Plant Syst Evol
DOI 10.1007/s00606-014-1067-z

ORIGINAL ARTICLE

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

Priscila Andressa Cortez · Ana Paula Souza Caetano · Sandra Maria Carmello-Guerreiro · Simone Pádua Teixeira

Received: 7 February 2014 / Accepted: 8 April 2014

Abstract *Miconia* is one of the largest exclusively Neotropical genera, inserted in the tribe Miconieae. Although the monophyly of the tribe has recently been recognized, the delimitation of its genera is considered quite arbitrarily defined, mainly due to the great diversity combined with little morphological and genetic characterization of its members. Recent findings have associated this diversity with polyploidy, apomixis and hybridization, mechanisms commonly found in the group. The conventional delimitation of sections in *Miconia*, which has been largely based on stamen morphology, has proved to be undoubtedly artificial; nevertheless, the potential taxonomic significance of anther wall and pollen ontogenetic characters within the genera *Miconia* has been little explored. Hence, this study intended to fill that gap in our knowledge, by investigating the anther wall and pollen development in six closely related species of *Miconia*: *M. albicans*, *M. fallax*, *M. latecrenata*, *M. paucidens*, *M. pepericarpa* and *M. stenostachya*. Routine techniques for both light and electronic microscopy were used to examine anthers and pollen grains in several developmental stages. The species studied here share several character states, such as the remarkable monocotyledonous anther wall development, the persistent endothecium in the mature anthers with no cell wall thickenings, the bilocular dehiscent anther and the psilate pollen exine. This study also reports for the first time bisporangiate anthers, crystals and proteinoplasts in tapetal cells for Melastomataceae, and ultrastructural features of anther and pollen wall for *Miconia*.

Keywords Anther · Pollen · Ontogeny · *Miconia* · Miconieae · Taxonomy

P. A. Cortez (✉)
Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, Rodovia Jorge Amado km 16, Ilhéus, BA 45662-900, Brazil
e-mail: priscilaacortez@gmail.com

P. A. Cortez · A. P. S. Caetano
Programa de Pós-Graduação em Biologia Vegetal, Departamento de Biologia Vegetal, Instituto de Biologia, Universidade Estadual de Campinas, Caixa Postal 6109, Campinas, SP 13083-970, Brasil

A. P. S. Caetano · S. M. Carmello-Guerreiro
Departamento de Biologia Vegetal, Instituto de Biologia, Universidade Estadual de Campinas, Rua Monteiro Lobato no 970, Campinas, SP 13083-970, Brazil

S. P. Teixeira
Departamento de Ciências Farmacêuticas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Avenida do Café, s/n, Ribeirão Preto, SP 14040-903, Brazil

Introduction

Miconia Ruiz & Pav. belongs to Melastomataceae Juss., order Myrtales, and is one of the largest exclusively Neotropical genera, with about 1,057 species (Goldenberg et al. 2013). Its members are ecologically important since their fleshy fruits are used as a food resource for local fauna (Magnusson and Sanaiotti 1987; Levey 1990; Stiles and Rosselli 1993; Figueiredo and Longatti 1997; Allenspach and Dias 2012) and some species can be used in natural or human-induced forest regeneration (Ellison et al. 1993; Baider et al. 2001). *Miconia* species are also useful as medicinal plants due to antibacterial agent of their secondary metabolites (Alves et al. 2000; Joffry et al. 2012; Pereira et al. 2013) and particularly interesting due to the occurrence of apomixis, a reproductive phenomenon that occurs in several of its members (Goldenberg and Shepherd

Published online: 09 May 2014

Table 1 Information of *Miconia* species examined and their collection sites

Species	Reproductive system	Collection sites	Geographical coordinates	Vouchers
M. albicans	Apomictic	Itirapina, SP, Brazil	22°15′10″S 47°49′22″W	P.A. Cortez et al. s/n (UEC 162376)
M. fallax	Apomictic	Itirapina, SP, Brazil	22°15′10″S 47°49′22″W	P.A. Cortez et al. s/n (UEC 162378)
M. latecrenata	Apomictic	Jundiaí, SP, Brazil	23°12′/23°21′S 46°30′/47°05′W	R. Goldenberg s/n (UEC 84180)
M. paucidens	Sexual	Itirapina, SP, Brazil	22°15′10″S 47°49′22″W	P.A. Cortez et al. s/n (UEC 162385)
M. pepericarpa	Sexual	Itirapina, SP, Brazil	22°15′10″S 47°49′22″W	P.A. Cortez et al. s/n (UEC 162384)
M. stenostachya	Apomictic	Itirapina, SP, Brazil	22°15′10″S 47°49′22″W	P.A. Cortez et al. s/n (UEC 162382)

1998; Goldenberg and Varassin 2001) and has important ecological and evolutionary implications.

Recent revisions have recognized the tribe Miconieae as monophyletic but not the majority of its large genera (Penneys and Judd 2005), since *Miconia*, *Clidemia*, *Leandra* and *Ossaea* are paraphyletic or polyphyletic (Michelangeli et al. 2004; Goldenberg et al. 2008; Martin et al. 2008). The dilemma in the circumscription of the genera within Miconieae may be a consequence of apomixis, hybridization and polyploidization, mechanisms frequently found in this group (Goldenberg and Shepherd 1998), making the interpretation of morphological data a very complex task. The poor morphological characterization of Miconieae (Goldenberg et al. 2008) considering the large number of species does not yet allow the use of morphological characters to access evolutionary relations among its species.

Features from anther wall and pollen development have been used for systematic purposes in angiosperms (Maheshwari 1950; Davis 1966), including Myrtales (Tobe and Raven 1983). Endothecium without fibrous cell wall thickenings and uninucleated tapetal cells, for example, are consistent embryological markers for Melastomataceae members (Tobe and Raven 1983; Schmid 1984; Medeiros and Morretes 1996; Goldenberg et al. 2003; Melhem et al. 2003), but in general, the embryological characters are still little explored in Miconieae (see Subramanyam 1942; Patel et al. 1984; Tobe and Raven 1984; Medeiros and Morretes 1996; Caetano et al. 2013a). Also, some developmental pollen characters have recently been employed to distinguish *Miconia* species with different modes of reproduction (Cortez et al. 2012; Caetano et al. 2013b).

The majority of reproductive characters (e.g., embryology) seems to be more conserved than the vegetative ones (Tobe 1989) and their association with molecular data would provide greater reliability in reconstructing the evolutionary history of a taxa. Hence, this research intended to study some aspects of anther wall and pollen development in six closely related species of *Miconia* (see Goldenberg et al. 2008): *M. albicans* (Sw.) Triana, *M. fallax* DC., *M. latecrenata* (DC.) Naudin, *M. paucidens* DC., *M. pepericarpa* DC. and *M. stenostachya* DC., and check for embryological patterns in the group.

Materials and methods

At least five individuals from each of six species of *Miconia* growing in natural populations in São Paulo state, southeast Brazil, were included in this study, conducted between 2007 and 2012. Samples from all collected species were identified and deposited in the Herbarium UEC (Table 1).

Anthers in several stages of development were removed from young buds and flowers and immediately fixed for 24 h in a solution with 80 mL L^{-1} glutaraldehyde, 250 mL L^{-1} paraformaldehyde (16.0 %) and 500 mL L^{-1} phosphate buffer (0.1 M, pH 6.8) (modified from Karnovsky 1965) and then transferred to a phosphate buffer (0.1 M, pH 6.8) solution. These initial procedures were followed by one of the techniques detailed below.

The anatomical analyses were carried out on anthers of all the six species after their dehydration using ethanol series. The anthers were embedded in glycol methacrylate (Historesin Leica) according to the manufacturer's specification. Cross and longitudinal sections, both between 1.0 and 3.0 μm thick were made on a RM2245 Leica rotary microtome using a tungsten knife. The sections were submitted to the following staining solutions: 12 g L^{-1} acetocarmine solution for better cytoplasm visualization, 4′,6-diamidino-2-phenylindole (DAPI) in phosphate buffered saline (PBS) for better nuclear visualization, 0.05 %

toluidine blue (CI 52040) in citrate buffer (pH 4.0) for phenolic compounds, Lugol solution for starch grains, xylidine Ponceau (pH 2.5) (C.I. 16150) for total proteins, Sudan black B (C.I. 26150) for lipids, periodic acid Schiff reaction (pararosanilin C.I. 42500) for structural carbohydrates, Ruthenium red solution for pectin, and aniline blue (pH 8.0) (C.I. 42755) using a fluorescence microscope equipped with an ultraviolet (UV) excitation filter for detection of callose wall deposition. Light polarization techniques were used to better observe crystals. Digital images were obtained under a BX 51 Olympus light microscope.

