

UNESP - UNIVERSIDADE ESTADUAL PAULISTA
CÂMPUS DE BOTUCATU
INSTITUTO DE BIOCIÊNCIAS

Filogenia, biogeografia e evolução de estruturas secretoras de
representantes da subtribo Cajaninae (Leguminosae,
Papilionoideae, Phaseoleae)

Wanderleia de Vargas Araujo

**Tese apresentada ao Instituto de
Biociências, Câmpus de Botucatu,
UNESP, para obtenção do título de
Doutor em Ciências Biológicas
(Botânica), AC: Sistemática Vegetal**

BOTUCATU – SP

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Resumo geral

Leguminosae é a terceira maior família dentre as Angiospermas, é cosmopolita, podendo ocorrer em florestas tropicais úmidas, florestas secas, savanas, regiões mediterrâneas e desérticas. Papilionoideae, subfamília com maior riqueza de espécies de Leguminosae, apresenta Phaseoleae como uma de suas tribos de maior importância econômica e alto número de espécies. Cajaninae, por sua vez, é a maior subtribo de Phaseoleae, destacando-se por apresentar aproximadamente 490 espécies distribuídas em 10 gêneros. Os representantes desta subtribo possuem distribuição pantropical, com apenas *Rhynchosia* e *Eriosema* ocorrendo nos neotrópicos. De modo geral, as espécies desta subtribo ocorrem em campos gramíneos, florestas tropicais secas, regiões semiáridas, áreas degradadas e ambientes propensos à passagem do fogo. A ocorrência das espécies de Cajaninae nestes diferentes ambientes pode estar relacionada à presença de estruturas secretoras, como glândulas vesiculares e tricomas de base bulbosa. Apesar do conhecimento sobre a existência destas estruturas peculiares neste grupo desde o século 19, nenhum estudo detalhado de anatomia, ultraestrutura ou estudos com enfoque evolutivo haviam sido realizados. A subtribo tem sido considerada monofilética, porém os poucos trabalhos filogenéticos existentes abordam Phaseoleae como um todo, possuindo uma amostragem muito baixa dos representantes de Cajaninae, o que não esclarece sua monofilia. Considerando o pouco conhecimento sobre as relações filogenéticas em Cajaninae, sobre as estruturas secretoras que são peculiares a este grupo e os poucos estudos abrangentes da subtribo, este estudo teve por objetivos: testar a monofilia da subtribo (e de seus gêneros), esclarecer a delimitação dos gêneros pertencentes à Cajaninae (especialmente *Rhynchosia* e *Eriosema*), conhecer tipos, ocorrência, composição química dos exsudatos, além do mecanismo de secreção das estruturas secretoras e reconstruir estados ancestrais baseados nas estruturas secretoras dos representantes da subtribo. Como resultados principais, foi obtido completo levantamento das estruturas secretoras ocorrentes na subtribo Cajaninae, descrição dos tipos de material secretados por estas glândulas e sua ultraestrutura, e uma abrangente filogenia, bem como a evolução ancestral das glândulas amplamente distribuídas neste grupo. É importante salientar que todos estes objetivos foram cumpridos com cobertura amostral significativa. Sendo assim é possível afirmar que: a glândula vesicular é unificadora do grupo; lipídeos e compostos fenólicos são os principais compostos secretados pelas estruturas secretoras da subtribo; estas substâncias agem principalmente na defesa contra herbívoros e contra possíveis danos causados pela exposição dos raios UV, aos quais estão sujeitos os representantes do grupo; além disso, as estruturas subcelulares estão diretamente ligadas aos compostos secretados pelas glândulas analisadas; quanto à filogenia, pode-se afirmar o monofiletismo da subtribo, assim como dos gêneros *Adenodolichos*, *Flemingia*, *Dunbaria*, *Cajanus* e *Eriosema*; e em relação a evolução ancestral das estruturas secretoras somente a glândula vesicular e o tricoma capitado estiveram presentes no ancestral hipotético de Cajaninae. Estudos de datação molecular e história biogeográfica da subtribo estão sendo desenvolvidos para melhor compreensão da sistemática do grupo, além disso mais estudos com enfoque sistemático

e taxonômico serão realizados, principalmente nos gêneros não monofiléticos da subtribo Cajaninae.

Palavras-chave: Fabaceae; Glândulas vesiculares; Idioblastos; Reconstrução ancestral; Sistemática vegetal; Tricomas de base bulbosa;

General abstract

Leguminosae is the third largest family among Angiosperms, is cosmopolitan, occurring in humid tropical forests, dry forests, savannas, Mediterranean and desert regions. Papilionoideae, a subfamily with the highest species richness of Leguminosae, presents Phaseoleae as one of its tribes of major economic importance and high number of species. Cajaninae, in turn, is the largest subtribe of Phaseoleae, standing out to present approximately 490 species distributed in 10 genera. The representatives of this subtribe have pantropical distribution, with only *Rhynchosia* and *Eriosema* occurring in the neotropics. The species of this subtribe occur in greenfields, dry tropical forests, semi-arid regions, degraded areas and environments prone to fire. The occurrence of Cajaninae species in these different environments may be related to the presence of secretory structures, such as vesicular glands and bulbous based-trichomes. Despite the knowledge about the existence of these peculiar structures in this group since the 19th century, no detailed study of anatomy, ultrastructure or evolutionary approach studies had been performed. The subtribe has been considered monophyletic, but the few existing phylogenetic works address Phaseoleae as a whole, having a very low sampling of Cajaninae representatives, which does not clarify its monophyly. Considering the little knowledge about the phylogenetic relationships in Cajaninae, on the secretory structures that are peculiar to this group and the few comprehensive studies of the subtribe, this study had as objectives: to test the subtribe monophyly (and its genera), to clarify the delimitation of genera belonging to Cajaninae (especially *Rhynchosia* and *Eriosema*), to know types, occurrence, chemical composition of the exudates, besides the mechanism of secretion of the secretory structures and to reconstruct ancestral states based on the secretory structures of the subtribe representatives. As main results were obtained a complete survey of the secretory structures occurring in the Cajaninae subtribe, a description of the types of material secreted by these glands and their ultrastructure, and a comprehensive phylogeny, as well as the ancestral evolution of the glands widely distributed in this group. It is important to note that all these objectives were met with significant sample coverage. Thus, it can be stated that: the vesicular gland is unifying the group; lipids and phenolic compounds are the main compounds secreted by the secretory structures of the subtribe; these substances act mainly in the defense against herbivores and against possible damages caused by the exposure of UV radiation, to which the representatives of the group are subject; in addition, the subcellular structures are directly linked to the compounds secreted by the glands analysed; As for phylogeny, it can be affirm that Cajaninae is monophyletic, as well as the genera *Adenodolichos*,

Flemingia, *Dunbaria*, *Cajanus* and *Eriosema*; and in relation to the ancestral evolution of the secretory structures only the vesicular gland and the capitate trichome were present in the hypothetical ancestor of *Cajaninae*. Molecular dating studies and biogeographic history of the subtribe are being developed to better understand the systematics of the group, in addition, more studies with a systematic and taxonomic focus will be carried out, mainly in the non-monophyletic genera of the *Cajaninae* subtribe.

Keywords: Ancestral reconstruction; Bulbous-based trichomes; Fabaceae; Idioblasts; Vesicular glands

Introdução geral

Leguminosae Juss., terceira maior família dentre as Angiospermas, compreende cerca de 770 gêneros, mais de 19.500 espécies e possui distribuição cosmopolita (Schrire et al. 2005; LPWG 2017). A importância econômica deste grupo de plantas se dá pela utilização da madeira como matéria-prima para móveis e instrumentos musicais; também são fonte de alimento, forrageiras, e indicadas para a recuperação de áreas degradadas; além disso, seus compostos secundários (resina, óleos e taninos) são amplamente extraídos para fabricação de medicamentos, corantes e vernizes (Lewis et al. 2005; LPWG 2017).

Esta família ocorre em florestas tropicais úmidas na África, América do Sul e Ásia, ainda dominam florestas secas e savanas em todo o trópico, e também ocorrem nas regiões mediterrâneas, desérticas e temperadas, até altas latitudes e elevações (Schrire et al. 2005). Leguminosae foi descrita pela primeira vez em *Familles des plantes* (Adanson 1763), e desde então as principais características morfológicas que delimitam a família, com algumas exceções, são: o ovário unilocular súpero, placentação parietal e fruto do tipo legume com suas variações (Lewis et al. 2005). A família é considerada monofilética e atualmente está dividida em seis subfamílias: Caesalpinioideae DC. (incluindo o clado Mimosoide), Cercidoideae LPWG, Detarioideae Burmeist., Dialioideae LPWG, Duparquetioideae LPWG e Papilionoideae DC. (LPWG 2017).

Papilionoideae é a subfamília mais diversa em número de gêneros e espécies de Leguminosae (Polhill 1994), com aproximadamente 500 gêneros e 14.000 espécies (LPWG 2017). As características morfológicas desta subfamília incluem: flor papilionada [com 5 pétalas, sendo: 1 pétala adaxial na posição média e mais externamente posicionada (estandarte/vexilo), 2 pétalas laterais (asas/alas), 2 pétalas que recobrem gineceu e androceu (pétalas da quilha/carena)]; sépalas unidas (pelo menos na base); radícula do embrião curva; e nódulos radiculares presentes (LPWG 2017). Estudos filogenéticos desenvolvidos com Leguminosae apontam a subfamília Papilionoideae como monofilética (Doyle et al. 2000; Pennington et al. 2001; Kajita et al. 2001; Herendeen et al. 2003; Wojciechowski 2003; Wojciechowski et al. 2004; LPWG 2017).

Papilionoideae possui 28 tribos (Lewis et al. 2005), dentre elas Phaseoleae, que se destaca por ser a mais importante economicamente, além de ser uma das maiores tribos em números de espécies, com aproximadamente 90 gêneros e 1.600 espécies (Schrire

2005). As principais características que definem este grupo de plantas incluem: eófilos simples e opostos, folhas trifolioladas com a base assimétrica, inflorescências em pseudorracemos, espessa camada de endexina sobre o grão de pólen, presença de estípelas e número cromossômico básico de 10 ou 11 (Lackey 1981; Bruneau et al. 1995).

Phaseoleae é considerada para ou polifilética segundo algumas análises filogenéticas moleculares (Bruneau et al. 1990, 1995; Doyle & Doyle 1993; Delgado-Salinas et al. 1993; Doyle et al. 1997, 2000; Kajita et al. 2001; Lee & Hymowitz 2001). Tradicionalmente está dividida em oito subtribos: Diocleinae, Phaseolinae, Cajaninae, Ophrestiinae, Clitoriinae, Kennediinae, Glycininae e Erythrinae (Lackey 1981). A classificação infratribal foi primeiramente circunscrita por Benthham (1837) e desde então não há um consenso com relação ao reconhecimento das subtribos e suas delimitações entre os sistemas taxonômicos propostos (Bruneau et al. 1995).

A classificação infratribal proposta por Lackey (1981), que reconheceu oito subtribos, é a que mais se aproxima daquela de Benthham (1837), que exclui a subtribo Galactieae e Rhynchosieae de Benthham (1837, 1865) e adiciona a subtribo Ophrestiinae. Já Baudet (1978) reconheceu três subtribos (Cajaninae, Glycininae e Phaseolinae) e estabeleceu vários grupos dentro destas subtribos; sendo alguns deles equivalentes às subtribos de outros estudiosos, como por exemplo, o grupo Kennediastreae que é análogo a Kennediinae de Benthham (1837) e Lackey (1981).

Alguns tratamentos anatômicos foram feitos na tribo Phaseoleae, principalmente aqueles referenciados nas obras de Metcalfe & Chalk (1950) e Solereder (1908). Dois trabalhos de destaque foram realizados por Debold (1892) e por Lackey (1978). Estes estudos mostraram a variação da morfologia de tricomas dentro de Phaseoleae, e assim separaram alguns gêneros pelo tipo e pela presença destes tricomas. No estudo de Lackey (l.c.), admite-se que Cajaninae é a subtribo mais “consistente” de Phaseoleae, por seus representantes possuírem estruturas secretoras exclusivas, genericamente denominadas glândulas vesiculares e tricomas de base bulbosa, que estão distribuídas por todo o corpo da planta (Fig. 1-3). Através de nossos estudos sabemos que glândulas vesiculares são as únicas estruturas exclusivas desta subtribo, ademais, Cajaninae também possui tricomas de base secretora em alguns dos seus representantes (Cândido et al. 2016; Vargas et al. 2018a). Além destas estruturas secretoras, Cajaninae possui outras características peculiares que unem o grupo, como: ausência de bractéolas e canavanina e presença de

leucoantocianinas (Baudet 1978; Lackey 1977, 1981), exceto no gênero *Adenodolichos* Harms.

Estudos combinando dados morfológicos e informações de análises filogenéticas são promissores para o entendimento de padrões de mudanças morfológicas (e.g. Fougère-Danezan et al. 2010; Nogueira et al. 2013) ou ainda para compreensão das relações entre famílias e gêneros (e.g. Anderberg e Stahl 1995; Redden et al. 2010; Soh & Panell 2011; Gomes-da-Silva et al. 2012; Monteiro et al. 2015). A reconstrução de caracteres trata-se da recuperação da informação sobre a modificação de pares de caracteres homólogos e diferentes entre si ao longo do tempo (Amorim 1994). Nosso estudo utilizou as estruturas secretoras como alvo para o melhor entendimento dos padrões de mudanças morfológicas, sendo assim podemos observar que as glândulas de *Cajaninae* se diversificaram diversas vezes dentro da subtribo, mostrando diversas homoplasias ao longo do cladograma.

A grande diversidade e riqueza de *Cajaninae* pode ser explicada por várias características, a exemplo da presença das estruturas secretoras, que desempenham papel importante para a inter-relação da planta com o ambiente. Estudos sobre a função secretora nas plantas, incluindo o conhecimento da composição química dos exsudatos e a atividade secretora em nível celular, podem fornecer dados importantes à compreensão da versatilidade funcional das glândulas e ajudar a compreender riqueza, diversificação e distribuição de um certo grupo de plantas (Roshchina & Roshchina 1993; Tresmondi et al. 2017). Através de nossas análises histoquímicas e ultraestruturais sabemos atualmente que as glândulas de *Cajaninae* secretam lipídeos e compostos fenólicos, estes exsudatos auxiliam este grupo de plantas na defesa contra herbívoros e contra possíveis danos causados pela radiação ultravioleta (Vargas et al. 2018b), eventos que ocorrem no ambiente em que os representantes de *Cajaninae* se distribuem.

Cajaninae é a maior subtribo de *Phaseoleae* em número de espécies (ca. 490), e está dividida em 10 gêneros, sendo eles: *Adenodolichos* (ca. 15 spp.), *Paracalyx* S.I. Ali (6 spp.), *Flemingia* Roxb. ex W.T. Aiton (ca. 30 spp.), *Carrissoa* Baker f. (1 sp.), *Chrysoscias* E. Mey. (3-4 spp.), *Cajanus* Adans. (34 spp.), *Dunbaria* Wight & Arn. (20 spp.), *Bolusafr* Kuntze (1sp.), *Eriosema* (DC.) Desv. (ca. 150 spp.) e *Rhynchosia* Lour. (ca. 230 spp.) (Lewis et al. 2005).

Os membros desta subtribo se distribuem desde a região paleotropical até regiões temperadas do Velho Mundo, além de ocorrerem também nas regiões neotropical e subtropical, principalmente em ambientes secos e quentes, como savanas e o Cerrado (Schrire 2005; Cândido 2018). A maioria das espécies de Cajaninae (ca. 400) está distribuída no Velho Mundo, com exceção de aproximadamente 90 espécies pertencentes aos gêneros *Eriosema* e *Rhynchosia*, que possuem distribuição pantropical. No Brasil, a subtribo está representada apenas pelos dois últimos gêneros, com aproximadamente 34 e 19 espécies, respectivamente (Cândido et al. 2014a, b, 2016; Fortuna-Perez et al. 2017; Bezerra et al. in press.).

Morfologicamente, os membros de Cajaninae se caracterizam por apresentar hábito subarbustivo a herbáceo, folhas trifolioladas (ou unifolioladas em *Eriosema* e *Rhynchosia*, ou pentafofoliadas em *Rhynchosia* e *Adenodolichos*), flores amarelas, alaranjadas a avermelhadas, dispostas geralmente em racemos laxos ou congestos e legumes, ora com duas sementes (em *Eriosema*, *Rhynchosia*, *Paracalyx*, *Flemmingia*, *Carrissoa* e *Chrysoscias*), ora com três ou mais sementes (em *Bolusafr*a, *Cajanus*, *Dunbaria* e *Adenodolichos*) (Gear 1970, 1978; Lackey 1981; Harms 1904).

Estudos moleculares de DNA de cloroplasto apontaram Cajaninae como monofilética (Bruneau et al. 1995; Egan et al. 2016; LPWG 2017), contudo vale ressaltar que estes estudos possuem amostragem muito baixa e nenhum deles é focado na subtribo, ou qualquer um dos seus gêneros. Através de análises de DNA das regiões de ITS (nuclear) e rpl32-trnL (UAG) e trnQ (UUG) -5'rps16 (cloroplasto), nossos resultados apontam que Cajaninae é monofilética, bem como os gêneros *Eriosema*, *Flemmingia* e *Adenodolichos*. Os táxons de Cajaninae compartilham muitas características morfológicas e isso pode esclarecer um pouco o arranjo dos táxons nas árvores filogenéticas. É importante salientar que nossa amostragem é ampla, com mais da metade dos representantes da subtribo, além de nossos estudos abordarem morfologia, anatomia, ultraestrutura e filogenia, reforçando nossas conclusões sobre a homogeneidade do grupo, contudo análises de filogenômica podem ser necessárias para um completo entendimento das relações entre os táxons do grupo.

Diante do exposto e considerando que a revisão bibliográfica mostra que existem estruturas secretoras peculiares a este grupo, primeiramente estudadas por Debold (1892) e Lackey (1972) em alguns táxons de Phaseoleae, o primeiro capítulo desta tese apresenta uma revisita aos tricomas foliares ocorrentes em Cajaninae, ampliando a amostragem

(164 táxons da subtribo) e aprofundando as descrições sobre cada tipo de estrutura secretora existente nesta subtribo [Revisiting the leaflet secretory structures in subtribe Cajaninae Benth. (Leguminosae, Phaseoleae)]. Este capítulo foi publicado eletronicamente na revista *International Journal of Plant Sciences* em agosto de 2018. Adicionalmente, nenhum estudo sobre a ultraestrutura e a histoquímica dos tricomas secretores de Cajaninae havia sido conduzido. Neste contexto, o segundo capítulo da presente tese tratou sobre as conexões existentes entre as secreções glandulares e as estruturas subcelulares de três principais glândulas de Cajaninae [Ultrastructure and secretion of glandular trichomes in species of subtribe Cajaninae Benth (Leguminosae, Phaseoleae)]. Este capítulo foi publicado eletronicamente na revista *Protoplasma* em setembro de 2018. Em relação ao pouco conhecimento sobre as relações filogenéticas na subtribo Cajaninae, o terceiro capítulo retratou a filogenia molecular de Cajaninae, com uma ampla amostragem, a fim de verificar sua monofilia e examinar as relações entre seus gêneros. Além disso, provemos a reconstrução de estados ancestrais das estruturas secretoras da subtribo [Filogenia e evolução de caracteres na subtribo Cajaninae (Leguminosae – Phaseoleae)].

Sendo assim, esta tese atendeu os objetivos propostos desde o seu projeto inicial:

- i) conhecer os tipos, a ocorrência, a composição química dos exsudatos e o mecanismo de secreção das estruturas secretoras de Cajaninae;
- ii) testar a monofilia da subtribo;
- iii) esclarecer a delimitação dos gêneros pertencentes a Cajaninae, especialmente *Rhynchosia* e *Eriosema*;
- e iv) reconstruir os estados ancestrais baseados nas estruturas secretoras dos representantes desta subtribo.

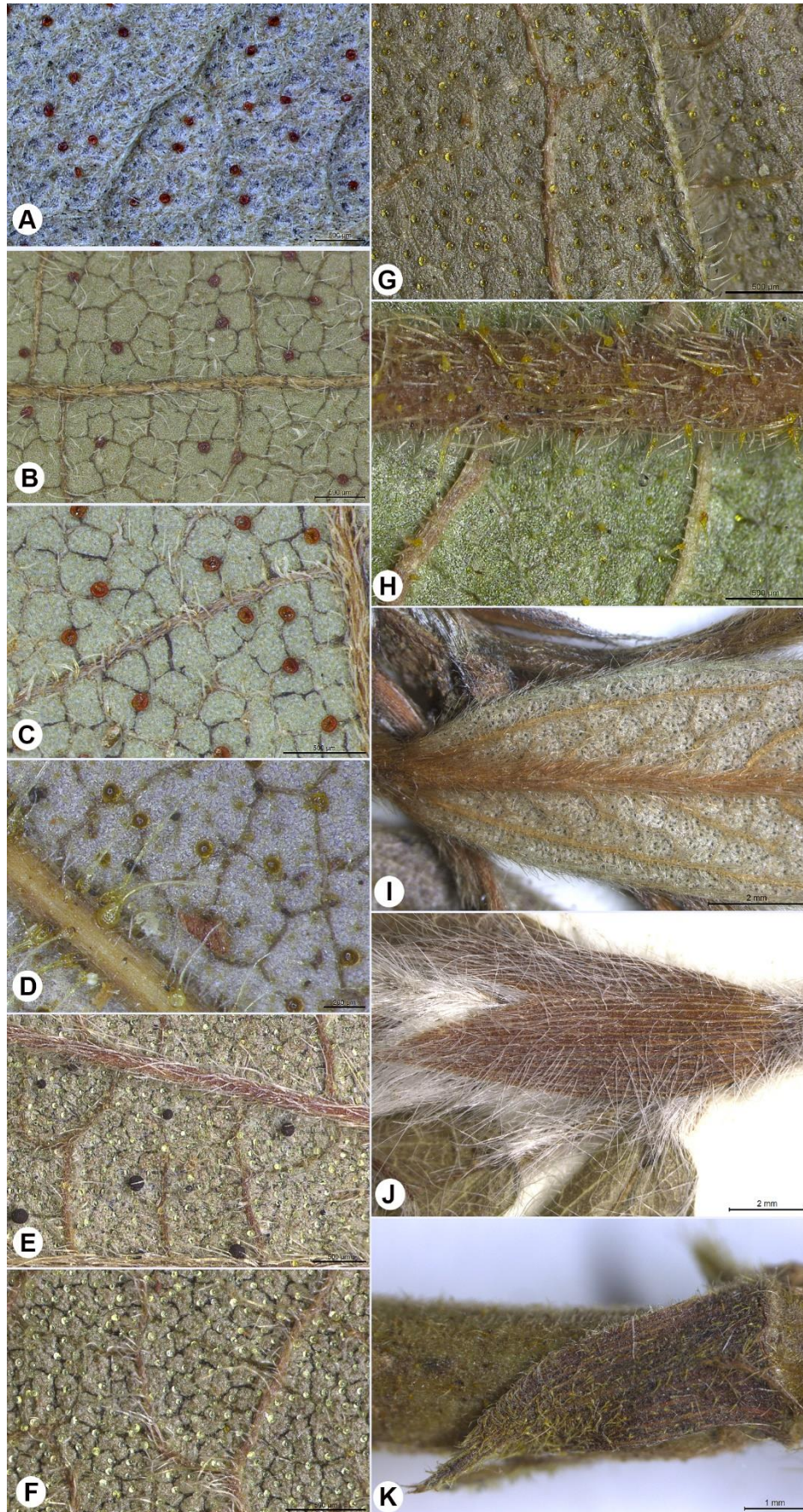


FIG 1: Tricomas glandulares e não glandulares em representantes da subtribo Cajaninae. A. glândulas vesiculares em face abaxial de folíolos de *A. baumii*, B. *A. punctatus*, C. *A.*

rhomboideus, E-F. *E. platycarpon*, G. *E. campestre* var. *delicatula*. D e H. glândulas vesiculares e tricomas de base bulbosa em face abaxial de folíolos *B. bituminosa* (D) e *E. campestre* var. *campestre* (H). I. tricomas tectores em face abaxial de folíolo de *E. violaceum*. J-K. Estípulas com tricomas tectores em *E. tacuaremboense* e glandulares *E. campestre* var. *campestre*.



FIG 2: Imagens de estereomicroscópio mostrando cobertura de tricomas tectores e glandulares sobre flores e inflorescências de representantes de Cajaninae. A. tricomas de base bulbosa sobre cálice de *B. bituminosa*. B-C. tricomas de base bulbosa sobre cálice de *E. campestre* var. *delicatula*. D. glândula vesicular sobre cálice de *E. campestre* var. *delicatula*. E-F. indumento amarelado em cálice de *E. obovatum*.

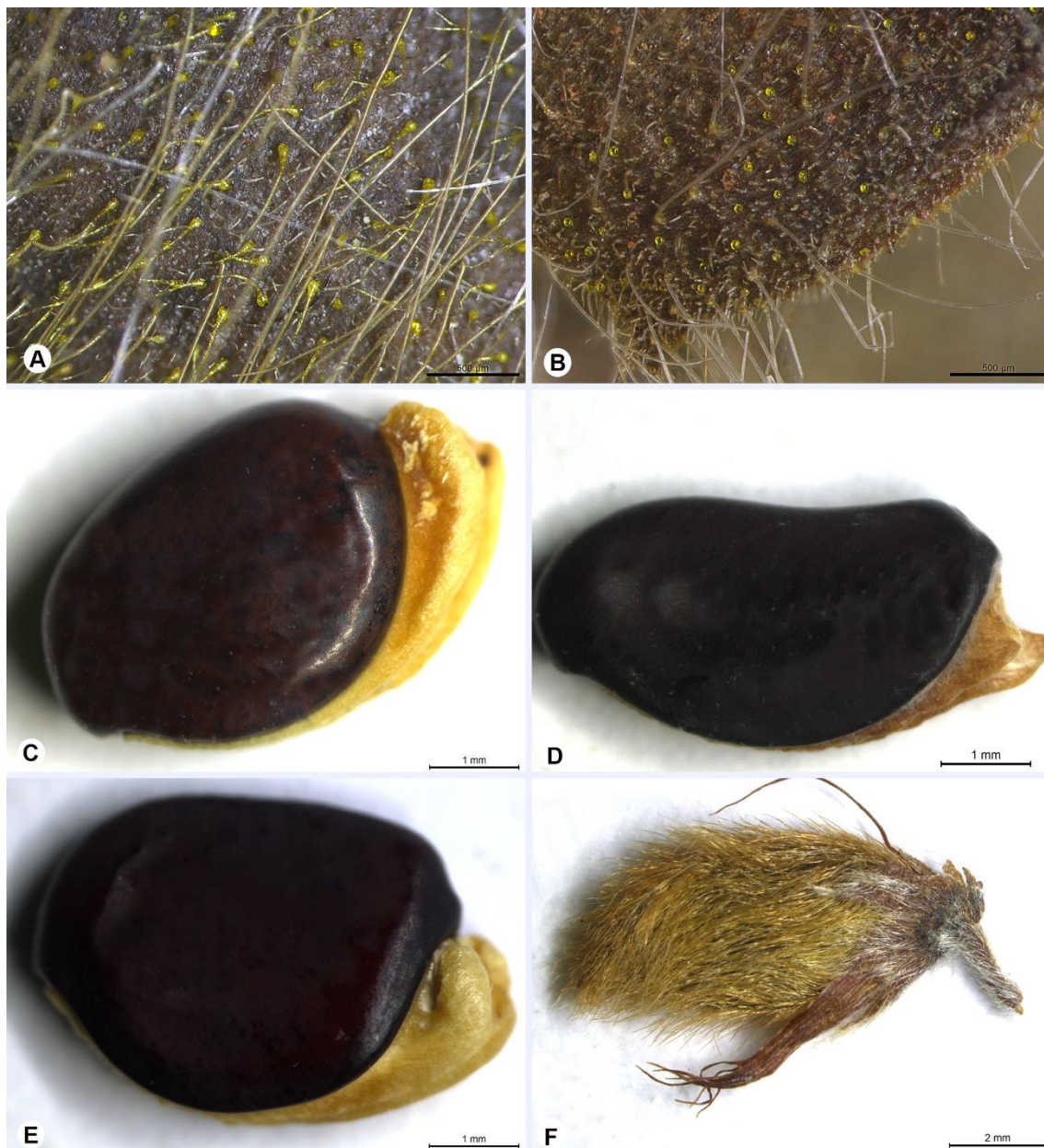


FIG 3: Imagens de estereomicroscópio mostrando morfologia das sementes e cobertura de tricomas tectores e glandulares sobre os frutos de representantes de Cajaninae. A. tricomas tectores e tricomas de base bulbosa em *E. campestre* var. *campestre*. B. tricomas tectores e glândulas vesiculares em fruto de *E. longifolium*. C-E. morfologia das sementes de *E. obovatum*, *E. platycarpon* e *E. tacuarembense*. F. fruto imaturo densamente indumentado de *E. violaceum*.

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Conforme estabelecido pelo Programa de Pós-Graduação em Ciências Biológicas (Botânica) do IBB, UNESP, os resultados obtidos durante a execução do projeto de doutorado foram reunidos em três capítulos, sendo que os dois primeiros já foram publicados e o terceiro está em via de submissão.

Capítulo 1: Revisiting the leaflet secretory structures in subtribe Cajaninae Benth. (Leguminosae, Phaseoleae) - (nas normas da revista International Journal of Plant Sciences)

Capítulo 2: Ultrastructure and secretion of glandular trichomes in species of subtribe Cajaninae Benth (Leguminosae, Phaseoleae) - (nas normas da revista Protoplasma)

Capítulo 3: Filogenia e evolução de caracteres na subtribo Cajaninae (Leguminosae – Phaseoleae) - (nas normas da revista Molecular Phylogenetics and Evolution)

Revisiting the leaflet secretory structures in subtribe Cajaninae Benth. (Leguminosae,
Phaseoleae)

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Shortened title: Revisiting the secretory structures of Cajaninae

KEYWORDS: anatomical traits; Fabaceae; glands; systematics; taxonomy; trichomes.

Abstract

Premise of the Research: Cajaninae is the most speciose subtribe of the legume tribe Phaseoleae, comprising ca. 490 species distributed in 10 genera. The group is characterized by peculiar secretory structures, such as bulbous-based trichomes and vesicular glands. The taxonomic importance of these secretory structures in the tribe Phaseoleae was considered in earlier publications, although few taxa in subtribe Cajaninae were included in those studies. To evaluate the secretory structures on and in the leaflets of representatives of subtribe Cajaninae, anatomical and micromorphological studies were performed.

Methodology: Cross sections of the leaflets were studied using light microscopy, Scanning Electron Microscopy (SEM) and trichome types were identified for 164 taxa in nine genera of Cajaninae.

Pivotal Results: A great diversity of trichome forms exists in the studied group, including capitate trichomes, secretory-base trichomes, bulbous-based trichomes, vesicular glands, and secretory idioblasts in the midrib. These are distributed on or in the leaflet blades of 164 taxa in nine genera belonging to Cajaninae and vary in morphology, abundance and occurrence. We describe, for the first time, the glands and idioblasts found within Cajaninae and can confirm that the vesicular glands are the sole type that can be considered as a unifying character of the subtribe.

Conclusions: Our findings suggest that caution is needed when interpreting the morphological variation of the types of secretory structures because within the same trichome type there can be different cell numbers and forms. The present report expands upon earlier findings and revisits, in detail, gland types reported in previous studies.

Introduction

Studies on secretory structures in Leguminosae have proven useful taxonomically, as reported over 100 years ago by Solereder (1908) for tannin sacs with brown contents, and by Lersten and Brubaker (1987); Turner (1986); Teixeira et al. (2000); Teixeira and Rocha (2009); Sartori and Tozzi (2002); Marquiasfável et al. (2007), and Silva et al. (2018), among others for nectaries, cavities and idioblasts in some genera of Papilionoideae.

Function, type, and structural studies of trichomes have been published for Leguminosae (especially Papilionoideae), e.g. Baudet (1973); Baudet and Marechal (1976); Kothari and Shah (1975); Flores and Epinoza (1977); Gupta and Murphy (1977); Leelavathi and Ramayya (1983); Marquiasfável et al. (2009); Sengsai et al. (2014); Marinho et al. (2015) and particularly Debold (1892) and Lackey (1978) for secretory trichomes in tribe Phaseoleae and Vargas et al. (2015) in *Rhynchosia* Lour. (in subtribe Cajaninae).

Throughout the Angiosperms, glandular trichomes can be found on all aerial plant-parts (Werker 2000) and display a great diversity in morphology, with variations in shape, size, structure, function, location, and pattern of development (Argyropoulou et al. 2010). Applied scientists and character evolutionists have studied glandular trichomes because their secreted products may have commercial value or because their morphology is diagnostic in systematic classification (Duke 1994; Ascensão et al. 1995). In previous studies of tribe Phaseoleae, a large part of the significant taxonomic data has focused on the variation in morphology of the trichomes, as shown in the works of Debold (1892) and Lackey (1978, 1981). Multicellular glandular trichomes are present throughout the tribe, whilst vesicular glands and bulbous-based trichomes are exclusive to subtribe Cajaninae (Lackey 1978, 1981).

A plant structure with vesicular glands is sometimes referred to as “glandular punctate”. Such glands consist of a spherical head and short stalk, sometimes inserted in an epidermal depression (Solereder 1908; Lackey 1978, 1981), and a trapezoidal to square basal cell (pers. obs.). Furthermore, in this type of gland all cells in its body break down to form a yellow glandular fluid (Uphof 1962). On the other hand, bulbous-based trichomes are composed of one to two basal cells, a group of cells that form the “bulb”, apically elongated cells, and slightly rounded terminal cell (Solereder 1908; Vargas et al. 2015). These two peculiar types of glandular trichome were described in the dissertation of Debold (1892), discussed as important secretory trichomes of tribe Phaseoleae (subtribe Cajaninae) by Solereder (1908), and later affirmed as exclusive to subtribe Cajaninae by Lackey (1978, 1981).

Cajaninae is the most speciose subtribe of Phaseoleae (comprising approximately 490 species) and is subdivided into 10 genera: *Adenodolichos* Harms (15 spp.), *Paracalyx* S.I.Ali (6 spp.), *Flemingia* Roxb. ex W.T. Aiton (30 spp.), *Chrysoscias* E. Mey. (3-4 spp.), *Carrissoa* Baker f. (1sp.), *Cajanus* Adans. (34 spp.), *Dunbaria* Wight and Arn. (20 spp.), *Bolusafr*a Kuntze (1 sp.), *Eriosema* (DC.) Desv. (150 spp.) and *Rhynchosia* (230 spp.) (Schrire 2005). Most genera are distributed in the Old World (Africa, Asia) and Oceania, except for the most speciose genera, *Eriosema* and *Rhynchosia* (comprising 150 and 240 species, respectively), which are pantropical (Schrire 2005). The members of the subtribe grow in warm, dry environments, with most occurring in savannas and Cerrado (Grear 1970, 1978; Schrire 2005).

Despite the richness and wide distribution of Cajaninae species, few anatomical studies of the subtribe have been published. Among them are the master’s thesis of Debold (1892), which treated a small number of species belonging to five genera; Lackey’s (1978) review which included 17 species in seven genera; the study by Vargas

et al. (2015) of a few species of *Rhynchosia*; and some general observations in the studies of Solereder (1908) and Metcalfe and Chalk (1950).

Considering that the secretory structures have been shown to be useful for the taxonomy of several groups of Leguminosae and that the subtribe Cajaninae is supported by the presence of exclusive types of secretory trichomes, a study with greater sampling and detailed analyses of these structures of different Cajaninae groups was considered to be timely. In this report we consider the secretory structures found in a sample of 164 taxa of nine genera currently included in Cajaninae and, in consequence, our study expands upon the earlier findings of Lackey (1978) and Debold (1892).

Material and methods

Plant material

We sampled 164 taxa of Cajaninae belonging to nine (of the total ten) genera in the subtribe (including all species of *Bolusafrá*, *Paracalyx*, and *Chrysoscias*, 60% of the species of *Dunbaria*, c. 51% of *Eriosema*, 50% of *Flemingia* and *Cajanus*, 40% of *Adenodolichos*, and c. 11% of *Rhynchosia*) (Table 1).

Most of the material was obtained from specimens in national and foreign herbaria, while a few taxa were collected fresh in the field. Vouchers of the newly collected plants were deposited in the Irina Delanova Gemtchújnicov Herbarium (BOTU) in the Institute of Biosciences of Botucatu, UNESP. A graph (Fig. 2) summarizes the occurrence and distribution of each type of secretory structure on/or in the leaflets of the Cajaninae taxa studied.

Light microscopy

Leaflets were first embedded in Histo-resin (Leica®) and cross sections of the middle portion of the leaflets were prepared using a rotary microtome. Sections (10µm

thick) were stained with Toluidine pH 4 (Vidal 1977) and mounted with Entellan®. Images were obtained using a Leica® digital camera linked to a light microscope.

Mature leaflets from herbarium specimens were rehydrated following the procedure of Smith and Smith (1942) and stored in 70% ethanol. Whenever available, up to three specimens were analysed for each taxon. Mature leaflets were collected from Cerrado vegetation (= savanna) in the states of São Paulo, Minas Gerais, Goiás and Mato Grosso do Sul. These leaflets were fixed in formalin-acetic acid-alcohol (FAA) for 24 hours (Johansen 1940).

Scanning Electron Microscopy (SEM)

For observations with SEM, samples were removed from herbarium specimens, mounted on aluminum stubs, sputter-coated with gold, examined with a FEI Quanta 200 scanning electron microscope (voltage of 12.5 kV) and documented using a digital image capturing system coupled to the electron microscope. These procedures were carried out in the Electron Microscopy Center of the Institute of Biosciences of Botucatu, UNESP.

