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

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ABSTRACT

Melastomataceae have porate anthers. However, unlike Solanaceae and many monocots, in which the poricidal dehiscence depends on the presence of a mechanical layer (often the endothecium), most members of Melastomataceae have no evident specialized layer related to the poricidal opening. The goal of this study was to characterize the tissues that form the apical pore of the anther in 10 *Miconia* species, which may help to understand the nearly unknown mechanism of anther dehiscence in this genus, considered to be one of the largest and most diverse New World genera. Before anthesis, the apical pores of all of the species are closed by a uniseriate epidermis, the cells of which lack a cuticle. In contrast, the epidermis of the remainder of the anther is covered by a thick, ornamented cuticle. Among Myrtales, the Melastomataceae form a clade with Alzateaceae, Crypteroniaceae and Penaeaceae, almost all of which have anthers with endothecium lacking wall thickenings. In these families, the endothecium may or may not be present in the mature anther, with degenerating cells in the latter case. Anther dehiscence does not depend on the endothecium as the mechanical layer, and this process is still not well understood. However, in the *Miconia* species studied here, the cuticle may prevent 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.

Introduction

Anther dehiscence is a complex process that involves regulated differentiation and degeneration of specific anther tissues, leading to its aperture and pollen grain presentation (Bonner and Dickinson, 1989; Cecchetti et al., 2013; Sanders et al., 2005). The anther dehiscence program starts after meiosis in microspore mother cell (Goldberg et al., 1993), and in general, it involves two main steps: the disruption of the septum, a tissue located between two adjacent locules, and the breakage of the stomium (Keijzer, 1987a). The stomium is a portion of the anther with structural features that change during development, allowing its aperture and pollen grain release (Hufford and Endress, 1989). It is often related to the endothecium, a subepidermal tissue whose cells are characterized by specific walls with lignified secondary thickenings (Davis, 1966). The endothecium plays a key role in anther dehiscence, although anthers that lack a fibrous endothecium are also able to dehisce (Cortez et al., 2014; Hermann and Palser, 2000; Marazzi et al., 2007).

The type of dehiscence is a conserved characteristic among angiosperms and may influence the pollination mechanism (Bernhardt, 1996; Buchmann, 1983; Endress, 1996a). The most common type of anther dehiscence in angiosperms is longitudinal (Endress, 1996b; Lersten, 2004), while valvate dehiscence is occasionally observed in members of Berberidaceae (Batygina, 2002), Hamamelidaceae (Hufford and Endress, 1989) and Magnoliidae (Endress and Hufford, 1989), and poricidal dehiscence is found in members of Ericaceae (Hermann and Palser, 2000; Stevens

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et al., 2004), Leguminosae (Marazzi et al., 2007), Melastomataceae (Renner, 1989) and Solanaceae (Bohs, 2005).

Poricidal dehiscence is a character-state with important adaptive value to pollinators capable of collecting pollen by the high frequency vibration of stamens (Larson and Barrett, 1999; Luca and Vallejo-Marín, 2013), and the involved mechanisms have been better studied in members of Ericaceae, Leguminosae and Solanaceae. Most Ericaceae species exhibit a dehiscence mechanism that does not depend on an endothecium or other fibrous tissue but instead depends on a specific so-called resorption tissue (Hermann and Palser, 2000). *Senna* species (Leguminosae) also lack an endothecium, and in most of them, poricidal dehiscence involves thick-walled hypodermal and subhypodermal cells (Marazzi et al., 2007). In some *Solanum* species (Solanaceae) with poricidal anthers, the dehiscence mechanism is related to the stomium and its surrounding cells, which also have thickened walls (García et al., 2008).

Melastomataceae, although considered an important family in number of species, wide geographical distribution and great variation of morphological features (APG III, 2009; Clausing and Renner, 2001; Renner, 1993), are scarcely studied in relation to this interesting anther dehiscence mechanism. The few studies on its representatives have focused on pollen grain (Patel et al., 1984; Cortez et al., 2014) and stamen morphology (Goldenberg et al., 2008), mainly with a taxonomic approach. The exception is the study on anther dehiscence in few species of *Miconia* Ruiz & Pav.: *M. cinnamomifolia* Naudin, *M. latecrenata* Naudin and *M. pusilliflora* Naudin, in which Goldenberg et al. (2003) suggested that dehiscence occurs due to the association between a non-striate epidermis present in the dehiscence region and the intersporangial septum.