The surfaces of *M. albicans*, *M. fallax*, *M. latecrenata*, *M. paucidens* and *M stenostachya* anthers and pollen grains were analyzed after being dehydrated using an ethanol series, critical-point dried using liquid CO_2 on a Balzers CPD030 apparatus and mounted on metallic stubs using double-sided carbon adhesive tape. Some anthers were carefully opened to expose the pollen grains, and then the material was gold coated on a Balzers SCD050 sputter coater. The observations and illustrations were made under a JEOL JSM-5200 scanning electron microscope.

The ultrastructural characteristics of anther wall and pollen grains were obtained for *M. albicans*, *M. paucidens* and *M. stenostachya*. The anthers were transferred from the phosphate buffer (0.1 M, pH 6.8) solution to a post-fixation solution (1.0 % osmium tetroxide in phosphate buffer) and, after dehydration through a series of graded acetone, the anthers were submitted to the infiltration and embedding procedures using Araldite 502 Polysciences resin, and the blocks obtained were sectioned using a diamond knife at 50 nm in a Reichert UltraCut S Leica. The sections were placed on nickel mesh grids, contrasted using both uranyl acetate (Watson 1958) and lead citrate (Reynolds 1963) for 15 min. The observations and illustrations were taken under an EM 208 Philips transmission electron microscope.

Fresh pollen grains were collected from opening anthers and submitted to the water content and germination tests. In the water content test, pollen grains were deposited on two groups of glass slides: in the first group, the pollen grains were immediately covered with a drop of water; in the second group, the pollen grains were immediately covered with a drop of immersion oil (modified from Dafni et al. 2005). After a few minutes, both slide groups were observed under a BX 51 Olympus light microscope and compared in relation to pollen grain size and shape. In the germination test, pollen grains were deposited in a nutritive medium containing 2.0 % colorless gelatin, 20.0 % sucrose, 0.01 % boric acid and 0.05 % calcium nitrate (modified from Santos and Mariath 1997). After an incubation period of 3 h at 25 °C in dark chamber, the pollen grains were observed under a BX 51 Olympus light microscope for pollen tube morphology. The pollen grains that successfully germinated were also submitted to the anatomical analysis detailed above.

Pollen grain terminology followed Hesse et al. (2009).

Results

Initial anther wall development and microsporogenesis

Five of the six species studied here present anthers with four microsporangia (Fig. 1a–e); only *Miconia latecrenata* have anthers composed by two microsporangia, one in each theca (Fig. 1f).

The early sporogenous tissue is massive and located in the central portion of the microsporangium; its cells are polygonal in shape, with electron-dense cytoplasm composed of mitochondria, dictyosomes, smooth endoplasmic reticulum and small vacuoles; some plasmodesmata can be seen connecting adjacent sporogenous cells (Fig. 2a, b). This sporogenous tissue is surrounded on its outer side by the epidermis (outer) and by the primary parietal layer (inner), both one-layer thick; the cells of the primary parietal layer divide periclinally to form the outer and the inner secondary parietal layers (Fig. 2c). The outer secondary parietal layer develops into the endothecium while the inner secondary parietal cells divide periclinally to form the outer middle layer and the inner tapetum layer (Fig. 2d–f).

Just before the beginning of meiosis, microspore mother cells develop from the sporogenous cells and a gradual deposition of a thick wall composed of callose occurs around each microspore mother cell in all the six species (Fig. 3a–c). The meiotic process occurs with strong irregularities in most of the anthers of *M. fallax*, *M. latecrenata* and *M. stenostachya* (Fig. 3d, e), and it was not observed in *M. albicans* anthers. Successful meiosis only occurs in *M. pepericarpa* and *M. paucidens* microspore mother cells, and in some anthers of *M. fallax* and *M. stenostachya*, resulting in four-nucleate syncytium (Fig. 3f); in this species, meiosis is followed by simultaneous cytokinesis, which gives rise to tetrahedral microspore tetrads (Fig. 3g, h), with each microspore still surrounded by the callose wall when the primexine deposition starts (Fig. 3h, i). From the pre-meiotic stage to cytokinesis, the anther wall in all the six species is composed of four or five layers: the epidermis (the outermost layer), endothecium, middle layers (one or two layers thick) and tapetum (the innermost layer); the middle layer may appear crushed due to the enlargement of the tapetal cells, which are secretory and uninucleate (Fig. 3a, c).

At the post-meiotic stage, all microspores—including those abnormally formed in the *M. fallax, M. latecrenata,*

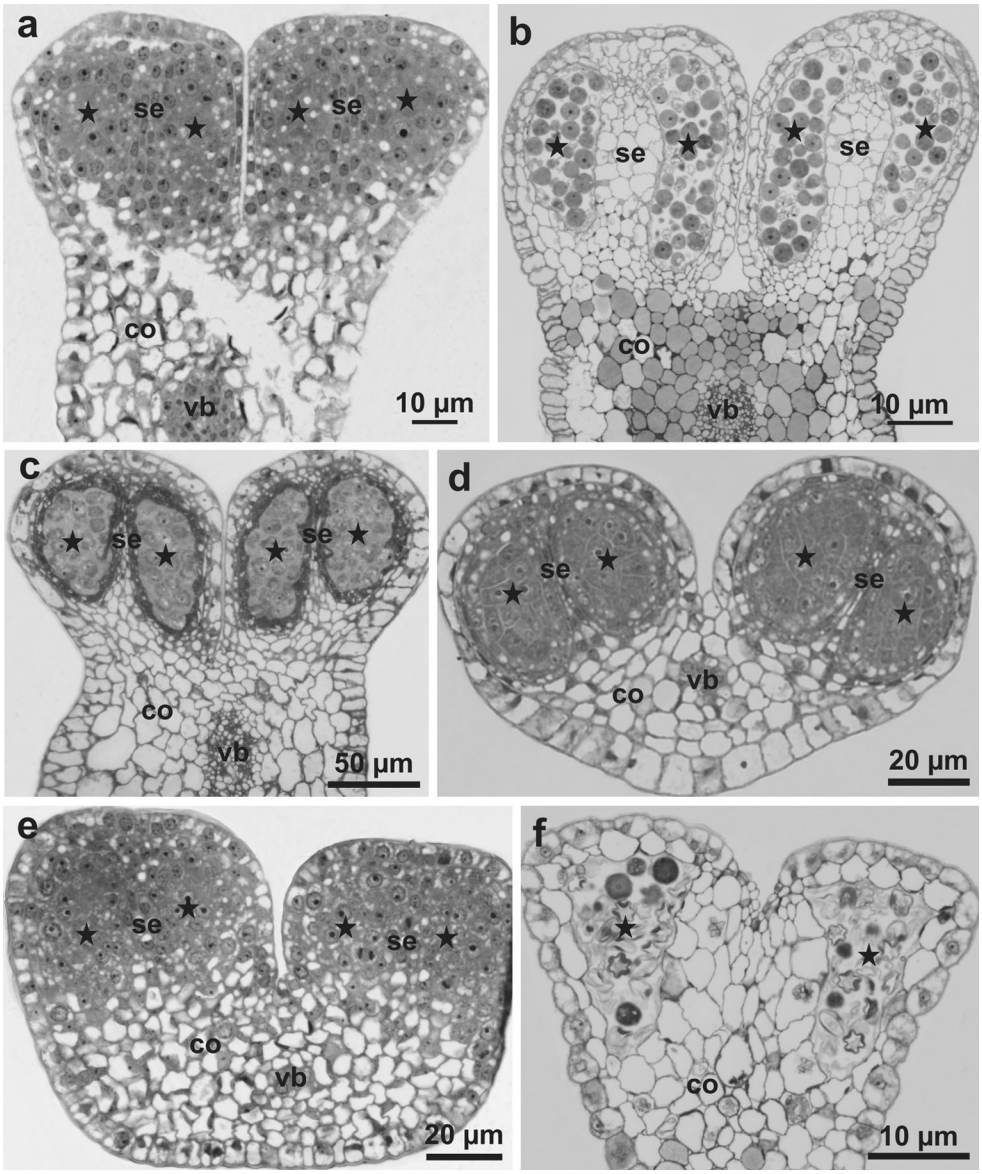

Fig. 1 Transversal section of *Miconia* anthers. **a**–**e** Tetrasporangiate anthers. **f** Bisporangiate anther. Symbols: *filled star* microsporangium, *co* connective, *se* septum, *vb* vascular bundle. **a** *M. albicans*, **b** *M. fallax*, **c** *M. paucidens*, **d** *M. pepericarpa*, **e** *M. stenostachya*, **f** *M. latecrenata*. **a**–**f** Light Microscopy

and *M. stenostachya* anthers—become free due to the callose wall dissolution, and a stratified wall is gradually formed along with the aperture delimitation around each microspore (Fig. 4a). At this stage, all *Miconia* species display the anther epidermal cells with thickened outer periclinal wall covered by a striate cuticle, except for the dehiscent pore, where the cuticle is absent (Fig. 4e, f); the endothecium cells enlarge and the tapetal cells start a gradual degradation (Fig. 4a–d, h); several Ubisch bodies are especially abundant in the locule-facing tapetal cells, close to the inner periclinal cell walls, forming the tapetal and peritapetal membranes (Fig. 4c, h). In *M. paucidens* anthers, proteinoplasts and druse crystals were observed in the tapetal cell cytoplasm (Fig. 4g, h).

