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Simone Cartaxo Pinto

UMA ABORDAGEM PALINOLÓGICA SOBRE A FAMÍLIA VITACEAE

Rio de Janeiro
2021

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Tese de Doutorado apresentada ao Programa de Pós-graduação em Ciências Biológicas (Botânica), Museu Nacional, da Universidade Federal do Rio de Janeiro, como parte dos requisitos necessários à obtenção do título de Doutor.

Orientadoras: Dra. Claudia Barbieri Ferreira Mendonça
Dra. Vania Gonçalves Lourenço Esteves

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DEDICATÓRIA

Dedico a minha família

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RESUMO

UMA ABORDAGEM PALINOLÓGICA SOBRE A FAMÍLIA VITACEAE

Simone Cartaxo Pinto

Orientadores: Prof^{a} Dr^{a} Claudia Barbieri Ferreira Mendonça

Prof^{a} Dr^{a} Vania Gonçalves Lourenço Esteves

Resumo da Tese de Doutorado submetida ao Programa de Pós-graduação em Ciências Biológicas (Botânica), Museu Nacional, da Universidade Federal do Rio de Janeiro - UFRJ, como parte dos requisitos necessários à obtenção do título de Doutor em Ciências Biológicas (Botânica).

A família Vitaceae possui ca. 900 espécies e 16 gêneros e está distribuída nas regiões temperadas, subtropicais e tropicais. Atualmente a família possui cinco tribos reconhecidas: Ampelopsideae, Cisseae, Cayratieae, Parthenocisseae e Viteae. As espécies são trepadeiras lenhosas ou videiras, e apresentam gavinhas, frequentemente opostas às folhas. Esse estudo teve como objetivo descrever as características polínicas de representantes das tribos de Vitaceae, indicar caracteres úteis para auxiliar na delimitação dos gêneros e espécies, buscando com isso fornecer dados para taxonomia da família, ampliando o conhecimento palinológico e contribuindo para futuros estudos palinotaxonômicos e evolutivos. O material estudado foi obtido de exsicatas proveniente de herbários: BHCB, JCT, MBM, NY, PACA, RB e US. Os grãos de pólen foram acetolisados, medidos, descritos e ilustrados sob microscopia de luz, as descrições realizadas com base em microscopia eletrônica de varredura foram realizadas com grãos de pólen não acetolizados. Para a análise multivariada utilizou-se os programas Fitopac e Pc-Ord e o mapeamento de caracteres foi realizado no programa Mesquite. Foram estudadas características polínicas como forma, tamanho, abertura e padrão de ornamentação da exina e a história evolutiva dos caracteres palinológicos. Algumas espécies de *Vitis* apresentam uma característica marcante em relação as outras espécies conseguindo separá-las. Pode-se observar que em todos os gêneros estudados a ornamentação apresentou uma grande diversidade. Os resultados mostraram que a morfologia polínica não auxilia na organização taxonômica da família. Os representantes dos oito gêneros estudados distribuídos nas cinco tribos podem ser separados em grupos polínicos de acordo com a morfologia apresentada, indicando que Vitaceae é euripolínca.

Palavras-chave: palinologia, análise multivariada, Vitaceae.

ABSTRACT

A PALINOLOGICAL APPROACH TO THE VITACEAE FAMILY

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Abstract Doctoral Thesis submitted to the Postgraduate Program in Biological Sciences (Botany), National Museum, Federal University of Rio de Janeiro - UFRJ, as part of the requirements for obtaining the title of Doctor of Biological Sciences (Botany).

The Vitaceae family has ca. 900 species and 16 genera and is distributed in temperate, subtropical and tropical regions. Currently the family has five recognized tribes: Ampelopsideae, Cisseae, Cayratieae, Parthenocisseae, and Viteae. The species are woody vines or vines, and have tendrils, often opposite the leaf. This study aimed to describe the pollen characteristics of representatives of the tribes of Vitaceae, indicate useful characters to help delimit genera and species, thus seeking to provide data for the family's taxonomy, expanding palynological knowledge and contributing to future palynotaxonomic and evolutionary studies. The material studied was obtained from exsicates from herbaria: BHCB, JCT, MBM, NY, PACA, RB and US. The pollen grains were acetolyzed, measured, described and illustrated under light microscopy, the descriptions based on scanning electron microscopy were performed with non-acetolyzed pollen grains. For the multivariate analysis, the Fitopac and Pc-Ord programs were used and the character mapping was performed using the Mesquite program. Pollen characteristics such as shape, size, opening and pattern of ornamentation of the exine and the evolutionary history of palynological characters were studied. The results showed that pollen morphology does not help in the taxonomic organization of the family. Some species of Vitis have a distinctive characteristic in relation to other species, managing to separate them. It can be observed that in all studied genera ornamentation showed great diversity. The representatives of the eight genera studied distributed in the five tribes can be separated into pollen groups according to the presented morphology, indicating that Vitaceae is eurypalinus.

Keywords: palynology, multivariate analysis, Vitaceae.

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ESTRUTURA DE TESE

O presente estudo teve como principal objetivo descrever os caracteres palinológicos das espécies da família Vitaceae. Estabelecendo relações da morfologia do pólen entre os gêneros e as tribos. Contribuindo, assim, com dados que possam auxiliar no estudo sistemático da família. A tese foi construída da seguinte forma: Introdução geral com três capítulos, seguido das considerações finais. Apresenta-se abaixo os capítulos que constituem a tese:

Capítulo 1 – “A palynological perspective on the tribe Viteae of the grape family (Vitaceae)”: este capítulo contribui para o conhecimento palinológico de algumas espécies não descritas para a tribo Viteae e fornece novas informações palinotaxonômicas sobre a família Vitaceae.

Capítulo 2 – “Contributions to the palynological study of selected species of *Cissus* L. (Vitaceae): a new appreciation of ornamentation”: este capítulo descreve a palinologia de espécies de *Cissus* e contribui com maiores informações sobre a palinotaxonomia do gênero.

Capítulo 3 – “Pollen analysis of representatives of the tribes Ampelopsideae, Cayratieae, and Parthenocisseae and evolutionary history of Vitaceae genera”: este capítulo amplia o conhecimento palinológico de representantes das tribos de Vitaceae e aborda estudo evolutivo.

INTRODUÇÃO GERAL

A família Vitaceae compreende 16 gêneros e cerca de 950 espécies distribuídas principalmente nas regiões pantropicais e apenas *Vitis* L., *Parthenocissus* Planch. e *Ampelopsis* Michx. ocorrem nas regiões temperadas no Hemisfério Norte (Lombardi 2000, Liu 2016, Lu et al. 2017, Soejima & Wen 2006, Wen 2007, Wen et al. 2007, 2013, 2014, 2018). A maioria dos taxons é encontrada em florestas, embora algumas espécies de *Cissus* L. e *Chyphostemma* (Planch.) Alston são registradas em savanas. *Ampelocissus* Planch., *Nothocissus* (Miq.) Latiff, *Cissus* e *Pterisanthes* Blume preferem planícies de florestas tropicais. *Parthenocissus*, *Ampelopsis* e *Vitis* são encontrados em regiões de montanhas de zonas temperadas, com algumas espécies em florestas montanas em média altitude de regiões subtropicais a tropicais. Os representates de *Tetrastigma* (Miq.) Planchon são bem desenvolvidos em florestas de planície, mas muitas espécies são de montanha e algumas ocupam habitats rochosos (Wen 2007).

A família é composta por plantas hermafroditas, polígamas monoicas a polígamas dioicas, trepadeiras lenhosas ou videiras; gavinhas simples, bífidas, ou 2-3 ou 4-12-ramificadas (em *Parthenocissus*), frequentemente opostas às folhas. Folhas simples, compostas, lobadas, compostas digitadas com 1-3 pinas, alternas, com glândulas peroladas. Inflorescências em panículas, corimbos, ou raramente espigas, frequentemente opostas às folhas, pseudo-terminais ou axilares (em *Cayratia* Juss. e *Tetrastigma*). Flores pequenas; cálice de 4-5(-7) sépalas; pétala valvar, 4-5(-7), anteras tetraesporangiadas, introrsas, deiscência longitudinal, grãos de pólen tricolporados; disco floral intraestaminal; ovário súpero, com um estilete simples, estigma discoide ou capitado, raramente 4 lóbulos (*Tetrastigma*); 2 óvulos por lóculo, fruto baga, 1-4 sementes (Lombardi 2000, Wen 2007).

O disco floral (grau de desenvolvimento do disco nectarífero intraestaminal e de sua adnação à parede do ovário) e a posição das inflorescências são características utilizadas na segregação dos gêneros em Vitaceae. Esses caracteres são informativos para a taxonomia da família segundo Lombardi (2000) e Wen et al. (2018).

A família tem grande importância econômica pela produção de uvas (frutas frescas), vinhos, sucos e uvas passas. Especialmente *Vitis vinifera* L., assim como outras espécies e híbridos de *Vitis*. Algumas espécies também são utilizadas como ornamentais (*Ampelopsis*, *Cissus*, *Parthenocissus*, *Rhoicissus* Planchon e *Tetrastigma*). *Cissus* é utilizado para tingimento de tecidos, principalmente por indígenas. Os nativos americanos utilizam folhas de algumas espécies de *Ampelopsis* e *Vitis* para uso medicinal. Algumas espécies também são importantes ecologicamente como cipós e videiras em florestas tropicais e temperadas, podendo dominar savanas tropicais e paisagens onde substratos calcários são predominantes (Lombardi 2000, Wen 2007, Lu et al. 2017).

Vitaceae (segundo o APG III 2009), pertence à ordem Vitales Juss. ex Bercht. & J. Presl, aparece separada dentro do clado Rosides e tem como grupo irmão Fabides e Malvides. No APG IV (2016) Vitales permanece isolado no clado Rosides, tendo como única família, Vitaceae, com 15 gêneros distribuídos entre duas subfamílias: Leeoideae (1) e Vitoideae (14) (Steve 2013). Alguns pesquisadores divergem dessa colocação, pois reconhecem Leeaceae como uma família irmã de Vitaceae classificando as duas famílias na ordem Vitales (Ma et al. 2021). Vitales aparece como grupo irmão de Saxifragales, Fabides e Malvides (Zhang et al. 2016).

De acordo com os estudos filogenéticos para família utilizando marcadores moleculares e dados morfológicos, vêm estabelecendo grupos irmãos dentro da família. Como observado nos trabalhos de Rosseto et al. (2001), Soejima & Wen (2006), Wen et al. (2007) foi registrado para a família a formação de grandes linhagens: *Cayratia-Cyphostemma-Tetrastigma*, Core *Cissus*, *Parthenocissus-Yua*, *Ampelocissus-Vitis*, *Ampelopsis s.l.* Recentemente essas análises foram se confirmando e a família foi dividida em cinco grandes linhagens: *Ampelocissus-Rhoicissus*, *Cissus*, *Cayratia-Cyphostemma-Terastigma*, *Parthenocissus-Yua* e *Vitis-Ampleocissus* (Fig. 1 – Ren et al. 2011, Zhang et al. 2015, Lu et al. 2017).

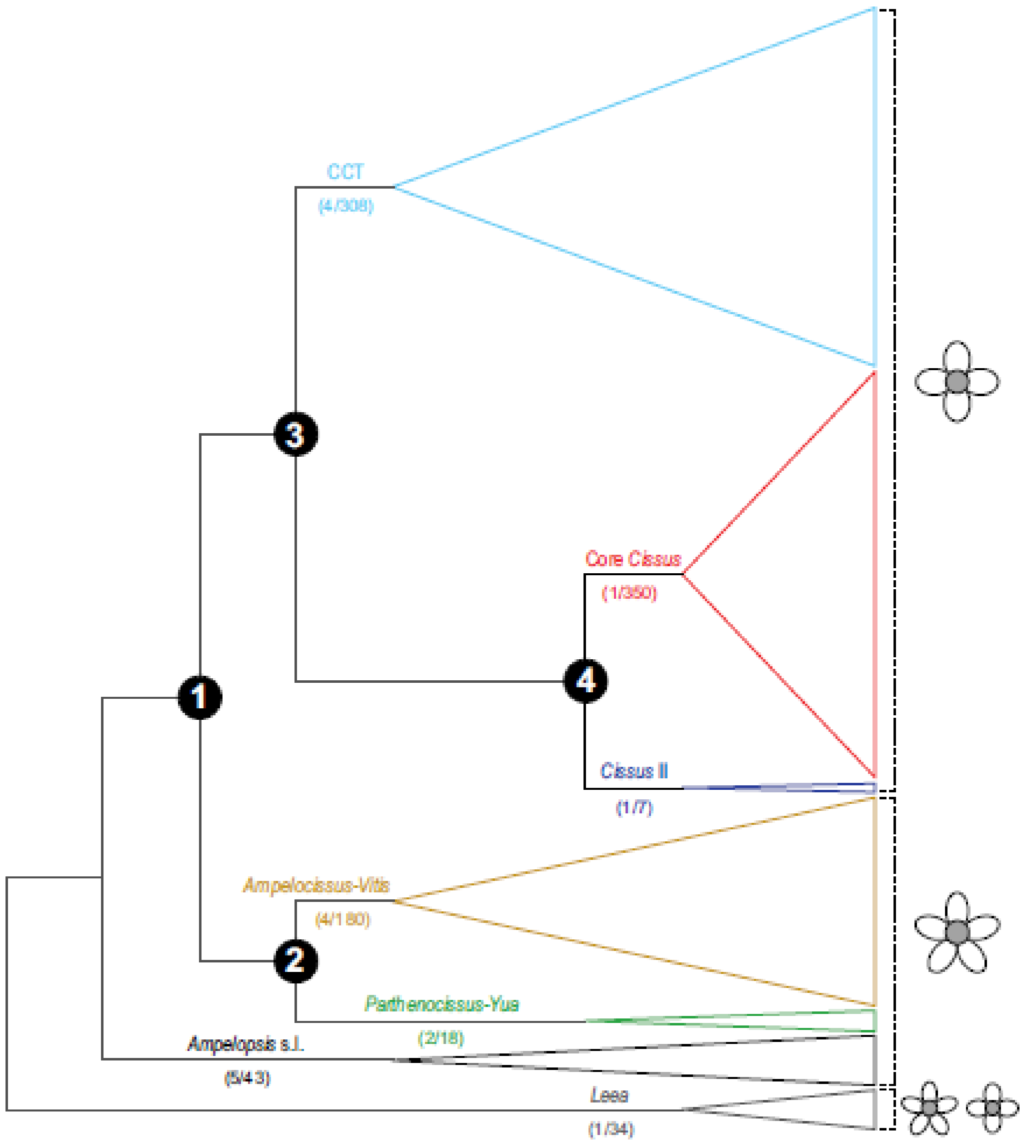
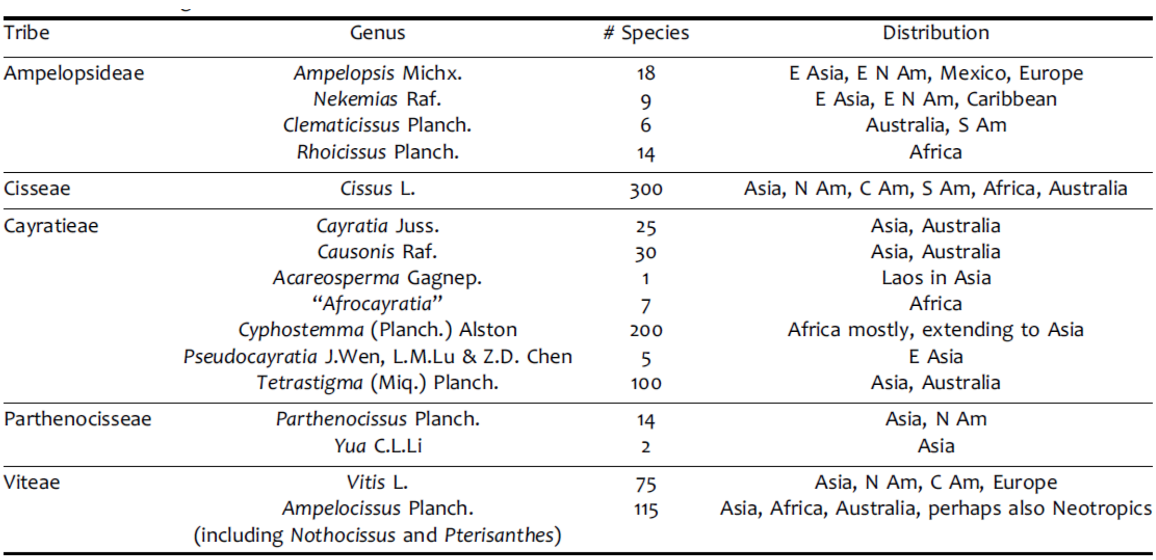


Fig. 1 - Suporte principal filogenético de Vitaceae com nomes das linhagens, Lu et al. (2018).

A partir das análises filogenéticas e morfológicas os 16 gêneros receberam uma nova classificação e foram distribuídos em cinco tribos: tribo Ampelopsideae J. Wen & Z.L. Nie; tribo Cisseae Rchb.; tribo Cayratieae J.Wen & L.M. Lu; tribo Parthenocisseae J. Wen & Z.D. Chen e tribo Viteae Dumort (Fig. 2 - Wen et al. 2018). Em um estudo recente Ma et al. (2021) analisando os dados nucleares e de plastídeos confirmam que a árvore produzida sustenta a nova classificação.



Tribe	Genus	# Species	Distribution
Ampelopsideae	*Ampelopsis* Michx.	18	E Asia, E N Am, Mexico, Europe
	Nekemias Raf.	9	E Asia, E N Am, Caribbean
	Clematicissus Planch.	6	Australia, S Am
	Rhoicissus Planch.	14	Africa
Cisseae	*Cissus* L.	300	Asia, N Am, C Am, S Am, Africa, Australia
Cayratieae	*Cayratia* Juss.	25	Asia, Australia
	Causonis Raf.	30	Asia, Australia
	Acareosperma Gagnep.	1	Laos in Asia
	"*Afrocayratia*"	7	Africa
	Cyphostemma (Planch.) Alston	200	Africa mostly, extending to Asia
	Pseudocayratia J.Wen, L.M.Lu & Z.D. Chen	5	E Asia
	Tetrastigma (Miq.) Planch.	100	Asia, Australia
Parthenocisseae	*Parthenocissus* Planch.	14	Asia, N Am
	Yua C.L.Li	2	Asia
Viteae	*Vitis* L.	75	Asia, N Am, C Am, Europe
	Ampelocissus Planch. (including *Nothocissus* and *Pterisanthes*)	115	Asia, Africa, Australia, perhaps also Neotropics

Fig. 2 – Tribos e gêneros de Vitaceae. Wen et al. (2018).

Os grãos de pólen possuem variações em sua estrutura, com isso o seu estudo pode ser utilizado em diversas áreas da ciência. Uma das suas aplicações é na taxonomia, pois os atributos polínicos apresentam valores morfológicos que permitem caracterizar família, gêneros e espécies. Esses caracteres contribuem com os estudos cladísticos, estabelecendo ligações entre as espécies. O estudo palinológico contribui para a análise do ponto de vista evolutivo e nos registros fósseis (Melhem et al. 2003).

Em Vitaceae são poucos os trabalhos palinológicos registrados e neles os grãos de pólen são descritos como tricolporados na maioria dos gêneros, a forma varia de oblata a prolata e a sexina é reticulada (Wen 2007).

Devido à falta de um maior conhecimento sobre a palinologia da família, esse estudo teve o propósito de analisar os grãos de pólen de espécies de Vitaceae, descrevendo a morfologia polínica das espécies estudadas, buscando uma correspondência com as tribos e/ou clados formados a partir das análises filogenéticas existentes, e uma das primeiras tentativas de mostrar a evolução dos grãos de pólen na família.

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Capítulo I

A palynological perspective on the tribe Viteae of the grape family (Vitaceae)

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Resumo

A tribo Viteae compreende cerca de 200 espécies e é representada pelos gêneros *Vitis* e *Ampelocissus*. *Vitis* é o gênero mais estudado dentro da família da uva (Vitaceae) devido à importância econômica global de seus frutos e produtos derivados. Este estudo examinou a palinologia das espécies de Ampelocissus e Vitis pertencentes à tribo Viteae para identificar caracteres polínicos que possam contribuir para a caracterização taxonômica e morfológica de Ampelocissus. Espécimes de pólen foram obtidos de herbários, tratados pelo método de acetólise, medidos, fotografados e descritos em microscopia óptica e eletrônica de varredura. Nas espécies analisadas, os grãos de pólen eram isopolares ou heteropolar, de tamanho médio, tricolporado, oblato esferoidal a subprolado. Os padrões de ornamentação diferiram entre as espécies, com exemplos de sexina bireticulada, microrreticulada, perfurada, reticulada e rugulada. Esses caracteres foram usados para o desenvolvimento de uma chave de pólen para os gêneros. A análise de componentes principais revelou que os caracteres quantitativos do pólen eram os mais importantes para a discriminação de grupo. Este estudo contribui para o conhecimento palinológico de algumas espécies não descritas e fornece novas informações palinotaxonômicas sobre a família Vitaceae.

Palavras-chave: caracterização morfológica, ornamentação, pólen, palinotaxonomia

Abstract

The tribe Viteae comprises about 200 species and is represented by the genera *Vitis* and *Ampelocissus*. *Vitis* is the most studied genus within the grape family (Vitaceae) because of the global economic importance of its fruits and derived products. This study examined the palynology of *Ampelocissus* and *Vitis* species belonging to the tribe Viteae to identify pollen characters that may contribute to the taxonomic and morphological characterization of *Ampelocissus*. Pollen specimens were obtained from herbaria, treated by the acetolysis method, measured, photographed, and described using light and scanning electron microscopy. In the analyzed species, pollen grains were isopolar or heteropolar, medium-sized, tricolporate, oblate spheroidal to subprolate. Ornamentation patterns differed among species, with examples of bireticulate, microreticulate, perforate, reticulate, and rugulate sexine. These characters were used for the development of a pollen key to the genera. Principal component analysis revealed that quantitative pollen characters were the most important for group discrimination. This study contributes to the palynological knowledge of some undescribed species and provides novel palynotaxonomic information on the family Vitaceae.

Keywords: *morphological characterization*, *ornamentation*, *pollen*, *palynotaxonomy*

The tribe Viteae Dumort is represented by the genera *Vitis* L. and *Ampelocissus* Planch., according to the taxonomic organization of Wen et al. (2018). Previous phylogenetic studies of the family Vitaceae identified that *Ampelocissus* and *Vitis* formed strongly supported clades (Liu et al. 2016). The tribe is characterized by thyrse inflorescences, resembling panicles with a spiral pattern (Wen et al. 2018). Genera occur in Asia, Africa, North and South Americas, Australia, and Europe (Liu et al. 2016; Lu et al. 2017; Wen et al. 2018). Only one *Vitis* species (*V. tiliifolia* Humb. & Bonpl. ex Schult.) occurs in South America (Lombardi 2007), and four *Ampelocissus* species can be found in Central America, Mexico, and the Caribbean (Lombardi 2000).

Vitis comprises ca. 75 species (Wen et al. 2018) and is the most studied genus of the family Vitaceae because of its great economic value (Zecca et al. 2012). *Vitis vinifera* L. is widely studied, as its highly valued fruits can be consumed fresh or used for juice extraction and wine production. In nature, wild grapes (heliophile lianas) usually grow along river banks. Currently, the few remaining communities of wild vines are fragmented and threatened with extinction (Terral et al. 2009).

According to the latest taxonomic classification, *Ampelocissus* comprises ca. 115 species, encompassing *Pterisanthes* Blume and *Nothocissus* (Miq.) Latiff. These taxa are nested within *Ampelocissus* in the phylogenetic tree. *Vitis* is related to *Ampelocissus* species occurring in the Neotropical region; the latter species may be transferred to *Vitis* in the future (Wen et al. 2018; Ma et al. 2021).

Palynologically, representatives of the tribe Viteae have been little studied. The literature generally focuses on the germination and viability of cultivated *Vitis* species (Jovanovic-Cvetkovic et al. 2016; Pereira et al. 2018). Previous studies have described the pollen morphology of the genus, such as Huang (1967), Chavez et al. (1996), Wang et al.

(2000), Punt et al. (2003), and Perveen and Qaiser (2008). However, to date, no study has focused on the palynology of *Ampelocissus*.

In recent years, palynological information has been used for taxonomic description of different families, exemplifying the importance of such investigations for distinguishing plant groups (Mendonça et al. 2010; Mezzonato-Pires et al. 2020). This characterization is possible because pollen grains vary greatly in morphology (e.g. Erdtman 1986). Morphological characters also contribute to cladistic studies aiming to establish links between species (Melhem et al. 2003). This study was carried out using material from representatives of *Ampelocissus* and *Vitis* that were fertile and available in the consulted herbaria, without considering their geographic distributions. The objectives were to characterize pollen attributes, investigate whether such characters contribute to the taxonomy of the genera, and describe, for the first time, the pollen morphology of *Ampelocissus*.

Material and methods

Pollen material

Pollen material was obtained from selected herbaria. Sample selection was based on the availability of material and flower fertility. It was not possible to consider the geographic distribution of samples. Twenty-six species were examined: nine of *Ampelocissus* and 16 of *Vitis*. The material was obtained from the following herbaria (acronyms according to Thiers 2019): BHCB, JCT, MBM, NY, PACA, and US.

Light microscopy

For light microscopy, anthers were acetolyzed using the method of Erdtman (1952) with the modifications proposed by Melhem et al. (2003). Samples were analysed within seven days of preparation (Salgado-Labouriau 1973). Measurements were performed under a light microscope at 40× and 100× magnification.

Scanning electron microscopy (SEM)

Non-acetolyzed pollen grains were mounted on stubs with carbon tape (Cartaxo-Pinto et al. 2017) and examined using a JEOL JSM 6390LV system at the Laboratory of Invertebrate Electron Microscopy, National Museum, Federal University of Rio de Janeiro, Brazil, and a FEI Quanta 450 field-emission SEM system at the Nanotechnology Characterization Center (CENANO), National Institute of Technology, Brazil.

Measurements of pollen grains

Pollen grains were measured in polar and equatorial views. A total of 25 measurements were randomly taken of the polar and equatorial diameters in equatorial view. Ten measurements were performed of the equatorial diameter in polar view, apocolpium region, aperture length and width, and thickness of exine layers. Data were analysed statistically, and results are presented as arithmetic mean, standard deviation of sample, standard deviation of mean, and 95% confidence intervals in tables. Microscope slides were deposited at the pollen collection of the Álvaro Xavier Moreira Laboratory of Palynology, Department of Botany, Museu Nacional, Universidade Federal do Rio de Janeiro, Brazil.

Terminology

Pollen size, shape, and sexine ornamentation were described following the terminology of Punt et al. (2007) and Hesse et al. (2009). The description of polar area and aperture size follows the classification developed by Faegri and Iversen (1966), which is based on polar area index.

Multivariate analysis

Character matrices were constructed from quantitative and qualitative pollen data and subjected to principal component analysis (PCA) using Fitopac software version 2.1 (Shepherd 2010). PCA results were plotted to identify differences in pollen characters among species. For convenience, species are abbreviated to the first letter of the generic epithet followed by the first letters of the specific epithet. Vector loadings and total cumulative variances are presented in tables and character matrices (Marinho 2017).

Results

General description of the tribe Viteae

Pollen grains were medium-sized, tricolporate, heteropolar (*Vitis*) or isopolar (*Ampelocissus* and *Vitis*), oblate spheroidal, prolate, prolate spheroidal, or subprolate. Surface ornamentation varied among species, with examples of bireticulate, microreticulate, perforate, reticulate, and rugulate sexine. These characters were used to construct a pollen key to the two genera.

Pollen key to Ampelocissus and Vitis *species*

1. Heteropolar pollen
    2. One pole with traces of muri and isolated elements and the other pole without these characteristics...*V. arizonica*
    2. One pole with muri higher, lumina wide with granules and apparent columella, elevating the pole in relation to the mesocolpium, and the other pole without these characteristics...*V. piasezkii*
1. Isopolar pollen grains
    3. Prolate or prolate spheroidal shape
        4. Pollen grains prolate
            5. Sexine bireticulate in the mesocolpium, perforate and reticulate in the apocolpium, muri narrow with lumina large and ornamented.............*V. riparia*
            5. Sexine rugulate in the mesocolpium with muri perforate, reticulate in the apocolpium with traces of muri and isolated elements......................*V. tiliifolia*
        4. Pollen grains prolate spheroidal
            6. Sexine reticulate
                7. Muri without ornamentation
                    8. 95% Confidence intervals of the polar diameter > 32.0 µm.........*A. indica*

8. 95% Confidence intervals of the polar diameter < 27.0 µm...*A. barbata*, *A. martini*, *V. baileyana*

7. Muri with ornamentation

9. Muri with microechinae..*A. frutescens*

9. Muri with striae..*A. tomentosa*

6. Sexine rugulate throughout the surface or rugulate in the mesocolpium and bireticulate in the apocolpium

10. Sexine rugulate throughout the surface.......................................*A. elegans*

10. Sexine rugulate in the mesocolpium and bireticulate in the apocolpium..*V. bourgaeana*

3. Pollen grains oblate spheroidal or subprolate

11. Pollen grains oblate spheroidal

12. Sexine microreticulate...*A. acapulcensis*

12. Sexine reticulate or rugulate

13. Sexine reticulate..*A. artemisiifolia*

13. Sexine rugulate...*A. ochracea*

11. Pollen grains subprolate

14. Sexine rugulate or bireticulate

15. Sexine rugulate in the mesocolpium and reticulate in the apocolpium..*V. cinerea*

15. Sexine bireticulate in the mesocolpium and reticulate in the apocolpium..*V. vinifera*

14. Sexine reticulate or microreticulate

16. Sexine reticulate

17. Sexine reticulate throughout the surface.........*V. flexuosa*, *V. vulpina*

17. Sexine microreticulate in the mesocolpium
    18. Apocolpium with traces of muri and isolated elements..*V. labrusca*
    18. Apocolpium without traces of muri and isolated elements...*V. monticola*
16. Sexine microreticulate
    19. Sexine microreticulate throughout the surface...........*V. munsoniana*
    19. Sexine reticulate or bireticulate in the apocolpium
        20. Sexine reticulate in the apocolpium and microreticulate in the mesocolpium..*V. acerifolia*
        20. Sexine bireticulate in the apocolpium
            21. Bireticulate in the apocolpium extending to the mesocolpium, mesocolpium microreticulate, muri straight and wide..*V. aestivalis*
            21. Bireticulate only in the apocolpium, with outer muri sinuate and narrow...*V. adstricta*

Description of Ampelocissus and Vitis pollen

Genus Ampelocissus - the following species were analysed: *A. acapulcensis* (Kunth) Planch. (Figure 1A–C), *A. artemisiifolia* Planch. (Figure 1D–F), *A. barbata* (Wall.) Planch. (Figure 1G–I), *A. elegans* Gagnep. (Figure 1J–L), *A. frutescens* Jackes (Figure 2A–C), *A. indica* Planch. (Figure 2D–F), *A. martini* Planch. (Figure 2G, H), *A. ochracea* Merr. (Figure 2I, J), and *A. tomentosa* (Roth) Planch. (Figure 2K, L).

Pollen size, polarity, amb, shape, and polar area - pollen grains medium in size and isopolar; amb subtriangular; shape oblate spheroidal in *A. acapulcensis*, *A. artemisiifolia*, and *A.*

ochracea and prolate spheroidal in the other species. Polar area very small in most species, small only in *A. elegans* (Table I).

Aperture - pollen grains 3-colporate, with colpi long and narrow in *A. elegans* and very long and narrow in the other species (Table III). Colpus length ranged from ca. 33.0 µm in *A. frutescens* to ca. 18.1 µm in *A. elegans*. Colpus width was greatest in *A. artemisiifolia* (ca. 3.3 µm) and smallest in *A. elegans* (ca. 1.0 µm). It was possible to identify an ornamented membrane (granules) in some colpi (Figures 1C, F, 2F). Endoaperture slightly lolongate in *A. indica* and *A. tomentosa*, circular in *A. elegans* and *A. frutescens*, lalongate in *A. ochracea*, and slightly lalongate in the other species (Table III).

Exine - three types of ornamentation were recorded (Table IV): (a) rugulate-perforate throughout the surface in *A. elegans* (Figure 1K, L) and *A. ochracea* (Figure 2I, J); (b) microreticulate in *A. acapulcensis* (Figure 1B, C); and (c) reticulate in *A. artemisiifolia* (Figure 1D, E, F), *A. barbata* (Figure 1G, I), *A. frutescens* (Figure 2C), *A. indica* (Figure 2E, F), *A. martini* (Figure 2H), and *A. tomentosa* (Figure 2K, L). In reticulate species, muri may be narrow, straight, perforate, without luminal ornamentation, as observed in *A. artemisiifolia*, *A. indica*, and *A. martini* (Figures 1D, 2E, F, H); wide, straight, perforate, and non-ornamented, as found in *A. barbata* (Figure 1G, I); wide, straight, with microechinae, as seen in *A. frutescens* (Figure 2C); or wide, straight, striate, with lumina granulate, as found in *A. tomentosa* (Figure 2L). Sexine simplicolumellate (Figure 2K). Exine thickness ranging from 2.0 to 3.2 µm. The sexine is thicker than the nexine in *A. artemisiifolia* and *A. ochracea*, less thick than the nexine in *A. acapulcensis*, *A. frutescens*, *A. indica*, and *A. martini*, and as thick as the nexine in *A. barbata* and *A. elegans* (Table III).