The trichome types were identified following the classification of Payne (1978); Theobald et al. (1979); Lackey (1977, 1978, 1981); Leelavathi and Ramayya (1983), and Cândido et al. (2016). A list of nomenclature equivalences was performed to clarify the terms used by us to describe the secretory structures of Cajaninae and the terminology found in the literature (Table 2).

Results

The secretory structures found in leaflets of the studied taxa include vesicular glands, bulbous-based trichomes, capitate trichomes, secretory-base trichomes, and idioblasts. These structures were evaluated according to their morphology, occurrence, and position in the leaflets: adaxial-abaxial surfaces for glandular trichomes and midrib region for idioblasts (Table 1).

Here we describe the main groups of secretory structures observed in representatives of *Cajaninae*, and summarize the variation observed in Figure 1. It is important to note that the same category of secretory structure may vary in shape and in the number of cells which constitute it.

Vesicular glands are the most frequent secretory structure in the *Cajaninae*, followed by secretory idioblasts in the leaflet midrib, and capitate trichomes (Fig. 2). Bulbous-based trichomes are the least frequent in the taxa studied (Fig. 2). Secretory-base trichomes were the only gland type that was not present in all genera analysed (see below).

- *Idioblasts* (Fig. 3)

The idioblasts found in *Cajaninae* are spherical cells (staining dark blue in Toluidine blue), located in the leaflet midrib, and distributed between the cells of the phloem or in the parenchyma (Fig. 3, arrowhead). In the mesophyll of all *Cajaninae* taxa, mainly in the paravenal and palisade parenchyma, several cells have brown contents (Fig. 3b, white arrow), but we do not recognize these as idioblasts. These are present in all genera of *Cajaninae*, but their presence varies between taxa and, thus, can have diagnostic value, differentiating between similar species (Table 1).

- *Vesicular glands* (Figs. 1 [A-E], 4, and 5 [A-B, F, I, J, L])

Foot (Fig. 4): 1- to 2-celled; trapezoidal form in most taxa; thin wall. Stalk (Fig. 4): Unicellular to absent. Body/Head (Fig 4 and 5): Multiseriate; multicellular; contents sparse; circular, oval or club-shaped; present a cavity in the center of the head or cells side by side forming the head of the glandular trichome; thin walled; cuticular vesicle covering the head of the gland; variable number of cells in the

head. The vesicular gland occurs more frequently on the abaxial surface of the leaflets (Fig. 2) and was present in almost all taxa analysed except for *Adenodolichos paniculatus* and *A. rupestris* (Table 1).

- *Bulbous-based trichomes* (Figs. 1 [F-J], 5 [D and G], and 6 [A-F])

Foot (Fig. 6D-E): 1-celled; without contents; thin walled. Stalk: Unicellular with trapezoidal shape or absent. Body (divided into the bulbous part, the neck and a terminal part) (Fig. 6A-F): Multiseriate; the basal part consists of a bulb and a long neck, and the terminal part is indistinct to 4-celled; the bulbous part is inflated, 4 to 8 cells in length and 2 to 5 cells in width in the middle region (Figs. 1F-J and 6B); the neck is longer than broad, 2 to 6 cells in length and 1 to 2 cells in width (Fig. 1F-J). Cells are usually broader than long in the bulbous part, and longer than broad in the neck. Terminal cell is rounded or conical, and sometimes not distinguishable (Fig. 6C). Bulbous-based trichomes are more unusual than vesicular glands but are distributed in all genera of *Cajaninae*, with their position on the surface of the leaflets varying amongst taxa (Table 1).

- *Secretory-base trichomes* (Figs. 1 [K-O] and 6 [G-I])

Foot (Fig. 6G-I): 1-celled; large and hexagonal; dense, flocculent contents stained pink by toluidine blue (Fig. 6G). Body (Fig 6G-I): Uniseriate; multicellular; 1 to 2 intermediary cells with thick, unlignified walls; 1 terminal cell filiform, elongated and acuminate. These secretory structures are very similar to non-glandular trichomes, except for their secretory base. They are common in the genera *Dunbaria*, *Eriosema*, *Flemingia* and *Rhynchosia* (Table 1).

- *Capitate trichomes* (Figs. 1 [P-X] and 6 [J-Q])

Foot: 1-celled; without contents; thin walled; usually rectangular to square or trapezoidal in shape. Stalk (6N-Q): Unicellular, thin walled; square to broader than long to absent. Body/Head: clavate; 2 to 5 cells in length, and 1 to 4 cells in width; 1 to 2-celled in the basal part of the head. They are common in all genera of Cajaninae (Table 1).

Discussion

This study presents the inventory and description of the leaflet secretory structures (idioblasts and trichomes) found in fully expanded leaflets of 164 taxa in nine genera of subtribe Cajaninae. This is a taxonomic and geographical representative sample of the approximately 490 species in 10 genera of Cajaninae.

Vesicular glands and bulbous-based trichomes had previously been reported in leaflets of a few taxa of Cajaninae (Debold 1892; Solereder 1908; Lackey 1978; Gear 1970, 1978), as well as in calyces, corollas and stems (Gear 1970, 1978), but there existed no detailed overview for the subtribe.

In the present study, all genera of Cajaninae have vesicular glands, bulbous-based trichomes, idioblasts, and capitate trichomes. Lackey (1978) asserted that Cajaninae was well supported morphologically as a subtribe due to the presence of the bulbous-based trichomes and vesicular glands. Although based on low sampling, studies of the phylogeny of the tribe Phaseoleae support the Cajaninae as monophyletic (Kajita et al. 2001; Wojciechowski et al. 2004; Egan et al. 2016).

Bulbous-based trichomes

Although the bulbous-based trichomes are present in all Cajaninae genera analysed here, they are not as frequent as vesicular glands. They are present in only one taxon of the genera *Adenodolichos* (*A. punctatus*), *Chrysoscias* (*C. grandiflora*),

Flemingia (*F. involucrata*), and *Paracalyx* (*P. somalorum*); in 4 to 6 taxa of *Cajanus* and *Dunbaria*; and in a maximum of ten taxa in the most speciose genera, *Eriosema* and *Rhynchosia*. This trichome type was not observed in Lackey's (1978) study of the genera *Flemingia*, *Eriosema*, and *Paracalyx*, likely due to low taxon sampling in that study.

We sampled six species of *Adenodolichos*, but only one has the bulbous-based trichomes, and only on the abaxial surface of the leaflets; four species have vesicular glands on both surfaces of their leaflets. This indicates that thorough sampling is needed to confirm the absence or presence of these secretory structures, certainly at the genus level. Based on the analysis of one specimen, Lackey (1977, 1978) stated that the genus *Adenodolichos* does not possess the distinctive glands of the Cajaninae and suggested that it should be taxonomically placed outside of Cajaninae and Phaseoleae. Three years later Lackey (1981) found bulbous-based trichomes on *Adenodolichos* leaflets and, due to the presence of these, he then considered the genus to be related to Cajaninae. Although *Adenodolichos* possesses bracteoles and canavavine (a free amino acid), unlike the other genera of Cajaninae, it was included in the subtribe (Lackey 1981). Currently, *Adenodolichos* appears inside subtribe Cajaninae in the phylogenetic study of Egan et al. (2016).

The bulbous-based trichomes and capitate trichomes (described as “bulbous hairs/bottle-shaped hairs” and “small capitate hairs”, respectively by Horner and Zindler-Frank 1982) are first developed on very young leaflets, so with leaf expansion the number of trichomes per unit area decreases (Horner and Zindler-Frank 1982). This might explain why these trichomes are observed less frequently than the vesicular glands which are present in almost all taxa sampled. Studies comparing the number of vesicular glands and bulbous-based trichomes in young and adult leaflets are, thus, desirable to obtain more complete data.

The term “uniseriate filiform obpyriform hairs” was considered to be synonymous with “bulbous glandular hairs” by Leelavathi and Ramayya (1983), who used this terminology to describe the bulbous-based trichomes. While working with trichomes in subfamily Papilionoideae, Leelavathi and Ramayya (1983) found this type of trichome in genera that do not belong to subtribe Cajaninae, such as *Alysicarpus* Neck. ex Desv., *Desmodium* Desv., and *Stylosanthes* Sw. This suggests that bulbous-based trichomes are not a morphological character that assists in diagnosing subtribe Cajaninae, in disagreement with the conclusion of Lackey (1978).

Capitate trichomes

The structures termed “stalked glands” and “unstaked glands” by Lackey (1978), and “uniseriate filiform capitate hairs” or “glandular hairs” by Leelavathi and Ramayya (1983) are similar to the structures that we here describe as capitate trichomes. Leelavathi and Ramayya (1983) found these structures to be common within the genera *Atylosia* Wight & Arn. (= *Cajanus*) and *Rhynchosia* of the Cajaninae, and also within other genera of Papilionoideae, including *Alysicarpus*, *Clitoria* L. and *Desmodium*.

According to Lackey (1977), the stalked glands are frequent in other taxa of tribe Phaseoleae and aid in supporting the relationship between the subtribes Phaseolinae, Kennediinae and Diocleinae. Lackey suggested that the presence of these glands in these three groups have an independent origin., but this relationship has not been confirmed by more recent phylogenetic studies (Kajita et al. 2001; Wojciechowski et al. 2004), nor by our anatomical studies in contrast to the findings of Lackey (1978), who observed this trichome type within Cajaninae only in *Atylosia trinervia* (DC.) Gamble (= *Cajanus trinervius*), we have observed these structures in almost all sampled representatives of the subtribe, including *Cajanus trinervius*. The trichome that Lackey (1977, 1978) called an “unstaked gland” he found present in almost all the representatives of Cajaninae, except

in *Fagelia bituminosa* (L.) DC. (= *Bolusafra bituminosa*), *Dunbaria villosa*, and *Endomallus spirei* Gagnep. (= *Cajanus goensis*). This contrasts with our findings that capitate trichomes are also present in these three species.

Vesicular glands

Vesicular glands found in the present study are widely cited as occurring only in Cajaninae in broader studies (Solereder 1908; Metcalfe and Chalk 1950) to more focused surveys (Debold 1892; Grear 1970, 1978; Lackey 1977, 1978; Vargas et al. 2015). Undoubtedly, this secretory structure is a character that assists in the taxonomic delimitation of Cajaninae.

Vesicular glands were referred to as “uniseriate filiform vesicular clavate hairs” by Leelavathi and Ramayya (1983). Although these authors did not cite the widely recognized term for this gland type, they studied several taxa of Papilionoideae and only found these secretory structures in *Atylosia scarabaeoides* (= *Cajanus scarabaeoides*), *Rhynchosia aurea* (Rottler) DC., and *R. minima*, further reinforcing the existence of this trichome type only in Cajaninae.

Vesicular glands were also treated as a synonym of “nectar glands” by Leelavathi and Ramayya (1983), although we could find no literature citing these glands as sugar secretors. Moreover, structurally they have no configuration of a nectar gland or any type of nectary.

To a certain extent, the vesicular gland can be compared to the uncommon cavitated secretory trichomes described for Leguminosae in *Bauhinia* L. s.s. (Marinho et al. 2015), *Hoffmannseggia* Cav. and *Caesalpinia* L. s.l. (Lersten and Curtis 1994, 1996), *Centrolobium* Mart. ex Benth. (Matos and Paiva 2012), *Indigofera* L. (Marquiefável et al. 2009), and some genera of the families Asteraceae (Carlquist 1959), Lythraceae

(Uphof 1962), and Rutaceae (Zhou et al. 2012). These secretory structures have a stalk that connects the secretory cavitated head to the epidermis, and the lumen can vary in size and shape; in some species it may resemble intercellular spaces (Lersten and Curtis 1994; Marinho et al. 2015). Based on this, the distinction between the vesicular gland and the uncommon cavitated secretory trichomes is very difficult to determine and will be resolved only with ontogeny and ultrastructure studies. We are currently working on the latter.

Secretory-base trichomes

The secretory-base trichomes were not present in all Cajaninae genera and are thus important diagnostic genus-level characters within the subtribe. These secretory structures apparently were neglected in earlier studies. We have recently described them as a new secretory structure for the tribe Phaseoleae (Cândido et al. 2016). “Multiseriate ventricose hairs,” as described by Leelavathi and Ramayya (1983), are a structure that morphologically resembles the secretory-base trichomes, but the former is not secretory (i.e., it is non-glandular) and the latter is. Secretory-base trichomes stand out as being a differentiating secretory structure among Cajaninae genera because they do not occur in *Adenodolichos*, *Bolusafr*a, *Cajanus*, and *Paracalyx*.

Idioblasts

Idioblasts are considered to be those cells that differ from neighboring cells by shape, size and content (Esau 1977). In the mesophyll of the Cajaninae representatives, mainly in the paravenal parenchyma or palisade parenchyma, several cells can be confused with idioblasts due to their brown content. According to Metcalfe and Chalk (1950), these are morphologically indistinguishable from their neighboring cells except that they contain accumulated contents. This applies to tannin cells, which are most conspicuous in herbarium material, as is the case for most of the material used in this

study. We do not consider these cells idioblasts because they are not isolated and differentiated from the other adjacent cells.

The internal secretory cells observed in some Cajaninae taxa differ from neighboring cells because they have a rounded shape and dense, dark blue contents. Therefore, we have classified them as secretory idioblasts. These secretory structures vary in content in the several species in which they occur, are common in Papilionoideae, and were described as tannin-sacs by Solereder (1908) for *Tephrosia* Pers., *Machaerium* Pers., and *Sophora* L., among others.

Secretory idioblasts in the midrib and smaller vessels are new findings for Cajaninae. We observe that the occurrence of idioblasts has diagnostic value for some taxa of Cajaninae, such as *Eriosema glabrum* Mart. ex Benth. and *E. elegans* Fort.-Perez and M.J. Silva. The systematic utility of the idioblast has already been shown in other studies, such as that of Metcalfe and Chalk (1950), in which idioblasts were referred to as pellucid dots in the leaf, common structures in *Lonchocarpus* Kunth., and of systematic significance at the infrageneric taxonomic level (Teixeira et. al. 2000). In addition, the occurrence of secretory idioblasts in the mesophyll of the leaflets is also of diagnostic value for *Zornia* J.F. Gmel. (Fortuna-Perez et al., 2012).

Conclusions

For the first time, the glands and idioblasts found in subtribe Cajaninae are described in detail. The secretory structures of this remarkable subtribe of tribe Phaseoleae previously had been little explored, despite their alleged diagnostic significance for the systematics of the group. No previous anatomical study of the subtribes of Phaseoleae has sampled so widely, with more than 30% of the species and 90% of the genera included.

In our study, the morphology and distribution of the secretory structures allow us to confirm that the vesicular glands are the only secretory type that can be considered as a unifying character of *Cajaninae*, and not the bulbous-based trichomes as was previously suggested by earlier authors. In addition, we note that bulbous-based trichomes had already been reported for other genera of *Papilionoideae* and that they should no longer be considered specific to *Cajaninae*.

Our findings suggest that caution is needed when interpreting the morphological variation of the types of secretory structures that can be found in *Cajaninae*. Within the same type of trichome, we can find different cell numbers, but this variation should not alone determine the recognition of new trichome types. Although we illustrate the morphological variations found in the same type of secretory trichome, we do not consider these small changes as a diagnostic feature.

Working with herbarium material is advantageous to obtain a good representative coverage of taxa. On the other hand, it has the disadvantage of not being able to investigate the histochemistry, ontogeny and ultrastructure, which could add interesting data for this work which aims to better understand the distribution, development and morphological variation of these secretory structures.

In summary, future studies with fresh samples of *Cajaninae* taxa will help to interpret the variations we identified in the secretory structures. Such studies open new perspectives on the taxonomic value (differentiating amongst morphologically similar *Cajaninae* taxa) and ecological function (providing information about the biology and the chemical nature of the exudate produced to better understand the function of these glands) of the secretory structures in this legume group. Studies involving the ultrastructure, histochemistry and evolutionary relationships of these secretory structures are already underway.

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Table 1. Taxa studied, vouchers used and distribution of secretory structures in *Cajaninae* leaflets

Species and infraspecific taxa	Voucher specimen (s)	Country	Vesicular	Gland	Bulbous- based trichomes	Capitate trichomes	Secretary- base trichomes	Idioblasts
			D	B				
<i>Adenodolichos baumii</i> Harms	MG Bingham 14158 (K); JP Laveridge 403 (PRE)	South Africa	+	+	-	-	-	+
<i>Adenodolichos kaessneri</i> Harms	R Devired (PRE 3590)	South Africa	-	+	-	-	-	-
<i>Adenodolichos paniculatus</i> (Hua) Hutch. & Dalziel	BO Daramola 257 (K); PK Rwaburindore 3860 (PRE)	Uganda	-	+	-	-	-	+
<i>Adenodolichos punctatus</i> (Micheli) Harms	EA de Leyser 26 (K); J Pawek 9896 (PRE)	Malawi	-	-	-	+	-	+
<i>Adenodolichos rhomboideus</i> Harms	HM Richards 5927 (K); EA Robinson 3794 (PRE)	Angola	-	+	-	-	+	+
<i>Adenodolichos rupestris</i> Verdc.	AA Bullock 2631 (K)	Zambia	-	-	-	-	+	+
<i>Bolusafrá bituminosa</i> (L.) Kuntze	C Cowell and N Jodamus 1174 (K); A Hafström and G Lindeberg (S 13-6627); A Hafström (S 13-6630)	South Africa	-	+	+	+	-	+
<i>Cajanus albicans</i> (Wight & Arn.) Maesen	KM Matthew 43616 (K)	India	-	+	-	-	+	-
<i>Cajanus cajan</i> (L.) Huth	OO Miller and JR Johnston 139 (P)	Venezuela	+	+	-	-	-	-
<i>Cajanus cajanifolius</i> (Haines) Maesen	LJG van der Maesen 2739 (K)	India	+	+	-	-	+	-
<i>Cajanus cinereus</i> (F. Muell. ex Benth.) F. Muell.	MJS Sands 4590 (K)	Australia	+	+	+	+	+	-
<i>Cajanus crassus</i> (Prain ex King) Maesen	HHD McLaren 11056 (K)	China	-	+	-	-	-	-
<i>Cajanus flavus</i> DC.	CGT Kotschy 266 (P)	Ethiopia	+	+	-	-	-	-
<i>Cajanus goensis</i> Dalzell	P Remanandan 4835 (K)		-	+	-	+	-	-
<i>Cajanus grandiflorus</i> (Benth. ex Baker) Maesen	G Forrest 18602 (K)	South Africa	-	+	-	-	+	+
<i>Cajanus indicus</i> Spreng.	HF Mooney 1946 (K)	India	+	+	-	-	+	-
<i>Cajanus lineata</i> (Wight & Arn.) Maesen	CJ Saldanha 15837 (K)	India	+	+	-	+	-	-
<i>Cajanus marmoratus</i> (R. Br. ex Benth.) F. Muell.	JR Maconochie 1730 (K)	Australia	-	+	-	-	+	+

<i>Cajanus platycarpus</i> (Benth.) Maesen	P Remanandan 4557 (K)	India	+	+	+	+	+	+	-	-	+
<i>Cajanus reticulatus</i> (Dryand.) F. Muell.	B Verdcourt and White 4870 (K)	New Guinea	+	+	-	-	+	-	-	-	-
<i>Cajanus rugosus</i> (Wight & Arn.) Maesen	LJG van der Maesen 4033 (K)	India	+	+	-	-	-	+	-	-	-
<i>Cajanus scarabaeoides</i> (L.) Thouars	AN Egan 13-0733 (K)	Thailand	+	+	-	-	-	+	-	-	+
<i>Cajanus trinervia</i> (DC.) Maesen	LJG van der Maesen 4159 (K)	Srilanka	+	+	-	-	+	+	-	-	-
<i>Cajanus volubilis</i> (Blanco) Blanco	P Boyce et al. 121 (K)	Indochina	-	+	-	-	-	+	-	-	+
<i>Chrysoscias calycina</i> E. Mey.	SL Williams 841 (K)	South Africa	-	+	-	-	+	-	-	-	+
<i>Chrysoscias grandiflora</i> E. Mey.	HT Scharf 1433 (K)	South Africa	-	+	+	+	+	+	-	-	+
<i>Chrysoscias parviflora</i> E. Mey.	E Esterhuysen 13330 (K)	South Africa	-	+	-	-	+	-	-	-	+
<i>Dunbaria bella</i> Prain	Chantaronothai et al. 854 (K)	Thailand	-	+	-	-	+	+	+	+	+
<i>Dunbaria cumingiana</i> Benth.	Kessler et al. 1186 (K)	Indonesia	-	+	+	+	+	-	-	+	-
<i>Dunbaria debilis</i> Baker	H Steimann 47798 (K)	New Guinea	-	+	-	+	+	-	-	-	+
<i>Dunbaria ferruginea</i> Wight & Arn.	Fagerlind and Klackenberg 10216 (K)	India	+	+	-	-	+	+	-	-	-
<i>Dunbaria fusca</i> (Wall.) Kurz	AN Egan 13-0808 (K)	Thailand	+	+	+	+	-	+	-	-	-
<i>Dunbaria glandulosa</i> Prain	K Larsen and SS Larsen (K 1452185)	Thailand	+	+	-	+	+	+	-	-	-
<i>Dunbaria incana</i> (Zoll. & Moritz) Maesen	Iboet 461 (K)	Sumatra	-	+	+	-	+	+	-	-	-
<i>Dunbaria podocarpa</i> Kurz	NV Du et al. 2604 (K)	Vietnam	+	+	+	+	+	+	-	-	+
<i>Dunbaria punctata</i> (Wight & Arn.) Benth.	Backer 20921 (K)	Arch. Madoera	+	+	+	+	+	+	-	-	-
<i>Dunbaria subrhombea</i> (Miq.) Hemsl.	J Klackenberg 1123 (S)	Laos	+	+	-	-	+	+	-	-	-
<i>Dunbaria truncata</i> (Miq.) Maesen	Backer 1907 (K)	Indonesia	+	+	-	-	-	+	-	-	-
<i>Dunbaria villosa</i> (Thunb.) Makino	J Klackenberg 1123 (K); BR Yinger et al. 2634 (K)	Korea	+	+	-	+	-	+	-	-	-
<i>Eriosema adamaouense</i> Jacq. - Fél.	H Jacques-Félix 8734 (S)	Cameroun	-	+	-	-	+	+	-	-	+
<i>Eriosema adamii</i> Jacq. -Fél.	JG Adam 14687 (P)	New Guinea	-	+	-	-	-	+	-	+	-
<i>Eriosema affine</i> De Wild.	E Emanuelsson 406 (S)		+	+	-	-	+	+	-	-	-
<i>Eriosema andohii</i> Milne-Redh.	R Demange 2670 (P)	South Africa	-	+	-	-	-	+	-	-	+
<i>Eriosema angolense</i> Baker f.	M Reekmans 6308 (K)	Nigeria	+	+	-	+	-	+	-	-	+

<i>Eriosema bauchiense</i> Hutch. & Dalziel	HM Richards 20643 (S)	Congo	+	+	-	-	+	+	-	-	+
<i>Eriosema brachyrhachis</i> Harms	AP Duarte and A Matos 8365 (NY)	Brazil	+	+	-	-	+	+	-	-	-
<i>Eriosema brevipes</i> Grear	AP Fortuna-Perez 2101 (BOTU)	Brazil	-	+	-	-	+	-	-	-	+
<i>Eriosema campestre</i> var. <i>campestre</i> Benth.	W Vargas 70 (BOTU)	Brazil	+	+	+	-	+	+	-	+	+
<i>Eriosema campestre</i> var. <i>macrophyllum</i> (Grear) Fortunato	AP Fortuna-Perez 1436 (OUPR); HS Irwin et al. 11593 (NY)	Brazil	-	+	+	-	+	+	-	+	+
<i>Eriosema virgatum</i> Benth.	(P02749688)	India	-	+	-	-	-	+	+	-	+
<i>Eriosema chinense</i> Vogel	ED Merrill 878 (SI)	China	+	+	-	-	-	+	-	-	+
<i>Eriosema chrysadenium</i> Taub.	FJ Breteler 11638 (S)	Tanzanie	-	+	+	-	-	+	-	+	-
<i>Eriosema congestum</i> Benth.	MJ Silva 7396 (UFG)	Brazil	+	+	-	-	+	-	-	-	-
<i>Eriosema cordatum</i> E. Mey.	CH Stirton 3898 (P); CH Stirton 489 (S); K Grieve 987 (PRE)	South Africa	-	+	-	-	-	+	-	-	-
<i>Eriosema crinitum</i> var. <i>fusiforme</i> (Rusby) Grear	HF Leitão Filho et al. 635 (UEC); A Flores and RA Rodrigues 539 (UEC)	Brazil	-	+	-	-	+	+	-	+	+
<i>Eriosema crinitum</i> var. <i>crinitum</i> (Kunth) G. Don	ES Cândido et al. 1075 (OUPR)	Brazil	-	+	+	+	-	+	-	+	+
<i>Eriosema crinitum</i> var. <i>discolor</i> Fortunato	J Correa Gomes Jr 1660 (JB); WR Anderson 7333 (UB)	Brazil	-	+	-	+	-	+	-	+	+
<i>Eriosema crinitum</i> var. <i>pulchellum</i> Benth.	W Mantovani 1177 (SP); Irwin et al. 21371 (SP)	Brazil	-	+	-	-	+	+	+	+	+
<i>Eriosema crinitum</i> var. <i>stipulare</i> (Benth.) Fortunato	MA Silva 4111 (HUTO)	Brazil	-	+	-	-	+	-	+	+	+
<i>Eriosema defoliatum</i> Benth.	ES Cândido 1500 (BOTU)	Brazil	+	+	-	-	+	+	-	-	+
<i>Eriosema distinctum</i> N.E. Br.	CH Stirton 708 (S)	South Africa	-	+	-	-	+	+	+	+	-
<i>Eriosema dregei</i> E. Mey.	A Abbott 9272 (K)	South Africa	-	+	-	-	+	+	-	-	+
<i>Eriosema elegans</i> Fort.-Perez & M.J. Silva	MJ Silva 7228 (UFG); AP Fortuna-Perez 2000 (BOTU)	Brazil	+	+	-	-	+	+	+	-	-
<i>Eriosema elliotii</i> Baker f.	RM Polhill 2405 (S)	Kenya	-	+	-	-	+	+	-	-	+
<i>Eriosema ellipticifolium</i> Schinz	CH Stirton 1441 (K)	South Africa	+	+	-	-	+	+	+	+	+
<i>Eriosema ellipticum</i> Welw. ex Baker	A Stolz 328 (S)	South Africa	-	+	-	-	-	+	-	-	+
<i>Eriosema erectum</i> Baker f.	HM Richards 15782 (S)	South Africa	+	+	-	-	+	+	+	+	-
<i>Eriosema flexuosum</i> Staner	E Milne-Redhead and P Taylor 8920 (S)	Congo	-	+	-	-	+	+	+	-	+

<i>Eriosema floribundum</i> Benth.	ES Cândido et al. 1090 (OUPR)	Brazil	-	+	-	-	+	+	-	-	-
<i>Eriosema glabrum</i> Mart. ex Benth.	ES Cândido et al. 1067 (OUPR)	Brazil	+	+	-	-	+	+	-	-	+
<i>Eriosema glomeratum</i> (Guill. & Perr.) Hook. f.	O Flock 356 (S)	Tanzanie	-	+	-	-	+	+	-	-	+
<i>Eriosema grandiflorum</i> (Schltdl. & Cham.) G. Don	HS Gentry 1837 (S)	Mexico	-	+	-	-	+	+	-	-	+
<i>Eriosema grearii</i> Cândido & Fort.-Perez	Guimarães 1196 (HRB)	Brazil	-	+	-	-	+	+	-	+	-
<i>Eriosema hasslerianum</i> Chodat	K Dinter 5750 (S)	Brazil	-	+	-	-	+	+	-	-	+
<i>Eriosema hatschbachii</i> Fort.-Perez & G.P.Lewis	AP Fortuna-Perez et al. 1401 (OUPR)	Brazil	-	+	+	-	+	+	-	-	-
<i>Eriosema heterophyllum</i> Benth.	ES Cândido et al. 1068 (OUPR)	Brazil	-	+	-	-	+	+	-	-	-
<i>Eriosema humbertii</i> Staner & de Craene	H Humbert 7659 (P)	Zaire	-	+	-	-	+	-	+	-	+
<i>Eriosema irwinii</i> Grear	AP Fortuna-Perez et al. 1467 (OUPR); ES Cândido 1501 (BOTU)	Brazil	-	+	+	+	+	+	-	-	+
<i>Eriosema juronianum</i> Staner & de Craene	JB Gillett 14464 (S)	Ethiopia	-	+	-	-	+	+	+	+	-
<i>Eriosema kraussianum</i> Meisn.	K Grieve 942 (PRE)	South Africa	+	+	-	-	+	+	-	-	-
<i>Eriosema macrostipulatum</i> Fort.-Perez, Cândido & M.J. Silva	AP Fortuna-Perez 1447 (OUPR); MJ Silva 4696 (UFG)	Brazil	+	+	-	-	+	+	-	-	-
<i>Eriosema laurentii</i> De Wild.	K Arnstein Lye s/n (K)	Uganda	+	+	-	-	+	+	+	-	-
<i>Eriosema laxiflorum</i> Harms	MJ Silva 4407 (UFG)	Brazil	+	+	-	-	+	+	-	-	-
<i>Eriosema lebrunii</i> Staner & De Craene	M Reekmans 8784 (S)	Burundi	-	+	-	-	+	+	+	+	+
<i>Eriosema lejeunei</i> Staner & De Craene	HF Mooney 5925 (S)	Ethiopia	-	+	-	-	+	-	+	-	-
<i>Eriosema longifolium</i> Benth.	AP Fortuna-Perez et al. 1442 (OUPR)	Brazil	-	+	-	-	+	+	-	-	-
<i>Eriosema montanum</i> Baker f.	A Stolz 222 (S)	Nigeria	-	+	-	-	-	+	+	+	-
<i>Eriosema multiflorum</i> B.L. Rob.	M Hahn 386 (P)	Mexico	+	+	-	-	+	+	-	-	+
<i>Eriosema parviflorum</i> E. Mey.	L Marques 120 (S)	Mozambique	-	+	-	-	+	+	-	-	-
<i>Eriosema pauciflorum</i> Klotzsch	A Hafstroms 579 (S)	South Africa	-	+	-	-	-	+	+	+	-
<i>Eriosema platycarpon</i> Micheli	DO Norris 17 (UB)	Paraguay	-	+	-	+	+	-	-	-	-
<i>Eriosema populifolium</i> Benth. ex Harv.	CH Stirton 1200 (S)	South Africa	-	+	-	-	-	+	+	+	+
<i>Eriosema preptum</i> C.H. Stirt.	HJ Venter and A Venter 10237 (S)	South Africa	+	+	-	-	+	+	+	+	+

<i>Eriosema procumbens</i> Benth. ex Baker	R Baron 4144 (S)	Madagascar	-	+	-	-	+	+	+	+	-
<i>Eriosema prorepens</i> Benth.	R Romero et al. 4653 (VIC); R Romero et al. 4551 (VIC)	Brazil	+	+	-	+	+	+	+	+	+
<i>Eriosema pulchellum</i> (Kunth) G. Don	CG Pringle 9748 (S)	Brazil	-	+	-	-	+	+	+	+	-
<i>Eriosema pycnanthum</i> var. <i>pycnanthum</i> Benth.	SG Rezende and MS Mendes 2088 (BHCB); FF Carmo 1957 (BHCB)	Brazil	+	+	-	-	+	+	-	+	+
<i>Eriosema pycnanthum</i> var. <i>veadeirense</i> Grear	HS Irwin et al. 12885 (NY)	Brazil	-	+	-	-	+	+	+	+	-
<i>Eriosema rhynchosoides</i> Baker	P Kuchar 25127 (S)	Tanzanie	-	+	-	-	+	+	+	+	+
<i>Eriosema riedelli</i> Benth.	Pedralli et al. 683 (UEC)	Brazil	-	+	-	-	+	-	-	-	+
<i>Eriosema robustum</i> Baker	JB Gillett 14595 (S)	Ethiopia	+	+	-	-	+	+	-	-	+
<i>Eriosema rossii</i> C.H. Stirt.	K Grieve 946 (PRE)	South Africa	+	+	-	+	+	+	-	+	+
<i>Eriosema rufum</i> (Kunth) G. Don var. <i>rufum</i>	W Vargas 12 (CGMS)	Brazil	+	+	+	-	+	-	+	+	+
<i>Eriosema simplicifolium</i> (DC.) G. Don	AP Fortuna-Perez 2102 (BOTU)	Brazil	+	+	-	-	+	+	-	-	+
<i>Eriosema simplicifolium</i> var. <i>micranthum</i> Grear	RS Rodrigues et al. 1386 (UEC)	Brazil	+	+	-	-	+	+	-	-	+
<i>Eriosema solignum</i> L. Mey	K Grieve 968 (PRE)	South Africa	+	+	-	-	+	+	-	-	+
<i>Eriosema sparsiflorum</i> Baker f.	A. Borgdan 3680 (S)	Tanzanie	-	+	-	-	+	+	+	+	-
<i>Eriosema speciosum</i> Welw. ex Baker	N Schinz 101 (S)	Angola	+	+	-	-	+	-	+	+	+
<i>Eriosema squarrosum</i> (Thunb.) Walp.	A Hafstrom and JPH Acock 616 (S)	South Africa	-	+	-	-	+	+	-	-	+
<i>Eriosema stenophyllum</i> Harms	LP Queiroz 15074 (HUEFS)	Brazil	-	+	-	-	-	-	-	-	+
<i>Eriosema strictum</i> Benth.	G Hatschbach 8643 (HB)	Brazil	+	+	+	-	-	+	-	-	+
<i>Eriosema tacuarembense</i> Arechav.	AP Fortuna-Perez et al. 1443 (OUPR)	Brazil	-	+	-	-	+	+	-	-	+
<i>Eriosema tozziae</i> Cândido & Fort.-Perez	LS Kinoshita et al. 02/158 (UEC)	Brazil	-	+	-	+	+	+	-	-	-
<i>Eriosema tuberosum</i> A. Rich.	Schimper 1202 (S)	Ethiopia	-	+	-	-	+	-	-	-	-
<i>Eriosema velutinum</i> Baker f. & Haydon	AA Bullock 2844 (S)	Congo	+	+	-	-	+	+	+	-	-
<i>Eriosema venulosum</i> Benth.	HS Irwin 11428 (P)	Brazil	-	+	-	-	+	+	-	-	+
<i>Eriosema violaceum</i> (Aubl.) G. Don	Ule 8160 (K)	Brazil	-	+	-	-	-	+	-	-	+
<i>Flemingia chappar</i> Buch. - Ham. ex Benth.	LJG van der Maesen 5062 (K)	India	+	+	-	-	+	+	-	-	-

<i>Flemingia faginea</i> (Guill. & Perr.) Baker f.	J Sihronen 125 (S)	Boromo	-	+	-	-	-	-	-	-	-
<i>Flemingia grahamiana</i> Wight & Arn.	WJ Hanekom 2315 (P)	South Africa	-	+	-	-	+	+	-	-	-
<i>Flemingia lineata</i> (L.) Roxb. ex W.T. Aiton	HF Mooney 3166 (K); M Ramos 41648 (P)	India/ Philippines	-	+	-	-	+	+	-	-	+
<i>Flemingia macrophylla</i> (Willd.) Kuntze ex Merr.	JM Delavay 3157 (P)	China	-	+	-	-	-	-	-	-	-
<i>Flemingia pauciflora</i> Benth.	JR Clarkson and VJ Nelder 7998 (K)	Australia	-	+	-	-	+	+	-	-	+
<i>Flemingia procumbens</i> Roxb.	Rarmilch 21515 (K)	India	-	+	-	-	-	+	-	-	-
<i>Flemingia sericans</i> Kurz	ID Cowie and KG Brenan 9083 (K)	Australia	+	+	-	-	+	+	+	+	+
<i>Flemingia trifoliatrum</i> Domin	M Evans 3012 (K)	Australia	-	+	-	-	+	+	-	-	+
<i>Flemingia bracteata</i> (Roxb.) Wight	LJG van der Maesen 4222 (K)	India	-	+	-	-	-	+	-	-	+
<i>Flemingia congesta</i> Roxb. ex W.T. Aiton	P Remanandan 4698 (K)	India	+	+	-	-	+	+	-	-	-
<i>Flemingia ferruginea</i> Wall. ex Benth.	K Larsen 9152 (K)	Thailand	+	+	-	-	+	+	-	-	-
<i>Flemingia involucrata</i> Benth.	TH Thai et al. 7703 (K); BR Maslin 5115 (K)	Vietnam	-	+	+	+	+	+	-	+	+
<i>Flemingia paniculata</i> Dieter.	LJG van der Maesen 4894 (K)	India	+	+	-	-	+	-	-	-	-
<i>Flemingia strobilifera</i> (L.) R. Br.	TB Worthington 7202 (K)	India	+	+	-	-	+	+	-	-	+
<i>Paracalyx balfourii</i> Ali	AG Miller et al. 8657 (K)	Socotra	-	+	-	-	+	+	-	-	+
<i>Paracalyx microphyllus</i> Ali	M Thulin et al. 10558 (K)	Somalia	+	+	-	-	+	+	-	-	+
<i>Paracalyx nogalensis</i> Ali	M Thulin and AM Warfa 5425 (K)	Somalia	-	+	-	-	+	+	-	-	+
<i>Paracalyx scariosus</i> (Roxb.) Ali	N Kameswara Rao 12 (K); LJG van der Maesen 1973 (K)	India	-	+	-	-	+	+	-	-	+
<i>Paracalyx schweinfurthii</i> Ali	O Cronk 3 (K)	Australia	+	+	-	-	+	+	-	-	+
<i>Paracalyx somalorum</i> Ali	I Friis 9993 (K)	Ethiopia	-	+	+	+	-	+	-	-	+
<i>Rhynchosia albissima</i> Gand.	EE Galpin 133121 (P)	South Africa	-	+	-	-	-	+	-	-	+
<i>Rhynchosia alluaudi</i> Saccl.	J Raynal 20795 (P)	Nigeria	-	+	-	-	+	+	-	-	-
<i>Rhynchosia ambacensis</i> (Hiern) K. Schum.	J Raynal 12914 (P)	Cameroun	-	+	-	-	-	+	-	-	+
<i>Rhynchosia breviracemosa</i> Hauman	A de Menezes 732 (P)	Angola	-	+	+	-	+	-	-	-	+
<i>Rhynchosia brunnea</i> Baker f.	RAA Oldeman 803 (P)	Gana	-	+	-	-	-	-	-	-	-

<i>Rhynchosia buettneri</i> Harms	P Fabrègues 3473 (P)	Costa do Marfim	+	+	-	-	+	-	-	-	+
<i>Rhynchosia bullata</i> (E. Mey.) Benth. ex Harv. & Sond.	JF Drège 1842 (P)	South Africa	-	+	-	+	-	-	+	+	+
<i>Rhynchosia chrysoscias</i> Benth. ex Harv.	Schuh et al. PBI SA03 H191 (NY)	South Africa	-	+	+	+	-	-	+	-	+
<i>Rhynchosia clausenii</i> Benth.	CR Martins 252 (UEC)	Brazil	+	+	-	-	-	+	+	+	+
<i>Rhynchosia cliffordii</i> Hutch. & E.A. Bruce	JB Gillett 4864 (P)	Somalia	-	+	+	+	-	-	-	-	+
<i>Rhynchosia clivorum</i> S. Moore	W Goetze 1130 (P)	Tanzanie	-	+	+	-	+	+	-	-	+
<i>Rhynchosia caribaea</i> (Jacq.) DC.	PCM Jansen 7339	Mozambique	-	+	-	-	-	+	-	-	-
<i>Rhynchosia difformis</i> (Elliott) DC.	S McDaniel 29983 (NY)	EUA	+	+	-	-	+	-	-	-	-
<i>Rhynchosia edulis</i> Griseb.	F França et al. 2164 (HUEFS)	Brazil	-	+	+	+	+	+	-	-	+
<i>Rhynchosia hauthalii</i> (Kuntze) Grear	TM Pedersen 2982 (NY)	Argentina	-	+	+	-	+	+	+	+	+
<i>Rhynchosia lateritia</i> Burkart	HA Keller et al. 4566 (UEC)	Brazil	+	+	-	-	+	-	-	-	+
<i>Rhynchosia leandrii</i> Du Puy & Labat	T Leandri and P Saboureaux 2830 (P)	Madagascar	+	+	-	-	-	+	-	-	+
<i>Rhynchosia madagascariensis</i> R. Vig.	M Bardot-Vaucoulon 743 (P)	Madagascar	-	+	+	+	+	+	-	-	+
<i>Rhynchosia minima</i> (L.) DC.	W Vargas 9 (CGMS); JRI Wood and P Pozo 25974 (UB); VC Lima 46 (IPA)	Brazil	+	+	-	-	+	+	-	-	-
<i>Rhynchosia phaseoloides</i> (Sw.) DC.	RB Pinto 500 (UEC)	Brazil	-	+	-	-	+	+	-	-	-
<i>Rhynchosia platyphylla</i> Benth.	J Raveratti et al. 640 (UB)	Brazil	-	+	+	+	+	+	-	-	+
<i>Rhynchosia pycnostachya</i> (DC.) Meikle	H Ern 2562 (P)	Togo	-	+	-	-	+	+	-	-	-
<i>Rhynchosia schomburgkii</i> Benth.	JA Steyermark 88376 (SP)	Venezuela	-	+	-	-	-	+	-	-	-
<i>Rhynchosia totta</i> (Thunb.) DC.	CH Stirton 14089 (PRE)	South Africa	-	+	-	-	+	+	-	-	-
<i>Rhynchosia usambarensis</i> Taub.	A Bogdan 1861 (K)	Kenya	-	+	-	-	+	+	-	-	-
<i>Rhynchosia volubilis</i> Lour.	F Konta 35508 (P); G Murata 13831 (NY); YN Xiong and K Yao 122 (NY)	Japan	-	+	-	-	-	+	+	-	+

1 = Vesicular glands; 2 = Bulbous-based trichomes; 3 = Capitate trichomes; 4 = Secretory-base trichomes; 5 = Idioblasts; D = Adaxial; B = Abaxial; M = Midrib; + = Present; - = Absent; Vouchers listed by collector, number, and herbarium acronym (abbreviations follow index herbariorum).