Final anther wall development and microgametogenesis

The final stage of the free microspore is marked by the formation of a large cytoplasmic vacuole, which coincides with the parietal positioning of the nucleus (Fig. 5a, b) and with the asymmetrical first mitosis (Fig. 5c). 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 (Fig. 5d, e). In some

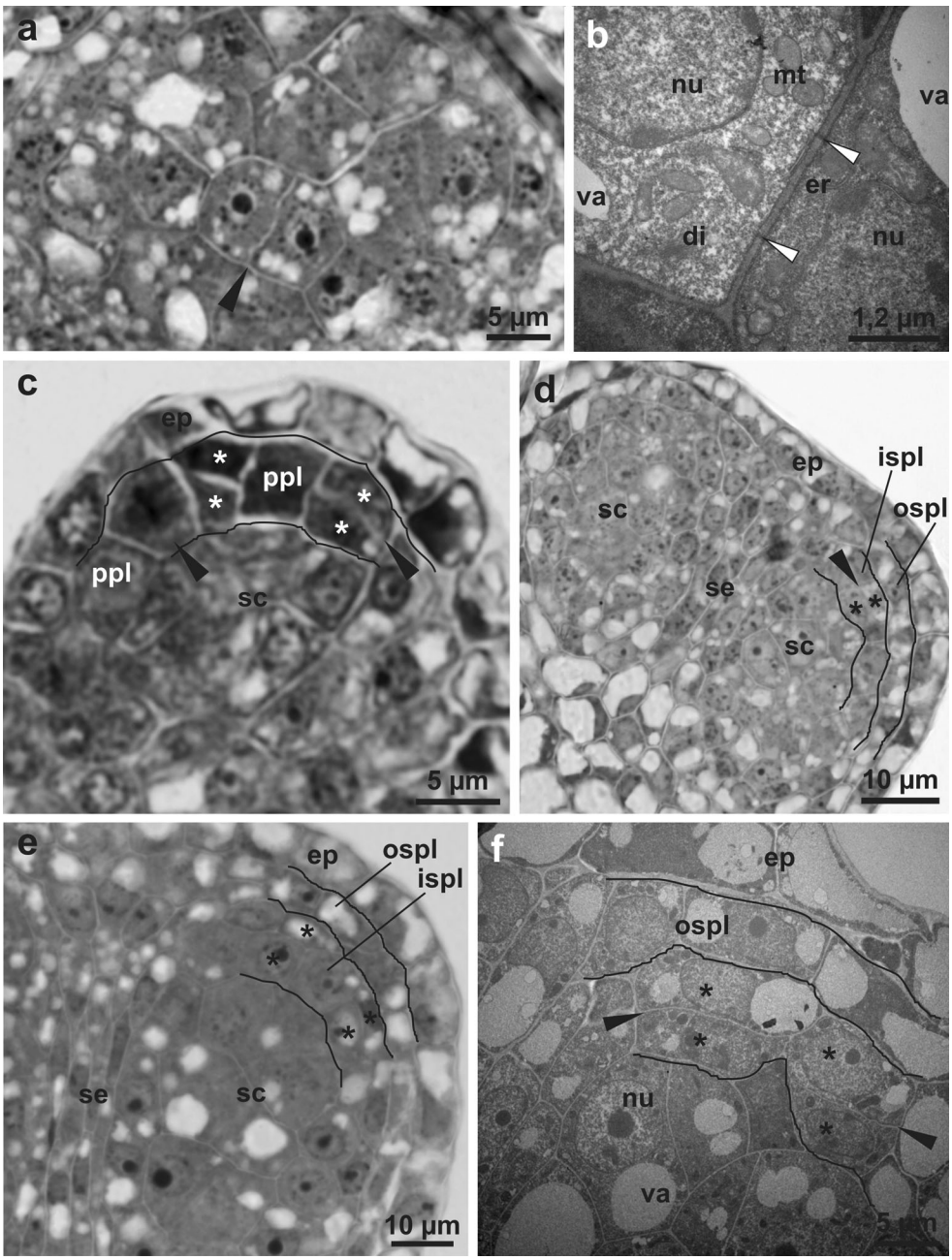

Fig. 2 Transversal sections of *Miconia* anthers in pre-meiotic stage. **a**, **b** Sporogenous cells. **c** Periclinal division in primary parietal layer (ppl). **d**–**f** Periclinal division in cells of the inner secondary parietal layer (ispl). Note that the cells of the outer secondary parietal layer (ospl) do not divide. Symbols: *open right triangle* plasmodesmata, *filled right triangle* mitotic division, *asterisk* sister cells, *di* dictyosome, *ep* epidermis, *er* endoplasmic reticulum, *mt* mitochondria, *nu* nucleus, *sc* sporogenous cell, *se* septum, *va* vacuole, **a** *M. fallax*. **b** *M. paucidens*. **c** *M. pepericarpa*. **d** *M. fallax*. **e**, **f** *M. albicans*. **a**, **c**–**e** Light microscopy, **b**, **f** transmission electron microscopy

microspores of *M. fallax* and *M. stenostachya*, this first mitosis also occurs but in an irregular way, leading to the formation of two similar-sized cells; in *M. albicans*, all the steps of gametogenesis were absent. In *M. paucidens* and in *M. fallax* and *M. stenostachya* with asymmetrical first division, the vegetative cell has electron-dense cytoplasm containing mitochondria, endoplasmic reticulum, dictyosomes and vesicles, in addition to the spherical and prominent nucleus, and generative cell has a few portions of cytoplasm containing mitochondria and a large spherical nuclei (Fig. 5e). The generative cell changes in shape from lenticular to spherical, and gradually detaches from the pollen wall and moves toward the center of the pollen grain, remaining totally inside the vegetative cell cytoplasm (Fig. 6a–d). The

