UNIVERSIDADE DE SÃO PAULO
FFCLRP - DEPARTAMENTO DE BIOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA COMPARADA

Tétrades e políades de Mimosa L. (Leguminosae): Um olhar sobre a diversidade e evolução do pólen

Tetrads and polyads in Mimosa L. (Leguminosae): An overview on pollen diversity and evolution

STEFANY LIAU KANG GOICOCHEA

Dissertação apresentada à Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto-USP, como parte das exigências para obtenção do título de Mestre em Ciências - Área: Biologia Comparada

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Orientador: Leonardo Maurici Borges

To my parents, my sister and my children Emma and Isaac.
My eternal gratitude and love.

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ABSTRACT

Mimosa L. has more than 500 species around the world, which have successfully adapted to the most varied ecosystems in their different forms of life, most of which are endemic. Despite the existence of innumerable studies at the macroscopic level, pollen morphology of *Mimosa* is known only to one-fourth of its more than 500 species (27.2%). The species already studied present variability as for the dispersion units, grain organization, ornamentation, and size, mainly. This work was divided in two parts: first, we studied the pollen of *Mimosa* species to understand if we have already reached the limits of known pollen diversity in the genus. Second, we mapped the most complete pollinic dataset to date over *Mimosa*'s phylogeny to understand how pollen evolved in the genus. Although the pollen of more than 400 species is still unknown, our work increased knowledge on the morphology of *Mimosa* pollen, and filled existing gaps related to its evolution.

KEYWORDS: Fabaceae, Mimosoid, Palynology, Pollen evolution, Piptadenia group

RESUMO

Mimosa L. possui mais de 500 espécies em todo o mundo, que se adaptaram com sucesso aos mais variados ecossistemas em suas diferentes formas de vida, a maioria das quais endêmicas. Apesar da existência de inúmeros estudos em nível macroscópico, a morfologia do pólen de *Mimosa* é conhecida apenas por um quinto de suas mais de 500 espécies (27,2%). As espécies já estudadas apresentam variabilidade quanto às unidades de dispersão, organização do grão, ornamentação e tamanho, principalmente. Este trabalho foi dividido em duas partes: na primeira, estudamos o pólen de espécies de *Mimosa* para entender se já atingimos os limites da diversidade pólen conhecida no gênero. Em segundo lugar, mapeamos o conjunto de dados polínicos mais completo até o momento sobre a filogenia de *Mimosa* para entender como o pólen evoluiu no gênero. Embora o pólen de mais de 400 espécies ainda seja desconhecido, nosso trabalho aumentou o conhecimento sobre a morfologia do pólen de *Mimosa*, e preencheu lacunas existentes relacionadas à sua evolução.

PALAVRAS-CHAVE: Fabaceae, Mimosoideae, Palinologia, Evolução do pólen, Grupo Piptadenia

OVERVIEW

Pollen grains organized in compound arrangements occur in approximately 40 plant families (Harder & Johnson, 2008). Among them, the Leguminosae stands out as one of the most diverse (Erdmant, 1945; 1952; Salgado-Labouriau, 1973; Silvestre-Capelato & Melhem, 1997). However, tetrads and polyads are in general restricted to the mimosoid legumes (Caesalpinioideae; VanCampo & Guinet, 1961; Guinet, 1969; Caccavari, 1985; Guinet & Ferguson, 1989; Jumah 1991; Guinet & Caccavari, 1992; Medina-Acosta et al., 2018), which includes ca. 17% of the family's diversity (ca. 3300 species; LPWG 2017). More specifically, the genus *Mimosa* L. has tetrads, bitetrads (8 cells) and polyads up to 12 cells (Sorsa 1969; Flores-Cruz et al., 2006; Lima et al., 2008) which also vary in respect to their relative organization (uniplanar, multiplanar or irregularly arranged), ornamentation (Flores-Cruz et al., 2006; Lima et al., 2008; Santos-Silva et al., 2013), and size (El Ghazali, 1997; Guinet 1969; Erdtman, 1971; Guinet & Caccavari, 1992; Silvestre-Capelato & Melhem, 1997; Caccavari, 2002; Da Luz et al., 2013). However, the pollen of many species within the genus is still unknow.

Besides being a useful tool in Taxonomy (Jiang et al., 2019; Melhem et al., 2003; Rico and Banks 2001; Santos-Silva,2013), pollen morphology can reveal patterns of the evolutionary history of species. However, it is important to acuratelly characterize pollen variation, particularly in groups with high structural and morphological variability (Caccavari, 2010), such as *Mimosa*.

CHAPTER I: **Pollen Morphology of *Mimosa* L.**

Mimosa Pollen Morphology

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Abstract:

The pollen morphology of the Leguminosae is widely diverse. However, it is also poorly known. For example, in the genus *Mimosa*, pollen was described for only one-fifth of its more than 500 species. The taxa already studied vary in dispersion units, grain disposition, ornamentation, and size. In this work, we described 45 *Mimosa* species (53 samples and for the first-time morphology of 39 species). The samples were studied under light and scanning electron microscopy. *Mimosa* presents tetrads as the main dispersion unit (4 cells), in smaller amounts of 8 cells and rarely the polyads of 12 to 16 cells. But also, varying in the disposition of cells into the dispersion unit and size of tetras/polyads. The ornamentation of the exine may be areolate or reticulated.

Keywords:

Palynology, *Mimosa*, Leguminosae, Fabaceae

Introduction:

The wide taxonomic diversity of the Leguminosae, which includes more than 19,000 species (Lewis et al. 2005, LPWG 2017) is also seen in its morphology (Barneby, 1991; Simon et al., 2011). However, even though the family's macromorphology has been extensively showcased in taxonomic works, we still lack knowledge on the phenotypic diversity of its microscopic traits, such as pollen. Although this diversity is seen also for pollen grains, most taxa have monads (Erdmant, 1945;1952; Silvestre-Capelato & Melhem, 1997, Salgado-Labouriau, 1973) and compound dispersal unities, in the form of tetrads and polyads. Among these features, aggregated pollen grains, a relatively rare condition in Angiosperms (Harder & Johnson, 2008), are mostly restricted to the Mimosoid clade (Caesalpinioideae), in which they predominate (VanCampo & Guinet, 1961; Guinet 1969; Caccavari, 1985; Guinet & Ferguson, 1989; Jumah 1991; Guinet & Caccavari, 1992; LPWG, 2017; Medina-Acosta et al. 2018). Within the clade, grains are aggregated in tetrads or polyads with 8, 12 or 16 cells, which also vary in respect to their relative organization (uniplanar, multiplanar or irregularly arranged), and other traits (Walker and Doyle 1975; Banks et al., 2010; Banks & Rudall 2016;). However, the pollen of many species within the clade is still unknown. For example, while the pollen of most *Stryphnodendron* Mart. (ca. 30 species; Luckow, 2005) have been documented (Guinet & Caccavari 1992; Caccavari 2002), we still don't have data for ca. 75% of *Mimosa*'s more than 500 species (Flores-Cruz 2006; Lima et al. 2008; Buril et al. 2010; Santos-Silva et al.2013; Cruz et al.2018; Medina-Acosta et al. 2018).

From what we already know, *Mimosa* has pollen grains dispersed in tetrads, mainly organized in uniplanar (rhomboidal or tetragonal) or multiplanar tetrads (tetrahedral or decussated), and 8, 12- or more rarely 16-celled polyads (Banks & Lewis, 2018; Buril, 2010; Doyle and Luckow 2003; Guinet, 1981; Harder and Johnson 2008; Jumah, 1991; Santos & Alves, 2010; Silvestre-Capelato & Melhem, 1997; Flores-Cruz, 2006; Lima et al. 2008). The exine is areolate, microverrucate or verrucae (Flores-Cruz et al. 2006; Lima et al. 2008; Santos-Silva et al. 2013), and size ranges from small to medium (El Ghazali, 1997; Guinet 1969; Erdtman, 1971; Guinet & Caccavari, 1992; Silvestre-Capelato & Melhem, 1997; Caccavari, 2002; Da Luz et al., 2013). At the same time, the genus shows relatively high levels of polymorphism. For example, some species may have up to 3 different types of organization (El Ghazali 1997, Sorsa 1969).

As a biologically important trait, pollen may reveal patterns of the evolutionary history of species and is, thus, an important information source for plant systematics (Jiang et al. 2019; Melhem et al. 2003; Rico and Banks 2001; Santos-Silva, 2013). However, it is important to accurately characterize pollen variation, particularly groups with high structural and morphological variability (Caccavari, 2010), such as *Mimosa*. Thus, considering that we know the pollen morphology of only a fraction of *Mimosa* and that polymorphisms are common in the genus. First, we describe pollen of 45 *Mimosa* species (53 specimens), 39 of which were never studied before. Second, given that known *Mimosa* pollen traits come from a restricted but taxonomically diverse sample, we ask if our sampling increments the current knowledge about the genus pollinic diversity.

Materials and Methods:

a. Plant material

We obtained pollen samples from 45 *Mimosa* species (53 specimens; Table1) deposited in three herbaria (CEN, ESA, and SPSC; acronyms according to Thiers, continuously updated). Of these, 39 had not been described palynologically before. All samples were stored in glacial acetic acid before the treatment for morphological analyses.

b. Palynological analyses

To clear the pollinic content, we used a modified version of the Ertdman Acetolysis method (Salgado-Laborau 1973; González & Mosquera 2016). These modifications aimed to avoid deformation or disaggregation of the delicate polyads. First, to avoid solution degradation during storage (Salgado-Labouriau, 1973), we prepared an acetolysis solution (9:1 acetic anhydride and sulphuric acid; Erdtman, 1952) for each sample. Then we centrifuged the samples at 2500 rpm for 5 minutes in glacial acetic acid 100% p.a. After discarding the supernatant, we added at least 5 ml of the acetolysis mixture to each sample, and slowly agitated them inside a water bath at 90°C for 2 minutes, shaking slowly. We again centrifuged the samples at 2500 rpm for 5 min and discard the supernatant. Then we added 46% alcohol and centrifuge at 2500 rpm for 5 min. This process was repeated with alcohols of different concentrations (70%, 90%, 97%) until obtaining signs of total purification (González & Mosquera, 2016).

For the optical microscopy analyses, we prepared permanent slides using colorless stained-glass varnish, which has a good refractive index, does not alter the chemical properties of the sample, and is cheaper than other chemicals (Paiva et al., 2006). We added a few drops of each sample to different glass slides and waited until they were completely dry. Later, we added two drops of varnish and placed the coverslip. Finally, we dried the slides for at least 24 hours before analysis.

Part of the samples were also treated for scanning electron microscopy (SEM). For that, after the acetolysis, they were centrifuged in pure water, at 2500 for 5min. After removing the supernatant, we centrifuged them at 2500 RPM for 5 minutes twice; first in an alcohol-acetone 1:1 solution and later in acetone 100%. We then dried the samples over silicon sheets at room temperature. Samples were then gold-coated (approximately 15 nm thick) in a BAE 250 deposition system (Balzers Instruments). The pressure of the deposition chamber was approximately $2X10^{-5}$ mbar. We let the samples cool for about an hour and, after that time, the pressure of the system was released. For MEV imaging, we used an LEO 440 electron microscope (Oxford).

We collected both qualitative and quantitative data from each sample. Qualitative data were: dispersion unit (number of grains), pollen arrangement, grain cohesion, outline, ornamentation, and aperture type. We also measured the larger and smaller diameters in at least 30 tetrads or polyads and exine thickness and pore diameter in 10 dispersal unities. Later we obtained the arithmetic mean and standard deviation for each measurement.

Results:

Pollen grains of the species studied here occur in tetrads (four cells, of variable organization: uniplanar -tetragonal or rhomboidal- or multiplanar -tetrahedral and decussed-), eight cells, and, more rarely, 12 cells. Considering the combination of pollen arrangement and outline, we observed 12 types of pollen aggregates (Figure 1).

In the case of tetrads, the same species can present several types of cell organization, although one (or two) forms predominate. Most species have ellipsoidal tetrads, except for *Mimosa adenocarpa* (Fig 2) and *M. aurivillus* var. *aurivillus (Fig 3)*, which are elliptical. On the other hand, polyads with 8 cells, organized in bitetrads (two tetrads in dissymmetric position, one over the other) and polyads with 12 cells, organized in tritetrads. The outline is generally elliptical, but spheroidal in some species. We must consider that the outline depends on the angle from where it was observed the tetrad/polyad (and therefore, it would be the angle of preference of the arrangement).

Tetrad/Polyad size ranges from small (10 to 25 µm) to medium (25 to 50 µm). *Mimosa adenocarpa* (Fig 2) shows the smallest (10 to 12.6 µm) and *M. diplotricha var. diplotricha* (Fig 2), the largest (30 to 40.2 µm). Exine ornamentation, when observed under optical microscopy, generally appears to be psilate, while in larger polyads, it is areolate or. Nonetheless, the more accurate SEM analyses point out for a verrucate or areolate exine ornamentation, of cerebroid aspect (Fig 2 – Fig 5). Areolae are very small and range from 0.2 µm to 0.5 µm in diameter.

All polyads are acalymmate with porate grains (Fig 2 – Fig 5), although the number of pores varies among species. Pores face each other in groups of three or four. Sometimes pores are difficult to be observed (even on SEM), due to grains' small size and position on tetrads. See Table 1 for a detailed description for each species.