Table 2. List of nomenclature equivalences with the terms used by us to describe the secretory structures of Cajaninae and the terminology found in the literature (with citations)

Terms used in the present work	Terms for equivalent secretory structures in the literature	Citation
Vesicular gland	Glandular punctate	Lackey (1981; 1978)
	uniseriate filiform vesicular clavate hairs	Leelavathi and Ramayya (1983)
	nectar gland	
Bulbous-based trichomes	Uniseriate filiform obpyriform hair	Leelavathi and Ramayya (1983)
	Bulbous hair/Bottle-shaped hairs	Horner and Zindler-Frank (1982)
Capitate trichomes	Uniseriate filiform capitate hair	Leelavathi and Ramayya (1983)
	Glandular hair	
	Stalked glands	
	Unstalked glands	Lackey (1977; 1978)
	Small capitate hairs	Horner and Zindler-Frank (1982)

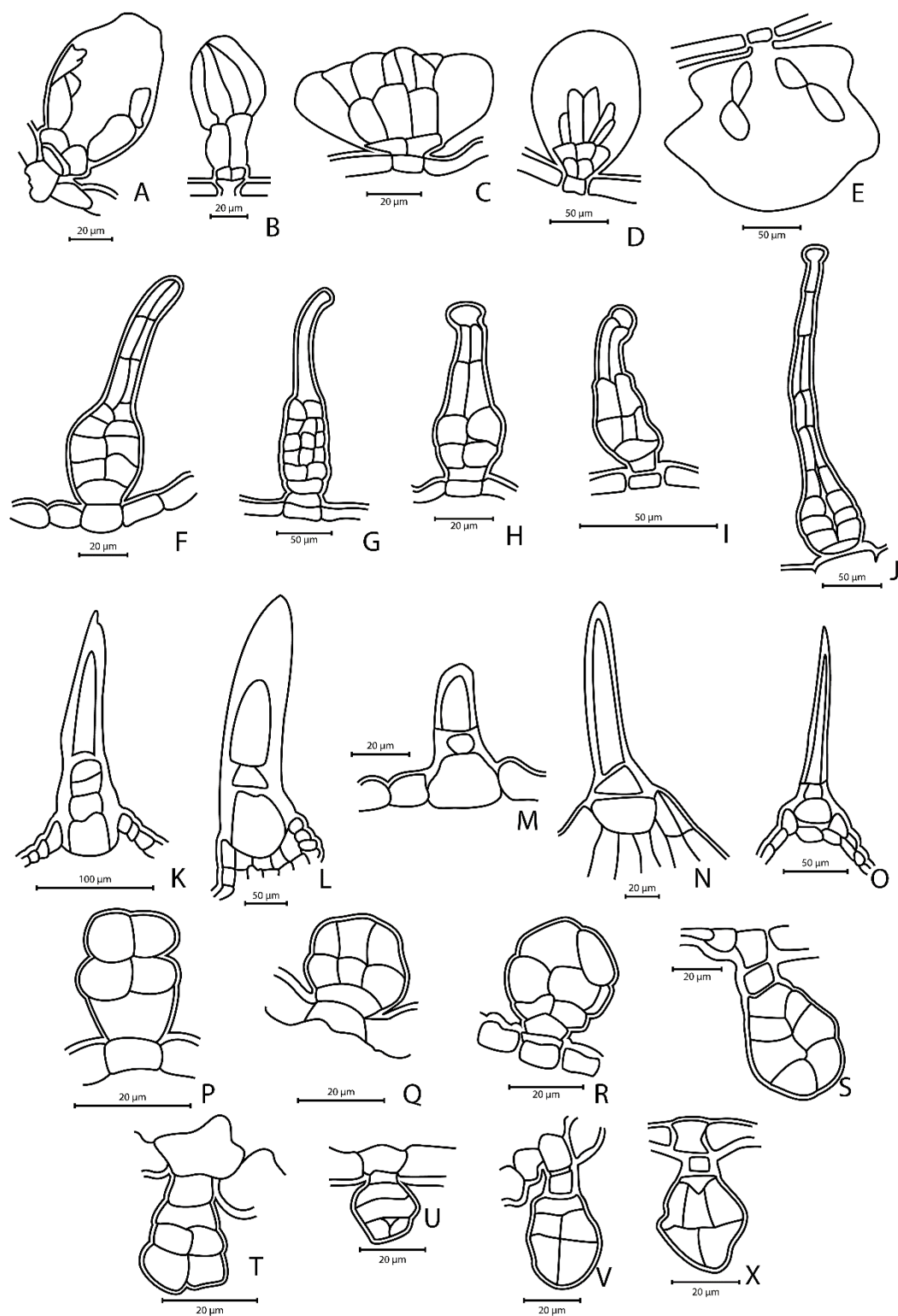


Figure 1. Morphological variation in the secretory trichomes of Cajaninae. A-E, vesicular glands. F-J, bulbous-based trichomes. K-O, secretory-base trichomes. P-X, capitate trichomes. The images of the trichomes E, S-X refer to the positioning in the abaxial face of the leaflets.

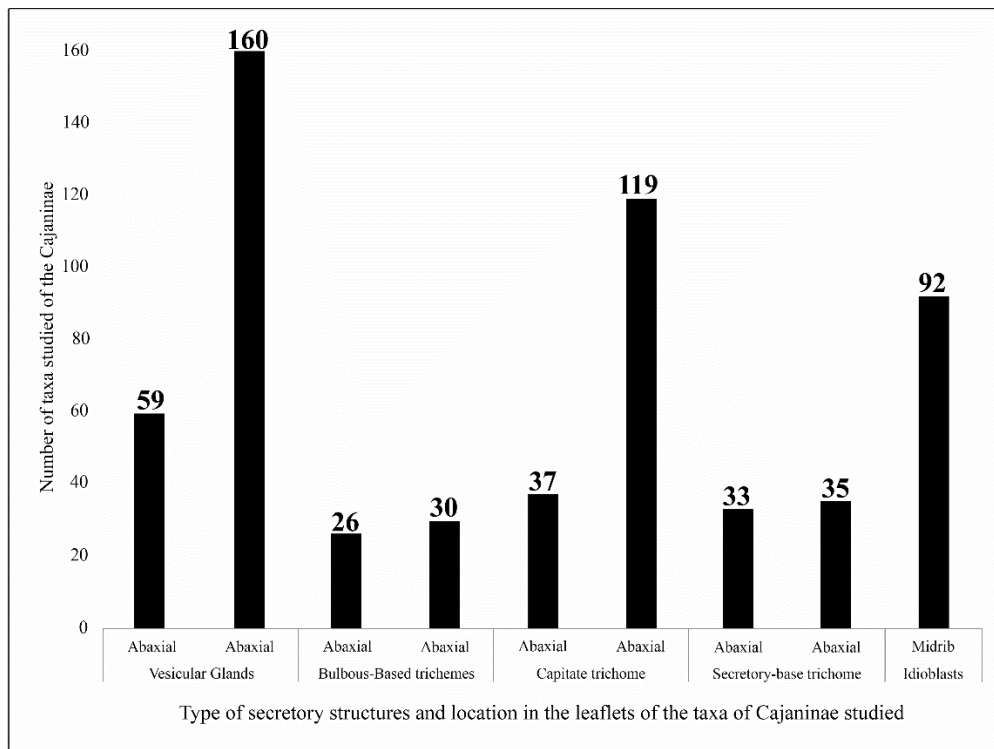


Figure 2. Graph showing the richness of each secretory structure on both surfaces of the leaflets of the taxa of Cajaninae studied. The Y axis represents number of taxa studied of the Cajaninae and X axis represents the type of secretory structures and location in the leaflets of the taxa of Cajaninae.

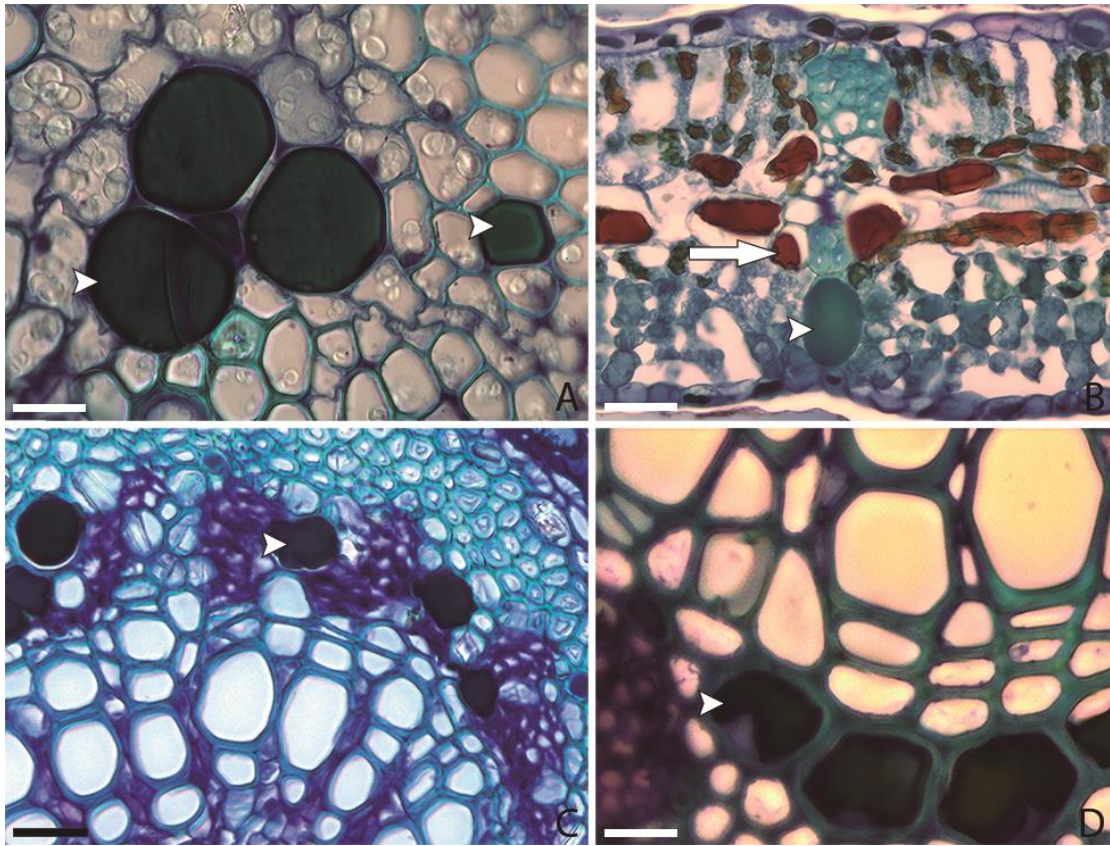


Figure 3. Photomicrographs of the idioblasts in Cajaninae representatives. A, secretory idioblasts (arrows) in *Eriosema defoliatum*. B, secretory (arrowhead) and non-secretory idioblasts (long arrow) in *Eriosema multiflorum*. C, secretory idioblasts (arrowhead) in *Eriosema robustum*. D, secretory idioblasts (arrowhead) in *Flemingia sericans*. Scale bars: 50µm (B-C); 20µm (A and D).

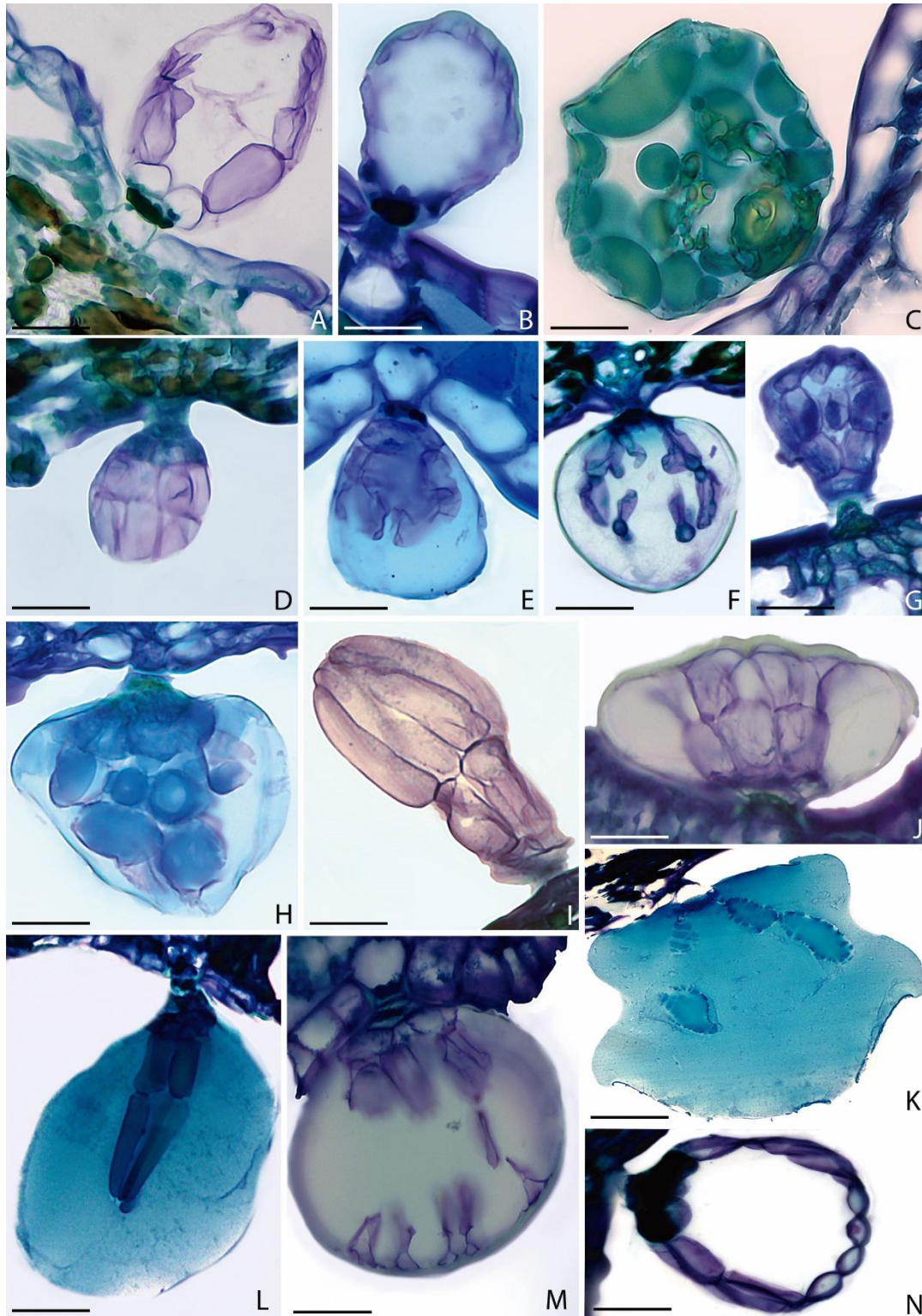


Figure 4. Photomicrographs of the vesicular glands in Cajaninae taxa. A, *Eriosema solignum*. B, *Eriosema simplicifolium* var. *micranthum*. C, *Eriosema kraussianum*. D, *Eriosema venulosum*. E, *Eriosema glabrum*. F, *Eriosema ellipticifolium*. G, *Eriosema velutinum*. H, *Eriosema montanum*. I, *Rhynchosia difformis*. J and M, *Eriosema bauchiense*. K, *Eriosema cordatum*. L, *Rhynchosia brunnea*. N, *Adenodolichos rhomboideus*. Scale bars: 50µm (K and L); 20µm (all others). The images of the trichomes D-F, H, K-N refer to the positioning in the abaxial face of the leaflets

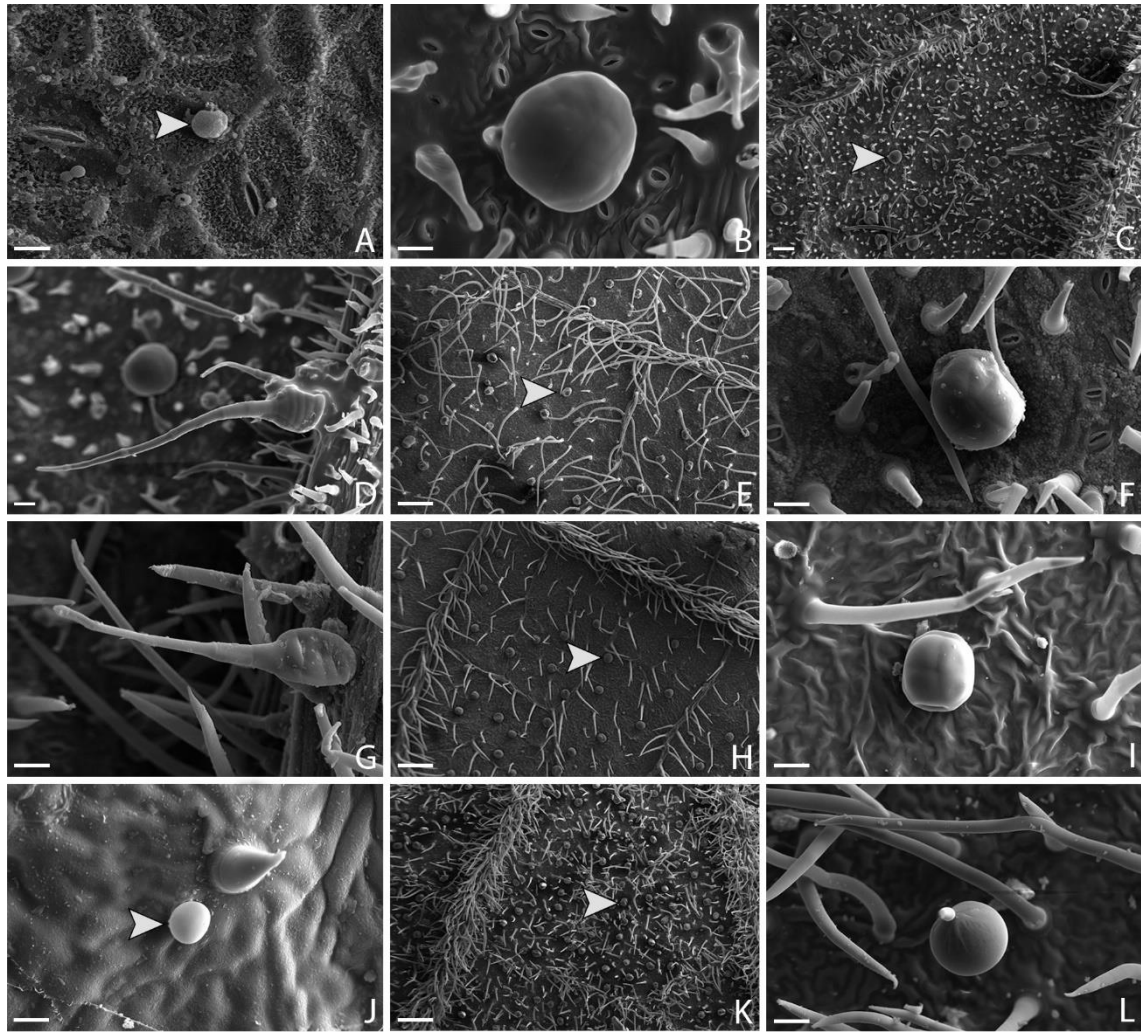


Figure 5. Scanning electron micrographs of the abaxial surface of the leaflets of Cajaninae taxa showing the secretory structures. A, vesicular gland (arrowhead) in *Adenodolichos paniculatus*. B-D: *Bolusafra bituminosa*. B, vesicular gland. C, overview of the leaflet showing the high density of vesicular glands (arrowhead). D, bulbous-based trichome. E, overview of the leaflet showing the vesicular glands (arrowhead) in *Cajanus volubilis*. F-G: *Chrysoscias grandiflora*. F, vesicular gland. G, bulbous-based trichome. H-I: *Dunbaria villosa*. H, overview of the leaflet showing the vesicular glands (arrowhead). I, vesicular gland. J, vesicular gland (arrowhead) in *Flemingia strobilifera*. K-L: *Paracalyx somalorum*. K, overview of the leaflet showing the high density of vesicular glands (arrowhead). L, vesicular gland with secretion. Scale bars: 200µm (H and K); 100µm (C and E); 20µm (B, D, F, G, I, L); 10µm (A and J).

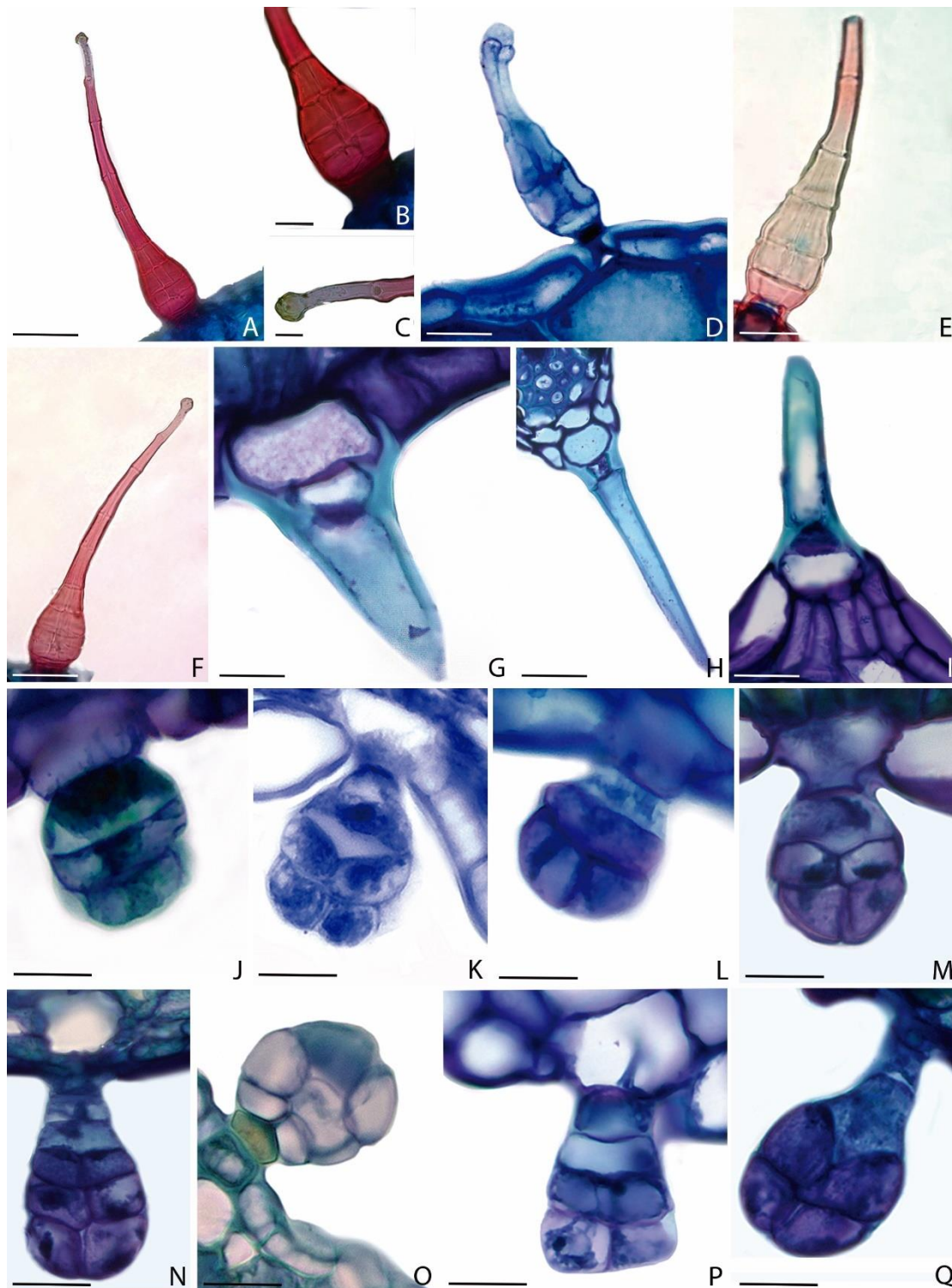


Figure 6. Photomicrographs of bulbous-based trichomes, secretory-base trichomes, and capitate trichomes in Cajaninae taxa. A-F, bulbous-based trichomes in: A-C and F, *Eriosema campestre* var. *campestre* (details of bulbous [B] and apical cell [C]). D, *Eriosema hatschbachii*. E, *Eriosema rufum* var. *rufum*. G-I, secretory-base trichomes in: G, *Eriosema sparsiflorum*. H, *Rhynchosia hauthalii*. I, *Rhynchosia clausenii*. J-Q, capitate trichomes in: J, *Eriosema solignum*. K, *Eriosema kraussianum*. L, *Eriosema campestre* var. *macrophyllum*. M, *Rhynchosia ambacensis*. N, *Rhynchosia alluaudi*. O, *Rhynchosia clivorum*. P, *Eriosema rossii*. Q, *Cajanus albicans*. Scale bars: 50µm (A, D, E, H and I); 20µm (B, F, G, J-Q); 10µm (C).

Ultrastructure and secretion of glandular trichomes in species of subtribe Cajaninae Benth. (Leguminosae, Phaseoleae)

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Abstract

The subtribe *Cajaninae* of papilionoid legumes has a pantropical distribution and comprises approximately 490 species. These species have diversified throughout dry environments where there are high temperatures and strong light. The subtribe stands out because all its representatives have vesicular glands. In addition, bulbous-based and capitate trichomes are important secretory structures present in all genera of the *Cajaninae*. We analysed the ultrastructure and histochemistry of these glandular trichome types in leaflets of the three species of the subtribe. Using transmission electron microscopy and histochemical analyses, we link the glandular secretions to subcellular structures. We here report for the first time the type of exudate and ultrastructure of the glands of subtribe *Cajaninae*. Terpenoids and phenolics were confirmed by histochemistry tests, and we observed that the organelles responsible for biosynthesis of oils are the most representative in these glands. Each glandular trichome showed particular ultrastructural features compatible with the compounds produced. We suggest that these glandular trichomes, with their respective exudates, act in defence against herbivory and against possible damage by ultraviolet radiation.

Keywords *Cajaninae* glands, Fabaceae, phenolic compounds, terpenoids

INTRODUCTION

Plants produce a wide variety of secretions, from aqueous solutions rich in salts, amino acids and sugars to more complex mixtures consisting of primary metabolites (e.g. proteins, polysaccharides) or primary and secondary metabolites (e.g. terpenoids, phenylpropanoids and alkaloids). Many exudates contain biologically active molecules that contribute to the evolutionary success of the species that produce them (Ascensão 2007), can help in the interactions of plants with their surrounding environment (Fahn 1979, 2002).

Secretions are produced by isolated cells or specialized multicellular structures that are extremely variable in morphology (Fahn 1979; Evert 2006; Ascensão 2007). In Leguminosae, many types of secretory structures in vegetative organs have been recorded, including glandular trichomes, secretory epidermal cells, extrafloral nectaries, idioblasts, canals, and cavities (Solereder 1908; Metcalfe and Chalk 1950, 1979, 1983; Pyykko 1966; Fahn 1979; Gregory and Baas 1989; Lersten and Curtis 1993, 1994, 1995, 1996; Sartori and Tozzi 2002; Fortuna-Perez 2012; Pereira et al. 2018).

Focussing on glandular trichomes, the Leguminosae displays a wide diversity of these structures, including: peltate glands in *Indigofera* L and *Centrolobium* Mart ex Benth. (Metcalfe and Chalk 1950; Matos and Paiva 2012), cavitated secretory trichomes in *Bauhinia* L. s.s. (Marinho et al. 2015), *Hoffmannseggia* Cav., *Caesalpinia* L. s.l. (Lersten and Curtis, 1994, 1996), and *Indigofera* L. (Marquiafével et al. 2009), uniseriate filiform capitate hairs in *Alysicarpus rugosus* (Willd.) DC., uniseriate filiform hairs in *Melilotus albus* Medik., uniseriate prickle-like hairs in *Vigna cylindrica* (L.) Skeels (Leelavathi and Ramayya 1983), and especially, capitate trichomes, bulbous-based trichomes, and vesicular glands in representatives of subtribe Cajaninae (Lackey 1978; Vargas et al. in press.).

Earlier published reports about the types of secretory structures occurring in Cajaninae maintained that the bulbous-based trichomes and vesicular glands are typical secretory structures of the subtribe (Debold 1892; Lackey 1978; Vargas et al. 2015; Vargas et al. in press.). The subtribe Cajaninae has a pantropical distribution, and its taxa are found in grasslands, seasonally dry tropical forests, semi-arid areas, disturbed areas, and fire-prone environments (Schrire 2005). Cajaninae comprise approximately 490 species in ten genera, with the two genera *Eriosema* (DC.) Desv. and *Rhynchosia* Lour. being the most speciose, with 150 and 230 species, respectively (Schrire 2005). These two genera possess the African and American continent as the main centers of diversity (Gear 1970).

Considering these micromorphological structures, the capitate trichomes consist of a short, single basal cell, usually rectangular to square in shape, a unicellular to absent stalk, and a clavate head (Vargas et al. in press.); while bulbous-based trichomes are composed of one to two basal cells supporting a group of cells that form the bulbous base, a number of apically elongated cells, and slightly rounded terminal cells (Solereder 1908; Vargas et al. 2015). The vesicular glands have a trapezoidal to square basal cell (Vargas et al. in press.), a narrow, short stalk supporting a large and spherical head surrounded by an amber-coloured exudate. These vesicular glands are frequently inserted into an epidermal depression, (Solereder 1908; Lackey 1978, 1981). Uphof (1962) described how in the vesicular glands all cells of the head break down to form the yellow glandular fluid. These various glandular trichomes are extensively distributed throughout the leaflets of taxa in the Cajaninae, and provide important diagnostic value (Vargas et al. in press.).

Capitate trichomes are widely encountered in tribe Phaseoleae (Lackey 1978), and in subtribe Cajaninae they occur in some species and their presence or absence can be used as a diagnostic character (Vargas et al. in press.). Bulbous-based trichomes also occur in all Cajaninae genera but are not an exclusive characteristic of the subtribe (Leelavathi and Ramayya 1983; Vargas et al. in press.). In almost all Cajaninae taxa, bulbous-based trichomes are less abundant than vesicular glands; nevertheless, they serve as a diagnostic character at infrageneric taxonomic rank in the subtribe (Vargas et al. 2015; Vargas et al. in press.). Vesicular glands are exclusively and abundantly distributed amongst Cajaninae taxa and they are a unifying character of the subtribe (Vargas et al. in press.), facilitating the prompt identification of any member of Cajaninae.

Given the anatomical variation of these glandular trichomes in Cajaninae and their taxonomic diagnostic value at infra-tribal and infra-generic levels, we considered that a combination of ultrastructural and histochemical analyses might provide support in understanding the functions of each gland type. Here we investigate and compare the histochemistry and ultrastructure of capitate trichomes, bulbous-based trichomes, and vesicular glands in a search for similarities or differences among them and we consider the potential function of these trichomes. *Rhynchosia minima* (L.) DC., *Eriosema rufum* (Kunth) G. Don and *Eriosema simplicifolium* (DC.) G. Don were selected as model species for this study.

MATERIALS AND METHODS

Plant material

Samples of young leaflets of *R. minima*, *E. rufum* and *E. simplicifolium* were collected from wild populations occurring in the Cerrado (Brazilian savanna) and forest vegetation of the States of São Paulo and Minas Gerais, Brazil (Table 1). Voucher specimens were deposited in the Irina Delanova Gemtchújnicov Herbarium (BOTU) in the Institute of Biosciences of UNESP, Botucatu, São Paulo, Brazil. The three types of trichomes most widely distributed in the Cajaninae leaflets (according to Vargas et al. in press.) were analysed in their secretory phase (= substance release) in three individuals of each species.

Histochemical tests

Fresh samples of leaflets of *R. minima*, *E. rufum* and *E. simplicifolium* or those fixed with formalin–acetic acid–alcohol (FAA) for 24 h and buffered formalin for 48 h (Lillie 1965) were hand-cut and subjected to the following treatments for histochemical analyses: Sudan IV (Pearse 1980) for total lipids, Nadi reagent (David and Carde 1964) for terpenoids, vanillin-hydrochloric acid (Mace and Howell 1974) for tannins, ferric chloride (Johansen 1940) for phenolic compounds, Dragendorff reagent (Svedsen and Verpoort 1983) for alkaloids, Bromophenol Blue (Mazia et al. 1953) for total proteins, tannic acid–ferric (Pizollato 1977) for mucilage, and Nile Blue (Cain 1947) for acidic lipids. The control samples were tested according to the specifications of each test. Micrographs were obtained using a light microscope (Olympus BX 41) linked to a digital camera in the Laboratory of Plant Anatomy of UNESP - Botucatu (LPAV).

Transmission electron microscopy

For transmission electron microscopy (TEM), samples of the middle portion of leaflets from *R. minima*, *E. rufum* and *E. simplicifolium* were removed and fixed for 24 h in 2.5% glutaraldehyde in 0.1

mol·L⁻¹ phosphate buffer (pH 7.3), after which the materials were post-fixed with 1% osmium tetroxide aqueous solution in the same buffer for 1 h at 25 °C. The samples were then dehydrated in an acetone series and embedded in Araldite resin (Machado et al. 2006). Ultrathin sections (70 nm) were stained with uranyl acetate and lead citrate (Reynolds 1963) and then examined with a transmission electron microscope (FEI Tecnai) at 80 kV at the Electron Microscopy Center of the Institute of Biosciences of Botucatu, UNESP.

RESULTS

Capitate trichomes and vesicular glands occurred on both leaflet surfaces of all studied taxa, except for *Eriosema rufum* that the capitate trichomes occurred only on the adaxial surface. Bulbous-based trichomes were present in *Rhynchosia minima* and *E. rufum* on the abaxial surface (Table 2).