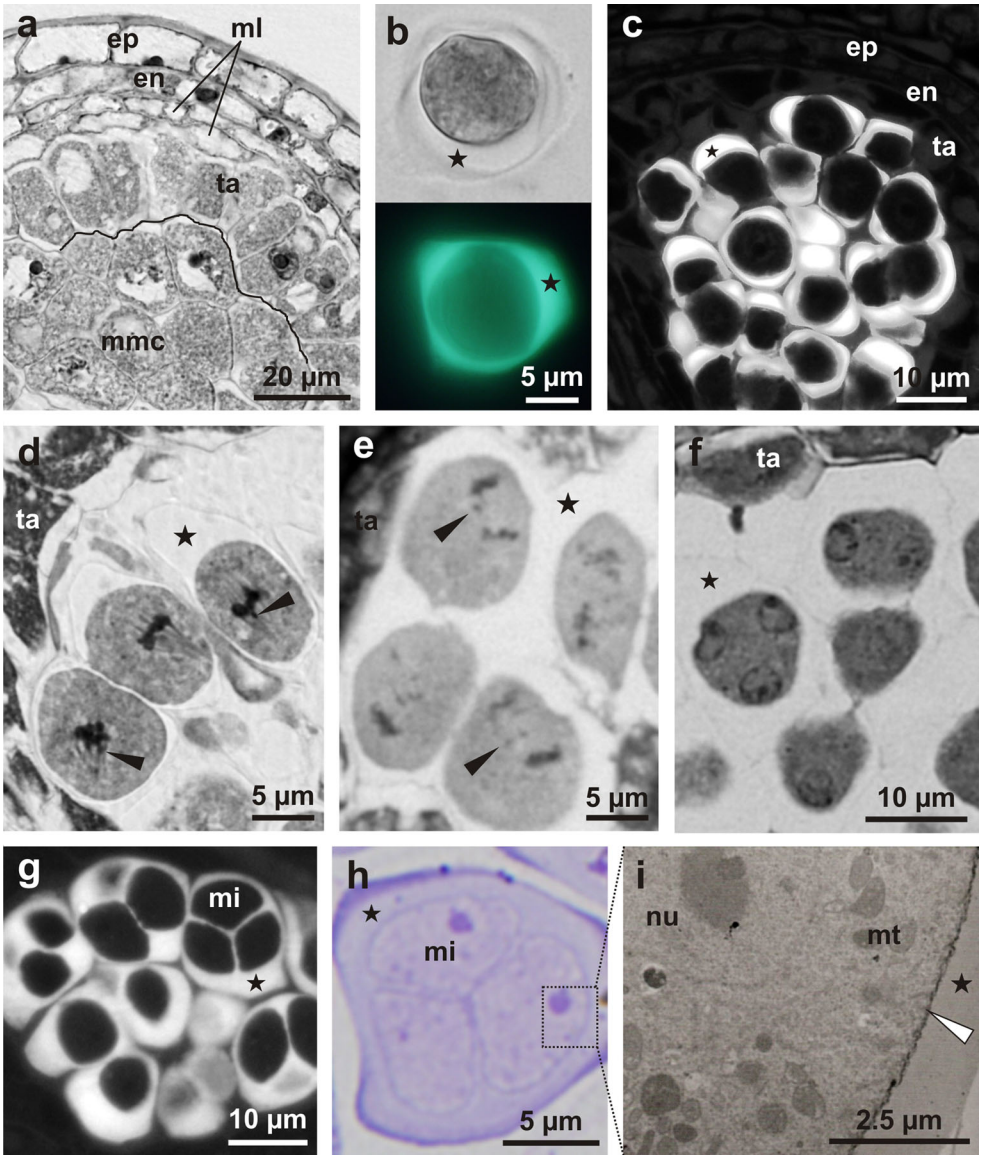

Fig. 3 Transversal sections of *Miconia* anthers during microsporogenesis. **a** Microspore mother cells (mmc) before callose wall deposition. **b**, **c** Isolated microspore mother cells after callose wall deposition. **d**, **e** Irregular meiotic figures. *Arrows* indicate precocious chromosome ascension in (**d**) and lagging chromosome in (**e**). **f** Tetranucleate syncytium surrounded by the callose wall. **g** Tetrahedral microspore tetrads originated by simultaneous cytokinesis. **h**, **i** Primexine deposition around microspores. Symbols: *filled star* callose wall, *open right triangle* primexine, *en* endothecium, *ep* epidermis, *mi* microspore, *ml* middle layer, *mt* mitochondria, *nu* nucleus, *ta* tapetum, **a** *M. albicans*, **b** *M. stenostachya*, **c** *M. latecrenata*, **d** *M. fallax* **e** *M. stenostachya*, **f** *M. pepericarpa*, **g** *M. paucidens*, **h**, **i** *M. stenostachya*. **a**–**h** Light microscopy, **i** transmission electron microscopy

generative cell progressively changes in shape from spherical to elongated (Fig. 6e), which occurs late and less frequently in *M. fallax* and *M. stenostachya*.

In all species except *M. albicans*, the mature pollen grains are shed in monads, which are small (c.a. 10 µm), prolate, heteroaperturate, with three colpori alternating with three pseudocolpi (Fig. 6f–h); the exine surface is perforate in *M. latecrenata* (Fig. 6i), granulate–verrucate in *M. paucidens* (Fig. 6j) and psilate in *M. stenostachya* (Fig. 6k). In *M. fallax*, the exine pattern is masked by the great amount of locular substances, possibly Ubisch bodies (Fig. 6l). *M. albicans* did not present well-formed pollen grain and for this, its exine surface was not analyzed. The released pollen grains of all species except *M. albicans* are bicellular and orthodox, differing in form and size between its dry and hydrated condition (Fig. 6m, n). In *M. paucidens* and *M. stenostachya*, the exine has an ektexine (outer) and an endexine (inner); the ektexine is composed by the discontinuous tectum, the

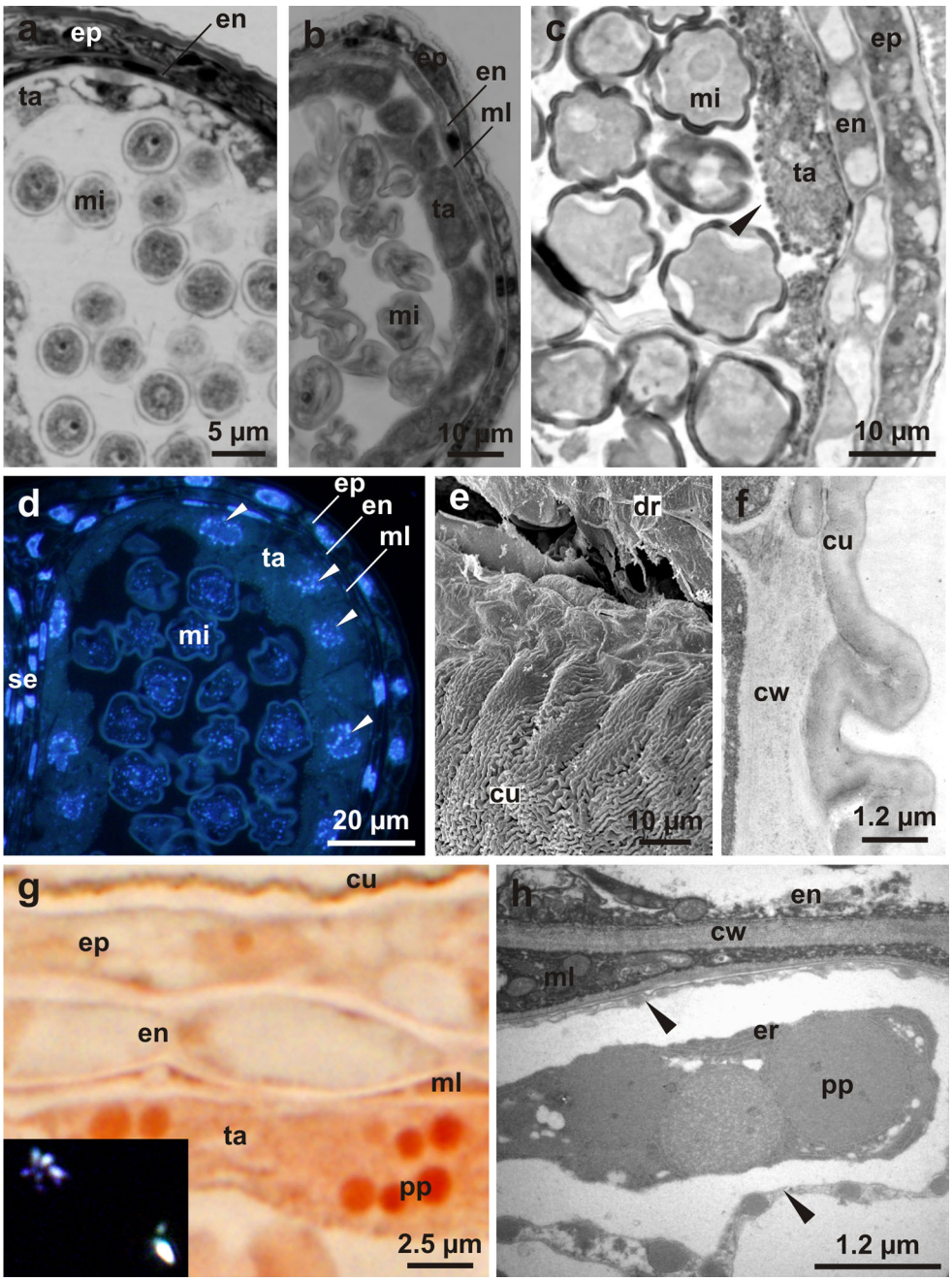

Fig. 4 Transversal sections of *Miconia* anthers during free microspore stage. **a–d** Free microspores and the beginning of tapetal cell degradation. Note the aggregated Ubisch bodies (*black arrow*) in (**c**) and tapetal cells with one nucleus (*white arrow*) in (**d**). **e**, **f** Anther epidermis in detail showing cuticle ornamentation. **g**, **h** Tapetal cells with proteinoplasts (pp) and crystals ("*inset*") inside the cytoplasm. Note the tapetal membrane (*black arrow*) in (**h**). Symbols: *cu* cuticle, *cw* cell wall, *dr* dehiscence region, *en* endothecium, *ep* epidermis, *er* endoplasmic reticulum, *mi* microspore, *ml* middle layer, *nu* nucleus, *pp* proteinoplast, *se* septum, *ta* tapetum. **a**, **c**, **g**, **h** *M. paucidens*, **b**, **e** *M. latecrenata*, **d** *M. stenostachya*, **f** *M. albicans*. **a–d**, **g** Light microscopy, **e** scanning electron microscopy, **f**, **h** transmission electron microscopy

columellate infratectum and the continuous foot layer; the endexine is compact and continuous; the lumen delimited by the columella is large, filled with pollenkitt, the appearance of which is electron dense; the intine is thin except in the colpore region, where it is very thick (Fig. 7a, b). At this stage, all *Miconia* species display epidermis and non-fibrous endothecium as parietal layers (Fig. 7c–f), and the tapetal membrane (Fig. 7g); all species display degeneration of the septum that divides the two pollen sacs of each theca,

