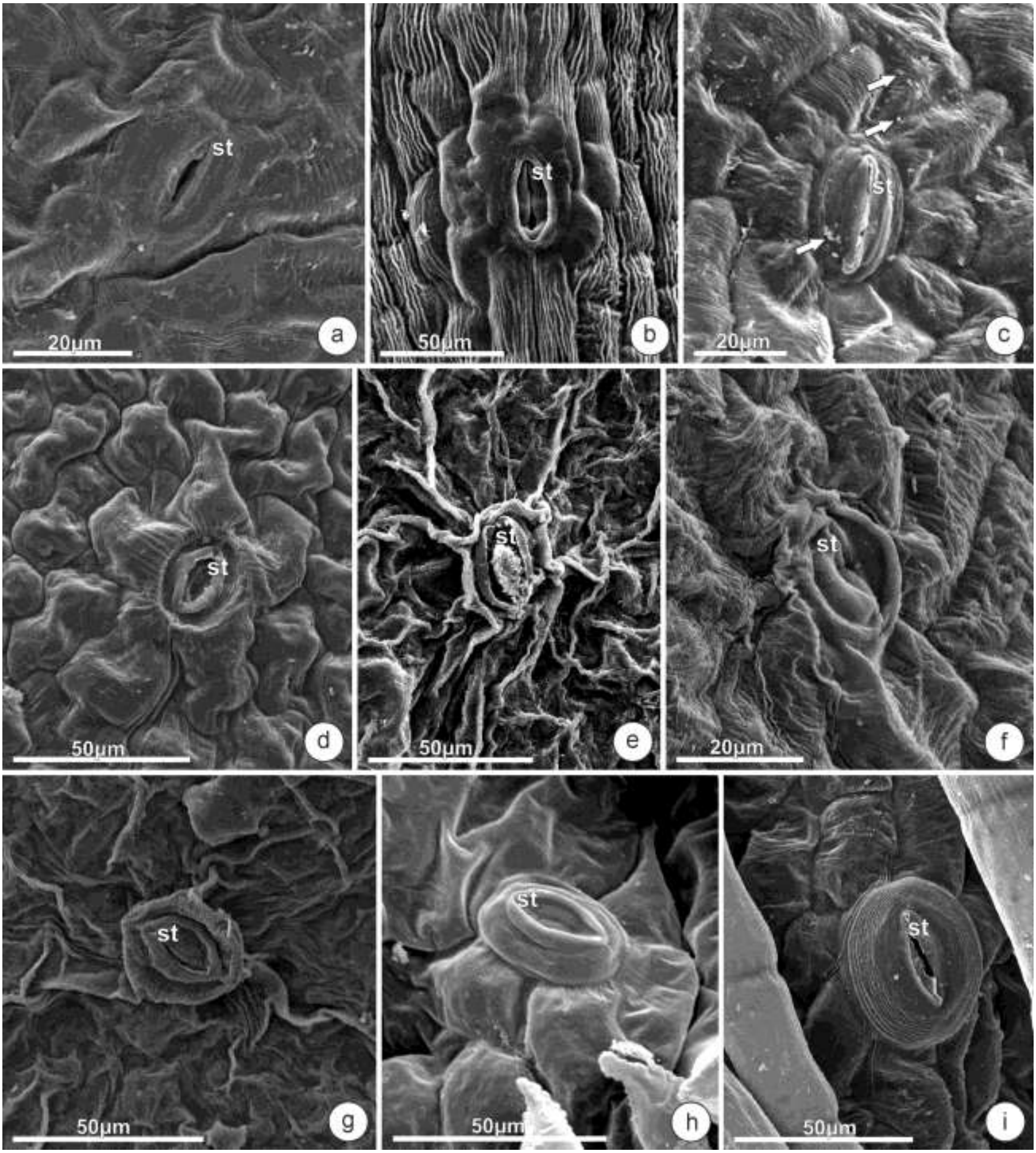


Fig. 9 SEM images of the corolla surface of Bignonieae species illustrating cuticular ornamentation. **a** *Fridericia platyphylla*, abaxial surface of the tube roof with short, thin curved folds confined to each cell. **b** *Adenocalymma nodosum*, adaxial surface of the tube floor with short, curved and randomly orientated folds restricted to each cell. **c** *Anemopaegma* sp., adaxial surface of the lobe floor with short, thick curved folds confined to each cell. **d** *Cuspidaria pulchra*, abaxial surface of the tube roof with slightly curved folds on the outer wall with radiating short folds across the anticlinal wall boundaries. **e** *Cuspidaria convoluta*, adaxial surface of the tube roof with strongly curved folds on the outer wall with radiating short folds across the anticlinal walls. **f** *Cuspidaria floribunda*, abaxial surface of the tube floor with reticulate folds on raised anticlinal walls. **g** *Cuspidaria floribunda*, adaxial surface of the tube roof with curved folds forming a dense reticulum on buttressed ridges. **h** *Fridericia florida*, abaxial surface of the tube roof with a dense, intricate network of long and short curved folds on buttressed ridges with deep sulci at their sides. **i** *Adenocalymma peregrinum*, adaxial surface of the tube floor with coarse reticulum of tall folds. **j** *Adenocalymma nodosum*, adaxial surface of the lobe floor with folded cuticle on papillae and papilla base. Note the epicuticular wax granules in the i–j. Note the slightly collapsed cells by the preparation method in all figures. Symbols: gl, glandular trichome; (arrows) epicuticular wax granules



and patches distributed on the corolla tube floor, following the same direction as the grooves towards the nectar chamber. The grooves on the tube floor may act as tactile nectar guides, which may be enhanced by osmophore scent emission, resulting in a combination of tactile and chemical traits leading towards the floral trophic resource.

In *Amphilophium mansoanum*, *Callichlamys latifolia* and *Fridericia cinnamomea* osmophores delineate the

◂**Fig. 10** SEM images of the corolla surface of Bignonieae species illustrating stomata. **a** *Adenocalymma nodosum*, ordinary stomata presenting a long, narrow aperture, with outer stomatal rim in the abaxial surface of the tube floor. **b** *Pyrostegia venusta*, prominent stomata with narrow, ellipsoidal aperture surrounded with outer stomatal rim, and smooth subsidiary cells in the adaxial surface of the tube floor. **c** *Callichlamys latifolia*, stomata with subsidiary cells radially arranged presenting wax granules in the adaxial surface of the tube floor. **d** *Cuspidaria sceptrum*, slightly sunken stomatum, with long, wide aperture and a distinct outer stomatal rim with short folds laterally extended in the abaxial surface of the tube floor. **e** *Anemopaegma arvense*, prominent stomata surrounded by curved cuticular folds on the subsidiary cells in the adaxial surface of the tube floor. **f** *Adenocalymma pedunculatum*, prominent stomata surrounded by curved cuticular folds on the subsidiary cells in the adaxial surface of the tube floor. **g** *Cuspidaria sceptrum*, protuberant stomata surrounded by curved cuticular folds on the subsidiary cells in the adaxial surface of the tube floor. **h** *Amphilophium mansoanum*, giant stomata with wide aperture and stomatal rim, raised by their subsidiary cells in the adaxial surface of the tube floor. **i** *Fridericia simplex*, giant, raised stomata with concentric rings of folds in the adaxial surface of the tube floor. Note the slightly collapsed cells by the preparation method in e–g. Symbols: st, stomata; (arrows) epicuticular wax granules

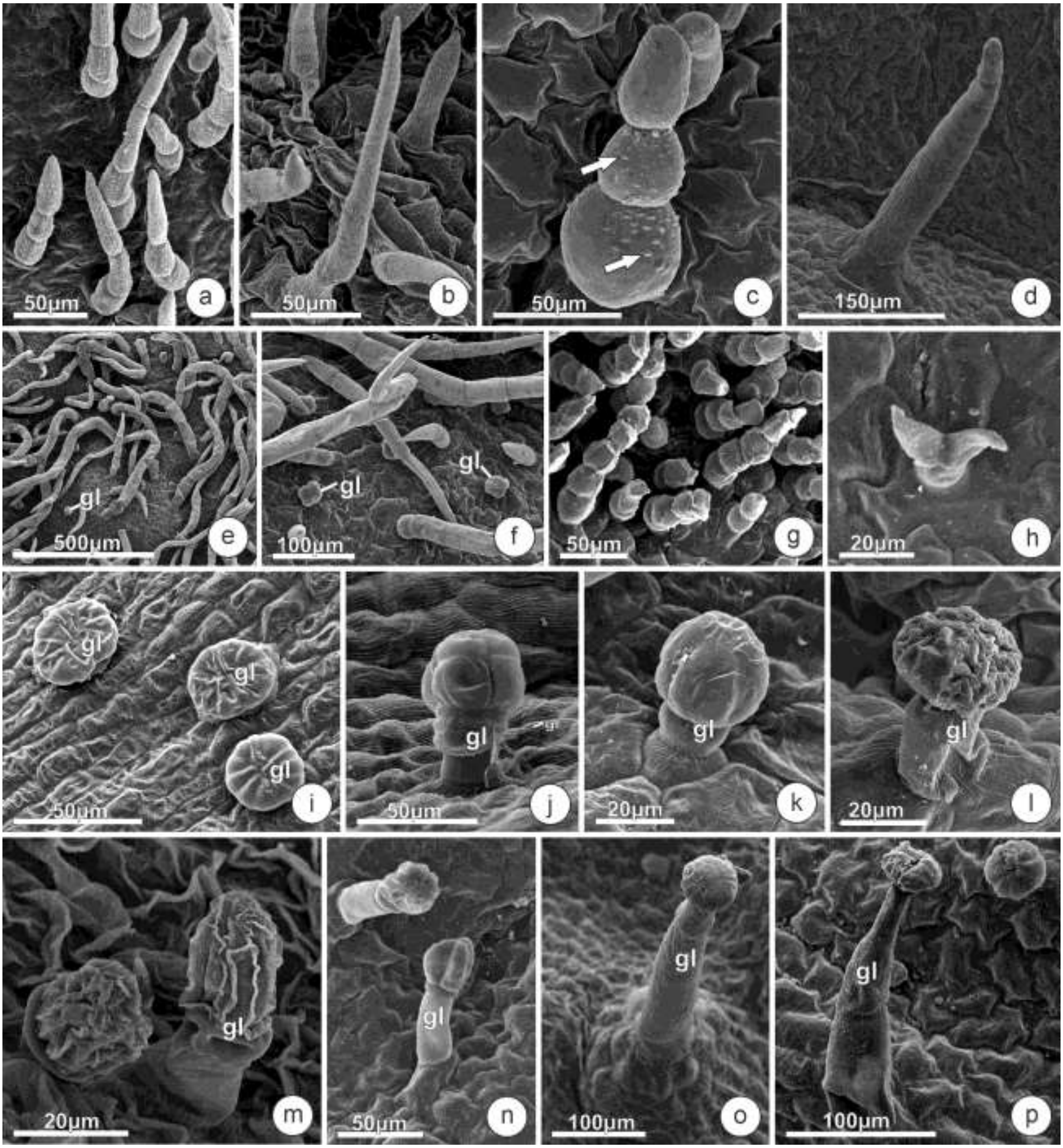
flower entrance, as a ring. Considering that these species are reported to be visited by medium- to large-sized bees (Gentry 1974a; Balduino et al. 2023), it is possible that osmophores may assist bees make a precise landing on these tubular flowers. Bumblebees, for example, can learn and discriminate spatial patterns of scent within flowers, especially if these patterns match visual ones (Lawson et al. 2018), as observed in *Cuspidaria* species (Tunes et al. 2021).

