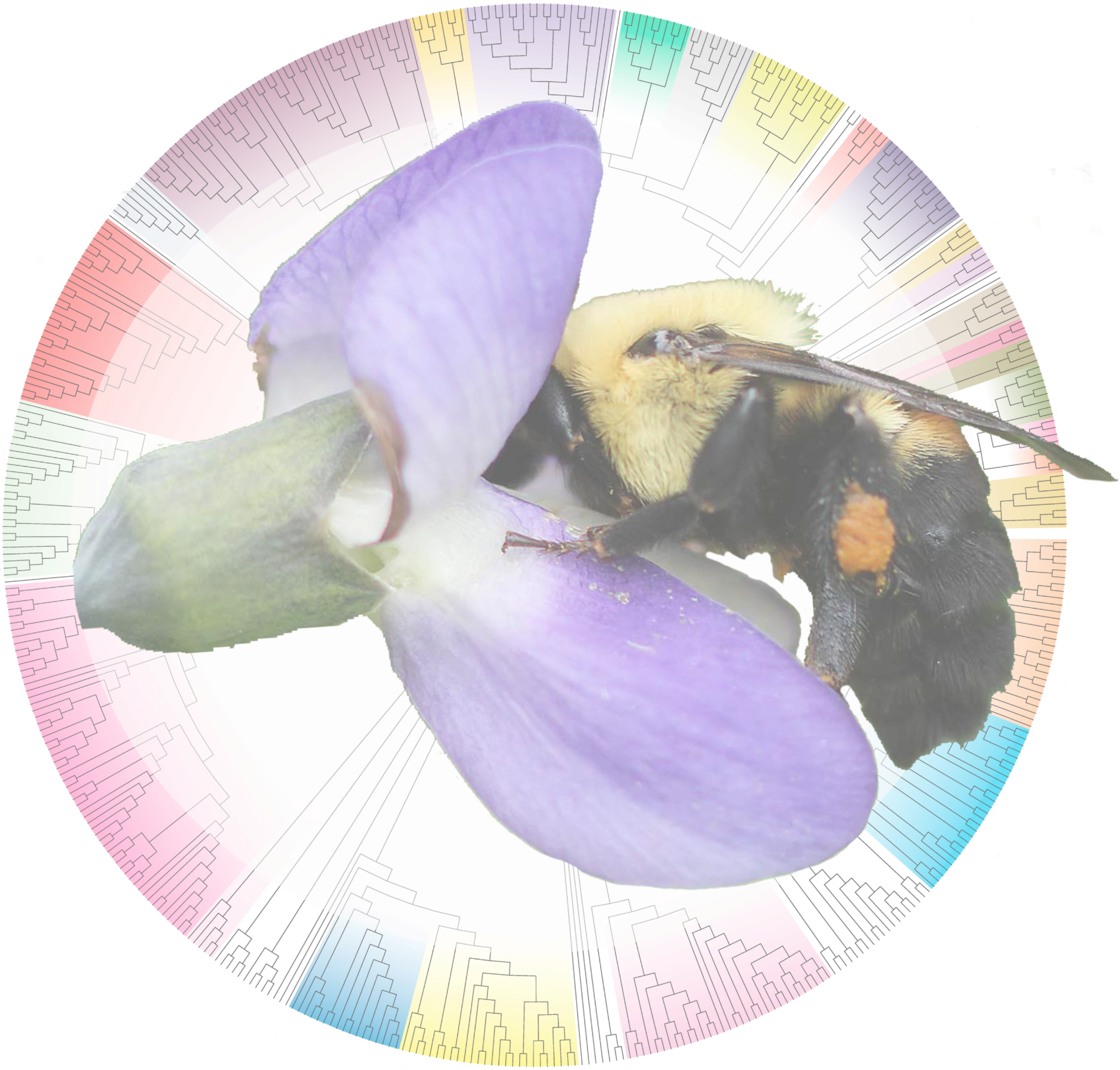
Cássia Cristina Sacramento Silva



PADRÕES MACROEVOLUTIVOS EM ARQUITETURA FLORAL:

REVELANDO O PAPEL DA MICROMORFOLOGIA DE PÉTALAS
NO SUCESSO EVOLUTIVO DE PAPILIONOIDEAE (LEGUMINOSAE)

Programa de Pós-Graduação em Biodiversidade e Evolução

Universidade Federal da Bahia

Padrões macroevolutivos em arquitetura floral:
revelando o papel da micromorfologia de pétalas
no sucesso evolutivo de Papilionoideae (Leguminosae)

Cássia Cristina Sacramento Silva

Tese apresentada ao Instituto de Biologia da Universidade Federal da Bahia para a obtenção do Título de Doutor em Biodiversidade e Evolução pelo Programa de Pós-Graduação em Biodiversidade e Evolução.

Orientador: Dr. Domingos Benício Oliveira Silva Cardoso

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Tese de Doutorado submetida ao Programa de Pós-Graduação em Biodiversidade e Evolução da Universidade Federal da Bahia como parte dos requisitos necessários à obtenção do título de Doutora na área de Biodiversidade e Evolução.

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Orientador e Presidente da banca

Para minhas mães Veralice e
Conceição, minhas certezas
nas horas de dúvida.
Com todo meu amor.

“Talvez não tenha conseguido fazer o melhor, mas lutei para que o melhor fosse feito. Não sou o que deveria ser, mas Graças a Deus, não sou o que era antes”.

(Martin Luther King, 1929 - 1968)

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RESUMO

A flor papilionada é a característica mais marcante que define as Papilionoideae, a subfamília taxonômica e mais ecologicamente diversa das Leguminosae. Este tipo de flor apresenta uma ampla gama de características relacionadas à atração e fidelidade dos polinizadores. Além dos mecanismos de polinização particulares associados às pétalas altamente diferenciadas, a presença de esculturas e *pockets* nas alas das flores papilionadas auxilia os polinizadores. Os *pockets* fornecem um mecanismo de "botão de pressão" entre as alas e da carena, mantendo-as juntas para que se movam como uma unidade quando um polinizador pousa. As esculturas atuam como apoio para os insetos, fornecendo uma superfície estruturada para que as abelhas se segurem enquanto buscam néctar. Embora tenhamos avançado bastante sobre os padrões macroevolutivos da arquitetura floral e sua contribuição para o sucesso evolutivo e ecológico das Papilionoideae, as microesculturas nunca foram exploradas no contexto de diversificação floral em leguminosas. Para isso é fundamental uma sistematização sobre a definição e caracterização mais detalhada da variação de esculturas e *pockets* num contexto da diversidade de flores e dos diferentes clados de Papilionoideae. Além disso, ainda não sabemos nada sobre as origens e mudanças evolutivas destas microestruturas. Neste trabalho, foram realizados estudos de flores coletadas em campo e preservadas em amostras de herbários, bem como dados disponíveis na literatura sobre a caracterização das alas em leguminosas. No total, foram analisados 2160 representantes da família Leguminosae, distribuídos em 1746 espécies de Papilionoideae divididas em 445 gêneros, totalizando 90% dos gêneros da subfamília avaliados quanto à presença e aos tipos de esculturas e *pockets*. Usamos microscopia óptica e eletrônica de varredura para revisar, caracterizar e descrever a variação de esculturas e *pockets* nas alas em todos os principais clados das Papilionoideae. Analisamos de três a cinco flores de 445 gêneros, a maioria dos quais eram de gêneros tropicais nunca descritos completamente em relação às esculturas e *pockets*. Reforçamos que o termo 'esculturas' seja usado para denotar as dobras epidérmicas na superfície da pétala que servem principalmente como apoio para insetos, e *pockets* para descrever as dobras ou invaginações da lâmina da pétala. Os dados obtidos foram utilizados para descrever a diversidade das esculturas e *pockets* nos clados; além disso, realizamos uma reconstrução de caracteres ancestral das esculturas e *pockets* com uma abordagem de máxima verossimilhança e uma filogenia de plastoma robustamente suportada das leguminosas

Papilionoideae para estimar as origens e transições evolutivas das esculturas de pétalas e *pockets*, com base nas análises comparativas das alas de mais de 445 gêneros. Nossos resultados aprofundam o conhecimento sobre a presença e variação das esculturas e *pockets* nos clados de Papilionoideae, destacando os complexos caminhos evolutivos que levaram à origem e às mudanças dessas estruturas. Ainda há lacunas no conhecimento sobre a função e os impactos da micromorfologia das pétalas na diversificação das Papilionoideae, para as quais os dados aqui levantados poderão oferecer suporte em estudos futuros. O conhecimento científico gerado neste trabalho também foi explorado em um contexto de divulgação científica voltado para o público infantojuvenil, com o objetivo de popularizar a ciência, aproximando o conhecimento produzido na academia para a sociedade em geral.

Palavras-chave: divulgação científica; esculturas; evolução; flor papilionada; *pockets*; reconstrução de caracteres ancestral.

ABSTRACT

The papilionate flower is the most distinctive feature that defines the Papilionoideae, the most taxonomically and ecologically diverse subfamily of the Leguminosae. This type of flower displays a wide range of characteristics closely related to pollinator attraction and fidelity. In addition to the specific pollination mechanisms associated with the highly differentiated petals, the presence of sculpturing and pockets on the wings of papilionate flowers assists pollinators. The pockets provide a "snap-button" mechanism between the wing and keel petals, holding them together so that they move as a unit when a pollinator lands. The sculpturing serves as support for insects, providing a structured surface for bees to grip while foraging for pollen and/or nectar. Although we have made considerable progress on the macroevolutionary patterns of floral architecture and its contribution to the evolutionary and ecological success of the Papilionoideae, micro-sculpturing has never been explored in this context of floral diversification in legumes. Therefore, a systematic approach to the definition and more detailed characterization of the variation in sculpturing and pockets in the context of floral diversity and the different clades of Papilionoideae is essential. Furthermore, we still know nothing about the origins and evolutionary changes of these microstructures in Papilionoideae flowers. In this study, we conducted studies on flowers collected in the field and preserved in herbarium samples, as well as data available in the literature on the characterization of wing petals in legumes. In total, 2,160 representatives of the Leguminosae family were analyzed, distributed across 1,746 species of Papilionoideae divided into 445 genera, totaling 90% of the subfamily's genera evaluated for the presence and types of sculpturing and pockets. We used light microscopy and scanning electron microscopy to review, characterize, and describe the variation of sculpturing and pockets on wing petals across all major clades of Papilionoideae. We analyzed three to five flowers from 445 genera, most of which were from tropical genera never fully described before in relation to sculpturing and pockets. We emphasize that the term 'sculpturing' should be used to denote the epidermal folds on the petal surface that primarily serve as support for insects, and pockets to describe the folds or invaginations of the petal blade. The data obtained were used to describe the diversity of sculpturing and pockets in the clades; in addition, we performed an ancestral reconstruction of sculpturing and pockets using a maximum likelihood approach and a robustly supported plastome phylogeny of Papilionoideae legumes to estimate the origins and evolutionary transitions of petal sculpturing and pockets based on comparative analyses of wing petals from over 414 genera. Our results deepen the knowledge about the presence and variation of sculpturing and pockets in Papilionoideae clades, highlighting the complex evolutionary paths that led to the origin and changes of these structures. There are still gaps in the knowledge about the function and impacts of petal micromorphology on the diversification of Papilionoideae, for which the data raised here may provide support in future studies. The scientific knowledge generated in this study was also explored in a scientific outreach context aimed at children and teenagers, with the goal of popularizing science and bringing the knowledge produced in academia closer to society in general.

Keywords: ancestral reconstruction; papilionate flower; pockets; scientific outreach; sculpturing.

INTRODUÇÃO GERAL

O surgimento das plantas com flores foi um importante evento na história da vida na Terra, as inovações promovidas por essa linhagem foram profundamente bem-sucedidas, o que propiciou novas oportunidades ecológicas intrínsecas como o surgimento de composições florestais e a enorme diversidade encontrada nas florestas tropicais (Benton et al., 2022). E oportunidades ecológicas extrínsecas, como a coevolução flor/polinizador que teve impacto em muitas linhagens de insetos principalmente as abelhas, besouros, borboletas; aos mamíferos através da oferta de alimentos principalmente para os herbívoros; e as samambaias do sub-bosque e fungos por proporcionar ambiente favorável (Benton et al., 2022; Li et al. 2019). Durante a origem e irradiação das angiospermas, mudanças evolutivas dramáticas aconteceram na arquitetura básica da flor (Sauquet et al. 2017; Specht & Bartlett 2009). Tornando a flor um dos resultados mais espetaculares de sucessivas inovações evolutivas ao longo do tempo, o que progressivamente levou a um aumento na sua complexidade estrutural (Endress 2006).

O surgimento de inovações como a simetria bilateral resultou em grandes irradiações em alguns clados (Endress 2006; Ricklefs & Renner 1994; Vamosi & Vamosi 2010, 2011). Tal traço define linhagens megadiversas das plantas com flores, por exemplo: Orchidaceae, Leguminosae e Lamiales (Endress 1999; Wessinger & Hileman 2020). A simetria floral não só participa da atratividade e beleza das flores, como também é um traço arquitetônico integrador que explica a surpreendente diversidade de formas das flores (Jabbour et al. 2009). Estudos comparativos de simetria e desenvolvimento molecular indicam que a simetria bilateral é uma condição prévia importante para que ocorra irradiação. A mudança na simetria floral impacta a eficiência de polinizadores e a transferência de pólen, que pode resultar em barreiras sexuais, manutenção da heterozigose e posterior especiação (Endress 1999, 2001, 2006, 2016; Jabbour et al. 2009; Specht & Bartlett 2009; Wessinger & Hileman 2020). Assim, como uma característica estrutural derivada associada à diversificação, a zigomorfia constitui uma inovação-chave (Jabbour et al. 2009).

A inovação-chave (*key innovation*) está entre os eventos podem moldar a diversidade (Donoghue & Sanderson 2015). O termo é definido como um traço ou uma característica que influencia na riqueza de espécies (*sensu* Vamosi & Vamosi 2011). Essas características podem ser responsáveis por abrir novas zonas adaptativas, associando o surgimento de um novo caráter de valor adaptativo à irradiação adaptativa (Donoghue & Sanderson 2015).

Existem diversos exemplos de irradiação adaptativa. O mais clássico deles são os tentilhões de Galápagos, objeto de estudos de C. Darwin na elaboração da teoria da seleção natural (Grant & Grant 2002, 2024); os peixes ciclídeos da África Oriental (Kocher 2004); os lagartos *Anolis* (Losos & Schneider 2009); e os beija-flores havaianos (Fleischer et al. 2008). No reino vegetal, a irradiação adaptativa também é um fenômeno importante e pode ser observada em vários grupos de plantas, incluindo as angiospermas (Magallón & Castillo 2009); como por exemplo as Cactaceae, que apresentam formas de vida especializadas em resposta ao estresse ambiental (Arakaki et al. 2011); e *Salvia*, o maior gênero entre as Lamiaceae, um exemplo de irradiação adaptativa em consequência de uma inovação-chave. *Salvia* apresenta grande diversidade estrutural, mas a modificação dos estames em alavancas é apontada como a inovação que promoveu a irradiação do grupo, que se diversificou junto com o polinizador (Claßen-Bockhoff et al. 2004). Assim como o gênero *Salvia*, as Papilionoideae também se diversificaram junto com insetos polinizadores (Arroyo 1981; Alemán et al. 2017) e apresentam grande diversidade na morfologia floral (Arroyo 1981; Stirton 1981; Crepet & Taylor 1985; Cardoso et al. 2012, 2013a).

As Papilionoideae são as mais taxonomicamente diversas entre as seis subfamílias das Leguminoseae (Tucker 2003; Cardoso et al. 2012; LPWG 2017), contando com cerca de 500 gêneros e 14.000 espécies (LPWG 2024). As Papilionoideae também impressionam pela importância econômica, diversidade e especialização da morfologia floral (Arroyo 1981; Stirton 1981; Crepet & Taylor 1985; Cardoso et al. 2012). A subfamília se diversificou junto com as Hymenoptera (Arroyo 1981; Alemán et al. 2017), o maior grupo de insetos polinizadores (Pennington et al. 2000) e exibe uma diversidade de mecanismos adaptativos voltados para a polinização (Arroyo 1981; Pennington et al. 2000). Assim, parte do sucesso evolutivo das Papilionoideae possivelmente é resultado de eventos de coevolução com as abelhas (Arroyo 1981; Stirton 1981; Crepet & Taylor 1985; Schrire 1989; Pennington et al. 2000).

A flor papilionada é um capítulo à parte na história evolutiva da família, sendo o traço morfológico característico das Papilionoideae. Com simetria zigomórfica a flor é considerada uma inovação-chave, uma vez que flores com essa simetria favorece uma maior precisão na transferência do pólen e restrição ao acesso aos recursos florais (Endress 1999, 2001; Sargent 2004). No entanto, nem todos os clados das Papilionoideae apresentam a flor altamente especializada com simetria bilateral e pétalas claramente diferenciadas (Cardoso et al. 2012, 2013a; Choi et al. 2022; Carvalho et al. 2023). Clados de diversificação inicial apresentam grande variação na morfologia floral, enquanto grupos mais derivados apresentam arquitetura floral conservada (Pennington et al. 2000; Cardoso et al. 2012). É nesse último grupo, onde a morfologia da flor papilionada parece ter se fixado e se tornado mais especializada, que encontramos a maior riqueza da subfamília: quase 70% dos gêneros de toda as Papilionoideae (Pennington et al. 2000; Wojciechowski et al. 2004; Cardoso et al. 2012, 2013a).

Na flor papilionada, cada parte desempenha um papel diferente durante a reprodução dos indivíduos. Enquanto o estandarte tem a função de atrair os polinizadores por meio do padrão de cores, a carena contribui para proteção para a coluna estaminal, e as duas alas, junto com a carena, atuam como plataforma de pouso para os polinizadores (Arroyo 1981). Stirton (1981) sugere que as alas possuem três funções diferentes: atração de polinizadores, alavanca para deprimir e suspender a carena, e plataforma de pouso para os polinizadores. É através dessa última função que a flor e o polinizador iniciam a interação primordial para o sucesso da polinização. Essa interação ocorre por meio da sinalização tátil promovida pelas células epidérmicas da pétala e percebida pelo polinizador através da *sensilla trichodea* nas pontas das antenas dos insetos ou pelos pés dos insetos após o pouso (Kevan & Lane 1985).

Basicamente, existem três tipos de células epidérmicas na corola: planas, cônicas e arredondadas (Whitney et al. 2011; Kraaij & van der Kooi 2020; Wilmsen et al. 2021). As formas celulares estão ligadas a diferentes funções (visual, tátil e olfativa) (Ojeda et al. 2009). As células epidérmicas planas são pouco frequentes na natureza (~10%), enquanto as células epidérmicas cônicas são encontradas em 75-80% das pétalas examinadas (Whitney et al. 2011; Kraaij & van der Kooi 2020; Wilmsen et al. 2021). As células epidérmicas cônicas (CEC) são uma característica definidora das pétalas, já que, em geral, esse tipo de célula não é encontrado em outros órgãos da planta, sendo usado para definir e detectar conversões homeóticas entre pétalas e outros órgãos florais (Whitney et al. 2011). Adicionalmente, as células epidérmicas da corola são recobertas por uma cutícula estruturada de aspecto rugoso, localizada na face adaxial da pétala ou orientada para potenciais polinizadores (Whitney et al. 2011). Kevan & Lane (1985) demonstraram que as abelhas podem reconhecer superfícies epidérmicas distintas, seja de outros indivíduos ou de outros órgãos vegetais, apenas pelo toque; essa habilidade pode permitir que os polinizadores se orientem na pétala e, portanto, funcione como um guia tátil para o recurso (Glover & Martin 1998; Whitney et al. 2011). Desse modo, constitui uma vantagem seletiva ter as diferentes pétalas identificadas com diferentes tipos de células, como acontece em Papilionoideae (Ojeda et al. 2009), e ainda mais ter um par de pétalas com estruturas especializadas para o pouso do polinizador.

Além das CEC, as flores papilionadas apresentam esculturas e *pockets*, que podem ser definidos como ornamentações exclusivas das alas de Papilionoideae (Arroyo 1981; Stirton 1981; Alemán et al. 2017). As esculturas e *pockets* foram estudados pela primeira vez por Schlieden & Vogel em 1839 (Stirton, 1981). Embora o fenômeno tenha sido descrito mais de 120 vezes ao longo do tempo, o trabalho mais representativo até o momento é o de Stirton (1981), onde o autor classificou as esculturas em lunar, lamelar e lunada-lamelar, e os *pockets*, descrevendo as estruturas quanto à posição, exposição, presença e ausência. O autor observou que essas estruturas são encontradas apenas na subfamília Papilionoideae. Embora ainda não tenha havido comprovação empírica da(s) função(s) dessas estruturas, elas oferecem uma superfície de suporte onde os polinizadores podem "cravar" as garras tarsais, permitindo à abelha aterrissar e encontrar o ângulo e a abordagem corretos para acessar a recompensa (Moyroud & Glover 2017). A polinização da flor papilionada apresenta sofisticados mecanismos para evitar visitantes pilhadores (Amaral-Neto et al. 2015). Logo, a importância da presença de esculturas e *pockets* nas pétalas laterais aumenta à medida que a flor se torna mais difícil de manipular, seja por serem verticalizadas ou por condições abióticas, como o vento (Whitney et al. 2011).

A presença de esculturas e *pockets* pode ter sido decisiva para o estabelecimento da relação entre o polinizador e a flor papilionada e representar duas inovações florais com papel significativo na irradiação adaptativa de plantas (Claßen-Bockhoff et al. 2004). Esse fenômeno pode ter contribuído para a diversidade das Leguminosae e de grupos com morfologia floral similar, que podem ter se beneficiado das relações já estabelecidas entre as abelhas e a flor papilionada (Uluer et al. 2022).

Embora tenha havido um grande avanço na caracterização das esculturas, especialmente com a adequação da nomenclatura e a inclusão de dados como a orientação e a posição dessas estruturas nas pétalas, a partir do trabalho de Stirton (1981). Muitos grupos neotropicais não foram amplamente incluídos na primeira publicação. Desde a revisão de Stirton (1981) houve mudanças na taxonomia e delimitação de gêneros. Diversos estudos filogenéticos em Papilionoideae com bases em dados moleculares revelaram muitas mudanças nas circunscrições tribais e posicionamentos genéricos (e.g., Pennington et al. 2000; Wojciechowski et al. 2004; Cardoso et al. 2012; 2013b; Ramos et al. 2016; LPWG, 2017; Choi et al. 2022), além de permitir estudos comparativos de variação morfológica entre clados (Cardoso et al. 2013a), e avanços recentes permitindo uma abordagem em escala genômica (e.g., Choi et al. 2022) têm permitido investigar a evolução das Papilionoideae.

Assim, existe a necessidade de fazer uma revisão dos conceitos tendo em vista ampla diversidade da morfologia floral em Papilionoideae (Polhill 1981; Lewis et al. 2005; Cardoso et al. 2013a; LPWG 2017). A partir de uma revisão mais completa, poderemos avançar na compreensão do papel das esculturas e *pockets*, bem como suas origens e mudanças ao longo do curso da história evolutiva das Papilionoideae.

Diante do exposto, este trabalho teve como objetivo geral caracterizar a variação da micromorfologia das alas em todos os principais clados de Papilionoideae, bem como reconstruir a evolução das diferentes microesculturas. A tese está estruturada em 4 capítulos. No **Capítulo 1** revisamos a presença e os tipos de esculturas e *pockets* em todos os principais clados de Papilionoideae, caracterizando e descrevendo estas microestruturas presentes nas alas. Além de ampliar o conhecimento anterior sobre a variação destas microestruturas, é apresentada uma visão mais completa e atualizada da distribuição das esculturas e *pockets*, tendo em vista os avanços recentes na delimitação e classificação filogenética em Papilionoideae.

No **Capítulo 2**, é abordada a evolução das esculturas e *pockets*, identificando a origem e mudanças evolutivas destas microestruturas ao longo da filogenia das Papilionoideae. Além disso, ao reconstruir também o surgimento e transições evolutivas da arquitetura floral das Papilionoideae, foi possível compreender melhor como as esculturas e *pockets* estão relacionadas com a morfologia da flor papilionada mais especializada. Neste capítulo, também são apresentados novos insights sobre as possíveis funções das esculturas e *pockets*.

Em consonância com as tendências atuais, além do genuíno interesse em tornar a ciência acessível a todos, os **Capítulos 3** e **4** foram desenvolvidos para publicação em revistas de divulgação cientifica, tanto em língua estrangeira quanto em português. Nestes capítulos, adequamos os resultados da pesquisa para uma linguagem não acadêmica, mais acessível a jovens de 11 a 15 anos.

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CAPÍTULO 1

REVISITING WING PETAL SCULPTURING AND POCKET VARIATION IN PAPILIONOID LEGUMES

Revisiting wing petal sculpturing and pocket variation in papilionoid legumes

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Abstract

The papilionate flower is the hallmark trait that characterizes the species-rich subfamily Papilionoideae of the Leguminosae. This flower type exhibits a wide range of features closely related to the attraction and fidelity of pollinators. In addition to particular pollination mechanisms associated with the highly differentiated petals, wing petal sculpturing and pocket found in the papilionate flowers of many papilionoid species aids visiting pollinators. Pockets provide a 'snap-button' locking mechanism between the wing and keel petals, by holding them together so that they usually move as one unit when a pollinator lands. The sculpturing acts as insect footholds by providing an elaborately structured surface for the bees to hold while accessing nectar. We used optical and scanning electron microscopy to review, characterize, and describe the variation of the wing petal sculpturing and pockets across all major clades of the Papilionoideae. We analyzed three to five flowers of 445 genera, most of which were from tropical genera never thoroughly described before with respect to sculpturing and pockets. We reinforce that the term 'sculpturing' be used for denoting the epidermal folds on the petal surface that primarily serve as insect footholds, and that the term 'pocket' should describe the folds or invaginations of the petal blade. Of the total genera analyzed, 195 only presented sculpturing and 41 only pockets, while 30 presented both. Sculpturing can be further classified as lamellate, lunate, and lunate-lamellate. The lamellate type, found in 65% of the genera with sculpturing, varies in appearance, ranging from discrete grooves to intricate epidermal parallel folds. This type is frequently observed in the NPAAA (non-protein amino acid-accumulating) clade. The lunate type, where the epidermal folds resemble a half-moon, accounts for about 23% of genera exhibiting sculpturing; it is primarily found in the Crotalarieae clade. The lunate-lamellate type, the least frequent at 11% of the genera with sculpturing, was recorded in the Amorpheae and Dalbergieae clades. The pocket displays a broad variation in depth, number, shape, and position. We have identified three main types: (i) the elongate pocket is oriented longitudinally on the petal and varies in depth and shape, with folded or entire margins, these restricted to one region or on various parts of the petal; (ii) the punctate pocket is hole-like and has a well-marked concavity with variation in depth and number; and (iii) the perpendicular pocket is oriented transversely on the petal and is deep. Among the pocket-bearing genera, the elongate shape is the most common (46%), followed by the punctate (36%) and perpendicular types (5.5%). Future research should focus on investigating how often this broad variation in wing petal sculpturing has evolutionarily shifted during the floral diversification of the Papilionoideae.

Keywords: epidermis cells; Fabaceae; floral architecture; petal micromorphology

Introduction

The flower is the most important defining structure of angiosperms. It is the result of successive evolutionary innovations over time that have led to its current structural complexity (Endress, 2006; Hernández-Hernández & Wiens, 2020; Li et al., 2019; Sauquet et al., 2017; Specht & Bartlett, 2009). The emergence of the flower was a significant evolutionary event in the history of life, opening new ecological and evolutionary opportunities for diverse forms of life (Li et al., 2019). The flower enables more efficient pathways for cross-pollination, ensuring the maintenance of heterozygosity and preventing inbreeding depression through pollination (Pinheiro et al., 2014; Wessinger & Hileman, 2020).

Pollination is an extremely complex event involving ecological, evolutionary, and morphological responses from the organisms involved and the surrounding environment (Barônio et al., 2016). Various strategies are employed by both sides to optimize gains and reduce energy costs. Plants optimize the amount of reward offered to genuine pollinators and impose barriers to pilfering visitors, while pollinators employ strategies to increase the exploitation of floral resources for their benefit (van der Kooi et al., 2021). Thus, plants and pollinators act as agents of natural selection, enhancing mechanical, morphological, behavioral, and/or physiological adjustments between flower and pollinator (Mackin et al., 2021; van der Kooi et al., 2021). The adjustments between flower and pollinator can be so refined that flower and pollinator coevolve (Hu et al., 2008; Lunau, 2004).

The coevolution between flower and pollinator has led to complex combinations of floral characteristics associated with pollinator attraction (Frachon et al., 2021). One of these morphological adjustments is bilateral symmetry, which, in addition to acting on flower attractiveness, also results in a surprising diversity of forms (Jabbour et al., 2009). Furthermore, bilateral symmetry selects pollinators with specific traits, thus improving pollen transfer efficiency (Wessinger & Hileman, 2020). The evolution of bilateral symmetry is closely associated with bee pollination, so any alteration in floral size and shape can impact resource acquisition and pollination efficiency (Jabbour et al., 2009). An example of modification in flowers with bilateral symmetry is keel flowers (*sensu* Westerkamp, 1997).

The keel flower is, by definition, a flower with bilateral or zygomorphic symmetry, pentamerous, with three types of petals (standard, wings, and keels), usually with connate floral parts (stamens and keel petals), and reproductive organs protected by the keel (Westerkamp, 1997). These flowers are referred to as an adaptive response to bees, which have evolved not only to attract the pollinator but also to protect the pollen (Uluer et al., 2022). The morphology of this flower requires its pollinators to have specific characteristics to access their rewards (Amaral-Neto et al., 2015).