Pollen key to Ampelocissus species

1. Pollen grains with sexine microreticulate..*A. acapulcensis*

1. Pollen grains with sexine rugulate-perforate or reticulate

    2. Pollen grains with sexine rugulate-perforate

        3. Polar area small, endoaperture circular...*A. elegans*

        3. Polar area very small, endoaperture lalongate..*A. ochracea*

    2. Pollen grains with sexine reticulate

        4. 95% Confidence intervals of the polar diameter ≤ 27.0 µm..*A. barbata*, *A. martini*

        4. 95% Confidence intervals of the polar diameter > 30.0 µm

            5. 95% Confidence intervals of the polar diameter = 39.0–40.2 µm, muri of the reticulum with supratectal microechinae..*A. frutescens*

            5. 95% Confidence intervals of the polar diameter < 38.0 µm

                6. Muri of the reticulum with striae and lumina with granules..........*A. tomentosa*

                6. Muri of the reticulum without these characteristics*A. artemisiifolia*, *A. indica*

Genus Vitis - the following species were analysed: *Vitis acerifolia* Raf. (Figure 3A, B), *V. adstricta* Hance (Figure 3C, D), *V. aestivalis* Michx. (Figure 3E, F), *V. arizonica* Engelm. (Figure 3G–I), *V. baileyana* Munson (Figure 3J), *V. bourgaeana* Planch. (Figure 3K, L), *V. cinerea* (Engelm.) Millardet (Figure 4A, B), *V. flexuosa* Thunb. (Figure 4C, D), *V. labrusca* L. (Figure 4E–H), *V. monticola* Buckley (Figure 4I, J), *V. munsoniana* J.H. Simpson ex Planch. (Figure 4K, L), *V. piasezkii* Maxim. (Figure 5A–C), *V. riparia* Michx. (Figure 5D–F), *V. tiliifolia* (Figure 5G, H), *V. vinifera* (Figure 5I–K), and *V. vulpina* L. (Figure 5L).

Pollen size, polarity, amb, shape, and polar area. — Pollen grains medium-sized, heteropolar in *V. arizonica* and *V. piasezkii* and isopolar in the other species. Heteropolarity was observed in *V. arizonica* (Figure 3G, H) and *V. piasezkii* (Figure 5A, B), characterized by differences in ornamentation between poles. Amb subcircular to subtriangular in isopolar and heteropolar species. Shape prolate in *V. riparia* and *V. tiliifolia*, prolate spheroidal in *V. baileyana*, *V. bourgaeana*, and *V. munsoniana*, and subprolate in the other species (Table I). Polar area small in most species and very small in *V. acerifolia*, *V. baileyana*, *V. bourgaeana*, *V. labrusca*, *V. monticola*, *V. munsoniana*, and *V. vulpina* (Table II).

Aperture - pollen grains 3-colporate, with colpi very long in *V. acerifolia*, *V. baileyana*, *V. bourgaeana*, *V. labrusca*, *V. monticola*, *V. munsoniana*, and *V. vulpina* and long in the other species (Table II). Colpus length ranged from ca. 27.2 µm in *V. arizonica* to ca. 16.8 µm in *V. munsoniana*. For colpus width, the greatest value was observed in *V. labrusca* (ca. 2.1 µm) and the smallest in *V. cinerea*, *V. flexuosa*, *V. riparia*, and *V. vinifera* (ca. 1.0 µm). Colpi narrow and slightly bifurcate at one end in *V. adstricta* (Figure 3C), *V. aestivalis* (Figure 3F), and *V. vinifera* (Figure 5J); margins ornamented and narrow, measuring about 1 µm. It was also possible to identify the presence of an ornate membrane (granules) in most species (Figures 3I, K, 4A, 5G). A costa was observed in *V. aestivalis* (Figure 3E). Endoaperture slightly lalongate in most species and circular in *V. arizonica*, *V. bourgaeana*, *V. piasezkii*, and *V. vulpina* (Table III).

Exine - exine 2 µm thick in all species, excepting *V. labrusca* and *V. munsoniana* (Table III). Sexine ornamentation varies across species. Ornamentation types are presented below (Table IV).

(1) Microreticulate in the mesocolpium, reticulate with lumina ornamented in the apocolpium: *V. acerifolia* (Figure 3A, B).

(2) Microreticulate in the central region of the mesocolpium, bireticulate only in the apocolpium and perforate, with outer muri sinuate and narrow: *V. adstricta* (Figure 3C, D).

(3) Microreticulate in the mesocolpium, bireticulate in the apocolpium extending to the mesocolpium, with perforations, muri straight and wide: *V. aestivalis* (Figure 3F).

(4) Microreticulate in the mesocolpium, reticulate in poles, with muri higher in one of the poles, lumina wide with granules and apparent columella, elevating one of the poles in relation to the mesocolpium: *V. piasezkii* (Figure 5A–C).

(5) Rugulate in the mesocolpium, reticulate with traces of muri and isolated elements in one pole and solely reticulate in the other pole: *V. arizonica* (Figure 3G–I).

(6) Rugulate in the mesocolpium, reticulate in the apocolpium, lumina with granules and apparent columella: *V. cinerea* (Figure 4A, B).

(7) Microreticulate and perforate on the entire surface, lumina without ornamentation: *V. munsoniana* (Figure 4K, L).

(8) Rugulate in the mesocolpium with muri perforate, reticulate in the apocolpium with traces of muri and isolated elements: *V. tiliifolia* (Figure 5G, H).

(9) Bireticulate in the apocolpium, rugulate and perforate in the mesocolpium: *V. bourgaeana* (Figure 3K, L).

(10) Bireticulate in the mesocolpium, perforate and reticulate in the apocolpium, muri narrow with lumina large and ornamented: *V. riparia* (Figure 5D–F).

(11) Bireticulate in the mesocolpium, perforate and reticulate in the apocolpium, lumina ornamented: *V. vinifera* (Figure 5I–K).

(12) Reticulate throughout the surface: *V. baileyana* (Figure 3J), *V. flexuosa* (Figure 4C), and *V. vulpina* (Figure 5L).

(13) Reticulate in the apocolpium, with traces of muri and isolated elements, lumina with granules and apparent columella, microreticulate in the mesocolpium: *V. labrusca* (Figure 4G, H).

(14) Reticulate and perforate in the apocolpium, muri wide and straight, lumina with granules, microreticulate in the mesocolpium: *V. monticola* (Figure 4I, J).

Pollen key to Vitis species

1. Heteropolar pollen grains

    2. One pole with traces of muri and isolated elements and the other pole without these characteristics..*V. arizonica*

    2. One pole with muri higher, lumina wide with granules and apparent columella, elevating the pole in relation to the mesocolpium, and the other pole without these characteristics..*V. piasezkii*

1. Isopolar pollen grains

    3. Prolate or prolate spheroidal shape

        4. Pollen grains prolate

            5. Sexine bireticulate in the mesocolpium, perforate and reticulate in the apocolpium, muri narrow with lumina large and ornamented................*V. riparia*

            5. Sexine rugulate in the mesocolpium with muri perforate, reticulate in the apocolpium with traces of muri and isolated elements..........................*V. tiliifolia*

        4. Pollen grains prolate spheroidal

            6. Sexine reticulate throughout the surface..*V. baileyana*

            6. Sexine rugulate in the mesocolpium and bireticulate in the apocolpium................ ....*V. bourgaeana*

    3. Pollen grains subprolate

        7. Sexine rugulate or bireticulate

            8. Sexine rugulate in the mesocolpium and reticulate in the apocolpium.................. ....*V. cinerea*

8. Sexine bireticulate in the mesocolpium and reticulate in the apocolpium...*V. vinifera*

7. Sexine reticulate or microreticulate

9. Sexine reticulate

10. Sexine reticulate throughout the surface.......................*V. flexuosa, V. vulpina*

10. Sexine microreticulate in the mesocolpium

11. Apocolpium with traces of muri and isolated elements............*V. labrusca*

11. Apocolpium without traces of muri and isolated elements...*V. monticola*

9. Sexine microreticulate

12. Sexine microreticulate throughout the surface..........................*V. munsoniana*

12. Sexine reticulate or bireticulate in the apocolpium

13. Sexine reticulate in the apocolpium and microreticulate in the mesocolpium...*V. acerifolia*

13. Sexine bireticulate in the apocolpium

14. Bireticulate in the apocolpium extending to the mesocolpium, mesocolpium microreticulate, muri straight and wide.....*V. aestivalis*

14. Bireticulate only in the apocolpium, with outer muri sinuate and narrow...*V. adstricta*

TABLE I - Measurements (in µm) of Viteae pollen grains in equatorial view ($n = 25$). $\bar{x}$- arithmetic mean; s- standard deviation; $s\bar{x}$- standard deviation of the mean; CI- confidence interval.

Species	Polar Diameter (PD)			Equatorial Diameter (ED)			
	Range	$\bar{x} \pm s\bar{x}$	C.I.95%	Range	$\bar{x} \pm s\bar{x}$	C.I.95%	P/E
Ampelocissus acapulcensis	32.5-37.5	34.8±0.31	34.1-35.4	33.7-37.5	36.1±0.27	35.6-36.7	0.96
A. artemisiifolia	32.5-37.5	34.5±0.28	34.0-35.1	32.5-35.0	34.6±0.19	34.2-35.0	1.00
A. barbata	25.0-27.5	26.3±0.25	25.8-26.8	22.5-27.5	25.7±0.27	25.1-26.2	1.02
A. elegans	22.5-27.5	24.8±0.28	24.2-25.4	22.5-27.5	23.8±0.29	23.2-24.4	1.04
A. frutescens	37.5-42.5	39.6±0.28	39.0-40.2	30.0-35.0	33.6±0.35	33.0-34.3	1.17
A. indica	30.0-35.0	32.9±0.28	32.3-33.5	30.0-32.5	31.0±0.25	31.5-31.5	1.06
A. martini	25.0-27.5	26.4±0.25	25.9-26.9	22.5-27.5	24.5±0.29	23.9-25.1	1.08
A. ochracea	22.5-27.5	24.9±0.23	24.4-25.4	22.5-27.5	24.9±0.34	24.2-25.6	1.00
A. tomentosa	27.5-35.0	31.0±0.32	30.3-31.7	25.0-32.5	30.2±0.35	29.5-31.0	1.02
Vitis acerifolia	22.5-27.5	23.8±0.29	23.2-24.4	22.5-25.0	23.7±0.25	23.2-24.2	1.00
V. adstricta	22.5-27.5	25.8±0.28	25.2-26.4	20.0-22.5	20.6±0.22	20.1-21.0	1.25
V. aestivalis	32.5-35.0	33.2±0.23	32.7-33.7	22.5-27.5	25.0±0.27	24.5-25.6	1.32
V. arizonica	27.5-32.5	28.8±0.29	28.2-29.4	20.0-25.0	22.2±0.26	21.6-22.7	1.29
V. baileyana	25.0-30.0	25.9±0.28	25.3-26.5	22.5-25.0	24.3±0.23	23.8-24.8	1.06
V. bourgeana	22.5-27.5	25.4±0.24	24.9-25.9	22.5-25.0	24.4±0.22	24.0-24.8	1.04

V. cinerea	25.0-27.5	25.9±0.24	25.4-26.4	20.0-22.5	20.4±0.19	20.1-20.8	1.27
V. flexuosa	25.0-27.5	25.8±0.24	25.3-26.3	20.0-22.5	21.4±0.25	20.9-21.9	1.20
V. labrusca	27.5-32.5	30.2±0.32	29.5-30.8	20.0-27.5	23.5-±0.35	22.8-24.2	1.28
V. monticola	25.0-30.0	27.8±0.30	27.2-28.5	22.5-25.0	22.9±0.19	22.5-23.3	1.22
V. munsoniana	20.0-22.5	21.0±0.25	20.5-21.5	20.0-22.5	21.4±0.25	20.9-21.9	0.98
V. piasezkii	25.0-27.5	26.4±0.25	25.9-26.9	20.0-22.5	21.2±0.25	20.7-21.7	1.24
V. riparia	32.5-35.0	33.2±0.23	32.7-33.7	22.5-25.0	23.9±0.25	23.4-24.4	1.39
V. tiliifolia	22.5-27.5	25.6±0.36	24.8-26.3	15.0-22.5	17.7±0.30	17.1-18.4	1.44
V. vinifera	27.5-32.5	30.1±0.23	29.6-30.6	22.5-27.5	24.5±0.29	23.9-25.1	1.23
V. vulpina	25.0-30.0	27.5±0.35	26.8-28.2	22.5-25.0	23.2±0.23	22.7-23.7	1.18

Table II. Measurements (in µm) of Viteae pollen grains in polar view (*n* = 10). EDPV- equatorial diameter in polar view; AS- apocolpium side; PAI- polar area index.

Espécies	Equatorial Diameter in Polar View (EDPV)		Side of Apocolpus (SA)		
	Range	$\bar{x}$	Range	$\bar{x}$	PAI
Ampelocissus acapulcensis	32.5-37.5	34.2	5.0-10.0	8.2	0.24
A. artemisiifolia	32.5-35.0	33.5	7.5-10.0	8.2	0.24
A. barbata	25.0-27.5	25.7	5.0-7.5	5.7	0.22
A. elegans	22.5-25.0	23.0	7.5-10.0	8.5	0.37
A. frutescens	30.0-35.0	31.7	5.0-7.5	6.5	0.20
A. indica	30.0-32.5	30.2	5.0-7.5	6.2	0.21
A. martinii	22.5-25.0	23.2	2.5-5.0	4.5	0.19
A. ochracea	25.0-27.5	25.5	5.0-7.5	5.5	0.21
A. tomentosa	30.0-32.5	31.0	5.0-7.5	6.0	0.19
Vitis acerifolia	20.0-25.0	22.7	2.5-5.0	4.6	0.20
V. adstricta	20.0-25.0	22.2	5.0-7.5	5.5	0.25
V. aestivalis	22.5-27.5	24.5	5.0-7.5	7.2	0.29
V. arizonica	20.0-25.0	22.0	5.0-10.0	6.2	0.28
V. baileyana	22.5-25.0	22.7	2.5-5.0	3.6	0.16
V. bourgeana	20.0-25.0	22.2	2.5-3.7	2.7	0.12
V. cinerea	20.0-22.5	20.7	6.2-7.5	7.2	0.35
V. flexuosa	20.0-22.5	21.5	5.0-7.5	6.0	0.28
V. labrusca	22.5-25.0	24.7	2.5-5.0	3.9	0.16
V. monticola	20.0-25.0	23.0	5.0-7.5	5.6	0.24
V. munsoniana	20.0-22.5	20.2	2.5-5.0	4.2	0.21
V. piasezkii	20.0-22.5	22.2	5.0-7.5	6.6	0.30
V. riparia	22.5-25.0	23.5	5.0-7.5	6.7	0.29
V. tiliifolia	17.5-22.5	20.2	5.0-7.5	6.7	0.33
V. vinifera	22.5-25.0	24.2	5.0-7.5	7.0	0.29
V. vulpina	22.5-25.0	23.0	3.7-5.0	4.5	0.20

Table III. Measurements (in µm) of apertures and exine layers of Viteae pollen grains (n = 10).

Species	Colpus		Endoaperture		Exine layers		
	length	width	length	width	exine	nexine	sexine
Ampelocissus acapulcensis	24.7	2.4	4.3	4.4	2.9	1.9	1.0
A. artemisiifolia	28.0	3.3	4.0	4.2	3.2	1.3	1.9
A. barbata	20.5	2.0	3.3	3.5	2.0	1.0	1.0
A. elegans	18.1	1.0	3.0	3.0	2.0	1.0	1.0
A. frutescens	33.0	2.8	4.0	4.0	2.9	1.8	1.1
A. indica	24.8	2.8	3.7	3.6	2.9	1.6	1.3
A. martini	22.2	1.2	2.2	2.4	2.6	1.6	1.0
A. ochracea	19.2	1.4	1.9	2.5	2.1	1.0	1.1
A. tomentosa	30.1	1.5	2.3	2.0	2.2	1.2	1.0
Vitis acerifolia	19.4	1.3	4.0	4.4	2.0	1.0	1.0
V. adstricta	21.9	1.1	3.1	3.2	2.0	1.0	1.0
V. aestivalis	23.0	1.3	3.8	4.0	2.0	1.0	1.0
V. arizonica	27.2	1.2	4.3	4.3	2.0	1.0	1.0
V. baileyana	18.7	1.5	3.6	4.0	2.0	1.0	1.0
V. bourgeana	22.3	1.7	2.3	2.3	2.0	1.0	1.0
V. cinerea	21.6	1.0	2.4	2.5	2.0	1.0	1.0
V. flexuosa	21.5	1.0	2.7	2.9	2.0	1.0	1.0
V. labrusca	20.4	2.1	2.6	2.9	1.8	1.1	0.7
V. monticola	24.6	1.2	2.0	2.0	2.0	1.0	1.0
V. munsoniana	16.8	1.3	2.2	2.7	1.8	0.9	0.9
V. piasezkii	22.0	1.1	3.7	3.7	2.0	1.0	1.0
V. riparia	26.4	1.0	3.2	3.2	2.0	1.0	1.0
V. tiliifolia	19.8	1.3	3.2	3.4	2.0	1.0	1.0
V. vinifera	21.3	1.0	3.7	4.0	2.0	1.0	1.0
V. vulpina	22.8	1.2	2.0	2.0	2.0	1.0	1.0

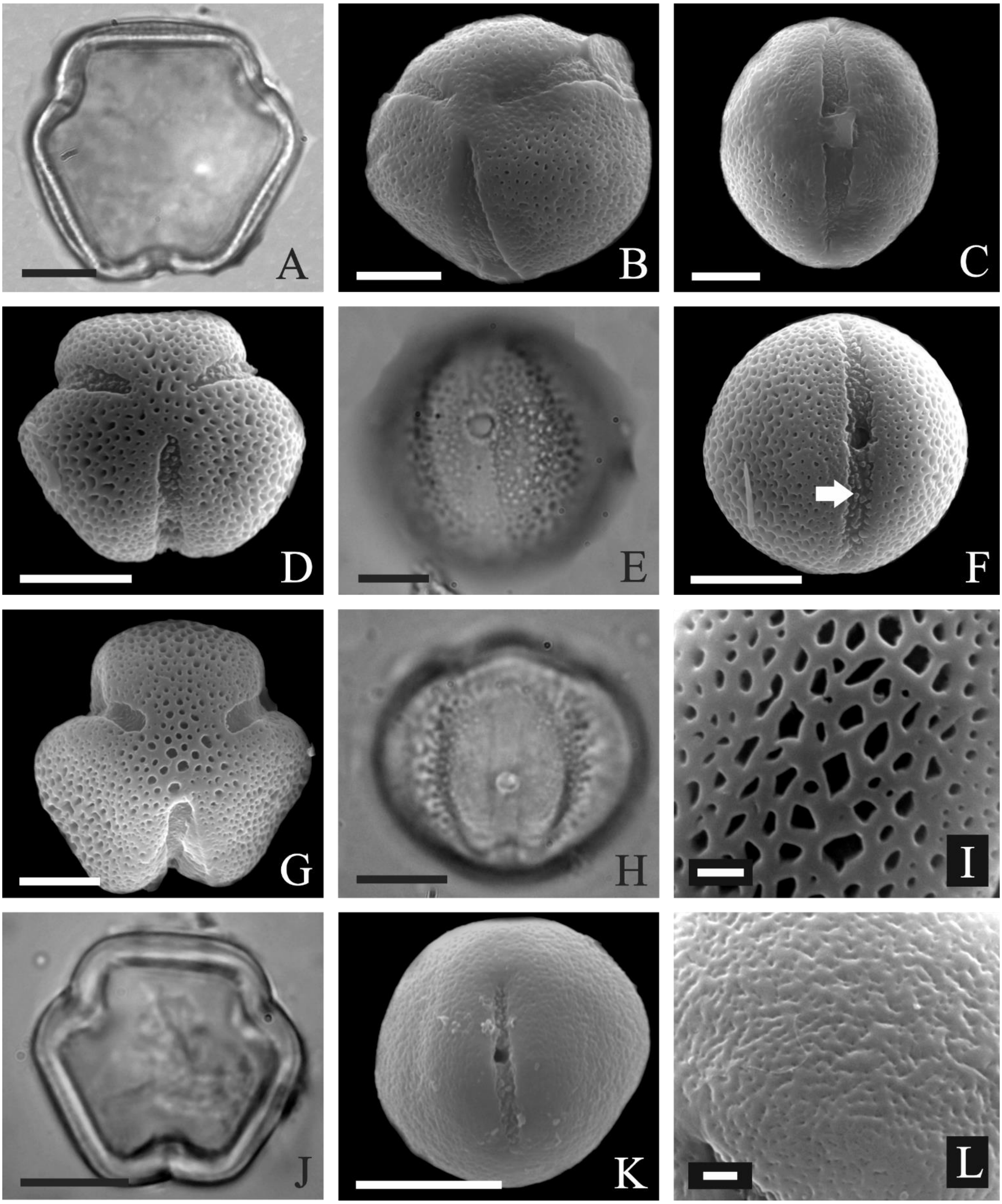


Figure 1. Light microscopy (LM) and scanning electron microscopy (SEM) images of *Ampelocissus* pollen grains. **A–C.** *A. acapulcensis*. **A.** Polar view (LM). **B.** Polar view (SEM). **C.** Equatorial view (SEM). **D–F.** *A. artemisiifolia*. **D.** Polar view (SEM). **E.** Equatorial view (LM). **F.** Equatorial view (SEM), ornate membrane (arrow). **G–I.** *A. barbata*. **G.** Polar view (SEM). **H.** Equatorial view (LM). **I.** Details of the surface (SEM). **J–L.** *A. elegans*. **J.** Polar view (LM). **K.** Equatorial view (SEM). **L.** Details of the surface (SEM). Bars: 1 µm (I, L), 5 µm (G), 10 µm (A, B, C, D, E, F, H, J, K).

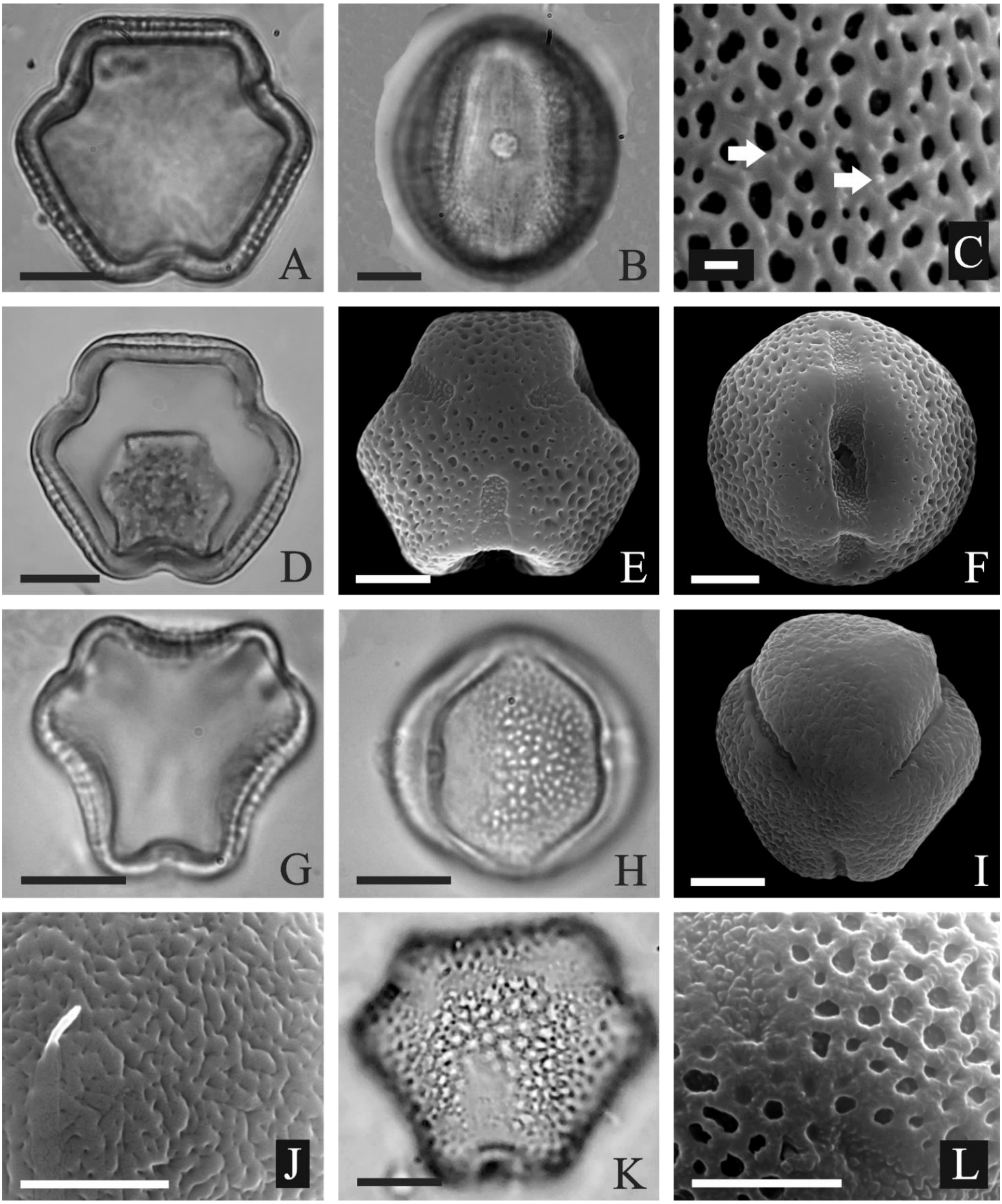


Figure 2. Light microscopy (LM) and scanning electron microscopy (SEM) images of *Ampelocissus* pollen grains. **A–C.** *A. frutescens*. **A.** Polar view (LM). **B.** Equatorial view (LM). **C.** Details of the surface (SEM), microechinae (arrow). **D–F.** *A. indica*. **D.** Polar view (LM). **E.** Polar view (SEM). **F.** Equatorial view (SEM). **G–H.** *A. martini*. **G.** Polar view (LM). **H.** Mesocolpium, details of the surface (LM). **I–J.** *A. ochracea*. **I.** Polar view (SEM). **J.** Details of the surface (SEM). **K–L.** *A. tomentosa*. **K.** Polar view (LM). **L.** Details of the surface (SEM). Bars: 1 µm (C), 4 µm (J), 5 µm (E, F, I, L), 10 µm (A, B, D, G, H, K).

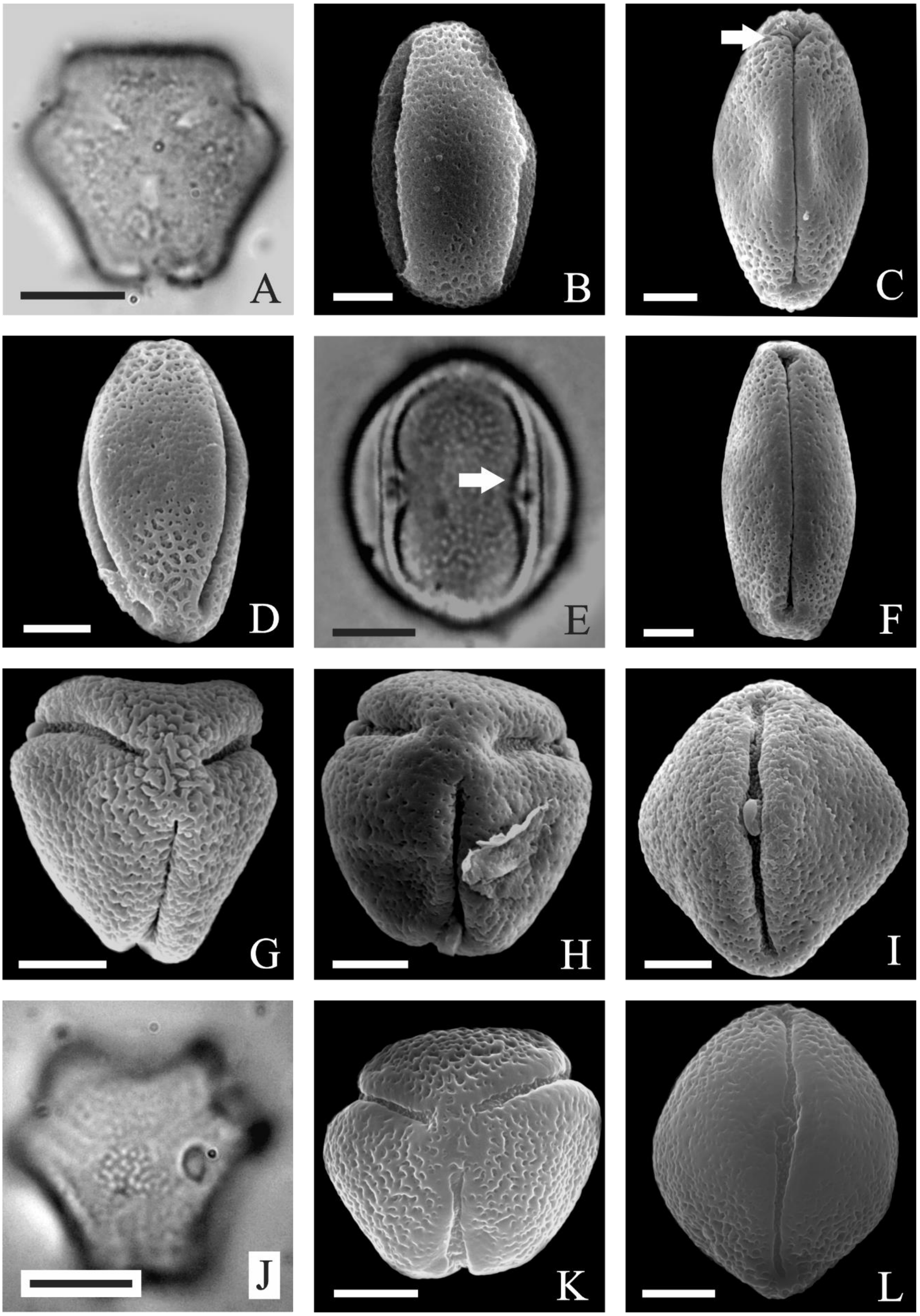


Figure 3. Light microscopy (LM) and scanning electron microscopy (SEM) images of *Vitis* pollen grains. **A–B.** *V. acerifolia* **A.** Polar view (LM). **B.** Equatorial view (SEM). **C–D.** *V. adstricta.* **C.** Equatorial view (SEM), bifurcate colpus (arrow). **D.** Mesocolpium, details of the surface (SEM). **E–F.** *V. aestivalis*. **E.** Equatorial view (LM), costa (arrow). **F.** Equatorial view (SEM). **G–I.** *V. arizonica*. **G, H.** View of the different poles (SEM). **I.** Equatorial view (SEM). **J.** *V. baileyana*. **J.** Polar view (LM). **K–L.** *V. bourgaeana*. **K.** Polar view (SEM). **L.** Equatorial view (SEM). Bars: 5 µm (B, C, D, F, G, H, I, K, L), 10 µm (A, E, J).