The monophyly of Melastomataceae is morphologically supported by anthers that open by pores, and the absence of a characteristic fibrous endothecium (i.e., with secondary thickening) appears to be an important phylogenetic marker (Clausing and Renner, 2001) present in the early diverging lineages of Olisbeoideae and *Pternandra* Jack sp. and absent in all other members. Anther dehiscence has been studied in few species (Wilson et al., 2011), and Melastomataceae are an interesting group to be used as a model for studies such as this one, because it represents one of a few families in which anther dehiscence is poricidal and not associated with a specialized mechanical tissue. Therefore, the aim of this study was to analyze the anther morphology before, during and after dehiscence in 10 non closely related species of *Miconia* (according to the phylogenetic tree proposed by Goldenberg et al., 2008) to elucidate the structural aspects associated with the anther dehiscence mechanism.

Materials and methods

Anther dehiscence investigations were performed using plants of 10 species of *Miconia* (Table 1, Figs. 1a–h) growing in natural populations from the southeast Brazil (Table 1) from 2007 to 2011.

Completely developed stamens, before and after anther dehiscence, were removed from young floral buds and flowers, respectively, of at least five individuals from each species. The samples were fixed for light and electron microscopy in a solution composed of 80 mL L^{-1} glutaraldehyde, 250 mL L^{-1} paraformaldehyde (16%) and 500 mL L^{-1} phosphate buffer (0.1 M, pH 6.8) for 24 h (modified from Karnovsky, 1965).

For light microscopy, the samples were dehydrated through ethanol series (Johansen, 1940) and embedded in glycol methacrylate (Historesin, Leica, Germany) according to the manufacturer's specification. Sections with 3.0 μm thickness were obtained using a rotary microtome (RM2245, Leica, Germany) and stained with toluidine blue at 0.05% in acetate buffer, pH 4.7. To better visualize the structure of the dehiscence region, some sections 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 (Johansen, 1940). A light polarizing filter was applied to better visualize the crystals. Observations and digital images were taken using a light microscope (BX51, Olympus, Japan) with a coupled digital camera (DP70, Olympus, Japan).

The samples for scanning electron microscopy were dehydrated through an ethanol series, critical-point dried using liquid CO_2 (CPD030, BAL-TEC, Liechtenstein), mounted on metallic stubs using double-sided carbon adhesive tape and coated with gold (SCD 050, BAL-TEC, Liechtenstein). Observations and digital images were taken using a scanning electron microscope (JSM-5200, JEOL, Japan) with a coupled digital camera.

Results

Each pre-dehiscent anther is formed by two thecae that are connected by a parenchymatous connective tissue. A vascular bundle is located in the central part of the connective tissue. Each theca consists of two adjacent pollen sacs, which are separated by the intersporangial septum, so the anther is tetrasporangiate (Fig. 2a). The intersporangial septum is parenchymatic with elongated cells, except by a portion near the epidermis in which the cells are smaller, isodiametric and with denser cytoplasm (Fig. 2b).

The pre-dehiscent anther wall is formed by the epidermis, endothecium, one or two middle layers and tapetum (Figs. 2c, d). The anther epidermal cells have a very thick outer periclinal wall, which is covered by a thick and rugose cuticle except in the apical region of the anther (Figs. 2c–f). This region corresponds to the apical pore (Figs. 3a–e). Just before the beginning of dehiscence, the anther wall is restricted to two layers, the epidermis and the endothecium, and the endothecium cell walls lack any thickening even near the pore region (Figs. 3f, g).

Shortly before anther dehiscence, the small and isodiametric cells of the intersporangial septum start degradation (Fig. 4a). The subsequent total septum breakage results in bilocular anthers (Figs. 4b–d).

Anther dehiscence starts 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, those with and those without a cuticle (Figs. 5a–i). Following this initial opening process, the epidermal cells lacking a cuticle located at the pore region degenerate completely leading to poricidal anther dehiscence (Figs. 6a–j). At the apical pore, the two locules converge in a unique chamber allowing all pollen grains to be released through a single opening (Fig. 6f).