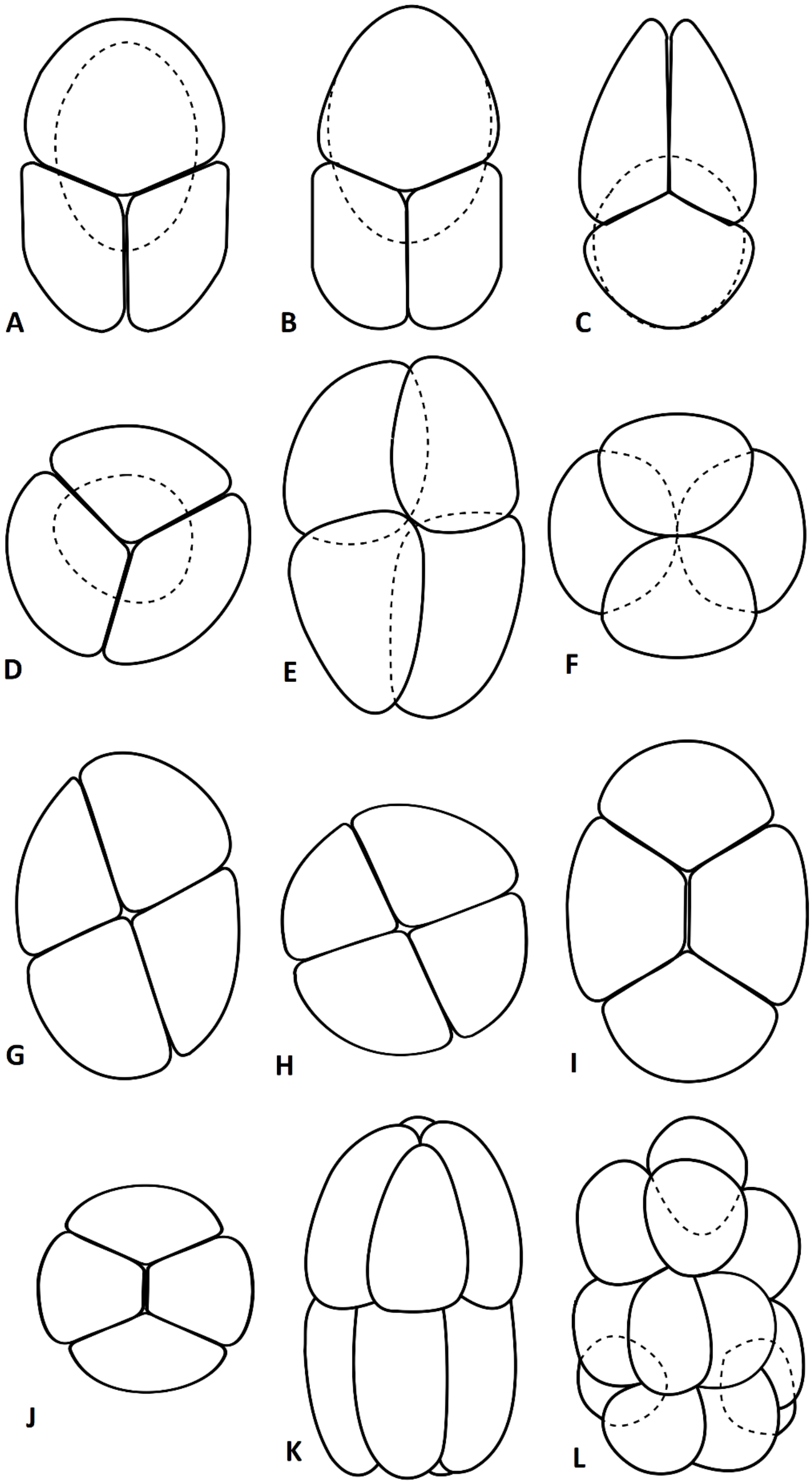

Figure 1 - Types of pollen organization observed in Mimosa: Tetrahedral elliptical (A), tetrahedral oval – subtype 1 (B), tetrahedral oval – subtype 2 (C), tetrahedral spheroidal (D), decussated elliptical (E), decussated spheroidal (F), tetragonal elliptical (G), tetragonal spheroidal (H), rhomboidal elliptical (I), rhomboidal spheroidal (J), 8 - polyad (K) and 12 - polyad (L).

Table 1. Main characteristics observed in the genus *Mimosa* belong to sections *Batocaulon, Calothamnos, Habbasia, Mimadenia* and *Mimosa*. Species with asterisk (*) had not been described palynologically before. In parentheses () of the organization are the least observed or rare information. Species with (+) are large (n < 25 µm).

Genus	Series	species and variety	n	Organization	Outline	Longer diameter (µm)	Shorter diameter (µm)	Voucher
Mimosa	*Glanduliferae*	*pithecolobioides*	12	irregular arrangement	elliptical	21.28 (22.74 ± 0.78)24.54	15.16 (17.63 ± 1.41) 21.32	A. P. Savassi-Coutinho 1217 (ESA 114163)
Mimosa	*Acantholobae*	*acantholoba**	8	bitetrads	elliptical	10.3 (12.8 ± 0.81) 14	7.3 (8.5 ± 0.65) 9.6	M. F. Simon 826 (CEN)
Mimosa	*Bimucronatae*	*bimucronata*	8	bitetrads	elliptical	10.6 (13 ± 1.12) 14.8	8.3 (9.2 ± 0.45) 10	M. Fedele s.n. (ESA 012850)
Mimosa	*Caesalpiniifolia*	*laticifera**	8/4	rhomboidal	elliptical	22 (24.8 ± 1.97) 29	14 (18.6 ± 2.86)23	V. C. Souza 15003 (ESA 44188)
Mimosa	*Ceratoniae*	*ceratonia var. pseudo-obovata**	8/4	bitetrads (tetrahedral tetrads)	elliptical	13.2 (14.1 ± 0.46) 14.9	10 (10.8 ± 0.58) 12.5	H.C. de Lima 279 (ESA 121223)
Mimosa	*Glandulosae*	*adenocarpa**	4	tetrahedral	elliptical	15.7 (19.2 ± 1.29) 21.6	12 (13.5 ± 0.95) 15.3	F. G. Pereira 288 (ESA 136183)
Mimosa	*Glandulosae*	*adenocarpa**	4	decussate (tetrahedral)	spheroidal	10.0(11.7)12.6	7.5(9.6)12.5	(C.F.C.R 2109 – SPF)
Mimosa	*Glandulosae*	*adenocarpa**	4	tetrahedral (decussate)	spheroidal	10.0(12.5)15.0	10.0(10.1)11.3	L.M. Borges 487 (SPF)
Mimosa	*Leiocarpae*	*pteridifolia*	4	tetrahedral (tetragonal)	elliptical	17.1 (19.4 ± 1.05) 21.5	10.7 (12.8 ± 0.9) 14.1	Y. Barros-Souza 37 (SPSC)

Mimosa	*Leiocarpae*	*tenuiflora*	4	decussate (tetrahedral)	elliptical	18.3 (21.3 ± 1.43) 24.06	11.4 (13.4 ± 1.1) 16	R. P. Lyra-Lemos 6998 (ESA 99145)
Mimosa	*Paucifoliatae*	*diplotricha var. diplotricha**	4	rhomboidal	elliptical	30 (34.1 ± 2.74) 40.2	23.7 (26 ± 1.21) 28.2	J. Kuntz 445 (ESA 116346)
Mimosa	*Paucifoliatae*	*gracilis var. capillipes (benth.) barneby**	4	decussate (rhomboidal)	elliptical	19.82 (23.78 ± 1.86) 27.11	15.04 (16.95 ± 1.50) 20.31	*L. M. Borges 1242 (SPF)*
Mimosa	*Plurijugae*	*invisa var. macrostachya*	4	rhomboidal	elliptical	19.82 (23.78 ± 1.86) 27.11	15.04 (16.95 ± 1.50) 20.31	L.V.B. Bufo & P.C. Sabadim s.n. (ESA 63960)
Mimosa	*Stipellares*	*cruenta**	4	tetrahedral	elliptical	12.9 (15 ± 1.09) 17.1	8.6 (9.5 ± 0.48) 10.6	J. A. Jarenkow 3672 (ESA 60484)
Mimosa	*Stipellares*	*strobiliflora**	4	rhomboidal (decussated)	elliptical	18.3 (20.1 ± 1.08) 22.5	10.8 (13.8 ± 1.08) 15.5	O.S. Ribas 3600 (ESA 83253)
Mimosa	*Quadrivalves*	*candollei*	4	rhomboidal (tetragonal, decussate)	elliptical	29.3 (31.6 ± 2.19) 35.58	23.09 (26.2 ± 1.99) 30.12	C. Delfini 234 (ESA 120662)
Mimosa	-	*aurivillus var. aurivillus**	4	tetrahedral (decussated)	spheroidal	12 (14 ± 0.84) 15.4	10.2 (11.2 ± 0.86) 12.8	R. M. Harley 27240 (ESA 114010)
Mimosa	-	*scabrella**	4	tetrahedral (decussated)	spheroidal	13 (13.9 ± 0.62) 15.6	8.5 (10.1 ± 0.79) 11.9	V. C. Souza 39542 (ESA 134747)
Mimosa	*Habbasia*	*pigra var. dehiscens**	4	decussate (rhomboidal)	elliptical	21.7 (23.7 ± 1.12) 27.2	13.8 (16.1 ± 1.39) 19.6	J. P. Souza 8103 (ESA 56761)
Mimosa	*Habbasia*	*pigra var. dehiscens**	4	decussate (rhomboidal)	elliptical	20.0(20.8)22.5	15.0(17.3)20.0	L.M. Borges 20 (SPF)

Mimosa	*Pachycarpae*	*adenotricha**	4	tetrahedral	elliptical	12.1 (15.9 ± 1.53) 17.6	9.5 (11.5 ± 0.78) 12.95	V. C. Souza 22449 (ESA 60954)
Mimosa	*Pachycarpae*	*adenotricha**	4	decussate (tetragonal, tetrahedral)	elliptical	12.5(16.7)18.7	8.8(12.4)15.0	R. Mello-Silva 3162 (SPF)
Mimosa	*Pachycarpae*	*albolanata var. brasiliana**	4	decussate (rhomboidal)	elliptical	17.3(20.4)25	12.5(13.1)15.0	N.T. Silva 57165 (SPF)
Mimosa	*Pachycarpae*	*capito**	4	decussate	elliptical	17.7(20.1)22.5	11.2(12.7)13.8	J.A. Ratter 2619 (SPF)
Mimosa	*Pachycarpae*	*claussenii var. claussenii**	4	decussate (tetragonal, tetrahedral)	elliptical	12.5(19.8)20.0	12.5(12.9)15.0	Pirani 1559 (SPF)
Mimosa	*Pachycarpae*	*claussenii var. claviceps**	4	decussate	elliptical	(23.0)+	(14.3)+	T.B. Cavalcante 1323 (SPF)
Mimosa	*Pachycarpae*	*claussenii var. megistophylla barneby**	4	decussate	elliptical	(25.0)+	(15.1)+	*L. M. Borges 1274 (SPF)*
Mimosa	*Pachycarpae*	*claussenii aff. var. prorsiseta barneby**	4	tetrahedral (decussate)	elliptical	(27.0)+	(18.0)+	Y. B. Souza 77 (SPSC)
Mimosa	*Pachycarpae*	*foliolosa var. brevibractea**	4	decussate (rhomboidal)	elliptical	17.5(20.1)22.5	12.0(13.0)15.0	L. Rossi (CFCR 1061 -SPF 22987)
Mimosa	*Pachycarpae*	*foliolosa subsp. brevibractea var. paranani**	4	rhomboidal (tetrahedral)	elliptical	18.19 (20.16 ± 0.88) 21.66	11.75 (12.96 ± 0.67) 14.15	Y. B. Souza 76 (SPSC)
Mimosa	*Pachycarpae*	*foliolosa var. franciscana**	4	decussate (rhomboidal)	elliptical	15.5(20.6)22.5	10,9(12.5)13.0	C. M. Sakuragui (CFCR 15241 – SPF)
Mimosa	*Pachycarpae*	*foliolosa var. multipinna**	4	decussate (rhomboidal)	elliptical	17.5(19.7)22.5	12.5(13.1)15.0	W. Thomas 5794 (SPF)
Mimosa	*Pachycarpae*	*foliolosa var. pachycarpa**	4	rhomboidal	elliptical	15.7 (18 ± 1.52) 22.4	10.5 (13.5 ± 1.82) 17.6	V. C. Souza 10280 (ESA 36094)
Mimosa	*Pachycarpae*	*foliolosa var. pubescens**	4	rhomboidal (tetrahedral)	elliptical	17.1 (19.1 ± 1.35) 21.6	11.5 (13.3 ± 0.92) 14.8	J. Paula-Souza 4270 (ESA 89811)

Mimosa	*Pachycarpae*	*foliolosa var. vernicosa**	4	decussate (tetragonal, tetrahedral)	elliptical	17,5(18.8)22.5	10(12.12)13.7	A. Furlan (CFCR 3208 – SPF 23415)
Mimosa	*Pachycarpae*	*foliolosa var. viscidula**	4	decussate	elliptical	18.7(20.6)22.5	11.3(13.0)16.0	D. C Zappi (CFCR 10502 – SPF 47248)
Mimosa	*Pachycarpae*	*laniceps**	4	decussate	elliptical	17.5(21.0)22.5	12.5(13.5)17.6	Irwin 24658 (SPF)
Mimosa	*Pachycarpae*	*laniceps**	4	decussate (tetrahedral)	elliptical	(25)+	(18)+	M.L. O. Trovó 439 (SPF)
Mimosa	*Pachycarpae*	*aff. laniceps barneby**	4	decussate	elliptical	(24)+	(15)+	L. M. Borges 1273 (SPF)
Mimosa	*Pachycarpae*	*maguirei**	4	decussate (tetrahedral)	elliptical	17.5(19.7)21.3	12.5(13.1)15.0	M.L.O. Trovó 417 (SPF)
Mimosa	*Pachycarpae*	*manidea**	4	tetrahedral	elliptical	11.2 (14 ± 1.25) 16.1	9 (10 ± 0.61) 11.5	Y. B. Souza 74 (SPSC)
Mimosa	*Pachycarpae*	*manidea**	4	decussate (tetrahedral)	elliptical	15.0(17.4)20.0	10.0(12,4)13.7	M.L.O. Trovó 462 (SPF)
Mimosa	*Pachycarpae*	*myrioglandulosa**	4	rhomboidal (tetragonal)	elliptical	14.8 (16.8 ± 1.31) 19.3	8.1 (11.3 ± 1.16) 13	Y. B. Souza 91 (SPSC)
Mimosa	*Pachycarpae*	*oedoclada**	4	tetrahedral (rhomboidal)	elliptical	15.1 (16.5) 17.9	9.9 (10.9) 12.2	Y. B. Souza 73 (SPSC)
Mimosa	*Pachycarpae*	*prorepens**	4	decussate (tetrahedral)	elliptical	12.5(16.9)18.0	10.0(11.8)12	Hatschback 60171 (SPF)
Mimosa	*Pachycarpae*	*rhodostegia**	4	decussate (rhomboidal)	elliptical	17.5(20.7)22.5	12.5(13.4)15.0	M.L.. Fonseca 590 (SPF)
Mimosa	*Pachycarpae*	*setosissima**	4	decussate (tetrahedral)	elliptical	15.0(17,2)20.0	10.0(12,1)18.7	Anderson 10236 (SPF)

