PRISCILA ANDRESSA CORTEZ

"DESENVOLVIMENTO NA ANTERA EM ESPÉCIES DE *Miconia* (MELASTOMATACEAE) COM DIFERENTES SISTEMAS REPRODUTIVOS"

Campinas, 2012

UNIVERSIDADE ESTADUAL DE CAMPINAS

INSTITUTO DE BIOLOGIA

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"DESENVOLVIMENTO NA ANTERA EM ESPÉCIES DE *Miconia* (MELASTOMATACEAE) COM DIFERENTES SISTEMAS REPRODUTIVOS"

Tese apresentada ao Instituto de Biologia para obtenção do Título de Doutor em Biologia Vegetal.

Orientadora: Profa. Dra. Simone de Pádua Teixeira

Co-Orientadora: Profa. Dra. Sandra Maria Carmello Guerreiro

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Dedico esta tese à Taís Mazzola e ao Flávio Cavalcante.

A Perfeição

"O que me tranquiliza é que tudo o que existe, existe com uma precisão absoluta. O que for do tamanho de uma cabeça de alfinete não transborda nem uma fração de milímetro além do tamanho de uma cabeça de alfinete. Tudo o que existe é de uma grande exatidão. Pena é que a maior parte do que existe com essa exatidão nos é tecnicamente invisível. Apesar da verdade ser exata e clara em si própria, quando chega até nós se torna vaga pois é tecnicamente invisível. O bom é que a verdade chega a nós como um sentido secreto das coisas. Nós terminamos adivinhando, confusos, a perfeição."

(Clarice Lispector. 1984. *In* A Descoberta do Mundo. Nova Fronteira, Rio de Janeiro, p. 226)

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Índice

Resumo

A principal forma de reprodução das angiospermas é a sexuada, que envolve obrigatoriamente a produção (gametogênese) e a fusão (fecundação) dos gametas haploides. Entretanto, um número expressivo de espécies se reproduz assexuadamente por meio de apomixia, na qual a meiose é ausente ou modificada e os embriões são formados sem que haja fecundação. Dois tipos básicos de apomixia são reconhecidos: um em que o embrião se origina no interior de um megagametófito com número cromossômico somático (não reduzido); e outro, em que não há formação do megagametófito e o embrião se origina de células do nucelo ou do tegumento do óvulo. Espécies apomíticas são relatadas com mais frequência entre as Poaceae, Asteraceae, Rosaceae e Rutaceae, famílias com grande importância econômica. No entanto, vários estudos têm revelado cada vez mais exemplares apomíticos entre as Melastomataceae, principalmente em *Miconia*, gênero parafilético que inclui cerca de 1050 espécies. Levando em consideração o grande número de grãos de pólen inviáveis produzidos pelas espécies apomíticas neste gênero e a frequente utilização da forma e do desenvolvimento dos estames no reconhecimento de relações evolutivas entre os membros do grupo, este trabalho teve como objetivos (1) comparar o desenvolvimento dos estratos parietais da antera e dos grãos de pólen de *Miconia albicans* (espécie apomítica sem produção de grãos de pólen), *M. stenostachya* (espécie apomítica com baixa porcentagem de grãos de pólen viáveis) e *M. paucidens* (espécie sexuada com alta porcentagem de grãos de pólen viáveis); (2) estudar as características estruturais e ultraestruturais relacionadas à inviabilidade dos grãos de pólen de *M. albicans* e *M. stenostachya*; e (3) analisar a relação entre a epiderme e o endotécio no mecanismo de deiscência das anteras das espécies apomíticas *M. albicans*, *M. fallax*, *M. leucocarpa*, *M. stenostachya* e *M. sellowiana*, e das espécies sexuais *M. chamissois*, *M. minutiflora*, *M. paucidens*, *M. pseudonervosa*, e *M. theaezans*. A estrutura, ultraestrutura e micromorfologia da parede da antera e dos grãos de pólen foram analisadas em anteras fixadas em solução contendo glutaraldeído e processadas segundo protocolos de rotina para microscopia de luz e microscopias eletrônicas de transmissão e varredura. A forma e a viabilidade dos grãos de pólen maduros e a forma do tubo polínico foram analisadas a partir de grãos de pólen frescos coletados de anteras de flores recém-abertas, submetidos a testes de hidratação e de germinação “in vitro”. A detecção dos principais grupos de substâncias químicas presentes nos vários estratos parietais da antera e nos grãos de pólen foi feita por meio da aplicação de reagentes e corantes específicos às seções finas e aos grãos de pólen frescos. Em *M. albicans*, *M. stenostachya* e *M. paucidens*, as anteras são tetrasporangiadas, o desenvolvimento da parede da antera é do tipo monocotiledôneo e os grãos de pólen não apresentaram substâncias de reserva ao longo do desenvolvimento; em *M.*

paucidens, as células do tapete apresentaram cristais e proteoplastos no citoplasma. A antera em estádio de deiscência é constituída pela epiderme e pelo endotécio não funcional. Em *M. albicans*, a ausência de figuras meióticas indica que a meiose é um evento raro nessa espécie; as anteras maduras apresentaram-se vazias ou contendo estruturas semelhantes a grãos de pólen com paredes mal formadas e sem conteúdo. Em *M. stenostachya*, irregularidades meióticas levaram à formação de micrósporos de diferentes tamanhos e em número maior que quatro; a porcentagem nula de germinação dos grãos de pólen de *M. stenostachya* indicou que mesmo os grãos de pólen corados e com parede bem formada são inviáveis. Em *M. paucidens*, os grãos de pólen são alongados e liberados da antera parcialmente desidratados, havendo a formação do tubo polínico após a hidratação proporcionada pelo contato com a superfície estigmática. Em todas as espécies analisadas, o mecanismo de deiscência envolve a degradação da epiderme da antera numa região em que a cutícula é ausente e não depende da ação do endotécio, já que este tecido não apresenta nenhum tipo de especialização que possa ser relacionada à deiscência das anteras.

Palavras Chave: Apomixia, deiscência poricida, endotécio, esterilidade masculina, histoquímica, irregularidades meióticas, Melastomataceae, *Miconia*, sistema reprodutivo, ultraestrutura.

Abstract

Most angiosperms reproduce sexually, with the production (gametogenesis) and fusion (fertilization) of the haploid gametes. However, some of them do so in an asexual way, called apomixis, a way of reproduction with the production of seeds which embryos are produced without fertilization of the egg cell. There as two main types of apomixis: one in which the embryo is formed inside the megagametophyte with somatic chromosome level (unreduced); and another one, in which there is no megagametophyte formation and the embryo is formed from ovule nucellar or tegument cells. Apomixis is often studied in Poaceae, Asteraceae, Rosaceae and Rutaceae families, probably due to its economical value. However, many studies have shown an increasing number in the tropical flora, mainly among *Miconia* (Melastomataceae), a paraphyletic genus with about 1050 species. Taking into account the amount of unviable pollen grains in apomictic *Miconia* and the importance of stamen morphology and development to the phylogenetic studies, this study aimed to (1) compare the anther and pollen grain development in *Miconia albicans* (apomictic species with lacks pollen grains), *M. stenostachya* (apomictic species with low percentage of viable pollen grains) and *M. paucidens* (sexual species with high percentage of viable pollen grains); (2) study the structure and ultrastructure characteristics related to the pollen grains development in *M. albicans* and *M. stenostachya*; and (3) study the relationship of the endothecium and the epidermis with the poricidal anther dehiscence mechanism in the apomicts *M. albicans*, *M. fallax*, *M. leucocarpa*, *M. stenostachya* and *M. sellowiana*, and in the sexual species *M. chamissois*, *M. minutiflora*, *M. paucidens*, *M. pseudonervosa*, and *M. theaezans*. Anthers in various stages of development were studied under light microscopy and scanning and transmission electron microscopes. The shape and the viability of mature pollen grains and the pollen tube shape were examined in fresh pollen grains obtained from mature anthers, which were submitted to hydration and "in vitro" germination testes. Tests for the main groups of chemical substances were applied to thin sections and to fresh pollen grains. *M. albicans*, *M. stenostachya* and *M. paucidens* have tetrasporangiated anthers, anther wall development following the "monocotyledonous" type, and pollen grains which lack reserve substances during all steps of development; in *M. paucidens*, crystals and proteoplasts were found inside the tapetal cell cytoplasm. The dehiscent anthers have epidermis and non-functional endothecium. The absence of meiotic figures in *M. albicans* may indicate that the meiosis is a rare event in this species; its mature anthers were also empty or with pollen grain-like structures, but completely empty and with abnormal pollen wall. The meiotic irregularities observed in *M. stenostachya* gave rise to the microspores with abnormal size (smaller or bigger than the normal ones) and often with more than four per tetrad; the null percentage of pollen grain germination in

M. stenostachya showed that even the well-stained pollen grains were unviable. *M. paucidens* showed elongated pollen grains that are released from anther in a partially dehydrated way, being the pollen tube originated after the pollen grain hydration when in contact with the stigmatic surface. In all of the species studied, the anther dehiscence mechanism is a consequence of cell degradation in a portion that lack cuticle being, for this, independent of the endothecium layer.

Keywords: Apomixis, poricide anther, endothecium, male sterility, histochemistry, meiotic irregularities, Melastomataceae, *Miconia*, reproductive system, ultrastructure.

Introdução geral

As angiospermas apresentam como principal forma de reprodução a sexuada, que envolve obrigatoriamente a produção e a fusão de gametas haplóides, e tem como consequência gerar variabilidade genética por meio de eventos de recombinação, segregação e singamia. Entretanto, um número expressivo de espécies apresenta a reprodução assexuada como uma forma natural alternativa ou obrigatória de reprodução, na qual indivíduos geneticamente semelhantes à planta-mãe (clones) são originados por meio da propagação vegetativa ou da apomixia (Maheshwari 1950, Cocucci 1969, Grant 1981, Nogler 1984, Went e Willemse 1984, Naumova 1993, Endress 1994, Richards 1997, Raven et al. 2005, Mariath et al. 2006).

Apomixia, em seu sentido restrito (consultar Asker e Jerling 1992, e Mogie 1992 para informações terminológicas), refere-se aos casos em que a reprodução se dá obrigatoriamente por meio da formação de sementes, sendo, por isso, considerada derivada dos sistemas reprodutivos sexuados. Em uma planta apomítica, a meiose é ausente ou modificada e a formação do embrião ocorre sem que haja fusão de gametas (Asker e Jerling 1992, Naumova 1993, Koltunow et al. 1995, Vielle-Calzada et al. 1996, Carman 1997, Richards 1997, Drews et al. 1998, Berthaud 2001, Grossniklaus et al. 2001b, Naumova 2008).

A nomenclatura empregada aos casos de apomixia é numerosa e confusa. Tal fato se deve, principalmente, à dificuldade de se reconhecer padrões que possam ser extrapolados aos vários grupos que contêm espécies apomíticas. Entretanto, dois tipos básicos e distintos de apomixia podem ser reconhecidos (Figura 1). Na apomixia gametofítica, um megagametófito (saco embrionário) com número cromossômico não reduzido é formado a partir de uma megasporócito (diplosporia) ou a partir de uma ou mais células somáticas do óvulo (aposporia); o embrião apomítico tem origem autônoma a partir da oosfera ou de outra célula do megagametófito. A apomixia esporofítica (embriogenia ou embrionia adventícia), não depende da formação do megagametófito, e o embrião apomítico se desenvolve diretamente a partir de células do nucelo ou do tegumento do óvulo (Nogler 1984, Naumova 1993, Richards 1997, Koltunow et al. 1995, Grossniklaus et al. 2001a, Mendes-Rodrigues et al. 2005).

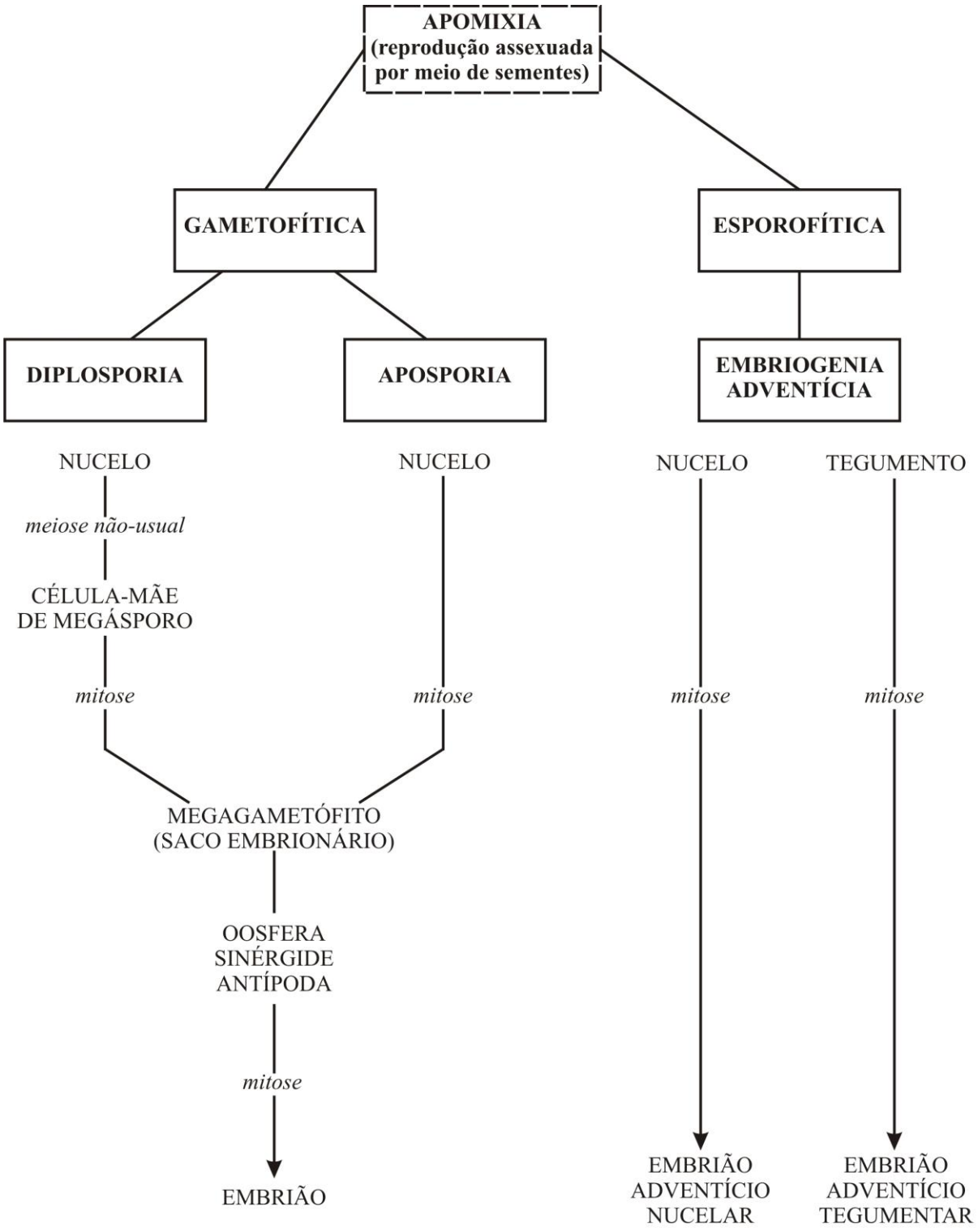

Figura 1. Tipos básicos de apomixia em angiospermas. Baseado em Nogler (1984), Naumova (1993), Richards (1997), Koltunow et al. (1995), Grossniklaus et al. (2001a), Mendes-Rodrigues et al. (2005).

Os primeiros estudos a relatarem a ocorrência da apomixia entre as angiospermas datam da metade do século XIX, e seu padrão de distribuição nas angiospermas indica origem independente (Asker e Jerling 1992, Grossniklaus et al. 2001a, Naumova 2008), sendo reconhecidas atualmente cerca de 400 espécies apomíticas, distribuídas em cerca de 50 famílias, com mais frequência em espécies de Poaceae, Asteraceae, Rosaceae e Rutaceae (Asker e Jerling 1992, Carman 1997, Richards 1997, Grossniklaus et al. 2001a, Berthaud 2001, Richards 2003, Naumova 2008). Vários estudos têm revelado que esse tipo de reprodução é também comum entre as Melastomataceae, particularmente na tribo Miconieae, na qual mais de 60% das espécies estudadas são classificadas como apomíticas (Goldenberg e Shepherd 1998).

Incluída na ordem Myrtales (Figura 2), Melastomataceae Juss. (Figura 3) compreende cerca de 4500 espécies distribuídas em 150-166 gêneros (Renner 2004), distribuídas principalmente em regiões tropicais e subtropicais (Figura 4). Cerca de três mil espécies em 107 gêneros podem ser encontradas nos Neotrópicos (Renner 1989). Seus representantes compõem a flora de diversas formações vegetais e apresentam diversidade de hábito, de arquitetura vegetativa e reprodutiva, e de tipos de indumento (Renner 1989), embora possam ser, com exceção de poucos gêneros, facilmente reconhecidas pela vascularização foliar paralelódroma e pela presença de estames falciformes. A produção de néctar é rara nos membros da família (Varassin et al. 2008) e grande parte das espécies é polinizada por abelhas que coletam os grãos de pólen das anteras por meio do movimento vibratório do corpo e das asas, um mecanismo conhecido como “buzz pollination” (Buchmann 1983, Renner 1989, Laroca 2003).

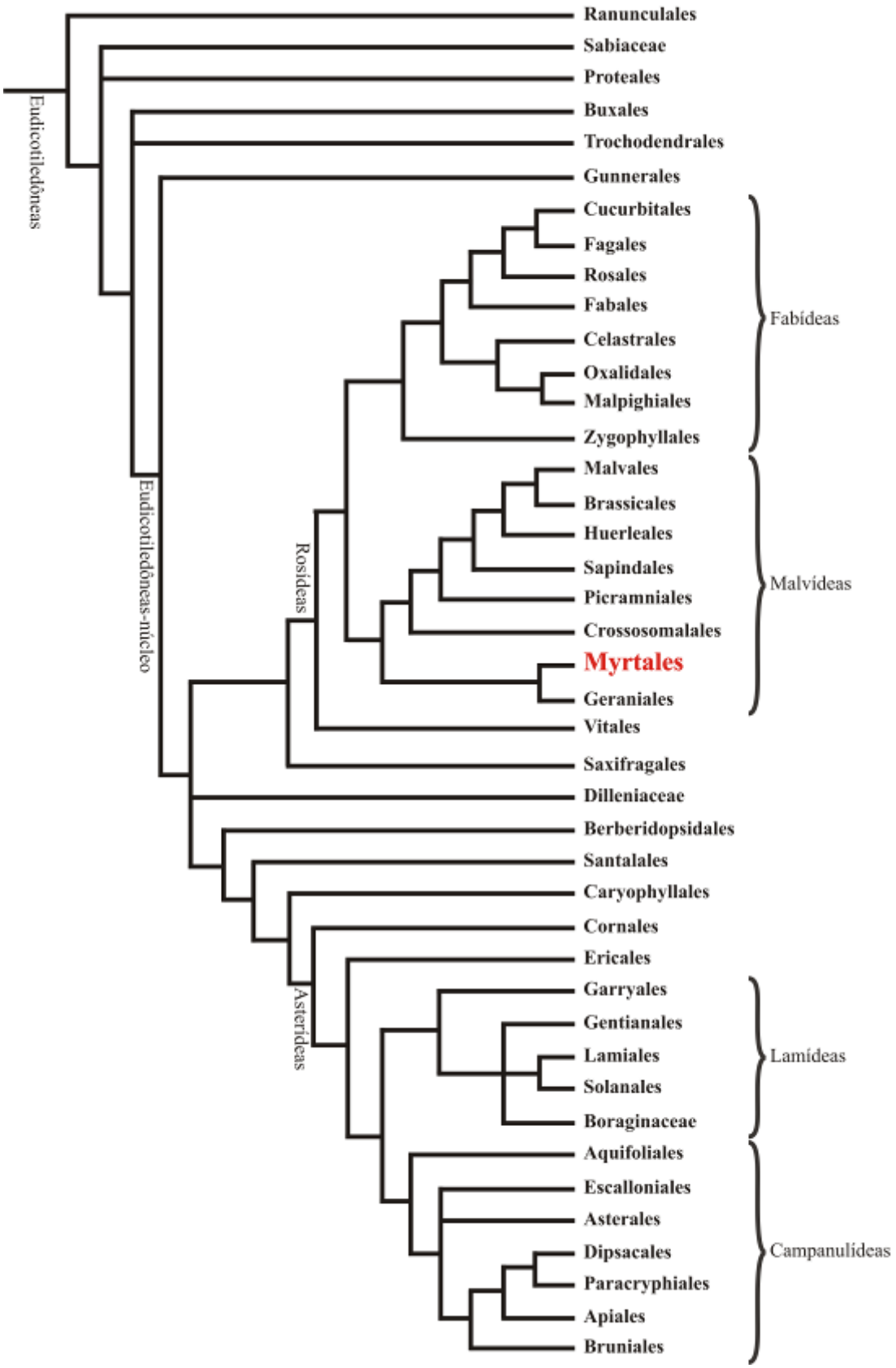

Figura 2. Cladograma mostrando as relações entre as angiospermas eudicotiledôneas, com destaque para a ordem Myrtales. Modificado de APG III (2009).

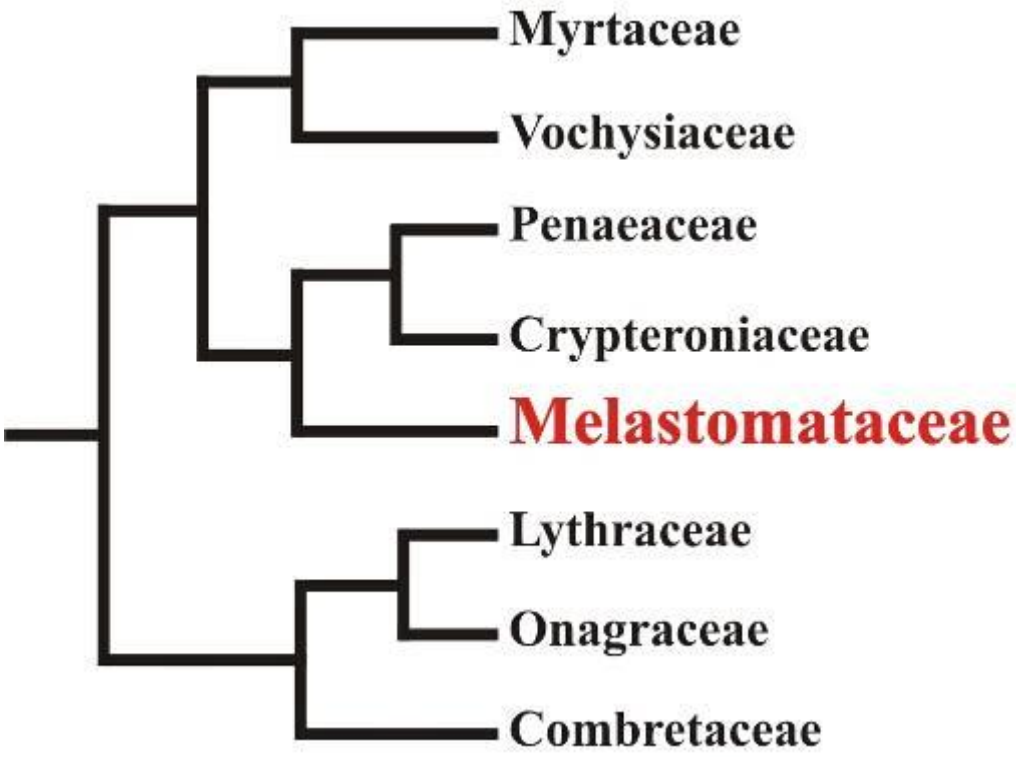

Figura 3. Cladograma mostrando as relações hipotéticas dentro da ordem Myrtales, com destaque para a família Melastomataceae. Modificado de Soltis et al. (2011).

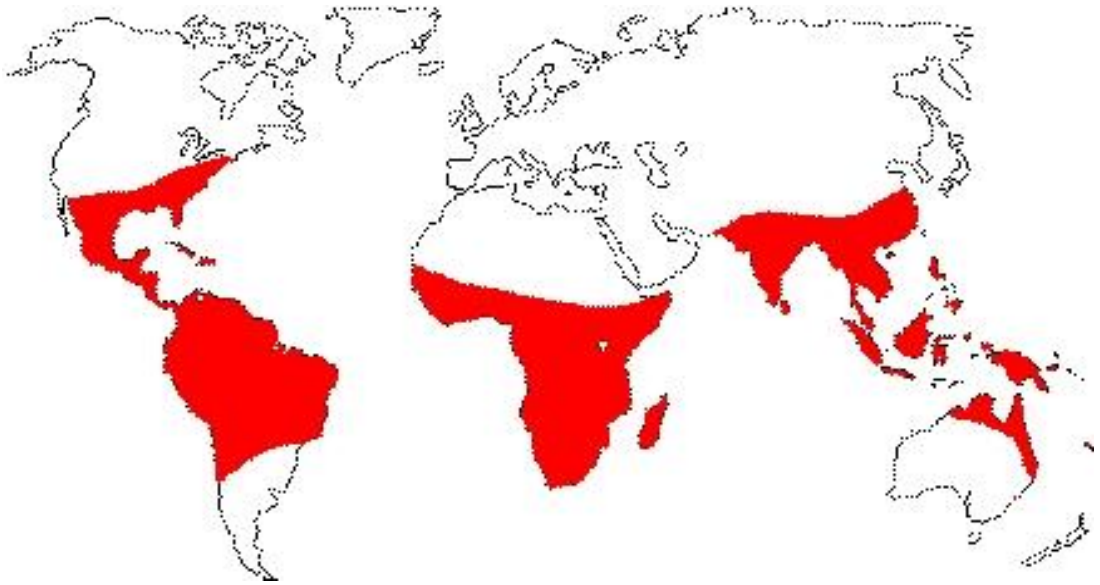

Figura 4. Mapa de distribuição de espécies da família Melastomataceae. Modificado de www.mobot.org/MOBOT/research/APweb.

A família Melastomataceae é considerada monofilética, condição apoiada tanto por caracteres morfológicos (Johnson e Briggs 1984, Renner 1993) quanto por sequências de DNA (Conti 1994, Clausing et al. 2000, Clausing e Renner 2001b, Renner et al. 2001, Michelangeli et al. 2004, Renner 2004) e está dividida em nove tribos, todas elas reconhecidas como provavelmente monofiléticas (Clausing e Renner 2001a, b).

Miconieae DC. é a tribo de Melastomataceae com maior número de espécies, cerca de 2200 em 30 gêneros (Renner 2004); é um clado exclusivamente Neotropical que possui como sinapomorfia os frutos do tipo baga (Judd et al. 2008), embora seus gêneros sejam, até certo ponto, arbitrariamente definidos (Goldenberg et al. 2008). Estudos recentes visando entender melhor as relações evolutivas no grupo indicam que a utilização conjunta de um grande número de caracteres morfológicos tem se mostrado fonte confiável e informativa das relações filogenéticas na tribo (Whiffin e Tomb 1972, Skean 1993, Groenendijk et al. 1996, Michelangeli 2000, Fritsch et al. 2004, Michelangeli et al. 2004, Penneys e Judd 2005, Judd 2007, Martin et al. 2008). Goldenberg et al. (2008) observaram que as linhagens em Miconieae (Figura 5) têm forte relação geográfica e que alguns taxa, historicamente referidos como de ampla distribuição são, na realidade, restritos a determinadas regiões, sendo os membros geograficamente distantes pertencentes a outros clados. Estudos dessa abrangência são particularmente importantes quando se trata de gêneros com grande número de espécies, como é *Miconia* Ruiz & Pav., maior gênero da tribo, com cerca de 1050 espécies (Judd et al. 2008), todas nativas das Américas.

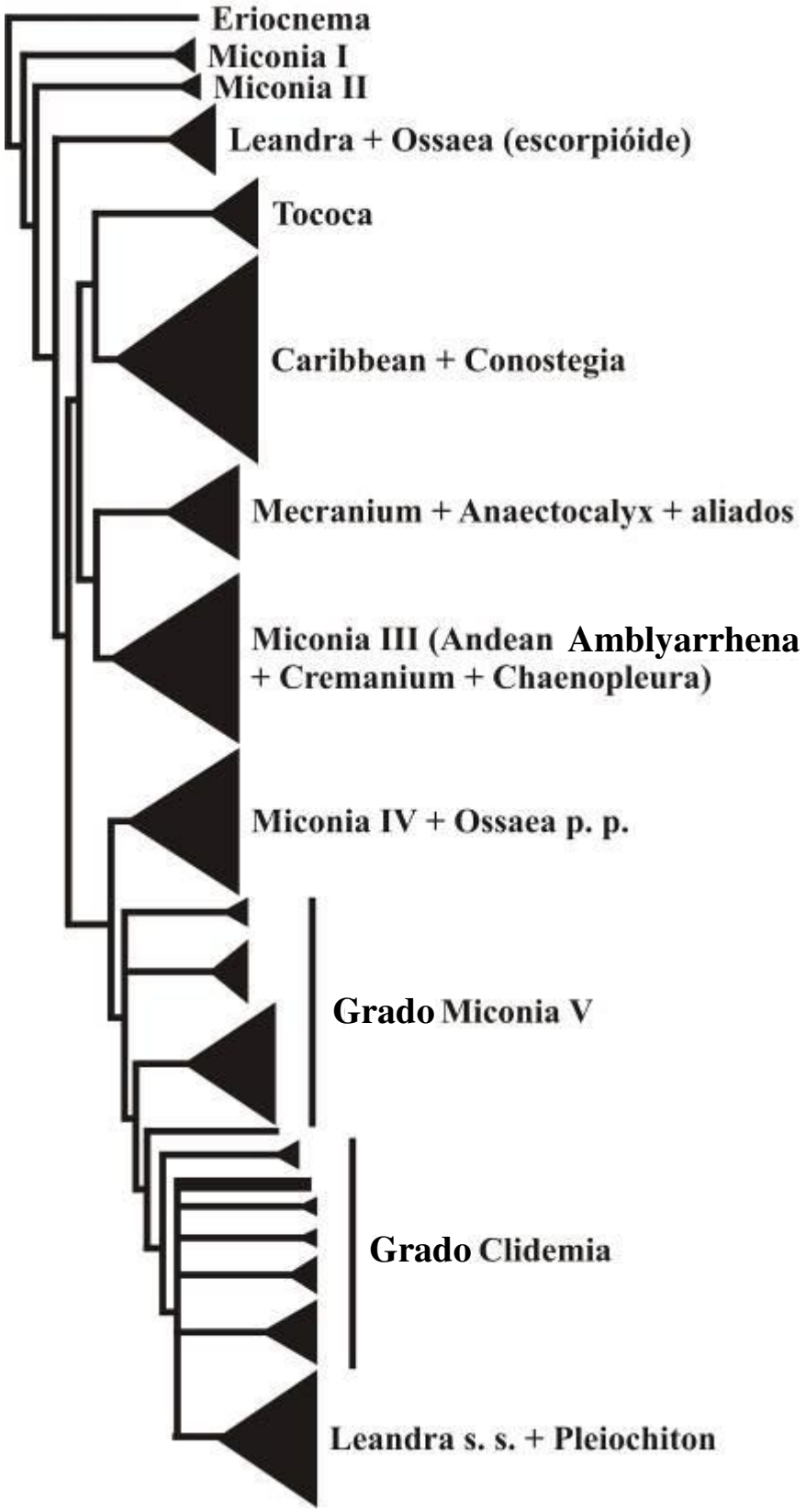

Figura 5. Filogenia da tribo Miconieae, resultantes da análise combinada entre sequências de *nr*ITS e *ndhF*. Modificado de Goldenberg et al. (2008).