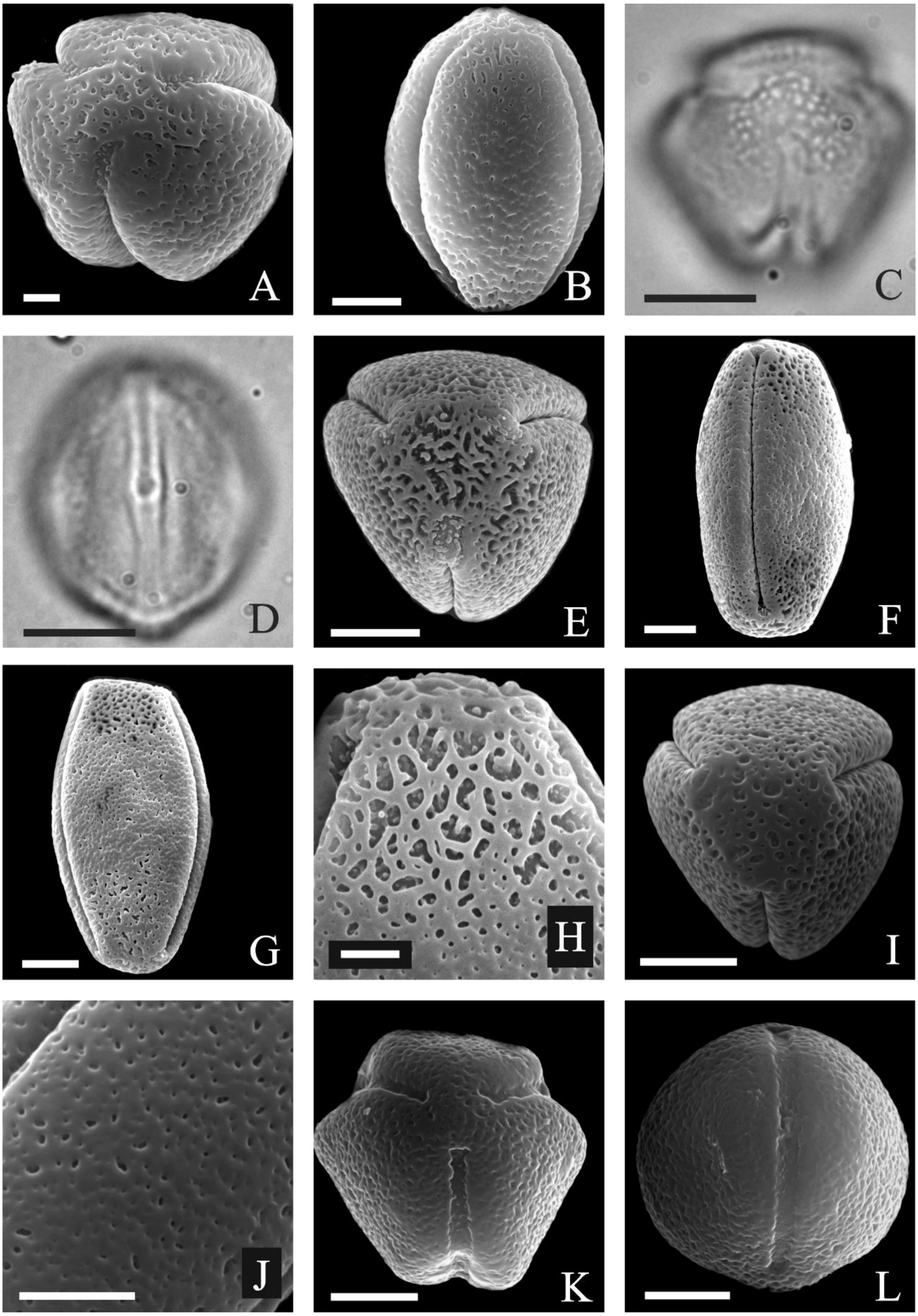


Figure 4. Light microscopy (LM) and scanning electron microscopy (SEM) images of *Vitis* pollen grains. **A–B.** *V. cinerea*. **A.** Polar view (SEM). **B.** Equatorial view (SEM). **C–D.** *V. flexuosa*. **C.** Polar view (LM). **D.** Equatorial view (LM). **E–H.** *V. labrusca*. **E.** Polar view (SEM). **F.** Equatorial view (SEM). **G.** View of the mesocolpium region (SEM). **H.** Details of the pole surface (SEM). **I–J.** *V. monticola.* **I.** Polar view (SEM). **J.** Details of the surface (SEM). **K–L.** *V. munsoniana*. **K.** Polar view (SEM). **L.** Equatorial view (SEM). Bars: 2 µm (A, H), 4 µm (J), 5 µm (D, E, F, G, I, K, L), 10 µm (C, D).

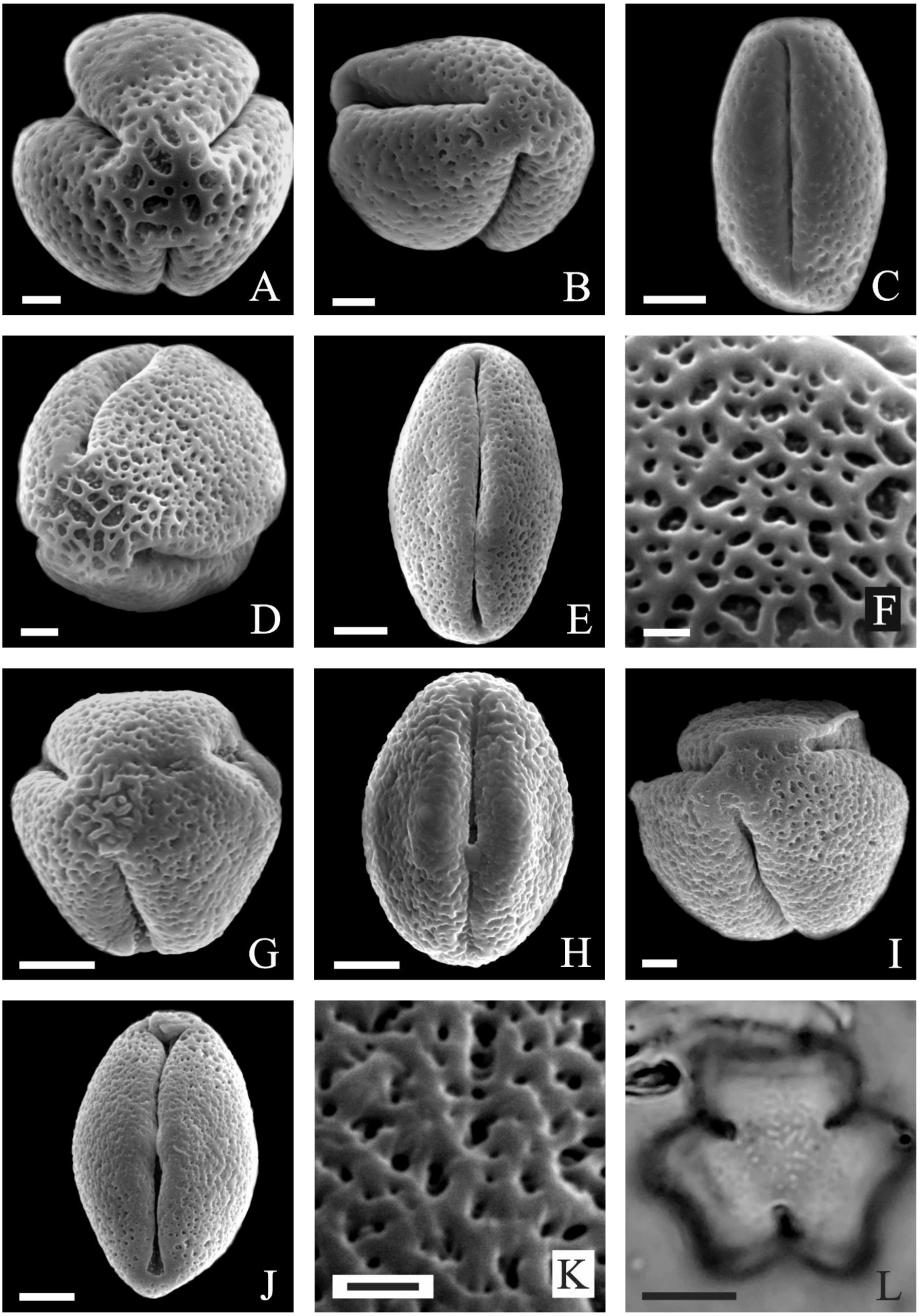


Figure 5. Light microscopy (LM) and scanning electron microscopy (SEM) images of *Vitis* pollen grains. **A–C.** *V. piasezkii*. **A, B.** View of different poles (SEM). **C.** Equatorial view (SEM). **D–F.** *V. riparia*. **D.** Polar view (SEM). **E.** Equatorial view (SEM). **F.** Details of the surface (SEM). **G–H.** *V. tiliifolia*. **G.** Polar view (SEM). **H.** Equatorial view (SEM). **I–K.** *V. vinifera*. **I.** Polar view (SEM). **J.** Equatorial view (SEM). **K.** Details of the surface (SEM). **L.** *V. vulpina*. **L.** Polar view (LM). Bars: 1 µm (F, K), 2 µm (A, B, D, I), 5 µm (C, E, G, H, J), 10 µm (L).

Data analysis (Tables V and VI, Figure 6)

PCA revealed two principal components (PCs), which together explained 66.50% of the total variance in the dataset. PC1 explained 41.78% of the variance. The following variables were the most relevant on the positive side of the PC1 axis: polar diameter, equatorial diameter, endoaperture width, exine, and equatorial diameter in polar view. *A. acapulcensis*, *A. artemisiifolia*, and *A. frutescens* were plotted far from other species because they loaded high on PC1. These species were influenced by the vectors equatorial diameter and equatorial diameter in polar view. The other species were dispersed and influenced by the vectors endoaperture length, colpus length, colpus width, apocolpium, and isopolarity. On the negative side of PC1, species were dispersed by the vectors polar diameter/equatorial diameter, polar area index, and heteropolarity. Heteropolarity influenced *V. arizonica* and *V. piasezkii*, which were plotted far from the other species. *V. vinifera* and *V. riparia* were influenced by polar area index, sharing the same position. *V. munsoniana* was polarized in relation to other species because of its association with polar diameter/equatorial diameter. The other species were dispersed on the negative side of PC1, without a strong influence of vectors.

PC2 explained 24.72% of the variance. The most significant variables plotted on the positive side of PC2 were colpus length, colpus width, apocolpium, polar diameter/equatorial diameter, polar area index, and heteropolarity. *V. arizonica* and *V. piasezkii* showed high similarity because of their association with heteropolarity. Colpus length, colpus width, and polar area index influenced *V. aestivalis*, *V. riparia, V. tiliifolia*, and *V. vinifera,* highlighting the similarity between species. The other species were dispersed on the positive side, without being related to the vectors. On the negative side of PC2, the vectors with the greatest representativeness were equatorial diameter, endoaperture width, and isopolarity. *A. martini*, *A. ochracea*, *A. tomentosa*, *V. baileyana*, *V. bourgaeana*, *V. labrusca*, *V. munsoniana*, and *V.*

vulpina were highly influenced by these vectors. The other species were dispersed on the negative axis and were not significantly associated with the vectors.

Table IV. Morphological characteristics of pollen grains of *Ampelocissus* and *Vitis* species (Vitaceae). OS- oblate spheroidal; P- prolate; PS- prolate spheroidal; S- subprolate; I- isopolar; H- heteropolar; Bir- bireticulate; Mic- microreticulate; Re- reticulate; Re-Perf- reticulate-perforate; Ru- rugulate; Ru-Perf- rugulate-perforate.

Species	Shape	Polarity	Surface with uniform ornamentation	Surface with different ornamentation		Poles with different ornamentation
				Apocolpium	Mesocolpium	
Ampelocissus acapulcensis	OS	I	Mic	---	---	---
A. artemisiifolia	OS	I	Re	---	---	---
A. barbata	PS	I	Re	---	---	---
A. elegans	PS	I	Ru-Perf	---	---	---
A. frutescens	PS	I	Re	---	---	---
A. indica	PS	I	Re	---	---	---
A. martini	PS	I	Re	---	---	---
A. ochracea	OS	I	Ru-Perf	---	---	---
A. tomentosa	PS	I	Re	---	---	---
Vitis acerifolia	PS	I	---	Re	Mic	---
V. adstricta	S	I	---	Bir	Mic	---
V. aestivalis	S	I	---	Bir	Mic	---
V. arizonica	S	H	---	Re	Ru	X
V. baileyana	PS	I	Re	---	---	---

V. bourgeana	PS	I	---	Bir	Ru	---
V. cinerea	S	I	---	Re	Ru	---
V. flexuosa	S	I	Re	---	---	---
V. labrusca	S	I	---	Re	Mic	---
V. monticola	S	I	---	Re-Perf	Mic	---
V. munsoniana	PS	I	Mic	---		---
V. piasezkii	S	H	---	Re	Mic	X
V. riparia	P	I	---	Re-Perf	Bir	---
V. tiliifolia	P	I	---	Re	Ru	---
V. vinifera	S	I	---	Re-Perf	Bir	---
V. vulpina	S	I	Re	---	---	---

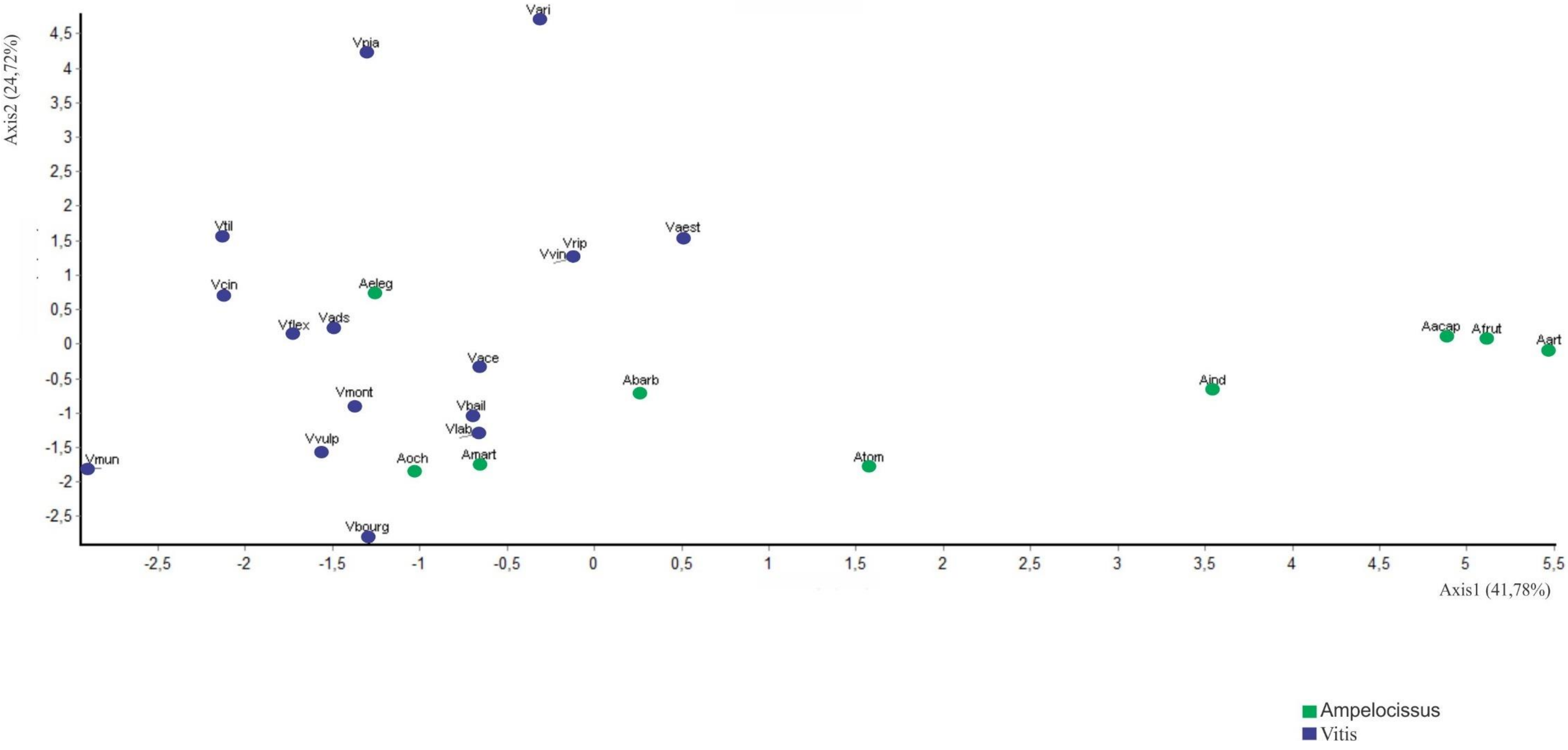
Table V. Cumulative variance and vector values for principal components of Viteae palynological characters.

Principal Axis	1	2
Cumulative Value%	41.78	24.72
Character	Axis	
Polar diameter (PD)	0.3657	0.0960
Equatorial diameter (ED)	0.4054	-0.1307
Length Endoaberture (LE)	0.3020	0.0936
Width endoaperture (WE)	0.3620	-0.1422
Length Colpus (LC)	0.2446	0.3461
Width Colpus (WC)	0.2090	0.3133
Exine (Ex)	0.3823	-0.0481
Equatorial diameter in polar view (EDPV)	0.4077	-0.0941
Apocolpus side (AS)	0.1864	0.3225
Polar diameter/Equatorial diameter P/E	-0.1391	0.3210
Polar area index (PAI)	-0.0759	0.3991
Isopolar (Iso)	0.0448	-0.4179
Heteropolar (Het)	-0.0448	0.4179

Table VI. Matrix of quantitative (metric) and qualitative (categorical) data of Viteae. PD- polar diameter; ED- equatorial diameter; CL- colpus length; CW- colpus width; EL- endoaperture length; EW- endoaperture width; Ex- exine; EDPV- equatorial diameter in polar view; SA- side of the apocolpium; PD/ED- polar diameter to equatorial diameter ratio; PAI- polar area index.

Species	Abreviation	PD	ED	CL	CW	EL	EW	Ex	EDPV	SA	PD/ED	PAI	ISO	HET
Ampelocissus acapulcensis	Aacap	34.8	36.1	24.7	2.4	4.3	4.4	2.9	34.2	8.2	0.96	0.24	1	0
A. artemisiifolia	Aart	34.5	34.6	28.0	3.3	4.0	4.2	3.2	33.5	8.2	1.0	0.24	1	0
A. barbata	Abarb	26.3	25.7	20.5	2.0	3.3	3.5	2.0	25.7	5.7	1.02	0.22	1	0
A. elegans	Aeleg	24.8	23.8	18.1	1.0	3.0	3.0	2.0	23.0	8.5	1.04	0.37	1	0
A. frutescens	Afrut	39.6	33.6	33.0	2.8	4.0	4.0	2.9	31.7	6.5	1.17	0.2	1	0
A. indica	Aind	32.9	31.0	24.8	2.8	3.7	3.6	2.9	30.2	6.2	1.06	0.21	1	0
A. martini	Amart	26.4	24.5	22.2	1.2	2.2	2.4	2.6	23.2	4.5	1.08	0.19	1	0
A. ochracea	Aoch	24.9	24.9	19.2	1.4	1.9	2.5	2.1	25.5	5.5	1.0	0.21	1	0
A. tomentosa	Atom	31.0	30.2	30.1	1.5	2.3	2.0	2.2	31.0	6.0	1.02	0.19	1	0
Vitis acerifolia	Vace	23.8	23.7	19.4	1.3	4.0	4.4	2.0	22.7	4.6	1.0	0.20	1	0
V. adstricta	Vads	25.8	20.6	21.9	1.1	3.1	3.2	2.0	22.2	5.5	1.25	0.25	1	0
V. aestivalis	Vaest	33.2	25.0	23.0	1.3	3.8	4.0	2.0	24.5	7.2	1.32	0.29	1	0
V. arizonica	Vari	28.8	22.2	27.2	1.2	4.3	4.3	2.0	22.0	6.2	1.29	0.28	0	1
V. baileyana	Vbail	25.9	24.3	18.7	1.5	3.6	4.0	2.0	22.7	3.6	1.06	0.16	1	0
V. bourgeana	Vbourg	25.4	24.4	22.3	1.7	2.3	2.3	2.0	22.2	2.7	1.04	0.12	1	0
V. cinerea	Vcin	25.9	20.4	21.6	1.0	2.4	2.5	2.0	20.7	7.2	1.27	0.35	1	0
V. flexuosa	Vflex	25.8	21.4	21.5	1.0	2.7	2.9	2.0	21.5	6.0	1.2	0.28	1	0
V. labrusca	Vlab	30.2	23.5	20.4	2.1	2.6	2.9	1.8	24.7	3.9	1.28	0.16	1	0
V. monticola	Vmont	27.8	22.9	24.6	1.2	2.0	2.0	2.0	23.0	5.6	1.22	0.24	1	0
V. munsoniana	Vmun	21.0	21.4	16.8	1.3	2.2	2.7	1.8	20.2	4.2	0.98	0.21	1	0
V. piasezkii	Vpia	26.4	21.2	22.0	1.1	3.7	3.7	2.0	22.2	6.6	1.24	0.30	0	1
V. riparia	Vrip	33.2	23.9	26.4	1.0	3.2	3.2	2.0	23.5	6.7	1.39	0.29	1	0
V. tiliifolia	Vtil	25.6	17.7	19.8	1.3	3.2	3.4	2.0	20.2	6.7	1.44	0.33	1	0
V. vinifera	Vvin	30.1	24.5	21.3	1.0	3.7	4.0	2.0	24.2	7.0	1.23	0.29	1	0
V. vulpina	Vvulp	27.5	23.2	22.8	1.2	2.0	2.0	2.0	23.0	4.5	1.18	0.20	1	0

Figure 6. Principal component analysis biplot (PCA) of Viteae species.



Discussion

Ampelocissus pollen

The main pollen morphological features that characterized the genus *Ampelocissus* were isopolarity, observed in all species, and variations in pollen shape and ornamentation. The pollen key developed in the present study to distinguish *Ampelocissus* species was based on qualitative (ornamentation and endoaperture type) and quantitative pollen data. Only *A. artemisiifolia* and *A. indica* could not be distinguished, suggesting palynological similarity. However, multivariate analysis showed that *A. indica* was not close to *A. artemisiifolia*, which formed a group with *A. acapulcensis* and *A. frutescens*. It should be noted that ornamentation type was not included in PCA, which could explain the differences between results.

Previous palynological studies on Vitaceae reported pollen grains medium (Roubik & Moreno 1991; Chávez et al. 1996; Carreira and Barth 2003; Patil 2006; Lu et al. 2012) and medium to large (Huang 1967; Reille 1967; Filice 1981; Melhem and Bissa 1985; Cartaxo-Pinto et al. 2016) in size. Other genera of the family were found to have a similar polarity to *Ampelocissus*, as evidenced by Roubik and Moreno (1991), Carreira and Barth (2003), Patil (2006), and Lu et al. (2012). Cartaxo-Pinto et al. (2017), in analysing *Cissus* species, recorded heteropolar and isopolar pollen grains. *Vitis*, as observed in the current study, was found to have heteropolar pollen.

The shape of *Ampelocissus* pollen ranged from oblate spheroidal to prolate spheroidal. There have been reports of variations in pollen shape among members of the family Vitaceae, ranging from oblate spheroidal to prolate (Filice 1981; Melhem and Bissa 1985; Roubik and Moreno 1991; Carreira and Barth 2003; Patil 2006; Cartaxo-Pinto et al. 2016). Lu et al. (2012), in studying the genus *Parthenocissus* Planch., found that pollen grains ranged from subprolate to perprolate. These results demonstrate that pollen shape is highly variable within the family.

In *Ampelocissus*, we identified three types of exine: rugulate-perforate, microreticulate, and reticulate. There is a great diversity of exine ornamentation in Vitaceae, as observed by Cartaxo-Pinto et al. (2017) when studying *Cissus*. Overall, according to previous research, exine ornamentation can be reticulate (Reille 1967; Melhem and Bissa 1985; Roubik and Moreno 1991; Carreira and Barth 2003), foveolate, microreticulate, or reticulate (Filice 1981), and foveolate or reticulate (Lu et al. 2012).

Recent phylogenetic studies on *Ampelocissus* suggest transferring Neotropical *Ampelocissus* species to the genus *Vitis* (Liu et al. 2016, Wen et al. 2018 and Ma et al. 2021). In the present study, it was found that the Neotropical species *Ampelocissus acapulcensis* was not grouped with *Vitis* species, not even when considering pollen ornamentation. Thus, pollen morphological characters do not support the transfer of this species to *Vitis*.

Geographically, it is not possible to determine whether Neotropical, African, Asian, or Australian species are distinct. Asian species have two types of pollen ornamentation, rugulate-perforate and reticulate; the pollen of Australian species also shows reticulate ornamentation, and that of Neotropical species is microreticulate. We highlight, however, that, because of the low number of analysed species, it was not possible to identify a pattern.

In the current phylogenetic tree, *Pterisanthes* and *Nothocissus* are nested within *Ampelocissus* (Wen et al. 2018, Ma et al. 2021). We were not able to confirm this assertion, as the pollen material of these two genera was not available in the consulted herbaria and there is no previous palynological study that can be used for comparison.

Given the lack of palynological studies on *Ampelocissus*, we compared our results with those of studies on previously investigated genera, such as *Cissus*. This allowed identifying relationships between genera of the family Vitaceae, mainly in the type and number of apertures and ornamentation.

Vitis pollen

In *Vitis*, the main palynological characters recorded were polarity, shape, and ornamentation, which varied between apocolpium and mesocolpium regions. Fourteen types of ornamentation were identified.

The pollen key organized in the present study used polarity (two taxa were heteropolar because of differences in ornamentation between poles), shape, and sexine ornamentation to differentiate species. Only two species could not be separated, *V. flexuosa* and *V. vulpina*. Multivariate analysis, performed using qualitative and quantitative data, revealed that both species were similar. *V. arizonica* and *V. piasezkii* were placed at the same level in the key and were plotted close to each other in the PCA plot. *V. riparia* and *V. vinifera* had the same type of pollen ornamentation and were grouped together; however, the species were placed at different levels in the pollen key because of differences in pollen shape. These findings are evidence of the robustness of the proposed key and multivariate analysis results.

In the present study, only medium-sized pollen grains were observed. Wang et al. (2000) assessed the palynological features of eight *Vitis* species and found that *V. cinerea*, *V. flexuosa*, and *V. piasezkii* had small pollen grains, differing from our results. Chavez et al. (1996) and Punt et al. (2003) also reported different results for *V. bourgaeana* and *V. vinifera* pollen size, respectively. Perveen and Qaiser (2008), on the other hand, only observed medium-sized pollen grains, in agreement with the present study.

Our analysis showed that *Vitis* pollen grains can be heteropolar or isopolar. In some studies, this character was not reported. Perveen and Qaiser (2008) observed that the evaluated species had isopolar pollen. Polarity is an important pollen character determined by the spatial orientation of microspores in the tetrad. Polarity is also directly related to the shape and location of apertures (Halbriter et al. 2018).

Pollen grains were subprolate in most species, but prolate and prolate spheroidal types were also observed. Similar findings were reported by Huang (1967), Chavez et al. (1996), Punt et al. (2003), and Perveen and Qaiser (2008). In the study of Wang et al. (2000), pollen shape ranged from oblate to perprolate and only *V. riparia* pollen was described as prolate.

The variation in *Vitis* pollen ornamentation observed in the current study underscored the importance of analysing both the apocolpium and mesocolpium for determination of sculptural patterns. Only *V. baileyana*, *V. flexuosa*, and *V. vulpina* showed homogenous ornamentation, characterized as reticulate throughout the surface. This ornamentation pattern was observed in *Cissus alata* Jacq., *C. fusifolia* Lombardi, *C. trigona* Willd. ex Schult. & Schult.f. (Cartaxo-Pinto et al. 2017) and *C. microcarpa* Vahl (Cartaxo-Pinto et al. 2021 in press). Such a variation had not yet been reported for the genus. According to Hesse et al. (2009), it is common to observe combinations of sculptural elements in pollen grains; therefore, it is typically necessary to use more than one term to describe ornamentation.

Data analysis and pollen key

According to the key to *Ampelocissus* and *Vitis* developed in the current study, there is no clear distinction between species of the two genera. For instance, *A. barbata, A. martini*, and *V. baileyana* were grouped on the basis of pollen shape, ornamentation, and confidence interval values. In comparing the results of the key with those of multivariate analysis, we observed that polarity was important in both analyses, as it separated *V. arizonica* and *V. piasezkii* (both heteropolar) from other species in the key and along the PC1 axis. *V. flexuosa* and *V. vulpina* were grouped because of the presence of reticulate sexine throughout the pollen surface; however, in the multivariate analysis, *V. vulpina* was closer to *A. martini*, *A. ochracea*, *A. tomentosa*, *V. baileyana*, *V. bourgaeana*, *V. labrusca*, and *V. munsoniana*.

The most consistent group formed by PCA comprised *A. acapulcensis*, *A. artemisiifolia*, and *A. frutescens*, which were highly similar in terms of equatorial diameter and equatorial diameter in polar view. In the pollen key, *A. acapulcensis* and *A. artemisiifolia* were close because of their oblate spheroidal pollen. Although *V. vinifera* and *V. riparia* shared the same position on the PCA plot because of their polar area index, they were far apart in the key because they differed in pollen shape. The other PCA vectors were not significant and did not form distinct groups along PC1.

V. aestivalis, *V. riparia*, *V. tiliifolia*, and *V. vinifera* were clustered together on the PC2 axis. According to the pollen key, however, only *V. riparia* and *V. tiliifolia* were close, because of their prolate pollen. The second group formed along PC2 comprised *A. martini*, *A. ochracea*, *A. tomentosa*, *V. baileyana*, *V. bourgaeana*, *V. labrusca*, *V. munsoniana*, and *V. vulpina*. In the pollen key, *A. martini*, *V. baileyana*, and *A. tomentosa* were close, as these species had the same pollen shape (prolate spheroidal) and ornamentation (reticulate). The other species were dispersed, as their characters were not significant.

Pollen keys separate species by characters until they are completely isolated. PCA was used for the formation of groups with similar characteristics. Few groups were formed, and most species were dispersed, given the low influence of pollen characters.

Conclusion

Palynological investigation of both genera of the tribe Viteae revealed that ornamentation is one of the most relevant pollen characters. Variations in pollen ornamentation were observed in *Ampelocissus* and *Vitis.* In some *Vitis* species, ornamentation differed between polar and mesocolpium regions, a feature not observed in *Ampelocissus*. Polarity and shape also differed between species. PCA showed that pollen diameter (quantitative) and polarity (qualitative) were the most influential variables. The palynological characters were not exclusive to genera, which hindered their separation; however, within each genus, most species could be identified by pollen attributes.

Although there was great within-genera variability, especially in pollen ornamentation, we observed differences between taxa of the tribe Viteae, supporting their separation into clades. *Vitis* species exhibited two types of pollen ornamentation, with differences between mesocolpium and apocolpium regions. By contrast, *Ampelocissus* species showed uniform exine ornamentation throughout the pollen surface. The results indicate that a more comprehensive study on the pollen ornamentation of the family is needed for better characterization of species and clades.

Specimens investigated

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Ampelocissus artemisiifolia Planch. China, Province Yun-Nar, M. Fabbé Delavay, no number, no date (NY).

Ampelocissus barbata Planch. China, Southern Anam, R. W. Squires 912, 05/VI/1932 (NY).

Ampelocissus elegans Gagnep. Malaysia, Singapore, Jun Wen 11685, 27/VIII/2010 (US3665178).

Ampelocissus frutescens Jackes. Australia, Northern Territory, Jabiru, J. Russel-Smith 852, 06/XII/1983 (JCT).

Ampelocissus indica (L.) Planch. Ceylon, Kalutara District, H. Huber 277, 23/IX/1977 (US2891219).

Ampelocissus martini Planch. Philippine, Semerara Island, Elmer D. Merril 4150, VI/1908 (NY).

Ampelocissus ochracea Merr. Philippine, Surigao, C. A. Wenzel 3407, 28/V/1928 (NY).

Ampelocissus tomentosa (Roth.) Planch. India, Mysore, Hassan District, C. L. Saldanha 13520, 05/V/1969 (US2794900).

Vitis acerifolia Raf., USA, Oklahoma, Donley Co., Jericho, Delzie Danares no number, 05/III/1956 (NY).

Vitis adstricta Hance. China, Jiangxi prov., Jiujiang Co., Tan Ce-Ming 9605117, 21/V/1996 (MBM238572).

Vitis aestivalis Michx. USA, Wisconsin, Richland Co., M. Nee 14990-a, 28/V/1977 (MBM117798).

Vitis arizonica Engelm. USA, Arizona, Gila Co., L.R. Landrum 9764 et al., 04/V/2000 (MBM258859).

Vitis baileyana Munson. USA, Virgina, Dickenson Co., J. Wen 12594, S. Ickert-Bond, 20/VI/2014 (US3684983).

Vitis bourgaeana Planch. Mexico, Veracruz, Yecustlas, Gutierrez B. 3995, 17/IV/1990 (NY).

Vitis cinerea (Engelm.) Millardet. USA, Alabama, Sumter Co., R. Kral 46920, 30/V/1972 (MBM148735).

Vitis flexuosa Thunb. China, Yunan, Henry A. 10549, no date (US457928).

Vitis labrusca L. USA, Pensilvania, Indiana, Frank W. Johnson, no number, 19/IX/1915 (NY).

Vitis monticola Buckley. USA, Texas, Kerr Co., Daniel Atha, Marie Greener 11753, 13/IV/2012 (NY).

Vitis munsoniana J.H. Simpson ex Planch. USA, Florida, Levy Co., Bruce & Joann Hansen 9616, 20/V/1982 (MBM079581).

Vitis piasezkii Maxim. China, Chingai, Xining, Luo Chong-Chun 1392, 22/V/1996 (MBM206830).

Vitis riparia Michx. USA. West Virgnia, Tucker Co., no collector (MBM100228).

Vitis tiliifolia Humb. & Bonpl. ex Schult. Loc. Mexico, Michoacán, San Antonio Villalongin. E. Martínez S. y Joseluis Villa Señor 446, 29/III/1982 (BHCB31981).

Vitis vinifera L. Brazil, Rio Grande do Sul, Porto Alegre, B. Rambo, SJ no date, 05/X/1951(PACA456).

Vitis vulpina L. USA, Washington D.C., Peggy Fleming 1113 31/V/1989 (US3204526).