The largest keel-flowered angiosperm lineage is the subfamily Papilionoideae of legumes (Leguminosae or Fabaceae). The high prevalence of this floral architecture among species in the subfamily has led these flowers to be called papilionate flowers (Uluer et al., 2022). In addition to the challenges posed by the keel flower described above, Papilionoideae exhibit different mechanisms of primary and secondary pollination arising from differences in size, color, shape, number, and arrangement of structures (Leppik, 1966; Arroyo, 1981; Stirton, 1981). These mechanisms ensure the accuracy and efficiency of pollen deposition, limiting resource waste and optimizing pollination, thus making the papilionate flower even more specialized (Alemán et al., 2022; Arroyo, 1981; Stirton, 1981; Uluer et al., 2022). The effectiveness of pollination is also influenced by the aggregations of flowers into different types of inflorescences,(Prenner, 2013), the number of flowers presented in these or open at any time, plus the spacing of rewards (Makino et al., 2006; Wyatt, 1982).

The corolla of the typical, specialized papilionate flower (or flag blossom, *sensu* van der Pijl, 1972) is composed of five petals organized in a dorsal to ventral arrangement, the standard (vexillum, banner), a pair of lateral petals, the wings, and a pair of ventral petals, the keel (Arroyo, 1981; Etcheverry, 2001; Stirton, 1981; Uluer et al., 2022). Each petal plays a role in pollen transfer (Arroyo, 1981; Etcheverry, 2001; Stirton, 1981): the standard primarily functions in pollinator attraction; the pair of keel petals protects the stamens and pistil and is part of the secondary pollen exposure mechanism along with the pair of wing petals (Arroyo, 1981; Stirton, 1981); and the pair of wing petals often help to attract pollinators, levering to depress and suspend the keel that results in a pollen triggering mechanism and landing platform for pollinators (Fig. 1a) (Etcheverry, 2001; Stirton, 1981). It is through this latter function that the flower and pollinator initiate the interaction that will be crucial for pollination success. This interaction is not yet fully understood, but it is known that the wing petals can have conical papillose cells, ornamented cuticle, sculpturing (Fig. 1a-b), and pockets (Fig. 1c), all of which are linked to flower pollination (Koch et al., 2008; Stirton, 1981; Whitney et al., 2011a).

The function and distribution of sculpturing and pockets on the wing petals have not yet been fully understood, nor has their variation and distribution among the different clades of the Papilionoideae. Although the papilionate floral architecture is a hallmark of the subfamily, it is noteworthy that the flowers in Papilionoideae vary greatly (Polhill, 1981; Pennington et al., 2000; Cardoso et al., 2012a). Such flowers may exhibit radial symmetry, with petals that are either completely absent or consist of five undifferentiated petals. The bilaterally symmetrical flowers of Papilionoideae may also have poorly differentiated petals or be restricted to just the adaxial standard petal, often accompanied by numerous free stamens (Cardoso et al., 2013b; Choi et al., 2022; Citerne et al., 2010; Prenner et al., 2015). Given this floral heterogeneity, it is expected that the different strategies and mechanisms of successful pollination would vary among the different lineages. Therefore, it is essential to scrutinize and systematize not just the general morphological variation in terms of floral architecture but also the nomenclature, shape, and distribution of sculpturing and pockets on the wing petals. Understanding the evolution of putatively innovative, highly specialized micromorphological traits, such as the sculpturing that ornament the keel flowers, is fundamental for gaining insights into the remarkable diversification history of ecologically and economically important angiosperm groups like the Papilionoideae (Aleman et al., 2017; Amaral-Netto et al., 2015; Uluer et al., 2022). A better understanding of the distribution of petal sculpturing and pocketing can provide additional insights into how flowers of the Papilionoideae have been so evolutionarily and ecologically successful. In this study, we have reviewed the presence and types of sculpturing and pockets across all Papilionoideae clades, by characterizing and describing the microstructures present on the wing petals.

Material and Methods

The pioneering study by Stirton (1981) analyzed 1156 species of Papilionoideae, establishing a significant foundation for subsequent research. This study not only consolidates existing data but also expands upon it, encompassing a broader and more diverse range of samples, particularly from previously unstudied tropical genera. Such expansion was made possible by the inclusion of new data from visits to herbaria and collecting expeditions conducted in various regions of Brazil.

Taxon sampling — Intending to encompass all the floral diversity of Papilionoideae, we established a minimum sampling of one species per genus. For the Leguminosae family, we analyzed a total of 2160 representatives across 1772 species. Of these, 2132 representatives belong to 1746 species and 445 genera out of the approximately 500 genera currently recognized in the Papilionoideae subfamily (LPWG, 2024). Within the Leguminosae, we also examined representatives from the subfamilies Caesalpinioideae, Cercidoideae, and Detarioideae to investigate the potential presence of sculpturings or pockets in other subfamilies. The materials analyzed were obtained through field collections, consultation of specialized literature, and rehydrated preserved specimens from the following herbaria: Alexandre Leal Costa Herbarium (ALCB), Rio de Janeiro Botanical Garden Herbarium (RB), and Feira de Santana State University Herbarium (HUEFS). Because some studies have revealed that different types of sculpturing may become evident only in the final stages of floral development (e.g., Leite et al., 2014a; McMahon and Hufford, 2005), we minimized the bias in floral development by always selecting apparently fully developed flowers, avoiding bud stages or apparent developmental stages. However, as most of the material studied consisted of herbarium specimens, we cannot definitively determine the stage of development of the over six thousand flowers analyzed (3 to 5 flowers per species).

For the use of the published material, preference was given to the author's description of the structures over images, illustrations, and photographs, when provided. In the absence of such images, the author's description was the primary reference considered (Supporting Information Table S1). Searches were conducted using the names of clades, tribes, and genera. Data obtained from the literature were adjusted based on the description of the structures adopted here (i.e., position and region of the petal where sculpturing/pockets occur) (Fig. 1c). Our dataset was also largely augmented by the use of Stirton's (1981) original database (Supporting Information Table S1).

The names of all sampled species and their status were checked in the Royal Botanic Gardens, Kew's Plants of the World Online (POWO, https://powo.science.kew.org), with the help of the expowo package (Zuanny et al., 2024). The abbreviation of herbarium names follows Thiers (2024). The full list of observed materials, as well as the bibliography used, is available in Supporting Information Table S1.

Standardized nomenclature used for wing petal sculpturing and pocket — The terminology for sculpturing and pockets adopted in this study follows the standardization proposed by Stirton (1981), encompassing the position and distribution of characters on the wing petal (Fig. 1c). In addition to the author's proposed nomenclature for pockets, we introduced a complementary classification to facilitate the identification and description of this structure in future research. Due to the complex morphology of the papilionate flower, determining the position of the wing petal surface (adaxial, abaxial) is not as straightforward as in more regular flowers. Therefore, we adopted the definition proposed by Amaral-Neto et al. (2015) and Cavallini-Speisser et al. (2021), where the surface of the wing petals facing the reproductive structures is considered the inner surface (adaxial), as opposed to the outer surface (abaxial).

The advances in the study of the petal epidermal cells have resulted in a varied nomenclature for their characterization (e.g., Alcorn et al., 2012; Bailes & Glover, 2018; Dyer et al., 2006; Kay et al., 1981; Ojeda et al., 2009, 2013; Whitney et al., 2009a, 2011b). Here, we follow the standard definitions proposed by Bailes & Glover (2018), Koch et al. (2008), Ojeda et al. (2009), and Whitney et al. (2009a). The terms used for the classification and description of cuticular folds were adapted from Barthlott et al. (2017) and Koch et al. (2008, 2009a, 2013), where we refined the concept of granular and striate into: (*i*) cuticle organization, including irregularly striate; parallel striate, and smooth; and (*ii*) density, for the visible aspect of cuticular folds, where they can be dense, when the cuticular folds present little or no space between them; sparse, when the spaces between the cuticular folds allow the observation of smooth regions of the cuticle; or rare, when the smooth region of the cuticle occupies more space than the cuticular folds.

Stereomicroscope observation and scanning electron microscopy **—** All herbarium material underwent a rehydration process in heated water and glycerin (30:1) until boiling for 30 seconds (adapted from Smith & Smith, 1942), whereas the field-collected specimens preserved in 70% alcohol were directly described. The petals (standard; wings, and keel) of each flower were separated, and both wing petals were analyzed under an Olympus® SZ61 stereomicroscope with an attached camera. Data on the presence/absence, types, position, display, orientation of sculpturing, and pockets on the wing petals, as well as data on shape, symmetry, resupination, and floral architecture type were organized into a data matrix in an Excel® spreadsheet.

To understand the composition of the sculpturing and pockets, we selected the most distinct types for analysis by scanning electron microscopy (SEM). The wing petals stored in 70% alcohol, were placed in increasing concentrations of ethanol (80%, 90%, and 100%) for a minimum of two hours at each concentration. For complete dehydration, the petals were placed in the chamber of the Leica© EM CPD030 drying equipment and subjected to a gradually increasing series of liquid CO_2 until reaching the critical point. They were then fixed on steel specimen holders ("stubs") with carbon adhesive tape and subsequently coated with gold (5-7 min) using the Balzers SCD 050 sputter coater (Bozzola & Russel, 1992). The samples were then analyzed and photographed using the JEOL JSM-6390LV scanning electron microscope (SEM).

Cuticular folds **—** To investigate potential differences in cuticular folds and cell types between the regions with sculpturing and pockets and the other regions of the blades of the wing petals, we analyzed the outer surface (abaxial) of the wings of 24 species, covering 22 different genera (Supporting Information Table S2). To ensure standardization of the observed areas, we examined the entire extent of the sculpturing and pockets, noting their variations when present. Similarly, in the regions without ornamentation, we focused our observation on the central portion, excluding vein areas, or on the distal portion of the petal next to the sculpturing or pockets , but without continuity with them (Supporting Information Table S2).

Figure preparation **—** We initially used the R (R Core Team, 2024) packages ggplot2 (Wickham, 2016) and circlize (Gu et al., 2014) to prepare the graphics, with further adjustments in Adobe Photoshop© to make the final composition. Minor aesthetic adjustments were also made on the SEM images, such as color standardization, brightness, and removal of image artifacts, using the commercial program Adobe Photoshop©. All such edits were made respecting the boundaries of the structures studied here, without altering any content. The final composition of the images was also organized in Adobe Photoshop©.

Results

The comparative analysis of the variation in structure and distribution of wing petal sculpturing and pocket across 1746 species and 445 genera from all main clades of the papilionoid legumes led us to systematize a standardized definition where sculpturing may be classified into lamellate, lunate or lunate-lamellate, whereas pockets may be elongate, transverse or punctate. These types are briefly described below, including examples of some representative taxa where they occur, but a more thorough, across-clade discussion is provided in the Discussion section.

***Sculpturing* —** Our investigation revealed that sculpturing always consists of epidermal folds of the petal, usually visible on only one side. Based on variation in sculpturing shapes, the sculpturing can be further classified as follows:

Lamellate. This sculpturing type resembles lines, wrinkles, or folds in the epidermis of the petals (Figs. 1a-c and 3a-e). The lines of the lamellae are not homogeneously organized (Fig. 3a-e). This type of sculpturing presents a great variation in the thickness of the lamellate crests and widths of the troughs and in how they are arranged among themselves. Lamellate sculpturing can be strongly folded inward into the petal, forming well-pronounced and close crests, with deep and scarcely visible valleys, as in the case of *Pueraria montana* (Lour.) Merr. (Fig. 3d) and *Dipteryx odorata* (Aubl.) Forsyth f. (Fig. 3e), or they can present well-defined crests, separated by visible valleys, as in *Dipteryx polyphylla* Huber (Fig. 1b - *H bar*). The folds can be smooth, with thin crests and superficial valleys forming discrete lines on the petal, as in *Cajanus cajan* (L.) Huth (Fig. 3c). Lamellate sculpturing may also present broad and sparse folds, resembling scars on the petals, as is the case of *Luetzelburgia auriculata* (Allemão) Ducke (Fig. 3b). In other cases, the folds may overlap with adjacent ones, as observed in *Robinia* sp. and *Pueraria montana* (Fig. 3a,d).

Lunate. In this type of sculpturing, the epidermal folding has a crescent-shaped format and generally the crescents overlap each other (Figs. 3f-j and 4b). This crescent moon, arch, or 'C' aspect is formed by the opening between the crest and the valley of the previous fold. The opening of lunate sculpturing is always directed towards the most proximal portion of the petal. Lunate epidermal folding, unlike lamellate, tends to organize itself into columns, delimited by the primary and secondary veins of the petal (Figs. 3f,h,i-j and 4b). Lunate sculpturing does not vary in format (crescent-moon-shaped), but the folding can vary in size and appearance, such as the long-lunate folding of *Pearsonia aristata* (Schinz) Dümmer (Fig. 3j), or shorter lunate folding found in *Lupinus leucophyllus* Douglas ex Lindl. (Fig. 3i), or even with a cup-shaped format also observed in *Pearsonia aristata* (Fig. 3f,j). Lunate sculpturing, due to its format, can appear as a small pocket, which sometimes leads to confusion in the description of these structures (e.g., Alemán et al., 2022; Swanepoel et al., 2015), but as we will see next, pockets are structurally different.

Lunate-lamellate. This sculpturing type characterizes the wing petals that show both lunate and lamellate sculpturing together (Figs. 3k-o and 4c). A gradual transition from lamellate to lunate sculpturing is often observed, as in *Nissolia vincentina* (Ker Gawl.) T.M.Moura & Fort.-Perez (Fig. 3m), or they can occupy distinct spaces, as found in *Hymenolobium janeirense* Kuhlm. (Fig. 3o).

***Pockets* —** While sculpturing often involves an epidermal modification of the petal, pockets can be defined as depressions, folds, or invaginations in the all tissues composing wing petal, observed on both sides of the petal (Fig. 4d-q). This variation is reflected in the three different types of pockets that we have described here: elongate, punctate, and transverse.

Elongate. The elongate pocket is characterized by a depression or fold in the petal. This depression or fold creates a concavity always located on the upper margin of the petal (Fig. 4d-h, k-l). This type shows the greatest diversity, varying in terms of depth, when the fold in the petal margin can have a smooth or more pronounced concavity (Fig. 4d-e, k-l); and region, when the pocket can be found in only one position of the petal or extend across multiple positions. When combined, we can observe the following pocket variation: (*i*) smooth depression throughout its extension, possibly present in one or more regions of the petal, as seen in *Hedysarum alpinum* L. (Fig. 4d-e); (*ii*) deeper depression point present in one or more regions of the petal (Fig. 4f-h); or (*iii*) folded margins (*sensu* Stirton, 1981) (Fig. 4k-l). This variation is usually formed by a fold and depression on the petal margin, with the level of the fold and the depth of the depression varying among the analyzed individuals, here represented by *Pachyrhizus erosus* (L.) Urb. (Fig. 4k-l). Another feature of the elongate pocket is that it never reaches the distal position of the petal (Supporting Information Table S1).

Transverse. This type of pocket is formed by the invagination of the petal, always located in the upper basal portion, having a shape like a transversal cut to the petal. The transverse pocket is represented here by *C. cajan* (Fig. 4p-q) and was observed in only a few species, mostly from within the Genistoid and NPAAA clades (Supporting Information Table S1).

Punctate. This type of pocket consists of small, well-defined concavities formed by the invagination of the wing petal. Punctate pockets can vary in number (1-2) and depth and can be classified as follows: (*i*) a single deep and well-defined concavity, as found in *Medicago sativa* L. (Fig. 4m-o); (ii) a single shallow concavity; (iii) two well-defined and shallow concavities, with *Astragalus convallarius* Greene (Fig. 4i-j) being one of the representatives. Except for type *iii*, punctate pockets are restricted to the upper basal region of the petal (Supporting Information Table S1).

Sculpturing and pockets in numbers **—** This study addressed a total of 2160 species of Leguminosae, of which 2132 representatives belong across all main clades of the subfamily Papilionoideae (Fig. 2), where 445 genera were described in relation to the ornamentation of their lateral petals. Only a few genera exhibited polymorphism, such as *Luetzelburgia* which possesses species with or without sculpturing (Supporting Information Table S1).

Sculpturing and pockets were not encountered in the flowers of Caesalpinioideae, Cercidoideae or Detarioideae analyzed here. Moreover, sculpturing and pockets were absent in all 39 Papilionoideae genera (237 analyzed representatives) lacking keel flowers or with radial floral symmetry, underscoring that these features are not just exclusive to the subfamily but are exclusive traits of papilionate flowers (Fig. 5, Supporting Information Table S1). The flowers with a nearly-papilionate architecture (*sensu* Cardoso et al., 2013b), on the other hand, exhibited sculpturing but not pockets (Fig. 5). From this group, 19 genera with 62 representatives were analyzed. The presence of pockets within the nearly-papilionate architecture was limited to 3 genera (*Baphia*, *Baphiastrum*, *Bracteolaria*) (Supporting Information Table S1).

Among the 1835 papilionate-flowered representatives analyzed here (86% of the total), 262 genera (1249 individuals) exhibited some type of sculpturing, while pockets were observed in 127 genera, representing 29% of the genera (Fig. 5).

We also observed that 175 genera (39%) showed no type of ornamentation on the lateral petals; 196 genera (44%) exclusively exhibited sculpturing on their wings; 44

genera (10%) exclusively displayed pockets; and 30 genera (7%) presented both sculpturing and pockets on their lateral petals (Fig. 6a).

A total of 170 (38%) genera had at least one representative with lamellate sculpturing. The lamellate sculpturing was the most common in our observations, followed by the lunate type, with 56 genera (~13%), and the lunate-lamellate type, with 27 genera (6%) (Fig. 6b).

Similarly, the analysis of pocket variation showed it is present in 127 genera. The most frequent pocket type was elongate, which is present in 58 (13%) genera, followed by the punctate type, present in 46 (10%) genera, and finally, the perpendicular type that is present in only 7 (1.6%) genera (Fig. 6c).

A total of 1955 specimens were analyzed with respect to their sculpturing and pocket position along the wing petal (Fig. 7a-b). The most common location of such petal ornamentations is the upper part of the petal (Fig. 7a-b). Sculpturing has a broader distribution compared to pockets, occurring both on the upper and lower parts of the petals (Fig. 7a). The presence of these structures on the upper portion is consistent, while their occurrence in the lower region is accompanied by their manifestation in the upper region, without exceptions (Table 1; Fig. 7a). Conversely, pockets occur exclusively on the upper part (Table 2; Fig. 7b). Sculpturing was frequently found in the basal region and extending to the central left portion (299 individuals); completely sculptured petals (upper, lower, basal, central, distal) were recorded in 52 representatives, while sculpturing on the upper and lower portions of the petal, in the central to distal region, were observed in only one representative. See also Table 1 for a full description of all other sculpturing distributions. Unlike sculpturing, pockets do not exhibit a wide distribution on the petal, often being restricted to the basal region, reaching a maximum at the central right region (Table 2; Fig. 7b). Most pockets were recorded in the basal position of the petal (148 individuals). See also Table 2 for the full description of pocket distribution.

Discussion

***Towards a standardized concept and delimitation of wing petal sculpturing and pocket* —** Schlieden and Vogel (1839) were pioneers in studies concerning the presence of structures found on the external surface of the wing petals of Papilionoideae flowers (Chung & Lee, 1991; Stirton, 1981). The authors named these structures '*alae faveolata-rugosae*', which means wings with small pitted like. Since then, several studies have cited these sculpturing patterns, using various terms to describe these structures such as lines, wrinkles, lamellae, lunate, and cavae, among others (Chung & Lee, 1991). For a long time, the absence of an adequate nomenclature made it difficult to describe the different microstructures present on the wings. Stirton (1981) published the first most comprehensive study describing wing petal sculpturing and pocket variation across the Papilionoideae. By examining the wing petals from a significant number of species, he proposed a standard terminology and discussed the possible functions of the sculpturing and pockets. Stirton's (1981) basic terminology involved position, type, and orientation of the sculpturing, and has been widely used (e.g., Alemán et al., 2017, 2022; Amaral-Neto et al., 2015; Cardoso et al., 2014; Etcheverry, 2001; Etcheverry et al., 2003, 2008; Etcheverry & Vogel, 2017; Leite et al., 2014b; Queiroz et al., 2010; Westerkamp & Weber, 1999), sometimes comprehensively (e.g., Boatwright, 2010; Chung & Lee, 1991; Egan & Pan, 2015; McMahon & Hufford, 2005; Sampaio et al., 2013) or more succinctly (e.g., Jongkind, 2003; Ohashi & Mill, 2000; van Wyk & Schutte, 1994).

In addition to its widespread figurative use, the term 'sculpturing' is employed in structural botany to designate the ultrastructure present in the superficial region of plant parts (Koch et al., 2009a). The term encompasses a variety of forms in different structures, such as leaves, cuticles, cells, petals, pollen grains, and seed coats (Akabari & Azizian, 2006; Davies & Winters, 1998; Dulberger, 1981; Halbritter et al., 2018; Jabeen et al., 2023; Koch et al., 2009a; Masinde, 2004; Rashid et al., 2023; Rudall & Campbell, 1999; Stpiczyńska & Stpiczyńska, 2001; Xiao et al., 2020). To define the sculpturing found on the wing petals of Papilionoideae, we have adapted the concepts of sculpturing and ornamentation from the works of Mayfield (2021) and Stirton (1981). Therefore, here we suggest designating wing sculpturing as the epidermal folds of various shapes (lamellate, lunate, lunate-lamellate), creating a three-dimensional composition on the external surface (abaxial) of the wing petals. Based on the work of Stirton (1981), we distinguish the epidermal folds into two categories, inspired by the shapes that the sculpturing assumes in paradermal view (Leite et al., 2014b; Etcheverry, 2001) in SEM (Fig. 1b - arrowhead and ‘H’ bar). The highest point of this fold is what we call a "crest", and the depressed area between the folds, we call a "valley".

Pockets are structures distinct from sculpturing, differing both in their shape and structure, as described above. Although there is still some confusion regarding the nomenclature (e.g., Alemán et al., 2022; Bailes & Glover, 2018), pockets are depressions, folds, or invaginations in the wing petal, occurring on both sides of the petal. Pockets also seem to play a crucial role in the reproductive success of papilionate flowers. Because they are often located near the petal margins, their role was initially thought to provide some support during the landing of visiting insects; however, some studies suggest different functions, for example, they can serve as tactile guides to nectar or provide greater support to the sides of the wing petals (Alemán et al., 2017, 2022; Etcheverry et al., 2008). In some cases they are part of the articulation of the auricles of the standard or appendages, if present, on the lower part of the standard above the claw which when swollen twist the wing petals into a horizontal position providing a landing stage. Stirton (1975) has provided a detailed description of such a process in the genus *Eriosema*.

Hierarchical surface design — Flowers tend to exhibit petals with conical papillose epidermal cells, which are generally covered by a structured cuticle called cuticular folds (Kay et al., 1981; Koch, 2010; Koch et al., 2008, 2013; Whitney et al., 2009a, 2009b, 2011a, 2011b). These structures are commonly found in papilionate flowers, with the presence of conical cells particularly associated with the wing petal (Ojeda et al., 2009). Therefore, we can assert that the wing petals of Papilionoideae present a complex structure, composed of sculpturing, pockets, and a variety of cell types, as well as cuticular folds (Fig. 8a-k). This complexity can be readily associated with the concept of hierarchical structure applied to surfaces Koch et al. (2008).

To identify and describe any pattern of interaction among these structures, we examined 22 genera (24 species), of which 17 species exhibit the same cellular pattern (papillose or tabular) in both ornamented and non-ornamented areas (Fig. 8a-e). The knob-like type of papillae was the most common, found in 15 sampled species in the region with sculpturing and 11 sampled species in the region without sculpturing (Fig. 8a, j). In six species, knob-like type papillae occur in both regions — with and without sculpturing. (Supporting Information Table S2). The tabular or papillose classification pertains to the shape of the cell perimeter, while flat, rugose, stepped, conical, lobular, or knob-like refers to how much the cell protrudes from its base (Bailes & Glover, 2018). Although the authors distinguish between the papillose knob-like and tabular rugose types based on the perimeter shape and degree of projection, these types can be easily confused, as the degree of cell projection varies considerably within these categories, leaving the definition much more reliant on cell shape.

Among the sampled species, 11 exhibited some differences in cell type. Of these, only *Pearsonia aristata*, *Laburnum alpinum* (Mill.) Bercht. & J.Presl, and *Lotus maritimus* L. showed greater contrast (in terms of shape and projection) between the epidermal cells forming sculpturing and pockets and the non-ornamented region (Supporting Information Table S2). We expected the epidermal cells of the sculpturing and pockets to differ from those in the non-ornamented regions, adding another level of tactile information to the sculpturing and pockets, given that these structures are related to insect landing (Alemán et al., 2017, 2022; Arroyo, 1981; Stirton, 1981). The plant epidermis is not a uniform tissue and tends to exhibit different cellular shapes and textures, usually related to the function it performs (Bailes & Glover, 2018; Koch et al., 2008). However, contrary to expectations, there seems to be a tendency for the cells composing the sculpturing and pockets to show some similarity to the non-ornamented region (Supporting Information Table S2). In papilionate flowers, each petal has cellular identity, with conical papillose cells generally associated with the standard and the wings (Fig. 8b). The presence of these cells in these petals is linked to their role in attraction, landing, and triggering the pollination mechanism (Bailes & Glover, 2018; Ojeda et al., 2009). As discussed by Stirton (1981), the wing petals present a diversity of "landscapes". In fact, the wing petals sometimes exhibit mosaics of cell types.

Several studies have investigated the patterns and functions of epidermal cells in Papilionoideae (e.g., Kay et al., 1981; Ojeda et al., 2009; Stirton, 1981; Bailes & Glover, 2018). The morphological diversity of the epidermis is largely related to pollination, either directly or indirectly. These functions include serving as visual or tactile signals to guide pollinators for landing and gripping under normal or adverse conditions (Fairnie et al., 2022; Ojeda et al., 2013; Whitney et al., 2009a, 2009b), production of volatile substances (Whitney et al., 2009a), keeping petals dry and clean (Koch et al., 2008; Whitney et al., 2011a, 2011b), or enhancing color and brightness (Kay et al., 1981; Moyroud & Glover, 2017; Papiorek et al., 2014).

In addition to sculpturing, pockets, and different cell types, there is also a third hierarchical level of organization: the microstructured cuticle also referred to as cuticular folding (Fig. 8f-k). This third hierarchical level completes the complex structuring of the papilionoid flower (Koch et al., 2009b). The dimensions and shape of the cuticle folds may vary depending on the plant's habitat, species, organ, and developmental stage (Voigt et al., 2012). In the analyzed individuals, the most frequent cuticular fold among the studied regions was the dense parallel striate (Fig. 8h; Supporting Information Table S2). Additionally, *Dipteryx punctata* (S.F.Blake) Amshoff, *Lupinus leucophyllus*, *Thermopsis montana* Nutt., and *Lotus maritimus* varied between parallel striate (Fig. 8h) and irregular striate (Fig. 8a, g) among the compared areas (Supporting Information Table S2). As for the species *Dipteryx odorata*, *Dipteryx punctata*, *Nissolia vincentina*, and *Pueraria* sp., we observed variation in the density of cuticular folds (Supporting Information Table S2). Widely occurring, the microstructured cuticle on floral petals holds significant functional importance, being responsible for maintaining wettability properties, stabilizing thin cell walls, self-cleaning, insect sliding, visible light reflection, and UV radiation absorption (Kakani et al., 2003; Kay et al., 1981; Koch, 2010).

The sampled species revealed a tendency for lamellate-type sculpturing to be formed by papillose knob-like cells (11 ind.), with dense cuticular folds (12 ind.) (Fig. 8g-h). As for the pockets, which had eight representatives here, regardless of type, they tend to be formed by tabular-shaped (Fig. 8c-e) cells with sparse parallel striations (5 ind.) (Fig. 8i-j, Supporting Information Table S2). Bailes & Glover (2018) found a similar trend in their observations on genetic mutants of *Vicia faba* L., where tabular striate cells were specifically found around what the authors call 'wing petal folds', suggesting that these structures may be related to some function in the pollination triggering mechanism. Additionally, flat cells (Fig. 8c, g) are not common in the visually active part of the petal, as this type of cell does not tend to be very bright (Moyroud & Glover, 2017). The presence of flat tabular cells in the region is also related to the exposure of these structures in the flower. The exposure or non-exposure of the sculpturing and pockets was a trait analyzed by Stirton (1981), where the sculpturing and pockets can be covered by the standard or by another part of the flower. In addition, flat or stepped tabular cells are related to floral traps, like those found in the pitchers of some carnivorous plant species. (Kraaij & van der Kooi, 2020).

The unique combination of cell types is presumably related to the role played by each region of the petal (Bailes & Glover, 2018). Thus, the presence of specific cell types in the region of the sculpturing and pockets may indicate a differentiated function of this area or even suggest that the wing petals are composed of distinct regions that may evolve independently of each other (Delpeuch et al., 2022).

We further observed that species of the genera *Luetzelburgia* and *Lupinus* exhibited cells in the sculpturing valley region with smooth cuticle and intensely striate crest cells, as also reported by Stirton (1981). Surprisingly, the species *Pearsonia aristata* displayed a contrasting pattern, with cells that are less striate and smooth at the apex of the crest, and more striate in the sculpturing valley region. In contrast to Stirton's (1981) report, the genera analyzed in this study did not present smaller and/or papillose cells in the crest region of the sculpturing.

Among-clade distribution and taxonomic significance of wing sculpturing and pocket — Stirton (1981) had already suggested the taxonomic significance of petal sculpturing and pocket. Other more recent studies have used sculpturing mainly for species identification (e.g., Arbainsyah & Adema, 2024; Britto & Senthilkumar, 2001; Chung & Lee, 1991; Etcheverry, 2001; Leite et al., 2014b). However, an updated and more comprehensive assessment of petal sculpturing and pocket across tribes and major clades of the papilionoid legumes was still lacking since Stirton's (1981) pioneering broad investigation. The advances in the phylogenetic systematics of the Papilionoideae based on molecular data during the past 20 years (e.g., Wojciechowski et al., 2004; Cardoso et al., 2012b; LPWG, 2017; Choi et al., 2022) have revealed many new changes in tribal circumscriptions and generic placements, in addition to enabling comparative studies of among-clade morphological variation (Cardoso et al., 2013a). Here, we have used a robustly resolved phylogenomic framework of the Papilionoideae (Fig. 2) to explore the distribution and taxonomic significance of sculpturing and pocket across the major clades of the subfamily.