Mimosa	*Pachycarpae*	*ulei var. ulei**	4	decussate (tetrahedral)	elliptical	17.5(18.4)20.0	7.5(11.4)12.5	Forzza 386 (SPF)
Mimosa	*Piresianae*	*piresii**	4	decussate (rhomboidal)	elliptical	17.5(20.4)22.5	12.5(14.9)16,3	R.R.Riberio 1200 (SPF)
Mimosa	*Setosae*	*paludosa*	4	tetrahedral	elliptical	17.1 (18.8 ± 1.34) 22.68	11.2 (13.2 ± 0.89) 15.1	Y. B. Souza 89 (SPSC)
Mimosa	*Setosae*	*paludosa*	4	decussate (rhomboidal)	elliptical	20.0(22.3)25	12.5(14.3)17.5	Pirani 2233 (SPF)
Mimosa	*Setosae*	*rupigena**	4	decussate (rhomboidal)	elliptical	17.5(19.5)21.3	12.5(13.4)15.0	J. Semir (CFCR 179 – SPF)
Mimosa	*Mimosa*	*dryandoides**	4	tetrahedral (decussated)	elliptical	11.7 (13.1 ± 0.84) 15.2	7.3 (8.7 ± 0.52) 9.8	J. M. 38180 (ESA 129629)

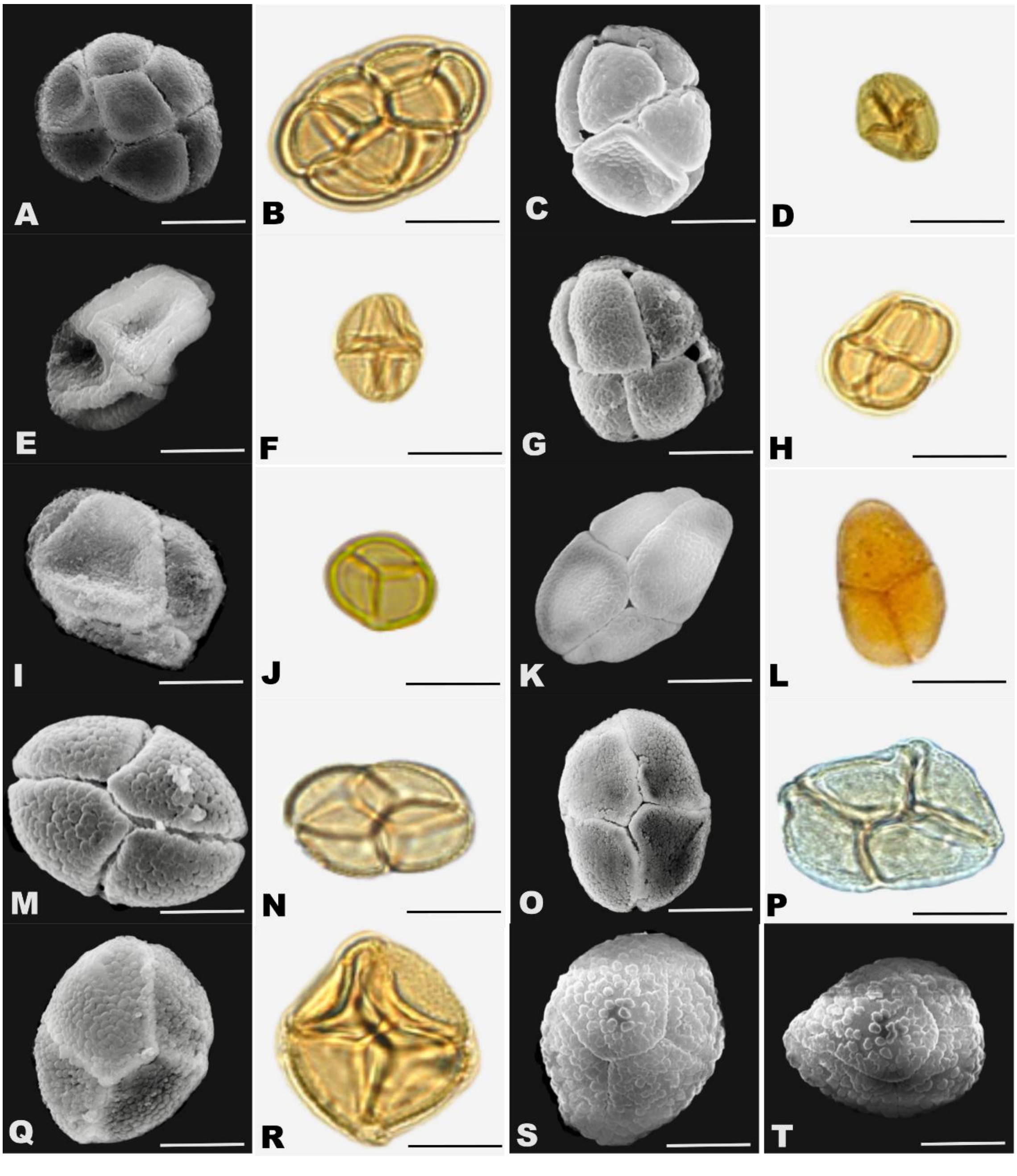

Figure 2 – Photomicrographs of *Mimosa* species. A-B. *M.pithecolobioides*. C-D. *M.acantholoba**. E. *M.laticifera**. F. *M. bimucronata*. G-H. *M. ceratonia var. pseudo-obovata**. I-J. *adenocarpa**. K-L. *M. pteridifolia*. M-N. *M. tenuiflora*. O-P. *M. diplotricha var. diplotricha**. Q-R. *M. invisa var. macrostachya*. S-T. *M. gracilis var. capillipes**. Species with asterisk (*) had not been described palynologically before.

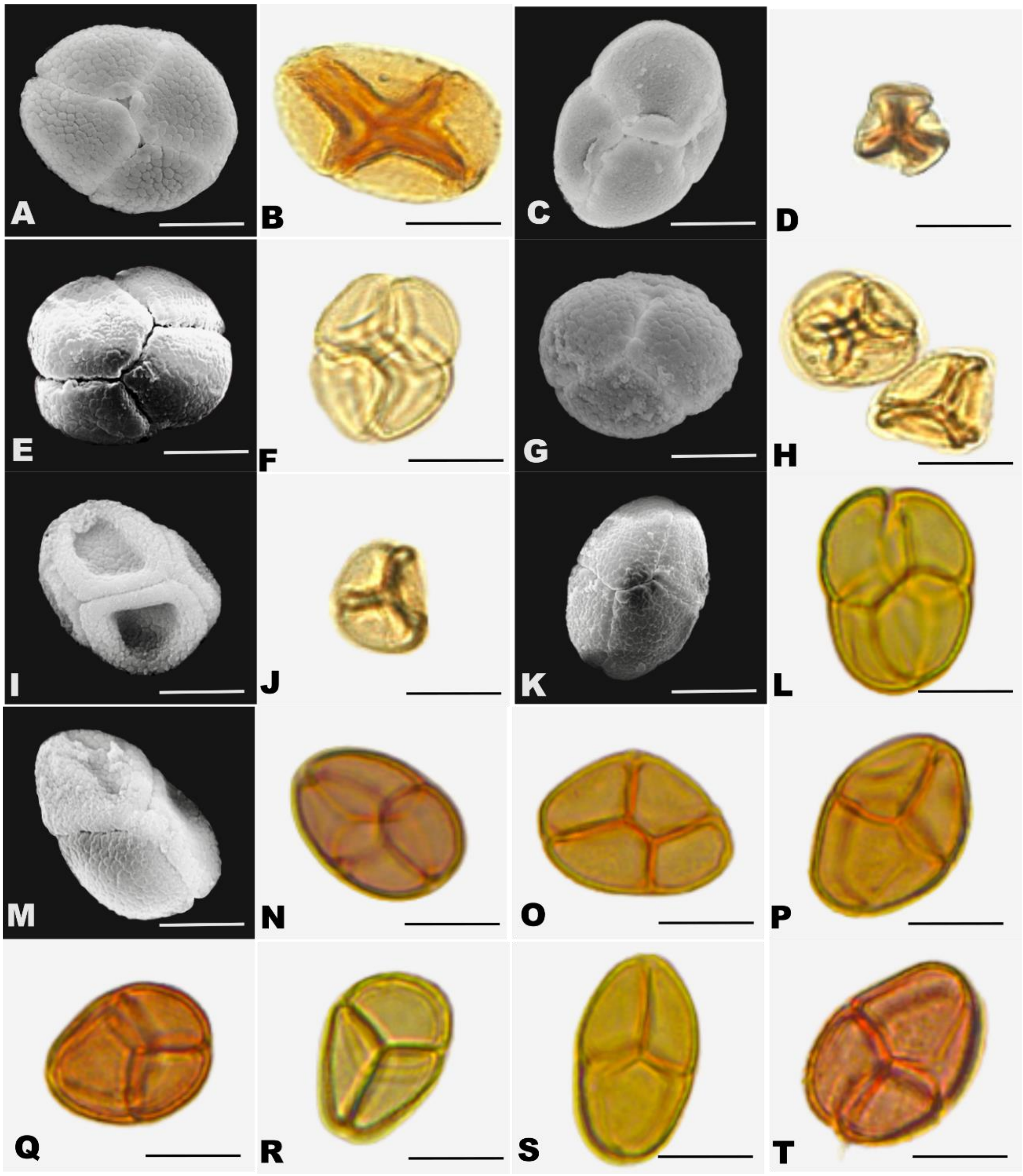

Figure 3 - Photomicrographs of *Mimosa* species. A-B. *M. candollei*. C-D. *M.* c*ruenta**. E-F. *M.* s*trobiliflora**. G-H. *M. aurivillus var. aurivillus**. I-J. *M.* s*cabrella**. K-L. *M. pigra var. dehiscens**. M-N. *M. adenotricha**. O-P. *M. albolanata var. brasiliana**. Q-R. *M. capito**. S. *M. claussenii var. claussenii**. T. *M.* claussenii var. claviceps*. Species with asterisk (*) had not been described palynologically before.

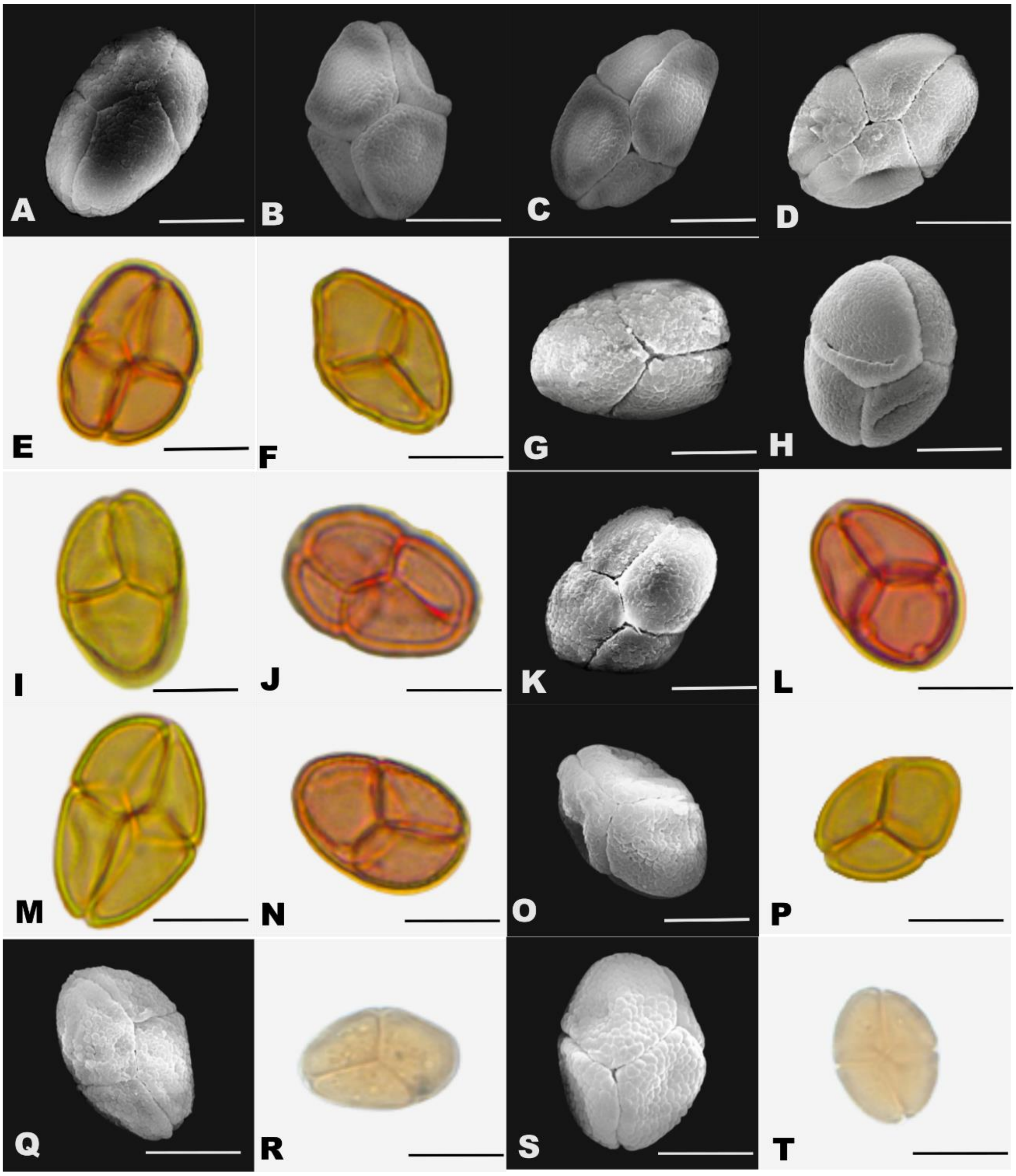

Figure 4 - Photomicrographs of *Mimosa* species. A. *M. claussenii var. megistophylla**. B-C. M. *claussenii aff. var. prorsiseta**. D. *M. foliolosa subsp. brevibractea var. paranani**. E. *M. foliolosa var. franciscana**. F. *M. foliolosa var. multipinna**. G. *M. foliolosa var. pachycarpa**. H. M. *foliolosa var. pubescens** I. *M. foliolosa var. vernicosa** J. *M. foliolosa var. viscidula**. K. *M. aff. laniceps**. L-M. *M. laniceps**. N. *M. maguirei**. O-P. *M. manidea**. Q-R. *M. myrioglandulosa**. S-T. *M. oedoclada**. Species with asterisk (*) had not been described palynologically befor