Estudos filogenéticos reconhecem *Miconia* como um gênero claramente parafilético (Judd e Skean 1991, Michelangeli et al. 2004, Martin et al. 2008), cuja classificação infragenérica, estabelecida por Cogniaux em 1891 e ainda hoje aceita, embora com reservas, inclui 11 seções (Tabela 1), definidas, sobretudo, com base em caracteres reprodutivos, principalmente aqueles relacionados à morfologia dos estames (Goldenberg et al. 2008). Espécies de *Miconia* ocorrem do sul do México até o norte da Argentina e Uruguai, sendo 276 delas encontradas no Brasil (Goldenberg 2010). As espécies deste gênero possuem flores completas, (3)4-5(8)-meras, actinomorfas ou com estames e estilete formando conjuntos zigomorfos; o androceu é frequentemente diplostêmone, com estames iguais ou com dois verticilos levemente desiguais, com anteras mais comumente deiscentes por um poro apical com conectivo portando ou não apêndices; o gineceu tem ovário (1)2-5-locular, ínfero ou semi-ínfero; uma ou várias sementes piramidais a ovais (Martins 2009).

Tabela 1. Seções de *Miconia* de acordo com Cogniaux (1891). Modificado de Goldenberg et al. (2008).

1. *Jucunda*
2. *Tamonea*
3. *Adenodesma*
4. *Octomeris*
5. *Laceraria*
6. *Miconia*
6.1. *Aplostachyae*
6.2. *Diplostachyae*
6.3. *Impetiolares*
6.4. *Glomeratiflorae*
6.5. *Seriatiflorae*
6.6. *Paniculares*
7. *Glossocentrum*
8. *Chaenanthera**
9. *Amblyarrhena* (incluindo *Hartigia*)
10. *Cremanium*
11. *Chaenopleura*

*Sinonimizada sob a seção *Hypoxanthus* por Goldenberg (2000).

É interessante notar que a ocorrência de populações apomíticas no gênero *Miconia* é frequente e pode ter relação direta com a grande especiação e o sucesso evolutivo observado no grupo, refletidos em sua ampla distribuição geográfica. Assim como para a tribo Miconieae, cerca de 60% das espécies do gênero *Miconia* estudadas quanto ao sistema reprodutivo foram

reconhecidas como apomíticas (Goldenberg 1994). Muitas delas são poliplóides, já que apresentam número cromossômico básico superior ao x=17 reconhecido para a tribo Miconieae (Solt e Wurdack 1980, Almeda 1997, Almeda e Chuang 1992, Goldenberg e Shepherd 1998). A relação entre poliploidia e apomixia em espécies de *Miconia* pode ser decorrente de uma possível origem híbrida para os membros do grupo (Goldenberg 2000).

Nesse contexto, estudos de desenvolvimento têm auxiliado a reconhecer anormalidades estruturais ocorrentes nos micrósporos (geração esporofítica) e/ou nos grãos de pólen (geração gametofítica), considerados indicadores consistentes da ocorrência de apomixia. Embora vários genes relacionados à inviabilidade polínica tenham sido identificados e caracterizados funcionalmente (Cigan et al. 2001, Fei e Sawhney 2001, Wilson et al. 2001, Kapoor et al. 2002, Lai et al. 2002, Sorensen et al. 2003), ainda são raros os estudos que descrevem as alterações morfológicas associadas a essas anormalidades.

Estudos estruturais e ultraestruturais de órgãos reprodutivos, sobretudo dos tecidos do óvulo e da antera diretamente relacionados à formação dos gametas femininos e masculinos, permitem reconhecer aspectos importantes relacionados ao sucesso reprodutivo de um táxon (Gibson e Diggle 1998, Ilarslan et al. 1999). Em linhagens estéreis, esses estudos têm mostrado que o fenômeno está, frequentemente, relacionado a alterações morfológicas e funcionais das células dos estratos parietais da antera, especialmente do tapete (Izhar e Frankel 1971, Bhandari 1984, Kronestedt-Robards e Rowley 1989, Johri et al. 1992, Loukides et al. 1995, Ilarslan et al. 1999), ou no próprio tecido esporogênico (Knox 1984, Peel et al. 1997). Tais alterações têm como causas principais as irregularidades meióticas das células-mãe de micrósporos, a nutrição deficiente dos micrósporos e/ou a má-formação da parede dos micrósporos (geração esporofítica) e/ou dos grãos de pólen (geração gametofítica).

Estudos ontogenéticos realizados em *Miconia albicans* (Cortez 2007) e em *M. pepericarpa* e *M. fallax* (Caetano 2010, Caetano et al. em preparação) forneceram informações morfológicas importantes a respeito da inviabilidade dos grãos de pólen, bem como permitiram reconhecer os estádios de desenvolvimento mais informativos, que se tornaram o foco principal de estudos posteriores, incluindo esta tese. Os resultados obtidos sobre a morfologia contribuirão para estudos em andamento que visam a entender as relações evolutivas entre os membros desse gênero diverso. A descrição de características relacionadas à esterilidade masculina em espécies de *Miconia*, muitas delas inéditas, poderá contribuir também com estudos com enfoque reprodutivo e ecológico do grupo, já que a existência de espécies sexuadas e apomíticas com diferentes porcentagens de aborto de grãos de pólen na população é particularmente interessante por permitir análises comparativas

dos caracteres ontogenéticos em um mesmo genótipo ou em genótipos diferentes de uma mesma população (Naumova e Vielle-Calzada 2001).

Se os resultados obtidos até o momento sobre o sistema reprodutivo das espécies de *Miconia* forem extrapolados à família Melastomataceae, podemos reconhecê-la como o maior complexo apomítico dentre as famílias até hoje estudadas. Entretanto, este "status" se mantém como suposição, já que apenas cerca de 5% das espécies descritas para a família foram estudadas quanto ao seu sistema reprodutivo. Dessa forma, este trabalho teve como objetivos (1) comparar o desenvolvimento dos estratos parietais da antera e dos grãos de pólen de *Miconia albicans* (espécie apomítica sem produção de grãos de pólen), *M. stenostachya* (espécie apomítica com baixa porcentagem de grãos de pólen viáveis) e *M. paucidens* (espécie sexuada com alta porcentagem de grãos de pólen viáveis) (Capítulo 1); (2) estudar as características estruturais e ultraestruturais relacionadas à inviabilidade dos grãos de pólen de *M. albicans* e *M. stenostachya* (Capítulo 2); e (3) analisar a relação entre a epiderme e o endotécio no mecanismo de deiscência das anteras das espécies apomíticas *M. albicans*, *M. fallax*, *M. leucocarpa*, *M. stenostachya* e *M. sellowiana*, e das espécies sexuais *M. chamissois*, *M. minutiflora*, *M. paucidens*, *M. pseudonervosa*, e *M. theaezans* (Capítulo 3).

Espécies Estudadas

As espécies incluídas neste estudo (Tabela 2, Figura 6) foram escolhidas com base nos estudos disponíveis sobre o sistema reprodutivo (Renner 1989, Goldenberg e Shepherd 1998) e posição filogenética (Goldenberg et al. 2008) de *Miconia*. A identificação das espécies foi auxiliada pela Dra. Angela Borges Martins, pelo Dr. Renato Goldenberg, e pelo Professor Jorge Yoshio Tamashiro, sendo utilizadas também chaves de identificação para o gênero (Goldenberg 2009).

Tabela 2. Espécies de *Miconia* incluídas neste estudo. [1]Seção *Cremanium*. [2]Seção (*Eu*)*Miconia*. [3]Seção *Glossocentrum*. [4]Seção *Hypoxanthus*. *Segundo Almeda e Chuang (1992).

Espécies	Sistema reprodutivo	Número cromossômico*	Posição filogenética	Local de coleta
Miconia theaezans[1]	sexual	n=17	Miconia III	Uberlândia-MG
Miconia albicans[2]	apomítica	2n=34, 48	Miconia IV + Ossaea	Campinas-SP Itirapina-SP Ubatuba-SP
Miconia stenostachya[2]	apomítica	n=ca26		
Miconia fallax[2]	apomítica	n=ca17		Itirapina-SP
Miconia minutiflora[3]	sexual	n=17		
Miconia pepericarpa[3]	sexual	?		
Miconia chamissois[2]	sexual	?	Miconia V	Uberlândia-MG
Miconia sellowiana[4]	apomítica	?		Jundiaí-SP
Miconia paucidens	sexual	?	(posição filogenética não estimada)	Itirapina-SP
Miconia leucocarpa	apomítica	?		
Miconia pseudonervosa	sexual	?		

1. *Miconia albicans* (Sw.) Triana, Trans. Linn. Soc. London 28: 116. 1871.

Arbustos até 2,5 m. Ramos, pecíolos, brácteas e hipanto densamente revestidos por indumento tomentoso, canescente ou ferrugíneo. Folhas com pecíolos; lâmina coriácea, elíptica, oblonga ou obovada, base arredondada a subcordada, ápice obtuso, agudo a curtamente acuminado, margem levemente ondulado-crenulada, nervuras acródromas basais ou ocasionalmente suprabasais; face adaxial das folhas jovens densamente tomentosa, depois glabra, face abaxial densamente tomentosa. Panículas escorpioides, terminais, piramidais a cilíndricas. Flores 5-meras; hipanto 2,5mm; cálice persistente, lacínias internas e externas fundidas, largamente triangulares; anteras brancas, uniporosas, conectivo prolongado abaixo das tecas, bastante espessado no dorso, nos antessépalos com projeção basal ampla, contínua da região ventral à dorsal, nos antepétalos com calcar dorsal alargado e com duas aurículas ventrais curtas; ovário 3-locular, glabro, estilete abruptamente alargado no ápice. Baga verde-jade quando madura, ca. 30 sementes. Ocorre desde o sul do México e Antilhas até o Paraguai e sul do Brasil (Paraná). No estado de São Paulo, ocorre em cerrados e vegetação secundária, inclusive litorânea. Coletada com flores e frutos praticamente durante todo o ano (Goldenberg 2009).

2. *Miconia chamissois* Naudin, Ann. Sci. Nat. Bot. sér. 3, 16: 179. 1850.

Arbustos ou árvores até 4,5m, glabros. Folhas com pecíolo; lâmina coriácea, oval-elíptica; base levemente atenuada a aguda ou arredondada, ápice acuminado, margem inteira, nervuras acródromas suprabasais, frequentemente assimétricas; ambas as faces glabras. Panículas terminais. Flores 5-

meras; hipanto ca. 2mm; cálice persistente, lacínias externas e internas fundidas, truncadas; anteras brancas, uniporosas, conectivo bastante espessado no dorso, nos antessépalos com projeção basal ampla, contínua da região ventral à dorsal, nos antepétalos com calcar dorsal, ambos com aurículas ventrais reduzidas; ovário 3-5-locular glabro, estilete levemente espessado no ápice. Baga atropurpúrea, ca. 30 sementes. Ocorre na Bolívia e Brasil, desde o Mato Grosso, Goiás, Piauí e Ceará até o Paraná. No estado de São Paulo, ocorre em locais alagados, geralmente em áreas de domínio de vegetação de cerrado. Coletada com flores entre janeiro e setembro e com frutos entre maio e novembro (Goldenberg 2009).

3. *Miconia fallax* DC., Prodr. 3: 181. 1828.

Arbustos até 2m; ramos, pecíolos, brácteas, bractéolas e hipanto densamente revestidos por indumento estrelado, canescente. Folhas sésseis a subsésseis; lâmina subcoriácea, ovalada, base arredondada a cordada, ápice arredondado a agudo, nunca acuminado, com múcron curto, margem levemente crenada e revoluta, nervuras acródromas basais; face adaxial glabra, abaxial densamente recoberta por indumento estrelado, canescente. Panículas escorpioides, terminais. Flores 5-meras; hipanto ca. 3mm; cálice persistente, lacínias internas e externas fundidas, triangulares e agudas; pétalas com margem ciliada; anteras amarelas, uniporosas, conectivo espessado no dorso e levemente calcarado na base; ovário 3-locular, glabro, estilete levemente espessado no ápice. Baga atropurpúrea, ca. 45 sementes. Ocorre desde o Peru, Venezuela e Guiana até o Paraguai e sudeste do Brasil. No estado de São Paulo, ocorre em cerrados. Coletada com flores entre setembro e novembro e com frutos entre outubro e janeiro (Goldenberg 2009).

4. *Miconia leucocarpa* DC., Prodr. 3: 182. 1828.

Miconia pohliana Cogn. In Mart., Eichler & Urb., Fl. bras. 14(4): 349. 1887.

Arbustos ou árvores até 5m; ramos jovens, pecíolos e hipanto densamente recobertos por indumento estrelado-furfuráceo a dendrítico-tomentoso, ocráceo. Folhas com pecíolo; lâmina coriácea, oval a oval-elíptica, base arredondada a cordada, ápice obtuso, agudo ou arredondado, mucronado, margem inteira ou denteado-ciliada, nervuras acródromas basais; face adaxial das folhas jovens estrelado-furfurácea, depois glabra, face abaxial moderada a densamente recoberta – mas sempre deixando visível a superfície da folha – por indumento estrelado-furfuráceo a dendrítico-tomentoso, ocráceo. Panículas de glomérulos, terminais. Flores 5-meras, ca. 2mm; cálice persistente, lacínias internas curtas, triangulares, externas muito reduzidas, inconspícuas; anteras brancas, uniporosas, conectivo longamente prolongado abaixo das tecas, giboso e com calcar dorsal curto e aurículas ventrais

curtas; ovário 3-locular, glabro, estilete levemente espessado no ápice. Baga arroxeada, 15-20 sementes. Ocorre em Goiás, Distrito Federal, Minas Gerais e São Paulo. No estado de São Paulo, ocorre em cerrados. Coletada com flores em julho e com frutos em julho e outubro (Goldenberg 2009).

5. *Miconia minutiflora* (Bonpl.) DC., Prodr. 3: 189. 1828.

Arbustos a árvores 1-6(-8)m; ramos e pecíolos esparsamento recobertos por indumento estrelado-furfuráceo, depois glabros, permanecendo alguns tricomas nos nós dos ramos e eixos das inflorescências. Folhas com pecíolo; lâmina membranácea, oblongo-lanceolada, base atenuada a arredondada, ápice acuminado até caudado, margem sinuosa revoluta, nervuras acródromas basais; ambas as faces das folhas jovens estrelado-furfuráceas, depois glabras. Panículas terminais. Flores 5-meras; hipanto 1-1,5mm; cálice caduco, lacínias internas arredondadas, cilioladas, externas constituídas por dentículos reduzidos; pétalas com ápice esparsamente ciliado-glanduloso; anteras 2mm ou 2,5mm, brancas, uniporosas, conectivo espessado no dorso, nos antessépalos com projeção basal arredondada, nos antepétalos com calcar dorsal curto, ventralmente biapendiculado; ovário 3-locular, glabro, estilete levemnete espessado no ápice. Baga atropurpúrea, 20-30 sementes. Ocorre desde o sul do México e Caribe até o sudeste do Brasil. No estado de São Paulo, ocorre em vegetação secundária, borda de florestas e cerrados. Coletada com flores em fevereiro e com frutos entre fevereiro e maio (Goldenberg 2009).

6. *Miconia paucidens* DC., Prodr. 3: 186. 1828.

Miconia langsdorffii Cogn. in Mart., Eichler & Urb., Fl. bras. 14(4): 232. 1887.

Arbustos ou arvoretas 1-3m; ramos jovens, pecíolos e hipanto esparsamente furfuráceos, depois glabros. Folhas com pecíolo; lâmina membranácea, lanceolada a oblongo-lanceolada, base atenuada a arredondada, ápice acuminado, margem levemente repanda, nervuras acródromas suprabasais ou basais; face adaxial glabra, abaxial furfurácea apenas sobre as nervuras. Panículas terminais. Flores 5-meras; hipanto 2,5-3mm; cálice caduco, verticilo interno truncado, lacínias externas longas, subuladas; filetes esparsamente glandulosos, anteras amarelas, uniporosas, conectivo nos antessépalos ligeiramnete prolongado, expandido na base e com aurículas ventrais curtas, nos antepétalos não expandido e com aurículas ventrais reduzidas; ovário 3-locular glabro, estilete filiforme, encurvado. Baga atropurpúrea, ca. 20 sementes. Ocorre em Minas Gerais, São Paulo, Paraná e Paraguai. No estado de São Paulo, ocorre em cerrados e florestas. Coletada com flores entre setembro e dezembro e com frutos durante quase o ano todo (Goldenberg 2009).

7. *Miconia pepericarpa* DC., Prodr. 3: 182. 1828.

Arbustos 2-3m ou árvores até 5m; ramos, pecíolos e hipanto recobertos por indumento denso, estrelado-lepidoto e furfuráceo, canescente a ocráceo, com raros tricomas dendríticos esparsos. Folhas com pecíolo; lâmina cartácea, oblongo-lanceolada, base arredondada, ápice acuminado, margem inteira revoluta, nervuras acródromas suprabasais, ocasionalmente basais; face adaxial das folhas jovens furfurácea, depois glabra, e face abaxial densamente recoberta por indumento estrelado-lepidoto. Panículas de glomérulos, pêndulas, terminais; ramos secundários curtos com apenas um glomérulo na extremidade. Flores 4-meras; hipanto ca. 1,5mm; cálice persistente, lacínias internas curtas, largamente triangulares a arredondadas, externas triangulares, agudas, muito reduzidas; anteras creme, uniporosas, conectivo curtamente prolongado abaixo das tecas, espessado no dorso, inapendiculado; ovário 2-3-locular, estrigoso no ápice, estilete levemente espessado no ápice. Baga azul-pálida, 2-3 sementes. Ocorre em Goiás, Distrito Federal, Bahia, Minas Gerais e São Paulo. No estado de São Paulo, ocorre em cerrados fechados e bordas de mata. Coletada com flores em novembro e dezembro e com frutos entre janeiro e julho (Goldenberg 2009).

8. *Miconia pseudonervosa* Cogn. in Mart., Eichler & Urb., Fl. bras. 14(4): 337. 1887.

Arbustos até 3m; ramos, pecíolos e hipanto densamente recobertos por tricomas seríceo-lanosos, ocasionalmente glandulares. Folhas com pecíolo 1,5-4cm; lâmina membranácea a cartácea, elíptica a elíptico-lanceolada, base cuneada a atenuada e curtamente decorrente, ápice agudo a acuminado, margem curtamente serreado-denteada e ciliada, nervuras acródromas suprabasais; face adaxial esparsamente hirsuta e densamente estrigosa sobre as nervuras, face abaxial densamente lanoso-vilosa, mas deixando a superfície da folha visível. Panículas de glomérulos, terminais, eixos frequentemente avermelhados. Flores 5-meras; hipanto 2-3mm; cálice persistente, lacínias internas truncadas, externas triangulares, agudas; anteras brancas, levemente corrugadas, uniporosas, conectivo inapendiculado; ovário 3-locular, setuloso no ápice, estilete filiforme. Baga alaranjada ou rosada quando jovem, azul pálida quando madura, ca. 100 sementes pequenas. Ocorre em Goiás, Distrito Federal, Minas Gerais, Rio de Janeiro e São Paulo. No estado de São Paulo, ocorre em locais alagados, em áreas de vegetação de cerrado e de floresta. Coletada com flores entre março e outubro e com frutos entre junho e janeiro (Goldenberg 2009).

9. *Miconia sellowiana* Naudin, Ann. Sci. Nat. Bot., sér. 3, 16: 206. 1850.

Arbustos 2m até árvores 15m; ramos, pecíolos e hipanto recobertos por indumento estrelado-furfuráceo, depois glabros. Folhas com pecíolo; lâmina cartácea a membranácea, lanceolada a oblongo-lanceolada, base atenuada e decorrente, ápice acuminado a caudado, margem distintamente serreada exceto no terço inferior, nervuras acródromas suprabasais, em geral unidas à base da nervura central por membrana, par marginal basal ocasionalmente presente; ambas as aces das folhas jovens e esparsamente estrelado-furfuráceas, depois glabras. Panículas terminais. Flores 5-meras; hipanto ca. 2mm; cálice caduco, lacínias internas membranáceas, arredondadas, cilioladas, externas triangulares, estreitas; anteras brancas, com poro muito amplo e inclinado (à semelhança de uma rima), atingindo ca. 1/3 do comprimento da teca, conectivo ligeiramente prolongado abaixo das tecas, com dois lobos ventrais pouco conspícuos; ovário 3-locular, papiloso no ápice, estilete espessado no ápice. Baga atropurpúrea, 6-9 sementes. Ocorre desde Goiás e Minas Gerais até o Rio Grande do Sul. No estado de São Paulo, ocorre em cerrados e formações florestais. Coletada com flores entre julho e setembro e com frutos entre junho e janeiro (Goldenberg 2009).

10. *Miconia stenostachya* DC., Prodr. 3: 181. 1828.

Arbustos até 2m; ramos, pecíolos, brácteas, bractéolas e hipanto densamente revestidos por indumento estrelado, canescente. Folhas com pecíolo; lâmina cartácea, oval-lanceolada a lanceolada, base obtusa, ápice arredondado a levemente acuminado, margem levemente crenada, nervuras acródromas basais; face adaxial glabra, abaxial densamente revestida por indumento estrelado, canescente. Panículas escorpioides, terminais. Flores 5-meras; hipanto ca. 3mm; cálice persistente, lacínias internas e externas fundidas, triangulares, agudas; pétalas com margem ciliada; anteras amarelas, uniporosas, conectivo espessado no dorso e levemente calcarado na base; ovário 3-locular glabro, estilete levemente espessado no ápice. Baga atropurpúrea, ca. 45 sementes. Ocorre desde o sul do México até a Bolívia e Sul do Brasil (Paraná). No estado de São Paulo, ocorre em áreas de cerrados. Coletada com flores e frutos durante praticamente o ano todo (Goldenberg 2009).

11. *Miconia theaezans* (Bonpl.) Cogn. in Mart., Eichler & Urb., Fl. bras. 14(4): 337. 1888.

Arbustos 1-2m a raramente arvoretas 7m, glabros ou ocasionalmente ramos e folhas jovens com indumento furfuráceo, esparso e caduco. Folhas com pecíolo; lâmina membranácea a subcoriácea, obovada a elíptica, raramente oval, base atenuada ou arredondada, ápice curtamente acuminado, margem serrado-ciliada, nervuras acródromas basais; face adaxial às vezes verrucosa. Panículas terminais. Flores 5-meras; hipanto 1,5-2mm; cálice persistente, lacínias internas arredondadas,

externas triangulares, mais curtas; anteras brancas, 4-porosas, conectivo prolongado, ventralmente bituberculado; ovário 3-locular, glabro, estilete levemente espessado no ápice. Baga azulada, depois atropurpúrea, ca. 40 sementes. Ocorre desde a América Central até o Sul do Brasil (Santa Catarina). No estado de São Paulo, ocorre em áreas brejosas e florestas ciliares. Coletada com flores e frutos praticamente o ano todo (Goldenberg 2009).

Figura 6. (**a-h**) Espécies de *Miconia* incluídas neste estudo. (**a**) *M. albicans*. (**b**) *M. chamissois*. (**c**) *M. fallax*. (**d**) *M. paucidens*. (**e**) *M. leucocarpa*. (**f**) *M. minutiflora*. (**g**) *M. pseudonervosa*. (**h**) *M. stenostachya*. (**i**) *M. sellowiana*. Fotos: Jorge Yoshio Tamashiro (a). Ana Paula de Souza Caetano (b, c, e, g, i). Priscila Andressa Cortez (d, f, h).

Locais de Coleta

As espécies de *Miconia* foram coletadas em cinco áreas de Cerrado localizadas nos municípios de Uberlândia-MG, Itirapina-SP e Campinas-SP, em uma área de Floresta Mesófila Estacional Semidecidual localizada no município de Jundiaí-SP, e em três áreas de Floresta Atlântica localizadas no município de Ubatuba-SP (Figura 7, Tabela 3).

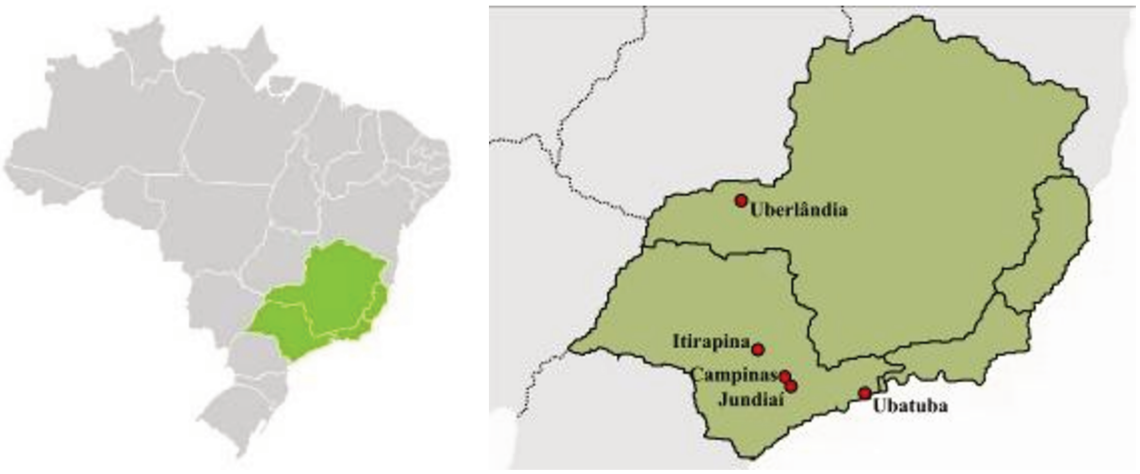

Figura 7. Mapa de locais de coleta das espécies de *Miconia* incluídas neste estudo.

Tabela 3. Coordenadas geográficas dos locais de coleta das espécies de *Miconia*.

Formação Vegetacional	Município	Localização	Coordenadas Geográficas
Cerrado	Uberlândia-MG	Parque do Sabiá	18º54'27,0"S e 48º14'27,0"W
	Itirapina-SP	Valério I	22º13'21,4"S e 47º51'15,5"W
		Pedregulho	22º14'24,8"S e 47º49'43,6"W
		Graúna	22º15'55,4"S e 47º47'50,5"W
	Campinas-SP	Laboratório Nacional de Luz Síncrotron	22º48'10,3"S e 47º03'18,3"W
Floresta Mesófila Estacional Semidecidual	Jundiaí-SP	Serra do Japi	23º14'S e 46º58'W
Floresta Atlântica	Ubatuba-SP	Praia da Picinguaba	23º22'17,8"S e 44º50'07,7"W
		Praia da Fortaleza	23º31'02,1"S e 45º10'00,2"W
		Ilha Anchieta	23º32'15,5"S e 45º03'48,1"W

Referências

Almeda F. 1997. Chromosome numbers and their evolutionary significance in some neotropical and paleotropical Melastomataceae. BioLlania Edicion Especial 6: 167-190.

Almeda F, Chuang TI. 1992. Chromosome numbers and their significance in some Mexican Melastomataceae. Systematic Botany 17: 583-593.

APG III (2009). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Botanical Journal of the Linnaean Society 161: 105-121.

Asker SE, Jerling L. 1992. Apomixis in plants. CRC Press, Boca Raton.

Berthaud J. 2001. Apomixis and the management of genetic diversity. *In* The flowering of apomixis: from mechanisms to genetic engineering (Savidan Y, Carman JG, Dresselhaus T, eds). CIMMYT, EU. Pp. 8-23.

Bhandari NN. 1984. The microsporangium. *In* Embryology of angiosperms (Johri BM, ed). Springer-Verlag, New York. Pp. 53-121.

Buchmann SL. 1983. Buzz pollination in angiosperms. *In* Handbook of experimental pollination (Jones CE, Little RJ, eds). Van Nostrand Reinhold, New York. Pp. 73-113.

Caetano APS 2010. Apomixia e reprodução sexuada em espécies de Miconia Ruiz & Pavon, Melastomataceae. Dissertação de Mestrado, Universidade Estadual de Campinas, Campinas-SP.

Caetano APS, Carmello-Guerreiro SM, Teixeira SP. Pollen development and viability reveal new insights on apomictic and sexual *Miconia* (Miconiaeae, Melastomataceae). Em preparação.