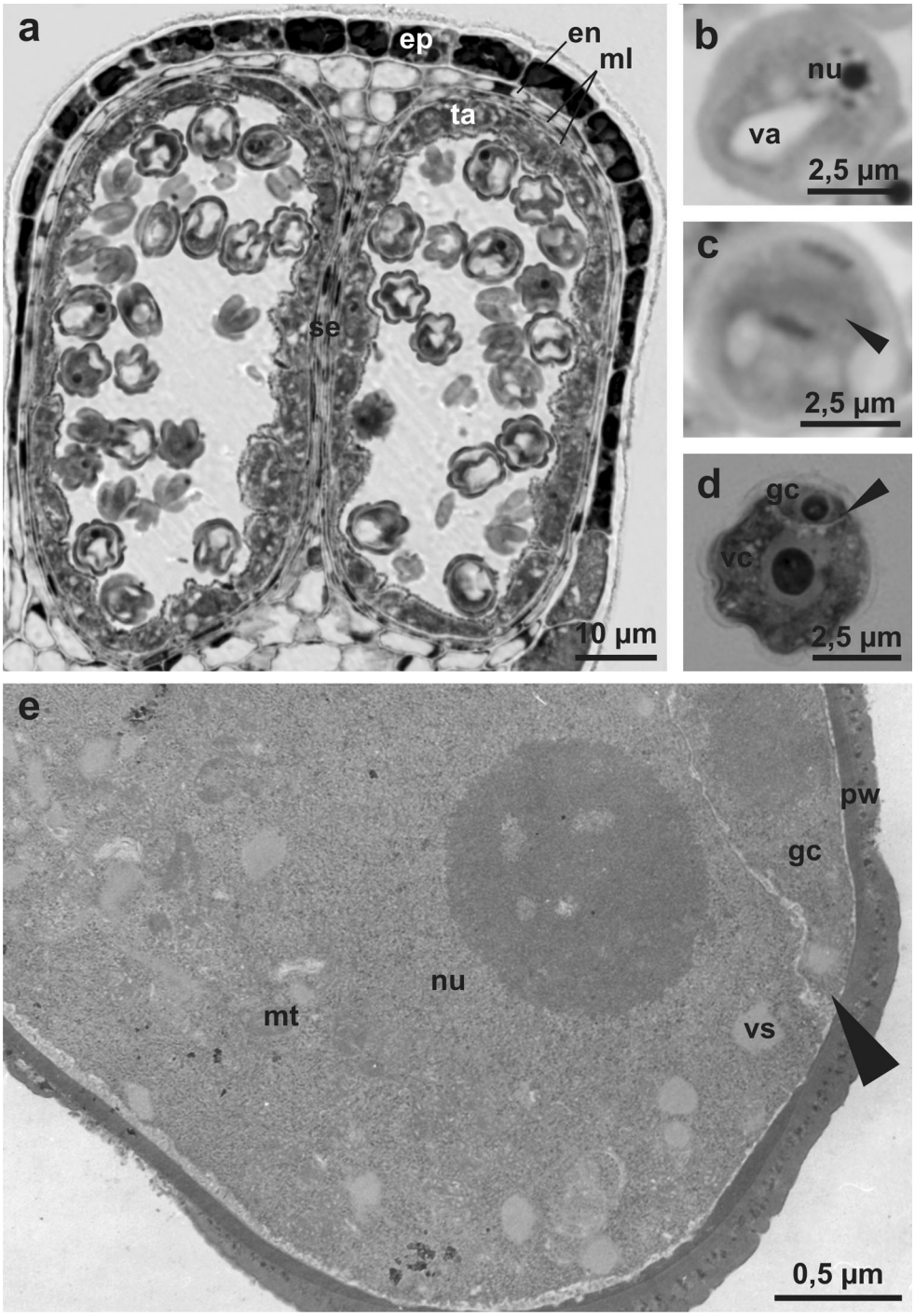

Fig. 5 Transversal sections of *Miconia* anthers during the first mitosis of microgametogenesis. **a**, **b** Vacuolated microspores. **c**, **d** Microspore first mitosis, giving rise to bicellular pollen grain. **e** Young pollen grain with one vegetative cell (vc) and one parietal generative cell (gc). Symbols: *filled right triangle* cell division, *en* endothecium, *ep* epidermis, *gc* generative cell, *ml* middle layer, *mt* mitochondria, *nu* nucleus, *pw* pollen wall, *ta* tapetum, *va* vacuole, *vc* vegetative cell, *vs* vesicle. **a** *M. pepericarpa*, **b** *M. stenostachya*, **c**, **e** *M. paucidens*, **d** *M. fallax*. **a**–**d** Light microscopy, **e** transmission electron microscopy

except *M. latecrenata*, because its mature anther becomes bilocular (Fig. 7c, d).

In *M. paucidens* and *M. pepericarpa*, the second mitotic division that gives rise to the two sperm cells occurs inside the pollen tube, which emerges from one of the three colpori (Fig. 7h); the sperm cells can be observed inside the pollen tube cytoplasm and callose plugs are also observed along the pollen tube (Fig. 7i).