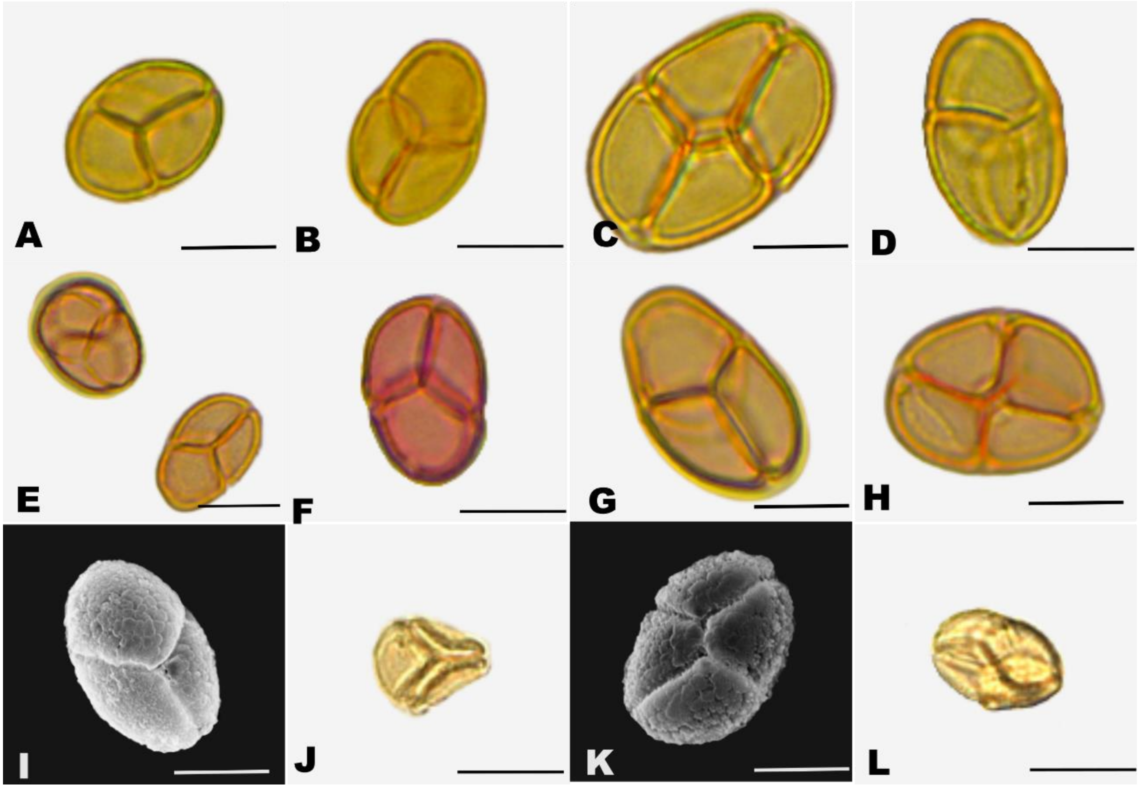

Figure 5 - Photomicrographs of *Mimosa* species. A-B*. M. prorepens*. C-D. M. aguapeia*. E. M. setosissima*. F. M. ulei var. ulei*. G-H. M. paludosa. I-L. M. dryandoides var. extratropica*. S*pecies with asterisk (*) had not been described palynologically before.

Discussion:

Here we have investigated pollen morphology of *Mimosa,* 39 had not been described palynologically before, raising the number of species for which pollinic data is known from 137 (27.2%) to 176 (33.2% of Mimosa species).

Features varied sufficiently for most of the species to be recognized for the morphology of their pollen grains (Jumah, 1991). The shapes and sizes of the polyads, the presence of apertures, and exine ornamentation (Medina-Acosta, 2018) may also be of taxonomic importance.

In the case of *Mimosa*, we observed that is partially heterogeneous, three types of occurrences exist: Tetrads, bitetrads and polyads. Tetrads being the most common (Van Campo & Guinet, 1961; Sorsa, 1969). Lima (2007) reported pollen organized in monads and dyads, however in our work they were not observed, but Simon (2011) suggest that these types of compound organization could be a mistake in observation. Variations also occur at the level of cellular organization; tetrads can be uniplanar (Rhomboid or tetragonal) or multiplanar (Decussate or tetrahedral).

Intra-specific polymorphisms on the organization of *Mimosa* pollen has already been reported (Medina-Acosta 2018), with up to 5 different types of organization combining type of pollen arrangements and outline in the same species (El Ghazali 1997). Our results showed 12 types of cell arrangements: Tetrahedral elliptical, tetrahedral oval – subtype 1, tetrahedral oval – subtype 2, tetrahedral spheroidal, decussated elliptical, decussated spheroidal, tetragonal elliptical, tetragonal spheroidal, rhomboidal elliptical, rhomboidal spheroidal, bitetrad (polyad with 2 tetrads) and tritetrad (Polyad with 3 tetrads), and some species has 2 types of them (or 3, rarely). Other studies (Caccavari, 1985; Lima et al. 2007) reported some of these types of organizations, but separately.

However, we must bear in mind that they studied smaller numbers of species of *Mimosa* (Sorsa 1969; Buril et al. 2010;), or they studied species of the same clade or clades that were very close phylogenetically (Flores-Cruz et al., 2006; Santos-Silva et al., 2013), or that have the same environment of occurrence (Lima et al. 2007); These factors could result in biased data.

The ornamentation is areolate or verrucate, or a mixture of them, with verrucae in the proximal side and areolae in the distal side. Regarding size, it goes from small to medium. The unique type of aperture reported in *Mimosa* is pore, varying in the number of them 3 – 4 pores for Cruz (2017) and Santos-Silva (2013), 5 pores for Flores-Cruz (2006) and 3-6 pores for Sorsa (1969) and El Ghazali (1997). And, In the case of our species, they presented 3-4 pores per cell, being able to comply with Garside's rule or Fisher's rule (Guinet 1981). It would be advisable to do more studies regarding this to determine if this characteristic is of taxonomic value. The principal problem with this characteristic is the size of pores, that generally almost imperceptible (Jumah 1991) even on MEV. Santos-Silva (2013) and Cruz (2017) mentioned that pores in bitetrads could not be seen. Being a rich genus, there is still a vast knowledge to unravel regarding the tetrads and polyads of *Mimosa*.

Conclusion:

The *Mimosa* pollen we studied here for the first time are homogeneous in respect to outline (predominantly elliptical), grains cohesion (all acalymmate), ornamentation (areolate or verrucate) and type of aperture (porate grains). On the other hand, dispersion units, grain arrangements, ornamentation and size vary among studied species. Regarding dispersion units and pollen arrangements, although tetrads predominate (multiplanar: tetrahedral or decussated; or uniplanar: rhomboidal or rarely tetragonal), some species have 8 cells organized in bitetrads, or 12 cells in three tetrads. Although ranging only from small to medium, size also varies among species.

Our study expanded the taxonomic breadth of *Mimosa* pollen diversity and showed that species never studied before fit the known pollinic disparity for the genus. However, as an eurypalynous group of wide geographic and ecological distribution, *Mimosa* likely includes more palynological diversity than the one highlighted until now. Thus, it is imperative we further study its pollen morphology, in order to uncover both intra- and interspecific variations through increased specimen and taxonomic sampling. Nonetheless, our results indicate that we may already know the general limits of pollen diversity in Mimosa.

Author Contributions:

“Conceptualization, S.L.G. and L.M.B.; methodology, S.L.G.; validation, G.L.C., S.L.G and R.F.B.; formal analysis, S.L.G and L.M.B.; investigation, S.L.G.; data curation, F.A.R.D.; writing—original draft preparation, S.L.G.; writing—review and editing, S.L.G. and L.M.B.; supervision, L.M.B.

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CHAPTER II: ***Mimosa* L. Pollen Evolution**

“*Mimosa* L. Pollen Evolution”

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Abstract:

Mimosa has more than 500 species around the world, which have successfully adapted to the most varied tropical and subtropical ecosystems in their different forms of life, most of which are endemic. In contrast to the high rate of studies on the systematics and macroscopic morphology of the group, little is known about its evolutionary patterns and even more so comparing them with palynological characters. The genus is classified in 5 sections of which not all are monophyletic and managed to make evolutionary inferences based on morphology, despite having correct in his inferences, it was not until almost 20 years later that they were verified at the molecular level (Simon et al., 2011). With the current phylogenetic tools, it is possible to find the affinities based on pollen morphology and that they are reflected in a phylogenetic hypothesis. Therefore, in this work we unite all the available data on the phylogeny and pollen morphology of 94 species of *Mimosa* and 11 species of the informal group *Piptadenia* as an external group in order to understand the evolutionary history of *Mimosa*. Our results corroborate what is proposed by Barneby (1991) and Simon et al. (2011), and we hope that future works will reveal information on the more than 73% for which palynological information is still not available and will allow the evolutionary history of the genus to be elucidated.

Keywords

Evolution, Palynology, *Piptadenia, Mimosa, Leguminosae*

Introduction

Pollen grains organized in compound arrangements occur in approximately 40 plant families (Harder & Johnson, 2008). Among these, Leguminosae shows an outstanding morphological diversity, pollen included (Erdmant, 1945;1952; Salgado-Labouriau, 1973; Silvestre-Capelato & Melhem, 1997). Despite variable in many aspects (Walker and Doyle 1975; Banks et al., 2010; Banks & Rudall 2016;), most legume pollen is dispersed as monads. Tetrads and polyads are in general restricted to mimosoid legumes (Caesalpinioideae; VanCampo & Guinet, 1961; Guinet 1969; Caccavari, 1985; Guinet & Ferguson, 1989; Jumah 1991; Guinet & Caccavari, 1992; Santos-Silva et al. 2013; LPWG, 2017; Medina-Acosta et al. 2018). More specifically, the genus *Mimosa* L. has tetrads, bitetrads (8 cells) and polyads up to 12 cells (Sorsa 1969; the monads and lads seen by Lima et al. 2007 are likely artefacts; see Simon et al. 2011).

The evolution of *Mimosa* pollen diversity has been a matter of debate. Based on Burkart's (1948) view of the genus evolution, Caccavari (1965) proposed that spheroidal tetrahedral tetrads with areolate ornamentation were primitive, and from which the verrucate tetragonal tetrads and the bitetrads derived. However, Barneby (1991) argued that the large and medium-sized polyads were in fact ancestral in relation to the small tetrads. A test of these opposing views confirmed that polyads are the plesiomorphic and tetrads apomorphic (Simon et al. 2011). However, we still don't know how other pollinic traits, such as ornamentation, size, and shape, evolved.

Mimosa pollen evolution also has taxonomic significance (Medina-Acosta 2018). For example, lineages otherwise treated as the same taxonomic group differ in number of grains on each dispersal unity (Santos-Silva et al. 2013). Although variation of dispersal unities is limited in the genus, other traits could be informative. However, previous tests integrating *Mimosa* pollen morphology, phylogeny, and classification are taxonomically limited (e.g. the non-monophyletic *Mimosa ser. Leiocarpae* Benth.).

Considering the current lack of knowledge on pollen evolution and taxonomic significance for a highly diverse genus, we ask (1) what the patterns of pollen evolution in *Mimosa* are*?* and (2) how these patterns relate to the lineages (Simon et al., 2011) and infra-generic categories (Barneby, 1991) currently recognized within the genus? To answer these questions, we optimized the largest palynological database to date over a well-sampled phylogenetic hypothesis for *Mimosa*.