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Capítulo II

Contributions to the palynological study of selected species of Cissus L. (Vitaceae): A new appreciation of ornamentation

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Resumo

Cissus é o maior gênero de Vitaceae, com cerca de 300 espécies amplamente distribuídas em regiões tropicais e temperadas. Atualmente, o gênero está inserido na tribo Cisseae por apresentar disco floral espesso e não dividido, flores com quatro pétalas, uma sementes por fruto, com calaza longa e linear. Este estudo investigou a morfologia polínica de 31 espécies de *Cissus* não descritas anteriormente, visando contribuir para a taxonomia do gênero e o entendimento dos caracteres polínicos. Amostras de pólen foram obtidas de herbários, submetidas à acetólise com ácido lático para microscopia de luz ou preparadas convencionalmente para microscopia eletrônica de varredura, medidas, fotografadas e descritas. Os grãos de pólen de *Cissus* eram de tamanho médio a grande, isopolares na maioria das espécies, tricolporados, forma prolato esferoidal a prolato, com ornamentação diversa. Os caracteres mais distintos do pólen foram tamanho, forma, polaridade e ornamentação da sexina. A ornamentação da sexina mostrou-se importante, uma vez que foram identificados seis padrões diferentes. Os dados quantitativos das 31 espécies descritas neste estudo e 27 espécies de um estudo anterior foram submetidos à análise de componentes principais e agrupamento hierárquico, porém esta análise não obteve uma resposta significativa para a formação de grupos em *Cissus*. Os achados podem contribuir para o conhecimento da palinologia das espécies de *Cissus* e aumentar o conhecimento sobre a ornamentação sexina.

Palavras-chave: análise multivariada, exina, polen

Abstract

Cissus is the largest genus of Vitaceae, with about 300 species widely distributed in tropical regions and temperate areas. Currently, the genus is inserted in the tribe Cisseae for presenting floral disc thick and undivided, flowers four-petaled, seeds one per fruit, with chalaza long and linear. This study investigated the pollen morphology of 31 *Cissus* species that have not been previously described, aiming to contribute to the taxonomy of the genus and the understanding of pollen characters. Pollen specimens were obtained from herbaria, subjected to lactic acid acetolysis for light microscopy or prepared conventionally for scanning electron microscopy, measured, photographed, and described. *Cissus* pollen grains were medium to large in size, isopolar in most species, tricolporate, prolate spheroidal to prolate in shape, with diverse ornamentation. The most distinct characters were pollen size, shape, polarity, and sexine ornamentation. Sexine ornamentation proved to be important, given that six different patterns were identified. Quantitative data from the 31 species described in this study and 27 species from a previous study were subjected to principal component analysis and hierarchical clustering, however this analysis did not obtain a significant answer for the formation of groups in *Cissus*. The findings may contribute to the knowledge of the palynology of *Cissus* species and increase knowledge about sexine ornamentation.

Keywords: exine, multivariate analysis, pollen

1 Introduction

The Vitaceae family comprises 16 genera and about 950 species distributed mainly in pantropical, temperate regions. Most taxons are found in forests, although some species are recorded in savannas (Wen 2007, Wen et al. 2018). From the phylogenetic and morphological analysis, the 16 genera received a new classification and were distributed into five tribes: Ampelopsideae J. Wen & Z.L. Nie; Cisseae Rchb.; Cayratieae J.Wen & L.M. Lu; Parthenocisseae J. Wen & Z.D. Chen and Viteae Dumort (Wen et al. 2018). In a recent study Ma et al. (2021) analyzing nuclear and plastid data confirmed that the tree produced supports the new classification.

Cissus L. is the largest genus of the family Vitaceae, with approximately 300 species widely distributed in tropical regions and some temperate zones (Jackes 1988; Ingruille et al. 2002; Lombardi 2000; Wen 2007; Wen et al. 2018). The genus occurs in Africa (ca. 135 species), the Americas (ca. 70 species), South Asia (ca. 85 species), and Australia (ca. 12 species) (Liu et al. 2013; Rodrigues et al. 2014).

Cissus species can be identified by leaf size and shape, trichome type and position, seeds (usually one per fruit), number of tendril branches, and stipule type (Jackes 1988; Lombardi 2000); inflorescences are cymose and opposite to leaves, flowers four-petaled, and the floral disc continuous and cupular (Soejima and Wen 2006; Wen 2007).

Phylogenetic studies on the genus suggest that *Cissus* species form a monophyletic group (Soejima and Wen 2006; Ren et al. 2011; Rodrigues et al. 2014; Zhang et al. 2015). On the basis of these studies, three clades were proposed: clade *C. striata*, whose member species (*C. granulosa* Ruiz & Pav., *C. simsiana* Schult. & Schult.f., *C. striata* Ruiz & Pav., and *C. tweediana* Baker) were transferred to the genus *Clematicissus* Planch. by Lombardi (2015); clade *C. trianae*, which includes Australian *Cissus* species and the neotropical species *C. trianae*; and the core *Cissus* clade, formed by African, Asian, and neotropical species

(Rossetto et al. 2001, 2002, 2007; Soejima and Wen 2006; Liu et al. 2013; Rodrigues et al. 2014). According to these phylogenetic studies, *Cissus s.str.* belongs to the tribe Cisseae (Wen et al. 2018). The tribe is monogeneric and is characterized by floral disc thick and undivided, flowers four-petaled, seeds one per fruit, with chalaza long and linear (Wen et al. 2018). The *C. trianae* clade is a sister group to the *Cissus* core, as supported by the phylogenetic analysis of Zhang et al. (2015). However, species of the *C. trianae* clade differ in seed morphology compared with species of the *Cissus* core clade, necessitating a review of their taxonomic position (Wen et al. 2018; Ma et al. 2021).

Palynology has been applied by several researchers as a taxonomic tool. Pollen grains differ in structure and morphology and can be used for taxonomic identification at the species, genus, and family levels (Melhem et al. 2003).

In addition to phylogenetic studies, recent palynological analyzes were found for the tribes of Vitaceae, among them we can mention Cartaxo-Pinto et al. (2021a) who evaluated two genera of the tribe Viteae (*Vitis* L. and *Ampelocissus* Planch.) indicating the importance of ornamentation in the identification of species belonging to these genera and Cartaxo-Pinto et al. (2021b) who analyzed representatives of the tribes Ampelopsideae, Cayratieae, and Parthenocissea. In this study, the authors trace the evolutionary history of pollen characters within the family using existing phylogenies. Continuing the work carried out, this article deals with the genus *Cissus*, inserted in the tribe Cisseae.

Cissus is one of the most palynologically studied genera of Vitaceae. Important contributions have been made by Huang (1967), Reille (1967), Straka and Simon (1967), Bonnefille and Riollet (1980), Filice (1981), Melhem and Bissa (1985), Roubik and Moreno (1991), Chávez et al. (1996), Carreira and Barth (2003), Melhem et al. (2003), and Patil (2006). However, these studies investigated only one or two species or focused on flora, demonstrating the need for more detailed research on the palynology of the genus. Only the study by Cartaxo-Pinto et al. (2017) was more comprehensive, portraying approximately 30 species occurring in South American.

Thus, this study aims to morphologically characterize the pollen grains of representatives of *Cissus* species and investigate characteristics that may contribute to the palynology of the genus, enriching the knowledge of species ornamentation and providing palynological data for a better understanding of the group.

2 Material and methods

2.1 Pollen analysis

In this study, 31 representatives of *Cissus s.str.* (*C. trianae* and core *Cissus* clades) were analyzed. The criteria for choosing species were availability of material in the visited herbaria, identification by specialists, and distribution in continents or countries different from those investigated in our previous study (Cartaxo-Pinto et al. 2017), which focused on taxa occurring in South America. Pollen grains were collected from fertile anthers of flowers in anthesis or flower buds in pre-anthesis. The pollen results recorded for the 27 species assessed by Cartaxo-Pinto et al. (2017) were used in this study for a more robust assessment of the genus. The species analyzed here represent about 20% of all *Cissus* species described so far.

Specimens were obtained from the following herbaria: BHCB, JCT, MBM, NY, RB, and US (see Thiers 2019). The list of material used in the study is attached (Appendix 1).

2.2 Light microscopy

For light microscopy, anthers were treated by lactic acid acetolysis (Raynal and Raynal 1971) and examined under a light microscope using 40× and 100× objectives within three days of preparation (Wanderley and Melhem 1991).

Microscope slides were deposited in the pollen collection of the Álvaro Xavier Moreira Palynology Laboratory, Department of Botany, National Museum of the Federal University of Rio de Janeiro, Brazil.

2.3 Scanning electron microscopy

Non-acetolyzed pollen samples were used for scanning electron microscopy (SEM). First, two or three anthers were removed from the flowers or flower buds of each specimen. Pollen grains were released from anthers and then mounted on metal stubs with double-sided carbon tape (Melhem et al. 2003). Samples were sputter-coated with gold for about 3 min and examined using a JEOL JSM 6390 LV scanning electron microscope and a FEI Quanta 450 FEG field emission scanning electron microscope.

2.4 Statistical treatment

For morphological analysis, pollen grains were randomly selected and measured. We obtained 25 measurements of polar diameter (PD) and equatorial (ED) diameter in equatorial view and 10 measurements of equatorial diameter in polar view (EDPV), apocolpium side (SA), polar area index (PAI), and length and width of the aperture in equatorial view. Exine thickness was measured in 10 pollen grains from each species. Data were treated statistically and are presented as arithmetic mean, standard deviation, and 95% confidence intervals (Tables 1, 2, 3).

2.5 Terminology

Pollen size, shape, and sexine ornamentation were described following the terminology of Erdtman (1952) and Punt et al. (2007). Descriptions of polar area and aperture size follow the classification established by Faegri and Iversen (1966) for PAI.

2.6 Multivariate analysis

Data were subjected to hierarchical cluster analysis (HCA) with the paired-group algorithm and principal component analysis (PCA) using a variance–covariance matrix. A matrix of quantitative pollen attributes (Table 4) of the 31 *Cissus* species described here and 27 species analyzed by Cartaxo-Pinto et al. (2017) was subjected to exploratory data analysis using PC-ORD version 5.31 (McCune and Mefford 2006).

An HCA dendrogram was constructed with the paired-group algorithm using Euclidean distance and Ward's linkage method. A cut-off of 80% was used as a measure of similarity between species (Marinho et al. 2014).

For the ordering of variables, PCA was performed using the variance–covariance matrix obtained from the means of morphometric data, which were square-root transformed for normalization. The first two principal component axes were considered for our dataset, which contained more than 20 species, as recommended by Henderson (2003), given that the components had a cumulative variance of more than 30%. The values of the vectors on each axis and the total cumulative variance are listed in tables, as is the character matrix. For better visualization of species distribution in the PCA plot, colors were assigned to species symbols using CorelDRAW software (2018).

3 Results

3.1 Size, polarity, amb, and shape

Pollen grains are medium in size (25–50 µm) in most species and large (50–100 µm) in *C. aralioides*, *C. barbeyana*, *C. cardiophylla*, and *C. integrifolia*; pollen isopolar in most species (*C. gossypiifolia*, Fig. 1A) and heteropolar in *C. adnata* (Fig. 1B) and *C. assamica*; amb subcircular or subtriangular. The shape of pollen grains is subprolate in most species, prolate in *C. adnata*, *C. arguta*, *C. barbeyana*, *C. floribunda*, *C. grisebachii*, *C. hastata*, *C. integrifolia*, *C. javana*, *C. microcarpa*, and *C. reniformis*, and prolate-spheroidal in *C. antarctica*, *C. oblonga*, *C. penninervis*, and *C. sterculifolia* (Table 1).

3.2 Aperture

Pollen grains are 3-colporate (Fig. 1C); colpus very long and polar area very small in most species and colpus long and polar area small in *C. anisophylla* and *C. sterculifolia* (Table 2, 3); parasyncolpate in *C. adnata* (Fig. 1B) and *C. assamica* (Fig. 1D). The largest colpus length is observed in *C. cardiophylla* (ca. 40.3 µm) and the smallest in *C. haematantha* (ca. 25.8 µm). The colpus is narrow in all species; the largest width was observed in *C. bosseri* (ca. 3.9 µm) and the smallest in *C. penninervis* (ca 1.5 µm) (Table 3), with margo narrow and ornamented (Fig. 1E). Colpus ends are tapered. Mesoaperture (found in an intermediate position between an ecto- and endoaperture) only in *C. cucurbitina* (12.8 × 4.2 µm) (Fig. 1f); aperture area only in *C. hypoglauca* (33.9 × 12.8 µm) (Fig. 1G-I). Colpus membrane granulate in most species (Fig. 1J, K).

The endoaperture is lalongate, with the largest length in *C. arguta* (ca. 6.7 µm) and the smallest in *C. amoena* (ca. 2.8 µm), the largest width in *C. cucurbitina* (ca. 13.3 µm) and the smallest in *C. sterculifolia* (ca. 5.7 µm) (Table 3); ends are rounded or tapered; median constriction only in *C. grisebachii* and *C. tiliacea* (Fig. 1J); presence of costa (a thickening of the nexine/endexine bordering an endoaperture or following the outline of an ectoaperture) in

C. adnata, *C. anisophylla*, *C. arguta*, *C. assamica*, *C. bosseri*, *C. floribunda* (Fig. 1L), *C. gossypiifolia*, *C. intermedia*, *C. javana*, and *C. obliqua*.

3.3 Exine

Pollen grains are semitectate, exine with thickness ranging from 2.0 to 3.8 µm. Sexine (1.0–2.2 µm) is generally thicker than nexine (1.0–1.8 µm); sexine as thick as nexine in 11 species (Table 3). The following sexine ornamentation patterns were observed:

Microreticulate - muri without perforations in *C. amoena*, *C. anisophylla*, *C. barbeyana*, *C. hastata*, *C. integrifolia* (Fig. 2A), and *C. reniformis* (Fig. 2B) and with perforations in *C. pulcherrima* (Fig. 2C) and *C. sterculifolia*.

Reticulate

- Muri narrow, sinuous, without perforations; lumina of varied shape tending to elongate: *C. adenocaulis* (Fig. 2D) and *C. quadrangularis*.
- Muri narrow, straight; lumina circular without perforations, without apparent columellae, without ornamentation: *C. antarctica* and *C. intermedia*.
- Muri narrow, straight, with perforations; lumina circular: *C. javana*.
- Muri narrow, straight; lumina elongate with granules and columellae apparent: *C. hypoglauca* (Fig. 2E).
- Muri narrow, straight, perforate; lumina circular: *C. oblonga*
- Muri very wide, sinuous, perforate; lumina narrow, elongate: *C. aralioides* (Fig. 2F) and *C. arguta*
- Muri wide, sinuous, elongate; lumina wide: *C. cardiophylla*, *C. cucurbitina*
- Muri wide, sinuous, perforate; lumina circular: *C. grisebachii*.
- Muri wide, straight; lumina circular: *C. gossypiifolia*
- Muri wide, straight, without striae, perforate at the poles; lumina small, circular: *C. bosseri* (Fig. 2G)

- Muri wide, straight, with striae and perforations originating from the lumina: *C. obliqua*
- Muri wide, striate, sinuous, with striae originating from the lumina: *C. inundata* (Fig. 2H)
- Muri wide, straight, without striae, perforate at the poles; lumina circular, granulate: *C. penninervis* (Fig. 2I)
- Muri wide, striate, perforate; lumina circular: *C. adnata*
- Reticulate at the poles, striate at the middle region of the mesocolpium, muri with striae transverse; lumina circular at the poles, small in the mesocolpium, elongate towards the poles: *C. microcarpa* (Fig. 2J-L)

Foveolate – perforate; tectum broad; lumina more or less circular: *C. tiliacea* (Fig. 3A-C)

Perforate – tectum striate over most of the surface, rugulate only near the broad tectum; lumina more or less circular: *C. floribunda* (Fig. 3D-F)

Rugulate – perforate: *C. haematantha* (Fig. 3G-I)

Rugulate-reticulate – muri narrow, sinuous: *C. assamica* (Fig. 3J-L)

Table 1. Measurements (in µm) of *Cissus* pollen grains in equatorial view (*n* = 25). $\bar{x}$: arithmetic mean; s_x: standard deviation of the mean; CI: confidence interval.

Species	Polar Diameter (PD)			Equatorial Diameter (ED)			P/E	Shape
	Range	$\bar{x} \pm s_{\bar{x}}$	C.I.95%	Range	$\bar{x} \pm s_{\bar{x}}$	C.I.95%		
Cissus adenocaulis	40.0-42.5	41.0±0.25	40.5-41.5	32.5-37.5	34.5±0.39	33.5-35.1	1.19	subprolate
C. adnata	35.0-40.0	38.2±0.27	37.6-38.7	25.0-27.5	26.0±0.25	25.5-26.5	1.47	prolate
C. amoena	32.5-40.0	36.9±0.36	36.1-37.4	25.0-30.0	28.1±0.30	27.5-28.7	1.31	subprolate
C. anisophylla	35.0-42.5	38.7±0.36	37.9-39.4	30.0-35.0	33.1±0.33	32.4-33.8	1.17	subprolate
C. antartica	35.0-40.0	37.1±0.24	36.6-37.6	30.0-37.5	34.2±0.31	33.5-34.8	1.08	prolate spheroidal
C. aralioides	47.5-52.5	50.3±0.36	49.5-51.0	35.0-40.0	38.1±0.36	37.3-38.8	1.32	subprolate
C. arguta	40.0-47.5	43.4±0.29	42.7-44.1	27.5-32.5	28.8±0.29	28.2-29.4	1.50	prolate
C. assamica	45.0-50.0	48.3±0.34	47.6-49.0	35.0-40.0	36.9±0.37	36.2-37.7	1.31	subprolate
C. barbeyana	52.5-60.0	56.5±0.38	55.7-57.3	35.0-40.0	38.2±0.34	37.5-38.9	1.48	prolate
C. bosseri	45.0-50.0	46.5±0.32	45.8-47.2	32.5-37.5	36.1±0.32	35.4-36.8	1.29	subprolate
C. cardiophylla	52.5-57.5	54.7±0.33	54.0-55.4	40.0-45.0	42.0±0.29	41.4-42.6	1.30	subprolate
C. cucurbitina	42.5-45.0	44.1±0.24	43.6-44.6	37.5-42.5	40.3±0.30	39.7-40.9	1.09	subprolate
C. floribunda	42.5-45.0	43.5±0.25	43.0-44.0	30.0-32.5	31.8±0.23	31.3-32.3	1.37	prolate
C. gossypiifolia	42.5-47.5	46.0±0.29	45.4-46.6	32.5-37.5	35.6±0.30	35.0-36.2	1.29	subprolate
C. grisebachii	47.5-55.0	49.8±0.46	48.9-50.8	32.5-42.5	36.7±0.40	35.9-37.5	1.36	prolate
C. haematantha	32.5-37.5	34.8±0.35	34.1-35.5	27.5-32.5	29.7±0.22	29.4-30.1	1.17	subprolate
C. hastata	40.0-45.0	41.6±0.28	41.0-42.2	30.0-32.5	30.9±0.24	30.4-31.4	1.34	prolate

C. hypoglauca	42.5-47.5	44.4±0.30	43.8-45.0	35.0-40.0	36.4±0.35	35.7-37.1	1.21	subprolate
C. intermedia	35.0-40.0	37.1±0.24	36.6-37.6	25.0-30.0	27.9±0.28	27.3-28.5	1.33	subprolate
C. integrifolia	47.5-52.5	50.4±0.24	49.9-50.9	35.0-40.0	36.5±0.32	35.8-37.2	1.38	prolate
C. inundata	45.0-50.4	45.9±0.28	45.3-46.5	37.5-40.0	38.2±0.23	37.7-38.7	1.20	subprolate
C. javana	42.5-47.5	46.7±0.28	46.1-47.3	32.5-35.0	33.6±0.25	33.1-34.1	1.39	prolate
C. microcarpa	37.5-42.5	40.6±0.39	39.8-41.4	27.5-32.5	29.7±0.26	29.1-30.2	1.37	prolate
C. obliqua	42.5-47.5	45.1±0.39	44.3-45.9	32.5-37.5	35.0±0.25	34.5-35.5	1.29	subprolate
C. oblonga	35.0-42.5	38.9±0.35	38.2-39.6	35.0-40.0	36.6±0.32	36.0-37.2	1.06	prolate spheroidal
C. penninervis	32.5-37.5	35.0±0.41	34.1-35.8	27.5-32.5	30.6±0.39	29.8-31.4	1.14	prolate spheroidal
C. pulcherrima	45.0-50.0	47.6±0.42	46.7-48.5	35.0-37.5	36.1±0.25	35.6-36.6	1.31	subprolate
C. quadrangularis	37.5-42.5	40.5±0.35	39.8-41.2	32.5-37.5	35.4±0.34	34.7-36.1	1.14	subprolate
C. reniformis	42.5-47.5	44.5±0.35	43.8-45.2	27.5-32.5	30.6±0.33	29.9-31.3	1.45	prolate
C. sterculifolia	32.5-35.0	33.4±0.24	32.9-33.9	30.0-35.0	33.1±0.26	32.6-33.6	1.01	prolate spheroidal
C. tiliacea	40.0-45.0	41.5±0.32	32.5-37.5	34.2-35.7	35.0±0.35	34.3-35.7	1.18	subprolate

Table 2. Measurements (in µm) of *Cissus* pollen grains in polar view (*n* = 10). EDPV, equatorial diameter in polar view; SA, apocolpium side; PAI, polar area index.

Species	Equatorial Diameter Polar View (EDPV)		Side Apocolpium (SA)		Polar Area Index (PAI)
	Range	$\bar{x}$	Range	$\bar{x}$	
C. adenocaulis	30.0-37.5	33.5	5.0-7.5	5.7	0.17
C. adnata	25.0-30.0	27.2	0.0-5.0	3.2	0.12
C. amoena	27.5-32.5	30.0	5.0-7.5	7.2	0.24
C. anisophylla	25.0-30.0	27.0	5.0-7.5	7.0	0.26
C. antartica	32.5-35.0	33.7	5.0-10.0	7.2	0.21
C. aralioides	35.0-40.0	38.7	6.2-10.0	8.4	0.22
C. arguta	27.5-30.0	29.0	5.0-7.5	7.0	0.24
C. assamica	35.0-40.0	37.5	5.0-7.5	5.8	0.15
C. barbeyana	35.0-37.5	36.0	5.0-7.5	6.5	0.18
C. bosseri	35.0-37.5	36.7	2.5-7.5	5.0	0.14
C. cardiophylla	42.5-45.0	43.0	7.5-10.0	8.1	0.19
C. cucurbitina	37.5-42.5	40.5	5.0-10.0	7.0	0.17
C. floribunda	32.5-35.0	33.2	5.0-7.5	5.9	0.18
C. gossypifolia	32.5-37.5	35.2	5.0-7.5	5.2	0.15
C. grisebachii	35.0-37.5	37.0	7.5-10.0	8.0	0.22
C. haematantha	27.5-32.5	29.7	2.5-5.0	3.0	0.10
C. hastata	30.0-32.5	31.7	5.0-7.5	5.6	0.18
C. hypoglauca	35.0-40.0	38.0	7.5-10.0	8.5	0.22
C. intermedia	25.0-30.0	28.7	2.5-5.0	4.7	0.16
C. integrifolia	35.0-40.0	38.0	5.0-7.5	6.0	0.16
C. inundata	37.5-42.5	39.5	5.0-7.5	6.5	0.16
C. javana	32.5-35.0	34.5	5.0-7.5	7.0	0.20
C. microcarpa	27.5-31.2	30.0	2.5-5.0	4.7	0.16
C. obliqua	32.5-37.5	34.5	5.0-7.5	7.2	0.21
C. oblonga	35.0-40.0	36.6	5.0-7.5	6.7	0.18
C. penninervis	27-5-32.5	30.2	2.5-5.0	4.1	0.14
C. pulcherrima	35.0-37.5	35.5	2.5-5.0	3.2	0.09
C. quadrangularis	35.0-37.5	35.5	2.5-5.0	4.7	0.13
C. reniformis	27.5-32.5	29.1	5.0-7.5	7.0	0.24

C. sterculifolia	30.0-35.0	32.0	7.5-10.0	9.0	0.28
C. tiliacea	32.5-37.5	35.0	5.0-7.5	6.7	0.19

Table 3. Measurements (in µm) of apertures and exine layers of *Cissus* pollen grains (*n* = 10).

Species	Colpus			Endoaperture		Exine layers		
	length	width	margo	length	width	exine	nexine	sexine
C. adenocaulis	31.9	2.9	1.0	5.5	6.9	2.0	1.0	1.0
C. adnata	30.2	2.0	1.0	3.8	9.5	2.8	1.0	1.8
C. amoena	29.6	2.2	0.7	2.8	9.8	2.0	1.0	1.0
C. anisophylla	32.9	3.0	0.8	4.6	10.3	2.6	1.6	1.0
C. antartica	29.2	2.4	1.0	4.8	9.4	2.0	1.0	1.0
C. aralioides	45.2	1.8	1.0	3.4	10.6	3.8	1.8	2.0
C. arguta	38.0	3.3	1.0	6.7	11.0	2.0	1.0	1.0
C. assamica	38.0	2.5	1.0	5.5	10.0	2.9	1.0	1.9
C. barbeyana	42.5	2.5	1.3	5.7	10.2	3.1	1.3	1.8
C. bosseri	36.5	3.9	1.0	6.3	11.8	2.9	1.8	1.1
C. cardiophylla	40.3	3.6	1.2	6.4	10.8	3.5	1.5	2.0
C. cucurbitina	34.0	3.8	1.2	6.0	13.3	2.2	1.0	1.2
C. floribunda	35.8	2.1	1.0	4.5	11.0	3.0	1.0	2.0
C. gossypifolia	36.5	2.3	1.0	5.3	10.1	2.0	1.0	1.0
C. grisebachii	38.0	1.9	1.0	4.1	10.2	2.0	1.0	1.0
C. haematantha	25.8	2.3	1.0	4.5	10.2	2.0	1.0	1.0
C. hastata	31.5	1.9	1.0	5.1	8.4	2.2	1.2	1.0
C. hypoglauca	34.4	2.0	1.0	4.0	8.6	3.1	1.2	1.9
C. intermedia	29.1	2.7	1.0	4.4	9.6	2.0	1.0	1.0
C. integrifolia	40.5	2.6	1.0	6.1	13.2	3.8	1.6	2.2
C. inundata	34.3	3.6	1.0	6.3	10.6	2.2	1.1	1.1
C. javana	34.8	3.8	1.1	5.3	9.6	2.9	1.1	1.8
C. microcarpa	30.1	2.0	1.0	3.4	9.4	2.1	1.0	1.1

C. obliqua	35.5	2.2	1.0	5.7	10.9	2.3	1.0	1.3
C. oblonga	30.7	2.8	1.1	5.8	10.7	2.1	1.1	1.0
C. penninervis	29.1	1.5	1.3	5.6	7.7	2.0	1.0	1.0
C. pulcherrima	36.3	2.8	1.1	6.4	9.7	2.0	1.0	1.0
C. quadrangularis	34.0	3.8	1.2	6.6	10.5	3.0	1.1	1.9
C. reniformis	37.4	3.0	1.0	5.3	12.5	2.8	1.2	1.6
C. sterculifolia	25.3	2.2	0.6	4.5	5.7	2.0	1.0	1.0
C. tiliacea	36.4	1.6	0.9	4.8	8.9	3.4	1.7	1.7

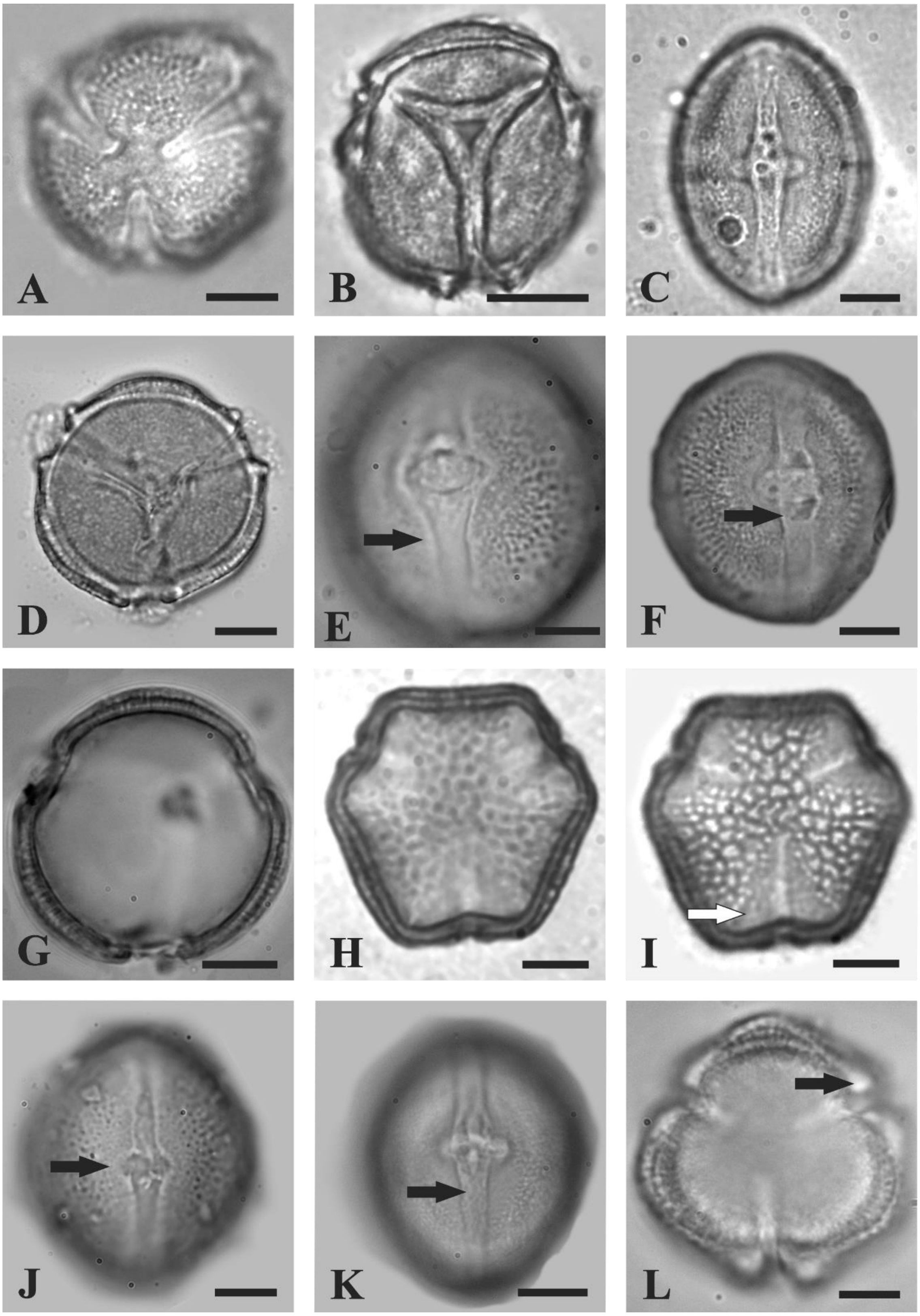


Fig. 1 Light micrographs of *Cissus* species. *C. gossypiifolia*: **A**. isopolar, polar view; *C. adnata*: **B.** heteropolar, polar view; *C. reniformis*: **C.** aperture type, colporus, equatorial view; *C. assamica*: **D.** parassincolpate, polar view; *C. cucurbitina*: **E.** margo in aperture (arrow), equatorial view; **F.** mesoaperture (arrow), equatorial view; *C. bosseri*: **G.** optical section, polar view; *C. hypoglauca*: **H.** first focus, polar view; **I.** second focus and apertural area (arrow), polar view; *C. tiliacea*: **J.** constriction in endoaperture (arrow), equatorial view; *C. anisophylla*: **K.** ornate membrane, equatorial view; *C. floribunda*: **L.** costa (arrow), polar view. Scale bars: 10 μm.