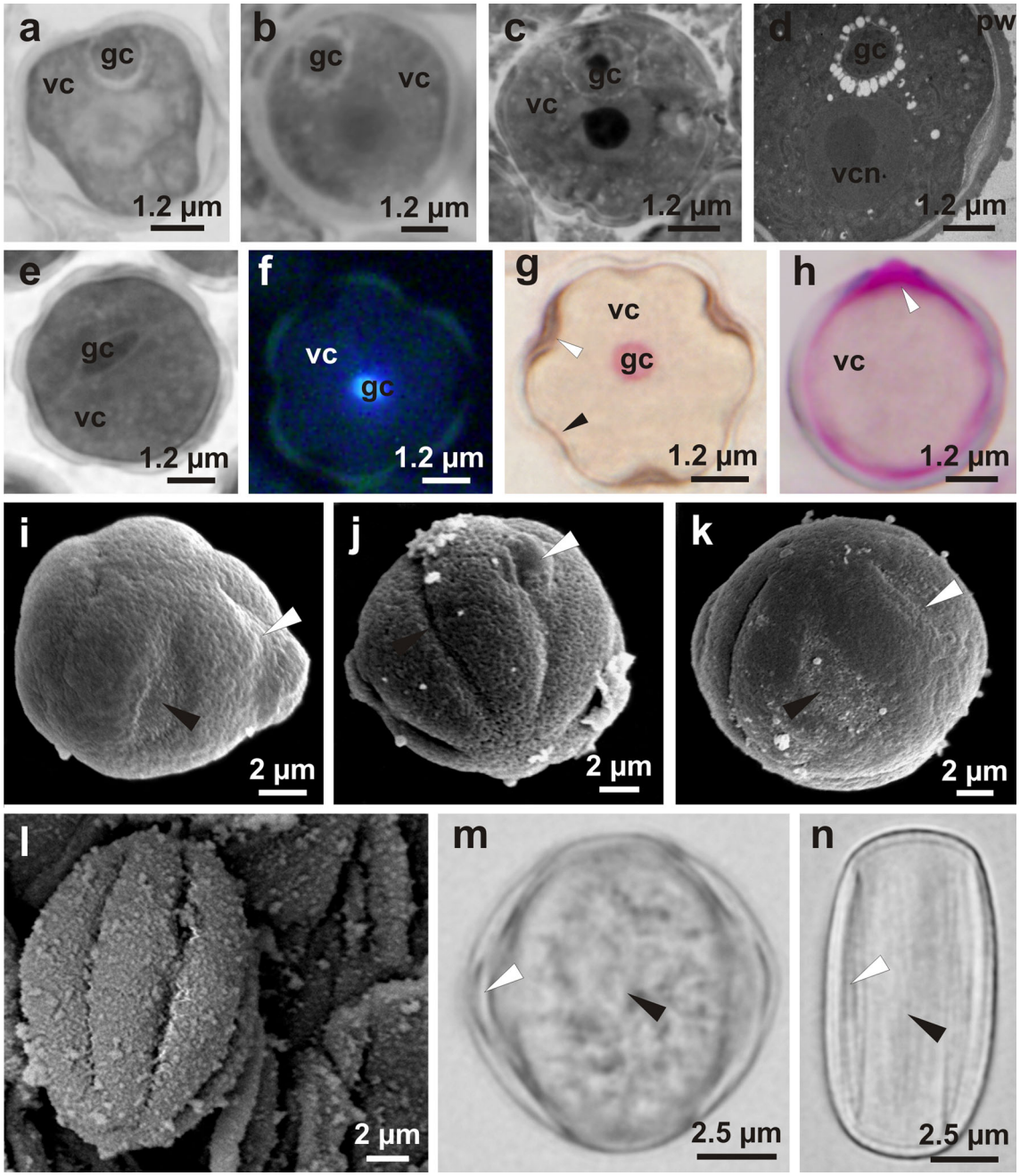

Fig. 6 Pollen grain development in *Miconia* species. **a**–**d** Detachment of the generative cell (gc) from the pollen wall (pw) and gradual rapprochement between generative cell (gc) and vegetative cell nucleus (vcn). **e** Mature bicellular pollen grain with elongated generative cell within the vegetative cell cytoplasm. **f** Generative cell (gc) nucleus evidenced by DAPI reaction. **g**, **h** Pollen grain apertures: three colpori (*white arrow*) alternating with three pseudocolpi (*black arrow*). Note the thicker intine at the colpori. **i**–**k** Exine surface pattern in mature pollen grains. **l** Exine surface masked by the great amount of locular substance. **m**, **n** Pollen grains just before liberation from mature anthers, in equatorial view. Note the different form and size of hydrated (**m**) and dehydrated (**n**) forms of the pollen grains. Symbols: *filled right triangle* colpi, *open right triangle* colpori, *gc* generative cell, *pw* pollen wall, *vc* vegetative cell, *vcn* vegetative cell nucleus. **a**, **b**, **d**, **f**–**h**, **j**, **m**, **n** *M. paucidens*, **c**, **l** *M. fallax*, **e** *M. pepericarpa*, **i** *M. latecrenata*, **k** *M. stenostachya*. **a**–**c**, **e**–**h**, **m**, **n** Light microscopy, **d** transmission electron microscopy, **i**–**l** scanning electron microscopy. **f**, **g** polar view, **h**–**l** oblique equatorial view, **m**, **n** equatorial view

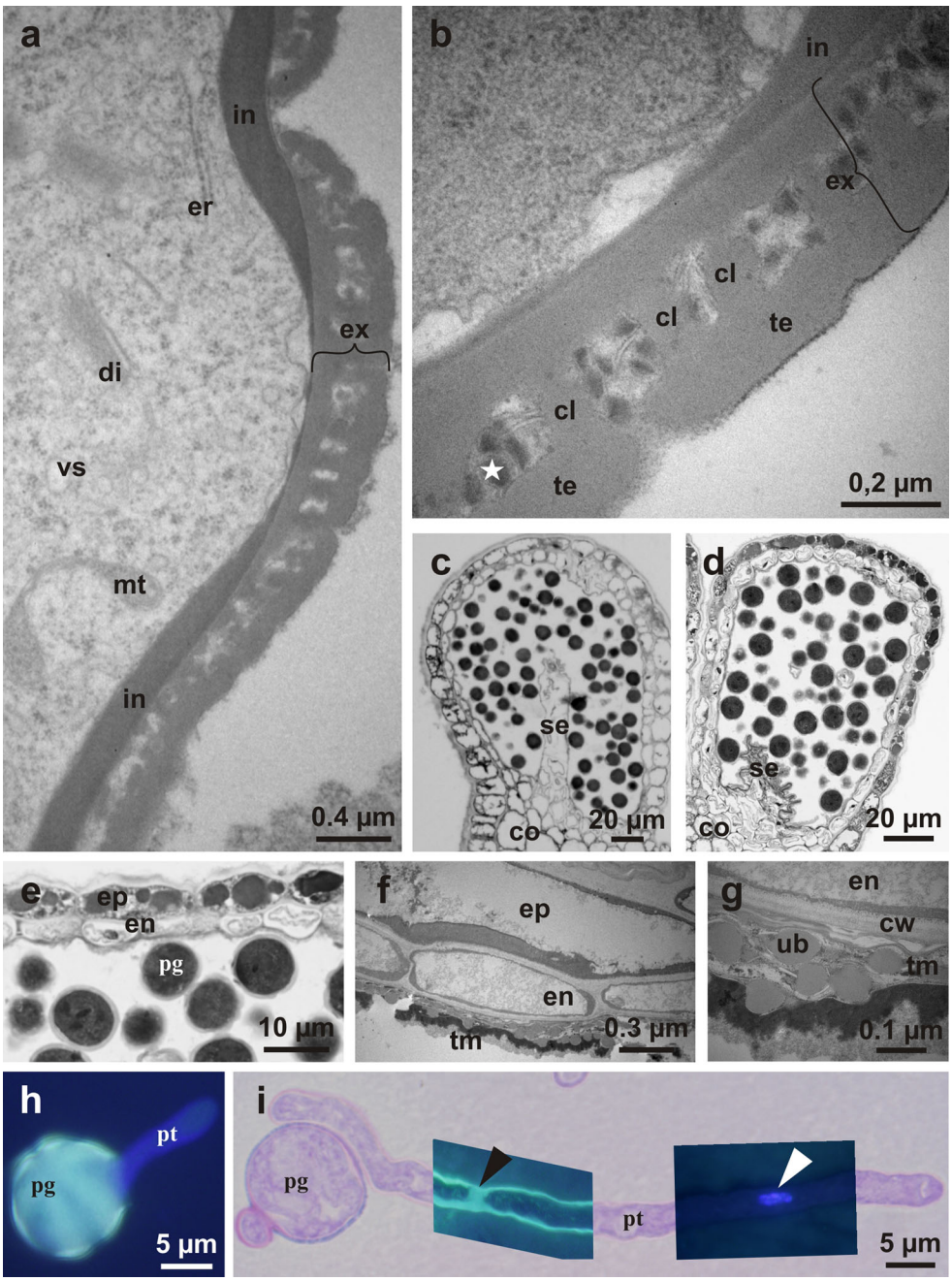

Fig. 7 Dehiscent anther and pollen grains in *Miconia* species. **a**, **b** Pollen wall composed of exine (ex) and intine (in). Note thicker intine at the apertures. **c**, **d** Septum degeneration determining the bilocular condition of the mature anther. **e**–**g** Anther wall composed of epidermis (ep), endothecium (en) and tapetal membrane (tm). **h**, **i** Germinating pollen grains. Note sperm cell nucleus (*white arrow*) and callose plugs (*black arrow*) whiting the pollen tube (pt). Symbols: *filled star* pollenkitt, *cl* columella, *co* connective, *cw* cell wall, *di* dictyosome, *en* endothecium, *ep* epidermis, *er* endoplasmic reticulum, *mt* mitochondria, *pg* pollen grain, *pt* pollen tube, *se* septum, *te* tectum, *tm* tapetal membrane, *ub* Ubisch body, *vs* vesicle. **a**, **c**, **f**–**i** *M. paucidens*, **b** *M. stenostachya*, **d**, **e** *M. pepericarpa*. **a**, **b**, **f**, **g** Transmission electron microscopy, **c**, **d**, **h**, **i** light microscopy

Discussion

The general structure of the pollen and anther wall development, observed in the six species of *Miconia* studied here (Table 2), are similar in relation to those seen in most angiosperms (Maheshwari 1950; Bhandari 1984; Lersten 2004). However, *Miconia* species share some character states from anther and pollen ontogeny which can be considered consistent embryological markers for members of the Melastomataceae, such as endothecium without