Carman JG. 1997. Asynchronous expression of duplicate genes in angiosperms may cause apomixis, bispory, tetraspory and polyembryony. Biological Journal of the Linnaean Society 61: 51-94.

Cigan AM, Unger E, Xu RJ, Kendall T, Fox TW. 2001. Phenotypic complementation of *ms45* maize requires tapetal expression of *MS45*. Sexual Plant Reproduction 14: 135-142.

Clausing G, Meyer K, Renner SS. 2000. Correlations among fruit traits and evolution of different fruits within Melastomataceae. Botanical Journal of the Linnaean Society 133: 303-326.

Clausing G, Renner SS. 2001a. Evolution of growth form in epiphytic Dissochaeteae (Melastomataceae). Organisms Diversity and Evolution 1: 45-60.

Clausing G, Renner SS. 2001b. Molecular phylogenetics of Melastomataceae and Memecylaceae: implications for character evolution. American Journal of Botany 88: 486-498.

Cogniaux AC. 1891. Melastomataceae *In* Monographiae phanerogamarum (de Candolle AP, de Candolle C, eds). G. Masson, Paris. 7: 1-1256.

Cocucci AE. 1969. El proceso sexuado en angiospermas. Kurtziana 5: 407-423.

Cortez PA. 2007. Embriologia de *Miconia albicans* (Sw.) Triana (Melastomataceae), espécie agamospérmica. Dissertação de Mestrado, Universidade Estadual de Campinas, Campinas-SP.

Conti E. 1994. Phylogenetic relationships of the Onagraceae and Myrtales: evidence from *rbcL* sequence data. Ph.D. Dissertation, University of Wisconsin, Madison.

Drews GN, Lee D, Christensen CA. 1998. Genetic analysis of female gametophyte development and function. Plant Cell 10: 5-18.

Endress PK. 1994. Diversity and evolutionary biology of tropical flowers. Cambridge University Press, Cambridge.

Fei HM, Sawhney VK. 2001. Ultrastructural characterization of male sterile33 (ms33) mutant in *Arabidopsis* affected in pollen desiccation and maturation. Canadian Journal of Botany 79: 118-129.

Fritsch PW, Almeda F, Renner SS, Martins AB, Cruz BC. 2004 Phylogeny and circumscription of the near-endemic Brazilian tribe Microlicieae (Melastomataceae). American Journal of Botany 91: 1105–1114.

Gibson PJ, Diggle PK. 1998. Flower development and male sterility in *Ocotea tenera*. Internation Journal of Plant Science 159: 405-417.

Goldenberg R. 1994. Estudos sobre a biologia reprodutiva de espécies de Melastomataceae de cerrado em Itirapina, SP. Dissertação de Mestrado. Universidade Estadual de Campinas, Campinas-SP.

Goldenberg R. 2000. O gênero *Miconia* Ruiz & Pav. (Melastomataceae): I. Listagens Analíticas, II. Revisão Taxonômica da Seção *Hypoxanthus* (Rich. Ex DC.) Hook. F. Tese de Doutorado. Universidade Estadual de Campinas, Campinas-SP.

Goldenberg R. 2009. *Miconia* Ruiz & Pav. *In* Flora fanerogâmica do estado de São Paulo (Wanderley MGL, Shepherd GJ, Melhem TS, Giulietti AM, Martins SE, eds). Vol. 6. Instituto de Botânica, São Paulo.

Goldenberg R. 2010. *Miconia In* Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. Disponível em: <http://floradobrasil.jbrj.gov.br/2010/FB009666>. Acesso em 13 de dezembro de 2011.

Goldenberg R, Shepherd GJ. 1998. Studies on the reproductive biology of Melastomataceae in "cerrado" vegetation. Plant Systematics and Evolution 211: 13-29.

Goldenberg R, Penneys DS, Almeda F, Judd WS, Michelangeli FA. 2008. Phylogeny of *Miconia* (Melastomataceae): patterns of stamen diversification in a megadiverse neotropical genus. International Journal of Plant Science 169: 963-979.

Goldenberg R, Caddah MK, Martin CV. 2010. Taxonomic notes on South American *Miconia* (Melastomataceae). Rodriguésia 61: S23-S28.

Grant V. 1981. Plant speciation. 2nd ed. Columbia University Press, New York.

Groenendijk JP, Bouman F, Cleef AM. 1996. An exploratory study on seed morphology of *Miconia* Ruiz and Pavon (Melastomataceae), with taxonomic and ecological implications. Acta Botanica Neerlandica 45: 323-344.

Grossniklaus U, Nogler GA, van Dijk PJ. 2001a. How to avoid sex: the genetic control of gametophytic apomixis. Plant Cell 13: 1491-1498.

Grossniklaus, U, Spillane C, Page DR, Köhler C. 2001b. Genomic imprinting and seed development: endosperm formation with and without sex. Current Opinion in Plant Science 4: 21-27.

Ilarslan H, Horner HT, Palmer RG. 1999. Genetics and cytology of a new male-sterile, female-sterile soybean mutant. Crop Science 39: 58-63.

Izhar S, Frankel R. 1971. Mechanism of male-sterility in *Petunia*. I. The relationship between pH, calase activity in the anthers and the breakdown of microsporogenesis. Theoretical Applied Genetics 41: 104-108.

Johri BM, Ambegaokar KB, Srivastava PS. 1992. Comparative embryology of angiosperms. Springer-Verlag, Berlin.

Johnson LAS, Briggs BG. 1984. Myrtales and Myrtaceae - a phylogenetic analysis. Annals of the Missouri Botanic Garden 71: 700-756.

Judd WS. 2007. Revision of *Miconia* sect. *Chaenopleura* (Miconieae, Melastomataceae) in the Greater Antilles. Systematic Botany Monographs 81:1-235.

Judd WS, Skean Jr, JD. 1991. Taxonomic studies in the Miconieae (Melastomataceae) IV. Generic realignments among terminal-flowered taxa. Bulletin of the Florida Museum of Natural History 36: 25-84.

Kapoor S, Kobayashi A, Takatsuji H. 2002. Silencing of the tapetum-specific zinc finger gene TAZ1 causes premature degeneration of tapetum and pollen abortion in petunia. Plant Cell 14: 2353-2367.

Knox RB. 1984. The pollen grain. *In* Embryology of angiosperms (Johri BM, ed). Springer-Verlag, New York. Pp. 197-271.

Koltunow AM, Bicknell RA, Chaudhury AM. 1995. Apomixis: molecular strategies for the generation of genetically identical seeds without fertilization. Plant Physiology 108: 1345-1352.

Kronestedt-Robards EC, Rowley JR. 1989. Pollen grain development and tapetal changes in *Strelitzia reginae* (Strelitziaceae). American Journal of Botany 76: 856-870.

Lai Z, Ma W, Han B, Liang L, Zhang Y, Hong G, Xue Y. 2002. An F-box gene linked to the self-incompatibility (S) locus of *Antirrhinum* is expressed specifically in pollen and tapetum. Plant Molecular Biology 50: 29-42.

Laroca S. 2003. Contribuição para o conhecimento das relações entre abelha e flores: coleta de pólen das anteras tubulares de certas Melastomataceae. Revista Floresta 5: 63-74.

Loukides CA, Broadwater AH, Bedinger PA. 1995. Two new male-sterile mutants of *Zea mays* (Poaceae) with abnormal tapetal cell morphology. American Journal of Botany 82: 1017-1023.

Maheshwari P. 1950. An introduction to the embryology of angiosperms. McGraw-Hill, New York.

Mariath JEA, Santos RP, Bittencourt Jr, NS. 2006. Flor. *In* Anatomia Vegetal (Appezzato-da-Glória B, Carmello-Guerreiro SM, eds). 2ª ed. Editora UFV, Viçosa-MG. Pp. 329-373.

Martin CV, Little D, Goldenberg R, Michelangeli FA. 2008. A preliminary phylogenetic analysis of the polyphyletic genus *Leandra* (Miconieae, Melastomataceae). Cladistics 24:315–327.

Martins AB. 2009. Melastomataceae. *In* Flora fanerogâmica do estado de São Paulo (Wanderley MGL, Shepherd GJ, Melhem TS, Giulietti AM, Martins SE, eds). Vol. 6. Instituto de Botânica, São Paulo.

Mendes-Rodrigues C, Carmo-Oliveira R, Talavera S, Arista M, Ortiz PL, Oliveira PE. 2005. Polyembryony and apomixis in *Eriotheca pubescens* (Malvaceae - Bombacoideae). Plant Biology 7: 533-540.

Michelangeli FA. 2000. A cladistic analysis of the genus *Tococa* (Melastomataceae) based on morphological data. Systematic Botany 25:211-234.

Michelangeli FA, Penneys DS, Giza J, Soltis D, Hils MH, Skean Jr, JD. 2004. A preliminary phylogeny of the tribe Miconieae (Melastomataceae) based on *nrITS* sequence data and its implications on inflorescence position. Taxon 53: 279-290.

Mogie M. 1992. The evolution of asexual reproduction in plants. Chapman & Hall, London.

Naumova TN. 1993. Apomixis in angiosperms: nucellar and integumentary embryony. CRC Press, Boca Raton.

Naumova TN, Vielle-Calzada JP. 2001. Ultrastructural analysis of apomitic development. *In* The flowering of apomixis: from mechanisms to genetic engineering (Savidan Y, Carman JG, Dresselhaus T, eds). CIMMYT, EU. Pp. 44-63.

Naumova TN. 2008. Apomixis and amphimixis in flowering plants. Cytology and Genetics 42: 179-188.

Nogler GA. 1984. Gametophytic apomixis. *In* Embryology of angiosperms (Johri BM, ed). Springer-Verlag, New York. Pp. 475-518.

Peel MD, Carman ZWL, Wang RRC. 1997. Meiotic anomalies in hybrids between wheat and apomitic *Elymus rectisetus*. Crop Science 37: 717-723.

Penneys DS, Judd WS. 2005. A systematic revision and cladistic analysis of *Charianthus* (Melastomataceae) using morphological and molecular characters. Systematic Botany 30:559-584.

Raven PH, Evert RF, Eichhorn SE. 2005. Biology of plants. 7th ed. WH Freeman and Company, New York.

Renner SS. 1989. A survey of reproductive biology in Neotropical Melastomataceae and Memecylaceae. Annals of the Missouri Botanical Garden 76: 496-518.

Renner SS. 1993. Phylogeny and classification of the Melastomataceae. Nordic Journal of Botany 13: 519-540.

Renner SS, Clausing G, Meyer K. 2001. Historical biogeography of Melastomataceae: the roles of tertiary migration and long-distance dispersal. American Journal of Botany 88: 1290-1300.

Renner SS. 2004 Bayesian analysis of combined chloroplast loci, using multiple calibrations, supports the recent arrival of Melastomataceae in Africa and Madagascar. American Journal of Botany 91:1427-1435.

Richards AJ. 1997. Plant breeding systems. 2nd ed. Chapman & Hall, London.

Richards AJ. 2003. Apomixis in flowering plants: an overview. Philosophical Transactions of the Royal Society of London - Biological Series 358: 1085-1093.

Skean JR. 1993. Monograph of *Mecranium* (Melastomatacae-Miconiae). Systematic Botany Monographs 39:1-116.

Solt ML, Wurdack JJ. 1980. Chromosome numbers in the Melastomataceae. Phytologia 47:199-220.

Soltis DE, Smith SA, Cellinese N et al. 2011. Angiosperm phylogeny: 17 genes, 640 taxa. American Journal of Botany 98: 704-730.

Sorensen AM, Krober S, Unte US, Huijser P, Dekker K, Saedler H. 2003. The *Arabidopsis* aborted microspores (AMS) gene encodes a MYC class transcriptional factor. The Plant Journal 33: 413-423.

Varassin IG, Penneys DS, Michelangeli FA. 2008. Comparative anatomy and morphology of nectar-producing Melastomataceae. Annals of Botany 102: 899-909.

Vielle-Calzada JP, Crane CF, Stelly DM. 1996. Apomixis: the asexual revolution. Science 5291: 1322-1323.

Went JL, Willemse MTM. 1984. Fertilization. *In* Embryology of angiosperms (Johri BM, ed). Springer-Verlag, New York. Pp. 273-317.

Whiffin T, Tomb AS. 1972. The systematic significance of seed morphology in the Neotropical capsular-fruited Melastomataceae. American Journal of Botany 59:411-422.

Wilson ZA, Morroll SM, Dawson J, Swarup R, Tighe PJ. 2001. The *Arabidopsis* MALE STERILITY1 (ML1) gene is a transcriptional regulator of male gametogenesis, with homology to the PHD-finger family of transcription factors. Plant Journal 28: 27-39.

Capítulo 1.

Anther wall and pollen development in *Miconia* species (Melastomataceae)

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Abstract

Miconia belongs to Melastomataceae, and is one of the largest Neotropical genera. *Miconia* is quite arbitrarily defined due to the great diversity and poor morphological and genetic characterization of its members. The conventional delimitation of sections in *Miconia* has been largely based on stamen morphology; nevertheless, the potential taxonomic significance of anther wall and pollen ontogenetic characters within Melastomataceae, especially in *Miconia,* has been little explored. Hence, this paper is intended to fill that gap in our knowledge, by studying the anther wall and pollen development in *Miconia albicans*, *M. stenostachya* and *M. paucidens*. Routine techniques for microscopy were used to examine anthers in several developmental stages collected from the above three species of *Miconia*. Differently from other members of the family, the anther wall development in the three species is of the monocotyledonous type. The endothecium is persistent in the mature anthers, but it lacks of cell wall thickness. The presence of druse crystals in the tapetal cells of *Miconia paucidens* was first described here, but their relation with the pollen development or anther dehiscence is still obscure.

Keywords: anther, pollen, ontogeny, *Miconia*, Melastomataceae

Introduction

Miconia belongs to Melastomataceae (order Myrtales) and is one of the largest Neotropical genera, with about 1050 species (Goldenberg et al. 2008). Within the tribe Miconieae, *Miconia* is quite arbitrarily defined due to the great diversity and little morphological and genetic characterization of its members (Judd 1986, 1989). In other groups within Melastomataceae, genera have been traditionally defined based on morphology and position of anther connective appendages, which are absent or reduced in Miconieae (Clausing and Renner 2001). Nevertheless, the conventional delimitation of sections in *Miconia* has been largely based on stamen morphology (Goldenberg et al. 2008).

The genus was first described by Ruiz and Pavon in 1794, with three species, and the last complete revision of the genus was done by Cogniaux in 1891, including 518 species in 11 sections (Table 1). Phylogenetic studies has shown that the genus is paraphyletic (Judd and Skean 1991; Michelangeli et al. 2004; Martin et al. 2008) and, although the relationships among the sections are considered obscure, the nuclear internal transcribed spacer and *ndh*F nucleotide sequence analysis for about 20% of the total species in the genus (Goldenberg et al. 2008) made possible to recognize

some stamen diversification patterns, which emphasizes the importance of this character in the evolution of the group.

Table 1. *Miconia* sections according to Cogniaux (1891), modified from Goldenberg et al. (2008). *Synonymized under section *Hypoxanthus* by Goldenberg (2000).

1. *Jucunda*
2. *Tamonea*
3. *Adenodesma*
4. *Octomeris*
5. *Laceraria*
6. *Miconia* 6.1. *Aplostachyae* 6.2. *Diplostachyae* 6.3. *Impetiolares* 6.4. *Glomeratiflorae* 6.5. *Seriatiflorae* 6.6. *Paniculares*
7. *Glossocentrum*
8. *Chaenanthera**
9. *Amblyarrhena* (including *Hartigia*)
10. *Cremanium*
11. *Chaenopleura*

The potential taxonomic significance of anther wall and pollen ontogenetic characters within Melastomataceae, especially in *Miconia,* has been little explored (see Tobe and Raven 1983 and Schmid 1984 for a critical review of the embryology of Myrtales). The poor systematic definition contrasts with the great number of species and floristic importance in the Neotropical flora. Hence, this paper is intended to fill that gap in our knowledge, by studying the anther wall and pollen development in *Miconia albicans*, *M. stenostachya* and *M. paucidens*. *M. albicans* and *M. stenostachya* belongs to the section *(Eu)Miconia*, and are considered in the clade Miconia IV (Goldenberg et al. 2008). *M. paucidens*, which belongs to the *Jucunda* tribe, was not included in their phylogenetic analysis, but species from this section that were studied always seemed to cluster with species from section *Miconia*. The data obtained in the present study were compared with those found in the literature to other members of the tribe Miconieae. The morphological characteristics that can contribute to knowledge of breeding systems are also stressed, since *M. albicans* and *M. stenostachya* are apomictic species and *M. paucidens* is a sexual species (Goldenberg and Shepherd 1998).

Material and Methods

Stamens in several stages of development were removed from young floral buds and flowers of at least five individuals of the three species, *Miconia albicans*, *M. stenostachya* and *M. paucidens*, growing in natural populations of São Paulo state, southeast Brazil, in cerrado areas from the municipalities of Itirapina (22º15'10" S and 47º49'22" W) and Campinas (22°54'20" S and 47°03'39" W), and in Atlantic Rain Forest from the municipalities of Ubatuba (23˚22'17" S and 44˚50'07" W), from 2007 to 2010.

Morphological features were initially verified from materials maintained in ethanol 70% and photographed under stereomicroscope equipped with a digital camera. Micromorphological study was carried on materials collected and immediately fixed in a solution composed of 80 mL L^{-1} glutaraldeyde, 250 mL L^{-1} paraformaldeyde (16%) and 500 mL L^{-1} phosphate buffer (0.1 M, pH 6.8) for 24 h (modified from Karnovsky 1965). After washing in the same buffer solution, the materials were dehydrated using ethanol series and submitted to the scanning electron microscope techniques. Some anthers were carefully opened to expose the pollen grains and the images were obtained in a scanning electron microscope (JSM 5200, Jeol).

The structural study and the detection of some chemical compounds were made from fixed anthers that were crushed against a glass slide and stained with 12 g L^{-1} acetocarmine solution for cytoplasm visualization, lugol solution for starch grains, xylidine de Ponceau (pH 2.5) (C.I. 16150) for total proteins, Sudan black B (C.I. 26150) for lipids, 0.05% toluidine blue (pH 4.0) (C.I. 52040) for phenolic compounds, periodic acid Schiff (PAS method) reaction (pararosanilin C.I. 42500) for structural carbohydrates, Ruthenium red solution for pectin, and 4′,6-diamidino-2-phenylindole (DAPI) in phosphate-buffered saline (PBS) for better nuclear visualization. To verify the deposition of callose wall, the anthers in tetrad stage were squashed onto a slide in a drop of aniline blue (pH 8.0) (C.I. 42755) and examined using fluorescence microscopy equipped with an ultraviolet (UV) excitation filter. The polarized light was applied to crystals observation. The tests above were also applied to the slides obtained from the structural analyses, after inclusion in plastic resin and sectioning in rotary microtome (2 µm thick). Digital images were obtained under a light microscope (BX 51, Olympus) with a digital camera.

For the ultrastructural studies, some fixed anthers were submitted to the routine transmission electron microscope techniques. The sections were observed and the images were taken using a transmission electron microscope (EM 208 and EM 301, Philips).

Fresh pollen grains were also submitted to water content and germination tests. In the water content test, some pollen grains were deposited over two groups of glass slides; in one group, the

pollen grains were immediately covered with a drop of water and, in another group, the pollen grains were covered with a drop of immersion oil; after a few minutes, the slides of the two groups were analyzed and compared in relation to the size and shape under a light microscope (Dafni et al. 2005). For the germination test we used a protocol adapted from Santos and Mariath (1997) and successfully applied to *Miconia paucidens*, a sexual species with high pollen fertility, used as a control. The germination medium contained 2% colorless gelatin, 20% sucrose, 0.01% boric acid and 0.05% calcium nitrate for optimal growth conditions was used; after incubation at room temperature (approximately 25° C) for 3 h in the dark, the pollen grains were examined under a light microscope for pollen tube formation observations. Pollen grains successfully germinated were submitted to the same procedures applied to structural studies.

Results

Anther Morphology

The three species have elongate anthers with one minute pore at the top (Figures 1a-c). Only the anthers of *Miconia paucidens* present glandular trichomes, located in the basal portion of the connective (Figure 1d).

Pre-Meiotic Stage

The four microsporangia of each anther are composed by the epidermis and the primary parietal layer, which surrounds the sporogenous tissue; the primary parietal cells divide periclinally to form both, the outer and the inner secondary parietal layers (Figure 2a). The outer secondary parietal layer develops itself into the endothecium while the inner secondary parietal cells divide periclinally to form the middle layer and the tapetum (Figure 2b).

The massive sporogenous tissue is located in the central portion of the microsporangium, with cells that are polygonal in shape; the cytoplasm is electron-dense, with few organelles as mitochondria, dictyosomes and endoplasmic reticulum, as well as small vacuoles; there are plasmodesmata connecting adjacent sporogenous cells (Figures 1h, i).

Meiotic Stage

At the meiotic stage, the anther wall is composed of four – in a few cases of five – layers: epidermis, endothecium, one or two middle layers and tapetum; soon before the beginning of meiotic process, the middle layer appears crushed by the tapetal cells enlargement; the tapetum is of the secretory type, with uninucleate cells (Figures 2a-e).

Each microspore mother cell undergoes a gradual deposition of a thick callosic wall (Figure 2f). The meiotic process is a rare event in *Miconia albicans* and occurs in an irregular way in the most of the *M. stenostachya* anthers (Figure 3a). Only in *M. paucidens* the meiotic process runs normally, resulting in a four-nucleate syncytium surrounded by the callose wall (Figures 3b, c). The cytokinesis is of the simultaneous type and gives rise to tetrahedral microspore tetrads (Figures 3d, e). The primexine starts to be formed when the microspores are still surrounded by the callose wall (Figure 3f).

Post-Meiotic Stage

In *Miconia stenostachya* and *M. paucidens*, the microspores become free after callosic wall dissolution at the same time that microspore wall is formed and the aperture is delimitated (Figure 4a). At this stage, the anther epidermal cells have a striate cuticle covering the thickened outer periclinal wall (Figures 4b-d), except by the dehiscence region where the cuticle is absent (Figure 4d). The tapetal cells of *M. paucidens* show proteoplasts and druse crystals (Figures 5a, b). In the three species the tapetal walls start a gradual degradation (Figure 5c); several Übisch bodies are especially abundant in the locule-facing, close to the inner periclinal cell walls, forming the tapetal and peritapetal membranes (Figures 5b, d).

Due to the *Miconia albicans* abnormalities, observed from meiotic stage, it is not possible to describe the following developmental stages of the microspores in this species as done for *M. stenostachya* and *M. paucidens*. Only in *M. albicans*, the tapetal cell cytoplasm degenerate but the cell walls remain at original sites due to the formation of an uncommon wall made of Übisch bodies aggregation (Figures 5e, f).

The final microspore stage is marked by the formation of a large vacuole, which coincides with the parietal position of the microspore nucleus (Figures 6a, b).

First Mitotic Stage

The degradation process observed in the tapetal cells is accelerated at the time of the microspores undergo the first mitotic division, which is absent in *Miconia albicans*; in *M. stenostachya*, some irregularities lead to the symmetric first mitosis; only in *Miconia paucidens* the first mitosis is asymmetric in the most of the microspore (Figure 6c). The asymmetrical cell division gives rise to bicellular pollen grains containing two unequal cells: a larger vegetative cell and a smaller, lenticular parietal generative cell (Figure 6d). The vegetative cell has electron-dense cytoplasm, containing mitochondria, endoplasmic reticulum, dictyosomes and vesicles besides the

spherical and prominent nucleus (Figure 6e). The generative cell is gradually detached from the pollen wall and moving towards the centre of the pollen grain, inside the vegetative cell cytoplasm (Figures 7a-d). The shape of the generative cell progressively changes from lenticular to spherical to elongate (Figures 7e, f), which occurs lately and less frequently in *M. stenostachya*; the generative cell has a few portion of cytoplasm containing mitochondria and a large spherical nuclei (Figure 6g).

The pollen grain has three colpori in an array alternated with three colpi (Figures 7h, j). The exine is psilate, collumelatae, with discontinuous tectum; the lumen delimited by the columella is large, filled with pollenkitt, which appearance is electron dense; the intine is thin except in the colpore region, where it is very thick (Figures 7h, i, 8a-c).

The releasing pollen grains are bicellular and orthodox or partially dehydrated (Figures 9a, b); at the time of anther dehiscence (Figure 9c, d), anther wall in the three species is composed by the epidermis and the endothecium. Endothecial cells are characterized by the lack of fibrous wall thickenings; sometimes the tapetal membrane is still present (Figures 9e, f). The septum that divides the two pollen sacs of a thecae degenerates and the mature anther may be considered billocular (Figure 9g).

Second Mitotic Stage

The mitotic division that gives raise to the two sperm cells occurs inside the pollen tube. The pollen tube emerges from one of the three colpore (Figure 9h); the sperm cells can be observed inside the pollen tube cytoplasm (Figure 9i); in some pollen tubes is possible to observe the callose plugs (Figure 9j).

Discussion

The general structure and ultrastructure of the pollen grains in several developmental stages, observed in *Miconia albicans, M. stenostachya* and *M. paucidens*, are similar in relation to those seen in most angiospermous species (Maheshwari 1950, Bhandari 1984, Lersten 2004). Moreover, these three species and other *Miconia* species share some character states from the anther and pollen grain ontogeny, which can be considered consistent embryological markers for members of the Melastomataceae (Table 2). Among them is the secretory (or glandular) and uninucleated tapetum, and tricolporate pollen grains; among the features not shared with other members of the group are the anther wall development type, the occurrence of an endothecium in mature anthers and the grouping of mature pollen grains in tetrads, the later only observed in *M. melanotricha* (Patel *et al.* 1984).

The interpretation of the anther wall development type in Melastomataceae deserves attention because *M. albicans*, *M. stenostachya* and *M. paucidens* exhibit the "monocotyledonous type", in which the outer secondary parietal layer gives raise directly to the endothecium while the inner secondary parietal layer divides periclinally to form the middle layer and the tapetum. In addition to the species cited above, information about this is only available for *Miconia cabucu* (Medeiros and Morretes 1996) and *Tococa guianensis* (Fernandes and Simão 2010), both showing the "dicotyledonous type", which is considered the most common type among angiosperms. In this type, both outer and inner secondary parietal layer cells divide periclinally to form the other anther wall layers. According to Schmid (1984), the Melastomataceae anther wall development is irregular or not characterized by the types proposed by Davis (1966) or by other researchers. The different developmental patterns observed in the anthers of phylogenetically related species are interesting since the majority of the development studies provide character states considered conservative, especially in their early stages. The scarcity of such information in a group as diverse as Melastomataceae is still a major obstacle to the evolutionary discussions in the group.

The absence of cell wall thickenings in the anther layers of *Miconia albicans*, *M. stenostachya* and *M. paucidens* was also described for *M. fallax* and *M. pepericarpa* (Caetano 2010), and for *M. cabucu* (Medeiros and Morretes 1996), corroborating the information compiled for Melastomataceae by Schmid (1984). But the endothecium in *M. cinnamomifolia*, *M. pusilliflora* and *M. latecrenata* was described as absent (Goldenberg *et al.* 2003). Such information, along with to the Clausing and Renner (2001) consideration that Melastomataceae species do not have endothecium may be merely due to their functional definition, since the endothecium is considered a mechanical layer specialized in the anther dehiscence mechanism (Batygina 2002).

Thus, we can consider two scenarios in *Miconia*: (1) absence of endothecium, whether it is considered a parietal layer specialized in the dehiscence mechanism and, therefore, with characteristic cell wall thickenings or (2) presence of endothecium, whether it is considered a layer originated from cellular divisions on the secondary parietal layer, but without the typical cell wall thickenings. The absence of endothecium, described in *M. cinnamomifolia, M. latecrenata* and *M. pusiliflora* (Goldenberg *et al.* 2003), may be due to the lack of information about the early steps of anther wall development, which creates doubts about the persistence of the middle layer instead of tapetum in the mature anthers of *M. latecrenata*. It also must be considered that Clausing and Renner (2001) did not include species of *Miconia* in their studies, which may have led these authors to assign to the family as a whole a state of character found only in members of some genus. This fact is quite common when there is little ontogenetic information for groups of great diversity, as in

Melastomataceae. In this case, one may suggest that the loss of mechanical function of the endothecium rather than its absence should be considered an important event in the evolution of Melastomataceae, possibly associated with the "buzz pollination", whereby the pollen grains are removed from the poricidal mature anthers by the vibrating movements executed by certain bees.

The druse crystals observed in the *Miconia paucidens* tapetal cells, the only sexual species included in this study, is an unpublished result for the whole order Myrtales. Tapetum cells with crystals are not commonly found in angiosperms, but were described in a few families, including Commelinaceae (Mepham and Lane 1969a, b) and Leguminosae (Buss and Lersten 1972), and their role in the anther is still unknown.

The exine ornamentation in *Miconia* may be a character of great importance in the phylogenetic studies, if extended to a large number of species. In the 16 *Miconia* species for which this kind of information is available, including the three species of this study, we found five different types of ornamentation (see Table 1). Variation in exine ornamentation was found in phylogenetically related species and can be considered an adaptive character, since these species exhibit similar reproductive system, often apomixis. Even if we consider only the sexual species, the type of evolutionary pressure exerted by pollinators could explain such variation, as observed in some Leguminosae species (Basso-Alves *et al.* 2011).

Importantly, much of the information obtained in this study is unique to the Melastomataceae. The scarcity and even the absence of information on most aspects of the Melastomataceae pollen grains, which surprised Patel *et al.* in 1984, still surprises us, especially when considering the large number of species of this group. The results obtained in this study show that some states of embryological characters are preserved in the group, especially those related to the anther wall, as the endothecium without cell wall thickenings and the uninucleate tapetum. Other character states, as the abnormal pollen grains, are more related to the reproductive systems of the species, since the irregular pollen ontogeny observed in *Miconia albicans* and in *M. stenostachya*, which are more severe in the first species, is determinant of the high rate of pollen abortion in these species.