In all developmental stages of the anther, few starch grains and druse crystals were observed inside the connective cells.

Discussion

The structural features observed in the selected *Miconia* species (Goldenberg et al., 2003; present study) show that the anther dehiscence mechanism in Melastomataceae differs from that of other taxa with poricidal anthers (see Bonner and Dickinson, 1989; Fahn, 1990; García et al., 2008; Hermann and Palser, 2000; Keijzer et al., 1996, 1987a; Marazzi et al., 2007). It is surprising that although most *Miconia* species are apomictic (Caetano et al., 2013; Cortez et al., 2012; Goldenberg and Shepherd, 1998; Goldenberg and Varassin, 2001; Santos et al., 2012), which means that fertilization is not necessary to produce viable embryos, anther dehiscence still occurs, even when

Table 1
Species analyzed of Miconia and its phylogenetic position on Miconieae tribe.

Phylogenetic position according to Goldenberg et al. (2008)	Species	Collection area in Brazil	Accession number
Miconia IV + Ossaea	*M. albicans*[2] (Sw.) Triana	Campinas, Itirapina, Ubatuba-SP	P.A. Cortez et al. s/n (UEC 162376)
	M. minutiflora[3] (Bonpl.) DC.	Itirapina-SP	P.A. Cortez et al. s/n (UEC 162375)
	M. pepericarpa[3] DC.	Itirapina-SP	P.A. Cortez et al. s/n (UEC 162384)
	M. stenostachya[2] DC.	Itirapina-SP	P.A. Cortez et al. s/n (UEC 162382)
	M. fallax[2] DC.	Itirapina-SP	P.A. Cortez et al. s/n (UEC 162378)
Miconia V	*M. chamissois*[2] Naudin	Itirapina-SP	A.P.S. Caetano et al. (UEC 178717)
	M. sellowiana[4] Naudin	Jundiaí-SP	P.A. Cortez et al. s/n (UEC 162380)
Not evaluated	*M. paucidens* DC.	Itirapina-SP	P.A. Cortez et al. s/n (UEC 162385)
	M. leucocarpa DC.	Itirapina-SP	P.A. Cortez et al. s/n (UEC 162377)
	M. pseudonervosa Cogn.	Itirapina-SP	M.K. Caddah 787 (RB, UPCB)