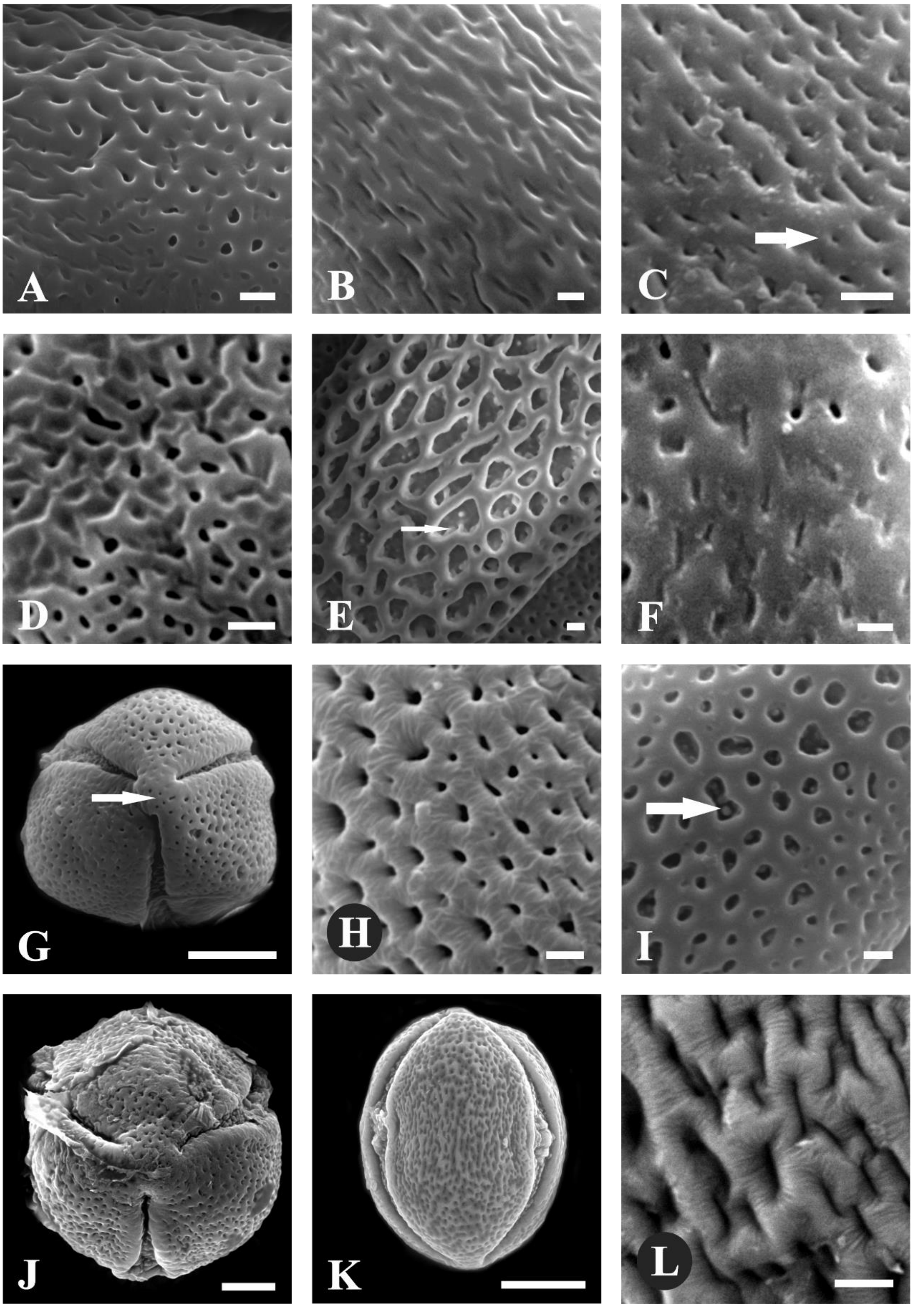


Fig. 2 Scanning electron micrographs of *Cissus* species. *C. integrifolia*: **A.** microreticulate; *C. reniformis*: **B.** microreticulate; *C. pulcherrima*: **C.** microreticulate with perforations (arrow); *C. adenocaulis*: **D.** reticulate, muri sinuous and narrow; *C. hypoglauca*: **E.** reticulate, muri straight and narrow, with columella apparent; *C. arguta*: **F.** reticulate, muri narrow with perforations (arrows); *C. bosseri*: **G.** reticulate, muri straight, wide, and perforate (arrow); *C. inundata*: **H.** reticulate, muri wide and perforate, striae originating from the lumen; *C. penninervis*: **I.** reticulate, perforations in the pole, with granules in the lumen (arrow), muri wide and smooth; *C. microcarpa*: **J.** reticulate in polar view; **K.** striate in the median region of the mesocolpium; **L.** muri striate. Scale bars: 1 µm (**A, C, D, E, F, H, J, L**); 10 µm (**B, G, I, K**).

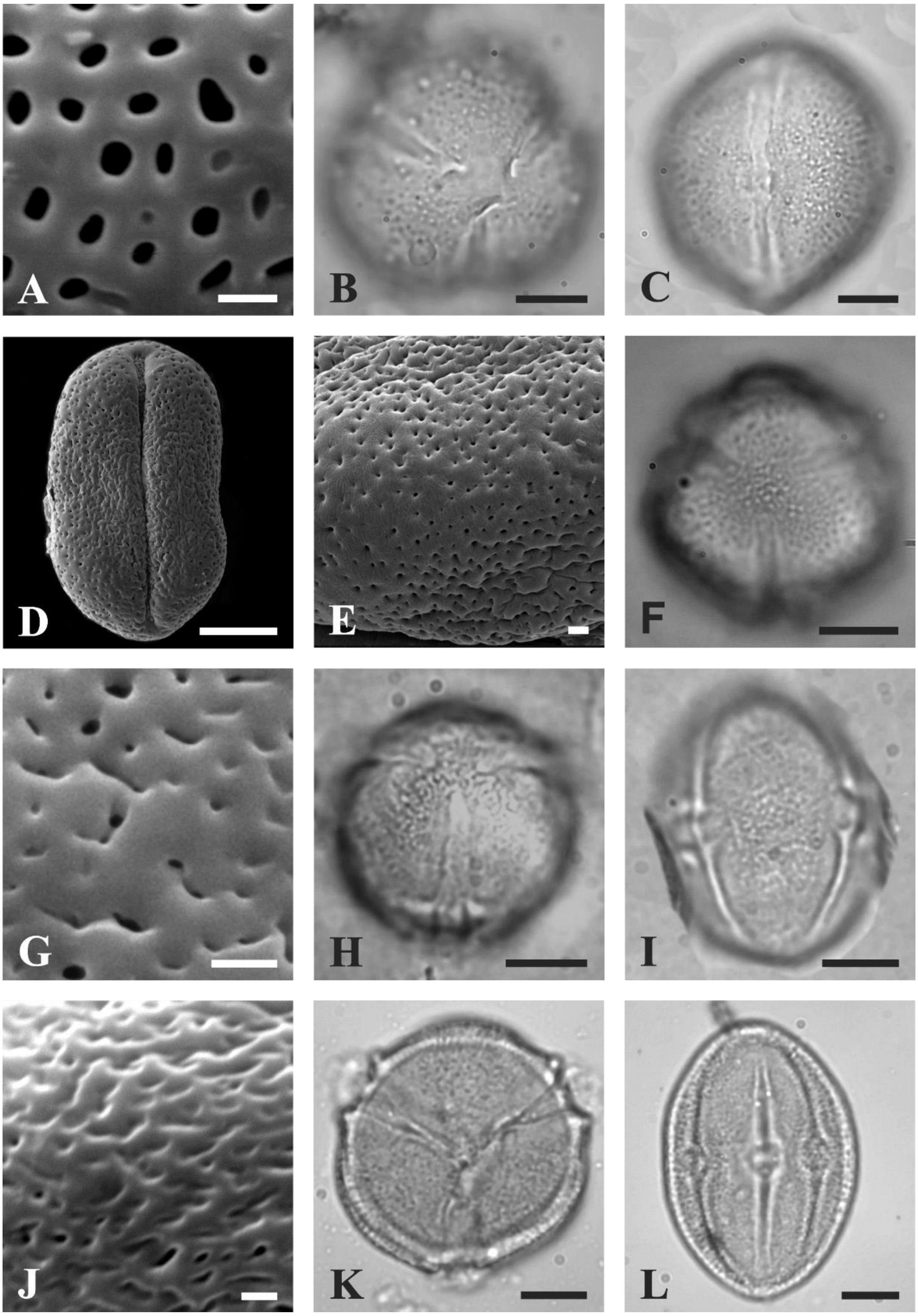


Fig. 3 Light (LM) and scanning electron micrographs (SEM) of *Cissus* species. *C. tiliacea*: **A.** foveolate, surface detail (SEM); **B.** polar view, surface (LM); **C.** equatorial view, surface (LM); *C. floribunda*: **D.** perforate with rugulae in the apertural region (SEM); **E.** perforate, muri wide, striate (SEM); **F.** polar view, surface perforate (LM); *C. haematantha*: **G.** rugulate-reticulate, surface detail (SEM); **H.** polar view, surface (LM); **I.** mesocolpium, surface (LM); *C. assamica*: **J.** rugulate with perforations, surface detail (SEM); **K.** polar view, surface (LM); **L.** equatorial view, surface (LM). Scale bars: 1 µm (**A, D, G, J**); 10 µm (**B, C, E, F, H, I, K, L**).

4 Multivariate analysis

4.1 Hierarchical cluster analysis

The HCA dendrogram of quantitative pollen data from *Cissus* species had a percentage chaining of 4.02 (Fig. 4). Using a cut-off of 80%, we obtained seven clusters. Cluster 1 was formed by 13 species: *C. adenocaulis*, *C. tinctoria*, *C. floribunda*, *C. hastata*, *C. trifoliata*, *C. paucinervia*, *C. xerophila*, *C. trianae*, *C. verticillata*, *C. amoena*, *C. anisophylla*, *C. arguta*, and *C. reniformis*. Cluster 2 contained the following six species: *C. antarctica*, *C. oblonga*, *C. spinosa*, *C. biformifolia*, *C. tiliacea*, and *C. sterculifolia.* Cluster 3 comprised eight species: *C. adnata*, *C. coccinea*, *C. campestris*, *C. microcarpa*, *C. neei*, *C. intermedia*, *C. haematantha*, and *C. penninervis*. Cluster 4 was formed by *C. erosa* and *C. glaucotricha*. Cluster 5 consisted of the following 12 species: *C. alata*, *C. pseudofuliginea*, *C. hypoglauca*, *C. cucurbitina*, *C. inundata*, *C. albida*, *C. paullinifolia*, *C. boliviana*, *C. javana*, *C. obliqua*, *C. paraensis*, and *C. grisebachii.* Cluster 6 was formed by nine species, *C. bahiensis*, *C. quadrangularis*, *C. subrhomboidea*, *C. bosseri*, *C. trigona*, *C. sulcicaulis*, *C. fusifolia*, *C. gossypiifolia*, and *C. pulcherrima.* Cluster 7 comprised eight species: *C. aralioides*, *C. cardiophylla*, *C. assamica*, *C. nobilis*, *C. integrifolia*, *C. descoingsii*, *C. barbeyana*, and *C. palmata.*

4.2 Principal component analysis

PCA explained 69.201% of the total variance in the dataset (Table 5).The first principal component explained 43.22% of the total variance. EDPV, ED, PD, SA, exine, and PAI were significant on the positive axis. EDPV, ED, and PD vectors influenced the species *C. aralioides*, *C. barbeyana*, *C. cardiophylla*, *C. descoingsii*, *C. integrifolia*, and *C. palmata* (Fig. 5), distancing them from the others. These species had the highest positive values on PC1, showing great similarity. The vectors also influenced the following species, albeit at a lower intensity: *C. alata*, *C. assamica, C. cucurbitina*, *C. grisebachii, C. hypoglauca*, *C. inundata*, *C. nobilis*, *C. paullinifolia*, *C. pseudofuliginea*, and *C. sulcicaulis* (Fig. 5). The other vectors plotted on the positive axis of PC1, although significant, did not markedly influence the other species, which remained scattered on the positive side of the PC1 axis (Table 5). On the negative side, the only representative vector was polar diameter/equatorial diameter ratio (P/E). However, the variable had a low cumulative index, not having a significant effect on species plotted on the negative side of PC1 (Table 5). *C. erosa* and *C. glaucotricha* were polarized by the influence of SA and PAI vectors, as the species had the highest negative values for the variables plotted on the positive side of the PC1 axis. The species *C. adnata*, *C. amoena*, *C. arguta*, *C. campestris*, *C. coccinea*, *C. haematantha*, *C. intermedia*, *C. microcarpa*, *C. neei*, *C. penninervis*, *C. trianae*, and *C. verticillata* were grouped together because of their negative values for EDPV, DE, and DP. Other species dispersed on the negative side of the PC1 axis were not strongly influenced by the vectors.

The second principal component explained 25.981% of the variance in the dataset (Table 5). The most significant variables on the positive side were exine, PD, P/E, ED, and EDPV. Exine and PD vectors influenced the species *C. erosa* and *C. glaucotricha*, which were polarized for exhibiting great similarity and the highest values for the variables (Fig. 5). The species *C. bahiensis*, *C. barbeyana*, *C. cardiophylla*, *C. descoingsii*, *C. integrifolia*, *C. palmata*, and *C. pulcherrima* were also influenced by the action of the vectors, although they were dispersed. The other species were not significantly influenced by the vectors. On the negative side of the PC2 axis, the representative vectors were SA and PAI. *C. sterculifolia* had the highest value on the negative axis, being polarized by the vectors. The variables also influenced *C. amoena*, *C. anisophylla*, *C. antarctica*, *C. arguta*, *C. oblonga*, *C. paraensis*, *C. paucinervia*, *C. spinosa*, and *C. xerophila*, indicating similarity between species. The other species did not show significant values and were not influenced by the vectors.

Table 4 Quantitative data matrix of *Cissus*.

Species	abreviations							
		PD	ED	EDPV	SA	PAI	P/E	Exine
Cissus adenocaulis	Caden	41.0	34.5	33.5	5.7	0.17	1.19	2.0
Cissus adnata	Cadn	38.2	26.0	27.2	3.2	0.12	1.47	2.8
*Cissus alata**	Cala	44.0	37.3	39.7	7.7	0.19	1.17	2.2
*Cissus álbida**	Calbi	45.4	35.5	36.2	6.5	0.18	1.27	2.7
Cissus amoena	Camoe	36.9	28.1	30.0	7.2	0.24	1.31	2.0
Cissus anisophylla	Canis	38.7	33.1	27.0	7.0	0.26	1.17	2.6
Cissus antartica	Cantar	37.1	34.2	33.7	7.2	0.21	1.08	2.0
Cissus aralioides	Carali	50.3	38.1	38.7	8.4	0.22	1.32	3.8
Cissus arguta	Cargu	43.4	28.8	29.0	7.0	0.24	1.5	2.0
Cissus assamica	Cassam	48.3	36.9	37.5	5.8	0.15	1.31	2.9
*Cissus bahiensis**	Cbahi	44.0	36.3	36.2	4.1	0.11	1.21	3.2
Cissus barbeyana	Cbarb	56.5	38.2	36.0	6.5	0.18	1.48	3.1
*Cissus biformifolia**	Cbifor	40.8	35.3	34.0	7.0	0.2	1.15	2.9
*Cissus boliviana**	Cboli	47.9	34.1	35.0	6.2	0.18	1.4	2.3
Cissus bosseri	Cboss	46.5	36.1	36.7	5.0	0.14	1.29	2.9
*Cissus campestri**	Ccamp	39.8	29.6	30.5	4.6	0.15	1.34	2.4
Cissus cardiophylla	Ccardi	54.7	42.0	43.0	8.1	0.19	1.3	3.5
*Cissus coccínea**	Ccocc	39.6	28.4	25.6	3.7	0.14	1.39	3.0
Cissus cucurbitina	Ccucur	44.1	40.3	40.5	7.0	0.17	1.09	2.2
*Cissus descoingsii**	Cdesc	50.3	41.2	41.5	6.0	0.14	1.22	2.9
*Cissus erosa**	Cero	40.4	29.2	25.0	0	0	1.32	3.0
Cissus floribunda	Cflori	43.5	31.8	33.2	5.9	0.18	1.37	3.0
*Cissus fusifolia**	Cfusi	45.6	34.1	35.2	5.5	0.15	1.33	2.6
*Cissus glaucotricha**	Cglau	37.5	31.9	33.6	0	0	1.17	2.4
Cissus gossypiifolia	Cgossy	46.0	35.6	35.2	5.2	0.15	1.29	2.0
Cissus grisebachii	Cgris	49.8	36.7	37.0	8.0	0.22	1.36	2.0
Cissus haematantha	Chaem	34.8	29.7	29.7	3.0	0.1	1.17	2.0
Cissus hastata	Chast	41.6	30.9	31.7	5.6	0.18	1.34	2.2
Cissus hypoglauca	Chypo	44.4	36.4	38.0	8.5	0.22	1.21	3.1
Cissus integrifolia	Cinteg	50.4	36.5	38.0	6.0	0.16	1.38	3.8
Cissus intermedia	Cinter	37.1	27.9	28.7	4.7	0.16	1.33	2.0
Cissus inundata	Cinun	45.9	38.2	39.5	6.5	0.16	1.2	2.2
Cissus javana	Cjavana	46.7	33.6	34.5	7.0	0.2	1.39	2.9
Cissus microcarpa	Cmicro	40.6	29.7	30.0	4.7	0.16	1.37	2.1
*Cissus neei**	Cneei	38.5	30.9	30.2	5.0	0.16	1.24	2.2
*Cissus nobilis**	Cnobi	48.9	38.1	39.0	6.0	0.15	1.28	2.8
Cissus obliqua	Cbliq	45.1	35.0	34.5	7.0	0.21	1.29	2.3
Cissus oblonga	Coblon	38.9	36.6	36.6	6.7	0.18	1.06	2.1
*Cissus palmata**	Cpalm	54.8	45.8	45.0	5.7	0.12	1.19	3.2
*Cissus paraensis**	Cparae	44.1	34.0	33.7	7.5	0.22	1.29	2.0
*Cissus paucinervia**	Cpauc	37.2	31.5	33.0	6.0	0.18	1.18	2.1
*Cissus paulliniifolia**	Cpaul	47.7	35.6	38.0	6.7	0.17	1.34	3.0
Cissus penninervis	Cpenn	35.0	30.6	30.2	4.1	0.14	1.14	2.0
*Cissus pseudofuliginia**	Cpseud	46.1	37.4	37.9	7.5	0.19	1.23	2.5
Cissus pulcherrima	Cpulc	47.6	36.1	35.5	3.2	0.09	1.31	2.0
Cissus quadrangularis	Cquad	40.5	35.4	35.5	4.7	0.13	1.14	3.0

Cissus reniformis	Crenif	44.5	30.6	29.1	7.0	0.24	1.45	2.8
_Cissus spinosa_*	Cspin	39.6	34.7	35.7	7.6	0.21	1.14	2.2
Cissus sterculifolia	Csterc	33.4	33.1	32.0	9.0	0.28	1.0	2.0
_Cissus subrhomboidea_*	Csubr	42.4	36.6	36.7	5.0	0.13	1.15	2.2
_Cissus sulcicaulis_*	Culci	44.2	37.4	37.0	5.7	0.15	1.18	3.2
_Cissus tiliacea_*	Ctilia	41.5	35.0	35.0	6.7	0.19	1.18	3.4
_Cissus tinctoria_*	Ctinc	39.6	34.2	32.1	5.0	0.15	1.16	2.7
_Cissus trianae_*	Ctria	33.7	33.0	33.0	5.0	0.15	1.02	2.3
_Cissus trifoliata_*	Ctrifo	40.9	29.3	32.5	5.5	0.17	1.39	2.6
_Cissus trigona_*	Ctrig	44.7	36.0	36.0	5.0	0.13	1.24	3.0
_Cissus verticillata_*	Cverti	42.8	25.4	33.9	5.4	0.15	1.68	2.3
_Cissus xerophyla_*	Cxero	37.9	32.0	33.6	6.2	0.18	1.18	2.3

The species represented by an asterisk (*) are from the work of Cartaxo-Pinto et al. (2017).

Table 5 Cumulative variance and vector values in principal component analysis of palynological characters of *Cissus.*

Principal Axis	1	2
Cumulative Variance	43.22%	25.981%
Character	Axis	
Polar diameter (PD)	0.8025	0.3900
Equatorial diameter (ED)	0.8818	0.1721
Equatorial diamete polar view (EDPV)	0.8894	0.1375
Side apocolpo (AS)	0.6354	-0.7401
Polar area index (PAI)I	0.4260	-0.8443
Polar diameter/equatorial diameter (P/E)	-0.0643	0.2417
Exine	0.4727	0.5468

Fig. 4 Combined dendrogram of quantitative data of *Cissus* species based on hierarchical cluster analysis.

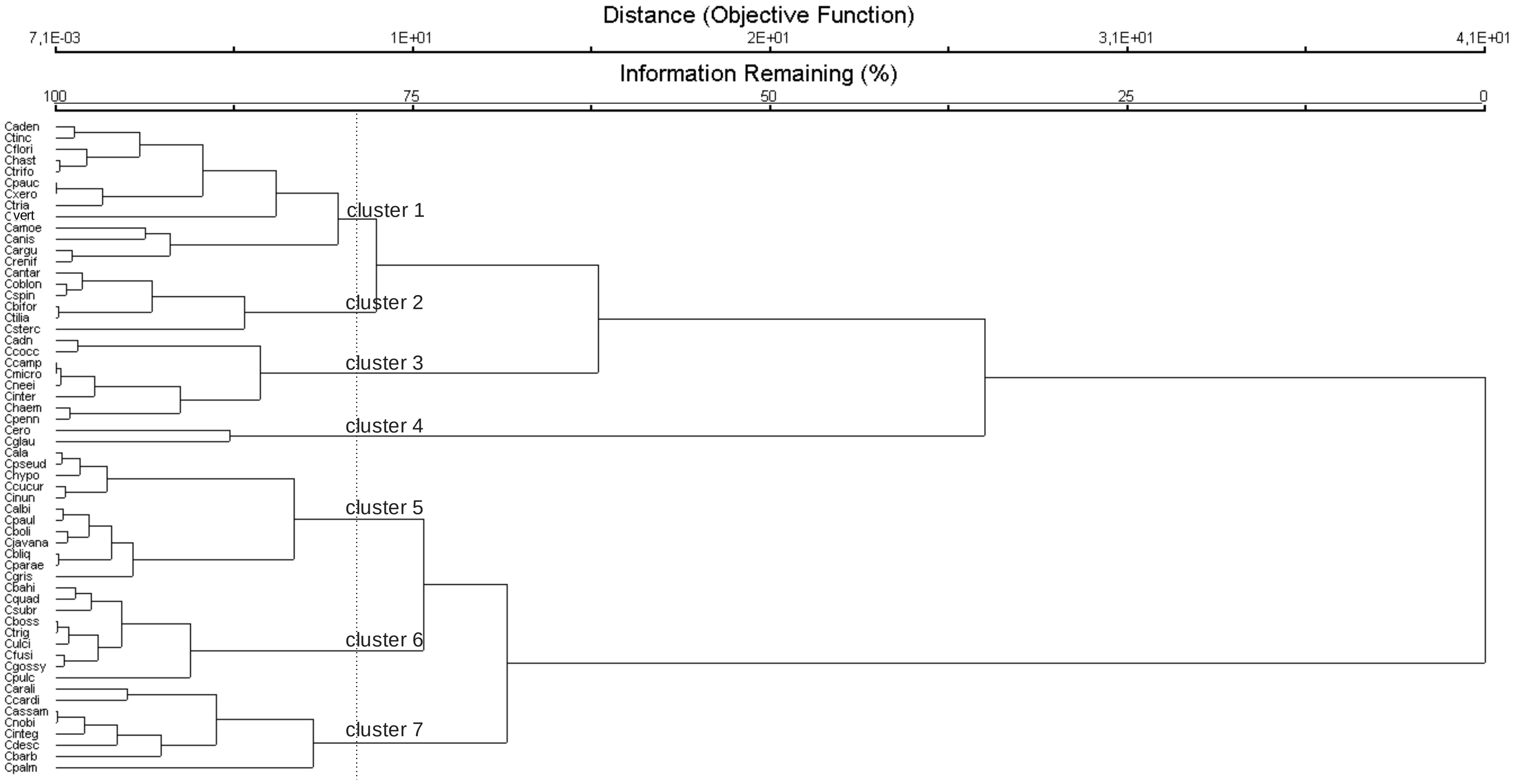
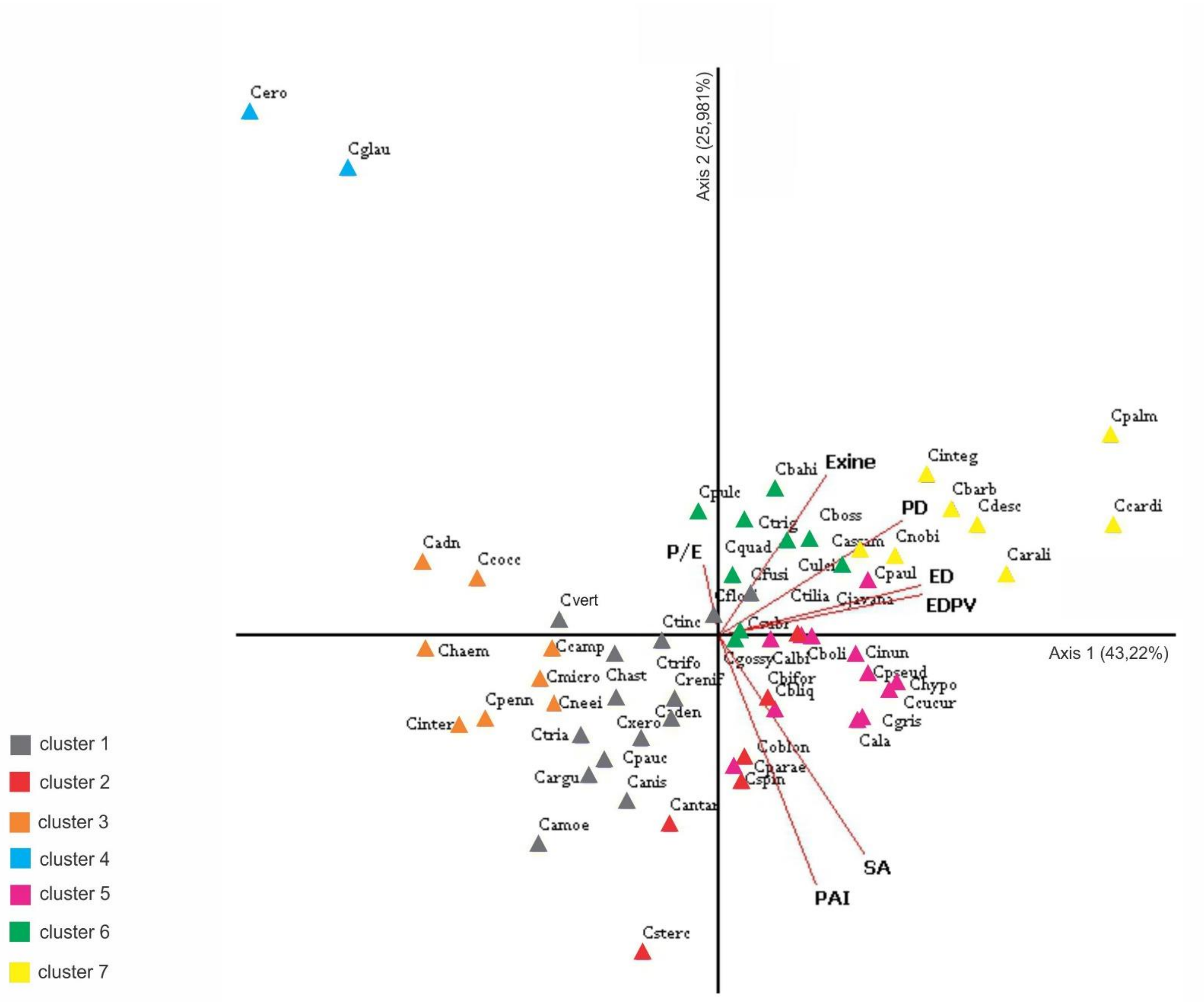


Fig. 5 Principal component analysis biplot of pollen characters of *Cissus* species.



5 Discussion

Most of the species analyzed in this study had medium-sized pollen grains. Large pollen grains were observed in *C. aralioides*, *C. barbeyana*, *C. cardiophylla*, and *C. integrifolia*. Such a variation in size was also reported by Huang (1967), Reille (1967), Filice (1981), Melhem and Bissa (1985), and Cartaxo-Pinto et al. (2017). However, Roubik and Moreno (1991), Chávez et al. (1996), Carreira and Barth (2003), and Patil (2006) observed only medium-sized pollen grains. Size was one of the most significant variables on the positive side of the PC1 axis, being influenced by PD, one of the variables used to describe pollen size in the present study.

Pollen shape ranged from prolate spheroidal to prolate, with subprolate pollen as the most frequent. The prolate shape is a synapomorphy in Vitaceae, derived from prolate spheroidal and subprolate pollen grains (Cartaxo-Pinto et al. 2021b). The oblate spheroidal to prolate pollen grains were identified by Cartaxo-Pinto et al. (2017). Filice (1981) and Carreira and Barth (2003) found that *Cissus* species other than those analyzed here had subprolate pollen grains. Other studies reported variations in shape. Melhem and Bissa (1985) and Roubik and Moreno (1991) reported subprolate to prolate pollen, whereas Patil (2006) identified oblate-spheroidal to subprolate pollen. Variations in shape can occur due to harmomegatic effects, that is, loss of liquids; this will depend on the method used for pollen preparation (Halbriter et al. 2018). Shape was not much significant in multivariate analysis. The P/E vector, which is an indicator of pollen shape, did not influence the grouping or dispersion of species on the PCA plot.

Regarding polarity, most species exhibited isopolar pollen grains. This attribute was also observed by Roubik and Moreno (1991) and Carreira and Barth (2003). However, heteropolarity was also observed here and in the study of Cartaxo-Pinto et al. (2017).

Our studies revealed six types of exine ornamentation. The most common pattern was reticulate. The reticulate pattern was considered a synapormophy, derived from microreticulate pollen grains and representing an evolutionary trend for Vitaceae (Cartaxo-Pinto et al. 2021b). Reticulate pollen grains were also observed by Reille (1967), Melhem and Bissa (1985), Roubik and Moreno (1991), and Carreira and Barth (2003). Foveolate, microreticulate, and reticulate ornamentation were reported by Filice (1981). Cartaxo-Pinto et al. (2017) identified five sexine ornamentation patterns (microreticulate, microreticulate-perforate, perforate, reticulate, and reticulate-perforate) and these patterns were also observed in the present study, including rugulate, rugulate-reticulate, and foveolate. Differences in apocolpium (reticulate) and mesocolpium (striate) ornamentation were observed in *C. microcarpa* pollen in the present study. These characteristics were also observed in the work mentioned above for C. alata, C. fusifolia and C. trigona. Although we see that most Australian species (C. antartica, C. hypoglauca, C. oblonga, C. cardiophylla and C. penninervis) have reticulated ornamentation (with variations), we cannot say that they form a group because other species in the Cissus core they also have, for the most part, reticulated ornamentation.

The ornamentation patterns found here confirm the variability among *Cissus* species, as also observed in the above-mentioned studies. The diversity of ornamentation found in *Cissus* pollen might be related to pollinator species. According to Yang et al. (2020), the more elaborate the pollen surface, the better the adherence of pollen grains, being associated with entomophilia. Little is known about pollination within the family, although there are reports of coleopteran, hymenopteran, and dipteran individuals pollinating the flowers of Vitaceae (Brantjes 1978; Lombardi 2000).

HCA revealed seven clusters, indicating similarity between species according to the quantitative characters analyzed. However, in the PCA plot, most species were dispersed along the axes, and few groups were formed. Cartaxo-Pinto et al. (2017) performed multivariate analysis, and established six groups using mainly the ornamentation attribute. Although exina ornamentation is very relevant, this characteristic did not allow the species to be grouped only by this attribute, requiring other characters such as shape and size.

As evidenced by multivariate analyses, the pollen characters evaluated did not allow the grouping of species according to the phylogenies proposed by Liu et al. (2013) and Rodrigues et al. (2014). The *C. trianae* clade, represented here by *C. trianae* (neotropical), *C. antarctica*, *C. cardiophylla*, *C. hypoglauca*, *C. oblonga*, *C. penninervis*, *C. reniformis*, and *C. sterculifolia* (Australian), was dispersed with species of the *Cissus* core. Lombardi (2007) established 15 informal groups for species of *Cissus* South American from morphological data, considering the groups proposed with the multivariate analysis carried out in this study, it is observed that most species were dispersed, not establishing a connection with the suggested groups.

Differences between existing taxonomic groups and results of multivariate analyzes are common when palynological characters are used. This can be proven, among others, in the study by Alzer et al. (2021) where data analysis did not agree with the existing phylogenetic relationships, showing that species from different sections remained in the same cluster.

Palynological analysis revealed that some *Cissus* species were homogeneous in terms of the number of openings and heterogeneous when considering pollen size, shape, polarity and ornamentation. The results also showed that some *Cissus* species showed important characteristics in some species, such as: mid-aperture, apertural area and presence of coast. The ornamentation was the most diverse character as six main patterns were identified.

The results of the current study, which also analyzed the dataset reported by Cartaxo-Pinto et al. (2017), allowed a broader assessment of the palynology of *Cissus*. Multivariate analysis of quantitative data demonstrated similarity between species, allowing the formation of clusters. However, the characters were not relevant for the construction of representative groups of *Cissus s.str*. Although the palynological study of *Cissus* does not support the existing phylogenies, palynology adds more data to the group and can contribute to morphological studies. The proposal of this study allowed the morphological characterization of the investigated species and contributed to the knowledge of the variation in ornamentation in *Cissus*.

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Appendix 1.

Material examined

Cissus adenocaulis Steud. ex A.Rich. (Fig. 2d) – Ruanda, Prefeture Ruhengeri, D'Arcy W. G, 01/IV/1974 (BHCB69950).

Cissus adnata Roxb. (Fig. 1b) – Australia, Queensland, Wilderness Lodge, Cape York, B. Jackes, 30/III/1994 (JCT).