Table 1 Anther and pollen available data of the *Miconia* species.

Species	Anther wall formation	Endothecium	Middle layer(s) persistence	Tapetum type	Mature Pollen Grain	Exine surface	Apertures	References
M. albicans	Monocotyledonous	Non-thickened	Ephemerous	Secretory and uninucleate	bicellular, monad	psilate	3-colpori and 3-pseudocolpi	Present study
M. alypifolia					-, monad	striate	Some 6-colporate with colpi and intercolpar concavities	Patel *et al.* 1984
M. argentea					bicellular, monad	-	-	Tobe & Raven 1984
M. cabucu	Dicotyledonous	Non-thickened	Ephemerous	Secretory and uninucleate	bicellular, monad	finely rugulate	3-colpori and 3-pseudocolpi	Medeiros & Morretes 1996
M. caesia					monad	With short, branched, cylindrical elements		Patel *et al.* 1984
M. candolleana					-, monad	psilate	3-colpori and 3-pseudocolpi	Melhem *et al.* 2003
M. cinnamomifolia	-	absent	Ephemerous	glandular				Goldenberg *et al.* 2003
M. fallax		Non-thickened	Ephemerous	Secretory and uninucleate	bicellular, monad			Caetano 2010
M. hondurensis					monad	Smooth-punctate with intercolpar concavities	Some 2-colporate, syncolpate with intercolpar concavities	Patel *et al.* 1984
M. latecrenata	-	absent	Ephemerous	glandular				Goldenberg *et al.* 2003
M. melanotricha					tetrad			Patel *et al.* 1984
M. paucidens	Monocotyledonous	Non-thickened	Ephemerous	Secretory and uninucleate	bicellular, monad	psilate	3-colpori and 3-pseudocolpi	Present study
M. pepericarpa		Non-thickened	Ephemerous	Secretory and uninucleate	bicellular, monad			Caetano 2010
M. pusilliflora	-	absent	Ephemerous	glandular				Goldenberg *et al.* 2003
M. rigidiuscula					-, monad	psilate	3-colpori and 3-pseudocolpi	
M. stenostachya	Monocotyledonous	Non-thickened	Ephemerous	Secretory and uninucleate	bicellular, monad	psilate	3-colpori and 3-pseudocolpi	Present study

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References

Basso-Alves JP, Agostini K, Teixeira SP. 2011. Pollen and stigma morphology of some *Phaseoleae* species (Leguminosae) with different pollinators. Plant Biology 13: 602-610.

Batygina TB. 2002. Embryology of flowering plants: terminology and concepts: generative organs of flowers. Science Pub Inc.

Bhandari NN. 1984. The microsporangium. *In* Embryology of angiosperms (Johri, B.M., ed). Springer-Verlag, New York. Pp. 53-121.

Buss PA, Lersten NR. 1972. Crystals in tapetal cells of the Leguminosae. Botanical Journal of the Linnean Society 65: 81-85.

Caetano 2010. Apomixia e reprodução sexuada em espécies de *Miconia* Ruiz & Pavon, Melastomataceae. Dissertação de mestrado. Universidade Estadual de Campinas, Campinas, SP.

Clausing G, Renner SS. 2001. Molecular phylogenetics of Melastomataceae and Memecylaceae: implications for character evolution. American Journal of Botany 88: 486-498.

Cogniaux A. 1891. Melastomataceae. In: A. & C. de Candolle (eds.). Monographiae Phanerogamarum. Paris, G. Masson.

Dafni A, Kevan PG, Husband BC. 2005. Practical pollination biology. Ontario, Enviroquest Ltd.

Davis GL. 1966. Systematic embryology of the angiosperms. Wiley & Sons, New York, London.

Fernandes WP, Simão DG. 2010. Desenvolvimento da parede da antera e do grão de pólen em Melastomataceae: *Rhynchanthera grandiflora* (Aubl.) DC. e *Tococa guianensis* Aubl. *In:* Resumos do VIII Encontro de Botânicos do Centro Oeste, Goiânia.

Goldenberg R 2000. O gênero *Miconia* Ruiz & Pav. (Melastomataceae): I. Listagens Analíticas, II. Revisão Taxonômica da Seção *Hypoxanthus* (Rich. Ex DC.) Hook. F. Tese de Doutorado. Universidade Estadual de Campinas, Campinas-SP.

Goldenberg R, Shepherd GJ. 1998. Studies on the reproductive biology of Melastomataceae in "cerrado" vegetation. Plant Systematics and Evolution 211: 13-29.

Goldenberg R, Teixeira SP, Martins AB. 2003. Anther dehiscence and circumscription of *Miconia* sect. *Hypoxanthus* (Melastomataceae). Kew Bulletin 58:1195-203.

Goldenberg R, Penneys DS, Almeda F, Judd WS, Michelangeli FA 2008. Phylogeny of *Miconia* (Melastomataceae): patterns of stamen diversification in a megadiverse neotropical genus. International Journal of Plant Science 169: 963-979.

Judd WS. 1986. Taxonomic placement of *Calycogonium squamulosum* (Melastomataceae: Miconieae). Brittonia 38: 238-242.

Judd WS. 1989. Taxonomic studies in the Miconiaeae (Melastomataceae): cladistic analysis of axillary-flowered taxa. Annals of the Missouri Botanical Garden. 76: 476-495.

Judd WS, Skean, JD. 1991. Taxonomic studies in the Miconieae (Melastomataceae). IV. Generic realignments among terminal-flowered taxa. Bulletin of the Florida Museum of Natural History, Biological Sciences 36: 25-84.

Karnovsky MJ. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in eletron microscopy. Journal of Cellular Biology 27: 137-138.

Lersten NR. 2004. Flowering plant embryology: with emphasis on economic species. Wiley-Blackwell.

Maheshwari P. 1950. An introduction to the embryology of angiosperms. McGraw-Hill, New York.

Martin CV, Little D, Goldenberg R, Michelangeli FA. 2008. A preliminary phylogenetic analysis of the polyphyletic genus *Leandra* (Miconieae, Melastomataceae). Cladistics 24: 315-327.

Medeiros JD, Morretes BL. 1996. The embryology of *Miconia cabucu* (Melastomataceae). Cytologia 61: 83-91.

Melhem TS, Cruz-Barros MAV, Corrêa AMS, Makino-Watanabe H, Silvestre-Capelato MSF, Esteves VLG. 2003. Variabilidade polínica em plantas de Campos do Jordão (São Paulo, Brasil). Boletim do Instituto de Botânica 16: 1-104.

Mepham RH, Lane GR. 1969a. Formation and development of the tapetal periplasmodium in *Tradescantia bracteata*. Protoplasma 68: 175-192.

Mepham RH, Lane GR. 1969b. Role of the tapetum in the development of *Tradescantia* pollen. Nature 221: 282-284.

Michelangeli FA, Penneys DS, Giza J, Soltis D, Hils MH, Skean DJD. 2004. A preliminary phylogeny of the tribe Miconieae (Melastomataceae) based on nrITS sequence data and its implications on inflorescence position. Taxon 53:279-290.

Patel VC, Skvarla JJ, Raven PH. 1984. Pollen characters in relation to the delimitation of Myrtales. Annals of the Missouri Botanical Garden 71: 858-969.

Santos RP, Mariath JEA. 1997. A simple method for fixing, dehydrating and embedding pollen tubes cultivated in vitro for optical and transmission electron microscopy. Biotechnic & Histochemistry 72: 315-319.

Schmid R. 1984. Reproductive anatomy and morphology of Myrtales in relation to systematics. Annals of the Missouri Botanical Garden 71: 832-835.

Tobe H, Raven PH. 1983. An embryological analysis of Myrtales: its definition and characteristics. Annals of the Missouri Botanical Garden 70: 71-94.

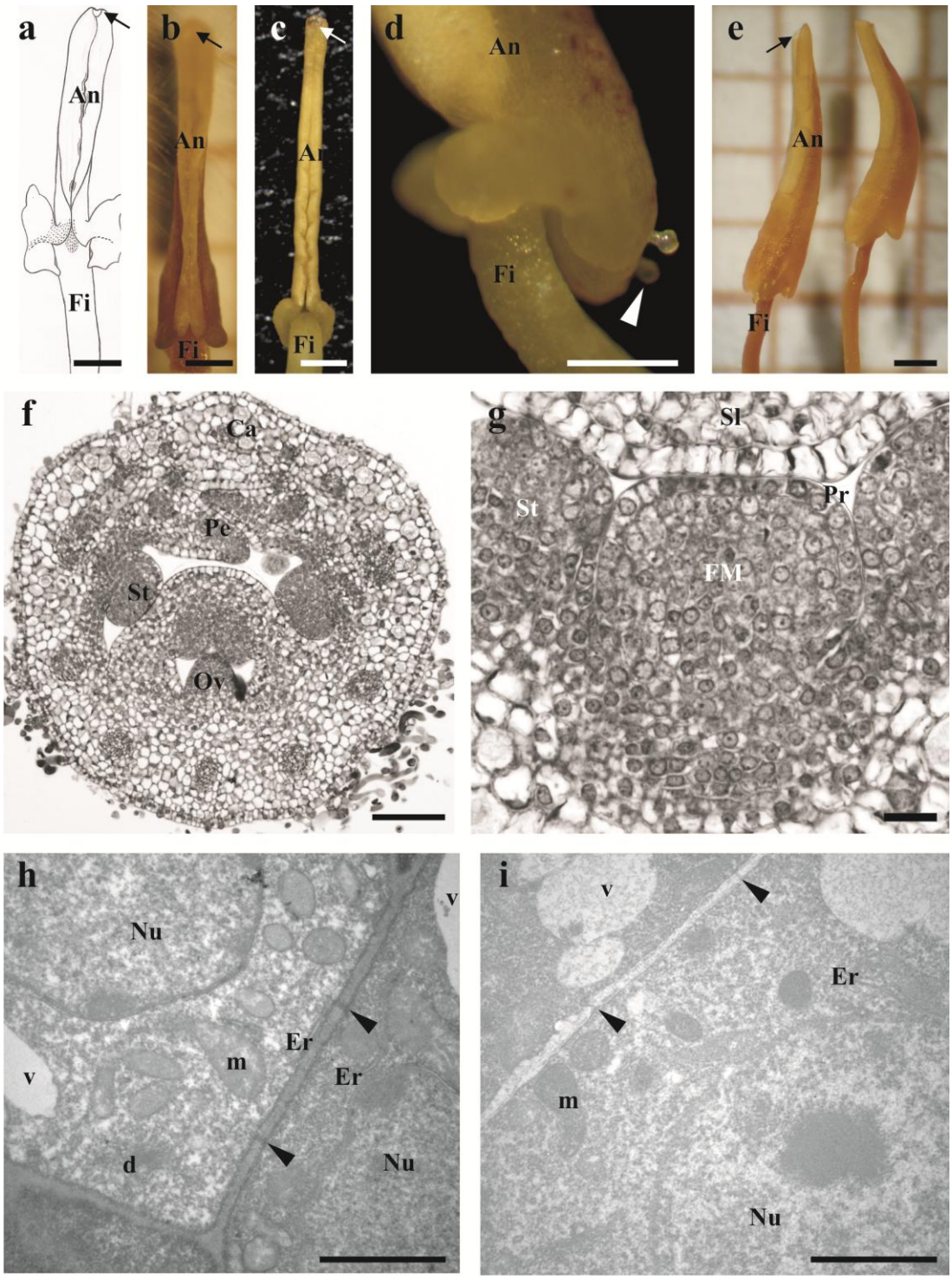

Figure 1 Anther of (**a, h, i**) *Miconia albicans*, (**b, e**) *Miconia stenostachya*, (**c, d, f, g**) *Miconia paucidens*. Note the glandular trichomes (arrowhead) in the connective base. (**e**) Alternate stamens have anther and filament of different size in the three species studied here. (**f, g**) Longitudinal section of bud flower in early developmental stage. Note the initial stamen development. (**g**) Early sporogenous cells development. Note the plasmodesmata (arrowhead) between adjacent cells. [Arrow: pore. An: anther. Ca: calice. d: dictyosome. Er: endoplasmic reticulum. Fi: filament. FM: fundamental meristem. m:mitochondria. Nu: nucleus. Ov: ovary. Pe: petal. Pr: protoderm. Sl: style. St: stamen. v: vacuole. Scale bars: 0,5mm in **a**-**e**, 50µm in **f,** 10µm in **g,** and 1µm in **h, i**].

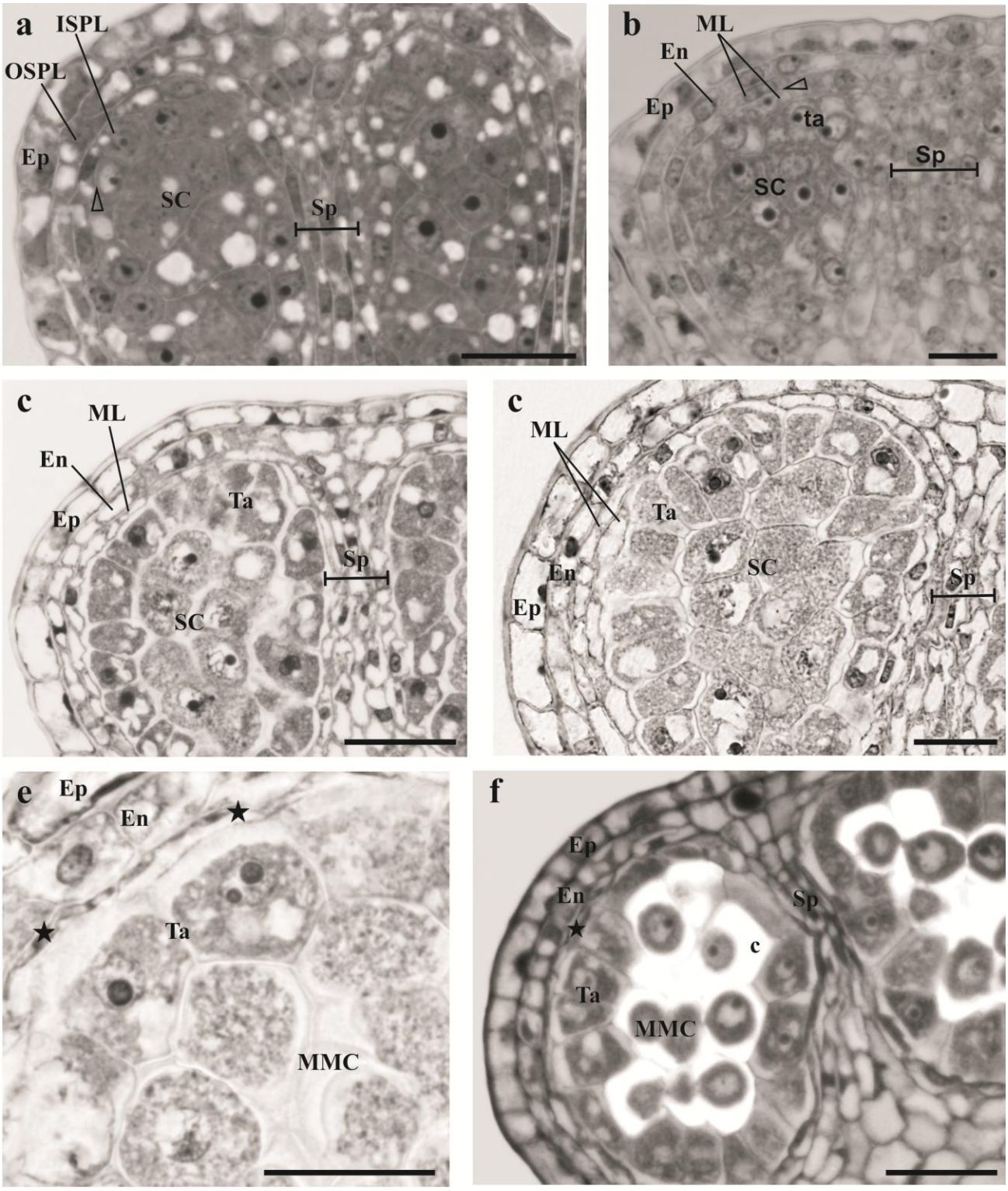

Figure 2 (**a-f**) Sequential developmental stages of *Miconia albicans* anthers. The same characteristics were also observed in *Miconia stenostachya* and *Miconia paucidens* anthers. [Arrow: pore. An: anther. Ca: calice. d: dictyosome. Er: endoplasmic reticulum. Fi: filament. FM: fundamental meristem. m:mitochondria. Nu: nucleus. Ov: ovary. Pe: petal. Pr: protoderm. Sl: style. St: stamen. v: vacuole. Scale bars: 20µm].

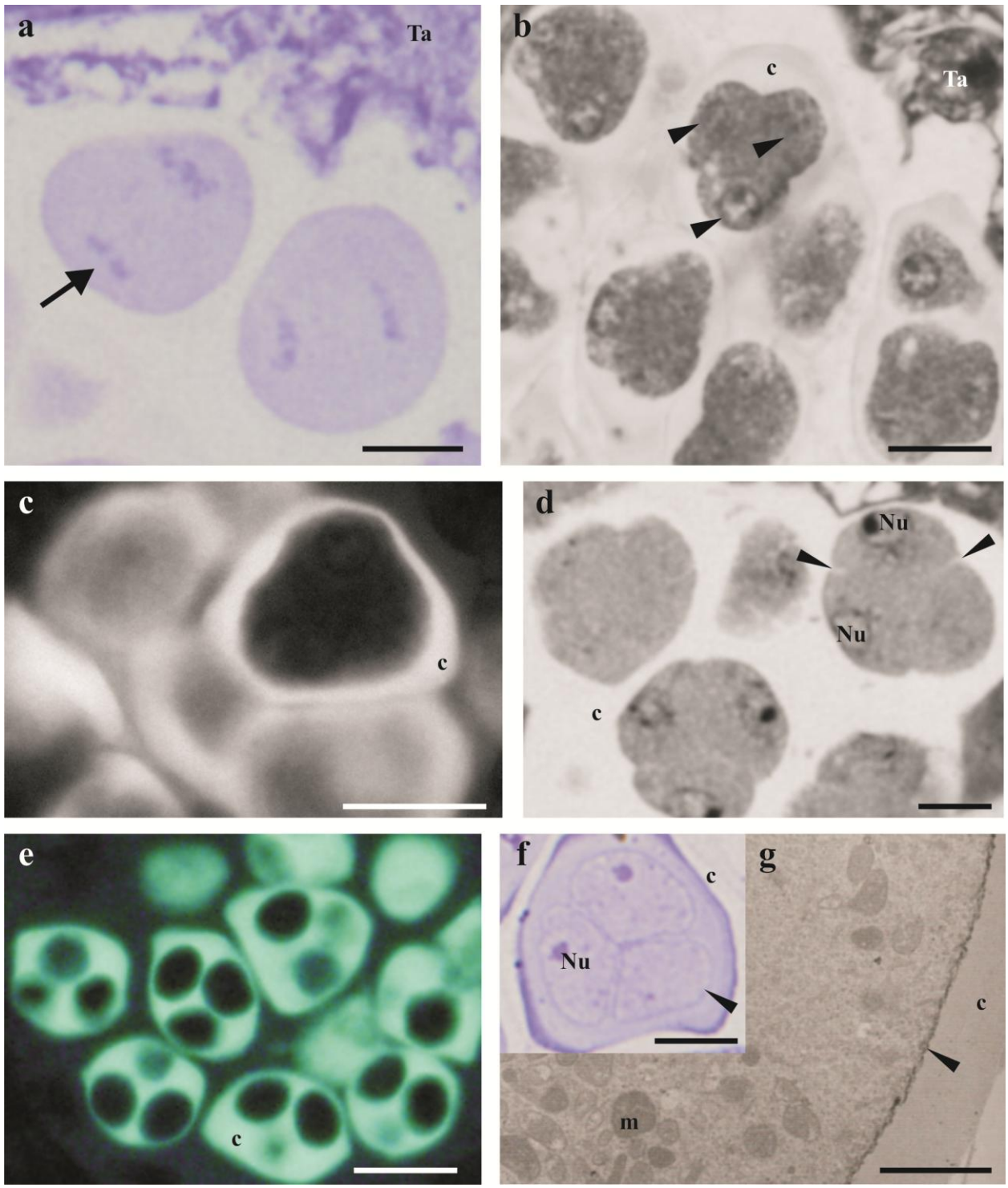

Figure 3 (**a-f**) Sequential steps of *Miconia stenostachya* (a, f, g) and *Miconia paucidens* (b-e) microsporogenesis. Microsporogenesis was not observed in *Miconia albicans* anthers. (**a**) Anaphase I showing set of chromosomes (arrow). (**b**, **c**) After the end of meiotic process, a syncythium with four nuclei (arrowhead) is formed from each microspore mother cell. (**d**, **e**) The cytokinesis initiates (arrowhead), resulting in the tetrahedral tetrad of microspores. (**f**, **g**) The primexine (arrowhead) is deposited when the microspore is still in a tetrad fashion. [c: callose wall. m: mitochondria. Nu: nucleus. Ta: tapetum. Scale bars: 5µm in **a**, **d**, 10µm in **b**, **c**, **e**, **f** and 1µm in **g**].

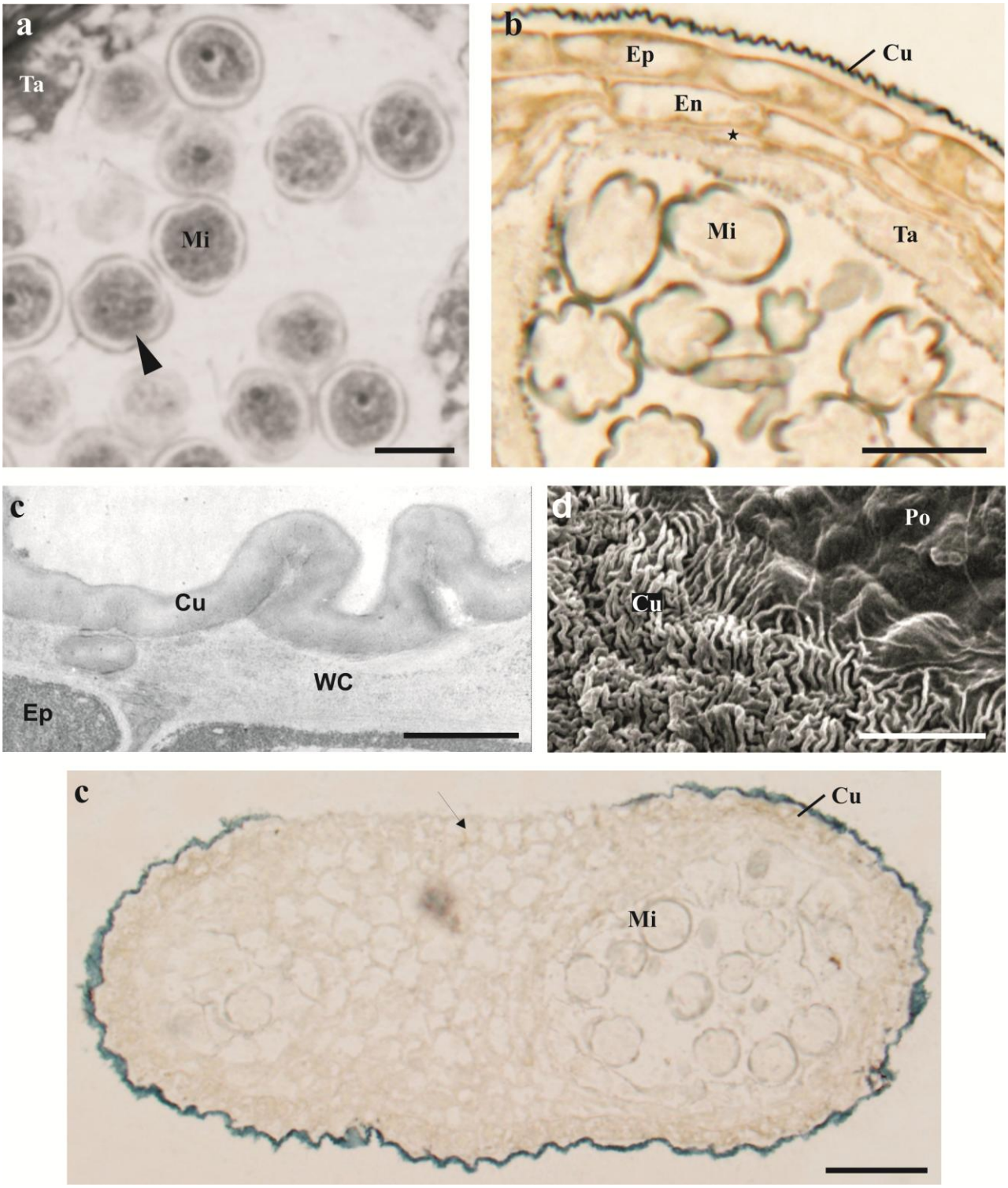

Figure 4 (**a**, **b**) Free microspores (Mi) in *Miconia paucidens* (a) and *M. stenostachya* (b). Note the initial formation of microspore wall (arrowhead). (**c**-**e**) Cuticle (Cu) in the epidermal (Ep) cells of *Miconia stenostachya* (c) and *Miconia paucidens* (d, e) anther wall. Note that cuticle is absent in the dehiscent region (Po, arrow) of the anther. [CW: cell wall. En: endothecium. Ta: tapetum. ★: middle layer. Scale bars: 5µm in **a**, 10µm in **b**, **d**, 1µm in **c** and 20µm in **e**].

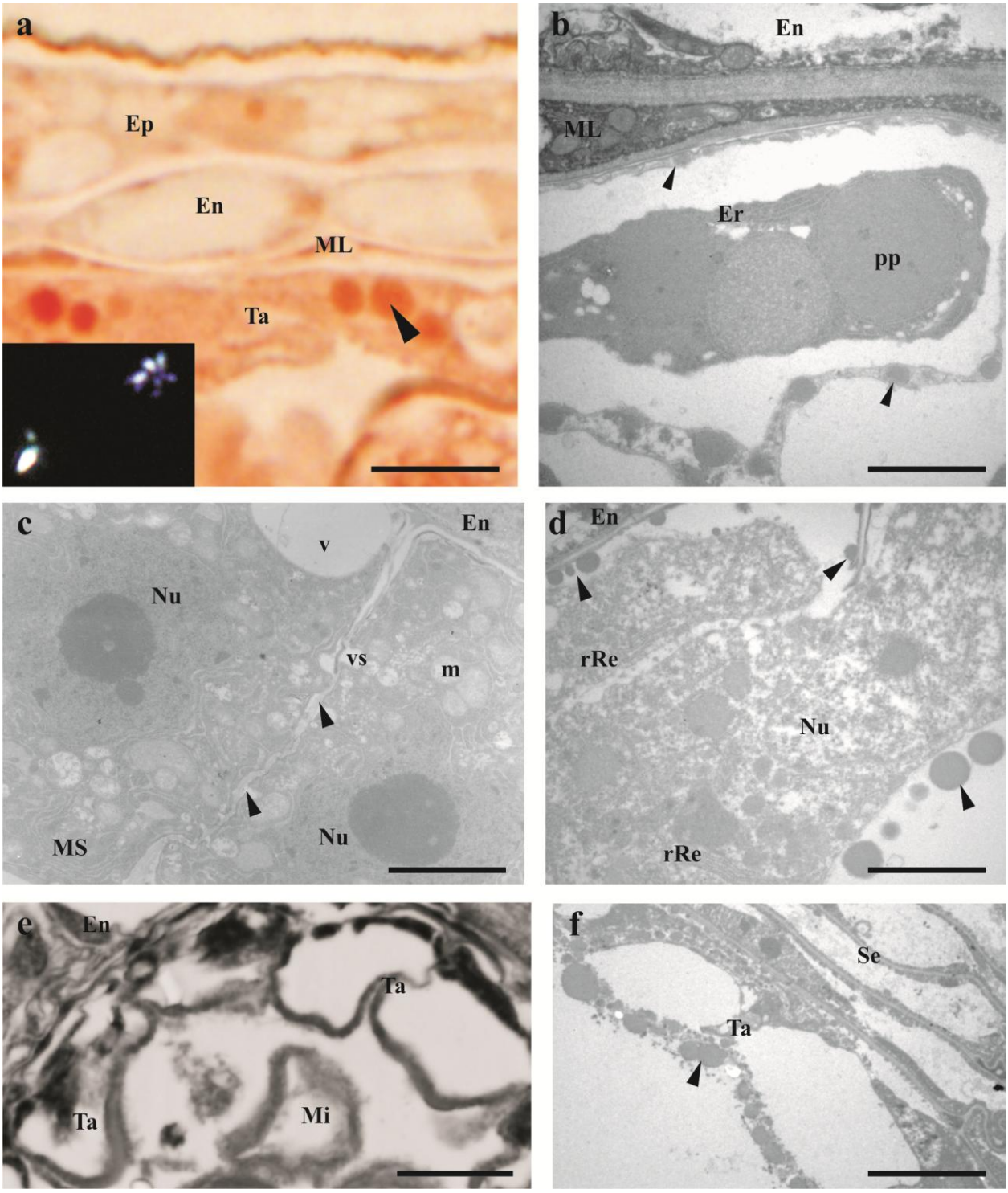

Figure 5 (**a**-**f**) Anther wall layers at the free microspore (Mi) stage. (**a**-**b**) Proteoplasts (arrowhead, pp) and druse crystals (inset) are present only in *Miconia paucidens*. (**c**, **d**) Cell wall (arrowhead) and cytoplasm degradation in *Miconia stenostachya* tapetum. (**e**, **f**) Abnormal wall formed by orbicules aggregation in *Miconia albicans* tapetum. [CW: cell wall. En: endothecium. Ep: epidermis. Er: endoplasmatic reticulum. m: mitochondria. ML: middle layer. Nu: nucleus. rEr: rough endoplasmic reticulum. Se: septum. Ta: tapetum. v: vacuole. vs: vesicle. Scale bars: 5µm in **a**, **e** and 1µm in **b**, **c**, **d**, **f**].