Additionally, the epidermal cell shape and the cuticle structure may create an array of floral visual signals other than color that can be of biological relevance in the interaction with the pollinator (van der Kooi et al. 2019; Kraaij and van der Kooi 2020). The presence of cuticular folds is common on the petal surfaces of the species studied (around 90% of species). These folds have been reported to cause incident light to be diffracted, resulting in the phenomenon of iridescence. This role in flower signaling to pollinators is still a matter of debate (Whitney et al. 2016; Moyroud

Table 4 Surface characterization, distribution of stomata, cuticular folds and trichome features on the corolla of the studied Bignonieae species.

Species	Surface F/Ada	Cuticular folds	Stomata distribution	Trichome distribution	Trichome types
Adenocalymma nodosum	Papillose	R and F (Ada/Aba)	R (Am), F (Hy)	R and F (Ada)	Glandular (capitate and peltate)
Adenocalymma pedunculatum	Papillose	R and F (Ada)	-	R (Ada), F (Ada), L (Ada)	Non-glandular/glandular (capitate and peltate)
Adenocalymma peregrinum	Papillose	R (Aba), F (Ada/Aba)	R (Ep)	R (Ada/Aba), F (Aba)	Non-glandular/glandular (capitate and peltate)
Amphilophium mansoanum	Papillose	-	R (Am)	R (Ada/Aba)	Non-glandular/glandular (capitate and peltate)
Anemopaegma arvense	Papillose	R and F (Aba)	R and F (Am)	R, F and L (Ada/Aba)	Glandular (capitate and peltate)
Anemopaegma sp.	Papillose	R (Aba), F (Ada)	-	R (Ada), F and L (Ada/Aba)	Glandular (capitate and peltate)
Callichlamys latifolia	Papillose	-	R (Hy), F (Ep)	R (Aba), V (Ada)	Non-glandular/glandular (peltate)
Cuspidaria convoluta	Papillose	R (Ada), F (Aba)	-	R and F (Ada/Aba), L (Ada)	Non-glandular/glandular (capitate and peltate)
Cuspidaria floribunda	Papillose	R (Ada), F (Aba)	-	R (Ada/Aba), L and F (Ada)	Non-glandular/glandular (capitate and peltate)
Cuspidaria pulchra	Papillose	R (Aba), F (Ada)	R (Ep), F (Hy)	R and L (Ada), F (Aba)	Non-glandular/glandular (capitate and peltate)
Cuspidaria sceptrum	Papillose	R and F (Aba)	R and F (Ep)	R, F and L (Ada)	Non-glandular/glandular (capitate and peltate)
Fridericia celastroides	Flattened	F (Ada)	F (Hy)	R and F (Ada/Aba), L (Ada)	Non-glandular/glandular (capitate and peltate)
Fridericia cinnamomea	Papillose	R (Ada/Aba)	-	R (Ada/Aba), F (Ada)	Non-glandular/glandular (capitate and peltate)
Fridericia conjugata	Papillose	R (Aba)	-	R and L (Ada), F (Ada/Aba)	Non-glandular/glandular (capitate and peltate)
Fridericia florida	Papillose	R (Aba)	-	R (Ada), L (Ada/Aba)	Non-glandular/glandular (capitate and peltate)
Fridericia platyphylla	Papillose	R (Aba)	-	R and L (Ada), F (Ada/Aba)	Non-glandular/glandular (capitate and peltate)
Fridericia simplex	Papillose	R (Aba)	F (Hy)	R and F (Ada/Aba), L (Ada)	Non-glandular/glandular (capitate and peltate)
Pyrostegia venusta	Flattened	R (Ada), F (Ada/Aba)	R (Hy), F (Am)	-	-

F tube floor, *R* tube roof, *L* corolla lobe, *Am* amphistomatic, *Ep* epistomatic, *Hy* hypostomatic, *Ada*, adaxial surface of the corolla, *Aba* abaxial surface of the corolla, (-) absent



et al. 2017). The presence of wax films has been associated with the glossy appearance of the corolla (Papiorek et al. 2014; van der Kooi et al. 2014, 2017 and references therein). Folded cuticular and epicuticular waxes on the petal surface may also play other important roles in flowers, such as protection against excessive incident light and wind, helping to

reduce the speed of water evaporation (Metcalfe and Chalk 1979), and increasing flower wettability (Bräuer et al. 2017). Additionally, the thickness of the cuticle observed in the species studied may be related to protection against desiccation (Wilkinson 1979). These characteristics help in the retention of water in the petal's internal tissues, slowing the loss of

◂ **Fig. 11** SEM images of the corolla surface of Bignonieae species illustrating trichomes. **a** *Fridericia conjugata*, simple, uniseriate, and multicellular non-glandular trichomes slightly curved and parallel to the epidermis surface in the adaxial surface of the tube floor. **b** *Cuspidaria convoluta*, non-glandular trichomes with thin bases and pointed apices in the adaxial surface of the tube roof. **c** *Fridericia platyphylla*, nodose non-glandular trichome with globose base on the adaxial sur- face of the tube floor. **d** *Cuspidaria sceptrum*, simple non-glandular trichome with rounded apical cell on the abaxial surface of the tube floor. **a–d** Warty ornamentation on the trichomes. **e–f** *Fridericia sim- plex*, long, non-glandular trichomes adpressed to the surface. In f, note the smooth surface. **g** *Amphilophium mansoanum*, corolineous non-glandular trichomes on the adaxial surface of the tube floor. **h** *Callichlamys latifolia*, multicellular, stalk two-armed trichome on the abaxial surface of the tube floor. **i** *Anemopaegma* sp., sessile peltate trichomes with flattened multicellular secretory head on the abaxial surface of the tube roof. **j** *Anemopaegma sp.*, short-stalked capitate trichomes with multicellular and biseriate bulbous heads on the adaxial surface of the tube floor. **k** *Fridericia simplex*, short-stalked capitate trichomes with multicellular and uniseriate ovoid heads on the adaxial surface of the tube roof. **l** *Adenocalymma peregrinum*, capitate glandular trichome slightly raised and formed by an inflated short stalk and flattened head on the adaxial surface of the tube roof. **m** *Cuspidaria convoluta*, capitate glandular trichomes formed by an inflated short stalk and bulbous head on the adaxial surface of the tube floor. **n** *Fridericia conjugata*, capitate glandular trichomes with multicellular bulbous head on the abaxial surface of the tube floor. **o–p** Glandular trichome with a thickened long stalk raised by a pedestal formed with radially arranged common epidermal cell, and a small multicellular, uniseriate flattened head in *Cuspidaria* species. Note the folded surface. **o** *Cuspidaria sceptrum*, tube floor. **p** *Cuspidaria pulchra*, tube roof. Note the slightly collapsed cells by the preparation method in i, m. Symbols: gl, glandular trichome; (arrows) warty ornamentation

water by transpiration—this being critical in a seasonally dry environment such as Cerrado (Oliveira and Marquis 2002). Additionally, micromorphology of the corolla surface, and especially the presence of papillae which we found in 94% of the species studied that were visited by bees, has been described as a specific tactile stimulus for bees (Kevan and Lane 1985; Bräuer et al. 2017). These features can constitute a surface structure suitable for bee attachment, so allowing bees to cling to the flower and to walk towards the nectar (Bräuer et al. 2017). On the other hand, the hummingbird-pollinated species, *Pyrostegia venusta*, had a flattened epidermal surface, which is expected for species with pollinators that have limited mechanical contact with the petals (Bräuer et al. 2017). Petal epidermal modification from papillose to flattening has been shown during the transition from insect to bird pollination and has been interpreted as an adaptively driven trait, functioning as a pro-bird and anti-bee trait (Ojeda et al. 2012, 2016).

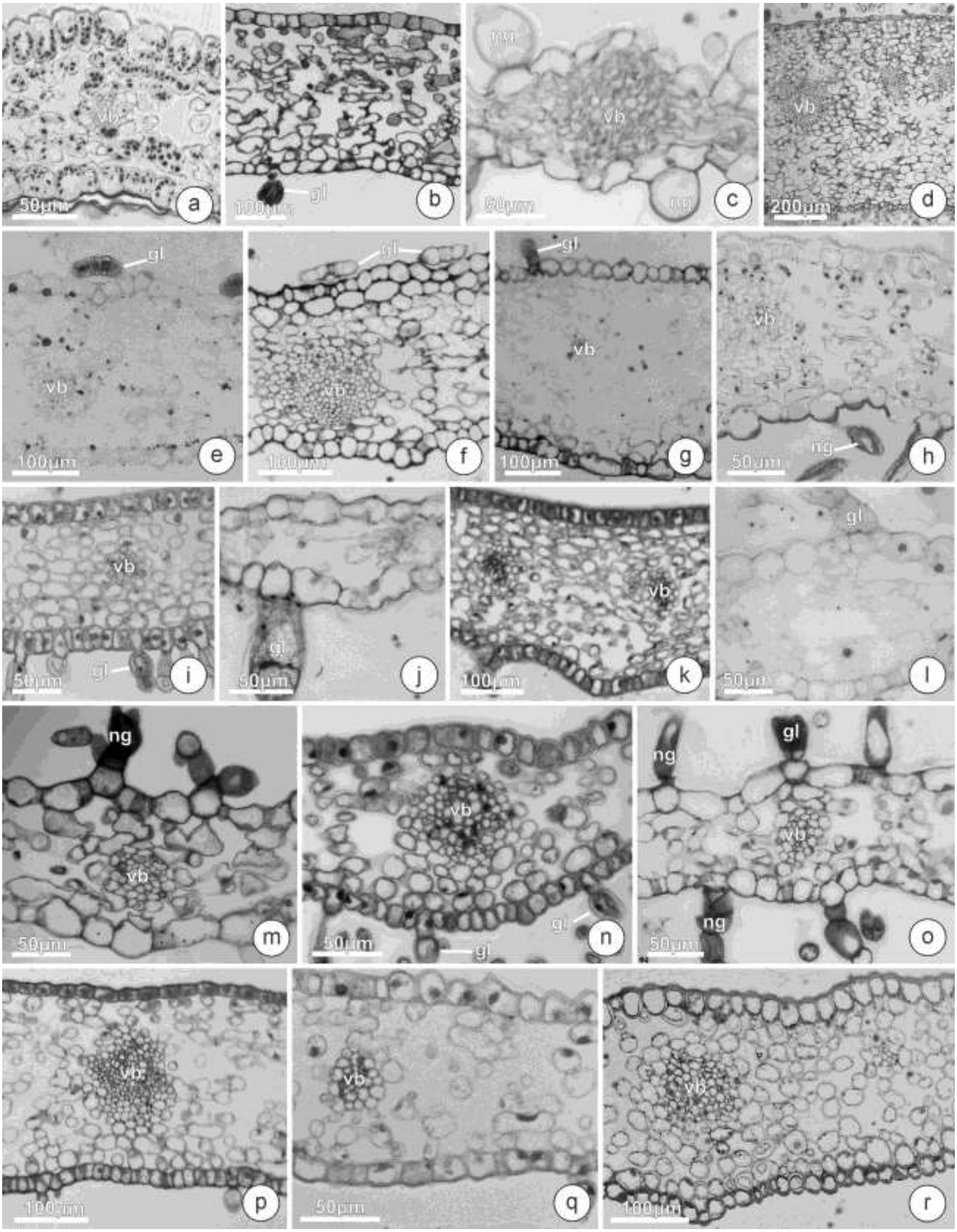
Most of the species studied here show sparse to dense indumenta composed by glandular and non-glandular trichomes on the corolla tube and lobes, and this is a widespread characteristic among Bignonieae members (Souza et al. 2010; Frazão et al. 2020; Lopes-Silva et al. 2022). The secretions produced by floral glandular trichomes can perform different functions, being able to act both in the defense against herbivores and pathogens and in the attraction of pollinators (see Nogueira et al. 2013).