The initial diversification of the Papilionoideae is marked by the evolution of a high floral diversity, including profound deviations from the typical papilionate architecture (Choi et al., 2022). This evolutionary trend appears to have been accompanied by a low presence of sculpturing and pockets in the early-branching clades of the subfamily, while in the more derived clades, characterized by papilionate flowers, these structures seem to be more prevalent. For example, the NPAAA (non-protein amino acid-accumulating) clade, the most species rich lineage of legumes, accounting for approximately 70% of the entire family's diversity (Cardoso et al., 2013a), may exhibit lamellate sculpturing and is the clade where pockets can be found in their full variation.

Below is a more detailed, clade-by-clade characterization of the distribution of petal sculpturing and pocket in the Papilionoideae. But for a full description of all small clades and other isolated monospecific lineages that are not featured here, see Supporting Information Table S1.

Angylocalyceae. In this subclade of the ADA clade, neither sculpturing nor pockets were observed. This subclade is largely characterized by floral architectures related to bird and bat pollination (Cardoso et al., 2012b, 2013a). As reported by Ojeda et al. (2016, 2013), changes in pollinators drive the evolution of floral traits, such as flower color and size, relative size and orientation of the petals, composition and quantity of nectar, and petal micromorphology. Given that sculpturing and pockets are related to bee pollination (Alemán et al., 2014, 2017; Etcheverry et al., 2008; Stirton, 1981), the absence of these structures in this clade may be related to its particular floral syndrome.

Dipterygeae. *Dipteryx* is the only genus in the clade that presents a wing pocket, whereas all analyzed species from the other genera present lamellate sculpturing, except for the non-papilionate flowered genus *Monopteryx* that does not present any type of sculpturing or pockets. Molecular phylogenies have strongly supported *Monopteryx* as sister to the remainder of the Dipterygeae (Cardoso et al., 2012b, 2013a; Carvalho et al., 2023), all of which are marked by an ancient floral conservatism involving papilionate architecture but with an elaborated modification of the calyx lobes (Carvalho et al., 2023). Interestingly, despite their highly evolutionarily conserved floral morphology, each of the Dipterygeae genera are distinct in their floral ontogenetic development (Leite et al., 2014a), in addition to having distinct types of wing sculpturing. Except for one specimen, the wing petals in the genus *Pterodon* are mostly marked by lunate sculpturing, whereas in *Taralea* they have lamellate sculpturing.

Amburaneae. No representative of the Amburaneae clade has pockets. The clade is known for its diversity in terms of floral morphology (Cardoso et al., 2013a). Yet only genera with papilionate flowers present sculpturing. For example, both the monotypic Amazonian genus *Petaladenium* and its phylogenetically closely related *Dussia* (Cardoso et al., 2015) have wing sculpturing. Particularly in the case of *Petaladenium*, the sculpturing is lamellate and with the appearance of small lines in the basal and central region of the petal. Prenner et al. (2015), in their floral development study of *Petaladenium urceoliferum* Ducke, discussed different hypotheses on possible connection of the unique fimbriate-glandular wing petals. Although they did not mention the importance of wing sculpturing, we suggest that it may also aid in the successful pollination mechanism of the species.

Swartzieae. No representative of this clade presents sculpturing or pockets. This was expected, given that their flowers only present a single, standard petal or no petal at all (Cardoso et al., 2013a).

Cladrastis clade. Petal sculpturing in species of the Cladrastis clade had already been extensively characterized by Chung & Lee (1991). Although having free stamens, all species in the clade have strongly bilateral, papilionate flowers. However, no genus in this clade presents pockets. The genus *Cladrastis*, except for the species *Cladrastis delavayi* (Franch.) Prain, has lunate sculpturing, while *Styphnolobium* does not present sculpturing.

Exostyleae. No representative of the Exostyleae clade has sculpturing or pockets. Indeed, most representatives of the clade present radially symmetrical flowers, except for bilaterally symmetrical flowered, although non-papilionate, genera *Zollernia* and

Uribea (Mansano et al., 2002; Cardoso et al., 2013a). Such non-papilionate floral architecture that largely marks the entire Exostyleae may explain the absence of wing petal sculpturing.

Vataireoid clade. No analyzed Vataireoid species presented pockets, whereas wing sculpturing, whenever present, is lamellate and found only in the genera *Luetzelburgia* and *Vatairea*. Because the crimped petals in *Luetzelburgia* species (Cardoso et al., 2014) may be confounded with the ridges and valleys of the sculpturing, describing the surface is sometimes a challenge. Lunate-lamellate sculpturing previously has been described for the genus by Cardoso et al., (2014). However, based on the concept used here, the sculpturing found in *Luetzelburgia* is more accurately described as lamellate (Fig. 3b).

Andira clade. The radially-symmetrical-flowered genus *Aldina*, as expected, did not present sculpturing or pockets. On the other hand, the papilionate genera *Andira* and *Hymenolobium* consistently displayed lamellate sculpturing, except for *H. grazielanum* H.C.Lima, which lacked sculpturing. The lamellate sculpturing in *Andira* was previously reported by Pennington (2003), in his taxonomic monograph of the genus. The species *A. humilis* Mart. ex Benth. and *A. multistipula* Ducke have elongate and punctate pockets, respectively. Elongate pockets were observed only in *Hymenolobium modestum* Ducke, amongst the species of genera studied.

Ormosieae. Both papilionate-flowered genera *Ormosia* and *Spirotropis* have wing sculpturing, while the Amazonian nearly-papilionate-flowered genus *Panurea* did not exhibit sculpturing or pockets. Among the studied species of the genus *Ormosia*, it was observed that the upper region of the wing petal tends to fold but without the formation of pockets. In this group, elongate pockets were observed only in *Ormosia bahiensis*

Monach. and *O. limae* D.B.O.S.Cardoso & L.P.Queiroz. *Ormosia costulata* (Miq.) Kleinhoonte and *O. friburgensis* Taub. ex Harms have lamellate sculpturing.

Brongniartieae. The Brongniartieae clade showed significant variation in the presence of sculpturing, yet whenever present, or absent, the character state was well-conserved within genera. Species of *Amphiodon*, *Behaimia*, *Cyclolobium*, *Harpalyce*, *Lamprolobium*, and *Tabaroa* did not exhibit sculpturing. On the other hand, when present, sculpturing is lamellate in the genera *Brongniartia*, *Haplormosia*, *Hovea*, *Limadendron*, *Plagiocarpus*, *Poecilanthe*, and *Templetonia*. As for the pockets, they were more diverse in type, even within species, as observed in *Harpalyce brasiliana* Benth., where one individual presented an elongate pocket, and a second specimen showed a punctate pocket with deep and well-defined cavity. *Limadendron amazonicum* (Ducke) Meireles & A.M.G.Azevedo, *Poecilanthe itapuana* G.P.Lewis, and *Poecilanthe ulei* (Harms) Arroyo & Rudd exhibited elongate pockets. As for the monotypic genus *Tabaroa* from the Brazilian Caatinga dry forests, we also confirmed the report of pockets as described earlier by Queiroz et al. (2010), when they newly described this genus.

Leptolobieae. No genus belonging to the Leptolobieae presented sculpturing or pockets. Genera in this clade are also marked by strongly contrasting floral architectures in terms of floral symmetry and petal differentiation (Cardoso et al., 2012a, 2013a). Despite such floral differences, Cardoso et al. (2012a) mentioned that the shared absence of sculpturing on the wing petals is a putative synapomorphy of the clade (Cardoso et al., 2013a).

Sophoreae. The presence of sculpturing is quite consistent in most of the analyzed genera of the Sophoreae clade. The only genera that did not present sculpturing were the ornithophilous-flowered *Anagyris* (Valtueña et al., 2007; Ortega-Olivencia & Catalán, 2009), the radially-symmetrical-flowered *Dicraeopetalum* (Cardoso et al., 2013a), as well as *Neoharmsia* and some species of *Sakoanala*. The genera *Ammodendron*, *Baptisia*, *Bolusanthus*, *Euchresta*, and *Thermopsis* exclusively exhibited lamellate sculpturing, which was predominant in the clade, comprising 45 species distributed among the mentioned genera. The lunate-lamellate sculpturing was the second most frequent type, accounting for 22 species distributed in the genera *Maackia* and *Sophora*. Lunate sculpturing was observed in the genera *Piptanthus* and *Salweenia*. In contrast to the sculpturing, pockets were only observed in five representatives of the genera *Ammodendron*, *Anagyris*, and *Thermopsis*. The relatively large variation in sculpturing type and position on the wing petals has also been taxonomically useful in the Sophoreae (Chung & Lee, 1991).

Podalyrieae. The Podalyrieae clade also showed great variation among the genera and within them. The radially-symmetrical-flowered genus *Cadia* did not present sculpturing as did the papilionate-flowered genera *Cyclopia* and *Virgilia.* Representatives of the genera *Amphithalea*, *Podalyria*, and *Xiphotheca* present lamellate sculpturing. The genus *Calpurnia* exhibited variation, where *C. aurea* (Aiton) Benth. varied from lamellate to lunate sculpturing, *C. glabrata* Brummitt may, or not, present sculpturing, and the species *C. intrusa* (R.Br.) E.Mey., *C. sericea* Harv., and *C. woodii* Schinz did not present any sculpturing at all. *Liparia* exhibited variation in the presence of sculpturing, where the lamellate type was only observed in *Liparia angustifolia* (Eckl. & Zeyh.) A.L.Schutte, *L. capitata* (Lam.) Thunb., *L. myrtifolia* Thunb., and *L. vestita* Thunb. Finally, *Stirtonanthus* presented one species with lunate sculpturing and another without sculpturing. Regarding pockets, they were observed only in some representatives of the genera *Calpurnia*, *Cyclopia*, *Liparia*, and *Podalyria*.

Crotalarieae. The genus in the clade that does not present sculpturing is the resupinate-flowered *Bolusia* and three species of *Aspalathus*. The genera *Leobordea*, *Listia*, *Robynsiophyton*, *Rothia*, *Wiborgiella*, and the species-rich *Crotalaria*, which although consistently presenting lunate sculpturing, authors such as Britto & Senthilkumar (2001) and Etcheverry (2001) proposed differentiating some species of the *Crotalaria* by using the region and position occupied by the sculpturing. They also used the number, size, exposure, and inclination of the sculpturing, as well as the presence of pockets and characteristics of the wings. This demonstrates that sculpturing and pockets can be important sources of taxonomic information. All remaining Crotalarieae genera presented species with distinct types of wing sculpturing. For example, *Aspalathus* and *Pearsonia* present species with lamellate, lunate, or lunate-lamellate sculpturing. Unlike *Crotalaria*, the other more speciose genus of the clade, *Aspalathus*, varied in terms of petal sculpturing (Stirton, 1981; Britto & Senthilkumar, 2001). On the other hand, the genera *Lebeckia*, *Lotononis*, and *Rafnia* exhibited individuals with lamellate and lunate sculpturing. Petal sculpturing proved a useful character to help discriminate the species of *Wiborgia*. The diversity of sculpturing is not accompanied by pockets, which has been observed only in some species of the genera *Aspalathus*, *Lotononis*, *Rafnia*, and *Robynsiophyton*.

Genisteae. Similarly to other Genistoid clades, all genera analyzed here presented sculpturing. We can readily separate some genera according to their types of sculpturing. The genera *Argyrocytisus*, *Argyrolobium, Calicotome*, *Chamaecytisus*, *Cytisus*, *Dichilus*, *Erinacea*, *Gonocytisus*, *Hesperolaburnum*, *Oberholzeria*, *Petteria*, and *Spartium* present lamellate sculpturing, with the exception being an individual of the species *Cytisus nigricans* L., where a lunate sculpturing was observed. *Adenocarpus*, *Cytisophyllum*, *Lupinus*, and *Melolobium* consistently have lunate sculpturing. The remaining Genisteae genera present a combination of lamellate and lunate sculpturing. Pockets were observed in the genera *Argyrolobium*, *Chamaecytisus*, *Dichilus*, *Genista*, *Lupinus*, *Melolobium*, *Spartium*, and *Ulex*, and less frequent in *Genista* and *Lupinus*. The association of osmophores with sculpturing in the Genistineae was explored by Adey (1978) and suggests a fruitful area of future research.

Amorpheae. The Amorpheae clade stands out for the evolution of radial symmetry or even for the simplification of papilionate flowers in most of its genera (Cardoso et al., 2013a; McMahon & Hufford, 2005). *Amorpha*, *Apoplanesia*, *Errazurizia*, and *Parryella* do not present sculpturing. On the other hand, the nearly-papilionate-flowered genera *Dalea*, *Marina*, and *Psorothamnus* present the three types of sculpturing. No genus of the clade exhibited pockets.

Dalbergieae. Except for the genera with non-papilionate flowers (*Acosmium*, *Inocarpus*, and *Riedeliella*), all remaining Dalbergioid genera present some type of sculpturing. Lunate sculpturing is the most common type across the dalbergioids, being found in 28 out the 39 analyzed genera. Only the genera *Dalbergia* and *Grazielodendron* present lunate-lamellate sculpturing. As for pockets, their presence was diffuse among the genera, which sometimes presented only one species with the structure (e.g., *Pterocarpus angolensis* DC.). Although the three types of pockets have been observed, the elongate type is more frequent in the clade.

Baphieae. This tribe has been shown to be the sister clade of the extremely biodiverse NPAAA clade (Cardoso et al., 2013a; Choi et al., 2022). Flowers in the tribe have scarcely differentiated lateral and abaxial petals. *Baphia*, *Bracteolaria*, and *Dalhousiea* present lamellate sculpturing. Pockets near the auricle region were observed in *Baphia*, *Baphiastrum*, and *Bracteolaria*.

Hypocalypteae. Among the analyzed species of this monogeneric tribe, *Hypocalyptus oxalidifolius* (Sims) Baill. was the only one to present sculpturing but did not present pockets. The remaining species presented pockets.

Mirbelioid clade. Most genera of this exclusively Australian clade, which encompasses the traditional tribes Mirbelieae and Bossiaeeae (Thompson, 2011; Barrett et al., 2021), have sculpturing on their wing petals. The absence of sculpturing was detected here in species *Bossiaea*, *Dillwynia*, *Gastrolobium*, *Isotropis*, and *Leptosema*. On the other hand, the presence of pockets in the tribe was relatively rare, with only a few species from certain genera identified as having these structures. Among them, *Chorizema*, *Daviesia*, and *Eutaxia*.

Indigofereae. Sculpturing and pocket were observed only in the genera *Phylloxylon* and *Indigofera*, respectively. However, given the high species richness and floral variation in the pantropically distributed genus *Indigofera* (Preez et al., 2023a, 2023b, 2024; Schrire, 2013; Schrire et al., 2009), a further scrutiny of wing sculpturing and pocket is warranted in the tribe. There is considerable variation in the shape, size and orientation of spurs of the keel petals which articulate with the wing petals and play a role in the explosive release as a unit once visited by pollinators.

Diocleae. In this clade of predominantly Neotropical lianas or lianescent plants (Queiroz et al., 2015), we observed variation regarding the presence of sculpturing. *Betencourtia*, *Dioclea*, *Galactia*, and *Lackeya* present sculpturing in all sampled species. Significant changes in floral traits are associated with the pollinator, ranging from alterations in corolla shape to its cellular composition (Kriebel, 2023; Ojeda, 2016). Although sculpturing and pockets are involved in pollination, these structures play different roles in the process. This can be observed in the resupinate-flowered genus *Canavalia*, and the hummingbird-pollinated genus *Bionia*, which has highly modified, long tubular, papilionate flowers. Both genera do not have any sculpturing, but a large part of the sampled species exhibited pockets that ranged from elongate to punctate. The absence of sculpturing in flowers where the wing petal loses its function as a landing platform reinforces the importance of sculpturing in the bee pollination system. In contrast, pockets seem to be involved in the pollen presentation mechanism (Alemán et al., 2022; Amaral-Neto et al., 2015). The occurrence of pockets appears to be more prevalent within the Diocleae, with 9 out of 15 genera exhibiting variations ranging from elongate to punctate. *Camptosema* and *Rhodopis* have no type of ornamentation on the lateral petals.

Core Millettioid clade. This highly diversified clade exhibits notable intrageneric variation concerning both sculpturing — found in *Aganope*, *Dahlstedtia*, *Deguelia*, *Derris*, *Lonchocarpus*, *Millettia*, *Muellera*, *Piscidia*, *Pongamia*, *Spatholobus*, and *Tephrosia* — and pockets, identified in *Aganope*, *Deguelia*, *Derris*, *Muellera*, and *Tephrosia.* In all these genera, at least one species showed variation in the presence or absence of sculpturing and pockets. It is worth noting that *Deguelia*, *Kunstleria*, *Platycyamus*, *Spatholobus*, and *Tephrosia* have elongate-type pockets, while *Muellera*, *Piscidia*, and *Pongamia* have punctate-type pockets. Although many genera exhibited variation in petal sculpturing and pockets, *Apurimacia*, *Arthroclianthus*, *Austrosteenisia*, *Chadsia*, *Mundulea*, *Philenoptera*, and *Schefflerodendron* stood out by having no observed variation.

Psoraleae. This clade was represented by 19 genera and 92 specimens, exhibiting exclusively lamellar sculpturings. The genera within the Psoraleae clade could be categorized into two groups: those showing no variation in the presence of sculpturings, including *Cologania*, *Neorautanenia*, *Otholobium*, *Pediomelum*, *Psoralea*, and *Pueraria*; and those displaying interspecific variation, such as *Amphicarpaea*, *Calopogonium*, and *Teramnus*. Additionally, *Teyleria* was the only genus with multiple species that consistently demonstrated the absence of sculpturing. Pockets were observed in ten genera. The genus *Pueraria* is particularly notable, as it exhibited pockets varying between punctate and elongate in all analyzed individuals. The elongate type was the most prevalent within the clade.

Phaseoleae. This clade was studied here by 69 specimens from across 26 genera. In this clade, we can clearly differentiate three groups among the studied individuals: (*i*) those that follow the trend observed in the NPAAA clade, the sculpturing in all Phaseoleae was predominantly lamellate, represented by the following genera: *Alistilus*, *Ancistrotropis*, *Cochliasanthus*, *Condylostylis*, *Dolichopsis*, *Nesphostylis*, *Oxyrhynchus*, *Ramirezella*, *Sigmoidotropis*, *Spathionema*, *Vatovaea* and *Vigna*; (*ii*) the genera that did not show consistency in the presence of sculpturing. These genera are: *Leptospron*, *Macroptilium* and *Phaseolus*; and (*iii*) the genera in which no sculpturing was observed in any of the analyzed species: *Decorsea*, *Dipogon*, *Helicotropis*, *Lablab*, *Macrotyloma*, *Mysanthus*, *Otoptera*, *Physostigma*, *Strongylodon* and *Wajira*. Regarding pockets, it was observed that 14 genera exhibited at least one species with pockets. All species within the genera *Lablab*, *Mysanthus*, and *Sigmoidotropis* displayed elongate-type pockets, with variation in the degree of pocket depth. The elongate pocket was the most common type in the sample, observed in 14 individuals. In contrast, only four specimens showed punctate-type pockets: *Condylostylis* and *Dipogon* each with one representative, and *Macrotyloma* with two representatives. It is important to note that the genera *Cochlianthus*, *Cochliasanthus*, *Condylostylis*, *Decorsea*, *Macroptilium*, *Physostigma*, *Ramirezella*, and *Wajira* have asymmetric flowers. Except for *Cochlianthus*, *Decorsea*, and *Physostigma*, all of these genera present some type of ornamentation on the wing petals, suggesting the maintenance of the function of these structures even in asymmetric flowers, mainly sculpturing, which is the most common ornamentation in the clade.

Desmodieae. This tribe, which is notable for its remarkable diversity and geographical distribution (Jin et al., 2019), was studied here by 24 genera comprising 41 specimens. The presence of sculpturing in this clade exhibited considerable variability, being detected in only 10 genera. Sculpturing is absent not just within genera, such as in species of *Phylacium* and *Pseudarthria*, but perhaps also within an entire genus, such as *Desmodium*, for which we have not observed any sculpturing among all studied species. Apart from *Lespedeza thunbergii* (DC.) Nakai, no pockets were observed in other *Desmodieae* genera.

Robinioid clade. This clade includes representatives of the traditional concept of the tribes Robinieae, Sesbanieae, and Loteae. The Robinioids were studied here by 74 individuals from across 22 genera (Supporting Information Table S1). Among them, *Coursetia* and *Poissonia* stood out by consistently presenting sculpturing. In contrast, *Olneya* did not present sculpturing or pockets, and in the remaining genera, presence of sculpturing varied among species. Elongate-type pockets were observed only in a few species of *Poissonia* and *Robinia*. Among the *Sesbania* species examined, only *Sesbania vesicaria* (Jacq.) Elliott did not exhibit floral sculpturing, and all species did not present any pockets. Among the genera traditionally classified within Loteae, *Anthyllis*, *Antopetitia*, *Dorycnopsis*, *Hammatolobium*, and *Hosackia* showed no sculpturing, with all but *Anthyllis* having only one representative. Elongate pockets were observed in *Coronilla*, *Hippocrepis*, *Lotus*, and *Tripodion*, while punctate pockets were identified in *Ornithopus*, *Scorpiurus*, and in two species of *Lotus*.

Inverted-Repeat-Lacking clade. This clade includes all genera traditionally classified in the tribes Astragaleae, Caraganeae, Fabaeae, Glycyrrhizeae, Hedysareae, and Wisterieae, and it was here studied by 220 samples from across 38 genera (Supporting Information Table S1). The presence of sculpturing in this clade is relatively low, with only 11 genera out of 16 species exhibiting sculpturing, all of which are lamellar. Variation among the genera with sculpturing was quite inconsistent, with the only exception being the two individuals of the genus *Padbruggea*. In contrast, pockets were more common, found in 106 species across 15 genera. Notable among these are *Astragalus*, *Lathyrus*, *Medicago*, *Oxytropis*, and *Vicia*, which showed a significant number of pockets in the studied samples. Notably, in *Astragalus*, no sculpturing was observed, and except for *Astragalus schmalhausenii* Bunge, all species in this genus have pockets. Punctate pockets were more frequent, with 56 representatives (34 in *Astragalus*), while elongate pockets were observed in 42 individuals (19 in *Astragalus*).

***Flower architecture and the distribution of sculpturing and pockets* —** Unlike other flowers classified as keel flowers, the papilionate flower of the Papilionoid legumes cannot be understood solely by its banner and keel structures (Westerkamp & Weber, 1999). Analyzing parts of a flower separately overlooks the fact that each part has a specific function and results from the evolutionary development of the entire organ (Stirton, 1981; Schrire, 1989). Therefore, it is essential to recognize the interdependence and integrated functionality of all parts, particularly the sculpturing and pocketing variation in wing petals, to fully understand their ecological roles within the organism.

Sculpturing and pockets were not encountered in the flowers of Caesalpinioideae, Cercidoideae and Detarioideae, thus confirming the observation that such structures are unique to the subfamily Papilionoideae (Chung & Lee 1991; Stirton 1981). Moreover, we found that sculpturing and pockets are always absent in Papilionoideae species lacking the highly specialized keel flowers (*sensu* Westerkamp, 1997). Out of the 39 genera (comprising 239 representatives) with non-papilionate flowers analyzed in this study, none displayed sculpturing or pockets. This finding underscores that these features are not just exclusive to the subfamily but are also unique traits of papilionate flowers (Fig. 6, Supporting Information Table S1).

The consistent absence of sculpturing and pockets in genera with non-papilionate flowers, as well as resupinate (e.g. *Centrosema*, *Clitoria*, and *Periandra*) or highly modified bird-pollinated (e.g. *Erythrina*; Bilbao et al., 2021) flowers, together with their more frequent presence in bilaterally symmetrical flowers, suggest that these wing traits may have emerged as part of a correlated evolution process between the complex papilionate flowers and their pollinating bees (Alemán et al., 2022; Amaral-Neto et al., 2015; Arroyo, 1981; Leppik, 1966; Stirton, 1981; Schrire 1989; Westerkamp, 1997; Westerkamp & Weber, 1999). Zygomorphic flowers, in general, already pose complex challenges for bees, with various floral traits such as petal morphology, arrangement of reproductive organs, and color patterns, working together for successful pollination (Assis, 2023). There still exist only a few tests and studies investigating the association between microstructures and floral macromorphology, as well as the precise contribution of these structures to plant-bee interactions (Bailes & Glover, 2018; Fairnie et al., 2022). In the context of Papilionoid legumes, the studies by Alemán et al. (2022), Amaral-Neto et al. (2015), Stirton (1981) and the current contribution have brought new advances in our understanding.

Conclusions and Future Perspectives

The types of sculpturing have been used as a source of taxonomic characters, mainly for species delimitation (Arbainsyah & Adema, 2024; Britto & Senthilkumar, 2001; Chung

& Lee, 1991; Etcheverry, 2001; Le Roux & Van Wyk, 2012; Stirton & Muasya, 2016; Swanepoel et al., 2015). Here, we show that some types of sculpturing can also serve taxonomically at clade level, such as for the Exostyleae and Leptolobieae, which are consistently characterized by the absence of sculpturing (Cardoso et al., 2012a; Cardoso et al., 2013a). However, an important aspect to note is the observed intraspecific variation, which may be related to the timing of the emergence of these structures during flower development. It previously has been reported that for some species, the folds only complete their development, becoming fully visible, in the stage preceding anthesis, as is the case for species of *Ruellia* in the Acanthaceae (Berry et al., 2023).

There are still several gaps in knowledge regarding the characterization, function, evolution, and impact of petal micromorphology in Papilionoideae and their potential effects on diversification. Although our approach focused on analyzing at least one species per genus —but for many genera exceeding this number, allowing us to observe some patterns with more confidence—a larger sample size is necessary to more securely describe patterns within clades. Careful description of both the presence or absence of sculpturing and pockets in taxonomic works can aid in understanding the distribution and function of these structures in papilionoid flowers. Currently, few taxonomic works comprehensively describe sculpturing and pockets.

Although we have significantly filled some gaps in our knowledge of sculpturing and pocketing variation, some open questions remain, particularly in an evolutionary and ecological context. These are outlined below:

(i) Estimation of evolutionary transitions. Currently, we have a favorable scenario for investigating the evolutionary aspects of characters, especially with advancements in phylogenetic studies in Papilionoideae, which have provided resolution and placement for many clades (e.g., Bello et al., 2022; Cardoso et al., 2012a, 2012b, 2013a, 2013b,

2015, 2017; Choi et al., 2022; LPWG, 2017; Queiroz et al., 2017; Ramos et al., 2016; Zhao et al., 2021). By leveraging the herein newly assembled micromorphological data with a robustly resolved Papilionoideae phylogeny, we would be able to understand how evolutionary shifts vs conservatism in wing petal sculpturing and pocketing have led to the ecological success of an important botanical group.

(ii) Empirical studies on reproductive biology and pollination ecology. Different pollinators show contrasting preferences for different types of petals, but the lack of observational tests prevents us from understanding the exact contribution of petal structures to plant-animal interactions (Fairnie et al., 2022). Several questions about the relationship between sculpturing and pockets with the pollinator are still open. For example: Does sculpturing offer only tactile cues or does it also somehow assist as a visual cue? What is the occurrence and position of osmophores in wing petals and what role do they play? Do different types of sculpturing (lamellate, lunate, lunate-lamellate) convey different types of messages to the pollinator? Is there a difference in pollination efficiency in the presence of sculpturing? Furthermore, such pollination investigations would enable us to understand the extent to which other traits on the outer surface of wing petals function similarly to sculpturing and pocketing, or whether they function synergistically when occurring together. For example, some species of *Aspalathus*, *Machaerium* and *Luetzelburgia* are known to have dense indumentum on the wing petal, a relatively rare feature among the Papilionoideae. Similarly, several genera such as *Luetzelburgia*, *Vataireopsis*, *Pterocarpus*, and *Tipuana*, have crimped petals.

(iii) Studies in corolla ontogeny and developmental genetics. Further floral ontogenetic studies and developmental genetics at gene expression level are needed to better understand the mechanisms of development of wing sculpturing and pocketing and the ease with which they can vary among clades. Identifying the genes involved in

the expression of their development and transformation would be useful for explaining their structural diversity across the Papilionoideae.

(iv) The geography and ecology of wing sculpturing and pocketing variation. Although we have mostly focused here on characterizing the morphology of wing sculpturing and pocketing across papilionoid clades, a perhaps exciting topic to explore is whether geographic and biome predilections can also explain the diversity and evolutionary shifts in wing sculpturing and pocketing variation (Stirton, 1981).

(v) Evolution of floral nanostructure. Moyroud et al. (2017) recognized that the evolution of floral nanostructures have evolved, on multiple independent occasions, and provide an effective degree of relative spatial disorder that generates a photonic signature that is highly salient to insect pollinators. Their observations suggest a fruitful new line of enquiry by trying to relate flower color to the cues provided by petal nanostructures attractive to pollinators.

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Declarations

Conflict of Interest The authors declare that there is no conflict of interest regarding the publication of this paper.

Data Availability Statement The authors confirm that all data supporting the findings of this study are available in its supplementary materials. All R scripts to reproduce the figures will be available by contacting the corresponding authors (CS, DC).

