

HIGOR ANTONIO DOMINGUES

**Palinotaxonomia e evolução de caracteres
polínicos de representantes do
clado informal Adesmia
(Leguminosae – Papilionoideae – Dalbergieae)**

Tese apresentada ao Instituto de Pesquisas Ambientais, da Secretaria de Meio Ambiente, Infraestrutura e Logística, como parte dos requisitos exigidos para a obtenção do título de DOUTOR em BIODIVERSIDADE VEGETAL E MEIO AMBIENTE, na Área de Concentração de Plantas Vasculares em Análises Ambientais.

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ORIENTADORA: DRA. CYNTHIA FERNANDES PINTO DA LUZ

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As noites passam e minha mente transmuta pensamentos em palavras. Trago histórias sobre sentimentos, devaneios e contos sobre mim mesmo, vagando pelos meus mundos.

Noutros momentos me pego lendo meus contos e percebo que passei a escrever cartas, para eu mesmo ler. Ao reviver cada momento, reescrevo minha história e entendo sobre cada universo que existe em mim.

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RESUMO

Dentre as angiospermas, Leguminosae é a maior família, a mais importante economicamente e merece atenção o clado informal Adesmia. Esse táxon está circunscrito dentre as dalbergioides (Papilionoideae) e é atualmente representado por quatro gêneros exclusivamente americanos (*Adesmia*, *Amicia*, *Nissolia*, *Poiretia*) e um gênero pantropical (*Zornia*). Esse clado que vem sendo amplamente estudado filogeneticamente, porém os estudos palinológicos são escassos. Sabendo que os grãos de pólen são uma importante ferramenta para a taxonomia e para elucidar a história evolutiva dos diversos grupos de angiospermas a presente tese almejou descrever a morfologia polínica bem como a ultraescultura e ultraestrutura da exina dos cinco gêneros que compõem o táxon utilizando a metodologia padrão para palinotaxonomia. Visando a robustez dos dados, foram também aplicadas análises estatísticas multivariadas além do mapeando dos caracteres palinológicos utilizando a filogenia mais recente. Foram analisados 145 espécimes distribuídos por 78 espécies dos cinco gêneros do clado. Através da palinologia foi possível a delimitação de espécies bem como de tipos polínicos dentro dos gêneros. Os tipos polínicos estabelecidos para os gêneros *Amicia* e *Nissolia*, utilizando a morfologia polínica e ultraescultura corroboram os cladogramas infragenéricos estabelecidos filogeneticamente auxiliando assim com dados morfológicos para o reconhecimento dos taxa. Todas as linhagens do clado Adesmia foram recuperadas com pelo menos um ou mais homoplasias/apomorfias, exceto pelo gênero *Adesmia*.

Palavras-chave: Dalbergieae, Fabaceae, morfologia polínica, palinologia, Papilionoideae.

ABSTRACT

Among the angiosperms, Leguminosae is the most diverse family and economically significant, and the Informal Adesmia Clade deserves attention. This taxon is circumscribed among the dalbergiods (Papilionoideae) and is currently represented by four exclusively American genera (*Adesmia*, *Amicia*, *Nissolia*, *Poiretia*) and one pantropical genus (*Zornia*). This clade has been widely studied phylogenetically and anatomically, but palynological studies are scarce. Knowing the pollen grains' importance for the taxonomy and elucidating the evolutionary history of the various groups of angiosperms, this thesis aimed to describe the pollen morphology and the ultrasculpture and ultrastructure of the exine of the five genera included in the taxa using the standard methodology for palinotaxonomy. Aiming for data robustness, multivariate analyses were also applied in addition to mapping palynological characters using the most recent phylogeny. Analyzed 145 specimens from 78 species of the five genera of the clade, and it was possible to delimit species and pollen types within genera. The pollen types established for the genera *Amicia* and *Nissolia*, using pollen morphology and ultrasculpture, corroborate the phylogenetically established infrageneric clades, thus helping with morphological data for the recognition of taxa. The description of the exine ultrastructure is new for the five genera of the Adesmia clade and is helpful for future studies of the phylogeny of the tribe Dalbergieae. All lineages of the Adesmia clade from the molecular phylogeny were recovered with at least one or more homoplasies/apomorphies, except by *Adesmia* genus.

Keywords: Dalbergieae, Fabaceae, palynology, Papilionoideae, pollen morphology.

Lista de siglas e abreviações

Ma	Milhões de anos
BFG	<i>The Brazil Flora Group</i>
LPWG	<i>Legume Phylogeny Working Group</i>
<i>sens. lat.</i>	<i>Sensu lato</i>
APG	<i>Angiosperm Phylogeny Group</i>
ss.	<i>Sensu stricto</i>
MO	Microscopia óptica
MEV	Microscopia eletrônica de varredura
MET	Microscopia eletrônica de transmissão
IBB	Instituto de Biociências
Av.	Avenida
pers. comm.	<i>Personal communication</i>
x	<i>Arithmetic mean</i>
Sx	<i>Average standard deviation</i>
s	<i>Sample standard deviation</i>
V%	<i>Coefficient of variability</i>
CI	<i>Confidence interval</i>
SEM	<i>Scanning electron microscopy</i>
TEM	<i>Transmission electron microscopy</i>
M	<i>Molar</i>
h	<i>Hours</i>
min	<i>Minutes</i>
nm	<i>Nanometers</i>
kV	<i>Kilovolt</i>
CEn	<i>Class of endoaperture</i>
WE	<i>Width of the endoaperture</i>
LE	<i>Length of endoaperture</i>
µm	<i>Micrometer</i>
P/E	<i>Polar axis/Equatorial axis (shape class)</i>
S	<i>Small size</i>
M	<i>Medium size</i>
N	<i>Nexine thickness</i>
S	<i>Sexine thickness</i>
USA	<i>United States of America</i>
PCA	<i>Principal component analysis</i>
PA	<i>Polar axis</i>
EA	<i>Equatorial axis</i>
CL	<i>Colpus length</i>
CW	<i>Colpus width</i>

EL	<i>Endoaperture length</i>
EW	<i>Endoaperture width</i>
Ex	<i>Exine thickness</i>
S/N	<i>Index of sexine and nexine thickness</i>
CA	<i>Cluster analysis</i>
e-translucent	<i>Electron-translucent</i>
e-dense	<i>Electron-dense</i>
UPGMA	<i>Unweighted Pair Group Method using Arithmetic averages</i>
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CNPq	Conselho Nacional de Desenvolvimento Científico e Tecnológico
FAPESP	Fundação de Amparo à Pesquisa do Estado de São Paulo
mya	<i>Millions of years</i>
DNA	<i>Desoxyribonucleic acid</i>
ITS	<i>Internal transcribed spacer</i>
matK	<i>Maturase K</i>
L	<i>Large size</i>
PT	<i>Pollen type</i>
vs	<i>Versus</i>
PALINO	Laboratório de Palinologia
IPA	Instituto de Pesquisas Ambientais
rDNA	<i>Ribosomal DNA</i>
BT	<i>Bootstrapping</i>
ZAP clade	<i>Zornia-Amicia-Poretia clade</i>

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INTRODUÇÃO GERAL

LEGUMINOSAE

Leguminosae Adans. é a terceira maior família das Angiospermas [Asteraceae (1^a), Orchidaceae (2^a)], apresentando cerca de 770 gêneros e mais de 19.500 espécies e a segunda maior em importância econômica, depois de Poaceae (LPWG 2017). A idade da família data entre 59 Milhões de Anos (MA) e 65 MA com uma rápida diversificação das linhagens durante o período Terciário (Bruneau et al. 2008; Lavin et al. 2005). No Brasil, é a família mais representativa com aproximadamente 255 gêneros e 3.040 espécies, sendo 18 e 1.586 endêmicos, respectivamente. Está presente em todos os domínios fitogeográficos brasileiros sendo a mais rica em espécies na Floresta Amazônica e Caatinga (Flora do Brasil 2020; BFG 2015).

O *Legume Phylogeny Working Group* (2017), propôs a classificação mais recente para as subfamílias de Leguminosae baseada em uma filogenia molecular abrangente. Caesalpiniodeae DC. como tradicionalmente circunscrita, não formava um grupo monofilético, com Mimosoideae DC. e Papilionoideae DC. se posicionavam dentro dela (LPWG 2013a). Assim, foi proposta a ampliação para seis subfamílias: Caesalpiniodeae foi desmembrada em cinco subfamílias monofiléticas [Duparquetioideae (1), Cercidoideae (2), Detarioideae (3), Dialioideae (4) e Caesalpiniodeae (5)] (LPWG 2013b, 2017). As “Mimosoideae” e o gênero *Chidlowia* Hoyle (antes Caesalpiniodeae) atualmente fazem parte do clado Mimosoide dentro da subfamília Caesalpiniodeae. Papilionoideae (6) se manteve como a maior das seis subfamílias, com aproximadamente 505 gêneros e 14.000 espécies cosmopolitas, distribuídas em 28 tribos sendo muitas de interesse econômico (Klitgaard & Lavin 2005, Lewis et al. 2005, LPWG 2017). Essa última subfamília é onde o grupo de enfoque da presente Tese está inserido.

Papilionoideae – Clado informal Dalbergioide

Em extensivas análises moleculares, as papilionoides formam um grupo monofilético que se divergiu das outras leguminosas em aproximadamente 45 a 50 Ma. Os representantes desta subfamília são reconhecidos morfológicamente principalmente pela orientação do hilo e pela iniciação unidirecional das sépalas (Doyle & Luckow 2003). Podem ser citados grandes grupos informais dentro de Papilionoideae, como as Papilionoideae basais, clados Baphioide, Dalbergioide *sens. lat.*, Gensitoide, Hologalegina, *inverted repeat-lacking clade*, Millettioide *sens. lat.*, Mirbelioide *sens. lat.* e Robinioide (Lewis et al. 2005).

Dalbergioide *sens. lat.* é um clado representante das linhagens de Papilionoideae que emergiram primeiro dentro da subfamília. Se não levarmos em conta a maior linhagem dentro das leguminosas papilionoideas (as *non protein amino acid accumulating*, que representam cerca de 70% das espécies dessa subfamília) as Dalbegoide *s.l.* representam 29% da diversidade dessa subfamília, com aproximadamente 1.370 espécies. Esse grupo é composto por dois outros clados: Amorpheae (formado por *Amorpha* e *Dalea*) e Dalbergieae (*Adesmia*, *Pterocarpus* e *Dalbergia*) (Cardoso et al. 2013)

Dalbergieae agrupa gêneros representantes anteriormente incluídos nas tribos Dalbergieae Bronn ex DC.; Aeschynomeneae (Benth.) Hutch.; Hedysareae subtribo Aeschynomeninae Benth.; Coronilleae subtribo Aeschynomene (Benth.) Schulze-Menz; Adesmieae (Benth.) Hutch.; Hedysareae subtribo Adesmiinae Benth., Hedysareae subtribo Patagoniinae Taub., Coronilleae subtribo Pagatoniinae (Taub.) Schulze-Menz e Desmodieae (Benth.) Hutch. Subtribo Bryinae (Klitgaard & Lavin 2005). O clado *Adesmia*, grupo foco do estudo desse trabalho, inclui o gênero *Adesmia* DC. (único membro da tribo Adesmieae *sensu* Polhill 1981) e mais quatro gêneros herbáceos, subarborescentes e arbustivos que faziam parte da tribo Aeschynomeneae: *Poiretia* Vent., *Amicia* Kunth, *Zornia* J.F. Gmel. e *Nissolia* Jacq.

Clado Adesmia

Esse clado pode ser reconhecido pelo conjunto de características morfológicas que incluem o hábito herbáceo, folhas com alguns folíolos opostos, pedicelos confluentes com o cálice e legumes lomentáceos (Lavin et al. 2001). É representado por aproximadamente 350 espécies com distribuição na região neotropical, exceto *Zornia* que é pantropical (Klitgaard & Lavin 2005).

Zornia possui como características diagnósticas as flores dispostas em inflorescências espiciformes com bractéolas peltadas, aos pares, protegendo cada flor (Sciamarelli 1996; Fortuna-Perez 2009). *Adesmia* pode ser reconhecido pela ocorrência de estames livres ou os dois superiores unidos a unguícula do estandarte, e pelos frutos do tipo lomento típico ou do tipo hemicraspédio (Miotto 1991). *Poiretia* apresenta geralmente de 1 a 4 folíolos, glândulas translúcidas, flores em racemos sem grandes bractéolas pareadas, e hábito frequentemente escandente (Rudd 1981, Müller 1984). *Amicia* possui androceu monadelfo, anteras uniformes, lomento lateralmente comprimido, hábito arbustivo ou herbáceo com folhas 4-folioladas, e cálice com glândulas redondas (Burkart 1939; Rudd 1981). *Nissolia* é caracterizada pelos frutos do tipo lomento, com o artículo terminal expandido, achatado, estéril, e do tipo alado ou pelo lomento alongado com artículos uniformes no tamanho (Burkart 1939; Rudd 1956, 1958, 1981).

Do ponto de vista filogenético, o estudo de Fortuna-Perez et al. (2021), com ênfase em estruturas secretoras, apresentou significativa contribuição para o conhecimento das relações evolutivas para o clado *Adesmia*. No entanto, deixou espaço para que questões filogenéticas infragenéricas e biogeográficas e para que demais estruturas fossem estudadas, já que podem estar relacionadas com fatores ambientais que guiaram a evolução do clado, como os grãos de pólen. Os caracteres polínicos apresentam bons resultados em estudos filogenéticos por serem mais estáveis que outras características morfológicas (Doyle 2005; Banks 2003; Penet et al. 2005; Thornhill & Crisp 2012, Banks & Rudal 2016; Grímsson et al. 2017). Os grãos de pólen apresentam vantagens por serem amplamente preservados nos registros fósseis de

distintos tempos geológicos em virtude da presença de esporopolenina em suas exinas e, portanto, bem estudados e documentados. Com isso, a investigação das ancestralidades e dos processos evolutivos dos diferentes grupos vegetais é facilitada tendo como base a unidade de dispersão polínica a partir da antera (mônades, tétrades, políades, etc), simetria e forma dos grãos, número, posição e caráter das aberturas, estrutura e escultura da exina dos grãos de pólen (Thanikaimoni 1983, Zavada 1983, Harley & Zavada 2000, Melhem et al. 2003, Lorscheitter 2006).

Palinologia em Angiospermas e Leguminosae

Por seu grande valor na taxonomia das Angiospermas, os caracteres polínicos são fontes de importantes informações nas propostas recentes de classificação do grupo, como revelam o APG II (2003), APG III (2009) e APG IV (2016). A variação no padrão numérico das aberturas polínicas não é aleatória nas Angiospermas, geralmente refletindo a filogenia dos grandes grupos (Penet et al. 2005). Em seus grupos mais basais (Doyle 2005) bem como em outras famílias mais derivadas como Myrtaceae (Thornhill & Crisp 2012) e Loranaceae (Grímsson et al. 2017) os caracteres polínicos combinados às análises moleculares possibilitaram delimitar os clados que compõem as famílias, empregando tipos polínicos diagnósticos e estados de caracteres ancestrais além de possibilitar a comparação de espécies atuais com morfoespécies fósseis. Palinologicamente, as eudicotiledôneas são conhecidas como o clado tricolpado sendo assim essa morfologia pode ser reconhecida como uma característica sinapomórfica para o grupo (Crane et al. 1995; Judd & Olmstead 2004).

Apesar do pólen tricolpado das eudicotiledôneas ser comum em Leguminosae, a variação das aberturas é ampla. Para sua atual classificação, que associou caracteres moleculares às características polínicas e outros caracteres morfológicos, químicos e cromossômicos, foi possível reconhecer palinologicamente as seis subfamílias de Leguminosae (LPWG 2017). As Cercidoideae apresentam grãos de pólen em mônades, 3-

colporados, 3-6-colpados, 3-porados, 3-pororados, 3-4-colporoidados ou inaperturados, raramente em tétrades, enquanto em Detarioideae são na sua maioria 3-colporados com uma variada ornamentação da sexina. Em Duparquetioideae as mônades são assimétricas com uma ectoabertura circundando o equador e duas endoaberturas circulares. Dialioideae apresenta mônades 3-colporadas, com sexina perfurada ou finamente reticulada, raramente com padrão estriado. Caesalpinoideae pode também apresentar mônades 3-colporadas ou tétrades, bitétrades ou políades poradas (maioria do Clado Mimosoideae), com sexina nunca estriada e, em Papilionoideae são mônades, 3-colporadas, 3-colpadas ou 3-poradas (faltou a citação para isso).

Mesmo antes da recente circunscrição da família, os palinólogos já haviam observado diferenças na estrutura e na função dos grãos de pólen em Caesalpinoideae *sensu lato* (Banks & Rudall 2016). Mais de 120 tipos morfológicos foram estabelecidos para Caesalpinoideae *sl*, pois apresenta uma alta diversidade na morfologia polínica, sendo 26 tipos em Cercidoideae, 76 em Detarioideae e, 19 em Caesalpinoideae *ss*. O opérculo também apresenta importante função para a taxonomia e funciona como uma estratégia contra a dissecação ambiental em alguns gêneros, cobrindo a abertura, mantendo até certo ponto a hidratação polínica e reduzindo seu tamanho. Está presente nos gêneros *Orphanodendron* Barneby & J.W. Grimes e *Parkinsonia* L. (Caesalpinoideae) e *Pilostigma* Tiegh. (Cercidoideae) e é amplamente distribuída nas Papilionoideae. A forma do colpo (sincolpo, parassincolpo), ornamentação supratectal e estrutura da exina (columelada, granular) funcionam na prevenção da ruptura das paredes dos grãos de pólen e apresenta valor taxonômico. Porém, a variação mais marcante entre os gêneros de Papilionoideae está no tipo apertural (Tewari & Nair 1979).

Pólen das adesmias

Espécies de representantes dos cinco gêneros do Clado Adesmia foram analisados em trabalhos palinológicos prévios. Esses estudos envolveram descrições em catálogos

palinológicos e melissopalinológicos (Carreira et al. 1996, Moreti et al. 2007a), bem como em publicações de cunho palinotaxonômico (Melhem 1966, Salgado-Labouriau 1973, Silvestre-Capelato & Melhem 1997, Moreti et al. 2007b, Buril et al. 2011, Luz et al. 2013, Silva et al. 2016) e taxonômico (Ohashi 1971, Pire 1974, Ferguson & Skvarla 1981). Ferguson & Skvarla (1981) verificaram que o tipo apertural tem maior valor que a escultura da sexina em Papilionoideae.

Compilando as descrições palinológicas disponíveis de aproximadamente 40 espécies, foi possível identificar que o Clado *Adesmia* é euripolínico, ou seja, com ampla variação morfológica. São sempre isopolares, 3-zonoaperturados, raros 4-zonoaperturados, com colpos com terminações afiladas, apresentando ou não constrição mediana, e podendo ter margem ou opérculo, com exina semitectada. Existem variações quanto ao tamanho dos grãos de pólen (pequenos, médios, grandes), tipo de abertura (colpado, colporado), tipo de endoabertura (lalongada, circular, lalongada, ausente) e ornamentação da sexina (perfurada, microrreticulada, reticulada, rugulado-microrreticulada). Outros caracteres que podem apresentar variação no clado *Adesmia* são: presença ou não de opérculo, padrão de ornamentação do opérculo e forma dos grãos de pólen, sendo esses caracteres importantes para a delimitação dos grupos (Melhem 1962, Furness & Rudall 2006).

Nesse cenário, visando testar se os dados palinológicos podem auxiliar na sistemática do clado *Adesmia*, pretendemos: 1) testar se a morfologia polínica permite o reconhecimento das espécies, ou mesmo dos gêneros *Adesmia*, *Amicia*, *Poiretia*, *Nissolia* e *Zornia*, 2) testar se a inclusão de caracteres morfológicos observados em conjunto com as análises moleculares visando a otimização de caracteres, poderá apontar quais características são úteis para o reconhecimento e delimitação de clados. 3) entender como se deu a evolução polínica no clado *Adesmia*, 4) testar se os padrões de evolução do pólen corroboram suas relações filogenéticas.

OBJETIVOS

Objetivo Geral

Realizar um estudo de otimização de caracteres morfopalinológicos no clado *Adesmia*, através de análises de mapeamento de caracteres.

Objetivos Específicos

- Descrever os grãos de pólen de representantes de *Adesmia*, *Amicia*, *Poiretia*, *Nissolia* e *Zornia*.
- Avaliar a significância dos caracteres palinológicos para definir tipos polínicos empregando análises multivariadas.
- Estabelecer estados de caracteres palinológicos do clado *Adesmia*.
- Realizar o mapeamento dos caracteres polínicos analisados na atual filogenia molecular disponível para o clado *Adesmia* (Fortuna-Perez et al. 2021).

MATERIAL E MÉTODOS

O tratamento foi delineado de acordo com a metodologia padrão para o estudo dos grãos de pólen sob microscopia óptica (MO) e microscopia eletrônica de varredura (MEV) e de transmissão (MET) e em análise estatístico-descritiva aplicada a Palinotaxonomia e otimização de caracteres. Maiores detalhes sobre os materiais e métodos utilizados serão detalhados no decorrer dos capítulos, que compõem a tese.

RESULTADOS

Os resultados são apresentados em cinco capítulos. Os três primeiros correspondem a artigos já publicados para os gêneros *Poiretia*, *Amicia* e *Nissolia*. O quarto capítulo é referente a palinologia de *Adesmia* e o quinto ao gênero *Zornia* em conjunto com a otimização dos caracteres palinológicos do Clado *Adesmia*.

Organização da tese

Capítulo I - *Pollen morphology, ultrasculpture and ultrastructure of Poiretia Vent.*

(Leguminosae - Papilionoideae - Dalbergieae - Adesmia informal clade) – publicado no periódico *Grana* (<https://doi.org/10.1080/00173134.2021.1984574>)

Capítulo II - *Palynology of Amicia Kunth. (Leguminosae – Papilionoideae – Dalbergieae –*

Informal Adesmia Clade) set in a systematic and phylogenetic context – publicado no

periódico *Grana* (<https://doi.org/10.1080/00173134.2022.2130011>)

Capítulo III - *Pollen study as a contribution to the taxonomy of Nissolia (Leguminosae –*

Papilionoideae – Dalbergieae – Adesmia Informal Clade) – publicado no periódico *Grana*

(<https://doi.org/10.1080/00173134.2023.2227190>)

Capítulo IV - *A new look at the taxonomy of Adesmia DC with a palinological approach*

(Leguminosae – Papilionoideae – Dalbergieae – Adesmia informal Clade) – a ser submetido no periódico *Grana*.

Capítulo V - *Palynology of Zornia J.F. sheds light on the phylogenetic relevance of pollen*

*grains in the Adesmia clade (Leguminosae – Papilionoideae – Dalbergieae) - a ser submetido no periódico *Perspective in Plant Ecology, Evolution and Systematics**

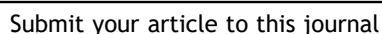
ISSN: (Print) (Online) Journal homepage: <https://www.tandfonline.com/loi/sgra20>

Pollen morphology, ultrasculpture and ultrastructure of *Poiretia* Vent. (Leguminosae – Papilionoideae – Dalbergieae – Adesmia informal clade)

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Pollen morphology, ultrasculpture and ultrastructure of *Poiretia* Vent. (Leguminosae – Papilionoideae – Dalbergieae – Adesmia informal clade)

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Abstract

Pollen morphology, ultrasculpture and ultrastructure were investigated for *Poiretia* in search of morphological characters of taxonomic importance using light microscopy as well as scanning and transmission electron microscopy. Additionally, principal components analysis was done to elucidate patterns of variation among the species. Pollen grains are small to medium size, isopolar, prolate spheroidal to prolate, 3-zonocolporate, colporus with operculum, membrane and margo are ornamented. Five pollen types are recognised and described based on mesocolpium exine ultrasculpture. Exine consists of a compact tectum, eutectate, tectate perforate to semitectate, the infratectum is collumellar and granular, foot layer discontinuous to absent in aperture region and endexine is continuous. A comprehensive description of the operculum, membrane ornamentations and the ultrastructure of exine were given for all species in the genus, nine of which were not previously analysed palynologically. Systematic implications of the palynological variations found in the literature and through light, scanning and transmission electron microscopical analyses were discussed. This article describes the importance of the characterisation of *Poiretia* pollen grains, which contributes to the systematics of the Adesmia informal clade. The class of endoaperture, ultrasculpture of mesocolpium and apocolpium, and ultrastructure of tectum, foot layer, and endexine support the eurypalynous status of *Poiretia* and are useful to recognise some species that have problematic delimitation (*P. latifolia*, *P. coriifolia*, *P. unifoliolata*, *P. mattogrossensis*, *P. elegans*, *P. marginata*) as well as groups of species.

Keywords: Dalbergioid, Fabaceae, palynology, palynotaxonomy, plant systematics

Poiretia Vent. is included in the Adesmia informal clade of the Dalbergieae tribe (Klitgaard & Lavin 2005) together with four other genera, *Adesmia* DC., *Amicia* Kunth, *Nissolia* Jacq., *Zornia* J.F. Gmel. (Fortuna-Perez et al. 2013, 2021). The genus is exclusively American and has 12 species, nine endemic to Brazil. Only *Poiretia punctata* (Willd.) Desv. is found in a wide geographic range, distributed from northern Mexico to Bolivia (Müller 1984). Most species of *Poiretia* that occur in Brazil

are found in the *Cerrado* region, including grassland and forest physiognomies (grasslands, highland rocky field, *cerrado lato sensu*) (Müller 1984; Perez 2020).

Poiretia is characterised by a frequent subshrub habit, leaves with one to four leaflets with translucent glands, and flowers in racemes and panicles, not bearing a large paired bract (Rudd 1981; Müller 1984). Recent molecular phylogenetic studies (Fortuna-Perez et al. 2021) that sampled 50% of the

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species, have demonstrated that the genus is monophyletic and most closely related to *Zornia* and *Amicia* forming the *Poiretia-Amicia-Zornia* clade characterised by secretory cavities in leaflets (Fortuna-Perez et al. 2021).

Distinguishing taxa of *Poiretia* based on external morphology is not straightforward due to overlap of characters among species (De Candolle 1825; Vogel 1838; Bentham 1859; Bentham & Hooker 1862–1863; Rudd 1972; Müller 1986; Mendes et al. 2019). As a result, specimens may be misidentified in herbaria (K. R. Mendes, pers. comm., 25 March 2020) and many anatomical, morphological and palynological studies have been carried out in search for potential diagnostic characters of taxonomic importance for dalbergioid legumes (Melhem 1966; Ohashi 1971; Pire 1974; Ferguson & Skvarla 1981; Fortuna Perez et al. 2012; Fortuna-Perez et al. 2013, 2021; Antonio-Domingues 2017; Antonio-Domingues et al. 2018a, 2018b, 2019; Mendes et al. 2019; Jiang et al. 2019; Lattar et al. 2020).

Leguminosae display the greatest palynological variation among angiosperms and pollen features are significant in the investigation of the relationships within papilionoid legumes and the Dalbergia tribe (Ferguson & Skvarla 1981; Antonio-Domingues 2017; Antonio-Domingues et al. 2019; Jiang et al. 2019; Lattar et al. 2020). Currently, however, only three of the 12 species of *Poiretia* have been palynologically documented, *P. angustifolia*, *P. latifolia* and *P. tetraphylla* (Melhem 1966; Ohashi 1971; Pire 1974; Lattar et al. 2020). The objective of this article is, therefore, to characterise pollen grains of all species of *Poiretia* to investigate whether palynology can contribute to the delimitation of species and/or species groups in this genus.

Material and methods

Flower buds were collected from 33 specimens belonging to the 12 species of *Poiretia* (*P. angustifolia*, *P. bahiana*, *P. coriifolia*, *P. crenata*, *P. elegans*, *P. latifolia*, *P. longipes*, *P. marginata*, *P. matto grossensis*, *P. punctata*, *P. tetraphylla*, and *P. unifoliolata*) from CGMS, K, NY and SP herbaria (acronyms following Thiers, continuously updated). All specimens examined are listed under Specimens Investigated. Samples were labelled with the abbreviation of the species epithet plus the last two numbers of the herbarium voucher. Two specimens without herbarium voucher were labelled with the first two collect number (see Table I, Specimen column).

Pollen grains were processed for acetolysis according to Erdtman (1960) with modifications by Mel-

hem et al. (2003). Measurements were made using an Olympus OSM-4 (10 ×) micrometre drum coupled to the eyepiece of an Olympus BX50 light binocular microscope. Polar axis and equatorial axis in equatorial view were measured for 25 specimens of each taxon and the results statistically analysed for arithmetic mean ($\bar{x}$), average standard deviation (Sx), sample standard deviation (s), coefficient of variability ($V\%$), and 95% confidence interval (CI). Ten measurements were also made for each taxon for length and width of colpus, length and width of endoaperture, thickness of nexine and sexine, and size of lumen and the arithmetic mean and the range for pollen grain size class and exine (sexine + nexine) was calculated. Photomicrographs were obtained using the Olympus BX50 with video camera (Olympus U-CMAD-2) and the Olympus Cell-Sens 1.5 software.

For scanning electron microscopy (SEM), non-acetolysed pollen grains were placed on a metal stub and sputter coated (Leica EM ACE 600 Vienna, Austria) with gold (80 nm). Samples were observed under a JEOL JSMIT300LV microscope (Tokyo, Japan) at 20 kV electron beam and images were digitised.

For transmission electron microscopy (TEM) mature anthers were removed from flower buds and fixed in a modified Karnovsky solution (Karnovsky 1965) for at least 24 h. They were subsequently rinsed with sodium cacodylate buffer (0.1 M) and immediately post-fixed in osmium tetroxide (1%) for 1 h. Samples were then rinsed in a saline solution (0.9% sodium chloride) and pre-stained with uranyl acetate (2.5%) followed by dehydration in an acetone series (35–100%), 20 min each. Samples were then gradually infiltrated in Spurr resin (Spurr 1969) and embedded in pure resin for 48 h, at 70 °C. Ultrathin section (60 nm) were obtained with an ultramicrotome (Sorvall Porter Blum MT2, Norwalk, CT, USA) deposited on copper grids and stained using uranyl acetate (2.5%) and lead citrate (Reynold 1963). Images were obtained under a transmission electron microscope (JEOL JEM 1400 Tokyo, Japan) at 80 kV.

Pollen terminology follows Punt et al. (2007) and Halbritter et al. (2018). TEM descriptions of exine infratectum follow Halbritter et al. (2018) and Ferguson and Skvarla (1983). To standardise descriptions of endoaperture types for *Poiretia* (Table II) a classification was established with CEn consisting of the width of the endoaperture (WE) divided by the length of endoaperture (LE).

$$CEn \frac{1}{4} \frac{WE}{LE}$$

Table I. *Poiretia* pollen grains dimentions (μm) in equatorial view observed light microscopy.

Specimen	Polar axis				Equatorial axis				<i>P/E</i>	Size	Colporus	Endoaperture		Exine	
	CI– ($x \pm Sx$)	CI+	<i>s</i>	<i>V%</i>	CI– ($x \pm Sx$)	CI+	<i>s</i>	<i>V%</i>	<i>x</i>	Range	Length–Width	Width (CEn)	Length	<i>N</i>	<i>S</i>
ang12	30.3 (30.9 $\pm$ 0.3)	31.5	1.4	4.6	23.4 (24.5 $\pm$ 0.5)	25.5	2.6	10.5	1.2	S–M	26.0–3.0	8.2 (1.1)	7.8	0.5	0.7
ang304	31.5 (32.0 $\pm$ 0.2)	32.4	1.0	3.2	24.0 (24.7 $\pm$ 0.3)	25.4	1.7	6.8	1.3	S–M	26.0–3.1	12.0 (1.3)	9.2	0.5	0.8
ang24	30,6 (31.4 $\pm$ 0.4)	32.9	1.9	6.0	24.8 (25.3 $\pm$ 0.2)	25.8	1.2	4.9	1.2	S–M	24.5–2.9	10.5 (1.4)	7.9	0.5	0.7
ang004	30.0 (30.4 $\pm$ 0.2)	30.9	1.2	3.9	23.5 (23.9 $\pm$ 0.2)	24.3	0.9	4.0	1.3	S–M	24.9–2.7	8.8 (1.2)	7.3	0.5	0.7
ang08	30.8 (31.4 $\pm$ 0.3)	31.9	1.4	4.4	25.5 (26.0 $\pm$ 0.2)	26.5	1.2	4.7	1.2	S–M	25.3–3.1	9.5 (1.3)	7.4	0.5	0.7
bah93	28.0 (28.4 $\pm$ 0.2)	28.8	1.0	3.4	24.7 (25.5 $\pm$ 0.4)	26.3	2.0	7.9	1.1	S–M	23.3–3.8	9.9 (1.3)	7.4	0.6	0.8
bah75	30.3 (30.6 $\pm$ 0.1)	30.9	0.7	2.3	23.6 (24.0 $\pm$ 0.2)	24.3	0.8	3.4	1.3	S–M	23.6–3.4	9.4 (1.2)	7.6	0.5	0.8
bah98	31.6 (32.0 $\pm$ 0.2)	32.3	0.9	2.7	25.0 (25.4 $\pm$ 0.2)	25.7	0.9	3.6	1.3	S–M	24.5–3.3	9.2 (1.2)	7.5	0.5	0.8
cor83	31.0 (32.3 $\pm$ 0.6)	33.5	3.1	9.5	23.2 (23.6 $\pm$ 0.2)	24.0	1.0	4.2	1.4	S–M	26.0–2.9	9.4 (1.3)	7.2	0.6	0.9
cor64	34.5 (35.2 $\pm$ 0.3)	35.8	1.6	4.4	27.4 (27.6 $\pm$ 0.1)	27.9	0.7	2.4	1.2	M	27.3–3.9	9.6 (1.1)	9.0	0.5	0.8
cor49	31.8 (32.4 $\pm$ 0.3)	32.9	1.4	4.3	25.0 (25.5 $\pm$ 0.2)	25.9	1.1	4.3	1.3	S–M	26.4–3.7	9.9 (1.3)	7.7	0.6	1.0
cre53	35.7 (36.4 $\pm$ 0.4)	37.1	1.8	4.8	27.8 (28.3 $\pm$ 0.2)	28.7	1.0	3.6	1.3	M	29.9–3.8	10.3 (1.3)	8.2	0.6	1.0
ele18	32.6 (33.1 $\pm$ 0.2)	33.5	1.1	3.4	23.9 (24.3 $\pm$ 0.2)	24.6	0.9	3.5	1.4	S–M	22.5–3.9	8.6 (1.2)	7.4	0.5	0.8
ele58	29.7 (30.4 $\pm$ 0.3)	31.1	1.7	5.6	24.0 (24.5 $\pm$ 0.3)	25.1	1.4	5.6	1.2	S–M	23.1–3.3	9.2 (1.4)	6.5	0.6	0.9
ele20	31.5 (31.9 $\pm$ 0.2)	32.4	1.1	3.5	23.6 (23.9 $\pm$ 0.2)	24.3	0.8	3.3	1.3	S–M	21.6–3.3	9.7 (1.3)	7.7	0.6	0.8
lat51	26.4 (26.7 $\pm$ 0.2)	27.1	0.8	3.1	22.1 (22.4 $\pm$ 0.2)	22.8	0.9	3.8	1.2	S–M	21.3–3.3	8.2 (1.2)	6.9	0.4	0.8
lat26	25.8 (26.6 $\pm$ 0.4)	27.4	2.0	7.4	22.1 (22.5 $\pm$ 0.2)	22.9	1.0	4.4	1.2	S–M	22.3–3.3	9.6 (1.2)	7.7	0.5	0.8
lon49	28.3 (28.7 $\pm$ 0.2)	29.0	0.9	3.3	23.7 (24.1 $\pm$ 0.2)	24.4	0.8	3.4	1.2	S–M	23.8–3.1	10.2 (1.4)	7.2	0.5	0.8
mar60	38.4 (39.3 $\pm$ 0.4)	40.2	2.2	5.6	26.5 (27.0 $\pm$ 0.2)	27.5	1.1	4.3	1.1	M	28.4–3.4	11.2 (1.4)	8.1	0.6	0.9
mar59	31.4 (32.1 $\pm$ 0.3)	32.7	1.6	4.9	25.5 (26.0 $\pm$ 0.2)	26.5	1.2	4.7	1.2	S–M	25.6–3.1	9.2 (1.3)	7.3	0.6	1.0
mat30	34.3 (35.0 $\pm$ 0.3)	35.7	1.7	4.9	25.0 (25.3 $\pm$ 0.1)	25.6	0.7	2.9	1.4	S–M	28.0–3.1	10.3 (1.2)	8.5	0.5	0.8
pun10	30.2 (30.7 $\pm$ 0.3)	31.3	1.4	4.5	24.6 (24.9 $\pm$ 0.2)	25.3	0.8	3.3	1.2	S–M	24.5–3.1	9.7 (1.1)	8.6	0.5	0.6
pun60	28.2 (28.7 $\pm$ 0.3)	29.3	1.3	4.6	23.5 (24.0 $\pm$ 0.2)	24.4	1.1	4.5	1.2	S–M	21.7–3.6	10.5 (1.6)	6.6	0.5	0.6
pun20	31.1 (31.7 $\pm$ 0.2)	32.2	1.2	3.9	24.9 (25.4 $\pm$ 0.3)	25.9	1.3	5.0	1.2	S–M	24.8–3.4	11.0 (1.3)	8.4	0.6	0.8
pun50	30.0 (30.6 $\pm$ 0.3)	31.1	1.3	4.3	23.4 (23.9 $\pm$ 0.2)	24.4	1.2	5.2	1.3	S–M	25.2–3.3	9.9 (1.5)	6.8	0.5	0.7
pun33	32.1 (33.1 $\pm$ 0.5)	34.2	2.6	7.8	26.6 (27.1 $\pm$ 0.3)	27.7	1.3	4.8	1.2	M	26.8–3.3	10.9 (1.3)	8.8	0.6	0.8
pun53	29.9 (30.7 $\pm$ 0.4)	31.6	2.0	6.6	23.4 (24.0 $\pm$ 0.3)	24.6	1.4	5.6	1.3	S–M	24.8–2.9	10.0 (1.6)	6.1	0.6	0.8
tet90	32.2 (32.7 $\pm$ 0.2)	33.1	1.0	3.1	24.4 (25.2 $\pm$ 0.4)	26.0	1.9	7.6	1.3	S–M	25.7–3.1	11.3 (1.6)	7.0	0.5	0.7
tet01	29.4 (30.2 $\pm$ 0.4)	30.9	1.9	6.3	21.4 (21.9 $\pm$ 0.2)	22.4	1.2	5.6	1.4	S–M	24.5–2.8	9.6 (1.3)	7.8	0.5	0.8
tet92	28.5 (28.9 $\pm$ 0.2)	29.3	1.0	3.3	22.3 (22.6 $\pm$ 0.2)	22.9	0.8	3.4	1.3	S–M	23.2–2.6	8,3 (1.4)	6.9	0.5	0.8
tet25	28.5 (28.9 $\pm$ 0.2)	29.3	1.0	3.5	25.0 (25.4 $\pm$ 0.2)	25.7	0.9	3.7	1.1	S–M	23.0–3.0	10.6 (1.5)	7.2	0.6	0.8
uni61	25.7 (26.2 $\pm$ 0.2)	26.7	1.2	4.8	21.9 (22.2 $\pm$ 0.1)	22.4	0.6	2.8	1.4	S–M	21.7–2.8	8.8 (1.4)	6.3	0.5	0.9
uni16	25.3 (26.1 $\pm$ 0.4)	26.8	1.9	7.2	21.6 (22.1 $\pm$ 0.2)	22.6	1.2	5.6	1.2	S–M	21.3–3.0	7.9 (1.3)	6.2	0.5	0.8

Specimens were identified by abbreviation of the epithet and the last two numbers of the herbarium voucher (see Appendix). Confidence interval at 95% of probability of the lowest sample values (CI–) and highest sample values (CI+), arithmetic mean (x), average standard deviation (Sx), sample standard deviation (s), coefficient of variability ($V\%$), small size (S), medium size (M).

Table II. Endoaperture classes of *Poiretia* endoaperture.

Class of endoaperture (CEn)	
Very lolongate	< 0.50
Lolongate	0.50–0.91
Circular	0.91–1.09
Lalongate	1.09–1.50
Very lalongate	> 1.50

The CEn consisting of the width of the endoaperture divided by its length (width/length).

To explore whether the characteristics of the pollen grains allowed for the grouping of species, a principal component analysis (PCA) was performed using FITOPAC (Shepherd 1996) and PC-ORD version 7 (McCune & Mefford 2016) software using nine metric variables (polar axis [PA], equatorial axis [EA], colpus length [CL] colpus width [CW], endoaperture length [EL], endoaperture width [EW], nexine thickness [N], sexine thickness [S = infratectum + tectum], exine thickness [Ex = N + S]), and also three classes/index (shape class [$P/E = PA/EA$], class of endoaperture [CEn], index of sexine and nexine thickness [S/N]).

To understand how *Poiretia* specimens relate to each other, based on pollen morphology, a cluster analysis (CA) was performed using the software PC-ORD version 7 (McCune & Mefford 2016). This produced a similarity dendrogram by calculating the Euclidean distance, based on the same seven PCA nine metric variables and the three classes/index.

Results

General description

Poiretia Vent. — Pollen grains are small to medium size, isopolar, prolate spheroidal to prolate, with amb circular to subtriangular, ellipsoidal in equatorial view (Figures 1, 3, 5, Table I), 3-zonocolporate, colporus with pointed apices. Colporus with fossulate, granulate, granulate-rugulate or granulate-fossulate operculum; granulate membrane and perforate margo (Figures 2A–I, 4A–I, 6A–F, 7A–F). Endoaperture is circular, lalongate to very lalongate, rarely lolongate (Table III), and protruding apertures and costae are present. Sexine from 1.1 to 2.5 thicker than nexine; exine 1.1–2.1 μm thickness (Table I). Aberrant pollen grains are present in a negligible presence in some specimens, presenting abnormal deviant size, in pollen shape and differences in the aperture number and arrangement.

Exine ultrastructure

Exine presents an electron-translucent (e-translucent) or electron-dense (e-dense) compact tectum that varies from eutectate (Figures 2M, 4L), tectate perforate (Figures 2N, 4M), to semitectate (Figures 2L, 6G). The tectum can be smooth or presents undulations, with an e-translucent or e-dense *pollenkitt* (Figures 2J–O, 4J–O, 6G, H, 7G–I, Table IV).

We observed a combination of two types of infratectal structures in all species: (1) granules organised in an irregular narrow row and (2) simple columellae, with some incomplete columellae (Figures 2J–O, 4J–O, 6G, H, 7G–I, Table IV). We also observed an e-translucent or e-dense infratectal *pollenkitt*.

The thick foot layer is compact, continuous with channels (Figures 2N, 7I) to discontinuous, thin and absent in the aperture region (Figures 4J–M, 6H). The thin endexine is compact-continuous, smooth. (Figures 2N, 7I). Closer to the aperture, the endexine is thick (costate), spongy with endocracks (endosculptured) (Figures 4O, 6H, 7G, H) and, in some cases, two-layered. The intine can be bilayered and presents an e-dense ektintine and an e-translucent endointine (Figures 2L, N, 4L, M, O, Table IV).

Exine ultrasculpture and pollen types

Sexine presents five different ornamentation patterns in the mesocolpium and apocolpium (Table III): reticulate (nanoreticulate, microreticulate and/or reticulate) (Figures 4A–C, F, G, 6C, 7A–C), nanoreticulate-perforate (Figure 4D, H, I), fossulate-perforate (Figure 6B, E–F), rugulate-perforate (Figure 2A–C), and perforate (Figure 5D–F). Five pollen types have been recognised based on mesocolpium exine ultrasculpture.

Type 1: rugulate-perforate mesocolpium. — Species: *Poiretia angustifolia* Vogel (Figures 1A, B, 2A–C, J, K). Pollen small to medium size (Table III), colporus with granulate-rugulate operculum; endoaperture lalongate to circular, rarely lolongate endoaperture; apocolpium rugulate-perforate.

Sexine from 1.2 to 2.1 times thicker than nexine; exine 1.2–2.1 μm thick. Tectum eutectate to tectate perforate with undulations. The infratectal structure presents granules organised in an irregular narrow row and simple columellae.

The thick foot layer is compact, with channels continuous to discontinuous and thin in the aperture areas. The endexine is compact-continuous, thin, smooth and costate two-layered in some areas and near to the aperture spongy and endosculptured.

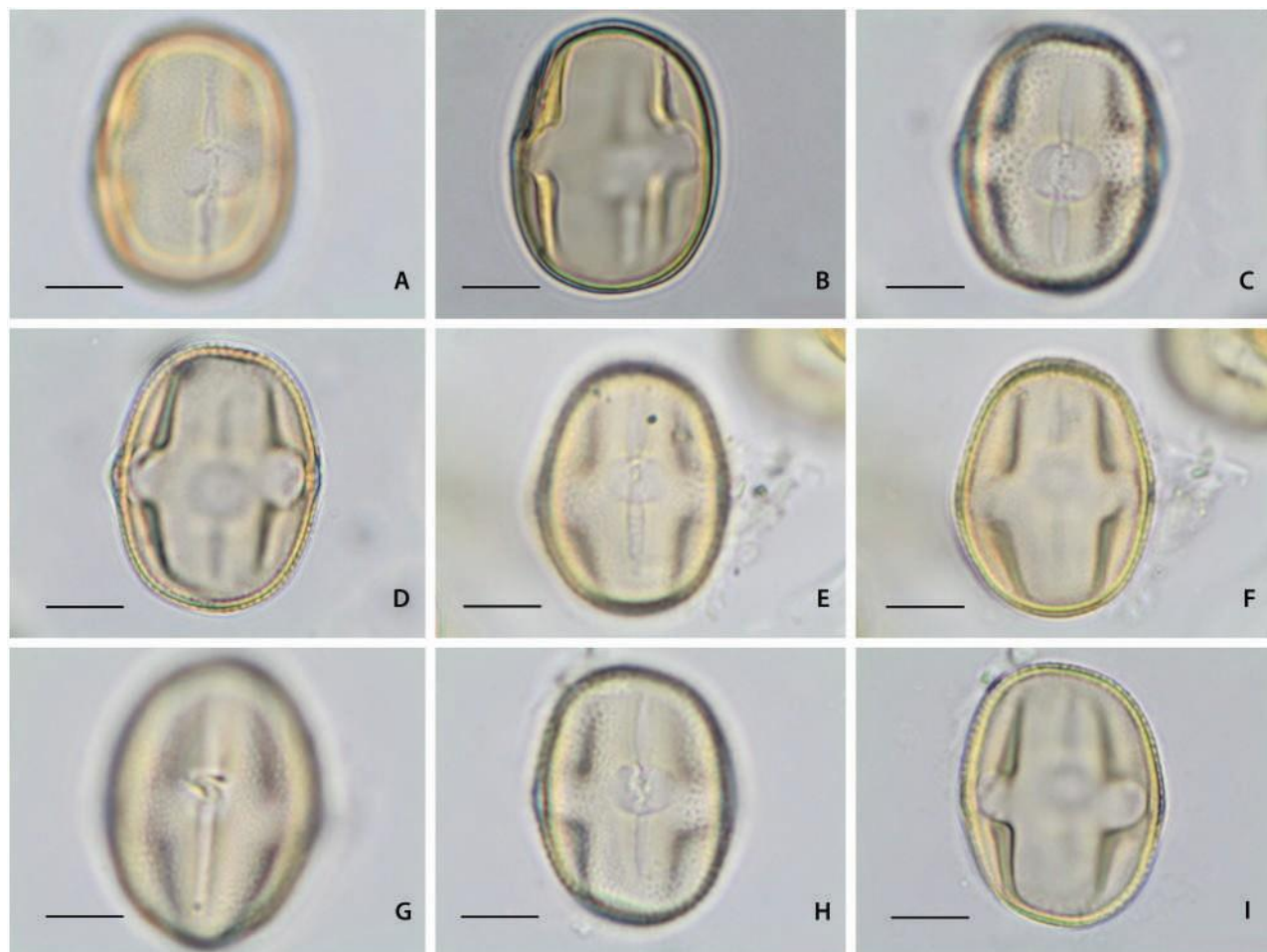


Figure 1. Light microscopy of *Poiretia* species in equatorial view: pollen types I and II. **A, B.** *Poiretia angustifolia*. **A.** Detail of the colporus and surface. **B.** Optical section. **C, D.** *Poiretia bahiana*. **C.** Detail of the colporus, surface and operculum. **D.** Optical section. **E, F.** *Poiretia coriifolia*. **E.** Detail of the colporus, surface and operculum. **F.** Optical section. **G.** *Poiretia crenata*. Detail of the colpus and endoaperture. **H, I.** *Poiretia elegans*. **H.** Detail of the colporus, surface and operculum. **I.** Optical section. Scale bars – 10 μm .

Type II: nanoreticulate-microreticulate mesocolpium. — Species: *Poiretia bahiana* Müll. Hal., *Poiretia coriifolia* Vogel., *Poiretia crenata* Cl. Müll., *Poiretia elegans* Müll. Hal., *Poiretia longipes* Harms., *Poiretia mattogrossensis* Müll. Hal., *Poiretia unifoliolata* Barreto ex Martins et Pedersoli (Figures 1C–H, 2D–I, L–O, 3A–F, 4A–O).

Pollen small to medium size (Table III), colporus with areolate-granulate or granulate (*Poiretia crenata*) operculum, endoaperture lalongate (*P. bahiana*, *P. mattogrossensis*) or lalongate to very lalongate (*P. longipes*, *P. unifoliolata*) or circular to lalongate (*P. coriifolia*, *P. crenata*, *P. elegans*). Apocolpium nanoreticulate (*P. bahiana*, *P. coriifolia*, *P. crenata*, *P. elegans*) or nanoreticulate-perforate (*P. longipes*, *P. mattogrossensis*, *P. unifoliolata*).

Sexine from 1.2 to 2.4 times thicker than nexine; exine 1.1–2.1 μm thick. Tectum semitectate to tec-

tate perforate with undulations. The infratectal structures presents simple collumelae and sparse granules somewhat organised in an irregular narrow row. In *Poretia bahiana*, *P. elegans*, *P. mattogrossensis* and *P. unifoliolata* these granules are present just in the structure of aperture area.

The thick foot layer is compact, with channels, from continuous to discontinuous, thin and absent near to the apertures (*Poiretia angustifolia*, *P. coriifolia*, *P. elegans*). The endexine is compact-continuous or discontinuous (*P. mattogrossensis*), thin smooth and costate, spongy, endosculptured near the apertures and bilayered in some areas. In *P. mattogrossensis* only, endexine is absent in polar areas and in *P. bahiana* compact near to the aperture and monolayered.

Type III: fossulate-perforate mesocolpium. — Species: *Poiretia latifolia* Vogel., *Poiretia tetraphylla* (Poir.) Burkart (Figures 5A–E, 6A–H).

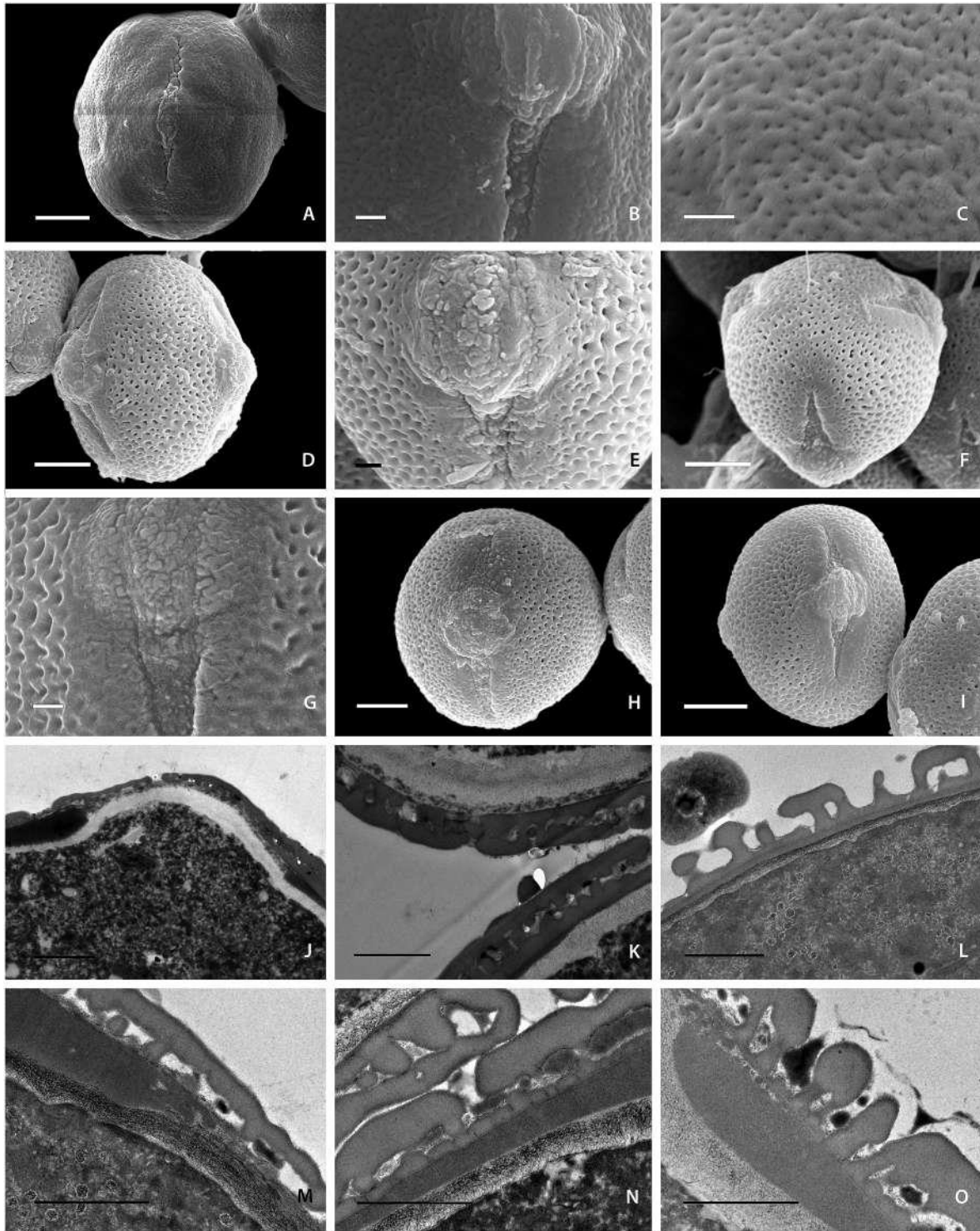


Figure 2. Scanning electron microscopy (A–I) and transmission electron microscopy (J–O) of *Poretia* species: pollen types I and II. A–C. *Poretia angustifolia*. A. Apertural area, equatorial view. B. Detail of apertural membrane of the colporus, margo and operculum, equatorial view. C. Detail of apocolpium regulate-perforate. D–F. *Poretia bahiana*. D. Mesocolpium and apertural area, equatorial view. E. Detail of apertural membrane of the colporus, margo and operculum, equatorial view. F. Nanoreticulate apocolpium. G. *Poretia coriifolia*. Detail of apertural membrane of the colporus, margo and operculum, equatorial view. H. *Poretia crenata*. Apertural area and mesocolpium, equatorial view. I. *Poretia elegans*. Apertural area and mesocolpium, equatorial view. J, K. *Poretia angustifolia*. Tectum eutectate to tectate perforate, infratectum types 1 and 2, foot layer continuous or discontinuous to absent in aperture area, endexine with endocrack, thicker and spongy near to the apertures. L, M. *Poretia bahiana*. Tectum semitectate to tectate perforate, infratectum types 1 and 2, foot layer continuous to discontinuous, compact endexine becomes spongy, thicker with endocrack in aperture area, intine bilayered (ektintine e-dense, endointine e-translucent). N. *Poretia coriifolia*. Tectum semitectate to tectate perforate, infratectum types 1 and 2, foot layer with channel, continuous to discontinuous, endexine compact, thinner to thicker, intine bilayered (ektintine e-dense, endointine e-translucent). O. *Poretia elegans*. Tectum tectate perforate, infratectum type 1 (but also presentes type 2), foot layer discontinuos, compact endexine becomes spongy, thicker, spongy and bilayered in aperture area. Scale bars – 5 μm (A, D, F, H, I), 2 μm (J), 1 μm (B, C, E, G, K–O).

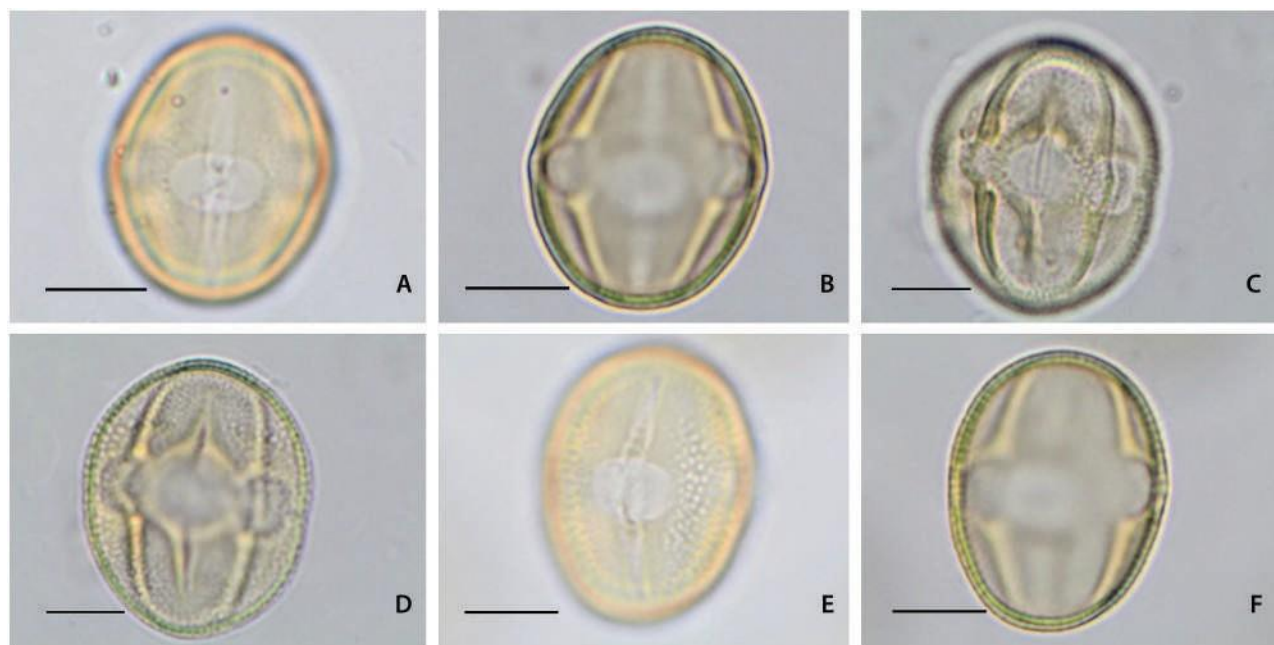


Figure 3. Light microscopy of *Poiretia* species in equatorial view: pollen type II. **A, B.** *Poiretia longipes*. **A.** Detail of the colporus, surface and operculum. **B.** Optical section. **C, D.** *Poiretia mattogrossensis*. **C.** Detail of the colporus and surface. **D.** Optical section. **E, F.** *Poiretia unifoliolata*. **E.** Detail of the colporus, surface and operculum. **F.** Optical section. Scale bars – 10 μm .