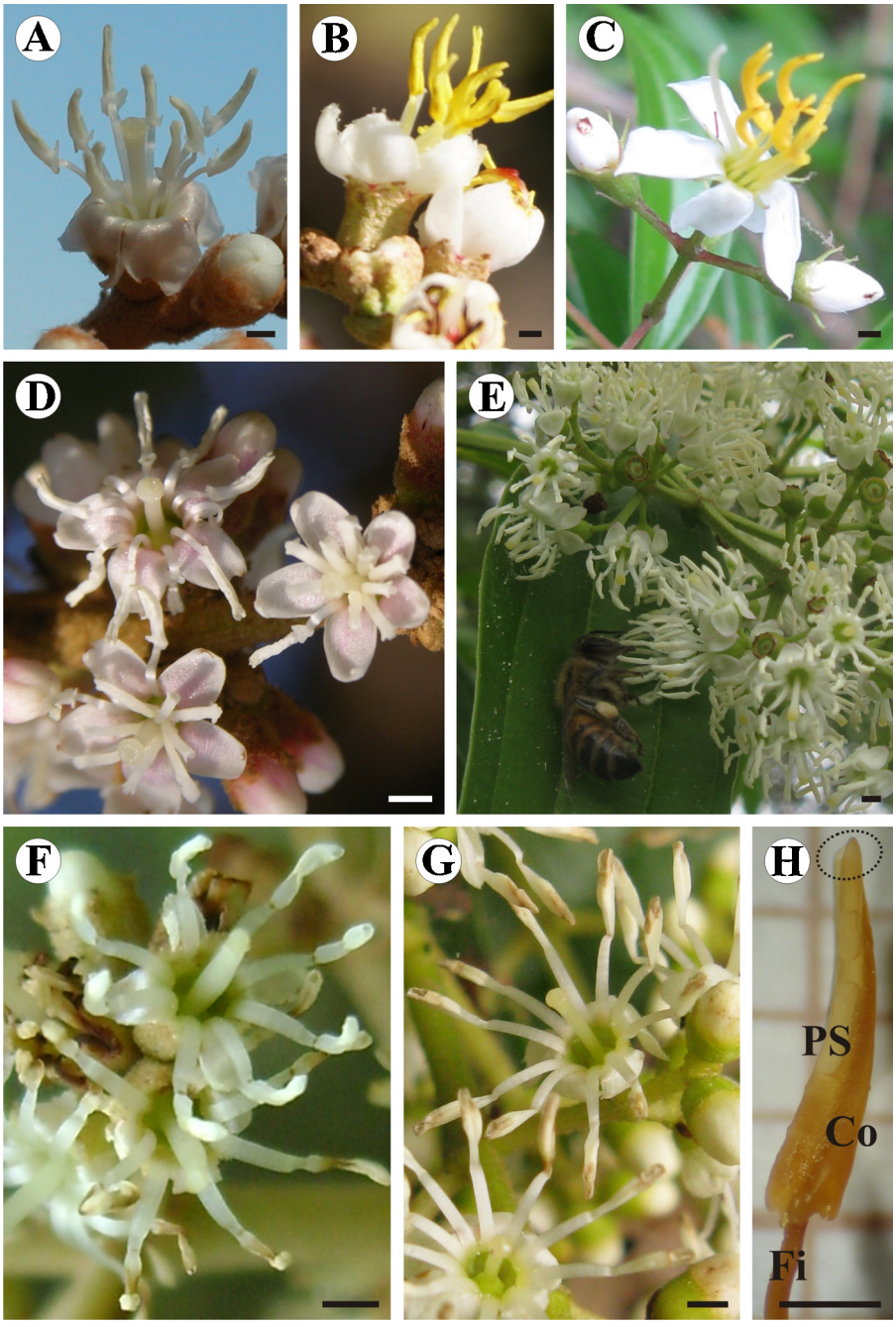

Fig. 1. Stamens from flowers of some *Miconia* species selected for this study. (A) *Miconia albicans*. (B) *M. fallax*. (C) *M. paucidens*. (D) *M. leucocarpa*. (E) *M. minutiflora*. (F) *M. pepericarpa*. (G) *M. sellowiana*. (H) *M. stenostachya*. Co = connective. Fi = filament. PS = pollen sac. Ellipse = anther apical pore. Scale bars: 1 mm.