Materials and methods

Taxonomic sampling and palynological data collection

Our sample includes 105 species, of which 94 belong to *Mimosa* (about a fifth of the species in the genus) and 11 to closely related genera in the mimosoid clade (*Piptadenia stipulacea* (Benth.) Ducke*, P. adiantoides* (Spreng.) J.F. Macbr.*, P. gonoacantha* (Mart.) J.F. Macbr.*, P. trisperma* (Vell.) Benth.*, Anadenanthera colubrina* (Vell.) Brenan*, Microlobius foetidus* (Jacq.) M. Sousa & G. Andrade*, Stryphnodendron adstringens* (Mart.) Coville*, Stryphnodendron obovatum* Benth.*, Parapiptadenia excelsa* (Griseb.) Burkart*, Pityrocarpa moniliformis* and *P. obliqua* (Pers.) Brenan. We obtained 945 palynological characters (Qualitatives: Grains number, Dispersal unit Organization, Bitetrad presence, Dispersal unit planes, Outline, SEM Ornamentation; an Quantitatives: pore number, mean longer diameter, mean shorter diameter) from the literature (Guinet & Caccavari 1992; Caccavari 2002; Flores-Cruz 2006; Lima et al. 2008; Buril et al. 2010; Santos-Silva et al.2013; Cruz et al.2018; Medina-Acosta et al. 2018; Ribeiro et al. 2018; chapter 1 of this work). To avoid bias due to different palynological methods used by different authors, we only considered data obtained with the same methods (SEM of samples treated with Ertdman's Acetolysis (1960).

Ancestral state inference

We made a tree with the phylogenies of *Mimosa* of Vasconcelos et al. 2020 and Simon et al. 2011 using Ape and dependencies in R program, we extracted the species without palynological data and left only the important ones. Before analyses, we updated all names in our dataset according to BFG 2020, GBIF.org, The Plant List (2010) and Tropicos.org. Using palynological data obtained from literature and our collections, we performed character state analyzes using Phytools and dependencies in R, considering that the rooting of our trees is in *Anadenanthera* Speg.

Subsequently, We made a binary matrix for each of the characteristics, with their respective character states. To deal with polymorphisms, we determined that all states present in them could occur (polymorphisms were treated as such, one of the states was not chosen randomly).

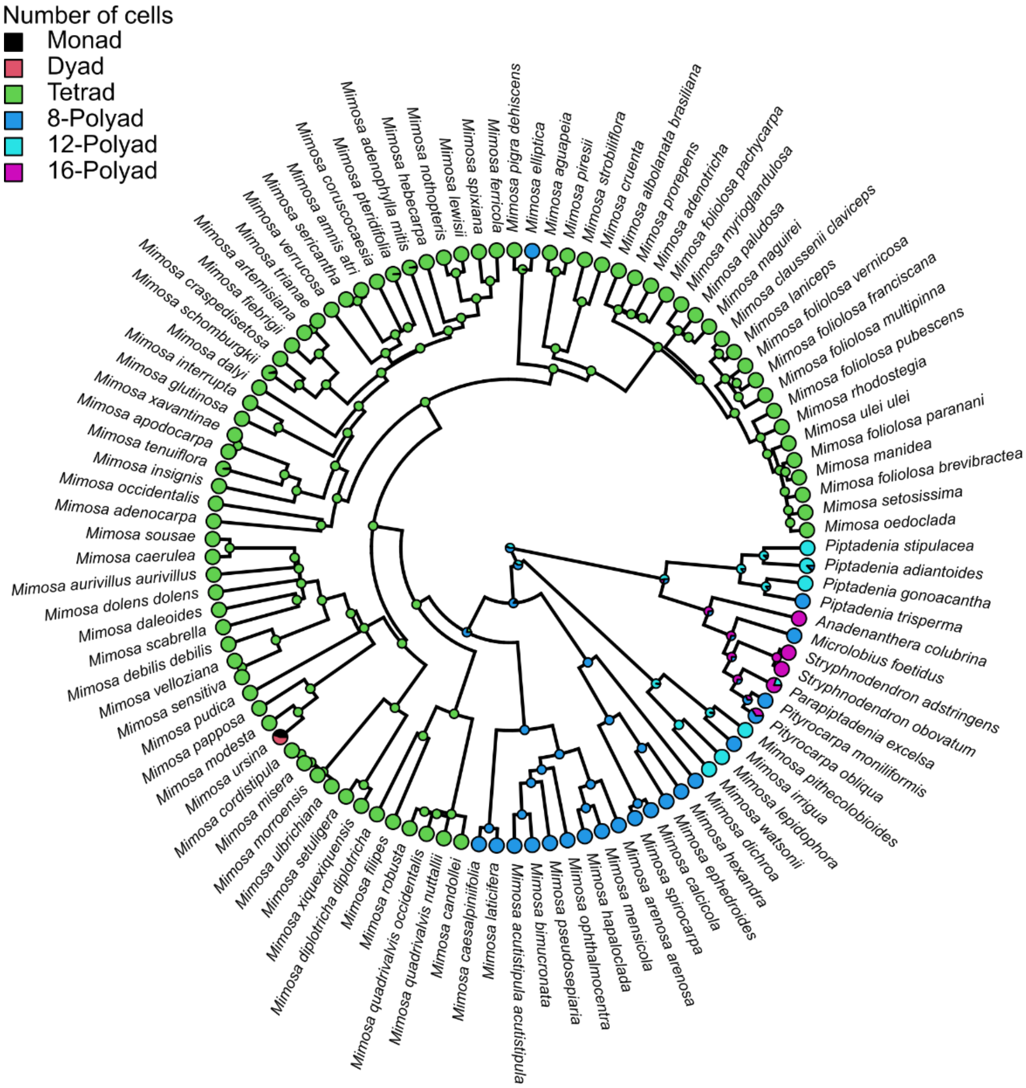
To deal with missing data, we determined that all character states would have the same chance of occurring. For data analysis, we run the stochastic mapping for each of the characteristics, using the tree and the respective binary matrix. Finally, we plot the tree with stochastic mapping, with colored circles indicating which character states are present in each node.

For continuous data, a vector was created for each of the analyzed characters. Each vector contains the respective character measures for each species. We ran the mapping of continuous characters, estimating states of internal nodes. For the case of missing data, the method used estimated values through. We plotted the mapped character tree, with the color of the branches indicating how the character size possibly varied along the phylogeny.

Results

Number of cells

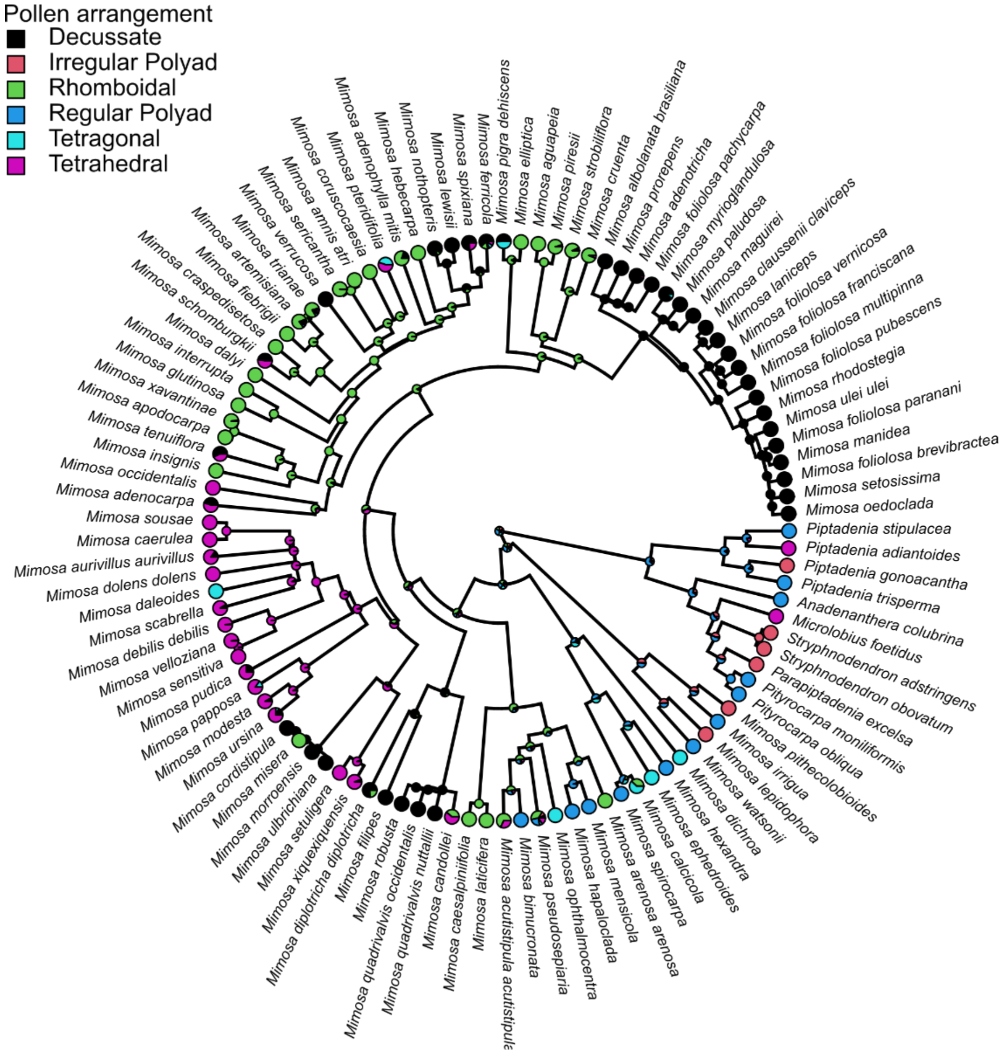
Our results show that the 16-cell polyads are a plesiomorphic character with respect to our focus group. From this characteristic, the 12 and 8 cell polyads are derived, the latter being the ancestral of the tetrads, which would become the derived character. In the clades that have 8 and 12 cells, we observed a lot of polymorphisms, which we could attribute to their polyphilia. We observed reversion for the 8-cell polyad state in *Mimosa* L., since this same character is observed in *Pityrocarpa* (Benth.) Britton & Rose.



Pollen arrangement

According to our trees, the polyads with irregular cellular organization could be the ancestral from which the polyads with regular organization would derive. Another option is that the irregular organization is synapomorphic for the *Mimosa* species of the basal clade and some species of *Microlobius C.Presl, Stryphnodendron* Mart. and *Anadenanthera* Speg. Among the characters of regular organization, we have tritetrads (12 cells), bitetrads and tetrads, mainly in *Mimosa*. Apparently, there may be convergence between some species of *Pityrocarpa* (Benth.) Britton & Rose and *Mimosa* with bitetrads.

For decussate tetrads there appear to be homoplasy, appearing in distant clades. The tetrahedral and rhomboidal tetrads seem to derive from an ancestor in common with the decussate tetrads. Being the rhomboidal tetrads the “most” apomorphic among all the tetrads.



Bitetrad presence

For 8-cell polyads in bitetrads, it is not clear whether it is convergence or synapomorphy. We believe that the ordering character in bitetrads could be more derived and that polyads with 8 cells would be the ancestral one.

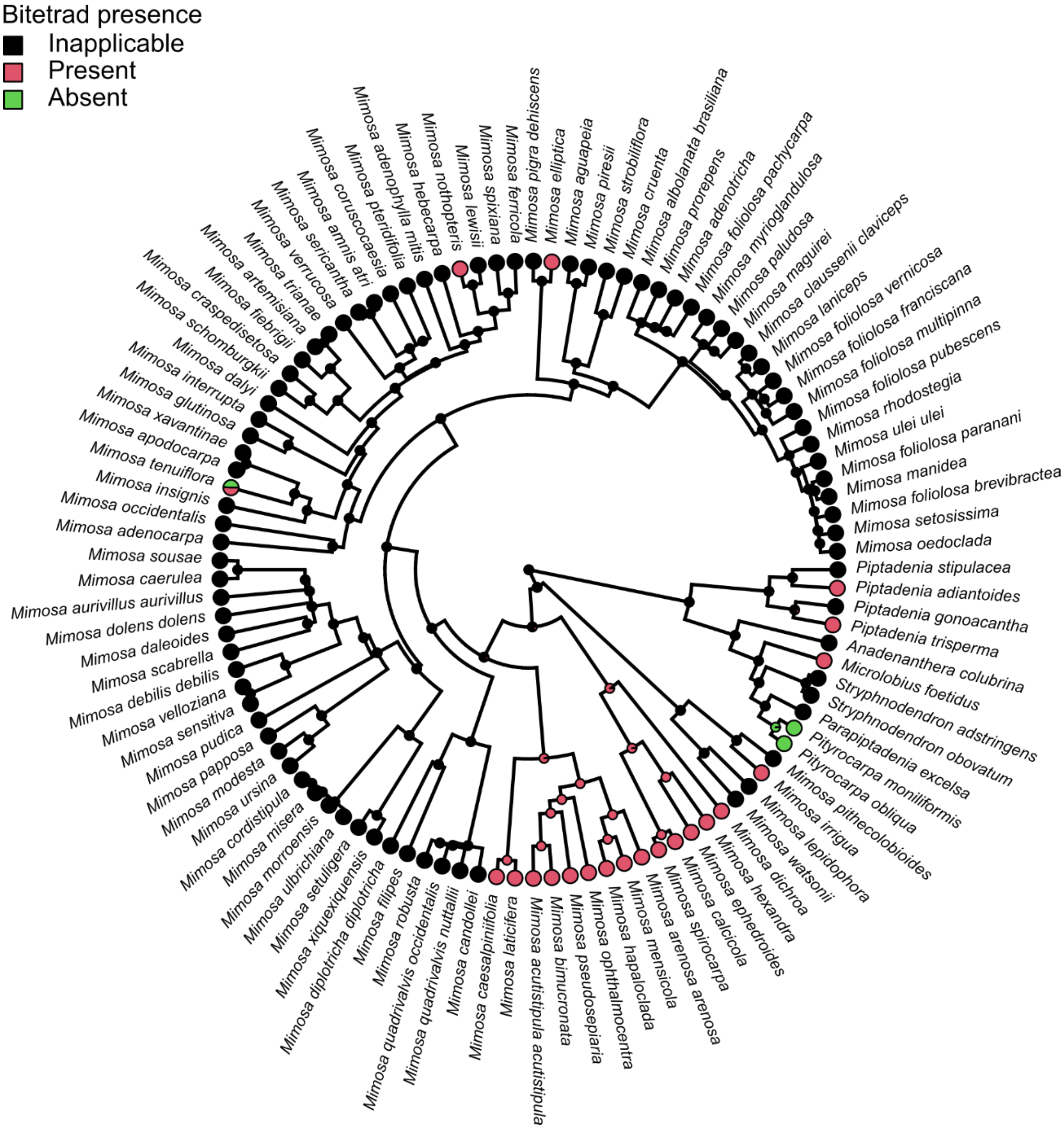


Figure 8. Optimization of Bitetrad presence (three-character states: Inapplicable, present and absent.