Through histochemical analysis, we verified that total lipids occurred in all glandular trichomes observed (image for vesicular gland Fig. 1a-b). Essential oils occurred only in bulbous-based trichomes (Fig. 1c) and acidic lipids in bulbous-based trichomes (Fig. 1d) and capitate trichomes. Phenolic compounds occurred in bulbous-based trichomes (Fig. 1e) and vesicular glands (Fig. 1f) only in *R. minima*. Proteins were detected in capitate trichomes and vesicular gland. Alkaloids, polysaccharides (mucilage), and tannins were not detected. The positive reaction was detected in different sites of these trichomes (Table 3; Fig. 1).

The capitate trichomes were composed of a basal cell and a multicellular head covered with a continuous thick cuticle (Fig. 2a). The basal cell was projected above the other cells of the epidermis and its side walls were covered by a thicker cuticle (Fig. 2a). The basal cell had a thin tangential wall, a parietal voluminous nucleus, a large central vacuole and a reduced cytoplasm (Fig. 2a). Rounded plastids, devoid thylakoid and with oil drops, mitochondria with well-developed cristae and a rough endoplasmic reticulum characterized the cytoplasm of this cell (Fig. 2b). The head cells had a square or rectangular shape and exhibited thin walls, a large central nucleus, vacuoles of different sizes and abundant cytoplasm (Fig. 2c-d). In the central region of the head the cells, where more than two cells are joined, and in the distal portion of anticlinal walls in contact with cuticle, the middle lamella was more electron dense and thickened (Fig. 2c-d). In these regions the middle lamella showed signs of dissolution (Fig. 2d-e) and accumulation of flocculated material was frequently observed at these sites. The cytoplasm of the head cells contained free ribosomes, polyribosomes, plastids, a rough endoplasmic reticulum (RER), mitochondria with developed cristae, and Golgi bodies (Fig. 2f). The plastids were polymorphic, devoid thylakoids, and had starch grain or lipid inclusions (Fig. 2f and 3a-b). The rough endoplasmic reticulum was extensive, and some profiles exhibited dilation (Fig. 3b). The Golgi bodies were composed of two to four elongated cisternae with few vesicles in the ends (Fig. 3b). Coated vesicles from RER occurred in the peripheral cytoplasm and juxtaposed to the plasmalemma, and showed signs of fusion with this membrane (Fig. 3c), clustered inside the multivesicular bodies located near the plasmodesmata, and scattered in the cytosol (Fig. 3d). In these cells the anticlinal walls had variable electron density with light areas interspersed with dark areas (Fig. 3a-b), having a loose aspect with gaps in some regions (Fig. 3e). In the distal region of the head, the cells showed RER proliferated adjacent to plasmalemma and dark materials accumulated in periplasmatic spaces along the outer periclinal walls (Fig. 3f). Dark material was seen also in the cell wall, inside the cuticular layer, and on the cuticle (Fig. 3g). The outer periclinal cell wall had a reticulated appearance and the cuticle

presented a large cuticular layer with electron dense ramifications that do not reach the proper cuticle (Fig. 3h).

The bulbous-based trichomes were composed of one to two basal cells, a 1-celled stalk (or this absent), a multicellular bulbous region, and a narrow-tapered neck region with a variable number of cells axially elongated and ending in a terminal swollen spathulate cell (Fig. 1c). The trichome was covered by a thick cuticle that gradually increases in thickness towards the base (Fig. 4a-b) and apex (Fig. 5d-f). Lipid inclusions were observed in the lateral walls of the cells of the bulbous region (Fig. 4b). The base was formed by two epidermal cells (proximal and distal) that were joined by very thin tangential walls (Fig. 4c) rich in plasmodesmata (Fig. 4d). The proximal cell was larger and characterized by a rounded shape, a large central vacuole, and reduced cytoplasm (Fig. 4a, c). Extensive RER, globular mitochondria, and ellipsoidal chloroplasts with oval starch grains, plastoglobules, and a peripheral reticulum occurred in this cell (Fig. 4d). The distal cell had a lenticular form, reduced size, vacuoles with different sizes, a larger nucleus, and abundant cytoplasm (Fig. 4c). The tangential wall between the distal cell and the cells of the bulb was relatively thicker and denser (Fig. 4c-d). The first cell of the bulbous region was rectangular, and the tangential walls joined to other bulb cells were thicker and denser (Fig. 4a, e). The other cells of the bulbous region were square in shape, thin-walled, and differed in relation to vacuole development depending of their position in the swollen base (Fig. 4a). Larger vacuoles and a reduced cytoplasm occurred in the cells of the “bulb” periphery (Fig. 4a), while the inner cells had a voluminous nucleus, few small vacuoles, and an abundant cytoplasm (Fig. 4e). The vacuoles were translucent and contained fibrillar material (Fig. 4f). Smooth and rough endoplasmic reticulum, mitochondria, plastids with starch grains, signs of degradation and osmiophilic globules, and vesicles occurred in the cytoplasm of the inner cells (Fig. 4f). The vesicles occurred mainly in peripheral cytoplasm or were juxtaposed to plasmalemma (Fig. 4f). The middle lamella in the corner of the inner cells became swollen and progressively showed signs of dissolution, giving rise to intercellular spaces (Fig. 4g). In these cells the plasmalemma was sinuous in outline and dark material occurred in the periplasmic space, the cell walls and intercellular spaces (Fig. 4g-h). The dissolution of the middle lamella progressed along the entire length of the cell and formed large tubular spaces between the cells. Accumulation of heterogeneous material was observed in these intercellular spaces (Fig. 4h and 5a). In the cytoplasm of all cells of the bulbous region oil drops occurred scattered in the cytoplasm, in contact with the tonoplast (Fig. 5b), and inside the plastids (Fig. 5c). The vacuoles contained lipid inclusions and membranous material (Fig. 5b). The cell walls presented an inner reticulate layer and an outer fibrous layer, with fibrils protruding into the cuticle (Fig. 5c). In the neck region the proximal cells had larger vacuoles and reduced cytoplasm (Fig 5d), while distal cells had abundant cytoplasm and poorly developed vacuoles, some of them filled with multilamellar bodies (Fig. 5e). Accumulation of dense material occurred in the periplasmic and intercellular spaces, cells walls, and on the cuticle (Fig. 5e). The terminal cell of the neck region exhibited a poly-lamellate outer wall, and there was an evident transversal line of breakage between this cell and the underlying neck cells (Fig. 5f). Wall ingrowths covered with plasmalemma, characterizing labyrinthic walls occurred in all extensions of the terminal cell of the neck region; microtubules arranged perpendicularly to the cell wall were common in this cell (Fig. 5g).

The vesicular glands consisted of a trapezoidal basal cell, a 1-celled stalk (or this absent), and a multicellular head with a central cavity (Fig. 6a). Frequently this gland was inserted in an epidermal

depression (Fig. 6a) and was covered with a thick continuous cuticle (Fig. 6c) that detached to form small sub-cuticular spaces (Fig. 6l). The basal cell had vacuoles of different sizes and abundant cytoplasm with mitochondria, and smooth endoplasmic reticulum (SER) (Fig. 6b). The stalk cell contained few small vacuoles, a dense cytoplasm with abundant mitochondria having well-developed cristae, RER, plastids with prominent starch grains, and scattered oil drops (Fig. 6b). The tangential wall was thicker and had numerous plasmodesmata connecting the basal and stalk cells (Fig. 6b). The central cavity developed by separation of the adjoining primary cell walls due to middle lamella dissolution (Fig. 6c-d), this a schizogenous process. The schizogenesis started in the corner of the inner head cells, advanced to other wall regions (Fig. 6e), and then reached the cuticle (Fig. 6f), resulting in the displacement of the head cells towards the periphery of the gland and in the formation of a broad lumen (Fig. 6g). Polyribosomes, rounded plastids devoid of thylakoids and containing oil drops (Fig. 6h), mitochondria with well-developed cristae, extensive RER, proliferate SER, and numerous vesicles occurred in the cytoplasm of the head cells (Fig. 6i). Oil drops occurred scattered in the cytosol (Fig. 6h), in the peripheral cytoplasm near the plasmalemma facing both the central cavity (Fig. 6j) and the outer periclinal wall (Fig. 6k), and inside the periplasmic space (Fig. 6k).

DISCUSSION

This study represents the first detailed ultrastructural and histochemical analyses of glandular trichomes of subtribe *Cajaninae*. The relationship between gland histochemistry, subcellular structures and the mechanisms of secretion for the three major gland types encountered in subtribe *Cajaninae* (capitate trichomes, bulbous-based trichomes, and vesicular glands) are considered. Despite the great diversity of glandular trichomes occurring in the *Leguminosae* (e.g., Solereder 1908; Metcalfe and Chalk 1950; Lackey 1978; Leelavathi and Ramayya 1983; Marquiasfável et al. 2009; Matos and Paiva 2012; Marinho et al. 2015; Vargas et al. 2015; Vargas et al. in press.) studies regarding the secretion processes and histochemistry of the secretory structures in legumes are rare.

The relationship between the histochemistry and subcellular organization of these trichomes helps us to better understand the probable functions of these glands in *Cajaninae*. Terpenoids, phenolic compounds (flavonoids and tannins), and alkaloids are examples of secondary metabolites present in the secretory structures of legumes (Wink 2003; Roshchina 2012). Of these groups of compounds, we observed terpenoids (essential oils) and phenolics in *Cajaninae*.

Among the main factors that influence lipidic and phenolic metabolism are soil moisture, light and temperature; these abiotic factors may induce changes in the nature and quantity of the metabolites produced (Gouinguéné and Turlings 2002; Ramakrishna and Ravishankar 2011; Sampaio et al. 2011; Singer et al. 2016). Species of the *Cajaninae* have diversified through “cerrado” and savanna environments (Grear 1970, 1978; Schrire 2005), where there is high light incidence, high temperatures, and low availability of ground water (Furley and Ratter 1988). Most likely, the lipids (essential oils and total lipids) and phenolic compounds produced by the glandular trichomes of the *Cajaninae* help this group of plants to survive in these environments because, as suggest by Gouinguéné and Turlings (2002), plants growing in dry environments invest more in the synthesis of defense compounds. According to these authors, when

water is available plants can invest more in growth and this mitigates against herbivory, whereas when less water is available growth is reduced and protection of the vegetative parts becomes more important.

Phenolic compounds may act against a variety of herbivores and pathogenic micro-organisms (Mazid et al. 2011), as well as various kinds of abiotic stresses (Feucht et al. 1994), including the absorption of ultraviolet light (Harborne 1977). In a brief review of phenolic compounds secreted by plants, Castro and Demarco (2008 and citations therein) mention that glandular trichomes secreting these natural compounds are present in the Asteraceae, Cucurbitaceae, Lamiaceae, Oleaceae, Orobanchaceae, and Verbenaceae families, but not in Leguminosae. However, we observed that *R. minima* produces these metabolites in its bulbous-based trichomes and vesicular glands. Given that phenolics are associated with resistance to drought, the identification of these substances in the glands of the studied species suggests a clear ecological function in the Cajaninae, and this will be particularly important during predicted periods of increasing drought as a consequence of global warming.

Among the three types of secretory structures here studied, only the vesicular glands have a stalk cell. It appears that these stalk cells have a different function to the head cells. Stalk cells generally have very large vacuoles, in contrast to the head secretory cells which have dense cytoplasm (Werker 2000), exactly as occurs in vesicular glands of the Cajaninae. The stalk cell has a thick cuticle and cell walls. Similarly, the basal cell in the glandular trichomes observed in this study also have thick walls. We hypothesize that lipid incrustation in the lateral walls of the stalk and in basal cells forms an apoplastic barrier which regulates directional transport of metabolites to the glandular cells above (Werker 2000; Lange and Turner 2013; Sadala-Castilho et al. 2016). An additional function of the thick stalk walls and the basal cells is to support the head of the gland, as seen in glands of the plant family Velloziaceae (Sadala-Castilho et al. 2016).

Considering the organelles observed in glandular trichomes of the Cajaninae, the smooth endoplasmic reticulum and plastids devoid of thylakoids and with lipid inclusions are common characteristics which have a function in the biosynthesis of oil (Schnepf 1974; Fahn 1979, 2000; Lange and Turner 2013; Sá-Haiad et al. 2015; Tresmondi et al. 2017), as seen in the oil secreting structures of other genera of the Leguminosae (Paiva and Machado 2007; Teixeira and Rocha 2009; Rodrigues et al. 2011; Matos and Paiva 2012; Rodrigues and Machado 2012). Lipid inclusions and osmiophilic material in cell walls, oil drops scattered in cytoplasm and on the inside of the plastids, vacuoles containing lipid inclusions and multilamellar bodies were all observed in some cells of the glandular trichomes studied. The TEM results are consistent with our histochemical tests. All secretory structures here studied (in the cuticle, subcuticular spaces, lateral walls, central cavity, and protoplasm) reacted positively to Sudan IV and Nile blue tests indicating the presence of lipophilic substances (Figueiredo et al. 2007). This type of secretion previously has been reported for other glandular trichomes in legume species (Matos and Paiva 2012; Marinho et al. 2015).

The occurrence of plastids with plastoglobules is another important feature of the cells in the glandular trichomes in the three studied species: *R. minima*, *E. simplicifolium*, and *E. rufum*. Plastoglobules associated with thylakoids act as a reservoir of the lipids (Lichtenthaler 1968); plastoglobules also have a

structural function, maintaining the shape of the lipid bodies and preventing their coalescence (Kessler and Vidi 2007). Additionally, a peripheral reticulum in the plastids was encountered in the basal cell (proximal) of the bulbous-based trichomes. The presence of thylakoids in these cells suggests that they have a photosynthetic function and are thus different from the cells of the other trichomes here studied. It is known that the peripheral reticulum increases the contact surface of the plastid with the cytoplasm and it appears to be involved in the movement of photosynthetic materials into and out of the plastid (Rosado-Alberio et al. 1968; Gracen et al. 1972). The peripheral reticulum probably functions in the transfer of the osmophilic material to the chloroplast envelope, from where it passes to the endoplasmic reticulum and finally to the plasmalemma (Werker and Fahn 1981).

We also observed Golgi bodies in the head cells of the capitate trichomes. The presence of these organelles, in association with RER, indicates secretion of carbohydrates and proteins (Hawes 2005). We confirmed this with a positive reaction in the protoplasm of these cells using bromophenol blue histochemical tests. In addition, in all glandular trichomes in the *Cajaninae* species studied there is a high density of ribosomes, polyribosomes and rough endoplasmic reticulum, and these could be related to the intense cytoplasmic enzyme synthesis needed for cell metabolism (Turner and Croteau 2004). A hypothesis is that this intense cytoplasmic activity assists in the release of the gland exudates and the dissolution of the middle lamella, a process which occurs in all the glands here studied in subtribe *Cajaninae* and which is necessary for the cell wall gaps to develop (Machado et al. 2006).

Signs of granulocrine and eccrine mechanisms of elimination of the secretion from the protoplast were observed in the three types of glands studied here. Evidence of this includes the presence of scattered oil drops in the cytoplasm and near plasmalemma, multivesicular bodies, and numerous vesicles (scattered in the cytoplasm and near plasmalemma). The presence of oil drops near the plasmalemma facing the intercellular spaces suggests an eccrine secretion mechanism. In this secretion mechanism the substance crosses the plasmalemma by active processes (Fahn 1979), then traverses the porous cell wall through the mechanical action of the protoplast with successive cycles of contraction and expansion, as suggested by Paiva (2016). On the other hand, the numerous coated vesicles and multivesicular bodies juxtaposed to the plasmalemma in the cells of the glandular trichomes of the *Cajaninae* indicate a granulocrine mechanism of secretion (Fahn 1979). In this process the membrane of the vesicles fuses with the plasmalemma and the vesicle content is released from the protoplast (Fahn 1979). Images suggesting that the secretion reach the periplasmic space by exocytosis (granulocrine secretion) were frequently observed in most cells of the *Cajaninae* secretory trichomes. Alternatively, the vesicles are eliminated by invaginations of the plasmalemma (Fahn 1979), this probably occurring in the terminal cell in the neck region of the bulbous-based trichomes where we observed labyrinthic walls with microtubules arranged perpendicularly. The labyrinthic walls characterize transfer cells (Evert 2006), a common feature in salt glands, nectaries (Fahn 1979), and glandular trichomes (Owen and Thomson 1991; Tozin and Rodrigues 2017). This structure enlarges the surface protoplast (Fahn 1979) and may facilitate release of the exudates (Gunning & Pate 1969).

Apparently, the cell walls in some cells of the *Cajaninae* secretory trichomes have variable electron density because the passage of material through them occurs. In addition, our results demonstrate

the involvement of the secretory cell walls in the release of exudates, as discussed by Tresmondi et al. (2017). The loose aspect of these cell walls is a feature that aids the passage of exudates, because the loosely arranged cellulose microfibrils produce a porous cell wall that allows material from the cytoplasm to pass through them (Evert 2006). The formation of intercellular spaces through the displacement of the cells results from the dissolution of the middle lamella. This dissolution starts where more than two cells are joined in the head of a capitate trichome or vesicular gland, and in the bulbous region in bulbous-based trichomes. The accumulation of secretion in the intercellular spaces already has been reported for some glandular trichomes (Baucher and Holzl 1959; Akers et al. 1978; Possobom and Machado 2017). In the vesicular glands, these spaces are larger, and at maturity it is possible to observe a wide space where the secretion is stored. This feature also was described for the peltate and cavitated trichomes of other Leguminosae taxa (Matos and Paiva 2012; Marinho et al. 2015). Furthermore, in the vesicular glands we observed the detachment of the cuticle from the external cell walls, a feature previously noted in several types of glandular trichomes (Amelunxen 1964; Schnepf 1968; Cornara et al. 2001; Duke and Paul 1993; Matos and Paiva 2012). The secretion from the protoplasm of the cells of the gland head most probably ends up being stored in the subcuticular space, the origin of which seems to be related to the dissolution of the pectic layer between the outer periclinal cell wall and the cuticle, as described in other gland types (Possobom and Machado 2017).

In glandular trichomes exudates release can occur via pores in the cuticle (Fahn 1979; Ascensão et al. 1999), by the rupture of the cuticle (Serrato-Valenti et al. 1997, Bisio et al. 1999), or even by diffusion through the cuticle (Serrato-Valenti et al. 1997). The release of exudates from the bulbous-based trichomes most likely occurs through the rupture of trichome cells by a line of breakage. A similar process also has been observed in some trichomes of the Asteraceae, where the breakage zone was described as a "line of weakness in the middle of the gland head" (Cornara et al. 2001), although the morphology of these glands in Asteraceae is completely different from the bulbous-based trichomes of the Cajaninae. In this peculiar glandular trichome of Cajaninae the line of breakage is in the terminal portion of the neck region and the gland breakage and gland exudate release is readily caused by herbivory. In vesicular glands and capitate trichomes, exudates exit from the glandular head through the intact cuticle. The cuticle of the Cajaninae glandular trichomes becomes loosely organized but remains undamaged. We observed electron-dense bands in the cuticle; these bands probably are microchannels, as observed by Ascensão et al. (1997) in the reticulate cuticle of peltate trichomes in Lamiaceae species. Microchannels have also been reported in cell walls, as in *Hibiscus pernambucensis* Arruda (Malvaceae) (Rocha and Machado 2009); the authors noted that these structures increase the porosity of the wall to macromolecules. Ascensão et al. (1997) suggested that microchannels constitute the exit routes of the volatile substances of peltate trichomes of *Leonotis leonurus* (L.) R.Br.

The vesicular glands of Cajaninae morphologically resemble the cavitated glands of *Bauhinia* L., described by Marinho et al. (2015), with similar formation of a large intercellular space in the cells of the head of these two secretory structures by dissolution of the middle lamella. Nevertheless, the two gland types are different because vesicular glands have a rounded shape and a single-celled foot. The formation of the head cells occurs side by side, followed by dissolution of the middle lamella and then separation of

the cells of the head. In the space formed by the dissolution of the lamella there is deposition of the lipophilic content secreted by the cells that have moved away, and the exudate exits from the glandular head through the cuticle. In contrast, Marinho et al. (2015) observed a lenticular shape and biseriate foot in the cavitated glands of *Bauhinia*, and the internal space in the head is formed by the separation of cells from the apex towards the base, after which trichome elongation occurs only due to the increase in cell volume. Moreover, the secretion process in cavitated glands occurs through rupture of the cuticle.

Secondary metabolites are adaptive traits that have been subjected to selective pressures during evolution and are important for survival due to their defence function (Wink 2003). Our results suggest that the glandular trichomes in *Cajaninae* and their secretory compounds function as a defence against herbivores and help to prevent damage by UV radiation. The low incidence of herbivory (personal observation Vargas, W.) and the survival capacity in environments with a high light intensity and high temperatures of taxa in the *Cajaninae* appear to be strongly associated with the presence of glandular trichomes.

Despite the apparent functional and ecological importance of secretory trichomes in this plant group, a greater sampling of species is needed across the wide geographical distribution of the *Cajaninae* before the full functional importance of glandular trichomes can be understood.

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Table 1. Voucher information for the studied taxa

Taxa	Locality	Vegetation	Collector	Herbarium
<i>Eriosema simplicifolium</i> (DC.) G. Don	Botucatu, São Paulo, Brazil	Cerrado, open grassland in rocky areas	W Vargas and DP Seixas 76; W.Vargas 81 and 82	BOTU
<i>Rhynchosia minima</i> (L.) DC.	Botucatu, São Paulo, Brazil	Cerrado, edge of dry forest	W Vargas 71, 83, 84	BOTU
<i>Eriosema rufum</i> (Kunth) G. Don var. <i>rufum</i>	Corinto, Minas Gerais, Brazil	"campos cerrados" and pastures on sandy soils	W Vargas et al. 80, 85, 86	BOTU

Table 2. Distribution of secretory structures in three species of Cajaninae

Species	Vesicular Glands		Bulbous-based trichomes		Capitate trichomes	
	Ad	Ab	Ad	Ab	Ad	Ab
<i>Eriosema simplicifolium</i> (DC.) G. Don	+	+	-	-	+	+
<i>Rhynchosia minima</i> (L.) DC.	+	+	-	+	+	+
<i>Eriosema rufum</i> (Kunth) G. Don var. <i>rufum</i>	+	+	-	+	+	-

+ = positive; - = negative; Ad = Adaxial; Ab = Abaxial

Table 3. Histochemical analysis in reaction sites in each cell of the secretory trichomes of three species of Cajaninae

Type of glandular trichomes		Capitate trichome		Bulbous-based trichome				Vesicular glands		
Histochemistry test	Compounds	Reaction site								
		Basal cell	Head cells	Basal cell	Bulb cells	Neck cells	Apical cell	Basal cell	Stalk cell	Head cells
Sudan Red	Total lipids	Lateral walls	Lateral walls; Cuticle	Lateral walls	Lateral walls; Cuticle	Lateral walls; Cuticle	Lateral walls; Cuticle	Lateral walls; Protoplasm	Lateral walls; Cuticle	Lateral walls; Cuticle; Subcuticular space; Central cavity
Nile Blue	Acidic lipids	Protoplast	Protoplast	-	Protoplast	-	-	-	-	-
Ferric chloride	Phenolic compounds	-	-	-	*Protoplast	*Intercellular space	-	*Protoplast	*Protoplast	*Protoplast
Bromophenol blue	Total proteins	Protoplast	Protoplast	-	-	-	-	Protoplast	Protoplast	Protoplast
Nadi reagent	Terpenes	-	-	-	-	Protoplast; Intercellular space	Protoplast	-	-	-

- = absence; * only in *Rhynchosia minima*

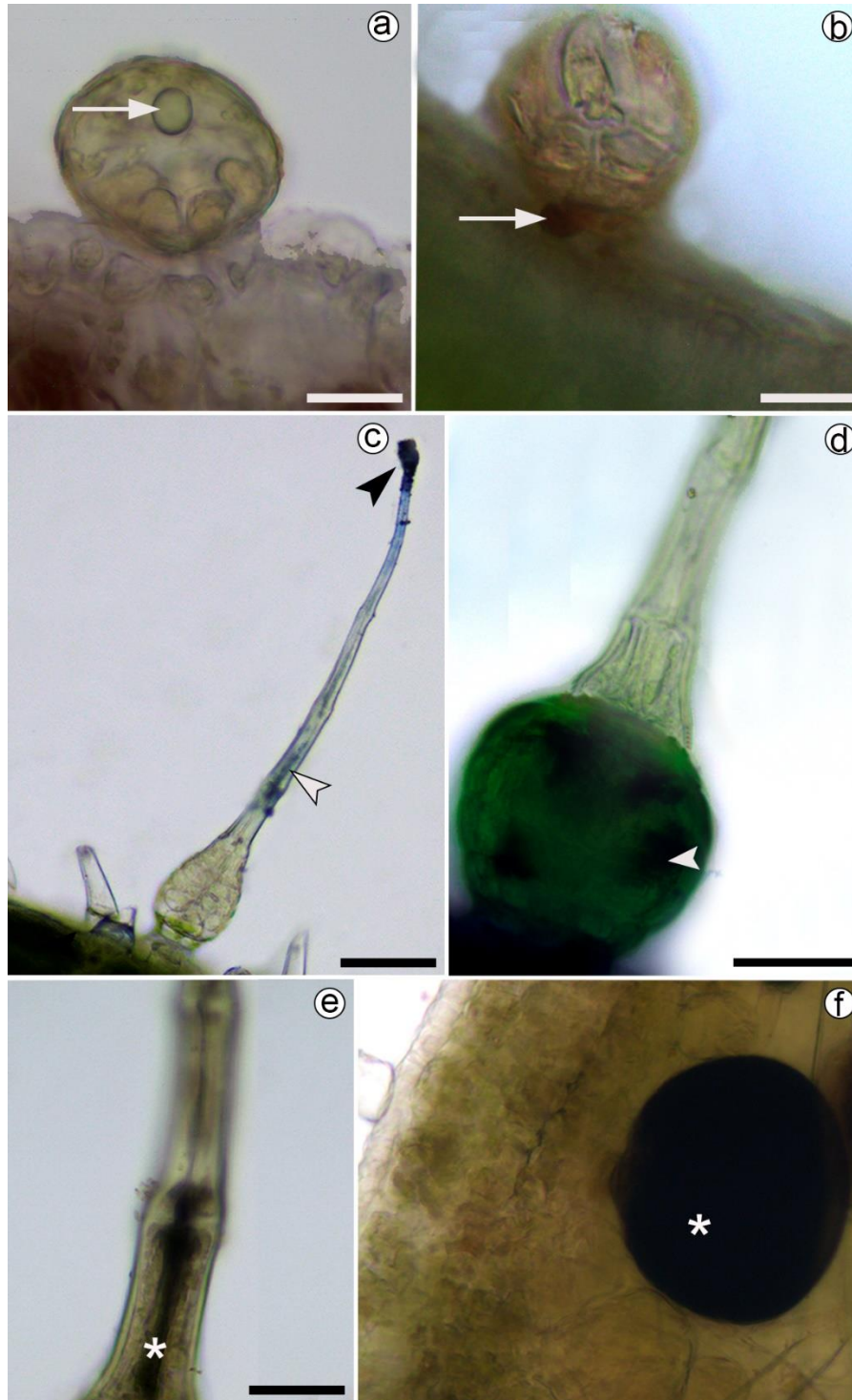


Fig. 1 Micrographs of the histochemical tests in Cajaninae glands in *E. simplicifolium* (a-b), *E. rufum* (c), and *R. minima* (d-f). a Oil drop (white arrow) identified by the histochemical Sudan IV test in central cavity of vesicular glands. b Lipid (white arrow) identified by the histochemical Sudan IV test in stalk of vesicular gland. c Essential oils in a terminal cell (black arrowhead) and proximal cells (white arrowhead) in neck region identified by the histochemical Nadi test of bulbous-based trichomes. d Acidic lipids (white arrowhead) identified by the histochemical Nile blue test in the cells of bulbous region of bulbous-based trichomes. e Phenolic compounds identified by the histochemical Ferric Chloride test in proximal cells in neck region (white asterisk) of bulbous-based trichomes. f and vesicular glands. Scale bars: a-f = 20 μ m.

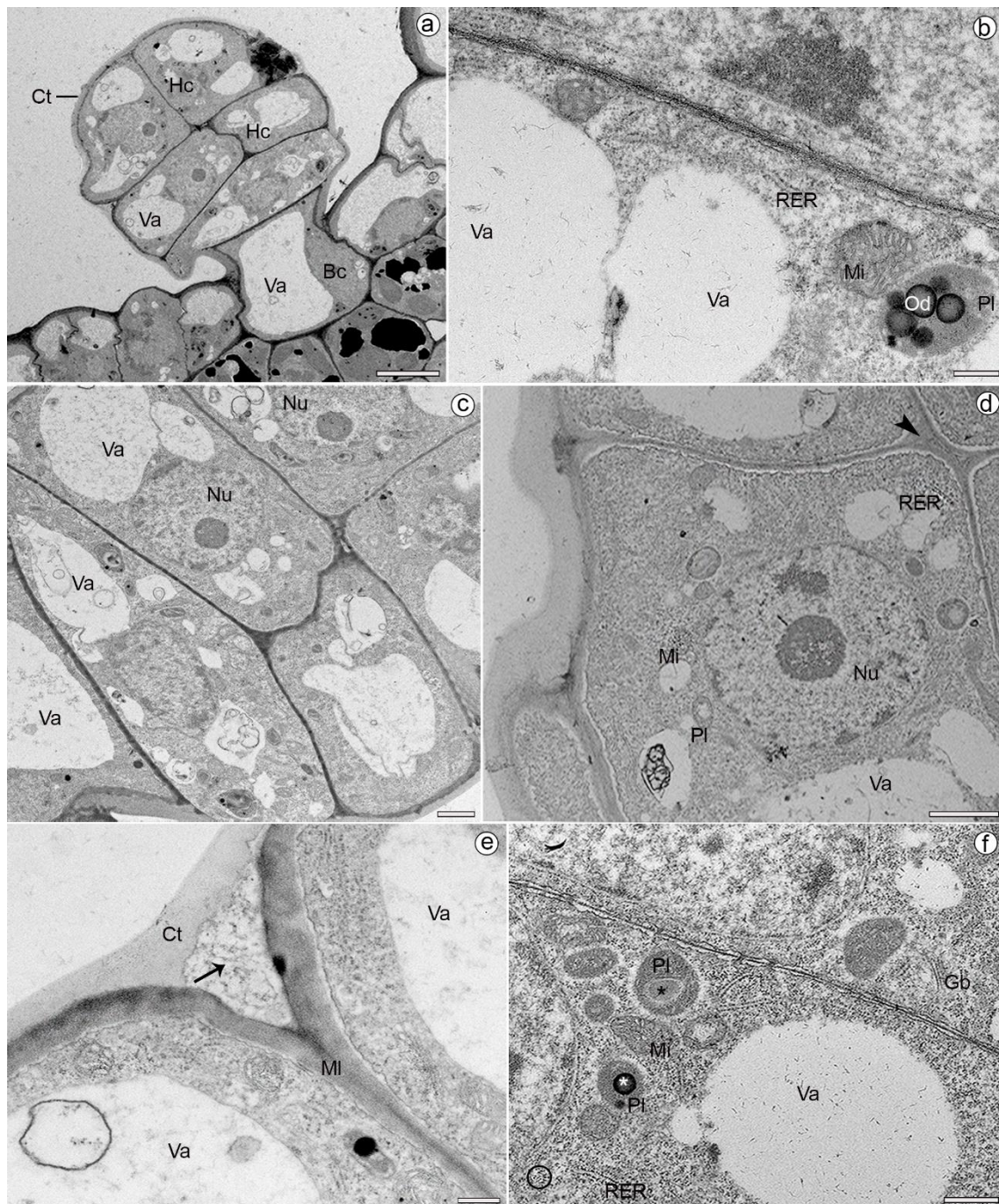


Fig. 2 TEM micrographs of capitulate trichomes in *Rhynchosia minima* (a-c, and e), *E. rufum* (f) and *Eriosema simplicifolium* (d). a Overview of a trichome with basal cell and multicellular head covered with a tick cuticle; basal cell project above the other cell of the epidermis, exhibiting a thin tangential wall, voluminous nucleus, and large vacuoles. b Basal cells with rounded plastids, devoid thylakoid and with oil drops, mitochondria with well-developed cristae, and rough endoplasmic reticulum. Head cells (c-f) c Cells with square or rectangular shape and exhibiting thin walls, large and central nucleus, vacuoles of different sizes and abundant cytoplasm. d Rough endoplasmic reticulum (RER), plastids, and mitochondria with developed cristae; cells in the distal portion with middle lamella more electron dense and tick (black arrowhead). e Middle lamella showing signs of dissolution and accumulation of flocculated material (black arrow). f Cytoplasm with free ribosomes, polyribosomes (black circle), plastids with starch grain (black asterisk) and lipid inclusion (white asterisk), rough endoplasmic reticulum, mitochondria, and Golgi bodies. (Bc Basal cell, Ct Cuticle, Gb Golgi bodies, Hc Head cell, Mi Mitochondria, Ml Middle lamella, Nu Nucleus, Od Oil drop, Pl Plastids, RER Rough endoplasmic reticulum, Va Vacuole). Scale bars: a = 5 μ m; b, f = 500nm; c-e = 2 μ m.

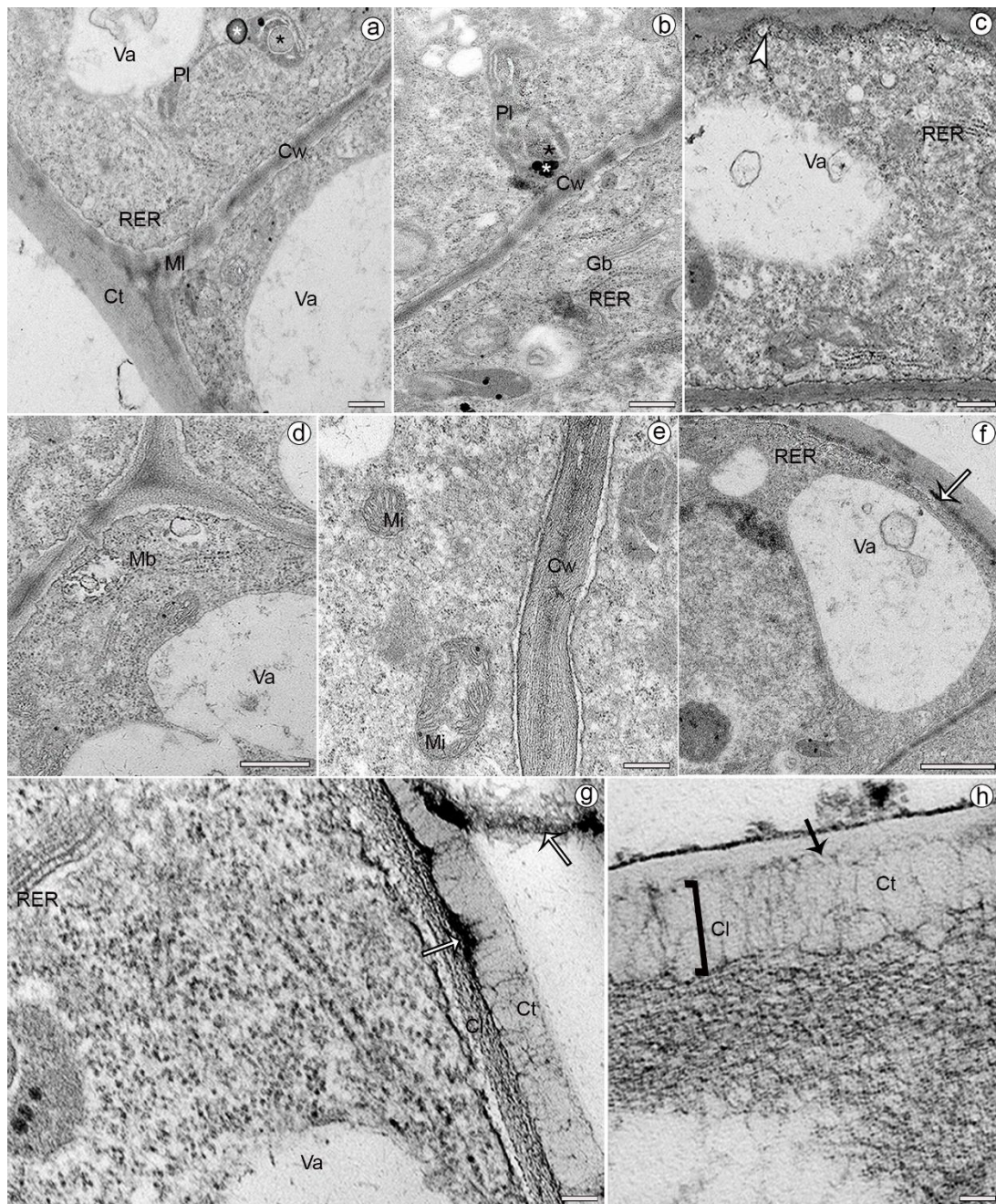


Fig. 3 TEM micrographs of the head cells of the capitate trichomes in *Rhynchosia minima* (b and f), *E. rufum* (a, and c-e) and *Eriosema simplicifolium* (g-h). a Anticlinal walls with variable electron density, polymorphic plastids devoid thylakoids, and with starch grains (black asterisk) and lipid inclusion (white asterisk). b Anticlinal walls with variable electron density, Golgi bodies with two to four cisternae, rough endoplasmic reticulum (RER), and polymorphic plastids with starch grain (black asterisk) and lipid inclusion (white asterisk). c Coated vesicles (white arrowhead) from RER in the peripheral cytoplasm and juxtaposed to the plasmalemma with signals of fusion with this membrane. d Multivesicular bodies located near the plasmodesmata and scattered in the cytosol. e Anticlinal walls with variable electron density exhibiting loose aspect, and mitochondria with well-developed cristae. f Cells in distal region showing RER adjacent to plasmalemma and dark materials (white arrow) accumulated in periplasmic space in outer periclinal walls. g Dark material (white arrow) in cell wall, inside cuticular layer, and on the cuticle. h Outer periclinal cell with reticulated aspect and cuticle exhibiting a large cuticular layer with electron dense ramifications that do not reach the proper cuticle (black arrow). (Cl Cuticular layer, Ct Cuticle, Cw cell wall, Gb Golgi bodies, Mb Multivesicular bodies, Mi Mitochondria, Ml Middle lamella, Pl Plastids, RER Rough endoplasmic reticulum, Va Vacuole). Scale bars: a-b and d-f = 500nm; c = 1 μ m; g = 200 nm; h = 100 nm.