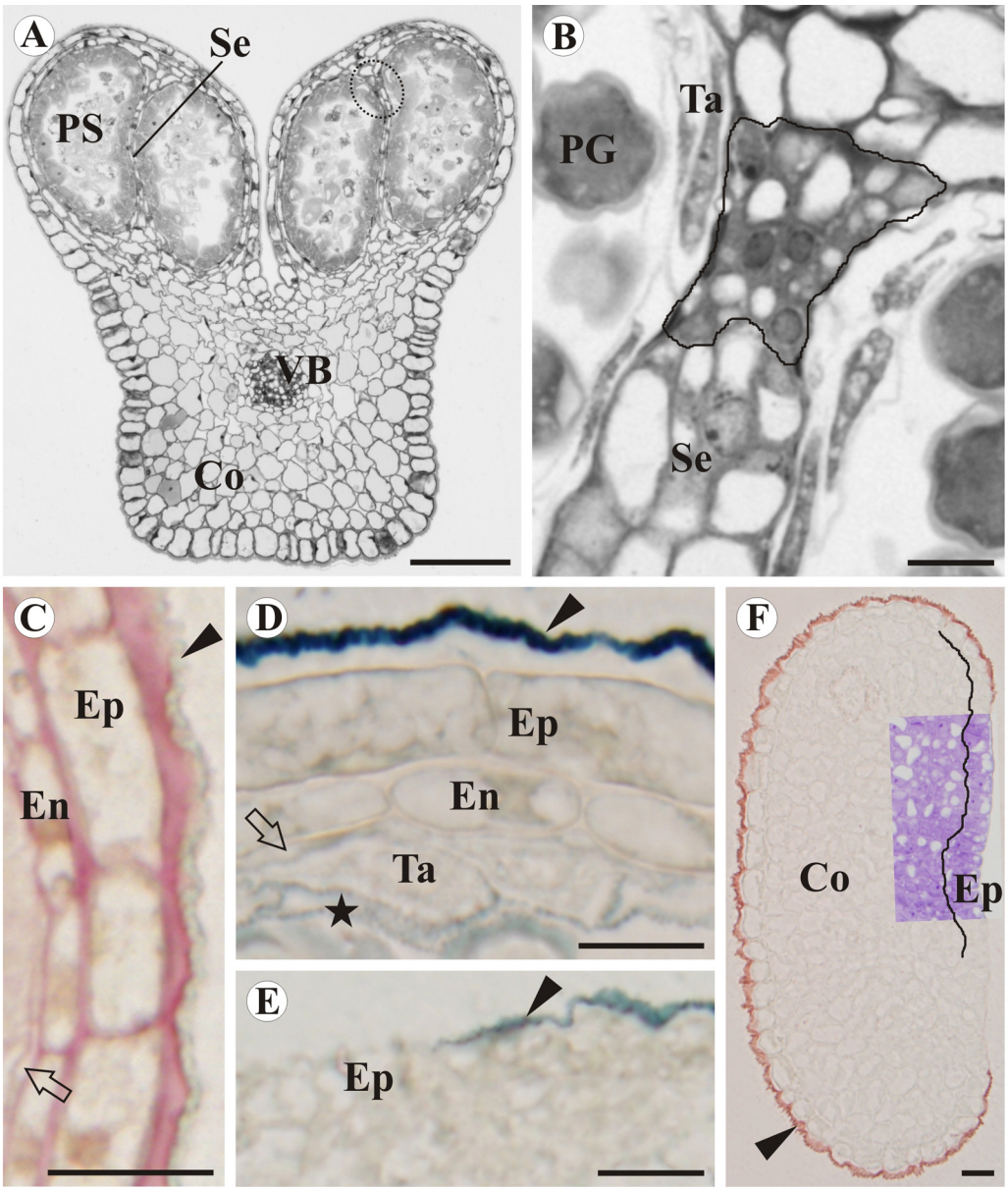

Fig. 2. Transverse sections of a *Miconia* immature anther under light microscopy. (A) Bilobed anther with parenchymatic connective (Co) and a single central vascular bundle (VB). Each theca bears two pollen sacs (PS) which are separated by a parenchymatous septum (Se), in which a portion (ellipse) can be detached by its cells, that are smaller and with denser cytoplasm. (B) Septum (Se) portion with cells that can be distinguished from others by their smaller size and denser cytoplasm. These cells, at the marked area, are responsible for the septum breakage. PG = pollen grain. Ta = tapetum. (C, D) Anther wall composed of epidermis (Ep), endothecium (En), middle layer (leaked arrow) and tapetum (Ta). Orbicules (star) can be observed attached to the inner cell walls of the tapetum. (E, F) Anther apex showing the connective tissue and the rugose cuticle (arrowhead), which is absent at the pore epidermis (Ep). Ruthenium red in (C), Sudan Black B in (D, E), and Sudan IV in (F). (A) *Miconia albicans*. (B–E) *M. paucidens*. (F) *M. stenostachya*. Scale Bars: 50 μm in (A); 10 μm in (B–F).

viable pollen grains are not produced and mature anthers are empty (personal observation). These data indicate that the poricidal mechanism is independent of pollinator action and pollen production.

The mechanism of anther dehiscence in the studied species of *Miconia* can be summarized in three stages: the intersporangial septum breakage, the gradual separation of the border region between the groups of epidermal cells with and without a cuticle and the subsequent and complete degeneration of the pore epidermal cells that lack cuticle. In general, the last stage occurs during or after anthesis, although anther opening was already observed within some floral buds. Dehiscent anthers before anthesis is observed in some angiosperm species (Keijzer, 1987a), and the implications of this observation for the reproductive mechanism are not yet understood.