Pollen small to medium size (Table III), colporus with areolate-granulate (*Poiretia latifolia*) or rugulate-granulate (*P. tetraphylla*) operculum, endoaperture lalongate (*P. latifolia*) or circular to lalongate (*P. tetraphylla*); apocolpium nanoreticulate (*P. latifolia*) or fossulate-perforate (*P. tetraphylla*).

Sexine from 1.2 to 2.5 times thicker than nexine; exine 1.1–1.5 μm thick. Tectum semitectate to tectate perforate with undulations. The infratectal structures present simple collumelae and sparse granules somewhat organised in an irregular narrow row. In *Poiretia tetraphylla* the granules are only present in the structure of aperture area.

The thick foot layer goes from continuous, compact and with channels, to thin and discontinuous in the aperture area. The endexine is compact-continuous, costate and near to the aperture is spongy, endosculptured and bilayered.

Type IV: microreticulate-reticulate mesocolpium. — Species: *Poiretia marginata* Cl. Mull (Figures 5F, G, 7A–C, G).

Pollen medium size (Table III), colporus with areolate-granulate operculum, lalongate endoaperture; apocolpium with nanoreticulate-microrreticulate ornamentation.

Sexine from 1.3 to 2.4 times thicker than nexine; exine 1.2–1.8 μm thick. Tectum semi tectate

to tectate perforate, compact with undulations. The infratectal structures presents simple collumelae and some sparse granules somewhat organised in an irregular narrow row in the aperture area.

The thick foot layer is continuous compact with channel and thin and discontinuous in aperture area. The endexine is thin compact-continuous, costate and near to the aperture is spongy, endosculptured and bilayered.

Type V: perforate mesocolpium. — Species: *Poiretia punctata* (Willd.) Desv. (Figures 5H, I, 7D–F, H, I).

Pollen small to medium size (Table III), colporus with areolate-granulate operculum, lalongate endoaperture; perforate apocolpium and mesocolpium.

Sexine from 1.1 to 1.8 times thicker than nexine; exine 1.1–1.5 μm thick. Tectum tectate perforate compact tectum with undulations. The infratectal structures present simple collumelae and some sparse granules somewhat organised in an irregular narrow row in the aperture area.

The thick foot layer is continuous compact, with channel and thin, and discontinuous in apertures area. The endexine is thin compact-discontinuous, endosculptured, costate and near to the aperture is spongy and bilayered.

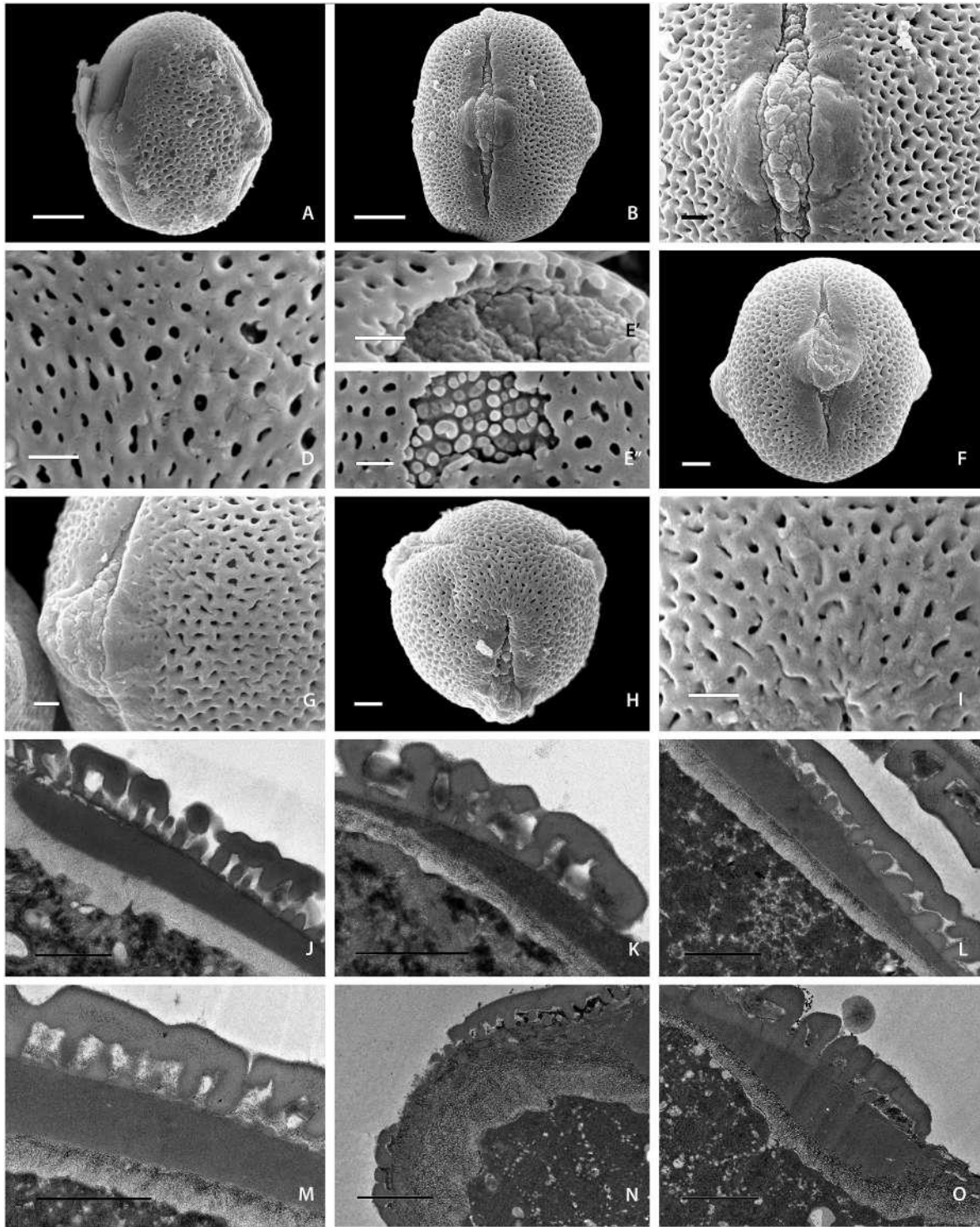


Figure 4. Scanning electron microscopy (A–I) and transmission electron microscopy (J–O) of *Poretia* species: pollen type II. **A.** *Poretia longipes*. Mesocolpium and apertural area, equatorial view. **B–E.** *Poretia mattogrossensis*. **B.** Apertural area, equatorial view. **C.** Detail of apertural membrane of the colporus, margo and operculum, equatorial view. **D.** Detail of apocolpium nanoreticulate-perforate. **E'**. section of the exine. **E''.** Detail of fractured exine showing the infratectal structures. **F–I.** *Poretia unifoliolata*. **F.** Apertural area, equatorial view. **G.** Detail of apertural membrane of the colporus, margo, operculum and mesocolpium area, equatorial view. **H.** General view of polar view. **I.** Detail of polar area. **J, K.** *Poretia longipes*. Tectum semitectate to tectate perforate, infratectum types 1 and 2, foot layer discontinuous, endexine compact (**J**) to spongy (**K**) with endocracks. **L, M.** *Poretia mattogrossensis*. Tectum tectate perforate (also presents eutectate and semitectate), infratectum types I and II, foot layer discontinuous, endexine thinner to thicker, intine bilayered (ektintine e-dense, endointine e-translucent). **N, O.** *Poretia unifoliolata*. Tectum tectate perforate, infratectum types I and II, foot layer discontinuous with channels, compact endexine becomes spongy, thicker with endocrack in aperture area, intine bilayered (ektintine e-dense, endointine e-translucent). Scale bars – 5 μ m (A, B, C–E), 1 μ m (G, I–O), 2 μ m (F, H).



Figure 5. Light microscopy of *Poiretia* species: pollen types III, IV and V. **A–C.** *Poiretia latifolia*. **A.** Detail of the colporus, surface and operculum, equatorial view. **B.** Optical section, equatorial view. **C', C''.** L.O. (high and low focus, respectively), polar view. **D, E.** *Poiretia tetraphylla*. Detail of the colporus, surface and operculum, equatorial view. **E.** Optical section, equatorial view. **F, G.** *Poiretia marginata*. **F.** Detail of the colporus, surface and operculum, equatorial view. **G.** Optical section, equatorial view. **H, I.** *Poiretia punctata*. **H.** Detail of the colporus, surface and operculum, equatorial view. **I.** Optical section, equatorial view. Scale bars – 10 µm (A, B, D–I), 2 µm (C).

Principal component analysis (PCA)

The two first axes (Figure 8, Table V) represent 59.55% of the total variability of the quantitative data analysed, showing six correlations of variables in each axis. The first axis represented 44.61% (> 0.0167): PA, EA, CL, CW, EL and N. The second component represented a smaller variance (14.94%), (> 0.0124): P/E, EW, S, E, S/N and CEn (Table V).

The largest pollen grains, represented by *Poiretia coriifolia* (cor64, cor49), *P. crenata* (cre53), *P. marginata* (mar56, mar60) and *P. matogrossensis* (mat30), associated with high values for the variables related to the first axis, were grouped on the top left side of the graph. *Poiretia angustifolia* (ang004, ang12), *P. bahiana* (bah75, bah98),

P. coriifolia (cor83), *P. elegans* (ele18, ele58), *P. latifolia* (lat26, lat51), *P. tetraphylla* (tet01, tet92), and *P. unifoliolata* (uni16, uni61) were grouped on the top right side of the graph and were associated with lower values for these variables, presenting smaller dimensions.

On the bottom left side of the graph, *Poiretia angustifolia* (ang304, ang08, ang24), *P. punctata* (pun10, pun20, pun33) and *P. tetraphylla* (tet90) showed the highest values regarding endoaperture width. On the bottom right side, *P. bahiana* (bah93), *P. elegans* (ele20), *P. longipes* (lon49), *P. punctata* (pun50, pun53) and *P. tetraphylla* (tet25) showed the highest values regarding to the class of endoapertures.

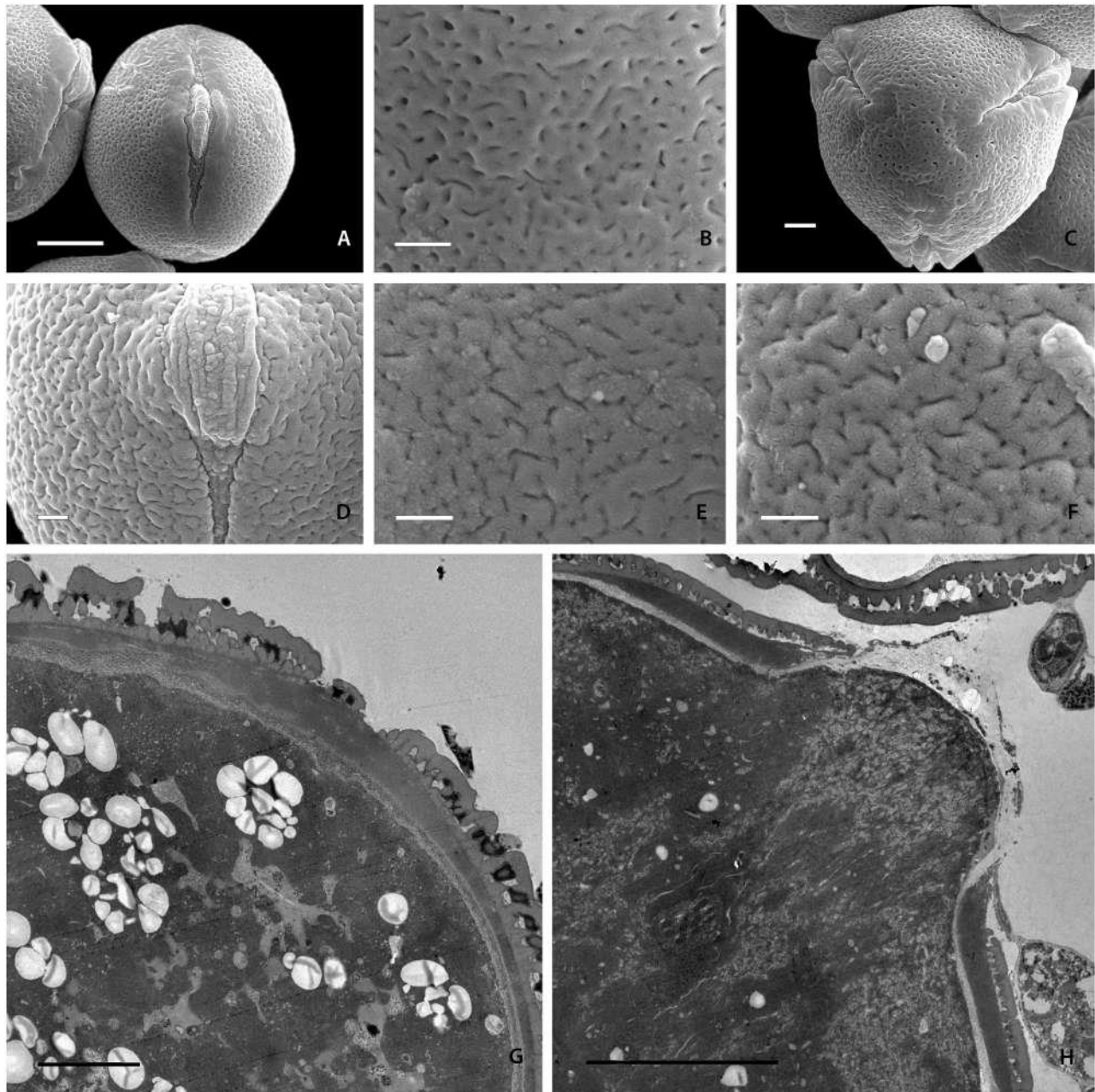


Figure 6. Scanning electron microscopy (A–F) and transmission electron microscopy (G, H) of *Poiretia* species: pollen type III. A–C. *Poiretia latifolia*. A. Apertural area, equatorial view. B. Detail of mesocolpium fossulate-perforate. C. Polar area. D–F. *Poiretia tetraphylla*. D. Detail of apertural membrane of the colporus, margo, operculum and mesocolpium region, equatorial view. E. Detail of mesocolpium fossulate-perforate. F. Detail of apocolpium fossulate-perforate. G. *Poiretia latifolia*. Tectum semitectate to tectate perforate, infratectum types I and II, foot layer discontinuous, compact endexine becomes spongy, thicker with endocrack in aperture area, intine bilayered (ektintine e-dense, endointine e-translucent); operculum with tectum, infratectum, foot layer thinner and thicker endexine. H. *Poiretia tetraphylla*. Tectum tectate perforate, infratectum types I and II, foot layer discontinuous to absent in aperture area, compact endexine becomes spongy, bilayered with endocrack in aperture area. Scale bars – 5 μm (A, H), 1 μm (B, D–F), 2 μm (C, G).

Cluster analysis (UPGMA and Euclidean distance)

The CA provided a similarity dendrogram for the studied specimens by calculation of the Euclidean distance (Figure 9). Two groups of specimens with 0% similarity were identified, one comprising the species

with the largest pollen grains (*Poiretia marginata* [mar60], *P. crenata* [cre53], *P. coriifolia* [cor64], *P. mattogrossensis* [mat30], *P. punctata* [pun33, pun20] and *P. angustifolia* [ang304]), and one accommodating the remainder species, all with smaller pollen grains (the smallest grains in *P. latifolia* [lat51, lat26])

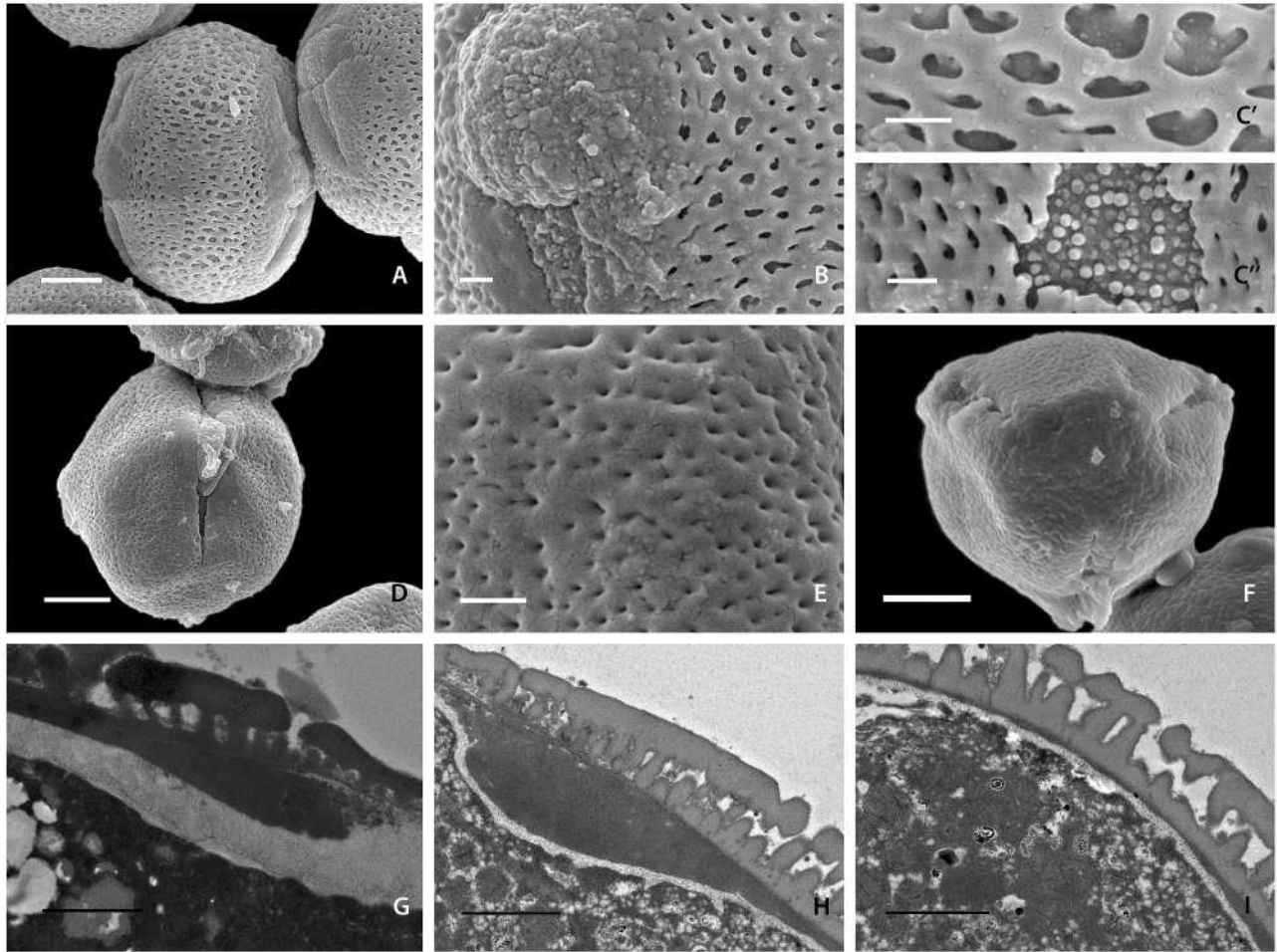


Figure 7. Scanning electron microscopy (A–F) and transmission electron microscopy (G–I) of *Poiretia* species: pollen types IV and V. A–C. *Poiretia marginata*. A. Mesocolpium, equatorial view. B. Detail of aperture membrane of the colporus, margo, operculum and mesocolpium area, equatorial view. C. Detail of mesocolpium microreticulate-reticulate. D–F. *Poiretia punctata*. D. Aperture area and mesocolpium, equatorial view. E. Detail of perforate mesocolpium. F. Polar area. G. *Poiretia marginata*. Tectum semitectate to tectate perforate, infratectum types I and II, foot layer continuous to discontinuous in aperture region, compact endexine become spongy, thicker with endocrack in aperture area. H, I. *Poiretia punctata*. Tectum tectate perforate, infratectum types I and II, foot layer continuous to discontinuous in aperture area, compact endexine becomes spongy, thicker with endocrack in aperture region. Scale bars – 5 µm (A, D, F), 1 µm (B, C, E, G–I).

and *P. unifoliolata* [uni61, uni16]). *P. bahiana* (bah75) and *P. elegans* (ele20) show almost 100% similarity, *P. longipes* (long49) and *P. tetraphylla* (tet25) presented more than 95% similarity, and *P. coriifolia* (cor83) and *P. marginata* (mar56) more than 90%.

In general, the specimens of each species show values of pollen similarity below 75%, indicating that there is significant variation in the pollen characteristics of these species. Exceptions seen in both PCA and CA are the *Poiretia punctata* (pun33, pun20) specimens that show 100% similarity, *P. angustifolia* (ang24, ang08) and (ang12, ang004) specimens with more than 95% similarity, *P. bahiana* (bah75, bah98) specimens more than 80% similarity, *P. unifoliolata* (uni61, uni16) specimens with about 85% similarity, and *P. tetraphylla* (tet01, tet92) specimens totalling almost 80% similarity.

Discussion

Pollen morphology and ultrasculpture

Earlier studies of *Poiretia* pollen were carried out for *P. angustifolia* (Melhem 1966), *P. latifolia* (Melhem 1966; Ohashi 1971; Pire 1974; Lattar et al. 2020) and *P. tetraphylla* (Pire 1974; Lattar et al. 2020). Results of these studies are supported by our analyses, although with some differences in the observation of sexine sculpture, presence or absence of operculum and endoaperture and size classes (Table VI). The operculum and membrane ornamentations are described for *Poiretia* for the first time here, and we also described the remaining nine species of the genus palynologically for the first time (Table VI).

Table III. Class of endoaperture and ultrasculpture of *Poiretia* pollen grains using scanning and transmission electron microscopy.

Species	Endoaperture	Operculum	Mesocolpium	Apocolpium
<i>P. angustifolia</i>	Lalongate, circular to lolongate*	Granulate-rugulate	Rugulate-perforete	Perforate-rugulate
<i>P. bahiana</i>	Lalongate	Areolate-granulate	Nanoreticulate-microreticulate	Nanoreticulate
<i>P. coriifolia</i>	Circular to lalongate	Areolate-granulate	Nanoreticulate-microreticulate	Nanoreticulate
<i>P. crenata</i>	Circular to lalongate	Granulate	Nanoreticulate-microreticulate	Nanoreticulate
<i>P. elegans</i>	Circular to lalongate	Areolate-granulate	Nanoreticulate-microreticulate	Nanoreticulate
<i>P. latifolia</i>	Lalongate	Areolate-granulate	Fossulate-perforate	Nanoreticulate
<i>P. longipes</i>	Lalongate to very lalongate	Areolate-granulate	Nanoreticulate-microreticulate	Nanoreticulate-perforate
<i>P. marginata</i>	Lalongate	Areolate-granulate	Microreticulate-reticulate	Nanoreticulate-microrreticulate
<i>P. matogrossensis</i>	Lalongate	Areolate-granulate	Nanoreticulate-microreticulate	Nanoreticulate-perforate
<i>P. punctata</i>	Lalongate	Areolate-granulate	Perforate	Perforate
<i>P. tetraphylla</i>	Circular to lalongate	Rugulate-granulate	Fossulate-perforate	Fossulate-perforate
<i>P. unifoliolata</i>	Lalongate to very lalongate	Areolate-granulate	Nanoreticulate-microreticulate	Nanoreticulate-perforate

*, Rarely.

Pollen of *Poiretia angustifolia* has a rugulate-perforate mesocolpium and apocolpium, lalongate to circular rare lolongate endoaperture and granulate-rugulate operculum (Table IV). Melhem (1966) was uncertain regarding the nature of the sexine sculpturing stating that ‘in high magnification sometimes it appears that there is some sculpture’ and our studies indicate that a rugulate-perforate ultra-sculpture is present. Additionally, the presence of a circular endoaperture and the granulate-rugulate operculum observed here was not described by Melhem (1966).

Our observations that *Poiretia latifolia* pollen is small to medium size with fossulate-perforate sexine, fossulate-perforate mesocolpium, nanoreticulate apocolpium, apertures with areolate-granulate operculum and perforate margo (Table IV), differ partially from information in the literature (Melhem 1966; Ohashi 1971; Pire 1974; Lattar et al. 2020). Also, the small to medium size pollen with lalongate endoaperture was not observed by Lattar et al. (2020), who described this species as having medium to large pollen grains with lolongate endoaperture. Moreover, large pollen was not observed among the 33 specimens analysed in the present study.

Poiretia tetraphylla pollen is small to medium size, colpore with rugulate-granulate operculum, circular to lalongate, mesocolpium and apocolpium fossulate-perforate (Table IV). Pire (1974) described the pollen of this species as of medium size and with

reticulate sexine, differing from our results, but confirmed the presence of lalongate endoaperture. Lattar et al. (2020) describe *P. tetraphylla* pollen grains as of medium size with lolongate endoaperture, colpore with psilate margo and with reticulate sexine, contrasting with our results.

Exine ultrastructure

The granular structure of the infratectum in the *Adesmia* informal clade is reported for the first time in the present study. Previous works have reported the granular structure of the Desmodieae, Indigofereae, Loteae, Millettieae, Phaseoleae, Psoraleae and Viciae tribes in the Papilionoideae subfamily (Ferguson & Skvarla 1983; Antonio-Domingues et al. 2019). The presence of granules combined with columellae represent an intermediary condition of the infratectum (Doyle 2005) and endexine thickness with a discontinuous or absent foot layer characterise a derived pollen grain morphology in the Papilionoideae (Ferguson & Skvarla 1983; Ridder-Numan & Ham 1997).

Our observations indicate a variable ultrastructure of tectum, foot layer and endexine for the *Poiretia* species. The infratectum structure represents valuable pollen characters (Doyle & Endress 2000; Doyle 2008). Therefore, adding detailed ultrastructural data to palynotaxonomical of the *Adesmia*

Table IV. Ultrastructure of *Poiretia* Vent. pollen grains using scanning and transmission electron microscopy.

Species	Tectum	Foot layer	Endexine	Intine
<i>P. angustifolia</i>	Eutectate-tectate perforate	Discontinuous-absent*, thin, channels	Compact-spongy*, costa*, endocracks, bilayered ⁻	Monolayered
<i>P. bahiana</i>	Semitectate-tectate perforate	Discontinuous-absent*, thin, channels	Compact-spongy*, costa*, endocracks bilayered ⁻	Bilayered
<i>P. coriifolia</i>	Semitectate-tectate perforate	Discontinuous-absent*, thin, channels	Compact-spongy*, costa*, endocracks, monolayered	Bilayered
<i>P. crenata</i>	—	—	—	—
<i>P. elegans</i>	Semitectate-tectate perforate	Discontinuous-absent*, thin, channels	Compact-spongy*, costa*, endocracks, bilayered ⁻	Bilayered
<i>P. latifolia</i>	Semitectate-tectate perforate	Discontinuous, thin, channels	Compact-spongy*, costa*, endocracks, bilayered*	Bilayered
<i>P. longipes</i>	Semitectate-tectate perforate	Discontinuous, thin, channels	Compact-spongy*, costa*, endocracks, bilayered*	Monoleayered
<i>P. marginata</i>	Semitectate-tectate perforate	Discontinuous*, thin, channels	Compact-spongy*, costa*, endocracks, bilayered*	Monoleayered
<i>P. matogrossensis</i>	Semitectate-tectate perforate	Discontinuous-absent*, thin, channels	Compact-spongy*, costa*, endocracks, bilayered ⁻ , absent ⁺	Bilayered
<i>P. punctata</i>	Tectate perforate	Continuous-discontinuous*, channels	Compact-spongy*, costa*, endocracks, bilayered ⁻ , discontinuous*	Monoleayered
<i>P. tetraphylla</i>	Semitectate-tectate perforate	Continuous-discontinuous*, channels	Compact-spongy*, costa*, endocracks, bilayered*	Bilayered
<i>P. unifoliolata</i>	Tectate perforate	Continuous-discontinuous*, channels	Compact-spongy*, costa*, endocracks, bilayered*	Bilayered
<i>P. angustifolia</i>	Eutectate-tectate perforate	Discontinuous-absent*, thin, channels	Compact-spongy*, costa*, endocracks, bilayered ⁻	Monoleayered
<i>P. bahiana</i>	Semitectate-tectate perforate	Discontinuous-absent*, thin, channels	Compact-spongy*, costa*, endocracks bilayered ⁻	Bilayered
<i>P. coriifolia</i>	Semitectate-tectate perforate	Discontinuous-absent*, thin, channels	Compact-spongy*, costa*, endocracks, monolayered	Bilayered
<i>P. crenata</i>	—	—	—	—
<i>P. elegans</i>	Semitectate-tectate perforate	Discontinuous-absent*, thin, channels	Compact-spongy*, costa*, endocracks, bilayered ⁻	Bilayered
<i>P. latifolia</i>	Semitectate-tectate perforate	Discontinuous, thin, channels	Compact-spongy*, costa*, endocracks, bilayered*	Bilayered
<i>P. longipes</i>	Semitectate-tectate perforate	Discontinuous, thin, channels	Compact-spongy*, costa*, endocracks, bilayered*	Monoleayered
<i>P. marginata</i>	Semitectate-tectate perforate	Discontinuous*, thin, channels	Compact-spongy*, costa*, endocracks, bilayered*	Monoleayered
<i>P. matogrossensis</i>	Semitectate-tectate perforate	Discontinuous-absent*, thin, channels	Compact-spongy*, costa*, endocracks, bilayered ⁻ , absent ⁺	Bilayered
<i>P. punctata</i>	Tectate perforate	Continuous-discontinuous*, channels	Compact-spongy*, costa*, endocracks, bilayered ⁻ , discontinuous*	Monoleayered
<i>P. tetraphylla</i>	Semitectate-tectate perforate	Continuous-discontinuous*, channels	Compact-spongy*, costa*, endocracks, bilayered*	Bilayered
<i>P. unifoliolata</i>	Tectate perforate	Continuous-discontinuous*, channels	Compact-spongy*, costa*, endocracks, bilayered*	Bilayered

—, Not conclusive; *, in apertural area, +, in polar area, -, in some regions.

informal clade is important and should be considered in future cladistic studies.

Implications of palynotaxonomy for the systematic of *Poiretia*

Poiretia species that have taxonomic conflicts in their delimitation (*P. latifolia*, *P. coriifolia*, *P. unifoliolata*, *P. matogrossensis*, *P. elegans*, *P. marginata*) show differences in pollen morphology, ultrasculpture and ultra-

structure that can contribute to a more precise diagnosis (Table VII). Showing the importance of the pollen grains in systematics of Leguminosae, mainly in the *Adesmia* informal clade, these species could be distinguished here by the class of endoaperture, ultrasculpture of mesocolpium and apocolpium and ultrastructure of tectum, foot layer and endexine.

Poiretia coriifolia and *P. unifoliolata* were previously classified as varieties of *P. latifolia* (Vogel 1838; Bentham 1859; Rudd 1972). In addition to differences in features such as class of endoaperture and pollen type

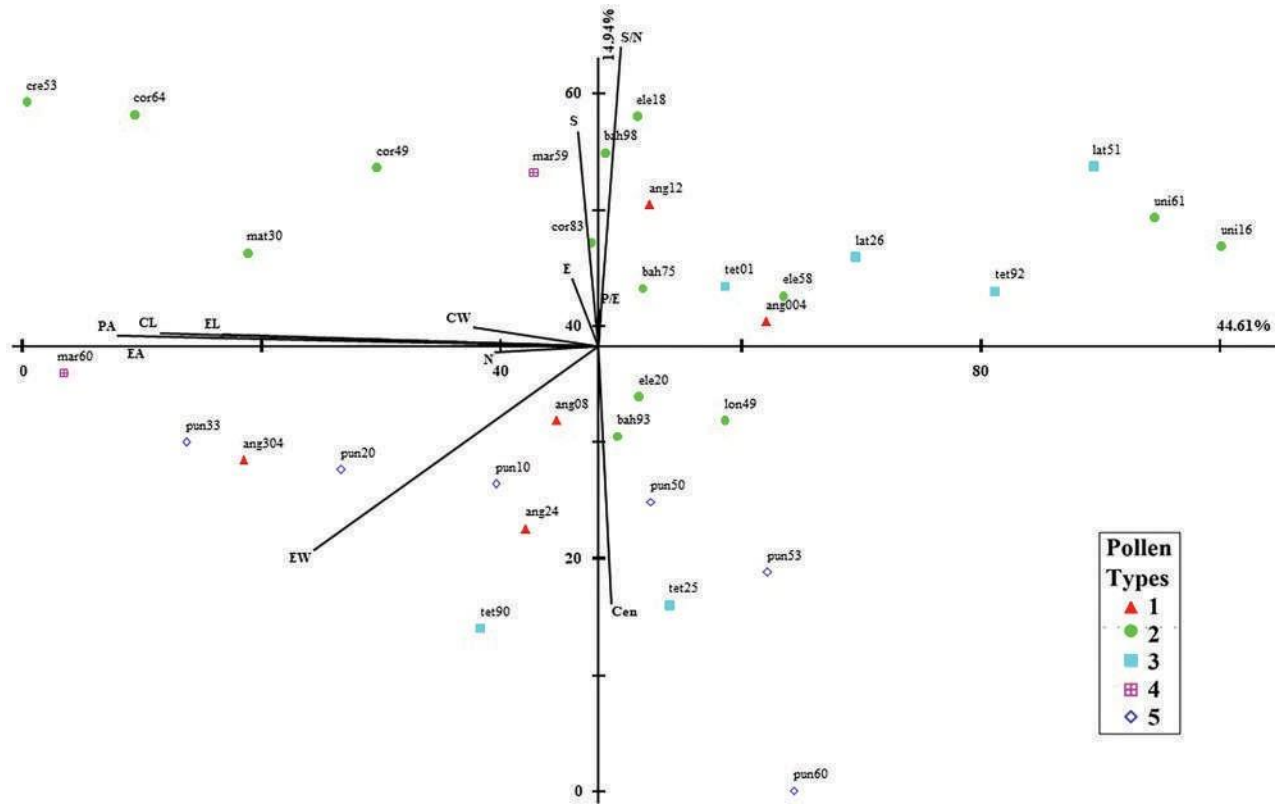


Figure 8. Biplot of principal component analysis for *Poiretia* specimens, using pollen grain metric variables and classes/index.

(Table VII) these three species also show differences according to the confidence interval of the equatorial polar axes (Table I) and all specimens of *P. coriifolia* (cor64, cor49, cor83) were grouped on the top left

Table V. Pearson and Kendall coefficients for pollen grain metric variables and classes/index of the first two axis of principal component analysis ordination in *Poiretia*.

Principal component		
Variables	Axis 1	Axis 2
PA	-0.4991	0.1256
EA	-0.3367	-0.0124
P/E	0.0167	0.1078
CL	-0.4437	0.1365
CW	-0.2270	0.1492
EL	-0.4591	0.1443
EW	-0.3906	-0.5745
CEn	0.0534	-0.4023
N	-0.0917	-0.0415
S	-0.0624	0.3495
Ex	-0.0602	-0.1689
S/N	0.0826	0.5161

PA, polar axis; EA, equatorial axis; CL, colpus length; CW, colpus width; EL, endoaperture length; EW, endoaperture width; N, nexine thickness; S, sexine thickness ($S = \text{infratectum} + \text{tectum}$); Ex, exine thickness ($\text{Ex} = N + S$); P/E = PA/EA; CEn, class of endoaperture, S/N, index of sexine and nexine thickness.

side of the graph, while the *P. latifolia* (lat26, lat51) and *P. unifoliolata* were on the top right (Figure 8). The specimens of *P. unifoliolata* (uni61, uni16) and *P. latifolia* (lat51, lat26) forming a similarity cluster lower than 50% and with *P. coriifolia* the similarity is lower than 10% (Figure 9). The recognition of these three taxa as species was made by Müller (1986) and it is reinforced when our palynological data is added to the data of Mendes et al. (2019) for the leaflet anatomy.

Likewise, specimens of *Poiretia mattogrossensis* and *P. elegans* are often mistakenly identified as *P. latifolia*. Furthermore, individuals of *P. mattogrossensis* may be misidentified as *P. marginata* and *P. elegans* may be misidentified as *P. coriifolia* (Mendes et al. 2019). *Poiretia latifolia* has type III pollen and differs in endoaperture, apocolpium, tectum, foot layer aperture area and endexine from *P. coriifolia*, *P. mattogrossensis* and *P. elegans*, which have type II pollen (Table VII). The specimens of *P. mattogrossensis* (mat30), *P. elegans* (ele18, ele20, ele58) and *P. latifolia* (lat51, lat26) were grouped between species in different places of the graph (Figure 8) and presented less than 10% of similarity (Figure 9); this low similarity supports the recognition of these species.

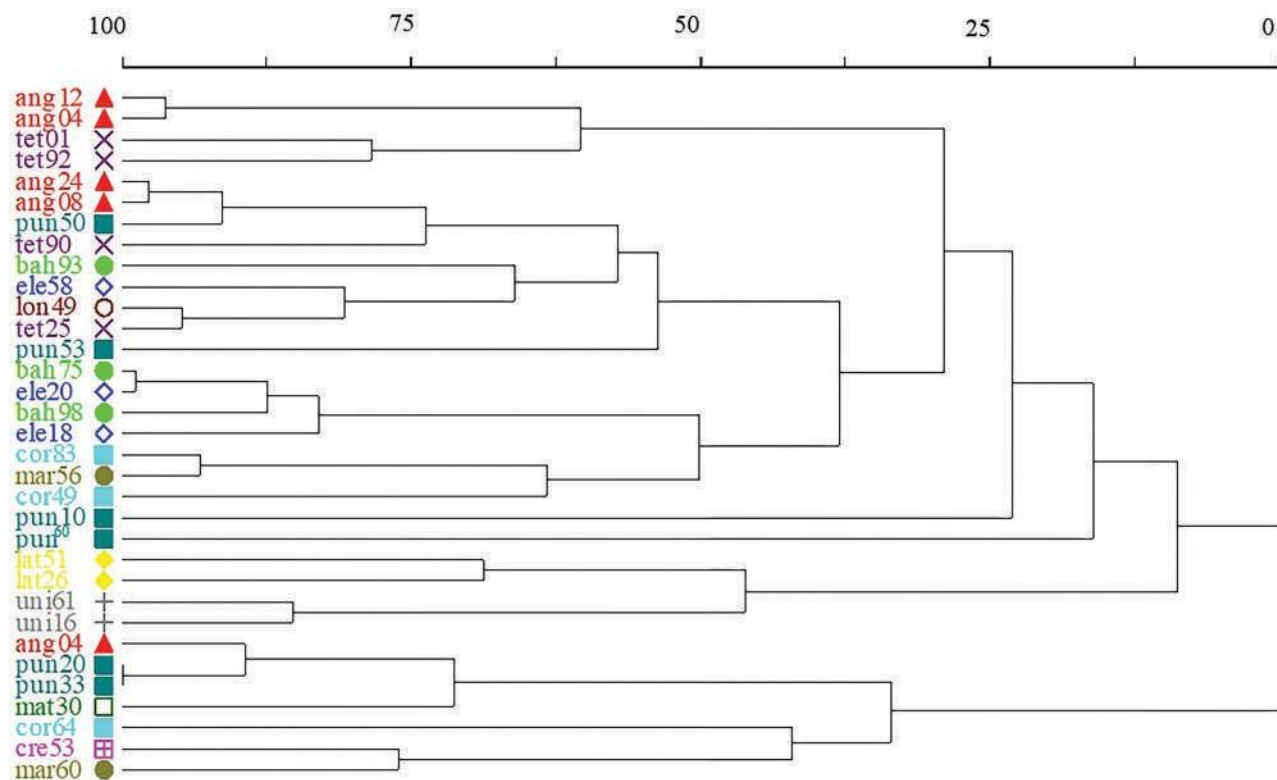


Figure 9. Dendrogram built from cluster analysis (Euclidean distance) for *Poiretia* specimens, information remaining (%).

Table VI. Compiled of pollen morphology by author in literature and comparison with the present study.

Specie	Author	Class of size	Class of endoaperture	Operculum ultraesclature	Sexine ultraesclature	
					Mesocolpium	Apocolpium
<i>P. angustifolia</i>	M 1966	Small to medium	Lalongate	—	Psilate ?	
	PS	Small to medium	Lalongate to circular, lolongate*	Granulate-rugulate	Rugulate-perforate	Rugulate-perforate
<i>P. latifolia</i>	M 1966	Small to medium	Lalongate	—	Reticulate	
	O 1971	Small to medium	Lalongate	—	Reticulate	
	P 1974	Medium	Circular	—	Reticulate	
	L 2020	Small to large	Lolongate	—	Reticulate	
	PS	Small to medium	Lalongate	Areolate- granulate	Fossulate-perforate	Microrreticulate
<i>P. tetraphylla</i>	P 1974	Medium	Lalongate	—	Reticulate	
	L 2020	Medium	Lolongate	—	Reticulate	
	PS	Small to medium	Circular to lalongate	Rugulate- granulate	Fossulate-perforate	Fossulate-perforate

—, The authors do not described this feature; ?, the author has doubt in this feature; M 1966, Melhem 1966; O 1971, Ohashi 1971; P 1974, Pire 1974; L 2020, Lattar et al. 2020; PS, present study.

*, Rarely.

Table VII. Pollen diagnostics characteristics to the problematic *Poiretia* species.

Specie	Class of endoaperture	pollen type	Apocolpium	Tectum	Foot layer in apertural area	Endexine
<i>P. coriifolia</i>	Circular to lalongate	Type II	Nanorreticulate	Semitectate-perforate	Absent	Bilayered
<i>P. elegans</i>	Circular to lalongate	Type II	Nanorreticulate	Semitectate-perforate	Absent	Bilayered
<i>P. latifolia</i>	Lalongate	Type III	Nanorreticulate	Semitectate-perforate	Discontinuos	Bilayered
<i>P. marginata</i>	Lalongate	Type IV	Nanoreticulate-microrreticulate	Semitectate-perforate	Descontinuos	Monoleayered
<i>P. mattogrossensis</i>	Lalongate	Type II	Nanorreticulate-perforate	Semitectate-perforate	Absent	Bilayered
<i>P. unifoliolata</i>	Lalongate to very lalongate	Type II	Nanoreticulate-perforate	Perforate	Discontinuos	Bilayered

Palynological contribution to the phylogenetically related genera of *Poiretia*

Earlier studies have documented common palynological characteristics among the taxa of *Poiretia* and *Zornia* (Melhem 1966; Silvestre-Capelato & Melhem 1997; Pire 1974; Carreira et al. 1996; Souza et al. 2004; Moreti et al. 2007; Buril et al. 2011; Luz et al. 2013; Matos et al. 2014). These two genera are phylogenetically closely related (Fortuna-Perez et al. 2013, 2021) and pollen morphological similarities such as size class, apertural type, presence of operculum, membrane, margo and ornamentation indicate that these features may unite the *Poiretia-Zornia* group (Fortuna-Perez et al. 2013). These two genera are phylogenetically closely related (Fortuna-Perez et al. 2013, 2021) and pollen morphological similarities such as size class, apertural type, presence of operculum, membrane, margo and ornamentation indicate that these features may unite the group (Fortuna-Perez et al. 2013). Thus, further studies of pollen characters optimisation of *Poiretia-Zornia* are needed in order to investigate the potential additional synapomorphies.

Conclusions

Pollen ultrastructure is described for the first time for *Poiretia*. The study is also the first ultrastructural account for a genus of the informal *Adesmia* informal clade. The new palynological data, as well as important contributions in the search of potential characteristics for the systematics of *Poiretia* and *Adesmia* clade. A diverse pollen morphology, ultrasculpture and ultrastructure was observed in the species analysed and contributes to the delimitation of some species with conflict in the delimita-

tion (*P. latifolia*, *P. coriifolia*, *P. unifoliolata*, *P. mattogrossensis*, *P. elegans*, and *P. marginata*) and group of species.

Varied classes of endoaperture, ultrasculpture and ultrastructure of tectum, foot layer and endexine support the eurypalynous status of *Poiretia*. The recognition of *P. mattogrossensis* and *P. elegans* as *P. latifolia* can be optimised with our palynotaxonomical data just as it happens among species *P. mattogrossensis/P. marginata* and *P. elegans/P. coriifolia*.

The palynological data support the morphological descriptions of the Dalbergieae tribe and can be used in future phylogenetic approaches to define the systematic and morphological studies of the *Poiretia* and the *Adesmia* informal clade.

As the pollen represents an important potential diagnosis tool, we recommend to review species belonging to other closely related genera of *Adesmia* clade using a palynological stand method.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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* Specimen used for illustrations

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Palynology of *Amicia* Kunth. (Leguminosae – Papilionoideae – Dalbergieae – Informal Adesmia clade) set in a systematic and phylogenetic context

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Abstract

Amicia is the least diverse genus of the Adesmia clade. It has a disjunct distribution with six endemic species restricted to the central Andes of South America and one species endemic to Mexico. The phylogeny and taxonomy of this genus have been extensively studied. Nevertheless, pollen data are still insufficiently known within a taxonomic context. In this study, we analysed all *Amicia* species using light, scanning, and transmission electron microscopy and we present phylogenetically useful palynological information to support taxonomic studies. Variation in pollen grain size and aperture features is used to delimit pollen types, which corroborate molecular and biogeographical data. Multivariate analysis reinforced the pollen type subdivisions and revealed novel diagnostic morphological features for two infrageneric taxa of *Amicia*. Our pollen results provide informative pollen characters to assist in the delimitation of *Amicia* species, which can be easily misidentified due to similarities in vegetative structures. *Amicia* pollen morphology reflects population isolation and divergence of *Amicia* lineages and provides critical features for future phylogenetic optimisation of the Adesmia clade.

Keywords: Central America, Dalbergia clade, Fabaceae, Mexico, pollen morphology, South America, ultrasculpture, ultrastructure

The American genus *Amicia* Kunth is the least diverse member of the informal Adesmia clade, which belongs to the tribe Dalbergieae of subfamily Papilionoideae in the Leguminosae (Lavin et al. 2001; Klitgaard & Lavin 2005). Based on plastid and nuclear ribosomal DNA analysis, *Amicia* and the genera *Poiretia* Vent. et *Zornia* J.F. Gmel. form the monophyletic *Zornia*-*Amicia*-*Poiretia* clade, which diverged within the larger Adesmia clade about 24.77 mya (Fortuna-

Perez et al. 2013, 2021). The clade is morphologically supported by the synapomorphy of secretory structures present and is diagnosed by the fruit type of articulated loments ornamented with crests, and by having mostly paripinnate leaves comprised of 2–4 leaflets (Fortuna-Perez et al. 2013, 2021; Särkinen & Hughes 2015).

Amicia is distinguished from closely related genera by a unique combination of characters: a strongly

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bilabiate calyx, straight rather than curved keel petals, and conspicuous kidney-shaped and basifixed stipules (Kunth 1824, Särkinen & Hughes 2015). The genus has a disjunct distribution with six species endemic to the central Andes of South America and one (*A. zygomeris* DC.) endemic to Mexico (Särkinen & Hughes 2015). Based on analysis of ITS and matK DNA markers, there are two infrageneric clades: the ‘Medicaginea clade’, which is robustly supported and comprises three species (*A. fimbriata* Harms ex Kuntze, *A. micrantha* Harms ex Kuntze, *A. medicaginea* Griseb), and the weakly supported ‘Lobbiana clade’, with four species (*A. zygomeris*, *A. andicola* (Griseb.) Harms, *A. glandulosa* Kunt, *A. lobbiana* Benth. ex Rusby). Additional morphological characterisation of the group members is needed to better understand their relationships (Särkinen & Hughes 2015).

Pollen grains of *Amicia* and other representatives of the informal *Adesmia* clade lack detailed study. Pollen grain morphology, ultrasculpture and ultrastructure have provided key taxonomic features for other taxa in the tribe Dalbegieae (Ferguson & Skvarla 1981; Antonio-Domingues 2017; Antonio-Domingues et al. 2018, 2019, 2022; Jiang et al. 2019; Lattar et al. 2020). Although scarce, previous studies have demonstrated that *Amicia* is the most palynologically diverse genus within the *Adesmia* clade. However, only four of the seven described species have been palynologically documented in the literature (Ohashi 1971; Pire 1974; Särkinen & Hughes 2015). Therefore, our study aimed to comprehensively characterise the pollen variation in all seven *Amicia* species in an attempt to recognise palynological patterns of taxonomic significance that might be useful for infrageneric and/or species delimitation.

Material and methods

Unopened mature flower buds were obtained from 13 herbarium specimens belonging to all seven species of *Amicia* (*A. andicola* [Griseb.] Harms, *A. fimbriata* Harms ex Kuntze, *A. glandulosa* Kunth, *A. lobbiana* Benth. ex Rusby, *A. medicaginea* Griseb., *A. micrantha* Harms ex Kuntze, and *A. zygomeris* DC.). Specimens were sampled from CGMS, K, NY and SP herbaria (acronyms follow Thiers 2021 [continuously updated]). All specimens examined are listed in the *Specimens Investigated* section. Samples were labelled with an abbreviation of the species epithet plus the last two numbers of the collection number on the herbarium voucher (see Table I, Species and specimen column).

Pollen grains were treated with the acetolysis method (Erdtman 1960), following the modifications proposed by Melhem et al. (2003). Measurements were performed under a light microscope using an Olympus OSM-4 (10×) micrometer drum coupled to the eyepiece of an Olympus BX50 binocular microscope. The polar axis and equatorial axis in equatorial view were measured on 25 randomly selected pollen grains from each taxon. These measurements were statistically analysed for the arithmetic mean ($\bar{x}$), average standard deviation (S_x), sample standard deviation (s), coefficient of variability ($V\%$), and 95% confidence interval (CI) (Zar 2010; Vieira 2011). Ten measurements of the main pollen morphometric parameters were also made: length and width of the colpus, length and width of the endoaperture (except for *A. zygomeris*), the thickness of the nexine and sexine layers. Exine measurements were made in the mesocolpium region. The arithmetic mean and range were calculated for each pollen grain size class and exine (sexine + nexine).

Photomicrographs were obtained using an Olympus BX 50 light microscope with an Olympus U-CMAD-2 video camera and Olympus CellSens 1.5 software. Permanent light microscopy (LM) slides were deposited in the pollen reference collection of Laboratório de Palinologia, Instituto de Pesquisas Ambientais, São Paulo State, Brazil.

To avoid the loss of the operculum and exine elements covering the aperture (Hesse & Waha 1989) for scanning electron microscopy (SEM), non-acetolysed pollen grains were washed and placed on a metal stub and sputter-coated (Leica EM ACE 600; Leica, Vienna, Austria) with gold (80 nm). Samples were observed under a JEOL JSMIT300LV microscope (Tokyo, Japan) operating at a 20 kV electron beam, and the images were digitised.

For transmission electron microscopy (TEM) mature anthers were removed from flower buds and fixed in a modified Karnovsky solution (Karnovsky 1965) for at least 24 h. They were subsequently rinsed with sodium cacodylate buffer (0.1 M) and immediately post-fixed in osmium oxide (1%) for 1 h. Samples were then rinsed in a saline solution (0.9% sodium chloride) and pre-stained with uranyl acetate (2.5%) followed by dehydration in an acetone series (35–100%), 20 min each. Samples were then gradually infiltrated in Spurr resin (Spurr 1969) and embedded in pure resin for 48 h, at 70 °C. Ultra-thin sections (60 nm) were obtained with an ultramicrotome (Sorvallter Blum MT2, Norwalk, CT, USA), deposited on copper

Table I. *Amicia* Kunth dimensions (µm) of pollen grains in equatorial view using light microscopy.

Specimen	Polar axis				Equatorial axis				P/ E	Size	Colporus Length – Width	Endoaperture Width (Cen) Length	Exine	
	CI– ($x \pm sx$)	CI+	s	V%	CI– ($x \pm sx$)	CI+	s	V%	x	Range			N	S
and58	34.1 (35.9 ± 0.9)	37.8	4.5	12.6	35.0 (36.3 ± 0.6)	37.6	0.6	8.9	1.0	M	27.9 – 5.5	13.2 (1.1) 11.9	0.8	1.5
and41	37.8 (38.5 ± 0.3)	39.1	1.6	4.1	32.5 (33.0 ± 0.2)	33.5	1.2	3.6	1.2	M	26.6 – 5.2	13.2 (1.0) 12.9	0.8	1.2
fim49	43.0 (44.2 ± 0.6)	45.4	3.4	7.7	39.2 (40.4 ± 0.6)	41.6	3.5	8.5	1.1	M	36.2 – 14.5	16.9 (1.0) 17.6	0.6	1.5
fim	44.3 (45.3 ± 0.5)	46.3	2.4	5.3	44.4 (46.0 ± 0.8)	47.7	4.0	8.6	0.7	M	36.9 – 19.6	20.6 (0.9) 22.0	0.8	1.5
gla62	28.5 (29.1 ± 0.3)	29.7	1.4	4.9	24.6 (24.9 ± 0.2)	25.2	0.8	3.3	1.2	S–M	24.4 – 4.4	10.1 (1.0) 10.0	0.7	1.3
lob46	29.4 (30.0 ± 0.3)	30.6	1.4	4.6	24.2 (24.7 ± 0.3)	25.2	1.3	5.1	1.2	S–M	24.9 – 4.0	9.9 (1.2) 8.0	0.6	1.0
lob26	33.5 (33.9 ± 0.2)	34.2	0.2	2.8	29.2 (29.6 ± 0.2)	30.0	1.0	3.3	1.1	M	29.5 – 6.5	13.2 (1.1) 12.1	0.6	0.9
lob30	32.0 (32.4 ± 0.2)	32.8	1.1	3.4	26.4 (26.8 ± 0.2)	27.3	1.1	4.1	1.2	M	27.0 – 5.6	12.8 (1.2) 11.1	0.5	0.8
med73	41.3 (42.2 ± 0.5)	43.2	2.5	5.9	42.4 (43.6 ± 0.6)	44.8	3.2	7.2	1.0	M	34.9 – 14.2	19.1 (1.0) 19.2	0.8	1.4
mic36	42.4 (43.7 ± 0.6)	44.9	3.0	6.9	40.0 (40.9 ± 0.4)	41.8	2.2	5.4	1.1	M	38.1 – 19.9	21.0 (1.0) 22.0	0.6	1.3
mic66	36.4 (37.6 ± 0.6)	38.7	2.8	7.5	34.5 (36.1 ± 0.8)	37.7	3.8	10.6	1.0	M	32.3 – 13.7	15.0 (0.9) 17.5	0.6	1.7
zyg37	48.4 (49.7 ± 0.6)	51.0	0.6	3.1	30.4 (30.9 ± 0.2)	31.4	1.1	3.7	1.6	M–L	42.6 – 8.8	Absent	0.7	1.5
zyg88	40.7 (41.6 ± 0.4)	42.4	2.1	5.0	32.9 (33.9 ± 0.5)	34.8	2.3	6.9	1.2	M	34.4 – 7.7	Absent	0.6	1.8

Note. Specimens were identified by abbreviation of the species epithet plus the last two numbers of the collection number on the field label of the herbarium specimen (see Specimens investigated). Confidence interval at 95% probability of the lowest sample values (IC–) and highest sample values (IC+), arithmetic mean (x), average standard deviation (sx), sample standard deviation (s), coefficient of variability ($V\%$).

grids and stained using uranyl acetate (2.5%) and lead citrate (Reynolds 1963). Images were obtained under a transmission electron microscope (JEOL JEM 1400; JEOL, Tokyo, Japan) at 80 kV.

Pollen terminology is based on Punt et al. (2007) and Halbritter et al. (2018). Pollen shape classes and amb types follow Erdtman (1952), and colpus length categories follow Mark et al. (2012). The endoaperture classes and index of sexine and nexine thickness were proposed by Antonio-Domingues et al. (2022). TEM descriptions of the exine infratectum follow the terminologies of Halbritter et al. (2018) and Ferguson and Skvarla (1983).

Principal component analysis (PCA) was performed to explore whether pollen grain characteristics allow for the grouping of species and specimens. Seven metric variables (polar axis [PA], equatorial axis [EA], colpus length [CL], colpus width [CW], nexine thickness [N], sexine thickness [S = infratectum + tectum], exine thickness [$Ex = N + S$]) and two classes/index (shape class [$P/E = PA/EA$], index

of sexine and nexine thickness [S/N]) were analysed for ordination using FITOPAC (Shepherd 1996) and PC-ORD version 7 (McCune & Mefford 2016).

To understand how *Amicia* specimens relate to each other, based on pollen morphology, a cluster analysis (CA) was performed using the software PC-ORD version 7 (McCune & Mefford 2016). This produced a similarity dendrogram by calculating the Euclidean distance based on the same seven PCA metric variables and the two classes/index.