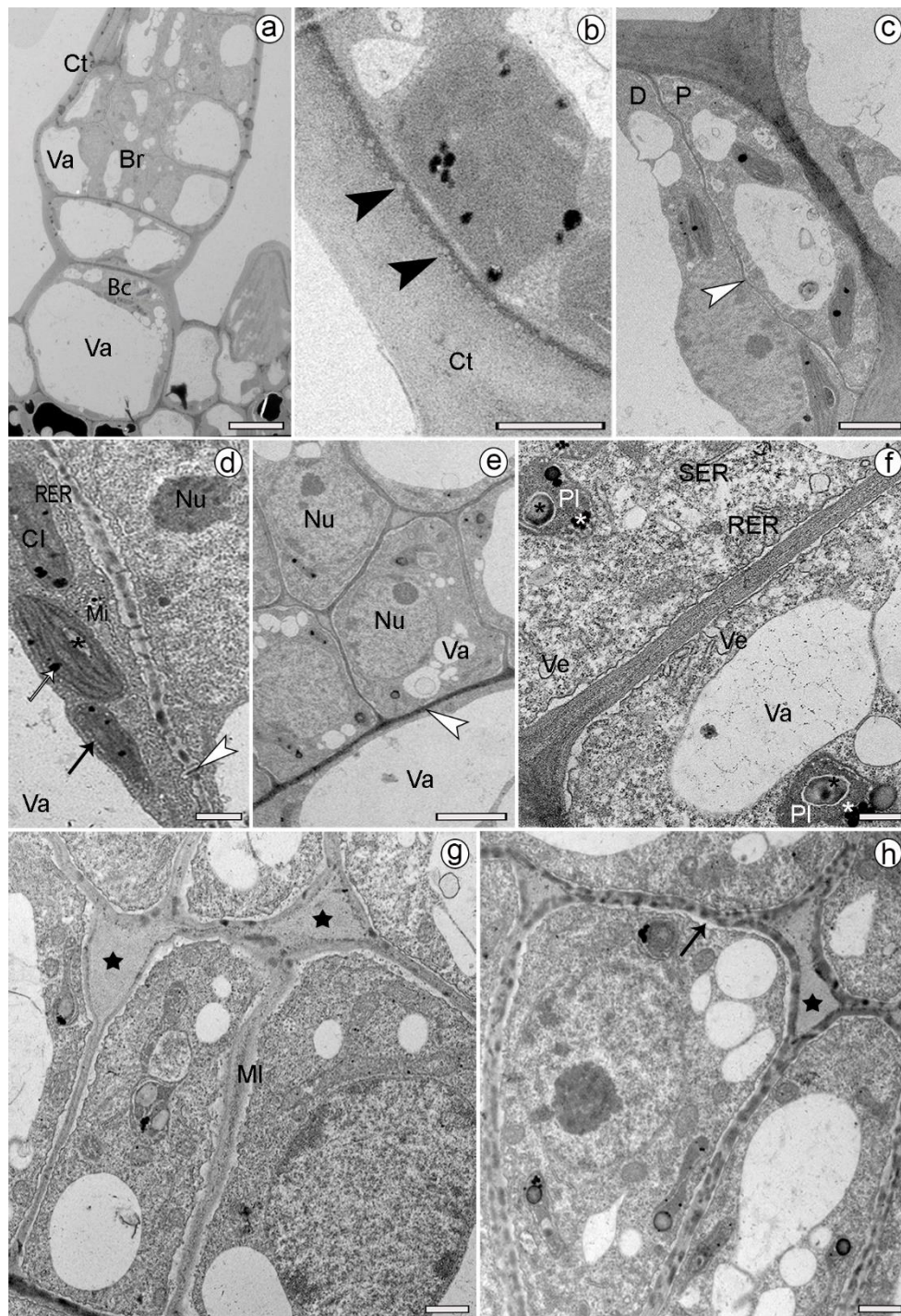


Fig. 4 Transmission electron micrographs (TEMs) of the bulbous-based trichomes in *Rhynchosia minima*. a Overview of the basal and bulbous region cells covered with thick cuticle; the basal cells are composed by proximal and distal cell, the proximal cell have large vacuole; the cells of the periphery of the bulbous regions have large vacuoles and reduced cytoplasm, while the inner cell of the bulbous regions have small vacuoles and abundant cytoplasm. b Lipid inclusions (black arrowhead) in the lateral walls of the cells of the bulbous region. *Basal cells (c-d)* c Detail of the thin tangential wall (white arrowhead) in the proximal and distal cell. d The proximal cell contain vacuole, rough endoplasmic reticulum (RER), mitochondria, and chloroplasts with starch grain (asterisk), plastoglobules (white arrow), and peripheral reticulum (black arrow); the distal cell contain larger nucleus, and abundant cytoplasm; plasmodesmata (white arrowhead) between proximal and distal cells. *Bulbous region (e-h)* e The rectangular first cell, with tick wall (white arrowhead), and large vacuole; the inner cells with square shape, thin-walled, and have voluminous nucleus, few and small vacuoles, and abundant cytoplasm. f Inner cells with smooth and rough endoplasmic

reticulum, plastids with starch grains (black asterisk) and osmiophilic globules (white asterisk), and vesicles in peripheral cytoplasm and near plasmalemma. g Inner cells with middle lamella becoming to dissolve and forming intercellular spaces (black stars). h Dark materials in periplasmic space (black arrow), cell walls and intercellular space (black star) in inner cells. (Bc Basal cells, Br Bulbous region, Cl Chloroplasts, Ct Cuticle, D Distal cell, Mi Mitochondria, MI Middle lamella, Nu Nucleus, P Proximal cells, PI Plastids, RER Rough endoplasmic reticulum, SER Smooth endoplasmic reticulum, Va Vacuole, Ve Vesicles). Scale bars: a-b, and d = 5 μ m; c and e= 500nm; f-g = 1 μ m; h = 2 μ m.

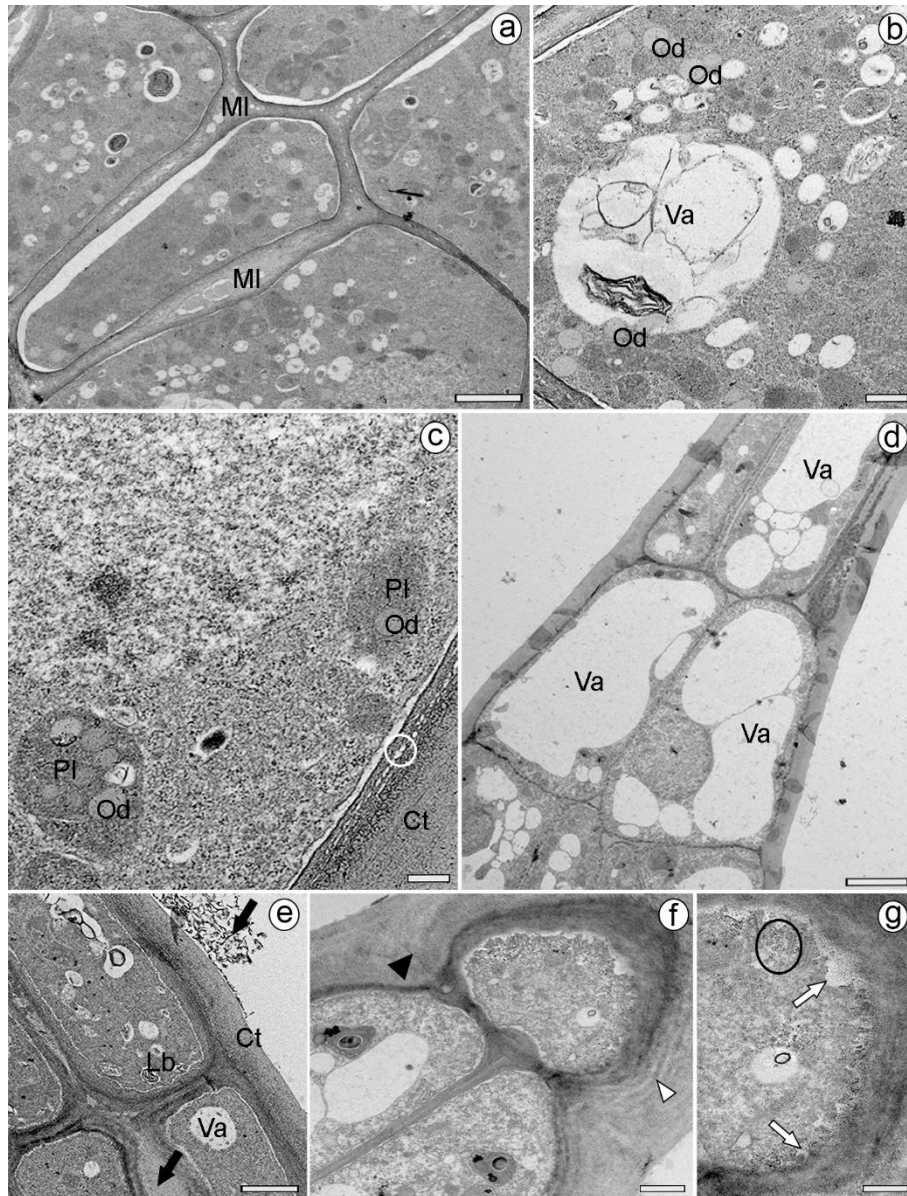


Fig. 5 Transmission electron micrographs (TEMs) of the bulbous-based trichomes in *Eriosema rufum* (a-c) and *Rhynchosia minima* (d-g). **Bulbous region** (a-c) a Progressive dissolution in the middle lamella forming large tubular space between inner cells. b Cells with oil drops scattered in the cytoplasm and in contact with the tonoplast. c Inner cells showing plastids with oil drops and cell walls presented an inner reticulate layer and outer fibrous layer (white circle), with fibrils protruding into the cuticle. **Neck region** (d-g) d Proximal cells with large vacuoles and reduced cytoplasm e Distal cell with abundant cytoplasm and few vacuoles exhibiting lamellar bodies. It is possible to observe that the secretion (black arrow) is accumulated in the periplasmic and intercellular space, cell walls, and on cuticle. f Terminal cell exhibiting poly-lamellate outer walls (white triangle), with evident transversal line of breakage (black triangle) between this cell and the underlying neck cells. g Labyrinthic walls (white arrow) in terminal cell and microtubules (black circle)

arranged perpendicularly to the cell wall. (Ct Cuticle, Lb Lamellar bodies, MI Middle lamella, Od Oil drops, Pl Plastids, Va Vacuole). Scale bars: a-b and d-g = 1 μ m; c = 5 μ m.

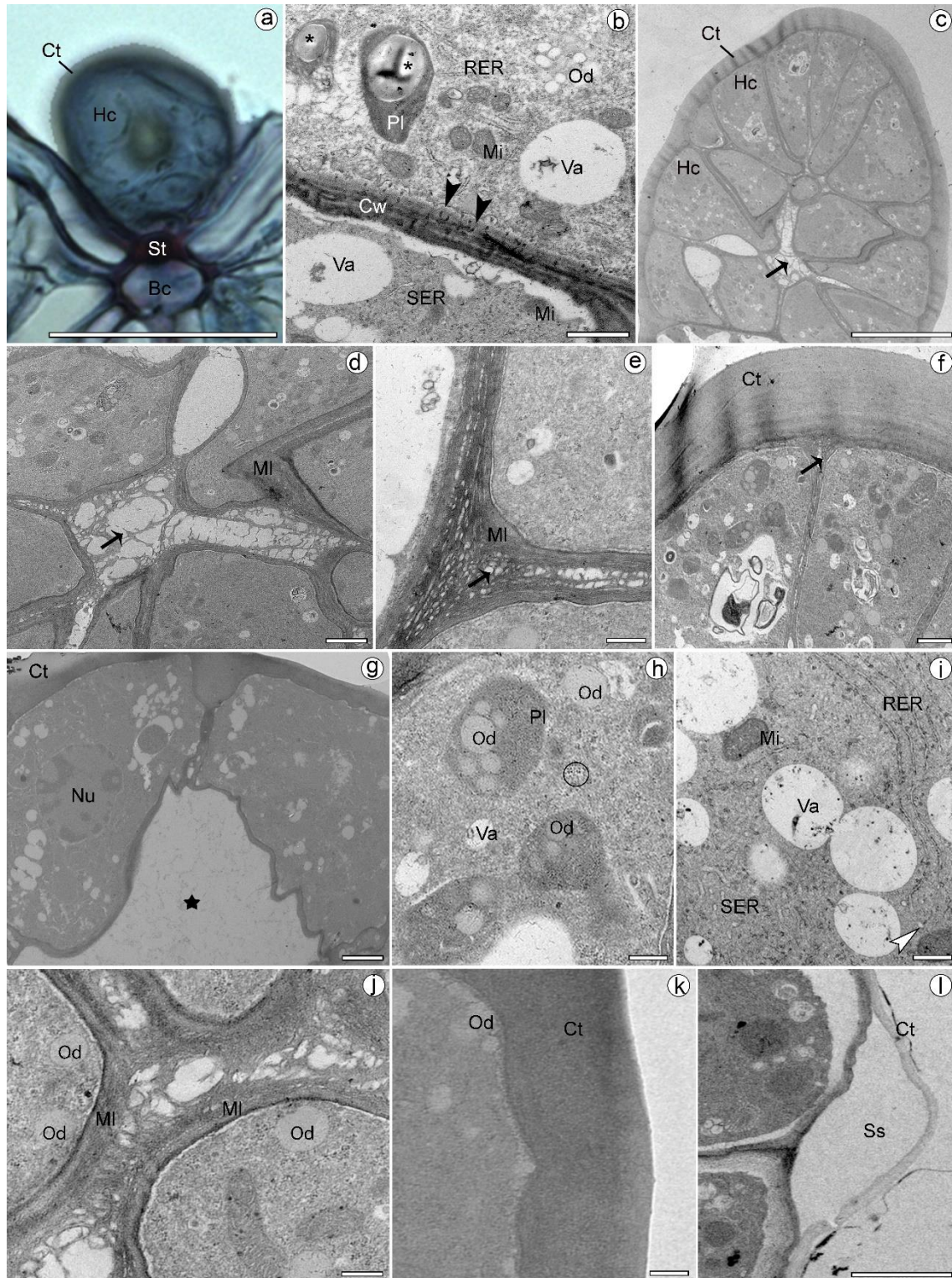


Fig. 6 Transversal section (a) and transmission electron micrographs (TEMs) (b-l) of the vesicular glands in leaflet of the *Eriosema simplicifolium*. a Vesicular gland with continuous cuticle, trapezoidal basal cell, a multicellular head with a central cavity, and inserted in an epidermal depression. b Detail of the basal and stalk cell: basal cell showing vacuoles with different size, mitochondria, and smooth endoplasmic reticulum (SER); stalk cell with few and small vacuoles, dense cytoplasm exhibiting mitochondria, rough endoplasmic reticulum (RER), plastids with starch grain (asterisk), and oil drop scattered in cytoplasm; thick tangential wall between basal and stalk cell with plasmodesmata (black arrowhead). c General view

of head cells exhibiting central cavity developed by separation of adjoining primary cells walls through the middle lamella dissolution (black arrow). d Detail of middle lamella in dissolution (black arrow) characterizing schizogenous process in the head cells. e Detail of middle lamella in dissolution (black arrow) started in the corner of the inner head cell, advancing to other wall regions. f Middle lamella in dissolution (black arrow) reaching the cuticle in an apical head cell. g Broad intercellular space (black star) formed through of the displacement of the head cells towards the periphery of the vesicular gland. h Detail of head cell showing small vacuoles, polyribosomes (black circle), oil drops scattered in cytoplasm, rounded plastids devoid of thylakoids and containing oil drops in head cell. i Detail of head cell showing mitochondria with well-developed cristae, extensive RER, proliferate SER, and numerous vesicles (white arrowhead). j Oil drop occurring in the peripheral cytoplasm near the plasmalemma facing the central cavity in head cells k Several oil drops occurring in the outer periclinal wall in the head cell l Formation of the subcuticular space in the region of the head of the gland. (Bc Basal cell, Ct Cuticle, Cw Cell wall, Hc Head cells, Mi Mitochondria, Ml Middle lamella, Nu Nucleus, Od Oil drop, Pl Plastids, RER Rough endoplasmic reticulum, SER Smooth endoplasmic reticulum, Ss Subcuticular space, St Stalk cell, Va Vacuole). *Scale bars:* a = 50 μm ; b and f = 1 μm ; c and l = 5 μm ; d, g and j = 2 μm ; e, h, i and k = 500nm.

Phylogeny and secretory structures evolution of the giant Cajaninae subtribe (Leguminosae, Papilionoideae, Phaseoleae)

Short title: Phylogeny and evolution of secretory structure of Cajaninae

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Abstract Cajaninae is the largest subtribe of the tribe Phaseoleae, comprising ten genera and close to 500 species, with an overall pantropical distribution. Morphologically representatives of the Cajaninae are quite uniform, being climbing herbs, shrubs or subshrubs, with trifoliolate leaves (some genera with 1 or 5 leaflets), lax or crowded racemose inflorescences, and having fruits commonly with two seeds (some genera have 5 or 6 seeds per fruit). Cajaninae is considered the most uniform subtribe of Phaseoleae for presenting peculiar secretory structures including bulbous-based trichomes and vesicular glands which occur in the majority of their representatives. A number of taxonomic studies indicate that the genera *Bolusafr* Kuntze, *Carrisoa* Baker f., and *Chrysoscias* E. Mey. should be recognised as synonyms of *Rhynchosia* Lour., but the studies have failed to fully elucidate the relationship between the two largest genera, *Eriosema* (DC.) Desv. and *Rhynchosia*. To test the monophyly of Cajaninae and clarify the phylogenetic relationships among the genera within the subtribe, especially *Eriosema* and *Rhynchosia*, which comprise 150 and 240 species, respectively, phylogenetic analyses using the DNA sequences of nuclear ITS and chloroplast *rpl32-trnL* (UAG) and *trnQ* (UUG) -5'rps16 were performed. A total of nearly 300 accessions of American, Asian, African and Australian species were included in the study. Results support the monophyly of the Cajaninae and indicate that *Rhynchosia* and *Eriosema* are sister groups. In addition, anatomical, micromorphological and ultrastructural studies were carried out on 163 taxa of Cajaninae (encompassing nine of the ten genera of the subtribe) to evaluate secretory structures in leaflets, and their evolutionary history. Several secretory structures were found, including bulbous-based and secretory-based trichomes (new observations for Papilionoideae), secretory idioblasts in the mesophyll and in midrib, and vesicular glands which are found in almost all species analysed, and vary in size and morphology. Reconstruction of ancestral characters, based on secretory structures, is being carried out to improve our understanding of the evolution and systematics of the Cajaninae.

Keywords *Eriosema*; *Rhynchosia*; *Cajanus*; phylogenetic reconstruction; anatomy

INTRODUCTION

The pantropical Cajaninae Benth. is the largest subtribe of the Phaseoleae and comprises ca. 500 species, distributed in ten genera, *Adenodolichos* Harms, *Bolusafr* Kuntze, *Cajanus* DC., *Carrissoa* Baker f., *Chrysoscias* E. Mey., *Dunbaria* Wight & Arn., *Paracalyx* S.I. Ali, *Flemingia* Roxb. ex W.T. Aiton, *Eriosema* (DC.) Desv., and *Rhynchosia* Lour. (Schrire 2005). A number of taxonomic studies indicate that the genera *Carrissoa* and *Chrysoscias* should be recognised as synonyms of *Rhynchosia* (Germishuizen 2000; Schrire 2005), and the species of *Paracalyx* were originally described in the genus *Cylista* Aiton, which is now a synonym of *Rhynchosia* (Ali 1968; Schrire 2005), but the studies have failed to fully elucidate the relationship between the two largest genera, *Eriosema* and *Rhynchosia*.

The genera of Cajaninae are mainly distributed in the tropical and subtropical regions of the Old World (Africa, Asia) and Oceania, except the biggest genera, *Eriosema* and *Rhynchosia* (holding 150 and 240 species, respectively), that are pantropical (Schrire 2005). The taxa occur mainly in seasonally dry tropical forest, grasslands, woody rootstock in fire-prone environments, along water courses in rocky areas, and Mediterranean montane sclerophyllous shrubland (fynbos) (Schrire 2005 – Figs 1a-c). Moreover, some genera occur in drought-prone environments seasonal and rocky or sandy shallow soils with low fertility (Candido 2018). These environments in neotropical region are referred to “campos rupestres”, in Africa a very similar vegetation is found in the Chimanimani mountains on the border Zimbabwe-Mozambique (Lock 2006), where one of the abundant genus is *Eriosema* (Timberlake et al., 2016). These types of vegetation are considered some of the most threatened ecosystems in the planet, mainly

by the mining, climate changes, agricultural and urban expansion (Milton et al. 1999; Martinelli 2007; Timberlake et al. 2016).

This subtribe includes many important genera used for human food (*Cajanus*: pigeon pea or gungo pea; *Flemingia*: underground tubers; *Eriosema* and *Rhynchosia*: famine foods; *Dunbaria*: related to *Cajanus* and may be useful in breeding plans of pigeon pea) (van der Maesen, 1998; Schrire 2005). Also used to medicine (*Eriosema kraussianum* Meisn., *Eriosema robustum* Baker, *Eriosema laurentii* De Wild.) for erectile dysfunction, cough, bacterial infections, infertility treatment and menopause (Burkill 1985; Ojewole 2007; Kokwaro 2009; Awouafack et al. 2013, 2008). The Cajaninae plants also be useful for green manure (the most of genera), handicraft (*Rhynchosia* with bicoloured seeds – Fig 1e) and cover crops (Schrire 2005). However, despite the importance of the taxa of this subtribe the relationships among the infratribal groups are still poorly known.

Cajaninae involves climbing herbs, vines, herbs, shrubs, and subshrubs (Fig 1d), with mainly trifoliolate leaves (or 1-5-foliolate), flowers arranged in raceme inflorescences (lax or crowded), petals frequently yellow (Fig 1g) or red, and fruits (a legume) with commonly two seeds (rare 3 or more – Figs 1e,f and h), bracteoles and canavanine absent (except in *Adenodolichos*) (Lackey 1977, 1981). The group is characterized by peculiar secretory structures such as bulbous-based trichomes and vesicular glands (Debold 1892; Lackey 1977, 1981; Solereder 1908; Vargas et al. 2018 – Figs 2-3). Moreover, novel secretory structures have been discovered recently for *Eriosema*, as secretory-based trichomes, which proved to be useful for the taxonomy of taxa (Cândido et al. 2016).

The secretory structures are so important for this group that Lackey (1981) used these characteristics to maintain *Adenodolichos*, the genus with the highest morphological

discrepancy of the subtribe, within *Cajaninae*. Unlike other genera of the subtribe, *Adenodolichos* has bracteoles, bearded style, and canavanine, but was maintained in *Cajaninae* because it has bulbous-based trichomes and vesicular glands, which were structures considered unique to the constituents of the subtribe (Lackey 1981).

Through studies with the morphology of *Cajaninae* genera, Lackey (1977, 1981) and Baudet (1978) suggested that the subtribe was a most consistent group of *Phaseoleae*. Supporting the results of these authors, *Cajaninae* was resolved as a monophyletic subtribe (Doyle & Doyle 1993; Bruneau et al. 1995; Egan et al. 2016; LPWG 2017), however in these studies the subtribe was represented by no more than 10 species. The data of chloroplast DNA of Bruneau et al. (1995) suggest that the subtribe originated from part of the major radiation of *Phaseoleae* (including *Desmodieae*) differently from *Clitoriinae*, *Ophrestiinae*, *Diocleinae*, *Neorudolphia* Britton, and *Rhodopis* Urb. Doyle et al. (2000) and Kajita et al. (2001) considered that the *Cajaninae* subtribe formed a basal polytomy with *Phaseolinae*, *Glycininae* and *Psoraleeae* clade. Recently, Egan et al. (2016) showed that *Cajaninae* plus subtribes *Psoraleeae*, *Glycininae*, *Erythrinae*, and the genus *Butea* Roxb. ex Willd. comprise core *Phaseoleae* (sensu Schrire 2005), the results of this study also showed *Cajaninae* as monophyletic in the analysis of chloroplast markers, but in the analyses with the AS2 nuclear marker, this subtribe can be monophyletic if includes the genus *Apios* Fabr. Besides that, in LPWG (2017) study, which used the chloroplast region *matK*, *Cajaninae* was considered monophyletic.

Based on the above considerations our aims were to provide the most robust phylogeny of the subtribe *Cajaninae* with wide sampling and also examine ancestral states for the presence of bracteoles, number of seeds per fruit, and secretory structures types. Therefore, our results were used to: (1) verify (or not) the monophyly of *Cajaninae*; b) to clarify the generic relationships among *Cajaninae* genera; and c) to reconstruct ancestral

characters related to morphology and secretory structures, that are so important to subtribe.

MATERIALS AND METHODS

Taxon Sampling – In this study c. 300 samples of taxa belonging to nine (of total ten) traditionally recognized genera in Cajaninae and covering all geographic areas of occurrence of the subtribe representatives around the world were included (Table 1). The monospecific genus *Carrissoa* (*C. angolense* Baker f.) was not included in this study, due to lack of available material for sequencing. Also were included one species of the genera *Centrosema*, *Camptosema*, and *Galactia* (all members of the Phaseoleae tribe) (Table 1). The outgroups were chosen based on previous systematic studies by Lavin et al. (2001) and Klitgaard & Lavin (2005) that showed them to be most closely related to Cajaninae. We analysed the nuclear ribosomal ITS-1 and two chloroplast regions (rpl32-trnL^(UAG) and trnQ^{(UUG)-5' rps16}). Most of material was obtained from specimens in national and foreign herbaria, while a few taxa were collected in the field and stored in silica gel. A complete list of GenBank accession numbers will be provided before the publication of this study.

DNA Extraction, Amplification, and Sequencing – Total DNA was extracted using the NucleoSpin plant II kit (Macherey-Nagel, Düren, Germany), according to the manufacturer's protocols with a modified protocol in the beginning of the procedure, adding 4 µL RNase A stock solution and for herbarium materials we left on the heater for about three hours. Herbaria and silica-dried material was grounded with liquid nitrogen or with a Geno/Grinder® 2010 machine (SPEX® SamplePrep LLC; for 2 minutes on speed 2). To amplify ITS region (including ITS1 and ITS2 spacer regions and the 5.8S ribosomal subunit) of nuclear ribosomal DNA (nrDNA) primer pairs 17SE and 26SE (Sun et al. 1994) were used. The PCR amplifications were prepared in 25 µL

reactions containing 2 μ L of 10 $\times$ PCR buffer, 1.5 μ L of 50 mM MgCl₂, 1 μ L of 2.5 mM dNTPs, 1 μ L of Kb Extender (Invitrogen), 1 μ L of DMSO 5%, 0.5 μ L of bovine serum albumin, 0.5 μ L of betaine, 2 μ L of each primer (5 pM), 0.2 μ L of Platinum Taq DNA polymerase (Invitrogen), and 1.5 μ L of DNA template, adjusted with de-ionized water. The ITS region was amplified according to the following program: 11 cycles using the touchdown strategy: 94°C for 3 min, 94°C for 1 min; -1°C per cycle as from 65°C; 72°C for 1 min; 15 cycles back to step 2, 94°C for 1 min; 50°C for 1 min; 72°C for 4 min; 21 cycles back to step 2; 72°C for 1 min and 15°C constant at the end of the reaction. The chloroplast DNA (cpDNA) rpl32-trnL and trnQ-5'rps16 intergenic spacers were amplified using primers, trnL^(UAG)/rpL32-F and trnQ^(UUG)/rpS16x1, respectively (Shaw et al. 2007). The rpl32-trnL and trnQ-5'rps16 intergenic spacers were amplified according to the following program: 35 cycles: 95°C for 1 min; 55°C for 1 min; 72°C for 1 min; final extension 72°C for 7min and 10°C constant at the end of the reaction. Following PCR cycling, the amplification was checked by agarose gel electrophoresis using 0.8% agarose gels. The PCR products were purified using the NucleoSpin gel and PCR clean-up kit (Macherey-Nagel, Düren, Germany). Purified PCR products were used as templates for sequencing with the primers used in the amplifications. DNA sequencing reactions were performed in an automatic sequencer ABI 3730xl (Macrogen Inc., Seoul, South Korea).

Phylogenetic analysis – Forward and reverse sequences were edited and aligned into contigs with Geneious version 8.1.9 (<http://www.geneious.com>, Kearse et al., 2012). A BLAST search (<http://blast.ncbi.nlm.nih.gov/>) was conducted for all sequences to check for possible contaminant DNA. Regions of DNA were aligned using MAFFT v7.369b (Katoh & Standley, 2013) under default setting, before the alignments were manually revised in AliView (Larsson, 2014). Our phylogenetic analysis are based on

Maximum likelihood (ML) approaches. ML analyses were performed using Randomized Accelerated Maximum Likelihood (RAxML), version 8.0.14 (Stamatakis, 2014). Were performed 1000 bootstrap replicates and searched for the best-scoring ML tree.

Evolution of Secretory Structures studies —To obtain a broad view of the variations of the secretory structures occurring in *Cajaninae* taxa, samples of 163 species (Table 1) were rehydrated following the procedure of Smith and Smith (1942) and stored in 70% ethanol. Leaflets were embedded in Historesin (Leica) and sectioned in a rotative microtome in mid portions of the midrib and terminal leaflets. Section, with 10 μ m were stained with Toluidine pH 4 (Vidal 1977). The documentation was prepared with the aid of an appropriate Leica photomicrography optical microscope. Leaflets from one herbarium specimens of each genus of *Cajaninae* were selected for analyses in scanning electron microscope (SEM). Samples were mounted on aluminum stubs, sputter-coated with gold, and examined with a FEI Quanta 200 SEM at the Eletron Microscopy Center in Institute of Biosciences (UNESP-Botucatu). Overall secretory structures classification were based on Lackey (1977; 1978; 1981), Payne (1978), Theobald et al. (1979), Leelavathi and Ramayya (1983), and Cândido et al. (2016).

The ancestral states were coded according to the presence or absence of vesicular glands, bulbous-based trichomes, secretory-based trichomes, capitate trichomes and idioblasts in midrib and mesophyll. The morphological traits were coded according to Schrire (2005) and Lackey (1981) using two states for number of seeds per fruit (being, 1: Two seeds and 2: three or more seeds), and presence and absence for bracteoles.

Ancestral states reconstruction on the nodes of the tree was conducted by Mesquite version 3.6 (Maddison and Maddison, 2018) by maximum likelihood method using concatenated tree of nuclear and chloroplast markers (277 OTU).

We provide a table (1) with taxa names, voucher specimens, locations, ancestral states, and markers used. Besides that, a table (2) with general features about Cajaninae genera, such as distribution range, environments of occurrence, ploidy level and uses, was carried out (according to Lackey, 1981; Schrire, 2005; Rice et al. 2014).

RESULTS

Our analyses included 228 taxa (285 samples) and 745 DNA sequences were newly generated, we implemented just ML analyses until this moment. Of the total taxa sampled, 237 were analysed for ITS, 257 for rpl32-trnL, and 251 for trnQ-5'rps16 (Table 1). Based on the ML analyses, using combined dataset of all markers (ITS, rpl32-trnL and trnQ-5'rps16), we showed that the subtribe Cajaninae is monophyletic (with high support, 100% BS – Figs 4g, 5b and 6). Besides that, the genera *Adenodolichos*, *Dunbaria*, *Cajanus* (all with high support, 100% BS – Figs 4g and 5b), *Eriosema* and *Flemingia* (with high and moderate support, 93% and 77% BS, respectively – Figs 4d and g), also emerged as monophyletic. After the clades formed by *Adenodolichos* and *Flemingia* was noted a clade that included genera *Dunbaria*, *Cajanus*, and species *Rhynchosia volubilis* and *R. dielsii* with moderate support (75% BS – Fig 4g).

The interspecific relationships within *Eriosema* were not resolved yet, and this genus appeared as sister group of *R. versicolor* Baker and *R. clivorum* S. Moore (with robust support 93% BS, Figs 4a-d). Apparently, there were two major geographic groups (American and African) within *Eriosema*, but with low support (low to 50%).

The genus *Rhynchosia* appeared as polyphyletic and divided in 5 lineages (Figs 4d-g and 5). One lineage of *Rhynchosia* (we called *Rhynchosia* I, Figs 4g and 5b) included just *R. volubilis* Lour. (type species of the genus) and *R. dielsii* Harms (100% BS), both with Asian distribution. The second lineage of *Rhynchosia* (*Rhynchosia* II, Figs 4d-f and 5) had weak support (68% BS), and was composed of American, Asian, and African

species. Other lineage of *Rhynchosia* (*Rhynchosia* III, Figs 4d and 5a) was weakly supported (54% BS) and included only African *Rhynchosia* species. The fourth lineage of this genus (*Rhynchosia* IV, Figs 4d and 5a) had good support (93% BS) and included just two African species of *Rhynchosia*, being the sister group of *Eriosema*. The last lineage that were considered in *Rhynchosia*, showed that the genera *Chrysoscias* and *Bolusafra* emerged as paraphyletic (70% BS, Figs 4b and 5a) nested inside *Chrysoscias-Bolusafra* group that is a sister of *Rhynchosia ferulifolia* Benth. ex Harv. and *R. capensis* Schinz. This group is formed by only species of South Africa. The genus *Paracalyx* also was resolved as paraphyletic and nested within the African species of *Rhynchosia* (Fig 4f).

About the reconstruction of ancestral characters analyses, the hypothetical ancestor of Cajaninae (Fig 7) probably did not possess bracteoles. Almost all Cajaninae retained this ancestral state, only *Adenodolichos* representatives that presented bracteoles (100% of clade *Adenodolichos*). Regarding the number of seeds per fruit, we observed that the hypothetical ancestor had most likely two seed in each legume. This state was retained in *Flemingia*, *Rhynchosia*, *Chrysoscias*, *Paracalyx*, and *Eriosema* (100% of each genus). The state more than three seeds per fruit was involved at least in four independent evolutionary events, in genera: *Adenodolichos*, *Dunbaria*, *Bolusafra*, and *Cajanus* (one event each genus - Fig 7).

Regarding to secretory structures it was inferred that hypothetical ancestor of Cajaninae most likely had vesicular glands and capitate trichomes (Fig 8). With very few exceptions, these glands are common in the vast majority of Cajaninae taxa (almost 100%). About bulbous-based and secretory-base trichomes probably the hypothetical ancestor did not have these structures. When these trichomes were considered, ML reconstruction showed several independent origins (Fig 9). The bulbous-based trichome

was present in all genera (at least one time for each genus), while secretory-base trichome was present in *Flemingia*, *Dunbaria*, *Rhynchosia*, and *Eriosema* (genus with the greatest abundance of occurrence). Idioblasts probably were not present in the hypothetical ancestor, regarding ML ancestral character state reconstruction (Fig 10). This secretory structure was present in almost all Cajaninae representatives, and also in two outgroup taxa (*Galactia striata* and *Camptosema grandiflorum*).

DISCUSSION

Our preliminary analyses with ML (nuclear and chloroplast sequences combined) strongly suggest the monophyly of the Cajaninae subtribe (Figs 4,5 and 6), corroborating previous studies done for the Leguminosae family (LPWG 2017) and tribe Phaseoleae as a whole (Doyle & Doyle 1993, Bruneau et al. 1995, Schrire 2005, Kajita et al. 2000, Egan et al. 2016), but the current study has a much larger sampling. According to the aforementioned studies (except Egan et al., 2016) all the representatives of this subtribe are monophyletic, however our study indicated that among Cajaninae genera only *Adenodolichos*, *Dunbaria*, *Cajanus*, *Flemingia*, and *Eriosema* remained monophyletic.

Our results positioned *Adenodolichos* as the sister group of the other members of Cajaninae (Fig 4g), as already stated by Bruneau et al. (1995). The monophyly of this genus can be interpreted through its unifying characteristics that had already been observed by Lackey (1977, 1981). This author considered *Adenodolichos* with a doubtful position in Cajaninae, for possessing canavanine and bracteoles, characteristics absent in the other representatives of this subtribe. However, the same author, later stated that *Adenodolichos* belonged to Cajaninae because it possesses bulbous-based trichomes and vesicular glands, which are peculiar structures of the subtribe.

The genus *Flemingia* was resolved as a sister clade of *Dunbaria*, *Cajanus*, *Rhynchosia volubilis*, and *R. dielsii* (Fig 4g). All these taxa have distribution mainly in

Asian continent (Lackey 1977; Schrire 2005), so this can explain the proximity among these genera. In contrast, the species of *Flemingia* are morphologically distinct from *Rhynchosia* because they have very robust leaves and inflorescences, but are similar with *Cajanus* and *Dunbaria* in number of seeds per fruit (+3, Table 2) and herbs/shrub habit (same in *Cajanus* Table 2) (Lackey 1977).

The dense sampling of *Rhynchosia* taxa, allowed to notice five different lineages of this genus (Figs 4d-g and 6), showing that is paraphyletic, as asserted by Egan et al. (2016) and LPWG (2017), but with minimal sampling and without the intention of discussing relations between the species of this genus. Of these five lineages considered by us, the first included *R. volubilis* (type species of the genus) and *R. dielsii*, the pair with Asian distribution (collected in Japan). Both species have some similar morphological features, such as climbing habit and ovate-deltoid to rhomboid leaflets (W. Vargas, personal observation). In our sampling were included three accessions of *R. volubilis* in order to avoid the possibility of uncertain placement of the type species due to a problem with sequence. For the first time these taxa were included in the phylogeny of Cajaninae.