Planes

Although our tree does not reflect this state of character (Uniplanar), we know that there are uniplanar polyads that were not considered in this study for the genera *Anadenanthera* and *Parapiptadenia*. Therefore, we could intuit that the uniplanar tetrads (in *Mimosa* ser. *Leiocarpae, Mimosa ser. Habbasia* and *Mimosa ser. Piresianae)*, are derived from the uniplanar polyads.

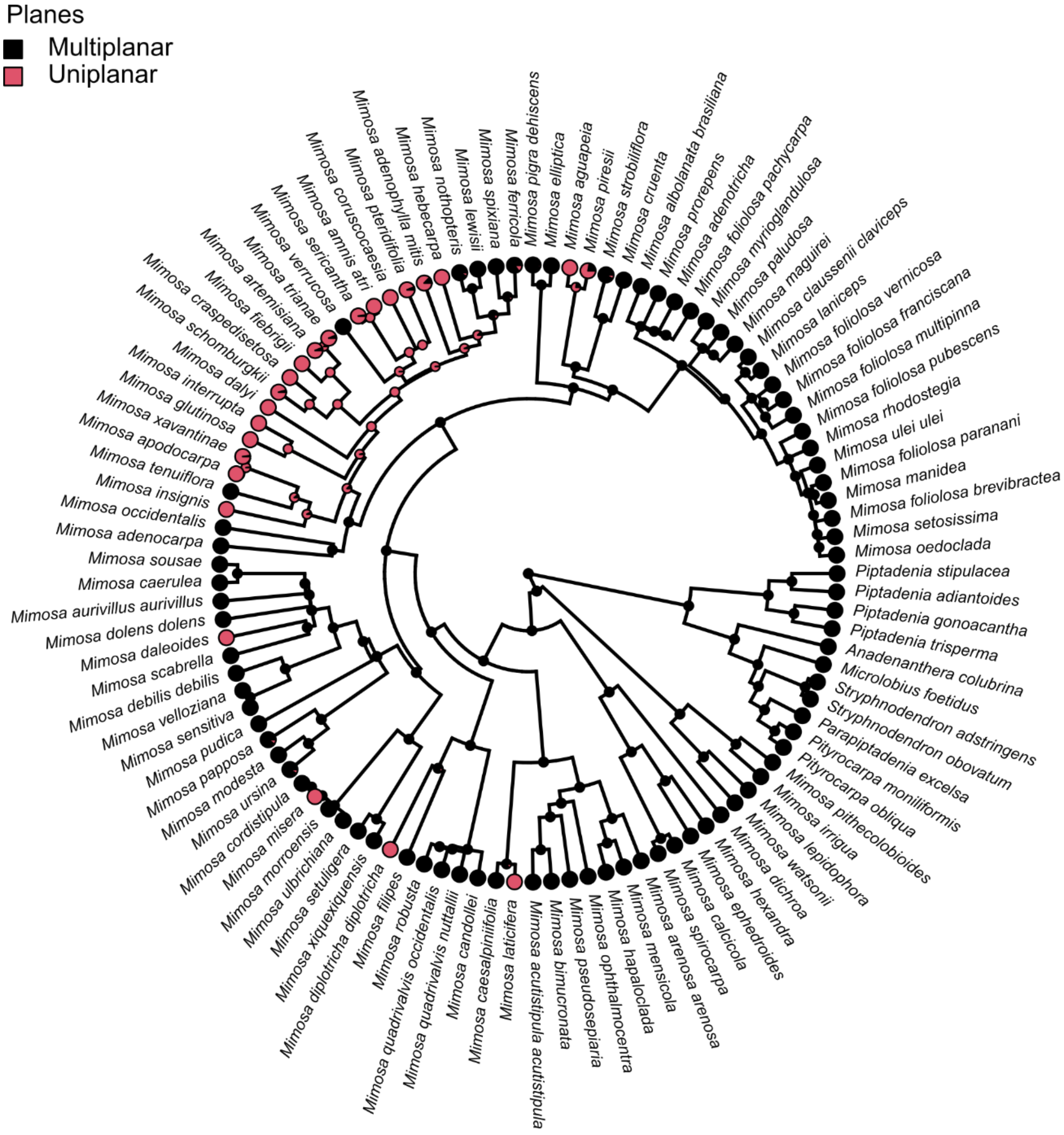


Figure 9. Optimization of planes (two-character states: Uniplanar and multiplanar

Outline

For this characteristic we must consider that the type of outline will depend on the angle from which the polyad or tetrad was observed, and the occurrence preference. Therefore, when analyzing these character states (which can be displayed up to 2 per species), we analyze the preference state. This being the case, we can say that the Oval and Elliptical would be the ancestral ones. On the other hand, For *Mimosa sect. Mimosa*, the circular-spheroidal outline is apomorphic, being homogeneous.

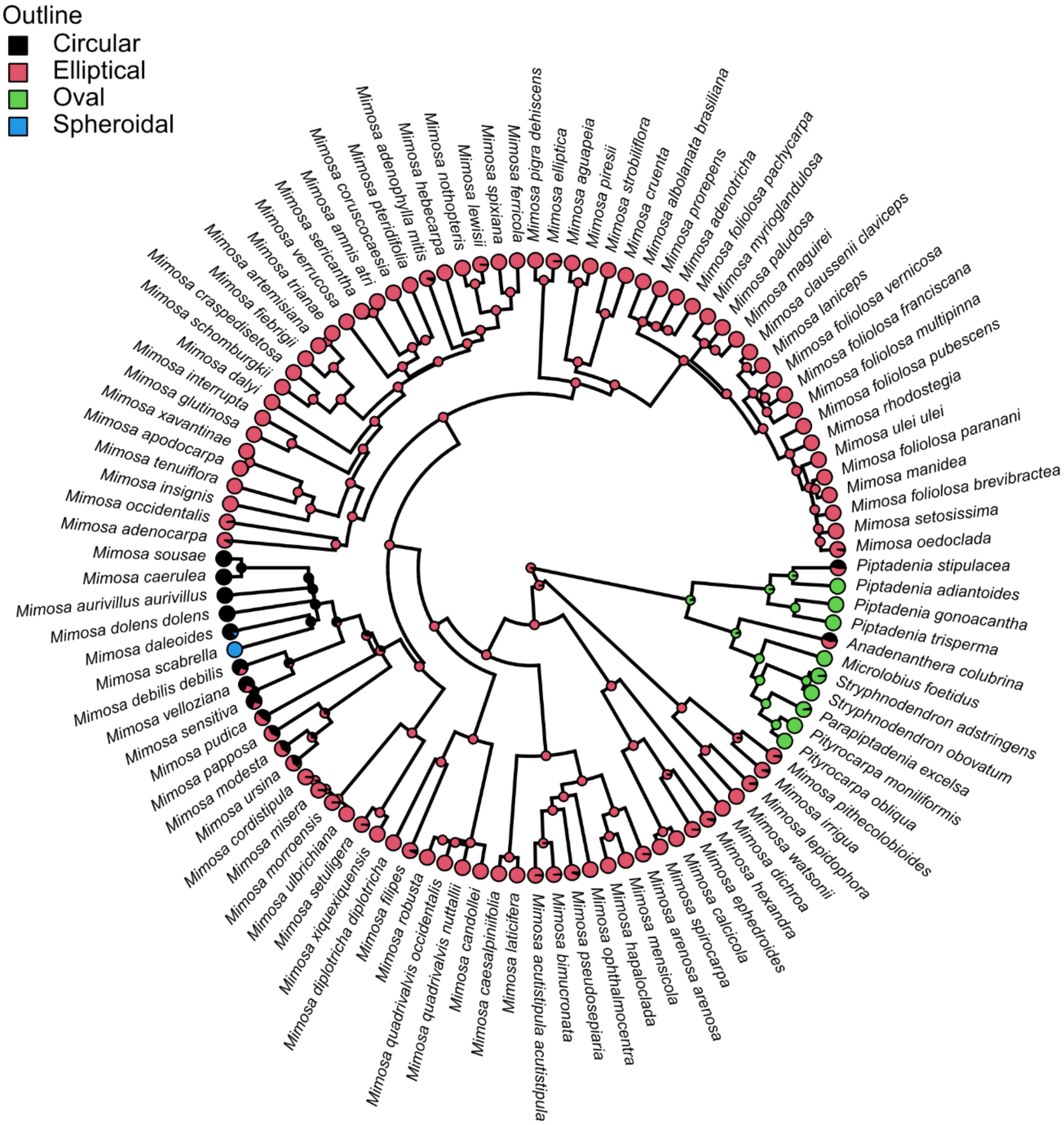


Figure 10. Optimization of outline (four-character states: Circular, elliptical, oval spheroidal)

Ornamentation

According to the data of our tree. The microverrucate state (In *Anadenanthera* Speg.) could be the ancestral of the synapomorphic states: verrucate and areolate, appearing as homoplastic character at least 4 times. In the case of the rugulate ornamentation, appears to be convergent, recorded at least 3 times independently, this ornamentation is observed in other Mimosoids and could be the plesiomorphic character. As expected, in the case of these characters, we observed homogeneity for the monophyletic series.

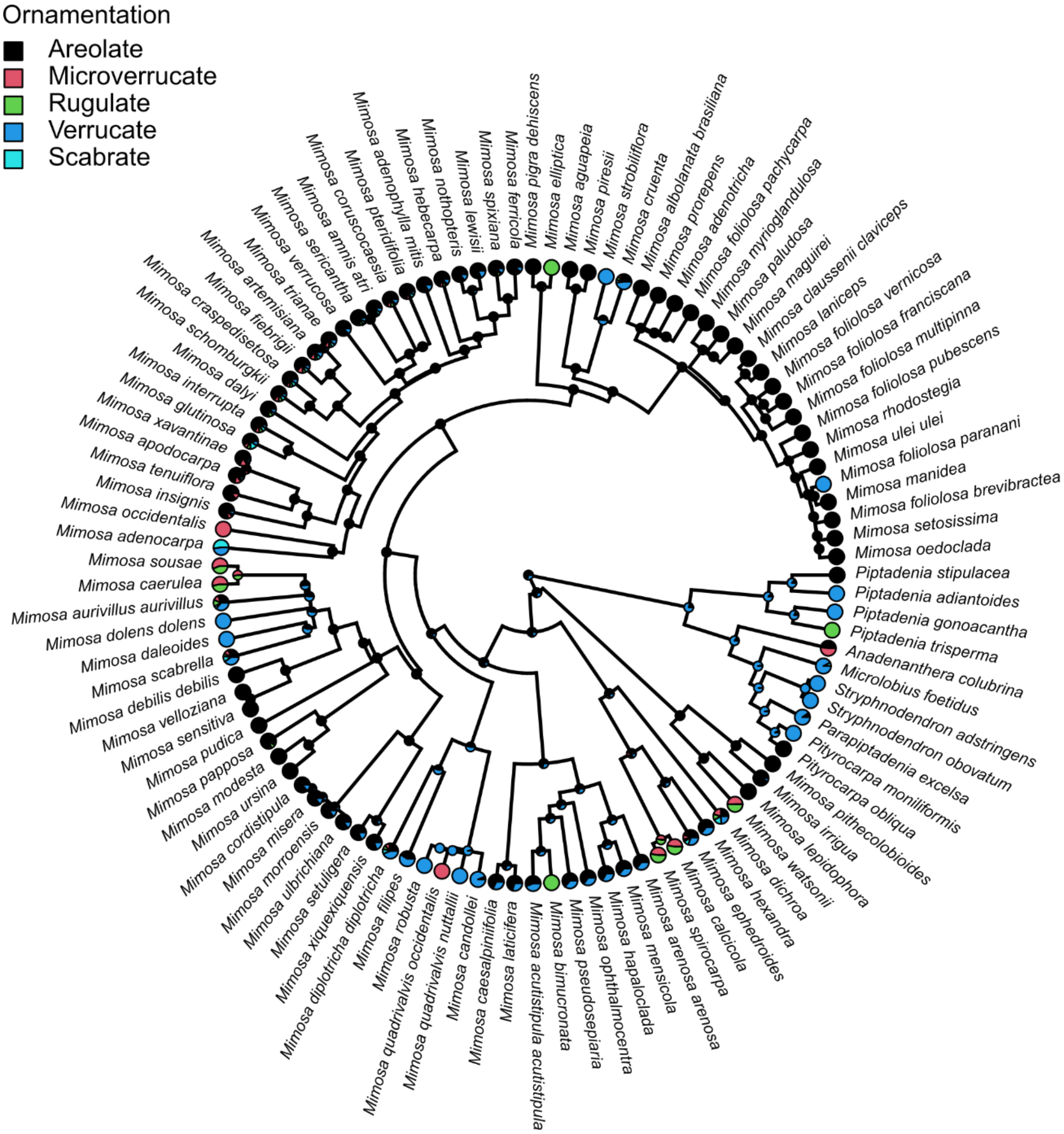


Figure 11. Optimization of ornamentation (five-character states: Areolate, microverrucate, regulate, verrucate and scabrate

Number of pores per cell

Mimosa L*.* appears to be homogeneous, generally having 3-4 pores. Despite not being shown in the tree, there are species of *Mimosa* with 6 pores, from which we can intuit that the high number of pores could be the derivate one of the other states. Cells with 4 pores appear in most species, being highly homoplastic. In the case of cells with 3 pores, it is a plesiomorphic character observed in species of the external group and preserved in *Mimosa*.