Results

General description of *Amicia* pollen grains

Pollen (Figures 1–5) grains in monads from small to large size, isopolar, oblate to prolate, amb circular, subtriangular or hexagonal (*Amicia fimbriata*) (Figure 3B), angulaperturate (Figures 1E, B, H, 3B, E) or planaperturate (*A. zygomeris*) (Figure 3K); polar area small to very small or rarely absent.

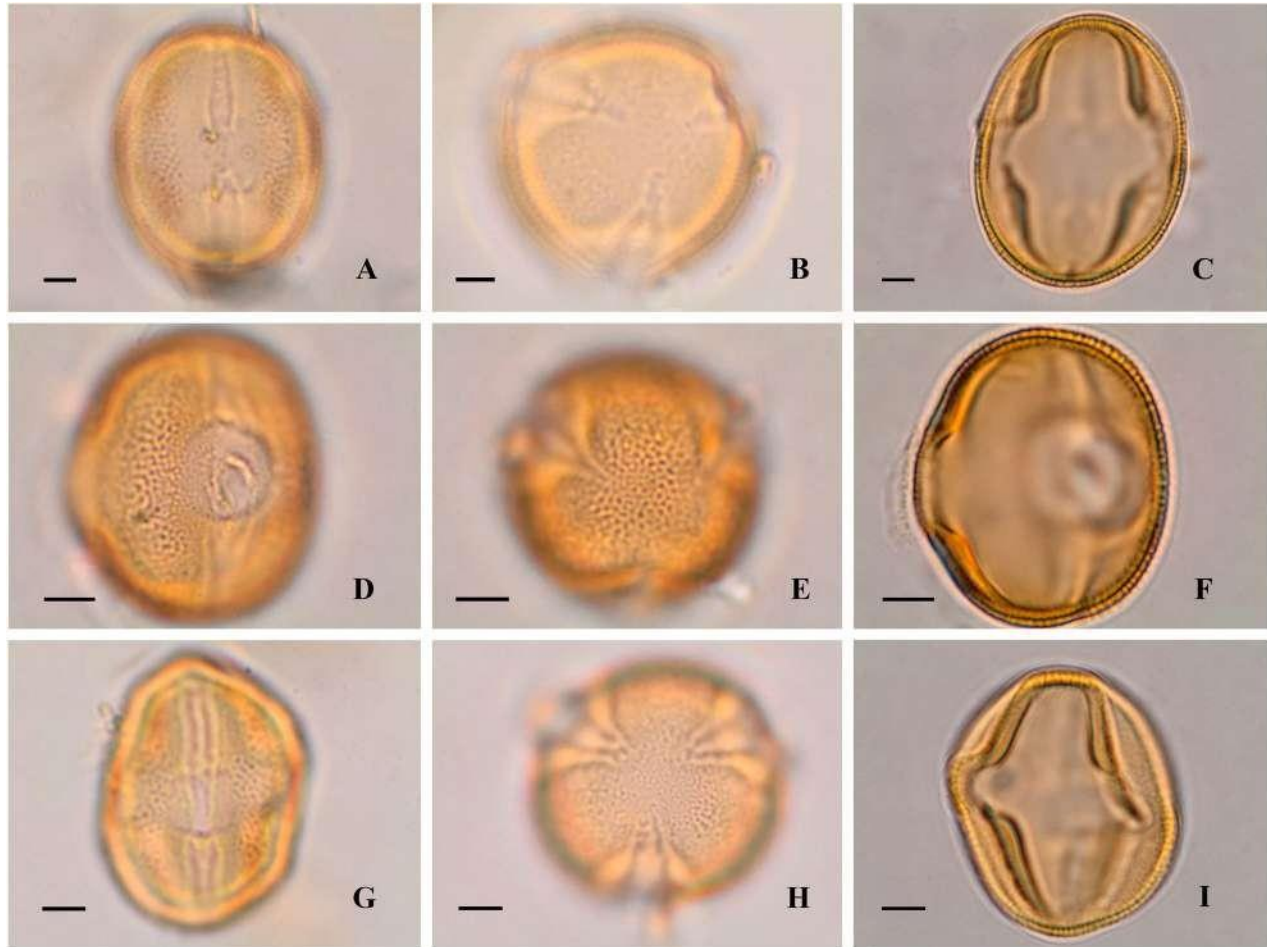


Figure 1. Light microscopy of *Amicia* DC. species: pollen type I subtype Ia. A–C. *Amicia andicola*. A. Detail of the colporus, endoaperture, operculum and surface, equatorial view. B. Apertures 3-colporate, long and pointed apices, colpuri and apocolpium surface. C. Optical section, equatorial view. D–F. *Amicia glandulosa*. D. Detail of the colporus, endoaperture, surface and operculum, equatorial view. E. Apertures 3-colporate, long and pointed apices and apocolpium surface. F. Optical section, equatorial view. G–I. *Amicia lobbiana*. G. Detail of the colporus, surface and operculum, equatorial view. H. Apertures 3-colporate, long and pointed apices and apocolpium surface. I. Optical section, equatorial view. Scale bars – 5 μm .

Predominantly 3-zonocolporate (Figures 1A, D, G, 3A, D, G) or 3-zonocolpate (*A. zygomeris*) (Figure 3J), with long to very long, wide to narrow, constricted or non-constricted colporus, with pointed (Figures 1B, E, H, 3E, H, K) or rounded apices (*A. fimbriata*) (Figure 3B). Colporus margo broad or narrow with different perforate (*A. lobbiana*) (Figure 2H) or psilate-perforate (Figures 2A, E, 4) ornamentation or absent margo (Figure 4B, E, H). An operculum is always present with areolate-granulate (Figures 2A, 4C, E, H), psilate-perforate (Figure 4K) or granulate (Figure 2E) ornamentation. Colporus membrane granulate (Figures 2A, E, H, 4B, E, H, K). Endoaperture circular, lolongate, lalongate or absent (*A. zygomeris*) (Figure 3J, L).

Sexine with semitectate, heterobrochate, nanoreticulate to microreticulate ornamentations (Figures 2,

G, F, 4C), network-like pattern with predominantly rounded lumina, straight to curved muri simple-columellate, continuous or interrupted, with wall connections at one level; or semitectate to tectate perforate sexine with rugulate-perforate (Figures 2B, 4F, I, L) or perforate (Figure 2C, I) ornamentations. Sexine 1.2–4.2 times thicker than the nexine, and sometimes thickened in the apocolpium or mesocolpium areas.

A summary of the measurements is shown in Table I and additional observations and descriptions are provided in Table II.

Exine ultrastructure

The exine has an electron translucent (e-translucent) or electron-dense (e-dense) compact tectum that varies from semitectate to tectate-perforate. The

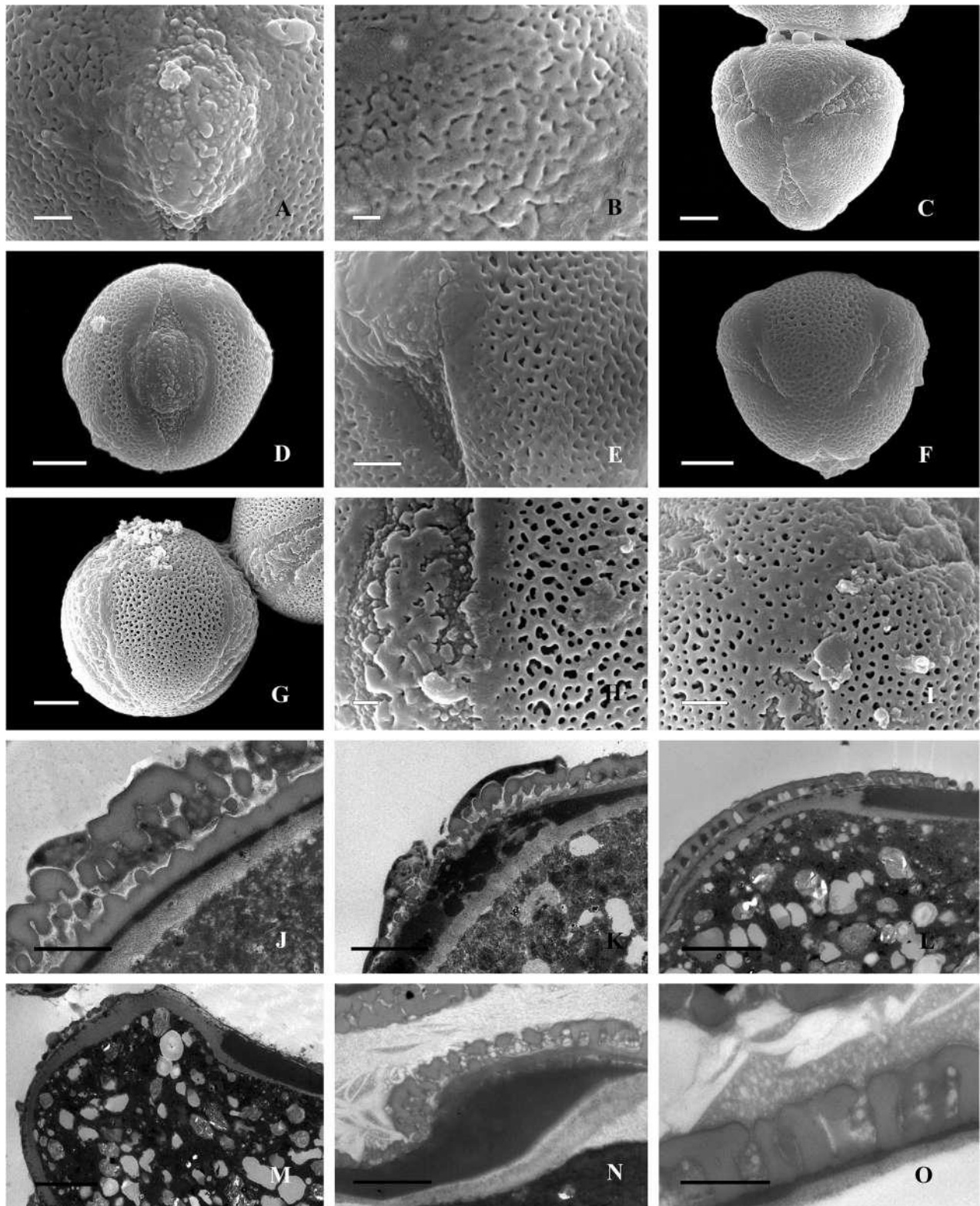


Figure 2. Scanning electron microscopy (A–I) and transmission electron microscopy (J–O) of *Amicia* DC. species: pollen type Ia subtype Ia. A–C. *Amicia andicola*. A. Detail of apertural membrane of the colporus, margo and operculum, equatorial view. B. Detail of rugulate-perforate mesocolpium. C. Perforate apocolpium. D–F. *Amicia glandulosa*. D. General view of apertural area, equatorial view. E. Detail of apertural membrane of the colporus, margo, operculum and mesocolpium, equatorial view. F. General view of nanoreticulate to microreticulate apocolpium. G–I. *Amicia lobbiana*. G. General view of nanoreticulate to microreticulate mesocolpium, equatorial view. H. Detail of apertural membrane of the colporus, margo, operculum and mesocolpium, equatorial view. I. Detail of perforate apocolpium. J, K. *Amicia andicola*. J. Section in a non-apertural area. K. Section in the apertural area. L, M. *Amicia glandulosa*. L. Section near to the aperture. M. Section in the apertural area. N, O. *Amicia lobbiana*. N. Section near to the aperture. O. Section in a non-apertural area. Scale bars – 5 μm (C, D, F, G), 2 μm (A, E, I, K–N), 1 μm (B, H, J, O).

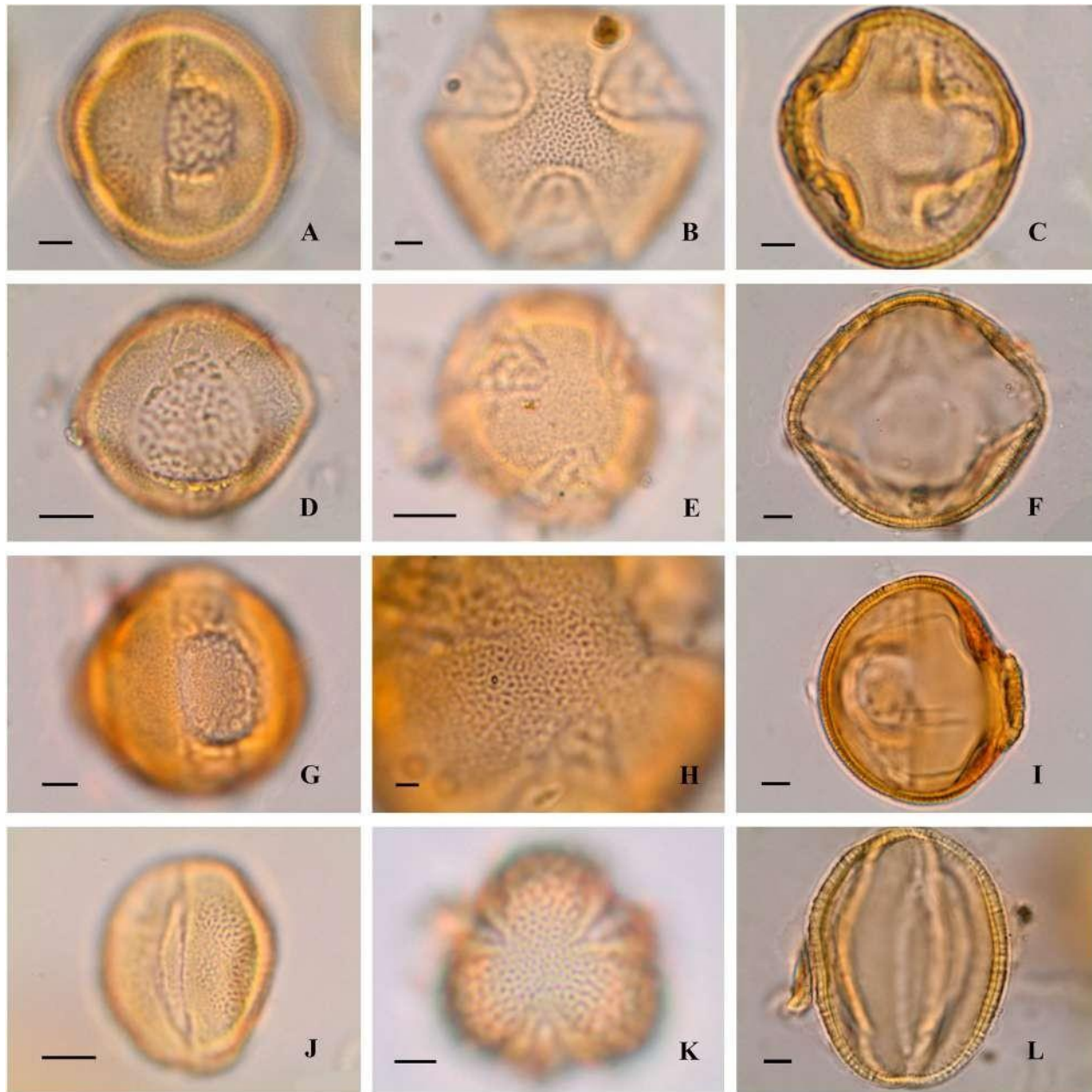


Figure 3. Light microscopy of *Amicia* DC. species: pollen type I subtype Ib (A–I) and type II (J–L). A–C. *Amicia fimbriata*. A. Detail of the colporus, endoaperture, operculum and surface, equatorial view. B. Apertures 3-colporate, very long and rounded apices, colpuri and apocolpium surface. C. Optical section, equatorial view. D–F. *Amicia medicaginea*. D. Detail of the colporus, endoaperture, surface and operculum, equatorial view. E. Apertures 3-colporate, very long and pointed apices and apocolpium surface. F. Optical section, equatorial view. G–I. *Amicia micrantha*. G. Detail of the colporus, surface and operculum, equatorial view. H. Apertures 3-colporate, very long and pointed apices and apocolpium surface. I. Optical section, equatorial view. J–L. *Amicia zygoensis*. J. Detail of the colporus, surface and operculum, equatorial view. K. Apertures 3-colporate, very long and pointed apices and apocolpium surface. L. Optical section, equatorial view. Scale bars – 10 μ m (D, J), 5 μ m (A–C, E–I, K, L).

tectum is smooth or undulated with an e-translucent or e-dense *pollenkitt* (Figures 2J–O, 5).

We observed two types of infratectal structures: (1) granules somewhat organised in a narrow and

irregular row; (2) simple complete and or incomplete columellae. Combinations of the two types occurred in all species analysed. In *Amicia lobbiana*, *A. medicaginea* and *A. micrantha* the granules are

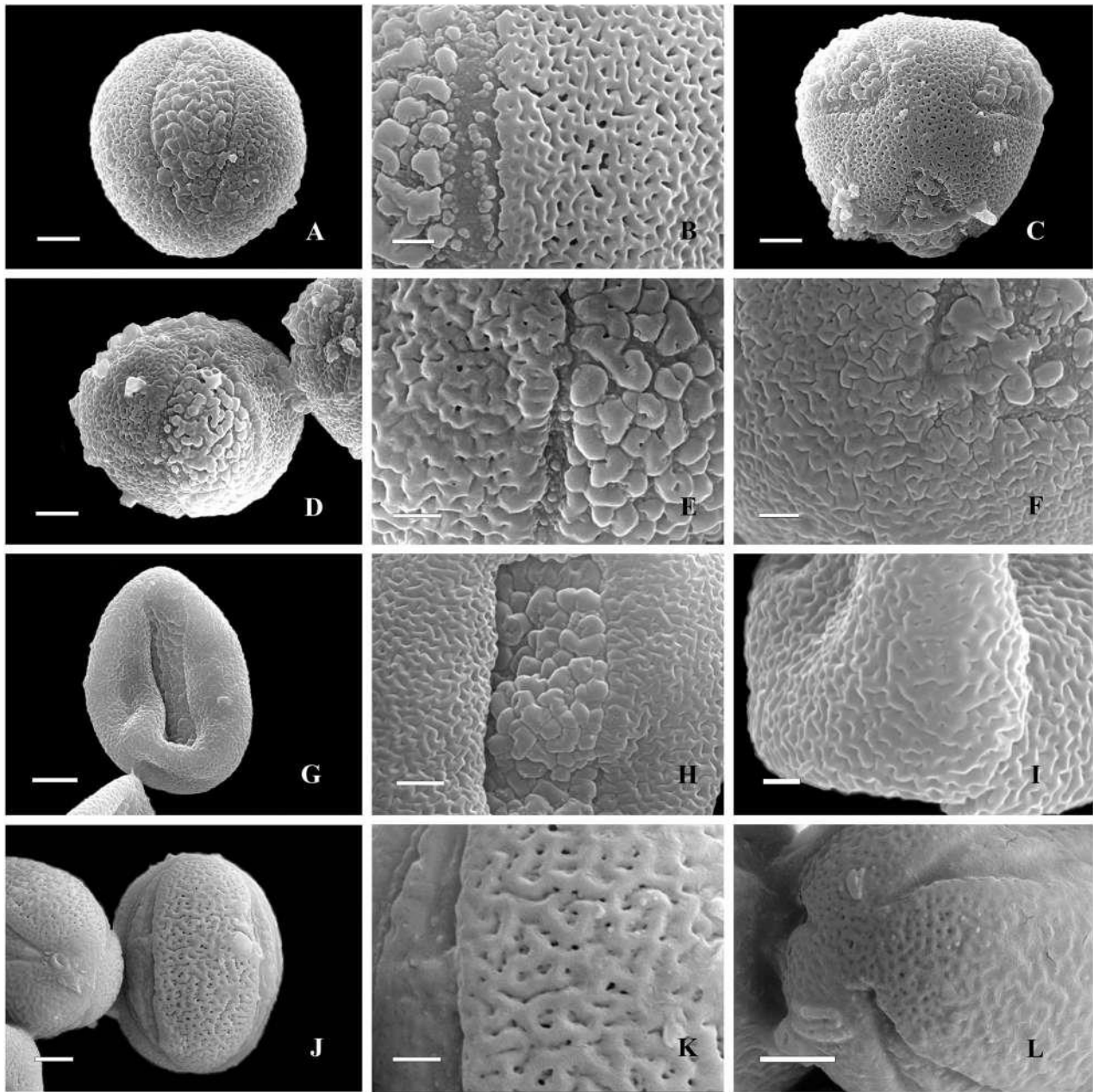


Figure 4. Scanning electron microscopy of *Amicia* DC. species: pollen type I subtype Ib (A–I) and type II (J–L). A–C. *Amicia fimbriata*. A. General view of apertural area, equatorial view. B. Detail of apertural membrane of the colporus, margo, operculum and mesocolpium, equatorial view. C. Detail of nanoreticulate to microreticulate apocolpium. D–F. *Amicia medicaginea*. D. General view of apertural area, equatorial view. E. Detail of apertural membrane of the colporus, margo, operculum and mesocolpium, equatorial view. F. Detail of rugulate-perforate apocolpium. G–I. *Amicia micrantha*. G. General view of apertural area, equatorial view. H. Detail of apertural membrane of the colporus, margo, operculum and mesocolpium, equatorial view. I. Detail of rugulate-perforate apocolpium. J–L. *Amicia zygomeres*. J. General view of mesocolpium area, equatorial view. K. Detail of apertural membrane of the colporus, margo, operculum and mesocolpium, equatorial view. L. General view of rugulate-perforate apocolpium. Scale bars – 5 μm (A, C, D, G, J), 2 μm (B, E, F, H, K, L), 1 μm (I).

present only near to the colpi. We also observed an e-translucent and e-dense infratectal *pollenkitt* (Figures 2J–O, 5).

The thin foot layer was compact and with channels, from continuous to discontinuous or absent in the apertural area, rarely irregular. The thick or

thin endexine was e-dense, compact-continuous, smooth or with endo-cracks. The endexine is compact-continuous, thick, costate and spongy near to the aperture, endo-sculptured and bi-layered. The intine is mono-layered, e-translucent or bi-layered (*Amicia lobbiana* and *A. medicaginea*) and

Table II. Morphology and ultrastructure of *Amicia* pollen grains using light and scanning electron microscopy.

Species	PT	Size	Endoaperture	Operculum	Membrane	Margo	Mesocolpium	Apocolpium
<i>Amicia andicola</i>	Ia	M	Circular to lalongate,	Areolate-granulate	Granulate	Psilate-perforate	Rugulate-perforate	Perforate
<i>Amicia fimbriata</i>	Ib	M-L*	Circular to lalongate	Areolate-granulate	Granulate	Absent	Nanoreticulate to microreticulate	
<i>Amicia glandulosa</i>	Ia	S-M	Circular	Granulate	Granulate	Psilate-perforate	Nanoreticulate to microreticulate	Perforate
<i>Amicia lobbiana</i>	Ia	S-M	Lalongate, circular*	Areolate-granulate	Granulate	Perforate	Nanoreticulate to microreticulate	Perforate
<i>Amicia medicaginea</i>	Ib	M	Circular	Areolate-granulate	Granulate	Absent	Rugulate-perforate	
<i>Amicia micrantha</i>	Ib	M	Lolongate, circular*	Areolate-granulate	Granulate	Absent	Rugulate-perforate	
<i>Amicia zygomeris</i>	II	M	Absent	Psilate-perforate	Granulate	Psilate-perforate	Rugulate-perforate	

PT, pollen type; S, small, M, medium; L, large; *, rarely.

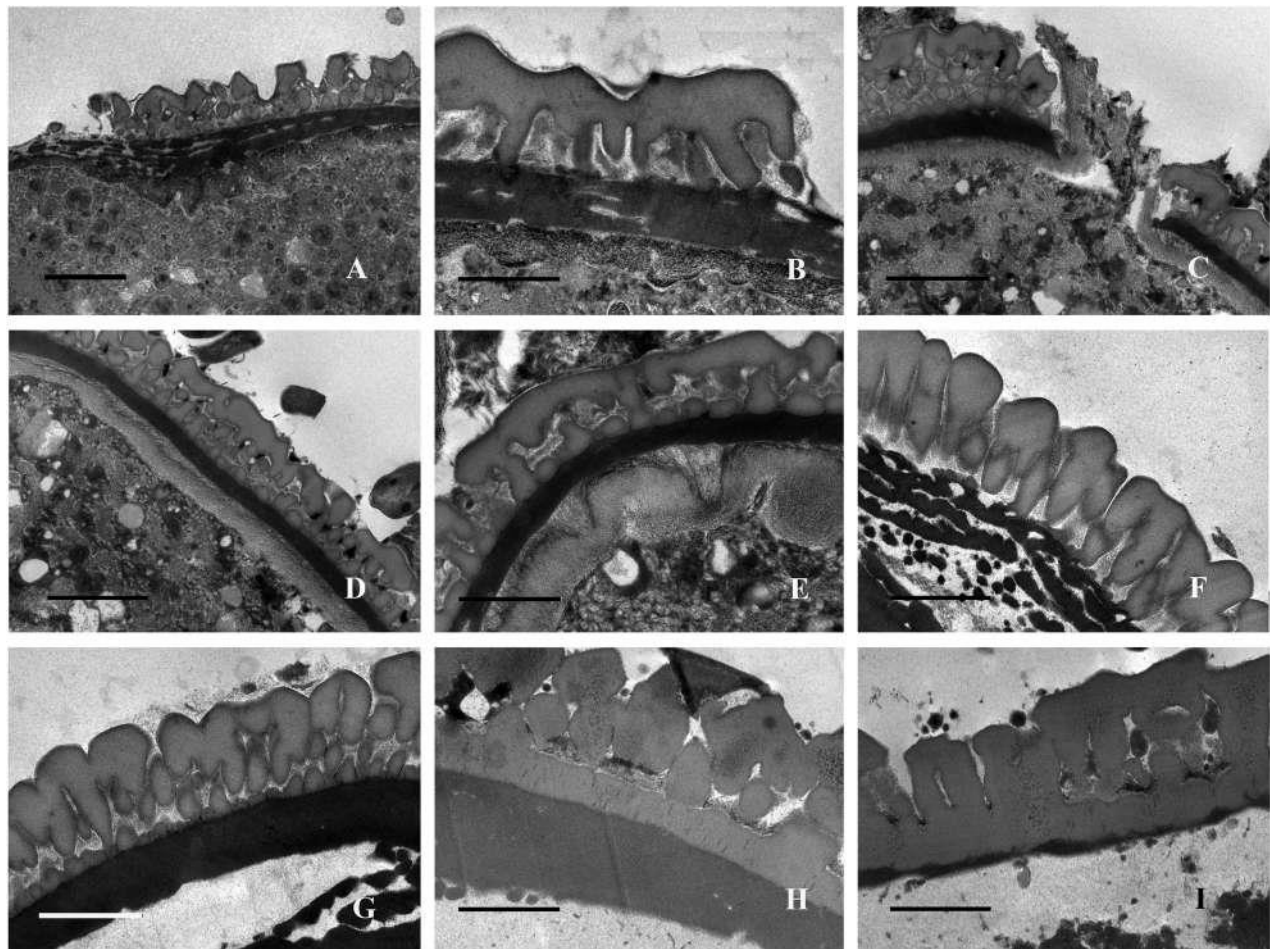


Figure 5. Transmission electron microscopy of *Amicia* DC. species: pollen type I subtype Ib (A–G) and type II (H, I). A, B. *Amicia fimbriata*. A. Section in the apertural area. B. Section near to the aperture. C–E. *Amicia medicaginea*. C. Section in the apertural area. D. Section near to the aperture. E. Section in a non-apertural area. F, G. *Amicia micrantha*. F. Section near to the aperture. G. Section in a non-apertural area. H, I. *Amicia zygomeris*. H. Section near to the aperture. I. Section in a non-apertural area. Scale bars – 2 µm (A, C, D), 1 µm (B, E–I).

has an e-dense ektintine and an e-translucent endointine (Figures 2J-O, 5).

Apertures and pollen types

Type I: Colporate. — Pollen grains 3-colporate, angulaperturate.

Subtype Ia: Lobbiana. — Pollen grain equatorial axis less than 36 μm ($x \leq 36 \mu\text{m}$), colporus length and width less than 30 and 8 μm respectively ($x < 30$ and 8 μm) and endoaperture length and width both less than 14 μm ($x < 14 \mu\text{m}$). Species: *Amicia andicola* (Griseb.) Harms., *A. glandulosa* Kunth., *A. lobbiana* Benth. ex Rusby. (Figures 1, 2).

Pollen grain small (*Amicia andicola*) or small to medium-sized, predominantly prolate spheroidal to subprolate, rarely suboblate or oblate-spheroidal (*A. andicola*) or prolate (*A. lobbiana*), with subtriangular to circular amb. (Figure 1B, E, H), ellipsoidal in equatorial view (Figure 1C, F, I). Pollen grains 3-zonocolporate, colporus narrow, large, with pointed apices (Figures 1B, E, H, 2E, F); operculum granulate (*A. glandulosa*) (Figure 2E) or areolate-granulate (Figure 2A, H); granulate colporus membrane (Figure 2A, E, F); colporus margo perforate (*A. lobbiana*) (Figure 2H) or psilate perforate (Figure 2A, E). Endoaperture circular (*A. glandulosa*) (Figure 1D) or circular to lalongate (Figure 1A, G). Mesocolpium rugulate-perforate (*A. andicola*) (Figure 2A) or nanoreticulate to microreticulate (Figure 2E, H) with apocolpium perforate (Figure 2B, E, I).

Sexine from 1.3–2.3 times thicker than nexine; exine 1.2–2.7 μm thick. Tectum semitectate to tectate-perforate (*Amicia andicola*) (Figure 2J, K) or tectate-perforate (Figure 2L-O). The infratectal structure presents simple incomplete columellae and some sparse granules somewhat organised in an irregular narrow row. In *A. lobbiana* the granules are present only in the apertural area. The thick foot layer ranges from continuous, compact, with channels, to thin and discontinuous in the apertural area. The endexine is compact-continuous, thin, costate, and spongy near to the aperture, endo-sculptured and bi-layered. Intine bi-layered with ektintine e-dense and endointine e-translucent (*A. lobbiana*) or mono-layered.

Subtype Ib: Medicaginea. — Pollen grain equatorial axis greater than 36 μm ($x \geq 36 \mu\text{m}$), colporus length and width greater than 30 and 8 μm respectively ($x > 30 \mu\text{m}$ and 8 μm) and endoaperture length and width both greater than 14 μm ($x > 14 \mu\text{m}$). Species: *Amicia fimbriata* Harms ex Kuntze.,

A. medicaginea Griseb., *A. micrantha* Harms ex Kuntze. (Figures 3A-I, 4A-I, 5H, I).

Pollen grains medium-sized, oblate to subprolate (*Amicia fimbriata*) or oblate-spheroidal to subprolate, with hexagonal (*A. fimbriata*) (Figure 3B) or circular amb. (Figure 3E), circular to ellipsoidal equatorial view. Pollen grains 3-zonocolporate, colporus broad, very large, with rounded (*A. fimbriata*) (Figure 3B) or pointed apices (Figure 3E, H); operculum areolate-granulate, colporus membrane granulate and margo absent (Figure 4B, E, F, H). Endoaperture circular (*A. medicaginea*) (Figure 3B) or circular to lalongate (Figure 3A, G). Mesocolpium and apocolpium nanoreticulate to microreticulate (*A. fimbriata*) (Figure 4B, C) or rugulate-perforate (Figure 4E, F, H, I).

Sexine from 1.5–3.1 times thicker than nexine; exine 1.7–2.6 μm thick. Tectum semitectate to tectate-perforate (*Amicia fimbriata*) (Figure 5A, B) or tectate-perforate (Figure 5C-G). The infratectal structures present simple incomplete columellae and some sparse granules somewhat organised in an irregular narrow row. In *A. medicaginea* the granules are present only in the apertural area (Figure 5C-E). Intine mono-layered (*A. fimbriata*) or bi-layered with ektintine e-dense and endointine e-translucent.

Type II: Zygomeris. — Pollen grains 3-colpate, planaperturate. Species: *Amicia zygomeris* DC. (Figures 3J-L, 4J-L, 5H, I).

Pollen grains medium to large-sized, subprolate to prolate, with subtriangular amb, ellipsoidal in equatorial view (Figure 3J-L). Colpus narrow, with psilate-perforate operculum, granulate membrane and psilate-perforate margo (Figure 4J-L); endoaperture absent (Figure 3J). Rugulate-perforate mesocolpium and apocolpium (Figure 4K, L).

Sexine from 1.9–4.2 thicker than nexine; exine 2.0–2.8 μm thick. Tectum perforate; the infratectal structures have simple incomplete and complete columellae and sparse granules somewhat organised in an irregular narrow row. The thick foot layer varies from discontinuous, compact, with a channel, to thin and discontinuous in the apertural area. The endexine is compact-continuous, thick, costate and spongy near to the aperture, endo-sculptured and bi-layered. Intine mono-layered, e-translucent (Figure 5H-E).

Principal component analysis (PCA)

The PCA showed the relationship between species based on seven metric variables and two classes/indices (Figure 6). Correlation coefficients are

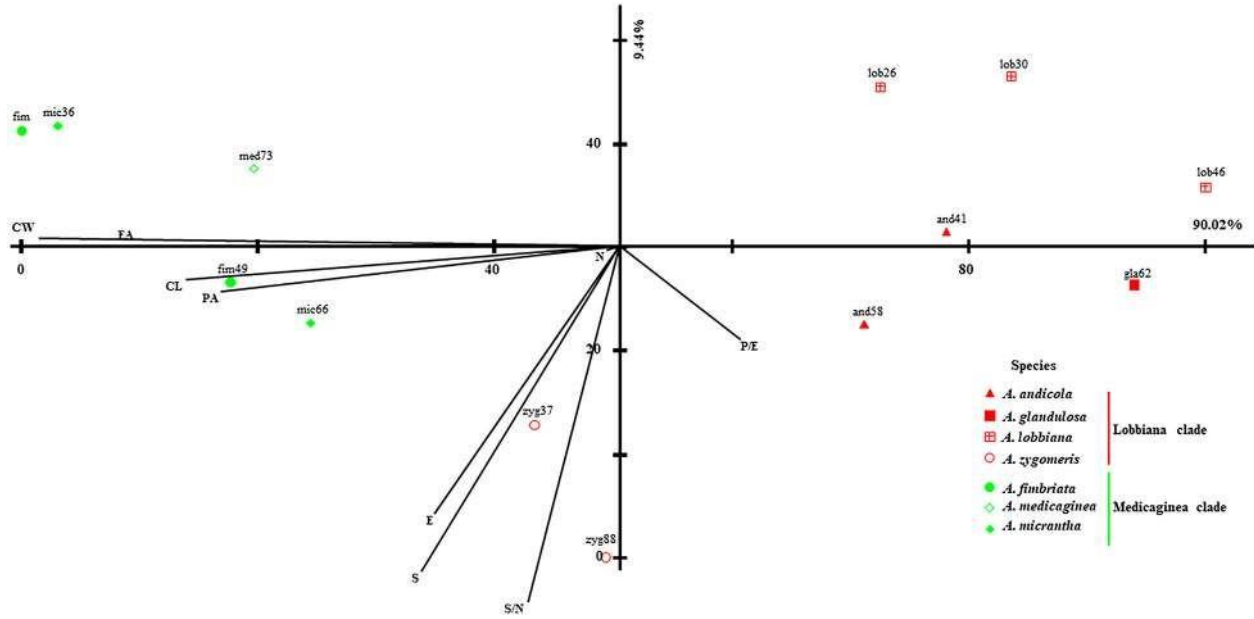


Figure 6. Principal component analysis biplot of the pollen grain metric variables and classes/indices of *Amicia* specimens.

shown in Table III. The first two axes accounted for 99.46% of the total variability of the quantitative data analysed. The first axis explained 90.02% of the variance and was mainly associated with CW, EA and CL. The second axis summarised 25.25% of the total variability, with *S/N*, *S*, *E*, *PA*, *P/E* and *N* being the variables that contributed most to this axis. The *N* was correlated with axis 2 but did not appear on the graph due to its low value (Table III).

Amicia fimbriata (fim49), *A. micrantha* (mic66), and *A. zygoensis* (zyg37, zyg88) were located on the bottom left side of the PCA due to their highest CL, *PA*, *E*, *S* and *S/N* values. *Amicia fimbriata* (fim), *A. micrantha* (mic36) *A. medicaginea* (med73) showed high EA, CW and low *P/E* and were located on the upper left side of the PCA.

Conversely, due to their lower CW and EA values, *Amicia andicola* (and58) and *A. glandulosa* (gla62) were placed on the bottom right side of the PCA. Specimens with the smallest pollen grains were located mainly on the upper right side of the graph. These specimens also had the lowest CL, *PA*, *E*, *S* and *S/N* values: *A. lobbiana* (lob26, lob30, lob46) and *A. andicola* (and41).

Cluster analysis (unweighted pair group method with arithmetic mean [UPGMA] and Euclidean distance)

We identified two groups of specimens with 0% similarity (Figure 7). One group comprised the

species with the smallest pollen grains and colpus dimensions (*Amicia andicola* [and48, and41], *A. glandulosa* [gla62] and *A. lobbiana* [lob46, lob26, lob30]), and the other, the remainder of species which had the largest pollen grains and the highest colpus values (*A. fimbriata* [fim, fim49], *A. micrantha* [mic66, mic36], *A. medicaginea* [med76], *A. zygoensis* [zyg37, zyg88]). *Amicia lobbiana* (lob26, lob30) specimens showed almost 100% similarity, while *A. andicola* (and58, and41) specimens had more than 90%. The other species,

Table III. Pearson and Kendall coefficients of pollen grain metric variables and classes/indices from the first two ordination axes of the principal component analysis (PCA) of *Amicia* species.

Principal components		
Variables	Axis 1	Axis 2
<i>PA</i>	−0.2273	−0.2295
<i>EA</i>	−0.3081	−0.0278
<i>P/E</i>	−0.0732	0.1894
<i>CL</i>	−0.2528	−0.2083
<i>CW</i>	−0.8617	−0.2992
<i>N</i>	−0.0157	−0.0259
<i>S</i>	−0.1231	−0.4647
<i>E</i>	−0.1232	−0.4355
<i>S/N</i>	−0.1053	−0.6095

PA, polar axis; *EA*, equatorial axis; *P/E* = *PA/EA*; *CL*, colpus length; *CW*, colpus width; *N*, nexine thickness; *S*, sexine thickness (*S* = infratectum + tectum); *E*, exine thickness (*EX* = *N* + *S*); *S/N*, index of sexine and nexine thickness.

except for *A. zygomeris* (65%), showed a pollen similarity greater than 75%.

Discussion

Pollen morphology, ultrastructure and ultrastructure of Amicia

Previous palynological analyses of *Amicia andicola*, *A. fimbriata*, *A. medicaginea*, and *A. zygomeris* (Ohashi 1971; Pire 1974) confirmed the circumscription of the species with an endoaperture of type I pollen and without an endoaperture of type II pollen. Our results also confirmed the type I pollen subtypes Ia and Ib based on the colporus and endoaperture characteristics, and measurements supported this subdivision. However, our results did not support previous results regarding the class of pollen size, endoaperture class and ultrastructure of operculum and sexine (Ohashi 1971; Pire 1974).

Table IV compiles our results and previous pollen descriptions of the analysed species found in the literature.

The exine ultrastructure has not been described previously for *Amicia* pollen grains. Antonio-Domingues et al. (2022) reported granules in the infratectum of *Poiretia*, a genus of the Adesmia clade phylogenetically related to *Amicia*. Previous studies have also observed the granular structure in the Papilionoideae subfamily (Ferguson & Skvarla 1983; Antonio-Domingues et al. 2019).

The combination of granules with columellae and the characteristic endexine thickness with a discontinuous or absent foot layer represents an intermediate infratectum condition and characterises a derived pollen structure in the subfamily (Ferguson & Skvarla 1983; Ridder-Numan & Ham 1997; Doyle 2008).

Palynotaxonomic implications for the systematics of the Lobbiana clade

The Lobbiana clade appears weakly supported in phylogenetic studies and comprises *Amicia andicola*, *A. glandulosa*, *A. lobbiana* and *A. zygomeris* (Särkinen & Hughes 2015). The Lobbiana clade (*sensu* Särkinen & Hughes 2015) can be recognised palynologically by the absence of endoaperture, except in *A. zygomeris* where the endoaperture is absent (= pollen type II). When present, the endoaperture length and width are less than 14 μm , equatorial axes less than 36 μm , and colporus length and width less than 30 μm and 8 μm , respectively (= pollen type Ib) (Figure 8). Except for *A. zygomeris* (bottom left side of PCA and 0% similarity with Lobbiana representatives), all Lobbiana clade species were grouped on the right part of the PCA (Figure 6) and presented more than 60% similarity (Figure 7).

The common type of aperture in Leguminosae is tricolporate with a small circular or lalongate endoaperture. The absence of endoaperture, as in *Amicia zygomeris*, is regarded as a trend towards

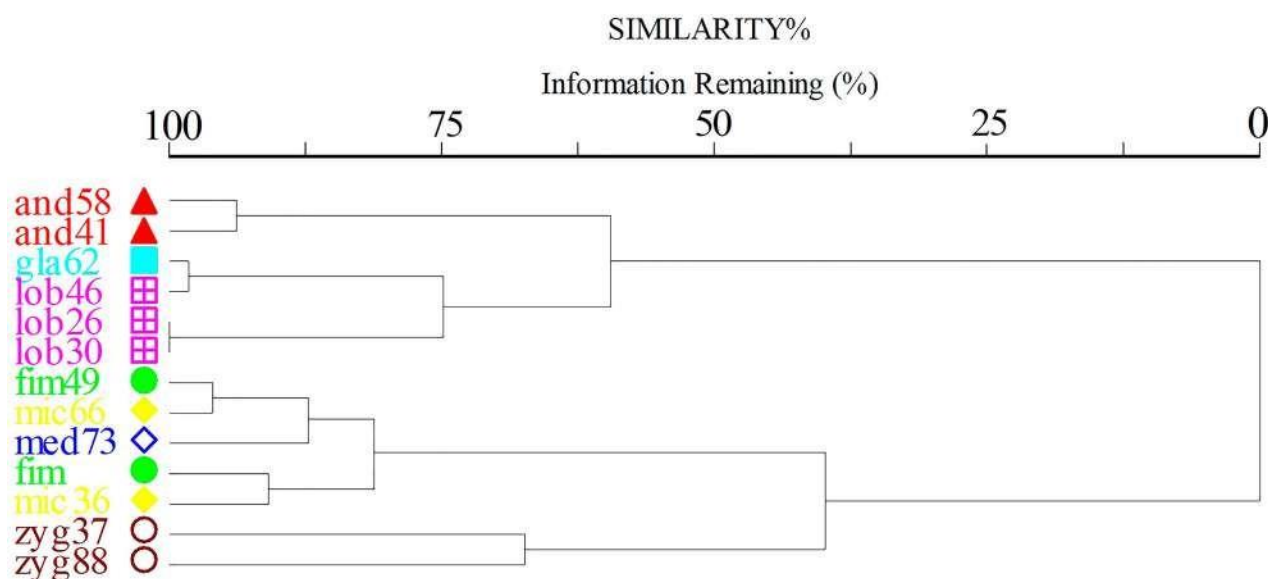


Figure 7. Dendrogram built from cluster analysis (Euclidean distance) for *Amicia* specimens, information remaining (%).

Table IV. Pollen morphology data from previous published studies compared with the present study.

Species	Author	Size class	Endoaperture class	Operculum ultrasculpture	Sexine ultrasculpture	
					Mesocolpium	Apocolpium
<i>Amicia andicola</i>	P 1974	L	Circular to lolongate	Reticulate	Heterobrochate	
	PS	M	Circular to lolongate	Areolate-granulate	Rugulate-perforate	Perforate
<i>Amicia fimbriata</i>	P 1974	L	Lolongate	Present	Heterobrochate	
	PS	M-l	Circular to lolongate	Areolate-granulate	Nanoreticulate to microreticulate	
<i>Amicia medicaginea</i>	P 1974	M	Lolongate	Present	Heterobrochate	
	PS	M	Circular	Areolate-perforate	Rugulate-perforate	
<i>Amicia zygoteris</i>	O 1971	M	Absent	Present	Heterobrochate	
	P 1974	L	Absent	Present	Heterobrochate	
	PS	M	Absent	Psilate-perforate	Rugulate-perforate	

L, large; M, medium; O 1971, Ohashi 1971; P 1974, Pire 1974; PS, present study.

specialisation (Ferguson & Skvarla 1981). Within the *Adesmia* clade, *A. zygoteris* and all species of *Zornia* also in the *Zornia-Amicia-Poiretia* clade did not present an endoaperture (Melhem 1966; Ohashi 1971; Pire 1974; Carreira et al. 1996; Silvestre-Capelato & Melhem 1997; Moreti et al. 2007; Buriel et al. 2011; Luz et al. 2013; Silva et al. 2016). *Amicia zygoteris* is the only species of *Amicia* in North America and is distinct in growth habit, androecium, and fruit morphology, as well as having a unique pollen morphology (Särkinen & Hughes 2015). *Amicia zygoteris* and *A. andicola* form a clade (Figure 8) and share the palynological characteristics of margo psilate-perforate and mesocolpium rugulate-perforate (Table II).

Populations of *Amicia lobbiana* from the Huánuco valley in central Peru have geographically disjunct populations in Bolivia that show the most pronounced intraspecific lineage divergences (Särkinen & Hughes 2015; Figure 8). One specimen from the Huánuco population (lob46) analysed in this study has approximately 75% similarity (Figure 7) with the other two specimens (lob26, lob30) belonging to other populations, which had 100% similarity (Figure 7). The Huánuco specimen has smaller axis, colporus and endoaperture dimensions than specimens from southern Peru and Bolivia (Table I), demonstrating that population isolation and divergent lineages in *A. lobbiana* are reflected in pollen morphology.

Palynotaxonomic implications for the systematics of the *Medicaginea* clade

Unlike the *Lobbiana* clade, the *Medicaginea* clade comprising *Amicia fimbriata*, *A. medicaginea*, and *A. micrantha* is strongly supported and has a distinctive diagnostic morphological character

(Särkinen & Hughes 2015). As shown in Figure 8, our results show that this clade is supported by a set of pollen characters, including equatorial axes greater than 36 μm , colporus length and width greater than 30 and 8 μm , respectively, and endoaperture length and width both greater than 14 μm (= pollen type Ib). All *Medicaginea* clade members were grouped on the left side of the PCA (Figure 6) and had more than 80% similarity (Figure 7), reinforcing the importance of pollen morphology and its close association with phylogenetic data (Figure 8).

Morphologically, *Amicia fimbriata* can be confused with *A. micrantha* (Särkinen & Hughes 2015). The nanoreticulate to microreticulate sexine ornamentation of the mesocolpium and apocolpium (vs rugulate-perforate in both regions of *A. micrantha*) represents an informative trait for species delimitation (Table II).

Conclusions

Characterising the pollen grains of all seven *Amicia* species has provided phylogenetically informative palynological data. Our study contributes data to the taxonomy of *Amicia* and the morphological characterisation of the infrageneric *Lobbiana* and *Medicaginea* clades.

Additionally, pollen morphology reflects population isolation of the divergent lineages of *Amicia lobbiana* from the Huánuco valley in central Peru and those from southern Peru and Bolivia. It also reflects the disjunct distribution of the genus in South America and Mexico. Variations in the presence and absence of an endoaperture, the size of the colporus and endoaperture and overall pollen size are the main morphological characteristics assisting recognition of the pollen types and

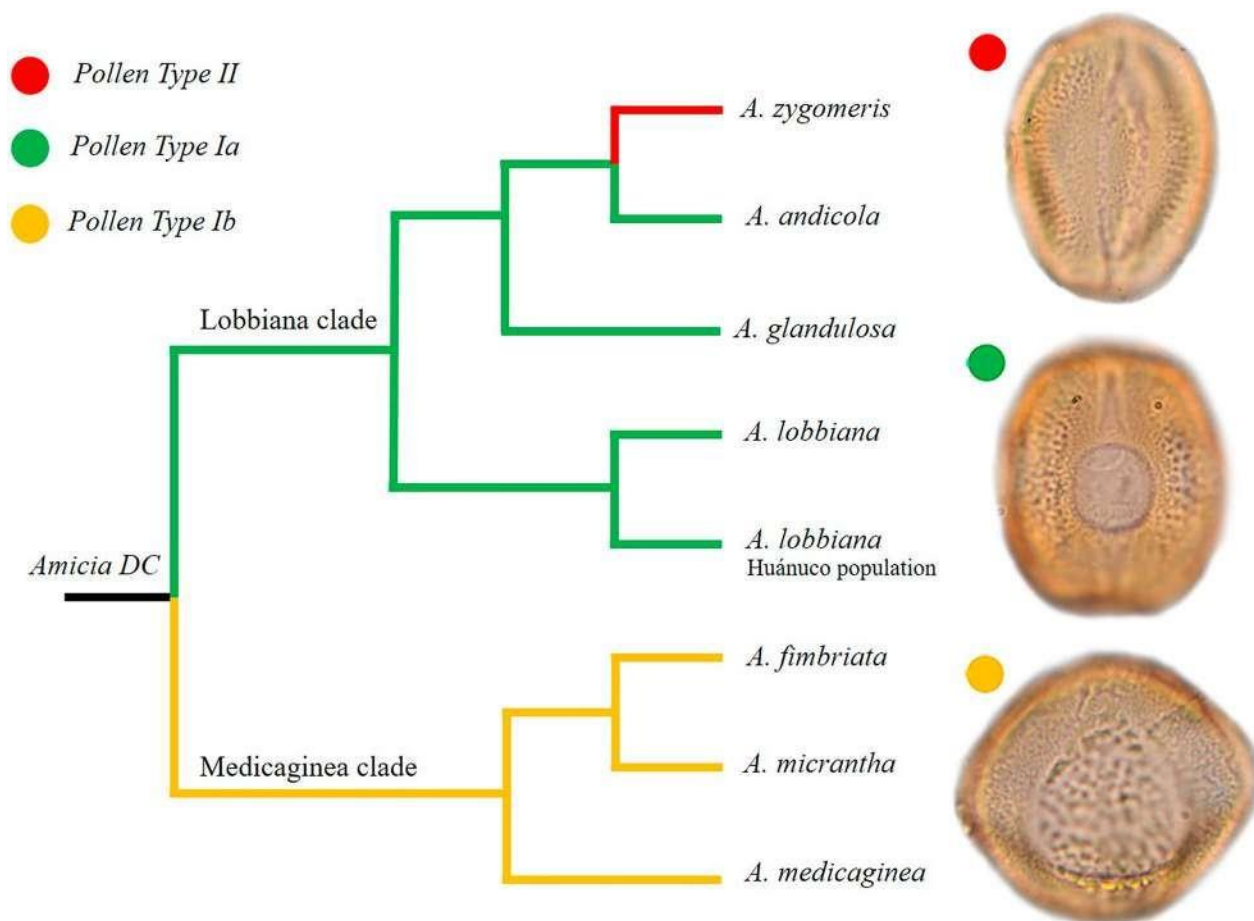


Figure 8. Phylogenetic tree for *Amicia* with pollen types coloured and pollen morphology mapped on to each species. The tree is redrawn from Särkinen and Hughes (2015). In pollen type II the endoaperture is absent and the pollen shows a narrow colpus, here represented by *A. zygomeris*. In pollen type Ib, the endoaperture is narrow and short and the pollen shows a narrow colpus, here represented by *A. glandulosa*. In pollen type IIB, the endoaperture and colporus are broad, here represented by *A. medicaginea*.

infrageneric taxa. Likewise, endoaperture type and ornamentation of the operculum, margo and sexine are important palynological diagnostic characters which aid species delimitation.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Specimens investigated

Amicia Kunth

Amicia andicola (Griseb.) Harms. Bolivia: Chuquisaca, Sucre. J.R.I. Wood 8092, 13 March 1994 (K 000556941). Bolivia: Chuquisaca, Oropeza. J.R.I. Wood 8097, 13 March 1994 (MO 100929458).

Amicia fimbriata Harms ex Kuntze. Bolivia: Chuquisaca, Zudañez. J.R.I. Wood 22400, 4 March 2066 (MO 100929849). Bolivia. Bridges *s.n.*, *s.d* (K).

Amicia glandulosa Kunth. Ecuador: Loja – Catamayo, La Toma. G.P. Lewis 3185, 22 April 1997 (NY 01912662).

Amicia lobbiana Benth. ex Rusby. Peru: Huánuco, Ambo. J. Aronson, P.E.B. Aronson 603, 4 August 1978 (MO 72746). Peru: Cusco, Paucartambo. T.E. Särkinen 2244, 9 May 2007 (MO 100929430). Bolivia. T Särkinen 2145, 7 April 2007 (E 274126).

Amicia medicaginea Griseb. Bolivia: Potosí, Cornelio Saavedra. G. Torrico, C.P.T. Pardo 475, 29 April 1993 (MO 3352473).

Amicia micrantha Harms ex Kuntze. Bolivia: Chuquisaca, Zudañez. J.R.I. Wood 23303, 31 March 2007 (MO 10092966). Bolivia: Santa Cruz, Valle Grande. M. Dematteis 3636, 9 March 2009 (K).

Amicia zygoteris DC. México: Puebla, Zacapoxtla. J.D. Amith 2687, 14 October 2015 (US 3740488). Mexico: Temascaltepec. G.B. Hinton 808, 27 May 1932 (K 000118237).

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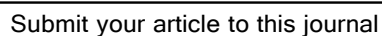
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The study of pollen grains as a contribution to the taxonomy of *Nissolia* (Leguminosae – Papilionoideae – Dalbergieae)

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Abstract

Nissolia has one of the most complex taxonomic histories of the informal *Adesmia* clade. In order to find additional taxonomic relevant characters for this genus, we studied the pollen morphology, ultrastructure, and ultrastructure of 21 species and a single variety of *Nissolia* using light, scanning, and electron transmission microscopy. Additionally, a principal component analysis was performed to elucidate patterns of quantitative data variation among species concerning the most recent molecular data. Three pollen types are described based on mesocolpium sexine ornamentation. Furthermore, pollen grain size and shape, class of endoaperture, ultrastructure of the operculum, membrane, and apocolpium sexine were informative pollen features for species recognition and delimitation in *Nissolia*. We conclude that pollen morphology and pollen types are related to species and phylogenetic distribution in *Nissolia*, providing valuable characters for taxonomic studies in the *Adesmia* clade.

Keywords: Chaetocalyx, *Dalbergia* clade, *Fabaceae*, informal *Adesmia* clade, pollen morphology

Nissolia Jacq. (Dalbergieae – Papilionoideae – Leguminosae) is a genus placed within the informal *Adesmia* clade (Klitgaard & Lavin 2005) with four other genera, *Adesmia* DC., *Amicia* Kunth, *Poiretia* Vent., and *Zornia* J.F. Gmel (Fortuna-Perez et al. 2013, 2021). Recently, a new taxonomic circumscription of *Nissolia* was published, including *Chaetocalyx* DC. as a new synonym (Moura et al. 2018b) as the result of extensive phylogenetic studies based on nuclear ribosomal and plastid data (Lavin et al. 2001; Pennington et al. 2004;

Fortuna-Perez et al. 2013; Iganci et al. 2013; Särkinen & Hughes 2015).

Nissolia comprises c. 32 species and a single variety (13 of them occurring in Brazil, and seven being endemics). The species of this genus morphologically recognised as woody, twining vines, with compound leaves with 5-17-leaflets, and a lomentaceous fruit with all articles fertile or with the terminal article forming a flattened sterile wing (rendering the fruit samaroid) (Rudd 1956, 1958; Moura et al. 2018b; Silva 2021). *Nissolia* is Neotropical and distributed

from southern United States (Arizona and Texas) to southern South America (Argentina and Uruguay), with two main centres of diversity in Mexico and Brazil (Moura et al. 2018b).

A majority-rule consensus tree of the *Adesmia* clade resulting from a Bayesian analysis of the rDNA ITS/5.8S evidenced *Nissolia* with two highly supported subclades, one predominantly Mexican and Central American with samaroid fruits and another South American with non-samaroid fruits. These clades equate to *Nissolia* and the previously recognised *Chaetocalyx* (Fortuna-Perez et al. 2021; Silva 2021).

Although pollen morphology, ultrasculpture, and ultrastructure represent important taxonomic characters in many papilionoid legumes (including Dalbergieae; Ferguson & Skvarla 1981; Antonio-Domingues 2017; Antonio-Domingues et al. 2019; Jiang et al. 2019; Lattar et al. 2020), only species of *Poiretia* and *Amicia* have previously been samples concerning these pollen characters in the *Adesmia* clade (Antonio-Domingues et al. 2022a, 2022b). Several authors have palynologically analysed various species of *Nissolia* (Ohashi 1971; Pire 1974; Carreira et al. 1996; Lattar et al. 2020), but their studies lacked any exine ultrasculpture and ultrastructure observations. Our study expands on previous research and adds new pollen data to the overall taxonomy of *Nissolia*.

Material and methods

Unopened mature flower buds were obtained from 45 specimens belonging to 21 species and a single variety of *Nissolia*: *N. acutifolia* (Vogel) T.M. Moura et Fort.-Perez, *N. bracteosa* (Rudd) T.M. Moura et Fort.-Perez, *N. brasiliensis* (Vogel) T.M. Moura et Fort.-Perez, *N. chacoensis* (Vanni) T.M. Moura et Fort.-Perez, *N. chiapensis* Rudd, *N. fruticosa* Jacq., *N. fruticosa* var. *guatemalensis* Jacq., *N. gentryi* Rudd, *N. klugii* (Rudd) T.M. Moura et Fort.-Perez, *N. latisiliqua* (Poir.) T.M. Moura et Fort.-Perez, *N. laxior* (B.L. Rob.) Rose, *N. leiogyne* Sandwith, *N. longiflora* (Benth. ex A. Gray) T.M. Moura et Fort.-Perez, *N. microptera* Poir., *N. nigricans* (Burkart) T.M. Moura et Fort.-Perez, *N. platycarpa* Benth., *N. pringlei* Rose, *N. schottii* (Torr.) A. Gray, *N. subulata* (Mackinder) I. Castro et Fort.-Perez, *N. tomentosa* (Gardner) T.M. Moura et Fort.-Perez, *N. vincentina* (Ker Gawl.) T.M. Moura et Fort.-Perez and *N. wislizeni* (A. Gray) A. Gray from the BOTU, HST, K, MBM, MO, NY, SP, UB and US herbaria (herbarium acronyms follow Thiers [continuously updated]). We follow the rules for herbarium sampling recently

proposed by Almeida et al. (2023), with all specimens examined being listed in the Specimens investigated section. Samples were labelled with a three-letter abbreviation of the species epithet plus the last two numbers of the herbarium voucher or collect number (see Table I, Specimen column).