In the second lineage of *Rhynchosia*, called group *Rhynchosia* II, there was the formation of a big grouping with species from Asia, Africa and America. This group is very diverse in relation to morphology, having habit from climbing to subshrubs, leaflets of various shapes and also including species of wide distribution, such as pantropical *R. minima*. In addition, in this large group the genus *Paracalyx* was nested. Egan et al. (2016) asserted that *Paracalyx* is sister of *Rhynchosia*, however suggest that a more comprehensive sampling would be needed to determine status and association of *Paracalyx*. Species of this genus were originally described in *Cylista* Aiton (Schrire 2005), but *Cylista* is synonymous with *Rhynchosia* (Schrire 2005). The representatives of

Paracalyx are morphologically similar to *Rhynchosia*, having fruits with two seeds (Table 2) and lobed calyx, very expanded and papyraceous after flowering (Lackey 1981).

Other lineage of *Rhynchosia*, called group *Rhynchosia* III, emerged along with *Bolusafr*a and *Chrysoscias* genera. These paraphyletic taxa appeared nested within an African group of *Rhynchosia* species and all species of this group have distribution in South Africa (data of voucher collection). *Bolusafr*a had already been reported as an allied to *Rhynchosia* and *Chrysoscias*, by Schrire (2005); and Germishuizen (2000) included *Chrysoscias* in the genus *Rhynchosia*. Morphologically *Bolusafr*a and *Rhynchosia* are very similar (Moteetee and Wyk 2006). This similarity was also observed by Lackey (1981), which described that *Bolusafr*a as a “viscid *Rhynchosia*-like vine”. Both are usually trifoliolate, have climbing habit, flowers stained, and lobes of calyx exceeding the corolla (Table 2).

The para or polyphyly of the genus *Rhynchosia*, although not previously tested by phylogenetic analysis (except for the work of Egan et al. 2016 and LPWG 2017), was already expected by us, because it is a genus very rich in number of species and is morphologically very diverse. This fact probably explains why *Rhynchosia* always have sectional classification, which is widely mentioned in works with representatives of this taxon (see Grear 1978, Moteetee et al. 2012, 2014, Manyelo 2014, Vargas et al. 2015, Jaca et al 2018). Although it is not our aim, through this work we noted that these sections were not maintained.

With high support the group of African species of *Rhynchosia* emerged as sister group of *Eriosema*, corroborated with some works with lower sampling of this genera (Doyle & Doyle 1993, Bruneau et al. 1995, Kajita et al. 2000). There is a tendency in the formation of geographic groups in *Eriosema*, since the majority of the representatives that occur in the American and African continents formed two separate larger groups.

Eriosema is the only one with geographic distribution restricted to tropical savannas (Cândido 2018) and appears to be the most recent lineage of the subtribe. No group was formed to explain the interspecific relationships within the clade *Eriosema*. Therefore, studies with different markers are needed to obtain better resolution inside this genus.

Regarding reconstruction of ancestral characters, the hypothetical common ancestor of Cajaninae most likely did not possess bracteoles and had two seeds per fruit. These two characters are taxonomically useful to recognize the Cajaninae genera, as more than three seeds are only found in *Adenodolichos*, *Dunbaria*, *Bolusafrax*, and *Cajanus*, as well as, bracteoles are seen only in *Adenodolichos*. Number of seeds in fruit was considered an important character in some groups of Desmodieae (Jabbour et al. 2018), these authors suggested that seed production may be linked to the adaptations to more effective dispersal or age of the individual. Although we have not yet performed the dating of Cajaninae members, it can be seen that in the early diverging lineages of cladogram, the number of seeds is lower (2 per fruit) than in the other lineages (more than 3 per fruit). Clearly this is a subject that should be addressed in future studies. On the other hand, as previously mentioned, the presence of bracteoles is so relevant, that Lackey (1977, 1981) considered *Adenodolichos* as anomalous inside Cajaninae, however this genus shares vesicular glands, which are important secretory structures to this subtribe (Vargas et al. 2018).

Probably the vesicular gland and capitate trichomes are the only secretory structures that were already present in hypothetical ancestor of Cajaninae. Both the bulbous-based trichome and the vesicular gland represent a significant part of the taxonomically important data for the subtribe, being evidenced by the exclusive presence of the vesicular gland in the representatives of Cajaninae (Lackey 1981; Vargas et al. 2018). Although the bulbous-based trichome is also considered as exclusive to Cajaninae,

it is already known that it occurs in members of other Phaseoleae, and even more distant groups, such as Dalbergieae or Desmodieae (Leelavathi and Ramayya 1983; Vargas et al. 2018).

We observed that the secretory structures of Cajaninae had evolved in several independent lineages, the same way as showed in the study of Nogueira et al. (2013) who studied trichomes in some taxa of Bignoniaceae. Corroborating Theobald (1979), that already verified that trichomes would have several independent origins, except on a very limited scale, within small groups. Besides that, the secretory structures of this subtribe, most likely, interact ecologically in survival capacity in environments with a high light intensity and high temperatures, habitats in which Cajaninae representatives are found (Vargas et al. 2018b), however this parameter remains unclear and needs to be tested in future studies, as well as the evolution patterns of trichomes in some clades and the trajectories of independent origins of each type of structure.

Lastly, this study represents the first and most comprehensive molecular phylogeny for giant Cajaninae. Additional studies are being carried out for paper with Bayesian inferences, in order to better understand the generic relationships of this group of plants, and about biogeographic history to determine divergence time estimation of the subtribe.

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Table 1. Taxa studied with voucher information, locations, markers, and ancestral states.

Espèce	ITS	trnq	rpl32	Continent	Country	Voucher	Herbarium	Vesicular Glands	Bulbous-Based trichomes	Capitate trichomes	Secretory-base trichome	Idioblasts	Bracteoles	Number of seeds per fruit
<i>Adendolichos baumii</i>	x	x		African	Zambia	M.G. Bingham 14158	K	1	0	1	0	1	1	2
<i>Adenodolichos baumii</i>	x	x	x	African	South Africa	JP Laveridge 403	PRE	1	0	1	0	1	1	2
<i>Adenodolichos punctatum</i>	x	x	x	African	Malawi	J Pawek 9896	PRE	1	1	1	0	1	1	2
<i>Adenodolichos rhomboideus</i>	x	x	x	African	~	EA Robinson 3794	PRE	1	0	1	0	1	1	2
<i>Bolusafra bituminosa</i>	x	x	x	African	South Africa	Adolf Hafström	S13-6630	1	1	1	0	1	0	2
<i>Bolusafra bituminosa</i>	~	x	x	African	South Africa	Adolf Hafström	S13-6623	1	1	1	0	1	0	2
<i>Bolusafra bituminosa</i>	x	x	x	African	South Africa	Adolf Hafström & Gösta Lindeberg	S13-6627	1	1	1	0	1	0	2
<i>Cajanus flavus</i>	x	x	~	African	Ethiopia	CGT Kotschy 266	P06892742	1	0	0	0	1	0	2
<i>Cajanus scarabaeoides</i>	x	x	x	African	Tanzania	H Faulkner 3202	P02723639	1	0	1	0	1	0	2
<i>Chrysoscias calycina</i>	x	x	x	African	South Africa	S.L. Williams 841	K	1	0	1	0	1	0	1
<i>Chrysoscias grandiflora</i>	x	x	x	African	South Africa	H.T. Scharf 1433	K	1	1	1	0	1	0	1
<i>Dumbaria vilosa</i>	x	x	x	African	Laos	J. Klackenberg 1123	S04-2090	1	1	1	0	0	0	2
<i>Eriosema affine</i>	~	x	x	African	Republic of Malawi	L.J. Brass 17426	NYBG	1	0	1	0	1	0	1
<i>Eriosema afzelii</i>	x	x	x	African	~	N Diarra 1391	SI	?	?	?	?	?	0	1
<i>Eriosema albo-griseum</i>	x	x	x	African	Angola	Anzancot de Menezes 1100	P03092903	?	?	?	?	?	0	1
<i>Eriosema andohii</i>	x	x	x	African	Mali	R Demange 2670	P00513344	1	0	1	0	1	0	1
<i>Eriosema angolense</i>	x	x	x	African	Burundi	M. Reekmans 6308	K	1	1	1	0	1	0	1
<i>Eriosema arachnoideum</i>	~	x	x	African	Tanzania	S Bidgood & I Darbyshire 5279	P03030277	?	?	?	?	?	0	1
<i>Eriosema bauchiense</i>	~	x	x	African	Democratic Republic of Congo	H. M. Richards 20643	S15-33274	1	0	1	0	1	0	1
<i>Eriosema buchananii</i>	~	x	x	African	Republic of Malawi	W. Nachamba & E.J. Tawakali 5	NYBG	?	?	?	?	?	0	1
<i>Eriosema buchananii</i>	x	x	x	African	Rwanda	H.G. Troupin 7480	NYBG	?	?	?	?	?	0	1

<i>Eriosema burkei</i>	x	x	x	African	South Africa	K Grieve 990	PRE	?	?	?	?	?	0	1
<i>Eriosema chrysadenium</i>	x	x	x	African	Tanzania	F. J. Breteler, 11638	S-A1318-84	1	1	1	1	1	0	1
<i>Eriosema cordatum</i>	x	x	x	African	South Africa	K Grieve 987	PRE	1	0	1	0	1	0	1
<i>Eriosema cordatum</i>	x	x	x	African	Natal	C.H. Stirton, 489	S15-33193	1	0	1	0	1	0	1
<i>Eriosema cordatum</i>	x	x	x	African	South Africa	CH Stirton 3898	P03092194	1	0	1	0	1	0	1
<i>Eriosema cordifolium</i>	x	x	x	African	Sudão do Sul	I. Friis & K. Vollescen 433	K	?	?	?	?	?	0	1
<i>Eriosema dregei</i>	x	x	x	African	South Africa	A. Albott 9272	K	1	0	1	0	1	0	1
<i>Eriosema ellipticifolium</i>	x	x	x	African	South Africa	C.H. Stirton 1441	K	1	0	1	1	1	0	1
<i>Eriosema ellipticum</i>	~	x	x	African	Mozambique	A. R. Torre 265	S15-33164	1	0	1	0	1	0	1
<i>Eriosema englerianum</i>	x	x	x	African	Zimbabwe	R. D. Bayliss 10499	S15-33156	?	?	?	?	?	0	1
<i>Eriosema erectum</i>	x	x	x	African	Congo	H. M. Richards 15782	S15-32977	1	0	1	1	0	0	1
<i>Eriosema flemingoides</i>	x	x	x	African	Ivory Coast	A. Fournier 145	NYBG	?	?	?	?	?	0	1
<i>Eriosema flexuosum</i>	x	x	x	African	Congo	E. Milne-Redhead & P. Taylor 8920	S15-33152	1	0	1	1	1	0	1
<i>Eriosema glomeratum</i>	~	x	x	African	Tanzania	Ossian Flock 356	S15-33084	1	0	1	0	1	0	1
<i>Eriosema gunniae</i>	x	x	x	African	South Africa	C.H. Stirton & Gernishuizen 6938	K	?	?	?	?	?	0	1
<i>Eriosema harmsianum</i>	x	x	x	African	South Africa	K. Dinter 5750	S12-10355	?	?	?	?	?	0	1
<i>Eriosema humbertii</i>	x	x	x	African	Ethiopia	J. B. Gillett 14464	S15-33055	1	0	1	1	1	0	1
<i>Eriosema humile</i>	x	x	x	African	Angola	D.J. Goyder 6273	K	?	?	?	?	?	0	1
<i>Eriosema juronianum</i>	~	x	x	African	Ethiopia	Ivar Ambjorn 372	S15-33058	1	0	1	1	0	0	1
<i>Eriosema kraussiana</i>	x	x	x	African	South Africa	K Grieve 942	PRE	1	0	1	0	1	0	1
<i>Eriosema kraussianum</i>	x	x	x	African	South Africa	James L. Sidey 1527	S13-6597	1	0	1	0	1	0	1
<i>Eriosema latifolium</i>	~	~	x	African	South Africa	C.H. Stirton 5599	K	?	?	?	?	?	0	1
<i>Eriosema laurentii</i>	x	x	x	African	Uganda	Kare Arnstein Lye s/n	K	1	0	1	1	1	0	1
<i>Eriosema lebrunii</i>	~	x	x	African	Burundi	M. Reekmans 8784	S15-32978	1	0	1	1	1	0	1
<i>Eriosema macrostipulum</i> var. <i>macrostipulum</i>	~	~	x	African	Burundi	M. Reekmans 4134	K	?	?	?	?	?	0	1
<i>Eriosema mirabilis</i>	~	x	x	African	Angola	R.G.N. Young 1107	NYBG	?	?	?	?	?	0	1

<i>Eriosema molle</i>	x	x	x	African	Togo	H Ern <i>et al.</i> 119	SI	?	?	?	?	?	0	1
<i>Eriosema montanum</i>	x	x	x	African	Mozambique	A. Stolz 222	S15-32914	1	0	1	1	1	0	1
<i>Eriosema monticola</i>	x	x	x	African	Togo	JM Lock 84/94	SI	?	?	?	?	?	0	1
<i>Eriosema naviculare</i>	x	x	x	African	South Africa	C.H. Stirton 9839	K	?	?	?	?	?	0	1
<i>Eriosema nutans</i>	x	x	x	African	South Africa	B.D. Schrire 2422	K	?	?	?	?	?	0	1
<i>Eriosema oblongum</i>	x	x	x	African	South Africa	R.F. Rand 590	NYBG	sn	?	?	?	?	?	0
<i>Eriosema opisenum</i>	~	x	x	African	Democratic Republic of the Congo	L. Liben 3673	NYBG	?	?	?	?	?	0	1
<i>Eriosema parviflorum</i>	x	x	x	African	Liberia	JG Adam 20593	P00582727	1	0	1	0	1	0	1
<i>Eriosema parviflorum</i>	x	x	x	African	Mozambique	Laurenco Marques 120	S15-33100	1	0	1	0	1	0	1
<i>Eriosema pauciflorum</i>	~	x	x	African	South Africa	Adolf Hafstrom & J. P. H. Acock 579	S13-6599	1	0	1	1	0	0	1
<i>Eriosema pauciflorum</i>	~	x	x	African	Zimbabwe	Erik Emanuelsson 620	S15-33180	1	0	1	1	0	0	1
<i>Eriosema pentaphyllum</i>	x	x	x	African	Zambia	H.M. Richards 8193	K	?	?	?	?	?	0	1
<i>Eriosema populifolium</i>	~	x	x	African	South Africa	C. H. Stirton 1200	S15-33310	1	0	1	1	1	0	1
<i>Eriosema preptum</i>	x	x	x	African	South Africa	Hendrik J. Venter & A. Venter 10237	S15-33314	1	0	1	1	1	0	1
<i>Eriosema prunelloides</i>	~	x	x	African	Zambia	D.B. Fanshame 8910	K	?	?	?	?	?	0	1
<i>Eriosema psoraleoides</i>	x	~	~	African	South Africa	Burkart, A. 24522	SI	?	?	?	?	?	0	1
<i>Eriosema rhyncosioides</i>	x	x	x	African	Tanzania	P. Kuchar 25127	S15-33421	1	0	1	1	1	0	1
<i>Eriosema robustum</i>	x	x	x	African	Ethiopia	J. B. Gillett 14595	S15-33424	1	0	1	0	1	0	1
<i>Eriosema rossii</i>	x	~	x	African	South Africa	K Grieve 946	PRE	1	1	1	1	1	0	1
<i>Eriosema shirensae</i> var. <i>adamii</i>	x	x	x	African	Guinea	Adam 14687	SI	?	?	?	?	?	0	1
<i>Eriosema solignum</i>	x	x	x	African	South Africa	K Grieve 968	PRE	1	0	1	0	1	0	1
<i>Eriosema</i> sp	x	x	x	African	South Africa	K Grieve 967	PRE	?	?	?	?	?	0	1
<i>Eriosema</i> sp	x	x	x	African	South Africa	K Grieve 1010	PRE	?	?	?	?	?	0	1
<i>Eriosema</i> sp	x	x	x	African	South Africa	K Grieve 1039	PRE	?	?	?	?	?	0	1
<i>Eriosema sparsiflorum</i>	x	x	x	African	Tanzania	A. Borgdan 3680	S15-33410	1	0	1	1	1	0	1

<i>Eriosema speciosum</i>	~	x	x	African	Angola	B. Fritzsche 101	S15-33406	1	0	1	1	1	0	1
<i>Eriosema spicatum</i>	x	x	x	African	Togo	JM Lock 84/49	K	?	?	?	?	?	0	1
<i>Eriosema squarrosom</i>	x	x	x	African	South Africa	Adolf Hafstrom & J. P. H. Acock 616	S13-6603	1	0	1	0	1	0	1
<i>Eriosema squarrosom</i>	x	x	x	African	South Africa	Ecklon & Zeyher	S15-33390	1	0	1	0	1	0	1
<i>Eriosema triformum</i>	~	x	x	African	Guinea	X. M. van der Burgt, Pierre Haba & Alphonse Traoré 1240	NYBG	?	?	?	?	?	0	1
<i>Eriosema tuberosum</i>	x	x	x	African	Ethiopia	Schimper 1202	S12-10356	1	0	1	0	1	0	1
<i>Eriosema vanderystii</i>	x	x	x	African	Zambia	S.M. Chisumpa SMC3	K	?	?	?	?	?	0	1
<i>Eriosema velutinum</i>	~	x	x	African	Congo	A. A. Bullock 2844	S15-33379	1	0	1	1	1	0	1
<i>Eriosema verdickii</i>	x	x	x	African	Nigeria	J Louve 2699	K	?	?	?	?	?	0	1
<i>Eriosema yongii</i>	x	~	~	African	Malawi	I.H. Patel 1432	K	?	?	?	?	?	0	1
<i>Flemingia grahamiana</i>	x	x	x	African	South Africa	WJ Hanekom 2315	P03130706	1	0	1	0	0	0	1
<i>Flemingia macrophylla</i>	x	x	x	African	Cameroon	P Mezeli 258	P03130671	1	0	0	0	0	0	1
<i>Paracalyx microphyllus</i>	x	x	x	African	Somalia	M. Thulin et al. 10558	K	1	0	1	0	1	0	1
<i>Paracalyx nogalensis</i>	x	x	x	African	Somalia	M. Thulin & A.M. Warfa 5425	K	1	0	1	0	1	0	1
<i>Paracalyx somalorum</i>	x	x	x	African	Ethiopia	I. Friis 9993	K	1	1	1	0	1	0	1
<i>Rhynchosia acuminatissima</i>	x	x	x	African	Papua New Guinea	W. Takeuchi 9284	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia adenodes</i>	x	x	x	African	South Africa	R.D.A. Bayliss 6409	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia albae-paulii</i>	x	x	x	African	Senegal	Berhaut 7495	P03452251	?	?	?	?	?	0	1
<i>Rhynchosia ambacensis</i>	x	x	x	African	Cameroon	J Raynal 12914	P03452241	1	0	1	0	1	0	1
<i>Rhynchosia androyensis</i>	x	x	x	African	Madagascar	L Alorge 2295	P00134988	?	?	?	?	?	0	1
<i>Rhynchosia androyensis</i>	x	x	x	African	Madagascar	D.J. DuPuy et al. M872	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia aureovillosa</i> var. <i>humbertii</i>	x	x	x	African	Rwanda	H.G. Troupin 5686	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia baukea</i>	x	x	x	African	Madagascar	M Peltier 1102	P0003676	?	?	?	?	?	0	1
<i>Rhynchosia breviracemosa</i>	~	x	x	African	Angola	Azancot de Menezes 732	P03452591	1	1	1	0	1	0	1

<i>Rhynchosia brunea</i>	x	x	x	African	Ghana	RAA Oldeman 803	P03452585	1	0	0	0	1	0	1
<i>Rhynchosia buchananii</i>	x	x	x	African	Republic of Malawi	K. Kaunda & R.B. Kwatha 632	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia buettneri</i>	x	x	x	African	Ivory Coast	Peyre de Fabrègues 3473	P03452583	1	0	1	0	1	0	1
<i>Rhynchosia capensis</i>	x	~	x	African	South Africa	N.S. Pillans 10165	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia chapelieri</i>	x	x	x	African	Madagascar	P Ranirison 699	P00460877	?	?	?	?	?	0	1
<i>Rhynchosia chrysoscias</i>	x	x	x	African	South Africa	Schuh, Cassis, Weirauch PBI_SA03_H191	NYBG	1	1	0	1	1	0	1
<i>Rhynchosia cliffordii</i>	x	x	x	African	Somalia	JB Gillett 4864	P03452613	1	1	0	0	1	0	1
<i>Rhynchosia clivorum</i>	x	x	x	African	South Africa	WJ Hanekom s/n	P03452609	1	1	1	0	1	0	1
<i>Rhynchosia debilis</i>	x	~	x	African	Ghana	J.M. Robertson 50	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia ferulifolia</i>	~	~	x	African	South Africa	H. Bolus 3775	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia ferulifolia</i>	x	x	x	African	South Africa	F.M. Leighton 1889	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia fleckii</i>	x	x	x	African	Namibia	R. Seydel 1806	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia hirsuta</i>	~	x	x	African	South Africa	R.D.A. Bayliss 8297	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia hirta</i>	x	x	x	African	Republic of Malawi	E.J. Tawakali & A.C. Chikuni 1672	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia hockii</i>	~	x	x	African	Rwanda	H.G. Troupin 3664	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia insignis</i>	x	x	x	African	Zimbabwe	R.D.A. Bayliss 10169A	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia luteola</i> var. <i>luteola</i>	x	x	x	African	Republic of Malawi	E.J. Tawakali & J.L. Balaka 1513	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia madagascariensis</i>	x	x	x	African	Madagascar	M Bardot-Vaucoulon 743	P00085422	1	1	1	0	1	0	1
<i>Rhynchosia malacophylla</i>	~	x	x	African	Tanzania	L. Ellemann 478	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia micrantha</i>	~	x	x	African	Tanzania	M.E. Archbold 1069	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia nyasica</i>	x	x	x	African	Zimbabwe	R.D.A. Bayliss 10129	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia nyikensis</i>	x	x	x	African	Republic of Malawi	W. Nachamba, I.H. Patel & E.J. Tawakali 78	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia puberula</i>	x	x	x	African	South Africa	R.D.A. Bayliss 4114	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia pycnostachya</i>	x	x	x	African	Togo	H Ern 2562	P03117017	1	0	1	0	1	0	1
<i>Rhynchosia resinosa</i>	x	x	x	African	Guinea	CCH Jongkind 8035	P030055348	?	?	?	?	?	0	1

<i>Rhynchosia resinosa</i>	~	x	x	African	Burundi	J. Lewalle 1643	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia stenodon</i>	x	x	x	African	South Africa	H.J. Venter & A. Venter 10216	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia sublobata</i>	x	x	x	African	Tanzania	S Bidgood <i>et al.</i> 6418	P03077547	?	?	?	?	?	0	1
<i>Rhynchosia totta</i>	x	x	x	African	South Africa	CH Stirton 14089	PRE	1	0	1	0	1	0	1
<i>Rhynchosia verdcourtii</i>	x	x	x	African	Tanzania	R Abdallah & K Vollesen 96/220	P03117948	?	?	?	?	?	0	1
<i>Rhynchosia versicolor</i>	x	x	x	African	Madagascar	Y Veyret 1329	P0003869	?	?	?	?	?	0	1
<i>Rhynchosia versicolor</i> subsp. <i>versicolor</i>	x	x	x	African	Madagascar	D.J. & B.P. Du Puy M322	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia viscosa</i>	x	x	x	African	Madagascar	J Bosser 21902	P03489949	?	?	?	?	?	0	1
<i>Rhynchosia woodii</i>	x	~	~	African	South Africa	F Willms s/n	K	?	?	?	?	?	0	1
<i>Eriosema brevipes</i>	x	x	x	American	Brazil	AP Fortuna-Perez 2101	BOTU	1	0	1	0	1	0	1
<i>Cajanus cajan</i>	x	x	x	American	Venezuela	OO Miller & JR Johnmston 139	P 02921261	1	0	1	0	1	0	2
<i>Camptosema grandiflorum</i>	x	x	x	American	Brazil	L.B. Santos e F.L.S. Ferreira 199	BOTU	0	0	0	0	1	1	2
<i>Centrosema virginianum</i>	x	x	x	American	Brazil	A. Amaral Jr. et al 306	BOTU	0	0	0	0	0	1	2
<i>Eriosema benthamianum</i>	x	x	x	American	Brazil	Coletada Chapada dos Veadeiros GO (Cândido 2)	BOTU	?	?	?	?	?	0	1
<i>Eriosema campestre</i> var. <i>campestre</i>	x	x	x	American	Brazil	AP Fortuna-Perez	BOTU	1	1	1	0	1	0	1
<i>Eriosema campestre</i> var. <i>delicatula</i>	x	x	x	American	Brazil	W Vargas 6	CGMS 33669	1	1	1	0	1	0	1
<i>Eriosema campestre</i> var. <i>macrophyllum</i>	x	x	x	American	Brazil	AP Fortuna-Perez 1436	OUPR 27049	1	1	1	1	1	0	1
<i>Eriosema campestre</i> var. <i>macrophyllum</i>	x	x	x	American	Brazil	HS Irwin et al. 11593	NY 7859	1	1	1	1	1	0	1
<i>Eriosema campestre</i> var. <i>pubescens</i>	x	x	x	American	Brazil	A flores & RS Rodrigues 711	UEC004744	?	?	?	?	?	0	1
<i>Eriosema congestum</i>	x	x	x	American	Brazil	MJ Silva 7396	UFG	1	0	1	0	1	0	1
<i>Eriosema crinitum</i>	x	x	x	American	Brazil	AP Fortuna-Perez 1439	BOTU	1	1	1	1	1	0	1
<i>Eriosema crinitum</i>	x	x	x	American	Brazil	ES Cândido <i>et al.</i> 1077	BOTU	1	1	1	1	1	0	1
<i>Eriosema crinitum</i>	x	x	x	American	Brazil	Andreza 579	UEC	1	1	1	1	1	0	1

<i>Eriosema crinitum</i> var. <i>crinitum</i>	x	x	x	American	Brazil	ES Cândido et al. 1075	OUPR 27914	1	1	1	1	1	0	1
<i>Eriosema crinitum</i> var. <i>stipulare</i>	x	x	x	American	Brazil	M Aparecida Silva 4111	HUTO 4727	1	0	1	1	1	0	1
<i>Eriosema cupreum</i>	x	~	~	American	Brazil	A. Glaziov 20888	S-R-9739	?	?	?	?	?	0	1
<i>Eriosema defoliatum</i>	x	x	x	American	Brazil	ES Cândido 1500	BOTU	1	0	1	0	1	0	1
<i>Eriosema diffusum</i>	x	x	x	American	Costa Rica	J. Bustamante, 202	S15-34044	?	?	?	?	?	0	1
<i>Eriosema elegans</i>	x	x	x	American	Brazil	MJ Silva 7228	UFG	1	0	1	0	1	0	1
<i>Eriosema elegans</i>	x	x	x	American	Brazil	AP Fortuna-Perez 2000	BOTU	1	0	1	1	1	0	1
<i>Eriosema floribundum</i>	x	x	x	American	Brazil	ES Cândido et al. 1090	OUPR 28325	1	0	1	0	1	0	1
<i>Eriosema glabrum</i>	x	x	x	American	Brazil	ES Cândido et al. 1067	OUPR 27545	1	0	1	0	1	0	1
<i>Eriosema glaziovii</i>	x	x	x	American	Brazil	AP Fortuna-Perez et al. 1457	OUPR 27070	?	?	?	?	?	0	1
<i>Eriosema grandiflorum</i>	x	x	x	American	Mexico	Howard Scott Gentry 1837	S15-33079	1	0	1	0	1	0	1
<i>Eriosema hatschbachii</i>	x	x	x	American	Brazil	AP Fortuna-Perez et al. 1401	OUPR 26605	1	1	1	0	1	0	1
<i>Eriosema heterophyllum</i>	x	x	x	American	Brazil	ES Cândido et al. 1068	OUPR 27546	1	0	1	0	1	0	1
<i>Eriosema irwinii</i>	x	x	x	American	Brazil	AP Fortuna-Perez et al. 1467	OUPR 27080	1	1	1	0	1	0	1
<i>Eriosema irwinii</i>	x	x	x	American	Brazil	ES Cândido 1501	BOTU	1	1	1	0	1	0	1
<i>Eriosema laxiflorum</i>	x	x	x	American	Brazil	M Silva 4407	UFG	1	0	1	0	1	0	1
<i>Eriosema laxiflorum</i>	x	x	x	American	Brazil	MJ Silva 5116	UFG	1	0	1	0	1	0	1
<i>Eriosema longiflorum</i>	x	x	x	American	Brazil	M Silva 4025	UFG	?	?	?	?	?	0	1
<i>Eriosema longifolium</i>	x	x	x	American	Brazil	AP Fortuna-Perez et al. 1442	OUPR 27055	1	0	1	0	1	0	1
<i>Eriosema macrostipulatum</i>	x	x	x	American	Brazil	AP Fortuna-Perez 1447	OUPR 27060	1	0	1	0	1	0	1
<i>Eriosema macrostipulatum</i>	x	x	x	American	Brazil	MJ Silva 4696	UFG	1	0	1	0	1	0	1
<i>Eriosema multiflorum</i>	x	x	x	American	Mexico	M Hahn 386	P02921305	1	0	1	0	1	0	1
<i>Eriosema palmeri</i> var. <i>palmeri</i>	x	x	x	American	Mexico	HS Gentry 2536	SI	?	?	?	?	?	0	1
<i>Eriosema platycarpon</i>	~	x	x	American	Paraguay	Troels Myndel Pedersen 4372	S15-34610	1	1	1	0	1	0	1
<i>Eriosema prorepens</i>	x	x	x	American	Brazil	R Romero et al. 4653	VIC 28255	1	1	1	1	1	0	1

<i>Eriosema prorepens</i>	x	x	x	American	Brazil	R Romero et al. 4551	VIC 28254	1	1	1	1	1	0	1
<i>Eriosema pulchellum</i>	x	x	x	American	Mexico	C. G. Pringle 9748	S15-33415	1	0	1	1	1	0	1
<i>Eriosema pycnanthum</i> var. <i>pycnanthum</i>	x	x	x	American	Brazil	FF Carmo 1957	BHCB 133893	1	0	1	1	1	0	1
<i>Eriosema pycnanthum</i> var. <i>pycnanthum</i>	x	x	x	American	Brazil	SG Rezende & MS Medens 2088	BHCB 117425	1	0	1	1	1	0	1
<i>Eriosema pycnanthum</i> var. <i>pycnanthum</i>	x	x	x	American	Brazil	T Mansur et al. 198	BHCB 149097	1	0	1	1	1	0	1
<i>Eriosema pycnanthum</i> var. <i>veadeirens</i>	~	x	x	American	Brazil	HS Irwin et al. 24471	NY 01019724	1	0	1	1	1	0	1
<i>Eriosema pynanthum</i> var. <i>veadeirens</i>	x	x	x	American	Brazil	HS Irwin et al. 12885	NY 7875	1	0	1	1	1	0	1
<i>Eriosema riedelii</i>	x	~	~	American	Brazil	Pedralli et al. 683	UEC004717	1	0	1	0	1	0	1
<i>Eriosema rufum</i> var. <i>rufum</i>	x	x	x	American	Brazil	W Vargas 12	CGMS 33675	1	1	1	0	1	0	1
<i>Eriosema simplicifolium</i> var. <i>micranthum</i>	x	x	x	American	Brazil	RS Rodrigues et al. 1386	UEC 124260	1	0	1	0	1	0	1
<i>Eriosema simplicifolium</i> var. <i>simplicifolium</i>	x	x	x	American	Brazil	AP Fortuna-Perez 2102	BOTU	1	0	1	0	1	0	1
<i>Eriosema simplicifolium</i> var. <i>simplicifolium</i>	x	x	x	American	Brazil	AP Fortuna-Perez	BOTU	1	0	1	0	1	0	1
<i>Eriosema stenophyllum</i>	x	x	x	American	Brazil	LP Queiroz 15074	HUEFS 165456	1	0	0	0	1	0	1
<i>Eriosema strictum</i>	x	~	~	American	Brazil	Hatschbach, G. 8643	HB	1	1	1	0	1	0	1
<i>Eriosema tacuarembense</i>	x	x	x	American	Brazil	AP Fortuna-Perez et al. 1443	OUPR 27056	1	0	1	0	1	0	1
<i>Eriosema tozziae</i>	x	x	x	American	Brazil	LS Kinoshita et al. 02/158	UEC 023815	1	1	1	0	1	0	1
<i>Eriosema violaceum</i>	x	~	~	American	Brazil	Ule 8160	K	1	0	1	0	1	0	1
<i>Galactia striata</i>	x	x	x	American	Brazil	W. Vargas & E.S. Cândido 72	BOTU	0	0	0	0	1	1	2
<i>Rhynchosia americana</i>	x	~	~	American		Fredholm 5399	SI	?	?	?	?	?	0	1
<i>Rhynchosia arenicola</i>	x	~	~	American	Brazil	Hatschbach, G. 2577	SI	?	?	?	?	?	0	1
<i>Rhynchosia balansae</i>	x	x	x	American	Brazil	G. Hatschbach et al. 74908	K	1	0	1	0	1	0	1
<i>Rhynchosia balansae</i> var. <i>balansae</i>	x	x	x	American	Brazil	A.L.B. Sartori et al 709	BOTU	1	0	1	0	1	0	1
<i>Rhynchosia caaguazuensis</i>	x	x	x	American	Paraguay	E. Hassler 11067	NYBG	?	?	?	?	?	0	1

<i>Rhynchosia calycosa</i>	x	~	~	American	Ecuador	L Holm-Nielsen & S leppensen 83	SI	?	?	?	?	?	0	1
<i>Rhynchosia caribaea</i>	x	~	~		American		SI	1	0	1	0	1	0	1
<i>Rhynchosia claussemi</i>	x	x	x	American	Brazil	CR Martins 252	UEC 137701	1	0	1	1	1	0	1
<i>Rhynchosia claussemi</i>		x	x	American	Brazil	EP Heringer	UB 6643	1	0	1	1	1	0	1
<i>Rhynchosia corylifolia</i>	x	x	x	American	Brazil	Fortuna-Perez, A.P. 1531	UEC	1	0	1	0	1	0	1
<i>Rhynchosia cytisoides</i>	x	x	x	American	United States	R.k. Godfrey 84172	NYBG 2622131	?	?	?	?	?	0	1
<i>Rhynchosia difformis</i>	x	x	x	American	United States	S. McDaniel 29983	NYBG 2622144	1	0	1	0	1	0	1
<i>Rhynchosia discolor</i>	x	x	x	American	Guatemala	J.J. Castillo & J.M. Vargas 2741	NYBG 87376	?	?	?	?	?	0	1
<i>Rhynchosia diversifolia</i>	x	x	x	American	Uruguai	Bonifacio, M. et al. 1803	SI	?	?	?	?	?	0	1
<i>Rhynchosia diversifolia</i> var. <i>diversifolia</i>	x	x	x	American	Argentina	A Krapovickas et al. 21474	P02749391	?	?	?	?	?	0	1
<i>Rhynchosia edulis</i>	x	x	x	American	Brazil	França, F. et al. 2164	HUEFS	1	1	1	0	1	0	1
<i>Rhynchosia elisae</i>	x	x	x	American	Mexico	O Tellez 12583	NY 00079857	?	?	?	?	?	0	1
<i>Rhynchosia hagenbeckii</i>	x	~	~	American	Argentina	M Micken 235	SI	?	?	?	?	?	0	1
<i>Rhynchosia hauthalii</i>	x	x	x	American	Argentina	TM Pedersen 2982	P03055337	1	1	1	1	1	0	1
<i>Rhynchosia hauthalii</i>	x	x	x	American	Argentina	T.M. Pedersen 2982	NYBG	1	1	1	1	1	0	1
<i>Rhynchosia lateritia</i>	x	x	x	American	Argentina	E.L. Ekman 1679	NYBG	1	0	1	0	1	0	1
<i>Rhynchosia lateritia</i>	x	x	x	American	Argentina	H.A. Keller, J. Pirelli, L. Ritter 4566	UEC	1	0	1	0	1	0	1
<i>Rhynchosia latifolia</i>	x	x	x	American	United States	D. Atha 12874	NYBG 2148474	?	?	?	?	?	0	1
<i>Rhynchosia leucophylla</i>	x	x	x	American	Brazil	W Vargas 10	CGMS 33673	1	0	1	0	1	0	1
<i>Rhynchosia lewisii</i>	x	x	x	American	Brazil	Guedes, M.L. et al 20795	UB	1	1	1	0	1	0	1
<i>Rhynchosia lineata</i>	x	~	~	American	Uruguai	Cabrera, A.L. & Zuloaga, F. 32380	SI	?	?	?	?	?	0	1
<i>Rhynchosia longeracemosa</i>	x	x	x	American	Mexico	M Souza 7851	SI	?	?	?	?	?	0	1
<i>Rhynchosia longeracemosa</i>	x	x	x	American	Guatemala	J.J. Castillo & A. Castillo 2718	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia macrocarpa</i>	x	x	x	American	Mexico	P. Tenorio L. et al. 10290	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia mantaroensis</i>	x	x	x	American	Peru	Reichlen 100	P02749567	?	?	?	?	?	0	1