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TABLES

Table 1 Frequency of the distribution of sculpturing on the wing petals of Papilionoideae legumes. For reference on the location and position, see Fig. 1c

Location	Position	Frequency
Absent	Absent	876
Upper	Basal	211
Upper, lower	Basal	13
Upper	Basal, central left	299
Upper, lower	Basal, central left	48
Upper	Basal, central right	217
Upper, lower	Basal, central right	73
Upper	Basal, central, distal	44
Upper, lower	Basal, central, distal	52
Upper	Central	63
Upper, lower	Central	4
Upper	Central left	30
Upper	Central right	10
Upper, lower	Central right	8
Upper	Central, distal	6
Upper, lower	Central, distal	1

Table 2 Frequency of the distribution of pockets on the wing petals of Papilionoideae legumes

Location	Position	Frequency
Absent	Absent	1770
Upper	Basal	148
Upper	Basal, central left	37
Absent	Basal, central right	1
Upper	Basal, central right	38
Upper	Central	11
Upper	Central left	7

FIGURES

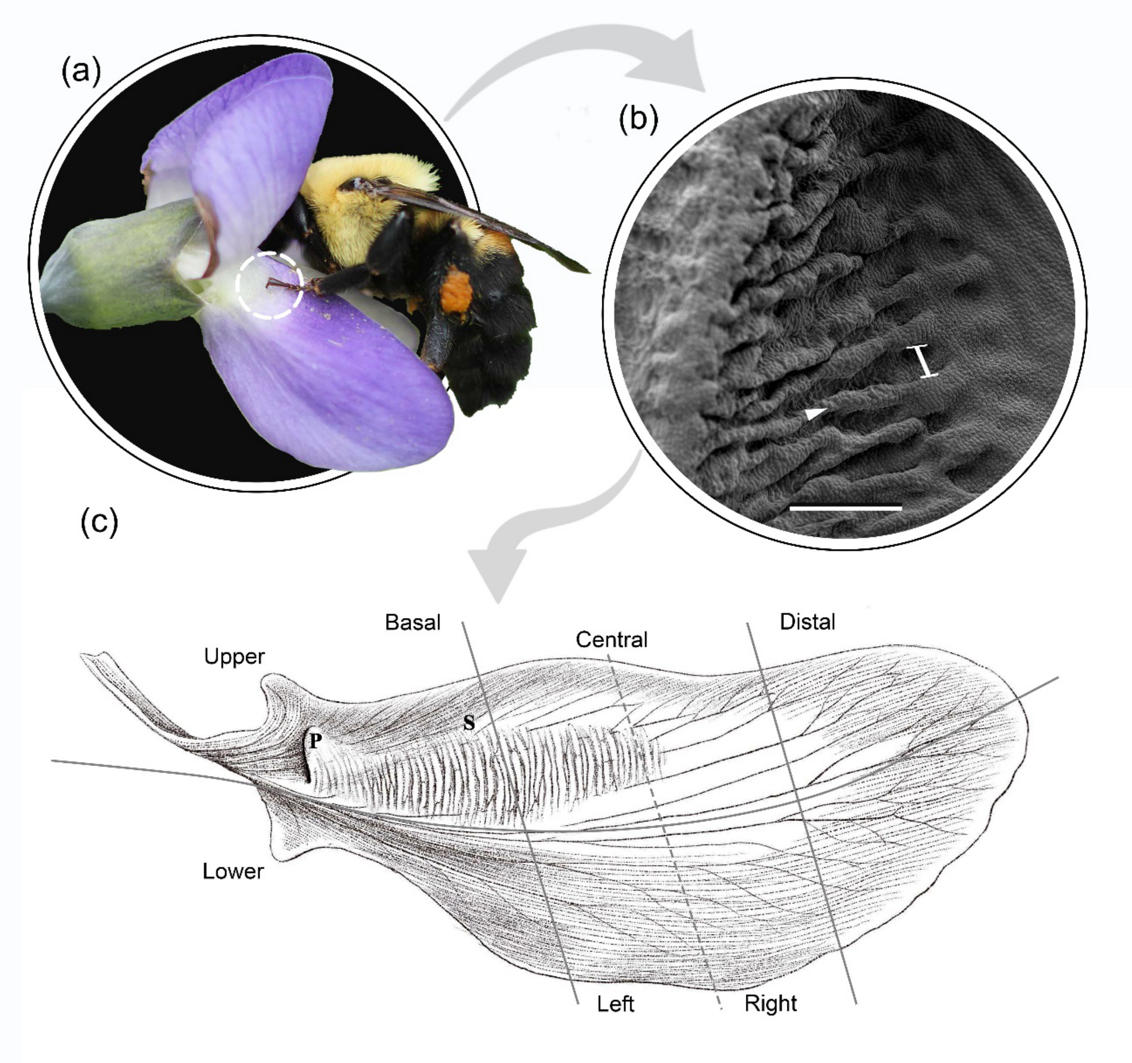


Fig. 1 Use of sculpturing in bee landing and scheme of regions and positions occupied by ornamentations. (**a**) Interaction between the pollinator and the papilionaceous flower, with a detail of the interaction between the bee's tarsal claws and the sculpturings present on the flower. (**b**) Detail of the lamellate type sculpturing found on the wing petal of *Baptisia australis*. (**c**) Schematic illustration of the terminology and observation model used to describe the sculpturing (S) and pockets (P) on the wing petals of Papilionoideae. **Legends**: arrowhead: crest of the sculpturing; *H*-bar: valley region of the sculpturings. **Scale bar** = 500 µm. The photo by Janet Davis shows a *Bombus griseocollis* bee holding the wing petal of a *Baptisia australis* flower (a). Illustration by Natanael Nascimento (c)

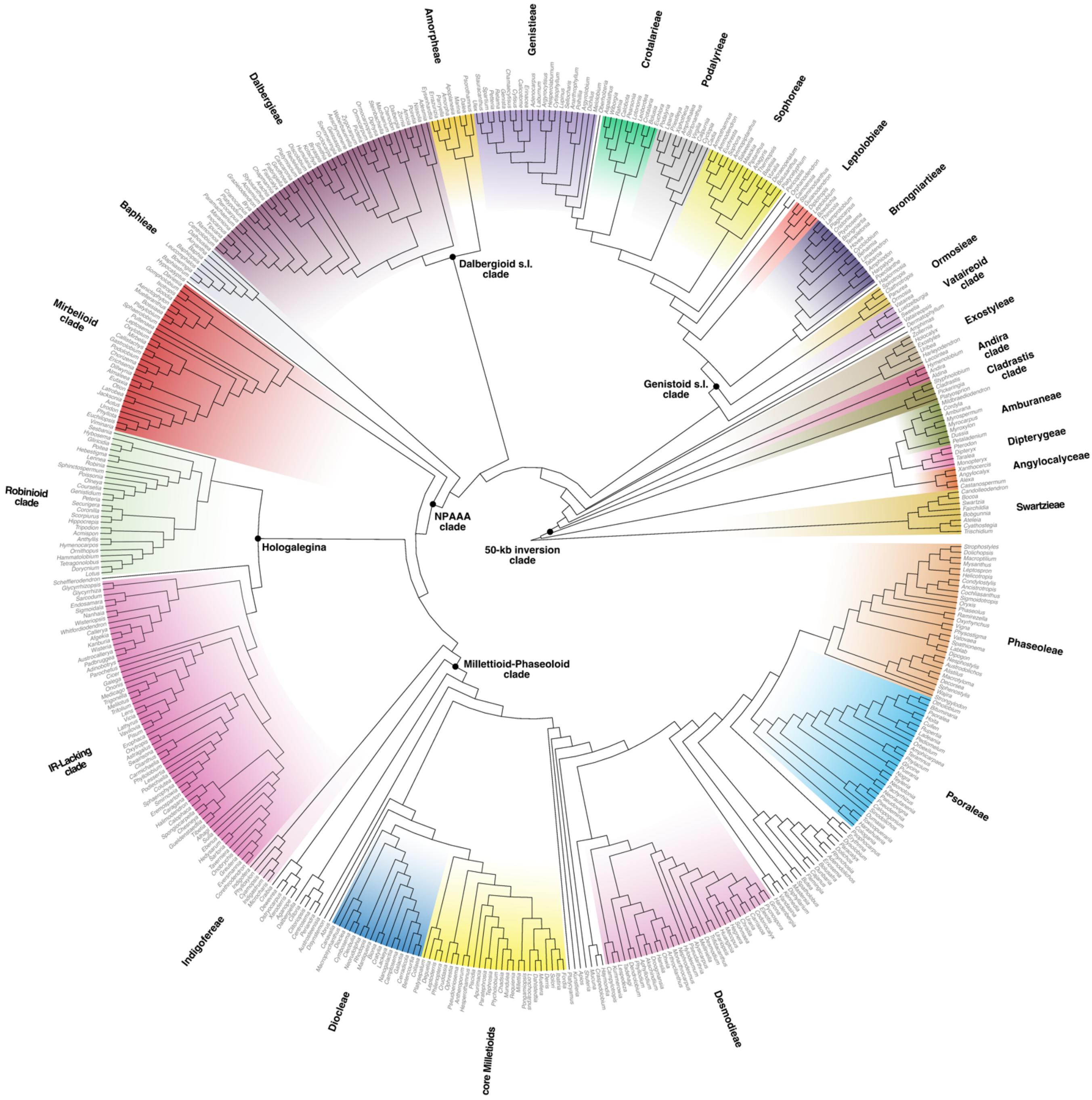


Fig. 2 Summary of the main clades and genus-level phylogenetic relationships in the Papilionoideae as derived from a maximum likelihood analysis of combined plastome and *matK* sequences, originally published by Choi et al. (2022)

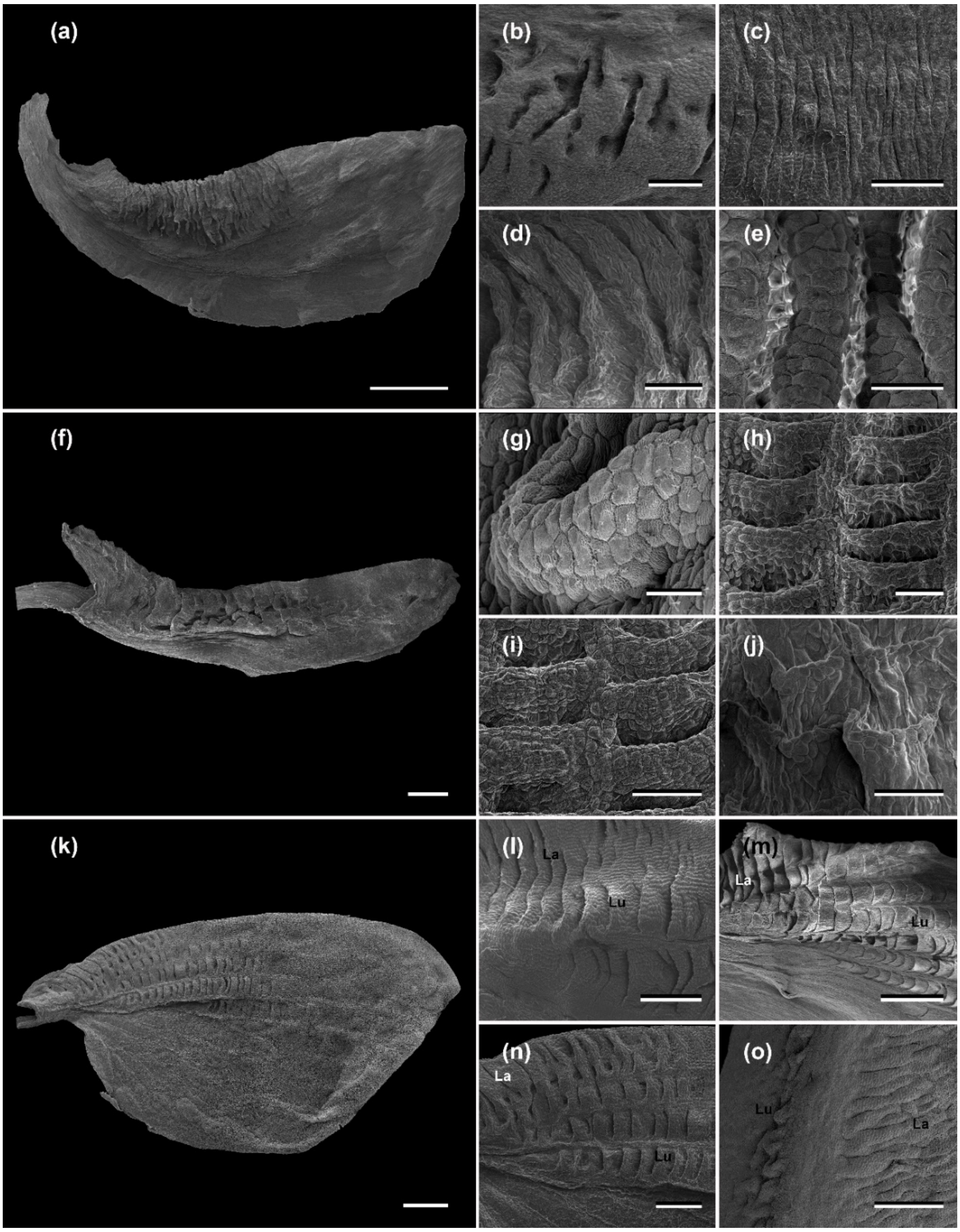


Fig. 3 Diversity of sculpturing on the wing petals of Papilionoideae. (**a-e**) Lamellate; (**f-j**) Lunate; (**k-o**) Lunate-lamellate. (**a**, **d**) Pronounced lamellae overlapping each other. (**b**) Broad lamellae, widely spaced. (**c**) Lamellae with faint ridges and shallow valleys, forming discrete lines. (**e**) Epidermal folds forming well-pronounced and closely spaced ridges with deep, barely visible valleys. (**f**, **j**) Long epidermal folds, cup-shaped, organized in two columns. (**g**) Short epidermal fold, non-overlapping, crescent-shaped. (**h-i**) Short epidermal folds, overlapping, crescent-shaped. (**k-l**, **n**) Discontinuous lunate-lamellate sculpturings, with lunate folds occupying the central region and lamellate folds near the petal margin. (**m**) Continuous lunate-lamellate epidermal folds, one following the other. (**o**) Lunate-lamellate sculpturings with different types of sculpturing occupying different regions of the petal. (**a**) *Robinia* sp. (**b**) *Luetzelburgia auriculata*. (**c**) *Cajanus cajan*. (**d**) *Pueraria montana*. (**e**) *Dipteryx odorata*. (**f**, **j**) *Pearsonia aristata*. (**g**, **o**) *Hymenolobium janeirense*. (**h**, **k**, **n**) *Zornia brasiliensis.* (**i**) *Lupinus leucophyllus*. (**l**) *Ormocarpum senoides*. (**m**) *Nissolia vincentina*. **Legend**: La = lamellate; Lu = lunate. **Bars**: (**a**, **e**) 1mm; (**b**) 125µm; (**c**, **f**, **k-m**, **o**) 500µm; (**d**, **n**) 200µm; (**g**) 50µm; (**h-j**) 100µm

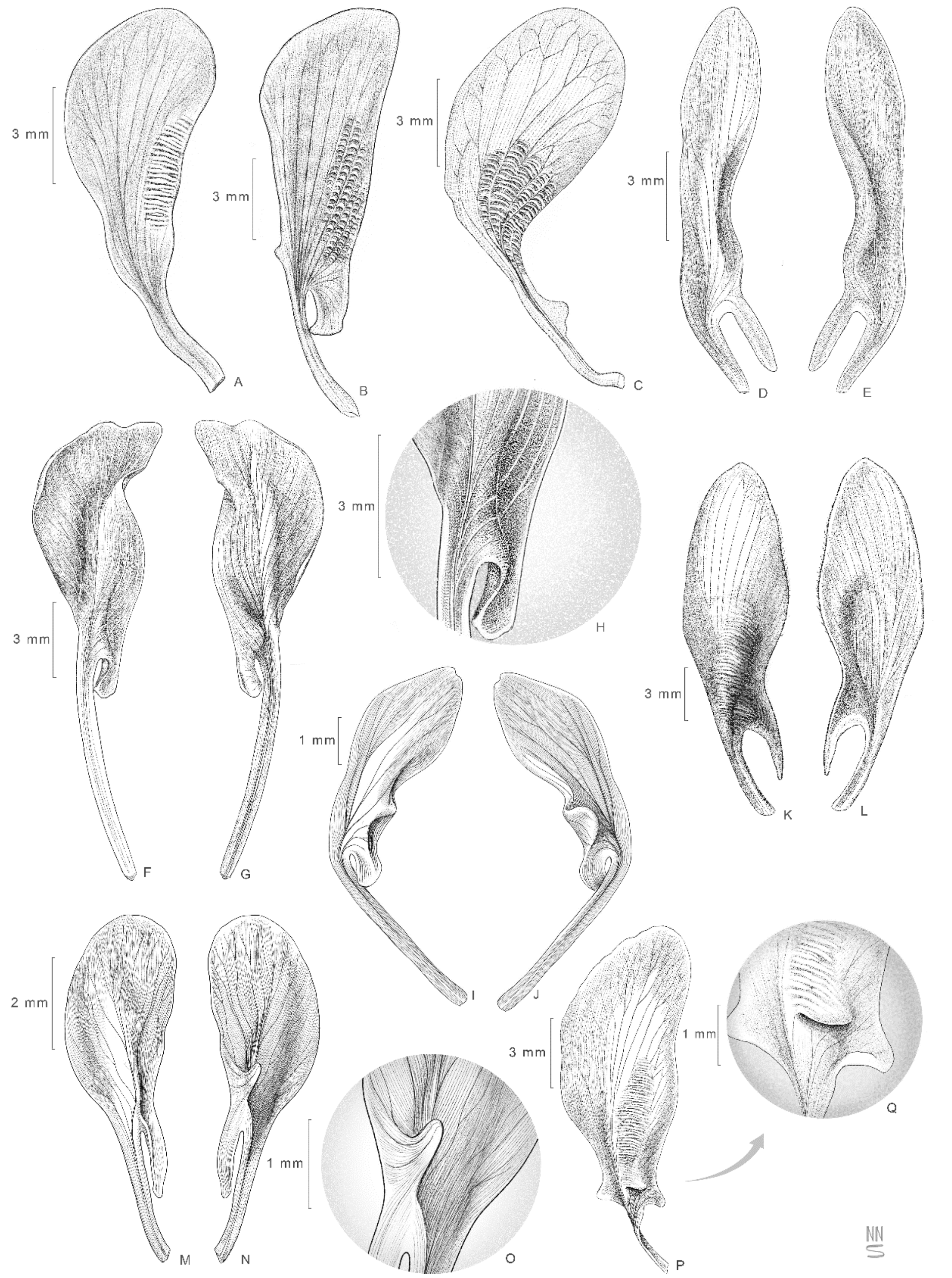


Fig. 4 Illustration of s and pockets in Papilionoideae (Leguminosae). (**a-c**) Sculpturing. (**d-q**) Pockets. (**a**) Lamellate. (**b**) Lunate. (**c**) Lunate-lamellate. (**d-h**, **k-l**) Elongate pocket. (**i-j**, **m-o**) Punctate pocket. (**p-q**) Transverse pocket. (**d-e**) Shallow elongate pocket - type *i*; (**d**) external face, (**e**) internal face. (**f-h**) Elongated pocket with a deeper point - type *ii*; (**f**) external face, (**g**) internal face, (**h**) detail of external face. (**i-j**) Punctate pocket with two concavities - type *iii*; (**i**) external face, (**j**) internal face. (**k-l**) Elongated pocket with folded edges - type *iv*. (**m-o**) Punctate pocket type *i*; (**m**) external face, (**n**) internal face, (**o**) detail of internal face. (**p-q**) Transverse pocket; (**q**) detail of the transverse pocket. (**a**) *Dolichopsis monticola*. (**b**) *Nissolia vicentina*. (**c**) D*iphysa carthagenensis*. (**d-e**) *Hedysarum alpinum*. (**f-h**) *Oxytropis besseyi*. (**i-j**) *Astragalus convallarius*. (**k-l**) *Pachyrhizus erosus*. (**m-o**) *Medicago sativa*. (**p-q**) *Cajanus cajan*. Illustration: Natanael Nascimento

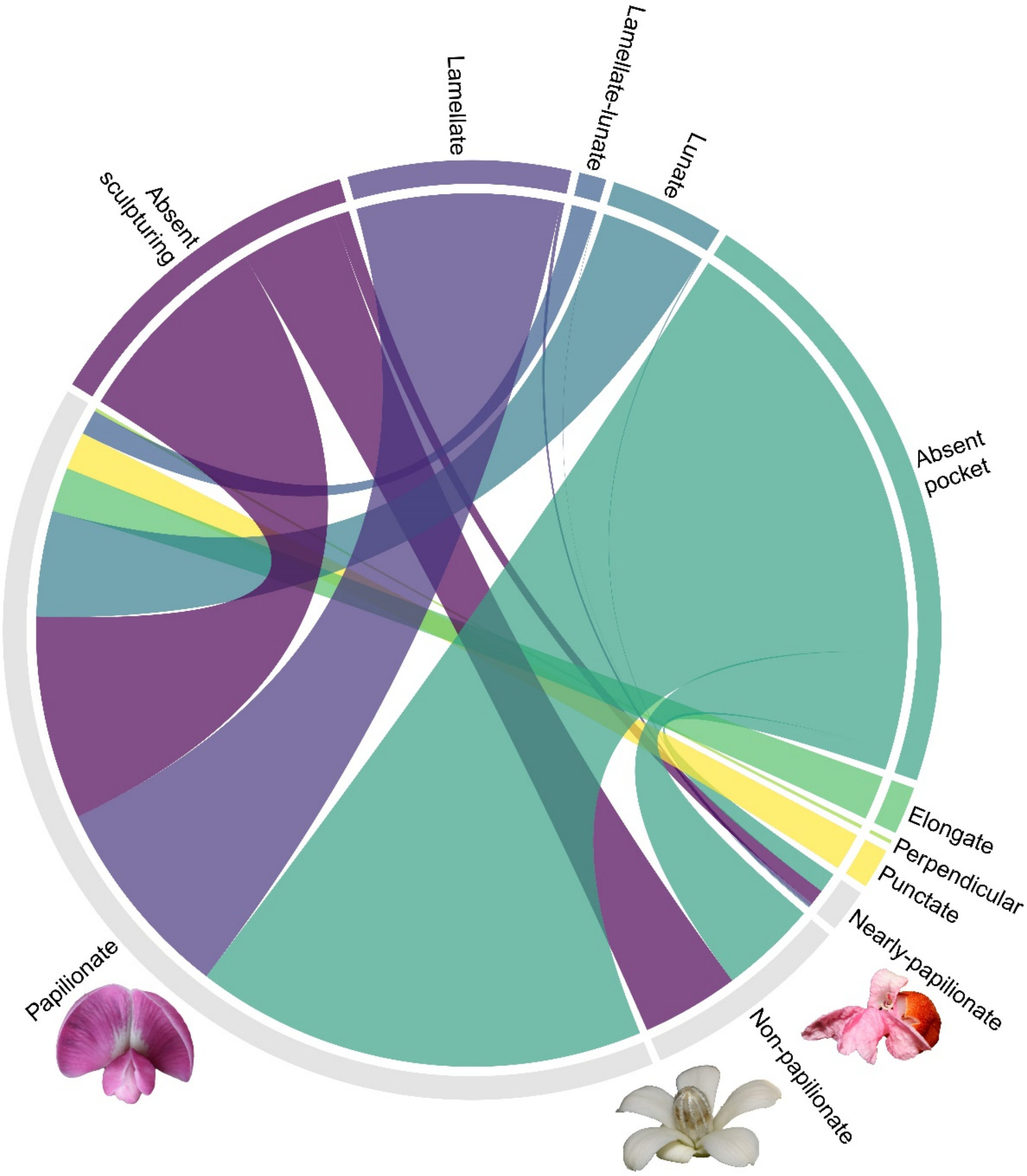


Fig. 5 Chord diagram illustrating the relationship between the different floral morphologies of Papilionoideae (Leguminosae) and the sculpturing and pockets. Colors are associated with the types of sculpturing and pockets, and the thickness of the chord represents the frequency with which each characteristic was found. Frequency values defining each chord: *Absent pocket* (nearly-papilionate = 55, non-papilionate = 264, papilionate = 1227); *Absent sculpturing* (nearly-papilionate = 41, non-papilionate = 264, papilionate = 552); *Elongate pocket* (nearly-papilionate = 0, non-papilionate = 0, papilionate = 121); *Lamellate* (nearly-papilionate = 10, non-papilionate = 0, papilionate = 553); *Lunate-lamellate* (nearly-papilionate = 1, non-papilionate = 0, papilionate = 68); *Lunate* (nearly-papilionate = 3, non-papilionate = 0, papilionate = 286); *Perpendicular pocket* (nearly-papilionate = 0, non-papilionate = 0, papilionate = 9); *Punctate pocket* (nearly-papilionate = 0, non-papilionate = 0, papilionate = 102)

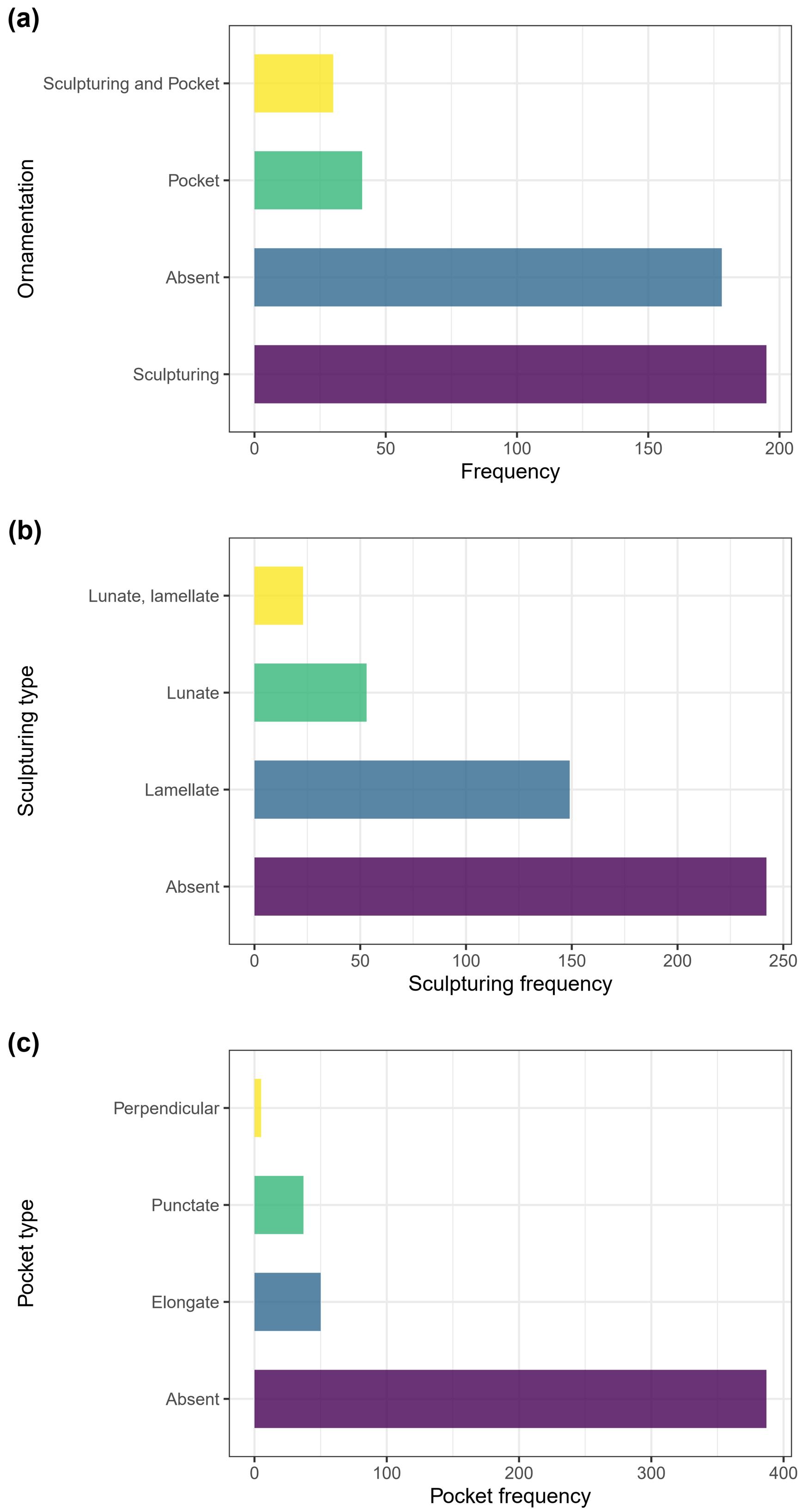


Fig. 6 Bar chart showing the frequencies of ornamentations in the genera of Papilionoideae (Leguminosae). (**a**) Frequency of occurrence and overlap of sculpturing and pockets among the analyzed genera. (**b**) Frequency of sculpturing types among the genera of Papilionoideae. (**c**) Frequency of different pocket types among the evaluated genera