Pollengrains were treated with the acetolysis method (Erdtman 1960), following the modifications proposed by Melhem et al. (2003). Measurements were made under a light microscope using an Olympus OSM-4 (10×) micrometer drum coupled to the eyepiece of an Olympus BX50 light binocular microscope. The polar and equatorial axes in equatorial views were measured on 25 randomly selected pollen grains from each specimen. These measurements were statistically analysed for the arithmetic mean ($\bar{x}$), average standard deviation ($S\bar{x}$), sample standard deviation (s), coefficient of variability ($V\%$), and 95% confidence interval (CI) (Vieira 2011; Zar 2010). Ten measurements of the prominent pollen morphometric parameters were also made in the length and width of the colpus, the length and width of the endoaperture, and the thickness of the nexine and sexine layers. Exine measurements were also taken in the mesocolpium region. The arithmetic means, and the range was calculated for each pollen grain size class and exine (sexine + nexine). Photomicrographs were obtained using an Olympus BX 50 light microscope with an Olympus U-CMAD-2 video camera and Olympus CellSens 1.5 software. Permanent light microscopy (LM) slides were deposited in the pollen reference collection of the Laboratório de Palinologia PALINO-IPA, Instituto de Pesquisas Ambientais, State of São Paulo, Brazil.

To avoid the loss of the operculum and exine elements covering the aperture (Hesse & Waha 1989), non-acetolysed pollen grains were washed and placed on a metal stub and sputter coated (Leica EM ACE 600; Leica, Vienna, Austria) with gold (80 nm) for scanning electron microscopy (SEM). Samples were observed under a JEOL JSMIT300LV microscope (Tokyo, Japan) under a 20 kV electron beam and the images were digitised.

For the transmission electron microscopy (TEM), mature anthers were removed from flower buds of four species of *Nissolia* (*N. longiflora*, *N. nigricans*, *N. subulata*, and *N. tomentosa*) and fixed in a modified Karnovsky solution (Karnovsky 1965) for at least 24 h. They were subsequently rinsed with sodium cacodylate buffer (0.1 M) and immediately post-fixed in osmium oxide (1%) for 1 h. Samples were then rinsed in a saline solution (0.9% sodium chloride) and pre-stained with uranyl acetate (2.5%), followed by dehydration in an acetone series (35–100%), 20 min each.

Table I. *Nissolia* Jacq. dimensions (μm) pollen grains in equatorial view using light microscopy.

Specimen	Polar axis				Equatorial axis				<i>P/E</i> <i>x</i>	Pollen size	Colporus Length-Width	Endoaperture		Exina	
	CI- ($x \pm Sx$)	CI+	<i>s</i>	<i>V%</i>	CI- ($x \pm Sx$)	CI+	<i>s</i>	<i>V%</i>				Width (Cen)	Length	<i>N</i>	<i>S</i>
acu07	30.0 (30.5 $\pm$ 0.2)	31.0	1.2	3.8	23.8 (23.3 $\pm$ 0.3)	27.7	1.3	5.5	1.3	S-M	26.2-4.0	9.0 (1.0)	9.4	0.6	1.0
brac43	39.9 (40.5 $\pm$ 0.3)	41.0	1.3	3.3	29.8 (29.5 $\pm$ 0.3)	30.1	1.5	5.2	1.4	M	34.2-3.1	11.7 (0.9)	12.9	0.6	0.9
brac34	35.8 (36.6 $\pm$ 0.4)	37.3	1.8	5.0	28.8 (29.5 $\pm$ 0.3)	30.2	1.7	5.6	1.2	M	30.1-4.0	13.1 (1.1)	12.0	0.5	0.8
bras00	40.2 (40.8 $\pm$ 0.3)	41.5	2.0	4.9	25.6 (26.4 $\pm$ 0.4)	27.2	2.4	8.9	1.6	M	34.0-3.6	11.3 (0.9)	12.1	0.5	1.1
bras25	40.2 (40.8 $\pm$ 0.3)	41.5	2.0	4.9	25.6 (26.4 $\pm$ 0.4)	27.2	2.4	8.9	1.6	M	31.7-4.3	11.5 (1.0)	12.1	0.6	1.0
bras57	38.2 (38.9 $\pm$ 0.3)	39.5	1.4	3.6	28.2 (28.7 $\pm$ 0.2)	29.2	1.2	4.3	1.4	M	32.7-3.9	11.0 (0.9)	11.7	0.6	1.1
cha66	29.2 (29.6 $\pm$ 0.1)	29.9	1.1	3.7	20.7 (20.9 $\pm$ 0.1)	21.0	0.5	2.2	1.4	S-M	23.6-4.2	8.0 (0.8)	9.9	0.5	0.7
chi42	23.7 (24.2 $\pm$ 0.3)	24.8	1.3	5.3	20.9 (21.5 $\pm$ 0.3)	22.1	1.4	6.5	1.1	S	18.4-3.1	8.9 (1.5)	6.2	0.5	1.0
fru16	22.7 (23.2 $\pm$ 0.2)	23.7	1.1	4.8	22.5 (22.8 $\pm$ 0.2)	23.1	0.8	3.4	1.0	S	19.6-3.5	9.5 (1.6)	3.1	0.5	0.8
fru48	24.3 (24.8 $\pm$ 0.2)	25.3	1.2	4.9	22.3 (23.1 $\pm$ 0.2)	23.3	1.2	5.0	1.1	S-M	19.8-3.3	9.1 (1.3)	7.3	0.5	0.8
fru71	22.6 (23.1 $\pm$ 0.2)	23.6	1.2	5.3	22.1 (22.7 $\pm$ 0.3)	23.3	1.6	6.9	1.0	S	18.8-3.2	9.5 (1.6)	6.0	0.6	1.0
fru56	23.1 (23.6 $\pm$ 0.2)	24.0	1.2	5.0	22.7 (23.0 $\pm$ 0.2)	23.4	0.9	4.0	1.0	S	19.0-3.6	10.8 (1.7)	6.3	0.5	0.8
fru73	23.0 (23.4 $\pm$ 0.2)	23.9	1.1	4.6	22.9 (23.2 $\pm$ 0.2)	23.5	0.8	3.5	1.0	S	18.0-3.6	10.3 (1.6)	6.7	0.6	0.9
gen70	23.4 (23.8 $\pm$ 0.2)	24.2	1.0	4.3	22.0 (22.3 $\pm$ 0.1)	22.5	0.7	2.9	1.1	S	20.3-3.2	9.3 (1.4)	6.7	0.5	0.9
klu60	32.4 (32.9 $\pm$ 0.2)	33.4	1.2	3.7	24.9 (25.4 $\pm$ 0.3)	26.0	1.4	5.4	1.3	S-M	26.0-3.7	9.5 (0.9)	10.4	0.6	1.1
klu75	31.7 (32.3 $\pm$ 0.3)	32.9	1.5	4.6	25.0 (26.0 $\pm$ 0.5)	26.9	2.4	9.2	1.3	M	28.6-3.9	10.2 (1.0)	10.1	0.6	1.1
lat73	27.1 (27.8 $\pm$ 0.3)	28.5	1.7	6.3	22.6 (23.1 $\pm$ 0.2)	23.6	1.2	5.0	1.2	S-M	23.6-4.6	9.3 (1.2)	7.5	0.6	0.9
lat49	34.6 (34.2 $\pm$ 1.3)	36.8	6.4	18.6	27.7 (29.3 $\pm$ 0.8)	31.6	4.0	13.7	1.2	M	26.9-4.6	11.4 (1.1)	11.0	0.6	1.1
lat92	25.8 (26.3 $\pm$ 0.2)	26.8	1.2	4.4	21.1 (21.4 $\pm$ 0.1)	21.6	0.7	3.1	1.2	S-M	22.2-4.0	7.8 (1.1)	6.9	0.6	1.1
lax63	22.8 (23.3 $\pm$ 0.2)	23.8	1.1	4.8	20.9 (21.3 $\pm$ 0.2)	21.7	0.9	4.2	1.1	S-M	18.1-2.8	8.1 (1.4)	6.0	0.6	0.9
lei70	22.8 (23.2 $\pm$ 0.2)	23.6	0.9	3.8	20.9 (21.2 $\pm$ 0.2)	21.5	0.8	3.6	1.1	S	16.3-2.8	9.2 (1.5)	6.2	0.5	0.9
lei87	25.4 (25.9 $\pm$ 0.2)	26.4	1.1	4.3	20.2 (20.5 $\pm$ 0.2)	20.9	0.8	4.0	1.3	S-M	22.2-3.3	8.1 (1.3)	6.6	0.5	0.7
lon01	33.6 (34.3 $\pm$ 0.4)	35.1	1.8	5.4	26.5 (26.9 $\pm$ 0.2)	27.4	1.1	4.1	1.3	M	29.3-4.9	9.9 (1.1)	9.4	0.6	1.3
lon07	33.7 (34.6 $\pm$ 0.4)	34.5	2.0	5.9	25.8 (26.4 $\pm$ 0.3)	27.8	1.4	5.4	1.3	M	28.0-5.5	11.6 (1.2)	9.8	0.6	1.1
lon30	39.1 (39.5 $\pm$ 0.2)	39.9	1.0	2.6	28.3 (28.6 $\pm$ 0.2)	29.0	1.0	3.4	1.3	M	28.7-4.9	11.7 (1.1)	10.6	0.5	1.0
mic68	27.1 (27.4 $\pm$ 0.1)	27.7	0.7	2.5	20.8 (21.1 $\pm$ 0.2)	21.4	0.8	3.6	1.3	S-M	20.7-2.4	9.0 (1.2)	7.6	0.5	0.7
mic50	22.0 (22.3 $\pm$ 0.1)	22.6	0.8	3.5	20.4 (20.7 $\pm$ 0.1)	21.0	0.7	3.5	1.1	S	18.6-3.3	5.9 (1.1)	5.6	0.5	0.7
nig04	34.3 (34.9 $\pm$ 0.3)	35.5	1.9	5.5	27.6 (28.2 $\pm$ 0.3)	28.9	2.1	7.6	1.2	M	31.4-4.9	11.8 (1.2)	4.9	0.6	1.1
nig93	30.0 (30.7 $\pm$ 0.3)	31.3	1.6	5.1	26.1 (26.6 $\pm$ 0.2)	27.1	1.2	4.4	1.2	M	26.0-5.0	11.0 (1.2)	9.0	0.7	1.2
nig83	33.0 (33.7 $\pm$ 0.3)	34.3	1.6	4.7	27.5 (27.8 $\pm$ 0.2)	28.1	0.8	2.8	1.2	M	29.9-4.4	10.2 (1.0)	9.9	0.5	1.1
nig36	32.8 (33.5 $\pm$ 0.3)	34.2	1.7	5.2	26.9 (27.5 $\pm$ 0.3)	28.1	1.5	5.2	1.2	M	28.7-4.9	10.9 ($\pm$)	9.0	0.6	1.4
pla14	22.7 (23.0 $\pm$ 0.1)	23.3	0.7	3.3	20.5 (20.8 $\pm$ 0.1)	21.1	0.7	3.5	1.1	S	20.4-3.6	9.2 (1.3)	9.8	0.5	0.8
pla90	23.6 (23.9 $\pm$ 0.1)	24.2	0.6	2.7	21.4 (21.8 $\pm$ 0.2)	22.2	0.9	4.3	1.1	S	19.2-3.5	9.1 (1.4)	6.8	0.6	0.9
pri66	22.5 (23.0 $\pm$ 0.2)	23.5	1.1	5.0	20.6 (20.9 $\pm$ 0.2)	21.2	0.8	3.6	1.1	S	17.2-3.0	8.6 (1.3)	6.7	0.5	0.8
sch01	22.1 (22.5 $\pm$ 0.2)	22.8	0.8	3.4	20.7 (21.1 $\pm$ 0.2)	21.4	0.8	3.9	1.1	S	18.4-3.0	8.0 (1.4)	5.6	0.6	0.8
sub44	37.8 (38.6 $\pm$ 0.4)	39.5	2.0	5.1	29.9 (30.7 $\pm$ 0.4)	31.4	1.9	6.0	1.3	M	31.6-4.3	12.5 (1.0)	12.3	0.7	1.6
sub98	36.6 (37.0 $\pm$ 0.2)	37.4	1.0	2.6	30.2 (30.6 $\pm$ 0.2)	31.0	1.0	3.2	1.2	M	31.1-4.4	10.7 (0.8)	14.2	0.5	0.8
tom04	24.6 (35.2 $\pm$ 0.3)	25.9	1.6	4.4	27.3 (28.0 $\pm$ 0.3)	28.5	1.5	5.3	1.3	M	31.0-4.0	12.1 (1.2)	10.3	0.6	1.1
tom64	39.2 (40.2 $\pm$ 0.5)	41.2	2.4	6.1	31.7 (33.3 $\pm$ 0.8)	35.0	3.9	11.0	1.2	M	34.0-4.2	11.3 (0.8)	14.5	0.6	1.0
vic73	29.9 (29.7 $\pm$ 0.2)	30.1	1.1	3.7	20.7 (21.3 $\pm$ 0.3)	21.9	1.4	6.6	1.4	S-M	22.7-3.9	8.9 (0.9)	9.9	0.6	0.8

(Continued)

Table 1. (Continued).

Specimen	Polar axis			Equatorial axis			P/E	Pollen size	Colporus Length-Width	Endoaperture		Exina	
	CI- (x ± SX)	CI+	s	V%	CI- (x ± SX)	CI+	x			Width (Cen)	Length	N	S
vic95	27.8 (28.2 ± 0.2)	28.7	1.1	4.0	20.5 (20.9 ± 0.2)	21.3	1.4	S-M	27.3-4.1	9.8 (1.2)	7.8	0.5	0.8
vic44	27.2 (28.0 ± 0.4)	28.8	2.0	7.0	19.3 (19.8 ± 0.2)	20.3	1.4	S-M	23.8-4.0	8.7 (1.1)	7.9	0.5	1.1
vic74	27.7 (28.1 ± 0.2)	28.5	1.0	3.7	20.4 (20.7 ± 0.1)	21.0	1.4	S-M	24.4-3.3	7.4 (0.9)	8.7	0.5	0.8
wis76	24.5 (24.9 ± 0.2)	23.5	1.0	3.9	21.0 (21.3 ± 0.1)	21.6	1.2	S	20.5-3.1	8.8 (1.5)	6.0	0.5	0.7
wis54	23.7 (24.0 ± 0.2)	24.4	0.8	3.4	20.4 (20.7 ± 0.1)	21.0	1.2	S	20.0-3.1	8.7 (1.4)	6.5	0.5	0.7

Note. Specimens were labelled with a three-letter abbreviation of the species epithet plus the last two numbers of the herbarium voucher or collect number (see Appendix). Confidence interval at 95% probability of the lowest sample values (CI-), arithmetic mean (x), average standard deviation (SX), sample standard deviation (s), coefficient of variability (V%).

Samples were then gradually infiltrated in Spurr resin (Spurr 1969) and embedded in pure resin for 48 h at 70 °C. Ultrathin sections (60 nm) were cut using an ultramicrotome (Sorvallter Blum MT2, Norwalk, CT, USA), deposited on copper grids and stained using uranyl acetate (2.5%) and lead citrate (Reynolds 1963). Images were obtained under a transmission electron microscope (JEOL JEM 1400; JEOL, Tokyo, Japan) at 80 kV.

Pollen terminology is based on Punt et al. (2007) and Halbritter et al. (2018). Pollen shape classes and amb types follow Erdtman (1952), and colpus length categories follow Mark et al. (2012). The endoaperture classes and index of sexine and nexine thickness were proposed by Antonio-Domingues et al. (2022a). TEM descriptions of the exine infratectum follow the terminologies of Halbritter et al. (2018) and Ferguson and Skvarla (1983).

A principal component analysis (PCA) was performed to explore whether pollen grain characters corroborate species and specimens grouping. Nine metric variables (polar axis [PA], equatorial axis [EA], colpus length [CL], colpus width [CW], endoaperture length [EL], endoaperture width [EW], nexine thickness [N], sexine thickness [$S = \text{infratectum} + \text{tectum}$], exine thickness [$E = N + S$]) and three classes/indexes (shape class [$P/E = PA/EA$], index of sexine and nexine thickness [S/N]) were analysed for ordination using FITOPAC (Shepherd 1996) and PC-ORD version 7 (McCune & Mefford 2016). To understand how *Nissolia* specimens relate to each other, based on pollen morphology, a cluster analysis (CA) was performed using the software PC-ORD version 7 (McCune & Mefford 2016). This produced a similarity dendrogram by calculating the Euclidean distance based on the same nine PCA metric variables and the three classes/indexes.

Results

General description of *Nissolia* pollen grains

Pollen grains in monads, small to medium-sized, radially symmetrical, isopolar, prolate spheroidal to prolate, amb circular to subtriangular, angulaperturate or planaperturate (Figures 1, 3, 5, 7, Table 1); polar area small to very small, 3-zonocolporate, with long to very long, narrow, constricted or non-constricted colporus (Figures 1A, D, G, J, M, 3A, D, G, J, M, 5A, D, G, J, M, 7A, D, G, J, M) with pointed apices (Figures 1B, E, H, K, N, 3B, E, H, K, N, 5B, E, H, K, N, 7B, E, H, K, N). Colpi margos are broad or narrow, psilate-perforate,

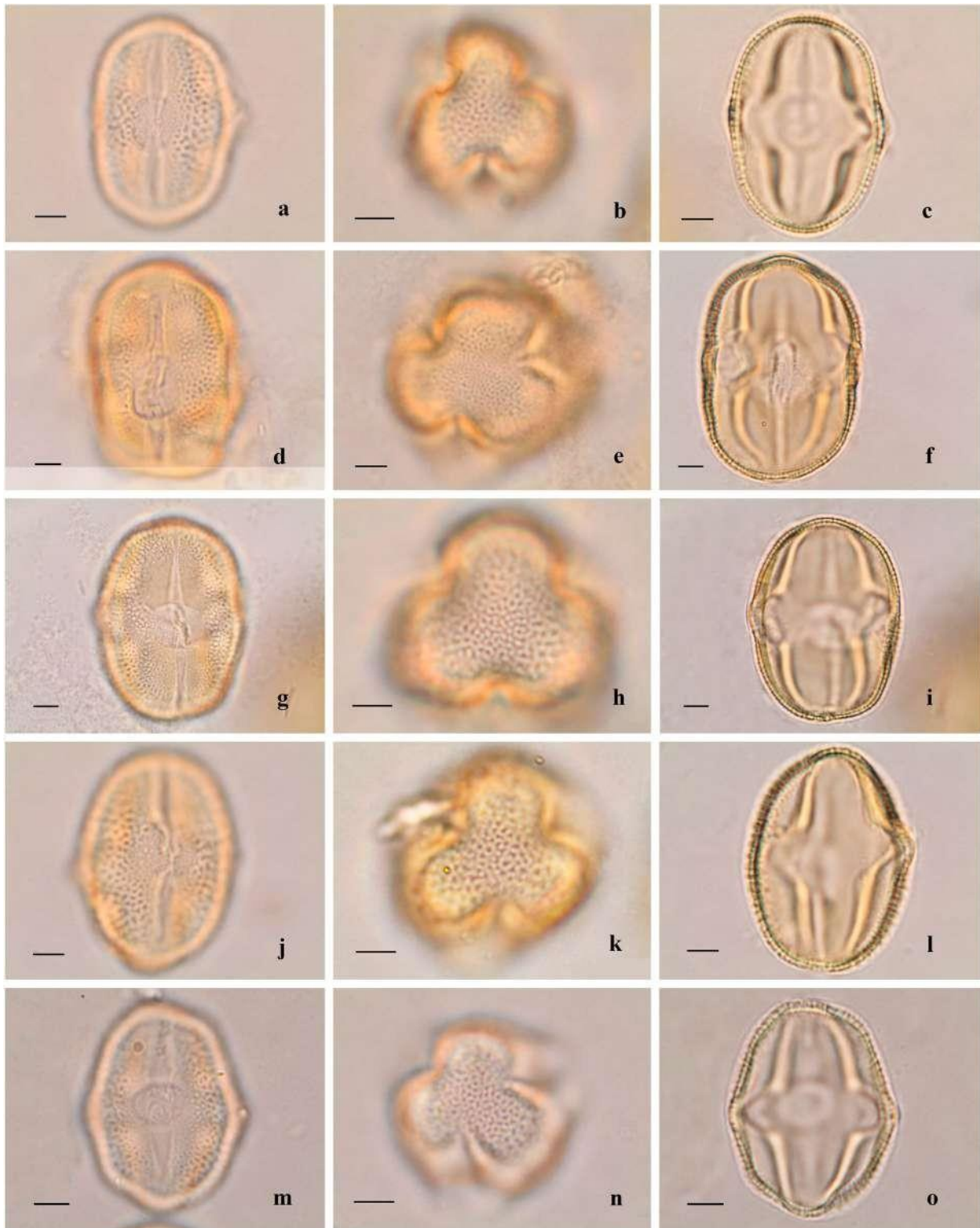


Figure 1. Light microscopy of *Nissolia* Jacq. species: pollen type Ia. A–C. *Nissolia acutifolia*. A. Detail of the colporus, endoaperture, operculum and surface, equatorial view. B. Apertures 3-colporate, long and pointed apices and apocolpium surface. C. Optical section, equatorial view. D–F. *Nissolia bracteosa*. D. Detail of the colporus, endoaperture, surface and operculum, equatorial view. E. Apertures 3-colporate, long and pointed apices and apocolpium surface. F. Optical section, equatorial view. G–I. *Nissolia brasiliensis*. G. Detail of the colporus, surface and operculum, equatorial view. H. Apertures 3-colporate, long and pointed apices and apocolpium surface. I. Optical section, equatorial view. J–L. *Nissolia kluggii*. J. Detail of the colporus, endoaperture and surface, equatorial view. K. Apertures 3-colporate, long and pointed apices and apocolpium surface. L. Optical section, equatorial view. M–O. *Nissolia latisiliqua*. M. Detail of the colporus, endoaperture, operculum and surface, equatorial view. N. Apertures 3-colporate, long and pointed apices and apocolpium surface. O. Optical section, equatorial view. Scale bars – 5 μ m.

prominent over the endoapertures, forming a protrusion (Figures 2E, H, K, 3B, E, K, 6B, E, H, N, 8K, E, H, K, N), creating a constriction in LM in the middle of the colpi on both sides of the touching margins (Figures 1A, D, J, 3A, J, 5A, G, 7A, D, G). The space/cavity formed below the domed sexine is devoid of nexine since the nexine delimits the endoapertural area's boundary, i.e. without fastigium. In *Nissolia gentryi* (Figure 6J, K) and *N. wislizeni* (Figure 4G, H), the colpi do not show any protrusion. An operculum with sparsely or densely granulate or areolate-granulate ornamentation or with rugulae and granules or an operculum absent. Colporus membrane sparsely or densely granulate, areolate-granulate or with microrugulate-granulate ornamentation. Endoaperture circular, lalongate, very lalongate, lolongate or very lolongate (Table I). Aberrant pollen grains infrequently occur in *Nissolia*, e.g. 2-colporate with a marked polar constriction or apolar pollen grains of abnormal size and 5-pantocolporate apertures (Figure 7F).

Sexine with heterobrochate, nanoreticulate to reticulate ornamentations and a network-like pattern with predominantly rounded lumina. Straight to curved muri, simple-columellate, continuous or interrupted, with wall connections at one level, or sexine with rugulate-perforate, perforate to fossulate-perforate ornamentations. Sexine 1.1–2.8 times thicker than the nexine and sometimes thickened in the apocolpium or mesocolpium areas (Table I). A summary of the measurements is shown in Table I, and additional observations and descriptions are provided in Table II.

Exine ultrastructure

The exine has an electron-translucent (e-translucent) and electron-dense (e-dense) compact tectum that varies from eutectate (Figure 9J–L), semitectate (Figure 9A, D, E) to tectate-perforate (Figure 9B, E). The tectum is smooth or undulated with an e-translucent or e-dense *pollenkitt*.

We observed two infratectal structures: (1) granules somewhat organised in a narrow, irregular row; (2) simple, complete and or incomplete columellar. Combinations of the two types occurred in the four species analysed. We also observed an e-translucent and e-dense infratectal *pollenkitt* (Figure 9D, G, H).

The thin foot layer was compact, with channels, and irregular, from continuous to discontinuous or absent in the apertural area (Figure 9C, E, I). The thick or thin endexine was e-dense, compact-continuous, smooth or with endo-cracks (Figure 9H, K). The endexine is compact-continuous to spongy, thick, costate near the aperture, endo-

sculptured and bi-layered (Figure 9J, K). The intine is bi-layered and has an e-dense ektintine and an e-translucent endointine (Figure 9A, B, G, I, J, L).

Exine ultrasculpture and pollen types

Sexine presents three different ornamentation patterns in the mesocolpium: reticulate (nanoreticulate, microreticulate and/or reticulate-perforate) (Figures 2, 4, 6A–C), rugulate-perforate (Figures 6D–O, 8A–L), and a dimorphic species with some perforate and fossulate-perforate mesocolpium ornamentation (Figure 8M–O). Three pollen types and three subtypes have been recognised based on mesocolpium exine ultrasculpture. Due to the absence of any SEM analysis for *Nissolia chaconsis*, this species was not included in any pollen type.

1. Type I. — nanoreticulate to reticulate mesocolpium.

1.1 Subtype Ia: nanoreticulate to microreticulate mesocolpium. — Species: *Nissolia acutifolia* (Vogel) T.M. Moura et Fort.-Perez, *N. bracteosa* (Rudd) T.M. Moura et Fort.-Perez, *N. brasiliensis* (Vogel) T.M. Moura et Fort.-Perez, *N. klugii* (Rudd) T.M. Moura et Fort.-Perez, *N. latisiliqua* (Poir.) T.M. Moura et Fort.-Perez, *N. longiflora* (Benth. ex A. Gray) T.M. Moura et Fort.-Perez, *N. nigricans* (Burkart) T.M. Moura et Fort.-Perez, *N. wislizeni* (A. Gray) A. Gray (Figures 1, 2, 3A–I, 4A–I, 9A–F).

Pollen grains are small to medium-sized (*Nissolia acutifolia*, *N. klugii*, *N. latisiliqua*) or medium-sized (*N. bracteosa*, *N. brasiliensis*, *N. longiflora*, *N. nigricans*, *N. wislizeni*), subprolate to prolate (*N. acutifolia*, *N. bracteosa*, *N. brasiliensis*, *N. longiflora*), prolate spheroidal to subprolate (*N. klugii*, *N. wislizeni*) prolate spheroidal to prolate (*N. latisiliqua*, *N. nigricans*); circular to ellipsoidal (*N. latisiliqua*, *N. longiflora*, *N. nigricans*) or ellipsoidal in equatorial view (*N. acutifolia*, *N. bracteosa*, *N. brasiliensis*, *N. klugii*, *N. wislizeni*).

Colporus with sparsely granulate operculum (*Nissolia acutifolia*), areolate-granulate operculum (*N. bracteosa*, *N. brasiliensis*, *N. latisiliqua*, *N. nigricans*, *N. wislizeni*) or without operculum (*N. klugii*, *N. longiflora*); microrugulate-granulate membrane (*N. acutifolia*), sparsely granulate (*N. bracteosa*, *N. brasiliensis*, *N. klugii*, *N. latisiliqua*, *N. longiflora*, *N. wislizeni*) or densely granulate (*N. nigricans*). Endoaperture lolongate, circular to lalongate (*N. bracteosa*), lalongate to very lalongate (*N. wislizeni*) or circular to lolongate (*N. acutifolia*, *N. brasiliensis*, *N. klugii*, *N. latisiliqua*, *N. longiflora*, *N. nigricans*, *N. wislizeni*).

Sexine from 1.2 to 2.9 times thicker than nexine; exine 1.1–2.9 μm thick; apocolpium nanoreticulate (*Nissolia longiflora*, *N. nigricans*), nanoreticulate to

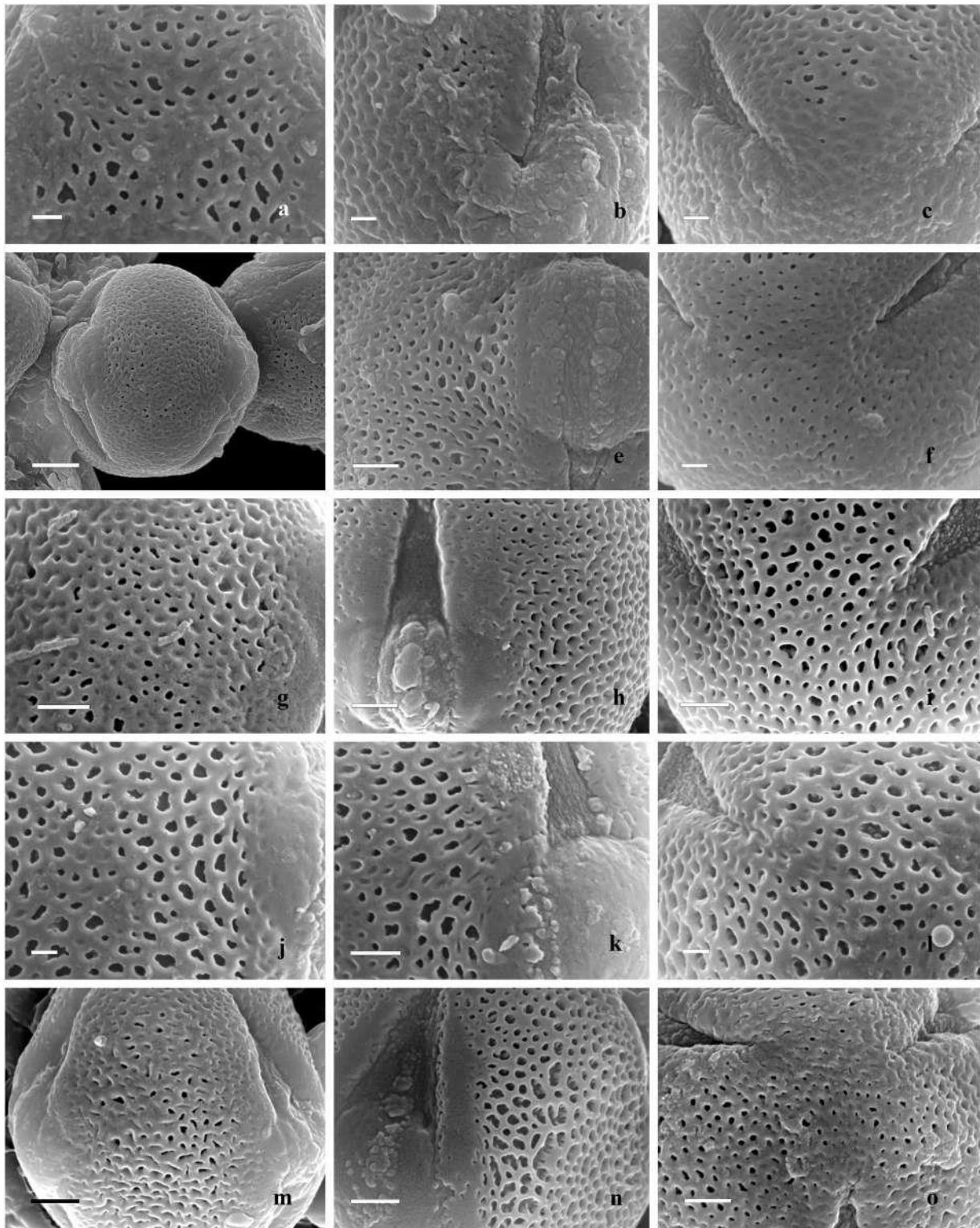


Figure 2. Scanning electron microscopy of *Nissolia* Jacq. species: pollen type Ia. A–C. *Nissolia acutifolia*. A. Detail of the mesocolpium, equatorial view. B. Detail of apertural membrane of the colporus, margo, operculum and mesocolpium, equatorial view. C. Detail of the apocolpium, polar view. D–F. *Nissolia bracteosa*. D. General view of the mesocolpium, equatorial view. E. Detail of apertural membrane of the colporus, margo, operculum and mesocolpium, equatorial view. F. Detail of the apocolpium, polar view. G–I. *Nissolia brasiliensis*. G. Detail of the mesocolpium, equatorial view. H. Detail of apertural membrane of the colporus, margo, operculum and mesocolpium, equatorial view. I. Detail of the apocolpium, polar view. J–L. *Nissolia klugii*. J. Detail of the mesocolpium, equatorial view. K. Detail of the colporus, endoaperture and surface, equatorial view. L. Detail of the apocolpium, polar view. M–O. *Nissolia latisiliqua*. M. General view of the mesocolpium, equatorial view. N. Detail of the colporus, endoaperture and surface, equatorial view. O. Detail of the apocolpium, polar view. Scale bars – 1 μm (A–C, F, J, L), 2 μm (E, G–I, K, M–O), 5 μm (D).

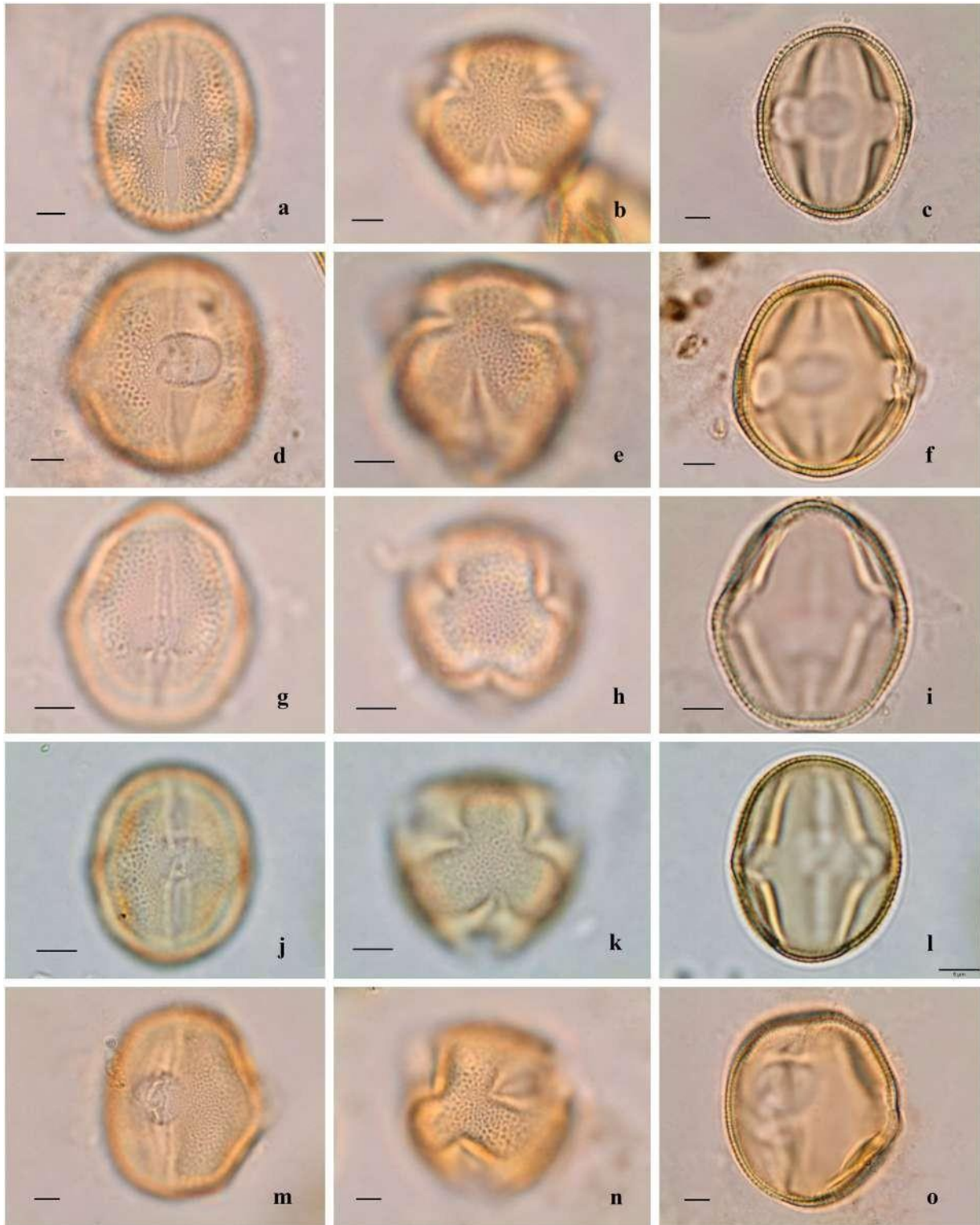


Figure 3. Light microscopy of *Nissolia* Jacq. species: pollen type 1a and 1b. A–C. *Nissolia longiflora*. A. Detail of the colporus, endoaperture and surface, equatorial view. B. Apertures 3-colporate, long and pointed apices and apocolpium surface. C. Optical section, equatorial view. D–F. *Nissolia nigricans*. D. Detail of the colporus, endoaperture, surface and operculum, equatorial view. E. Apertures 3-colporate, long and pointed apices and apocolpium surface. F. Optical section, equatorial view. G–I. *Nissolia wislizeni*. G. Detail of the colporus, surface and operculum, equatorial view. H. Apertures 3-colporate, long and pointed apices and apocolpium surface. I. Optical section, equatorial view. J–L. *Nissolia leiogyne*. J. Detail of the colporus, endoaperture and surface, equatorial view. K. Apertures 3-colporate, long and pointed apices and apocolpium surface. L. Optical section, equatorial view. M–O. *Nissolia subulata*. M. Detail of the colporus, endoaperture, operculum and surface, equatorial view. N. Apertures 3-colporate, long and pointed apices and apocolpium surface. O. Optical section, equatorial view. Scale bars – 5 μ m.

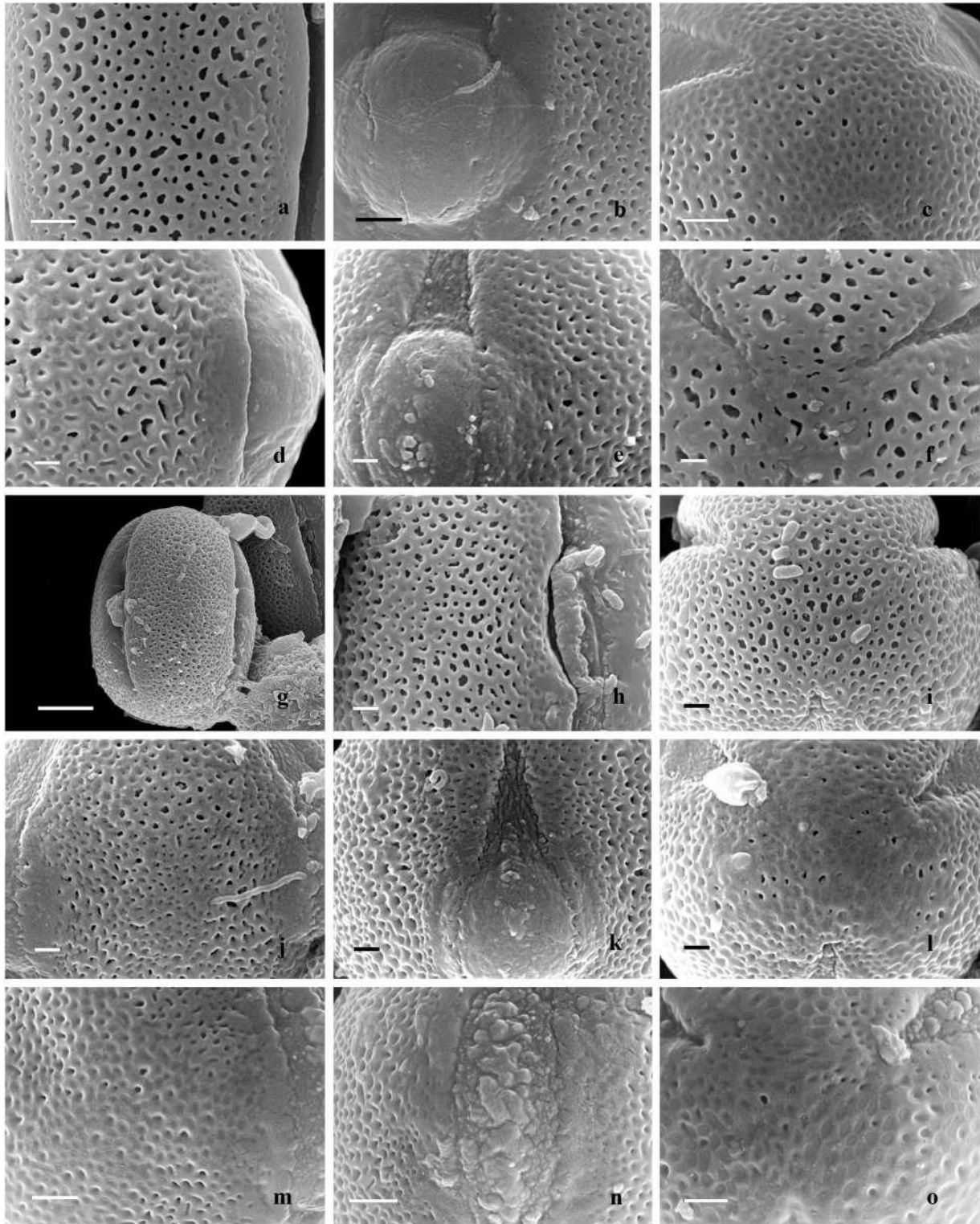


Figure 4. Scanning electron microscopy of *Nissolia* Jacq. species: pollen type Ia and Ib. A–C. *Nissolia longiflora*. A. Detail of the mesocolpium and colporus margo, equatorial view. B. Detail of apertural membrane of the colporus, margo and mesocolpium, equatorial view. C. Detail of apocolpium, polar view. D–F. *Nissolia nigricans*. D. Detail of the mesocolpium and colporus margo, equatorial view. E. Detail of apertural membrane of the colporus, margo, operculum and mesocolpium, equatorial view. F. Detail of apocolpium, polar view. G–I. *Nissolia wislizeni*. G. General view of the mesocolpium, equatorial view. H. Detail of apertural membrane of the colporus, margo, operculum and mesocolpium, equatorial view. I. Detail of apocolpium, polar view. J–L. *Nissolia leiogyne*. J. Detail of the mesocolpium and colporus margo, equatorial view. K. Detail of apertural membrane of the colporus, margo, operculum and mesocolpium, equatorial view. L. Detail of apocolpium, polar view. M–O. *Nissolia subulata*. M. Detail of the mesocolpium and colporus margo, equatorial view. N. Detail of apertural membrane of the colporus, margo, operculum and mesocolpium, equatorial view. O. Detail of apocolpium, polar view. Scale bars – 1 μm (D–F, H–L) 2 μm (A–C, M–O), 5 μm (G).

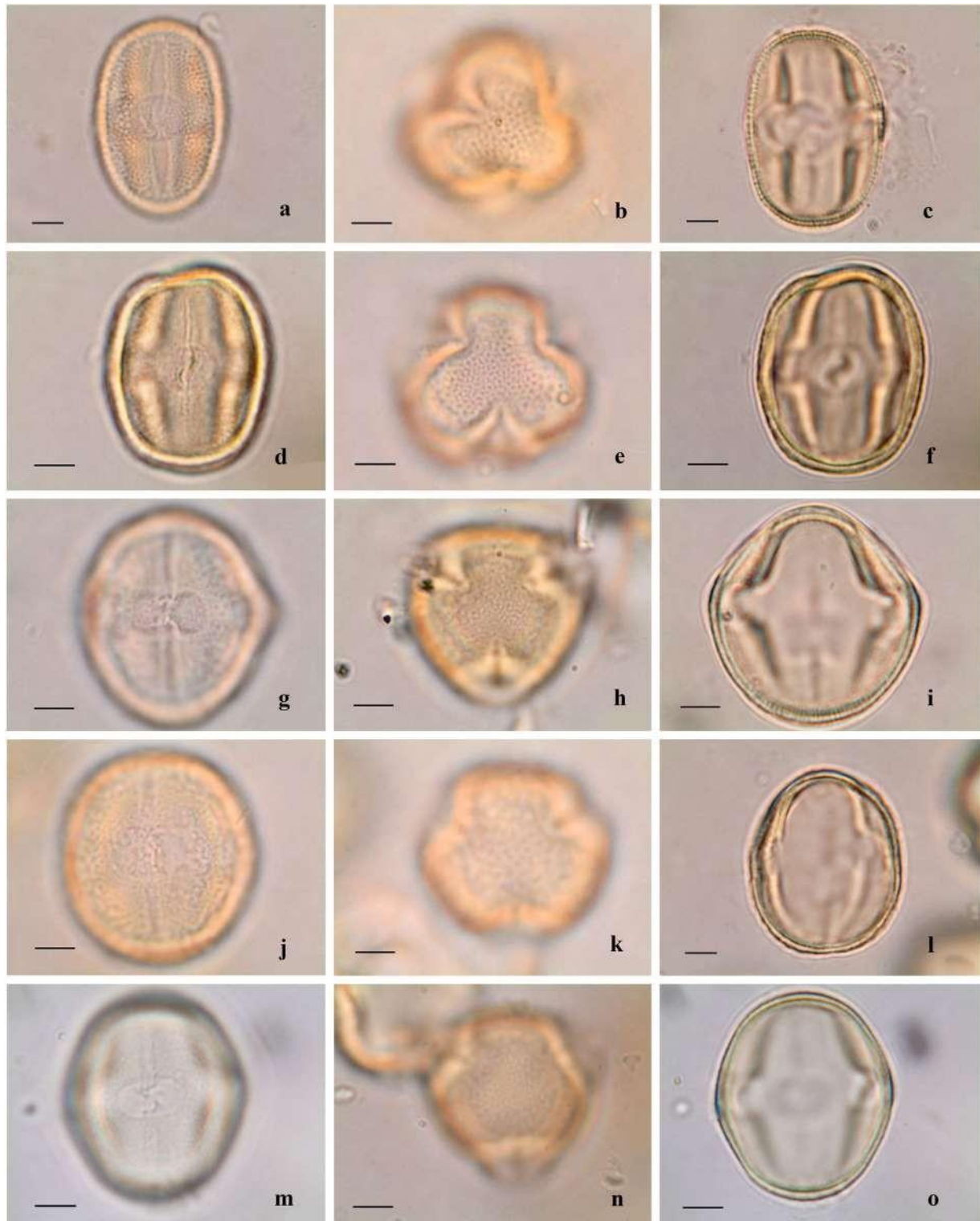


Figure 5. Light microscopy of *Nissolia* Jacq. species: pollen type Ic and II. A–C. *Nissolia vincentina*. A. Detail of the colporus, endoaperture and surface, equatorial view. B. Apertures 3-colporate, long and pointed apices and apocolpium surface. C. Optical section, equatorial view. D–F. *Nissolia chiapensis*. D. Detail of the colporus, endoaperture, surface and operculum, equatorial view. E. Apertures 3-colporate, long and pointed apices and apocolpium surface. F. Optical section, equatorial view. G–I. *Nissolia fruticosa*. G. Detail of the colporus, surface and operculum, equatorial view. H. Apertures 3-colporate, long and pointed apices and apocolpium surface. I. Optical section, equatorial view. J–L. *Nissolia gentryi*. J. Detail of the colporus, endoaperture, operculum and surface, equatorial view. K. Apertures 3-colporate, long and pointed apices and apocolpium surface. L. Optical section, equatorial view. M–O. *Nissolia laxior*. M. Detail of the colporus, endoaperture, operculum and surface, equatorial view. N. Apertures 3-colporate, long and pointed apices and apocolpium surface. O. Optical section, equatorial view. Scale bars – 5 μ m.

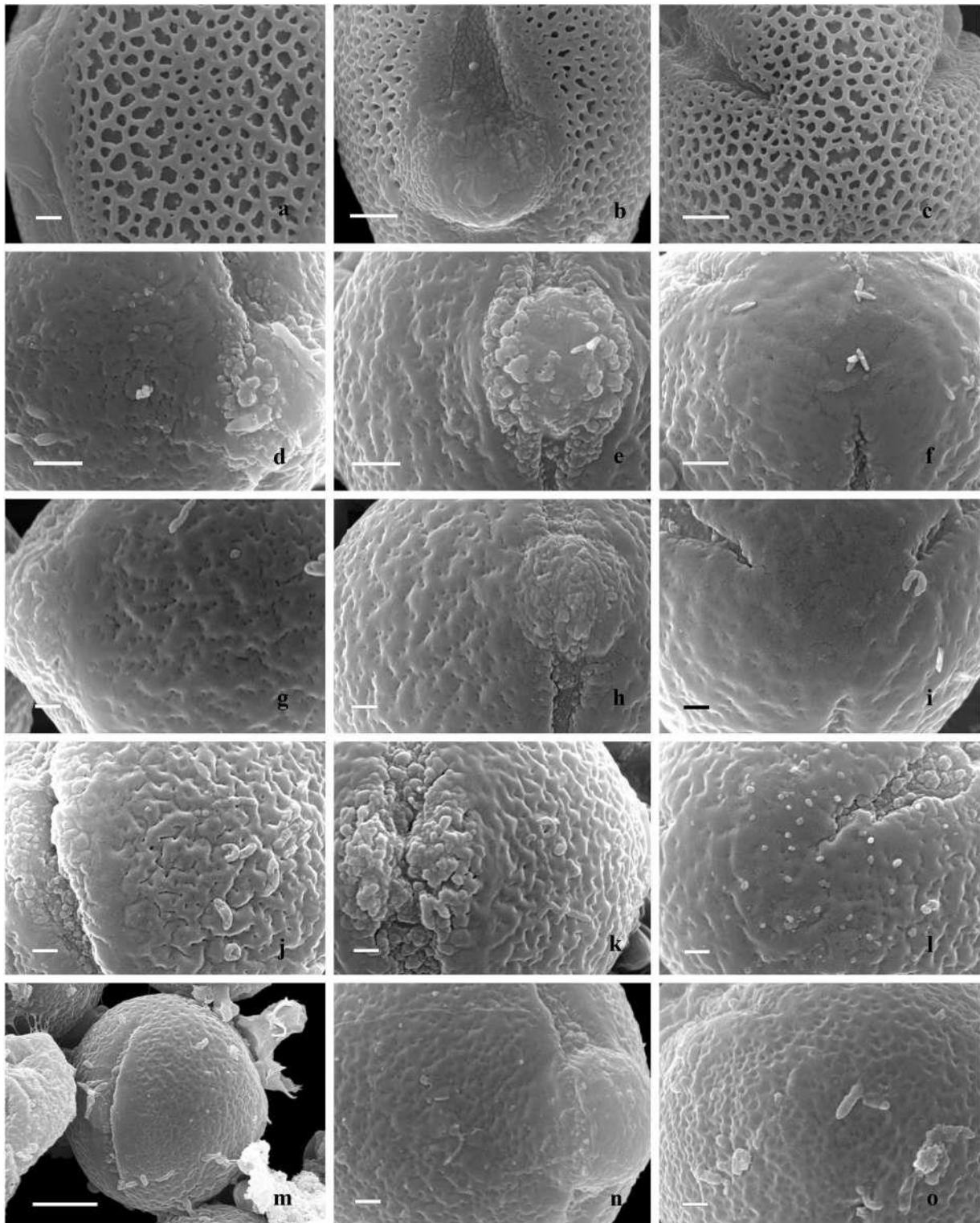


Figure 6. Scanning electron microscopy of *Nissolia* Jacq. species: pollen type Ic and II. A–C. *Nissolia vincentina*. A. Detail of the mesocolpium and colporus margo, equatorial view. B. Detail of apertural membrane of the colporus, margo, operculum and mesocolpium, equatorial view. C. Detail of apocolpium, polar area. D–F. *Nissolia chiapensis*. D. Detail of the mesocolpium and colporus margo, equatorial view. E. Detail of apertural membrane of the colporus, margo, operculum and mesocolpium, equatorial view. F. Detail of apocolpium, polar area. G–I. *Nissolia fruticosa*. G. Detail of the mesocolpium and colporus margo, equatorial view. H. Detail of apertural membrane of the colporus, margo, operculum and mesocolpium, equatorial view. I. Detail of apocolpium, polar area. J–L. *Nissolia gentryi*. J. Detail of the colporus, endoaperture, operculum and surface, equatorial view. K. Detail of apertural membrane of the colporus, margo, operculum and mesocolpium, equatorial view. L. Detail of apocolpium, polar area. M–O. *Nissolia laxior*. M. General view of the mesocolpium, equatorial view. N. Detail of apertural membrane of the colporus, margo and mesocolpium, equatorial view. O. Detail of apocolpium, polar area. Scale bars – 1 μm (A, G–L, N, O), 2 μm (B, C–F), 5 μm (M).

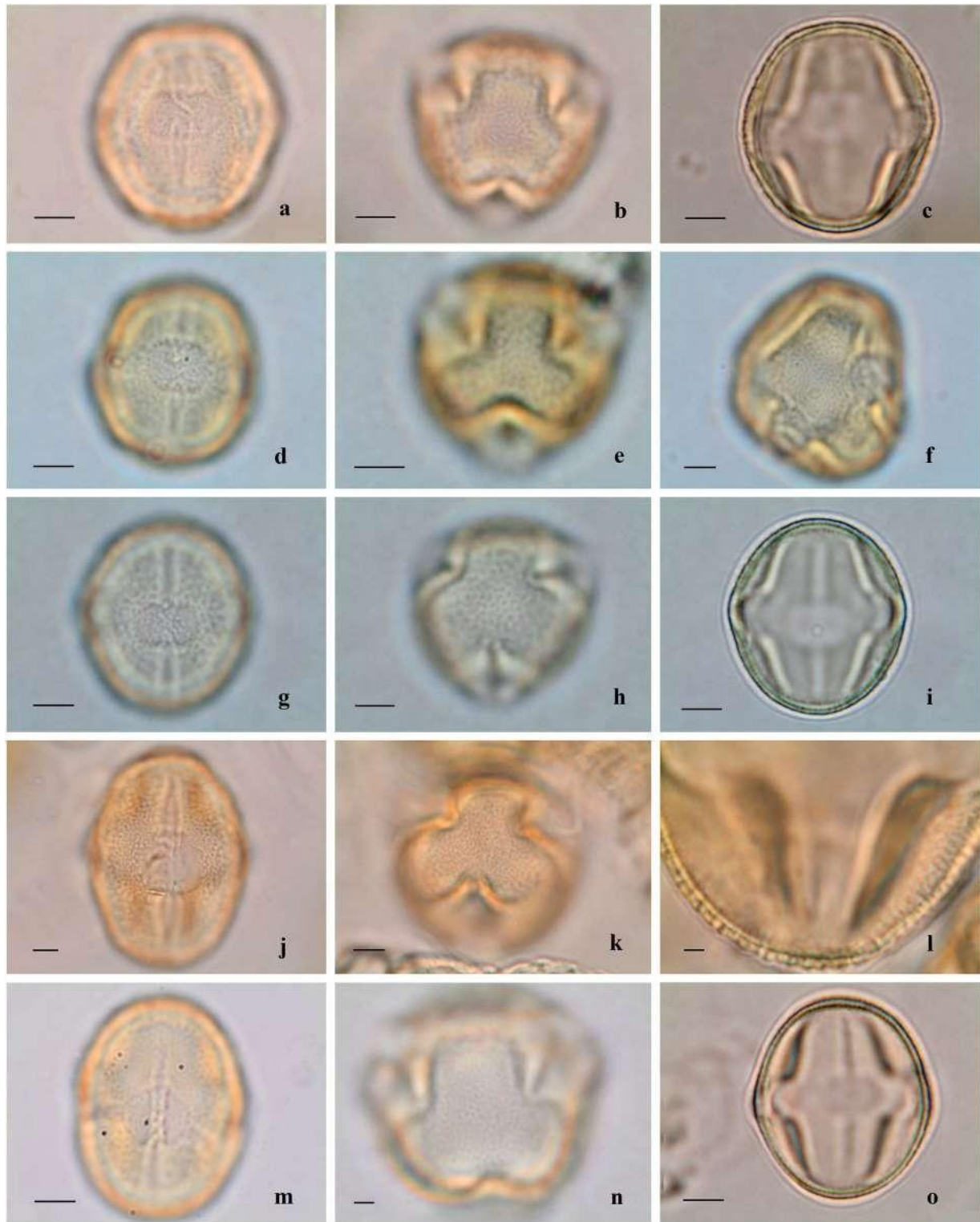


Figure 7. Light microscopy of *Nissolia* Jacq. species: pollen type II and III. A–C. *Nissolia platycarpa*. A. Detail of the colporus, endoaperture and surface, equatorial view. B. Apertures 3-colporate, long and pointed apices and apocolpium surface. C. Optical section, equatorial view. D–F. *Nissolia pringlei*. D. Detail of the colporus, endoaperture, surface and operculum, equatorial view. E. Apertures 3-colporate, long and pointed apices and apocolpium surface. F. Abnormal pollen grain, apolar. G–I. *Nissolia schottii*. G. Detail of the colporus, surface and operculum, equatorial view. H. Apertures 3-colporate, long and pointed apices and apocolpium surface. I. Optical section, equatorial view. J–L. *Nissolia tomentosa*. J. Detail of the colporus, endoaperture, operculum and surface, equatorial view. K. Apertures 3-colporate, long and pointed apices and apocolpium surface. L. Optical section, equatorial view. M–O. *Nissolia microptera*. M. Detail of the colporus, endoaperture, operculum and surface, equatorial view. N. Apertures 3-colporate, long and pointed apices and apocolpium surface. O. Optical section, equatorial view. Scale bars – 2 μm (L, N), 5 μm (A–K, M, O).