Cissus amoena Gilg & M.Brandt – Cameroon, Prov. Southwest, Vicinity of Mundemba, Ndian division, D. W. Thomas 6829, 25-30/III/1987 (US).

Cissus anisophylla Lombardi (Fig. 1k) – Equador, Tingo & LaMana, Prov Cotopaxi, E. Schupp 107, 15/XII/1979 (NY).

Cissus antarctica Vent. – Australia, Queensland, W Bunya Mountain, L. Winsor, 13/XII/1977 (JCT).

Cissus aralioides (Welw. ex Baker) Planch. (Fig. 2f)– Ghana, Amedzofe, Haal J. 40012, 28/IX/1969 (US).

Cissus arguta Hook. f. – Ghana, Eastern, Krobo Hill, D. K. Harder, D. Abbiw, J. Amponsah, A. Welsing 2967, no date (US3351159).

Cissus assamica (M.A.Lawson) Craib (Fig. 1d, 3j-l) – India, Mysore, Hassan District, Saldanha C. J. 15415, 28/X/1969 (US).

Cissus barbeyana De Wild. & T.Durand – Gabon, Ogooue-Lolo, Forest des Abeilles, J. Dibata 1131, 27/VII/1996 (US).

Cissus bosseri Desc. (Fig. 1g, 2g) – Madagascar, Toliara, P. B. Phillipson 2883, 14/XII/1988 (US).

Cissus cardiophylla (F.Muell.) Jackes – Australia, Queensland, Horseshoe Bay, Magnetic Island, B. Jackes, 24/I/1978 (JCT).

Cissus cucurbitina Standl. (Fig. 1e -f) – El Salvador, Carlos Renson 334, no date (US).

Cissus floribunda Planch. (Fig. 3d-f)– Madagascar, Ranomafana, G. E. Schatz, J. S. Miller 2455, 05/XII/1988 (US).

Cissus gossypiifolia Standl. (Fig. 1a) – Mexico, Petatlan, E. Martínez S. 5145, 23/X/1983 (BHCB).

Cissus grisebachii Planch. – Cuba, Habana, Vedado, Bro. León 5040, 06/V/1915 (US).

Cissus haematantha Miq. (Fig. 3g-i)– Brazil, Roraima, G. T. Prance, W. C. Steward, J. F. Ramos, O. P. Monteiro, 13/III/1971 (BHCB).

Cissus hastata Miq. – Australia, Queensland, Tully R., E. M. Jackes, 17/III/1980 (JCT).

Cissus hypoglauca A.Gray (Fig. 2e)– Australia, New South Wales, B. J. Lepschi, J. C. Mowat 1947, 11/XII/1993 (MBM).

Cissus intermedia A.Rich. – Porto Rico, Cabo Rojo, Pedermales, Dr. Alain and Perfa Liegier, 23-24/VI/1977 (NY).

Cissus integrifolia Planch. (Fig. 2a) – Malawi, Reg. Mulanja, Loc. Machemba Hill, J. L. Balaka, Dr. J. M. Seyania, M. Maunda 825, 17/XI/1904 (NY).

Cissus inundata (Baker) Planch. (Fig. 2h)– Brazil, Minas Gerais, Mun. Joaquim Felicio, E. L. Borba (BHCB44918).

Cissus javana DC. – Papua New Guinea, Morobe Province, W. Takeuchi, D. Ama 16270, 18/V/2002 (US).

Cissus microcarpa Vahl (Fig. 2j-l)– Nicaragua, Depto. Granada, Isla El Carraco, E. Martínez S. 1546, Russ Rivieri, 11/VIII/1982 (BHCB).

Cissus obliqua Ruiz & Pav. – Equador, Prov. Azuay, Molleturo, Guayaquil, X. Correlo, C. Bonifaz 3341, 04/X/1994 (BHCB).

Cissus oblonga (Benth.) Planch. – Australia, Queensland, Running River Gorge, M. Downie, 28/IX/1976 (JCT).

Cissus penninervis Planch. (Fig. 2i)– Australia, Queensland, Paluma, B. Jackes, 30/XI/1986 (JCT).

Cissus pulcherrima Vell. (Fig. 2c) – Brazil, Espirito Santo, D. Sucre 8451, 04/III/1972 (RB).

Cissus quadrangularis L. – Ceylon, Hambantota to Tissamaharama, S. H. Sohmer 8853, 19/XI/1973 (NY).

Cissus reniformis Domin (Fig. 2b) – Australia, Queensland, Townsville, R. Cumming, 25/XI/1984 (JCT).

Cissus sterculifolia Planch. – Australia, Western Australia, Victoria Park, J. B. Williams, 16/XI/1964 (JCT).

Cissus tiliacea Kunth. (Fig. 3a-c) – Mexico, Michoacán, Punguato, Arsène, Bro. G. 10008, VI/1910 (US).

Capítulo III

Pollen analysis of representatives of the tribes Ampelopsideae, Cayratieae, and Parthenocisseae and evolutionary history of Vitaceae genera

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Resumo

A família Vitaceae possui ampla distribuição, ocorrendo em climas temperados, subtropicais e tropicais. Os membros da família das uvas se desenvolvem em diferentes tipos de vegetação. Atualmente, a família inclui cinco tribos reconhecidas: Ampelopsideae, Cisseae, Cayratieae, Parthenocisseae e Viteae. Este estudo descreve a morfologia polínica de representantes de Ampelopsideae, Cayratieae e Parthenocisseae. As outras tribos não foram descritas porque já foram objeto de estudos anteriores. O objetivo foi avaliar a relação entre as cinco tribos usando análise multivariada e a reconstrução da evolução dos caracteres polínicos usando filogenias existentes. Espécimes representativos das três tribos foram obtidos de herbários, tratados pelo método de acetólise, medidos, fotografados e descritos em microscopia de luz e eletrônica de varredura. Os resultados mostraram que os grãos de pólen são de tamanho médios, isopolares, tricolporados e subprolatos a prolatos. A ornamentação varia entre as espécies. A análise multivariada foi baseada em caracteres quantitativos, dos quais diâmetros polínicos, lado apocolpum e índice de área polar foram os mais significativos. O rastreamento de caracteres do pólen mostrou que o tamanho, a forma e a ornamentação são uma tendência evolutiva dentro da família. Esta investigação amplia o conhecimento palinológico de representantes de tribos Vitaceae, contribuindo para futuros estudos palinotaxonômicos e evolutivos.

Palavras-chave: morfologia polínica, análise multivariada, evolução, Vitaceae

Abstract

The family Vitaceae has a wide distribution, occurring in temperate, subtropical, and tropical climates. Vine members of the family thrive in different types of vegetation. Currently, the family includes five recognized tribes: Ampelopsideae, Cisseae, Cayratieae, Parthenocisseae, and Viteae. This study describes the pollen morphology of representatives of Ampelopsideae, Cayratieae, and Parthenocisseae. The other tribes were not described because they have been the subject of previous studies. The aim was to assess the relationship between the five tribes using multivariate analysis and reconstruct the evolution of pollen characters using existing phylogenies. Specimens representing the three tribes were obtained from herbaria, treated by the acetolysis method, measured, photographed, and described using light and scanning electron microscopy. Results showed that pollen grains are medium in size, isopolar, tricolporate, and subprolate to prolate. Ornamentation varies between species. Multivariate analysis was based on quantitative characters, of which pollen diameters, apocolpium side, and polar area index were the most significant. Tracing of pollen characters showed that size, shape, and ornamentation are an evolutionary trend within the family. This investigation expands the palynological knowledge on representatives of Vitaceae tribes, contributing to future palynotaxonomic and evolutionary studies.

Keywords: pollen morphology, multivariate analysis, evolution, Vitaceae

1. Introduction

Vitaceae is widely distributed and occurs in temperate, subtropical, and tropical climate regions. The taxonomy of the family has been extensively studied, mainly because of the economic importance of grapes (*Vitis vinifera* L.), which are one of the most consumed fruits in the world (Lombardi 2000; Wen 2007; Zhang et al. 2015; Zhang et al. 2016; Ickert-Bond et al. 2018). Members of the family differ from other angiosperms in having leaf-opposed tendrils. This characteristic enables Vitaceae vines to thrive in different types of vegetation (Wen et al. 2018).

Molecular phylogenetic and morphological studies on the family identified clades with a common organization between species. Soejima and Wen (2006), Rosseto et al. (2007), Wen and Nie (2007), and Nie et al. (2012) noted that the genera *Ampelopsis* Michx. and *Rhoicissus* Planch. and the complex *Cissus striata* Ruiz & Pav. form a monophyletic group. The genus *Cissus* L. is found in clades that divide the South American species of the *C. striata* complex, Australian species, and the core *Cissus* clade (Soejima and Wen 2006; Rosseto et al. 2007; Trias-Blasis et al. 2012; Rodrigues et al. 2014). Currently, the complex *C. striata*, comprising *C. granulosa*, *C. simsiana*, *C. tweediana*, and *C. striata*, was transferred to *Clematicissu*s (Lombardi 2015). *Cayratia* Juss., *Cyphostemma* (Planch.) Alston, and *Tetrastigma* Planch. form a clade within which *Cyphostemma* and *Tetrastigma* are monophyletic and *Cayratia* is paraphyletic. Asian species form a sister clade to *Tetrastigma*, and African species form a well-supported sister clade to *Tetrastigma* (Soejima and Wen 2006; Rosseto et al. 2007; Wen and Nie 2007; Ren et al. 2011). A strongly supported clade comprising Asian and North American taxa of *Vitis* form a monophyletic group (Soejima and Wen 2006). In an investigation based on molecular analysis, Ren et al. (2011) identified a large clade consisting of *Ampelocissus* Planch. *Vitis* L., *Nothocissus* (Miq.) Latiff, *Pterisanthes* Blume, *Parthenocissus* Planch., and *Yua* CL Li. The clade was divided into two subclades, one including *Ampelocissus*, *Vitis*, *Nothocissus*, and *Pterisanthes* and the other comprising *Parthenocissus* and *Yua*.

These initial investigations on the phylogeny of the family resulted in the definition of five major lineages: *Ampelopsis–Rhoicissus*, *Cissus*, *Cayratia–Cyphostemma–Tetrastigma*, *Parthenocissus–Yua*, and *Vitis–Ampelocissus* (Zhang et al. 2015; Lu et al. 2017). Based on this system, Wen et al. (2018) used morphological and molecular characters to organize the family into five tribes: Ampelopsideae J. Wen & Z.L. Nie, Cisseae Rchb.; Cayratieae J. Wen & L.M. Lu, Parthenocisseae J. Wen & Z.D. Chen, and Viteae Dumort.

Palynology, a branch of science devoted to the study of pollen morphology, generates relevant data that can be used in applied research (Applied Palynology) or contribute to cladistic and evolutionary studies (Melhem et al. 2003). Palynological investigations on the family Vitaceae are not very comprehensive, with few studies available (Cartaxo-Pinto et al. 2017, 2021a in press). This study aimed to describe the pollen morphology of some Vitaceae species, examine the relationship between tribes using data analysis, and make a first attempt at tracing the evolutionary history of pollen characters within the family using existing phylogenies.

2. Material and methods

2.1. Pollen analysis

The pollen material was obtained from collections of the following herbaria (acronyms according to Thiers 2019): BHCB, JCT, MBM, NY, and US. Sample selection was based on material availability and flower fertility. Thirteen species representing three tribes were examined.

2.2. Light microscopy

For light microscopy, anthers were acetolyzed by the method of Erdtman (1952) as modified by Melhem et al. (2003). Pollen grains were measured within 7 days of preparation (Salgado-Labouriau 1973). Measurements were made under a light microscope at 40× and 100× magnification. Photomicrographs were taken using a Canon PowerShot G6 digital camera connected to a Zeiss Axiostar Plus microscope. Illustrations register pollen grains in polar and equatorial views, describing in detail the sexine ornamentation pattern and, whenever possible, the apertures.

The microscope slides used in the study were deposited in the pollen collection of the Álvaro Xavier Moreira Laboratory of Palynology, Department of Botany, National Museum, Federal University of Rio de Janeiro, Brazil.

2.3. Scanning electron microscopy

For scanning electron microscopy, unacetolyzed grains were mounted onto stubs with carbon tape (Cartaxo-Pinto et al. 2017) and analyzed using a JEOL JSM 6390 LV apparatus at the Laboratory of Invertebrate Electron Microscopy of the National Museum and a high-resolution field-emission scanning electron microscope (Quanta 450 FEG, FEI Company) at the National Institute of Technology, CENANO, Brazil.

2.4. Measurement of pollen grains

Pollen grains were measured in polar and equatorial views. Twenty-five random measurements of the polar and equatorial diameters in equatorial view were performed. Ten random measurements were taken of the equatorial diameter in polar view, apocolpium side, aperture length and width in equatorial view, and exine layers. Data were analyzed statistically, and results are presented as arithmetic mean, standard deviation of mean, and 95% confidence intervals. Statistically treated data are presented in tables.

2.5. Terminology

Pollen size, shape, and sexine ornamentation pattern were described following the terminology of Punt et al. (2007) and Hesse et al. (2009). Descriptions of the polar area and aperture size follow the classification proposed by Faegri and Iversen (1966) for polar area index.

2.6. Multivariate analysis

Multivariate analysis allows to assess the joint relationships of data organized into groups (James and McCulloch 1990). In the current study, multivariate techniques were used to analyze pollen character data for the studied species. Clustering may reveal independent groups being sharp or robust. Measures of sharpness should be used to determine the most adequate degree of separation between groups. Here, principal component analysis (PCA) was applied to the variance–covariance matrix.

A matrix was constructed using quantitative pollen data from 99 Vitaceae species, 86 of which were described by Cartaxo-Pinto et al. (2017, 2021a in press, 2021b unpublished data). The matrix was subjected to exploratory data analysis using PC-ORD software version

5.0 (McCune and Mefford 2006). To better organize the data, we abbreviated the species names to the first letter of the genus followed by the first four or five letters of the specific epithet (Appendix 1).

For commensurable variables, data were analyzed by PCA of the variance–covariance matrix constructed using the mean morphometric measurements of pollen grains. As recommended by Henderson (2003), we considered the first three axes of the total variance for datasets with more than 20 species, provided that the first three axes accumulated more than 30% of the variance. Given that there is no consensus on the most adequate values for multivariate analysis in palynological studies, we adopted the threshold values recommended for ecological studies (McGarigal et al. 2000). Results were plotted on a two-dimensional graph representing the first and second principal components. The pollen character matrix, total cumulative variance, and values of vectors along each axis are presented in tables.

2.7. Mapping of palynological characters

To visualize the evolution of Vitaceae pollen, we mapped pollen characters using the phylogenetic hypothesis of Ma et al. (2021). For the genus *Cissus*, the phylogenetic relationships previously established by Rodrigues et al. (2014) were used to compose the character matrix (Table 5). We sought to analyze the evolution of the following pollen characters: shape, size, and apocolpium and mesocolpium ornamentation. Palynological data were obtained from the literature and used to assess relationships between species of Vitaceae and those of its sister group (Appendix 2). The pollen matrix was analyzed using Mesquite software version 2.75 (Maddison and Maddison 2011). Using the parsimony method, we allocated species in the clade *Cissus* as a schematic proposal to illustrate their relationship (well supported by molecular data) with other genera of the family.

Consensus between phylogenetic relationships of the base tree were optimized on a cladogram. The consensus tree was constructed using Mesquite software version 2.75 (Maddison and Maddison 2011). Pollen character states were mapped on the phylogenetic tree by reconstruction of the evolutionary history of the character states of analyzed taxa (terminal nodes). The parsimony method was used to generate trees for each character. The evolutionary history of palynological characters is represented by different colors for each character state. Trees are presented in figures, with captions indicating the color of each ancestral state trace.

3. Results

3.1. Pollen analysis

3.1.1. Tribe Ampelopsideae

We analyzed pollen grains of species representing three genera of the tribe Ampelopsideae: *Ampelopsis* Michx.: *Ampelopsis grossedentata* (Hand.-Mazz.) WT. Wang (Plate 1, Figures 1–3); *Clematicissus* Planch.: *Clematicissus angustissima* (F. Muell.) Planch. (Plate 1, Figures 4–6), *Clematicissus granulosa* (Ruiz & Pav.) Lombardi (Plate 1, Figures 7–9), and *Clematicissus opaca* (F. Muell.) Jackes & Rossetto (Plate 1, Figures 10–12); and *Rhoicissus* Planch.: *Rhoicissus capensis* Planch. (Plate 2, Figures 1–3) and *Rhoicissus revoilii* Planch. (Plate 2, Figures 4 and 5).

Size, polarity, amb, shape, and polar area. Pollen grains medium in size; isopolar; amb subtriangular in *R. revoilii* and subcircular in the other species; shape subprolate in *A. grossedentata* and *R. revoilii*, prolate spheroidal in *C. angustissima* and *R. capensis*, prolate in *C. opaca*, and spheroidal in *C. granulosa* (Table 1). Polar area small only in *A. grossedentata* and very small in the other species (Table 2).

Aperture. Pollen grains 3-colporate; colpi long in *A. grossedentata* and very long in most species (Table 3). The largest colpus length was observed in *R. capensis* (ca. 41.7 µm) and the smallest in *C. angustissima* (ca. 28.8 µm); the largest width was found in *C. granulosa* (2.7 µm) and the smallest in *A. grossedentata* (1.2 µm). Colpi are narrow (Table 3), with narrow margins in all species (ca. 1.0 µm). It was possible to identify the presence of granulated margins in *C. angustissima*, *C. granulosa* (Plate 1, Figures 5 and 9-arrow), and *R. capensis* (Plate 2, Figure 2). The endoaperture is almost circular in *C. opaca* and lalongate in the other species (Table 3).

Exine. The sexine is as thick as the nexine in *A. grossedentata*, *C. opaca*, and *R. revoilii* and thicker than the nexine in *C. angustissima*, *C. granulosa*, and *R. capensis* (Table 3). Muri almost 1.5 µm wide in *C. angustissima*. The ornamentation pattern of each species is described below.

i. *A. grossedentata*, *C. opaca*: sexine microreticulate across the entire surface with perforations in the pole and, muri sinuous, lumina elongate (Plate 1, Figures 2, 3 and 12).

ii. *C. angustissima*: sexine reticulate, heterobrochate, with muri perforate, straight, and wide, lumen large, elongate, ornamented (granules), columellae apparent (Plate 1, Figure 6).

iii. *C. granulosa*: sexine reticulate, heterobrochate, with muri perforate, straight, and wide, lumen small, predominantly circular, without ornamentation, without columellae apparent (Plate 1, Figure 9).

iv. *R. capensis*, *R. revoilii*: sexine microreticulate, muri straight, lumina circular (Plate 2, Figures 1, 3 and 5).

3.1.2. Tribe Cayratieae

We analyzed only pollen of *Cayratia japonica* (Thunb.) Gagnep. (Plate 2, Figures 6 and 7).

Size, polarity, amb, shape, and polar area. Pollen medium-sized; isopolar; amb subcircular; shape prolate spheroidal (Table 1); polar area small (Table 2).

Aperture. Pollen 3-colporate; colpi long, narrow; endoaperture almost circular (Table 3).

Exine. Sexine microreticulate, muri straight (Plate 2, Figure 6), sexine thicker than the nexine (Table 3).

3.1.3. Tribe Parthenocisseae

We analyzed pollen grains of species of the genus *Parthenocissus* Planch. only: *Parthenocissus heptaphylla* (Planch.) Britton (Plate 2, Figures 8–10), *Parthenocissus* *himalayana* (Royle) Planch. (Plate 2, Figures 11 and 12), *Parthenocissus inserta* (A. Kern.) Fritsch (Plate 3, Figures 1–3), *Parthenocissus quinquefolia* (L.) Planch. (Plate 3, Figures 4 and 5), *Parthenocissus tricuspidata* (Siebold & Zucc.) Planch. (Plate 3, Figures 6–8), *Parthenocissus vitacea* (Knerr) Hitchc. (Plate 3, Figures 9 and 10).

Size, polarity, amb, shape, and polar area. Pollen grains medium-sized; isopolar; amb subcircular in *Parthenocissus vitacea*, subtriangular in *Parthenocissus heptaphylla* and *P. quinquefolia*; shape prolate (Table 1). Polar area small in *P. tricuspidata* and very small in the other species (Table 2).

Aperture. Pollen 3-colporate; colpi long only in *P. tricuspidata* (Table 2) and very long in the other species. The largest colpus length was observed in *P. heptaphylla* (42.8 µm) and the smallest in *P. himalayana* (34.3 µm); the largest colpus width was found in *P. vitacea* (2.7 µm) and the smallest in *P. tricuspidata* (1.9 µm). Colpi are narrow (Table 3), with margins narrow and ornamented with granules in all species (Plate 3, Figures 7 and 11). The endoaperture is almost circular in *P. tricuspidata* and lalongate in the other species (Table 3).

Exine. Sexine reticulate in most species and foveolate only in *P. inserta*. Sexine less thick than the nexine in *P. heptaphylla*, *P. himalayana*, *P. inserta*, and *P. vitacea* and thicker than the nexine in *P. quinquefolia* and *P. tricuspidata* (Table 3). The ornamentation pattern of each species is described below.

i. *P. heptaphylla*: sexine reticulate, muri straight, large, lumen predominantly circular, without ornamentation (Plate 2, Figure 9).

ii. *P. himalayana*: sexine microreticulate, muri straight, lumen circular (Plate 2, Figure 12).

iii. *P. inserta*: sexine foveolate, muri straight, wide, lumen large, rounded, ornamented, perforations sparse (Plate 3, Figures 1 and 3).

iv. *P. quinquefolia*: sexine reticulate, muri straight, narrow, lumen predominantly circular, without ornamentation (Plate 3, Figure 5).

v. *P. tricuspidata*: sexine reticulate, muri with sparse perforations, lumen elongate, large, without ornamentation, pole with remains of isolated muri (white arrow), free bacula (black arrow) and granules between muri (white arrow and black contour); mesocolpium with more uniform muri, muri wide, straight, perforate, lumina wide (ca. 2 μm), granulate, columellae apparent (Plate 3, Figures 6 and 8).

vi. *P. vitacea*: sexine reticulate, muri straight, wide, lumen elongate, without ornamentation, perforations sparse (Plate 3, Figures 10 and 12).

Table 1. Measurements (in µm) of pollen grains of Vitaceae species in equatorial view (n = 25). $\bar{x}$- arithmetic mean; $s\bar{x}$- standard deviation of the mean; CI- confidence interval.

Tribe		Polar Diameter (PD)			Equatorial Diameter (ED)				
	Species	Range	$\bar{x} \pm s\bar{x}$	C.I.95%	Range	$\bar{x} \pm s\bar{x}$	C.I.95%	P/E	Shape
Ampelopsideae	*Ampelopsis grossedentata*	37.5-42.5	39.0±0..32	38.3-39.7	27.5-32.5	29.9±0.37	29.1-30.7	1.30	subprolate
	Clematicissus angustissima	27.5-35.0	32.7±0.45	31.8-33.6	27.5-35.0	32.2±0.48	31.2-33.2	1.02	prolate spheroidal
	C. granulosa	35.0-40.0	38.0±0.29	37.4-38.6	35.0-40.0	37.9±0.34	37.2-28.6	1.00	spheroidal
	C. opaca	37.5-42.5	39.8±0.28	39.2-40.4	25.0-30.0	28.1±0.33	27.4-27.8	1.42	prolate
	Rhoicissus capensis	45.0-52.5	48.0±0.41	47.1-49.0	40.0-47.5	44.0±0.35	43.3-44.7	1.10	prolate spheroidal
	R. revoilii	37.5-42.5	39.9±0.30	39.3-40.5	30.0-35.0	33.2±0.31	32.3-34.0	1.20	subprolate
Cayrateae	*Cayratia japonica*	32.5-35.0	33.4±0.18	33.1-33.8	28.7-32.5	31.3±0.29	30.7-32.0	1.07	prolate spheroidal
Parthenocisseseae	*Parthenocissus heptaphylla*	45.0-50.0	48.8±0.29	48.2-49.4	27.5-32.5	30.3±0.41	29.4-31.1	1.61	prolate
	P. hymalayana	37.5-45.0	41.5±0.32	40.8-42.2	30.0-35.0	31.2±0.29	30.6-31.8	1.33	prolate
	P. inserta	45.0-50.0	48.2±0.34	47.5-48.9	32.5-37.5	36.3±0.29	35.7-37.0	1.33	prolate
	P. quinquefolia	40.0-47.5	43.0±0.35	42.3-43.7	27.5-30.0	28.4±0.24	28.0-29.0	1.51	prolate
	P. tricuspidata	42.5-47.5	45.4±0.30	44.8-46.0	30.0-32.5	31.6±0.24	31.1-32.1	1.44	prolate
	P. vitacea	42.5-47.5	45.7±0.31	45.1-46.3	32.5-37.5	34.2±0.28	33.6-35.0	1.34	prolate

Table 2. Measurements (in µm) of pollen grains of Vitaceae species in polar view (*n* = 10). EDPV- equatorial diameter in polar view; AS- apocolpium side; PAI- polar area index.

Tribe	Species	Equatorial Diameter Polar View (EDPV)		Apocolpium Side (AS)		
		Range	$\bar{x}$	Range	$\bar{x}$	PAI
Ampelopsideae	*Ampelopsis grossedentata*	27.5-32.5	30.2	5.0-10.0	7.7	0.26
	Clematicissus angustissima	27.5-32.5	30.0	5.0-7.5	6.7	0.22
	C. granulosa	35.0-40.0	37.5	5.0-6.2	5.1	0.14
	C. opaca	27.5-30.0	29.5	5.0-7.5	6.5	0.22
	Rhoicissus capensis	37.5-45.0	42.1	5.0-10.0	7.3	0.17
	R. revoilii	32.5-35.0	34.1	5.0-7.5	7.2	0.21
Cayratieae	*Cayratia japonica*	28.7-32.5	31.2	8.0-12.5	9.4	0.30
Parthenocisseae	*Parthenocissus heptaphylla*	30.0-35.0	32.0	5.0-7.5	5.5	0.17
	P. hymalayana	30.0-32.5	31.0	5.0-7.5	5.5	0.18
	P. inserta	35.0-40.0	37.2	5.0-7.5	6.5	0.18
	P. quinquefolia	27.5-32.5	29.2	5.0-7.5	5.5	0.19
	P. tricuspidata	30.0-32.5	32.7	7.5-12.5	10.2	0.31
	P. vitacea	32.5-35.0	34.0	5.0-10.0	7.0	0.21

Table 3. Measurements (in µm) of apertures and exine layers of pollen grains of Vitaceae species (*n* = 10).

Tribe	Species	Colpus		Endoaperture		Exine layers		
		length	width	length	width	exine	nexine	sexine
Ampelopsideae	*Ampelopsis grossedentata*	32.6	1.2	4.6	6.3	2.0	1.0	1.0
	Clematicissus angustissima	28.8	1.8	5.1	7.1	1.9	0.9	1.0
	C. granulosa	30.6	2.7	6.1	8.1	3.4	1.5	1.9
	C. opaca	32.8	1.6	4.4	4.8	2.0	1.0	1.0
	Rhoicissus capensis	41.7	1.3	8.0	9.3	3.0	1.0	2.0
	R. revoilii	36.0	1.8	4.7	6.7	2.2	1.1	1.1
Cayratieae	*Cayratia japonica*	23.5	2.2	3.4	3.1	2.4	1.0	1.4
Parthenocisseae	*Parthenocissus heptaphylla*	42.8	2.1	4.8	6.9	2.9	1.9	1.0
	P. hymalayana	34.3	2.5	3.5	7.8	1.9	1.9	1.0
	P. inserta	39.5	2.5	5.4	7.0	2.9	1.9	1.0
	P. quinquefolia	35.7	2.3	5.0	6.1	3.0	1.2	1.8
	P. tricuspidata	36.3	1.9	6.2	6.7	2.1	1.0	1.1
	P. vitacea	38.9	2.7	4.5	7.5	2.8	1.7	1.1

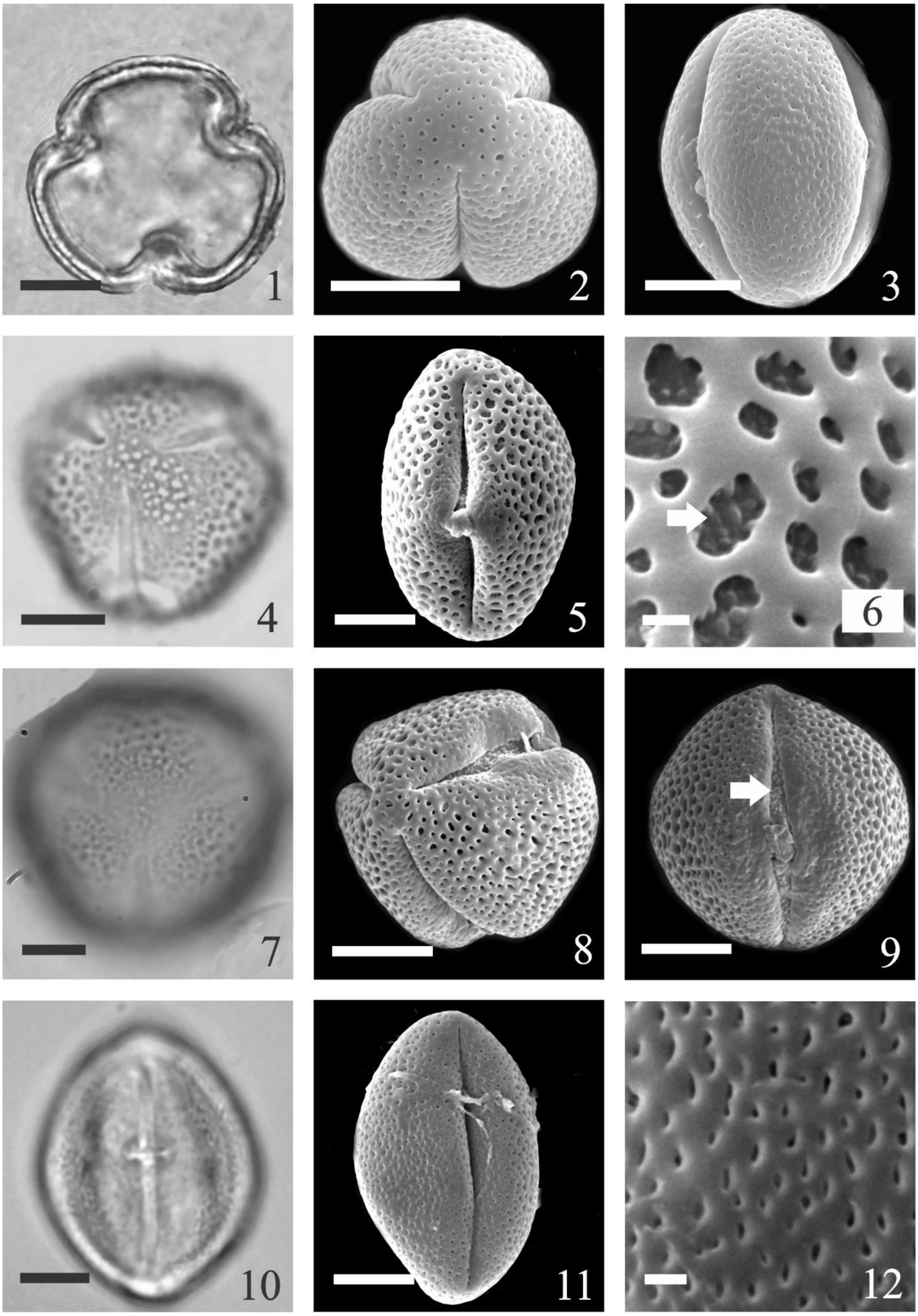


Plate 1. Photomicrographs and eletromicrographs of pollen grains. Tribe Ampelopsideae. *Ampelopsis grossedentada* – polar view: 1. optical section, 2. general aspect and surface; 3. mesocolpus view: ornamentation. *Clematicissus angustissima* - polar view: 4. general aspect, surface; equatorial view: 5. general aspect and aperture. 6. detail of the ornamentation, granules in the lumen (arrow). *C. granulosa* - polar view: 7, 8. general aspect and surface; equatorial view: 9. aperture and membrane with granules (arrow). *C. opaca* - equatorial view: 10, 11. general aspect and aperture; 12. detail of the ornamentation. Scale bar: 10 µm (1-5, 7-11), 1 µm (6, 12).