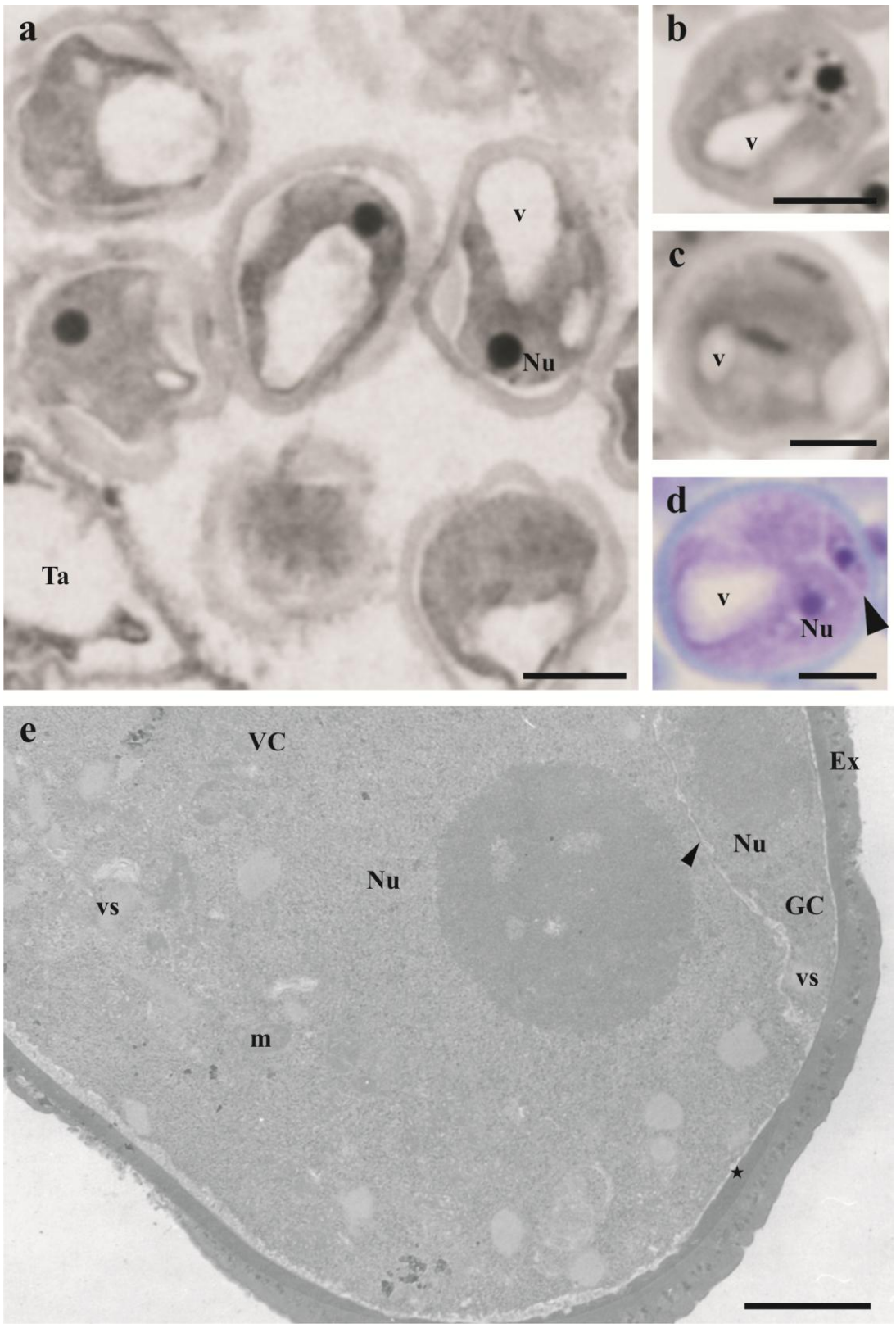

Figure 6 (**a**) Free vacuolated microspores in *Miconia paucidens* (a). (**b**-**e**) Microgametogenesis in *Miconia paucidens* (b, d) and *Miconia stenostachya* (c, e). Note the parietal position of the generative cell (arrowhead). [Ex: exine. GC: generative cell. m: mitochondria. Nu: nucleus. Ta: tapetum. v: vacuole. VC: vegetative cell. vs: vesicle. ★: intine. Scale bars: 5µm in **a**-**d** and 1µm in **e**].

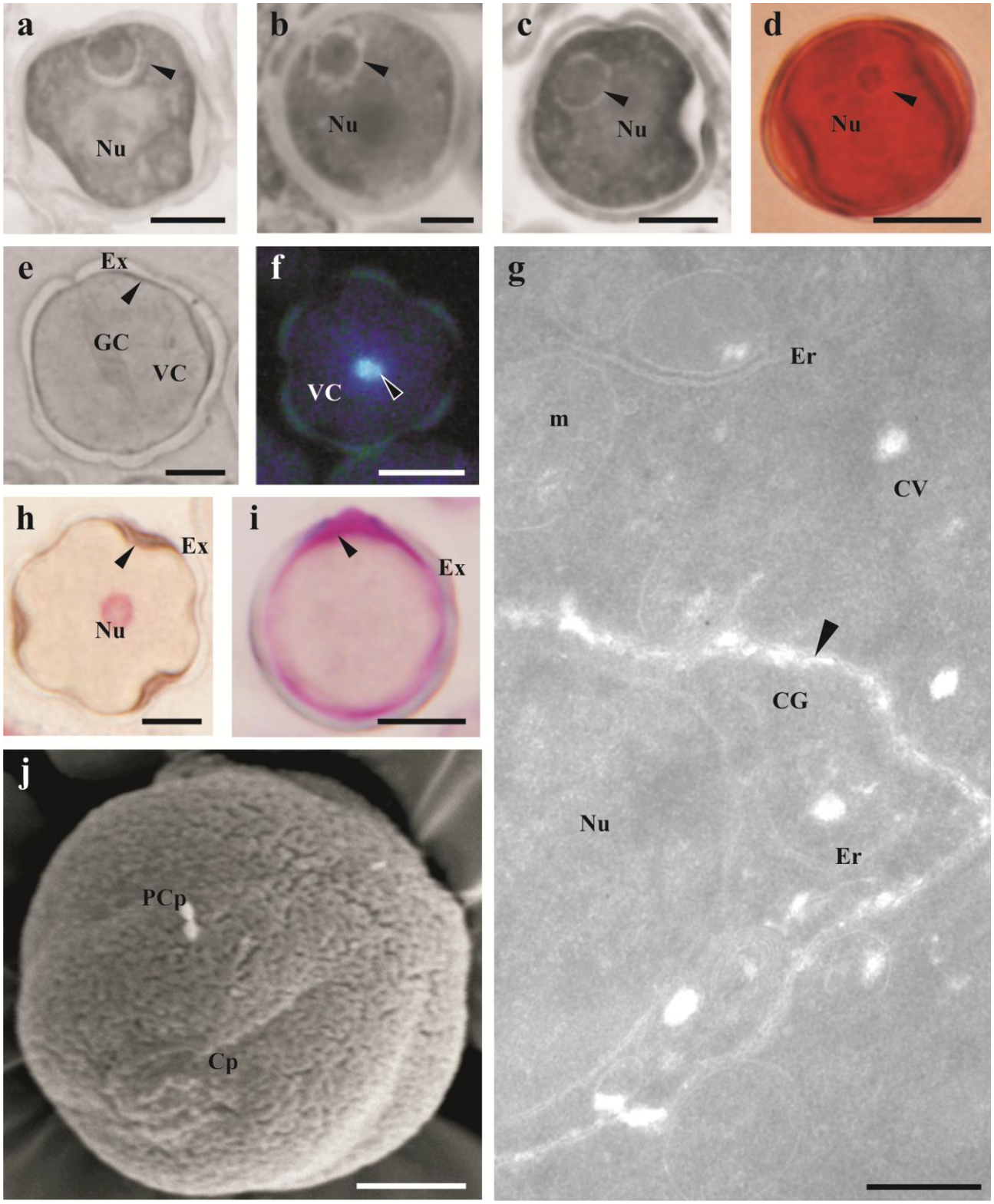

Figure 6 (**a-g**) Bicellular pollen grains in *Miconia stenostachya* (a, b, d) and *Miconia paucidens* (c, e-j). Note the intine (arrowhead) in e, h and i. [Cp: colpi. PCp: pseudocolpi. Er: endoplasmic reticulum. Ex: exine. GC, ►: generative cell. m: mitochondria. Nu: nucleus. VC: vegetative cell. Scale bars: 5µm in **a**-**e**, **h**, **i**, 10µm in **f**, 1µm in **g** and 3µm in **j**].

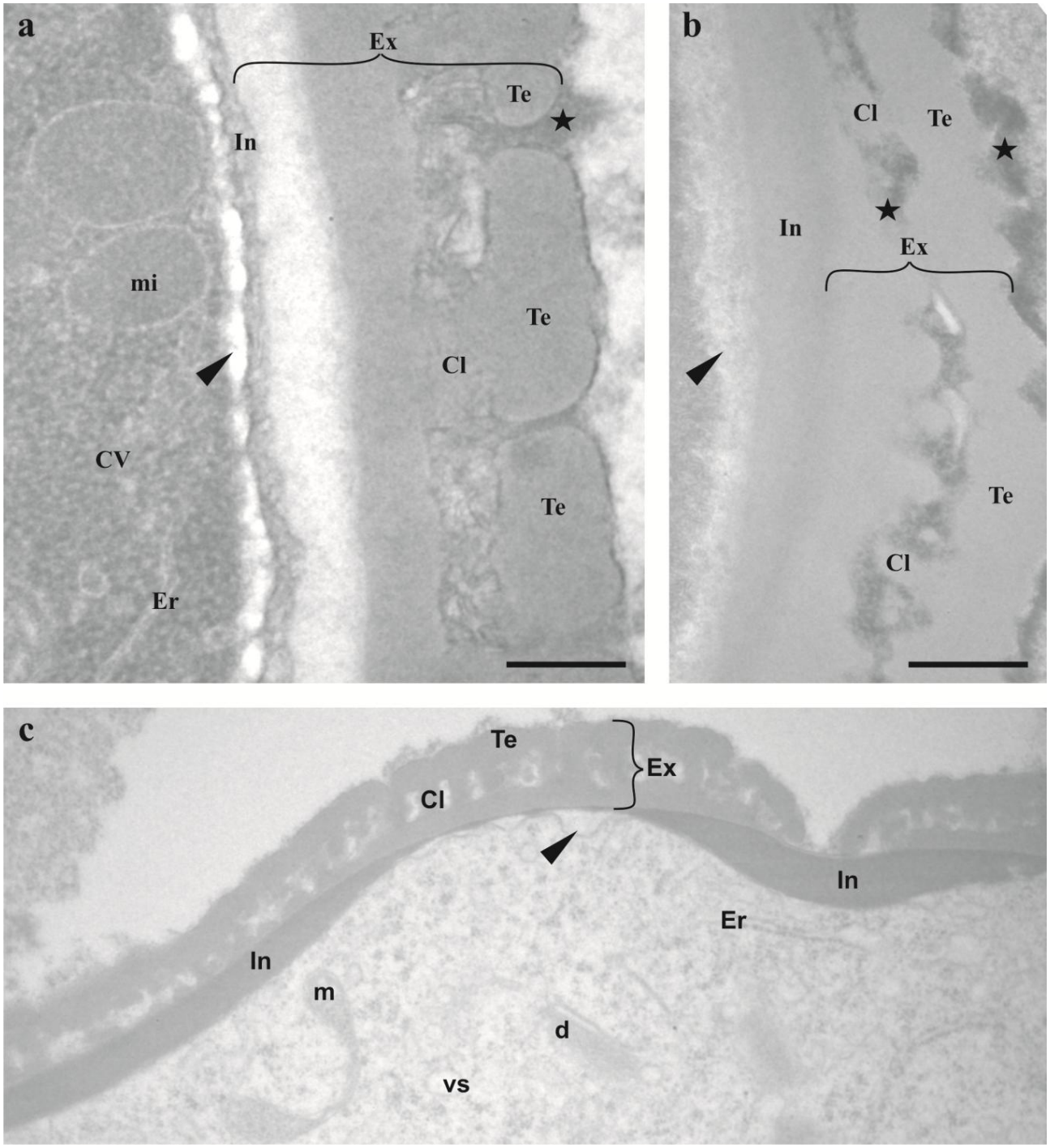

Figure 7 (**a-c**) Mature pollen grains wall in *Miconia paucidens* (a) and *Miconia stenostachya* (b, c). [Cl: columella. CV: vegetative cell. d: dictyosome. Er: endoplasmic reticulum. Ex: exine. In: intine. m: mitochondria. rEr: rough endoplasmic reticulum. Te: tectum. vs: vesicle. ►: plasmatic membrane. ★: "pollenkit". Scale bars: 1µm].

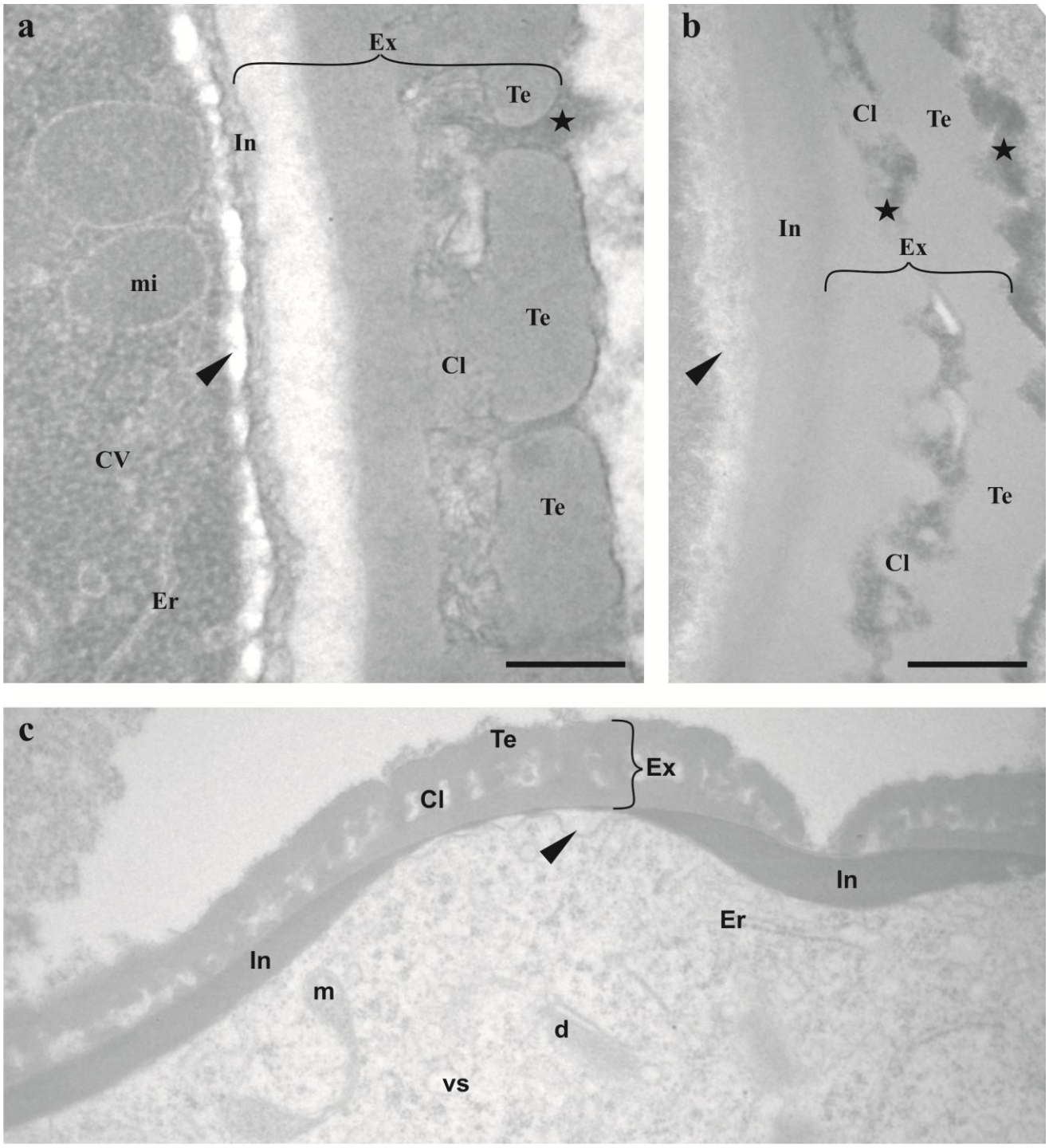

Figure 8 (**a**, **b**) Mature pollen grains in *Miconia paucidens*. Note the different form when dehydrated in a and after hydration in b. (**c**-**f**) Dehiscent anther. *M. paucidens* in c, *M. albicans* in d and *M. stenostachya* in e. Note the tapetal membrane in e (arrowhead) and f (TM). (**g**) Transversal section of the *M. paucidens* mature anther. Note the bilocullar appearance after septum (Sp) degradation. (h-j) Pollen tube (PT) in *Miconia paucidens*. Note the sperm cells nuclei (arrowhead) in i and one callose plug (arrow) in j. [Co: connective. Ep: epidermis. En: endothecium. PG: pollen grain. Po: pore. PS: pollen sac. VB: vascular bundle. Scale bars: 10µm in **a**, **e**, **i**, 5µm in **b**, **h**, **j**, 100µm in **c**, **j**, 50µm in **d** and 1µm in **f**].

Capítulo 2.

Morphological approach of male sterility in *Miconia* (Melastomataceae) species

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Abstract

Pollen abortion occurs in virtually all species and often does not prejudice reproductive success. However, large numbers of abnormal pollen grains, known as male sterility are characteristic of some species. Species with low pollen grain production are frequent among the genus *Miconia*, in which partial and complete male sterility is often related to apomixis. In this work, we compared the morphology of pollen grains over several developmental stages in *Miconia* species with different rates of male sterility. Our aim is to improve the knowledge of mechanisms that lead to male sterility in this ecologically important tropical group. Routine techniques for microscopy were used to examine anthers in several developmental stages collected from the apomictic species *Miconia albicans* and *M. stenostachya*. *M. albicans* has more severe abnormalities than *M. stenostachya*, and its microspores and pollen grains do not exhibit a stratified exine. *M. stenostachya* showed lagging chromosomes at anaphase I, and many nuclei originating from meiotic process were fused instead of giving rise to two daughter cells. Pollen grains of different sizes were observed in *M. stenostachya*, and a normal stratified exine and an intine were present even in the aborted pollen grains. Mitosis was symmetric, resulting in pollen grains with two similar cells. Our data indicate that meiosis is most strongly affected in *Miconia albicans,* and that meiotic irregularities lead to male sterility in *Miconia stenostachya*. Notably, abnormalities in *M. stenostachya* did not reach pollen wall formation, suggesting little or no tapetum involvement in male sterility in this species.

Keywords: male sterility, *Miconia*, microsporogenesis, microgametogenesis, pollen development, apomixis

Introduction

The reproductive success of the plants depends on the production of viable male and female gametes, as the new sporophyte originates from their fusion (Lersten, 2004). However, some species can produce viable embryos even in the absence of viable pollen grains through a mechanism called apomixis (Koltunow *et al.*, 1995). As apomixis is a useful mechanism for crop management, many studies have focused on economically important plants, generally growing under controlled conditions. However, knowledge about the cytology of structures related to apomixis, the pollen grain and the embryo sac, and their ontogeny in natural population of apomictic species is still scarce.

Among tropical plant families, Melastomataceae is one of the most rich in the number of apomictic species, particularly in the tribe Miconieae, in which about 60% of the studied species reproduce by means of apomixis (Goldenberg and Shepherd, 1998). In *Miconia* species, apomixis is almost always related to low levels of pollen viability; at least in some species, these abnormalities are due to meiotic irregularities, such as bridges and lagging chromosomes (Goldenberg and Shepherd, 1998).

Structural and ultrastructural studies have revealed that male sterility has multiple causes. The phenotypic manifestations of sterility are diverse, including absence of stamens, abortion of pollen at any stage of its development, absence of anther dehiscence or defects in pollen tube formation (Budar, 2001). Such pollen abnormalities may occur during ontogeny in virtually all angiosperm species, but most of them do not impair reproductive success. Some of the genes related to male sterility are now known, and phenotypic analysis of mutants has provided useful clues for understanding the events that lead to pollen abortion. Some of these gene mutations also affect other tissues in the anther, mainly the vascular tissue and the tapetum (Goldberg *et al*., 1993; Alves-Ferreira *et al*., 2007; Zhu *et al*., 2010).

Although structural and ultrastructural studies have provided useful information about male sterility in plants, they are still scarce in Melastomataceae species. Therefore, the goal of this study was to investigate structural and ultrastructural features of the pollen abortion in two apomictic species of *Miconia* that have low levels of pollen fertility (Goldenberg and Shepherd, 1998). Specifically, we set out to understand the steps that give rise to pollen abortion in these two apomictic species from natural populations. Observations were made over various stages of microsporogenesis and microgametogenesis. Determining the morphological consequences of irregularities during ontogeny of the pollen grains in natural populations that are characterized by low numbers of viable pollen grains, a phenomenon considered rare among angiosperms (Laser and

Lersten, 1972), is important for both structural and ecological studies. Moreover, understanding microspore development in different apomictic species will extend our knowledge of the important structural changes that can occur during pollen production.

Materials and Methods

Field investigations were performed using plants of the two apomictic species, according to Goldenberg and Shepherd (1998), *Miconia albicans* (Sw.) Triana and *M. stenostachya* DC., growing in natural populations of cerrado in southeast Brazil, from the municipalities of Itirapina (22º15'10" S and 47º49'22" W) and Campinas (22°54'20" S and 47°03'39" W), both in the São Paulo state, from 2007 to 2010.

Stamens in several stages of development were removed from young floral buds and flowers of at least five individuals of the two species. They were immediately fixed in a solution of 80 mL L^{-1} glutaraldeyde, 250 mL L^{-1} paraformaldeyde (16%) and 500 mL L^{-1} phosphate buffer (0.1 M, pH 6.8) for 24 h (modified from Karnovsky, 1965). Routine techniques were followed to analyze the samples using a light microscope (BX 51A, Olympus) and transmission (CM 100, Philips) and scanning (JSM 5200, Jeol) electron microscopes.

To visualize the morphology of the pollen grains and to detect some particular chemical compounds, some of the fixed anthers were crushed against a glass slide and stained with the following: 12 g L^{-1} acetocarmine solution for cytoplasm visualization, lugol solution for starch grains, xylidine de Ponceau (pH 2.5) (C.I. 16150) for total proteins, Sudan black B (C.I. 26150) for lipids, 0.05% toluidine blue (pH 4.0) (C.I. 52040) for phenolic compounds, periodic acid Schiff (PAS method) reaction (pararosanilin C.I. 42500) for structural carbohydrates, Ruthenium red solution for pectin and 4′,6-diamidino-2-phenylindole (DAPI) in phosphate-buffered saline (PBS) for better nuclear visualization. To verify the deposition of callose wall, anthers in tetrad stage were crushed on a slide in a drop of aniline blue (pH 8.0) (C.I. 42755) and examined using a fluorescence microscope equipped with an ultraviolet (UV) excitation filter. The above stains were also applied to the slides obtained from the structural analyses.

Fresh pollen grains were submitted to a germination test using a protocol adapted from Santos and Mariath (1997) and successfully applied to *Miconia paucidens*, a sexual species with high pollen fertility, as a control. A germination medium containing 2% colorless gelatin, 20% sucrose, 0.01% boric acid and 0.05% calcium nitrate was used for optimal growth conditions. After incubation at room temperature (approximately 25°C) for 3 h in the dark, pollen grains were examined under a light microscope to observe pollen tube formation.

Results

The development of *Miconia albicans* and *M. stenostachya* anthers proceeds normally until the microspore mother cell stage (see Cortez et al., Capítulo 1).

Miconia albicans

All microspores formed from microspore mother cell were abnormal. They had degenerating cytoplasms, and two or more nuclei per cell or were completely empty; these abnormalities are always accompanied by anomalous microspore wall development (Figures 1a, b). In addition, we observed some orbicules of surprisingly large size (Figure 1c), which result from the fusion of smaller ones (Figure 1d). The orbicules were also fused above the tapetal walls, mainly in the periclinal wall in contact with the locule, forming a compact wall that differed from the usual peritapetal membrane (Figures 1a-c).

Although the tapetum degeneration was normal, the tapetal cell architecture was maintained at the free microspore stage for some time due to the wall formed by the fused orbicules (Figures 2a-c). The mature anther walls, prior to dehiscence, were composed of the epidermis and the endothecium, as well as the orbicules that remained attached to the endothecium periclinal inner wall after the total tapetal cell degradation (Figure 2d). Mature pollen sacs showed only abnormal structures, with several rates of degeneration in the cytoplasm and nucleus, along with anomalous size and shape, or total absence of cell contents (Figure 2d). Most anthers were completely empty or only filled with an amorphous substance that was stained similar to orbicules and exine (Figure 2e), and some showed one or more obliterate pollen sacs (Figure 2f). Inside the pollen sacs, we found some abnormal microspores that remained in a dyad or tetrad arrangement due to the fusion of their walls (Figures 3a-e). Only few bicellular pollen grains were observed, but even these were abnormal (Figures 3f, g). In addition to the presence of some microspores and pollen grains with cell contents, the anomalous wall structure indicates that they were not properly formed (Figures 3f-j).

Miconia stenostachya

Miconia stenostachya showed several meiotic irregularities, including lagging chromosomes (Figure 4a) at anaphase I and II. Many of the syncytia resulting from the completion of meiosis I and II had four nuclei of different sizes, more than four nuclei per syncytium (Figure 4b) or showed evidence nuclei fusion (Figure 4c). In some cases, cytokinesis gave rise to dyads in addition to tetrads (Figure 4d). The lagging chromosomes that did not migrate toward the two poles at anaphase I (unbalanced chromosome segregation) were enclosed in an extra nucleus at interkinesis, resulting

in tetrads or polyads with one or more micronuclei (Figures 5a-e). Callose wall deposition appeared to proceed normally and surrounded even the micronuclei (Figures 5a-d). Some of the microspores of the tetrad showed more than one nucleus (Figure 5f). Some tetrads exhibited microspores with several levels of cytoplasm and nuclear disintegration (Figure 5g) or cytoplasmic connections between adjacent microspores (Figure 5h).

Free microspores were observed inside the anther loculus after callose wall disintegration. Some anthers had very dense contents inside the locule in the free microspore stage, and orbicules began to accumulate above the inner periclinal wall of the tapetum (Figure 6a). Microspores with more than one nucleolus per nucleus (Figures 6b, c), with more than one nucleus per cell or with cytoplasm degradation (Figure 6d) were common. Some microspores with anomalous exine staining (Figure 6e) or of different sizes (Figures 6d, f) were also observed. Protein, lipids, starch grains and polysaccharides were not detected as reserve substances inside the microspore cytoplasm. Even the microspores with degenerating cytoplasms exhibit normal sporoderms, which were stratified in the intine and exine (Figure 6f).

The mitotic division that gives raise to the vegetative and generative cells of bicellular pollen grains occurs synchronously with tapetal cell disintegration and is not always asymmetric; consequently, the formation of pollen grains with two cells of the same size was common (Figures 7a, b). Even in the cases where mitosis was asymmetric, pollen grains were abnormal due to the presence of more than one nucleus inside the generative and/or vegetative cells (Figure 7a, inset). The vegetative and generative cells also showed ultrastructural abnormalities as disturbances in organelles, the endomembrane system and the plasma membrane (Figures 7c, d).

Prior to dehiscence, the mature anther wall is composed of the epidermis and the endothecium, as well as the orbicules that remain attached to the endothecium periclinal inner wall after complete tapetal degradation. Most cellular structures found inside the mature pollen sacs showed the following structural abnormalities: spherical or anomalous-shaped generative cells (Figures 8a-c), degenerating cytoplasm (Figure 8d), supernumerary nuclei inside the cytoplasm (Figure 8e), cytoplasmic connections between adjacent structures (Figure 8f), totally empty structures despite the presence of normal exine (Figures 8g, h, m, o) and disturbed pollen wall morphology (Figures 8i-k). Ultrastructural disturbances were also observed in the cytoplasms of both vegetative and generative cells of mature bicellular pollen grains (Figures 8m-o).

Despite the presence of an apparently normal cytoplasm in some pollen grains, which were positively stained with acetic carmine, no pollen grain was able to develop a pollen tube in our in vitro experiments of pollen germination (Figure 8l).

Discussion

The normal early developmental stages of pollen observed in the anthers of *Miconia albicans* and *M. stenostachya* indicates that the abnormalities culminating in the male sterility occur during meiotic processes in both species. The few abnormalities observed in the microspore mother cell stage probably did not compromise pollen grain production, as they have also been observed in *Miconia* species with high levels of pollen viability (Cortez PA and Caetano APS, personal observations).

Despite the great number (more than 500) of anthers in the presumably meiotic stage analyzed, the meiotic process was never observed in *Miconia albicans,* indicating that meiosis is a rare event in this species or even absent in the most of its anthers. The presence of some abnormal microspores in the mature anthers of *M. albicans*, most of them in tetrahedral shape, may indicate that some kind of division, meiotic or mitotic, can still occur but in an abnormal way. This is supported by the structural similarities of some microspore abnormalities observed between *M. albicans* and *M. stenostachya*.

Some meiotic irregularities observed in this study of *Miconia stenostachya* were also reported by Goldenberg and Shepherd (1998). The micronuclei observed in the tetrad cells are a consequence of lagging chromosomes, as cytokinesis and the last callose deposition around each microspore occurred even in the resulting small nuclei. Many studies of male sterility indicate that some errors during meiosis are a frequent cause of male sterility in both natural and manipulated plants (Bertasso-Borges and Coleman, 2005; Bohdanowicz *et al*., 2005; Risso-Pascoto *et al*., 2005; Calisto, 2008). The irregularities result in unbalanced microspores, which arrests in development. These errors may result from reduced cohesion of sister chromatids at metaphase I and metaphase II (Roeder, 1997; Dawe, 1998) or defects in the spindle apparatus (Jiang *et al*., 2009).