Another interesting feature we observed in the corollas of these Bignonieae species was the presence of stomata with differential distributions. The accumulation and transpiration of water are necessary for the corolla to open and to remain open; on the other hand, the water accumulated in the open corolla must be lost if it is to close. The stomata are the only candidates that might cause these processes (Azad et al. 2007). Thus, the differential distribution of stomata observed in the corollas of the species studied (amphistomatic, epistomatic and hypostomatic) may indicate differences in the degree of water accumulation and transpiration during corolla movement, which is dependent on the thicknesses and temperatures on both surfaces (Azad et al. 2007). In addition, a supposed role of the stomata in the release of volatiles is still under con- sideration (Maoz et al. 2020).

The petals mesophyll of the Bignonieae species studied contained abundant intercellular spaces, characterizing aerenchyma (Kay et al. 1981). The efficiency of the mesophyll in reflecting light varies among species and is directly associated with the degree of mesophyll cell differentiation and the number of cell layers (Kay et al. 1981; van der Kooi et al. 2016). Starch grains which form a continuous layer in the subepidermis of the petal are a common feature of most species participating in light reflection (Kay et al. 1981; van der Kooi et al. 2019). The aerenchymatous mesophyll typical of petals reflects both ultraviolet and visible light (Kevan and Lane 1985). In general, the abundant lipid droplets found in corolla tissues of Bignonieae species may be ultraviolet absorbing pigments, usually represented by flavones or flavonols (Kay et al. 1981). In Cerrado vegetation, over 70% of plant species show UV-absorbing flowers regardless of their human-visible color; a trend also conformed for Bignonieae species (Tunes et al. 2021).

Conclusion

This study found that the distribution patterns of the osmophores in the Bignonieae species examined were markedly different. These differences were not clearly associated with specific histological traits, e.g., within pattern [A], there are histological differences in osmophore composition. Future studies with a greater number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◂**Fig. 12** Light microscopic images of anatomical features of the corollas of Bignonieae species. **a**–**r** Cross-sections showing the single-layered epidermises of both corolla surfaces, mesophyll with loose structure and vascular bundles. **a** *Adenocalymma nodosum*, papillose epidermis and parenchyma cells with starch grains identified with Lugol reagent. **b** *Adenocalymma pedunculatum*, epidermis with slightly convex cells on both surfaces and glandular trichome on the abaxial surface. **c** *Adenocalymma peregrinum*, epidermis with papillae and globose base of non-glandular trichomes, and thin mesophyll. **d** *Amphilophium mansoanum*, epidermis with abundant trichomes, and thick mesophyll containing several vascular bundles. **e**–**f** Epi- dermis with papillae and peltate trichomes on the adaxial surface in *Anemopaegma arvense* (**e**) and *Anemopaegma* sp. (**f**). **g** *Callichlamys latifolia*, papillose epidermis on the adaxial surface and flattened rectangular cells on the abaxial surface. **h** *Cuspidaria convoluta*, papillose epidermis on both surfaces alternating with trichomes on the abaxial surface. **i** *Cuspidaria floribunda*, palisade epidermises on both surfaces, glandular trichomes on the abaxial surface, and parenchyma cells with a relatively more compact arrangement. **j** *Cuspidaria pulchra*, papillose epidermis on both surfaces, conspicuous trichome on the abaxial surface, and thin mesophyll. **k** *Cuspidaria sceptrum*, palisade epidermis on both surfaces, and thick mesophyll. **l** *Fridericia celastroides*, papillose epidermis, and mesophyll with a loose structure. **m** *Fridericia cinnamomea*, papillose epidermis alternating with trichomes on the adaxial surface, and mesophyll with a loose structure. **n** *Fridericia conjugata*, papillose epidermis on both surfaces, and loose mesophyll. **o** *Fridericia florida*, glandular trichomes on both surfaces, and thin, loose mesophyll. **p** *Fridericia platyphylla*, epidermis composed of rectangular cells on the adaxial surface and by tall cells alternating with trichomes on the abaxial sur- face. **q** *Fridericia simplex*, papillose cells on both surfaces, and loose mesophyll. **r** *Pyrostegia venusta*, epidermis composed of ovoid cells covered with thick cuticle on both surfaces, and thick mesophyll. Symbols: gl, glandular trichome; ng, non-glandular trichome; vb, vascular bundle

of Bignonieae species and pollinator behavioral assays may help interpret the variations we identified here. As expected, all bee-visited species showed textured corolla surfaces, associated in the literature with bee attachment, as well as with tactile stimuli which guide the bees towards the nectar. Our study encourages further exploration of how such flower properties contribute to interactions with their pollinator species.

Acknowledgements We are grateful to Professor Lúcia Garcez Lohmann for plant species identification, to Daniel Tancler for preparing schematic flower drawings, and to the Electron Microscopy Center at the Institute of Bioscience—UNESP for assistance in processing the materials and the provision of equipment for electron microscopy.

Author contribution Silvia Rodrigues Machado and Elza Guimarães conceived and designed the research. Karise Mamede Macedo and Letícia de Almeida Gonçalves carried out field observation, the sampling, and the laboratory work. Silvia Rodrigues Machado, Elza Guimarães, Priscila Tunes, Karise Mamede Macedo, and Yve Canaveze carried out the data analyses and wrote the original draft. Silvia Rodrigues Machado, Elza Guimarães, Priscila Tunes, and Yve Canaveze reviewed and edited the manuscript.

Funding This work was supported by the 'Coordenação de Aperfeiçoamento de Pessoal de Nível Superior'—Brazil (CAPES), Finance Code 001 and by Grant #2021/13392–0, São Paulo Research Foundation (FAPESP- Process number 2021–13392-0) to SRM. EG [Grant number 312799/2021–7] and SRM [Grant number 308982/2020–7] receive scholarships from The National Council for Scientific and Technological Development (CNPq).

Data availability All data generated or analyzed in this study are included in this published article.

Declarations

Conflict of interest The authors declare no competing interests.

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Corolla structural and histochemical characterization in three bee-pollinated *Handroanthus* species (Bignoniaceae)

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Abstract

In this work, we investigated the corolla of three arboreal species of *Handroanthus*, a Bignoniaceae genus that grow in the Brazilian Cerrado and attract medium- to large-sized bees. With a scanning electron microscope, we analyzed the microstructure of the corolla surface, and with an optical microscope, we characterized the structure and histochemistry of the corolla tissues of *H. coronatus*, *H. impetiginosus*, and *H. serratifolius* flowers. In the three species, the corolla was composed of uniseriate epidermis composed of conical-papillate cells, aerenchymatous mesophyll, and collateral vascular bundles. Rough surfaces, striated and folded cuticles, glandular and non-glandular trichomes, and elevated stomata were observed in the three species, which may be useful characters in the taxonomy of the group. Secretory tissues in different regions of the corolla of the three species varied in organization, with *H. coronatus* exhibiting more complex structures formed by epidermal cells and underlying parenchymatic cells, suggesting the occurrence of typical osmophore in this species; *H. impetiginosus* showing only secretory epidermis with glandular and non-glandular trichomes, and *H. serratifolius* displaying a palisade secretory epidermis along the entire length of the corolla tube. Starch grains, lipid droplets, and terpenoids (except in *H. serratifolius*) were found in the corolla tissues of the three species, being more abundant in the secretory tissues, suggesting their involvement in the production of floral scent. The characteristics found in the corolla of *Handroanthus* species, taken together, may increase their attractiveness to pollinators while also providing protection to the flowers.

Keywords: Bignoniaceae, corolla anatomy, histochemistry, petal micromorphology, secretory tissues.

Introduction

Handroanthus Mattos is a Bignoniaceae genus composed of around 30 species distributed mainly in Central and South America, which extends from the Atlantic Forest, Amazon Forest, and dry seasonal habitats such as the Cerrado and Caatinga (BFG 2015). Brazil is considered the center of diversity for the genus, with 30 species described, 15 of which are Cerrado (BFG 2015). The flowers of *Handroanthus* species are zoophilous and are pollinated mainly by medium- to large-sized bees primarily belonging to the Euglossini tribe (Gandolphi and Bittencourt Jr 2010, Acra et al. 2012, Silva 2015).

The genus is characterized by large gamopetalous corolla (8-10 cm long), predominantly yellow (pink to magenta in only four species), tubular-infundibuliform to tubular-campanulate, base fleshy and apex membranous, externally densely glandular, internally villous, trichomes simple (Grose and Olmstead 2007). Glandular and non-glandular trichomes with diverse morphologies, ranging from sessile to pedicellate were described in the petals of *Handroanthus* species (Grose 2009, Zapater et al. 2009, Acra et al. 2012, Brasil and Guimarães-Brasil 2018). Comparative studies of the morphological traits of corollas, which comprises sometimes only slightly differing species, may be important for the taxonomy and phylogeny of the Bignoniaceae taxa (e.g., Alcantara and Lohmann 2010, Fonseca et al. 2016).