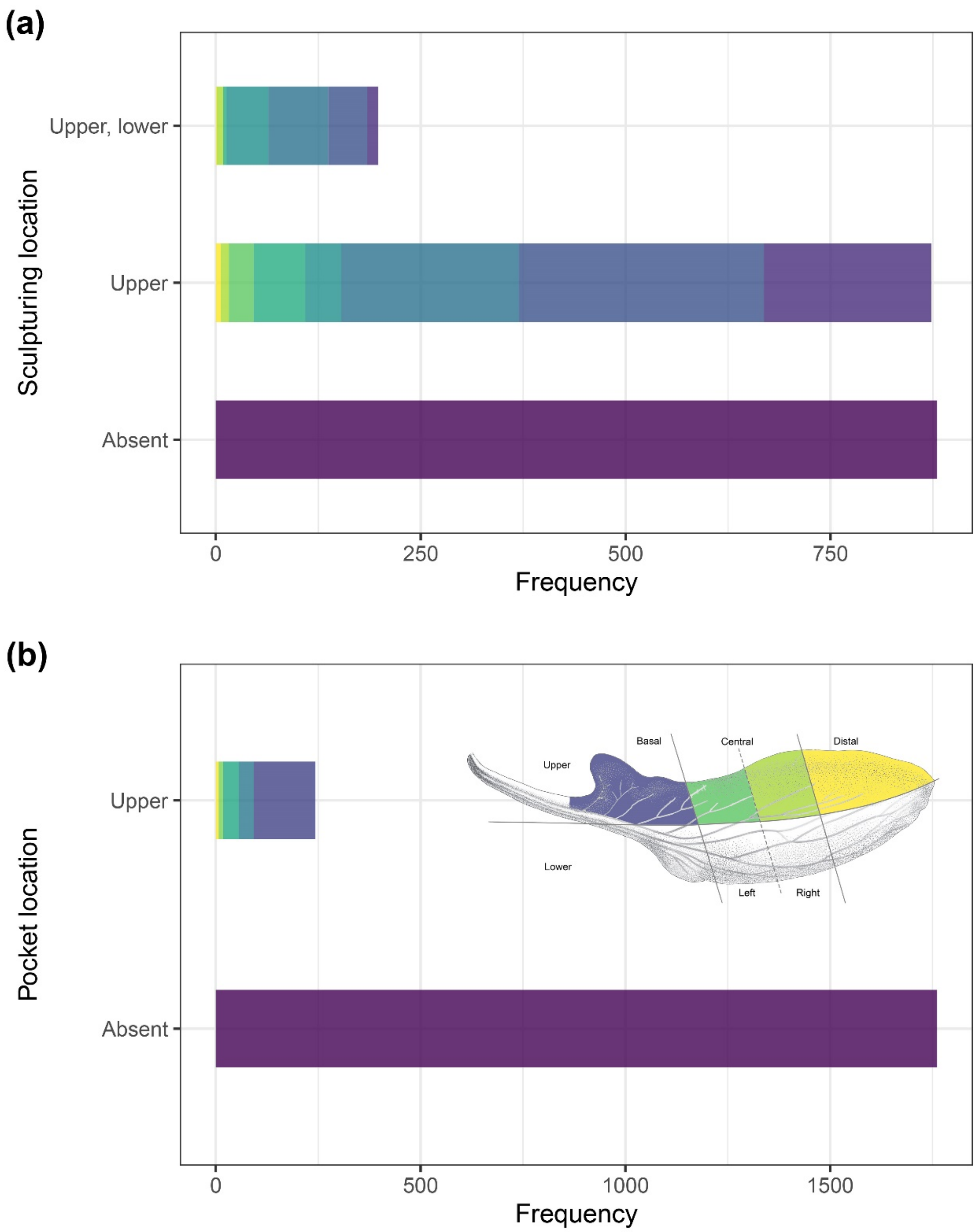


Fig. 7 Bar chart showing the frequency of occurrence of sculpturing and pockets on the wing petal. (**a**) Frequency of sculpturing locations on the wing petal. (**b**) Frequency of pocket locations on the wing petals of Papilionoideae

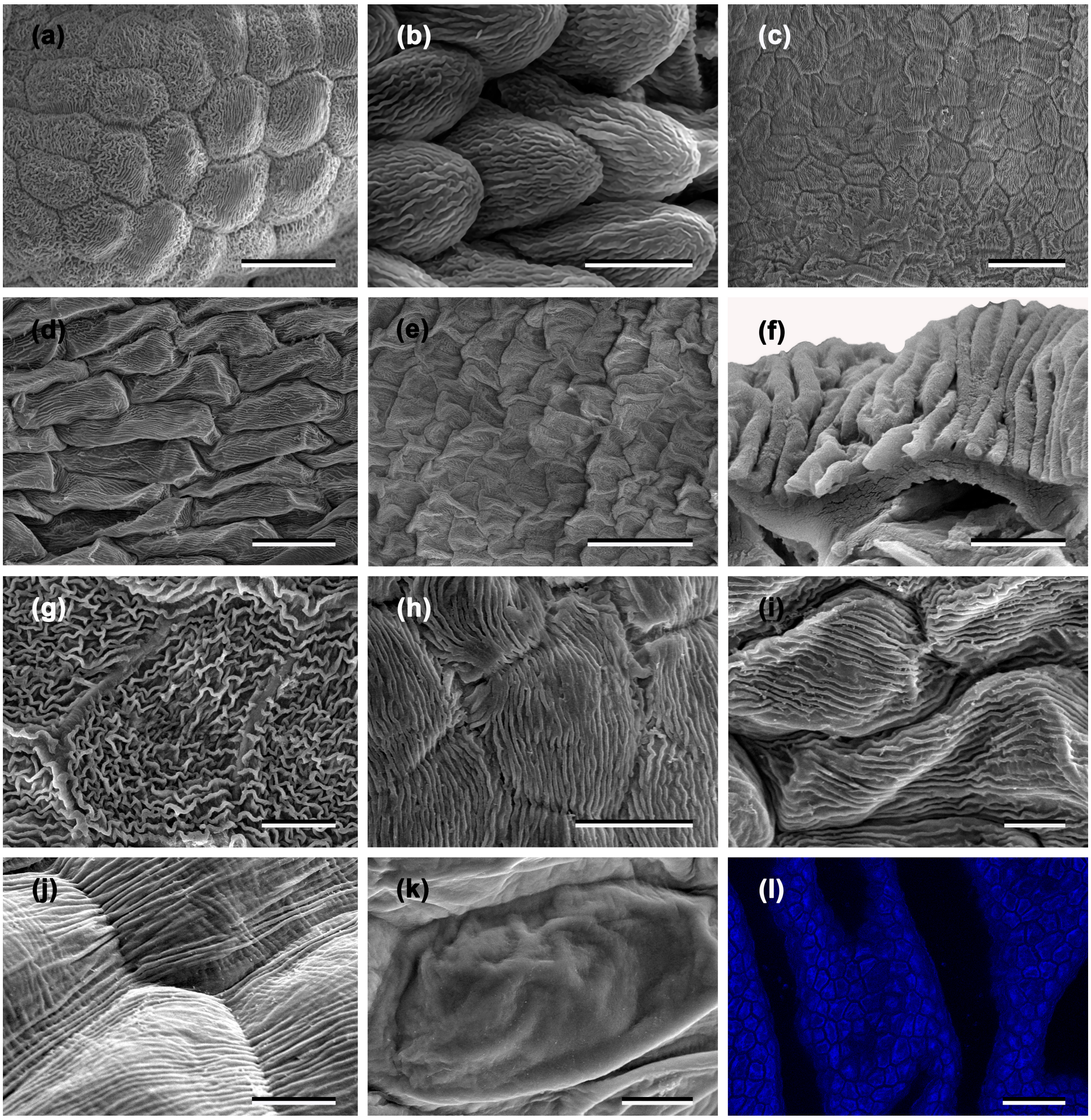
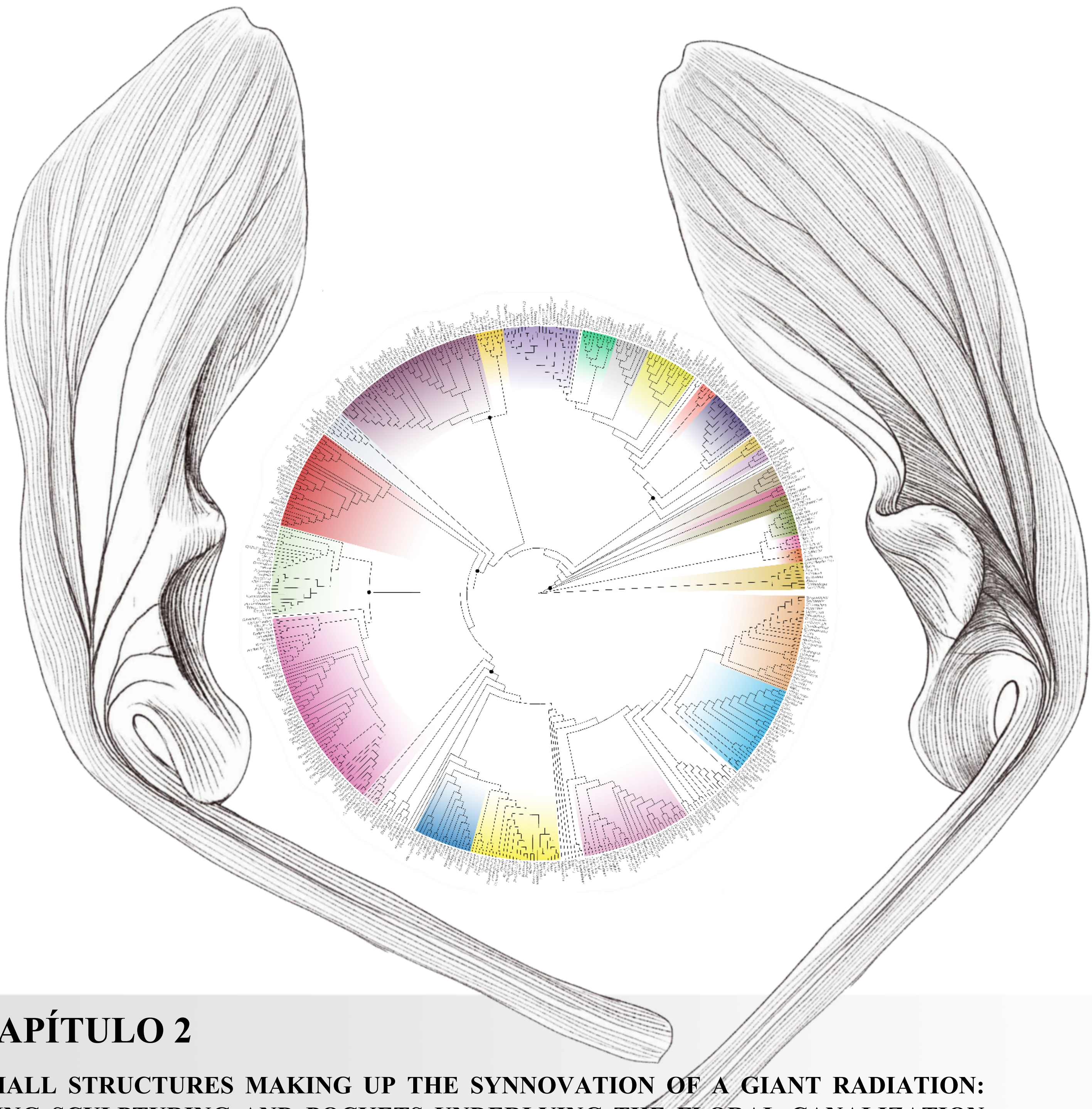


Fig. 8 Diversity of cell types and cuticular folds in the region of pockets and sculpturing on the wing petals of Papilionoideae. (**a**, **j**) Knob-like papillose cells. (**b**) Conical papillose cells. (**c**, **g**) Flat tabular cells. (**d**, **i**) Rugose tabular cells. (**e**) Stepped tabular cells. (**f**) Cross-sectional view of cuticular folds. (**a**, **c**, **g**) dense irregularly striate cuticular folds. (**b**, **h**) Parallel striate dense cuticular folds. (**i**) Parallel sparse cuticular folds. (**j**) Parallel rare cuticular folds. (**k**) Smooth cuticle. (**l**) 3D sculpturing with confocal - Leica© SP8. (**a**) *Andira fraxinifolia*. (**b**) *Zornia brasiliensis*. (**c**) *Nissolia vincentina*. (**d**, **i**) *Oxytropis sericea*. (**e**) *Lotus maritimus*. (**f**, **l**) *Luetzelburgia auriculata*. (**g**) *Laburnum alpinum*. (**h**) *Pearsonia aristata*. (**j**) *Collaea cipoensis*. (**k**) *Astragalus arizonicus*. B**ars**: (**a**, **c-d**) 50μm. (**b**, **i-k**) 10μm. (**e,** g, **l**) 100 μm. (**f**) 5μm. (**h**) 200μm

ONLINE SUPPLEMENTALS

Table S1 Dataset containing information on 2160 species of Leguminosae, with detailed descriptions of the presence and type of sculpturings and pockets on the wing petals of Papilionoideae, as well as other floral characteristics. Non-papilionate for flowers that do not have a corolla with a variable number, are absent, or undifferentiated; Nearly-papilionate for flowers with 5 free petals and mainly without differentiation (sensu Cardoso et al., 2013b); Papilionate for flowers with five petals differentiated into a dorsal standard (or vexillum), two lateral wings, and a pair of ventral keel petals (sensu Westerkamp, 1997). Available at: https://dx.doi.org/10.6084/m9.figshare.27377229

Table S2 Variation of epidermal cells and cuticular folding on the external (abaxial) surface of the wing petals in the Papilionoideae legume, as well as a comparison between regions with and without sculpturing and pockets. Comparison between regions with and without sculpturing and pockets. Available at: https://dx.doi.org/10.6084/m9.figshare.27377226



CAPÍTULO 2

SMALL STRUCTURES MAKING UP THE SYNNOVATION OF A GIANT RADIATION: WING SCULPTURING AND POCKETS UNDERLYING THE FLORAL CANALIZATION AND EVOLUTIONARY SUCCESS OF THE PAPILIONOID LEGUMES

Small structures making up the synnovation of a giant radiation: wing sculpturing and pockets underlying the floral canalization and evolutionary success of the papilionoid legumes

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Abstract

Small morphological changes in flowers can lead to significant diversification, allowing lineages to adapt to new ecological niches. The wing petals in many Papilionoideae species have conical epidermal cells and a microstructured cuticle, as well as sculpturings and pockets, which play a role in pollination mechanisms. These features help bees recognize, navigate to, and land on flowers, improving pollen collection efficiency. Here, we used a maximum likelihood approach and a robustly supported plastome phylogeny of the Papilionoideae legumes to estimate the origins and evolutionary transitions of petal sculpturing and pockets, based on the comparative analyses of wing petals of over 2000 specimens from across 1700 species and 414 genera. We established a minimum sampling of one species per genus and 3 to 5 flowers per species. We found that sculpturings and pockets arose independently at least 103 and 93 times, respectively. Lamellar sculpturing is widely distributed, whereas lunate and lunate-lamellar are restricted to a few clades. Resupination of flowers and crimped petals add complexity to the understanding of the functions and evolution of the various characteristics of Papilionoid flowers. Despite their role in plant-pollinator interactions, the distribution of the pockets is irregular, particularly in the NPAAA clade (Non-protein amino acid-accumulating). The evolution of sculpturing suggests a complex evolutionary dynamic in early papilionoid diversification. Sculpturings emerged early and concurrently with the papilionate flower, then they were lost and gained multiple times independently.
Keywords: ancestral reconstruction; Fabaceae; flower; key innovation; micromorphology; Papilionoideae.

Introduction

Diversification is central in evolutionary biology, resulting from the balance between speciation and extinctionis key to understanding how biodiversity varies (Morlon 2014; Wiens 2011; Rabosky 2017; Díaz and Malhi 2022). Various events can shape biodiversity, including the emergence or modification of a trait that allows an organism to exploit new resources or increase the efficiency with which these resources are used (Gillespie et al. 2001; Yoder et al. 2010; Vamosi and Vamosi 2011). These changes can facilitate the escape from environmental pressures, enhance individual fitness, or promote reproductive and ecological specialization (Hunter 1998; Claßen-Bockhoff et al. 2004). Such traits can open new adaptive zones, linking the emergence of a trait to adaptive radiation (Donoghue and Sanderson 2015). These morphological characteristics are known as key innovations (Sanderson and Donoghue 1994; Vamosi and Vamosi 2011; Donoghue and Sanderson 2015).

Floral morphological traits such as zygomorphic (bilateral) symmetry (Sargent 2004), nectar spurs (Wessinger and Hileman 2020), and heterostyly have been proposed as factors contributing to the diversification in different angiosperm clades (Ricklefs and Renner 1994; Endress 2006; Vamosi and Vamosi 2010, 2011; Hernández-Hernández and Wiens 2020; Wessinger and Hileman 2020). However, it has been suggested that neither geographical nor biological characteristics alone can determine diversification. Instead, certain traits or combinations of them, known as synnovation, may promote diversification within a specific geographical context (De Queiroz 2002). This concept takes into account the ecological and evolutionary context, integrating multiple biotic and abiotic factors, where two or more innovations interact, resulting in properties or functional benefit, a phenomenon known as confluence (Donoghue and Sanderson 2015).

Evolutionary innovations in flowers have favored high speciation rates and broad ecological tolerances, factors that have contributed to species diversity (Specht and Bartlett

2009; Li et al. 2019; Mohanty et al. 2022). Studies on symmetry and molecular development have indicated that bilateral symmetry is an important prerequisite for radiation to occur (Vamosi and Vamosi 2010; Endress 2016; Wessinger and Hileman 2020). Changes in floral symmetry can also lead to shifts in pollinators and specialization in pollen transfer, which may establish reproductive barriers, helping maintain heterozygosity and thus leading to speciation (Endress 1999, 2001, 2006; Wessinger and Hileman 2020). As a derived architectural trait associated with diversification, zygomorphy constitutes a key innovation (Jabbour et al. 2009).

The bilaterally symmetrical keel flowers (*sensu* Westerkamp 1997) are characterized by three distinct types of petals — standard, wings, and keel — and contain reproductive organs enclosed by the keel. These flowers typically exhibit connate floral parts, including the stamens and keel petals (Uluer et al. 2022). Found in at least 11 angiosperm families, keel flowers are particularly prominent in the Leguminosae family (Westerkamp 1997; Uluer et al. 2022), especially in the Papilionoideae subfamily, where over 70% of its members display this floral morphology (Tucker 2003), including the megadiverse genus *Astragalus*, the most species-rich of all flowering plants (Moonlight et al. 2024). Due to its high prevalence, the keel flower is commonly referred to as the papilionate flower within this subfamily (Uluer et al. 2022). Although the papilionate flower is the characteristic morphological trait of the papilionoid legumes, not all clades exhibit flowers with bilateral symmetry and clearly differentiated petals (Arroyo 1981; Crepet and Taylor 1985; Tucker 2003; Cardoso et al. 2012). There is significant diversity in flower shape, particularly in the early diverging lineages, ranging from actinomorphic (radial), pentamerous flowers with undifferentiated petals to zygomorphic flowers with slightly distinct petals, flowers without petals or restricted to the standard petal, with free and often numerous stamens (Pennington et al. 2000; Lewis et al. 2005; Cardoso et al. 2013a; LPWG 2017). This variation generally results from surprisingly uniform floral ontogenies (Tucker 2003). As the author points out, most papilionate flowers are radially symmetrical during the early to mid stages of development (Tucker, 2002). In taxa that exhibit zygomorphy at anthesis, these changes occur late in development. Taxa that remain radially symmetrical at anthesis are considered neotenous, as they lack the final developmental stages that would result in zygomorphy (Pennington et al. 2000).

Although Papilionoideae exhibit various pollination systems, such as ornithophily and chiropterophily, bee pollination is the most predominant in this subfamily (Arroyo 1981). The high specialization of the papilionate flowers (Tucker 2003; Cardoso et al. 2013a; Uluer et al. 2022) is tightly associated with bee pollination, resulting in complex mechanisms that range from precise pollen deposition on the pollinator's body to reducing pollen pilfering by non-pollinating visitors (Arroyo 1981; Uluer et al. 2022). In this context, each floral part of Papilionoideae is involved in an intricate mechanism to protect the pollen and reward only the pollinating bees. The standard petal primarily serves to attract pollinators; the keel protects stamens and pistil, and the wings function both to attract pollinators and act as levers to depress and lift the keel, while also serving as a landing platform for pollinators alongside the keel (Darwin 1858; Leppik 1966; Arroyo 1981; Stirton 1981). These functions change only when the papilionate flowers are resupinate, i.e., the flower is reversed or inverted in position, so as the standard appears upside down (Arroyo 1981) and functions as an insect landing platform.

In addition to these specialized functions, the wing petals of many Papilionoideae species exhibit additional structures. A distinctive feature of these petals is the conical epidermal cells (Ojeda et al. 2009). These cells are found in approximately 80% of angiosperm species (Kay et al. 1981; Whitney et al. 2011a, b; Kraaij and van der Kooi 2020; Wilmsen et al. 2021) and are used to define the perianth and detect homeotic transformations between petals and other floral organs (Whitney et al. 2011a). Additionally, the epidermal cells are covered by a structured cuticle with a rough appearance, located on the adaxial face of the petal, oriented towards potential pollinators (Whitney et al. 2011a). The micromorphology of the wing petal surface enhances bee foraging by providing a surface for the insect to "grip" (Moyroud and Glover 2017). The importance of these structures increases as the flower becomes more difficult to manipulate due to vertical orientation or abiotic conditions such as wind (Whitney et al. 2011b; Alcorn et al. 2012). In addition to conical epidermal cells and the microstructured cuticle, the wing petals also exhibit sculpturing and pockets (Sacramento et al. 2024, **Chapter 1**). Studies have shown that bees can recognize distinct epidermal surfaces in Asteraceae, whether from other individuals or other plant organs, by touch alone (Kevan and Lane 1985). This ability allows pollinators to orientate themselves on the petal, and thus, the combination of these structures may function as a tactile guide to the resource (Kevan and Lane 1985; Glover and Martin 1998; Whitney et al. 2011a), either pollen or nectar or both.

Small changes in form can have significant functional implications and facilitate lineages moving into a new ecological sphere, where they can diverge freely from competition with related species (Hunter 1998). The acquisition of simple structures, such as sculpturing and pockets, which have important functions for papilionate flowers, such as potential fidelity between flower and pollinator and the activation of pollination mechanisms (Stirton 1981; Hunter 1998; Amaral-Neto 2015; Aléman et al. 2022), may represent a significant evolutionary gain for these flowers. Given their functionality, these structures could represent a floral innovation, playing a significant role in their adaptive radiation. Based on this, our hypotheses were: (i) Sculpturing emerged together with papilionate flowers, being mainly present at the origin of the flower in the NPAAA (Non-protein amino acid-accumulating) clade, where the papilionate flower stabilized; (ii) Pockets will be present in clades/genera that have pollination mechanisms where the wing-keel complex needs to reset, and absent in mechanisms that can only be triggered once.

Although the evolutionary role of sculpturing and pockets is not yet fully clear, the objective of this work is to bridge the gap in micromorphological knowledge of flowers and consider their impacts on the subfamily. To this end, this study aims to understand the evolution of sculpturing and pockets by identifying the distribution of these structures throughout the phylogeny of Papilionoideae; to note how these structures are present or not in modifications of the papilionate flowers, and to determine at which point in the phylogeny sculpturing and pockets appeared on the lateral petals of Papilionoideae.

Material and Methods

***Trait and phylogenetic sampling* —** The data on wing petal ornamentations were obtained from a comprehensive sample of 2132 species of the Papilionoideae (Sacramento et al. 2024, **Chapter 1**), encompassing 445 of the nearly 500 described genera in the subfamily (LPWG 2024). We selected the following traits for ancestral estimation: (*i*) *Flower shape:* was defined as non-papilionate for flowers that do not have a corolla with a variable number, are absent, or indistinct; nearly-papilionate for flowers with 5 free petals but mainly without any differentiation (*sensu* Cardoso et al. 2013b) (Fig. 1A-B); and papilionate for flowers with five petals differentiated into a dorsal standard (or vexillum), two lateral wings, and a pair of ventral keel petals (*sensu* Westerkamp, 1997) (Fig. 1C, E). States: (non-papilionate = 0, nearly-papilionate = 1, papilionate = 2). (*ii*) *Sculpturing:* epidermal folds, usually visible on the outer surface of the petal (Fig. 1A-B) (Sacramento et al. 2024, **Chapter 1**). States: (absent = 0, present = 1). (*iii*) *Sculpturing type*: This was determined following Sacramento et al. (2024, **Chapter 1**). Absent: wing petals without sculpturing; Lamellate: for sculpturing in lines, wrinkles, or folds in the epidermis of the petals (Fig. 1D); Lunate: where the epidermal folding has a crescent-shaped format, with the crescents generally overlapping each other; Lunate-lamellate: where lunate and lamellate sculpturing occur together. States: (absent = 0, lamellate = 1, lunate = 2, lunate-lamellate = 3). (*iv*) *Pockets:* depressions, folds, or invaginations in the wing petal, observed on both sides of the petal (Fig. 1E-G) (Sacramento et al. 2024, **Chapter 1**). States: (absent = 0, present = 1).

To estimate the evolutionary transitions of flower architecture and wing petal sculpturing and pockets, we used a densely sampled, robust phylogeny of Papilionoideae, representing the phylogenetic relationships of 478 genera within the subfamily, as originally published by Choi et al. (2022). This phylogeny was derived from a maximum likelihood analysis that combined 39 fully sequenced plastomes with 478 *matK* sequences so as to cover all main clades of the papilionoid legumes. After pruning genera without available trait data and those with trait data that were not represented in the Choi et al. (2022) Papilionoideae phylogeny, the dataset analyzed for ancestral estimation included 2101 specimens from across 1700 species and 414 genera.

Ancestral state estimation **—** To estimate the ancestral trait evolution, we used the R scientific computing environment (R Core Team 2024) to perform a maximum likelihood approach implemented in the corHMM function from the corHMM package version 2.8, as described by Beaulieu et al. (2013) and Boyko and Beaulieu (2021). The corHMM function is designed to detect hidden phylogenetic factors that may influence the evolutionary processes of observed characters while controlling for phylogenetic bias. Additionally, it allows for the inclusion of terminals with unknown data (Kriebel et al. 2023), assigning probabilities to these gaps according to Felsenstein's (2004) method. This feature is particularly important for the reconstruction of nodes; however, the results will be reported based solely on the available data, with missing data being excluded from the analysis.

Evolutionary models were fitted using the fitDiscrete function from the 'geiger' package, version 2.0.11 (Pennell et al. 2014). Three distinct models were tested: the Equal Rates (ER) model, the All Rates Different (ARD) model, and the Symmetric (SYM) model. Each model was fitted individually for all analyzed traits, including flower shape, presence of sculpturing, type of sculpturing, and presence of pockets.

To determine the model that best fit the data, we used the Akaike Information Criterion corrected for small sample sizes (AICc) (Hurvich and Tsai 1989). The AICc is a widely recognized tool for model selection, allowing the comparison of complex models with a penalty for the number of parameters (Burnham and Anderson 2002). The results indicated that the ARD model provided the best fit among all tested models, as evidenced by the lowest AICc scores. This suggests that ARD, which allows for different transition rates between states, is more suitable for describing the evolution of the observed traits in this study (Table 1). The results indicated that the ARD model, which allows for different transition rates between states, provided the best fit for the following traits: sculpturing, pockets, and flower shape, among all tested models. The SYM model, which assumes that transition rates between all states are equal and symmetric, provided the best fit only for the types of sculpturing.

The trees with mapped ancestral states were plotted using R software with the ggplot2 package (Wickham 2016) and the ape package, version 5.7-1 (Paradis and Schliep 2019). For aesthetic purposes, minor adjustments were made, including color uniformity, brightness correction, and artifact removal, using Adobe Photoshop©. All edits were performed meticulously, adhering to the boundaries of the studied structures and preserving their original characteristics. The final organization of the images was also completed in Adobe Photoshop©.

Results

Evolution of flower shape **—** The ancestral flower of Papilionoideae most likely was not papilionate (Fig. 2). The early diverging clades exhibit non-papilionate flowers, and this trait is largely retained within groups such as Amburaneae, Amorpheae, Exostyleae, Leptolobieae, and Swartzieae, with few exceptions. The nearly papilionate flower shape independently emerged at least 13 times, typically from ancestors with non-papilionate flowers, as seen in Amorpheae, Angylocalyceae, the Vataireoid clade, and Leptolobieae. The Baphieae clade is unique in having a most recent common ancestor (MRCA) with nearly papilionate flowers that shifted from an ancestor with non-papilionate flowers. All members of the Baphieae clade, except *Baphiastrum*, possess nearly papilionate flowers. Additionally, there are two independent origins of this flower shape in the NPAAA clade, in the genera *Leptosema* and *Erythrina* (Fig. 2). Papilionate flowers, which are more prevalent throughout the phylogeny, arose at least 24 times independently. In the early stages of diversification (*sensu* Cardoso et al. 2013a), papilionate flowers appeared at least 16 times in genera belonging to clades with a diverse flower shape. Papilionate flowers emerged in the MRCA of Brongniartieae, Crotalarieae, Dalbergieae, Genistieae, Podalyrieae, Sophoreae, and became established at the MRCA in NPAAA clade.

Evolution of wing petal sculpturing **—** Our ancestral state estimation supports the MRCA of Papilionoideae as not possessing petal sculpturing and that it was lacking in the deep nodes of the Papilionoideae. Our results indicate that 29 genera distributed throughout the phylogeny have an intermediate likelihood (0.5 - 0.7) of developing sculpturing and that these structures arose independently at least 103 times during the evolution of Papilionoideae. Of these, 5 independent origins occurred in the MRCA of eight different clades: Crotalarieae, Dalbergieae, Genistieae, Mirbelioid, Phaseoleae, Podalyrieae, Sophoreae, as well as several unplaced NPAAA genera (Fig. 3). Within these clades, some genera exhibited a reversal to a state without sculpturing. In the Crotalarieae clade, the genus *Bolusia* displays a reversal to the ancestral state, without sculpturing, as did some species of *Aspalathus*. In the Dalbergieae clade, the genera *Acosmium*, *Geissaspi*s, *Inocarpus*, and *Riedeliella* did not present sculpturing. Additionally, seven genera within this clade showed an intermediate likelihood of absence of sculpturing (0.5), indicating variation among species (Supplementary Table S1). In the clades Genistieae and Mirbelioid, only one genus in each clade lacked sculpturing: *Sellocharis* in Genistieae and *Leptosema* in the Mirbelioids. Moreover, each of these clades displayed five genera with variation in the presence of sculpturing. In the unplaced genera of the NPAAA clade, sculpturing appeared in an ancestral member of the clade, with the genus *Dunbaria* showing a reversal to the state without sculpturing and two other genera exhibiting variation among species. In the Phaseoleae clade, a MRCA displayed sculpturing with a probability greater than 0.8. In this clade, the genera *Bituminaria*, *Cullen*, *Dumasia*, *Pseudovigna*, and *Teyleria* reverted to the ancestral state, without sculpturing, along with some species from five other genera within the clade (Fig. 3). In the Podalyrieae clade, the genera *Cadia* and *Virgilia* exhibited a reversal to the state without sculpturing, along with five genera that exhibited a moderate likelihood (0.5) of possessing sculpturing. The Sophoreae clade also had two genera with a reversion to the state without sculpturing - *Anagyris* and *Dicraeopetalum*. In this clade, only the genus *Sophora* showed an intermediate likelihood (0.5) of developing sculpturing (Fig. 3).