<i>Rhynchosia mantaroensis</i>	x	x	x	American	Peru	A. Sagástegui et al. 15255	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia melanocarpa</i>	x	x	x	American	Brazil	AP Fortuna-Perez	BOTU	1	1	1	0	1	0	1
<i>Rhynchosia melanocarpa</i>	x	x	x	American	Brazil	DC Giacometti s.n	UB 8469	1	1	1	0	1	0	1
<i>Rhynchosia minima</i>	x	x	x	American	Brazil	W Vargas 9	CGMS 33672	1	0	1	0	0	0	1
<i>Rhynchosia minima</i>	~	x	x	American	Brazil	K Santos 4703	UEC	1	0	1	0	0	0	1
<i>Rhynchosia minima</i>	x	x	x	American	Brazil	VC Lima 46	IPA 48459	1	0	1	0	0	0	1
<i>Rhynchosia minima</i>	x	x	x	American	Bolivia	JRI Wood & P Pozo 25974	UB	1	0	1	0	0	0	1
<i>Rhynchosia nainceckensis</i>	x	x	x	American	Bolivia	M. Nee 50484	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia nipensis</i>	~	x	x	American	Cuba	J.P. Carabia 3719	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia pallida</i> var. <i>boliviana</i>	x	x	x	American	Bolivia	M. Nee 36388	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia parvifolia</i>	x	x	x	American	United States	W.C. Brumbach 9455	NYBG 2622383	?	?	?	?	?	0	1
<i>Rhynchosia phaseoloides</i>	x	x	x	American	Brazil	RB Pinto 500	UEC	1	0	1	0	0	0	1
<i>Rhynchosia platyphylla</i>	x	x	x	American	Brazil	J Raveratti et al. 640	UB	1	1	1	0	1	0	1
<i>Rhynchosia precatória</i>	x	~	x	American	Mexico	A. Reyes-Garcia et al. 6211	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia pringlei</i>	~	x	x	American	Mexico	H.D. Ripley & R.C. Barneby 14, 675	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia prostrata</i>	x	x	x	American	Mexico	C.A. Purpus 3205	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia pyramidalis</i>	x	x	x	American	Honduras	S. Palma 346	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia quercetorum</i>	x	x	x	American	Costa Rica	W.A. Haber 3674	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia reniformis</i>	x	x	x	American	United States	SW Leonard & AE Radford 1547	P03055298	?	?	?	?	?	0	1
<i>Rhynchosia reticulata</i>	x	x	x	American	Brazil	Queiroz, L.P. et al. 1314	HUEFS	?	?	?	?	?	0	1
<i>Rhynchosia reticulata</i> var. <i>kuntzei</i>	x	x	x	American	Brazil	F França 3175	UB	?	?	?	?	?	0	1
<i>Rhynchosia rojasii</i>	x	x	x	American	Paraguay	EM Zardini & M Vera 45368	P03055342	?	?	?	?	?	0	1
<i>Rhynchosia rojasii</i>	x	x	x	American	Brazil	Groppo Jr., M. 414	HUEFS	?	?	?	?	?	0	1
<i>Rhynchosia schomburgkii</i>	~	x	x	American	Guyana	J. Ollerton et al 201	KEW	1	0	1	0	1	0	1
<i>Rhynchosia senna</i>	x	x	x	American	United States	K.C. Rice 1428	NYBG 2626831	?	?	?	?	?	0	1

<i>Rhynchosia swartzii</i>	x	x	x	American	United States	D.S. & H.B. Correll & J. Popenoe 48124	NYBG 2626961	?	?	?	?	?	0	1
<i>Rhynchosia tarphanta</i>	x	x	x	American	Mexico	T.S. Cochrane, M.A. Wetter & M. Rosales C. 11, 782	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia tomentosa</i>	x	x	x	American	United States	R. Dale Thomas & A. Dunn 157, 685	NYBG 2627103	?	?	?	?	?	0	1
<i>Rhynchosia volubilis</i>	x	x	x	Asian	Japan	F Konta 35508	P03055339	1	0	1	1	1	0	1
<i>Cajanus cajanifolia</i>	x	x	x	Asian	India	L.J.G. van der Maesen 2739	K	1	0	1	0	1	0	2
<i>Cajanus rugosus</i>	x	x	x	Asian	India	L.J.G. van der Maesen 4033	K	1	0	1	0	1	0	2
<i>Cajanus scarabaeoides</i>	x	x	x	Asian	Thayland	A.N. Egan 13-0733	K	1	0	1	0	1	0	2
<i>Cajanus trinervia</i>	x	x	x	Asian	Sri Lanka	L.J.G. van der Maesen 4159	K	1	0	1	0	1	0	2
<i>Dunbaria bella</i>	x	x	x	Asian	Thayland	Chantaronothai et al 854	K	1	0	1	1	1	0	2
<i>Dunbaria fusca</i>	x	x	x	Asian	Thayland	A.N. Egan 13-0808	K	1	1	1	0	0	0	2
<i>Dunbaria glandulosa</i>	x	x	x	Asian	Thayland	Kai Larsen & Supee S.	K149	1	1	1	0	0	0	2
<i>Dunbaria villosa</i>	x	x	x	Asian	South Korea	B.R. Yinger et al. 2634	K	1	1	1	0	0	0	2
<i>Eriosema chinense</i>	x	~	~	Asian	Philippines	Merrill, E.D. 878	SI	1	0	1	0	1	0	1
<i>Flemingia congesta</i>	x	x	x	Asian	India	P. Remanandan 4698	K	1	0	1	0	1	0	1
<i>Flemingia ferruginea</i>	x	x	x	Asian	India	Kai Larsen 9152	K	1	0	1	0	1	0	1
<i>Paracalyx balfourii</i>	~	~	x	Asian	Yemen	A.G. Miller et al. 8654	K	1	0	1	0	1	0	1
<i>Paracalyx scariosa</i>	~	x	x	Asian	India	L.J.G. van der Maesen 1973	K	1	0	1	0	1	0	1
<i>Paracalyx scariosa</i>	x	x	x	Asian	India	N. Kameswara Rao 12	K	1	0	1	0	1	0	1
<i>Rhynchosia avensis</i>	x	x	~	Asian	Thayland	D Kerr 4642	P02749192	?	?	?	?	?	0	1
<i>Rhynchosia craibiana</i>	x	x	x	Asian	China	DE Boufford et al. 3114	P03055317	?	?	?	?	?	0	1
<i>Rhynchosia dielsii</i>	x	x	x	Asian	Japan	K. Yao 9152	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia himalensis</i>	~	x	x	Asian	India	R.R. Stewart 21264	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia malacophylla</i>	x	x	x	Asian	Yemen	R. Spellenberg 7535	NYBG	?	?	?	?	?	0	1
<i>Rhynchosia volubilis</i>	x	x	x	Asian	Japan	G. Murata 13831	NYBG	1	0	1	1	1	0	1

<i>Rhynchosia volubilis</i>	x	x	x	Asian	Japan	Y.N. Xiong & K. Yao 122	NYBG	1	0	1	1	1	0	1
<i>Cajanus cinerea</i>	x	x	x	Oceania	Australia	Martin J.S. Sands	K	1	1	1	0	1	0	2
<i>Cajanus marmoratus</i>	x	x	x	Oceania	Australia	J.R. Moconochie 1730	K	1	0	1	0	1	0	2
<i>Cajanus reticulatus</i> var. <i>grandiflora</i>	x	x	x	Oceania	Papua New Guinea	Verdcourt, Huxley & White 4870	K	?	?	?	?	?	0	2
<i>Flemingia involucrata</i>	~	~	x	Oceania	Australia	B.R. Maslin 5115	K	1	1	1	1	1	0	1
<i>Flemingia pauciflora</i>	x	x	x	Oceania	Australia	J.R. Clarkson & V.J. Nelder 7998	K	1	0	1	0	1	0	1
<i>Flemingia sericea</i>	x	x	x	Oceania	Australia	J. Cowie & K.G. Brennam 9083	K	1	0	1	1	1	0	1
<i>Flemingia trifoliastrum</i>	x	~	x	Oceania	Australia	Evans M. 3012	K	1	0	1	0	1	0	1
<i>Paracalyx schweinfurthii</i>	x	x	x	Oceania	Australia	Omentin Cronk 3	K	1	0	1	0	1	0	1

Table 2: General features of Cajaninae genera (from: Lackey 1981; Schrire 2005; Rice et al. 2014)

Genera	Number of species/sample ratio	Habit	Seed number	Leaflet number	Ploidy level/chromosome number	Distribution range	Environments of occurrence	Notes
<i>Adenodolichos</i> Harms	20spp./4	Herbs or shrubs	3+	3-5	2n (22)	Africa (Zambezian region and Sudanian Africa)	Seasonally dry tropical woodland, scrub, wooded grassland and grassland, often appearing after fire.	Used for human food, medicine and cordage
<i>Paracalyx</i> S.I. Ali	6spp./6	Herbs	2	3	2n (22)	NE Africa; Indo-China	Seasonally dry tropical forest, woodland, thicket, bushland and scrub, often along water courses and in rocky areas.	All species were original described in the genus <i>Cylista</i> Aiton (1789), which is now a synonym of <i>Rhynchosia</i>
<i>Bolusafra</i> Kuntze	1sp./1	Herb or subshrub	3+	3	2n (22)	Africa (South Africa)	Mediterranean montane sclerophyllous shrubland (fynbos), often along streamsides.	Allied to <i>Rhynchosia</i> and <i>Chrysoscias</i> in subtribe Cajaninae.
<i>Carrisoa</i> Baker f.	1sp./0	Subshrub	2	3	-	Africa (Angola)	Seasonally dry tropical open bushland and scrub, or along watercourses, with woody rootstock in fire-prone environments.	Considered to be a <i>Rhynchosia</i>
<i>Chrysoscias</i> E. Mey.	3-4spp/2	Herbs or subshrubs	2	3	-	Africa (South Africa)	Mediterranean sclerophyllous shrubland (fynbos) on sandstones slopes.	The genus has been included in <i>Rhynchosia</i> in South Africa literature.
<i>Dunbaria</i> Wight & Arn.	20spp/5	Climbing herbs or subshrubs	3+	3	2n (20-22)	SE Asia (Indo-China to S China, Indian Subcontinent, Malasia and Papuasias to E Asia); N Australia	Seasonally dry tropical forest, thicket, scrub and grassland, from moist to semi-arid areas mainly in the tropics.	Used for green manure and medicine, so may be useful in breeding programmes of pigeon pea (related to <i>Cajanus</i>)

<i>Cajanus</i> DC.	34spp./9	Herbs or shrubs	3+	3	2n (22)	SE Asia (Indian subcontinente, China, E Asia, Indo-China, Malesia and Papuasias) N Australia, W Africa	Seasonally dry tropical forest to grassland, often in rocky or partly disturbed areas.	Used for human food, pasture legumes, forage, green manure, cover crop and in medicine.
<i>Flemingia</i> Roxb. ex W.T. Aiton	30-35spp./8	Herbs or shrubs	2	3	2n (22)	SE Asia (Indian subcontinente, China, E Asia, Indo-China, Malesia and Papuasias) N Australia, Africa	Seasonally dry tropical forest, wodland, wooded grassland and grassland, sometimes near swamps and streams, also in disturbed and waste areas.	Used as cover crops, green manure, medicine, human food (tubers) and an Orange dye.
<i>Eriosema</i> (DC.) Desv.	150spp./117	Herbs or subshrubs	2	1-3	2n (20-24)	Africa (included Madagascar) America and SE Asia to Australia	Seasonally dry tropical or subtropical forest margins, wodland, thicket and wooded grassland, or open grassland in rocky or swampy areas, or in old cultivations and waste ground.	Used as human famine food, medicine and fish poison.
<i>Rhynchosia</i> Lour.	230spp./103	Herbs, vines or subshrubs	2	3-5	2n (22)	Pantropical (Africa-Madagascar; in tropical and subtropical America; Asia to N Australia)	Seasonally dry forest, forest margins, wodland, thicket, wooded grassland, subshrubland grassland, often in open rocky areas or along streams and disturbed areas.	Many species are pyrophytes; Used as pasture plants, seeds also used as weights or as narcotics, handicraft, plant also used as famine foods.



Fig. 1: Diversity of habitats and features of some Cajaninae taxa. A. African savannah in Umtamvuna Nature Reserve (KwaZulu-Natal), South Africa. B-C. Brazilian cerrado in Parque Nacional Chapada dos Veadeiros (Alto Paraíso de Goiás), Brazil (B) and Parque Estadual do Rio Preto (São Gonçalo do Rio Preto), Brazil (C). D. subshrub habit of *E. glabrum*. E. bicoloured seeds of *R. melanocarpa*. F. fruit with two seeds in *F. macrophylla*. G. flower of *Paracalyx* sp. H. fruit with more than three seeds in *Cajanus cajan*. Photographs by Charles Stirton (A); E.S. Cândido (B); Luísa Bezerra (C-E); Ana Paula Fortuna-Perez (F-H)

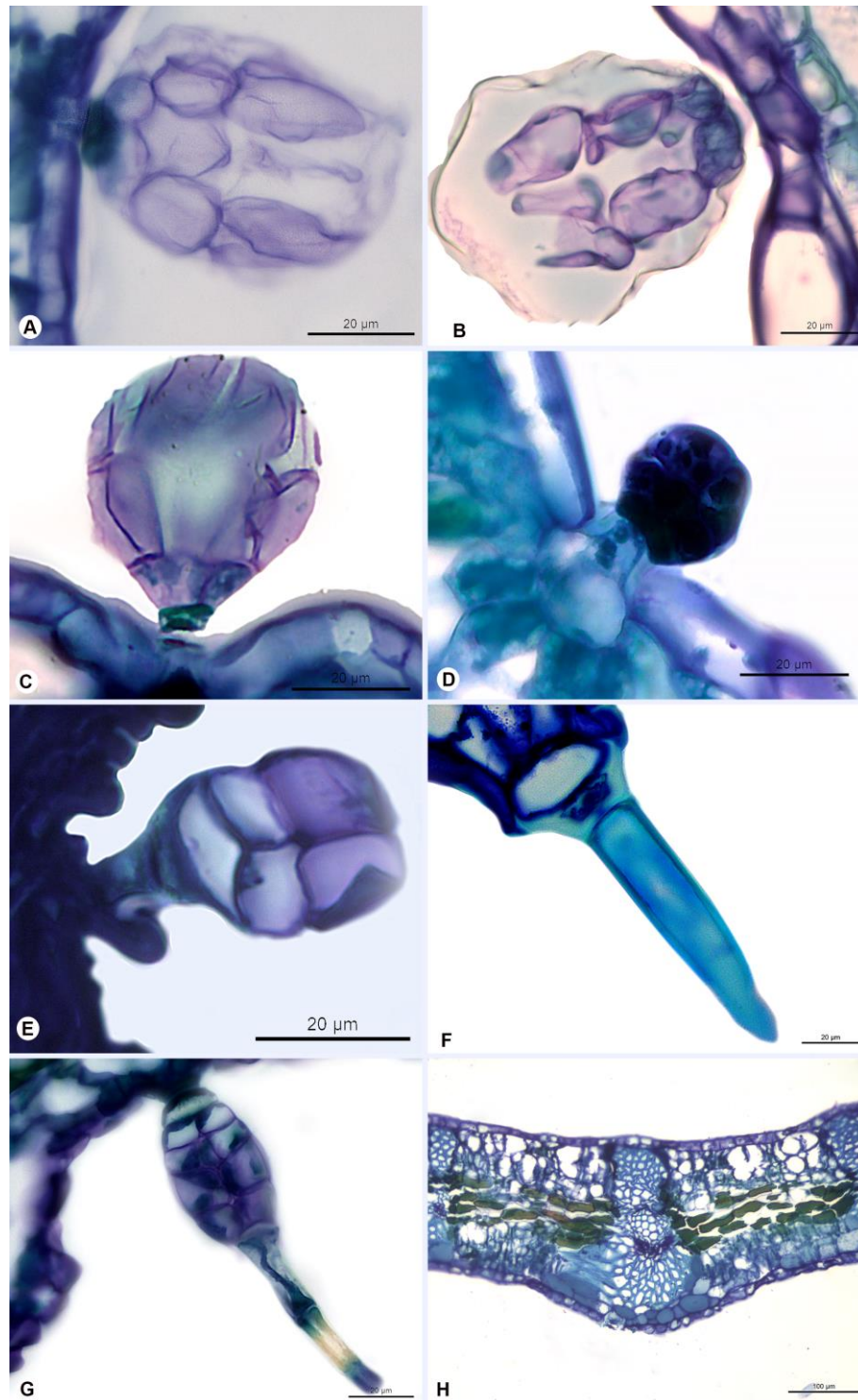


Fig. 2: Photomicrographs showing the different types of secretory structure occurring in Cajaninae representatives. A. vesicular glands in *E. solignum*, B. *E. kraussianum* and, C. *E. pycnanthum* var. *pycnanthum*. D. capitate trichome in *E. crinitum* var. *crinitum* and *E. R. usambarensis*. F. secretory-base trichome in *D. bella*. G. bulbous-based trichome in *Cajanus cinerea*. H. idioblasts in mesophyll of *E. stenophyllum*.

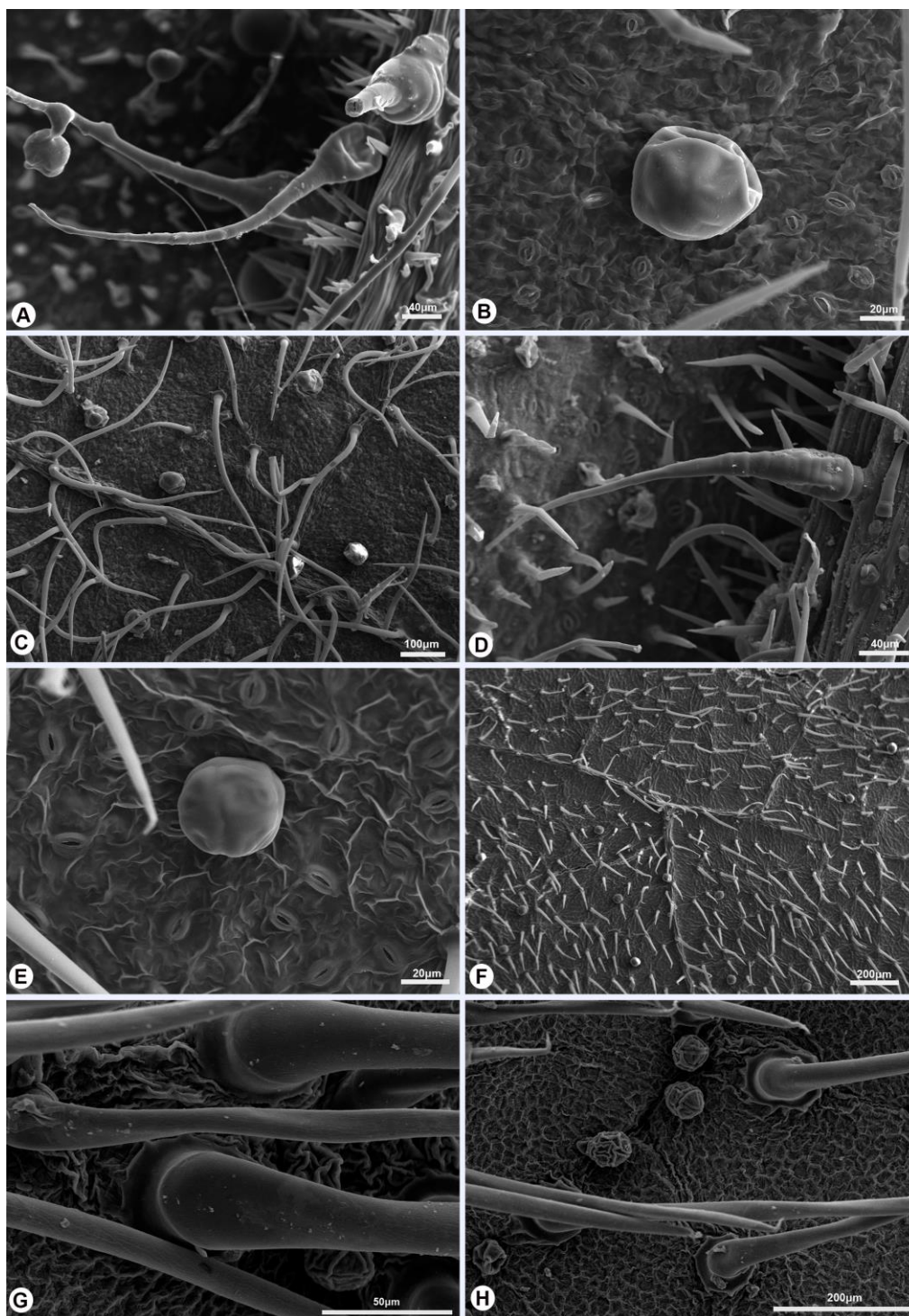


Fig. 3: SEMs of the abaxial surface of the leaflets of Cajaninae taxa, showing the secretory structures. A. bulbous-based trichomes of *B. bituminosa*. B. vesicular gland and C. overview of the leaflet showing the high density of non-glandular trichome and some vesicular glands in *Cajanus volubilis*. D. bulbous-based trichome in *Chrysoscias grandiflora*. E. vesicular gland and F. overview of the leaflet showing the high density of non-glandular trichome and some vesicular glands in *D. villosa*. G. secretory-base trichomes and H. capitate trichomes in *E. grearii*.

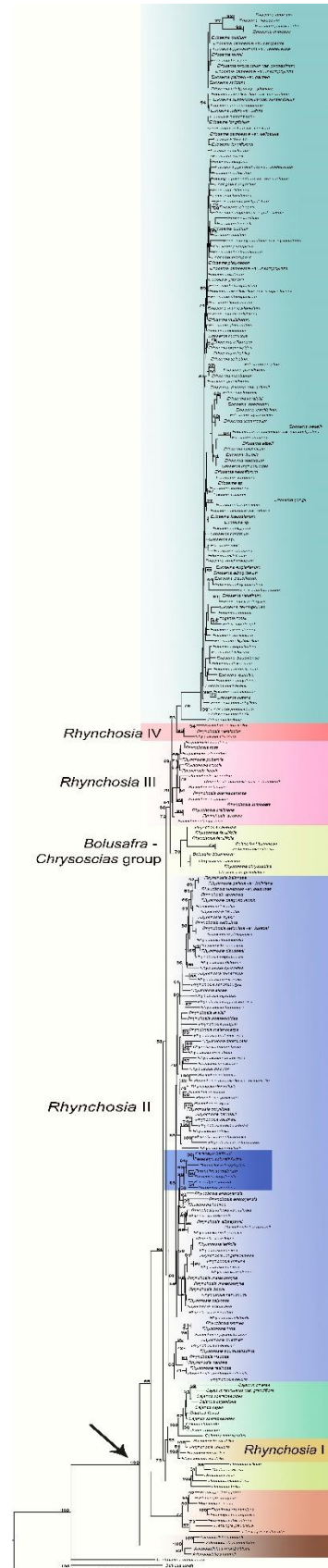


Fig. 4: Phylogenetic tree of the Cajaninae from Maximum Likelihood analysis of the ITS/5.8S and cpDNA dataset (rpl32-trnL and trnQ-5'rps16).



Fig. 4a (partitioned): Phylogenetic tree of the Cajaninae from Maximum Likelihood analysis of the ITS/5.8S and cpDNA dataset (rpl32-trnL and trnQ-5'rps16). Numbers along branches are posterior probabilities values.

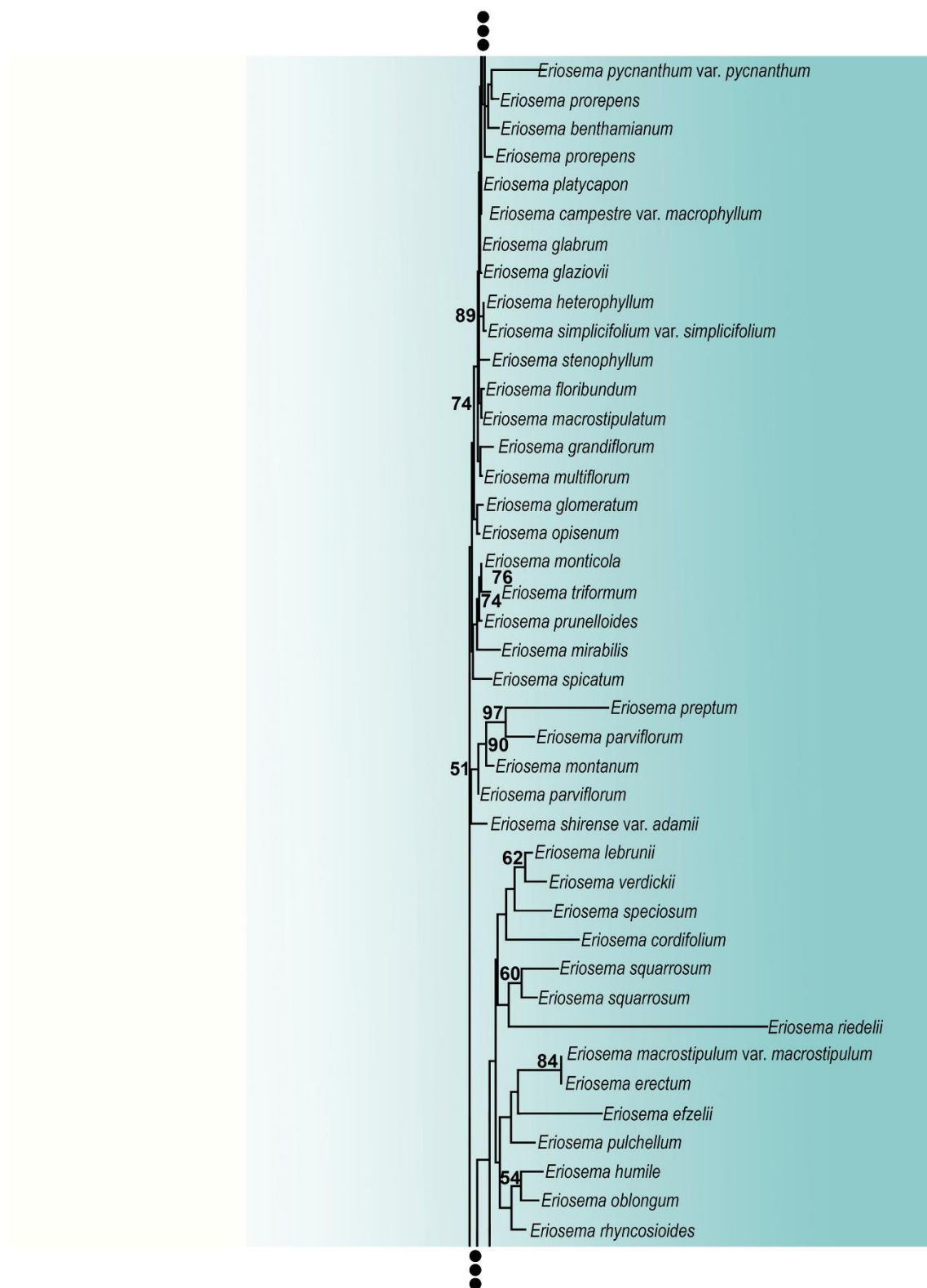


Fig. 4b (partitioned): Phylogenetic tree of the Cajaninae from Maximum Likelihood analysis of the ITS/5.8S and cpDNA dataset (rpl32-trnL and trnQ-5'rps16). Numbers along branches are posterior probabilities values.

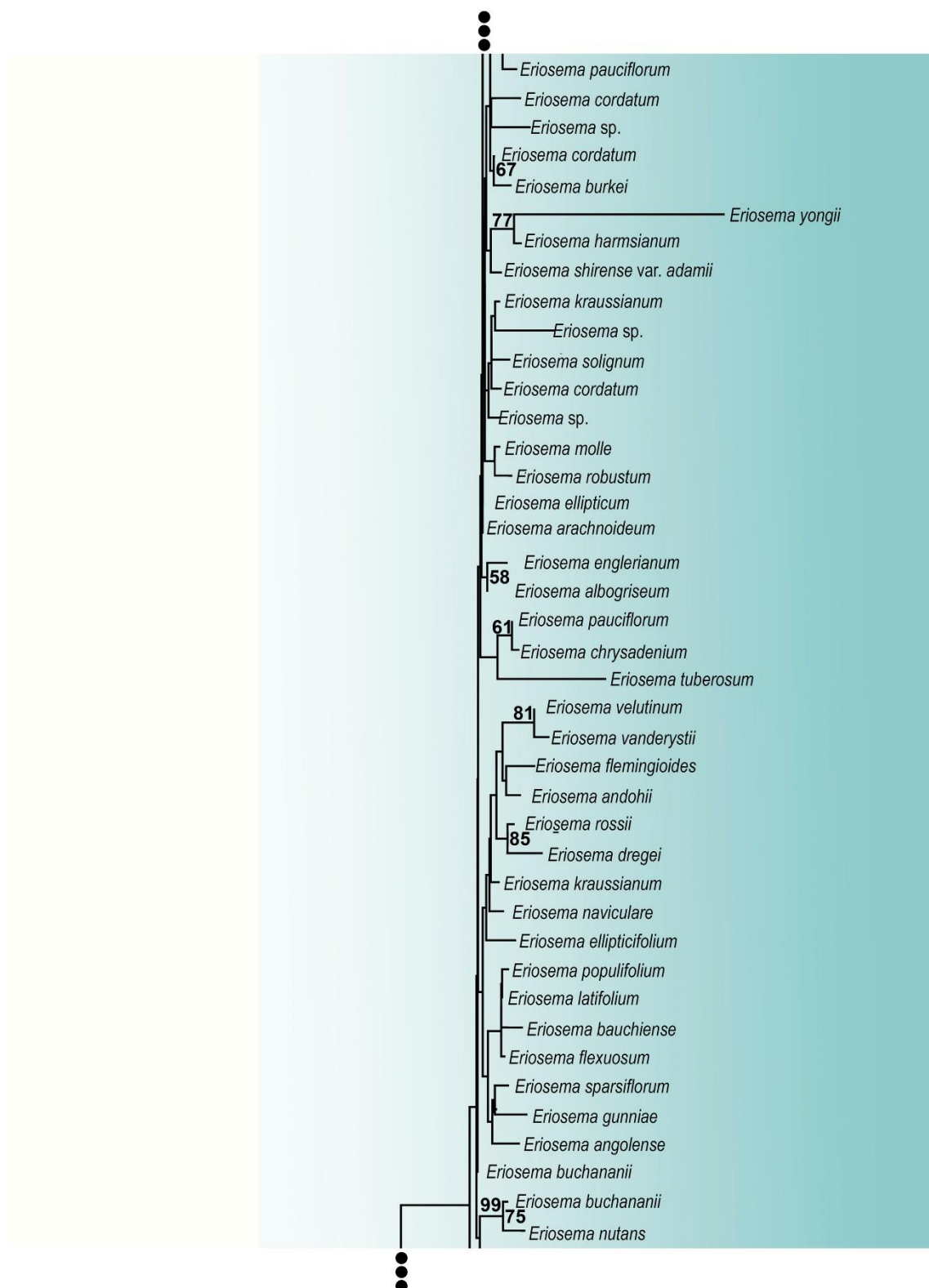


Fig. 4c (partitioned): Phylogenetic tree of the Cajaninae from Maximum Likelihood analysis of the ITS/5.8S and cpDNA dataset (rpl32-trnL and trnQ-5'rps16). Numbers along branches are posterior probabilities values.

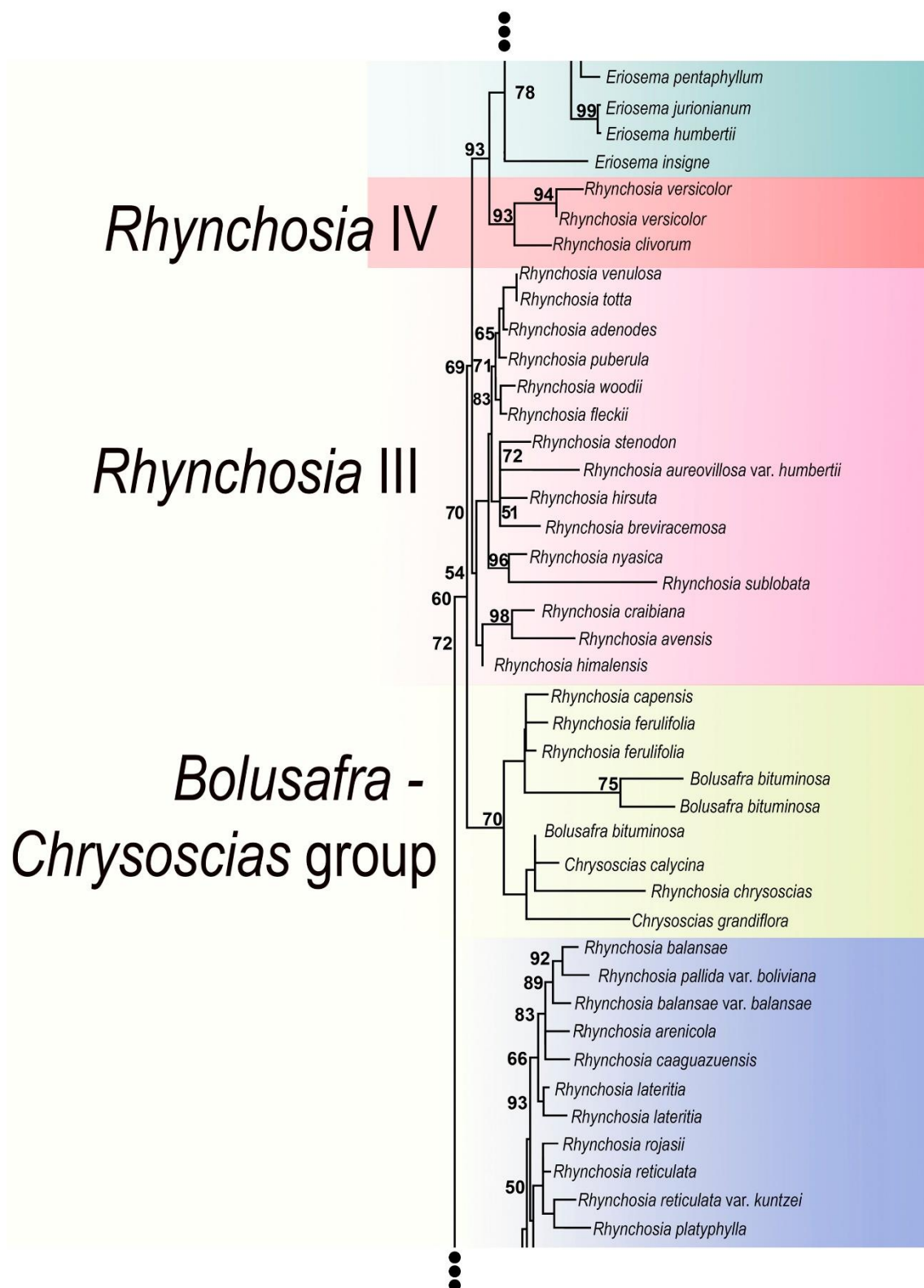


Fig. 4d (partitioned): Phylogenetic tree of the Cajaninae from Maximum Likelihood analysis of the ITS/5.8S and cpDNA dataset (rpl32-trnL and trnQ-5'rps16). Numbers along branches are posterior probabilities values.

Rhynchosia II

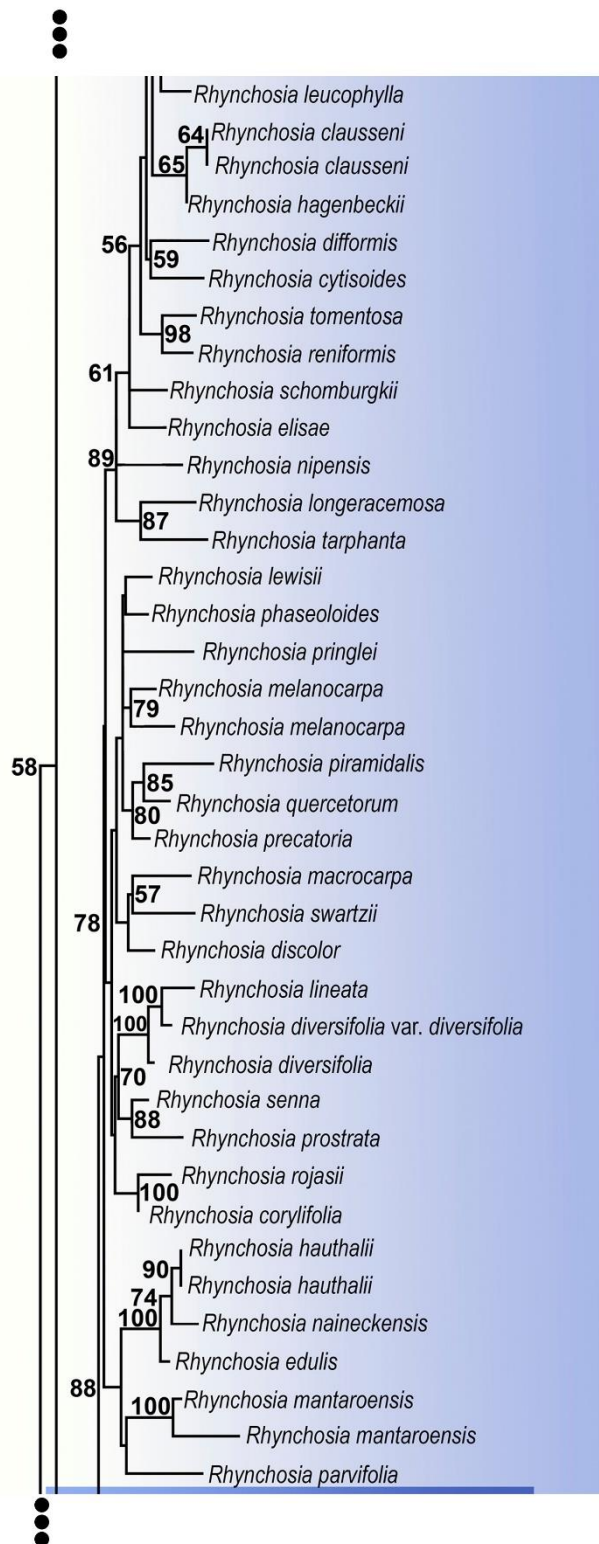


Fig. 4e (partitioned): Phylogenetic tree of the Cajaninae from Maximum Likelihood analysis of the ITS/5.8S and cpDNA dataset (rpl32-trnL and trnQ-5'rps16). Numbers along branches are posterior probabilities values.