Table 2 Comparative anther and pollen features of Melastomataceae species

Tribe	Species	Sporangium number	Anther wall development type	Occurrence of crystal in the tapetum	Locule number in dehiscent anther	Releasing pollen grain	Exine sculpturing type	Pollen grain aperture number
Miconieae	*Leandra cordifolia*[a]	4				Monad		6 Apertures
	Miconia albicans[b]	4	Monocotyledonous	–	2	–	–	–
	M. alypifolia[c]					Monad	Striate	3-Colpori and 3-pseudocolpi
	M. argentea[d]					Monad 2-cellular		
	M. cabucu[e]	2	Dicotyledonous		2	Monad 2-cellular	Finely rugulate	3-Colpori and 3-pseudocolpi
	M. caesia[c]					Monad	Short, branched, cylindrical elements	3-Colpori and 3-pseudocolpi
	M. candolleana[f]					Monad	psilate	3-Colpori and 3-pseudocolpi
	M. chamissois[g]	4			2	Monad 2-cellular		
	M. fallax[b]	4	Monocotyledonous	–	2	Monad 2-cellular		3-Colpori and 3-pseudocolpi
	M. hondurensis[c]					Monad	Smooth–punctate	3-Colpori and 3-pseudocolpi
	M. latecrenata[b]	2	Monocotyledonous	–	2	Monad	Perforate	3-Colpori and 3-pseudocolpi
	M. melanotricha[c]					Tetrad	Granular–verrucate	3-Colpori and 3-pseudocolpi
	M. paucidens[b]	4	Monocotyledonous	Druse type	2	Monad 2-cellular	Granulate–verrucate	3-Colpori and 3-pseudocolpi
	M. pepericarpa[b]	4	Monocotyledonous	–	2	Monad 2-cellular		3-Colpori and 3-pseudocolpi
	M. rigidiuscula[g]					Monad	Psilate	3-Colpori and 3-pseudocolpi
	M. stenostachya[b]	4	Monocotyledonous	–	2	Monad 2-cellular	Psilate	3-Colpori and 3-pseudocolpi

Symbols: – = absent, empty cells = missing information

[a] Subramanyam (1942)

[b] Present study

[c] Patel et al. (1984)

[d] Tobe and Raven (1984)

[e] Medeiros and Morretes (1996)

[f] Melhem et al. (2003)

[g] Caetano et al. (2013a)

fibrous cell wall thickenings and secretory (or glandular) tapetum with uninucleated cells (Tobe and Raven 1983; Schmid 1984; Medeiros and Morretes 1996; Goldenberg et al. 2003; Melhem et al. 2003; Caetano et al. 2013a, b). Other structural features, particularly those related to the pollen grain development of *M. albicans, M. fallax* and *M. stenostachya* are related with the reproductive system of those species, as recently reported by Cortez et al. (2012) and Caetano et al. (2013b).

The "monocotyledonous type" (sensu Davis 1966) described here for the anther wall development of *Miconia* species is remarkable and indicates that Melastomataceae members present distinct patterns for this character, since *Tibouchina cerastifolia* (tribe Melastomeae) and *M. cabucu*

(tribe Miconieae) exhibit the "dicotyledonous type" (Medeiros and Morretes 1996; Medeiros and Ross 1996), which is considered the most common type among angiosperms (Davis 1966). In the "monocotyledonous type" of anther wall development, the outer secondary parietal layer gives rise directly to the endothecium while the inner secondary parietal layer divides periclinally to form the middle layer and the tapetum. 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 that are considered conservative, especially in their early stages. Therefore, the occurrence of "dicotyledonous" and "monocotyledonous" types in Melastomataceae, and particularly in Miconieae, emphasizes that this character state can provide some base for future taxonomic delimitation in this tribe.

Bisporangiate anthers described here in *M. latecrenata* are the first report for Melastomataceae. The number of microsporangia in early developmental stages of anthers may be an interesting character state at the generic level since Melastomataceae is a group recognized as presenting predominantly tetrasporangiate (Tobe and Raven 1983; Medeiros and Morretes 1996; Medeiros and Ross 1996; Caetano et al. 2013a, b) or, less frequently, polysporangiate (Baumgratz et al. 1996) anthers. The druse crystals observed in tapetal cells of *M. paucidens* are also the first report for Melastomataceae being described only for Commelinaceae (Mepham and Lane 1969a, b) and Leguminosae (Buss and Lersten 1972), with unknown role. Both features deserve much attention and detailed investigations in a greater number of related species in Miconieae to evaluate their evolutionary value for the group.

Another character state shared by *Miconia* species is the presence of a layer ontogenetically recognized as endothecium, although devoid of cell wall thickenings (see Schmid 1984; Medeiros and Morretes 1996; Caetano et al. 2013a, b; present study—Table 2). Absence of an endothecium as a layer in mature anthers was described for *M. cinnamomifolia*, *M. pusilliflora* and *M. latecrenata* (Goldenberg et al. 2003). In *M. latecrenata*, Goldenberg et al. (2003) observed the persistence of the outer middle layer even after the pollen grains are completely developed, which contradicts the results obtained here for the same species, since we observed that the sub-epidermal and persistent layer are derived ontogenetically as endothecium. The structural and ontogenetic characteristics of the endothecium are important for the discussion on phylogeny of Melastomataceae since its absence has been considered as a synapomorphy for the entire family, except Olisbeoideae (Clausing and Renner 2001). Based on a purely functional, not in an ontogenetical point of view, we can correctly consider that the absence of endothecium in Melastomataceae species is a strong morphological marker because the endothecium is defined as a mechanical layer with distinct structural specializations, closely related to the anther dehiscence mechanisms (Batygina 2002). Nevertheless, this type of information should be carefully considered since endothecium with some kind of cell wall thickening was observed in *Tococa guianensis* (DG Simão, pers. comm.), a species also included in Miconieae. This fact is quite common when there is little ontogenetic information for species-rich groups like Melastomataceae.

Miconia albicans, M. fallax, M. latecrenata and *M. stenostachya* are apomictic species with null or very low amount of viable pollen grains (Goldenberg and Shepherd 1998; Goldenberg and Varassin 2001; Cortez et al. 2012; Caetano et al. 2013a, b). The structural features observed during the developmental stages of *M. latecrenata* pollen grains are similar to those reported for *M. albicans* and *M. stenostachya* (Cortez et al. 2012); so we can expect that *M. latecrenata* exhibits at least some similar meiotic irregularities as the cause of its pollen abortion. The irregularities during pollen grain development may also be the cause of the difficulties in recognizing the morphological patterns in some species, as in *M. hondurensis* (Patel et al. 1984, figures 35D, E) and *M. alypifolia* (Patel et al. 1984, figures 36A–C), since some pollen grains of these two species were morphologically similar to the apomictic abnormal pollen grains (Cortez et al. 2012). For the two sexually reproducing species, *M. pepericarpa* and *M. paucidens*, the pollen grain features are similar to those reported by Barth and Barbosa (1975), Patel et al. (1984), Santos et al. (1997) and Cruz-Barros et al. (2007) for the majority of the Melastomataceae species.

Despite phylogenetic proximity, it is remarkable the variation in the exine pattern surface among the studied species of *Miconia*, which can be related to the pressures exerted by different pollinators (Hesse 2000). The pollen wall structure and ultrastructure reported here for *M. paucidens* are the first for the whole *Miconia* and similar to those reported for the closely related species *Tococa stephanotricha* (Patel et al. 1984) but the evolutionary significance of these kinds of characters in Melastomataceae still depends on wider studies.

It is noteworthy that much of the information obtained in this study is unique for 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 surprise us today, especially when considering the large number of species in this group. The results obtained here show that some states of embryological characters are conserved in the group, especially those related to the anther wall, such as the endothecium without

cell wall thickenings and the uninucleate tapetum. Other character states, such as the abnormal pollen grains, are more related to the reproductive systems of the species since the irregular pollen ontogeny observed in *Miconia albicans*, *M. stenostachya* (Cortez et al. 2012) and *M. fallax* (Caetano et al. 2013b) is determinant for the high rate of pollen sterility in these species.

Acknowledgments We thank JY Tamashiro (IB, Unicamp) for help with the fieldwork, MDS Ferreira (FMRP, USP) for help with the ultrastructural procedures and Dewey Litwiller (University of Saskatchewan, Saskatoon, Saskatchewan, Canada) for the English revision and anonymous reviewers for their suggestions and comments. This study was financially supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (Fapesp—process numbers 2007/52030-0, 2008/10793-0 and 2010/15077-0), by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq—process numbers 301960/2009-7 and 302204/2012-1).