The cytoskeletal defects reported by Jiang *et al*. (2009) can also generate abnormalities during meiosis, which affect the formation of vegetative and generative cells of the bicellular pollen grain. In this stage, asymmetric division generates two cells of not only different sizes but with different cellular fates. This step is crucial because it determines that one cell will be involved in pollen tube formation and the other in the production of male gametes needed for fertilization. We may expect that if normal fuse fiber behavior is necessary for cell fate commitment, dysregulation in this process can lead to pollen abortion. Determining whether and how this contributes to pollen abortion in these species is an important direction for future studies.

The symmetrical mitosis observed in both studied species of *Miconia* has been recorded for *Glycine max* (Leguminosae) and is considered to be one of the mechanisms that divert the

gametophytic pathway to the embryogenic one, resulting in embryos (or callus) rather than pollen grains (Cardoso, *et al*.; 2004). In fact, we found some abnormal pluricellular structures inside the locular space of both species, but nothing that could be interpreted as a somatic embryo derived from male cells (androgenesis). The formation of microspores with more than one nucleus, as observed in *M. stenostachya*, was also reported in *Glycine max* as a consequence of normal meiosis but absent cytokinesis, resulting in cenocitic tetrads with sporodermis development and posterior abortion (Laser and Lersten, 1972).

The relationship between the genomes of the parental species has great influence on the determination of chromosome pairing and recombination processes, and thus the extent of meiotic irregularities and gamete viability (de Jong *et al*., 1993). Indeed, somatic hybrids are generally less fertile, which is a major problem for sexual reproduction (Pijnacker *et al*., 1992). Meiotic irregularities may be related to hybridization and have important evolutionary consequences. The chromosome counts for *Miconia albicans* showed different numbers (2n = 34 and 2n = 48), perhaps because the samples were from different regions (Goldenberg and Shepherd, 1998). The number obtained for *M. stenostachya* (n = ~26) suggests that this species may be triploid in the studied population. Goldenberg and Shepherd (1998) also reported a strong indication of hybrid origin for this species based on observation of meiotic irregularities. In the same areas where *M. stenostachya* and *M. albicans* were collected, other *Miconia* species are present, including *M. fallax*, *M. rubiginosa* and *M. leucocarpa*. Interestingly, *M. fallax* is also apomictic and probably diploid (n = ~17) according to Goldenberg and Shepherd (1998) or polyploid (2n > 60) according to Caetano *et al*. (unpublished data), and its vegetative and reproductive structures are very similar to those of *M. stenostachya*. Therefore, if *M. stenostachya* is truly a hybrid in origin, *M. fallax* is a strong candidate for a sister-clone (R. Goldenberg, personal communication). Another interesting fact is that *M. fallax* pollen grains, unlike *M. stenostachya* pollen grains, were able to germinate when manually deposited at the stigma surface of *M. albicans*, although in an abnormal way (personal observations).

The complexity of the events involved in meiosis suggests that the genes are tightly regulated in this process, many of which have been studied (Consiglio *et al*., 2003). The majority of the genes are present as dominant alleles (Kaul and Murphy, 1985). However, there is only limited knowledge as to how sporocytes differentiate and how meiosis is initiated and regulated in plants. From genetic evidence, it is known that different genes are involved in male (Hulskamp *et al*., 1997; Yang *et al*., 1999) and female meiosis (Byzova *et al*., 1999; Siddiqi *et al*., 2000), and only a few of them are common to both processes (Couteau *et al*., 1999; Grelon *et al*., 2001). We do not yet know

whether megasporogenesis is also abnormal in *M. albicans* and *M. stenostachya.* The absolute absence of fertilization in *M. albicans* may indicate the absence of meiosis during megasporogenesis, as the formation of a diploid embryo sac is a requirement for the production of diploid embryos.

Unlike *Miconia stenostachya*, in which abnormalities are restricted to the cytoplasm, the abnormal pollen wall structures observed in *M. albicans* indicate a more severe type of male sterility. Beyond the inner polysaccharide intine, the pollen wall normally has an outer sculptured thick exine that is composed mainly of sporopollenin (Scott, 1994). In some male sterile species, pollen wall abnormalities may be related to precocious callose wall degradation (Worall *et al*., 1992; Tsuchiya *et al*. 1995), abnormal primexine formation (Ariizumi *et al*., 2005) or defective sporopollenin synthesis or polymerization (Ariizumi *et al*., 2003). In *Actinidia deliciosa* (Actinidiaceae), the sexine and nexine are abnormal or absent (Biasi *et al*., 2001), and in *Arabidopsis thaliana* (Brassicaceae) abnormal exine ornamentation is due to post-meiotic defects (Taylor *et al*., 1998). Because of the rarity of normal tetrads in *M. albicans*, we were not able to identify which kind of abnormality was responsible for abnormal pollen wall formation, but we can exclude the defective sporopollenin synthesis since orbicules were present. The primexine forms around microspores, between the callose wall and the microspore plasma membrane, at the tetrad stage (Owen *et al*., 1995) as a scaffold for sporopollenin deposition. Regardless of the causes of abnormal primexine formation, we can consider it as a strong candidate responsible for abnormal pollen wall formation in *M. albicans*. Factors that are responsible for species-specific exine pattern are still poorly understood (Ariizumi *et al*., 2008).

The inability of the apparently viable pollen grains of *M. albicans* and *M. stenostachya* to germinate using germination test conducted *in vitro* with thousands of pollen grains, along with the lack or low number of pollen grains naturally deposited over the stigmatic surface of these species, strongly suggest that sexual reproduction is not crucial in these species. Thus, we can conclude that both *M. albicans* and *M. stenostachya* are completely male sterile at least in the studied populations. This conclusion is very significant, as some authors have questioned whether obligate apomixis exists (Asker, 1979).

Polyploidization is considered a major evolutionary force in plants, being of fundamental importance to angiosperm diversification (Soltis *et al*., 2004; Soltis *et al*., 2009). As a result, the application of species concepts has become problematic (Soltis *et al*., 2007). Otto and Whitton (2000) suggest that polyploidyzation may be the single most common mechanism of sympatric speciation in plants. Molecular studies of polyploids formation indicate that intragenomic

rearrangement and altered gene regulatory relationships can contribute to evolutionary flexibility (Soltis *et al*., 2009). In addition to the absence of meiosis and fertilization, this may explain why apomictic groups are so diverse, as observed in *Miconia* genus. Despite advances in the understanding of polyploidy, the consequences of genetic, physiologic and ecological changes for polyploid plants in natural populations are essentially unknown (Soltis *et al*., 2004).

The present study illustrates that morphological tools can shed light on apomictic reproduction in wild plants. Our data indicate that although the pollen grain cytoplasm maintains its affinity for cytological staining, the apparatus that allows the successful growth of the pollen tube does not necessarily do so, and so even stained pollen grains may be nonviable. The ecological significance of this mode of reproduction among Melastomataceae is unclear, but we note that the wide distribution of the apomictic species in Brazil indicates the evolutionary success of this type of asexual reproduction.

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References

Alves-Ferreira M, Wellmer F, Banhara A, Kumar V, Riechmann JL, Meyerowitz EM (2007). Global expression profiling applied to the analysis of *Arabidopsis* stamen development. Plant Physiol 145: 747-762.

Ariizumi T, Hatakeyama K, Hinata K, Sato S, Kato T, Tabata S *et al*. (2003). A novel male-sterile mutant of *Arabidopsis thaliana*, *faceless pollen-1*, produces pollen with a smooth surface and an acetolysis-sensitive exine. Plant Mol Biol 53:107-116.

Ariizumi T, Hatakeyama K, Hinata K, Sato S, Kato T, Tabata S *et al.* (2005). The *HKM* gene, which is identical to the *MS1* gene of *Arabidopsis thaliana*, is essential for primexine formation and exine pattern formation Sex Plant Reprod 18: 1-7.

Ariizumi T, Kawanabe T, Hatakeyama K, Sato S, Kato T, Tabata S *et al*. (2008). Ultrastructural characterization of exine development of the *transient defective exine 1* mutant suggests the existence of a factor involved in constructing reticulate exine architecture from sporopollenin aggregates. Plant Cell Physiol 49: 58-67.

Asker S (1979). *Progress in apomixis research*. Hereditas 91: 231-240.

Bertasso-Borges MS, Coleman JR (2005). Cytogenetics and embryology of *Eupatorium laevigatum* (Compositae). Genet Mol Biol 28: 123-128.

Biasi R, Falasca G, Speranza A, De Stradis A, Scoccianti V, Franceschetti *et al*. (2001). Biochemical and ultrastructural features related to male sterility

in the dioecious species *Actinidia deliciosa*. Plant Physiol Biochem 39: 395-406.

Bohdanowicz J, Szczuka E, Swierczynska J, Sobieska J, Koscinska-Pajak, M (2005). Distribution of microtubules during regular and disturbed microsporogenesis and pollen grain development in *Gagea Lutea* (L.) Ker.-Gaw. Acta Biol Crac Ser Bot 47: 89-6.

Budar F, Pelletier G (2001). Male sterility in plants: occurrence, determinism, significance and use. C R Acad Sci III 324: 543-550.

Byzova MV, Franken J, Aarts MGM, Almeida-Engler J, Engler G, Mariano C *et al.* (1999). *Arabidopsis STERILE APETALA*, a multifunctional gene regulating inflorescence, flower, and ovule development. Gene Dev 13: 1002-1014.

Calisto V, Fuzinatto VA, Message HJ, Mendes-Bonato AB, Boldrini KR, Pagliarini MS *et al.* (2008). Desynapsis and precocious cytokinesis in *Brachiaria humidicola* (Poaceae) compromise meiotic division. J Genet 87: 27-31.

Cardoso MB, Kaltchuk-Santos E, Mundstock EC, Bodanese-Zanettini MH (2004). Initial segmentation patterns of microspores and pollen viability in soybean cultured anthers: indication of chromosome doubling. Braz Arch Biol Technol 47: 703-712.

Consiglio F, Conicella C, Monti L, Carputo D (2003). Highlights of meiotic genes in *Arabidopsis thaliana*. Afr J of Biotechnol 2: 516-520.

Couteau F, Belzile F, Horlow C, Grandjean O, Vezon D, Doutriaux MP (1999). Random chromosome segregation without meiotic arrest in both male and female meiocytes of a *dmc1* mutant of *Arabidopsis*. Plant Cell 11: 1623-1634.

Dawe RK (1998). Meiotic chromosome organization and segregation in plants. Annu Rev Plant Physiol Plant Mol Biol 49: 371-395.

Goldberg RB, Beals TP, Sanders PM (1993). Anther development: basic principles and practical applications. Plant Cell 5: 1217-1229.

Goldenberg R, Shepherd GJ (1998). Studies on the reproductive biology of Melastomataceae in "cerrado" vegetation. Plant Syst Evol 211: 13-29.

Grelon M, Vezon D, Gendrot G, Pelletier G (2001) *AtSPO11-1* is necessary for efficient meiotic recombination in plants. EMBO J 20: 589-600.

Hulskamp M, Nikesh SP, Grini P, Schneitz K, Zimmermann I, Lolle SJ, Pruitt RE (1997). The *STUD* gene is required for male-specific cytokinesis after telophase II of meiosis in *Arabidopsis thaliana*. Dev Biol 187: 114-124.

Jiang H, Wang F, Wu Y, Zhou X, Huang X, Zhu J *et al*. (2009). MULTIPOLAR SPINDLE 1 (MPS1), a novel coiled-coil protein of *Arabidopsis thaliana*, is required for meiotic spindle organization. Plant J 59: 1001-1010.

Jong AJ de, Schmidt ED, de Vries SC (1993). Early events in higher-plant embryogenesis. Plant Mol Biol 22: 367-77.

Kaul MLH, Murphy TGK (1985). Mutant genes affecting higher plant meiosis. Theor Appl Genet 70:449-466.

Koltunow AM, Bicknell RA, Chaudhury AM (1995). Apomixis: molecular strategies for the generation of genetically identical seeds without fertilization. Plant Physiol 108: 1345-1352.

Laser KD, Lersten NR (1972). Anatomy and cytology of microsporogenesis in cytoplasmic male sterile angiosperms. Bot Rev 38: 425-454.

Lersten NR (2004). Flowering plant embryology - with emphasis on economic species. Blackwell Publishing: Iowa.

Otto SP, Whitton J (2000). Polyploid incidence and evolution. Ann Rev Genet 34: 401-437.

Owen HA, Makaroff CA (1995). Ultrastructure of microsporogenesis and microgametogenesis in *Arabidopsis thaliana* (L.) Heynh. Ecotype Wassilewskija (Brassicaceae). Protoplasma 185: 7-21.

Pijnacker LP, Ferwerda MA, Mattheij WM (1992). Microsporogenesis in three tetraploid somatic hybrids of potato and their di(ha)ploid fusion partners. Theor Appl Genet 85: 269-273.

Risso-Pascotto C, Pagliarini MS, Valle CB, Jank L (2005). Symmetric pollen mitosis I and suppression of pollen mitosis II prevent pollen development in *Brachiaria jubata* (Gramineae). Braz J Med Biol Res 38: 2005.

Roeder GS (1997). Meiotic chromosomes: it takes two to tango. Genes Dev 11: 2600-2621.

Santos RP, Mariath JEA (1997). A Simple method for fixing, dehydrating and embedding pollen tubes cultivated in vitro for optical and transmission electron microscopy. Biotech Histochem 72: 315-319.

Scott RJ (1994). Pollen exine: the sporopollenin enigma and the physics of pattern. In: Scott RJ, Stead MA (eds) Molecular and cellular aspects of plant reproduction. University Press: Cambridge. Pp 49-81.

Siddiqi I, Ganesh G, Grossniklaus U, Subbiah V (2000). The *DYAD* gene is required for progression through female meiosis in *Arabidopsis*. Development 127: 197-207.

Soltis DE, Soltis PS, Tate JA (2004). Advances in the study of polyploidy since plant speciation. New Phytol 161: 173-191.

Soltis DE, Soltis PS, Schemske DW, Hancock JF, Thompson JN, Husband BC *et al*. 2007. Autopolyploidy in angiosperms: have we grossly underestimated the number of species? Taxon 56: 13-30.

Soltis DE, Albert VA, Leebens-Mack J, Bell CD, Paterson AH, Zheng C *et al*. (2009). Polyploidy and angiosperm diversification. Am J Bot 96: 336-348.

Taylor PE, Glover JA, Lavithis M, Craig S, Singh MB, Knox RB *et al.* (1998). Genetic control of male fertility in *Arabidopsis thaliana*: structural analyses of postmeiotic developmental mutants. Planta 205: 492-505.

Tsuchiya T,Toriyama K, Yoshikawa M, Ejiri S, Hinata K (1995). Tapetum-specific expression of the gene for an endo-b-1,3-glucanase causes male sterility in transgenic tobacco. Plant Cell Physiol 36: 487-494.

Worall D, Hird DL, Hodge R, Paul W, Draper J, Scott R (1992). Premature dissolution of the microsporocyte callose wall causes male sterility in transgenic tobacco. Plant Cell 4: 759-771.

Yang M, Hu Y, Lodhi M, McCombie WR, Ma H (1999). The *Arabidopsis SKP1-LIKE1* gene is essential for male meiosis and may control homologue separation. Proc Natl Acad Sci USA 96: 11416-11421.

Zhu Y, Dun X, Zhou Z, Xia S, Yi B, Wen J *et al.* (2010). A separation defect of tapetum cells and microspore mother cells results in male sterility in *Brassica napus*: the role of abscisic acid in early anther development Plant Mol Biol 72: 111-123.

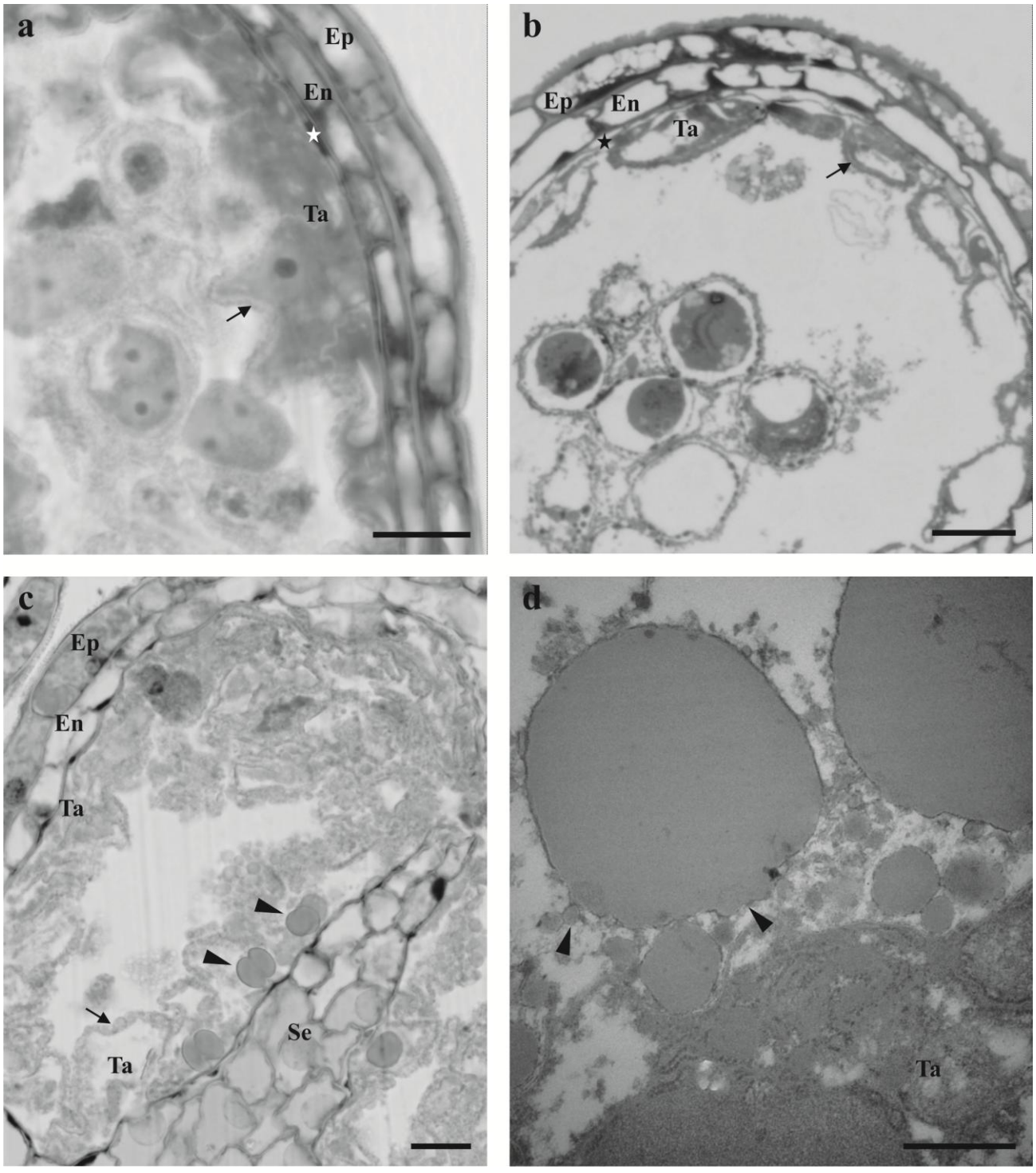

Figure 1 Abnormalities in *Miconia albicans*. (**a**, **b**) Transversal section of the anther with abnormal microspores inside the loculus. The orbicules are aggregated over the tapetum (Ta) cell walls (arrow). (**c**) Bigger orbicules (arrowhead) and anomalous tapetum (Ta) wall (arrow). (**d**) Smaller orbicules aggregates to form the bigger ones (arrowhead) inside the tapetum (Ta) cytoplasm. [En: endothecium. Ep: epidermis. Se: septum. ★: middle layer. Scale bars: 10µm in **a**-**c** and 1µm in **d**].

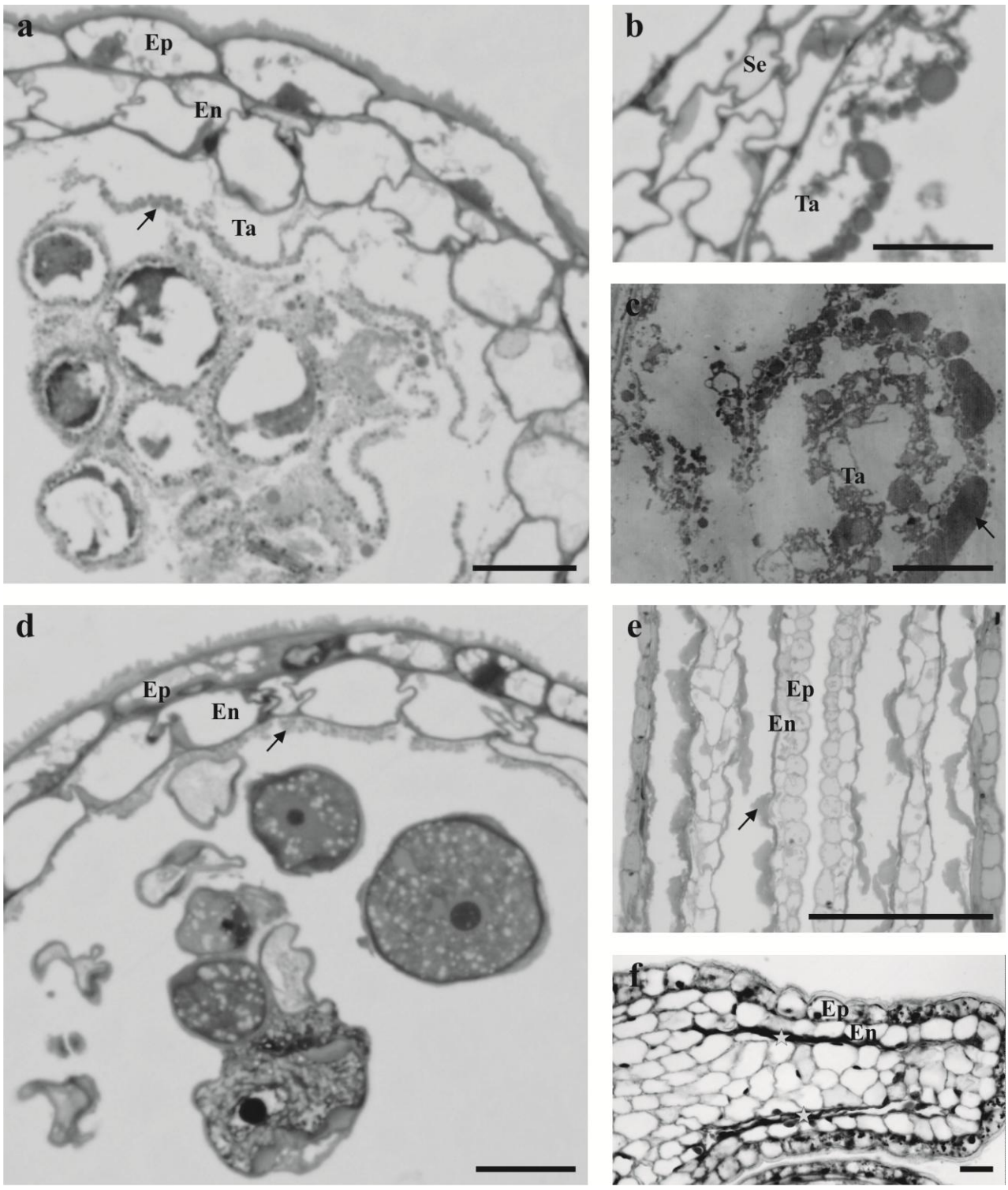

Figure 2 Abnormalities in *Miconia albicans*. (**a**-**c**) Transversal section of an anther showing abnormal microspores and anomalous tapetum (Ta) wall (arrow). (**d**) The orbicules are aggregated over the endothecium (En) walls after total tapetum degradation (arrow). (**e**) Longitudinal section of an empty mature anther showing anther wall composed by the epidermis (Ep) and endothecium (En) besides the presence of amorphous substance inside the pollen sac (arrow). (**f**) Transversal section of a mature anther with obliterate pollen sacs (stars). [Se: septum. Scale bars: 10µm in **a**, **b**, **d**-**f** and 1.2µm in **c**].

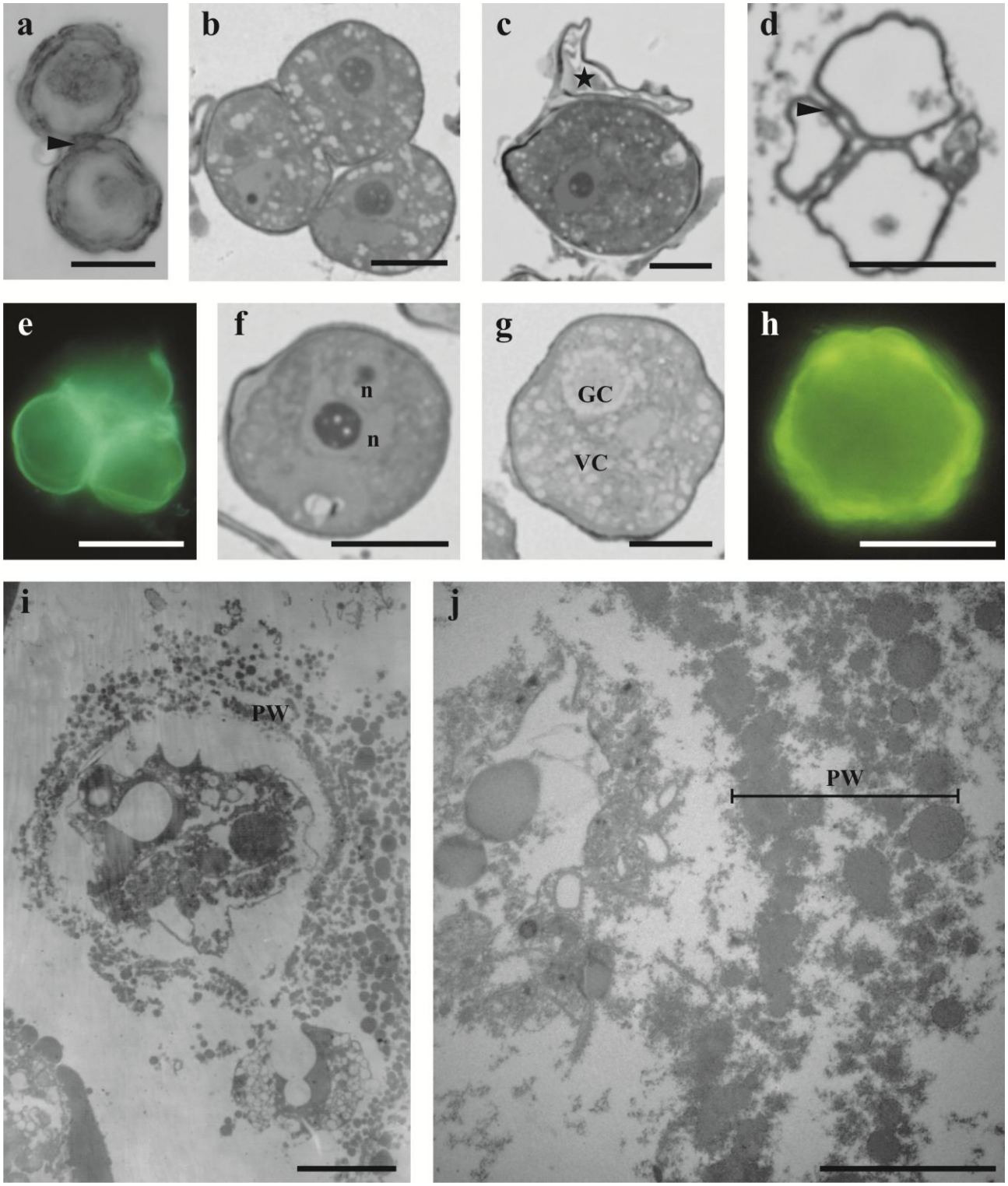

Figure 3 Abnormal cell structures in mature anthers of *Miconia albicans*. (**a**-**e)** Cell structures originated by exine fusion (arrowhead). Some of the structures degenerates (★). (**f**) Cell structure with two nucleus (n). (**g**) Abnormal bicellular pollen grain with the vegetative cell (VC) and the generative cell (GC). (**h**-**j**) Aborted pollen grain with abnormal pollen wall (PW). [Scale bars: 10µm in **a**-**h**, 1.2µm in **i** and 3µm in **j**].