During the evolution of papilionate flowers, the Angylocalyceae, Exostyleae, Leptolobieae, and Swartzieae clades retained the state without sculpturing in all their sampled representatives. Additionally, the Robinioid clade is noteworthy, as all genera within it also retained the state without sculpturing (i.e., the ancestral characteristic), with the sole exception being the genus *Sesbania*, which exhibited variation between species, either having or lacking sculpturing (see Supplementary Table S1).

Lamellate sculpturing evolved independently at least 74 times during the evolution of Papilionoideae (Fig. 4), with 56 of these instances occurring within the NPAAA clade. Additionally, at least 30 genera exhibited variation among the species evaluated, with some species displaying lamellate sculpturing while others lacked any sculpturing, resulting in a moderate likelihood (0.5) of sculpturing occurring. The genus *Pterodon* is the only one to exhibit species with either lamellate or lunate sculpturing. Among the genera that displayed more than one type of sculpturing, six showed lamellate or lunate-lamellate patterns. While most instances of lamellate sculpturing originated within specific genera, there were also origins at the MRCA of the Crotalarieae, Genistieae, Sophoreae, and Podalyrieae clades, as well as at the node leading to the Mirbelioid clade.

The analysis of ancestral nodes reveals significant variations among different clades. In the Crotalarieae clade a transition from lamellate to lunate sculpturing is observed in the MRCA that gave rise to the genera *Aspalathus*, *Calobota*, *Lotononis*, *Pearsonia*, *Rafnia*, and *Wiborgia* (Fig. 4). The Genistieae clade includes the genera *Adenocarpus*, *Cytisophyllum*, and *Lupinus*, which have lunate sculpturing, while the genus *Sellocharis* has reverted to unsculpted petals. In addition to these genera, the clade also encompasses *Anarthrophyllum*, *Argyrolobium*, *Cytisus*, *Genista*, *Laburnum*, *Retama*, *Stauracanthus*, and *Ulex*, which show variation in sculpturing types among species/individuals (Supplementary Table S1). In contrast, within the Sophoreae clade, the genera *Anagyris* and *Dicraeopetalum* have reverted to unsculpted petals. Variation among species in the clade is restricted to the genera *Maackia* and *Sophora*. Furthermore, the genera *Piptanthus* and *Salweenia* exhibit independent origins of lunate sculpturing.

The Podalyrieae clade shows variation among the genera *Amphithalea*, *Calpurnia*, *Liparia*, and *Stirtonanthus*, which exhibit differences in both the type and presence of sculpturing. In contrast, the genera *Cadia*, *Cyclopia*, and *Virgilia* exhibit a reversion to the state without sculpturing. Unlike other clades, the Mirbelioid clade did not show variation in the type of sculpturing, exhibiting only lamellate sculpturing. Variation in this clade was observed through the absence of sculpturing in the genus *Leptosema* and the presence of sculpturing in some representatives of the genera *Dillwynia* and *Gastrolobium*.

Unlike lamellate sculpturing, lunate sculpturing is less distributed in the phylogeny of Papilionoideae. Lunate sculpturing independently emerged at least nine times, with seven occurrences in genera from the Cladrastis, Crotalarieae, Genistieae, and Sophora clades. Two genera, *Lotononis* and *Pterodon*, exhibited both lamellate and lunate sculpturing, each with a 0.5 likelihood. Similarly, the genus *Stirtonanthus* displayed an equal distribution between the presence of lunate sculpturing and its absence. Additionally, four genera, *Calpurnia*, *Cytisus*, *Dalea*, and *Ulex*, exhibited three-character states, each with a 0.33 probability.

Lunate sculpturing originated in one of the ancestral nodes of the Crotalarieae clade, which gave rise to the genera *Aspalathus*, *Calobota*, *Lotononis*, *Pearsonia*, *Rafnia*, and *Wiborgia*. This origin occurred within a clade whose ancestor exhibited lamellate sculpturing, as previously mentioned. The second branch of the clade, where the genera *Bolusia* and *Crotalaria* are found, shows an ancestor with the likelihood of displaying both lamellate and lunate sculpturing, with lunate sculpturing being more probable (~60%). The genera in this clade are highly diverse in terms of sculpturing types, particularly the genus *Aspalathus*, which showed significant variation in the presence and type of sculpturing, with 0.25 for each character state (Fig. 4). Stirton (1981) proposed a framework outlining potential evolutionary pathways of sculpturing in *Aspalathus*. Another origin of lunate sculpturing occurred in the ancestor of the Dalbergieae clade (Fig. 4). Within this clade, there were four reversions to the ancestral state in the genera *Acosmium*, *Geissaspis*, *Inocarpus*, and *Riedeliella*, which exhibit flowers without sculpturing. Additionally, the genera *Centrolobium*, *Cyclocarpa*, *Geoffroea*, and *Platypodium* displayed independent origins for lamellate sculpturing. The genera *Diphysa* and *Platymiscium* show a 0.5 likelihood of having lamellate and lunate-lamellate sculpturing. Similarly, *Chapmannia* and *Pictetia* have species with lunate sculpturing as well as species without sculpturing. The genera *Adesmia*, *Ctenodon*, *Poiretia*, and *Zornia* exhibit variation between lunate and lunate-lamellate sculpturing. In addition, the genera *Dalbergia*, *Nissolia*, *Pterocarpus*, and *Stylosanthes* show a 0.33 proportion of different sculpturing type combinations.

Finally, lunate-lamellate sculpturing has only one independent origin, in the genus *Grazielodendron*, which belongs to the Dalbergieae clade. However, this type of sculpturing appears in 24 genera, with proportions of 0.5, 0. 33, and 0.25 in the Amorpheae, Crotalarieae, Dalbergieae, Genistieae, Sophoreae, and Vataireoid clades (Fig. 4).

***Evolution of wing petal pocketing* —** The appearance of pockets was infrequent throughout the evolution of Papilionoideae. Excluding missing data, pockets emerged at least 29 times in a scattered and independent manner across various genera belonging to different clades. Nevertheless, we identified 64 genera with a 0.5 probability of having evolved pockets. (Supplementary Table S1). Throughout the Papilionoideae evolution, the clades Swartzieae, Cladrastis, Exostyleae, Vataireoid, Leptolobieae, and Amorpheae did not exhibit any reversal of the ancestral condition of pocket absence. The reconstruction also indicates that the MRCA of the sister clades Phaseoleae and Psoraleeae had a high probability (> 0.8) of possessing pocket, although several genera within these clades do not exhibit pockets, showing a reversion to the MRCA condition (Fig. 5). The Phaseoleae and Psoraleeae clades were the only ones where the MRCA was recovered with a high probability of having developed pockets in all phylogeny of the Papilionoideae.

Discussion

Flower shape and the evolution of wing petal sculpturing and pockets **—** The early diversification history of the Papilionoideae is characterized by various evolutionary changes in floral structure (Pennington et al. 2000; Cardoso et al. 2012, 2013a; Ramos et al. 2016). During the early stages of Papilionoideae evolution, flowers exhibit significant morphological variability, ranging from radial or polysymmetric flowers with indistinct petals to zygomorphic flowers with slightly distinct petals, which may be absent or limited to the adaxial standard petal, with often numerous free stamens (Pennington et al. 2000; Cardoso et al. 2012). It was in this context of floral diversity that the papilionate flower envolved at least 18 times before appearing in deeper nodes, such as in the ancestor of the Brongniartieae clade. Floral sculpturing and pockets are found exclusively on the wing petals of representatives of the most diverse subfamily of Leguminosae, the Papilionoideae (Stirton 1981; Alemán et al, 2017; Sacramento et al. 2024, **Chapter 1**). Although sometimes subtle and even absent in many papilionoid clades, these structures exhibit remarkable variation in form and distribution on the wing petals (Sacramento et al. 2024, **Chapter 1**). While their functional and ecological roles have not been empirically determined, these structures are known not only to contribute to the morphological diversity within the subfamily but also to be closely associated with plant-pollinator interactions (Stirton 1981; Amaral-Neto et al. 2015; Alemán et al. 2017, 2022).

The evolutionary persistence of sculpturing and pockets in Papilionoideae flowers that deviate from the typical papilionate flower, such as those exhibiting resupination, is very intriguing. Resupination is characterized by a 180º twist of the flowers during development, positioning the flower upside down (Gottsberger et al. 1988; Amaral-Neto et al. 2015; Harley et al. 2017; Cardoso et al. 2024). This phenomenon, extensively studied in orchids, also occurs in 14 other families of angiosperms (Cardoso et al. 2024), including Leguminosae subf. Papilionoideae. In particular, this subfamily includes a group of plants with resupinate flowers. Interestingly, despite the loss of the wing petals' function as a landing platform, some genera retain sculpturing and pockets on these petals. With resupination, the sculpturing on the wings also loses its function as a tactile track, as the standard petal takes over the landing platform role. Although the presence of pockets is not prominent among resupinate flowers, they remain functional in the wing and keel interaction even after the flower vertical axis has shifted through 180º, suggesting a role for these structures in the pollination mechanism, as explained by Amaral-Neto et al. (2015). Notably, of the 18 studied genera with resupinate flowers, only *Andira*, *Coursetia*, and *Platycyamus* exhibited lamellate sculpturing in all analyzed species. The genus *Hymenolobium*, along with the genus *Liparia*, showed interspecific variation. It is worth noting that the MRCA of the genera with resupinate flowers and sculpturing did not exhibit sculpturing, which independently emerged in these three genera. *Bolusia* was the only genus with resupinate flowers whose MRCA exhibited sculpturing, indicating an evolutionary loss of sculpturing (Figs. 3 and 4). Identifying the moment when resupination emerged in the evolution of Papilionoideae may help understand whether the sculpturing was maintained as a deep homology (*sensu* Scotland 2010) or if it arose after the floral twist and the loss of its original function. The maintenance of sculpturing in resupinate flowers could also be explained if, in addition to providing tactile cues to pollinators, the sculpturing also serves other functions, such as acting as visual cues.

Stirton (1981) suggested that sculpturing might serve as a support mechanism for pollination due to the formation of epidermal folds in papilionate flowers. Although flowers with crimped petals do not exhibit these epidermal folds, the crimping mimics the valleys and ridges created by the epidermal sculpturing (Fig. 1A-B). The folds formed by these 'wrinkled' petals can be more accurately compared to those observed by Berry et al. (2023) in *Ruellia* (Acanthaceae), as they involve all petal tissues and are visible on both surfaces of the petal, in contrast to sculpturing, which is generally only visible on the abaxial surface

(Sacramento et al. 2024, **Chapter 1**). The Genistoid genus *Camoensia*, as well as the clade Leptolobieae, which includes genera with emblematic crimped petals, such as *Bowdichia* and *Diplotropis*, did not exhibit sculpturing or pockets. In contrast, genera such as *Luetzelburgia* and *Platypodium* that have flowers with crimped petals displayed lamellate sculpturing. Although the lamellate sculpturing here has a simpler appearance, with few lines distributed across the petals. (Figs. 3 and 4). Although less common, pockets are also observed in flowers with crimped petals (some examples include *Leptosema*, *Lotus*, *Platypodium*, *Pterocarpus*, *Tipuana*, and *Vicia*).

Evolution of wing sculpturing across papilionoid clades — The papilionate flower independently arose 17 times in the early diversification clades: ADA, Andira, Cladrastis, Exostyleae, Ormosieae, Swartzieae, and the Vataireoids. In these same clades, sculpturing also emerged independently in 10 genera. In all these instances, the emergence of sculpturing occurred alongside the evolution of the papilionate flower. The simultaneous emergence of sculpturing and papilionate flowers can also be observed in the MRCA of the early diversification clades that gave rise to the Crotalarieae, Dalbergieae, Genistieae, Podalyrieae, and Sophoreae (Figs. 1 and 2). This co-occurrence suggests a possible evolutionary synergy or a requirement that drove the correlated evolution of these traits. For clades such as Brongniartieae and most of the NPAAA clade, our reconstruction did not recover the presence of sculpturing in deep ancestral nodes, except for the Mirbelioid and Phaseoleae clades, whose MRCA exhibited sculpturing.

Initially, our hypothesis suggested that sculpturing could have played a crucial role in the fixation of papilionate flowers, especially in the NPAAA clade, where the highest species diversity within the subfamily is found (Wojciechowski et al. 2004; Cardoso et al. 2012). It is in these groups that the papilionate flower architecture appears to have become evolutionarily fixed and more specialized (Pennington et al. 2000; Cardoso et al. 2013a). However, our ancestral estimation did not provide evidence to support this relationship for this clade. These findings suggest that other factors may have been decisive in the evolution of papilionate flowers. Additionally, the sampling did not recover data from the deep nodes of the sculpturing in the NPAAA clade, which may be a consequence of sampling limitations. To overcome this limitation, it is essential to expand the database and include information on the as yet unsampled genera. This expansion will enable a more robust and detailed analysis, providing greater clarity on the distribution and evolution of the sculpturing throughout evolutionary history of the clade.

During the early diversification phase, the floral morphology of Papilionoideae exhibited high lability (Choi et al. 2022), accompanied by a low frequency of sculpturing. Our reconstruction confirms the observation made by Sacramento et al. (2024, **Chapter 1**), which states that, in addition to sculpturing being absent in non-papilionate flowers (Stirton 1981), it is also less frequent in nearly-papilionate flowers, which predominantly have non-papilionate-flowered ancestors.

The absence of sculpturing observed as a reversion in the MRCA is often associated with changes in pollinators, as seen in *Anagyris*, a genus recognized as ornithophilous (Valtueña et al. 2007; Ortega-Olivencia and Catalán 2009), or in the genus *Alexa*, which is bat-pollinated (Cardoso et al. 2012, 2013a). It can also result from changes in flower shape, as found in *Dicraeopetalum* and *Cadia*, with their radial flowers (Citerne et al. 2010; Cardoso et al. 2013a). Changes in pollinators have a significant impact on floral morphology (Uluer et al. 2022), influencing the evolution of floral traits such as flower color and size, petal size and orientation, nectar composition and quantity, and petal micromorphology (Ojeda et al. 2013, 2016). Since sculpturing seems to be associated with bee pollination (Stirton 1981; Etcheverry et al. 2008; Alemán et al. 2014, 2017), changes in this system may also impact the floral morphology of Papilionoideae, similar to the effects on 25% of angiosperms (Wyatt 1982; Uluer et al. 2022).

The evolution of sculpturing types across the Papilionoideae shows significant lability, to the extent that few clades can be characterized by a specific type of sculpturing, as the genera exhibit interchangeable types. The clades Agylocalyceae, Exostyleae, Leptolobieae, and Swartzieae are notable for the absence of sculpturing in their representatives. Surprisingly, all the genera within the NPAAA clade that exhibited sculpturing had the lamellate type. However, since not all genera in this clade display sculpturing and some have not yet been evaluated, we cannot define the clade as exclusively possessing lamellate sculpturing. Finally, lunate-lamellate sculpturing appears in the reconstruction only alongside lamellate or lunate, suggesting that lunate-lamellate sculpturing might represent an intermediate state rather than a distinct type of sculpturing. In our analyses, the only exception was the genus *Grazielodendron*, which showed only lunate-lamellate sculpturing. However, only one individual was observed for this genus. Intraspecific variation was reported in other genera by Sacramento et al. (2024, **Chapter 1**), thus increasing the number of individuals, even from the same species, can help confirm the more frequent type of sculpturing for the genus. It has been noted by McMahon and Hufford (2005) and Berry et al. (2023) that the sculpturing of some species emerges only in the final stages of floral development, and, therefore, flowers with lunate-lamellate sculpturing might indicate that the flower is still developing. In this case, the most developed type of sculpturing observed would be the predominant sculpturing type.

The function of sculpturing — The characteristics of plant surfaces play crucial roles in various biological functions. However, many of these assignments are based on hypotheses that still need to be experimentally tested (Riglet et al. 2021). This is particularly true in the case of the sculpturing and pockets of the Papilionoideae. There is a lack of empirical experiments regarding the function(s) performed by these structures, as well as the impact of these structures on the reproductive biology of the Papilionoideae. Although there is no experimental data , there seems to be general agreement that the sculpturing is related to the landing of insects, especially bees (Alemán et al. 2017, 2022; Arroyo 1981; Etcheverry 2001; Etcheverry et al. 2003, 2008; Stirton 1981; Tucker 2003). Other functions attributed to sculpturing include providing greater resistance to the wing petals in asymmetric flowers. The sculpturing in this region makes petals thicker, suggesting an additional function of reinforcement or support. In these flowers, due to the position the sculptures occupy, they also appear to serve as guides to the nectar (Etcheverry 2001; Etcheverry et al. 2003, 2008).

The properties of a surface are rarely determined by a single character but more likely arise from the combination of three-dimensional features at different scales (Riglet et al. 2021). As there are still few studies exploring the function of sculpturing beyond serving as a structure for pollinating insects to grip, we speculate (based on the literature), that a parallel can be drawn between the functions of sculpturing and conical epidermal cells. Both structures are thought to provide tactile cues and/or mediate the holding potential of an insect when landing on a flower (Kraaij & van der Kooi 2020).

Contrary to the sculpturing and pockets found in Papilionoideae, the papillose or conical cells have been extensively studied in recent years (e.g., Alcorn et al. 2012; Kay et al. 1981; Ojeda et al. 2013; Reed et al. 2022; Whitney et al. 2009a, 2009b, 2011a, 2011b; Wilmsen et al. 2021). Part of the advancement in the study of papillose or conical cells is explained by the widespread occurrence of these structures in angiosperms (Kay et al. 1981; Whitney et al. 2011a). In contrast, sculpturing and pockets are confined to the wing petals of Papilionoideae. Curiously, initial studies of conical papillose cells, as well as sculpturing and pockets, pointed to similar roles: providing tactile signals and/or mediating insect holding capacity when landing on the flower (Kevan and Lane 1985; Kraaij and van der Kooi 2020; Stirton 1981). We, therefore, combine our observations on sculpturing with data available in the literature to discuss some similarities between these different structures.

The role of conical papillose cells has already been described and empirically tested by several authors, and their function during the landing of pollinators, especially bees, is well established (Alcorn et al. 2012; Costa et al. 2017; Kevan and Lane 1985; Song et al. 2020; Whitney et al. 2009b, 2011a). Stirton (1981) suggested that sculpturing would serve as a support point for pollination, an idea corroborated by several authors (e.g., Alemán et al. 2022; Arroyo 1981; Etcheverry 2001; Etcheverry et al. 2008) or based on field observations (e.g., Etcheverry 2001), although no rigorous scientific experiment has been published to date. Berry et al. (2023) concluded, as had Stirton (1981), that for funnel-shaped or tubular flowers of Acanthaceae (species of *Neuracanthus* and *Ruelliea*), 'petal folds' would provide a better grip surface for the pollinator, an idea previously discussed by Hawkeswood and Sommung (2016) and Tripp and Manos (2008). The sculpturing in Papilionoideae, the petal folds in Acanthaceae, and the conical papillose epidermal cells, in general, are present in the petal region that serves as a landing platform for bees (Alemán et al. 2017; Arroyo 1981; Berry et al. 2023; Stirton 1981; Whitney et al. 2009a, 2011a). Although at different levels of organization (tissue and cells) and structurally different, these floral features in various groups may represent convergent floral adaptations for specific functional groups of pollinators, especially bees. The convergent floral adaptations are explained by the fact that functional parts can evolve independently along different pathways and at different rates in response to pollinator-mediated selection (Dellinger 2020).

Papilionate flowers have conical papillose epidermal cells (Bailes and Glover 2018; Kay et al. 1981); more than that, papillose cells are distinctive features of petal structure, as reported by Ojeda et al. (2009). The presence of sculpturing on wing petals reinforces that these structures are related to insect landing and petal holding. Papilionate flowers require strong, agile, and specialized pollinators capable of manipulating the flower, triggering pollination mechanisms, and accessing rewards (Amaral-Neto et al. 2015; Etcheverry and Vogel 2017; Westerkamp and Weber 1999).

The sculpturing might also function in roles still unknown in the flower-pollinator relationship. One of these possible functions is to provide visual cues, in a similar way e to the conical papillose cells (Reed et al. 2022; Whitney et al. 2009a, 2011a; Wilmsen et al. 2021). The sculpturing is formed by epidermal folding, and the mesophyll of these structures has spaces filled with air (aerenchyma) (Etcheverry 2001; Leite et al. 2014). Regions of the petal with a similar organization to that of the sculpturing create a difference in the refractive indices of the petal tissues and therefore a strong reflection and dispersion of light, contributing to the shiny appearance of the petals (Cavallini-Speisser et al. 2021; Kay et al. 1981; van der Kooi and Stavenga 2019). The color patterns on the petals are not only the result of pigment production. They are also influenced by the position of these pigments in the mesophyll or even in the epidermis, by the variation in the shape and texture of the cells, both on the surface and inside the petal. All these variables affect the path of light, potentially altering the visual appearance of the petal (Fairnie et al. 2022; Kay et al. 1981). Just like conical papillose cells, sculpturing can function as multi-sensory billboards, providing visual and tactile cues for pollinators before and after landing (Whitney et al. 2011b). As bees also learn from visual cues (Alcorn et al. 2012), allowing them to identify the target from a distance, sculpturing would provide an advantage for both the insect, reducing energy expenditure during foraging, and the plant, ensuring visibility and selection from afar.

In recent years, studies on conical papillose epidermal cells have advanced significantly, revealing their role in the reproductive success of plants. We predict that future research on the floral micromorphology of Papilionoideae will point towards other possible functions for the sculpturing of the wing petals of papilionate flowers.

Evolution of wing pocketing across papilionoid clades **—** The low occurrence of pockets among the species analyzed was reflected in the reconstruction of this trait in the phylogeny. Similar to sculpturing, pockets are present in papilionate flowers and, though infrequent, are also observed in some genera with nearly-papilionate flowers (Fig. 5). The occurrence of pockets in 93 genera, arising independently, suggests a high lability of this trait. This pattern of independent emergence across multiple clades is further supported by the fact that, of these 93 genera, 64 exhibit both intraspecific and interspecific variation. Surprisingly, the Psoraleeae and Phaseoleae clades have a MRCA with the presence of pockets with a likelihood greater than 0.8, making this the only deep node recovered in our reconstruction for the pockets. These clades share a common ancestor where pockets evolved, as well as a similar distribution (Li et al. 2013; Bello et al. 2022). Some representatives of Phaseoleae have flowers that differ from the typical papilionate flower. Certain representatives of this clade have strongly asymmetrical flowers with highly modified petals, showing strong associations between the wings and keel, and a narrow beak-shaped tube to the keel causing it to coil (Etcheverry et al. 2008). Asymmetrical flowers are effective and precise in placing and retrieving pollen on the bodies of pollinators (Westerkamp 1993; Etcheverry 2001; Etcheverry et al. 2008; Etcheverry and Vogel 2017). Genera in the Phaseoleae clade that have asymmetrical flowers a, such as *Vigna* and *Macroptilium*, have lost pockets during evolution. In contrast, some species of the genus *Phaseolus*, which have asymmetrical flowers maintain a reproductive system involving pollinators, retain pockets (Fig. 5) (Etcheverry et al. 2008; Etcheverry and Vogel 2017). Representatives of the Psoraleeae clade are widely distributed geographically, mainly in temperate biomes, and their habits vary from herbs to small trees, with flowers predominantly blue or purple (Bello et al. 2022). Similar to the Phaseoleae clade, the Psoraleeae clade also includes genera with cleistogamous species, such as the genus *Cullen*, on which there were no pockets in the individuals analyzed. Additionally, species of this genus have a wing-keel relationship that always includes some degree of petal fusion, just above the petals claws and dorsal to the auricle of the wings (Grimes 1997). The presence of structures that assist partial petal fusion between the wings and the keel petals could lead to pockets being functionally dispensable. Given that both clades, Phaseoleae and Psoraleeae, exhibit significant floral morphological diversity and some ecological similarities (Li et al. 2013; Bello et al. 2022), a detailed genus-by-genus analysis of these sister clades would be valuable for helping to understand the emergence of pockets and reversions to a non-pocket state. These clades could serve as excellent models for studying the origin and variation of pockets. In this study, the frequency of pockets was significantly less than that of sculpturing, albeit with variation observed among the studied genera (Sacramento et al. 2024, **Chapter 1**). It is important to determine the origin of this variability, the timing of pocket emergence, and whether any of the pocket types characterized by Sacramento et al. (2024, **Chapter 1**) exhibit phenotypic variation. Increased sampling would provide a better understanding of the origins and evolution of pockets.

Floral sculpturing and pockets, though often subtle in appearance, play important roles in the morphology and ecology of Papilionoideae flowers. These structures not only contribute to the morphological diversity within the subfamily but also seem to be closely linked to plant-pollinator interactions, suggesting specific adaptive functions. In the following paragraphs we will explore the potential functions of these features.

The function of pockets — Although there is still some confusion regarding the terminology (e.g., Alemán et al. 2022; Bailes and Glover 2018), pockets are structures distinct from petal sculpturing, differing both in their shape and structure, as described above. Pockets also seem to play a crucial role in the reproductive success of papilionate flowers. Because they are often located near the petal margins, their role was initially thought to provide some support during the landing of visiting insects; however, some studies suggest different functions.

A notable distinction between sculpturing and pockets is that the latter tend to have flat-shaped epidermal cells. These cells do not exhibit the same efficiency in light reflection from the mesophyll as conical papillose cells do, and probably for this reason, flat epidermal cells are not as common in petals as conical papillose cells. When present, they are usually in regions less visible to insects (Glover and Martin 1998; Kraaij and van der Kooi 2020; Kay et al. 1981). As is the case with some pockets, their position on the petals and their peculiar shape makes them less visible. However, the flat cells in the pockets appear to be a response not only to their location on the petal but also to the pollination mechanisms (Alemán et al. 2022; Amaral-Neto et al. 2015; Bailes and Glover 2018). Bailes and Glover (2018) identified stepped tabular striate cells around what they termed 'petal folds' (pocket) of the wing, suggesting that the straighter shape of the cells may be related to the pollination mechanism of *Vicia faba*, facilitating 'sliding' between the wing and keel petals.

Papilionate flowers are often described as complex flowers (Arroyo 1981; Etcheverry 2001; Etcheverry et al. 2008; Etcheverry and Vogel 2017). The morphological diversity of these flowers is highly integrated for successful pollination (Itagaki et al. 2020). The differentiated petals perform functions that are interchangeable only in the case of resupinate flowers (Arroyo 1981). This entire architecture is thought to have evolved as a means of protecting nectar and pollen (Arroyo 1981; Amaral-Neto et al. 2015). This complexity is attributed, for example, to bilaterally symmetrical (zygomorphic) floral symmetry (Arroyo 1981; Endress 2001; Etcheverry and Vogel 2017; Harley et al. 2017; Itagaki et al. 2020) and to highly elaborate petals that form a complex floral plane (Leppik 1966; Arroyo 1981;

Alemán et al. 2022). In addition to the floral morphological diversity exhibited by papilionate flowers, these flowers display distinct pollination mechanisms (Arroyo 1981; Etcheverry et al. 2008; Alemán et al. 2014, 2022; Etcheverry and Vogel 2017). These arose due to differences in floral architecture, such as size, shape, color, number, arrangement, ability to secrete nectar, and produce scents (Darwin 1858; Leppik 1966; Arroyo 1981; Stirton 1981; Etcheverry et al. 2008). Through highly specialized mechanisms, papilionate flowers ensure an efficient deposition on stigmas and reception from anthers of pollen grains (Westerkamp 1997; Etcheverry et al. 2008).

Four different types of pollination mechanisms are recognized, each related to a special floral architecture: brush, valvular, pump, and explosive (Arroyo 1981; Stirton 1981; Alemán et al. 2014, 2022; Etcheverry and Vogel 2017). The pollination mechanism 'brush type', characterized by secondary pollen presentation, where the stigma and the pollen-laden stylar brush emerge from the keel in response to the insect’s ventral landing on the flower. In this way, pollen donation and reception are concentrated in the same general location (Westerkamp 1997; Etcheverry et al. 2008; Etcheverry and Vogel 2017; Alemán et al. 2022). The valvular mechanism allows the keel and wings to be entirely free or moderately connected (Arroyo 1981). In this mechanism, the pollinator moves over the wing-keel complex, allowing the stamens and stigma to touch its body; when the pollinator leaves the flower, the perianth parts return to their original position, ready for a new insect visit (Alemán et al. 2022). In the pump mechanism, pollen is expelled through an opening at the tip of the keel and pushed out by the anthers and the style, releasing small quantities of pollen with each pollinator visit (Arroyo 1981; Alemán et al. 2022). Finally, in the explosive mechanism, the staminal column and stigma emerge abruptly from the keel when it is pressed, and the pollen is sprayed over a large part of the insect's body (Arroyo 1981).

The valvular, pump, and brush mechanisms require multiple visits to remove all the pollen (Etcheverry et al. 2012; Alemán et al. 2022). In contrast, in the explosive mechanism, due to its characteristics described above, nearly all the pollen is released in a single visit, and the flower generally does not receive more visits because the mechanism cannot be triggered again (Galloni et al. 2008; Alemán et al. 2014, 2022). Connections between floral parts are present in many Papilionoideae species (Arroyo 1981; Westerkamp 1997; Westerkamp and Weber 1999; Alemán et al. 2022). In this study, we have focused on the connection between the wings and keel petals mediated by the presence of pockets. For some pollination mechanisms the pockets provide the petals with a connection that is strong enough and, at the same time, flexible enough to allow the temporary displacement of the petals while the insect moves within the flower, allowing the petals to return to their initial position, leaving the flower ready to receive other pollinators (Alemán et al. 2022; Amaral-Neto et al. 2015). The connections between floral parts are common in many species of Papilionoideae, such as the interactions between wings and keels facilitated by auricles and claws (Alemán et al. 2022; Arroyo 1981; Westerkamp 1997). The wing and keel interaction plays an important role in pollination, allowing pollinators to land on the flower and providing precise insect-petal manipulation to activate the pollination mechanism (Westerkamp and Weber 1999). These connections are important because the way petals interact can determine the pollinator's activity (Tucker 2003).