Besides, the shape, color, and symmetry of the flowers, and the very subtle features of the petals, especially the distribution and shape of the epidermal cells, increase the attractiveness of a corolla (Glover and Martin 2002). The high frequency of the conical-papillate epidermal cells within angiosperms and their almost universal restriction to the petals argue for an adaptive explanation involving the function of these petal epidermal cells in pollinator attraction (Glover and Martin 2002, Baudino et al. 2007). This specialized cell morphology may attract pollinators by enhancing the color of the corolla (which would contrast strongly with vegetative organs), creating a sparkling effect, creating an intrafloral microclimate, or providing tactile cues (Kay et al. 1981, Glover and Martin 2002, Willmer 2011). Pollinators may be drawn to a flower by the roughness of the cuticle, the shape and arrangement of epidermal cells, and the profiles of the outer (periclinal) and lateral (anticlinal) cell walls (Alcorn et al. 2012, Ojeda et al. 2016). Flowers with conical epidermal cells attract bees of different sizes, while those with flat-shaped epidermal cells are associated with birds and bats (Papiorek et al. 2014, Ojeda et al. 2016, Bräuer et al. 2017). In turn, Kraaij and van der Kooi (2020) found no correlation between the microstructure of the corolla and interactions with floral visitors. Regarding the internal organization of the petal mesophyll (and its variations), there is no conclusive evidence that it acts in the interaction with floral visitors (Alcorn et al. 2012, Ojeda et al. 2016).

It has been known for many years that different parts of a flower, particularly the corolla, can emit volatile compounds, and researchers have emphasized the relevance of this phenomenon for pollinator attraction (Vogel 1990, Effmert et al. 2006, Gervasi and Schiestl 2017). It is possible for the petals to make and release volatile compounds in the secretory epidermis, with or without glandular trichomes, and in osmophores (Effmert et al. 2006). Osmophores, also known as scent glands, are complex structures that consist of multiple layers including a secretory epidermis and one to several layers of subepidermal cells, and typically contain lipid droplets and starch granules (Effmert et al. 2006). They usually face toward the adaxial side of the perianth and display a bullate, rugose, pileate, conical, or papillate epidermis (Effmert et al. 2006).

To access the morphofunctional characteristics of the corolla in bee-pollinated *Handroanthus* species, in this study we characterized the surface microstructure, anatomy, and histochemistry of the corolla in the flowers of three *Handroanthus* species that grow in the Brazilian Cerrado, a neotropical savanna. At the end of this study, we intend to answer the following questions: i) Are the characteristics of the corolla surface shared by the three species since they are all pollinated by bees? ii) Is the organization of the secretory tissues common to the three species? iii) Are the corolla surface and secretory structures' anatomical and histochemical characteristics consistent with the expected for bee-pollinated species?

Materials and methods

Plant species, collection site, and sampling

Flowers from three species belonging to *Handroanthus* Mattos (Table 1; Fig. 1) were collected in the Cerrado (Brazilian savanna), state of Goiás, Brazil (13–17°S, 47–50°W). A map was constructed using QGIS 2.8.2 software (Quantum GIS Development Team) based on the geographic coordinates obtained during the collection period (Fig. 2). Sampling was conducted throughout the flowering seasons of each species, from October 2020 to February 2022. The specimens were archived at the UFG Herbarium at the Federal University of Goiás, with duplicate specimens stored at the "Irina Delanova de Gemtchujnicov" (BOTU Herbarium) within the Department of Biodiversity and Biostatistics at the Institute of Biosciences in Botucatu, UNESP, Botucatu, São Paulo.

Microscopic examination of corolla

For each species, flowers from five inflorescences of at least three individuals per species were sampled for morphological, anatomical, and histochemical study. For micromorphological, anatomical and histochemical characterization samples were taken from the tube floor, tube roof, and corolla lobes (Fig. 3).

For characterization of the petal surface under scanning electron microscopy (SEM), the samples were fixed in 2.5% (v/v) glutaraldehyde (0.1 $mol \cdot L^{-1}$ phosphate buffer, pH 7.2). Subsequently, they were dehydrated through a graded ethanol series of 7, 5, 15, 30, 50, 70, 90, and 100% (twice at each concentration for 10 min), then dried at the critical point (Model CPD 030; BAL-TEC AG, Balzers, Liechtenstein), using liquid CO_2. The samples from the corolla tube and lobes were mounted on aluminum stubs and subsequently coated with a 10-nanometer-thick layer of gold (Model SCD 050) for 160 seconds at a current of 50 mA. The material was examined using a Quanta 200 SEM (Fei Company, FEI, Gräfelfing, Germany) operating at an accelerating voltage of 20 kV.

For corolla tissues characterization in light microscopy (LM), the samples were initially fixed in neutral-buffered formalin (NBF) for 48 hours for anatomical and histochemical studies (Lillie, 1965). Subsequently, the specimens were preserved in 70% ethanol, dehydrated in an ethyl series, and embedded in glycol methacrylate historesin (Leica Microsystems Inc., Heidelberger, Germany). Thin corolla cross sections (5 µm) were prepared with a rotary microtome (Leica 2245; Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany). The sections were stained with a 0.05% (w/v) aqueous solution of toluidine blue for 1 minute at 60 °C on a hot plate (Feder and O’Brien 1969). They were mounted on glass slides with synthetic resin (Entellan; New, Merck, Darmstadt, Germany). Cuticle thickness was measured by examining cross-sectional photos at 400x magnification with ImageJ software (Schindelin et al. 2012). Values are reported as minimum and maximum measurements (n = 3 sections per species and 10 fields per section).

Histochemical tests were performed on cross sections (about 5µm thick), from fixed and embedded samples as previously described, and subjected to the following treatments: Lugol's solution to detect starch grains (Johansen 1940); α-dimethyl-p-phenylenediamine (NADI) reagent was used to detect terpenes (David and Carde 1964); Sudan IV in 70% (w/v) ethanolic solution for lipid localization (Johansen, 1940) and a 10% (w/v) aqueous ferric chloride ($FeCl_3$) solution to detect phenolic compounds (Johansen 1940). We followed the recommended control procedures for all histochemical tests as per the original authors' guidelines.

The slides were examined using an optical microscope (Leica DM500; Leica Mikrosysteme Vertrieb GmbH) with an integrated digital camera (Leica ICC 50).

Results

Microstructure of the corolla's surface

Under the SEM, the surface of the corolla of the three species examined showed common epidermal cells of variable sizes and shapes, different degrees of undulations of the anticlinal walls, cuticular ornamentation, stomata, and trichomes with variable morphologies (Fig. 4-5, Table 2).

Based on the curvatures and projections of the outer periclinal wall, the corolla surface was classified as conical-papillate (Fig. 4-5). Papillae ranged from slightly convex (Fig. 4 a-f) to rounded (Fig.4 i-k; 5a-b, g-h) to dome-shaped papillae (Fig. 4l), half spheres (Fig. 4l; 5d), and to conical (Fig. 4m, o). The undulation of the anticlinal walls varied from straight (Fig. 4c-d), to curved (Fig. 4k-m), to slightly undulate (Fig. 5b, g). The cuticle varied from smooth (Fig. 4c-d) to striate (Fig. 4b, e, h-p; 5a-h). Cuticular folds varied from thin (Fig. 4b, e) to thick (Fig. 4h, n-p; 5e-f), occurring as short curved bands confined to each cell (Fig. 4b, m-n; 5a, d) to long, curved or sinuous, continuous and parallel folds distributed longitudinally over the whole corolla surface (Fig. 4e, h; 5e-f). Slightly curved folds on the outer wall with radiating short folds across the anticlinal wall boundaries (Fig. 4b, e, h; 5a), curve folds around the stomata (Fig. 4d, f; 5c), around the papillae (Fig. 5d) and tall folds forming a dense reticulum (Fig. 4n) more prominent around trichomes (Fig. 4o-p) and stomata (Fig. 5h-i). Epicuticular wax granules were observed on the smooth cuticles of *H. coronatus* (Fig. 4c-d, f).

Stomata were observed on the petals of *H. coronatus* and *H. serratifolius* (Table 2). Stomata were clearly visible on the abaxial surface of the tube roof (Fig. 4c-d) and on the adaxial surface of the tube floor in *H. coronatus* (Fig. 4f); in *H. serratifolius* stomata were found on the adaxial surface of the tube roof (Fig. 5b-c) and tube floor (Fig. 5g-i). No stomata were found in *H. impetiginosus*. Two morphological types of stomata were identified: raised stomata surrounded by curved cuticular folds in the subsidiary cells in *H. coronatus* (Fig. 4d) and regular stomata with a long and wide opening presenting a peristomatic edge in *H. serratifolius* (Fig. 5b-c, h-i).

Non-glandular trichomes were found in *H. coronatus* (Fig. 4a) while glandular trichomes were abundant in *H. impetiginosus* (Fig. 4g, i-k, o-p) and *H. serratifolius* (Fig. 4j).

Non-glandular trichomes were long, simple, uniseriate, and multicellular type, featured by having a tinny base attached to multi-cellular stalks by a narrow constriction, pointed apical cells, and linear warty ornamentations (Fig. 4a). Two types of glandular trichomes were identified: peltate and capitate. Both types showed variation in their macroscopic morphology. Capitate glandular trichomes with uniseriate stalk exhibiting variations in the numbers and sizes of stalk cells and the shape of the secretory head (globose to ovoid) with linear warty ornamentations occurred in *H. impetiginosus* (Fig. 4g, i-k, o-p). Sessile peltate trichomes with spherical secretory head covered with smooth cuticles were identified in *H. serratifolius* (Fig. 5j). The distribution of each trichome type in the regions and surfaces of corollas was summarized in the Table 2.

Anatomical characteristics of the corolla

Cross sections of the corolla tube in the three studied species revealed a uniseriate epidermis comprised of slightly convex (Fig. 6a-c) to conical-papillate of irregular sizes and variable distance between them (Fig. 6d-h; l-m, q), with the outer periclinal walls variable in curvature, often interspersed with trichomes (Fig. 6e, i-k). The epidermis was composed mostly of square to rectangular, slightly convex cells on the adaxial and abaxial surfaces in the tube roof (Fig. 6a) and tube floor (Fig. 6b-c) in *H. coronatus*, rounded to conical-papillate cells in the tube roof (Fig. 6e) and tube floor (Fig. 6f-g) in *H. impetiginosus*, and tall, narrow cells in a palisade arrangement with an outer periclinal wall slightly conical featuring a palisade epidermis in all analyzed regions of the corolla of *H. serratifolius* (Fig. 6l-o). Conical epidermal cells occurred invariably in the corolla lobe in the three species (Fig. 6d, h, and q). Epidermal cells with a voluminous nucleus and a well-defined nucleolus were evident in *H. impetiginosus* (Fig. 6g) and *H. serratifolius* (Fig. 6m-q). Subepidermal cells with a dense content and voluminous nucleus occurred in *H. coronatus* (Fig. 6c). The cuticle, in cross sections, was classified as thin (between 0.08 - 0.11 µm) in *H. coronatus* (Fig. 6a-d) and *H. impetiginosus* (Fig. 6e-h), and thick (0.23 µm) in *H. serratifolius* (Fig. 6l-q). The mesophyll of the petals from the three species consisted of thin-walled cells varying in shape and size, which were arranged in various layers (Fig. 6). Intercellular spaces of varying sizes (Fig. 76-h, l, n, o) were present among these loosely arranged cells. The petals contained collateral vascular bundles (Fig. 6d, f, h, l, n, q) of varying thicknesses, and with greater xylem development.