References

Allenspach N, Dias MM (2012) Frugivory by birds on *Miconia albicans* (Melastomataceae), in a fragment of cerrado in São Carlos, southeastern Brazil. Braz J Biol 72:407–413. doi:10.1590/S1519-69842012000200024

Alves TMA, Silva AF, Brandão M, Grandi TSM, Smânia EFA, Smânia A Jr, Zani CL (2000) Biological screening of Brazilian medicinal plants. Mem Inst Oswaldo Cruz, Rio de Janeiro 95:367–373. doi:10.1590/S0074-02762000000300012

Baider C, Tabarelli M, Mantovani W (2001) The soil seed bank during Atlantic Forest Regeneration in southeast Brazil. Rev Bras Biol 61:35–44. doi:10.1590/S0034-71082001000100006

Barth MO, Barbosa AF (1975) Catálogo sistemático dos pólens das plantas arbóreas do Brasil Meridional XIX—Melastomataceae. Mem Inst Oswaldo Cruz 73:101–108

Batygina TB (2002) Embryology of flowering plants: terminology and concepts: generative organs of flowers. Science Publishers, New Hampshire

Baumgratz JFA, Souza MLDR, Woodgyer EM, NicLughada EM (1996) Polysporangiate anthers: described for the first time in Melastomataceae. Kew Bull 51:133–144

Bhandari NN (1984) The microsporangium. In: Johri BM (ed) Embryology of angiosperms. Springer, New York, pp 53–121

Buss PA, Lersten NR (1972) Crystals in tapetal cells of the Leguminosae. Bot J Linn Soc 65:81–85. doi:10.1111/j.1095-8339.1972.tb00925.x

Caetano APS, Simão DG, Carmo-Oliveira R, Oliveira PE (2013a) Diplospory and obligate apomixis in *Miconia albicans* (Miconieae, Melastomataceae) and an embryological comparison with its sexual congener *M. chamissois*. Plant Syst Evol 299:1253–1262. doi:10.1007/s00606-013-0793-y

Caetano APS, Teixeira SP, Forni-Martins ER, Carmello-Guerreiro SM (2013b) Pollen insights into apomictic and sexual *Miconia* (Miconieae, Melastomataceae). Int J Plant Sci 174:760–768. doi:10.1086/669927

Clausing G, Renner SS (2001) Molecular phylogenetics of Melastomataceae and Memecylaceae: implications for character evolution. Am J Bot 88:486–498

Cortez PA, Carmello-Guerreiro SM, Teixeira SP (2012) Understanding male sterility in *Miconia* species (Melastomataceae)—a morphological approach. Aust J Bot 60:506–516

Cruz-Barros MAV, Corrêa AMS, Gasparino EC, Paes VB (2007) Flora Polínica da Reserva do Parque Estadual das Fontes do Ipiranga (São Paulo, Brasil). Família: 90-Melastomataceae Hoehnea 34:531–552

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

Davis GL (1966) Systematic embryology of the angiosperms. Wiley, New York

Ellison AM, Denslow JS, Loiselle BA, Brénes DM (1993) Seed and seedling ecology of Neotropical Melastomataceae. Ecology 6:1733–1749

Figueiredo RA, Longatti CA (1997) Ecological aspects of a Melastomataceae by marmosets and howler monkeys (Primates: Platyrrhini) in a semideciduous forest of southeastern Brazil. Rev Ecol Terre Vie 52:3–8

Goldenberg R, Shepherd GJ (1998) Studies on the reproductive biology of Melastomataceae in "cerrado" vegetation. Plant Syst Evol 211:13–29. doi:10.1007/BF00984909

Goldenberg R, Varassin IG (2001) Sistemas reprodutivos de espécies de Melastomataceae da Serra do Japi, Jundiaí, São Paulo, Brasil. Rev Bras Bot 24:283–288. doi:10.1590/S0100-84042001000300006

Goldenberg R, Teixeira SP, Martins AB (2003) Anther dehiscence and circumscription of *Miconia* sect. *Hypoxanthus* (Melastomataceae). Kew Bull 58:1195–1203

Goldenberg R, Penneys DS, Almeda F, Judd WS, Michelangeli FA (2008) Phylogeny of *Miconia* (Melastomataceae): patterns of stamen diversification in a megadiverse neotropical genus. Int J Plant Sci 169:963–979. doi:10.1086/589697

Goldenberg R, Almeda F, Caddah MK, Martins AB, Meirelles J, Michelangeli FA, Weiss M (2013) Nomenclator botanicus for the neotropical genus *Miconia* (Melastomataceae: Miconieae). Phytotaxa 106(1):1–171. doi:10.11646/phytotaxa.106.1.1

Hesse M (2000) Pollen wall stratification and pollination. Plant Syst Evol 222:1–17. doi:10.1007/BF00984093

Hesse M, Halbritter H, Weber M, Buchner R, Frosch-Radivo A, Ulrich S (2009) Pollen terminology: an illustrated handbook. Springer, New York

Joffry SM, Yob NJ, Rofiee MS, Affandi MMR, Meor M, Suhaili Z, Othman F, Akim AMD, Desa MNM, Zakaria ZA (2012) *Melastoma malabathricum* (L.) Smith ethnomedicinal uses, chemical constituents, and pharmacological properties: a review. Evid Based Complement Altern Med 2012:1–48. doi:10.1155/2012/258434

Karnovsky MJ (1965) A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J Cell Biol 27:137A–138A

Lersten NR (2004) Flowering plant embryology: with emphasis on economic species. Wiley-Blackwell, Iowa. doi:10.1002/9780470752685

Levey DJ (1990) Habitat-dependent fruiting behavior of an understorey tree, *Miconia centrodesma*, and tropical tree fall gaps as keystone habitats for frugivores in Costa Rica. J Trop Ecol 6:409–420. doi:10.1017/S026646740000479x

Magnusson W, Sanaiotti TM (1987) Dispersal of *Miconia* seeds by the rat *Bolomys lasiurus*. J Trop Ecol 3:277–278. doi:10.1017/S0266467400002169

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

Martin CV, Little DP, Goldenberg R, Michelangeli FA (2008) A phylogenetic evaluation of *Leandra* (Miconieae, Melastomataceae): a polyphyletic genus where the seeds tell the story, not the petals. Cladistics 24:315–327. doi:10.1111/j.1096-0031.2007.00185.x

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

Medeiros JD, Ross AL (1996) Aspectos do microsporângio, da microsporogênese e do gametófito masculino de *Tibouchina cerastifolia* (Naud.) Cogn. (Melastomataceae). Biotemas 9:5–14

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). Bol Inst Bot 16:1–104

Mepham RH, Lane GR (1969a) Formation and development of the tapetal periplasmodium in *Tradescantia bracteata*. Protoplasma 68:175–192. doi:10.1007/BF01247903

Mepham RH, Lane GR (1969b) Role of the tapetum in the development of *Tradescantia* pollen. Nature 221:282–284. doi:10.1038/221282a0

Michelangeli FA, Penneys DS, Giza J, Soltis D, Hils MH, Skean D Jr (2004) A preliminary phylogeny of the tribe Miconieae (Melastomataceae) based on nrITS sequence data and its implications on inflorescence position. Taxon 53:279–290. doi:10.2307/4135608

Patel VC, Skvarla JJ, Raven PH (1984) Pollen characters in relation to the delimitation of Myrtales. Ann Mo Bot Gard 71:858–969. doi:10.2307/2399170

Penneys DS, Judd WS (2005) A systematic revision and cladistic analysis of *Charianthus* (Melastomataceae) using morphological and molecular characters. Syst Bot 30:559–584. doi:10.1600/0363644054782125

Pereira MC, Pádua RM, Paula RC, Nascimento MFA, Soares LF, Isidório RG, Nelson DL (2013) In vitro antiprotozoal activity and cytotoxicity of *Microlicia crenulata* (DC) Mart extract. Int J Med Arom Plants 3:423–429

Reynolds ES (1963) The use of lead citrate at high pH as an electron-opaque stain for electron microscopy. J Cell Biol 17(1):208–212

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

Santos FAR, Souza MLD, Makino-Watanabe H, Borges HBN, Goldenberg R (1997) Palinotaxonomia de espécies brasileiras do género *Ossaea* DC. (Melastomataceae). Polibotánica 5:1–12

Schmid R (1984) Reproductive anatomy and morphology of Myrtales in relation to systematics. Ann Mo Bot Gard 71:858–969

Stiles FG, Rosselli L (1993) Consumption of fruits of the Melastomataceae by birds: how diffuse is coevolution? In: Fleming TH, Estrada A (eds) Frugivory and seed dispersal: ecological and evolutionary aspects. Kluwer Academic, Dordrecht, pp 57–73

Subramanyam K (1942) Gametogenesis and embryogeny in a few members of Melastomataceae. J Indian Bot Soc 21:69–85

Tobe H (1989) The embryology of angiosperms: its broad application to the systematic and evolution study. Bot Mag 102:351–367. doi:10.1007/BF02488572

Tobe H, Raven PH (1983) An embryological analysis of Myrtales: its definition and characteristics. Ann Mo Bot Gard 70:71–94. doi:10.2307/2399008

Tobe H, Raven PH (1984) The number of cells in the pollen of Melastomataceae. Bot Gaz 97:131–136. doi:10.1007/BF02488152

Watson ML (1958) Staining of tissue sections for electron microscopy with heavy metals. J Biophys Biochem Cy 4:5–8