Alemán et al. (2022) described the possible implications of this connection in the various pollination mechanisms of Papilionoideae. In their detailed and informative description, the authors describe a pocket, but refer to it as a sculpture. However, based on the morphological descriptions provided by Sacramento et al. (2024, **Chapter 1**) for these structures and the description provided by Alemán et al. (2022) , we believe they were referring to pockets. Nevertheless, the author's interpretation of the relationship between pollination mechanisms and different types of pockets is very useful. The punctate pockets (Fig. 1 E-G) described by Sacramento et al. (2024, **Chapter 1**) are responsible for a strong connection between the wings and the keel and are associated with species that can receive multiple pollinator visits, and have the brush, valvular, and pump pollen presentation mechanisms. In contrast, elongate pockets (*sensu* Sacramento et al. 2024, **Chapter 1**) offer a looser connection between the wing and keel and may be related to mechanisms that are triggered only once, epitomized by the explosive pollination mechanism. The pockets seem to be a simple, yet sophisticated, mechanism of interaction between the keel and wing petals. The pockets enable the coordinated movement of the wing-keel complex, exposing the staminal column as the pollinator moves over or within the flower (Alemán et al. 2017). The pockets may also be involved in an interaction with the standard petal, helping to lift it vertically. This is certainly the case in *Eriosema* and *Psoralea* subgenus *Psoralea*, where the pockets articulate with the swellings of the appendages at the base of the inner surface of the standard (Stirton 1975).

Folds in lateral petals were reported in *Delphinium anthriscifolium* (Ranunculaceae) by Zhang et al. (2024), where the authors demonstrated that the folds in the lateral petals were caused by a greater expansion in cell width, accompanied by folds in the cell wall in the adaxial epidermis. Ranunculaceae is one of the families that, along with Acanthaceae, Commelinaceae, Geraniaceae, Leguminosae, Onagraceae, Sapindaceae, Solanaceae, Trigoniaceae, and Tropaeolaceae, present keel flowers (Uluer et al. 2022; Westerkamp 1997). The folds in the lateral petals of Papilionoideae and Ranunculaceae are distinct and serve different functions. In the latter, the authors relate these folds to the asymmetric bending of the petals.

Each type of mechanism requires a certain amount of force; only specialized pollinators will be able to manipulate the flower and successfully access the rewards (Etcheverry and Vogel 2017). To access the floral resources, a bee will need all six legs to push down the wings and force back the standard to reveal the floral reward, while this process must also ensure the automatic return of the pollen-presenting structures into the keel, except in the explosive pollination mechanism (Gottsberger et al. 1988; Westerkamp and Weber 1999; Amaral-Neto et al. 2015). Pockets are infrequent and highly variable within genera and clades of Papilionoideae (Sacramento et al. 2024, **Chapter 1**). Significant variation in the presence of pockets occurs in clades that have different pollination mechanisms, such as in Genisteae (pump mechanism) and Phaseoleae (brush mechanism). Our reconstruction did not confirm our original hypothesis, and no clear trend was observed indicating that pockets are related to specific pollination mechanisms. Currently, the relationship between pockets and pollination mechanisms, as well as their intrageneric variation, is not fully understood , leaving room for investigations that could deepen our future understanding.

Conclusions and Future Perspectives

The results of this study add to our understanding of the evolution of floral sculpturing and pockets in the Papilionoid legumes, highlighting the complex evolutionary pathways leading to origin and changes of these structures within the subfamily. Resupination and crimped petals have emerged as significant factors, suggesting both vestigial and adaptive functions. The consistent presence of lamellate sculpturing in resupinate flowers, even after the loss of its tactile function, raises questions about the persistence and evolutionary importance of these structures. The function of visual cues may explain the emergence or retention of sculpturing in resupinate flowers, where epidermal folds, along with the wing petals, lose their primary function as landing platforms for insects. Behavioral studies similar to those conducted on conical epidermal cells (Kay et al. 1981; Whitney et al. 2009a, 2009b; Alcorn et al. 2012; Papiorek et al. 2014) could provide greater insight into the functions of sculpturing in the pollination of papilionate flowers. Some initial hypotheses regarding the evolution and relationship between sculpturing and pockets were not confirmed, and our findings emphasize the need for further investigation to elucidate the specific functions of these features in more detail. Moreover, the observed variation in floral sculpturing and the limited distribution of pockets suggest that specific ecological and morphological factors, still poorly understood, may play a crucial role in the evolution of these structures.

Since Stirton (1981), three types of sculpturing have been described and classified: lamellate, lunate, and lunate-lamellate. However, phylogenetic reconstructions have raised questions about whether the lunate-lamellate is truly a distinct form of sculpturing or merely a developmental phase in the sculpturing process. Anatomical studies and ontogenetic studies, not only of the sculpturing but also of the pockets, would help to elucidate issues related to the formation of these structures.

Additionally, an approach that incorporates a dated phylogeny and the reconstruction of ancestral environments would help elucidate the mechanisms involved in the evolution of sculpturing and pockets in papilionate flowers. Furthermore, an integrated approach that considers other characteristics of the papilionate flower may provide new insights into the synnovation and coevolution among important traits of Papilionoideae, such as pollen presentation mechanisms, pollinator type, floral morphological characteristics such as asymmetric flowers, and the combination of functions, would bring new insights into the evolution of such an important plant clade.

Another important aspect to address is that, although a significant gap has been filled by studying 414 genera (representing 83% of Papilionoideae genera), there are still 66 genera that have never been investigated. Taxonomic gaps in clades, such as Brogniartieae and Sophoreae, and particularly in the NPAAA clade, which has a higher number of genera without data (54), need to be filled. This clade is highly significant for the subfamily, as it is where the papilionate flower has been evolutionarily fixed, and it has high species richness and ecological importance (Fig. 2; Cardoso et al. 2013a; Choi et al. 2022). Filling these gaps will greatly enhance our understanding of the extraordinary floral diversification within the papilionoid legumes.

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TABLES

Table 1: Akaike Information Criterion (AIC) values resulting from the model fitting analysis for different morphological traits in Papilionoideae. The lowest AIC value, indicating the best-fitting model, is highlighted. ARD: All Rates Different model; ER: Equal Rates model; and SYM: Symmetric model.

Trait	**Akaike Information Criterion (AIC)**		
	ARD	ER	SYM
Flower shape	**258**	331	314
Pocket	**1001**	1056	1046
Sculpturing	**1133**	1175	1158
Sculpturing type	2331	2046	**1802**

FIGURES

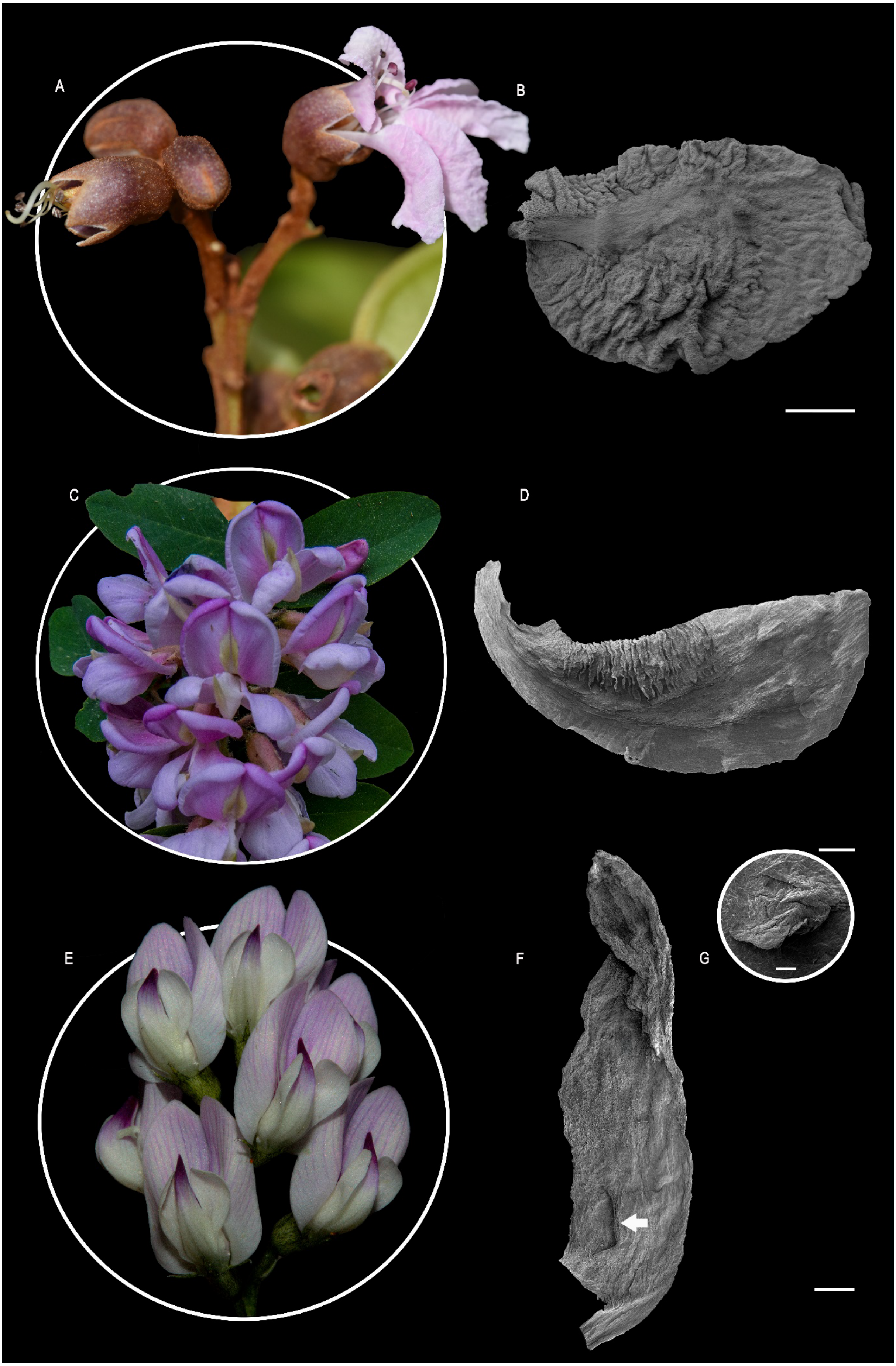


Fig. 1. Variation of wing petal ornamentation in bilaterally symmetrical flowers of the Papilionoideae (Leguminosae). **A-B**. *Diplotropis*. **C-D**. *Robinia*. **E-G**. *Astragalus*. **A.** Nearly papilionate flower with crimped petals. **B.** Crimped wing petal. **C, E**. Papilionate flower. **D**. Outer surface of the wing (abaxial) with lamellate sculpturing, where folds may overlap with adjacent ones. **F**. Outer surface of the wing (abaxial) with punctate pocket featuring a single deep and well-defined concavity (arrow). **G**. Inner surface of the wing (adaxial); detail of the inner surface of the pocket. **Scale bars: B**, **D**, **F**: 100µm; **G**: 50 µm. **Photos: A**, **E**: Domingos Cardoso. **C**. Martin Wojciechowski. **B** from *L. P. de Queiroz 16161* (HUEFS); **D** from *S. Dreveck 1145* **(**RB); **F-G** from *S. J. Harvey s/n* (HUEFS no. 262940).

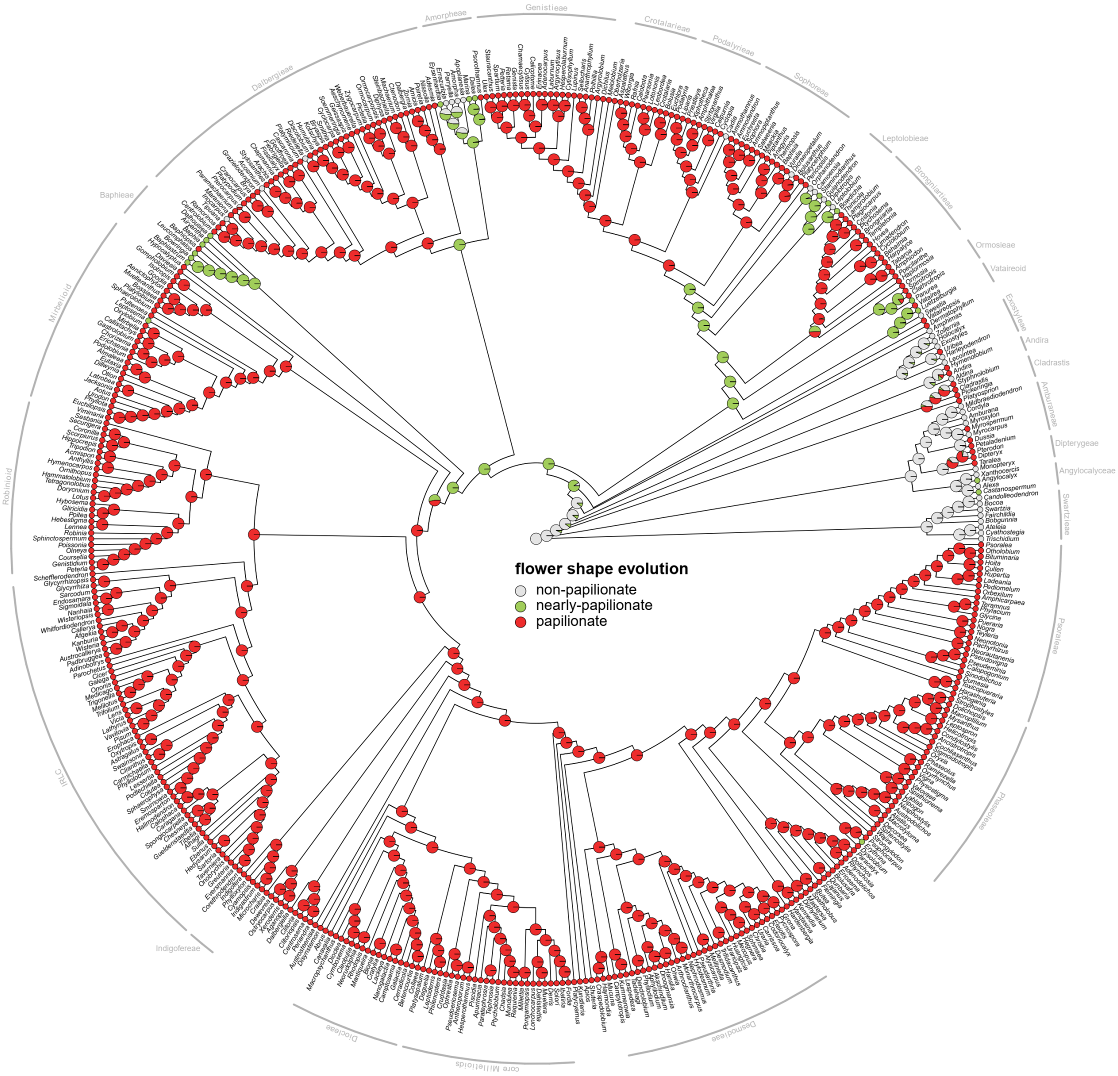


Fig. 2. Ancestral estimation of flower shape across a genus-level Papilionoideae phylogeny based on combined plastome and *matK* sequence data. The ancestral estimation used the AIC-selected ARD (All Rates Different) evolution model. The pie charts at each internal node indicate the probability of presence or absence of pockets. Terminals in gray indicate that pockets are unknown for that genus and so they were assigned with a 0.33 probability.

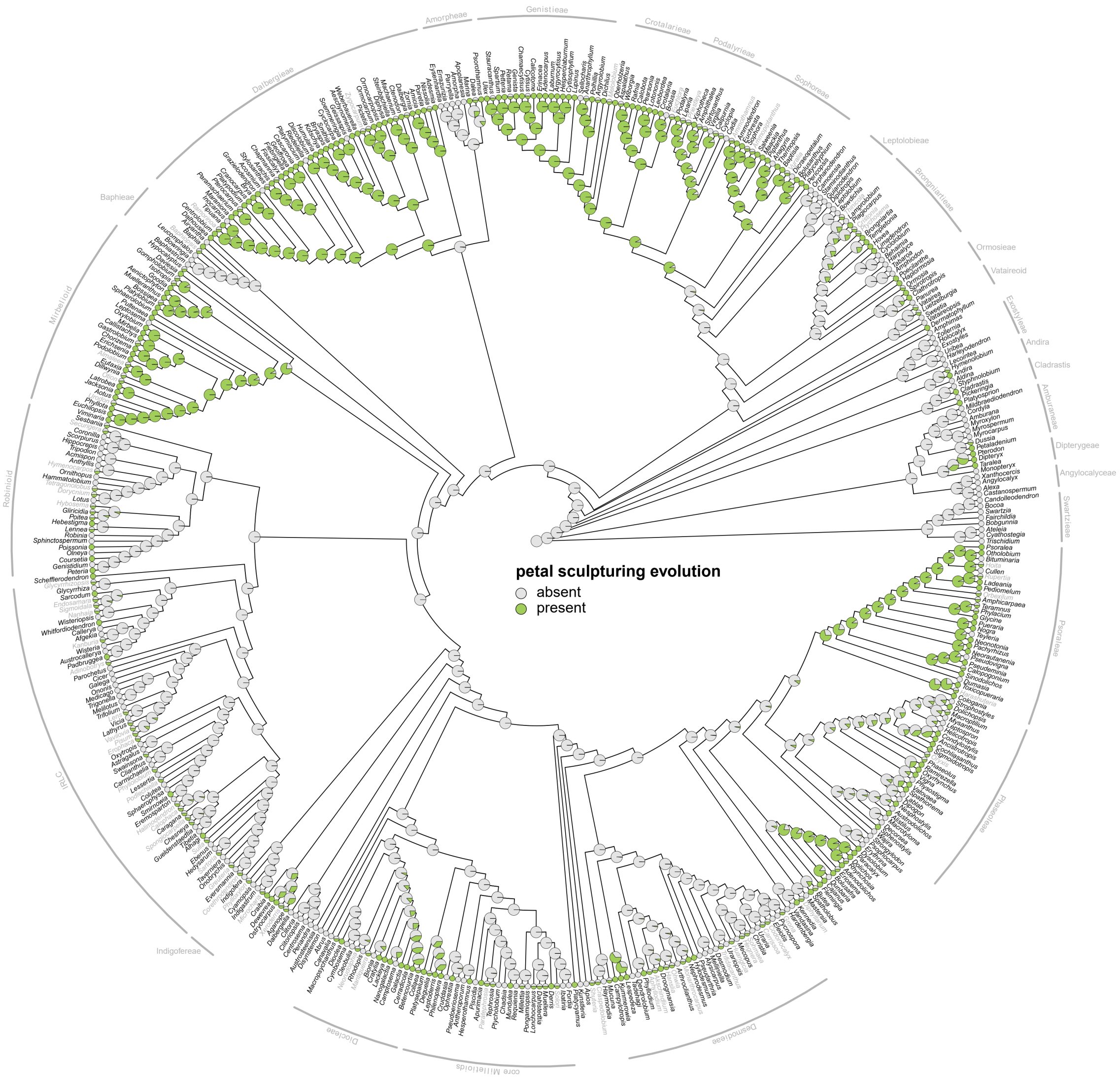


Fig. 3. Evolution of wing petal sculpturing across a genus-level Papilionoideae phylogeny based on combined plastome and *matK* sequence data. The ancestral estimation used the AIC-selected ARD (All Rates Different) evolution model. The pie charts at each internal node indicate the probability of presence or absence of pockets. Terminals in gray indicate that pockets are unknown for that genus and so they were assigned with a 0.5 probability.

Fig. 4. Evolution of types of wing petal sculpturing across a genus-level Papilionoideae phylogeny based on combined plastome and *matK* sequence data. The ancestral estimation used the AIC-selected SYM (Symmetrical) evolution model. The pie charts at each internal node indicate the probabilities of the presence or absence of sculpturing types. Gray terminals indicate that the sculpturing types are unknown for that genus and have therefore been assigned a probability of 0.25.

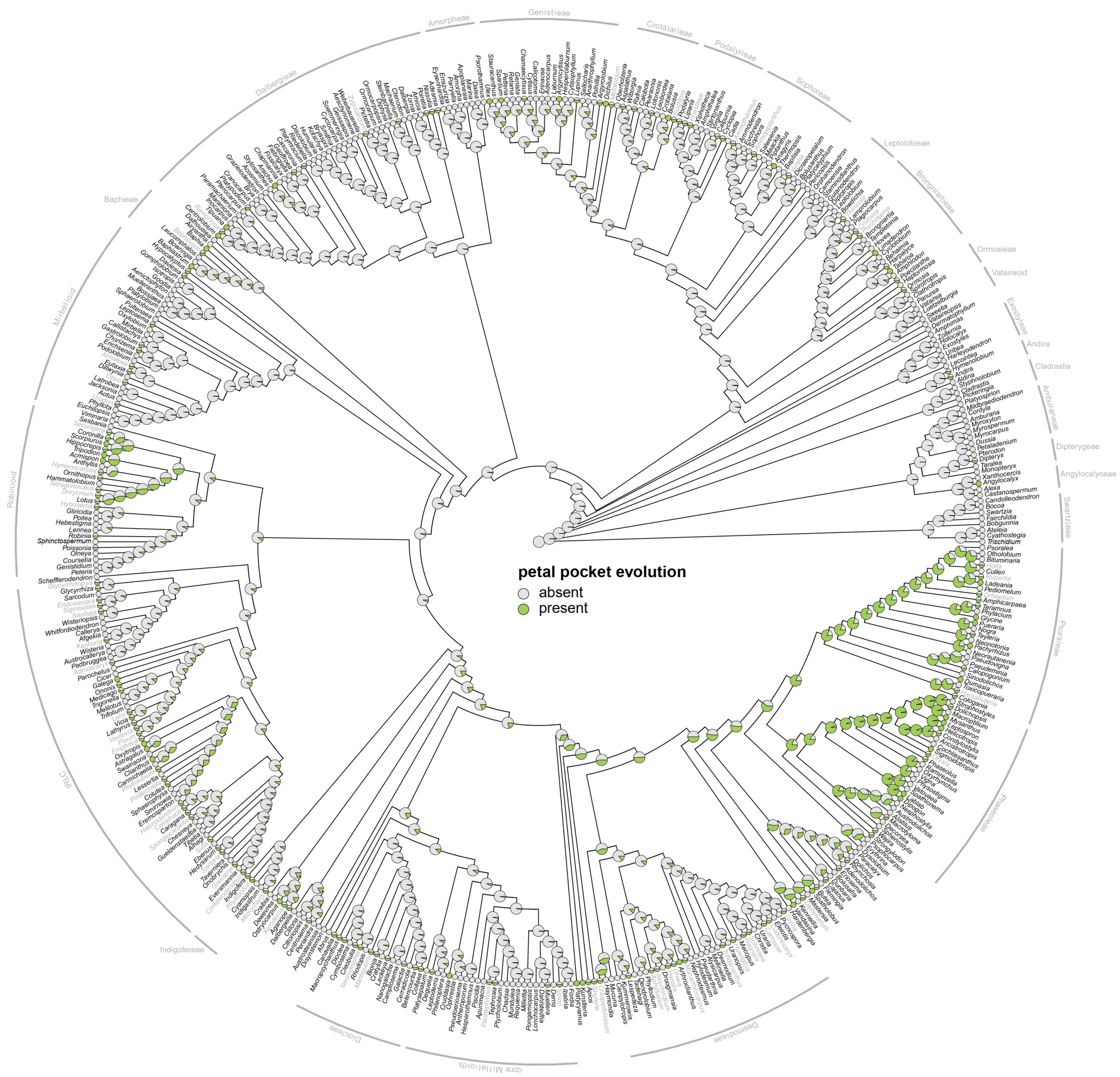
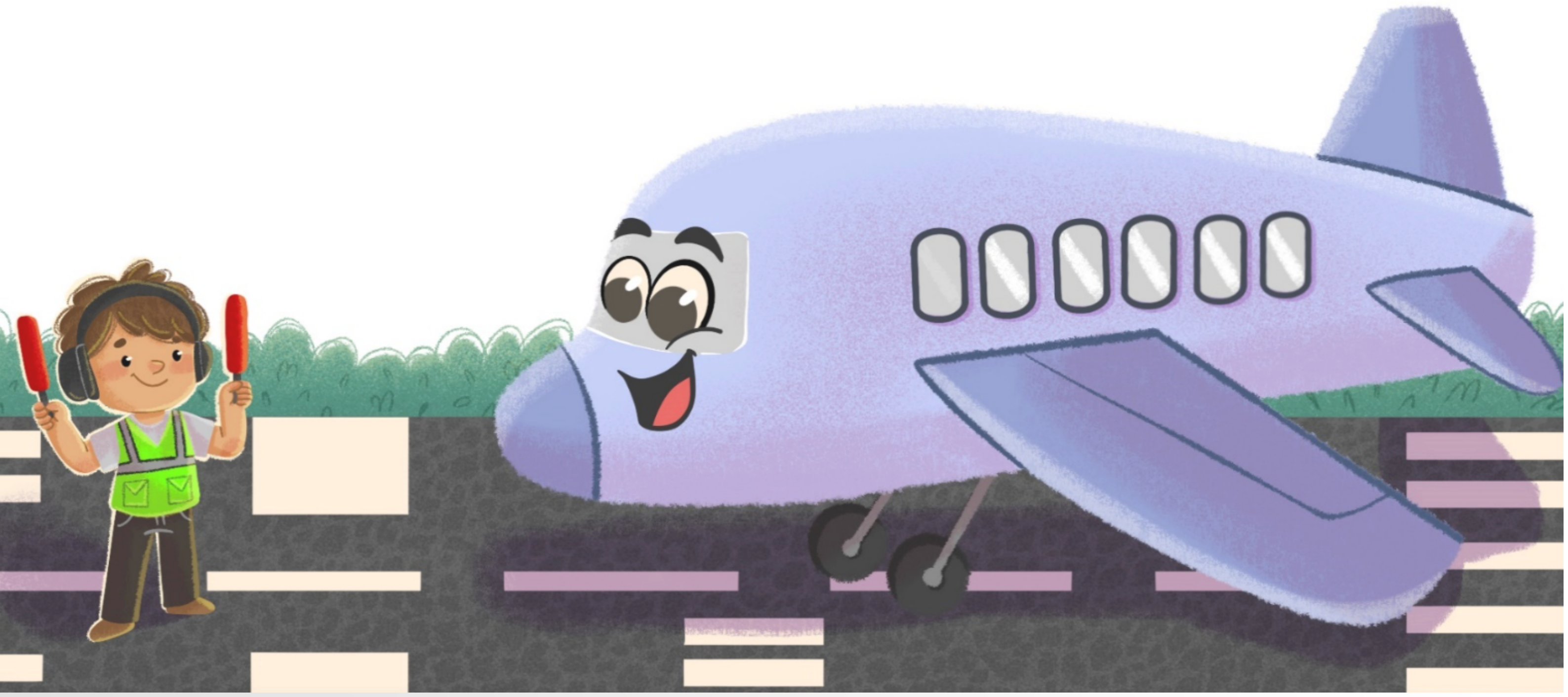


Fig. 5. Evolution of petal pocketing across a genus-level Papilionoideae phylogeny based on combined plastome and *matK* sequence data. The ancestral estimation used the AIC-selected ARD (All Rates Different) evolution model. The pie charts at each internal node indicate the probability of presence or absence of pockets. Terminals in gray indicate that pockets are unknown for that genus and so they were assigned with a 0.5 probability.

ONLINE SUPPLEMENTALS

Table S1. Dataset used to estimate the evolutionary transitions of the micromorphology of sculpturing and pockets present in the wing petals of Papilionoideae (Leguminosae). **Legend:** Flower shape: non-papilionate = 0, nearly-papilionate = 1, papilionate = 2; Sculpturing: absent = 0, present = 1; Sculpturing type: absent = 0, lamellate = 1, lunate = 2, lunate-lamellate = 3; and Pockets: absent = 0, present = 1. **Available at:** 10.6084/m9.figshare.27377520



CAPÍTULO 3

HOLES IN THE LANE: HIDDEN CLUES IN FLOWER POLLINATION

Holes in the Lane: Hidden Clues in Flower Pollination

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Keywords: Coevolution; bee; landing; sculpturing; tactile clues.

Abstract

We will explore what the landing of an airplane has in common with the landing of a bee on a flower. Both airplanes and bees are vehicles that traverse a path on the ground to reach their destinations. Although it may seem surprising, bees face more challenges when landing than airplane pilots do. However, bees are amazing "machines" engineered by millions of years of co-evolution with flowers, allowing them to easily overcome any obstacles during pollination. Here, we compare the fascinating similarities between the structures and mechanisms used on an airport runway and the hidden sculpturings present on the delicate wing petals of a pea flower.

The pollination

Pollination is a complex ecological event of utmost importance for all life on Earth. This process occurs when pollen grains, produced in the structures of flowers (stamens), are transferred to the organ (pistil). The pollination depends on so-called pollinators, which can be living (biotic) or nonliving (abiotic) agents. Among biotic pollinators, bees are predominant. About two-thirds of all existing plants depend on bees for reproduction. Bees are a highly diverse group of organisms that have a well-developed vision for colors, exhibit high learning abilities, and need floral resources – heat, nectar, oil, perfume, floral partsand resin – to survive. Another important aspect of bee pollination is that they generally land on petals. This behavior is crucial for the bees, as it allows them to spend less energy searching for floral resources essential for their survival and species maintenance. For flowers, this interaction reduces pollen grain losses, as the landing provides greater accuracy in placing pollen on the bee's body. [1].

Petal and landing strip

Structures as distinct as flower petals and airport runways surprisingly possess similarities that go beyond being regions for landing and takeoff for winged entities. For example, airport runways feature various visual signals to guide pilots during landings and takeoffs. Similarly, flowers signal with colors, stripes, spots, and scents to attract bees. In airports, the correct positioning of an aircraft for passenger boarding and disembarking is facilitated by ground controllers and ground markings that guide the pilot (Fig. 1). Likewise, in flowers, bees must position their bodies according to the structure predefined by the flower. This ensures that the bees gain access to resources and that the flower achieves efficient pollen deposition. This precise positioning of the bee's body on the flower is assisted by different hidden microtextures present on the petals (Fig. 1).