Histochemical characterization of the corolla

In *H. coronatus* the adaxial surface of the corolla tube floor exhibited a single-layer epidermis accompanied by subepidermal parenchyma cells that was ovoid to tangentially elongated, arranged in two to three layers, and with a bulky nucleus (Fig. 6c). Furthermore, the adaxial surface of the corolla lobe had epidermal papillate cells with a large nucleus and dense content (Fig. 6d). In the *H. impetiginosus* the adaxial surface of the tube floor (Fig. 6f-g) and of the lobe (Fig. 6h) were composed by single-layered secretory epidermis with conical-papillate cells that were easy to detect the large nucleus and denser content. Secretion residues occurred on the epidermis of the corolla lobe in *H. coronatus* (Fig. 6d) and tube floor *of H. impetiginosus* (Fig. 6f-g). In *Handroanthus serratifolius* the palisade epidermis exhibited secretory features all over the corolla places, such as a very large nucleus, many cytoplasmic granulations and a fully developed vacuole (Fig. 6m, o-q). Glandular trichomes alternating with epidermal secretory cells (Fig. 6i-k) were seen in *H. impetiginosus*.

Using Lugol's solution to stain the starch grains showed that they were evenly spread at the base of the secretory epidermal cells in *H. coronatus* (Fig. 7a), *H. impetiginosus* (Fig. 7b) and *H. serratifolius* (Fig. 7c), making a continuous layer of very small granules (Fig. 7d). In addition, larger starch grains occurred in mesophyll cells (Fig. 7a, c-d). Starch grain residues were found at the base of epidermal cells of the *H. impetiginosus* (Fig. 7b). The corolla cells of all three species contained lipids, with a particularly high concentration in the epidermal cells (Fig. 7e). Terpenes were observed as small droplets in the cell protoplast and inclusions inside the cell walls, stained in blue, of the epidermis and subepidermal parenchyma in *H. coronatus* (Fig. 7f-g). Terpenes were observed in the protoplast of epidermal cells (Fig. 7h) and glandular trichomes (Fig. 7i) in *H. impetiginosus*. *H. serratifolius* did not react to the NADI test (data not shown). Table 3 shows the results of the histochemical tests for each area of the petal examined.

Discussion

In this work, we studied the corolla of three *Handroanthus* species that grow in the Brazilian Cerrado, a neotropical savanna, and attract medium- to large-sized bees. With a scanning electron microscope, we analyzed the microstructure of the corolla surface, and with a light microscope, we characterized the structure and histochemistry of the floral tissues. We observed similar surfaces in the three *Handroanthus* species, with corolla rough surfaces, striate and folded cuticles, glandular and non-glandular trichomes, and raised stomata. However, the species varied regarding the secretory features of corolla portions, being that *H.*

coronatus corolla was made up of a secretory epidermis and underlying parenchyma layers, while the *H. impetiginosus* corolla showed only a secretory epidermis and *H. serratifolius* exhibited a palisade epidermis with secretory characteristics throughout the entire length of the corolla tube. The secretory tissues reacted to tests for starch grains, lipids, and terpenoids (except *H. serratifolius*) suggesting their involvement in floral scent production.

The putative link between corolla structure and pollinator attractiveness

The corolla of the three bee-pollinated *Handroanthus* species studied share some microstructural features. These included a conical-papillate surface, cuticular striations, and folds, which reflect light and can help pollinators to find the flowers. In a study that examined the microstructure of the corolla of 18 species of Bignonieae, 94% were found to exhibit some form of conical or papillate epidermal cells; the species visited by bees had papillate epidermis, while the single hummingbird-pollinated species presented a flat epidermis (Macedo et al. 2023 The shape and distribution of the epidermal cells, together with the rough cuticle, can give flowers different visual cues that may enhance the likelihood of cross-pollination by effectively guiding bee visits (Frankie et al. 1976, Schlindwein et al. 2014, Naczk et al. 2018). Furthermore, the papillae may act as a specific tactile stimulus for bees (Kevan and Lane 1985, Bräuer et al. 2017). These features may create a structured surface that makes it easier for bees to attach themselves and move towards the nectar (Bräuer et al. 2017). Only *H. impetiginosus* had epicuticular waxes on the corolla surface, which may give the corolla a glossy appearance (Papiorek et al. 2014, van der Kooi et al. 2014, 2017).

The petals in the three *Handroanthus* species have aerenchyma in the mesophyll, as observed in other Bignoniaceae species (Macedo et al. 2023). An aerenchymatous, unpigmented reflective mesophyll in petals usually reflects light and plays a key role in determining the texture of flowers (Kay et al. 1981, Zhang et al. 2008). But there isn't enough proof yet to say for sure that the way the petal mesophyll is organized affects how it interacts with flower visitors (Kraaij and van der Kooi 2020). To find out more about this, experiments are needed (Alcorn et al. 2012, Ojeda et al. 2016).

Secretory tissues and secretions in the corolla

In the *Handroanthus* studied species, the secretory cells are mostly conical to papillate, their contents are denser, their nuclei are larger and rounder, and the vacuoles are more developed than those of non-secretory epidermal cells. These features are typical of scent-producing cells (Fahn 1979, Evert 2006). On the other side, secretory epidermal cells do not always differ from those of other floral parts (Stern et al. 1987, Vogel 1990), as is the case in *H. serratifolius*. In this species, the epidermal cells throughout the entire length of the corolla have secretory characteristics. In addition, positive reactions for lipids and terpenes in *H. coronatus*, suggest the involvement of these cells in scent production (Baudino et al. 2007, Tolke et al. 2020, Macedo et al. 2023). Lipid droplets were present in the protoplast and on the surface of the epidermal cells, without or with small accumulations of terpenes inside the cells. Lipid droplets in conical-papillate epidermal cells, probably are lipid-protein mixtures, that contain essential oils to be released and lipids like fatty acids and triacylglyderides (Effmert et al. 2006). The accumulation of starch grains in the pre-secretory stage and their hydrolyzation at anthesis is a common feature of scent-producing cells and represents a source of energy for scent production (Vogel 1990). Residues of secreted material were more evident on the corolla tube surface of *H. impetiginosus*. The fact that NADI had no reaction showed that there were no terpenes in the *H. serratifolius* epidermis's secretory cells. It is possible that the poor histochemical detection of terpenes within the secretory cells is due to the fact that the floral scent is released and produced repeatedly without accumulation on the organ's surface (Vogel 1990, Lynch et al. 2020). Terpenes volatilization probably occurs via walls and cuticular diffusion in the studied *Handroanthus* species, a mechanism reported in various plant species (Vogel 1990, Stern et al. 1987, Curry et al. 1988, Stpiczynska 1993, 2001, Macedo et al. 2023), as no pores were observed in the cuticle.

The structure-function relationships of the corolla play a crucial role in protecting flowers

In addition to the implications for attracting pollinators, the microstructure of the petal surface, including striations and folds in the cuticle and epicuticular waxes, may also play other roles in flowers. These functions involve protecting against radiation and wind, helping to decrease water evaporation, and enhancing the flower's wettability (Bräuer et al. 2017). Furthermore, a rough cuticle may be associated with protection against desiccation (Wilkinson 1979). These features help to keep water within the petal's inner tissues, slowing water loss through evaporation. This may be especially important in a seasonally dry environment such as the Cerrado (Oliveira and Marquis 2002).

The occurrence of glandular and non-glandular trichomes that vary in size and shape in the three study *Handroanthus* species, have also been reported for reproductive organs of other Bignoniaceae species (Guimarães et al. 2008, Macedo et al. 2023). Non-glandular trichomes may help protect plants from predators and keep water from evaporating too quickly (Juniper and Jeffree 1983). On the other hand, glandular trichomes on flowers can produce volatiles belonging to fatty acid derivatives, terpenoids, phenylpropanoids/benzenoids and other amino acid derivatives, which comprise a chemically diverse group of plant metabolites that serve multiple functions, such as attracting pollinators, but also protecting plants from herbivory, defending plants against pathogenic microorganisms, and attracting enemies of flower antagonists (Dötterl and Gershenzon 2023). The presence of oils in the corolla cells of the studied *Handroanthus* species makes it more fluid and may help keep it from drying out (Schreiber and Schönherr 1993; Schreiber 2010). We observed stomata in the corollas of *H. coronatus* and *H. serratifolius*, and the role of stomata in the corolla is a matter of consideration. Water accumulation and evaporation of water are critical for the petals to unfold and remain turgid. As the water accumulated in the petal evaporates, the corolla closes. According to Azad et al. (2007), stomata are the only structures associated with these physiological processes. Maoz et al. (2020) have discussed the likely role of stomata in the release of volatiles. Additionally, these three species, as well as others from the same family, had collateral vascular bundles immersed in the petals' mesophyll of the petals. There were also more xylem cells than phloem cells (Bittencourt Jr. 1995, Macedo et al. 2023). This characteristic has been associated with greater amounts of water transport, both when the petals are expanding at anthesis and when they dry later (Dickison, 1990).

Conclusion

We believe that the results of this study represent an advance in our knowledge of the diversity of the microstructure of the corolla surface and anatomy of the petals of bee-pollinated Bignoniaceae species. In particular, they can also help us understand how different characteristics of the corolla surface can act at the interface with the environment, both in the interaction with pollinators and flower protection.

Credit authorship contribution statement

Sílvia Rodrigues Machado: Conceptualization; Funding acquisition; Methodology; Writing – original draft; Writing – review & editing. **Elza Guimarães**: Conceptualization; Methodology; Formal analysis; Writing – review & editing**. Karise Mamede Macedo**: Investigation; Methodology; Writing – original draft. **Yve Canaveze**: Methodology; Artwork; Writing – review & editing.

Declaration of competing interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability All data generated or analyzed in this study are included in the published article.

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Figure captions

Figure 1. Species of *Handroanthus* sampled in the Cerrado Domain. (a, b) *H. coronatus.* (c, d) *H. impetiginosus*. (e, f) *H. serratifolius*.

Figure 2. Map of collection areas (Cerrado Domain, in the state of Goiás, Brazil). *H. coronatus* = Red diamond*; H. impetiginosus* = Green diamond*; H. serratifolius* = Blue diamond.

Figure 3. Representation of a *Handroanthus* flower. **(a)** Frontal view of a flower indicating the floor and roof portions of the corolla tube. (**b).** Floral tube cross section showing the adaxial and abaxial surfaces (Adapted from Macedo et al. 2023. Redrawn).