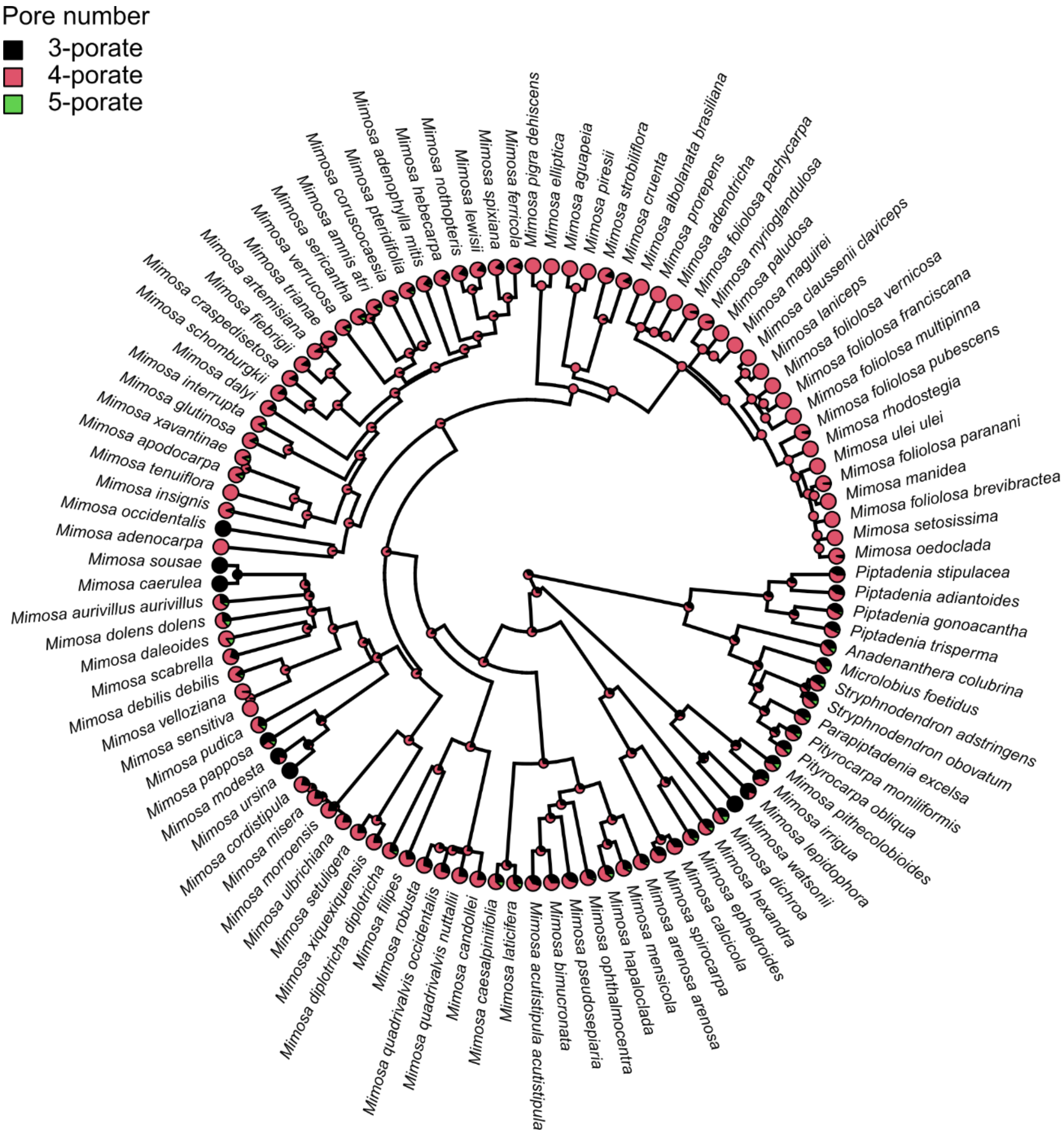


Figure 12. Optimization of pore number (three-character states 3-porate, 4-porate and 6-porate)

Longer diameter

The size of the tetrads and polyads is quite homogeneous for the *Mimosa* series. The ancestral state is the medium size, being conserved in some series of *Mimosa*. The derived character would be the small size, widely distributed as a synapomorphic character. Since *Mimosa robust*a R. Grether is the only species in our tree that has large size, we could that this would be a reversal and that despite not appearing here, we know that there are other species of the outer group with large polyads. Coincidentally, the ornamentation can be related to the size in some series of our tree, but it is not completely decisive.

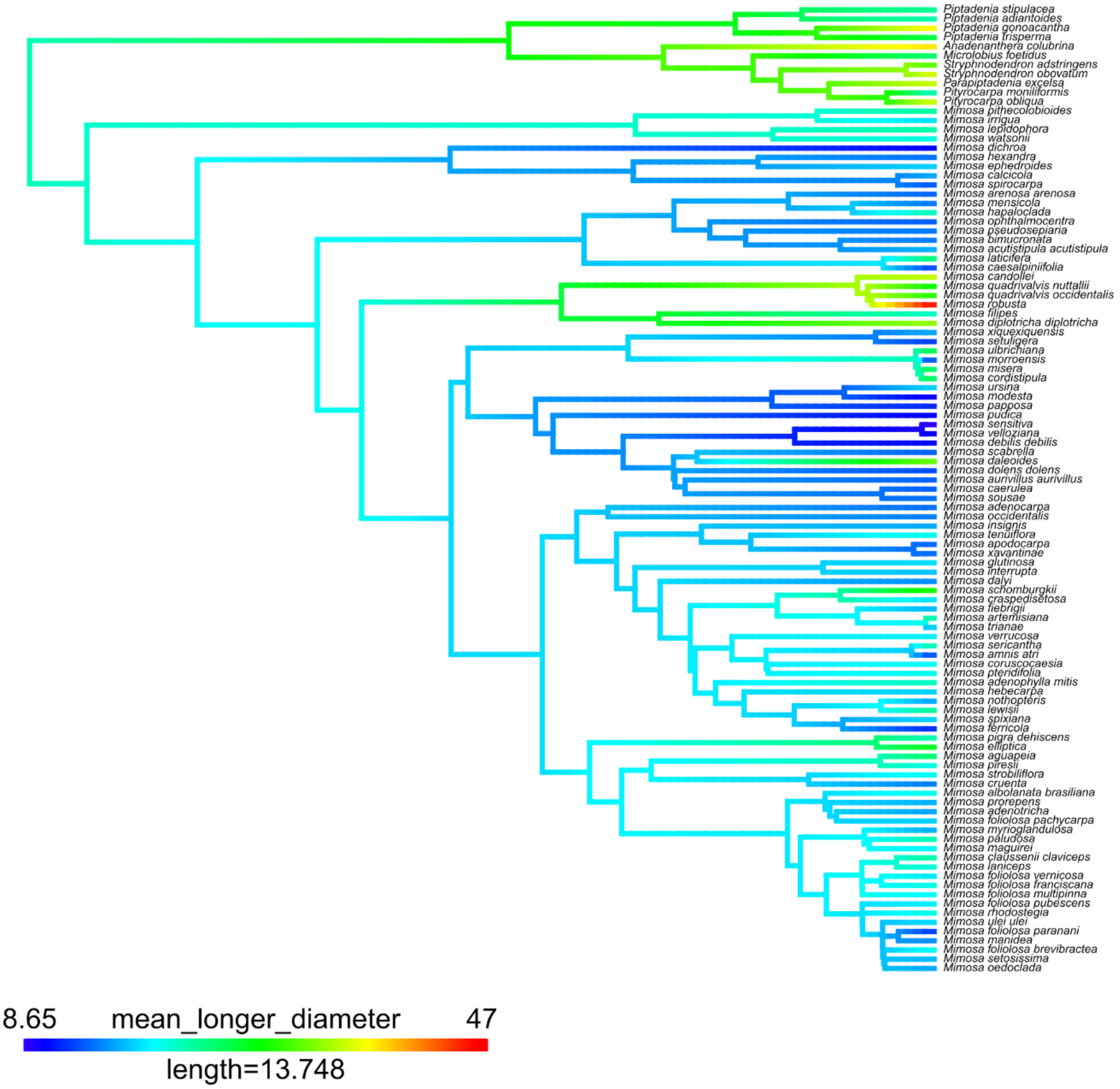


Figure 13. Optimization of mean of longer diameter.

Shorter diameter

Medium-sized polyads / tetrads are expected to have uniform largest and smallest diameters. However, the smaller diameter is directly related to the contour of the dispersion unit. Therefore, by relating these data, we obtain that the ancestral state could be an ellipsoidal polyad of medium size. And the synapomorphic state would be spheroidal / circular tetrads of small size. Large size could be an independent change.

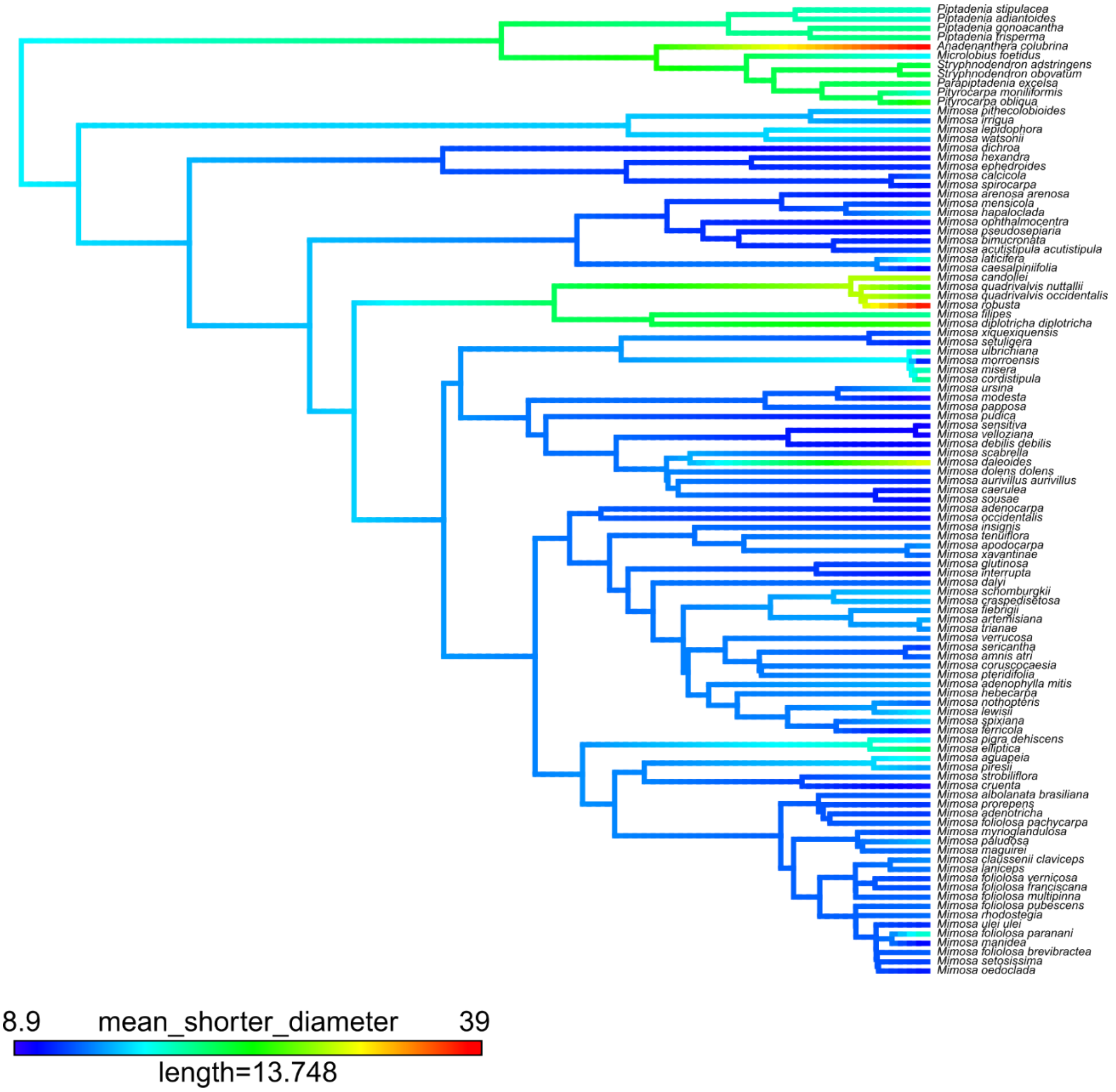


Figure 14. Optimization of mean of shorter diameter.