Another aspect shared between petals and runways is that both must remain dry; petals because of the deterioration caused by water, as well as the loss of brightness and dilution of resources (perfume and nectar) [2]. Runways must remain dry because water accumulation can form a water layer that may cause aircraft to skid [3]. Petals and runways must also provide safety during landing and takeoff. Petals aid bees in landing through cells with a conical shape that is coated with a water-repellent substance called cutin that composes the cuticle. The combination of conically shaped cells and the cuticle forms a texture on the petal that allows bees to grip - tactile runways[2] (Fig. 1). Similarly, in airports, the runway pavement must have microtextures, provided by small grooves in the asphalt. This ensures that where the aircraft tires contact the asphalt surface, there is adhesion between them, creating resistance to skidding and facilitating control during landings and takeoffs (Fig. 1)[3].

Difficulty landing

The differences between petals and runways are notable. An airport runway is always in a horizontal, static position, with weather conditions carefully monitored to prioritize flight safety. In contrast, petals are not always horizontal; they can be slightly inclined, with degrees of inclination varying up to complete verticalization (Figs. 2 and 3). Additionally, flowers are not static like paved runways. They are influenced by weather conditions, especially wind, making bee landing on these structures even more challenging.

Some plants increase the challenge for bees, especially those with bilaterally symmetrical flowers (Fig. 2). Under these conditions, the floral shape further restricts the access and movement of pollinators. Among the flowering plants that exhibit this characteristic, those known scientifically by the name Papilionoideae stand out for their innovations resulting in an even more complex floral structure. Their floral shape favors bees with specific skills, particularly strong and skillful bees, promoting greater precision in pollen transfer and, consequently, an increase in pollination efficiency.

A special flower

The papilionate floral architecture (Figs. 2 and 3) that is typical of the Papilionoideae is composed of five petals that are highly differentiated into three types – standard, wings, and a keel (Fig. 2) – each with a specific function during the pollination process. The standard petal often attracts pollinators, while the pair of wing petals serve as a landing platform and lever, and the pair of keel petals protect the reproductive organs, preventing the exposure of pollen grains.

The wing petals of the papilionate flower serve as landing strips, and therefore, exhibit all the previously described features for attracting bees and ensuring the safety of their landing. In addition to these features, the wing petals also provide additional safety resources: sculpturing and pockets (Fig. 3).

The sculpturing and pockets are adornments on the petal (Fig. 3). Sculpturing is formed by superficial folds of the petal epidermis, while pockets result from the folding of the petal itself (Fig. 3). Sculpturing can be classified as lamellate, lunate, and lunate-lamellate, and can be found in any region of the wing petal. On the other hand, pockets can be elongated, punctate, or transversal, and are present only in the upper region of the petal, extending from the base up to, at most, halfway along the petal. These structures serve distinct functions, both related to pollination. Sculpturing acts as climbing aids that bees use to grip the flower (Fig. 3). These supports are particularly important when the flower is upright or moving with the wind. Conversely, pockets appear to function in maintaining the connection between the wing petals and the keel petals under the weight and movement of the bee, thus aiding in pollen release. Additionally, due to their position on the petals, pockets also serve as footholds for the bees' feet.

The sculpturing and pockets can be compared to macrotextures, small fissures that ensure grip on the runway where the aircraft tires contact the pavement surface [3]. This function is very similar to that of sculpturing and pockets in Papilionoideae flowers. These adornments are unique to this group of flowering plants, raising questions about the role of these structures in the evolutionary history of one of the world's largest botanical groups in terms of species diversity. Just as in airport runways, in botany, small structures guide giants.

Glossary

Bilateral symmetry – A condition in which floral parts (sepals, petals, and reproductive structures) can be divided into two similar halves along a only central cutting plane.

Cuticle – Also known as wax. It covers the epidermal cells of various plant organs, typically composed of lipid polymer, giving the cuticle its hydrophobic properties.

Papilionoideae – The scientific name for a large and diverse group of plants of the legume family. This group includes morphologically diverse, yet closely related plants such as peas, peanuts, chickpeas, soybeans, and beans.

Pockets – Depressions, folds, or invaginations of the wing petal.

Sculpturing – Epidermal folds of various shapes that amalgamate, creating a three-dimensional composition on the outer surface of the wing petals.

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Figure 1. Illustration of bees landing on flowers with bilateral symmetry, including a detail of papillary cells indicating the position of the bee on the flower; and the landing of an airplane. Illustration: Graziela Andrade (@grazielandrade).

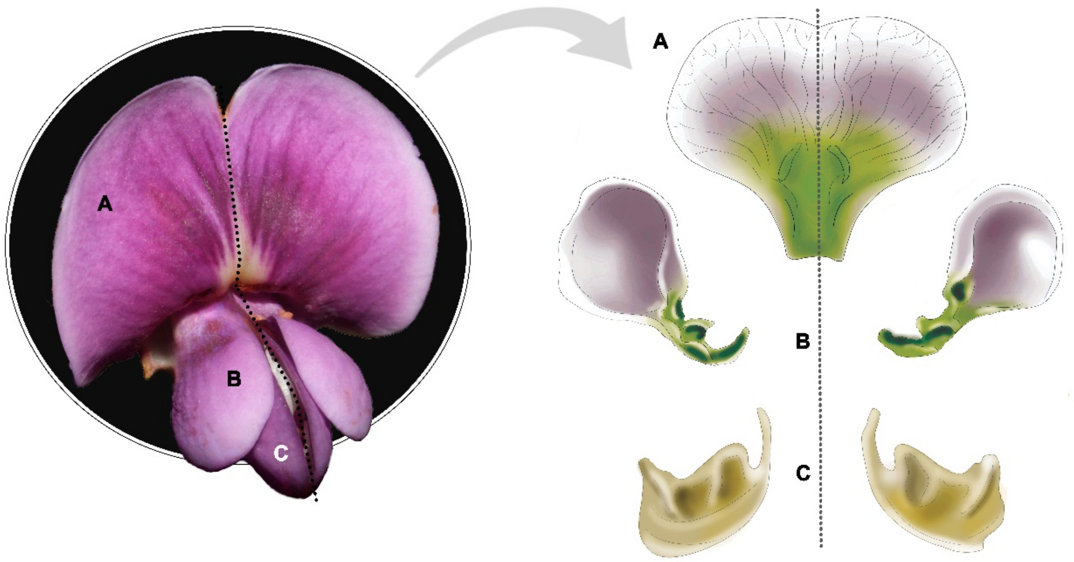

Figure 2. Structure of the bilaterally symmetrical flower of the Papilionoideae legumes, showing its five petals differentiated into three types: A. Standard. B. Wings. C. Keel. Image: Domingos Cardoso. Illustration: Gustavo Ramos.

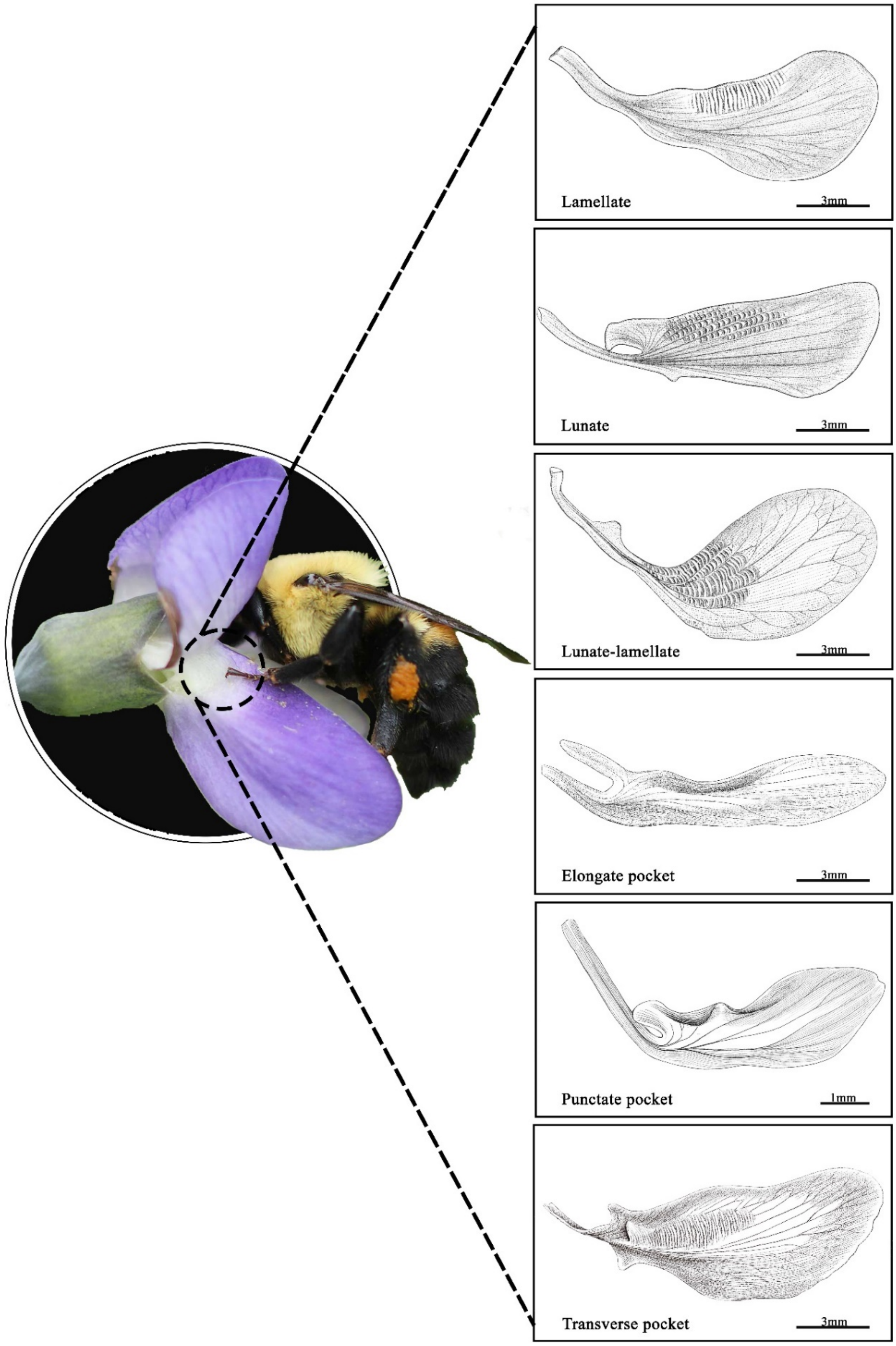

Figure 3. Diversity of sculpturings and pockets found in the wing petals of Papilionoideae legumes. Image: Janet Davis. Illustration: Natanael Nascimento.

An author biography

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CAPÍTULO 4

PISTAS OCULTAS NA POLINIZAÇÃO DAS FLORES

Pistas ocultas na polinização das flores

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Resumo

Pistas de aeroportos e pétalas de flores apresentam semelhanças surpreendentes. Além de região de pouso e decolagem para entes alados, pistas e pétalas apresentam estratégias para que esses entes pousem e decolem em segurança. Para isso, as pistas revestidas e asfaltadas de aeroportos e as delicadas pétalas de muitas flores lançam mão de estratégias similares para o sucesso dos pousos e decolagens. Alguns grupos de plantas com flores apresentam recursos extras para facilitar não só o pouso, mas a permanência dos polinizadores nas suas pétalas, como é o caso das flores do feijão. Chamada por cientistas de flores papilionadas, essas flores apresentam modificações em resposta ao principal polinizador, as abelhas. Entre as modificações exclusivas do grupo, a presença de determinadas estruturas, chamadas de esculturas e depressões nas pétalas das alas, principal responsável pelo pouso dos insetos, é um exemplo de traço morfológico que fortalece a relação flor e polinizador.

Palavras-chave: Coevolução; abelhas; pistas táteis; pouso; microevolução

O evento

A polinização é um evento ecológico complexo e de extrema importância para toda a vida na Terra. Este processo ocorre quando o grão de pólen, produzido nas estruturas masculinas das flores (estames), é transferido para o órgão feminino conhecido como pistilo [1]. Essa transferência pode ocorrer entre diferentes flores de uma mesma planta, entre flores de plantas diferentes ou ainda dentro de uma única flor, sempre da mesma espécie. A polinização depende dos chamados agentes polinizadores, que podem ser feita por seres vivos (por exemplo: mamíferos, répteis e insetos), ou abióticos/não vivos (vento, água). A reprodução da maioria das plantas terrestres ocorre por meio da polinização. É por causa da polinização que surgem frutos, sementes e novas plantas, que servem de alimento para muitos seres vivos, como humanos, porcos, macacos, coelhos, onças, borboletas e muitos outros. Sem polinização, esses animais não teriam o que comer e não conseguiriam sobreviver.

Entre os agentes polinizadores bióticos, os insetos são os mais frequentes na natureza. Cerca de dois terços de todas as plantas existentes dependem de insetos para se reproduzir. Dentre estes, as abelhas se destacam por terem a visão bem desenvolvida para cores; apresentarem um grande número de espécies dentre os quais alguns grupos apresentam altas habilidades em aprendizado e ter sua sobrevivência diretamente ligada às flores (por exemplo, acesso a calor, néctar, óleo, perfume, peças florais, pólen e resina). Outro aspecto importante na polinização por abelhas é que, geralmente, esses insetos pousam nas pétalas, e esse comportamento é crucial, tanto para os insetos quanto para as flores. Insetos que pousam nas flores gastam menos energia em busca de recursos florais que serão utilizados para a sobrevivência e manutenção da espécie. Para as plantas, insetos que pousam em suas flores reduzem as perdas do grão de pólen, pois o pouso permite à flor uma maior precisão na colocação de pólen no corpo do polinizador [2].

Pistas e pétalas

Surpreendentemente estruturas tão distintas como pétalas de flores e pistas de aeroportos possuem semelhanças que vão além de ser região de pouso e decolagem para entes alados. As pétalas das flores funcionam como pistas de pouso para as abelhas graças aos sinais visuais exibidos pelas pétalas (cores, manchas, texturas diferentes), por vezes invisíveis aos olhos humanos. Da mesma forma, as pistas de pouso e decolagem nos aeroportos também apresentam diversos sinais visuais para orientar os pilotos para pousos e decolagens seguros. Após aterrissar nas pétalas das flores, o inseto deve posicionar o corpo para acessar o recurso e assim ter o grão de pólen colocado em seu corpo. Isso ocorre com a ajuda de diferentes texturas presentes nas pétalas (Fig. 1). Já nos aeroportos, no solo, o piloto pode contar com os controladores de pista e com as marcações no solo para direcionar a aeronave. (Fig. 1). Tanto as pétalas quanto as pistas devem se manter secas. As pétalas para evitar adoecimento da flor, perda de brilho e diluição do perfume ou néctar (perfume e néctar) [3]. Já as pistas precisam que a água escoe rapidamente para evitar a aquaplanagem, ou seja, a derrapagem devido à presença de lâmina d’água na pista [4]. Ambas as estruturas devem oferecer segurança durante o pouso e decolagem. Enquanto as pétalas auxiliam o pouso de insetos por meio de células cônicas cobertas por cristas cuticulares, onde esses insetos podem se agarrar (pistas táteis) [3] (Fig. 1, detalhe), as pistas de pouso nos aeroportos ao redor do mundo empregam uma estratégia semelhante. Pequenas ranhuras no pavimento garantem a aderência da pista onde ocorre o contato entre os pneus da aeronave e a superfície, criando resistência à derrapagem e facilitando o controle durante pousos e decolagens. Essas estruturas também são responsáveis por prevenir a aquaplanagem, pois auxiliam no escoamento da água [4].

Os desafios do pouso

Diferentemente de uma pista de pouso em aeroportos, que está sempre em posição horizontal e estática, com condições climáticas cuidadosamente monitoradas, as pétalas de flores nem sempre estão em posição horizontal. Elas podem apresentar desde leves graus de inclinação até um giro de 180º. Além disso, as flores não são estáticas como as pistas pavimentadas; as flores balançam ao sabor dos ventos, tornando o pouso nessas estruturas ainda mais desafiador.

As células da epiderme em formato cônico, cobertas por cristas cuticulares, auxiliam no pouso das abelhas, oferecendo tanto pistas táteis como pistas visuais (Fig. 1). Essas células são percebidas pelas abelhas nas pontas das antenas, ou através dos pés após o pouso [5]. Em uma flor em posição vertical ou sob efeito de ventos, essas células assumem importância ainda maior. As células epidérmicas cônicas geralmente estão localizadas na parte da pétala voltada para onde o polinizador pousará, fornecendo assim uma superfície que facilita o inseto 'agarrar' a pétala, permitindo o pouso mesmo em condições mais desafiadoras [3]. A importância dessas células é tão grande que estão presentes em 85% das plantas com flores (angiospermas), e são encontradas em grupos botânicos importantes como a família das margaridas (Asteraceae) e das orquídeas (Orchidaceae).

Algumas plantas aumentam o desafio para as abelhas, especialmente aquelas com flores com simetria bilateral[1] (Figs. 2-3). Nessas condições, o formato mais estreito da flor restringe ainda mais o acesso e a movimentação dos polinizadores. Dentre as plantas que apresentam essa característica, as Papilionoideae[2] , pertencentes à família das leguminosas, se destacam por apresentar um grande número desse tipo de flor. Esse formato floral favorece insetos com habilidades específicas, principalmente abelhas fortes e habilidosas, promovendo maior precisão na transferência de pólen e, consequentemente, aumento na eficiência da polinização.

A grande maioria das Papilionoideae é reconhecida pelas suas flores papilionadas (Fig. 2), sendo composta por cinco pétalas distintas em três tipos – estandarte, alas e carena – cada uma com uma função específica durante o processo de polinização. A pétala estandarte atrai os polinizadores, enquanto o par de pétalas das alas serve como plataforma de pouso e alavanca, e o par de pétalas da carena protege os órgãos reprodutivos, impedindo a exposição dos grãos de pólen (Fig. 2). As alas da flor papilionada servem como pistas de pouso e, portanto, apresentam todas as características descritas anteriormente para atração, bem como para a segurança do pouso das abelhas. Além dessas características, as pétalas das alas também fornecem recursos adicionais de segurança: esculturas[3] e depressões[4] (Fig. 3).

A escultura e as depressões são estruturas da pétala (Fig. 3). A escultura é formada por dobras na superfície da pétala, na epiderme, enquanto os depressões resultam do dobramento de toda a pétala (Fig. 3). A escultura pode ser classificada como lamelar, lunar e lunar-lamelar, e pode ser encontrada em qualquer região da pétala da ala [6,7]. Por outro lado, as depressões podem ser alongadas, pequenos pontos ou transversais, e estão presentes apenas na região superior da pétala, estendendo-se da base até, no máximo, a metade da pétala. Essas estruturas desempenham funções distintas, mas relacionadas à polinização. A escultura atua como auxílio de escalada que as abelhas usam para agarrar a flor (Fig. 2). Esses suportes são particularmente importantes quando a flor está ereta (Fig. 3) ou se move com o vento. Por outro lado, os depressões parecem funcionar na manutenção da conexão entre as as alas e aa carena sob o peso e o movimento do inseto, auxiliando assim na liberação do pólen. Além disso, devido à sua posição nas pétalas, os depressões também servem como apoios para os pés das abelhas.

As esculturas e depressões podem ser comparados a pequenas fissuras que garantem a aderência na pista onde os pneus da aeronave entram em contato com a superfície do pavimento [3]. Essa função é muito semelhante à da escultura e dos depressões nas flores de Papilionoideae. Embora esculturas e depressões não sejam encontrados em todos os representantes de Papilionoideae não existe registro dessas estruturas em outra peça floral que não seja as pétalas das alas de Papilionoideae. A exclusividade e localização levanta questões sobre o papel dessas estruturas na história evolutiva de um dos maiores grupos botânicos do mundo em termos de diversidade de espécies. Assim como nas pistas de aeroportos, na botânica, pequenas estruturas guiam gigantes.

Glossário

[1]**Simetria bilateral** – Uma condição na qual as partes florais (sépalas, pétalas e estruturas reprodutivas) podem ser divididas em duas metades semelhantes por um plano de corte central.

[2]**Papilionoideae** – O nome científico de um grupo grande e diverso de plantas da família das leguminosas. Este grupo inclui plantas morfologicamente diversas, mas intimamente relacionadas, como ervilhas, amendoins, grão-de-bico, soja e feijões.

[3]**Escultura** – Dobras epidérmicas de várias formas que se amalgamam, criando uma composição tridimensional na superfície externa das pétalas das alas.

[4]**Depressões** – Depressões, dobras ou invaginações da pétala da ala.

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Figura 1. Ilustração do pouso da abelha na flor com simetria bilateral, detalhe das células papilosas indicando a posição que a abelha deve ocupar na flor; e o pouso de um avião. Ilustração: Graziela Andrade (@grazielandrade).

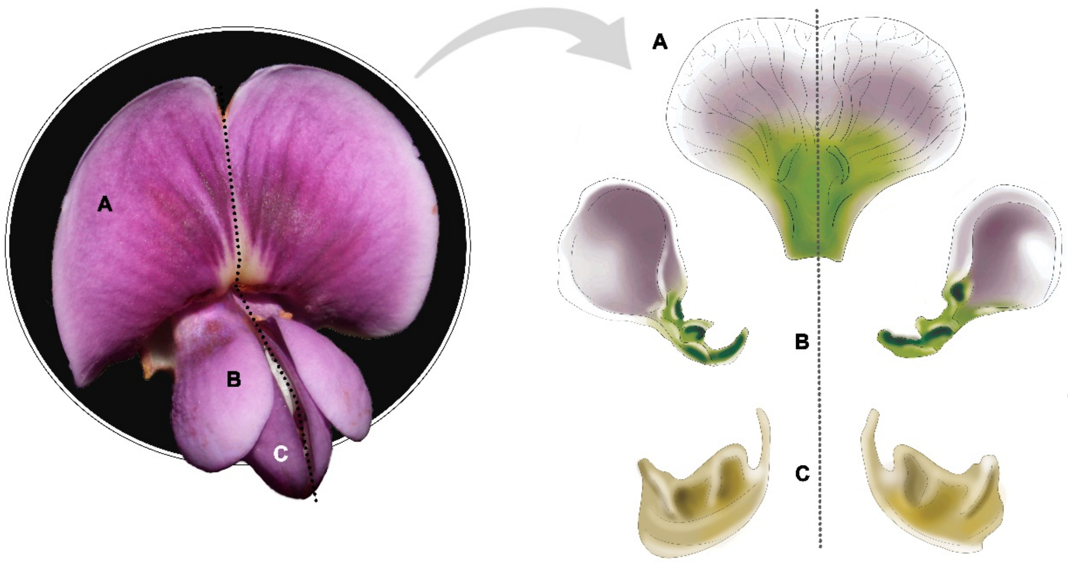

Figura 2. Estrutura da flor com simetria bilateral das Papilionoideae. Detalhe mostrando suas cinco pétalas diferenciadas em três tipos: A. Estandarte. B. Alas. C. Quilha. Imagem: Domingos Cardoso. Ilustração: Gustavo Ramos

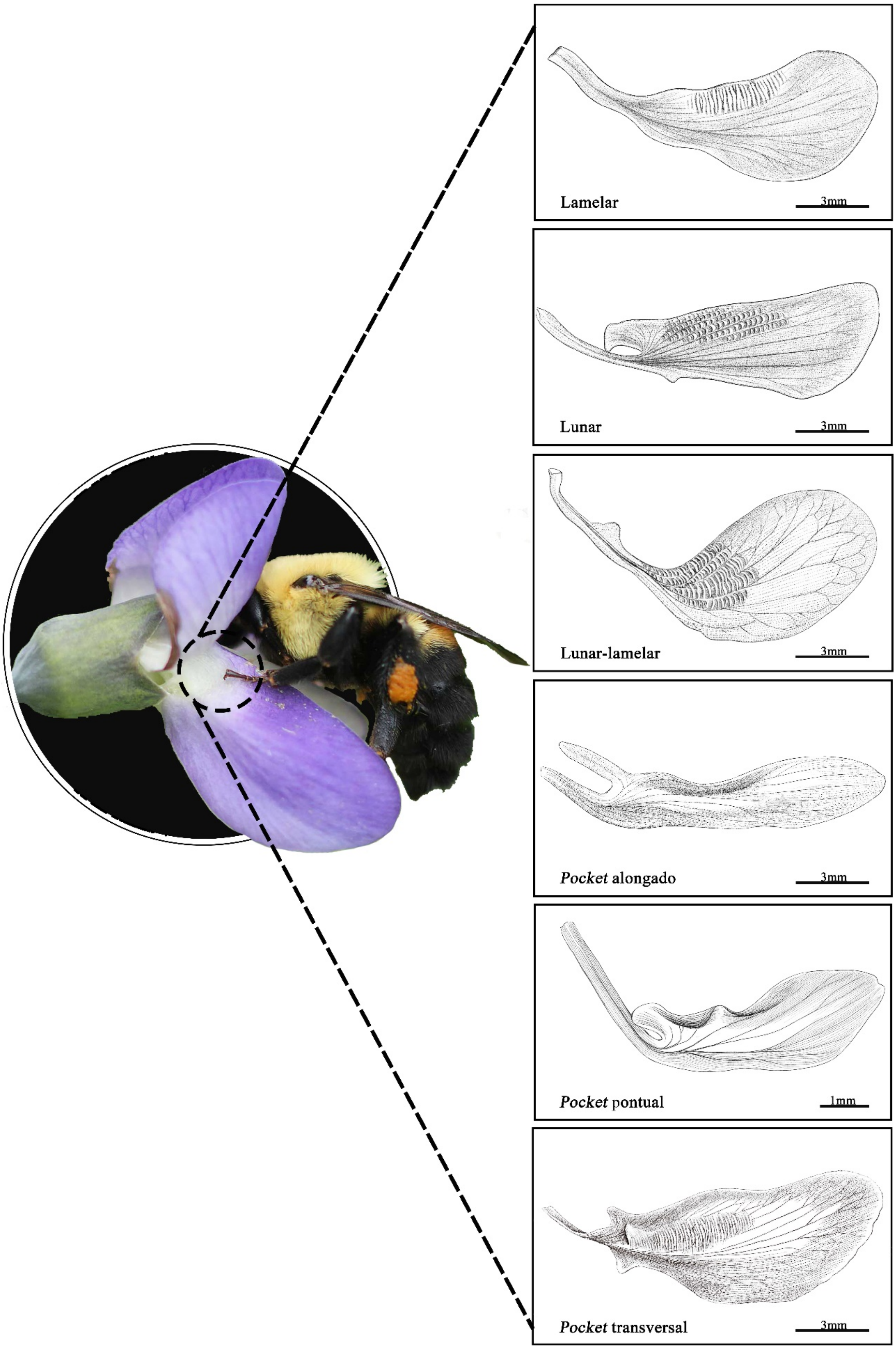

Figura 3. Diversidade de esculturas e pockets encontrados nas pétalas das asas das leguminosas Papilionoideae. Imagem: Janet Davis. Ilustração: Natanael Nascimento.

CONCLUSÃO GERAL

A flor papilionada é a característica que define a subfamília Papilionoideae, a mais diversa em termos taxonômicos e ecológicos dentro das Leguminosae. Esse tipo de flor apresenta uma ampla gama de características intimamente relacionadas à atração e fidelidade dos polinizadores. Além dos mecanismos específicos de polinização associados às pétalas altamente diferenciadas, as esculturas e os *pockets* presentes nas alas das flores papilionadas desempenham um papel importante no auxílio aos polinizadores. Os pockets atuam como um mecanismo de 'botão de pressão' entre as pétalas das asas e da quilha, mantendo-as unidas para que se movimentem em conjunto quando o polinizador pousa. As esculturas, por sua vez, fornecem suporte ao criar uma superfície texturizada, permitindo que os insetos se fixem enquanto procuram pólen e/ou néctar.

Nesta tese, analisamos flores coletadas em campo, exemplares preservados em herbários e dados da literatura sobre a caracterização das alas nas leguminosas. No total, foram analisados 445 gêneros, o que representa 90% dos gêneros da subfamília. Microscopia óptica e eletrônica de varredura foram utilizadas para revisar, caracterizar e descrever a variação das esculturas e *pockets* das alas em todos os principais clados da subfamília. Apesar dos avanços consideráveis no estudo dos padrões macroevolutivos da arquitetura floral e sua contribuição para o sucesso evolutivo e ecológico das Papilionoideae, a microescultura petalar nunca havia sido explorada no contexto da diversificação floral das leguminosas. Portanto, foi fundamental a utilização de uma abordagem sistemática para definir e caracterizar detalhadamente a variação das esculturas e *pockets* no contexto da diversidade floral e nos diferentes clados de Papilionoideae, bem como explorar as origens e as mudanças evolutivas dessas microestruturas nas flores da subfamília.

A diversidade estrutural e suas múltiplas origens independentes ao longo da evolução das Papilionoideae indicam um cenário evolutivo dinâmico, com ganhos e perdas dessas estruturas ocorrendo repetidamente em diferentes clados. As esculturas, em particular, mostraram-se altamente diversas morfologicamente, com os tipos lamelar, lunar e lunar-lamelar distribuídos de forma desigual nos clados, enquanto os *pockets* também apresentaram variações marcantes em sua forma e frequência, sugerindo uma função adaptativa complexa, possivelmente relacionada à interação planta-polinizador. A evolução dessas características revela um caminho intricado e multifacetado, com ressupinação das flores e pétalas onduladas adicionando mais complexidade às suas funções.

Os resultados aprofundaram o conhecimento sobre a presença e variação de esculturas e *pockets* nos clados de Papilionoideae, destacando os caminhos evolutivos complexos que levaram à origem e às modificações dessas estruturas. Existem ainda lacunas no entendimento sobre o papel e impacto da micromorfologia das pétalas na diversificação dessa subfamília, e os dados aqui obtidos podem fornecer base para futuros estudos. O conhecimento científico gerado também foi explorado em contextos de divulgação científica voltada para crianças e adolescentes, com o objetivo de aproximar a ciência produzida nas academias da sociedade em geral.