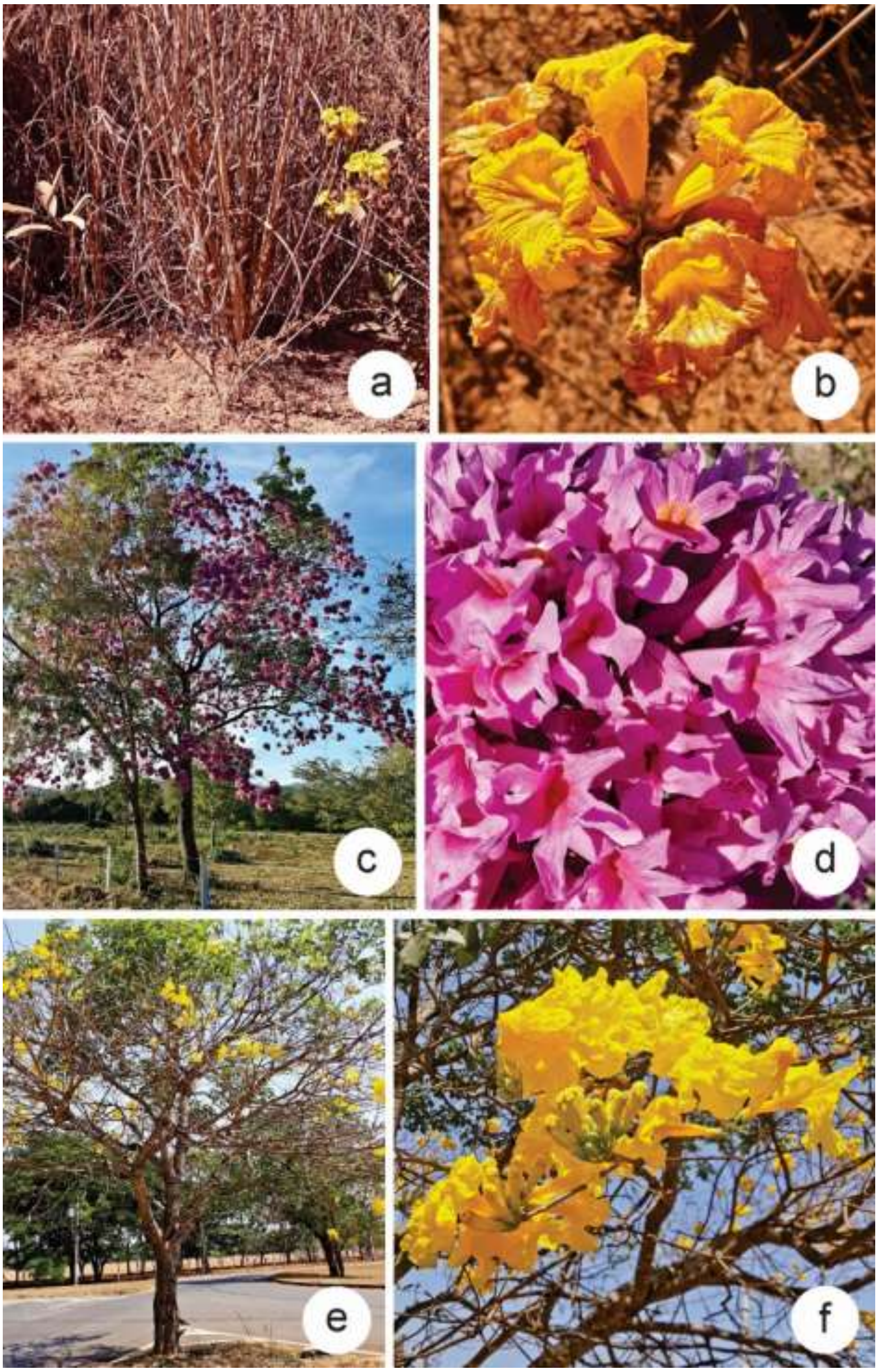
Figure 4. SEM images of the corolla surface of *Handroanthus* species. **a-f** *H. coronatus*; **g-p** *H. impetiginosus*. **(a-b)** Adaxial surface of the tube roof with non-glandular trichome (a) and cuticular striations confined to each cell (b). **(c-d)** Abaxial surface of the tube roof with smooth cuticle (c), epicuticular wax granules (d) and stomata presenting a long and wide opening, with an external stomatal border (d). **(e-f)** Tube floor, with cuticular striations on the abaxial surface (e), epicuticular wax granules and juxtaposed stomata presenting a long and narrow opening, with an external stomatal border on the adaxial surface (f). **(g-h)** tube roof, with capitate glandular trichomes with a single globose secretory head and curved folds on the outer walls on the adaxial surface (g), and striate cuticle with strongly curved folds on the outer wall and short radiating folds on the anticlinal walls on the abaxial surface (h). **(i-p)** tube floor; adaxial surface (i-l), showing multicellular capitate glandular trichomes with globose to ovoid secretory head (i-k) and dome-shaped papillae (l, asterisk). **(m-p)** conical papillae with short, curved cuticle striae confined to each cell (m), rough surface with thick cuticular folds (n), sessile (o) and stalked (p) glandular trichome with ovoid secretory head in the abaxial surface. *Symbols*: gl, glandular trichome; ng, non-glandular trichome; st, stomata.

Figure 5. SEM images of the corolla surface of *Handroanthus serratifolius*. **(a-d)** Tube roof, cuticular striation with strongly curved folds on the outer wall on the abaxial surface (a), rounded papillae covered with thin striations (b), raised stomata surrounded by cuticular curved folds in subsidiary cells (c) and half- sphere papillae with thin striae (d) in adaxial surface. **(e-j)** Tube floor, cuticular folds forming a dense, intricate network with short straight

to curved folds forming deep sulci on the abaxial surface (e), long, continuous and parallel striae over the whole tube floor abaxial surface (f). **(g-j)** Papillate cells and raised stomata surrounded by cuticular curved folds in subsidiary cells (g-i), and peltate glandular trichome (j) on the adaxial surface. *Symbols*: st, stomata; gl, glandular trichome; hs, half-sphere papillae.

Figure 6. Anatomical features of the corolla in cross-sections of *Handroanthus* species. **a-d**, *H. coronatus*; **e-k**, *H. impetiginosus*; **l-q**, *H. serratifolius*. **(a)** Tube roof, note uniseriate epidermis with rectangular, slightly convex cells. **(b-c)** Tube floor, slightly convex to papillate cells in both the epidermis surfaces (b), and subepidermal cells with denser content and voluminous nucleus (c). **(d)** Corolla lobe, with conical epidermal cells in both the surfaces. Note residues of secretion (arrowhead). **e** tube roof, with conical cells and non-glandular trichome in the adaxial surface. **(f-g)** Tube floor, globose to conical cells in the adaxial surface; note residues of secretion on the epidermis surface (arrowhead), and dense content and voluminous nucleus in the epidermal cells (g). **(h)** Corolla lobe, with larger conical-pappilate cells in the adaxial surface. **(i-k)** Tube floor, glandular trichomes in the abaxial surface of *H. impetiginosus*. **(l-m)** Tube roof, conical-papillate secretory epidermis in the adaxial surface, palisade epidermis in the abaxial surface, mesophyll with loose structure and collateral vascular bundles (l). **(m)** Detail of the conical epidermal cells with voluminous nucleus and evident nucleolus. **(n-p)** Tube floor, palisade epidermis with slightly papillate secretory cells in the both surfaces (n), detail of the secretory epidermal cells with spherical and voluminous nucleus with evident nucleoli and large vacuole in the adaxial (o) and abaxial surfaces (p). **(q)** Corolla lobe, larger conical epidermal cells in the adaxial surface. Note collateral vascular bundles (d, f, h, l, n, q). *Symbols*: ep, epidermis; gl, glandular trichome; nu, nucleus; pa, parenchyma; vb, vascular bundles.