Septum breakage

The first step of anther dehiscence, intersporangial septum breakage, is an important process because it allows pollen grains to form in the two microsporangia of each theca released once again through the same unique apical pore. This process is commonly observed in anthers of angiosperms with different types of dehiscence (Keijzer, 1987a; Venkatesh, 1955; D'Arcy, 1996; Nelson et al., 2012) and is a consequence of enzymatic cell lysis, most likely related to a programmed cell death mechanism (Sanders et al., 2005). The concept that pollen enlargement can contribute to or even cause septum breakage is doubtful as discussed by Keijzer et al. (1996), who suggested that at least the final push for septum breakage is given by the pollen grain enlargement, a phenomenon not observed in *Miconia* members.

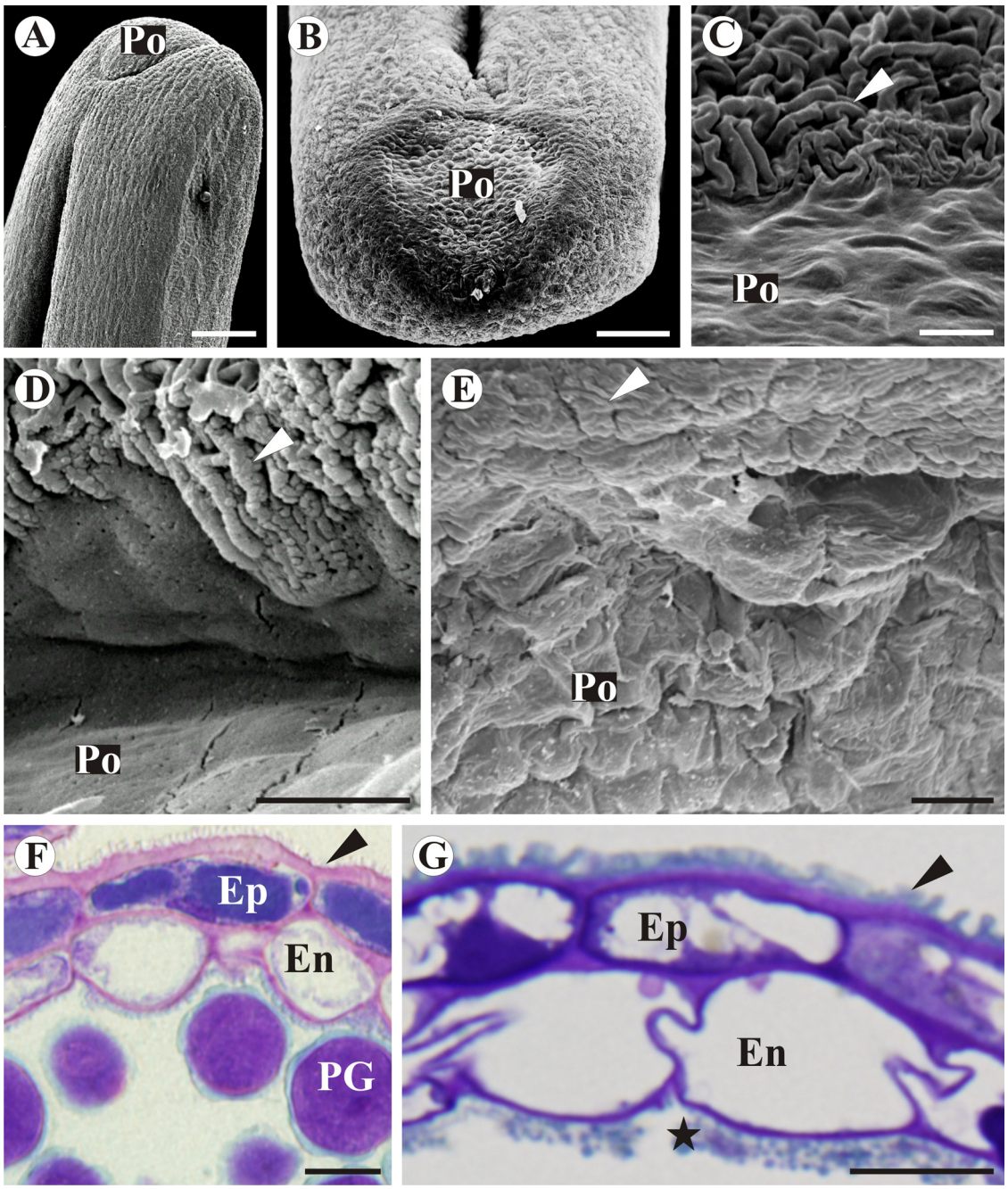

Fig. 3. Pre-dehiscent mature anthers in *Miconia*. (A–E) SEM micrography of anther apex, showing the well delimited pore (Po), whose epidermal cells lack a cuticle. Note the rugose cuticle (arrowhead) covering the remainder of the anther epidermal cells. (F, G) Transverse section under light microscopy showing the anther wall composed of epidermis (Ep) and endothecium (En). Note the rugose cuticle (arrowhead) covering the epidermal cells, the endothecium, which lacks wall thickening, and the orbicules (star). (A, G) *Miconia paucidens*. (B) *M. albicans*. (C) *M. chamissois*. (D) *M. fallax*. (E) *M. pseudonervosa*. (F) *M. pepericarpa*. Scale bars: 30 μm in (A, B); 50 μm in (C); 10 μm (D–G).

In *Miconia* species, septum breakage initiates after the degradation of a group of cells that are specified early in anther development characterized by small size and a large nucleus. Therefore, the initiation of septum breakage is clearly not a consequence of mechanical forces but rather of cell lysis, because the absence of endothecium and empty anthers formed due to total pollen abortion still result in total septum disruption (Cortez et al., 2014). Interestingly, the presence of these cells along the entire length of the anther allows septum degradation to occur from the tip to the basal portions of the anther, and all pollen grains, even those formed in the most basal part of the anther, are released through the apical pore.

This mechanism is especially important for *Miconia* species, in which the anthers are very long and the pollen release depends on buzz-pollinating bees (Renner, 1989).