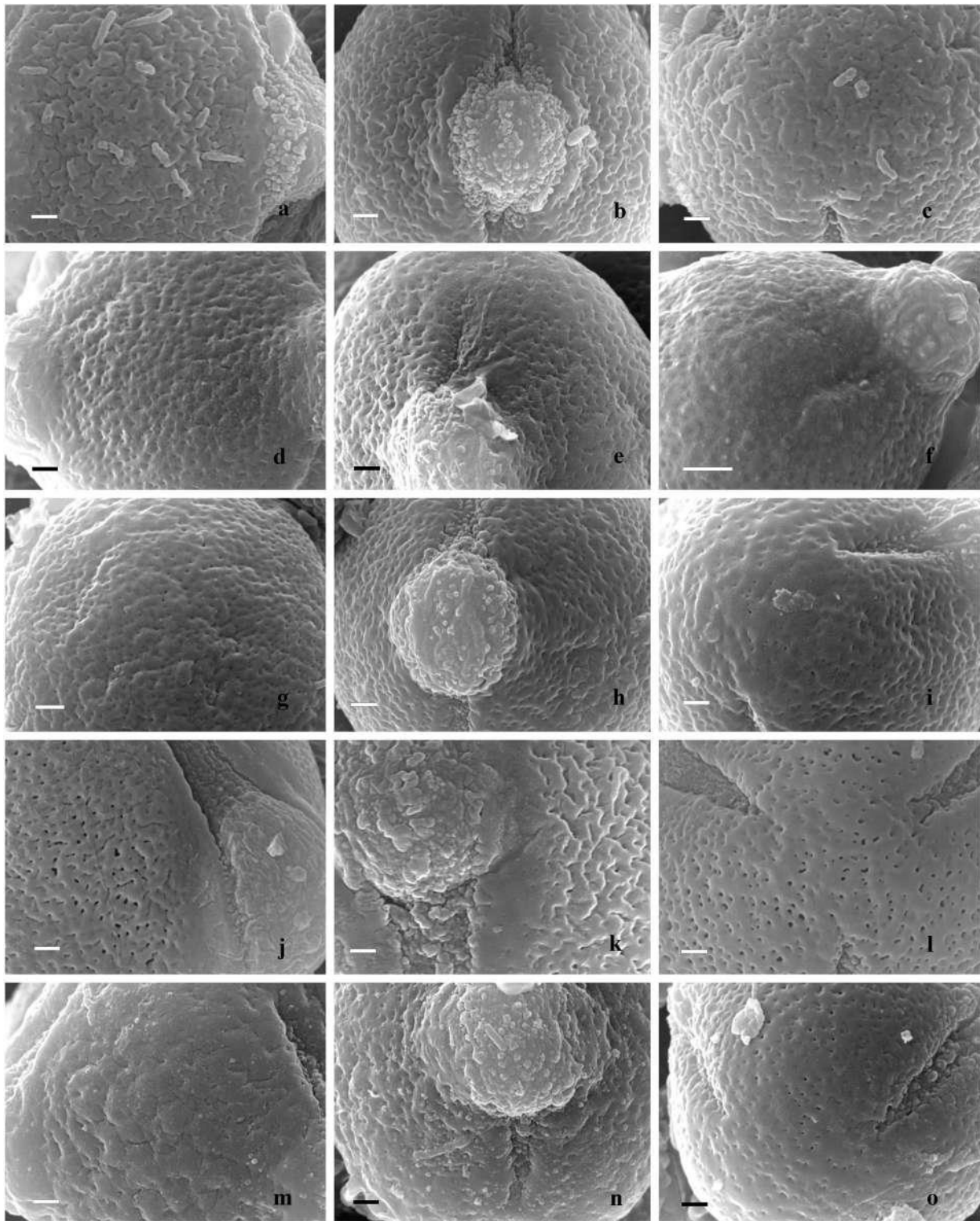


Figure 8. Scanning electron microscopy of *Nissolia* Jacq. species: pollen type II and III. A–C. *Nissolia platycarpa*. A. Detail of the mesocolpium and colporus margo, equatorial view. B. Detail of apertural membrane of the colporus, margo, operculum and mesocolpium, equatorial view. C. Detail of apocolpium, polar view. D–F. *Nissolia pringlei*. D. Detail of the mesocolpium and colporus margo, equatorial view. E. Detail of apertural membrane of the colporus, margo, operculum and mesocolpium, equatorial view. F. Detail of apocolpium, polar view. G–I. *Nissolia schottii*. G. Detail of the mesocolpium, equatorial view. H. Detail of apertural membrane of the colporus, margo, operculum and mesocolpium, equatorial view. I. Detail of apocolpium, polar view. J–L. *Nissolia tomentosa*. J. Detail of apertural membrane of the colporus, margo, operculum and mesocolpium, equatorial view. K. Detail of apertural membrane of the colporus, margo, operculum and mesocolpium, equatorial view. L. Detail of apocolpium, polar view. M–O. *Nissolia microptera*. M. Detail of the mesocolpium and colporus margo, equatorial view. N. Detail of apertural membrane of the colporus, margo, operculum and mesocolpium, equatorial view. O. Detail of apocolpium, polar view. Scale bars – 1 μm (A–E, G–O), 2 μm (F).

Table II. Morphology and ultrastructure of *Nissolia* pollen grains using light and scanning electron microscopy.

Species	PT	Size	Endoaperture	Operculum	Membrane	Apocolpium
<i>N. acutifolia</i>	Ia	S-M	Circular to lolongate	Granulate ⁻	Rugulate-granulate	Nanoreticulate to microreticulate
<i>N. bracteosa</i>	Ia	M	Lolongate, circular to lalongate	Areolate-granulate	Granulate ⁻	Nanoreticulate-perforate
<i>N. brasiliensis</i>	Ia	M	Circular to lolongate	Areolate-granulate	Granulate ⁻	Nanoreticulate to microreticulate
<i>N. chacoensis</i>	—	S-M	Circular to lolongate	—	—	—
<i>N. chiapensis</i>	II	S-M	Lalongate to very lalongate	Areolate-granulate	Granulate ⁺	Rugulate-perforate
<i>N. fruticosa</i>	II	S	Lalongate to very lalongate	Rugulate-areolate-granulate	Granulate ⁺	Rugulate-perforate
<i>N. gentryi</i>	II	S-M*	Circular to lolongate	Areolate-granulate	Granulate ⁺	Psilate-perforate
<i>N. klugii</i>	Ia	S-M	Circular to lolongate	Absent	Granulate ⁻	Nanoreticulate to microreticulate
<i>N. latisiliqua</i>	Ia	S-M	Circular to lalongate	Areolate-granulate	Granulate ⁻	Nanoreticulate-perforate
<i>N. laxior</i>	II	S	Lalongate to very lalongate	Absent	Granulate ⁻	Nanoreticulate-perforate
<i>N. leiogyne</i>	Ib	S	Lalongate to very lalongate	Areolate-granulate	Granulate ⁻	Nanoreticulate
<i>N. longiflora</i>	Ia	M	Circular to lolongate	Absent	Granulate ⁻	Nanoreticulate
<i>N. microptera</i>	III	S-M	Circular to lalongate	Areolate-granulate	Areolate-granulate	Psilate-perforate
<i>N. nigricans</i>	Ia	M	Circular to lalongate	Areolate-granulate	Granulate ⁺	Nanoreticulate
<i>N. platycarpa</i>	II	S	Lalongate to very lalongate	Granulate ⁺	Granulate ⁺	Rugulate-perforate
<i>N. pringlei</i>	II	S	Lalongate to very lalongate	Granulate ⁻	Granulate ⁻	Psilate-perforate
<i>N. schottii</i>	II	S	Lalongate to very lalongate	Rugulate-areolate-granulate	Granulate ⁺	Psilate-perforate
<i>N. subulata</i>	Ib	M	Circular to very lolongate, lalongate*	Areolate-granulate	Granulate ⁺	Nanoreticulate
<i>N. tomentosa</i>	II	M	Lolongate, lalongate*	Rugulate-areolate-granulate	Granulate ⁺	Psilate-perforate
<i>N. vicentiana</i>	Ic	S-M	Lolongate, circular to lalongate	Areolate-granulate	Rugulate-granulate	Nanoreticulate to microreticulate
<i>N. wislizeni</i>	Ia	S	Lalongate to very lalongate	Areolate-granulate	Granulate ⁺	Nanoreticulate to microreticulate

PT, pollen type; S, small; M, medium; ⁻, sparsely; ⁺, densely; *, rarely; —, not conclusive.

microreticulate (*N. acutifolia*, *N. brasiliensis*, *N. klugii*, *N. wislizeni*) nanoreticulate-perforate (*N. bracteosa*, *N. latisiliqua*).

1.2 Subtype Ib: nanoreticulate mesocolpium. — Species: *Nissolia leiogyne* Sandwith, *N. subulata* (Mackinder) I. Castro et Fort.-Perez (Figures 3J–O, 4J–O, 9G–I).

Pollen grains small (rarely medium [*Nissolia leiogyne*]) or medium (*N. subulata*) sized, prolate spheroidal to subprolate (*N. leiogyne*) or subprolate to prolate (*N. subulata*) ellipsoidal in equatorial view. Colporus with areolate-granulate operculum, sparsely granulate (*N. leiogyne*) or densely granulate (*N. subulata*) membrane. Endoaperture lalongate to very lalongate (*N. subulata*) or very lolongate to circular, rarely lalongate (*N. subulata*). Sexine 1.2 to 2.8 times thicker than nexine; exine 1.1–2.5 µm thick. Mesocolpium and apocolpium nanoreticulate.

1.3 Subtype Ic: nanoreticulate-reticulate mesocolpium.

— Species: *Nissolia vincentina* (Ker Gawl.) T.M. Moura et Fort.-Perez. (Figures 5A–C, 6A–C).

Pollen grains from small to medium-sized subprolate to prolate, ellipsoidal in equatorial view. Colporus with an areolate-granulate operculum, microrugulate-granulate membrane and psilate-perforate margo. Endoaperture lolongate, circular to lalongate, and protruding apertures present.

Sexine 1.1 to 2.4 times thicker than nexine, and exine 1.2–1.7 µm thick. Mesocolpium and apocolpium nanoreticulate-reticulate.

2. Type II: rugulate-perforate mesocolpium. —

Species: *Nissolia chiapensis* Rudd, *N. fruticosa* Jacq., *N. gentryi* Rudd, *N. laxior* (B.L. Rob) Rose, *N. platycarpa* Benth., *N. pringlei* Rose, *N. schottii* (Torr.) A. Gray, *N. tomentosa* (Gardner) T.M. Moura et Fort.-Perez. (Figures 5D–O, 6D–O, 7A–L, 8A–L, 9J–L).

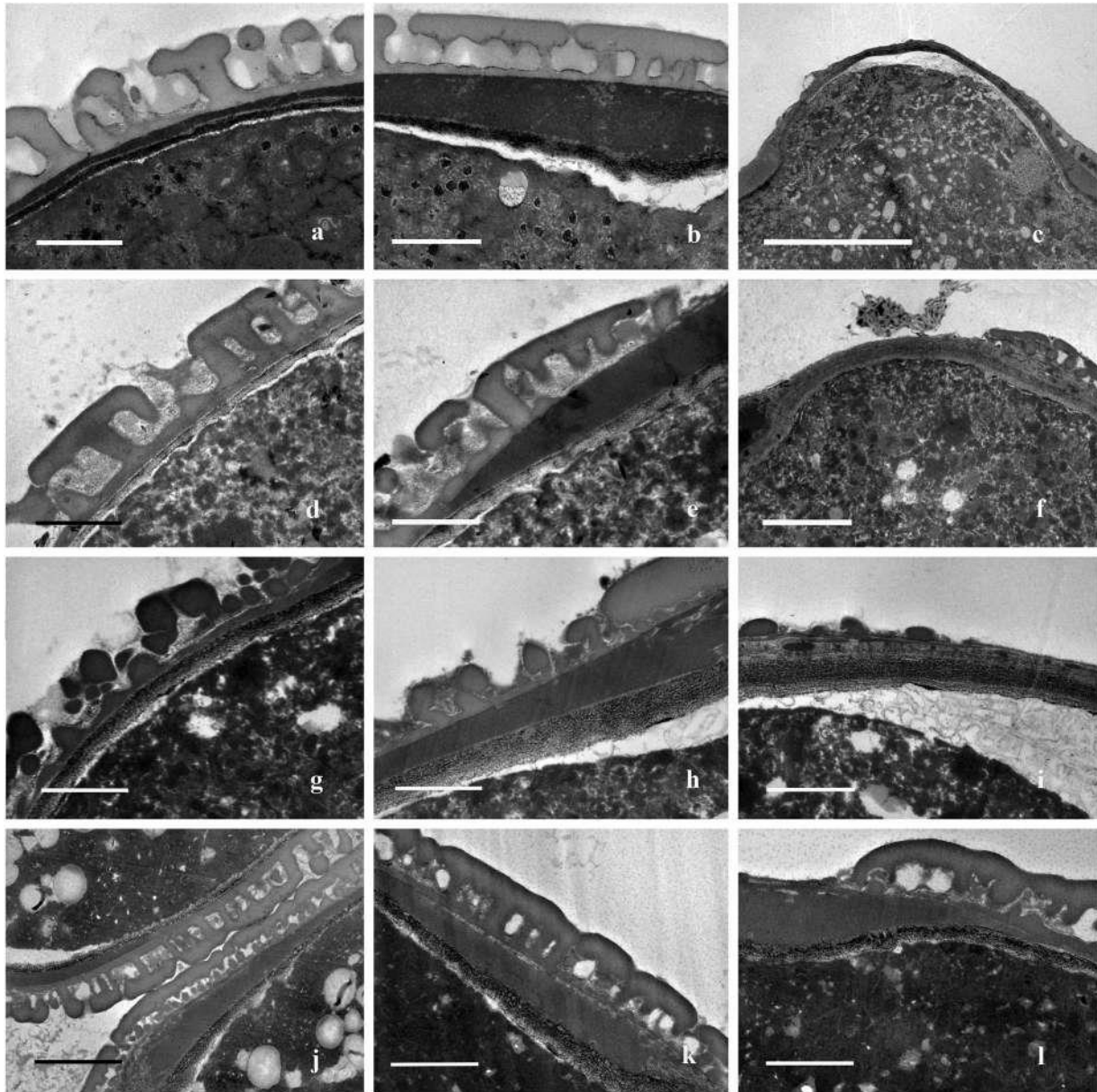


Figure 9. Transmission electron microscopy of *Nissolia* Jacq. species. A–C. *Nissolia longiflora*. A. Section in a non-apertural area. B. Section near to the aperture. C. Section in the apertural area. D–F. *Nissolia nigricans*. D. Section in a non-apertural area. E. Section near to the aperture. F. Section in the apertural area. G–I. *Nissolia subulata*. G. Section in a non-apertural area. H. Section near to the aperture. I. Section near to the aperture. J–L. *Nissolia tomentosa*. J. Section in a non-apertural area. K. Section near to the aperture. L. Section near to the aperture. Scale bars – 1 µm (A, B, D, E, G–I, K, L), 2 µm (F, J), 5 µm (C).

Pollen grains small (*Nissolia fruticosa* [rarely medium], *N. gentryi*, *N. laxior*, *N. platycarpa*, *N. pringlei*, *N. schottii*), from small to medium (*N. chiapensis*) or medium-sized (*N. tomentosa*), radially symmetric, isopolar, prolate spheroidal (*N. gentryi*, *N. laxior*, *N. platycarpa*, *N. schottii*), prolate spheroidal to subprolate (*N. chiapensis*), oblate spheroidal to subprolate (*N. fruticosa*), prolate spheroidal to prolate (*N. pringlei*,

N. tomentosa), ellipsoidal (*N. chiapensis*, *N. laxior*, *N. tomentosa*) or circular to ellipsoidal (*N. fruticosa*, *N. gentryi*, *N. platycarpa*, *N. pringlei*, *N. schottii*) in equatorial view.

Colporus with sparsely areolate-granulate (*Nissolia chiapensis*), densely areolate-granulate (*N. gentryi*), densely granulate (*N. platycarpa*), sparsely granulate (*N. pringlei*) rugulate-areolate-granulate (*N. fruticosa*, *N. schottii*, *N. tomentosa*) with

operculum or without operculum (*N. laxior*); densely granulate (*N. chiapensis*, *N. fruticosa*, *N. platycarpa*, *N. schottii*, *N. tomentosa*), sparsely granulate (*N. laxior*, *N. pringlei*) or densely areolate-granulate (*N. gentryi*) membrane. Endoaperture lalongate, rarely lalongate (only *N. tomentosa*) or lalongate to very lalongate.

Sexine from 1.1 to 2.2 times thicker than nexine; exine 1.1–1.8 μm thickness; apocolpium rugulate-perforate (*Nissolia chiapensis*, *N. fruticosa*, *N. platycarpa*), psilate-perforate (*N. gentryi*, *N. pringlei*, *N. schottii*, *N. tomentosa*) or nanoreticulate-perforate (*N. laxior*).

3. Type III: dimorphic fossulate-perforate or psilate-perforate mesocolpium. — Species: *Nissolia microptera* Poir. (Figures 7M–O, 8M–O).

Pollen grains small to medium-sized, radially symmetric, isopolar, prolate spheroidal to subprolate, and circular to ellipsoidal in equatorial view. Colporus with areolate-granulate operculum and membrane. Endoaperture circular to lalongate and protruding apertures present.

Sexine from 1.1 to 2.0 times thicker than nexine; exine 0.9–1.2 μm thick. Dimorphic ornamentation: ultrasculpture I – mesocolpium fossulate-perforate and apocolpium psilate-perforate;

ultrasculpture II – mesocolpium and apocolpium psilate-perforate.

Principal component analysis (PCA)

The PCA showed the relationship between species based on eight metric variables and three classes/indexes (Figure 10). Correlation coefficients are shown in Table III. The first two axes accounted for 83.91% of the total variability of the quantitative data analysed. The first axis explained 72.34% of the variance and was mainly associated with PA, EA and CL. The second axis summarised 11.56% of the total variability, with *P/E*, CW, EW, EL, *N*, *S*, *E*, *S/N* and Cen (class of endoaperture) being the variables that contributed most to this axis.

Nissolia latisiliqua (lat49), *N. longiflora* (lon01, lon07, lon30), *N. nigricans* (nig04, nig36, nig83, nig96), *N. subulata* (sub44), *N. tomentosa* (tom04) had the largest pollen grains and were located on the bottom left side of the PCA associated with high values EA, PA, CL, EW, CW, *S*, *E*, *N* and *S/N*. *Nissolia bracteosa* (brac34, brac43), *N. brasiliensis* (bras00, bras25, bras57), *N. chacoensis* (cha66), *N. klugii* (klu60, klu75), *N. vincentina* (vin73), *N. subulata* (sub98) and *N. tomentosa* (tom64) showed high EL and *P/E* and were located on the

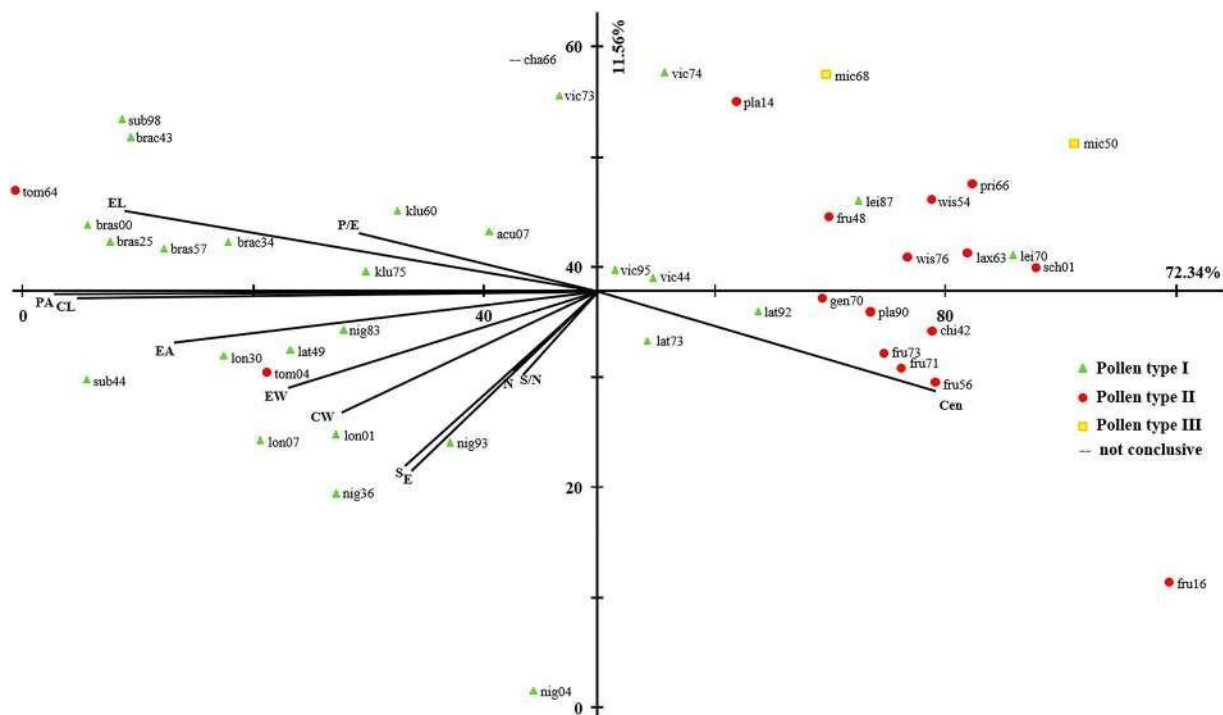


Figure 10. Principal component analysis biplot of the pollen grain metric variables and classes/indexes of *Nissolia* specimens.

Table III. Pearson and Kendall coefficients of pollen grain metric variables and classes/indices from the first two ordination axes of the principal component analysis (PCA) of *Nissolia* species.

Principal components		
Variables	Axis 1	Axis 2
PA	−0.4355	−0.0689
EA	−0.2714	−0.2361
P/E	−0.0941	0.1166
CL	−0.4608	−0.1261
CW	−0.2207	−0.3820
EW	−0.2411	−0.3381
EL	−0.5791	0.6005
N	−0.0328	−0.0796
S	−0.1291	−0.3074
E	−0.1241	−0.3067
S/N	−0.0614	−0.1630
Cen	0.1907	−0.2601

PA, polar axis; EA, equatorial axis; P/E = PA/EA; CL, colpus length; CW, colpus width; EW, endoaperture width; EL, endoaperture length; N, nexine thickness; S, sexine thickness (S = infratectum + tectum); E, exine thickness (E = N + S); S/N, index of sexine and nexine thickness; Cen, class of endoaperture.

upper left side of the PCA, and have a lolongate endoaperture.

Due to their high Cen values, *Nissolia chiapensis* (chi42), *N. fruticosa* (fru16, fru56, fru71, fru73), *N. gentryi* (gen70), *N. latisiliqua* (lat73, lat92) and *N. platycarpa* (pla90) were placed on the bottom right side of the PCA with a lalongate to very lalongate endoaperture. Specimens with the smallest pollen grains were located mainly on the upper right side of the graph. These specimens also had the lowest EA, PA, CL, EW, CW, S, E, N and S/N values: *N. fruticosa* (fru48), *N. laxior* (lax63), *N. leiogyne* (lei70, lei87), *N. microptera* (mic50, mic68), *N. platycarpa* (pla14), *N. pringlei* (pri66), *N. vincentina* (vin44, vin74, vin95), *N. schottii* (sch01) and *N. wislizeni* (wis76, wis54).

Cluster analysis (UPGMA and Euclidean distance)

We identified two groups of specimens with 0% similarity. One group had a similarity greater than 13%. It comprised the species with the largest pollen grains and endoaperture dimensions (*Nissolia acutifolia* [acu07], *N. klugii* [klu60, klu75], *N. latisiliqua* [lat49], *N. longiflora* [lon01, lon30, lon07], *N. nigricans* [nig04, nig83, nig36, nig93], *N. brasiliensis* [bras00, bras57, bras25], *N. bracteosa* [brac34, brac43], *N. subulata* [sub44, sub98], *N. tomentosa* [tom04, tom64]). The other group had similarity greater than 25%, and the remainder of the species had the smallest pollen grains and the lowest endoaperture values (*N. chacoensis* [cha66], *N. vincentina* [vin73, vin74, vin95, vin44],

N. latisiliqua [lat73, lat92], *N. platycarpa* [pla14, pla90], *N. chiapensis* [chi42], *N. fruticosa* [fru16, fru48, fru56, fru71, fru73], *N. laxior* [lax63], *N. leiogyne* [lei70, lei87], *N. pringlei* [pri66], *N. gentryi* [gen70], *N. wislizeni* [wis76, wis54], *N. microptera* [mic50, mic68]).

Discussion

Pollen morphology, ultrasculpture and ultrastructure of Nissolia

Previous palynological analyses of eight *Nissolia* species (*N. brasiliensis*, *N. fruticosa*, *N. latisiliqua*, *N. longiflora*, *N. pringlei*, *N. tomentosa*, *N. vincentina*, and *N. wislizeni*) provided data for comparison with the pollen analyses of the present study (Ohashi 1971; Pire 1974; Carreira et al. 1996; Lattar et al. 2020). Some of our results support previously published palynological data, highlighting differences in pollen size, endoaperture class, colporus details, presence of an operculum and its ornamentation and sexine sculpture. We compiled the pollen morphology described in the literature for comparison with our results (Table IV) (Ohashi 1971; Pire 1974; Carreira et al. 1996; Lattar et al. 2020).

The operculum presence and ornamentation type help differentiate individual species and species groups in the various pollen types and subtypes (Table II). These are essential pollen features in other papilionoid legumes (Antonio-Domingues et al. 2018, 2019, 2022a, 2022b, 2022c). Most of the species of *Nissolia* analysed

had an operculate colporus, except *N. klugii*, *N. laxior*, and *N. longiflora*, which had a sparsely granulate membrane covering the colporus. Five operculate species studied (*N. fruticosa*, *N. latisiliqua*, *N. longiflora*, *N. pringlei*, and *N. tomentosa*) have a membrane covering the colporus (Table IV) (Ohashi 1971). We recognise these species as having operculate pollen grains due to the aperture having a distinctly delimited, isolated sexine structure, and the aperture membrane usually represents the nexine (Punt et al. 2007).

The operculum covering the aperture is lost after acetolysis since it is loosely connected with the central part of the exine (Hesse & Waha 1989) and could explain the recorded absence of an operculum in the literature in species diagnosed as operculate in our study (Pire 1974; Carreira et al. 1996; Lattar et al. 2020) (Table IV). The ultrasculpture description of the *Nissolia* pollen exine is novel palynological data for the genus and the informal *Adesmia* clade.

Palynotaxonomic implications for the systematics of *Nissolia*

Differences in pollen morphology and ultrasculpture for some *Nissolia* species that have been difficult to delimit taxonomically might contribute to more precise taxonomic diagnoses (Moura et al. 2018b; Silva 2021). Comments about these taxonomic conflicts and related observations about pollen are presented later.

Specimens of *Nissolia bracteosa* are often mistakenly identified as *N. brasiliensis* (Silva 2021). Both species show pollen type Ia but differ by the lolongate, circular to lalongate endoaperture and nanoreticulate-perforate apocolpium in *N. bracteosa* versus the circular to lolongate endoaperture and nanoreticulate to microreticulate apocolpium in *N. brasiliensis* (Table II). The two species resemble *N. stipulata* (Silva 2021), but this species was not analysed in our study.

Nissolia brasiliensis morphologically resembles *N. longiflora* (Silva 2021) but differs by having an

Table IV. Pollen morphology data from previous published studies compared with the present study.

Species	Author	Size class	Endoaperture class	Operculum ultrasculpture	Sexine ultrasculpture	
					Mesocolpium	Apocolpium
<i>N. brasiliensis</i>	L 2020	M	Lolongate	—	Reticulate	
	PS	M	Circular to lolongate	Granulate ⁻	Nanoreticulate to microreticulate	
<i>N. fruticosa</i>	O 1971	S	'almost rounded'	—	Microreticulate	
	P 1974	M	Lalongate	—	Rugulate-reticulate-perforate	
	C 1996	M	Presente	—	Microreticulate	
	PS	S-M*	Lalongate to very lalongate	Rugulate-areolate-granulate	Rugulate-perforate	
<i>N. latisiliqua</i>	O 1971	S	'rounded to elliptic'	—	Microreticulate	
	P 1974	M	Lolongate	—	Heterobrochate	
	PS	M	Circular	Areolate-Perforate	Nano to microreticulate	Nanoreticulate-perforate
<i>N. longiflora</i>	O 1971	M	'almost rounded'	—	Reticulate	
	P 1974	L	Circular	—	Reticulate	
	L 2020	L	Lolongate	—	Reticulate	
	PS	M	Absent	Granulate ⁻	Nano to microreticulate	Nanoreticulate
<i>N. pringlei</i>	O 1971	S	'rounded to elliptic'	—	Reticulate	
	P 1974	M	Lalongate	—	Rugulate-reticulate-perforate	
	PS	S	Lalongate to very lalongate	Granulate ⁻	Rugulate-perforate	
<i>N. tomentosa</i>	O 1971	M	'rounded or slightly elongated meridionally'	—	Microreticulate	
	PS	M	Lolongate, lalongate*	Rugulate-areolate-granulate	Rugulate-perforate	
<i>N. vincentina</i>	P 1974	M	Circular to lalongate	—	Rugulate-reticulate-perforate	
	C 1996	M	Present	—	Microreticulate	
	B 2011	M	Elliptic	—	Microreticulate	
	PS	S-M	Lolongate, circular to lalongate	Areolate-granulate	Nanoreticulate to reticulate	
<i>N. wislizeni</i>	P 1974	M	Lalongate	—	Rugulate-reticulate-perforate	
	PS	M	Lalongate to very lalongate	Areolate-granulate	Nanoreticulate to microreticulate	

L, large; M, medium; S, small; *, rarely; -, sparsely; —, not described; PS, present study; O 1971, Ohashi 1971; P 1974, Pire 1974; C 1996, Carreira et al. 1996; L 2020, Lattar et al. 2020.

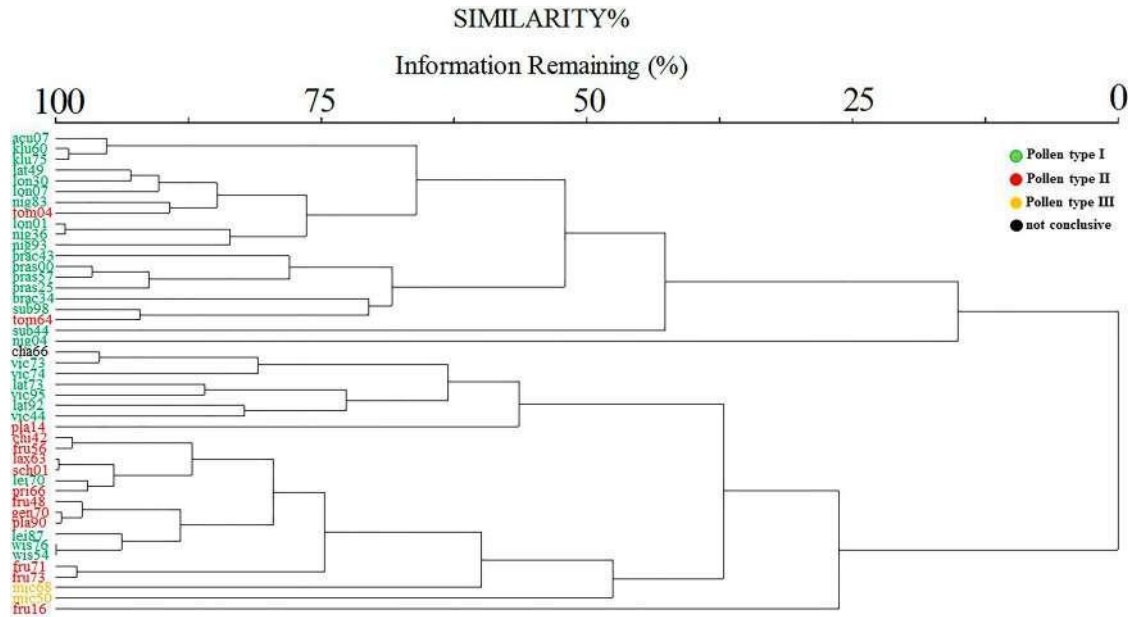


Figure 11. Dendrogram built from cluster analysis (Euclidean distance) for *Nissolia* specimens, information remaining (%).

areolate-granulate operculum and nanoreticulate-perforate apocolpium sexine versus the absence of an operculum and a nanoreticulate apocolpium in *N. longiflora* (Table II).

Nissolia latisiliqua and *N. fruticosa* are similar in having large leaflets and short calyx tubes; but they are palynologically very distinct (Moura et al. 2018b; Silva 2021). *Nissolia fruticosa* shows small pollen grains, a lalongate to very lalongate endoaperture, rugulate-areolate-granulate operculum, densely granulate membrane, and rugulate-perforate apocolpium and mesocolpium versus small to medium-sized pollen grains, a circular to lalongate endoaperture, areolate-granulate operculum, sparsely granulate membrane and nanoreticulate to microreticulate mesocolpium and nanoreticulate-perforate apocolpium in *N. latisiliqua*.

Nissolia species geographical and molecular distribution correlate with the mesocolpium ornamentation and pollen types. The 11 species included in the molecular study by Fortuna-Perez et al. (2021) are palynologically described showing a correlation between the pollen ultrasculpture type and the samaroid fruit clade and the non-samaroid fruit clade (Figure 12).

Except for *Nissolia fruticosa* (native) and *N. tomentosa* (endemic), all other native Brazilian species analysed (*N. acutifolia* [endemic], *N. bracteosa* [endemic], *N. brasiliensis*, *N. fruticosa*, *N. klugii*, *N. longiflora*, *N. nigricans*, *N. subulata* [endemic], and *N. vincentina*) have nanoreticulate

to reticulate mesocolpium sexine (pollen type I). Three other non-Brazilian species were included in the pollen type I: one South American (*N. latisiliqua* [Colombia, Ecuador]) and two North American (*N. leiogyne* [Mexico], *N. wislizeni* [United States, Mexico]) (Moura et al. 2018b). All species included in pollen type II (rugulate-perforate mesocolpium) or III (dimorphic fossulate-perforate or psilate-perforate mesocolpium) are from North and Central America except for two South American species (*N. fruticosa* and *N. tomentosa*).

Figure 12 shows that the non-samaroid fruit clade can be palynologically recognised by nanoreticulate to reticulate sexine mesocolpium (pollen type I). Members of this clade were located on the left side of the graph (Figure 10) except for *N. vincentina* (sca94, sca44, sca74) and *N. latisiliqua* (lat24, lat92) (right side of the graph) and had a 95 and 50% similarity (Figure 11), except for one specimen of *N. nigricans* (25% of similarity), *N. vincentina* (vin73, vin74, vin95, vin44), and *N. latisiliqua* (lat24, lat92) (0% of similarity). These *N. vincentina* and *N. latisiliqua* specimens formed a group with a more significant similarity above 50% (Figure 11). The samaroid fruit clade members were located on the upper right of the PCA (Figure 10), with similarity more significant than 50% (Figure 11). They had pollen types I, II and III (Figure 12).

Recent phylogenetic studies accessing pollen morphology demonstrate their systematic value to

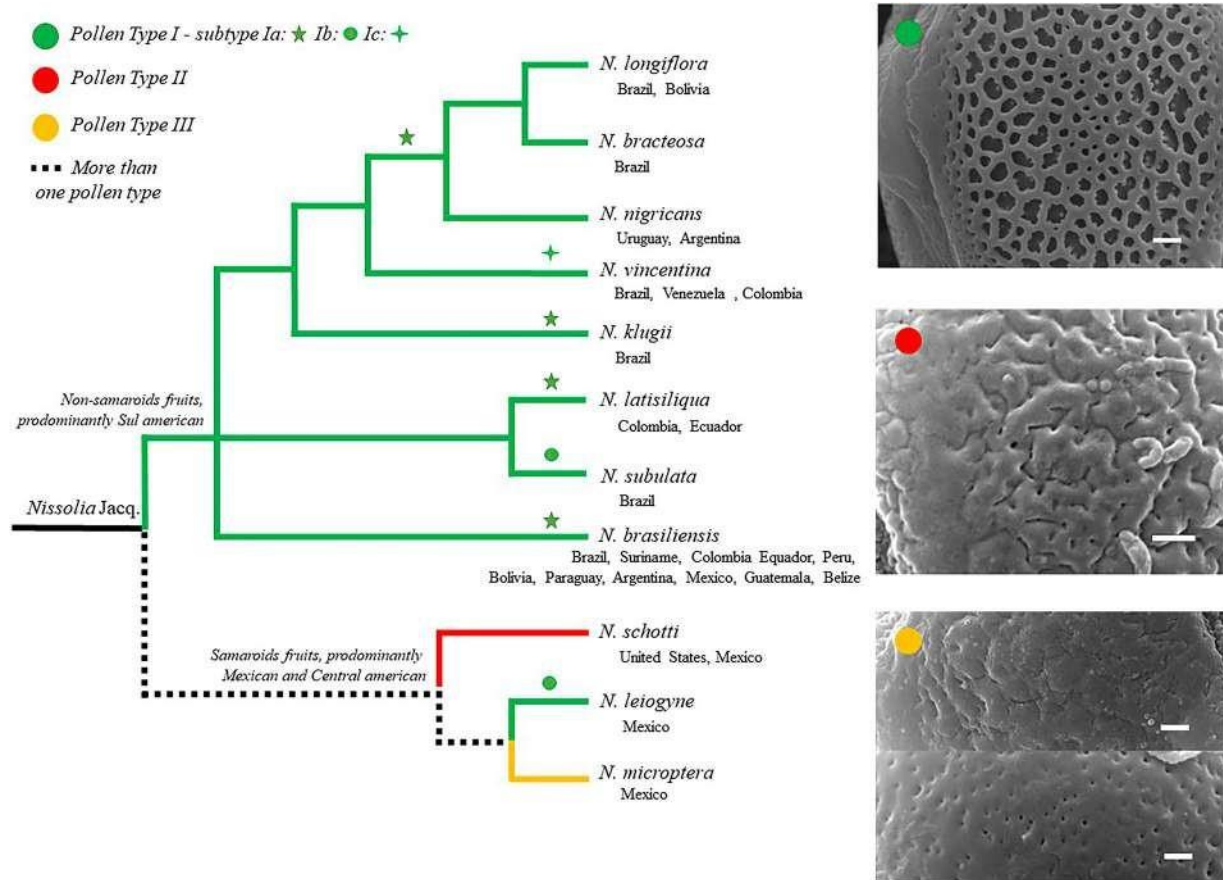


Figure 12. Phylogenetic tree for *Nissolia* with pollen types coloured and pollen morphology mapped onto each species. The tree is redrawn from Fortuna-Perez et al. (2021). In pollen type I, the sexine mesocolpium had nanoreticulate to reticulate ornamentation that varies from nanoreticulate-microreticulate (subtype Ia), nanoreticulate (subtype Ib) to nanoreticulate-reticulate (subtype Ic), here represented by *N. vincentina*. In pollen type II, the sexine mesocolpium had rugulate-perforate ornamentation, here represented by *N. gentryi*. In pollen type III, the sexine mesocolpium had dimorphic fossulate-perforate or psilate-perforate ornamentation, here represented by *N. microptera*. Scale bars – 1 µm.

the infrageneric classification of plant genera, such as *Amorimia* W.R.Anderson (Malpighiaceae) and *Mucuna* Adans. (Leguminosae) (Almeida et al. 2017; Moura et al. 2018a). The same pattern can be observed for *Nissolia*, in which both subclades recovered within this genus show distinctive pollen morphologies. The samaroid and non-samaroid subclades of *Nissolia* are vegetatively virtually indistinguishable (Moura et al. 2018b). However, these subclades are reproductively very distinct, with one subclade showing loments with sterile, winglike, flattened terminal articles, distributed from Mexico to Central America, while species of the other subclade show loments with equal articles, lacking the winglike terminal article, mostly occurring in South America (Rudd 1956, 1958; Särkinen & Hughes 2015; Moura et al. 2018b). Future systematic studies should

further explore additional characters for the *Nissolia* subclades, but for the time being, it is clear to us that fruit morphology, geographical distribution, and pollen grains might be used to propose a new infrageneric classification for the genus with both subclades being described as subgenera.

Conclusions

Our study describes the pollen morphological variation of 21 species of *Nissolia* and presents novel taxonomically significant pollen information for 18 species. More specifically, the mesocolpium sexine ultrasculpture data aided the taxonomy of *Nissolia*, and the morphological characterisation of the infrageneric samaroid and non-samaroid fruit clades reflected their species distribution. Variations in pollen grain size, class of endoaperture, presence or

absence of operculum and its ultrasculpture, corollary membrane and apocolpium ornamentation are the main pollen characters contributing to the recognition of taxonomically problematic species. A study on the pollen character evolution might shed some light on understanding this genus's evolutionary history.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Specimens investigated

Nissolia Jacq.

Nissolia acutifolia (Vogel) T.M. Moura et Fort.-Perez. Brazil: Bahia, Barreira. L. Coradin 1204, 26 July 1978 (NY 585507).

Nissolia bracteosa (Rudd) T.M. Moura et Fort.-Perez. Brazil: Piauí, Bom Jesus do Guruguá. L.P. Félix 7754, 18 May 1997 (HST 6334). Brazil: Maranhão, Porto Moutinho. G. Hatschbach 384004, 28 March 1976 (MBM 41943).

Nissolia brasiliensis (Vogel) T.M. Moura et Fort.-Perez. Brazil: Mato Grosso do Sul, Corumbá. A. Pott 10957, 29 March 2003 (BOTU32700). Brazil: Paraná, Graíra. M. Kirizawa et al. 723, 18 March 1982 (SP 178257). Brazil: Minas Gerais, Ituiutaba. A. Macedo 1783, 20 March 1949 (SP 84425).

Nissolia chacoensis (Vanni) T.M. Moura et Fort.-Perez. Bolivia: Santa Cruz, Cordillera. A.F. Fuertes et al. 2283, 12 February 1998 (MO 1322066).

Nissolia chiapensis Rudd. Chiapas, Mexico, D.E. Breedlove 55042, 7 November 1981 (NY).

Nissolia fruticosa Jacq. Mexico: Oaxaca. C.R. Torres 5573, 24 July 1984 (NY). Paraguay: Alto Paraguay. A.L. Woolston 1067, 21 March 1959 (SP 85056). Mexico: El Palmar. D.E. Breedlove, 52171, 1 August 1981 (NY).

Nissolia fruticosa var. *guatemalensis* Jacq. Bolivia: Santa Cruz, Vallegrande. M. Nee 37386, 28 December 1988 (SP 238148). Bolivia: Santa Cruz, Andrés Ibáñez. M. Nee 34292, 1 March 1987 (SP 238116).

Nissolia gentryi Rudd. Mexico: Sonora. A.L. Reina et al. 98-270, 17 March 1998 (NY).

Nissolia klugii (Rudd) T.M. Moura et Fort.-Perez. Brazil: Acre, Seringal Porongaba. DC Daly 6778, 29 May 1991 (NY 476760). Peru: Amazonas, Bagua. N. Jaramillo 251, 2 August 1994 (MO 916075).

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Nissolia leiogyne Sandwith. Mexico, Guerrero. G.B. Hinton 6291, 14 July 1934 (K 222387). Mexico: Jalisco, E.J. Lott 7861, 18 August 1991 (K 1306170).

Nissolia longiflora (Benth. ex A. Gray) T.M. Moura et Fort.-Perez. Brazil: São Paulo, Pirajuí. G. Edwall 51, July 1905 (SP 13307). Brazil: São Paulo, Botucatu. C.J. Campos 16, 25 May 1974 (BOTU 8901). Bolivia: Santa Cruz, Nuflo de Chavez. H. Michael 53044, 17 May 2005 (MO 3178230).

Nissolia microptera Poir. Mexico, Jalisco Tequila, E. Palmer 388, August 1886 (K001306150). Mexico, Michoacan, 11 October 2008 (K 1306268).

Nissolia nigricans (Burkart) T.M. Moura et Fort.-Perez. Argentina: Misiones, Sta. Ana, Candelaria. O. Morrone et al. 1162, 10 October 1996 (MO 2188393). Brazil: Paraná, Catanduvas. G. Hatschbach, P. Pelanda, 35141, 10 October 1974 (SP 141236). Argentina: Misiones, Iguazú. O. Ricardo et al. 2955, 17 December 1991 (MO 3175683). Brazil: Paraná, Catanduvas. G. Hatschbach & P. Pelanda, 35141, 10 October 1974 (MBM 23104).

Nissolia platycarpa Benth. Mexico: Nuevo León, Monterrey. H.D. Ripley 13257, 22 October 1963 (US 2483114). Mexico, Saltillo. R.A. Preston-Mafham 190, 1978 (K).

Nissolia pringlei Rose. Mexico: Puebla. Pedro Tenorio L. 15289, 19 October 1988 (K 1306166).

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A new look at the taxonomy of *Adesmia* DC. with a palynological approach
(Leguminosae – Papilionoideae – Dalbergieae – *Adesmia* Informal Clade)

Running title: Palynotaxonomy of *Adesmia*

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Adesmia is the largest taxa of the *Adesmia* informal clade and the most species-rich genus of Leguminosae in temperate South America. The pollen morphology, ultrasculpture, and ultrastructure of 25 species of *Adesmia* using light, scanning, and electron transmission microscopy were studied with the aim of adding new data to the systematic of the genus. Additionally, a principal components analysis was performed to elucidate patterns of quantitative data variation among species with respect to the most recent molecular data. We provided a novel data to the genus and a comprehensive description of the pollen grains and sexine ultrasculpture with descriptions regarding the exine ultrastructure. Furthermore, we suggest the delimitation of two pollen types based on dimensions of the equatorial and polar axis to help in futures palynotaxonomical treatments and character optimization utilizing pollen characters.

Key-words: Dalbergia Clade, Fabaceae, Palynology, Pollen Morphology, South America.

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The *Adesmia* DC. is the largest taxa of the *Adesmia* informal clade and the most species-rich genus of Leguminosae in temperate South America (Lavin et al. 2001, Klitgaard & Lavin 2005). It is represented by c. 250 species distributed from north of Peru and south of Brazil to *Tierra del Fuego* once the diversification centres the Chilean-Argentine Cordillera and the Argentine semi-arid regions (Burkart 1967; Iganci et al. 2013; Monteiro et al. 2021a, 2021b). *Adesmia* has a complex taxonomical history and is recognized by trees to subshrubs with pinnate leaves, yellow flowers with free stamens

or the two upper ones joined to the unguiculate standard, and a typical lomentum fruit or hemicraspedic (Miotto 1991).

The most complete and recent taxonomical treatment was performed by Burkart (1967) who defines the infrageneric taxa and provide biogeographic discussions. Henceforth, other studies were done to species of selected countries and/or specific regions or about specific series of the genus (Ulibarri 1980, 1982, 1984, 1986; Miotto 1991; Davyt & Izaguirre 1996; Miotto & Weachter 1996; Ulibarri & Burkart 2000; Mihoc et al. 2006; Igance et al. 2013). Based on morphology, such as the presence/absence of spines, is formally circumscribed in two subgenera (*Adesmia* subgen. *Adesmia* and *Adesmia* subgen. *Acanthadesmia* Burkart) and 43 series that needs to be phylogenetically tested (Burkart 1967; Lewis et al. 2005; Iganci et al. 2013).

Another taxonomical toll that is important and need to be tested is the palynology of the *Adesmia* genus. The pollen morphology, ultrasculpture and ultrastructure of Leguminosae, with special attention to Dalbergieae tribe, reveals informative characters to the taxonomy and systematic of these group and many genera were not studied with these approach (Ferguson & Skvarla 1981; Antonio-Domingues 2017; Antonio-Domingues et al. 2018, 2019, 2022a, 2022b, 2023 (in press); Jiang et al. 2019; Lattar et al. 2020). About the pollen studies of *Adesmia* genus, previous authors reveal valuable characters in size and shape of pollen grains, endoaperture type and ornamentation of sexine and margo (Ohashi 1971; Pire 1974; Espeche-María et al. 2018; Lattar 2020). Therefore, to understand how the pollen grains can contribute to future studies in taxonomy, systematic and phylogenetic of *Adesmia* we aimed to recognise species' palynotaxonomical patterns.

Material and methods

Unopened mature buds were obtained from 38 herbarium specimens belonging to the 25 species of *Adesmia* (*A. ameghinoi*, *A. araujoii*, *A. arillata*, *A. bicolor*, *A. boronoides*, *A. ciliata*, *A. filipes*, *A. incana*, *A. lanata*, *A. latifolia*, *A. miraflorensis*, *A. muricata*, *A. obovata*, *A. paranensis*, *A. parviflora*, *A. punctata*, *A. pampeana*, *A. reitziana*, *A. riograndensis*, *A. rocinhensis*, *A. sulina*, *A. trijuga*, *A. tristis*, *A. vallsii*, *A. volckmannii*). Specimens were sampled from CGMS, K, MBM, NY, SP and UEC herbaria (acronyms follow Thiers, continuously updated). All specimens examined are listed in the *Specimens Investigated* section. Samples were labelled with a three-letter abbreviation of the species epithet plus the last two numbers of the herbarium voucher or collect number (see Table I, Specimen column).

Pollen grains were treated by the acetolysis method (Erdtman 1960), following the modifications proposed by Melhem et al. (2003). Measurements were performed under the light microscope (LM) using an Olympus OSM-4 (10 ×) micrometre drum coupled to the eyepiece of an Olympus BX50 binocular microscope. The polar axis and equatorial axis in equatorial view were measured on 25 randomly selected pollen grains from each taxon. These measurements were statistically analysed for the arithmetic mean ($\bar{x}$), average standard deviation (S_x), sample standard deviation (s), coefficient of variability ($V\%$), and 95% confidence interval (CI) (Vieira 2011; Zar 2010). Ten measurements of the main pollen morphometric parameters were also made: length and width of the colpus, length and width of the endoaperture, the thickness of the nexine and sexine layers, and size of the reticulum lumen. Exine measurements were performed in the mesocolpium region. The arithmetic mean and range were calculated for each pollen grain size class and exine (sexine + nexine).

Photomicrographs were obtained using an Olympus BX 50 light microscope with an Olympus U-CMAD-2 video camera and Olympus CellSens 1.5 software. Permanent LM slides were deposited in the pollen reference collection (Palynotheca) of Laboratório de Palinologia PALINO-IPA, Instituto de Pesquisas Ambientais, São Paulo state, Brazil.

For scanning electron micrographs (SEM), non-acetolysed pollen grains were washed and placed on a metal stub and sputter-coated (Leica EM ACE 600 Vienna-Austria) with gold (80 nm). Samples were observed under a JEOL JSMIT300LV microscope (Tokyo, Japan) operating at a 20 kV electron beam, and the images were digitised.

For transmission electron microscopy (TEM) mature anthers were removed from flower buds and fixed in a modified Karnovsky solution (Karnovsky 1965) for at least 24 h. They were quently rinsed with sodium cacodylate buffer (0.1 M) and immediately post-fixed in osmium oxide (1%) for 1 h. Samples were then rinsed in a saline solution (0.9% sodium chloride) and pre- stained with uranyl acetate (2.5%) followed by dehydration in an acetone series (35–100%), 20 min each. Samples were then gradually infiltrated in Spurr resin (Spurr 1969) and embedded in pure resin for 48 h, at 70 °C. Ultrathin section (60 nm) were obtained with an ultramicrotome (Sorvallter Blum MT2, Norwalk, CT, USA) deposited on copper grids and stained using uranyl acetate (2.5%) and lead citrate (Reynold 1963). Images were obtained under a transmission electron microscope (JEOL JEM 1400 Tokyo, Japan) at 80 kV.

Pollen terminology was based on Punt et al. (2007) and Halbritter et al. (2018). Pollen shape classes and amb types followed Erdtman (1952), and colpus length categories were according to Mark et al. (2012). The endoaperture classes and index of sexine and nexine thickness were proposed by Antonio-Domingues et al. (2022). TEM descriptions of the exine infratectum followed Halbritter et al. (2018) and Ferguson and Skvarla (1983) terminologies.

Principal component analysis (PCA) was performed to explore whether pollen grain characteristics allowed for grouping genera and species. Nine metric variables (polar axis [PA], equatorial axis [EA], colpus length [CL] colpus width [CW], endoaperture length [EL], endoaperture width [WE], nexine thickness [N], sexine thickness [S = infratectum + tectum], exine thickness [Ex = N + S]) and three classes/indices (shape class [P/E = PA/EA], endoaperture class [Cen = EW/EL], index of sexine and nexine thickness [S/N]) were analysed using FITOPAC (Shepherd 1996) and PC-ORD version 7 (McCune & Mefford 2016).

Results

Pollen morphology and ultrasculpture

Adesmia ameghinoi: pollen grains are small sized, prolate spheroidal, circular to ellipsoidal in equatorial view. Colporus with granulate operculum and membrane and psilate-perforate margo. Endoaperture circular to lalongate, absent aperture protruding. Sexine 1.2 to 1.9 times thicker than nexine, exine 1.2 – 1.4 μm thick. Mesocolpium and apocolpium nanoreticulate-perforate (Figures 1A-C, 2A-C).

Adesmia araujoi: pollen grains are small sized, prolate spheroidal, circular to ellipsoidal in equatorial view. Endoaperture circular to lalongate and aperture protruding are present. Sexine 1.3 to 2.0 times thicker than nexine, exine 1.3 – 1.6 μm thick. Mesocolpium and apocolpium (Figures 1D-F).

Adesmia arillata: pollen grains are small sized, prolate spheroidal, circular in equatorial view. Colporus with areolate-granulate operculum, granulate membrane and psilate-perforate margo. Endoaperture lalongate and aperture protruding are present. Sexine 1.0 to 1.7 times thicker than nexine, exine 1.0 – 1.5 μm thick. Mesocolpium and apocolpium nanoreticulate (Figures 1G-I, 2D-F)

Adesmia bicolor: pollen grains small sized, prolate spheroidal, circular in equatorial view. Endoaperture circular to lalongate and aperture protruding are present. Colporus with areolate-granulate operculum, granulate membrane and psilate-perforate margo. Sexine 1.3 to 1.6 times thicker than nexine, exine 1.1 – 1.4 μm thick. Mesocolpium and apocolpium nanoreticulate-perforate (Figures 1J-L, 2G-I)

Adesmia boronoides: pollen grains are small sized, prolate-spheroidal to subprolate, circular in equatorial view. Endoaperture circular to lalongate and aperture protruding are present. Colporus without operculum, granulate membrane and psilate-perforate margo. Sexine from 1.7 to 2.5 times thicker than nexine; exine 1.6 – 1.9 μm thick. Mesocolpium and apocolpium nanoreticulate-perforate (Figures 1M-O, 2J-L).

Adesmia ciliata: pollen grains are small to medium sized, prolate spheroidal, circular to ellipsoidal in equatorial view. Endoaperture circular to lalongate and aperture protruding are present. Colporus with areolate operculum, granulate membrane and psilate-perforate margo. Sexine from 1.1 to 2.0 times thicker than nexine; exine 1.0 – 1.6 μm thick. Mesocolpium nano to microreticulate and apocolpium nanoreticulate (Figures 3A-C, 4A-C).

Adesmia filipes: pollen grains are medium sized, prolate spheroidal, circular to ellipsoidal in equatorial view. Colporus with areolate-granulate operculum, granulate membrane and psilate-perforate margo. Endoaperture lalongate, absent aperture protruding. Sexine from 1.2 to 1.8 times thicker than nexine; exine 1.1 – 1.5 μm thick. Mesocolpium nano- to microreticulate and apocolpium microreticulate (Figures 3D-F, 4D-F).

Adesmia incana: pollen grains are small to medium sized, oblate spheroidal to prolate spheroidal, circular to ellipsoidal in equatorial view. Colporus with areolate-granulate operculum, granulate membrane and psilate-perforate margo. Endoaperture very

lalongate and aperture protruding are present. Sexine from 1.2 to 1.8 times thicker than nexine, exine 1.6 – 1.8 μm thick. Mesocolpium nano- to microreticulate and apocolpium nanoreticulate-perforate (Figures 3G-I, 4G-I).

Adesmia lanata: pollen grains are small sized, oblate-spheroidal to prolate-spheroidal, circular to ellipsoidal in equatorial view. Colporus with areolate-granulate operculum, granulate membrane and psilate-perforate margo. Endoaperture circular to lalongate and aperture protruding are present. Sexine from 1.3 to 1.9 times thicker than nexine; exine 1.4 – 1.7 μm thick. Mesocolpium and apocolpium nanoreticulate-perforate (Figures 3J-L, 4J-L).

Adesmia latifolia: pollen grains are small sized, prolate-spheroidal to subprolate, circular to ellipsoidal in equatorial view. Colporus with granulate operculum and membrane, and psilate-perforate margo. Endoaperture circular to lalongate, rarely lalongate and aperture protruding are present. Sexine from 1.2 to 2.0 times thicker than nexine; exine 1.2 – 1.5 μm thick. Apocolpium and mesocolpium nanoreticulate-perforate (Figures 3M-O, 4M-O).

Adesmia miraflloensis: pollen grains are small sized, rarely medium, prolate-spheroidal, circular to ellipsoidal in equatorial view. Colporus with granulate operculum, granulate membrane and psilate-perforate margo. Endoaperture lalongate and aperture protruding are present. Sexine from 1.4 to 4.0 times thicker than nexine, exine 1.5 – 1.6 μm thick. Mesocolpium nano- to microreticulate-perforate and apocolpium nanoreticulate-perforate (Figures 5A-C, 6A-C).

Adesmia muricata: pollen grains are small to medium sized, oblate-spheroidal, circular to ellipsoidal in equatorial view. Colporus with granulate operculum and membrane, and psilate-perforate margo. Endoaperture circular to very lalongate and aperture protruding

are present. Sexine from 1.2 to 2.1 times thicker than nexine, exine 1.2 – 1.8 μm thick. Mesocolpium nano- to microreticulate and apocolpium perforate (Figures 5D-F, 6D-F).