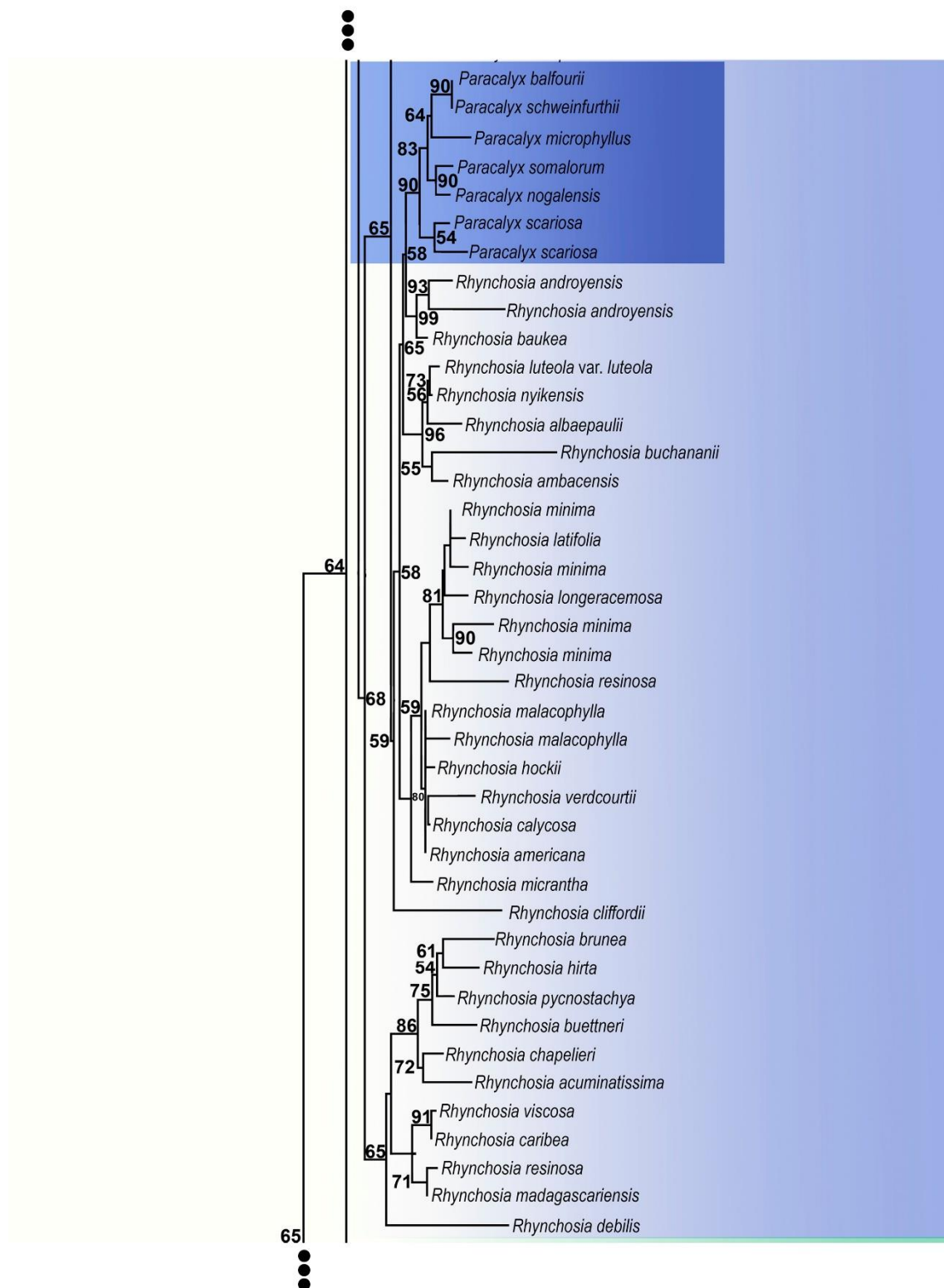


Fig. 4f (partitioned): Phylogenetic tree of the Cajaninae from Maximum Likelihood analysis of the ITS/5.8S and cpDNA dataset (rpl32-trnL and trnQ-5'rps16). Numbers along branches are posterior probabilities values.

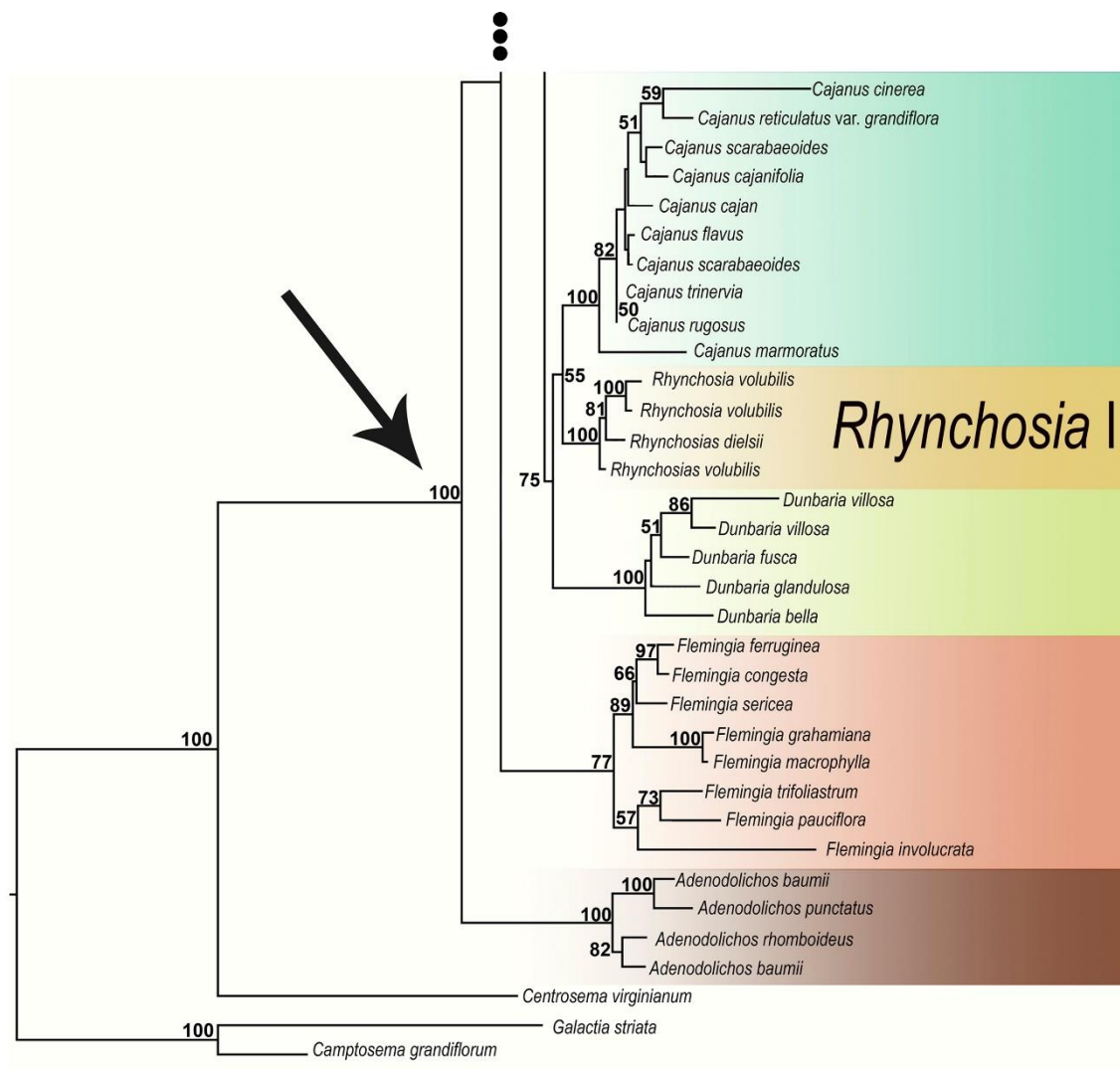


Fig. 4g (partitioned): Phylogenetic tree of the Cajaninae from Maximum Likelihood analysis of the ITS/5.8S and cpDNA dataset (rpl32-trnL and trnQ-5'rps16). Numbers along branches are posterior probabilities values.

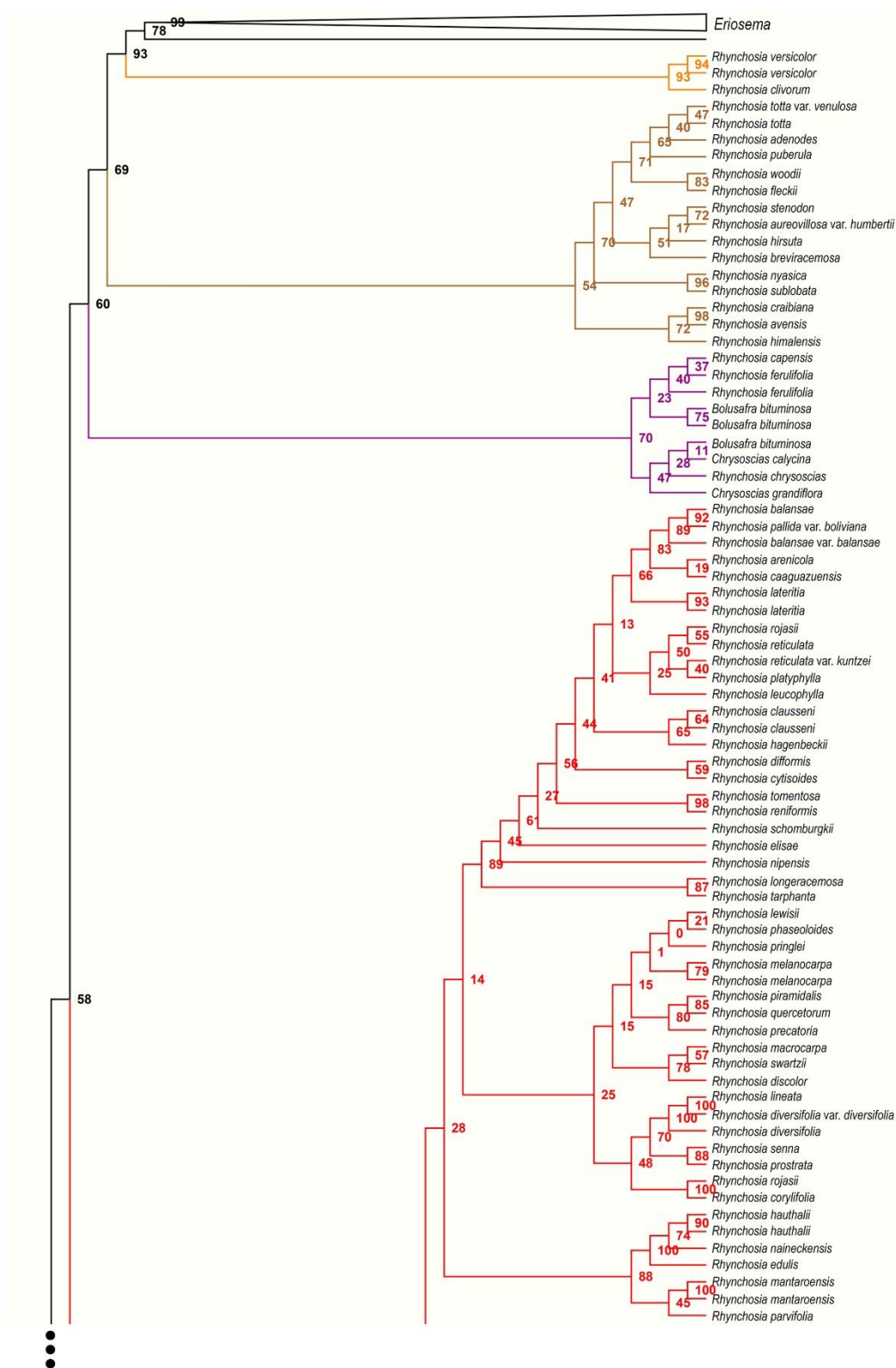


Fig. 5a (partitioned): Cladogram of the Cajaninae from Maximum Likelihood analysis of the ITS/5.8S and cpDNA dataset (rpl32-trnL and trnQ-5'rps16) with *Eriosema* genus collapsed. Numbers along branches are posterior probabilities values.

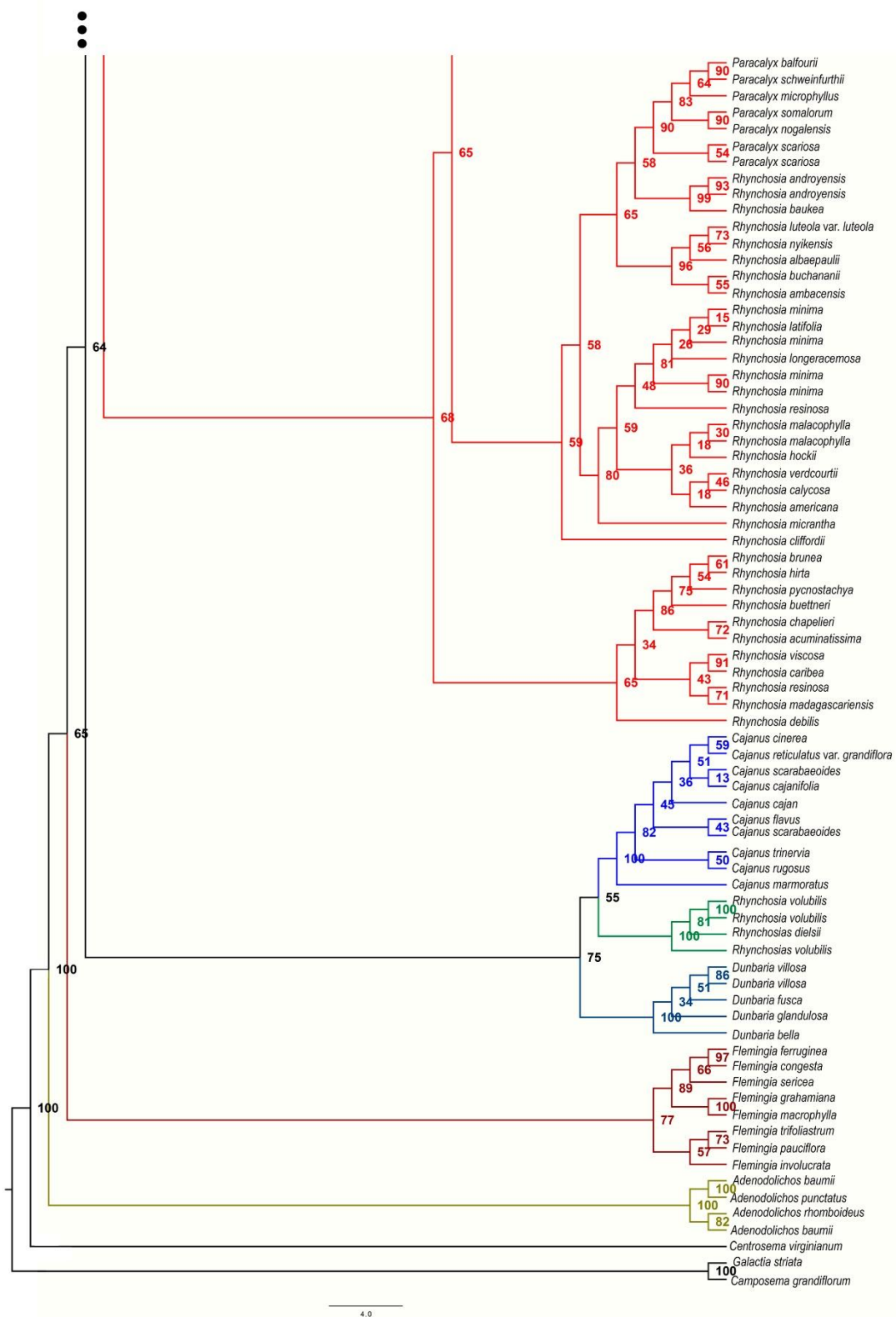


Fig. 5b (partitioned): Cladogram of the Cajaninae from Maximum Likelihood analysis of the ITS/5.8S and cpDNA dataset (rpl32-trnL and trnQ-5'rps16) with *Eriosema* genus collapsed. Numbers along branches are posterior probabilities values.

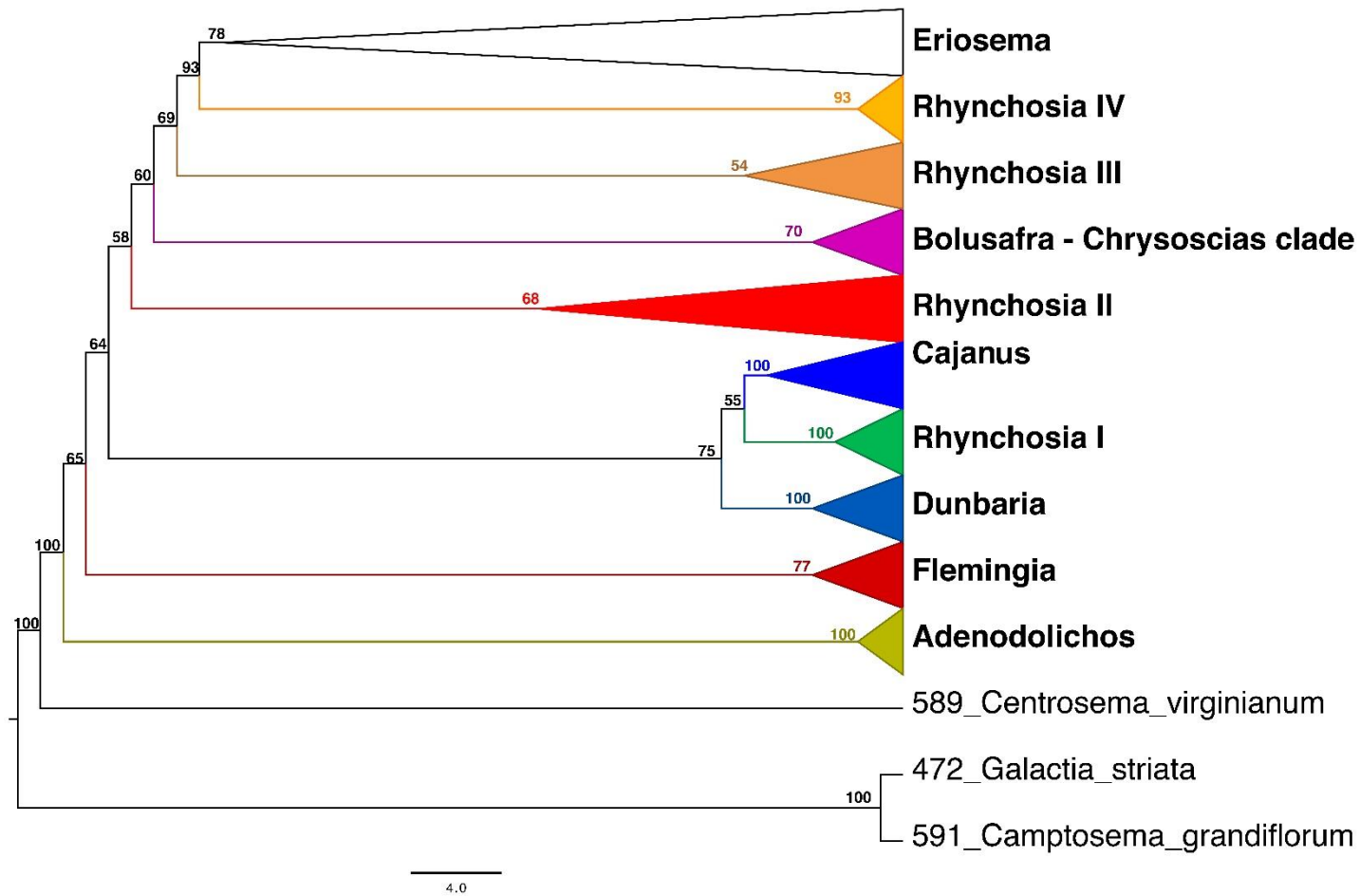


Fig. 6: Cladogram of the Cajaninae from Maximum Likelihood analysis of the ITS/5.8S and cpDNA dataset (rpl32-trnL and trnQ-5'rps16) with all genera collapsed. Numbers along branches are posterior probabilities values.

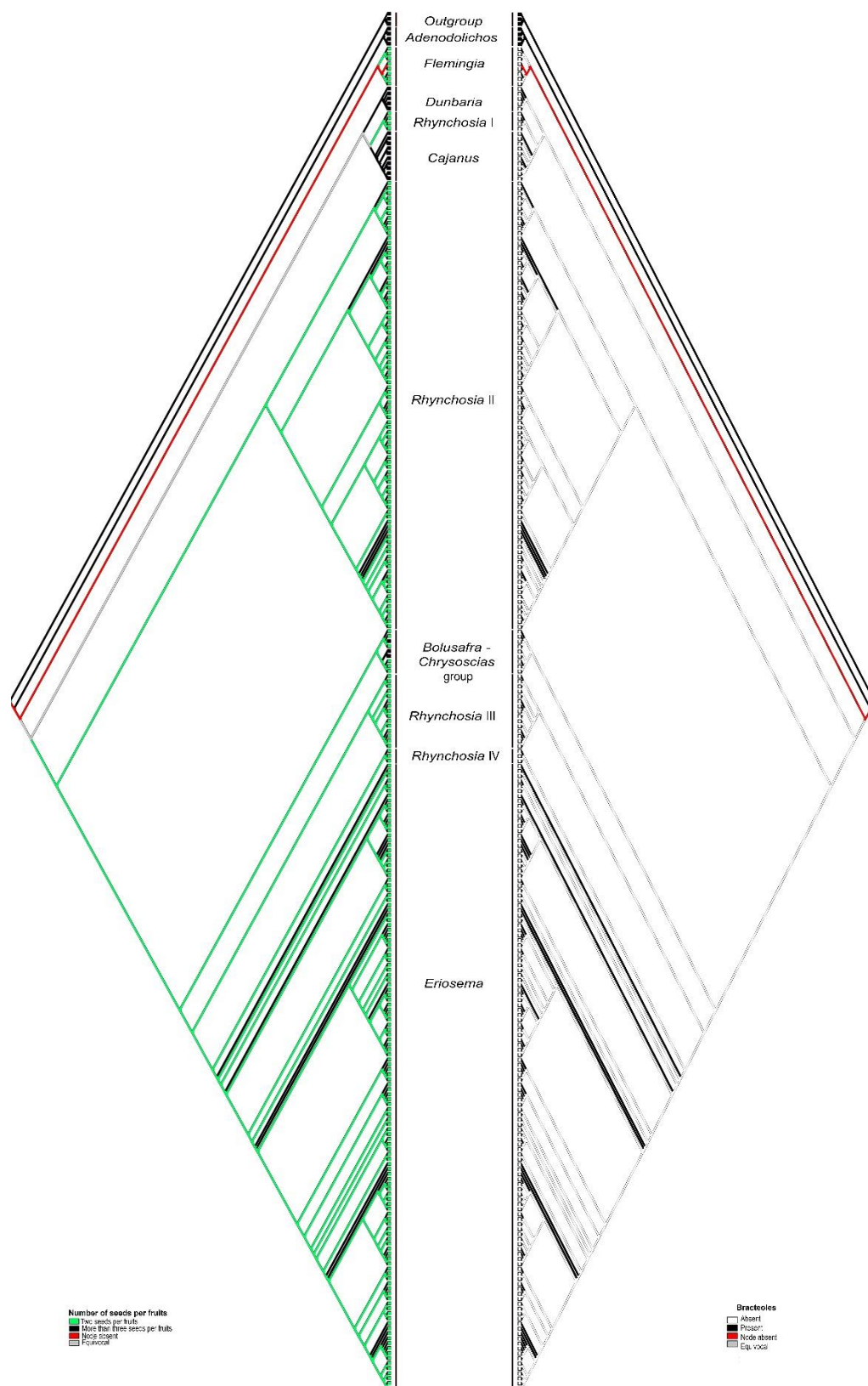


Fig. 7: Evolution of number of seeds per fruits and bracteoles in the Cajaninae subtribe inferred under Maximum Likelihood analysis of the ITS/5.8S and cpDNA dataset (rpl32-trnL and trnQ-5' rps16).

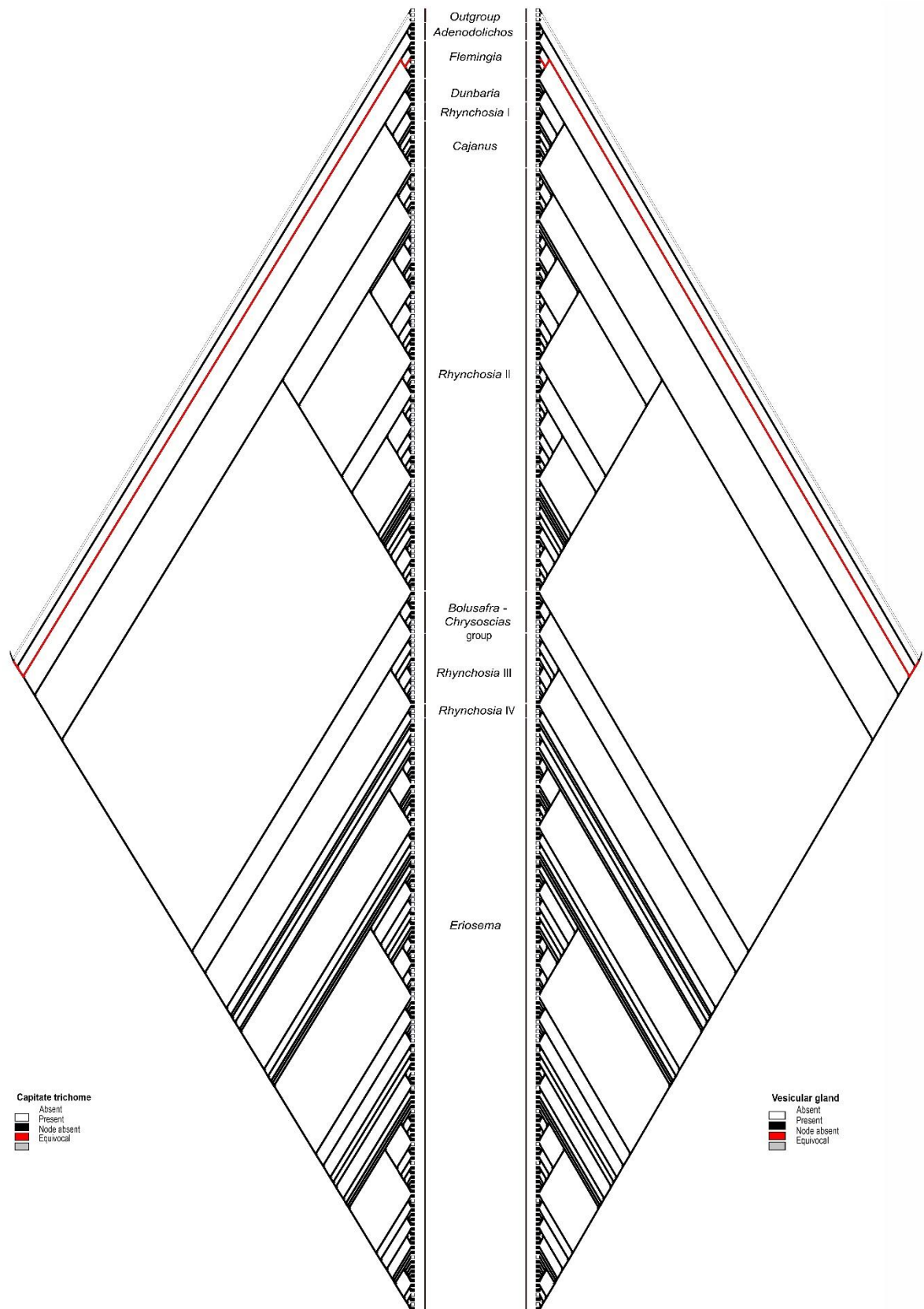


Fig. 8: Evolution of capitulate trichomes and vesicular glands in the Cajaninae subtribe inferred under Maximum Likelihood analysis of the ITS/5.8S and cpDNA dataset (rpl32-trnL and trnQ-5'rps16).

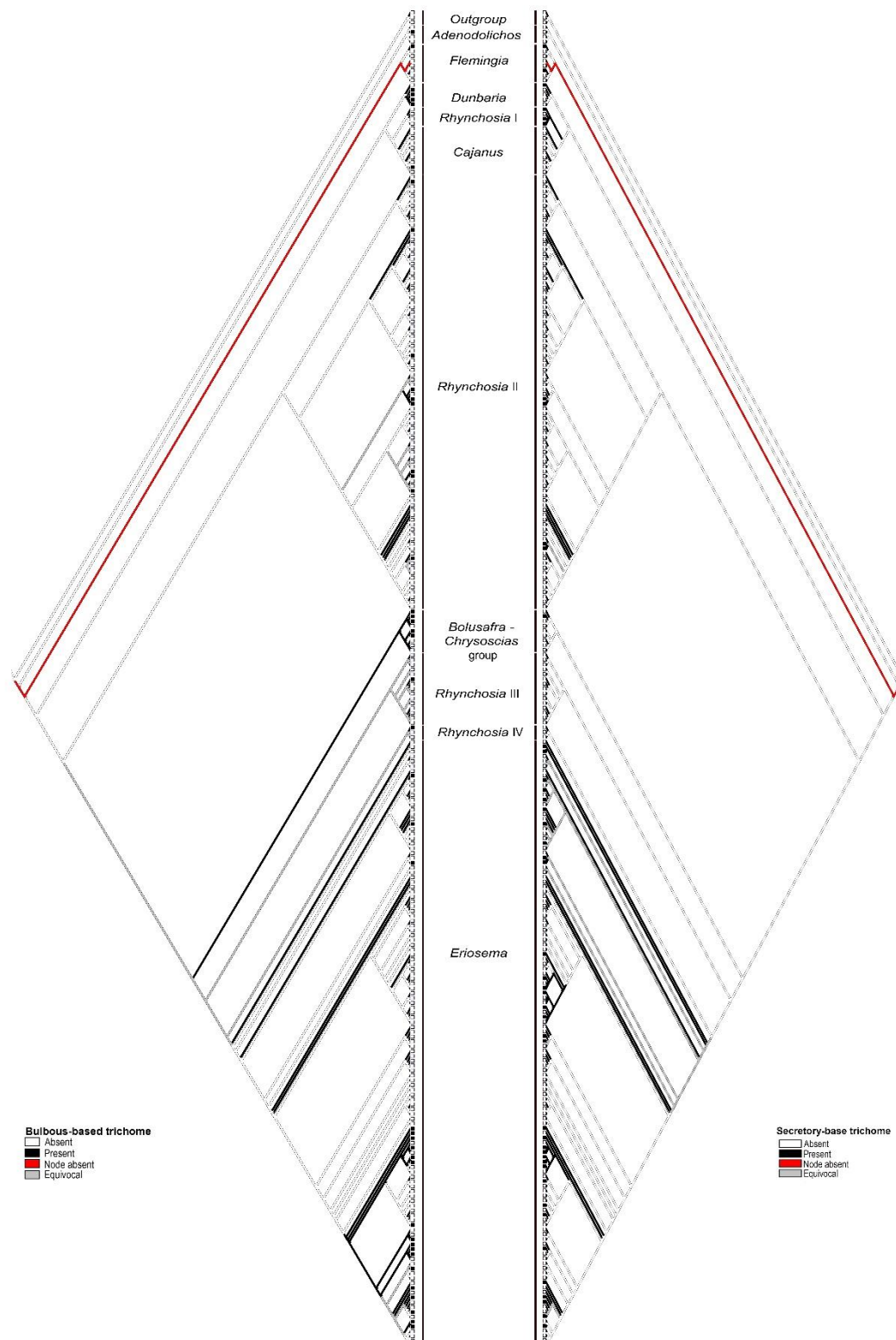


Fig. 9: Evolution of bulbous-based and secretory-base trichomes in the Cajaninae subtribe inferred under Maximum Likelihood analysis of the ITS/5.8S and cpDNA dataset (rpl32-trnL and trnQ-5'rps16).

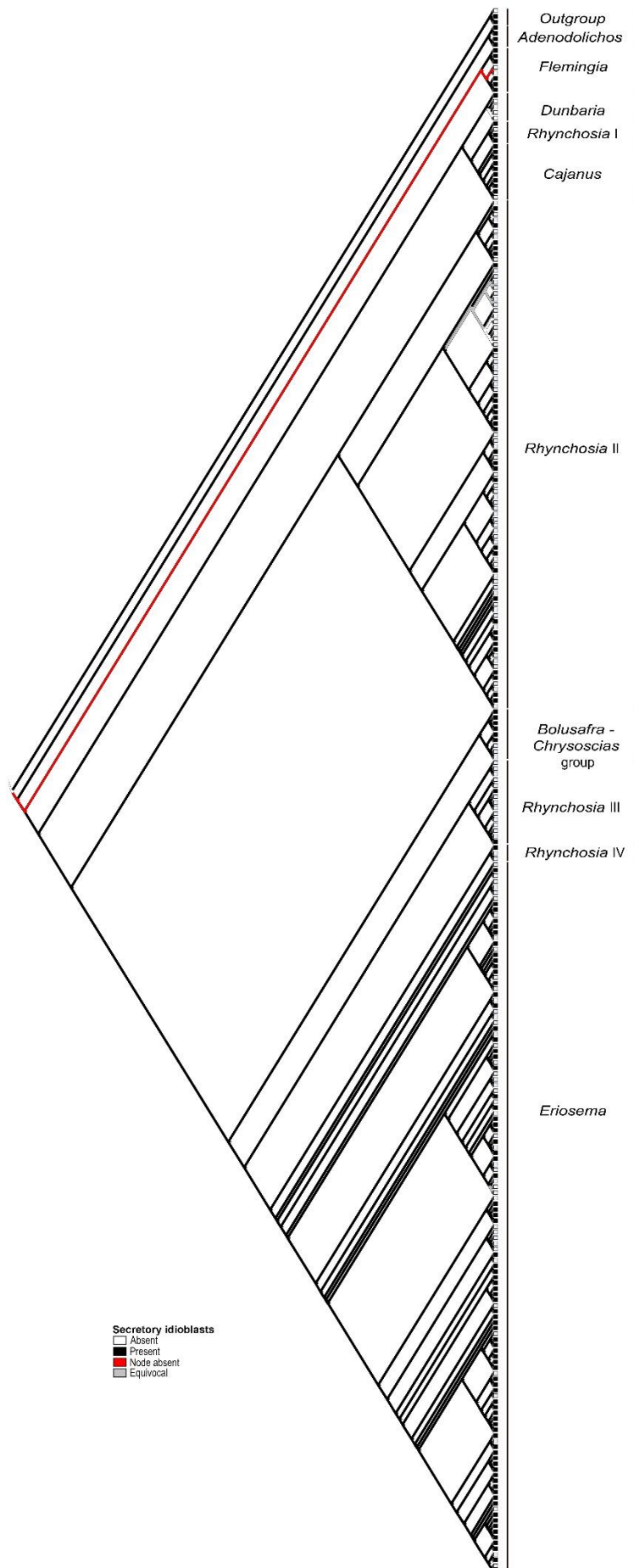


Fig. 10: Evolution of secretory idioblasts in the Cajaninae subtribe inferred under Maximum Likelihood analysis of the ITS/5.8S and cpDNA dataset (rpl32-trnL and trnQ-5'rps16).

Considerações finais

Esta tese incluiu um completo levantamento das estruturas secretoras ocorrentes na subtribo *Cajaninae*, investigação sobre os tipos de material secretados por estas glândulas e sua ultraestrutura, e uma abrangente filogenia, bem como uma reconstrução da evolução das glândulas amplamente distribuídas neste grupo. É importante salientar que todos estes objetivos foram cumpridos com cobertura amostral significativa.

As estruturas secretoras de *Cajaninae* estão distribuídas de modo geral em quase todos os representantes desse grupo, sendo elas: glândula vesicular, tricoma de base bulbosa, tricoma capitado, tricoma de base secretora e idioblastos secretores. Os dois primeiros tipos de glândulas já haviam sido anteriormente mencionados em trabalhos de sistemática sobre a subtribo e desde o seu descobrimento, no século 19, foram incluídas como uma das características mais importantes para o reconhecimento e sustentação de *Cajaninae*.

Nosso trabalho pode contribuir significativamente com a ciência, pois além de revisitar estas glândulas que já haviam sido descritas para o grupo, com nossos dados podemos discutir o tipo de composto secretado por elas, as estruturas subcelulares envolvidas no processo de secreção e principalmente a função e importância que elas desempenham para o grupo, que se diversificou em ambientes com muita exposição luminosa e raios UV, como as savanas e cerrados.

Com a conclusão deste trabalho podemos afirmar que *Cajaninae*, bem como *Adenodolichos*, *Flemingia*, *Dunbaria*, *Cajanus* e *Eriosema* são grupos monofiléticos, sendo este último o gênero com a mais recente diversificação dentro da subtribo, necessitando de mais estudos filogenéticos com diferentes marcadores para obter melhor

compreensão do relacionamento dos seus táxons. Além disso, *Chrysoscias*, *Bolusaфра*, *Paracalyx* e *Rhynchosia* são grupos não monofiléticos, levantando a necessidade de mais estudos sistemáticos com estes táxons, e também em relação aos aspectos nomenclaturais dos mesmos. Estudos de datação molecular e história biogeográfica da subtribo estão sendo desenvolvidos para melhor compreensão da sistemática do grupo.