Pore opening

It is worth noting that *Miconia* species showed no other specialization besides the absence of a cuticle in the epidermal cells of the anther pore region. This fact demonstrates the fundamental role of the epidermis in the anther dehiscence of *Miconia* species (Goldenberg et al., 2003; present study), which has not been found for other angiosperm groups. As also previously described in

Miconia cinnamomifolia, *M. latecrenata* and *M. pusilliflora* (Goldenberg et al., 2003), an epidermal layer in the apical region of the anther (=pore) contradicts Clausing and Renner's statement (2001, pages 495 and 496) 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 in the epidermis from the pore region is responsible for anther dehiscence in *Miconia*, because the cuticle provides mechanical strength and rigidity to the cell, especially after anther exposure to the low humidity of the environment outside the flower. Thus, we can functionally compare the pore region of *Miconia* species to the stomium found in anthers of *Solanum esculentum* (Bonner and Dickinson, 1989), which breaks open because it is composed of more fragile epidermal cells compared to other cutinized epidermal cells. Therefore, the mechanism that ultimately leads to the pore opening in *Miconia* species depends on specific tissue desiccation after anthesis, and the dehiscence mechanism is a consequence of more complex processes.

The dehydration of specific cells is one but not the only event leading to anther opening; however, this process occurring during the anthesis is essential for dehiscence as discussed by Keijzer (1987a, 1996) and Pacini et al. (2006). In *Miconia* species, differential dehydration between the two portions of the anther occurs, and the more fragile epidermal cells of the pore region, which are not covered by a cuticle, disrupt anther opening. Moreover, physiological studies are needed to investigate whether anther dehiscence also depends on more complex mechanisms of water reabsorption in the connective and filament tissues (see Bonner and Dickinson, 1990; Matsui et al., 2000; Stadler et al., 1999), because we observed that some anthers in all species studied here were open before anthesis, i.e. at a time when the anthers were not yet exposed to the dissecting environment. This type of question is interesting for