Adesmia obovata: pollen grains are small to medium sized, prolate-spheroidal to subprolate, ellipsoidal in equatorial view. Colporus with areolate-granulate operculum, granulate membrane and psilate-perforate margo. Endoaperture circular to lalongate and aperture protruding are present. Sexine from 0.9 to 1.5 times thicker than nexine, exine 1.0 – 1.5 μm thick. Mesocolpium nano- to microreticulate-perforate and apocolpium perforate (Figures 5G-I, 6G-I).

Adesmia paranensis: pollen grains are small to medium sized, prolate-spheroidal, circular to ellipsoidal in equatorial view. Colporus with areolate-granulate operculum, granulate membrane and psilate-perforate margo. Endoaperture circular to lolongate and aperture protruding are present. Sexine from 1.2 to 2.2 times thicker than nexine, exine 1.4 – 2.0 μm thick. Mesocolpium nano- to microreticulate and apocolpium nanoreticulate-perforate (Figures 5J-L, 6J-L).

Adesmia parviflora: pollen grains are small sized, rarely medium, oblate-spheroidal, circular to ellipsoidal in equatorial view. Colporus with areolate-granulate operculum, granulate membrane and psilate-perforate margo. Endoaperture lalongate and aperture protruding are present. Sexine 1.1 to 1.8 times thicker than nexine; exine 1.2 – 1.6 μm thick. Mesocolpium nano- to microreticulate and apocolpium perforate (Figures 5M-O, 6M-O).

Adesmia pampeana: pollen grains are small sized, prolate-spheroidal to subprolate, circular to ellipsoidal in equatorial view. Colporus with areolate-granulate operculum, granulate membrane and psilate-perforate margo. Endoaperture lolongate and aperture protruding are present. Sexine from 1.2 to 2.0 times thicker than nexine; exine 1.3 – 1.5

µm thick. Mesocolpium nano- to microreticulate and apocolpium nanoreticulate (Figures 7A-C, 8A-C).

Adesmia punctata: pollen grains are small to medium sized, prolate-spheroidal to subprolate, ellipsoidal in equatorial view. Colporus with areolate-granulate operculum, granulate membrane and psilate-perforate margo. Endoaperture circular to lolongate and aperture protruding are absent. Sexine 1.5 to 2.2 times thicker than nexine, exine 1.4 – 1.6 µm thick. Mesocolpium nanoreticulate-perforate and apocolpium perforate (Figures 7D-F, 8D-F).

Adesmia reitziana: pollen grains are small to medium sized, subprolate to prolate, circular to ellipsoidal in equatorial view. Colporus with areolate-granulate operculum, granulate membrane and psilate-perforate margo. Endoaperture lolongate and aperture protruding are present. Sexine from 1.3 to 2.3 times thicker than nexine; exine 1.4 – 2.1 µm thick. Mesocolpium nano- to microreticulate and apocolpium nanoreticulate-perforate (Figures 7G-I, 8G-I).

Adesmia riograndensis: pollen grains are small to medium sized, prolate-spheroidal to subprolate, ellipsoidal in equatorial view. Colporus with areolate-granulate operculum, granulate membrane and psilate-perforate margo. Endoaperture lolongate and aperture protruding are not present. Sexine from 1.2 to 1.8 times thicker than nexine; exine 1.2 – 1.4 µm thick. Mesocolpium and apocolpium nanoreticulate-perforate (Figures 7J-L, 8J-L).

Adesmia rocinhensis: pollen grains are small to medium sized, oblate-spheroidal to prolate-spheroidal, circular in equatorial view. Colporus with granulate operculum and membrane, and psilate-perforate margo. Endoaperture circular to lalongate and aperture protruding are present. Sexine from 1.3 to 1.8 times thicker than nexine; exine 1.3 – 1.6

µm thick. Mesocolpium nano- to microreticulate and apocolpium nanoreticulate (Figures 7M-O, 8M-O).

Adesmia sulina: pollen grains are small to medium sized, oblate-spheroidal to prolate-spheroidal, circular to ellipsoidal in equatorial view. Colporus with areolate-granulate operculum, granulate membrane and psilate-perforate margo. Endoaperture lalongate, circular to lolongate and aperture protruding are present. Sexine from 1.6 to 2.1 times thicker than nexine, exine 1.4 – 1.8 µm thick. Mesocolpium nano- to microreticulate and apocolpium nanoreticulate-perforate (Figures 9A-C, 10A-C).

Adesmia trijuga: pollen grains are small to medium sized, oblate-spheroidal to prolate-spheroidal, circular in equatorial view. Colporus with areolate-granulate operculum, granulate membrane and psilate-perforate margo. Endoaperture circular to lalongate and aperture protruding are absent. Sexine from 1.4 to 2.0 times thicker than nexine; exine 1.4 – 1.6 µm thick. Mesocolpium nanoreticulate and apocolpium nanoreticulate-perforate (Figures 9D-F, 10D-F).

Adesmia tristis: pollen grains are small sized, prolate-spheroidal, circular to ellipsoidal in equatorial view, colporus with areolate operculum, granulate membrane and psilate-perforate margo. Endoaperture circular to lolongate and aperture protruding are absent. Sexine from 1.2 to 1.9 times thicker than nexine; exine 1.3 – 1.7 µm thick. Mesocolpium nano- to microreticulate and apocolpium nanoreticulate (Figures 9G-I, 10G-I).

Adesmia vallsii: pollen grains are small to medium sized, prolate-spheroidal, circular in equatorial view. Colporus with granulate operculum and membrane, and psilate-perforate margo. Endoaperture lalongate, circular to lolongate and aperture protruding are present. Sexine from 1.3 to 1.8 times thicker than nexine; sexine 1.3 – 1.6 µm thick. Mesocolpium nanoreticulate and apocolpium perforate (Figures 9J-L, 10J-L).

Adesmia volckmannii: pollen grains are small to medium sized, prolate-spheroidal, circular to ellipsoidal in equatorial view. Colporus with areolate-granulate operculum, granulate membrane and psilate-perforate margo. Endoaperture circular to lalongate and aperture protruding are present. Sexine from 1.2 to 1.6 times thicker than nexine; sexine 1.2 – 1.5 μm thick. Mesocolpium nano- to microreticulate and apocolpium nanoreticulate-perforate (Figures 10M-O, 11M-O).

Pollen ultrastructure

Adesmia mirafllorensis: exine presents an e-translucent undulate compact tectum, tectate-perforate with supratectal e-dense pollen kit. The infratectal structures is e-translucent, present simple and complete columellae and e-dense infratectal pollen kit. The thick e-translucent foot layer goes from continuous with channels to discontinuous in apertural area. The e-dense endexine is compact-continuous, thin and costate, and near to the aperture is spongy, thick and endosculptured. Monolyered e-translucent intine (Figure 11A-C).

Adesmia punctata: exine presents an e-translucent undulate compact tectum, tectate-perforate to semitectate with supratectal e-dense pollen kit. The infratectal structures is e-translucent, present granules somewhat organised in a narrow and irregular row and some simple and complete to incomplete columellae, and e-dense infratectal pollen kit. The thick e-translucent foot layer goes from continuous with channels to discontinuous in apertural area. The e-dense endexine is compact-continuous, thin and costate, and near to the aperture is spongy, thick and endosculptured. Monolyered e-translucent intine (Figure 11D-F).

Adesmia rocinhensis: exine presents an e-translucent to e-dense undulate compact tectum, thath varies from eutectate, tectate-perforate to semitectate with supratectal e-dense

pollen kit. The infratectal structures is e-translucent, present simple, complete and incomplete columellae, some isolated granules are observed and e-dense infratectal pollen kit. The thick e-translucent foot layer goes from continuous with channels to discontinuous in apertural area. The e-dense endexine is compact-continuous, thin and costate, and near to the aperture is spongy, thick and endosculptured. Monolayered e-translucent intine (Figure 11G-I).

Principal component analysis (PCA)

The PCA showed the relationship between species based on nine metric variables and three classes/indices (Figure 12). Correlation coefficients are shown in Table III. The first two axes accounted for 93.74% of the total variability of the quantitative data analysed. The first axis explained 78.46% of the variance and was mainly associated with PA, CL, N, S, E and S/N. The second axis summarised 15.28% of the total variability, with EA, P/E, CW, EW, EL and Cen being the variables that contributed most to this axis.

Adesmia reitziana (rei89, rei72, rei58), *A. paranensis* (para28, para93), *A. punctata* (pun62), *A. trijuga* (trij67), *A. araujoii* (ara92), *A. filipis* (fil63) and *A. riograndensis* (rio71) represents the largest polar axis, colporus length and endoaperture length were located on the upper left side of the PCA associated with high values of PA, CL, EL, P/E. *Adesmia volckmannii* (vol19), *A. vallsii* (val33), *A. muricata* (mur27), *A. obovata* (obo*), *A. sulina* (sul55, sul77), *A. ciliata* (cil10), *A. incana* (inc10*), *A. trijuga* (trij06*), *A. miraflorensis* (mir12*) showed high EA, EW, CE, Cen, E, S and N were located on bottom left of PCA and represent the thicker exine layers and lalongate endoaperture.

Due to their lowest Cen, CW, EW and EA values *Adesmia latifolia* (lat63, lat00, lat01), *A. pampeana* (pam72), *A. filipes* (fil65) and *A. ameghinoi* (ame79*) were placed on the upper right side of the PCA with lalongate endoaperture. Conversely, lalongate endoaperture, represented by *A. parviflora* (par83), *A. muricata* (mur47), *A. bicolor*

(bic63), *A. tristis* (tris52, tris76), *A. ciliata* (cil62, cil92), *A. arillata* (ari63), *A. boronoides* (bor*), *A. lanata* (lan70) were placed on the bottom right side of the PCA.

Cluster analysis (UPGMA and Euclidean distance)

We identified two groups of specimens with 0% similarity (Figure 13). One group had similarity greater than 40% and comprised the species with the smallest pollen grains and smallest colporus dimensions [*Adesmia ameghinoi* (ame79*), *A. arillata* (ari63), *A. boronoides* (bor*), *A. lanata* (lan70), *A. filipes* (fil65), *A. latifolia* (lat01), *A. pampeana* (pam72)]. The other group had similarity greater than 25% and remainder of species which had the largest pollen grains and largest colporus dimensions [*A. araujoi* (ara92), *A. obovata* (obo*), *A. muricata* (mur27, mur47), *A. sulina* (sul55, sul77), *A. vallsii* (val33), *A. filipes* (fil63), *A. paranaensis* (para23, para63), *A. reitziana* (rei48, rei72, rei89), *A. trijuga* (trij67, trij06*), *A. volkmannii* (vol19*), *A. punctata* (pun62), *A. bicolor* (pun63), *A. latifolia* (lat63, lat00), *A. ciliata* (cil62, cil92, cil10), *A. tristis* (tris52, tris76), *A. miraflorensis* (mir12*), *A. incana* (inc10*) and *A. parviflora* (par83)].

Discussion

Previous palynological analyses of *Adesmia bicolor*, *A. muricata*, *A. latifolia*, *A. punctata* and *A. trijuga* were carried by Ohashi (1971), Pire (1974), Espeche-María et al. (2018) and Lattar et al. (2020). The results of these studies are supported by our analyses, although with some differences in the classification of pollen size, class of endoaperture, operculum presence, ornamentation and sexine ultrasculpture. Table IV compiles our results and previous pollen descriptions of the analysed species found in the literature.

In the present study, we described 18 species of the genus palynologically for the first time. In the Brazilian territory presents 18 native species and we study 15 of that and

the remaining species occurs in Argentina, Bolivia, Uruguay, Peru and Chile (Flora e Funga do Brasil 2021; Monteiro et al. 2021a, 2021b).

The presence of operculum and their ornamentations are described for *Adesmia* for the first time here. The absent operculum was observed here only in two species (*A. araujoi*, *A. boronoides*) and the remaining species presents granulate, areolate or areolate-granulate and it can be useful for species delimitation. The operculum absence in all species in the early studies can be explained by using acetolysed pollen to describes this aperture element (Hesse & Waha 1989). Operculate pollen grains are widespread in Leguminosae and show relevant ecological and physiological functions and these are important pollen features in the taxonomy of other Dalbergioid (Antonio-Domingues et al. 2018, 2019, 2022a, 2022b, 2022c, 2023 in press).

In the present study we do not delimit pollen types according to the pollen morphology and ultrasculpture of the *Adesmia* species but to futures studies we present some observations hereafter.

The arithmetic mean of pollen grain equatorial and polar axis goes from 16.9 to 24.9 μm and 18.5 and 28.8 μm (Table I), respectively, and it is the most important variable in first two axes of the PCA (Table III). All species with arithmetic mean of pollen grain equatorial and polar axis smaller than 20.2 μm and 21.4 μm [*A. ameghinoi*, *A. arillata*, *A. boronoides*, *A. filipis* (only fil65), *A. lanata*, *A. latifolia*, *A. pampeana*] (Table I) are in the right side of the graph (Image 12). Except by one specimen [*A. latifolia* (lat00)] all these specimens are in a cluster with similarity greater than 40% (Image 13). Of these species, only *A. arillata* and *A. latifolia* are Brazilian native and the others five do not occur in this national territory.

The other group of species present pollen grains equatorial and polar axis greater than 21.1 μm and 21.7 μm (Table I), respectively, and present similarity about 25%

(Figure 13). This groups include five non-Brazilian species (*A. miraflorensis*, *Adesmia obovata*, *A. parviflora*, *A. trijuga*, *A. volckmannii*) and 13 Brazilians native (*A. araujoi*, *A. bicolor*, *A. ciliata*, *A. incana*, *A. muricata*, *A. paranensis*, *A. punctatata*, *A. reitziana*, *A. riograndensis*, *A. rocinhensis*, *A. sulina*, *A. tristis*, *A. vallsii*).

Considering this, we suggest the delimitation of two pollen types to the *Adesmia* species. The pollen type I includes species with arithmetic mean of pollen grain equatorial and polar axis smaller than 20.5 μm and 21.5 μm and the pollen type II to the species with the arithmetic mean of pollen grain equatorial and polar axis greater than 20.5 μm and 21.5 μm .

Conclusions

Our study describes the pollen morphological variation of 25 species of *Adesmia*, and presents novel taxonomically significant pollen information for 18 species. In particular, the size of the equatorial and polar axis is important palynological data which may contribute with future palynotaxonomy studies of *Adesmia*. A study of character evolution related to pollen is now needed to better understand the evolutionary history of the genus and the *Adesmia* clade.

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The authors report no conflict of interest.

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Figure and table captions

Figure 1. Light microscopy of *Adesmia* DC. species. **A-C.** *Adesmia ameghinoi*. **A.** Detail of the colporus, endoaperture, operculum and surface, equatorial view. **B.** Apertures 3-colporate, long and pointed apices, colpori and apocolpium surface. **C.** Optical section, equatorial view. **D-F.** *Adesmia araujoii*. **D.** Detail of the colporus, endoaperture, surface and operculum, equatorial view. **E.** Apertures 3-colporate, long and pointed apices and apocolpium surface. **F.** Optical section, equatorial view. **G-I.** *Adesmia arillata*. **G.** Detail of the colporus, surface and operculum, equatorial view. **H.** Apertures 3-colporate, long and pointed apices and apocolpium surface. **I.** Optical section, equatorial view. **J-L.** *Adesmia bicolor*. **J.** Detail of the colporus, surface and operculum, equatorial view. **K.** Apertures 3-colporate, long and pointed apices and apocolpium surface. **L.** Optical section, equatorial view. **M-O.** *Adesmia boronoides*. **M.** Detail of the colporus, surface and operculum, equatorial view. **N.** Apertures 3-colporate, long and pointed apices and apocolpium surface. **O.** Optical section, equatorial view. Scale bars – A-C, H, I, K, L, N, O = 2 µm; D-G, J, M = 5 µm.

Figure 2. Scanning electron microscopy of *Adesmia* DC. species. **A-C.** *Adesmia ameghinoi*. **A.** Detail of mesocolpium. **B.** Detail of apertural membrane of the colporus, margo and operculum, equatorial view. **C.** Detail of apocolpium. **D-F.** *Adesmia arillata*. **D.** Detail of mesocolpium. **E.** Detail of apertural membrane of the colporus, margo and operculum, equatorial view. **F.** Detail of apocolpium. **G-I.** *Adesmia bicolor*. **G.** Detail of mesocolpium. **H.** Detail of apertural membrane of the colporus, margo and operculum, equatorial view. **I.** Detail of apocolpium. **J-L.** *Adesmia boronoides*. **J.** Detail of mesocolpium. **K.** Detail of apertural membrane of the colporus, margo and operculum, equatorial view. **L.** Detail of apocolpium. Scale bars – A-D, F-K = 1 μm ; E, L = 2 μm .

Figure 3. Light microscopy of *Adesmia* DC. species. **A-C.** *Adesmia ciliata*. **A.** Detail of the colporus, endoaperture, operculum and surface, equatorial view. **B.** Apertures 3-colporate, long and pointed apices, colpori and apocolpium surface. **C.** Optical section, equatorial view. **D-F.** *Adesmia filipis*. **D.** Detail of the colporus, endoaperture, operculum and surface, equatorial view. **E.** Apertures 3-colporate, long and pointed apices, colpori and apocolpium surface. **F.** Optical section, equatorial view. **G-I.** *Adesmia incana*. **G.** Detail of the colporus, endoaperture, operculum and surface, equatorial view. **H.** Apertures 3-colporate, long and pointed apices, colpori and apocolpium surface. **I.** Optical section, equatorial view. **J-L.** *Adesmia lanata*. Detail of the colporus, surface and operculum, equatorial view. **K.** Apertures 3-colporate, long and pointed apices and apocolpium surface. **L.** Optical section, equatorial view. **M-O.** *Adesmia latifolia*. **M.** Detail of the colporus, surface and operculum, equatorial view. **N.** Apertures 3-colporate, long and pointed apices and apocolpium surface. **O.** Optical section, equatorial view. Scale bars – D-F, J-L, N = 2 μm ; A-C, G- I, M, O = 5 μm .

Figure 4. Scanning electron microscopy of *Adesmia* DC. species. **A-C.** *Adesmia ciliata*. **A.** General view of mesocolpium. **B.** Detail of apertural membrane of the colporus, margo and operculum, equatorial view. **C.** Detail of apocolpium. **D-F.** *Adesmia filipis*. **D.** Detail of mesocolpium. **E.** Detail of apertural membrane of the colporus, margo and operculum, equatorial view. **F.** Detail of apocolpium. **G-I.** *Adesmia incana*. **G.** Detail of apertural membrane of the colporus, margo, operculum and mesocolpium. **H.** General view of apertural area. **I.** Detail of apocolpium. **J-L.** *Adesmia lanata*. **J.** Detail of mesocolpium. **K.** Detail of apertural membrane of the colporus, margo and operculum, equatorial view. **L.** Detail of apocolpium. **M-O.** *Adesmia latifolia*. **M.** Detail of mesocolpium. **N.** Detail of apertural membrane of the

colporus, margo and operculum, equatorial view. **O.** General view of apocolpium. Scale bars – B, C, E, J–N = 1 μm ; D, F, G, I = 2 μm ; A, H, O = 5 μm .

Figure 5. Light microscopy of *Adesmia* DC. species. **A–C.** *Adesmia miraflorensis*. **A.** Detail of the colporus, endoaperture, operculum and surface, equatorial view. **B.** Apertures 3-colporate, long and pointed apices, colpori and apocolpium surface. **C.** Optical section, equatorial view. **D–F.** *Adesmia muricata*. **D.** Detail of the colporus, endoaperture, operculum and surface, equatorial view. **E.** Apertures 3-colporate, long and pointed apices, colpori and apocolpium surface. **F.** Optical section, equatorial view. **G–I.** *Adesmia obovata*. **G.** Detail of the colporus, endoaperture, operculum and surface, equatorial view. **H.** Apertures 3-colporate, long and pointed apices, colpori and apocolpium surface. **I.** Optical section, equatorial view. **J–L.** *Adesmia paranensis*. Detail of the colporus, surface and operculum, equatorial view. **K.** Apertures 3-colporate, long and pointed apices and apocolpium surface. **L.** Optical section, equatorial view. **M–O.** *Adesmia parviflora*. **M.** Detail of the colporus, surface and operculum, equatorial view. **N.** Apertures 3-colporate, long and pointed apices and apocolpium surface. **O.** Optical section, equatorial view. Scale bars – K = 2 μm ; A–J, L–O = 5 μm .

Figure 6. Scanning electron microscopy of *Adesmia* DC. species. **A–C.** *Adesmia miraflorensis*. **A.** Detail of mesocolpium, apertural membrane of the colporus, margo and operculum, equatorial view. **B.** General view of apertural area. **C.** Detail of apocolpium. **D–F.** *Adesmia muricata*. **D.** Detail of mesocolpium, apertural membrane of the colporus, margo and operculum, equatorial view. **E.** General view of apertural area. **F.** Detail of apocolpium. **G–I.** *Adesmia obovata*. **G.** Detail of mesocolpium. **H.** Detail of apertural membrane of the colporus, margo and operculum, equatorial view. **I.** Detail of apocolpium. **J–L.** *Adesmia paranensis*. **J.** Detail of mesocolpium apertural membrane of the colporus, margo and operculum, equatorial view. **K.** General view of apertural area. **L.** Detail of apocolpium. **M–O.** *Adesmia parviflora*. **J.** Detail of mesocolpium. **K.** Detail of mesocolpium apertural membrane of the colporus, margo and operculum, equatorial view. **L.** Detail of apocolpium. Scale bars – A, C, D, J, L, M, O = 1 μm ; F, G–I, N = 2 μm ; B, E, K = 5 μm .

Figure 7. Light microscopy of *Adesmia* DC. species. **A–C.** *Adesmia pampeana*. **A.** Detail of the colporus, endoaperture, operculum and surface, equatorial view. **B.** Apertures 3-colporate, long and pointed apices,

colpori and apocolpium surface. **C.** Optical section, equatorial view. **D-F.** *Adesmia punctata*. **D.** Detail of the colporus, endoaperture, operculum and surface, equatorial view. **E.** Apertures 3-colporate, long and pointed apices, colpori and apocolpium surface. **F.** Optical section, equatorial view. **G-I.** *Adesmia reitziana*. **G.** Detail of the colporus, endoaperture, operculum and surface, equatorial view. **H.** Apertures 3-colporate, long and pointed apices, colpori and apocolpium surface. **I.** Optical section, equatorial view. **J-L.** *Adesmia riograndensis*. **J.** Detail of the colporus, endoaperture, operculum and surface, equatorial view. **K.** Apertures 3-colporate, long and pointed apices, colpori and apocolpium surface. **L.** Optical section, equatorial view. **M-O.** *Adesmia rocinhensis*. **M.** Detail of the colporus, endoaperture, operculum and surface, equatorial view. **N.** Apertures 3-colporate, long and pointed apices, colpori and apocolpium surface. **O.** Optical section, equatorial view. Scale bars – B, C, K = 2 μm ; A, D-J, L-O = 5 μm .

Figure 8. Scanning electron microscopy of *Adesmia* DC. species. **A-C.** *Adesmia pampeana*. **A.** Detail of mesocolpium, apertural membrane of the colporus, margo and operculum, equatorial view. **B.** General view of apertural area. **C.** Detail of apocolpium. **D-F.** *Adesmia punctata*. **D.** Detail of mesocolpium, equatorial view. **E.** Detail of mesocolpium, apertural membrane of the colporus, margo and operculum, equatorial view. **F.** Detail of apocolpium. **G-I.** *Adesmia reitziana*. **G.** Detail of mesocolpium, equatorial view. **H.** Detail of mesocolpium, apertural membrane of the colporus, margo and operculum, equatorial view. **I.** Detail of apocolpium. **J-L.** *Adesmia riograndensis*. **J.** Detail of mesocolpium, equatorial view. **K.** Detail of mesocolpium, apertural membrane of the colporus, margo and operculum, equatorial view. **L.** Detail of apocolpium. **M-O.** *Adesmia rocinhensis*. **M.** Detail of mesocolpium, equatorial view. **N.** Detail of mesocolpium, apertural membrane of the colporus, margo and operculum, equatorial view. **O.** Detail of apocolpium. Scale bars – A-D, F, G-L, N = 1 μm ; E, M, O = 2 μm .

Figure 9. Light microscopy of *Adesmia* DC. species. **A-C.** *Adesmia sulina*. **A.** Detail of the colporus, endoaperture, operculum and surface, equatorial view. **B.** Apertures 3-colporate, long and pointed apices, colpori and apocolpium surface. **C.** Optical section, equatorial view. **D-F.** *Adesmia trijuga*. **D.** Detail of the colporus, endoaperture, operculum and surface, equatorial view. **E.** Apertures 3-colporate, long and pointed apices, colpori and apocolpium surface. **F.** Optical section, equatorial view. **G-I.** *Adesmia tristis*. **G.** Detail of the colporus, endoaperture, operculum and surface, equatorial view. **H.** Apertures 3-colporate, long and pointed apices, colpori and apocolpium surface. **I.** Optical section, equatorial view. **J-**

L. *Adesmia vallsii*. **J.** Detail of the colporus, endoaperture, operculum and surface, equatorial view. **K.** Apertures 3-colporate, long and pointed apices, colpori and apocolpium surface. **L.** Optical section, equatorial view. **M-O.** *Adesmia volkmani*. **M.** Detail of the colporus, endoaperture, operculum and surface, equatorial view. **N.** Apertures 3-colporate, long and pointed apices, colpori and apocolpium surface. **O.** Optical section, equatorial view. Scale bars – B, C = 2 μm ; A, D-O = 5 μm .

Figure 10. Scanning electron microscopy of *Adesmia* DC. species. **A-C.** *Adesmia sulina*. **A.** General view of apertural area and mesocolpium. **B.** Detail of mesocolpium, apertural membrane of the colporus, margo and operculum, equatorial view. **C.** Detail of apocolpium. **D-F.** *Adesmia trijuga*. **D.** Detail of mesocolpium. **E.** Detail of mesocolpium, apertural membrane of the colporus, margo and operculum, equatorial view. **F.** Detail of apocolpium. **G-I.** *Adesmia tristis*. **G.** Detail of mesocolpium and margo, equatorial view. **H.** Detail of mesocolpium, apertural membrane of the colporus, margo and operculum, equatorial view. **I.** Detail of apocolpium. **J-L.** *Adesmia vallsii*. **J.** General view of the mesocolpium and apocolpium. **K.** Detail of mesocolpium, apertural membrane of the colporus, margo and operculum, equatorial view. **L.** Detail of apocolpium. **M-O.** *Adesmia volkmani*. **M.** Detail of mesocolpium, equatorial view. **N.** Detail of mesocolpium, apertural membrane of the colporus, margo and operculum, equatorial view. **O.** Detail of apocolpium. Scale bars – B, C, E, F, G, I, K, L = 1 μm ; M-O = 2 μm ; A, D, J = 5 μm .

Figure 11. Transmission electron microscopy of *Adesmia* DC. species. **A-C.** *Adesmia miraflorensis*. **A.** Section in general view. **B.** Section in a non-apertural area. **C.** Section near to the aperture. **D-F.** *Adesmia punctata*. **D.** Section in general view. **E.** Section in a non-apertural area. **F.** Section in the apertural area. **G-I.** *Adesmia rocinhensis*. **G.** Section in general view. **H.** Section in a non-apertural area. **I.** Section near to the aperture. Scale bars – B, C, E, H = 1 μm ; = 2 μm ; A, D, F, G = 5 μm .

Figure 12. Principal component analysis biplot of the pollen grain metric variables and classes/indices of *Adesmia* specimens.

Figure 13. Dendrogram built from cluster analysis (Euclidean distance) for *Adesmia* specimens, information remaining (%).

Table I. *Adesmia* DC. dimensions (μm) pollen grains in equatorial view using light microscopy.

Note. Specimens were labelled with a three-letter abbreviation of the species epithet plus the last two numbers of the herbarium voucher or collect number (see Appendix). Confidence interval at 95% probability of the lowest sample values (IC⁻) and highest sample values (IC⁺), arithmetic mean ($\bar{x}$), average standard deviation (sx), sample standard deviation (s), coefficient of variability (V%).

Table II. Morphology and ultrasculpture of *Adesmia* pollen grains using light and scanning electron microscopy.

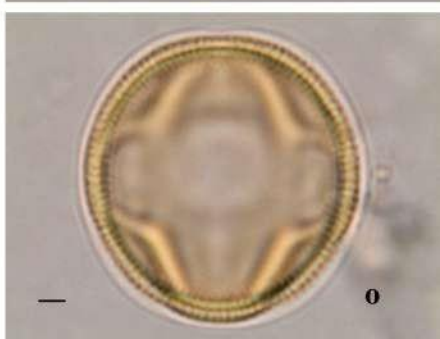
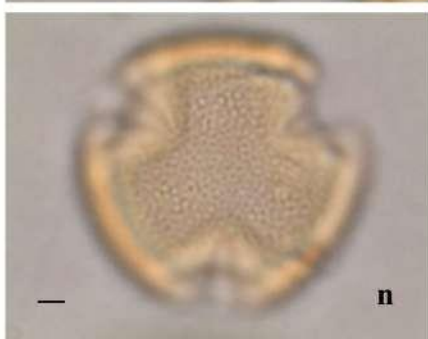
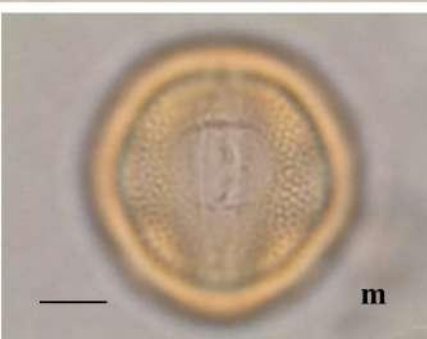
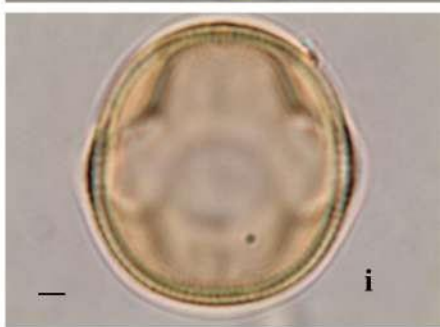
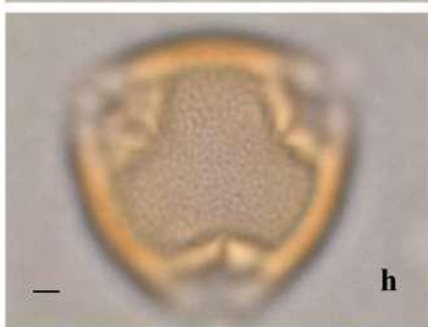
PT, pollen type; S, small, M, medium; ⁻sparsely, ⁺densely, *rarely, --not conclusive.

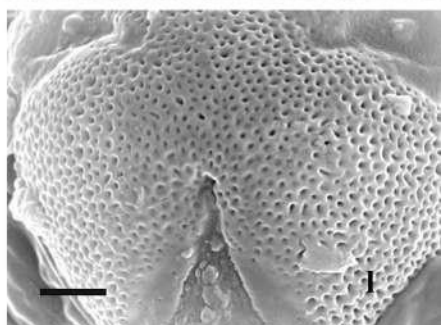
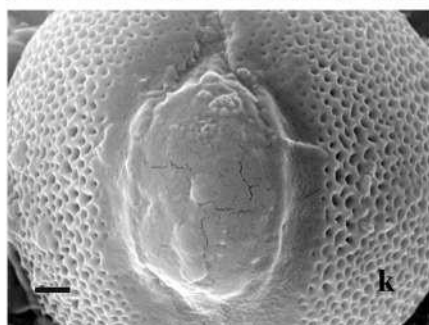
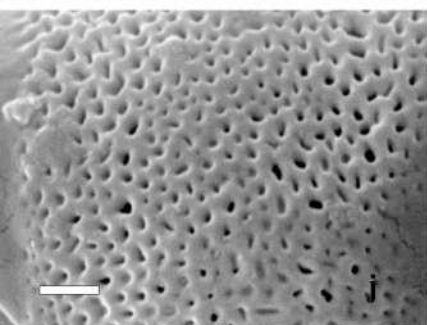
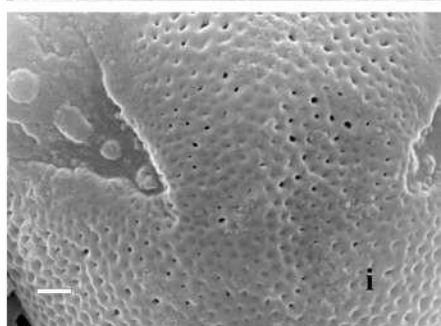
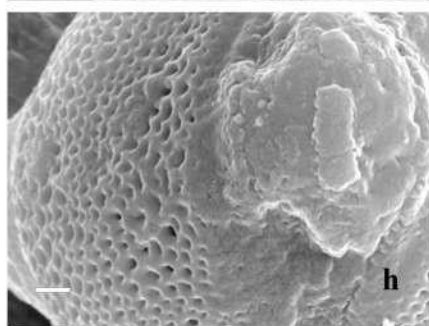
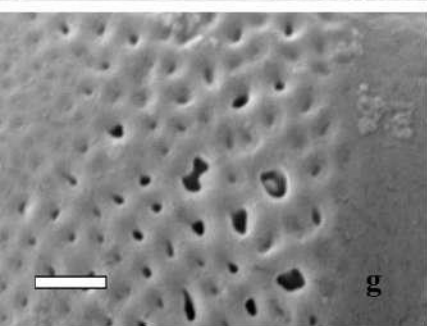
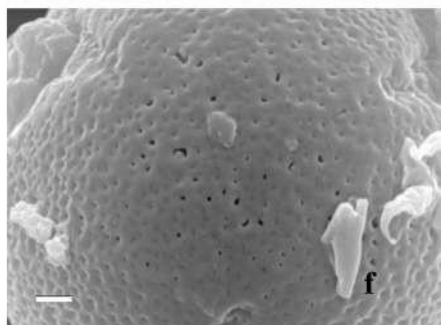
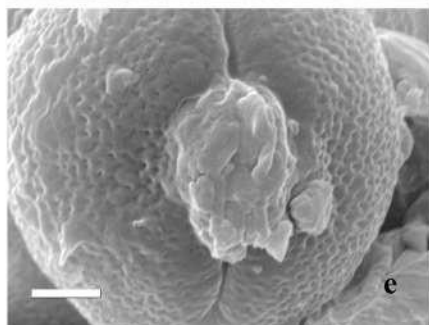
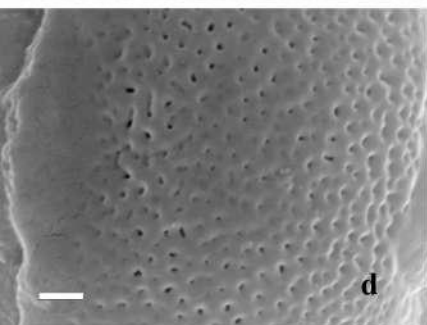
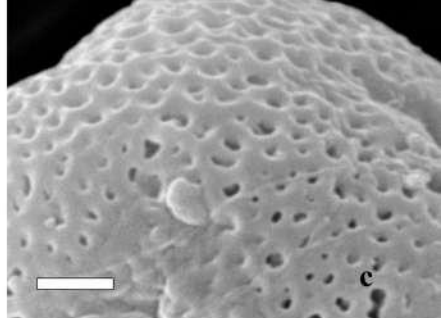
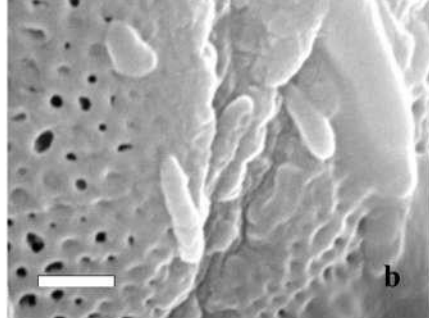
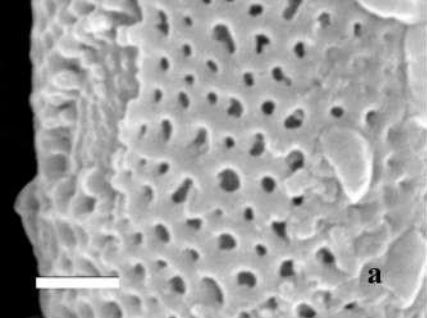
Table III. Pearson and Kendall coefficients of pollen grain metric variables and classes/indices from the first two ordination axes of the principal component analysis (PCA) of *Adesmia* species.

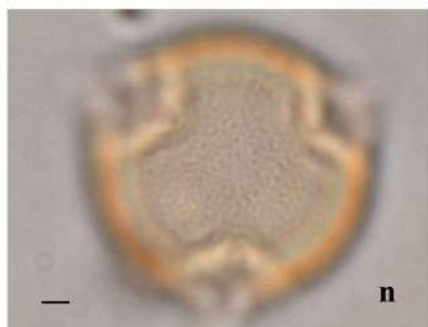
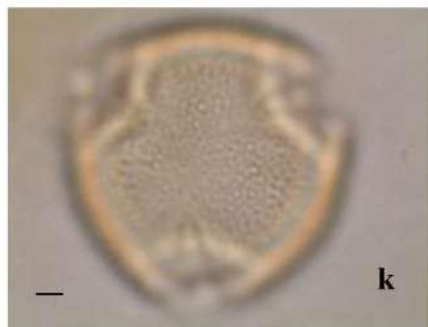
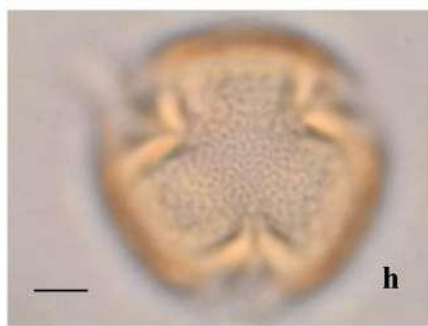
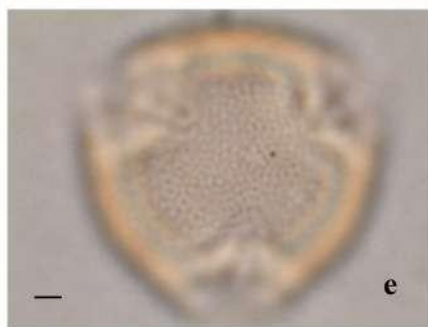
PA, polar axis; EA, equatorial axis; P/E = PA/EA; CL, colporus length; CW, colporus width; EW, endoaperture width; EL, endoaperture length; N, nexine thickness; S, sexine thickness (S = infratectum + tectum); E, exine thickness (Ex = N + S); s/n, index of sexine and nexine thickness; Cen, class of endoaperture.

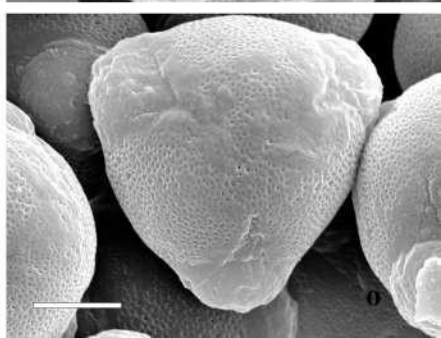
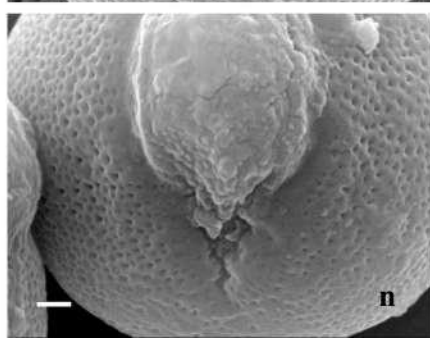
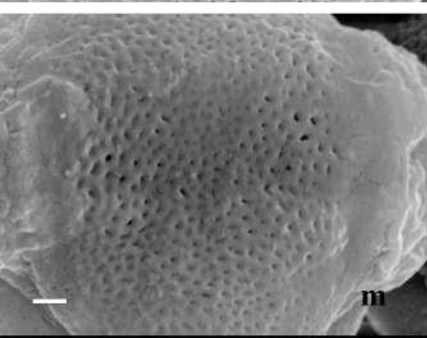
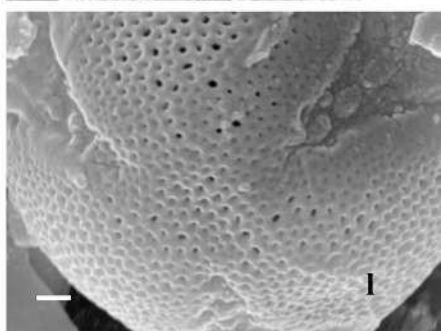
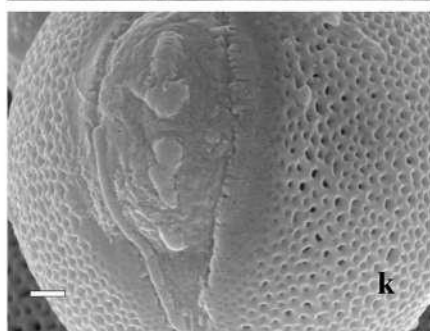
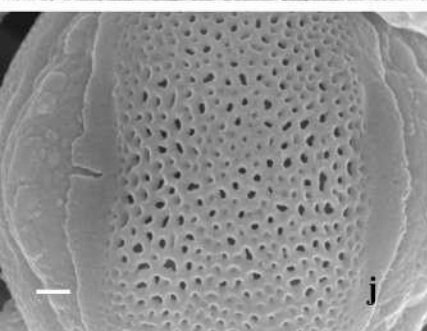
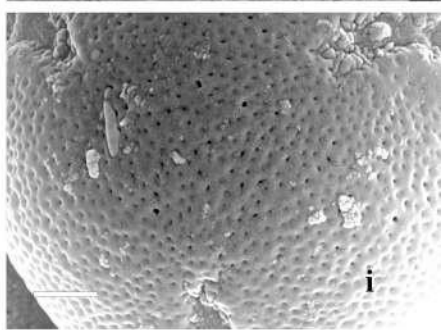
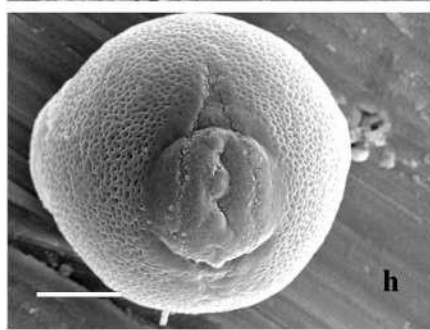
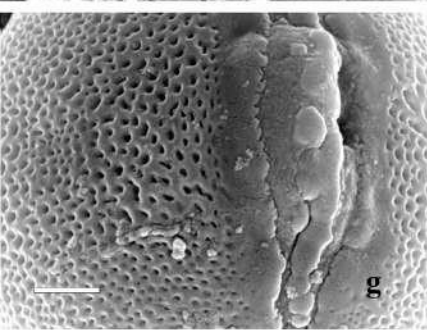
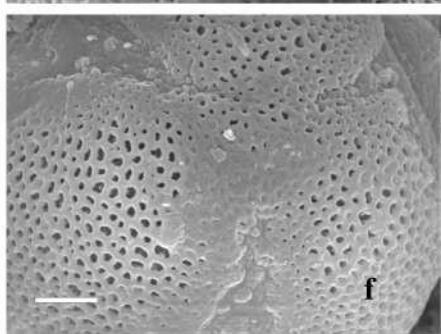
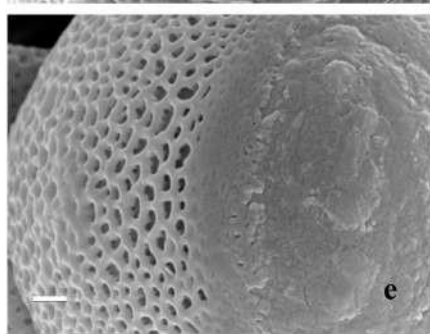
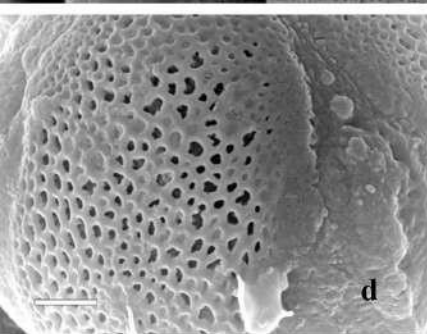
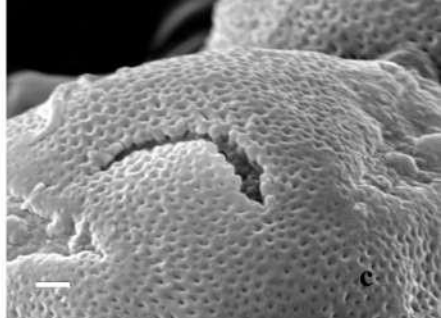
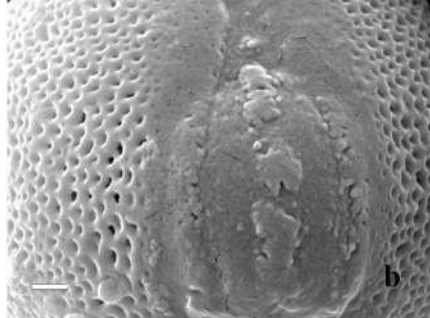
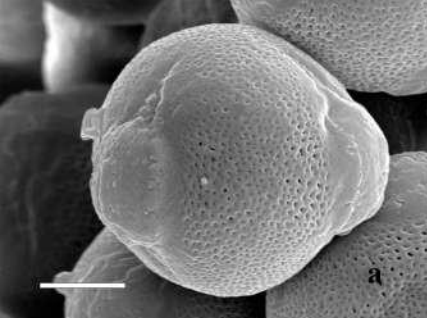
Table IV. Pollen morphology data from previous published studies compared with the present study.

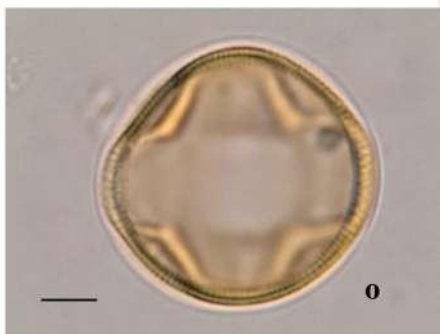
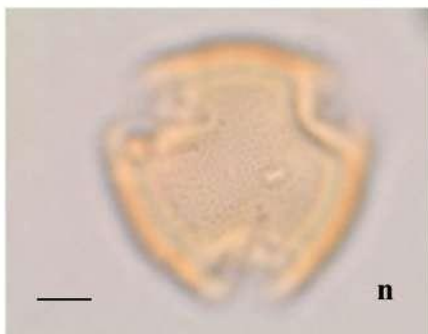
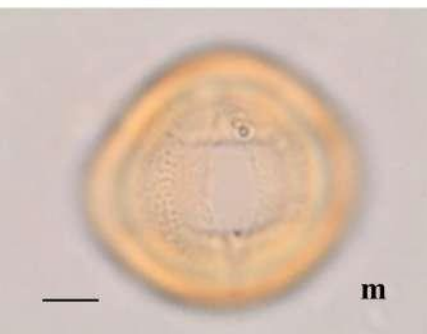
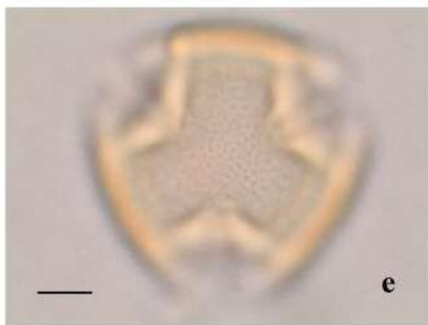
L, large; M, medium; S, small; PS, present study; *, rarely; -, sparsely; --, not descibed; O 1971, Ohashi 1971; P 1974, Pire 1974; E 2018, Espeche-María et al. (2018); L 2020, Lattar et al 2020.

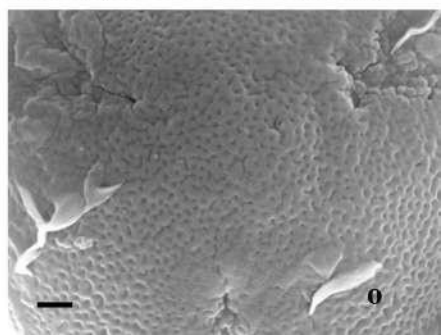
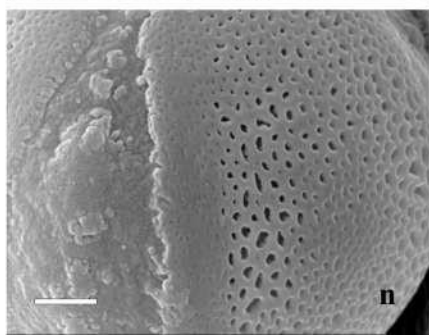
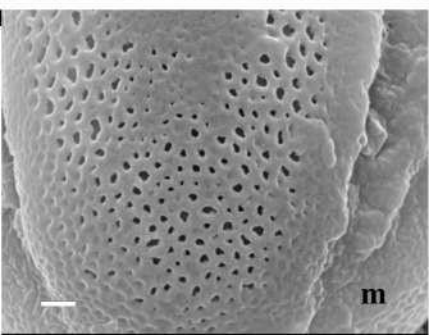
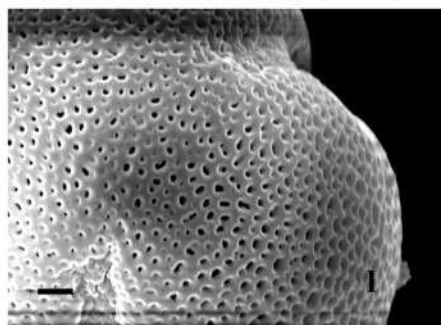
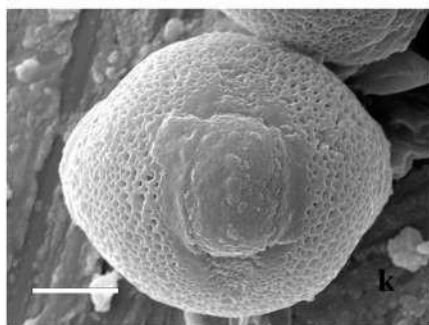
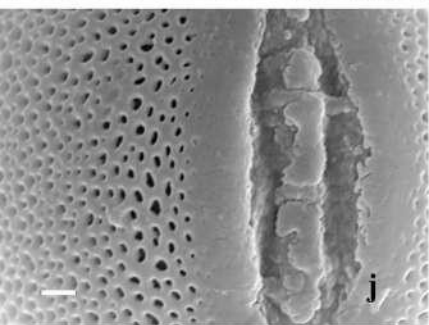
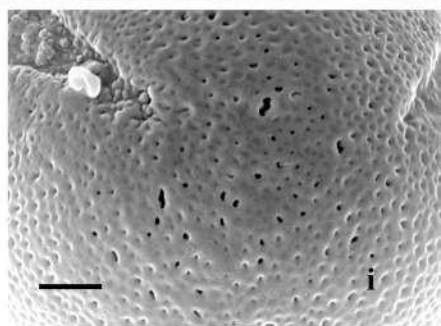
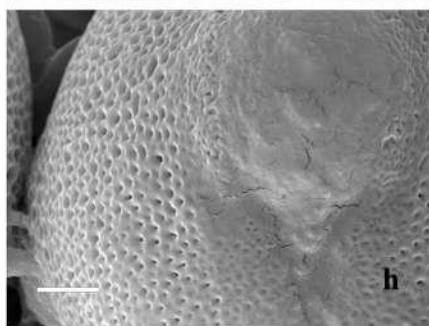
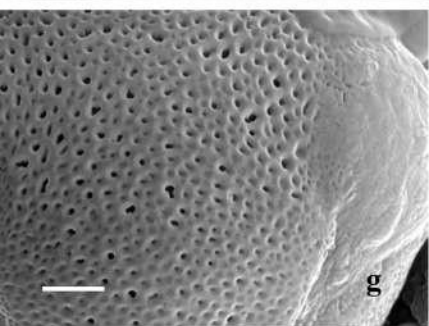
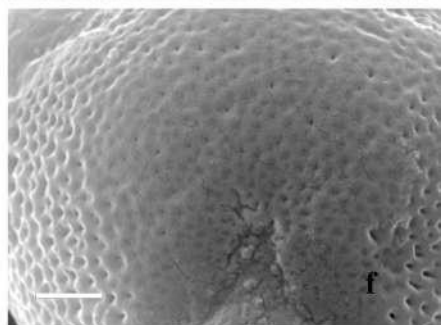
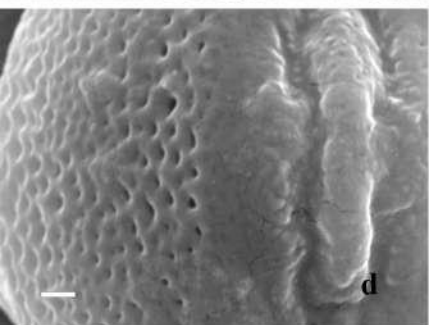
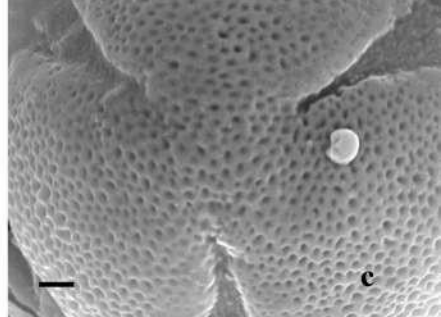
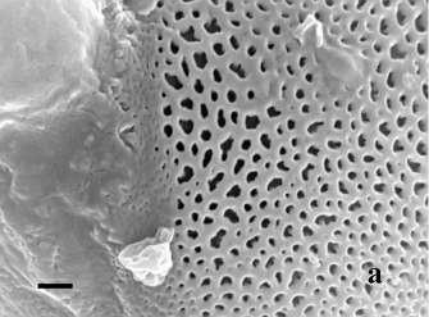


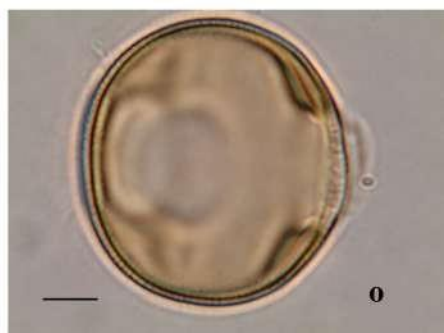
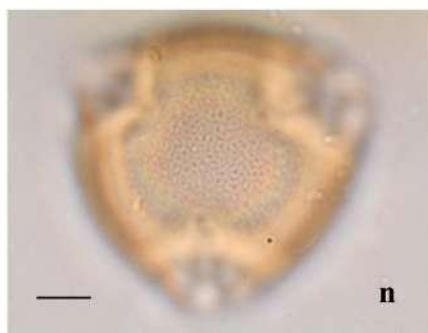
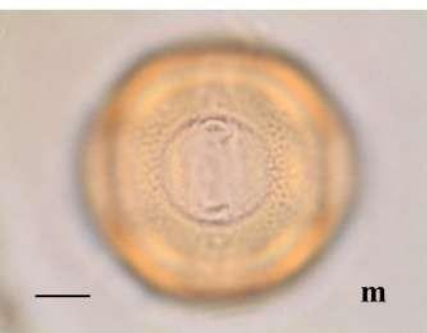
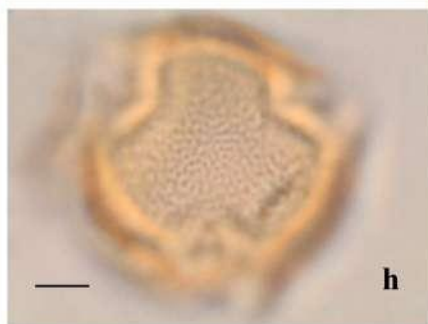
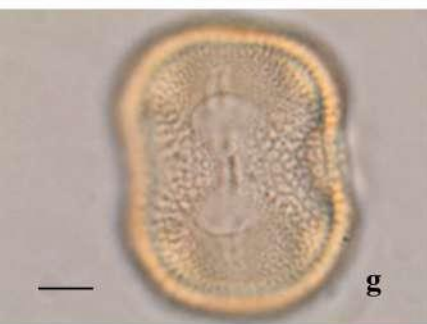
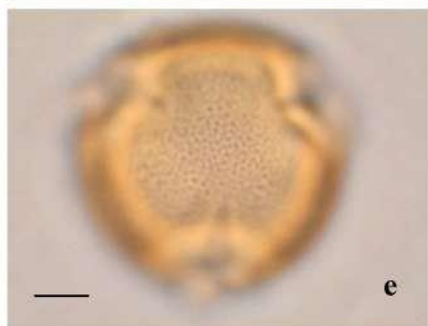