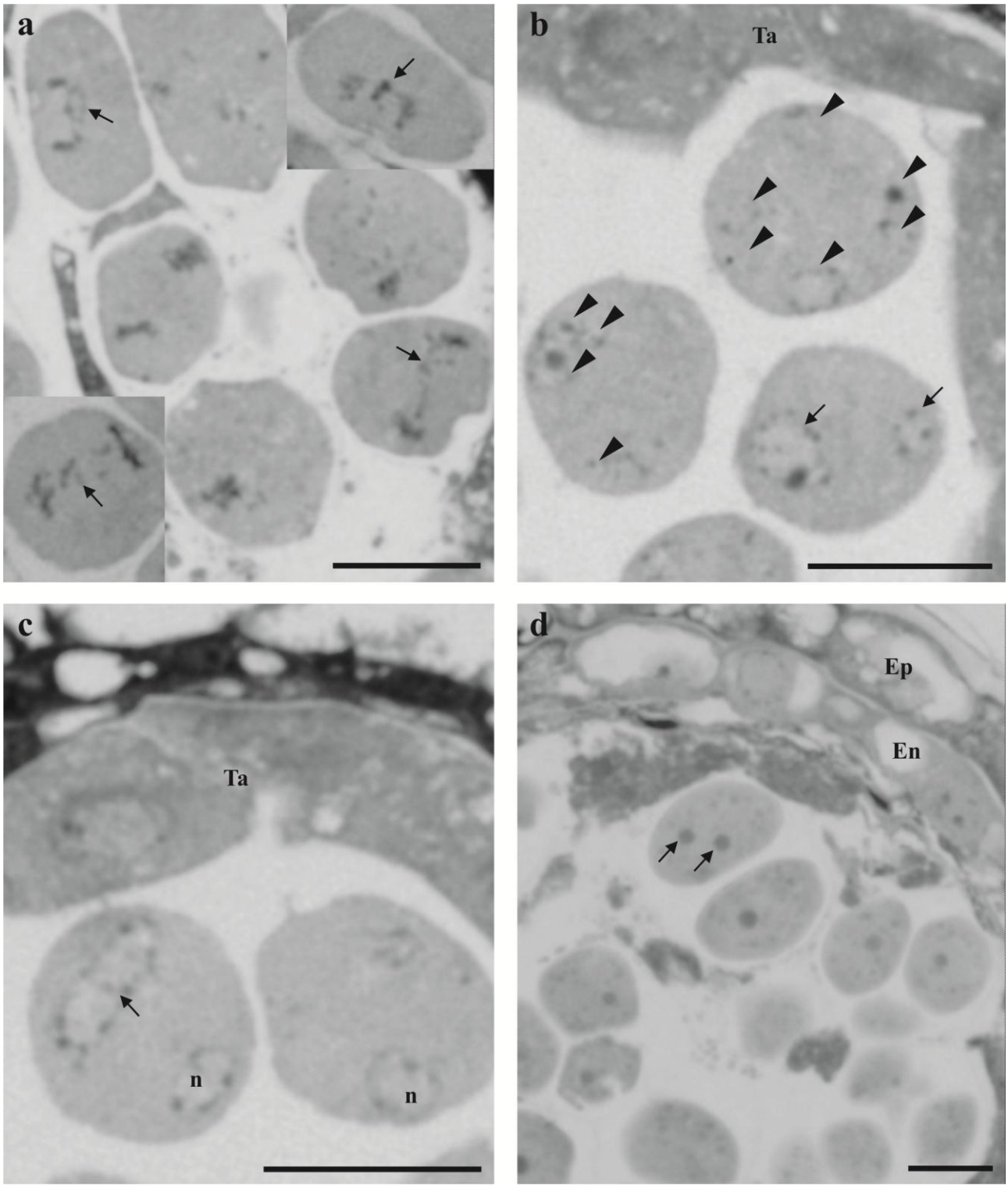

Figure 4 Meiotic irregularities in *Miconia stenostachya*. (**a**) Lagging chromosomes (arrow) at anaphase. (**b**) Abnormal syncytia, with supernumerary (more than usual four) nuclei (arrowhead), some of them with different sizes (arrow). (**c**) Nuclear fusion (arrow) in syncytia. (**d**) Dyad originated after irregular cytocinesis. Note the presence of two nuclei (arrow) in the microspore. [En: endothecium. Ep: epidermis. Ta: tapetum. Scale bars: 10µm].

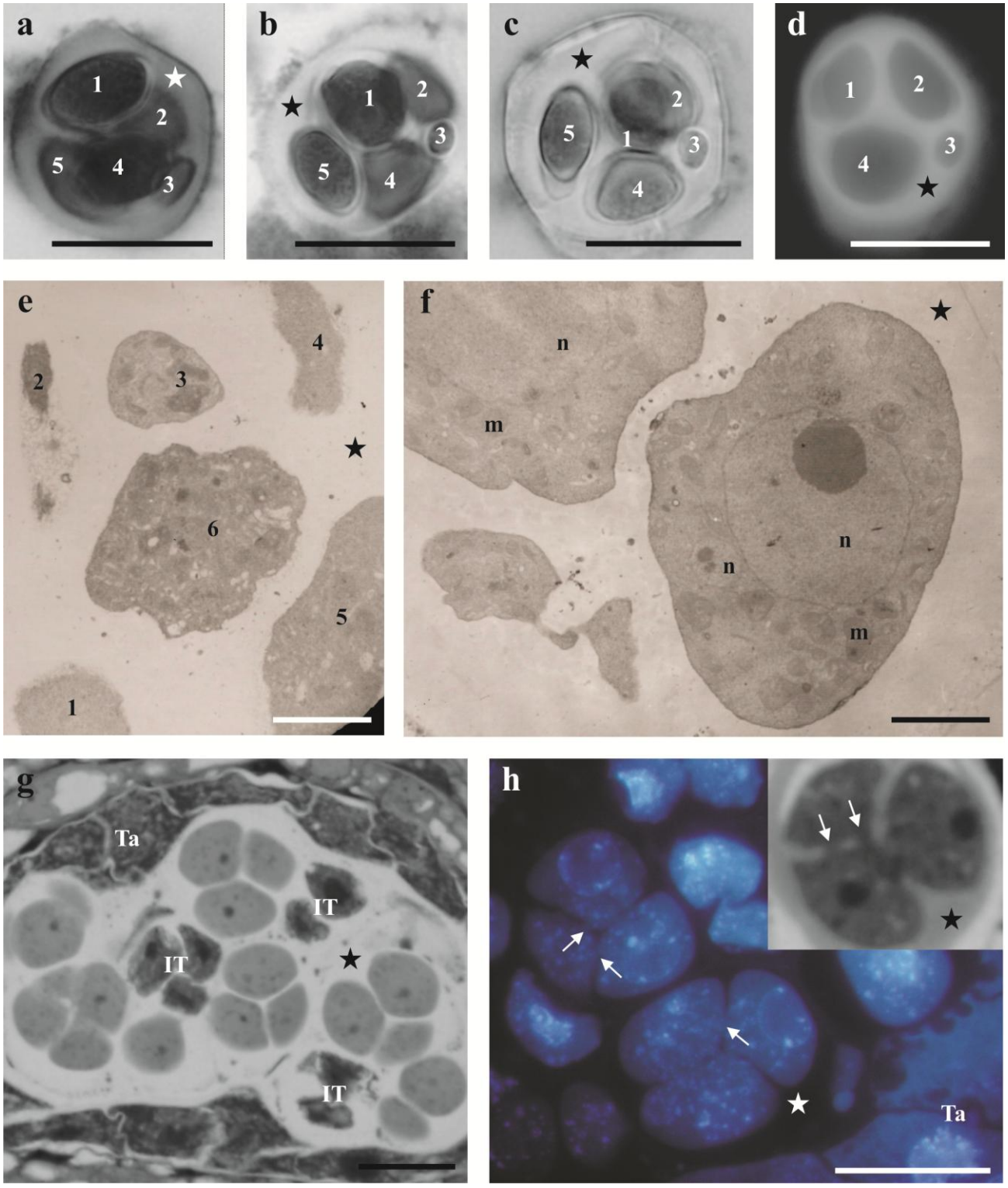

Figure 5 Microspore tetrads in *Miconia stenostachya*. (**a**-**e**) Abnormal tetrads with micronuclei (3). (**f**) Mitochondria (m) and nuclei (n) in the microspore of a tetrad. (**g**, **h**) Transversal sections of the anther with both, intact and degenerating (IT) tetrads. Some tetrads show cytoplasmic connections among adjacent microspores (arrow). [Ta tapetum. ★: callose wall. Scale bars: 10µm in **a**-**d**, **g**, **h** and 0.9µm in **e**, **f**].

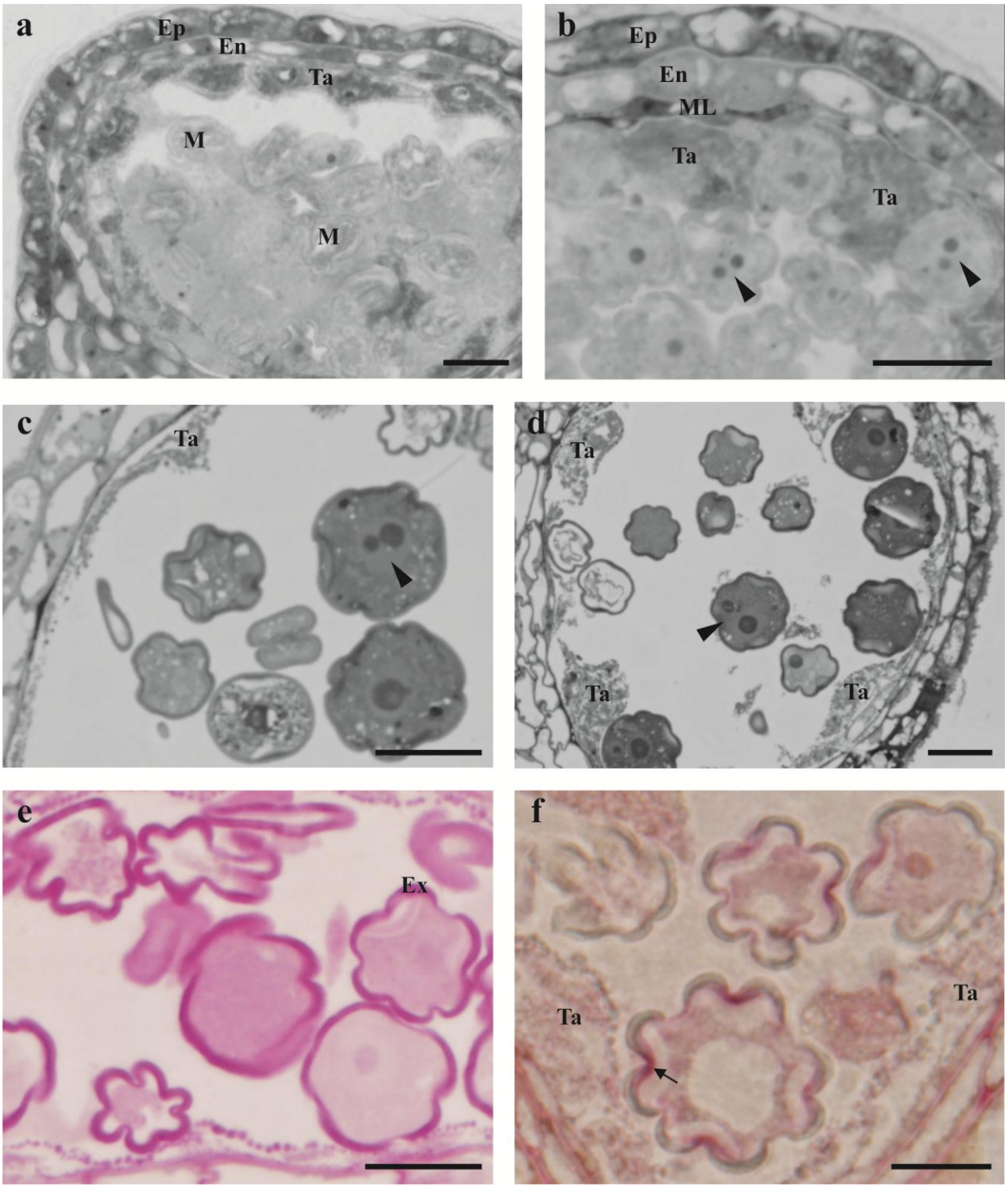

Figure 6 Transversal section of *Miconia stenostachya* anther showing free microspores. (**a**) Abnormal microspores (M) surrounded by dense locular fluid. (**b-d**) Microspore with more than one nucleoli (arrowhead). Note the presence of microspores of different size (**e**) Unusual positive reaction for PAS in the exine (Ex). (**f**) Unusual number of apertures in one big microspore. Note the positive reaction in the intine (arrow). [En: endothecium. Ep: epidermis. ML: middle layer. Scale bars: 10µm].

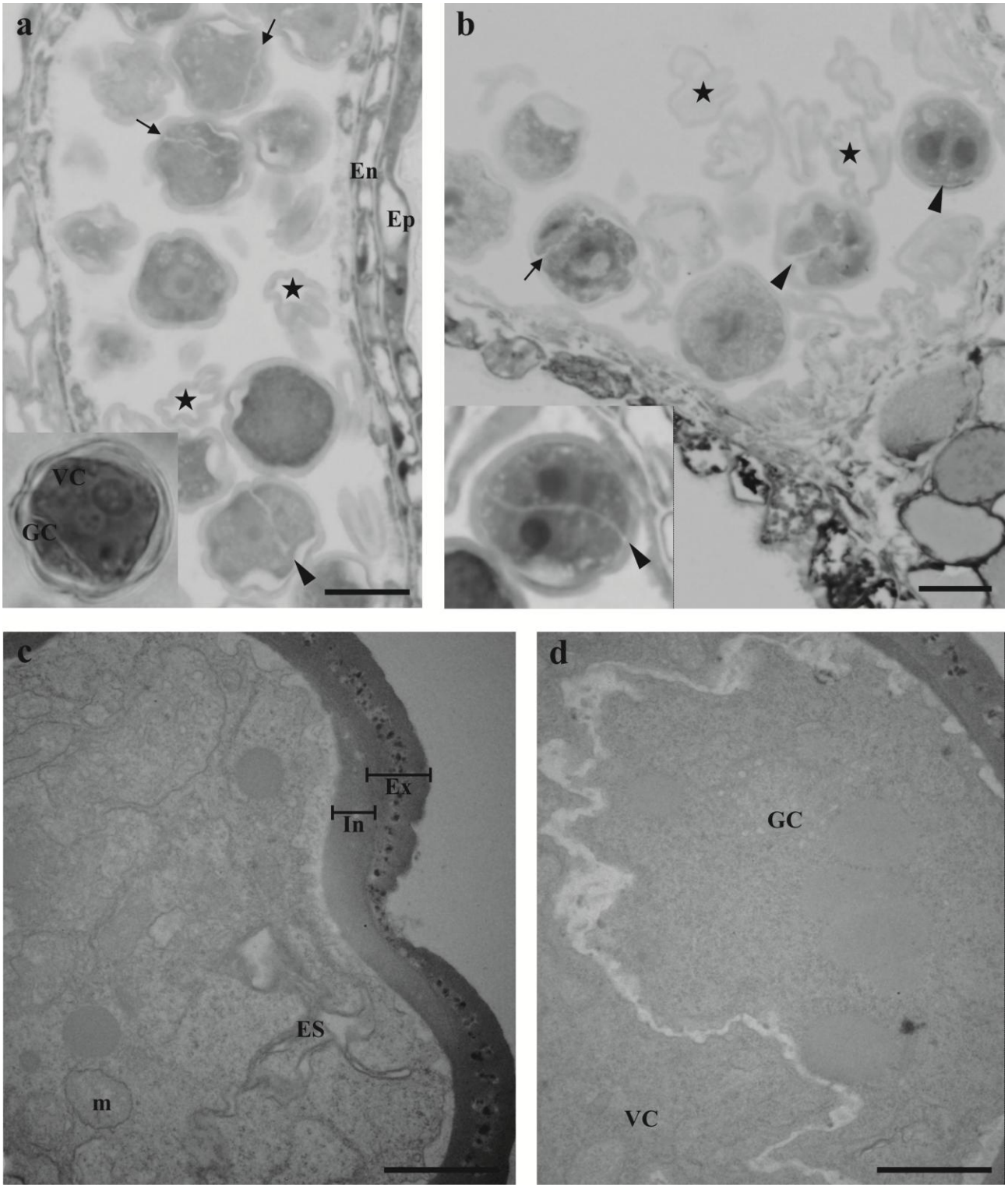

Figure 7 Microgametogenesis in *Miconia stenostachya*. (**a**, **b**) Transversal section of anther showing abnormal asymmetric (arrow) and symmetric (arrowhead) mitosis, besides empty structures (★). (**c**, **d**) Abnormal pollen grains with normally structured exine (Ex) and intine (In). [En: endothecium. Ep: epidermis. ES: endomembrane system. GC: generative cell. VC: vegetative cell. m: mitochondria. Scale bars: 10µm in **a**, **b** and 1µm in **c**, **d**].

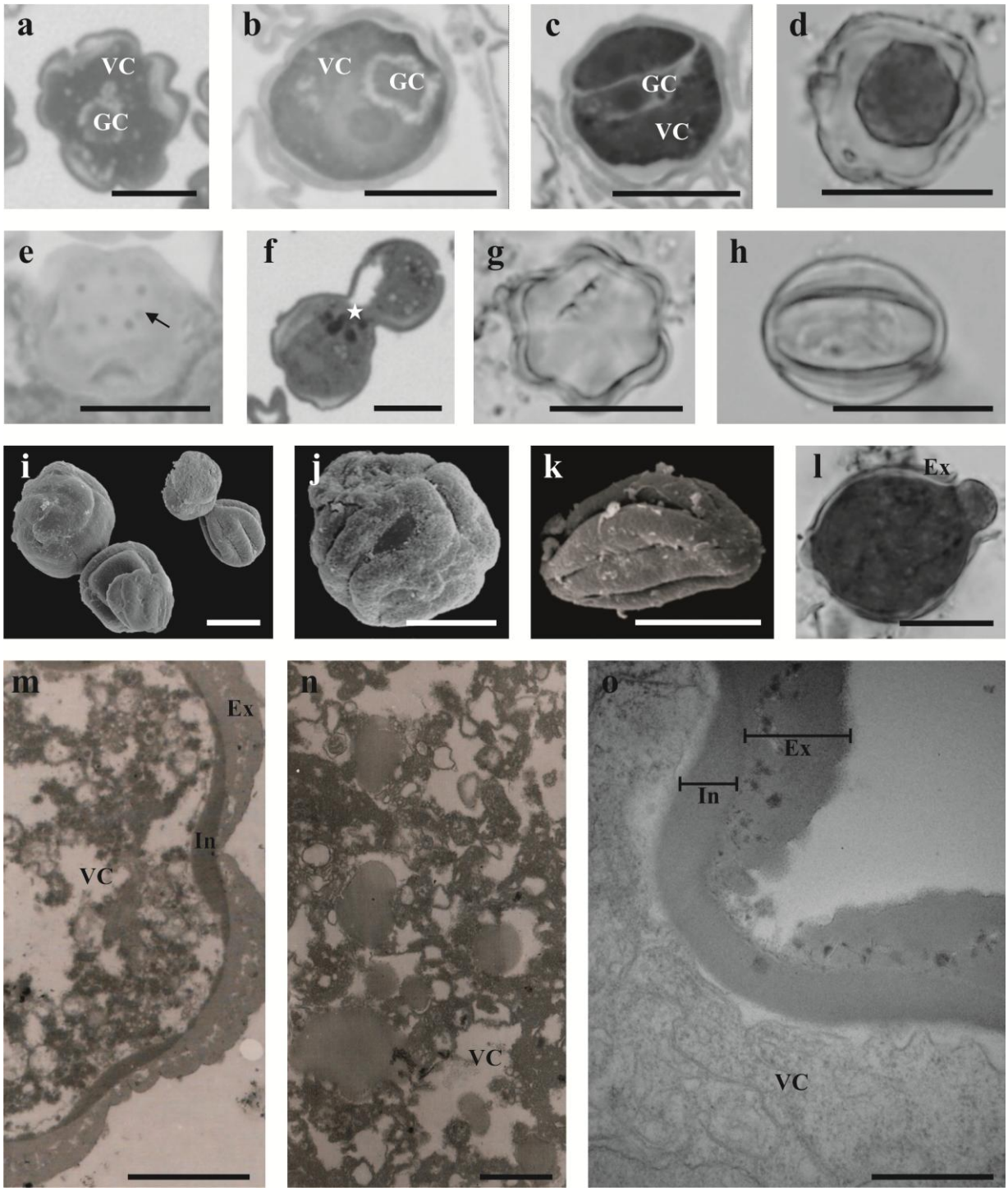

Figure 8 Abnormal cell structures found in mature anthers of *Miconia stenostachya*. (**a**-**c**) Abnormal pollen grains with vegetative cell (VC) and generative cell (GC). (**d**) Cellular structure with degenerating cytoplasm. (**e**) Cell structure with supernumerary nuclei (arrow). (**f**) Cytoplasmic connection (★) between two adjacent bicellular pollen grains. (**g**, **h**) Empty cell structure with normal exine and apertures. (**l**) Pollen grain with stained cytoplasm and normal exine (Ex) but unviable due to its inability to form the pollen tube. (**m-o**) Abnormal pollen grains with degenerating cytoplasm but pollen wall normally structured in exine (Ex) and intine (In). [GC: generative cell. VC: vegetative cell. m: mitochondria. Scale bars: 10µm in **a**-**l**, 1.7µm in **m**, 0.5µm in **n** and 1µm in **o**].

Capítulo 3.

Elucidating the mechanism of poricide anther dehiscence in *Miconia* species (Melastomataceae)

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Abstract

Melastomataceae species have porate anthers; however, unlike Solanaceae and many monocots, in which the pore dehiscence depends on the presence of a mechanical layer, often the endothecium, most members of the Melastomataceae have no evident specialized layer related to the pore opening. Moreover, the anther structure is remarkable since in Melastomataceae stamen features are important to both phylogenetic and ecological studies due to their morphological variation. The goal of this study was to characterize the tissues that form the apical pore of the anther in several *Miconia* species, which may help us to understand the almost unknown mechanism of anther dehiscence in these megadiverse genera. The anthers were fixed in a modified Karnovsky solution and studied under light and scanning electron microscopes according to routine protocols. Coloration tests were applied to observe the occurrence of cuticle covering epidermal cells. Before the anthesis of the flowers, the apical pores of all of the species were closed by a uniseriate epidermis, the cells of which lack a cuticle. In contrast, the epidermis of the rest of the anther was covered by a thick, ornamented cuticle. Among Myrtales families, the Melastomataceae seems to form a clade with Alzateaceae, Rhynchocalycaceae, Penaeaceae and Oliniaceae, almost all of which have anthers with endothecium lacking wall thickening. In these families, the endothecium may or may not be present in the mature anther, with degenerating cells in the latter case. The anther aperture is not dependent on the presence of the endothecium as the mechanical layer, and the process of anther dehiscence is still unknown. However, in the *Miconia* species studied here, the presence of a cuticle may be a way of preventing tissue dehydration, and the pore opening seems to be due to the passive process of dehydration taking place only in the pore region due to the absence of the cuticle.

Keywords: anther dehiscence, pore, not thickened endothecium, *Miconia*

Introduction

Anther dehiscence is a complex process which involves a regulated differentiation of several tissues of the anther, leading to its aperture and pollen grain presentation (Bonner & Dickinson 1989). The anthers may open by longitudinal slits, through valves or pores, and the type of dehiscence is considered a conservative character among angiosperms. The most common type of anther dehiscence in angiosperms is longitudinal, which involves a sequence of the follow events: degeneration of the middle layer and tapetum, endothecium cell wall lignification, septum cell degradation and breakage of stomium cells (Goldberg et al. 1993, Sanders et al. 1999). The valvar

dehiscence is occasionally observed in members of Berberidaceae (Batygina 2002) and Lauraceae (Judd et al. 1998), while several families may be characterized by the poricidal anthers, including Ericaceae (Lersten 2004), Melastomataceae (Clausing and Renner 2001) and Solanaceae (Lersten 2004).

The poricidal dehiscence has been better studied in Ericaceae and Solanaceae and is considered a character-state with important adaptive value to pollinators capable of collecting pollen by high frequency vibration of stamens (Buchmann and Buchmann 1981, Larson and Barrett 1999). In *Lycopersicon esculentum* (Solanaceae - Bonner and Dickinson 1989), the dehiscence mechanism involves several kinds of specializations, like the presence of oxalate crystals in the cells of the anther septum, lignified thickening in the endothecium cell wall located next to the pore region, and stomium formation (Bonner & Dickinson 1989, Fahn 1990). Additional studies have shown that the stomium is essential for anther dehiscence and its ablation leads anthers to fail to dehisce (Beals and Goldberg 1997), resulting in sterile plants (Sanders at al. 2005, Yang *et al.* 2007).

Melastomataceae, although considered an important family in number of species (ca. 4570) (Renner 1993, APG 1998), wide geographical distribution (Clausing and Renner 2001) and a great variation of morphological features, is scarcely studied in relation to the anther dehiscence. The few studies on their representatives have especially focused on pollen grain (Patel et al. 1984) and stamen morphology (Goldenberg et al. 2008), mainly with taxonomic bias. In a study about anther dehiscence in some *Miconia* species, Goldenberg et al. (2003) found that the dehiscence mechanisms depends on the association between the non-striate epidermis which covers the dehiscence area and the druse crystals of the septum located between the two thecae. The mechanism of dehiscence in species lacking any type of crystals in the septum or near the dehiscence area is still unknown.

The monophyly of Melastomataceae is supported morphologically by anthers that open by pores, and by acrodromal foliar venation (Clausing and Renner 2001). Interestingly, the occurrence of an endothecium appears to be an important phylogenetic marker among Melastomataceae and sister clades, as well as within Melastomataceae. However, this contradicts preliminary findings in *Miconia albicans*, *M. stenostachya* and *M. paucidens* (Cortez et al.), *M. pepericarpa* and *M. fallax* (Caetano et al.) and *M. cabucu* (Medeiros and Morretes 1996), in which endothecium cells lack wall thickenings. Nonetheless, even in these species, the role of the endothecium in the dehiscence mechanism is unknown.

Miconia is the largest genus in Melastomataceae (Goldenberg 2000), and the phylogenetic relation among the species is still obscure (Goldenberg et al. 2008). Moreover, many species are

apomictics, which means *a priori* that pollination is not necessary to produce viable embryos; nevertheless, even when viable pollen grains are not produced, the anther still dehisces, indicating that the aperture mechanism is independent of pollinator action. In the species in which viable pollen grains are successfully produced, pollen release is dependent on the pollinator action, in the so-called “buzz pollination”, as observed in the majority of Melastomataceae species.

Therefore, the aim of this study was to analyze the morphology of anthers before, during and after dehiscence of 11 not closely related species of *Miconia* (according to the phylogenetic tree proposed by Goldenberg et al. 2008) in order to elucidate the role of endothecium and epidermis in the anther dehiscence mechanism.

Materials and Methods

Field investigations were performed using plants of 11 species of *Miconia* (Table 1, Figures 1a-h) growing in natural populations from the municipalities of Itirapina-SP (22º15'10" S and 47º49'22" W), Campinas-SP (22°54'20" S and 47°03'39" W), Jundiaí-SP, Ubatuba-SP, Uberlândia-MG, southeast Brazil, from 2007 to 2011.

Table 1. *Miconia* species selected for this study. [1]Section Cremanium. [2]Section (Eu)Miconia. [3]Section Glossocentrum. [4]Section Hypoxanthus.

Phylogenetic position according to Goldenberg *et al.* (2008)	Species	Area of study
Miconia III	*Miconia theaezans*[1]	Uberlândia-MG
Miconia IV + Ossaea	*Miconia albicans*[2]	Campinas-SP, Itirapina-SP, Ubatuba-SP
	Miconia minutiflora[3]	Itirapina-SP
	Miconia pepericarpa[3]	Itirapina-SP
	Miconia stenostachya[2]	Itirapina-SP
	Miconia fallax[2]	Itirapina-SP
Miconia V	*Miconia chamissois*[2]	Uberlândia-MG
	Miconia sellowiana[4]	Jundiaí-SP
Not estimated phylogenetic position	*Miconia paucidens*	Itirapina-SP
	Miconia leucocarpa	Itirapina-SP
	Miconia nervosa	Itirapina-SP

Mature stamens, before and after anther dehiscence, were removed from young floral buds and flowers, respectively, of at least five individuals from each species. They were immediately fixed in a solution composed of 80 mL L^{-1} glutaraldeyde, 250 mL L^{-1} paraformaldeyde (16%) and 500 mL L^{-1} phosphate buffer (0.1 M, pH 6.8) for 24 h (modified from Karnovsky, 1965). Standard protocols were followed, and the samples were analyzed under light microscope (BX 51A, Olympus) and under scanning (JSM 5200, Jeol) electron microscope. To better visualize the structure of the dehiscence region, some sections obtained from fixed stamens were stained with Sudan black B (C.I. 26150) and Sudan IV (C.I. 26105) for cuticle detection and with lugol solution for starch grains. Selected sections were observed under polarized light for crystals and starch grains detection. Digital images were taken using an Olympus BX 51 light microscope.

Results

Each pre-dehiscent anther is formed by two thecae bound together by the parenquimatic connective tissue, in the central portion of which is located a central vascular bundle; at this point, each theca consists of two adjacent pollen sacs, which are separated by the septum, so the anther is tetrasporangiate (Figure 2a). When the anther epidermal cells reach maximal enlargement, the cells have a very thick outer periclinal wall, which are covered by a thick and rugose cuticle except near the apical region; in this region, located at the distal portion of the anther, the epidermal cells are smaller and lack cuticle corresponding to the apical pore (Figures 2b-f, g-k). Just before the

beginning of dehiscence, the anther wall is restricted to two layers, the outer epidermis and the inner endothecium; the endothecium lacks any cell wall thickening even near the apical pore region (Figures 2e,f).

The anther starts to dehisce when the cells located at the margin of the pore have lost their rigidity and there is a gradual separation between the two groups of epidermal cells (Figures 3a-i). Following this initial opening, the epidermal cells lacking cuticle in the pore degenerate completely leading to the anther dehiscence (Figures 4a-k). In *M. theaezans*, the degeneration of the epidermal cells of the pore is not complete and the septum persistence gives the appearance of a four-porate anther (Figures 4g,h).

Concomitantly with the anther dehiscence, the septum between the two adjacent pollen sacs start degradation, initially near a portion containing several small, isodiametric cells (Figure 5a,b); as a result, after the total septum degradation, the anther becomes bilocullar (Figure 5c,d).

Discussion

The mechanism of anther dehiscence in the studied species of *Miconia* involves the gradual separation of the border region between the groups of epidermal cells with and without cuticle and subsequent degeneration of cells in the second group. Ordinarily, such a mechanism operates during or after the flower anthesis, although some of the anthers from the floral buds already have the pore open. The presence of an endothecium lacking wall thickenings, i.e., an atypical endothecium, leads to the conclusion that its functional significance in the species studied here differs from that commonly attributed to species with longitudinal dehiscence and even to those species with poricidal dehiscence and wall thickened endothecium, as in Solanaceae (Bonner and Dickinson 1989, Fahn 1990). Specializations observed in *Miconia* species studied until now (Goldenberg *et al.* 2003 and present study) suggest that a mechanism of anther dehiscence, different of that already reported for other species with poricidal dehiscence, is operating in Melastomataceae.