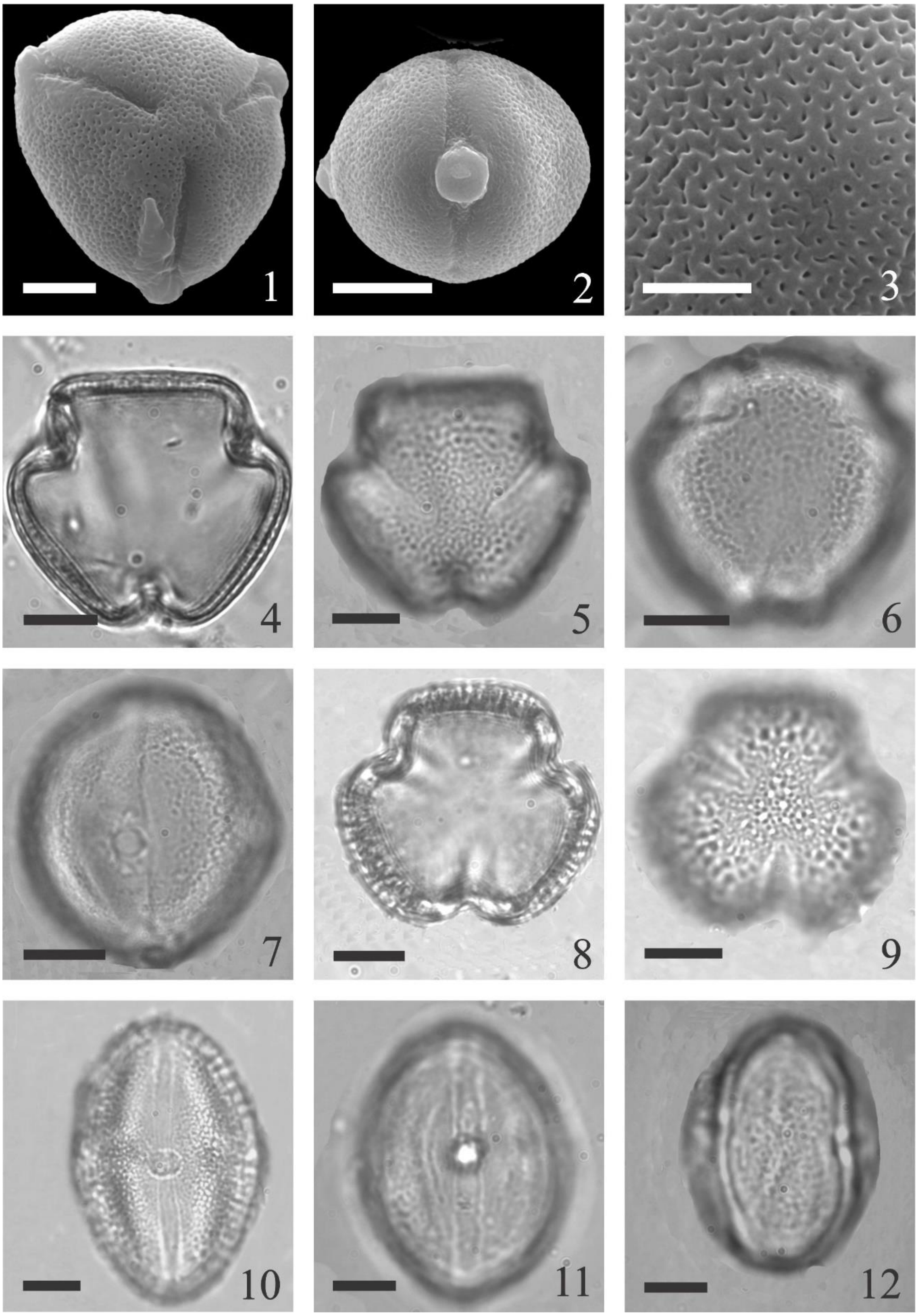


Plate 2. Photomicrographs and eletromicrographs of pollen grains. Tribe Ampelopsideae. *Rhoicissus capensis* - polar view: 1. general aspect and surface; equatorial view: 2. general aspect, aperture; 3. detail of the ornamentation. *R. reviolii* – polar view: 4. optical section, 5. general aspect, surface. Tribe Cayratieae. *Cayratia japonica* - polar view: 6. general aspect, surface; equatorial view: 7. general aspect, aperture. Tribe Parthenocisseae. *Parthenocissus heptaphylla* - polar view: 8. optical section, 9. general aspect, surface; equatorial view: 10. general aspect, aperture. *P. hymalayana* - equatorial view: 11. general aspect, aperture; mesocolpus view: 12. ornamentation. Scale bar: 10 µm (1, 2, 4-12), 5 µm (3).

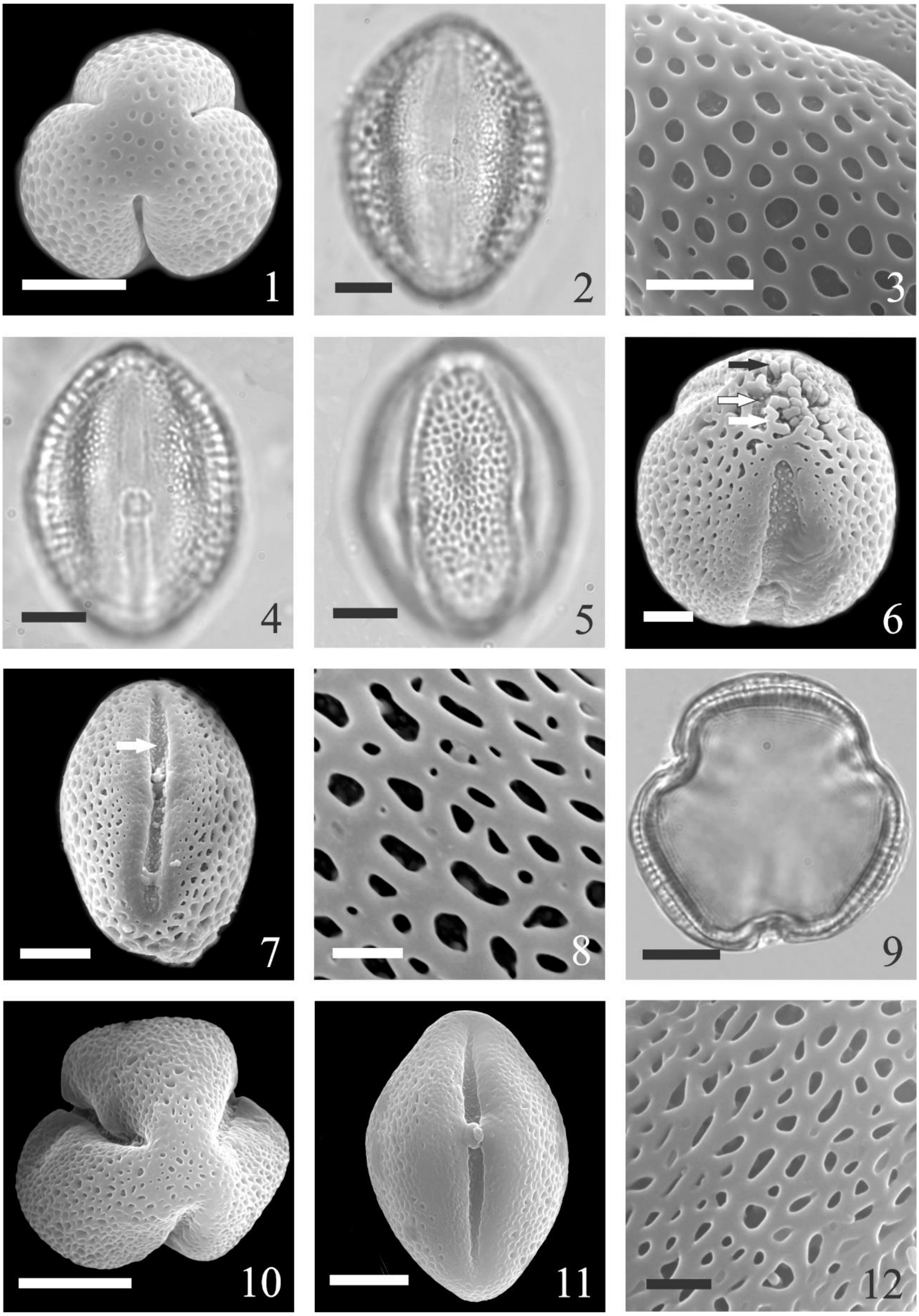


Plate 3. Photomicrographs and eletromicrographs of pollen grains. Tribe Parthenocisseae. *Parthenocissus inserta* – polar view: 1. general aspect, surface; equatorial view: 2. general aspect, aperture; 3. detail of the ornamentation. *P. quinquefolia* - equatorial view: 4. general aspect, aperture, 5 general aspect, surface. *P. tricuspidata* - polar view: 6. general aspect, discontinuous walls (arrow), lumen granules (arrow); equatorial view: 7. general aspect, aperture, ornate membrane (arrow); 8. detail of the ornamentation. *P. vitacea* - polar view: 9. optical section, 10. general aspect, surface; equatorial view: 11. general aspect, aperture; 12. detail of the ornamentation. Scale bar: 10 μm (1, 2, 4, 5, 9-11), 5 μm (6, 7), 2 μm (3, 8, 12).

3.2. Data analysis

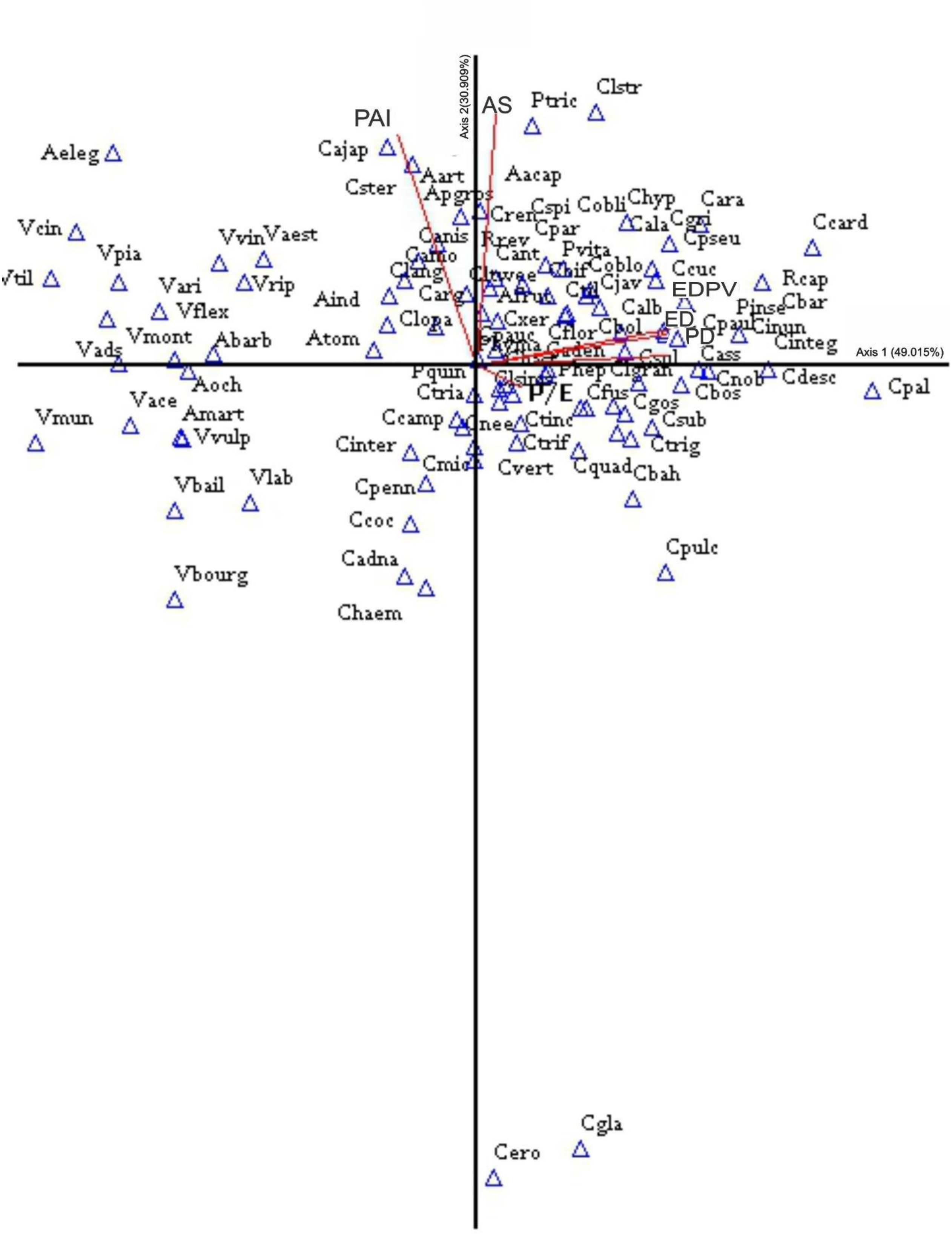
PCA explained 79.924% of the total variance in the dataset (Table 4). As shown by exploratory data analysis, the first principal component (PC) explained 49.015% of the total variance. The most significant variables along the positive axis were equatorial diameter in polar view, polar diameter, and equatorial diameter. The vectors equatorial diameter in polar view and equatorial diameter influenced *Cissus cardiophylla*, *C. descoingsii*, *C. palmata*, and *Rhoicissus capensis*; these species were far from the others because of their higher positive PC1 values, showing high similarity between each other. However, the vector equatorial diameter in polar view influenced the following species more markedly: *Cissus alata*, *C. albida*, *C. aralioides*, *C. assamica*, *C. bahiensis*, *C. barbeyana*, *C. boliviana*, *C. bosseri*, *C. cucurbitina*, *C. fusifolia*, *C. gossypiifolia*, *C. grisebachii*, *C. hypoglauca*, *C. integrifolia*, *C. inundata*, *C. javana*, *C. nobilis*, *C. obliqua*, *C. oblonga*, *C. paullinifolia*, *C. pseudofuliginea*, *C. pulcherrima*, *C. quadrangularis*, *C. spinosa*, *C. subrhomboidea*, *C. sulcicaulis*, *C. tiliacea*, *C. trigona*, *Clematicissus granulosa*, *C. striata*, and *Parthenocissus inserta* (Figure 1). The other vectors of the positive axis were not as significant, and the other species were dispersed along the axis (Table 4). Along the negative axis, the most significant vector was polar area index, but it had no significant effect on the evaluated species (Figure 1). *Ampelocissus barbata*, *A. elegans*, *A. martini*, *A. ochracea*, *Vitis acerifolia*, *V. adstricta*, *V. aestivalis*, *V. arizonica*, *V. baileyana*, *V. bourgaeana*, *V. cinerea*, *V. flexuosa*, *V. labrusca*, *V. monticola*, *V. munsoniana*, *V. piasezkii*, *V. riparia*, *V. tiliifolia*, *V. vinifera*, and *V. vulpina* had negative values for the significant variables of the positive axis (polar diameter, equatorial diameter, and equatorial diameter in polar view). Other species dispersed along the negative side of PC1 were less influenced by these vectors.

The second PC explained 30.909% of the variance in the dataset (Table 4). The most significant variables along the positive axis were apocolpium side and polar area index. The species *Ampelocissus acapulcensis*, *A. artemisiifolia*, *A. elegans*, *Ampelopsis grossedentata*, *Cayratia japonica*, *Cissus aralioides*, *C. cardiophylla*, *C. grisebachii*, *C. hypoglauca*, *C. sterculifolia*, *Clematicissus striata*, and *Parthenocissus tricuspidata* were influenced by the vector apocolpium side because they had the greatest PC2 values and high similarity (Figure 1). The position of the other species along the positive side of the PC2 axis was not strongly influenced by the vectors (Table 4). On the negative axis, the only representative vector was polar diameter/equatorial diameter. However, the vector was not significant for the species on the negative PC2 axis because it had a low cumulative index (Table 4). *Cissus erosa* and *C. glaucotricha* were polarized by the influence of the vector apocolpium side, as they showed the largest negative values for the variable. A similar behavior was observed for the species *Cissus adnata*, *C. bahiensis*, *C. coccinea*, *C. haematantha*, *C. penninervis*, *C. pulcherrima*, *Vitis baileyana*, *V. bourgaeana*, and *V. labrusca*, which were plotted close to each other because of the influence of the variable apocolpium side (Figure 1). Other species plotted on the negative side of PC2 were dispersed under the action of the vector apocolpium side.

Table 4. Cumulative variance and vector values of principal component analysis (PCA) using palynological characters from Vitaceae.

Principal Axis	**1**	**2**
Cumulative Variance (%)	49.015	30.909
Characters	**Axis**	
Polar Diameter (PD)	0.5560	0.0257
Equatorial Diameter (ED)	0.5499	0.0871
Equatorial Diameter in Polar View (EDPV)	0.5607	0.1030
Side of the apocolpo (SA)	0.0582	0.7294
Polar Area Index (PAI)	-0.2304	0.6670
Shape (P/E)	0.1324	-0.0653

Figure 1. Two-dimensional graph representing Vitaceae species in principal component analysis (PCA).



3.3. Pollen character states (Tables 5 and Appendix 2)

Using the phylogenetic tree and the distribution of character states among the observed (terminal) taxa, we reconstructed character states at ancestral nodes. The results are described below.

Size (Figure 2). Representatives of Vitaceae have medium-sized pollen grains, representing a plesiomorphic character state, shared with the ancestral state of the sister group Leeaceae. Large pollen size is an apomorphy in the clade *Cissus*, derived from medium-sized pollen; it arises independently in *Cissus aralioides* Planch. and *Cissus cardiophylla* Standl.

Shape (Figure 3). Pollen shape is a highly variable character in Vitaceae genera. Oblate-spheroidal, spheroidal, subprolate, prolate, and prolate spheroidal pollen grains occur in representatives of the family. Pollen grains with prolate spheroidal and subprolate shapes are a plesiomorphic condition in Vitaceae. Spheroidal pollen grains characterize an autapomorphy in *Cyphostemma bainesii* (Hook.f.) Desc., whereas oblate-spheroidal pollen represents an autapomorphy in *Ampelocissus ochracea* Merr. Prolate pollen grains are considered a synapomorphy in Vitaceae, derived from prolate spheroidal and subprolate pollen.

Ornamentation. In Vitaceae, pollen ornamentation patterns are highly diverse, particularly with regard to apocolpium and mesocolpium regions, with significant morphological elements in different regions.

Regarding the apocolpium region (Figure 4), microreticulate pollen represent a plesiomorphic condition in the clade *Cissus* and the species *Rhoicissus revoilii* and *Clematicissus opaca.* Pollen grains with reticulate apocolpium are an evolutionary trend in Vitaceae, characterized as a synapomorphy derived from microreticulate apocolpium. Reticulate-perforate ornamentation is an apomorphy that arose independently in *Cissus*, derived from microreticulate-perforate pollen grains. The diversification of ornamentation patterns in the apocolpium region can also be observed in autapomorphies in Vitaceae.

Bireticulate-perforate pollen grains are an autapomorphy in *Vitis* L. (*Vitis aestivalis* Michx.). An autapomorphy can also be observed in *Cissus verticillata* (L.) Nicolson & C.E.Jarvis, with perforate apocolpium, and in *Cissus*, with foveolate ornamentation, both derived from microreticulate grains. Pollen with rugulate-perforate ornamentation is a synapomorphy in the genus *Ampelocissus* Planch.

In the mesocolpium region (Figure 5), microreticulate ornamentation is a plesiomorphic condition shared with the sister group of Vitaceae, Leeaceae. Reticulate mesocolpium is a synapomorphy, derived from microreticulate pollen, representing an evolutionary trend in the family. Diversity in ornamentation patterns can also be observed in apomorphies and autapomorphies of character states. In *Vitis riparia* A.Gray, bireticulate ornamentation is an autapomorphy, derived from reticulate pollen grains. In *Cissus verticillata*, perforate ornamentation also represents an autapomorphy, which is derived from microreticulate pollen. The reticulate-perforate type emerged independently in *Cissus*, being derived from microreticulate pollen. Mesocolpium with foveolate ornamentation is also derived from the microreticulate type, characterizing an apomorphy in *Cyphostemma bainesii* and *Cissus tiliacea* Kunth. In *Vitis aestivalis*, there was a reversal to microreticulate ornamentation following a symplesiomorphic trend in pollen of Vitaceae and Leeaceae. In *Ampelocissus*, rugulate-perforate ornamentation is an apomorphy derived from reticulate pollen grains. Microreticulate-perforate pollen occurs in *Cissus trianae* Planch. and *Cissus neei* Croat, representing an apomorphy derived from microreticulate pollen.

Table 5. Matrix of pollen characters of Vitaceae species.

Species	**shape**	**size**	**mesocolpium**	**apocolpium**
Ampelocissus elegans	2	0	7	7
Ampelocissus ochracea	0	0	7	7
Ampelopsis grossedentata	3	0	2	3
Cissus adnata	1	0	5	5
Cissus anisophylla	3	0	2	2
Cissus aralioides	3	1	5	5
Cissus bahiensis	3	0	2	2
Cissus boliviana	1	0	2	2
Cissus campestri	1	0	2	2
Cissus cardiophylla	3	1	5	5
Cissus erosa	1	0	2	2
Cissus hastata	1	0	2	2
Cissus intermedia	3	0	5	5
Cissus javana	1	0	5	5
Cissus neei	3	0	3	3
Cissus obliqua	3	0	5	5
Cissus paulliniifolia	1	0	2	2
Cissus penninervis	2	0	5	6
Cissus pulcherrima	3	0	2	2
Cissus tiliacea	3	0	1	1
Cissus tinctoria	3	0	2	2
Cissus trianae	2	0	3	3
Cissus trifoliata	1	0	6	6
Cissus verticillata	1	0	4	4
Clematicissus angustissima	2	0	5	5
Clematicissus opaca	1	0	2	2
Parthenocissus quinquefolia	1	0	5	5
Rhoicissus revoilii	3	0	2	2
Vitis aestivalis	3	0	2	8
Vitis flexuosa	3	0	5	5
Vitis riparia	1	0	0	0
Vitis vulpina	3	0	5	5
Leea indica	3	0	2	2
Leea rubra	2	0	2	2
Cyphostemma bainesii	4	0	1	3
Parthenocissus henryana	1	0	5	5
Parthenocissus heptaphylla	1	0	5	5
Parthenocissus vitacea	1	0	5	5
Parthenocissus dalzieli	1	0	5	5
Parthenocissus tricuspidata	1	0	5	5
Yua thomsoni	1	0	5	5

Figure 2 - Evolutionary tracing of the size character state in the consensus tree of the phylogenetic hypotheses of Rodrigues et al. (2014) and Ma et al. (2021).

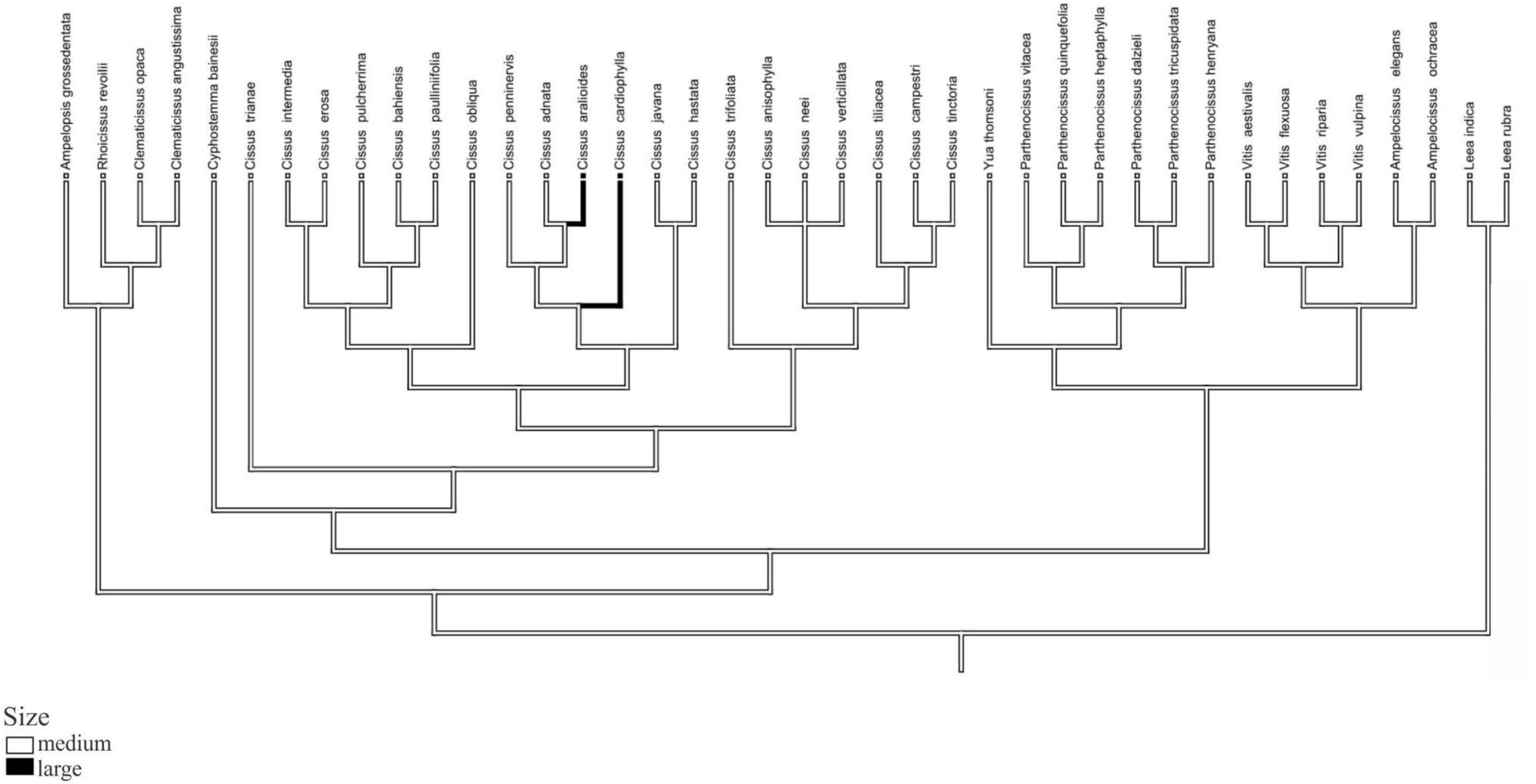


Figure 3. Evolutionary tracing of the shape character state in the consensus tree of the phylogenetic hypotheses of Rodrigues et al. (2014) and Ma et al. (2021).

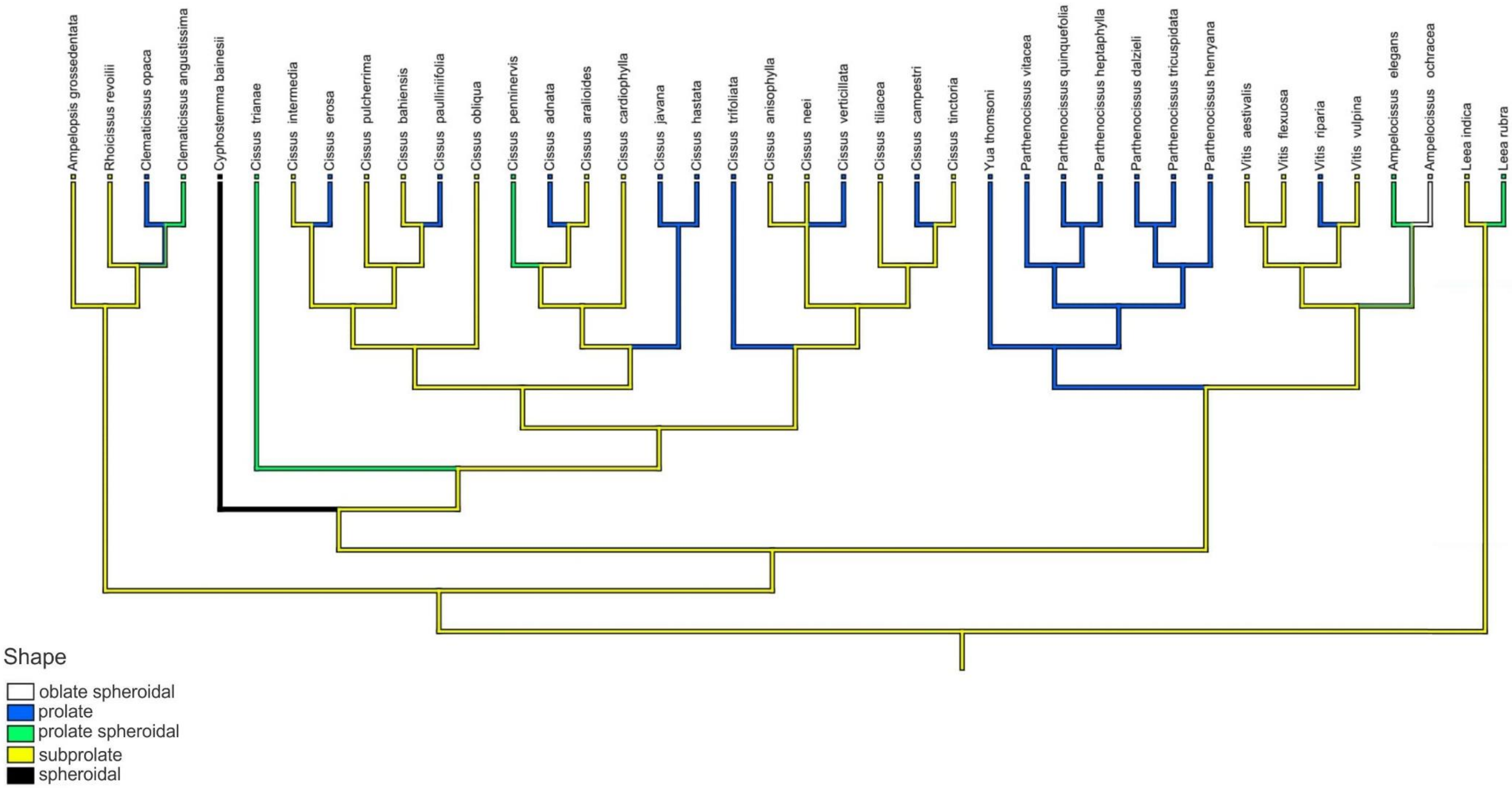


Figure 4. Evolutionary tracing of the apocolpium character state in the consensus tree of the phylogenetic hypotheses of Rodrigues et al. (2014) and Ma et al. (2021).