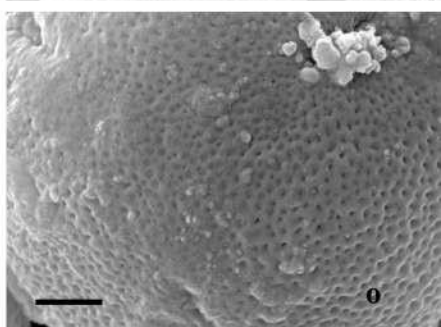
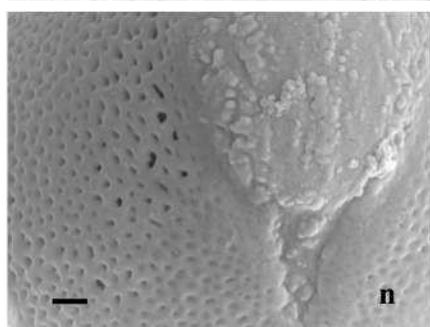
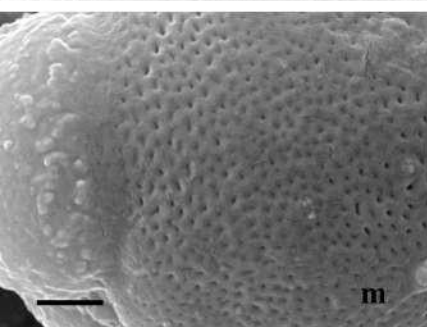
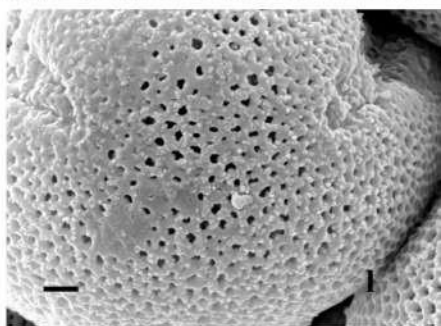
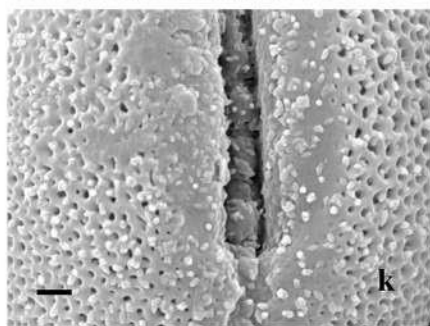
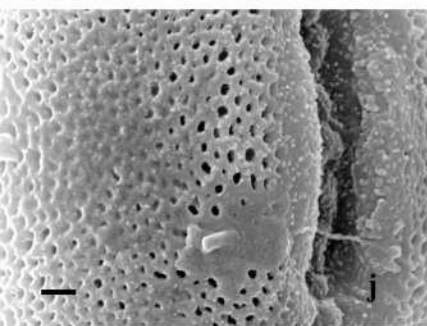
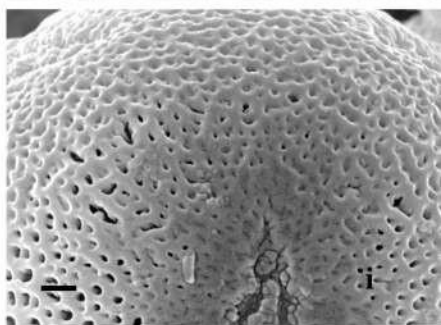
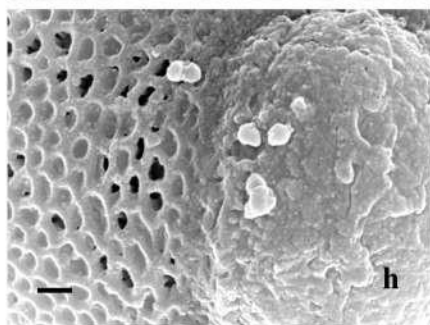
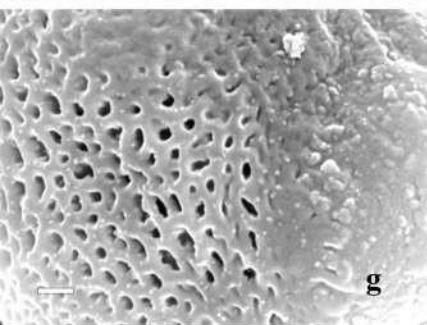
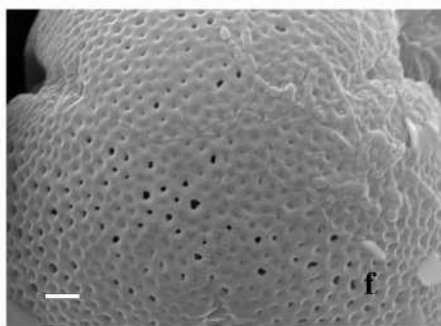
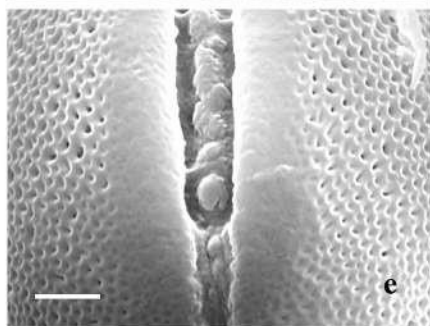
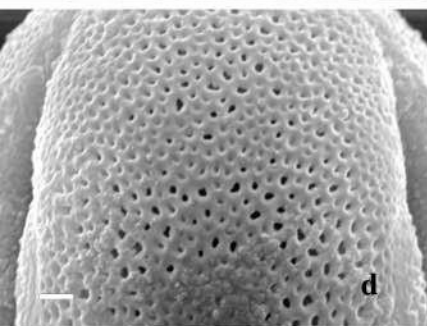
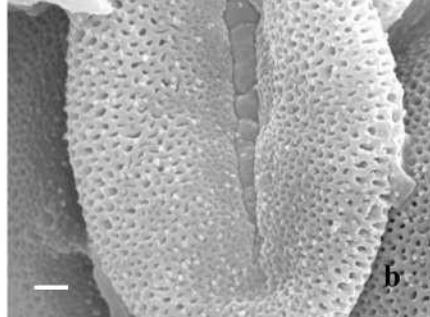
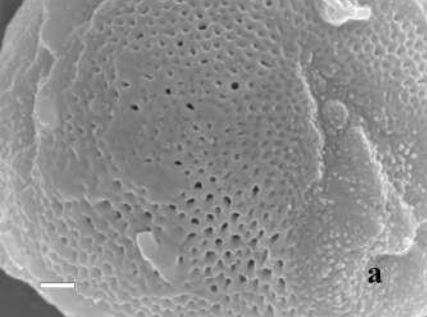


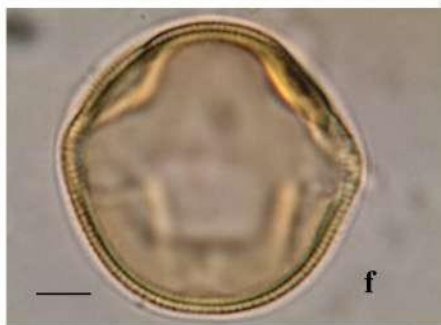
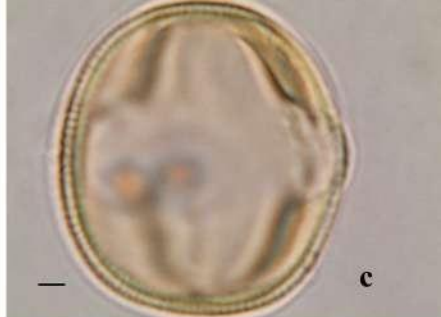


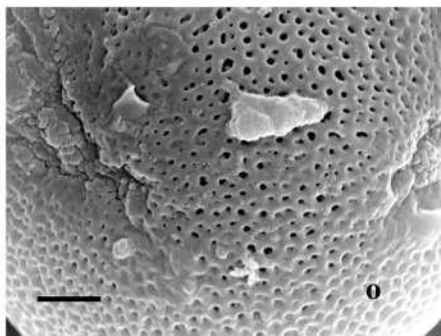
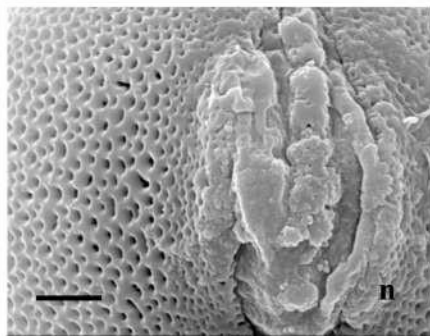
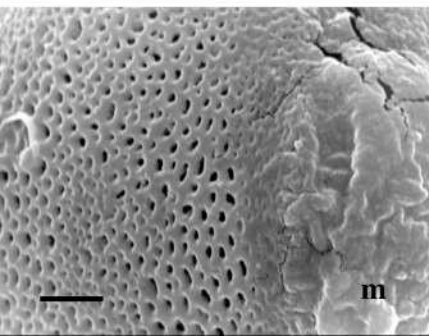
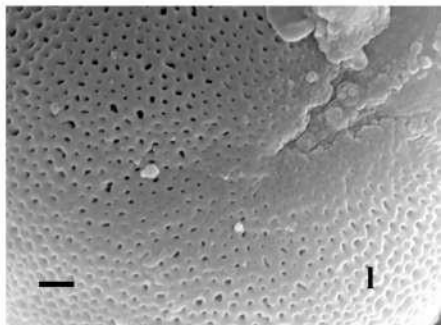
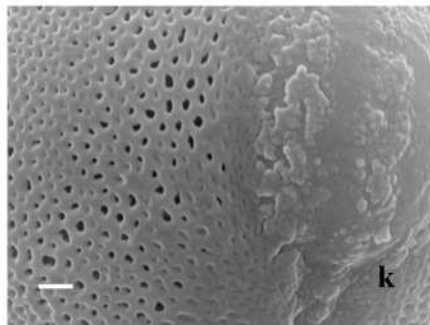
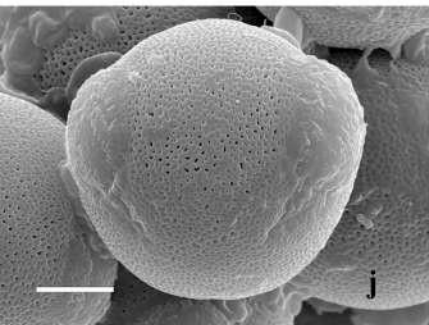
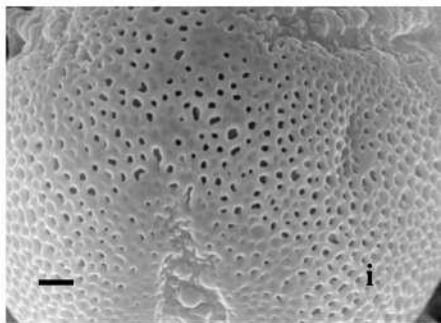
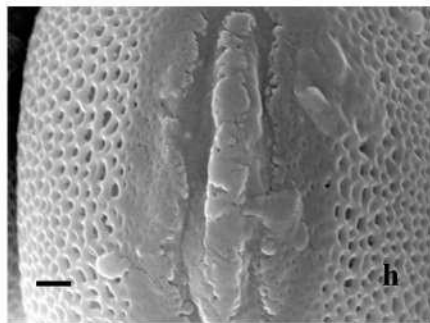
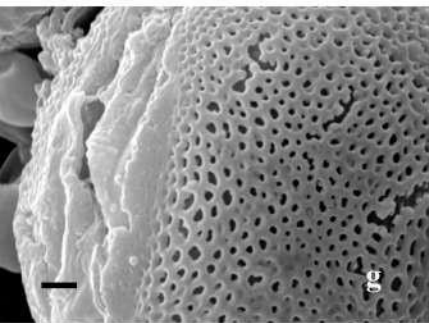
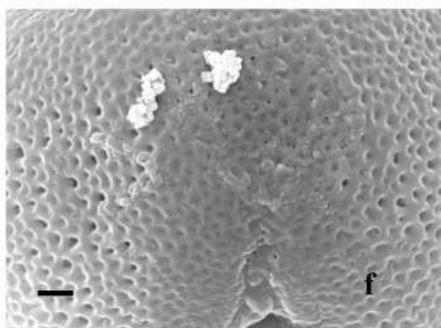
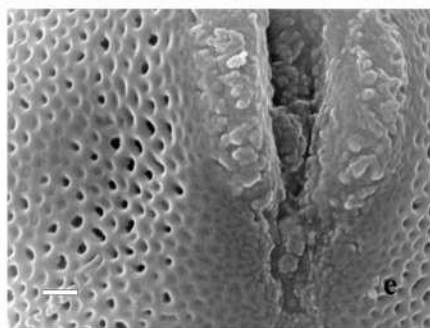
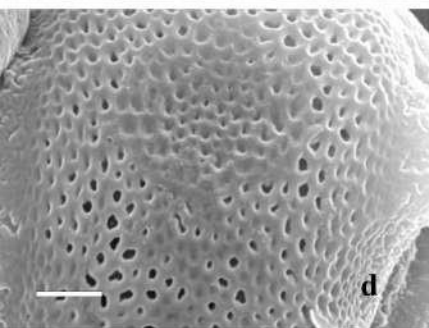
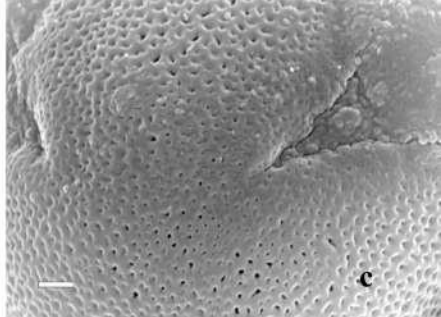
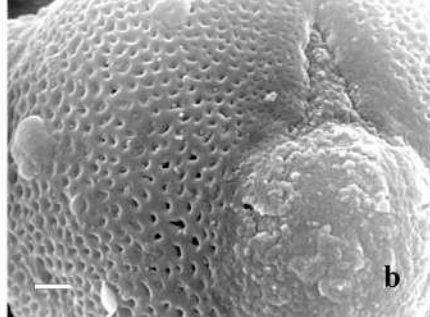
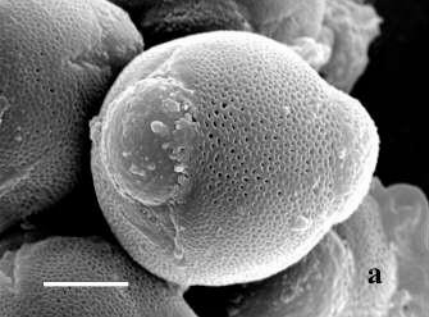


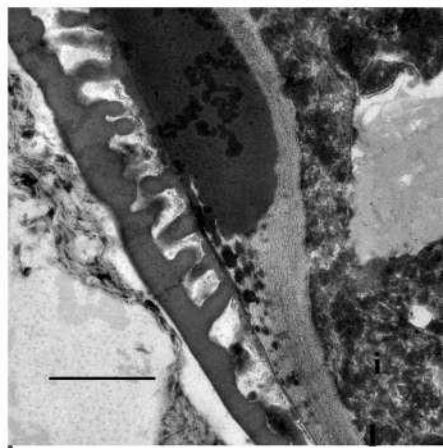
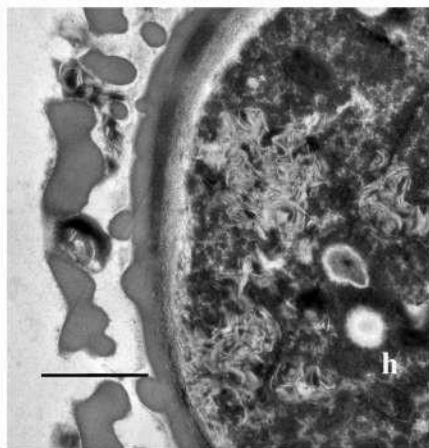
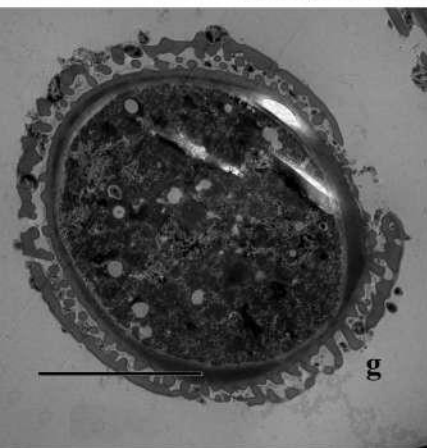
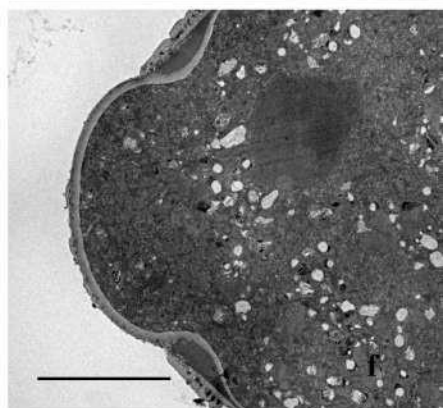
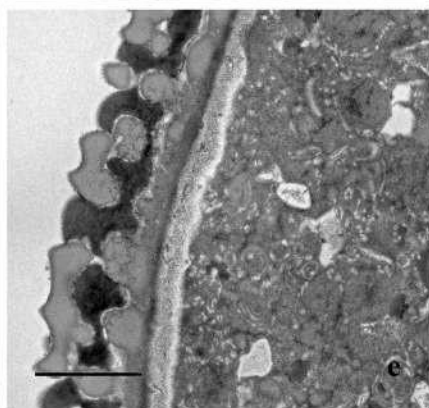
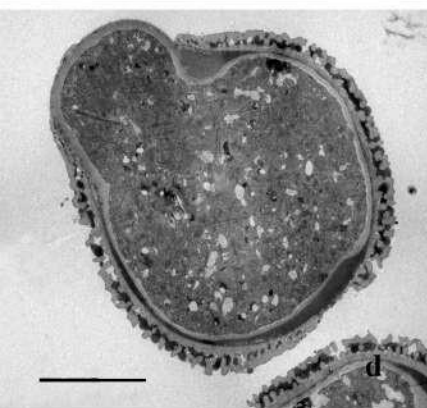
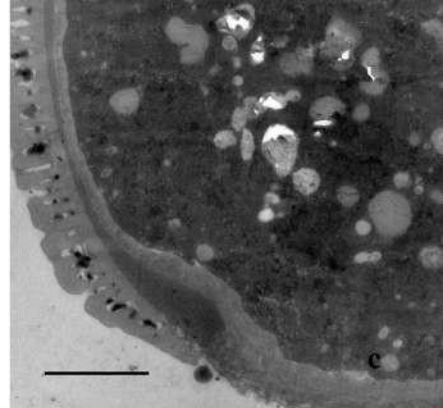
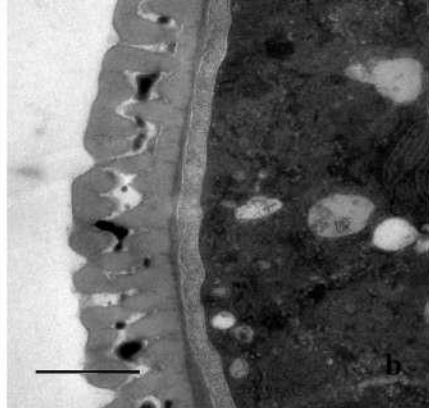
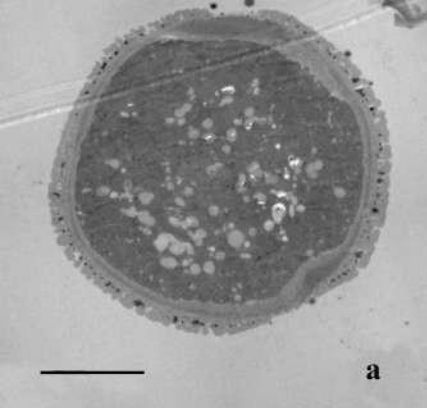












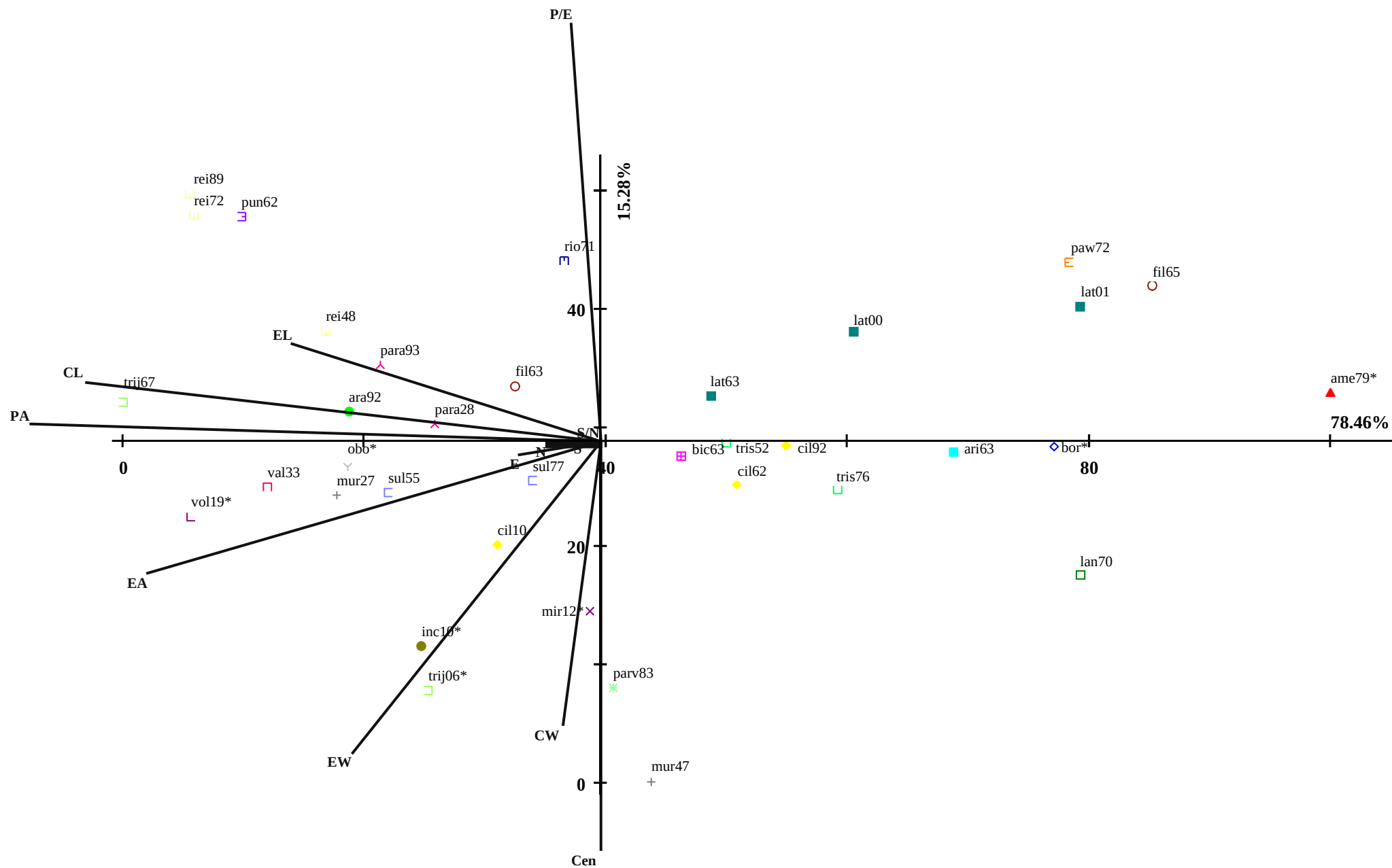
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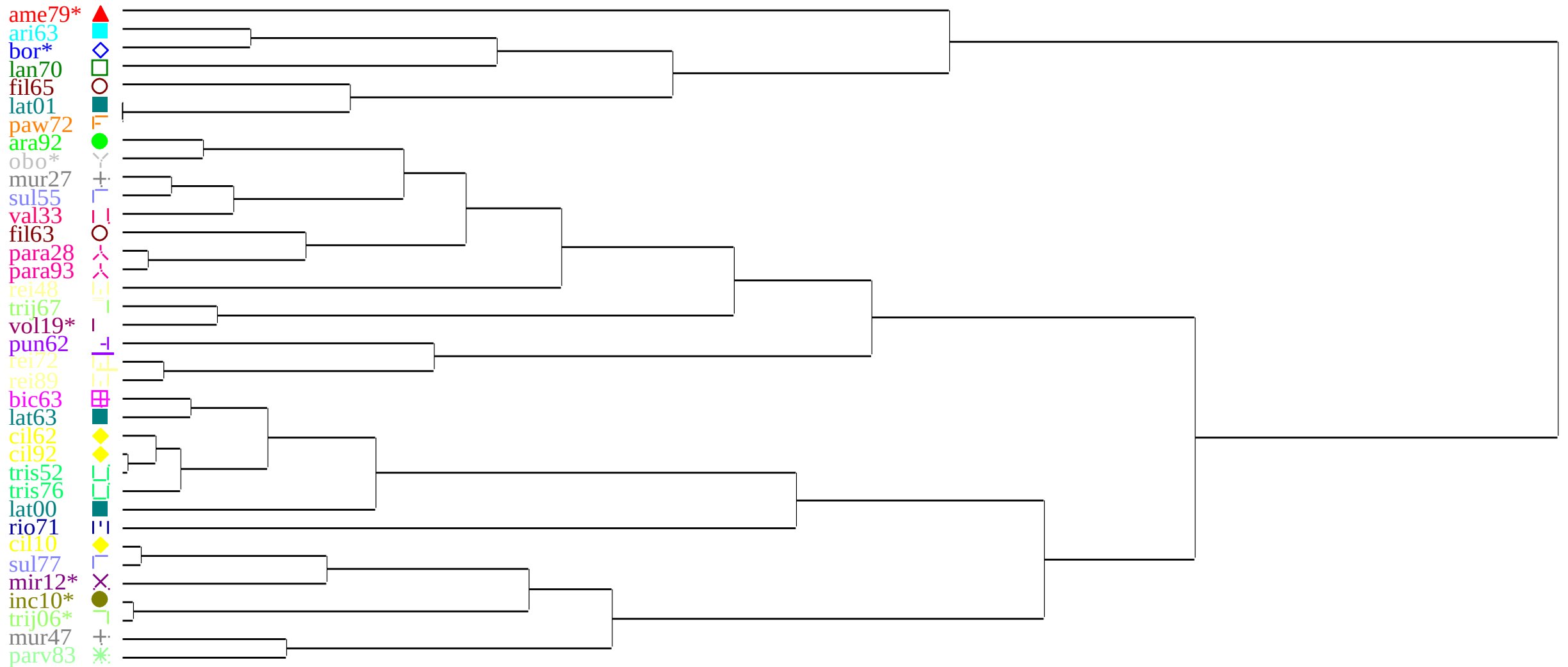


Table I. *Adesmia* DC. dimensions (μm) pollen grains in equatorial view using light microscopy.

Specimen	Polar Axis				Equatorial Axis				P/E	Size	Colporus	Endoaperture		Exina	
	CI - ($x \pm sx$)	CI +	s	V%	CI - ($x \pm sx$)	CI +	s	V%	x	Range	Length - Width	Width (Cen)	Length	N	S
ame79*	18.2 (18.5 $\pm$ 0.1)	18.7	0.6	3.4	16.6 (16.9 $\pm$ 0.1)	17.1	0.7	3.9	1.1	S	15.2 – 3.3	7.5 (1.1)	6.8	0.5	0.8
ara92	24.4 (24.7 $\pm$ 0.1)	25.0	0.7	2.9	23.0 (23.3 $\pm$ 0.2)	23.6	0.8	3.5	1.1	S	20.0 – 3.1	10.2 (1.0)	9.9	0.6	0.9
ari63	21.1 (21.4 $\pm$ 0.1)	21.6	0.6	2.8	19.3 (19.6 $\pm$ 0.1)	19.8	0.7	3.5	1.1	S	17.4 – 2.5	9.4 (1.2)	7.5	0.6	0.8
bic63	23.2 (23.5 $\pm$ 0.1)	23.8	0.7	3.1	21.8 (22.1 $\pm$ 0.1)	22.3	0.6	2.9	1.1	S	18.6 – 3.5	9.2 (1.0)	9.1	0.5	0.7
bor*	20.5 (20.8 $\pm$ 0.2)	21.2	0.9	4.2	18.9 (19.3 $\pm$ 0.2)	19.6	0.9	4.5	1.2	S	16.3 – 3.1	8.4 (1.1)	7.4	0.6	1.2
cil10	24.3 (24.7 $\pm$ 0.2)	25.0	0.8	3.1	23.2 (23.6 $\pm$ 0.2)	23.9	0.8	3.6	1.0	S	19.5 – 3.7	10.8 (1.2)	9.3	0.5	0.7
cil23	27.2 (27.6 $\pm$ 0.2)	28.1	1.1	4.0	24.9 (25.3 $\pm$ 0.2)	25.7	1.0	3.9	1.1	S-M	20.8 – 3.8	11.2 (1.1)	10.2	0.5	0.8
cil62	21.7 (22.2 $\pm$ 0.2)	22.6	1.1	5.0	21.4 (21.9 $\pm$ 0.2)	22.6	1.1	5.2	1.0	S	18.5 – 3.4	9.8 (1.0)	9.8	0.6	0.8
cil92	22.1 (22.4 $\pm$ 0.2)	22.7	0.8	3.4	20.9 (21.2 $\pm$ 0.1)	21.4	0.6	3.0	1.1	S	18.2 – 3.7	9.1 (1.0)	9.2	0.6	0.9
fil63	24.1 (24.3 $\pm$ 0.1)	24.6	0.6	2.5	22.5 (22.8 $\pm$ 0.1)	23.0	0.6	2.6	1.1	S	20.2 – 3.8	9.3 (0.8)	11.2	0.6	0.8
fil65	18.9 (19.1 $\pm$ 0.1)	19.3	0.6	3.0	17.5 (17.7 $\pm$ 0.1)	17.9	0.6	3.3	1.1	S	17.5 – 2.9	7.3 (0.8)	9.0	0.5	0.7
inc10*	24.8 (25.1 $\pm$ 0.2)	25.5	0.8	3.2	24.5 (24.9 $\pm$ 0.2)	25.2	0.8	3.4	1.0	S	19.4 – 4.0	11.6 (1.3)	9.0	0.7	1.0
lan70	19.4 (19.6 $\pm$ 0.1)	19.9	0.7	3.5	19.9 (20.2 $\pm$ 0.1)	20.5	0.7	3.7	1.0	S	15.5 – 3.6	9.5 (1.2)	8.1	0.6	0.9
lat00	22.0 (22.3 $\pm$ 0.2)	22.7	0.8	3.5	16.9 (19.9 $\pm$ 0.2)	20.3	0.9	4.7	1.1	S	18.1 – 3.3	8.2 (0.8)	9.6	0.5	0.8
lat01	20.6 (20.7 $\pm$ 0.1)	20.9	0.4	1.8	18.1 (18.3 $\pm$ 0.1)	18.5	0.4	2.4	1.1	S	16.2 – 2.8	7.4 (0.8)	9.6	0.5	0.8
lat63	23.5 (23.7 $\pm$ 0.1)	23.9	0.5	2.1	20.9 (21.1 $\pm$ 0.1)	21.3	0.6	2.7	1.1	S	18.9 – 2.8	9.1 (1.1)	8.5	0.5	0.9
mir12*	23.5 (23.8 $\pm$ 0.1)	24.0	0.6	2.5	22.5 (22.8 $\pm$ 0.1)	23.1	0.7	3.7	1.0	S	18.5 – 4.5	11.8 (1.3)	9.3	0.6	1.0
mur27	25.9 (26.4 $\pm$ 0.3)	27.0	1.3	5.0	24.2 (24.7 $\pm$ 0.2)	25.1	1.1	4.5	1.1	S-M	20.1 – 3.2	10.4 (1.0)	10.2	0.6	1.1
mur47	22.2 (22.5 $\pm$ 0.1)	22.8	0.7	2.9	24.2 (24.4 $\pm$ 0.1)	24.7	0.6	2.6	0.9	S	17.1 – 3.9	12.4 (1.4)	8.7	0.5	0.9
obo*	26.2 (26.8 $\pm$ 0.3)	27.3	1.3	4.8	23.2 (23.7 $\pm$ 0.3)	24.3	1.3	5.6	1.1	S-M	20.8 – 3.8	10.5 (1.2)	8.9	0.6	0.8
para28	24.5 (25.3 $\pm$ 0.4)	26.0	1.8	7.1	23.0 (23.2 $\pm$ 0.1)	23.4	0.5	2.1	1.1	S-M	20.8 – 3.6	10.0 (1.0)	10.0	0.6	1.0
para93	25.6 (26.0 $\pm$ 0.2)	26.3	0.8	3.0	22.5 (22.8 $\pm$ 0.2)	23.1	0.8	3.4	1.1	S-M	21.2 – 3.6	10.1 (1.0)	10.9	0.6	1.0

parv83	22.4 (22.7 ± 0.1)	23.0	0.7	3.3	24.2 (24.5 ± 0.1)	24.7	0.6	2.3	0.9	S	17.5 – 4.1	11.3 (1.1)	10.3	0.6	0.8
paw72	20.7 (20.9 ± 0.1)	21.2	0.6	3.0	17.9 (18.2 ± 0.2)	18.5	0.8	4.1	1.1	S	16.5 – 3.1	6.8 (0.7)	9.8	0.6	0.9
pun62	27.6 (28.0 ± 0.2)	28.4	1.0	3.5	22.3 (22.6 ± 0.1)	22.9	0.8	3.5	1.2	S-M	23.1 – 2.8	9.1 (0.9)	10.4	0.5	1.0
rei48	26.8 (27.3 ± 0.2)	27.7	1.1	4.0	22.9 (23.6 ± 0.3)	24.3	1.7	7.3	1.2	S-M	20.4 – 3.0	9.0 (0.8)	11.2	0.7	1.2
rei72	27.8 (28.2 ± 0.2)	28.6	0.9	3.2	22.7 (23.2 ± 0.2)	23.7	1.2	5.2	1.2	S-M	22.4 – 3.3	9.1 (0.7)	12.6	0.6	0.9
rei89	28.1 (28.6 ± 0.3)	29.2	1.3	4.7	21.8 (22.3 ± 0.2)	22.7	1.0	4.6	1.3	S-M	22.4 – 3.0	9.9 (0.8)	12.8	0.6	0.9
rio71	24.9 (25.4 ± 0.2)	25.8	1.2	4.6	20.9 (21.2 ± 0.2)	21.6	0.8	4.0	1.2	S-M	19.7 – 3.3	8.2 (0.8)	10.7	0.6	0.8
roc98	26.2 (26.6 ± 0.2)	27.0	0.9	3.5	24.5 (25.8 ± 0.2)	26.2	1.0	3.9	1.0	S- M	21.0 – 3.9	11.3 (1.0)	11.5	0.6	0.9
sul55	24.9 (25.3 ± 0.2)	25.8	1.1	4.4	23.6 (24.3 ± 0.3)	24.9	1.5	6.3	1.0	S-M	20.4 – 3.5	10.5 (1.0)	10.6	0.6	1.0
sul77	23.9 (24.3 ± 0.2)	24.7	1.0	4.1	22.5 (22.9 ± 0.2)	23.3	1.0	4.3	1.1	S	19.7 – 3.4	10.4 (1.0)	9.9	0.5	1.0
trij06*	24.9 (25.2 ± 0.1)	25.4	0.6	2.5	24.9 (25.2 ± 0.2)	25.6	0.9	3.4	1.0	S-M	18.5 – 4.5	11.8 (1.3)	9.3	0.6	1.0
trij67	28.5 (28.8 ± 0.2)	29.1	0.8	2.7	23.6 (24.1 ± 0.3)	24.2	1.3	5.3	1.2	S-M	22.4 – 3.5	11.5 (1.1)	10.5	0.6	0.9
tris52	22.9 (23.1 ± 0.1)	23.3	0.5	2.3	21.3 (21.6 ± 0.1)	21.9	0.7	3.3	1.1	S	18.2 – 3.1	9.3 (1.0)	9.5	0.6	1.0
tris76	21.4 (21.7 ± 0.1)	21.9	0.6	2.8	21.0 (21.3 ± 0.1)	21.5	0.6	2.7	1.0	S	17.5 – 3.1	9.5 (1.0)	9.3	0.6	0.9
val33	25.8 (26.1 ± 0.2)	26.5	0.9	3.4	24.5 (24.8 ± 0.1)	25.1	0.7	2.9	1.1	S-M	21.4 – 3.6	11.2 (1.0)	11.3	0.5	0.9
vol19*	27.2 (27.9 ± 0.3)	28.5	1.6	5.8	25.0 (25.4 ± 0.2)	25.9	1.0	3.9	1.1	M	21.3 – 3.4	12.3 (1.2)	10.0	0.6	0.8

Note. Specimens were identified by abbreviation of the epithet and the last two numbers of the herbarium voucher (see Appendix). Confidence Interval at 95% of probability of the lowest sample values (IC-) and highest sample values (IC+), arithmetic mean ($\bar{x}$), average standard deviation (s_x), sample standard deviation (s), coefficient of variability (V%).

Species	PT	Size	Endoaperture	Operculum	Mesocolpium	Apocolpium
<i>Adesmia ameghinoi</i>		S	Circular to lalongate	Granulate	Nanoreticulate-perforate	Nanoreticulate-perforate
<i>Adesmia araujoi</i>		S	Circular to lalongate	Absent	--	--
<i>Adesmia arillata</i>		S	Lalongate	Areolate-granulate	Nanoreticulate	Nanoreticulate
<i>Adesmia bicolor</i>		S	Circular to lalongate	Areolate-granulate	Nanoreticulate-perforate	Nanoreticulate-perforate
<i>Adesmia boronoides</i>		S	Circular to lalongate	Absent	Nanoreticulate-perforate	Nanoreticulate-perforate
<i>Adesmia ciliata</i>		S-M	Circular to lalongate	Areolate	Nano- to microreticulate	Nanoreticulate
<i>Adesmia filipes</i>		M	Lolongate	Areolate-granulate	Nano- to microreticulate	Nanoreticulate
<i>Adesmia incana</i>		S-M	Very lalongate	Areolate-granulate	Nano- to microreticulate	Nanoreticulate-perforate
<i>Adesmia lanata</i>		S	Circular to lalongate	Granulate	Nanoreticulate-perforate	Nanoreticulate-perforate
<i>Adesmia latifolia</i>		S	Circular to lolongate	Granulate	Nanoreticulate-perforate	Nanoreticulate-perforate
<i>Adesmia miraflorensis</i>		S-M*	Lalongate	Granulate	Nano- to microreticulate-perforate	Nanoreticulate-perforate
<i>Adesmia muricata</i>		M	Circular to very lalongate	Granulate	Nano- to microreticulate	Perforate
<i>Adesmia obovata</i>		S-M	Circular to lalongate	Areolate-granulate	Nano- to microreticulate-perforate	Perforate
<i>Adesmia paranensis</i>		S-M	Circular to lolongate	Areolate-granulate	Nano- to microreticulate	Nanoreticulate-perforate
<i>Adesmia parviflora</i>		S-M*	Lalongate	Areolate-granulate	Nano- to microreticulate	Perforate
<i>Adesmia pawpeana</i>		S	Lolongate	Areolata-granulate	Nano- to microreticulate	Nanoreticulate
<i>Adesmia punctata</i>		S-M	Circular to lolongate	Areolate-granulate	Nanoreticulate-perforate	Perforate
<i>Adesmia reitziana</i>		S-M	Lolongate	Areolate-granulate	Nano- to microreticulate	Nanoreticulate-perforate

<i>Adesmia riograndensis</i>	S-M	Lolongate	Areolate-granulate	Nanoreticulate-perforate	Nanoreticulate-perforate
<i>Adesmia rocinhensis</i>	S-M	Circular to lalongate	Granulate	Nano- to microreticulate	Nanoreticulate
<i>Adesmia sulina</i>	S-M	Lalongate, circular to lalongate	Areolate-granulate	Nano- to microreticulate	Nanoreticulate-perforate
<i>Adesmia trijuga</i>	S-M	Circular to lalongate	Areolate-granulate	Nanoreticulate	Nanoreticulate-perforate
<i>Adesmia tristes</i>	S	Circular to lalongate	Areolate	Nano- to microreticulate	Nanoreticulate
<i>Adesmia vallsii</i>	S-M	Lalongate, circular to lalongate	Granulate	Nanoreticulate	Perforate
<i>Adesmia volckmannii</i>	S-M	Lalongate	Areolate-granulate	Nano- to microreticulate	Nanoreticulate-perforate

Table II. Morphology and ultrasculpture of *Adesmia* pollen grains using light and scanning electron microscopy. PT, pollen type; S, small, M, medium; ¯sparsely, +densely, *rarely, --not conclusive.

Table III. Pearson and Kendall coefficients of pollen grain metric variables and classes/indices from the first two ordination axes of the principal component analysis (PCA) of *Adesmia* species

Variables	Principal components	
	Axis 1	Axis 2
PA	– 0.6738	0.2678
EA	– 0.4751	– 0.5843
P/E	– 0.0046	0.0394
CL	– 0.4660	0.3603
CW	– 0.0283	– 0.1750
EW	– 0.2266	– 0.5777
EL	– 0.2252	0.2893
N	– 0.0041	– 0.0030
S	– 0.0055	– 0.0029
E	– 0.0140	– 0.0128
s/n	– 0.0005	0.0004
Cen	– 0.0004	– 0.0851

PA, polar axis; EA, equatorial axis; P/E = PA/EA; CL, colpus length; CW, colpus width; EW, endoaperture width; EL, endoaperture length; N, nexine thickness; S, sexine thickness (S = infratectum + tectum); E, exine thickness (Ex = N + S); s/n, index of sexine and nexine thickness; Cen, class of endoaperture,

Table IV. Pollen morphology data from previous published studies compared with the present study. M, medium; S, small; PS, present study; *, rarely; ¯, sparcely; --, not descibed; O 1971, Ohashi 1971; P 1974, Pire 1974; E 2018, Espeche María et al. 2018; L 2020, Lattar et al 2020.

Species	Author	Size Class	Endoaperture class	Operculum	Sexine ultrasculpture	
					Mesocolpium	Apocolpium
<i>A. bicolor</i>	L 2020	S	Lolongate	Absent	Reticulate	
	PS	S	Circular to lalongate	Areolate-granulate	Nanoreticulate-perforate	
<i>A. muricata</i>	O 1971	S	Lalongate	Absent	Reticulate	
	P 1974	M	Lolongate	Absent	Reticulate	
	E 2018	S	Circular	Absent	Microreticulate	
	PS	M	Circular to very lalongate	Granulate	Nano- to microreticulate	Perforate
<i>A. latifolia</i>	L 2020	S	Lolongate	Absent	Reticulate	
	PS	S	Circular to lolongate	Granulate	Nanoreticulate-perforate	
<i>A. punctata</i>	L 2020	S	Lolongate	Absent	Reticulate	
	PS	S-M	Circular to lolongate	Areolate-granulate	Nanoreticulate-perforate	Perforate
<i>A. trijuga</i>	E 2018	S-M	Circular	Absent	Microreticulate	
	PS	S-M	Circular to lalongate	Areolate-granulate	Nanoreticulate	Nanoreticulate-perforate

Palynology of *Zornia* J.F. sheds light on the phylogenetic relevance of pollen grains in the *Adesmia* clade (Leguminosae – Papilionoideae – Dalbergieae)

Running title: Pollen evolution of the *Adesmia* clade (Leguminosae)

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Abstract

The *Adesmia* clade representatives were extensively studied in the last year regarding the pollen morphology and ultrastructure. However, *Zornia* remains understudied to complete a study on this clade's evolution and mapping of pollen characters. Aiming to understand the patterns of palynological evolution in the *Adesmia* clade, we characterise the pollen morphology and ultrasculpture variation of 15 species of *Zornia*. Using the most recent phylogenetic framework presented to the taxa, we scored and coded 16 micromorphological characters to test for secondary homologies. A comprehensive pollen characterisation permits a description of *Zornia* as stenopalynous with some differences in pollen grains size and ultrasculpture of the operculum, margo and sexine. All lineages of the *Adesmia* clade from the molecular phylogeny were recovered with at least one or more homoplasies/apomorphies, except by *Adesmia* genus.

Keywords: Character-mapping, Dalbergia Clade, Fabaceae, Pollen Morphology, Ultrasculpture.

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Leguminosae Juss. is the third most species-rich family of Angiosperms with substantial economic value and a complex taxonomical history (LPWG 2017; Lewis et al. 2005). To understand the systematics of these families, studies on pollen grains of Leguminosae and their subfamilies have proved helpful to taxonomy at different hierarchical levels. The palynology of the family demonstrated different types of dispersal units, pollen size and shape, number, positions and configuration of the apertures, sexine ornamentation and exine thickness and structure (Ferguson & Skvarla 1981; Banks & Rudall 2016; Jiang et al. 2019; Lattar et al. 2020; LPWG 2017; Tewari & Nair 1979).

In Papilionoideae legumes, pollen aperture variation represents an essential feature in the systematics and is helpful in character evolution (LPWG 2017). Regarding the informal Adesmia clade (dalbergioids), the most recent pollen grains analysis revealed variation in pollen morphology, ultrasculpture and ultrastructure. It provided phylogenetically informative palynological data for *Amicia*, *Poiretia*, *Nissolia* and *Adesmia* (Antonio-Domingues et al. 2022a, 2022b, 2023 in press, 2023 unpublished data). However, a character-mapping analysis of pollen grains was not performed.

Pollen grains descriptions of a few species of *Zornia* are available in the literature. However, to improve pollen character optimisation, some information is incomplete regarding the ultrasculpture of the sexine, ultrastructure and the aperture elements, which demonstrated taxonomic value in palynological studies of the Adesmia clade. More than less incongruity of the descriptions among these authors were observed (Buriel et al. 2011; Luz et al. 2013; Matos et al. 2014; Melhem 1966; Moreti et al. 2007; Ohashi 1971; Pire 1974; RCPol [continuously updated]; Silva et al. 2016; Silvestre-Capelato & Melhem 1997).

Thus, this study aimed to comprehensively characterise the pollen morphology, ultrastructure and ultrasculpture variation of 15 species of *Zornia* to recognise

palynological patterns and use the phylogenetic framework presented by Fortuna-Perez et al. (2021) to understand the patterns of pollen evolution in the *Adesmia* clade. We scored and coded 16 micromorphological characters to test for secondary homologies.

Material and methods

Palynological analysis

Unopened mature buds were obtained from 18 herbarium specimens belonging to the 15 species of *Zornia* [*Z. brasiliensis* Vogel., *Z. adenophora* (Domin) Mohlenbr., *Z. areolata* Mohlenbr., *Z. bracteata* J.F. Gmel., *Z. capensis* Pers., *Z. contorta* Mohlenbr., *Z. crinita* (Mohlenbr.) Vanni, *Z. thymifolia* Domin., *Z. gardneriana* Moric., *Z. glochidiata* Rchb. ex DC., *Z. harmsiana* Standl., *Z. myriadena* Benth., *Z. pardina* Mohlenbr., *Z. setosa* Baker f. and *Z. virgata* Moric.]. Specimens were sampled from K, SP and MBM herbaria (acronyms follow Thiers 2021, [continuously updated]). All specimens examined are listed in the *Specimens Investigated* section. Samples were labelled with the abbreviation of the species epithet and the last two numbers of the herbarium voucher (see Table I, Species and Specimen column).

Pollen grains were treated with the acetolysis method (Erdtman 1960), following the modifications proposed by Melhem et al. (2003). Measurements were made under a light microscope using an Olympus OSM-4 (10 ×) micrometer drum coupled to the eyepiece of an Olympus BX50 light binocular microscope. The polar and equatorial axes in equatorial views were measured on 25 randomly selected pollen grains from each specimen. These measurements were statistically analysed for the arithmetic mean ($\bar{x}$), average standard deviation (S_x), sample standard deviation (s), coefficient of variability ($V\%$), and 95% confidence interval (CI) (Vieira 2011; Zar 2010). Ten measurements of the prominent pollen morphometric parameters were also made in the length and width

of the colpus, the length and width of the endoaperture, and the thickness of the nexine and sexine layers. Exine measurements were made in the mesocolpium region. The arithmetic means, and the range was calculated for each pollen grain size class and exine (sexine + nexine). Photomicrographs were obtained using an Olympus BX 50 light microscope with an Olympus U-CMAD-2 video camera and Olympus CellSens 1.5 software. Permanent light microscopy (LM) slides were deposited in the pollen reference collection of the *Laboratório de Palinologia PALINO-IPA, Instituto de Pesquisas Ambientais*, São Paulo State, Brazil.

To avoid the loss of the operculum and exine elements covering the aperture (Hesse & Waha 1989), for scanning electron microscopy (SEM), non-acetolysed pollen grains were washed and placed on a metal stub and sputter coated (Leica EM ACE 600; Leica, Vienna, Austria) with gold (80 nm). Samples were observed under a JEOL JSMIT300LV microscope (Tokyo, Japan) under a 20 kV electron beam and the images were digitised.

Pollen terminology is based on Punt et al. (2007) and Halbritter et al. (2018). Pollen shape classes and amb types follow Erdtman (1952), and colpus length categories follow Mark et al. (2012). The endoaperture classes and index of sexine and nexine thickness were proposed by Antonio-Domingues et al. (2022a).

Principal component analysis (PCA) was performed to explore whether pollen grain characteristics allow for the grouping of species and specimens. Seven metric variables (polar axis [PA], equatorial axis [EA], colpus length [CL], colpus width [CW], nexine thickness [N], sexine thickness [S = infratectum + tectum], exine thickness [Ex = N + S]) and two classes/indexes (shape class [P/E = PA/EA], index of sexine and nexine thickness [S/N]) were analysed for ordination using FITOPAC (Shepherd 1996) and PC-ORD version 7 (McCune & Mefford 2016). To understand how *Zornia* specimens relate to each

other, based on pollen morphology, a cluster analysis (CA) was performed using the software PC-ORD version 7 (McCune & Mefford 2016). This produced a similarity dendrogram by calculating the Euclidean distance based on the same nine PCA metric variables and the three classes/indexes.

Character-mapping

The consensus phylogenetic tree presented by Fortuna-Perez et al. (2021) was pruned on Mesquite 2.73 (Maddison and Maddison 2006) to show only the groups sampled in our study and used for character mapping pollen micromorphology. We scored palynological data from the literature for the genera *Adesmia*, *Amicia*, *Poiretia*, and *Nissolia* (Antonio-Domingues et al. 2022a, 2022b, 2023 in press, 2023 unpublished data) to include in our sampling the remaining genera of the informal *Adesmia* clade. Character coding followed the recommendations of Sereno (2007) for morphological analyses. Primary homology hypotheses (De Pinna 1991) were proposed for pollen shape, size, ornamentation, exine structure and apertures. Sixteen micromorphological characters were scored for informal *Adesmia* clade and outgroups (Table V). We sampled *Tipuana tipu* (Benth.) Kuntze and *Pterocarpus rohrii* Vahl from the palynological literature for the character-mapping analyses based on Barth (1964), Santos et al. (2022), Trigo and Garcia (1990), Pino and Díez (1990) and RCPOL (continuously update). All characters were optimised on the concatenated tree using the Maximum Likelihood function (mk1 model) using Mesquite 2.73 (Maddison and Maddison 2006) and visualised on Winclada (Nixon 1999).

Results

General Description of Zornia pollen grains

Zornia adenophora: pollen grains are small to medium-sized, oblate spheroidal to subprolate. Colpus with areolate-granulate operculum, granulate membrane, psilate-perforate margo and aperture protruding are absent. Sexine from 1.4 to 2.0 times thicker than nexine; exine 1.6 – 1.9 μm . Mesocolpium nano- to microreticulate-perforate and nanoreticulate-perforate apocolpium (Figures 1A-C, 2A-C).

Zornia areolata: pollen grains are small to medium sizes, subprolate to prolate. Colpus with areolate-granulate operculum, granulate membrane, psilate-perforate margo and aperture protruding are absent. Sexine from 1.3 to 1.8 times thicker than nexine; exine 1.3 – 1.5 μm . Mesocolpium nano- to microreticulate and apocolpium nanoreticulate-perforate (Figures 1D-F, 2D-F).

Zornia bracteata: pollen grains are small to medium-sized, subprolate to prolate. Colpus with areolate-granulate operculum, granulate membrane, psilate-perforate margo and aperture protruding are absent. Sexine from 1.5 to 2.3 times thicker than nexine; exine 1.5 – 1.9 μm . Mesocolpium micro- to reticulate apocolpium nano- to microreticulate (Figures 1G-I, 2G-I).

Zornia brasiliensis: pollen grains are small to medium-sized, subprolate to prolate. Colpus with psilate operculum, granulate membrane, psilate-perforate margo and aperture protruding are absent. Sexine from 1.3 to 2.0 times thicker than nexine; exine 1.4 – 1.8 μm . Mesocolpium nano- to microreticulate-perforate and apocolpium perforate (Figures 1J-L, 2J-L).

Zornia capensis: pollen grains are small to medium-sized, prolate. Colpus with rugulate-perforate operculum, granulate membrane, psilate-perforate margo and absent aperture protruding. Sexine from 1.3 to 2.0 times thicker than nexine; exine 1.5 – 1.7 μm . Mesocolpium nano- to microreticulate and apocolpium nanoreticulate-perforate. (Figures 1M-O, 2M-O)

Zornia contorta: pollen grains are small to medium-sized, prolate. Colpus with areolate-granulate operculum, granulate membrane and psilate-perforate margo. Sexine 1.5 to 2.3 times thicker than nexine; exine 1.4 – 1.8 μm . Mesocolpium nano- to microreticulate and apocolpium nanoreticulate-perforate (Figures 3A-C, 4A-C).

Zornia crinita: pollen grains are small to medium-sized, subprolate to prolate. Colpus with perforate operculum, granulate membrane and rugulate-perforate margo. Sexine 1.4 to 2.0 times thicker than nexine; exine 1.6 – 2.1 μm . Mesocolpium nano- to microreticulate-perforate and apocolpium nanoreticulate-perforate (Figures 3D-F, 4D-F).

Zornia thymifolia: pollen grains are small to medium-sized, subprolate to prolate. Colpus with psilate operculum, granulate membrane and psilate perforate margo. Sexine 1.5 to 2.0 times thicker than nexine; exine 1.3 – 1.5 μm . Mesocolpium and apocolpium nanoreticulate-perforate (Figures 3G-I, 4G-I).

Zornia gardneriana: pollen grains are small to medium-sized, subprolate to prolate. Colpus with psilate-perforate operculum, granulate membrane and psilate-perforate margo. Sexine from 1.6 to 2.6 times thicker than nexine; exine 1.7 – 2.3 μm . Mesocolpium nano- to microreticulate and apocolpium nanoreticulate-perforate (Figures 3J-L, 4J-L).

Zornia glochidiata: pollen grains are small to medium-sized, prolate spheroidal to prolate. Colpus with areolate-granulate, granulate membrane and psilate-perforate membrane.

Sexine from 1.5 to 2.3 times thicker than nexine; exine 1.6 – 2.1 μm . Mesocolpium rugulate-perforate, rugulate and apocolpium rugulate-perforate (Figures 3M-O, 4M-O).

Zornia harmsiana: pollen grains are small-sized, prolate spheroidal to subprolate. Colpus with psilate operculum, granulate membrane and psilate-perforate margo. Sexine from 1.3 to 2.1 times thicker than nexine; exine 1.2 – 1.7 μm . Mesocolpium nano- to reticulate and apocolpium psilate (Figures 5A-C, 6A-C).

Zornia myriadena: this species was analysed only in SEM. Colpus with areolate-perforate operculum, granulate membrane and psilate-perforate margo. Mesocolpium nano- to microreticulate and apocolpium nanoreticulate-perforate (Figure 6D-F).

Zornia pardina: pollen grains are small to medium-sized, subprolate to prolate. Colpus with psilate operculum, granulate membrane and psilate-perforate margo. Sexine from 1.3 to 1.7 times thicker than nexine; exine 1.5 – 2.0 μm . Mesocolpium nano- to microreticulate-perforate and apocolpium nanoreticulate-perforate (Figures 5D-F, 6G-I).

Zornia setosa: pollen grains are small to medium-sized, prolate spheroidal to subprolate. Colpus with psilate-perforate operculum, granulate membrane and psilate-perforate margo. Sexine from 1.2 to 2.7 times thicker than nexine; exine 1.7 – 1.9 μm . Mesocolpium nano- to microreticulate and apocolpium nanoreticulate-perforate (Figures 5G-I, 6J-L).

Zornia virgata: pollen grains are medium-sized, subprolate to prolate. Colpus with psilate-perforate operculum, granulate membrane and psilate-perforate margo. Sexine from 1.7 to 2.6 times thicker than nexine; exine 2.1 – 2.6 μm . Mesocolpium nano- to microreticulate and apocolpium nanoreticulate-perforate (Figures 5J-L, 6M-O).

Principal component analysis (PCA)

The PCA showed the relationship between species based on seven metric variables and two classes/indices (Figure 6). Correlation coefficients are shown in Table III. The first two axes accounted for 88.14% of the total variability of the quantitative data analysed. The first axis explained 74.31% of the variance and was mainly associated with EA, CW, and N. The second axis summarised 13.83% of the total variability, with PA, P/E, CL, S, E and S/N being the variables that contributed most to this axis.

Zornia pardina (par65), *Z. crinita* (cri567*), *Z. areolata* (are9215), *Z. bracteata* (bra610*) and *Z. thymifolia* were located on the upper left side of the PCA due to their highest CL, PA and EA values. *Zornia virgata* (vir46, vir61) and *Z. adenophora* (ade7101*) showed high and low P/E and were located on the bottom left side of the PCA.

Conversely, due to their lower CW, N, S, E, S/N and high P/E values, *Zornia capensis* (cap10032), *Z. contorta* (con64) and *Z. brasiliensis* (bras24, bras372) were placed on the upper right side of the PCA. Specimens with the smallest pollen grains were located mainly on the bottom right side of the graph. These specimens also had the lowest CL, PA, EA, and P/E values: *Z. gardneriana* (gar35), *Z. glochidiata* (glo15012), *Z. setosa* (set23740), *Z. brasiliensis* (bras072) and *Z. harmsiana* (har25).

Cluster analysis (UPGMA and Euclidean distance)

We identified two groups of specimens with 0% similarity. One group with similarity greater than 75% comprised two *Zornia virgata* (vir46, vir61) with the largest values of pollen grains, colpus dimensions and exine thickness. Inversely, the remainder species which smaller pollen grains, colporus dimensions and exine thickness had similarity greater than 55%: *Z. Adenophora* (ade7001*), *Z. areolate* (are9215), *Z. thymifolia* (thy20), *Z. crinita* (cri567*), *Z. pardina* (par65), *Z. bracteata* (bra610*), *Z. capensis*

(cap10032), *Z. contorta* (con64*), *Z. gardneriana* (gar35), *Z. glochidiata* (glo15012), *Z. setosa* (set23740) and *Z. brasiliensis* (bras072, bras372, bras24) [except by *Zornia harmsiana* (har25) with about 35%].