Figure 7. Histochemical features of the corolla in cross sections of *Handroanthus* species. **a** *H. coronatus*, tube floor; **b** *H. impetiginosus*, tube roof; **c-d** *H. serratifolius*, corolla lobe; **e** *H. serratifolius*, tube floor; **f-l** *H. coronatus*, tube roof; **h-i** *H. impetiginosus*, corolla lobe (k) and tube floor. **(a-d)** Starch grains (arrowhead) identified with Lugol’s reagent. **e.** Lipid droplets (arrowhead) detected with Sudan IV. **(f-l)** Terpenes (inclusions inside the protoplast and cell walls stained in blue, arrowhead) identified with NADI reagent.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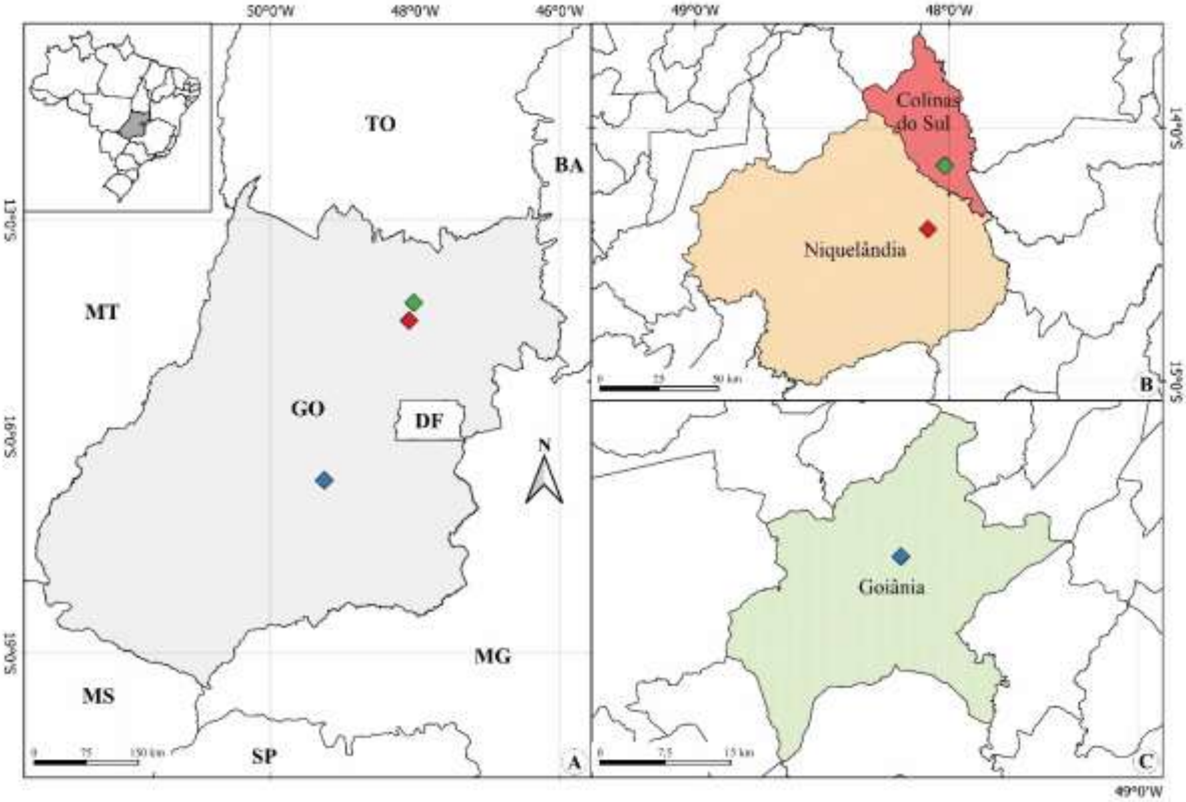
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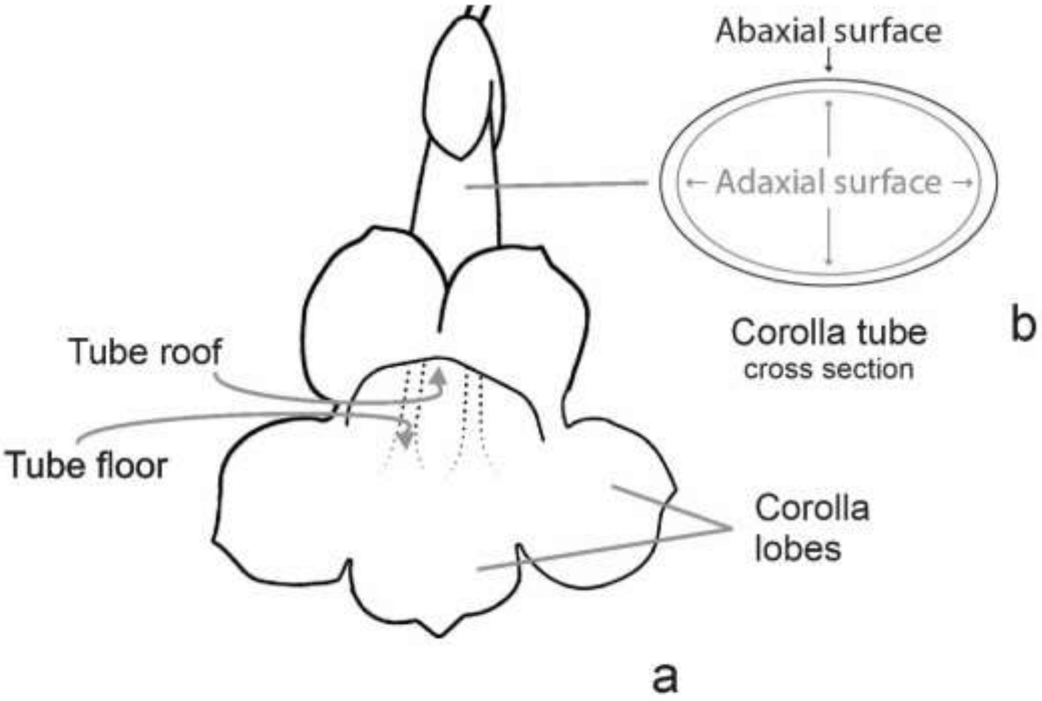
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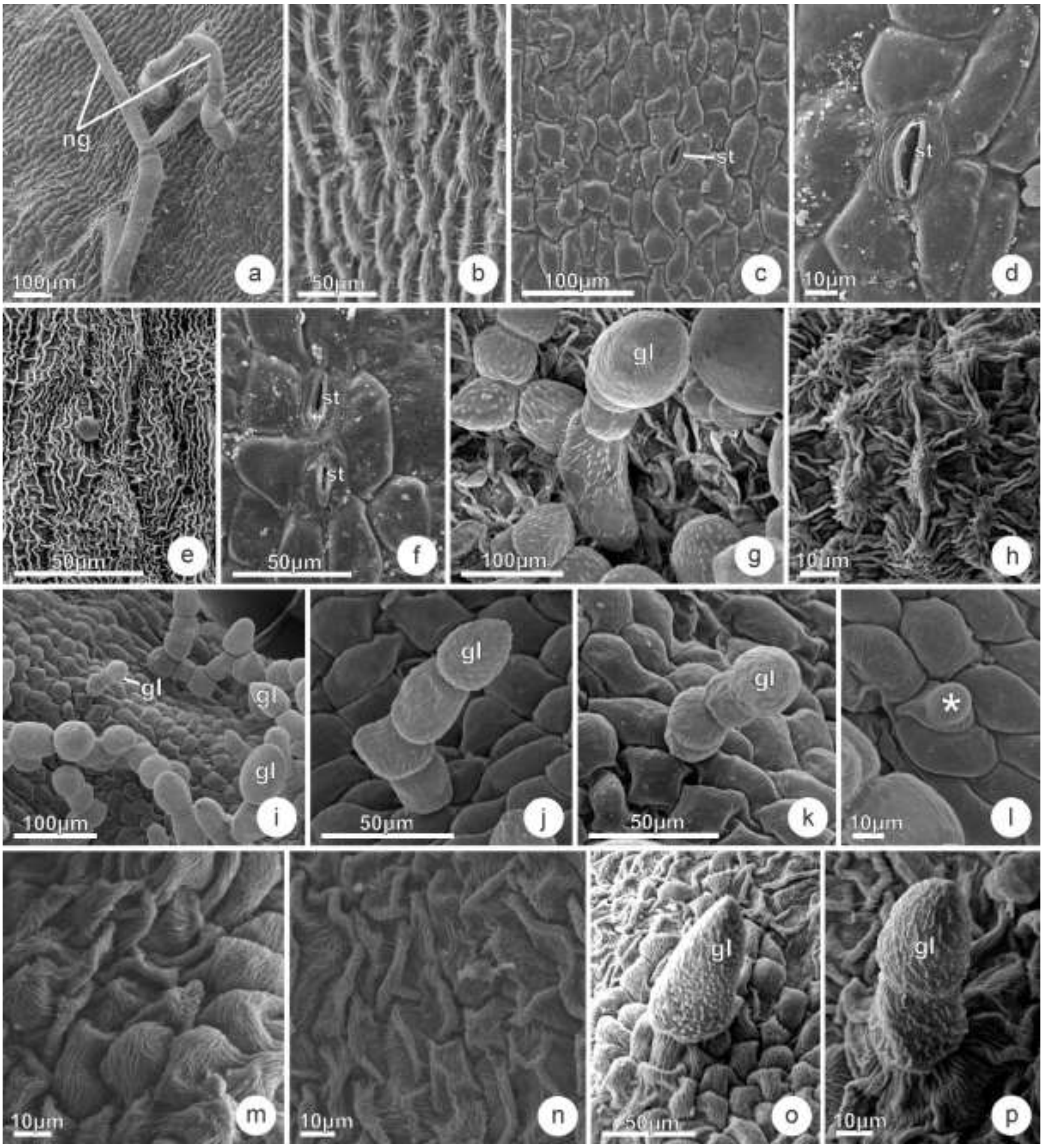
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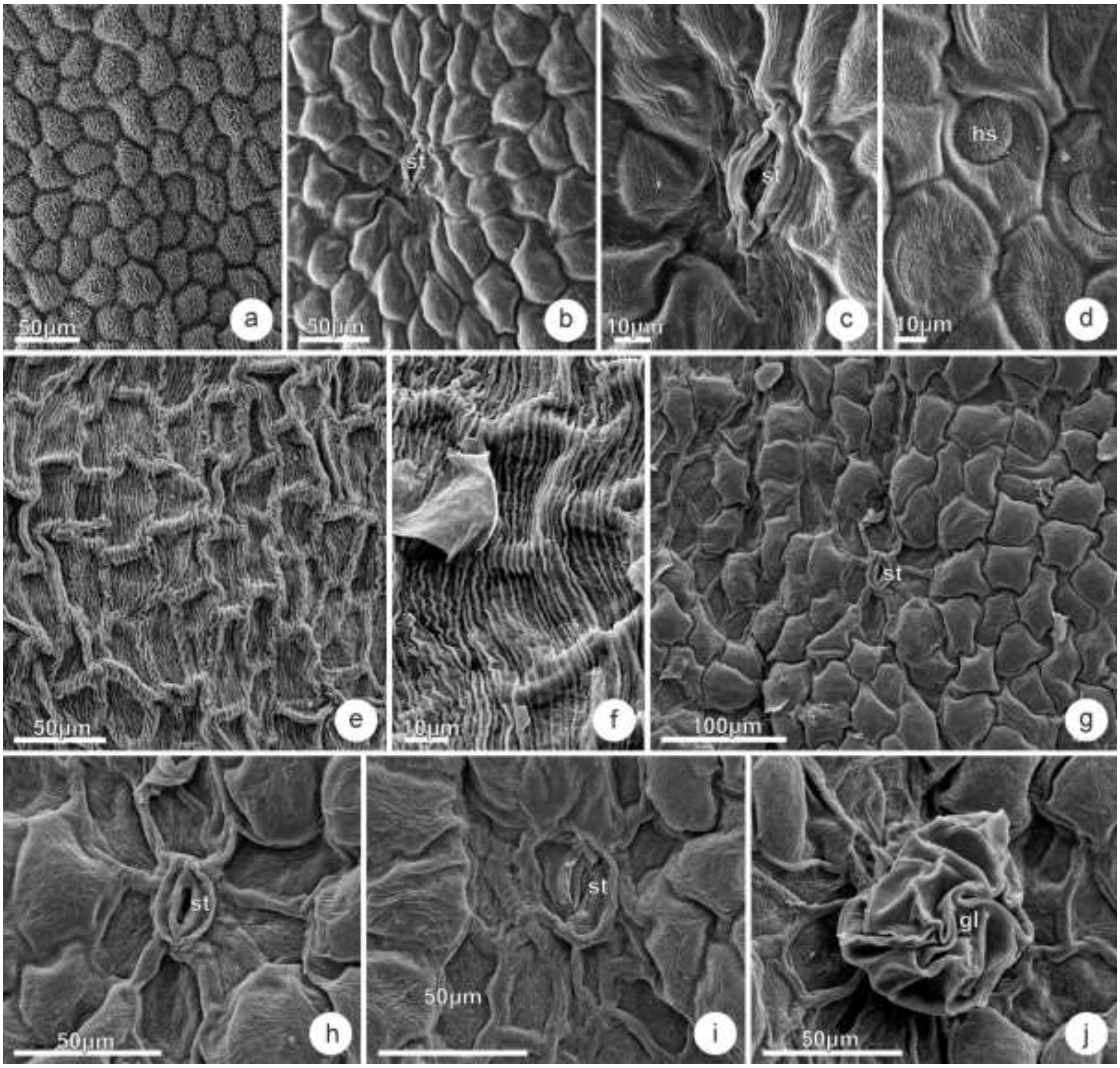
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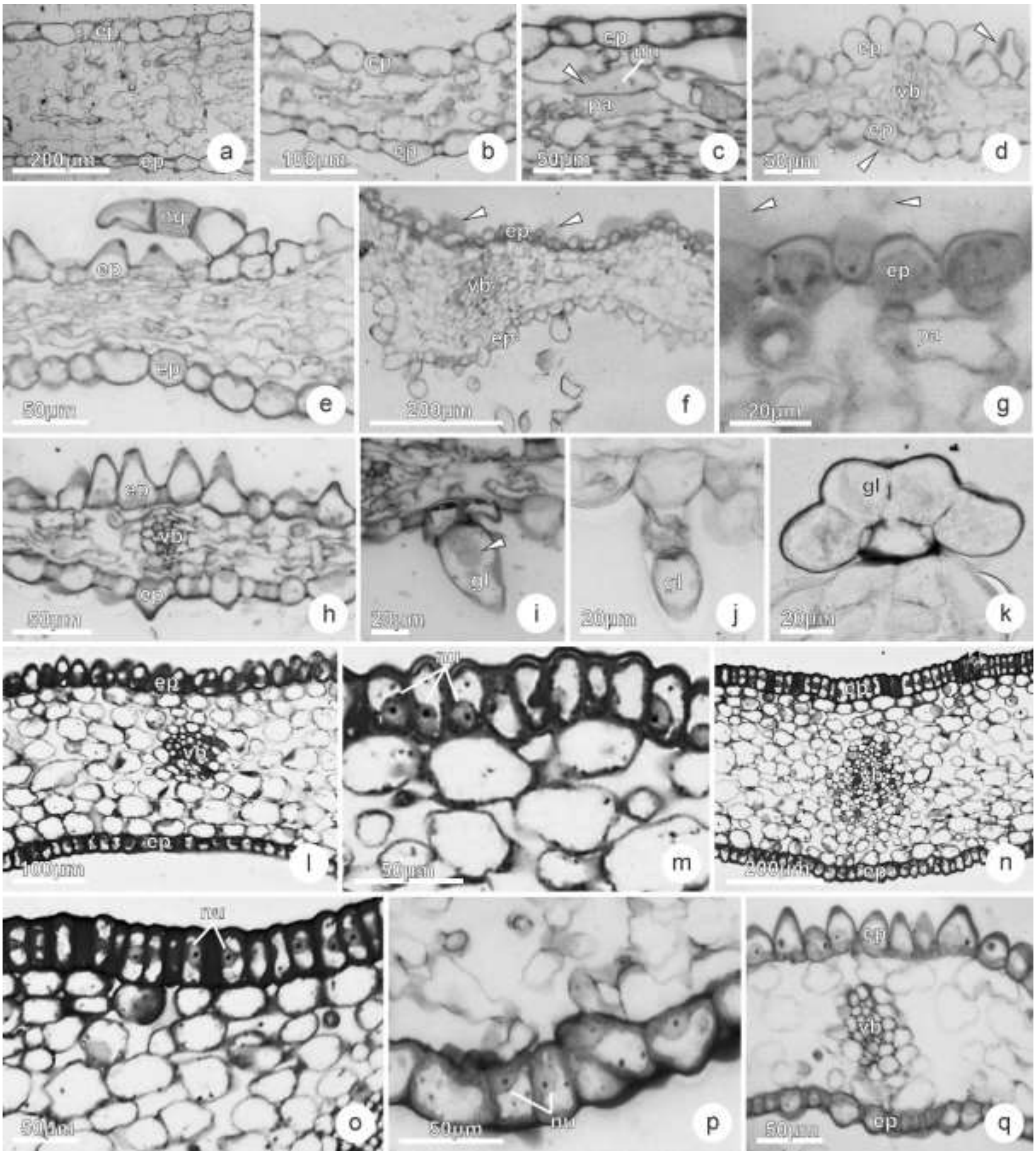
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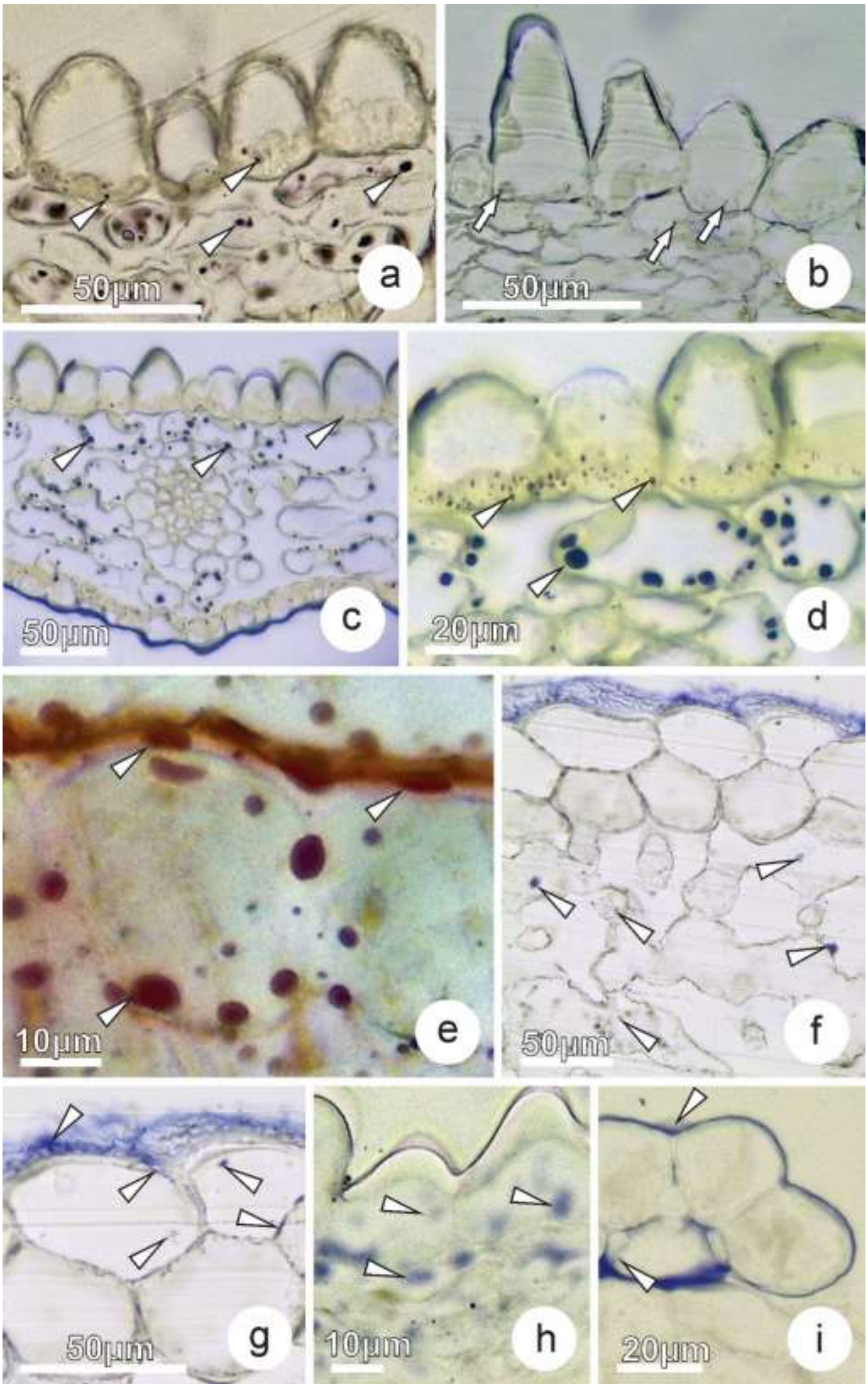
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Table captions