Exine thickness

Thin exine appears to be the conserved ancestral, *Mimosa* L. being quite homogeneous for that state. Thick exine is quite homoplastic, making it difficult to relate it to any other character state for *Mimosa.*

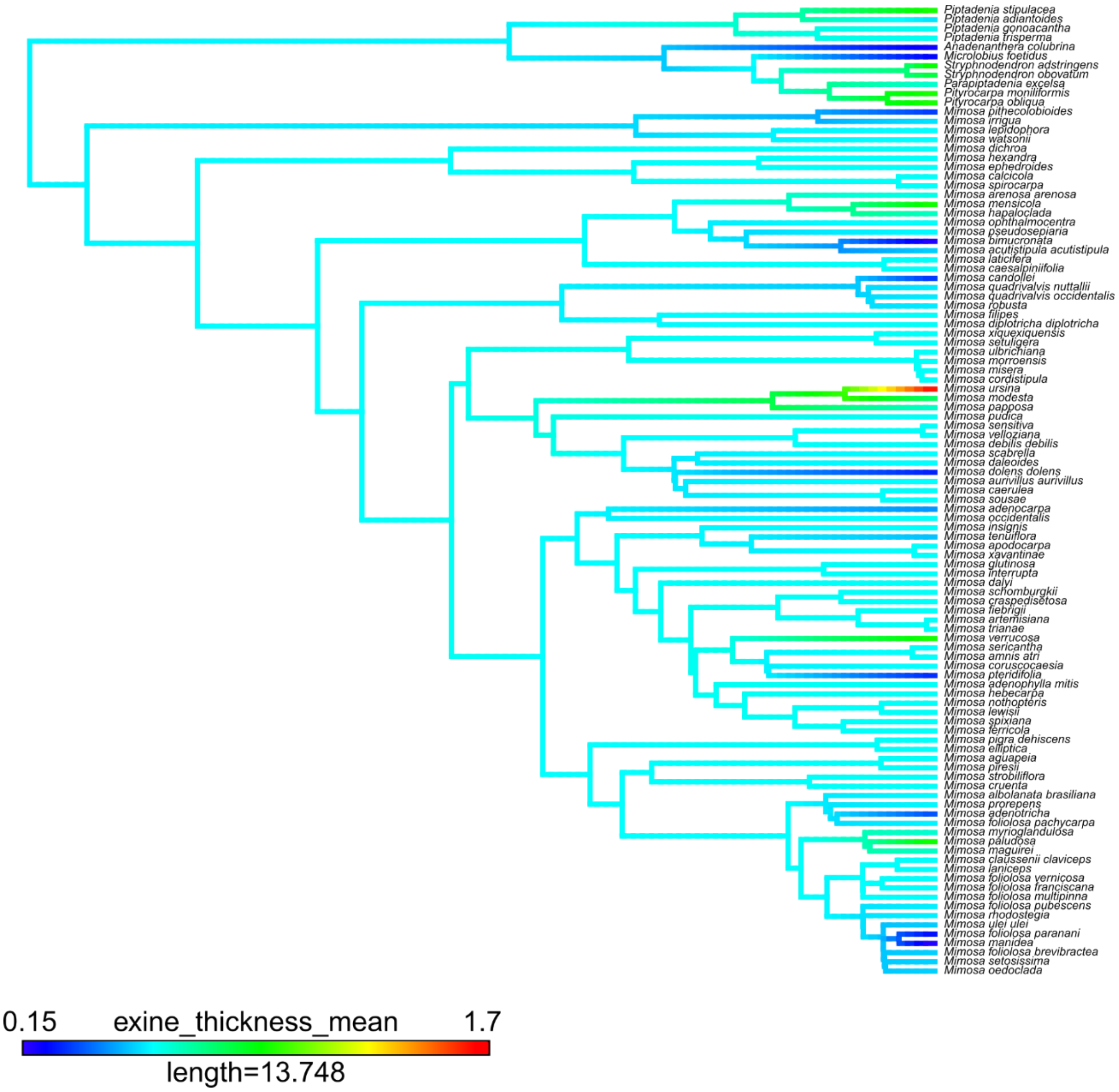


Figure 15. Optimization of mean of exine thickness.

Table 1. Relations between monophyletic series (Simon 2011) and homogeneity of pollen characters in them Modified of Simon (2011). – Series not sampled in Simon's study (2011). * Series without data in this study. Series with insufficient data (Only 1 specie per serie).

SECTION / SERIE	**MONOPHYLETIC**	**POLLEN MORPHOLOGY**
Mimadenia Barneby		
Myriadeniae Barneby	Yes	*
Glanduliferae Benth.	No	no
Revolutae Barneby	Monotypic	*
Nothacaciae Barneby	yes	*
Batocaulon Dc.		
Distachyae Barneby	no	Sampling data
Andinae Barneby	unresolved	*
Acanthocarpae Benth.	Unresolved	*
Acantholobae Barneby	Yes	*
Borales Barneby	No	Sampling data
Leiocarpae Benth.	No	No
Bimucronatae Barneby	No	no
Leucaenoideae Barneby	Unresolved	*
Rubicaules Benth.	No	*
Fagaranthae Barneby	Undersampled	*
Bahamenses Barneby	Monotypic	*
Farinosae Barneby	No	*
Ephedroideae Benth.	-	*
Echinocaulae Barneby	Monotypic	*
Paucifoliatae Benth.	No	Sampling data
Glandulosae (Benth) Barneby	No	Sampling data
Stipellares Benth	Yes	Yes

Auriculatae Barneby	monotypic	*
Caesalpiniifoliae Benth	Yes	Yes
Ceratoniae Barneby	undersampled	*
Cordistipulae Barneby	Yes	Yes
Campicolae Barneby	Undersampled	*
Filipedes Barneby	No	Sampling data
Quadrivalves Barneby	No	no
Plurijugae Karstn	no	*
Calothamnos Barneby		
-	unresolved	yes
Habbasia Dc.		
Setosae Barneby	No	Sampling data
Pachycarpae Benth	No	yes
Habbasia Dc.	Yes	yes
Bipinnatae Dc.		*
Neptunioideae Barneby	No	Sampling data
Rondonianae Barneby	-	*
Rojasianae Barneby	undersampled	*
Pseudocymosae Hassler	-	*
Piresianae Barneby	-	Sampling data
Mimosa L.		
Myriophyllae Benth.	unresolved	*
Mimosa L.	No	No
Modestae Benth.	no	no

Discussion

This result agrees with the overall homogeneity of *Mimosa* pollen (Flores-Cruz, 2006), is quite stable across the genus evolution mainly in monophyletic series. Our trees show that the 16-cell polyads are a plesiomorphic character (Barneby 1991) with respect to our focus group, the 12 and 8 cell polyads in *Mimosa* are derived. Although this has been noted before (Simon et al. 2011), here we showed that the plesiomorphic state is not 8 (Simon et al. 2011), but 12-celled polyads. This change stems from our outgroup selection, which better reflects the relationship between *Mimosa* and *Piptadenia* (Simon et al. 2016; LPWG 2017; Ribeiro et al. 2018). Indeed, 12-celled polyads apear to be a morphological synapomorphy for the clade including these two genera (Ribeiro et al. 2018).

Burkart (1948) proposed that "The *Pudicae subseries* (Within *Mimosa ser. Mimosa*) are the most evolved within the *Mimosa* genus and *Mimosa sect. Habbasia* would be the least advanced" based on the number of leaflets, their increase in size and the sensitivity of the leaves. Despite his work not having studied pollen grains to infer evolutionary patterns, it inspired the works of Caccavari, who also studied *Mimosa* species from Argentina and added knowledge through the palynological description of these species. A little over 30 years ago, Caccavari (1985; 1986) described 54 *Mimosa* species registered for Argentina and found 5 types of tetrads (small spheroidal tetrahedral, medium-sized tetrahedral, flattened decussate with circular or elliptical contour, ellipsoidal or oviform decussate and flattened tetragonal) and one type of polyad (bitetrads), and related them to the type of ornamentation, verrucate were the tetrahedral of medium size and the tetragonal flattened, the others were generally areolate. In addition, she proposed spheroidal tetrahedral tetrads with areolate ornamentation as the most primitive, from which derived the verrucate tetragonal tetrads and the bitetrads. And she stated that the *Mimosa* section was the ancestral and the *Mimosa sect. Habbasia* was the most derived. It is possible that the Caccavari's hypothesis has arisen when interpreting results in a reduced sampling group and that species belonging to non-monophyletic clades have been selected. A few years later, this proposal was contested by Barneby (1991) who believed polyads were plesiomorphic due to their presence in related genera belonging to the *Piptadenia* group. The presence of polyads may be associated with reduced pollen production in the anther and sporadic visits by pollinators (Harder & Johnson, 2008), so cell aggregation can make pollination more efficient and reduce the dehydration in difficult environments (Banks & Rudall, 2016).

It was common to observe in our results the presence of more than two types of pollen arrangements, so the polyads with irregular cellular organization could be plesiomorphic and the polyads with regular organization would derive, but it is not very clear because the species are polymorphic, there are reports of both characters for the same species (Lima et al., 2008). To prove correctly and make a more consistent inference of evolution of the presence of bitetrads (Regular organization of 8-polyads), we would need to study more other species of the outer group that present this characteristic of the genera: *Piptadenia, Pityrocar*pa and *Microlobius,* in contrast with the irregular 8-polyads.

Regarding the size of the polyads, we hypothesize that the ancestral state is the medium size and that the small size is the derived state. Despite not having been studied with respect to phylogeny before, these sizes have already been recorded by other authors, showing that small size is the most common in *Mimosa*, there are several species with medium size (Lima, 2008) This size parameter was also observed by Buril (2010) for the other members of the Piptadenia group. However, Sorsa (1969) made a much broader study of the Mimosoids, encompassing genera that generally possess small (10-25) and medium (25-50) size polyads, with exceptions in the genera *Parkia* R. Br., *Amblygonocarpus* Harms*, Piptadeniopsis* Burkart, *Serianthes* Benth*., Albizia* Durazz*., Pseudosamanea* Harms, *Lysiloma* Benth. and *Pithecellobium* Mart. large (50-100) and the genera *Calliandra* Benth. and *Inga Mill.*, very large (100-200), coincidentally, species with largest polyads do not belong to the Piptadenia group and are phylogenetically distant. Thus, we have that the thin exine is derived and the thick exine would be the ancestral one; on the other hand, the pores are the most advanced opening in contrast to the colpi and that the more evolved, it tends to have a greater number of pores (they went from 3 to 5 or 6 pores). Something that was hypothesized by Sorsa (1969) and that our results contradict, is that larger cells in Mimosoids, are more derivate. In contrast, we do not study the size of each cell but the size of the dispersion unit. And in our phylogenetic tree we observe a trend of decrease in size: small sizes would be the derivate.

For some groups it may be congruent that monophyletic series are homogeneous in their pollen morphology, as expected. Likewise, generally non-monophyletic series will be heterogeneous with respect to number of grains, cell organization, ornamentation, and size. However, we must carefully observe these series, which in some cases may have homogeneous morphology (Ex: *Mimosa ser. Pachycarpa* and *Mimosa sect. Calothamnos*), and the causes could be related to the habitat and the type of pollinator, although it has not yet been fully verified.

Conclusion:

Mimosa was homogeneous for some characteristics, mainly presenting the same categorical characteristics by monophyletic clades, varying only in the continuous data given. In non-monophyletic clades we observe heterogeneity, could see that the states are reflected in the phylogenetic tree, as expected Therefore we conclude that the 16 cell polyads are the plesiomorphic state, the 12 is the ancestral in *Mimosa* and 8 cell polyads would be derived. The tetrads would be the apomorphic state. About cell organization, we consider that the irregular arrangements are plesiomorphic and the regular ones would be derived, being possible to observe homoplasy. In the case of ornamentation, we consider that rugulate is plesiomorphic and the microverrucate state (In *Anadenanthera*) could be the ancestral of the synapomorphic states: verrucate and areolate come. In the case of pores, we observe high level of homoplasy for 4 pores, and probably 5-6 pores are derivate. The size small and medium of tetrads and polyads are derivate, other members of Piptadenia group have medium polyad but is evident the reduction of size per each grain. In *Mimosa* is common observe tetrads with grains very small being part of cellular arrangements. We also observe that some characters are related, such as ornamentation, to the size and the outline with the smallest diameter, but that they are not definitive for some groups. Therefore, our results agree partially with that proposed by Barneby (1991) and Simon et al. (2011) regarding cell number characters and refute what was hypothesized by Caccavari (1988), showing that a high number of cells is the plesiomorphic character and polyads with 12 cells are the ancestral for *Mimosa*. Furthermore, we conclude that it is necessary to continue studying the evolution of the other morphopollinic characters in order to clarify evolutionary doubts of this very diverse genus.

Author Contributions:

“Conceptualization, S.L.G. and L.M.B.; methodology and validation, G.L.C., R.F.B, S.L.G and Y.B.S.; formal analysis, S.L.G and L.M.B.; investigation, S.L.G.; data curation, F.A.R.D.; writing—original draft preparation, S.L.G.; writing—review and editing, S.L.G. and L.M.B.; supervision, L.M.B.

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GENERAL DISCUSSION

Mimosa pollen is quite stable across the genus evolution. Our work agrees with the overall homogeneity of *Mimosa's* pollen (Flores-Cruz, 2006), mainly in monophyletic series. The shapes and sizes of the polyads, the presence of apertures, and exine ornamentation (Medina-Acosta 2018) may also be of taxonomic importance.

Our results showed 12 types of cell arrangements: Tetrahedral elliptical, tetrahedral oval – subtype 1, tetrahedral oval – subtype 2, tetrahedral spheroidal, decussated elliptical, decussated spheroidal, tetragonal elliptical, tetragonal spheroidal, rhomboidal elliptical, rhomboidal spheroidal, bitetrad (polyad with 2 tetrads) and tritetrad (Polyad with 3 tetrads), and some species has 2 types of them (or 3, rarely). Other studies (Caccavari, 1985; Lima et al. 2007) reported some of these types of organizations, but separately. Our trees show that the 16-cell polyads are a plesiomorphic character (Barneby 1991) with respect to our focus group, the 12 and 8 cell polyads in Mimosa are derived. Although this has been noted before (Simon et al. 2011), here we showed that the plesiomorphic state is not 8 (Simon et al. 2011), but 12-celled polyads. This change stems from our outgroup selection, which better reflects the relationship between *Mimosa* and *Piptadenia* (Simon et al. 2016; LPWG 2017; Ribeiro et al. 2018). Indeed, 12-celled polyads apear to be a morphological synapomorphy for the clade including these two genera (Ribeiro et al. 2018).

In the case of ornamentation, we consider that rugulate is plesiomorphic and the microverrucate state (In *Anadenanthera*) could be the ancestral of the synapomorphic states: verrucate and areolate come. In the case of pores, we observe high level of homoplasy for 4 pores, and probably 5-6 pores are derivate. The size small and medium of tetrads and polyads are derivate, other members of Piptadenia group have medium polyad but is evident the reduction of size per each grain. Furthermore, we conclude that it is necessary to continue studying the evolution of the other morphopollinic characters in order to clarify evolutionary doubts of this very diverse genus.

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