Character-mapping

All lineages from the molecular phylogeny were recovered with at least one or more homoplasies/apomorphies, except for the genus *Adesmia*. *Tipuana tipu* and *Pterocarpus rohrii* were recovered supported by two synapomorphies regarding the ultrasculpture of the membrane (psilate-scabrate) and margo (microreticulate). The *Adesmia* clade was recovered supported by three homoplasies regarding the colporus length (20 - 30 μm), operculum ultrasculpture (areolate-granulate) and nexine thickness ($> 0.5 \mu\text{m}$). The genus *Adesmia* + ZAP clade (*Zornia* + *Amicia* + *Poiretia*) were recovered supported by a single homoplasy regarding the endoaperture length ($> 10 \mu\text{m}$). The ZAP clade was recovered supported by two homoplasies regarding the measurement of the polar axis (25 - 30 μm) and the equatorial axis (25 - 30 μm).

Poiretia was recovered supported by a single homoplasy regarding the nexine thickness ($< 0.5 \mu\text{m}$). The *Amicia* + *Zornia* clade was recovered by four homoplasies regarding the colporus width (5 - 10 μm), endoaperture width (absent), sexine thickness ($> 0.5 \mu\text{m}$) and nexine thickness ($> 0.5 \mu\text{m}$) and a single synapomorphy regarding the endoaperture class (absent). *Amicia* was recovered supported by two homoplasies regarding the endoaperture width ($> 10 \mu\text{m}$) and endoaperture class (circular). *Zornia* was recovered supported by three homoplasies regarding the pollen grain form (prolate), endoaperture length (absent) and operculum ultrasculpture (psilate-perforate).

Discussion

Palynological analysis

Of the species analysed here, previous palynological analyses were performed on *Zornia bracteata*, *Z. brasiliensis*, *Z. myriadena* and *Z. virgata* by Ohashi (1971), Pire (1974), Carreira et al. (1996), Moreti et al. (2007), Buril et al. (2011) and Silva (2016). The major of the results of these studies is supported by our analyses, although with some differences in the classification of pollen size, aperture type (colpus/colporus), operculum presence and size of reticulum. Table IV compiles our results and previous pollen descriptions of the analysed species in the literature.

One significant divergence is about the pollen aperture. In the present study, all *Zornia* representatives are 3-colpate (absent endoaperture), confirmed by the significant studies of pollen grains to the genus (Ohashi 1971; Pire 1974; Buril et al. 2011; Silvestre-Capelato & Melhem 1997; Luz et al. 2013). The presence of endoaperture (colporate) has been early reported both for species analysed here and for species not analysed (Carreira et al. 1996; Moreti et al. 2007; RCPol [continuously updated]; Silva 2016) and is probably a morphological artefact caused by acetolysis (Hesse & Waha 1989).

The operculum absence is another divergence between the present and early studies (Carreira et al. 1996; Moreti et al. 2007; Buril et al. 2011; Silva et al. 2016). This mistake is already related and discussed by other *Adesmia* clade representatives (Antonio-Domingues et al. 2022a, 2022b, 2023 in press, 2023 unpublished data) and is a morphological artefact caused by acetolysis or a wrong interpretation of the operculum and membrane (Hesse & Waha 1989).

Character-mapping analysis and pollen evolution

The character mapping analysis evidenced that most of the 16 palynological characters and their states scored in this study were recovered as homoplasies. This pattern of highly homoplastic palynological characters is typical in angiosperms, with all early diverging

lineages (i.e., Amborellales, Nymphaeales, Austrobaileyales, Magnoliids, and Monocots) showing monosulcate pollen grains (Lu et al. 2015). In Fabales, twelve homoplastic character states were consistent with ancestral states of this clade. These include dispersal unit, polarity, size, aperture number, aperture position, aperture structure, ectoaperture shape, aperture membrane ornamentation, presence of an operculum, presence of supratectal elements, supratectal element shape, and infratectum structure (Jiang et al. 2019).

The character mapping analysis could not recover homoplasies/apomorphies only for *Adesmia* among all the sampled genera. It is probably a reflection of the significant number of species in this genus (206 spp.) in relation to the number of species sampled in this study (14 spp.) (POWO 2023). Increasing the number of species of *Adesmia* sampled for palynological and phylogenetic datasets will likely evidence few homoplasies/apomorphies circumscribing this genus.

Based on the plastid and nuclear ribosomal DNA region, *Amicia* and the genera *Poiretia* and *Zornia* form the monophyletic clade Zornia-Amicia-Poiretia, which diverged within the *Adesmia* clade about 24.77 Ma ago (Fortuna-Perez et al. 2013, 2021). This clade is morphologically supported by the secretory cavities as synapomorphy and diagnosed by the same fruit type, articulated lomentos ornamented with crests and mainly paripinnate leaves with 2–4 leaflets (Fortuna-Perez et al. 2013, 2021; Särkinen & Hughes 2015). ZAP clade was palynological supported by two homoplasies regarding the measurement of the polar axis (25 - 30 μm) and the equatorial axis (25 - 30 μm), reinforcing this clade palynologically.

The aperture reveals important features in other pollen evolution studies of Leguminosae (Banks et al. 2014; LPWG 2017). In the *Adesmia* clade, only *Zornia* and *Amicia zygomeris* present 3-colpate pollen grains (Antonio-Domingues et al. 2022b).

Two homoplasies regarding the aperture recovered the *Amicia* + *Zornia* clade [colporus width (5 - 10 μm), endoaperture width (absent)] plus two regarding the sporoderm [sexine thickness ($> 0.5 \mu\text{m}$) and nexine thickness ($> 0.5 \mu\text{m}$)] and a single synapomorphy regarding the endoaperture class (absent).

Conclusions

A comprehensive pollen characterisation permits the description of *Zornia* as stenopalynous with some differences in pollen grain size and ultrasculpture of the operculum, margo and sexine. All lineages of the *Adesmia* clade were recovered with at least one or more homoplasies/apomorphies, except by *Adesmia*. Pollen morphology is highly informative for the *Adesmia* clade, even though most characters were recovered as homoplastic, which seems to be a pattern for Fabales.

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Disclosure statement

The authors reported no potential conflict of interest.

Specimens investigated

Zornia J.F. Gmel. (asterisk [*] only for SEM images)

Zornia brasiliensis Vogel. Brazil: Bahia, Caetité. Roque N *et al.* CFCR14964, 8 March 1994 (SP 312372). Brazil: Bahia. Torrend C 261, September 1936 (SP37124). Brazil: Paraíba, Pocinhos. Miranda AM, Félix LP 1858, 08 August 1994 (SP 332072).

Zornia adenophora (Domin) Mohlenbr. (7101)

Zornia areolata Mohlenbr. (9215)

Zornia bracteata J.F. Gmel. (610)

Zornia capensis Pers. (10032)

Zornia contorta Mohlenbr. Elrich R. 64K

Zornia crinita (Mohlenbr.) Vanni. (567)

Zornia fhyimifolia Domin. (K001088420)

Zornia gardneriana Moric. Brazil: Piauí. E. Ule 7185, January 1907 (K 000205335).

Zornia glochidiata Rchb. ex DC. Brummit *et al* 15012 K

Zornia harmsiana Standl. Brazil: Bahia, Remanso. L. Coradin *et al.* 5934, 22 June 1983 (K000205325).

Zornia myriadena Benth. *

Zornia pardina Mohlenbr. Brazil: Minas Gerais, Paracatu. A. Krapovickas & C.L. Cristóbal 42907, 12 January 1989 (K 000624365)

Zornia setosa Baker f. (23740)

Zornia virgata Moric. Brazil: Goiás, Alto Paraíso. G. Hatschbach *et al.* 70685, 8 May 2000 (MBM 248346). Brazil: Goiás, Abadiânia. G. Hatschbach 36693, 22 May 1975 (MBM37561)

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Figure and table captions

Figure 1. Light microscopy of *Zornia* J.F. species. **A-C.** *Zornia adenophora*. **A.** Detail of the colporus, endoaperture, operculum and surface, equatorial view. **B.** Apertures 3-colpate, long and pointed apices, colpori and apocolpium surface. **C.** Optical section, equatorial view. **D-F.** *Zornia areolata*. **D.** Detail of the colporus, endoaperture, surface and operculum, equatorial view. **E.** Apertures 3-colpate, long and pointed apices and apocolpium surface. **F.** Optical section, equatorial view. **G-I.** *Zornia bracteata*. **G.** Detail of the colporus, surface and operculum, equatorial view. **H.** Apertures 3-colpate, long and pointed apices and apocolpium surface. **I.** Optical section, equatorial view. **J-L.** *Zornia brasiliensis*. **J.** Detail of the colporus, surface and operculum, equatorial view. **K.** Apertures 3-colpate, long and pointed apices and apocolpium surface. **L.** Optical section, equatorial view. **M-O.** *Zornia capensis*. **M.** Detail of the colporus, surface and operculum, equatorial view. **N.** Apertures 3-colporate, long and pointed apices and apocolpium surface. **O.** Optical section, equatorial view. Scale bars – H, K, N = 2 μm ; A-H, I, J, L, M, O = 5 μm .

Figure 2. Scanning electron microscopy of *Zornia* J.F. species. **A-C.** *Zornia adenophora*. **A.** Detail of apertural membrane of the colporus, margo and operculum, equatorial view. **B.** Detail of mesocolpium. **C.** Detail of apocolpium. **D-F.** *Zornia areolata*. **D.** Detail of apertural membrane of the colporus, margo and operculum, equatorial view. **E.** Detail of mesocolpium. **F.** Detail of apocolpium. **G-I.** *Zornia bracteata*. **G.** Detail of apertural membrane of the colporus, margo and operculum, equatorial view. **H.** Detail of mesocolpium. **I.** Detail of apocolpium. **J-L.** *Zornia brasiliensis*. **J.** Detail of apertural membrane of the colporus, margo, operculum and mesocolpium, equatorial view. **K.** Detail of mesocolpium. **L.** Detail of apocolpium. **M-O.** *Zornia capensis*. **M.** Detail of apertural membrane of the colporus, margo and operculum, equatorial view. **N.** Detail of mesocolpium. **O.** Detail of apocolpium. Scale bars – L = 1 μm ; A-K, M, N = 2 μm ; O = 5 μm .

Figure 3. Light microscopy of *Zornia* J.F. species. **A-C.** *Zornia contorta*. **A.** Detail of the colporus, endoaperture, operculum and surface, equatorial view. **B.** Apertures 3-colpate, long and pointed apices, colpori and apocolpium surface. **C.** Optical section, equatorial view. **D-F.** *Zornia crinita*. **D.** Detail of the colporus, endoaperture, surface and operculum, equatorial view. **E.** Apertures 3-colpate, long and pointed apices and apocolpium surface. **F.** Optical section, equatorial view. **G-I.** *Zornia thymifolia*. **G.** Detail of the colporus, surface and operculum, equatorial view. **H.** Apertures 3-colpate, long and pointed apices

and apocolpium surface. **I.** Optical section, equatorial view. **J-L.** *Zornia gardneriana*. **J.** Detail of the colporus, surface and operculum, equatorial view. **K.** Apertures 3-colpate, long and pointed apices and apocolpium surface. **L.** Optical section, equatorial view. **M-O.** *Zornia glochidiata*. **M.** Detail of the colporus, surface and operculum, equatorial view. **N.** Apertures 3-colporate, long and pointed apices and apocolpium surface. **O.** Optical section, equatorial view. Scale bars – B = 2 μm ; A, C-O = 5 μm .

Figure 4. Scanning electron microscopy of *Zornia* J.F. species. **A-C.** *Zornia contorta*. **A.** Detail of apertural membrane of the colporus, margo and operculum, equatorial view. **B.** Detail of mesocolpium. **C.** Detail of apocolpium. **D-F.** *Zornia crinita*. **D.** Detail of apertural membrane of the colporus, margo, mesocolpium and operculum, equatorial view. **E.** Detail of mesocolpium. **F.** Detail of apocolpium. **G-I.** *Zornia thymifolia*. **G.** Detail of apertural membrane of the colporus, margo and operculum, equatorial view. **H.** Detail of mesocolpium. **I.** Detail of apocolpium. **J-L.** *Zornia gardneriana*. **J.** Detail of apertural membrane of the colporus, margo, operculum and mesocolpium, equatorial view. **K.** Detail of mesocolpium. **L.** Detail of apocolpium. **M-O.** *Zornia glochidiata*. **M.** Detail of apertural membrane of the colporus, margo and operculum, equatorial view. **N.** Detail of mesocolpium. **O.** Detail of apocolpium. Scale bars – A, B, H, I, J, K, O = 1 μm ; C-G, L, M, N = 2 μm .

Figure 5. Light microscopy of *Zornia* J.F. species. **A-C.** *Zornia harmisiana*. **A.** Detail of the colporus, endoaperture, operculum and surface, equatorial view. **B.** Apertures 3-colpate, long and pointed apices, colpori and apocolpium surface. **C.** Optical section, equatorial view. **D-F.** *Zornia pardina*. **D.** Detail of the colporus, endoaperture, surface and operculum, equatorial view. **E.** Apertures 3-colpate, long and pointed apices and apocolpium surface. **F.** Optical section, equatorial view. **G-I.** *Zornia setosa*. **G.** Detail of the colporus, surface and operculum, equatorial view. **H.** Apertures 3-colpate, long and pointed apices and apocolpium surface. **I.** Optical section, equatorial view. **J-L.** *Zornia virgata*. **J.** Detail of the colporus, surface and operculum, equatorial view. **K.** Apertures 3-colpate, long and pointed apices and apocolpium surface. **L.** Optical section, equatorial view. Scale bars – A-C, H = 2 μm ; D-G, I-L = 5 μm .

Figure 6. Scanning electron microscopy of *Zornia* J.F. species. **A-C.** *Zornia harmisiana*. **A.** Detail of apertural membrane of the colporus, margo, operculum and mesocolpium, equatorial view. **B.** Detail of mesocolpium. **C.** Detail of apocolpium. **D-F.** *Zornia myriadena*. **D.** Detail of apertural membrane of the colporus, margo, mesocolpium and operculum, equatorial view. **E.** Detail of mesocolpium. **F.** Detail of

apocolpium. **G-I.** *Zornia pardina*. **G.** Detail of apertural membrane of the colporus, margo, mesocolpium and operculum, equatorial view. **H.** Detail of mesocolpium. **I.** Detail of apocolpium. **J-L.** *Zornia gardneriana*. **J.** Detail of apertural membrane of the colporus, margo, operculum and mesocolpium, equatorial view. **K.** Detail of mesocolpium. **L.** Detail close to the apocolpium. **M-O.** *Zornia glochidiata*. **M.** Detail of apertural membrane of the colporus, margo and operculum, equatorial view. **N.** Detail of mesocolpium. **O.** Detail of apocolpium. Scale bars – A-F, J-L = 1 μm ; G-I, M-O = 2 μm .

Figure 7. Principal component analysis biplot of the pollen grain metric variables and classes/indices of *Zornia* specimens.

Figure 8. Dendrogram built from cluster analysis (Euclidean distance) for *Zornia* specimens, information remaining (%).

Figure 9. Molecular phylogeny and pollen character-mapping of informal Adesmia clade (Leguminosae – Papilionoideae - Dalbergieae) pruned from Fortuna-Perez et al. (2021). Character mapping tree - green circles represent apomorphies (synapomorphies and autapomorphies); white circles represent homoplasies. Numbers above circles represent the number of the pollen character; numbers below circles represent the number of the pollen character state reconstructed.

Table I. *Zornia* J.F. pollen grains dimensions (μm) in equatorial view using light microscopy.

Note. Specimens were identified by abbreviation of the epithet and the last two numbers of the herbarium voucher (see Appendix). Confidence Interval at 95% of probability of the lowest sample values (CI-) and highest sample values (CI+), arithmetic mean ($\bar{x}$), average standard deviation (sx), sample standard deviation (s), coefficient of variability (V%).

Table II. Pollen morphology and ultrasculpture of *Amicia* using light and scanning electron microscopy.

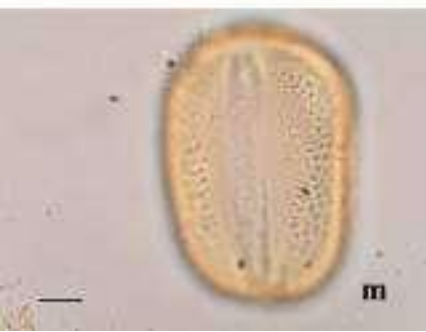
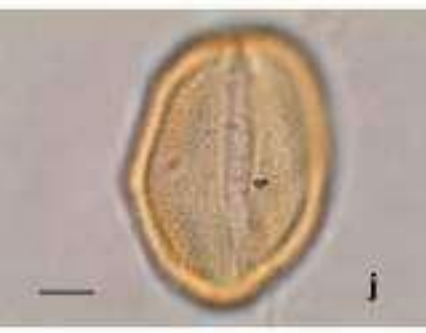
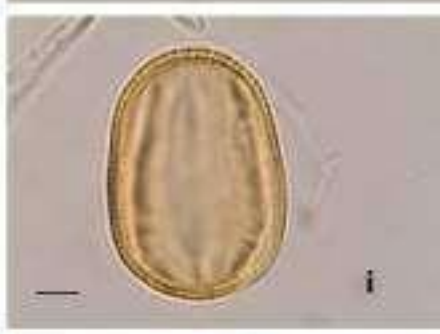
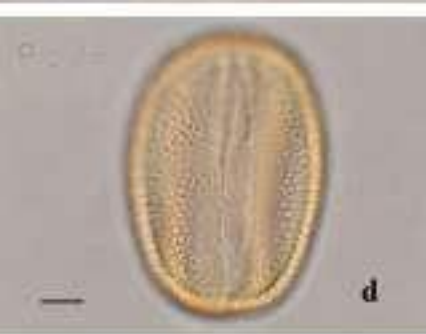
PT, pollen type; Pollen size: S, small, M, medium; L, large; *rarely

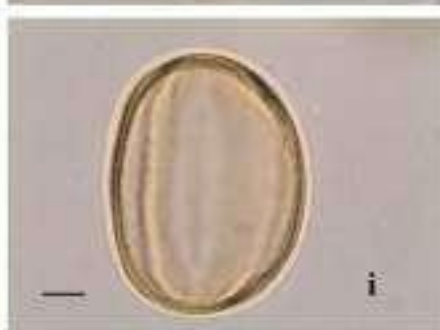
Table III. Pearson and Kendall coefficients for pollen grain metric variables and classes/index from the first two ordination axes of the principal component analysis (PCA) of *Zornia* species.

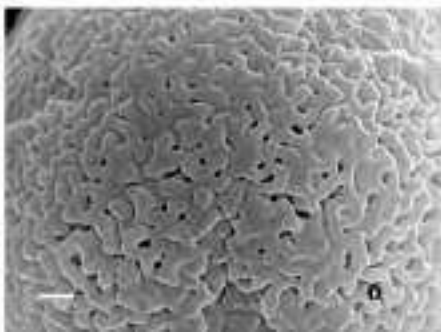
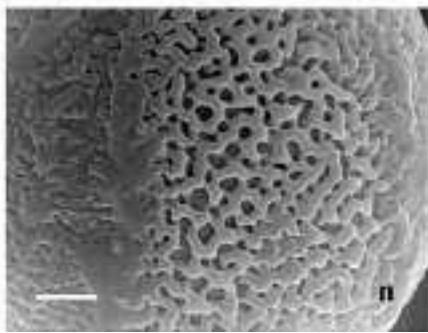
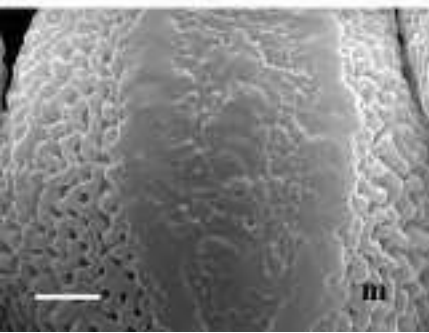
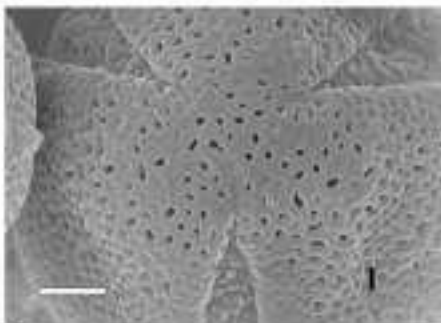
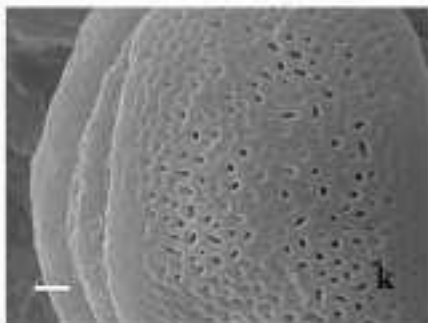
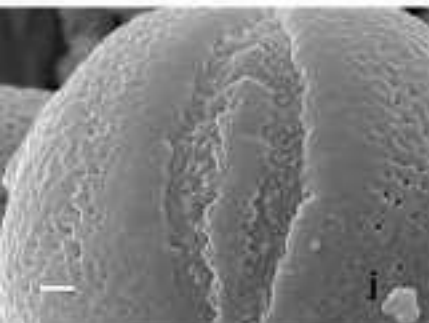
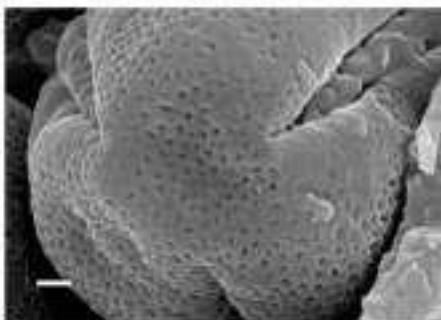
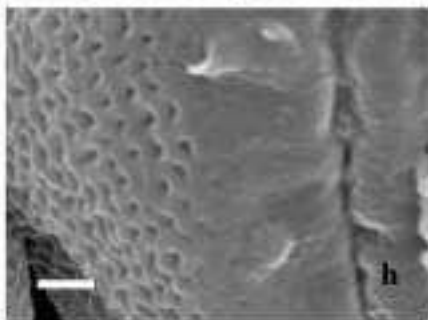
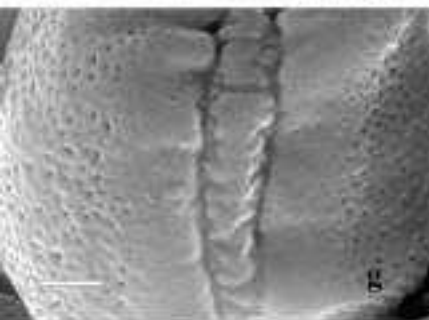
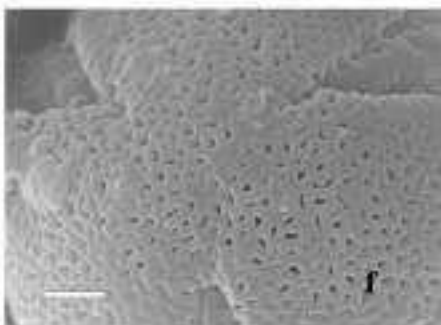
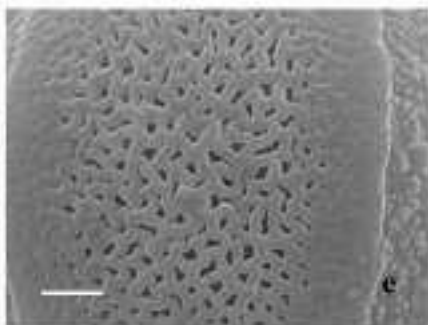
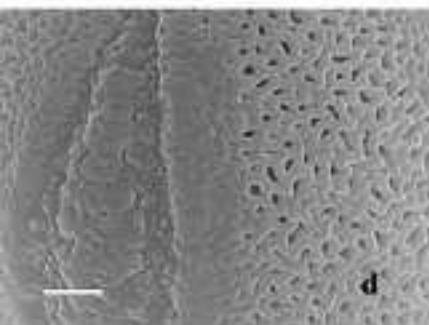
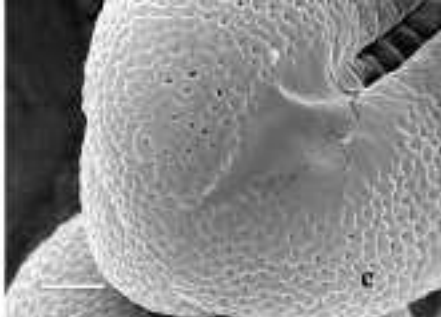
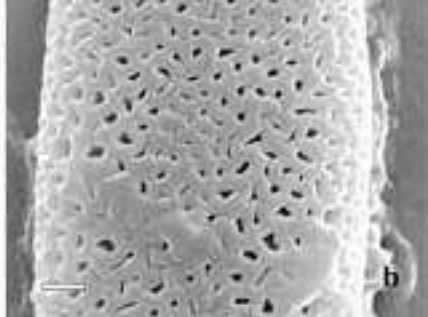
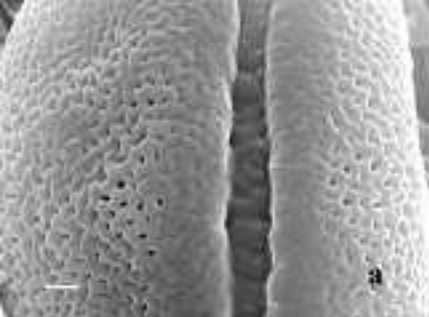
PA, polar axis; EA, equatorial axis; $P/E = PA/EA$; CL, colpus length; CW, colpus width; N, nexine thickness; S, sexine thickness ($S = \text{infratectum} + \text{tectum}$); E, exine thickness ($Ex = N + S$); s/n , index of sexine and nexine thickness.

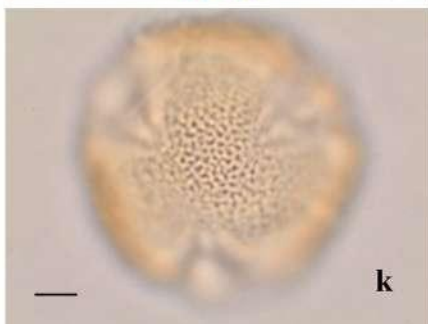
Table IV. Summary of pollen morphology of *Amicia* Kunth according to some authors in compared to the results obtained in this research. L, large; M, medium; O 1971, Ohashi 1971; P 1974, Pire 1974; **PS**, present study.

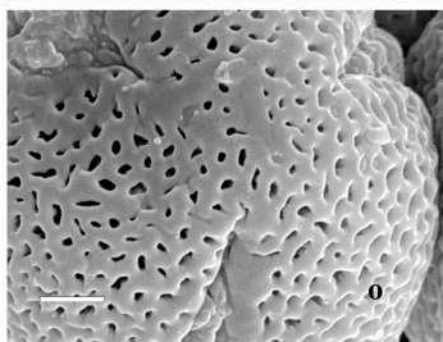
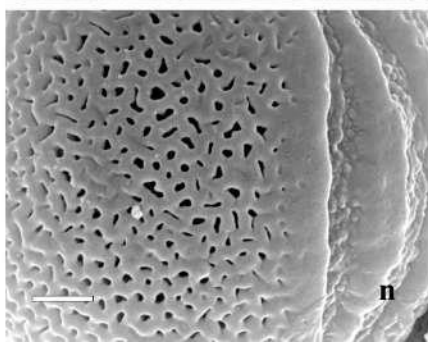
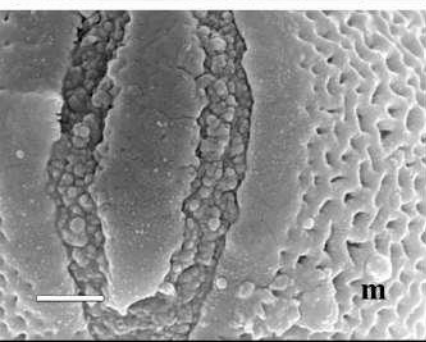
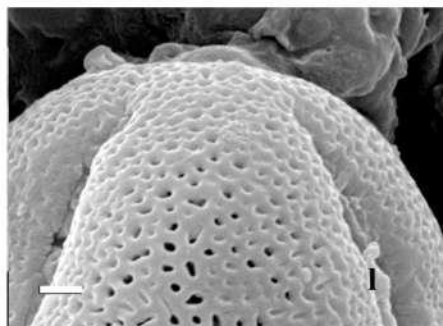
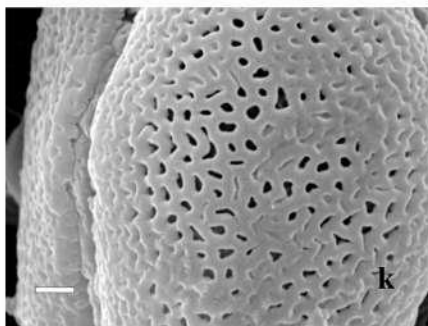
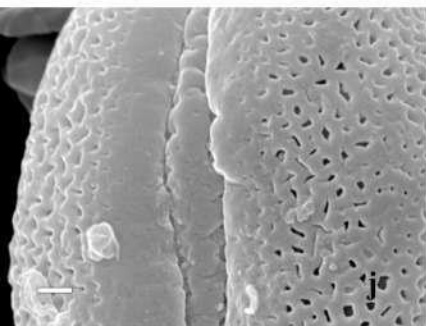
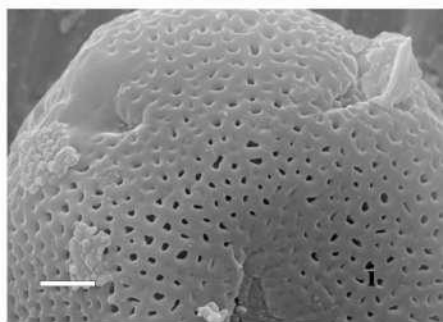
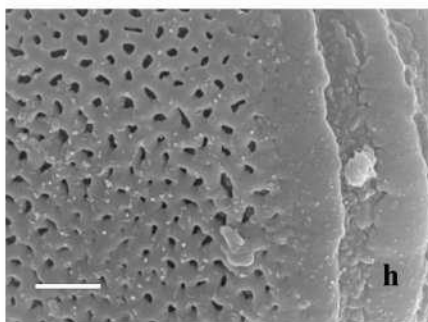
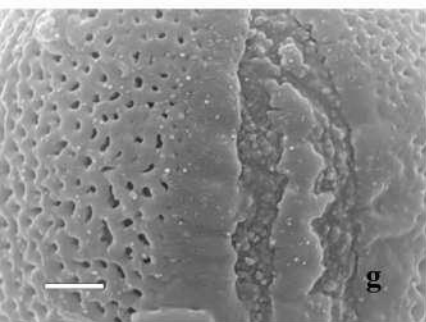
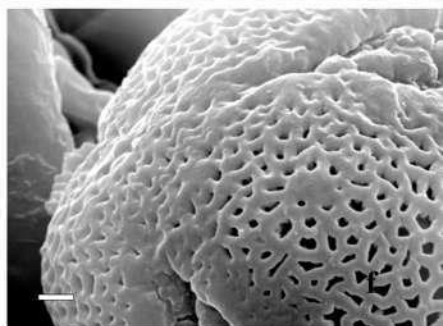
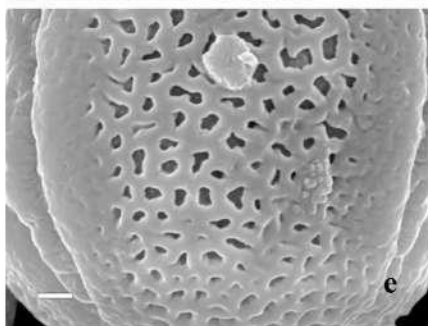
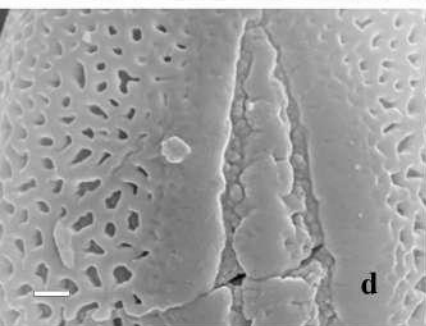
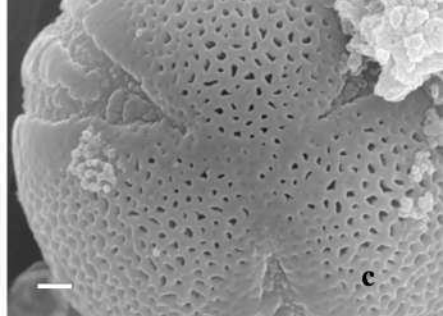
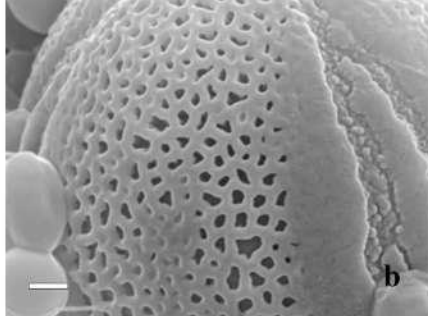
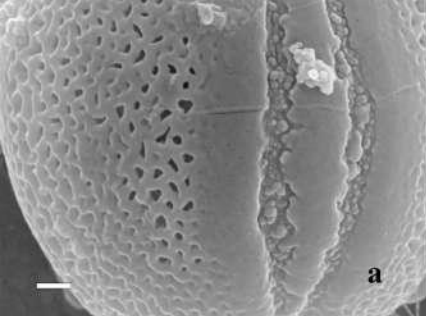
Table V. List of morphological characters of pollen grains and their character states for the species of informal *Adesmia* clade and outgroups sampled.

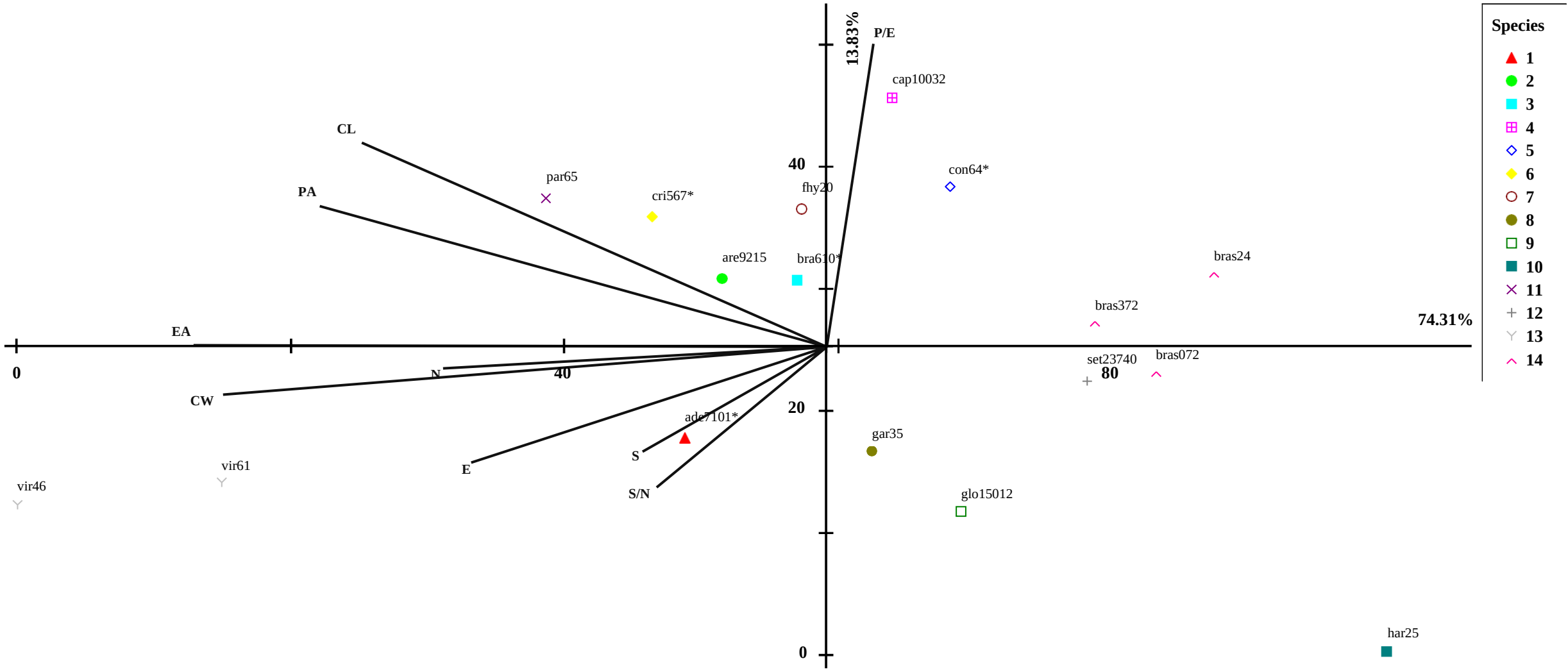












SIMILARITY%

154

Infor

100

75

50

25

0

ade7101

are9215

fil20

cri567

par65

bra610

cap10032

con64

gar35

glo15012

set23740

bras072

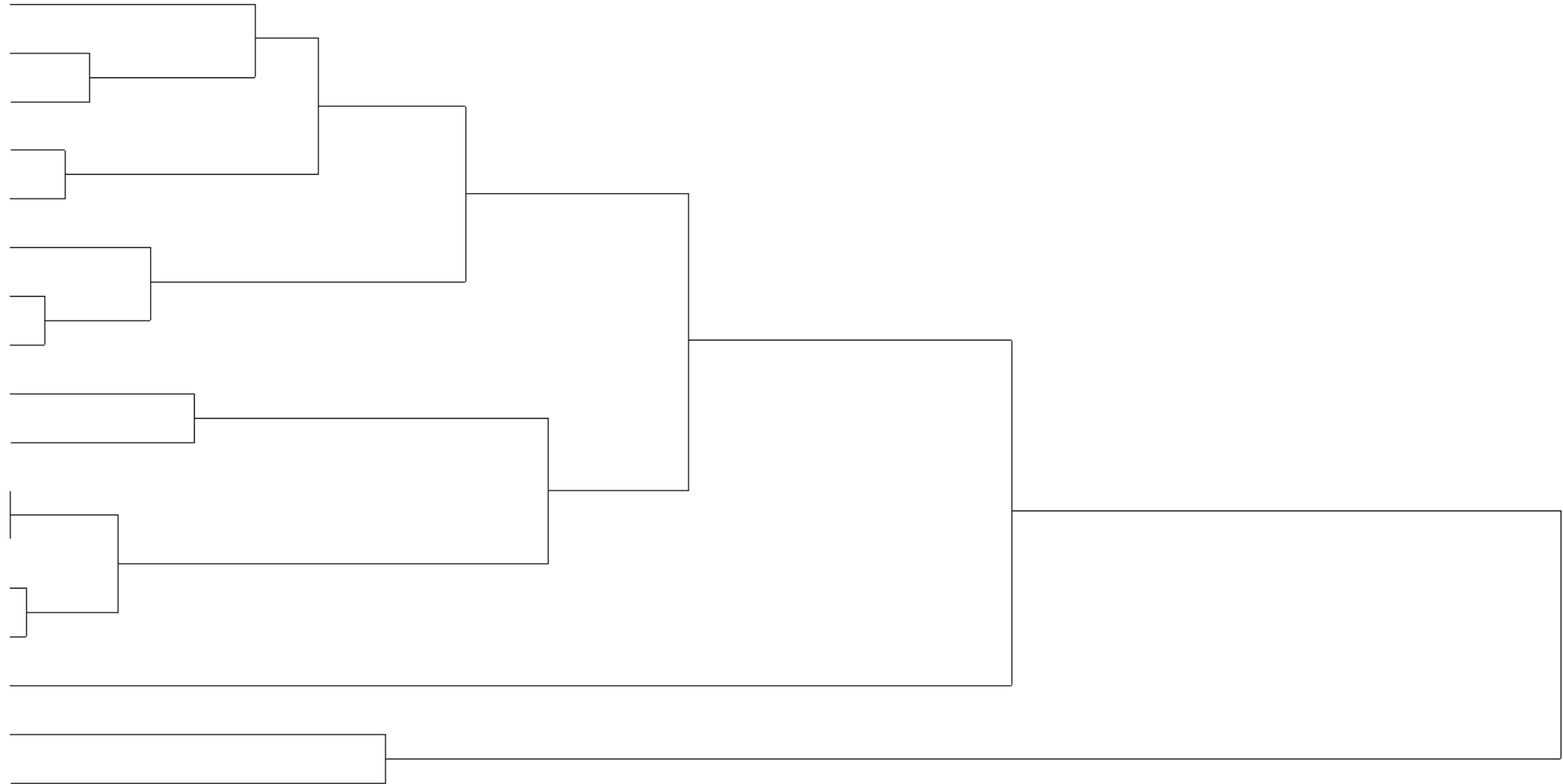
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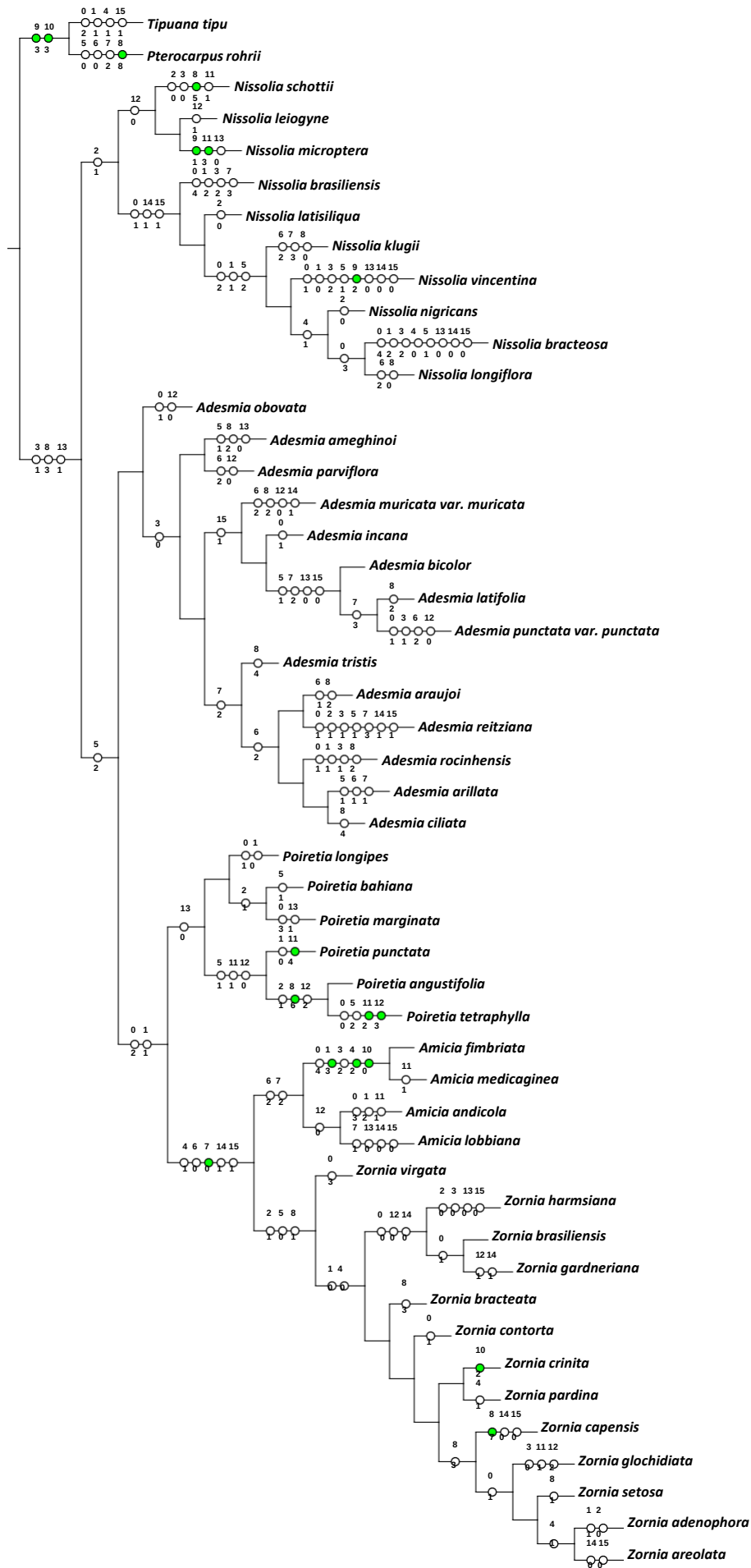


Table I. *Zornia* J.F. Gmel. (μm) pollen grains in equatorial view using light microscopy. ($\pm$)

Specimen	Polar Axis				Equatorial Axis				P/E	Polen	Colpus		Exine	
	CI - ($x \pm sx$)	CI +	s	V%	CI - ($x \pm sx$)	CI +	s	V%	x	Size	Length - Width		N	S
ade7101*	26.9 (27.5 $\pm$ 0.3)	28.2	1.6	5.7	24.3 (25.2 $\pm$ 0.5)	26.2	2.3	9.1	1.1	S – M	24.1 – 5.2		0.7	1.1
are9215	29.9 (29.8 $\pm$ 0.3)	30.4	1.5	5.0	23.2 (23.6 $\pm$ 0.2)	24.0	1.1	4.6	1.3	S – M	25.1 – 5.3		0.6	0.9
brac610*	30.1 (30.7 $\pm$ 0.3)	31.2	1.3	4.1	21.7 (22.2 $\pm$ 0.2)	22.7	1.2	5.5	1.3	S – M	25.3 – 4.3		0.6	1.1
bras24	26.4 (27.0 $\pm$ 0.3)	27.5	1.3	5.0	17.2 (17.5 $\pm$ 0.8)	17.9	0.8	4.5	1.5	S – M	21.2 – 3.2		0.6	1.0
bras072	24.7 (25.4 $\pm$ 0.4)	26.2	1.9	7.4	19.0 (19.5 $\pm$ 0.2)	19.9	1.1	5.7	1.3	S – M	20.3 – 3.5		0.6	1.0
bras372	26.0 (26.7 $\pm$ 0.3)	27.5	1.7	6.4	17.6 (18.3 $\pm$ 0.3)	19.0	1.7	9.2	1.5	S – M	21.7 – 3.8		0.6	1.0
cap10032*	30.8 (31.3 $\pm$ 0.2)	31.8	1.2	3.9	21.8 (22.2 $\pm$ 0.2)	22.6	0.9	4.2	1.4	S – M	27.2 – 3.6		0.6	1.0
con64*	29.2 (29.9 $\pm$ 0.3)	30.5	1.5	5.0	20.9 (21.3 $\pm$ 0.2)	21.7	0.9	4.4	1.4	S – M	25.5 – 3.5		0.6	1.1
cri567*	32.7 (33.6 $\pm$ 0.4)	34.5	2.2	6.6	23.5 (24.0 $\pm$ 0.3)	24.6	1.4	5.7	1.4	S – M	27.1 – 4.6		0.7	1.2
thy20	30.6 (31.1 $\pm$ 0.3)	31.7	1.4	4.6	22.5 (23.1 $\pm$ 0.3)	23.6	1.4	6.1	1.4	S – M	25.2 – 4.5		0.5	0.9
gar35	27.4 (27.8 $\pm$ 0.2)	28.2	1.0	3.8	21.2 (21.7 $\pm$ 0.2)	22.1	1.1	4.9	1.3	S – M	22.5 – 4.0		0.7	1.4
glo15012	27.2 (27.7 $\pm$ 0.2)	28.1	1.1	3.9	20.7 (21.1 $\pm$ 0.2)	21.5	0.9	4.5	1.3	S – M	19.0 – 4.3		0.6	1.2
har25	19.5 (19.9 $\pm$ 0.2)	20.4	1.2	6.0	16.7 (17.1 $\pm$ 0.2)	17.6	1.1	6.6	1.2	S	16.0 – 3.6		0.5	1.0
par65	33.3 (34.1 $\pm$ 0.4)	34.9	1.9	5.7	24.0 (24.4 $\pm$ 0.2)	24.8	1.0	4.1	1.4	S – M	28.8 – 5.3		0.7	1.1
set23740*	25.8 (26.2 $\pm$ 0.2)	26.7	1.1	4.1	20.2 (20.8 $\pm$ 0.3)	21.3	1.3	6.3	1.3	S – M	20.8 – 3.4		0.7	1.1
vir46	34.6 (35.7 $\pm$ 0.5)	36.8	2.7	7.6	28.9 (29.4 $\pm$ 0.2)	29.9	1.1	3.9	1.2	M	29.8 – 9.1		0.8	1.2
vir61	34.2 (35.2 $\pm$ 0.3)	35.4	1.7	4.8	26.9 (27.5 $\pm$ 0.3)	28.1	1.5	5.3	1.3	M	27.4 – 7.2		0.8	1.6

Note. Specimens were identified by abbreviation of the epithet and the last two numbers of the herbarium voucher (see Appendix). Confidence Interval at 95% of probability of the lowest sample values (IC-) and highest sample values (IC+), arithmetic mean (x), average standard deviation (sx), sample standard deviation (s), coefficient of variability ($V\%$).

Table II. Pollen morphology and ultrasculpture of *Zornia* pollen grains using light and scanning electron microscopy. PT, pollen type; S, small, M, medium; ⁻sparsely, ⁺densely, ^{*}rarely, --not conclusive.

Species	Size	Operculum	Margo	Mesocolpium	Apocolpium
<i>Zornia adenophora</i>	S-M	Areolate-granulate	Psilate-perforate	Microreticulate-perforate	Nanoreticulate-perforate
<i>Zornia areolata</i>	S-M	Areolate-granulate	Psilate-perforate	Nano- to microreticulate	Nanoreticulate-perforate
<i>Zornia bracteata</i>	S-M	Areolate-granulate	Psilate-perforate	Micro- to reticulate	Nano- to microreticulate
<i>Zornia brasiliensis</i>	S-M	Granulate	Psilate-perforate	Nano- to microreticulate-perforate	Perforate
<i>Zornia capensis</i>	S-M	Rugulate-perforate	Psilate-perforate	Nano- to microreticulate	Nanoreticulate-perforate
<i>Zornia contorta</i>	S-M	Areolate-granulate	Psilate-perforate	Nano- to microreticulate	Nanoreticulate-perforate
<i>Zornia crinita</i>	S-M	Perforate	Rugulate-perforate	Nano- to microreticulate-perforate	Nanoreticulate-perforate
<i>Zornia thymifolia</i>	S-M	Psilate	Psilate-perforate	Nanoreticulate-perforate	Nanoreticulate-perforate
<i>Zornia garderiana</i>	S-M	Psilate-perforate	Psilate-perforate	Nano- to microreticulate	Nanoreticulate-perforate
<i>Zornia glochidiata</i>	S-M	Areolate-granulate	Psilate-perforate	Rugulate-perforate	Rugulate-perforate
<i>Zornia harmesiana</i>	S	Psilate	Psilate-perforate	Nano- to reticulate	Psilate
<i>Zornia myriadeana</i>	--	Areolate-perforate	Psilate-perforate	Nano- to microreticulate	Nanoreticulate-perforate
<i>Zornia pardina</i>	S-M	Psilate	Psilate-peforate	Nano- to microreticulate-perforate	Nanoreticulate-perforate
<i>Zornia setosa</i>	S-M	Psilate-perforate	Psilate-perforate	Nano-to microreticulate	Nanoreticulate-perforate
<i>Zornia virgata</i>	M	Psilate-perforate	Psilate-perforate	Nano-to microreticulate	Nanoreticulate-perforate

Table III. Pearson and Kendall coefficients of pollen grain metric variables and classes/indices from the first two ordination axes of the principal component analysis (PCA) of *Zornia* species

Variables	Principal components	
	Axis 1	Axis 2
PA	– 0.3654	0.4468
EA	– 0.4145	0.4676
P/E	0.0364	0.2141
CL	– 0.3690	0.6093
CW	– 0.6705	– 0.4412
N	– 0.1207	– 0.0659
S	0.1260	0.2229
E	– 0.2108	– 0.2799
s/n	– 0.1100	– 0.2322

PA, polar axis; EA, equatorial axis; P/E = PA/EA; CL, colpus length; CW, colpus width; N, nexine thickness; S, sexine thickness (S = infratectum + tectum); E, exine thickness (Ex = N + S); s/n, index of sexine and nexine thickness.

Table IV. Pollen morphology data from previously published studies compared with the present study. M, medium; S, small; PS, present study; --, not described; O 1971, Ohashi 1971; P 1974, Pire 1974; C 1996, Carreira et al. 1996; M 2007, Moreti et al. 2007; B 2011, Buril et al. 2011; S 2016, Silva et al. 2016.

Species	Author	Size Class	Aperture	Operculum	Sexine ultrasculpture	
					Mesocolpium	Apocolpium
<i>Z. bracteata</i>	O 1971	S-M	3- colpate, anastomosed at poles	Present	Microreticulate	
	PS	S-M	3-colpate, non-anastomosed	Areolate-granulate	Micro- to reticulate	Nano- to reticulate
<i>Z. drasiliensis</i>	M 1966	S-M	3- colpate with membrane and margo	Present	Reticulate	
	C 1996	M	3-colporate	Absent	Microreticulate	
	M 2007	M	3-colporate	Absent	Reticulate	
	B 2011	S-M	3- colpate, granulate membrane	Absent	Microreticulate	
	S 2016	M	3-colporate	Absent	Microreticulate, homobrochate	
	PS	S-M	3-colpate, granulate membrane, psilate-perforate margo	Granulate	Nano- to microreticulate-perforate	Perforate
<i>Z. myriadena</i>	B 2011	S-M	3- colpate, granulate membrane	Absent	Microreticulate	
	PS	--	3-colpate, areolate perforate membrane	Areolate-perforate	Nano- to microreticulate	Nanoreticulate-perforate
<i>Z. virgata</i>	P 1974	M	3-colpate	Slightly reticulate	Reticulate, homobrochate	
	PS	M	3-colpate	Psilate-perforate	Nano- to microreticulate	Nanoreticulate-perforate

Table V. List of morphological characters of pollen grains and their character states for the species of informal *Adesmia* clade and outgroups sampled.

Character code	Character States
Character 0	pollen grain, polar axis, measurement: (0) < 25 μm , (1) 25 - 30 μm , (2) 25 - 30 μm , (3) 30 - 35 μm , (4) > 40 μm
Character 1	pollen grain, equatorial axis, measurement: (0) < 25 μm , (1) 25 - 30 μm , (2) 30 - 35 μm , (3) > 40 μm
Character 2	pollen grain, form, index: (0) subspheroidal, (1) prolate
Character 3	aperture, colporus, length, measurement: (0) < 20 μm , (1) 20 - 30 μm , (2) > 30 μm
Character 4	aperture, colporus, width, measurement: (0) < 5 μm , (1) 5 - 10 μm , (2) > 10 μm
Character 5	aperture, endoaperture, length, measurement: (0) absent, (1) < 10 μm , (2) > 10 μm
Character 6	aperture, endoaperture, width, measurement: (0) absent, (1) < 10 μm , (2) > 10 μm
Character 7	aperture, endoaperture, class, index: (0) absent, (1) lalongate, (2) circular, (3) lolongate
Character 8	aperture, operculum, presence and ultrasculpture: (0) absent, (1) psilate to perforate, (2) granulate, (3), areolate-granulate, (4) areolate, (5) rugulate-areolate-granulate, (6) rugulate-granulate, (7) rugulate-perforate, (8) present
Character 9	aperture, membrane, ultrasculpture: (0) granulate, (1) areolate-granulate, (2) rugulate-granulate, (3) psilate-scabrate
Character 10	ornamentation, margo, ultrasculpture: (0) absent, (1) psilate-perforate, (2) rugulate-perforate, (3) microreticulate
Character 11	ornamentation, mesocolpium, ultrasculpture: (0) nano- to reticulate, (1) rugulate-perforate, (2) fossulate-perforate, (3) dimorphic, (4) perforate
Character 12	ornamentation, apocolpium, ultrasculpture: (0) psilate-perforate, (1) nano- to reticulate, (2) rugulate-perforate, (3) fossulate-perforate
Character 13	sporoderm stratification, nexine, measurement: (0) < 0.5 μm , (1) > 0.5 μm
Character 14	sporoderm stratification, sexine, measurement: (0) < 0.5 μm , (1) > 0.5 μm
Character 15	sporoderm stratification, exine, measurement: (0) < 1.5 μm , (1) > 1.5 μm

Table VI. List of homoplasies and apomorphies (including synapomorphies and autapomorphies) recovered for all lineages in this study.

Lineages	Homoplasies	Apomorphies
<i>Tipuana</i> + <i>Pterocarpus</i> clade		Membrane psilate-scabrate; margo microreticulate
<i>Adesmia</i> clade	colporus length 20 - 30 μm ; operculum areolate-granulate; nexine thick > 0.5 μm	
<i>Nissolia</i>	pollen grain prolate	
<i>Adesmia</i> + ZAP Clade	endoaperture length > 10 μm	
<i>Adesmia</i>		
ZAP Clade	polar axis 25 - 30 μm ; equatorial axis 25 - 30 μm	
<i>Poretia</i>	nexine < 0.5 μm	
<i>Amicia</i> + <i>Zornia</i>	colporus width 5 - 10 μm ; endoaperture width absent; sexine > 0.5 μm ; nexine > 0.5 μm	endoaperture class absent
<i>Amicia</i>	endoaperture width > 10 μm , endoaperture class circular	
<i>Zornia</i>	pollen grain prolate; endoaperture length absent, operculum psilate-perforate	

CONCLUSÃO

Foi possível analisar e descrever palinologicamente os grãos de pólen dos cinco gêneros (*Adesmia*, *Amicia*, *Poiretia*, *Nissolia* e *Zornia*) utilizando microscopia ótica e eletrônica de varredura e de transmissão. Os padrões palinológicos do clado *Adesmia* foram reconhecidos e foi possível perceber que o grupo é predominantemente euripolínico assim como seus cinco gêneros, exceto por *Zornia*, que é estenopolínico. Os dados da micromorfologia dos grãos de pólen possibilitaram auxiliar na sistemática dos gêneros como também para as espécies aqui estudadas assim como inferir a evolução das características palinológicas dentro do grupo. Nossos dados da evolução de caracteres palinológicos, juntamente com os estudos já existentes para os grãos de pólen, morfológicos, anatômicos e moleculares, permitem uma melhor delimitação dos representantes do clado *Adesmia* bem como suportar futuras análises para a família Leguminosae, subfamília Papilionoideae tribo Dalbergieae.

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