Table 1. *Handroanthus* species collected in the Cerrado Domain in the state of Goiás, Brazil and the respective phytophysiognomies and geographic coordinates where they were collected.

SPECIES	PHYTOPHYSIOGNOMY	GEOGRAPHIC COORDINATES
Handroanthus coronatus (Proença & Farias) Farias	Cerrado *lato* sensu	14º24.9910'S and 48º5.0120'W
Handroanthus impetiginosus (Mart. ex DC.) Mattos	Cerrado *lato* sensu; anthropized area	14º9.5460'S and 48º1.6420'W
Handroanthus serratifolius (Vahl) S.O. Grose	Cerrado *lato* sensu and anthropized cerrado	16º36.5933'S and 49º15.4017'W

Table 2. Surface characterization, cuticular striae, stomata distribution and trichome features on the corolla of three *Handroanthus* species. F = tube floor; R = tube roof; L = corolla lobe; Ada = adaxial surface of the corolla; Aba = abaxial surface of the corolla; (-) = absent.

Species	**Surface**	**Cuticular striae**	**Stomata distribution**	**Trichome distribution**	**Trichome types**
Handroanthus coronatus	Papillate	R and F (Ada)	R and F (Aba/Ada)	R (Ada)	Non-glandular
Handroanthus impetiginosus	Papillate	R and F (Aba/Ada)	-	R (Ada), F (Ada/Aba), L (Ada)	Non-glandular and Glandular (capitate and peltate)
Handroanthus serratifolius	Papillate	R and F (Aba/Ada)	R and F (Aba/Ada)	F (Ada)	Glandular (peltate)

Table 3. Histochemical tests in the corolla of three species of *Handroanthus* and respective portions in which the compounds were detected. E = epidermis; P = parenchyma; VB= vascular bundle; GL= glandular trichome; (-) = no detection.

Species	Starch grains	Lipid drops	Terpenes
Handroanthus coronatus	E, P, VB	E, P	E, P
Handroanthus impetiginosus	E	E, P, GL	E, P, GL
Handroanthus serratifolius	E, P, VB	E, P	-

4. CONSIDERAÇÕES FINAIS

Diante dos resultados obtidos na presente tese, concluímos que os caracteres estruturais e histoquímicos observados nas pétalas das 18 espécies estudadas de Bignonieae e três espécies de *Handroanthus*, tais como a presença e padrões de distribuição de osmóforos, geometria das células epidérmicas (achatada/papilosa), ausência/presença de tricomas glandulares, rugosidade/textura da cutícula e organização do mesofilo (compacto/aerenquimatoso) estabelecem um conjunto de características intimamente ligadas a interação entre flores e polinizadores.

Nas espécies estudadas de Bignonieae, as regiões da corola coradas com Vermelho Neutro, interpretadas como sendo osmóforos, efetivamente correspondem a locais de síntese e emissão de voláteis caracterizadas por epiderme secretora e camadas subepidérmicas portadoras de lipídios, grãos de amido e terpenos. Algumas observações merecem destaque, como as variações no padrão de osmóforos entre espécies visitadas por abelhas incluindo variações dentro do mesmo gênero de Bignonieae; superfície de corola rugosa ou texturizada, interpretada como sendo um facilitador da fixação e movimento das abelhas em direção ao recurso floral.

Para *Handroanthus*, através de análises estrututrais e histoquímicas, as regiões interpretadas como osmóforos eram compostas por epiderme unisseriada com células cônico-papiladas, mesofilo aerenquimatoso e feixes vasculares colaterais. Além de grãos de amido, gotículas lipídicas e terpenóides (exceto em *H. serratifolius*) que foram encontrados nos tecidos da corola das três espécies, sendo mais abundantes nos tecidos secretores, sugerindo seu envolvimento na produção de perfume floral. Superfícies rugosas, cutículas estriadas e dobradas, tricomas glandulares e não glandulares e estômatos elevados foram observados nas três espécies, o que pode ser caracteres úteis na taxonomia do grupo.

Os resultados obtidos até o presente indicam um avanço considerável no conhecimento da diversidade micromorfológica, anatômica e histoquímica das pétalas em representantes de Bignoniaceae e *Handroanthus* e podem auxiliar na interpretação das variações nas características da corola e relações funcionais entre flores e polinizadores, além de suporte a estudos taxonômicos.