It is worth noting that no stomium or another specialization was observed in the anther pore of *Miconia* species, which could be considered an essential structure to the anther dehiscence (Beals and Goldberg 1997). Such a fact demonstrates the fundamental role of the epidermis in the anther dehiscence of *Miconia* species (present study, Goldenberg et al. 2003). In these species the anther dehiscence occurs due to a differential drying between the two portions of the anther; the more fragile epidermal cells of the pore region disrupt and the pore opens. However, physiological studies are needed to decide whether anther dehiscence also depends on more complex mechanisms of water reabsorption in the connective tissues and filament, since we observed that some anthers of all

species studied here presented the pore open before flower anthesis, i.e., at a time when the anthers were not yet exposed to the environment (personal observation).

As previously described in *Miconia pusilliflora, M. laticrenata* and *M. cinnamomifolia* (Goldenberg et al. 2003), the presence of an epidermal layer in the anther apical region (= pore) in *Miconia* studied here also contradicts Clausing and Renner's statement (2001) that "Melastomataceae pores develop in a patch at the tip of the anthers, where the epidermis is reduced and exposed mesophyll dries out and shrivels up." The lack of a cuticle and presence an epidermis in the pore region is responsible for anther dehiscence in *Miconia*, as the cuticle provides mechanical strength and rigidity to the cell (Bonner and Dickinson 1989). Thus, we can compare the pore region of *Miconia* species to the stomium found in *Lycopersicon esculentum* anthers (Bonner and Dickinson 1989), which breaks because it is composed of more fragile epidermal cells compared to other cutinized epidermal cells. Therefore, the mechanism that leads to anther dehiscence cannot be regarded as solely dependent on tissue desiccation but may be considered a consequence of several tensile forces which result from specific cell contents, cell wall thickness and/or cell lyses (Bonner and Dickinson 1989, Matsui et al. 2000).

Acknowledgements

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References

Angiosperm Phylogeny Group. 1998. An ordinal classification for the families of flowering plants. Annals of the Missouri Botanical Garden 85: 531-553.

Batygina TB. 2002. Embryology of Flowering Plants: Terminology and Concepts: Generative Organs of Flowers. Science Pub Inc.

Beals TP, Goldberg RB. 1997. A novel cell ablation strategy blocks tobacco anther dehiscence. The Plant Cell 9: 1527-1545.

Bonner LJ, Dickinson HG. 1989. Anther dehiscence in *Lycopersicon esculentum* Mill. I. structural aspects. New Phytologist 113: 97-115.

Buchmann SL, Buchmann MD. 1981. Anthecology of *Mouriri myrtilloides* (Melastomataceae: Memecyleae), an oil flower in Panama. Biotropica 13: 7-24.

Clausing G, Renner SS. 2001. Molecular phylogenetics of Melastomataceae and Memecylaceae: implications for character evolution. American Journal of Botany 88: 486-498.

Fahn A. 1990. Plant anatomy. 4th ed. Pergamon Press, Oxford.

Goldberg RB, Beals TP, Sanders PM. 1993. Anther development: basic principles and practical applications. The Plant Cell 5: 1217-1229.

Goldenberg R. 2000. O gênero *Miconia* Ruiz & Pav. (Melastomataceae): I. Listagens Analíticas, II. Revisão Taxonômica da Seção *Hypoxanthus* (Rich. Ex DC.) Hook. f. Tese de Doutorado. Universidade Estadual de Campinas, Campinas-SP.

Goldenberg R, Teixeira SP, Martins AB. 2003. Anther dehiscence and circumscription of *Miconia* sect. *Hypoxanthus* (Melastomataceae). Kew Bulletin 58:1195-203.

Goldenberg R, Penneys DS, Almeda F, Judd WS, Michelangeli FA 2008. Phylogeny of *Miconia* (Melastomataceae): patterns of stamen diversification in a megadiverse neotropical genus. International Journal of Plant Science 169: 963-979.

Karnovsky MJ. 1965. A formaldehyde-glutaraldeyde fixative of high osmolality for use in electron microscopy. Journal of Cell Biology 27: 137A-138A.

Larson BMH, Barrett SCH. 1999. The pollination ecology of buzz-pollinated *Rhexia virginica* (Melastomataceae). American Journal of Botany 86: 502-511.

Matsui T, Omasa K, Horie T. 2000**.** Mechanism of anther dehiscence in rice (*Oryza sativa* L.). Annals of Botany 84: 501-506.

Medeiros JD, Morretes BL. 1996. The embryology of *Miconia cabucu* (Melastomataceae). Cytologia 61: 83-91.

Patel VC, Skvarla JJ, Raven PH. 1984. Pollen character in relation to the delimitation of Myrtales. Annals of the Missouri Botanical Garden 71: 858-969.

Renner SS. 1993. Phylogeny and classification of the Melastomataceae. Nordic Journal of Botany 13: 519-540.

Sanders PM, Bui AQ, Weterings K, McIntire KN, Hsu Y, Lee PY, Truong MT, Beals TP, Goldberg RB. 1999. Anther developmental defects in *Arabidopsis thaliana* male-sterile mutants. Sexual Plant Reproduction 11: 297-322.

Yang C, Xu Z, Song J, Conner K, Barrena GV, Wilson ZA. 2007. *Arabidopsis* MYB26/MALE STERILE35 regulates secondary thickening in the endothecium and is essential for anther dehiscence. The Plant Cell 19: 534-548.

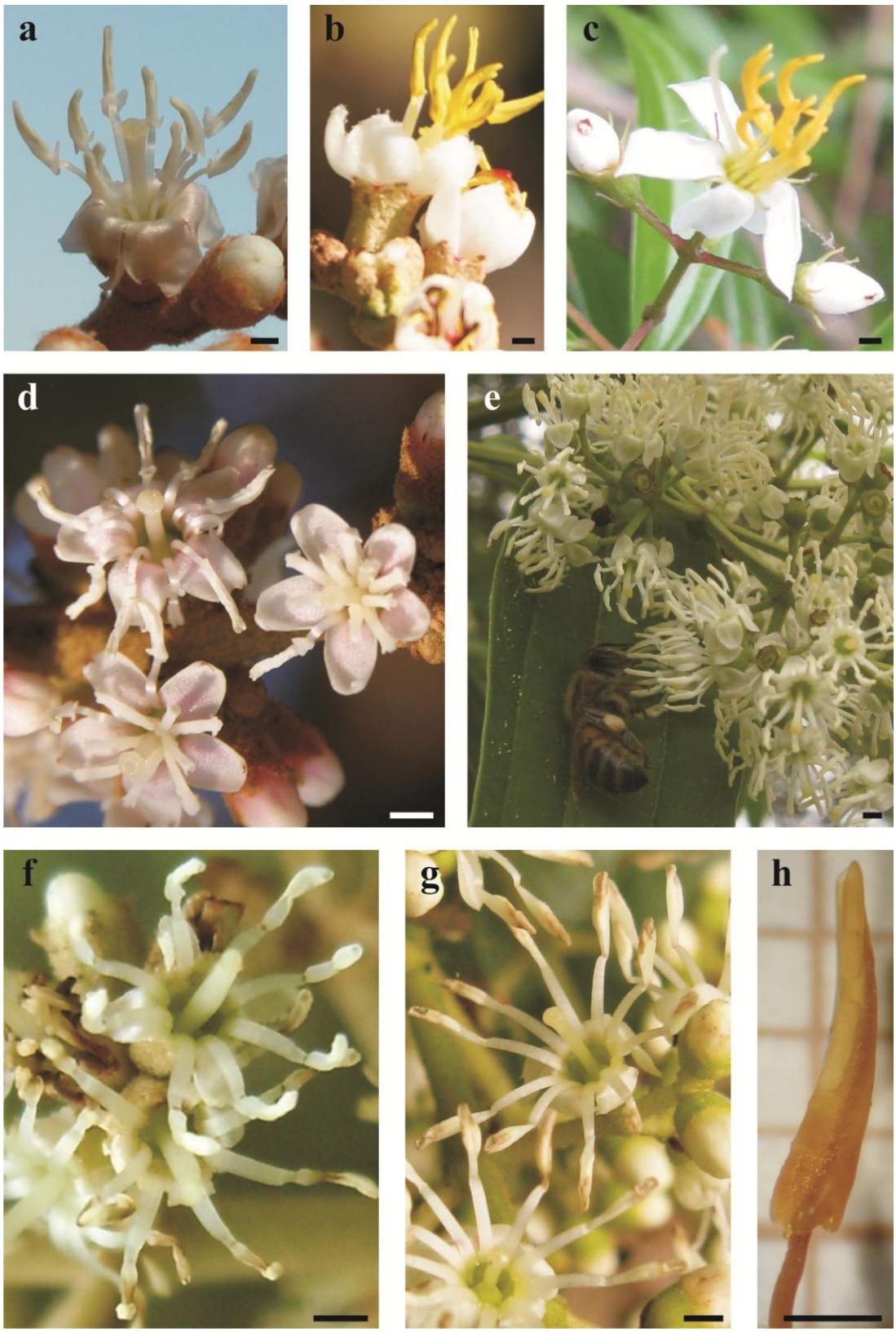

Figure 1 Flowers of *Miconia* species selected for this study. (**a**) *Miconia albicans*. (**b**) *Miconia fallax*. (**c**) *Miconia paucidens*. (**d**) *Miconia leucocarpa*. (**e**) *Miconia minutiflora*. (**f**) *Miconia pepericarpa*. (**g**) *Miconia sellowiana*. (**h**) *Miconia stenostachya*. [Scale bars = 1 mm].

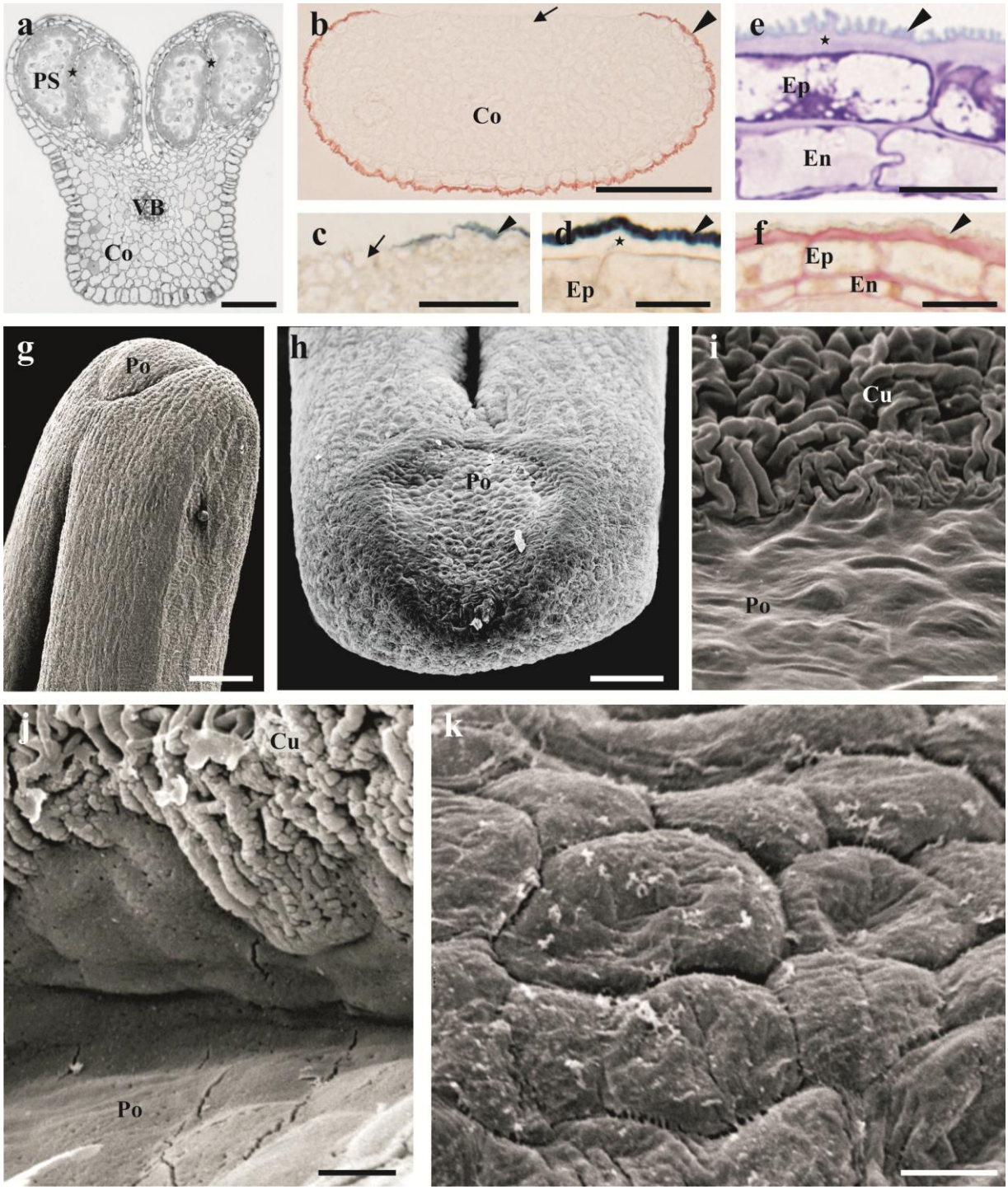

Figure 2 Pre-dehiscent anthers of several *Miconia* species. (**a-f**) Transversal section of a mature anther. Note the cuticle (arrowhead) covering epidermal cells, and its absence in the pore region (arrow). (**g, h**) Apical portion of the anthers showing the well delimited pore region (Po). (**i, j**) Neighboring region between pore region (lacking cuticle) and the rest of anther (cuticle covering epidermal cells). Note the ornamentation of the cuticle (Cu). (**k**) Pore region in detail. Note the epidermal cells without cuticle. [**a**, **h**: *Miconia albicans*. **b**: *M. stenostachya*. **c**-**g**: *M. paucidens*. **i**: *M. chamissois*. **j**: *M. fallax*. **k**: *M. theaezans*. Co: connective. Ep: epidermis. En: endothecium. PS: pollen sac. Se: septum. VB: vascular bundle. ★: cell wall. Scale bars: 50 µm in **a**, **b**, **i**, 20 µm in **c**, 10 µm in **d**-**f**, 100 µm in **g**, **h,** 30 µm in **j** and 5 µm in **k**].

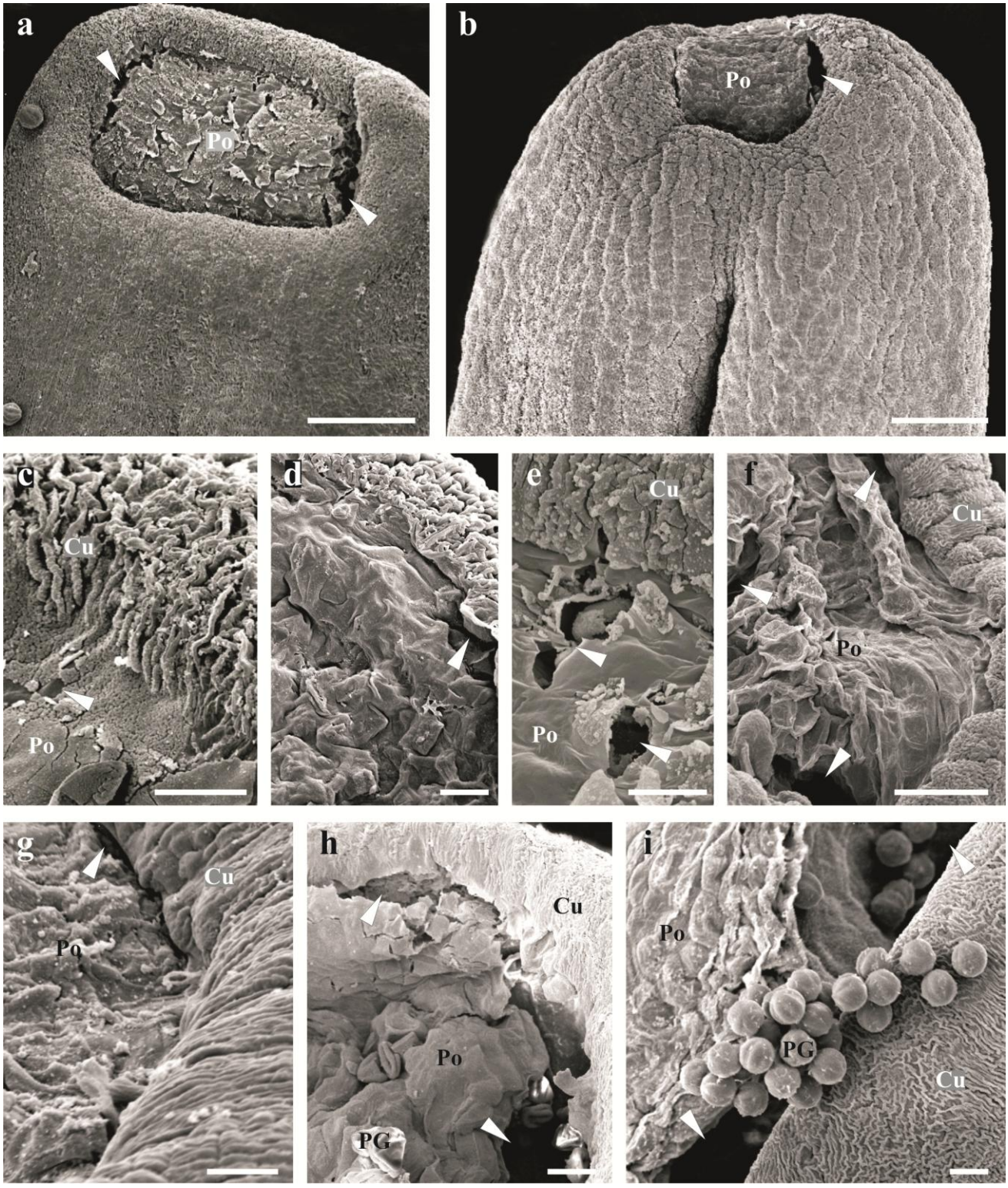

Figure 3 Initial stage of pore aperture (arrowhead) in *Miconia* species. (**a, c, d**) *Miconia fallax*. (**b, e**) *M. chamissois*. (**f**) *M. minutiflora*. (**g**) *M. pseudonervosa*. (**h**) *M. stenostachya*. (**i**) *M. theaezans* [Cu: cuticle. PG: pollen grain. Po: pore region. Scale bars: 100 µm in **a**, **b**, 10µm in **c**-**e**, **g**, **h** and 30 µm in **f**].

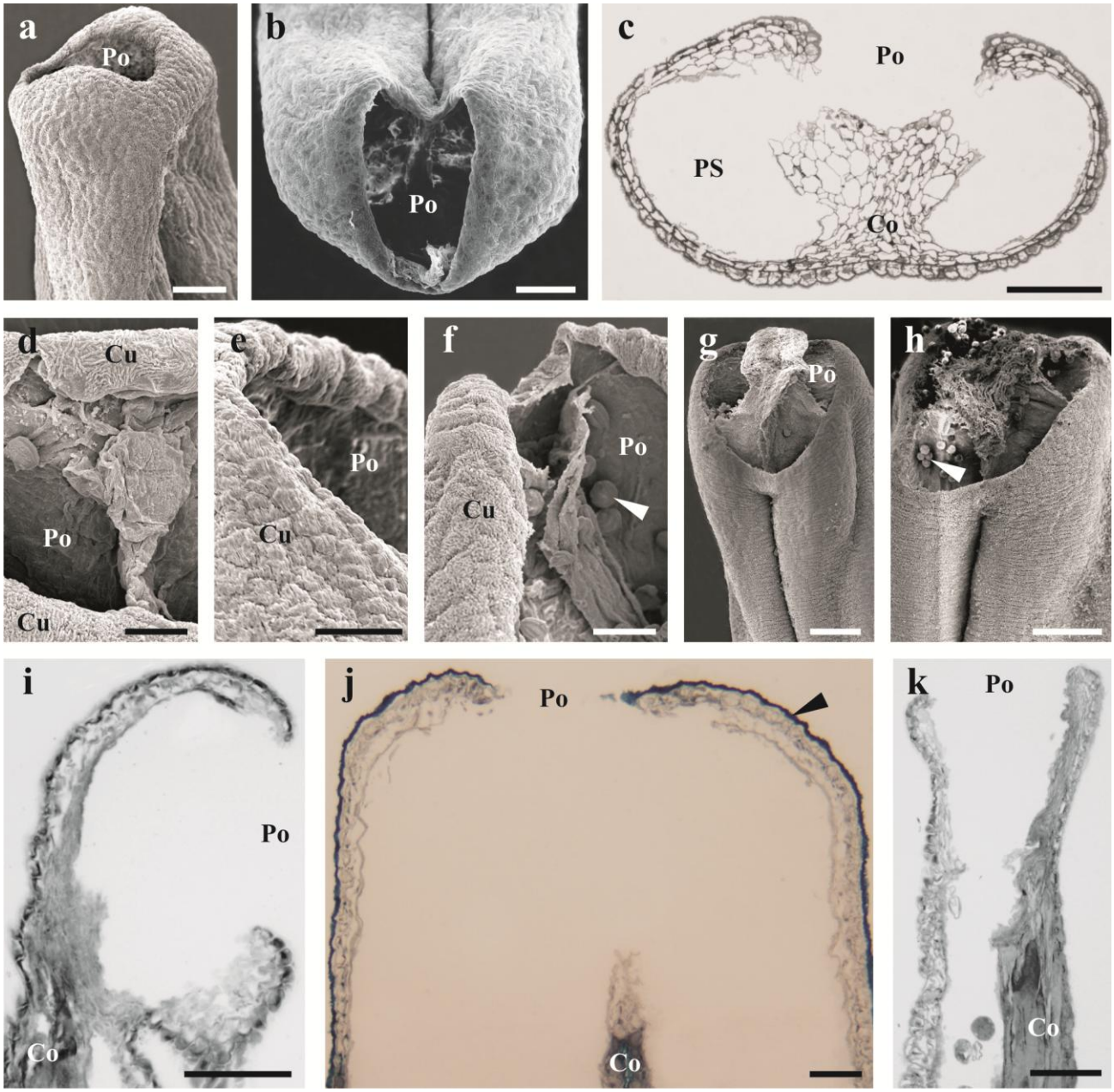

Figure 4 Dehiscent anthers of *Miconia* species. (**a, b**) Apical portion of the anthers. (**c**) Transversal section of the apical portion of the anther showing an opened pore. (**d-h**) Apical portion of the anthers in detail. Note the presence of pollen grains (arrowhead) in some anthers and the four porate-like anthers in **g** and **h**. (**i-k**) Longitudinal section of the anthers showing an opened pore. [**a**, **f:** *Miconia chamissois*. **b**, **c**: *M. albicans*. **d**, **i**: *M. stenostachya*. **e**: *M. pseudonervosa*. **g**, **h**: *M. theaezans*. **j**: *M. paucidens*. **k**: *M. fallax*. Arrowhead: cuticle. Co: connective. Cu: cuticle. En: endothecium. Ep: epidermis. Po: pore. PS: pollen sac. Se: septum. Scale bars: 100 µm in **a**, **b**, **g**, **h**, 50 µm in **c**, **i**, **k**, 30 µm in **d-f** and 20 µm in **j**].

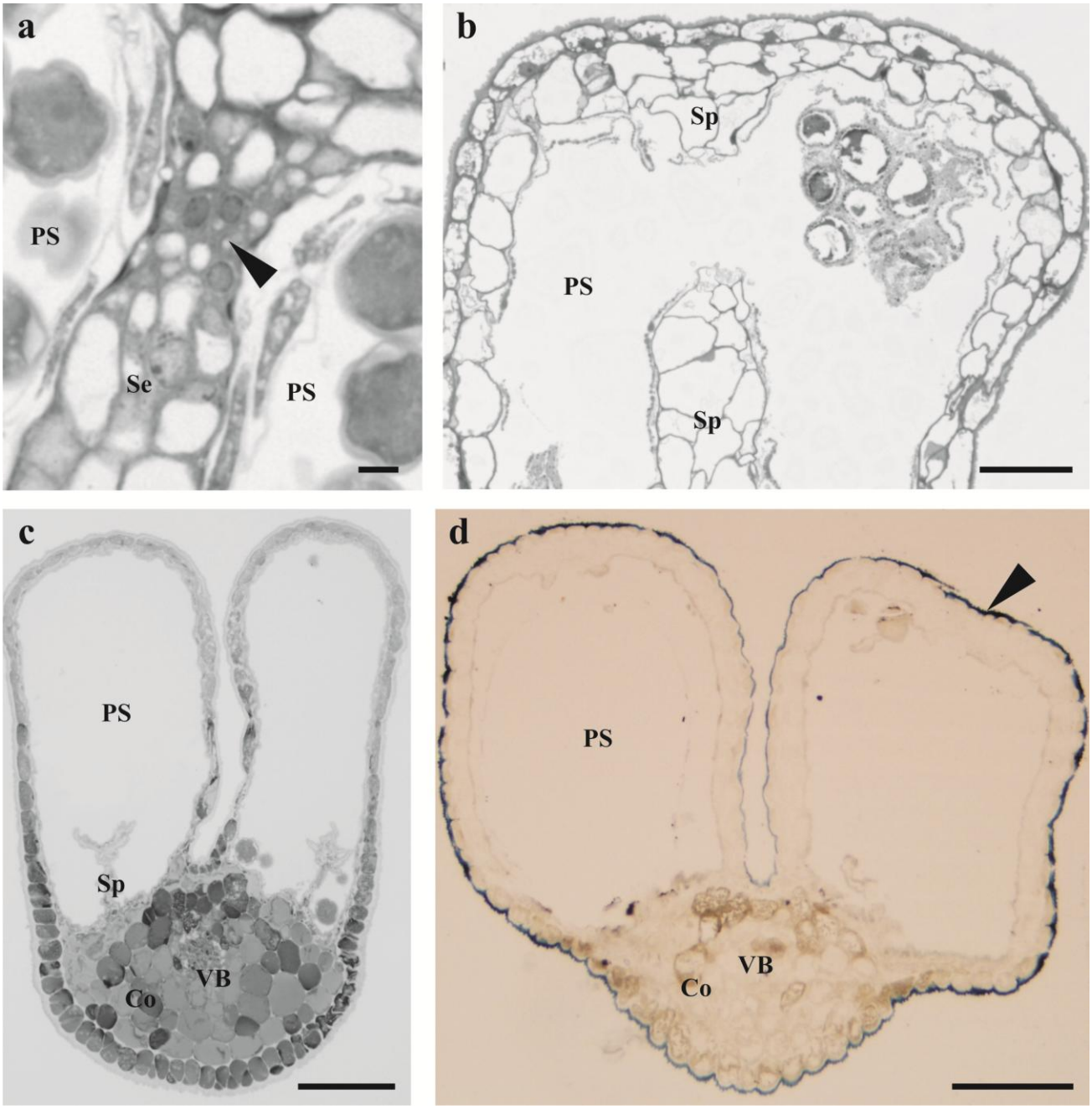

Figure 5 Transversal section of the anther showing septum degeneration during anther dehiscence in *Miconia* species. (**a**) Detail of the septum portion formed by different cells (arrowhead). (**b**) Initial septum degeneration. (**c, d**) Billocular mature anthers resulting from total septum degeneration. Note the cuticle covering the epidermis (arrowhead) [**a**, **c**: *Miconia paucidens*. **b**: *M. albicans*. **d**: *M. stenostachya*. Co: connective. PS: pollen sac. Sp: septum. VB: vascular bundle. Scale bars: 5µm in **a**, 20µm in **b** and 50µm in **c**, **d**].

Considerações Finais

A inclusão de espécies de *Miconia* com diferentes sistemas reprodutivos permitiu reconhecer que alguns estados de caráter, como grãos de pólen anormais, estão mais relacionados ao tipo de reprodução que à história evolutiva das espécies. O estudo em várias espécies do mesmo grupo permitiu reconhecer características que, embora sejam distintas daquelas observadas na maior parte das espécies de um determinado grupo (p.e. angiospermas), são compartilhadas por espécies de determinado subgrupo (p.e. *Miconia*).

O estudo comparativo de espécies com produção de grãos de pólen viáveis e inviáveis permitiu identificar o estádio de desenvolvimento em que as anormalidades ocorrem (ou têm início) e alguns aspectos relacionados às suas causas. Aparentemente, nas espécies de *Miconia* incluídas neste e em outros estudos ainda em andamento, o aborto dos grãos de pólen ocorre durante a microsporogênese, em decorrência de irregularidades meióticas. A relação entre tais anormalidades e a poliploidia ainda precisa ser melhor estudada em espécies apomíticas.

O estudo do desenvolvimento de orgãos reprodutivos é importante por permitir a identificação dos tipos celulares que dão origem a determinadas estruturas, e suas características mais marcantes. No caso particular deste trabalho, o estudo ontogênico permitiu verificar que o endotécio está presente em *Miconia albicans*, *M. stenostachya* e em *M. paucidens*, embora sem exibir os espessamentos de parede que típicos de sua função como camada mecânica relacionada à deiscência das anteras. O estudo micromorfológico da região do poro das anteras de *Miconia* permitiu reconhecer a presença de epiderme não cuticularizada na região do poro. A fragilidade dessa região devido à ausência de cutícula, em relação às regiões próximas cuticularizadas, pode ser suficiente para permitir a abertura do poro, visto que nenhuma outra especialização foi observada na antera. Se apenas a desidratação da epiderme da região do poro é responsável pela deiscência das anteras de *Miconia* ou se há algum mecanismo ativo relacionado ainda demanda estudos fisiológicos complementares.