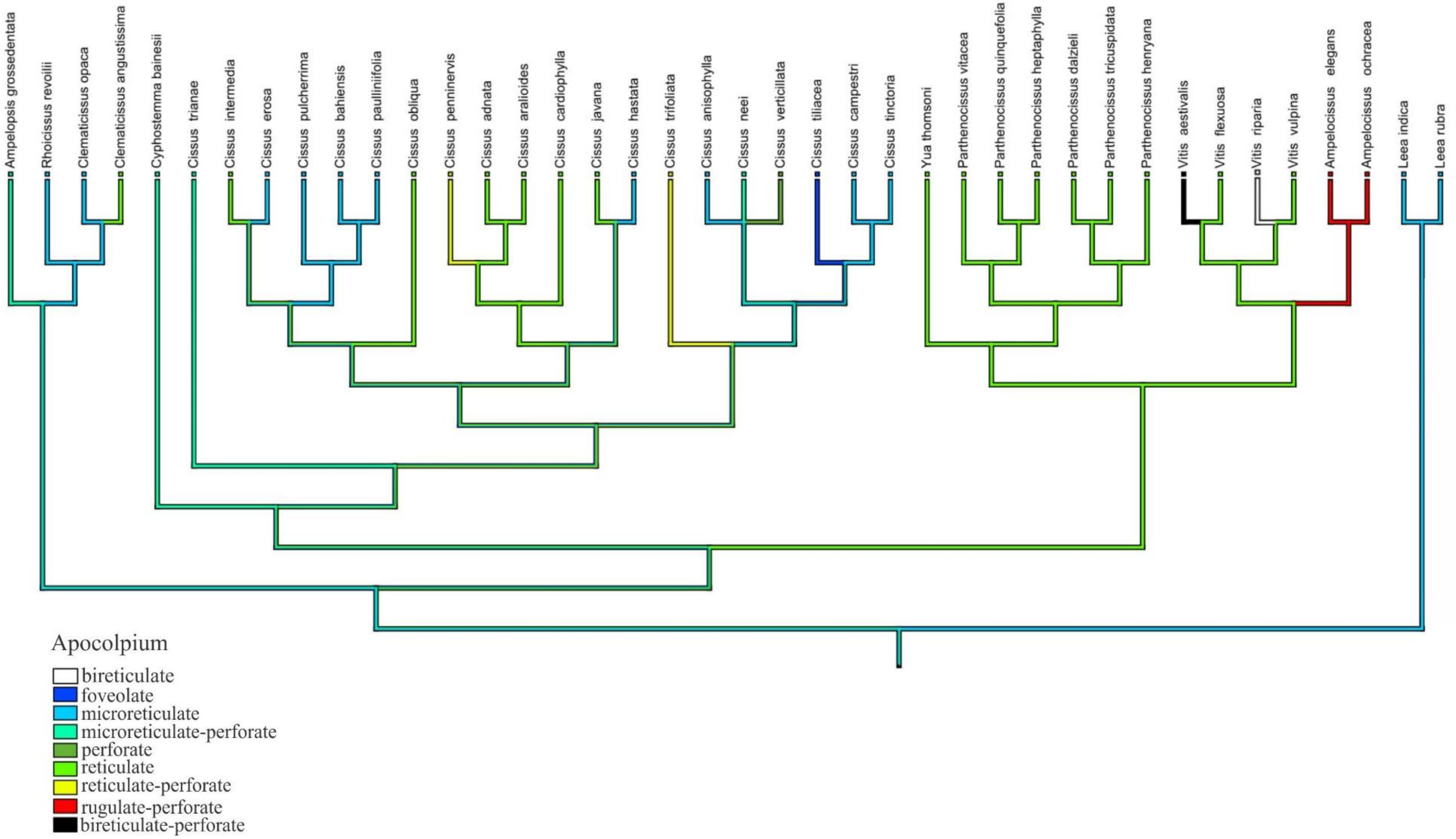
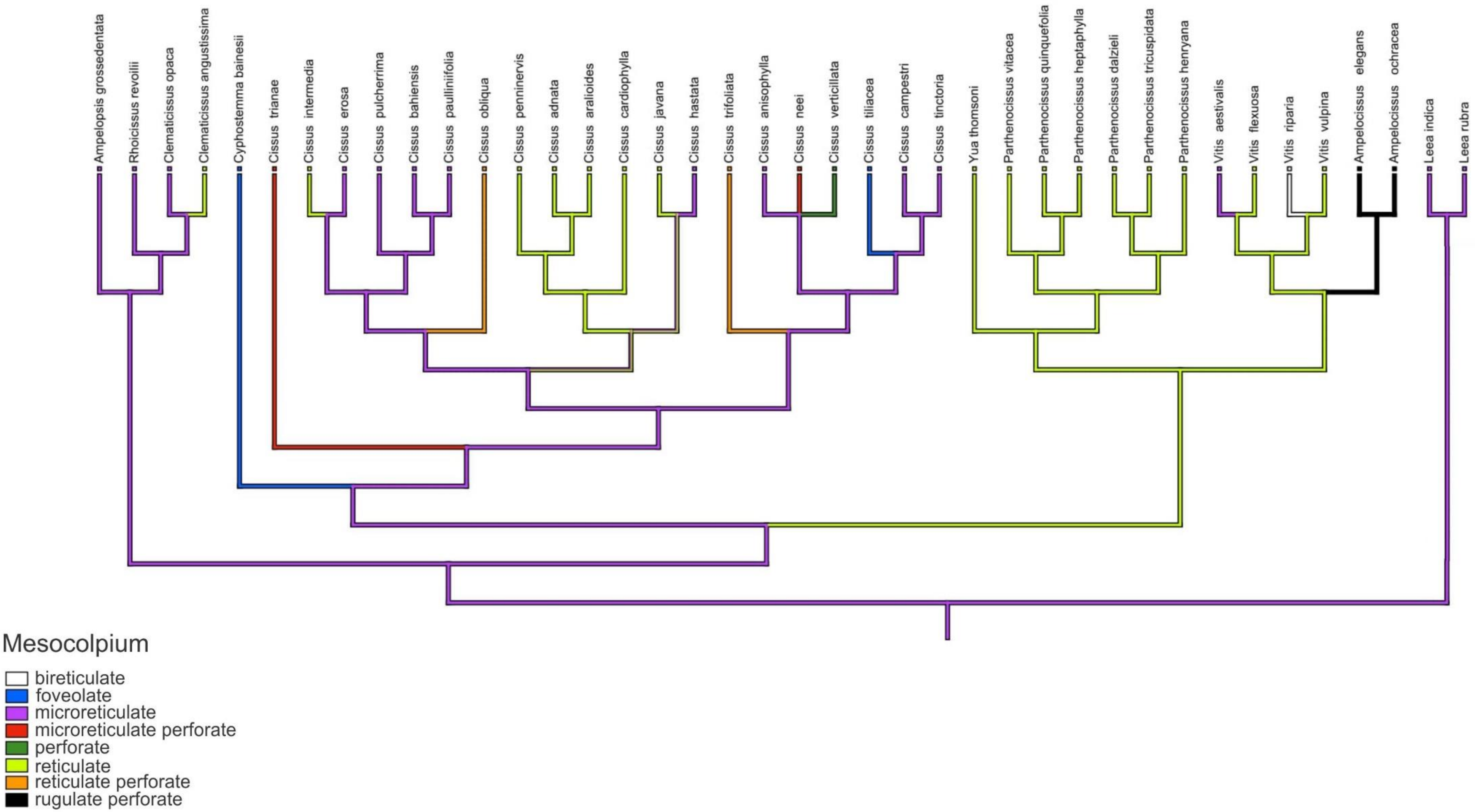


Figure 5. Evolutionary tracing of the mesocolpium character state in the consensus tree of the phylogenetic hypotheses of Rodrigues et al. (2014) and Ma et al. (2021).



4. Discussion

4.1. Pollen analysis

Analysis of species of the tribes Ampelopsideae, Cayratieae, and Parthenocisseae showed that pollen grains are homogeneous in terms of aperture, polarity, and size and heterogeneous in terms of shape and ornamentation.

The number and type of aperture observed here (3-colporate) has been described for the family in most studies (Huang 1967; Bonnefille and Riollet 1980; Filice 1981; Roubik and Moreno 1991; Chavez et al. 1996; Carreira and Barth 2003; Perveen and Qaiser 2008; Lu et al. 2012; Cartaxo-Pinto et al. 2017). Only Patil (2006) reported *Cissus* species with triporate pollen grains. In the current study, the genus was found to have only tricolporate pollen. Hesse et al. (2009) and Halbritter et al. (2018) stated that aperture number, type, and position are genetically determined characteristics and that no changes occur within the same species, although variations in aperture number may be observed.

According to Punt et al. (2007), polarity is related to tetrad orientation or aperture distribution. Here, we identified isopolar pollen grains. This characteristic was observed by Carreira and Barth (2003) and Perveen and Qaiser (2008), who carried out a general analysis of the family. Cartaxo-Pinto et al. (2017, 2021a in press) reported isopolar and heteropolar pollen in species of *Cissus* L. and *Vitis* L.

In analyzing pollen size, it is necessary to consider the measurement method used. As discussed by Walker and Doyle (1975), depending on the method of sample preparation, pollen size may be an unstable, reversible character. Pollen size is not only subject to natural variation, it may also vary depending on the degree of hydration and preparation method (Salgado-Labouriau et al. 1965; Hesse et al. 2009; Halbritter et al. 2018). This study confirms the variation in pollen size in Vitaceae: medium-sized pollen grains were observed in *Parthenocissus*, as also described by Lu et al. (2012); small to medium pollen were described by Huang (1967); Wang et al. (2000), and Cartaxo-Pinto et al. (2021b unpublished data); and medium (Perveen and Qaiser 2008; Patil 2006) and medium to large were reported in *Cissus* (Cartaxo-Pinto et al. 2017, 2021a in press).

Pollen shape varies greatly in Vitaceae, ranging from prolate-spheroidal to prolate (Huang 1967), subprolate (Filice 1981), oblate to perprolate (Wang et al. 2000), oblate-spheroidal and subprolate (Patil 2006), prolate-spheroidal to prolate (Perveen and Qaiser 2008), and oblate-spheroidal to prolate (Cartaxo-Pinto et al. 2017). In the *Parthenocissus* species studied by Lu et al. (2012), pollen grains were found to be prolate (most species), subprolate, and perprolate. In the present study, prolate pollen was observed in all *Parthenocissus* species.

Exine ornamentation is a very informative character, highly valuable for statistical and/or evolutionary analyses. Here, microreticulate ornamentation was the most common, followed by reticulate and foveolate. Previous reports of ornamentation patterns of the family show that this characteristic may vary greatly. Foveolate and reticulate pollen were recorded in *Parthenocissus* (Lu et al. 2012); foveolate, reticulate, microreticulate, perforate, and rugulate in *Cissus* (Filice 1981; Cartaxo-Pinto et al. 2017, 2021b unpublished data); reticulate and rugulate in *Vitis* (Wang et al. 2000); and perforate, reticulate, rugulate, microreticulate, and bireticulate in *Ampelocissus* and *Vitis* (Cartaxo-Pinto et al. 2021a in press). Furthermore, *Vitis* pollen were found to differ in ornamentation between poles. Other studies reported psilate, scabrous, reticulate, foveolate, and rugulate ornamentations in Vitaceae genera (Huang 1967; Roubik and Moreno 1991; Chavez et al. 1996; Carreira and Barth 2003; Perveen and Qaiser 2008).

4.2. Data analysis

Principal component analysis revealed that the most significant variables on the positive axis of PC1 were equatorial diameter in polar view, polar diameter, and equatorial diameter. These vectors allowed forming groups with similar characteristics, mainly influenced by equatorial diameter in polar view and equatorial diameter. One group comprised *Cissus cardiophylla*, *C. descoingsii*, *C. palmata*, and *Rhoicissus capensis*. Equatorial diameter in polar view greatly influenced *Cissus* species; however, it was not possible to delimit species of the genus. The high concentration of species belonging to the tribe Viteae on the negative side of PC1 is noteworthy (Wen et al. 2018); this finding can be attributed to vectors plotted along the positive PC1 axis. Apocolpium side was the vector that had the greatest similarity in the positive side of the PC2 axis; however, the character did not allow the formation of groups for genus delimitation. The PCA graph showed that most species were dispersed and that few taxa had similar quantitative characters.

4.3. Evolutionary history of pollen grains in Vitaceae

The family Vitaceae has about 950 species distributed in 16 genera (Ma et al. 2021). The taxonomic classification of the family and its phylogenetic relationships have been reorganized in recent years, currently including five tribes: Ampelopsideae, Cayratieae, Cisseae, Parthenocisseae, and Viteae (Wen et al. 2018). In this new reorganization, the monogeneric family Leeaceae was considered a sister to Vitaceae (Soltis et al. 2011). A reconstruction of phylogenetic relationships within the family was recently proposed by Ma et al. (2021), who used molecular data of nuclear genes and plastids based on the Hyb-Seq approach. In this new approach, cupular floral disc is an ancestral state, discrete floral disc is a derived state in the tribe Parthenocisseae, and adnate floral disc is a synapomorphy in the tribe

Viteae. Other character states, such as inflorescence type in Vitaceae and trichome type, provided important information and new synapomorphies for the group.

Although researchers have sought morphological contextualization based on molecular data, there is still no strongly supported approach for combining morphological and molecular sets in Vitaceae. Of the morphological characters contextualized by the authors (Ma et al. 2021), pollen grains have not been addressed in phylogenetic analyses. However, palynological studies of the family have made relevant contributions, given that palynological morphology is an important tool for taxonomic identification (Huang 1967; Reille 1967; Straka and Simon 1967; Bonnefille and Riollet 1980; Filice 1981; Melhem and Bissa 1985; Roubik and Moreno 1991); Chávez et al. 1996; Carreira and Barth 2003; Melhem et al. 2003; Patil 2006; and Cartaxo-Pinto et al. 2017).

According to Wortley and Scotland (2006), inferring morphological data on a molecular phylogenetic basis is a possible way to understand evolutionary aspects in the absence of a phylogeny combined with morphological and molecular data. As discussed by Gonçalves-Esteves et al. (2021), this phylogenetic hypothesis is supported by logical probability, not by statistical probability. Thus, we contextualized pollen morphology in Vitaceae using already established and well-supported kinship relationships.

In the current analysis, most pollen grains of Vitaceae representatives were medium-sized, this being considered a plesiomorphic character. Marinho (2017), in analyzing Sapindaceae, subordinated to the order Rosideae, in which Vitaceae is inserted, identified that medium pollen grains are an evolutionary trend in the family, derived from small pollen grains. According to Walker and Doyle (1975), pollen size is a reversible character in most angiosperms. By contrast, Doyle and Endress (2000) stated that pollen size is a continuous character. It should be noted that the preparation method and time between acetolysis and measurement might contribute to changes in size (Salgado-Labouriau et al. 1965). Although this character may vary according to the method used, as mentioned by the cited authors, it is necessary for species diagnosis (Halbritter et al. 2018).

Doyle (2005) reported that the spheroidal shape is a plesiomorphic condition directly related to pollen size. Yang et al. (2020), in investigating pollen evolution in Lamiids, observed a correlation between wet mesophytic habitat and oblate shape. In Vitaceae, morphological variations in *Vitis* were related to the different habitat types recorded by Ickert-Bond et al. (2018) in anatomical studies of leaves. There is no report of a relationship between habitat and such variations; however, Zhang et al. (2017) suggested a correlation between plant growth and pollen shape. Walker and Doyle (1975) explained that methodological differences in pollen preparation may lead to differences in shape. Nevertheless, the great diversity of pollen shapes in Vitaceae reveals that this character state is important to understand the evolutionary history of pollen morphology in the family.

Walker and Doyle (1975) showed that microreticulate ornamentation and its variations have been an evolutionary trend in most angiosperms. Given that such trends are the most probable, the observations of Yang et al. (2020) on the evolution of pollen grains in Lamiids may be applied to Vitaceae. The authors suggested that perforate and reticulate tecta were ancestral to other forms of ornamentation, such as striate, rugulate, striate-reticulate, and areolate, considered to be derived characters.

There is a significant correlation between ornamentation and the pollination type of plants (Yang et al. 2020). In Vitaceae, there are records of insects as pollinating agents (Branties 1978; Chen 2009). Perspectives on the evolution of pollination syndromes indicate a starting point to understand the evolution of ornamentation types in Vitaceae. A hypothesis for this great diversification in pollen sculpture might be related to coevolution between insects and Vitaceae species. Therefore, studies on floral and reproductive biology are necessary to establish new and important correlations for a better understanding of the evolution of pollen ornamentation and pollination syndromes.

5. Conclusion

Palynological analysis showed that species representing the different tribes of Vitaceae display a pattern of aperture number and type, pollen size, shape, and ornamentation that is consistent within the family, even though there is a great diversity of pollen shapes and ornamentations. The analyzed data indicated that it is not possible to separate Vitaceae tribes based on pollen characters. Multivariate analysis of species described in the current and previous studies showed that pollen diameters were significant but did not form groups related to the tribes. PCA was only informative for the tribe Viteae. The reconstruction of the evolutionary history of pollen grains using existing phylogenies (Rodrigues et al. 2014; Ma et al. 2021) showed that palynological data for the family are poorly explored in phylogenetic analyses. However, the results obtained contribute to increase the knowledge of pollen in Vitaceae, offering data for future phylogenetic studies. This analysis is the first attempt to track the evolution of pollen characters in Vitaceae.

Specimens investigated

Ampelopsis grossedentata (Hand.-Mazz.) WTWang – China, Mt. Mangshan, Yizhang Co., Hunan Prov., Xiao Bai-Zhong 3679, 09/VII/2004 (MBM 312934)

Cayratia japonica (Thunb.) Gagnep – Australia, Queensland, Bluewater Forestry, N of Townsville, B. Jackes, 24/IX/1978, (JCT).

Clematicissus angustissima (F.Muell.) Planch. – Australia, N of Binnu, WA. E. M. Jackes, 30/I/1982 (JCT).

Clematicissus granulosa (Ruiz & Pav.) Lombardi – Peru, Junín, Prov. Tarma, Dist. Huasahuasi, Carpapata, Nuñez, O. V. 3439, 16/XII/1951 (US2121613).

Clematicissus opaca (F.Muell.) Jackes e Rossetto -Australia, Queensland, Brisbane, Rchb., no date (MBM 102357).

Parthenocissus heptaphylla (Planch.) Britton – USA, Texas, Harris, B. B. 703, no date (US1466815).

Parthenocissus himalayana (Royle) Planch. – China, Xizang Prov., Yigong, Tibet-MacArthur 2820, J. Wen, Z. Nie, L Xie, Y. Nio, G. Li, F. Yang, S. Lutz, J. Von de Veire, 25/VI/2009 (US3560060).

Parthenocissus inserta (A. Kern.) Fritsch – USA, New York, Bronx, John Alcian 14, 14/VII/2015 (NY).

Parthenocissus quinquefolia (L.) Planch. – USA, Tenesse, McMinn Co., J. Wen 120024, 25/VI/2011 (US3666116).

Parthenocissus tricuspidata (Siebold & Zucc.) Planch. – Brazil, Paraná, Curitiba, A. Dunaiski Jr 10, 20/XII/1990 (BHCB 37613).

Parthenocissus vitacea (Knerr) Hitchc. – USA, Coconino, T. F. Daniel 1596, 26/VI/1981 (NY)

Rhoicissus capensis Planch. -South /africa, /Transvaal, Farm Rustfontein, Umgebung, H. J. Schlieben 7551 (US2272381)

Rhoicissus reviolii Planch. – Kenya, Coast Arabuko, Sokoke Fr, Mida track, Robertson S. A. 6960, 20/VI/1994 (US3343847).

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Appendix 1. Matrix of quantitative (metric) data in Vitaceae.

Species	Abreviation	PD	ED	EDPV	AS	PAI	P/E
Ampelocissus acapulcensis	Aacap	34.8	36.1	34.2	8.2	0.24	0.96
Ampelocissus artemiisifolia	Aart	34.5	34.6	33.5	8.2	0.24	1.0
Ampelocissus barbata	Abarb	26.3	25.7	25.7	5.7	0.22	1.02
Ampelocissus elegans	Aeleg	24.8	23.8	23.0	8.5	0.37	1.04
Ampelocissus frutecens	Afrut	39.6	33.6	31.7	6.5	0.2	1.17
Ampelocissus indica	Aind	32.9	31.0	30.2	6.2	0.21	1.06
Ampelocissus martini	Amart	26.4	24.5	23.2	4.5	0.19	1.08
Ampelocissus ochracea	Aoch	24.9	24.9	25.5	5.5	0.21	1.0
Ampelocissus tomentosa	Atom	31.0	30.2	31.0	6.0	0.19	1.02
Ampelopsis grossedentata	Apgros	39.0	29.9	30.2	7.7	0.26	1.3
Cayratia japonica	Cajap	33.4	31.3	31.2	9.4	0.3	1.07
Cissus adenocaulis	Caden	41.0	34.5	33.5	5.7	0.17	1.19
Cissus adnata	Cadna	38.2	26.0	27.2	3.2	0.12	1.47
Cissus alata	Cala	44.0	37.3	39.7	7.7	0.19	1.17
Cissus albida	Calb	45.4	35.5	36.2	6.5	0.18	1.27
Cissus amoena	Camo	36.9	28.1	30.0	7.2	0.24	1.31
Cissus anisophylla	Canis	38.7	33.1	27.0	7.0	0.26	1.17
Cissus antartica	Cant	37.1	34.2	33.7	7.2	0.21	1.08
Cissus aralioides	Cara	50.3	38.1	38.7	8.4	0.22	1.32
Cissus arguta	Carg	43.4	28.8	29.0	7.0	0.24	1.5
Cissus assamica	Cass	48.3	36.9	37.5	5.8	0.15	1.31
Cissus bahiensis	Cbah	44.0	36.3	36.2	4.1	0.11	1.21
Cissus barbeyana	Cbar	56.5	38.2	36.0	6.5	0.18	1.48
Cissus biformifolia	Cbif	40.8	35.3	34.0	7.0	0.2	1.15
Cissus boliviana	Cbol	47.9	34.1	35.0	6.2	0.18	1.4
Cissus bosseri	Cbos	46.5	36.1	36.7	5.0	0.14	1.29
Cissus campestri	Ccamp	39.8	29.6	30.5	4.6	0.15	1.34
Cissus cardiophylla	Ccard	54.7	42.0	43.0	8.1	0.19	1.3
Cissus coccinea	Ccoc	39.6	28.4	25.6	3.7	0.14	1.39
Cissus cucurbitina	Ccuc	44.1	40.3	40.5	7.0	0.17	1.09
Cissus descoingsii	Cdesc	50.3	41.2	41.5	6.0	0.14	1.22
Cissus erosa	Cero	40.4	29.2	25.0	0.0	0.0	1.32
Cissus floribunda	Cflor	43.5	31.8	33.2	5.9	0.18	1.37
Cissus fusifolia	Cfus	45.6	34.1	35.2	5.5	0.15	1.33
Cissus glaucotricha	Cgla	37.5	31.9	33.6	0.0	0.0	1.17
Cissus gossypiifolia	Cgos	46.0	35.6	35.2	5.2	0.15	1.29
Cissus grisebachii	Cgri	49.8	36.7	37.0	8.0	0.22	1.36
Cissus haematantha	Chaem	34.8	29.7	29.7	3.0	0.1	1.17
Cissus hastata	Chast	41.6	30.9	31.7	5.6	0.18	1.34
Cissus hypoglauca	Chyp	44.4	36.4	38.0	8.5	0.22	1.21
Cissus integrifolia	Cinteg	50.4	36.5	38.0	6.0	0.16	1.38
Cissus intermedia	Cinter	37.1	27.9	28.7	4.7	0.16	1.33
Cissus inundata	Cinun	45.9	38.2	39.5	6.5	0.16	1.2
Cissus javana	Cjav	46.7	33.6	34.5	7.0	0.2	1.39
Cissus microcarpa	Cmic	40.6	29.7	30.0	4.7	0.16	1.37
Cissus neei	Cnee	38.5	30.9	30.2	5.0	0.16	1.24
Cissus nobilis	Cnob	48.9	38.1	39.0	6.0	0.15	1.28
Cissus obliqua	Cobli	45.1	35.0	34.5	7.0	0.21	1.29
Cissus oblonga	Coblo	38.9	36.6	36.6	6.7	0.18	1.06
Cissus palmata	Cpal	54.8	45.8	45.0	5.7	0.12	1.19
Cissus paraensis	Cpar	44.1	34.0	33.7	7.5	0.22	1.29

Cissus paucinervia	Cpauc	37.2	31.5	33.0	6.0	0.18	1.18
Cissus paulliniifolia	Cpaul	47.7	35.6	38.0	6.7	0.17	1.34
Cissus penninervis	Cpenn	35.0	30.6	30.2	4.1	0.14	1.14
Cissus pseudofuliginia	Cpseu	46.1	37.4	37.9	7.5	0.19	1.23
Cissus pulcherrima	Cpulc	47.6	36.1	35.5	3.2	0.09	1.31
Cissus quadrangularis	Cquad	40.5	35.4	35.5	4.7	0.13	1.14
Cissus reniformis	Cren	44.5	30.6	29.1	7.0	0.24	1.45
Cissus spinosa	Cspi	39.6	34.7	35.7	7.6	0.21	1.14
Cissus sterculifolia	Cster	33.4	33.1	32.0	9.0	0.28	1.0
Cissus subrhomboidea	Csub	42.4	36.6	36.7	5.0	0.13	1.15
Cissus sulcicaulis	Csul	44.2	37.4	37.0	5.7	0.15	1.18
Cissus tiliacea	Ctil	41.5	35.0	35.0	6.7	0.19	1.18
Cissus tinctoria	Ctinc	39.6	34.2	32.1	5.0	0.15	1.16
Cissus trianae	Ctria	33.7	33.0	33.0	5.0	0.15	1.02
Cissus trifoliata	Ctrif	40.9	29.3	32.5	5.5	0.17	1.39
Cissus trigona	Ctrig	44.7	36.0	36.0	5.0	0.13	1.24
Cissus verticillata	Cvert	42.8	25.4	33.9	5.4	0.15	1.68
Cissus xerophyla	Cxer	37.9	32.0	33.6	6.2	0.18	1.18
Clematicissus angustissima	Clang	32.7	32.2	30.0	6.7	0.22	1.02
Clematicissus granulosa	Clgran	38.0	37.9	37.5	5.1	0.14	1.0
Clematicissus opaca	Clopa	39.8	28.1	29.5	6.5	0.22	1.42
Clematicissus simsiana	Clsims	38.2	32.6	34.0	5.5	0.16	1.17
Clematicissus striata	Clstr	38.7	40.2	39.0	10.5	0.26	0.96
Clematicissus tweediana	Cltwee	39.1	32.7	31.5	6.5	0.21	1.19
Parthenocissus heptaphylla	Phep	48.8	30.3	32.0	5.5	0.17	1.61
Parthenocissus himalayana	Phyma	41.5	31.2	31.0	5.5	0.18	1.33
Parthenocissus inserta	Pinse	48.2	36.3	37.2	6.5	0.18	1.33
Parthenocissus quinquefolia	Pquin	43.0	28.4	29.2	5.5	0.19	1.51
Parthenocissus tricuspidata	Ptric	45.4	31.6	32.7	10.2	0.31	1.44
Parthenocissus vitacea	Pvita	45.7	34.2	34.0	7.0	0.21	1.34
Rhoicissus capensis	Rcap	48.0	44.0	42.1	7.3	0.17	1.1
Rhoicissus revoilii	Rrev	39.9	33.2	34.1	7.2	0.21	1.2
Vitis acerifolia	Vace	23.8	23.7	22.7	4.6	0.2	1.0
Vitis adstricta	Vads	25.8	20.6	22.2	5.5	0.25	1.25
Vitis aestivalis	Vaest	33.2	25.0	24.5	7.2	0.29	1.32
Vitis arizonica	Vari	28.8	22.2	22.0	6.2	0.28	1.29
Vitis baileyana	Vbail	25.9	24.3	22.7	3.6	0.16	1.06
Vitis bourgeana	Vbourg	25.4	24.4	22.2	2.7	0.12	1.04
Vitis cinerea	Vcin	25.9	20.4	20.7	7.2	0.35	1.27
Vitis flexuosa	Vflex	25.8	21.4	21.5	6.0	0.28	1.2
Vitis labrusca	Vlab	30.2	23.5	24.7	3.9	0.16	1.28
Vitis monticola	Vmont	27.8	22.9	23.0	5.6	0.24	1.22
Vitis munsoniana	Vmun	21.0	21.4	20.2	4.2	0.21	0.98
Vitis piasezkii	Vpia	26.4	21.2	22.2	6.6	0.3	1.24
Vitis riparia	Vrip	33.2	23.9	23.5	6.7	0.29	1.39
Vitis tiliifolia	Vtil	25.6	17.7	20.2	6.7	0.33	1.44
Vitis vinifera	Vvin	30.1	24.5	24.2	7.0	0.29	1.23
Vitis vulpina	Vvulp	27.5	23.2	23.0	4.5	0.2	1.18

Appendix 2. Palynological data - bibliographical references. PD – polar diameter; ED – equatorial diameter; EDPV – equatorial diameter in polar view; AS- apocolpium side; PAI- polar area index; P/E polar / equatorial ratio.

Outgroup - genera/species	Palynological data source
Leeaceae	
Leea indica (Burm. f.) Merr.	Nair et al. (2018)
Leea rubra Blume ex Spreng.	
Vitaceae – genera/species	
Cyphostemma bainesii (Hook.f.) Desc.	Halbritter and Buchner (2016)
Parthenocissus dalzieli Gagnep.	Lu et al. (2012)
Parthenocissus henryana (Hemsl.) Graebn. ex Diels & Gilg	
Yua thomsoni (M.A. Lawson) C.L. Li	

CONSIDERAÇÕES FINAIS

O trabalho apresentado foi um dos mais abrangentes sobre a morfologia polínica da família Vitaceae. Buscando representar a maioria das espécies na família, analisando os dados para que fosse possível classificar os gêneros e as tribos de acordo com os caracteres mostrados.

A palinologia mostrou que os grãos de pólen de Vitaceae são homogêneos quanto à unidade de dispersão (mônades), quanto ao tipo e número de abertura (3-colporados), no entanto os grãos de pólen são heterogêneos quanto ao tamanho, à forma, à polaridade e à ornamentação. O tipo de ornamentação é a característica mais relevante, mostrando que as espécies podem apresentar o mesmo tipo de ornamentação em toda a superfície ou podem ter ornamentações diferentes no apocolpo e no mesocolpo. Essa variedade nos caracteres morfológicos indicam o caráter euripolínico para a família.

A forma e o tamanho apresentaram uma variação entre os gêneros. Apenas em *Parthenocissus* a forma prolata foi identificada para todas as espécies. Tanto a forma quanto o tamanho são características que podem ser alteradas de acordo com a metodologia de preparo, com isso podem sofrer alterações em suas medidas. No entanto, nesse estudo a metodologia e o tempo para a obtenção das medidas se mantiveram dentro do estabelecido por Salgado Labouriau (1973).

A polaridade é um caráter estabelecido geneticamente, quando os grãos de pólen ainda se apresentam em sua condição de tétrade. Quando se analisou essa característica observou-se que *Cissus adnata*, *C. assamica*, *Vitis arizonica e V. piasezkii* apresentaram heteropolaridade e as outras espécies foram descritas como isopolares. A heteropolaridade nas espécies de *Cissus* foi determinada pela parasincolpia, enquanto nas espécies de *Vitis* foi marcada por ornamentação diferente nos pólos.

A partir do que foi descrito sobre o tipo de ornamentação da exina em Vitaceae, foi possível organizar as espécies em grupos. Os grupos formados foram: grupo 1 com espécies em que a superfície apresenta o mesmo padrão de ornamentação, grupo 2 com espécies que possuem dois padrões de ornamentação (apocolpo e mesocolpo) e um terceiro grupo onde as espécies têm ornamentação diferente nos pólos e no mesocolpo.

O grupo 1 engloba os gêneros *Ampelocissus, Cayratia,* maioria de espécies de *Cissus, Clematicissus, Parthenocissus, Rhoicissus* e *Vitis baileyana* , *V. flexuosa, V. munsoniana* e *V. vulpina*. Dentro desse grupo pode-se formar subgrupos, pois os grãos de pólen apresentam mais de um elemento em sua ornamentação, podendo ser: foveolado com perfuração (*Cissus tiliacea, Parthenocissus inserta*); microrreticulado com perfuração (*Ampelopsis grossedentata, Cissus pulcherrima, C. sterculifolia, Clematicissus opaca, Vitis munsoniana*); perfurado-estriado (*Cissus floribunda*); reticulado com estrias (*Ampelocissus tomentosa*); reticulado com microespinhos (*Ampelocissus frutescens*); reticulado-estriado com perfuração (*Cissus adnata, C. bosseri, C. obliqua*); reticulado com perfuração (*Ampelocissus artemisiifolia, A. barbata, A. indica, A. martini, Cissus aralioiodes, C. arguta, C. grisebachii, C. javana, C. oblonga, C. penninervis, Clematicissus angustissima, C. granulosa, Parthenocissus tricuspidata, P. vitacea*); rugulado com perfuração (*Ampelocissus elegans, A. ochracea, Cissus haematantha*); rugulado-reticulado (*Cissus assamica*).

O segundo grupo reuniu espécies com os grãos de pólen onde a superfície exibe ornamentação em regiões diferentes dos grãos de pólen. Essa característica foi observada, principalmente, no gênero *Vitis*, e visualizado em *Cissus floribunda* e *C. microcarpa*. A partir desse tipo de ornamentação foi possível a formação de subgrupos que é descrito a seguir: microrreticulado no mesocolpo e reticulado no apocolpo (*Vitis acerifolia, V. labrusca, V. monticola*); microrreticulado no mesocolpo e birreticulado no apocolpo (*Vitis adstricta, V. aestivalis*); rugulado no mesocolpo e reticulado no apocolpo (*Vitis cinerea, V. tiliifolia*);

rugulado no mesocolpo e birreticulado no apocolpo (*Vitis bourgeana*), birreticulado no mesocolpo e reticulado no apocolpo (*Vitis riparia, V. vinifera*); estriado no mesocolpo e reticulado no apocolpo (*Cissus microcarpa*).

A ornamentação descrita para o terceiro grupo aparece com características marcantes para essas espécies de *Vitis*, pois é possível observar que a superfície dos pólos são diferentes e ainda apresentam o mesocolpo com outro padrão de ornamentação. Essas espécies são separadas da seguinte forma: microrreticulada no mesocolpo e reticulada nos pólos, com um dos pólos apresentando o reticulo elevado (*Vitis piasezkii*); rugulada no mesocolpo e reticulada nos pólos, e um dos pólos apresentando elementos isolados e resquícios de muro (*Vitis arizonica*).

Com base nesse amplo estudo em Vitaceae observou-se particularidades em alguns gêneros: em *Cissus* as aberturas de algumas espécies apresentam caracteres informativos como mesoabertura (*C. cucurbitina*), presença de área apertural (*C. hypoglauca*), constricção mediana na endoabertura (*C. grisebachii* e *C. tiliacea*) e presença de costa (*C. adnata, C. anisophylla, C. arguta, C. assamica, C. bosseri, C. floribunda, C. gossypiifolia, C. intermedia, C. javana* e *C. obliqua*). Em *Vitis* observa-se que o padrão da sexina variou de acordo com as regiões (apocolpo e mesocolpo) dos grãos de pólen.

Quando se observa a análise multivariada onde foram reunidas as espécies analisadas neste trabalho e as do estudo anterior (Cartaxo-Pinto et al. 2017), constatou-se que os gêneros não se agrupam, somente *Vitis* e *Ampelocissus* estabelecem certa proximidade. Identificou-se que os dados quantitativos são mais informativos quando realizada a análise do componente principal.

A história evolutiva dos grãos de pólen em Vitaceae foi a primeira tentativa desse tipo de análise na família. Os resultados aqui fornecidos podem ser inseridos em trabalhos futuros, contribuindo com a taxonomia da família.

O estudo palinológico em Vitaceae apresentou uma grande diversidade, principalmente, quando analisou-se a ornamentação. Entretanto, somente, a morfologia polínica não se mostrou útil para resolver a organização taxonômica da família.