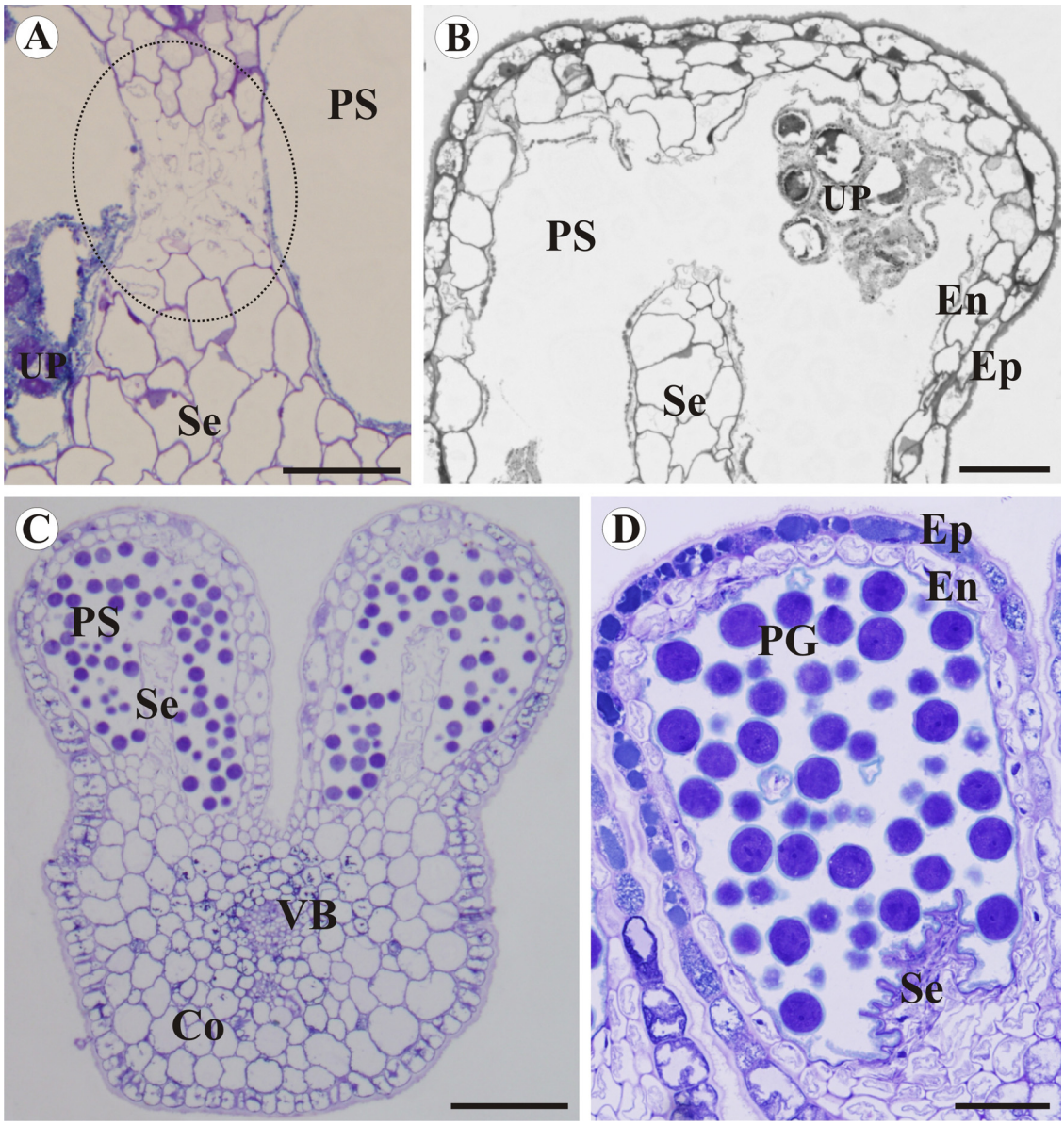

Fig. 4. Transverse sections of pre-dehiscent mature anthers in *Miconia* under light microscopy. (A–C) First steps of septum (Se) degradation. Note that cell lysis starts at the differentiated cells (ellipse) and progress through the entire septum. Abbreviations: Co = connective, En = endothecium, Ep = epidermis, PS = pollen sac, UP = unviable pollen grain, VB = vascular bundle. (D) After total septum (Se) breakage, the theca has a single pollen sac full of pollen grains (PG). Abbreviations: En = endothecium, Ep = epidermis. (A, B) *M. albicans*. (C) *M. paucidens*. (D) *M. pepericarpa*. Scale bars: 20 μm in (A, B, D); 100 μm in (C).

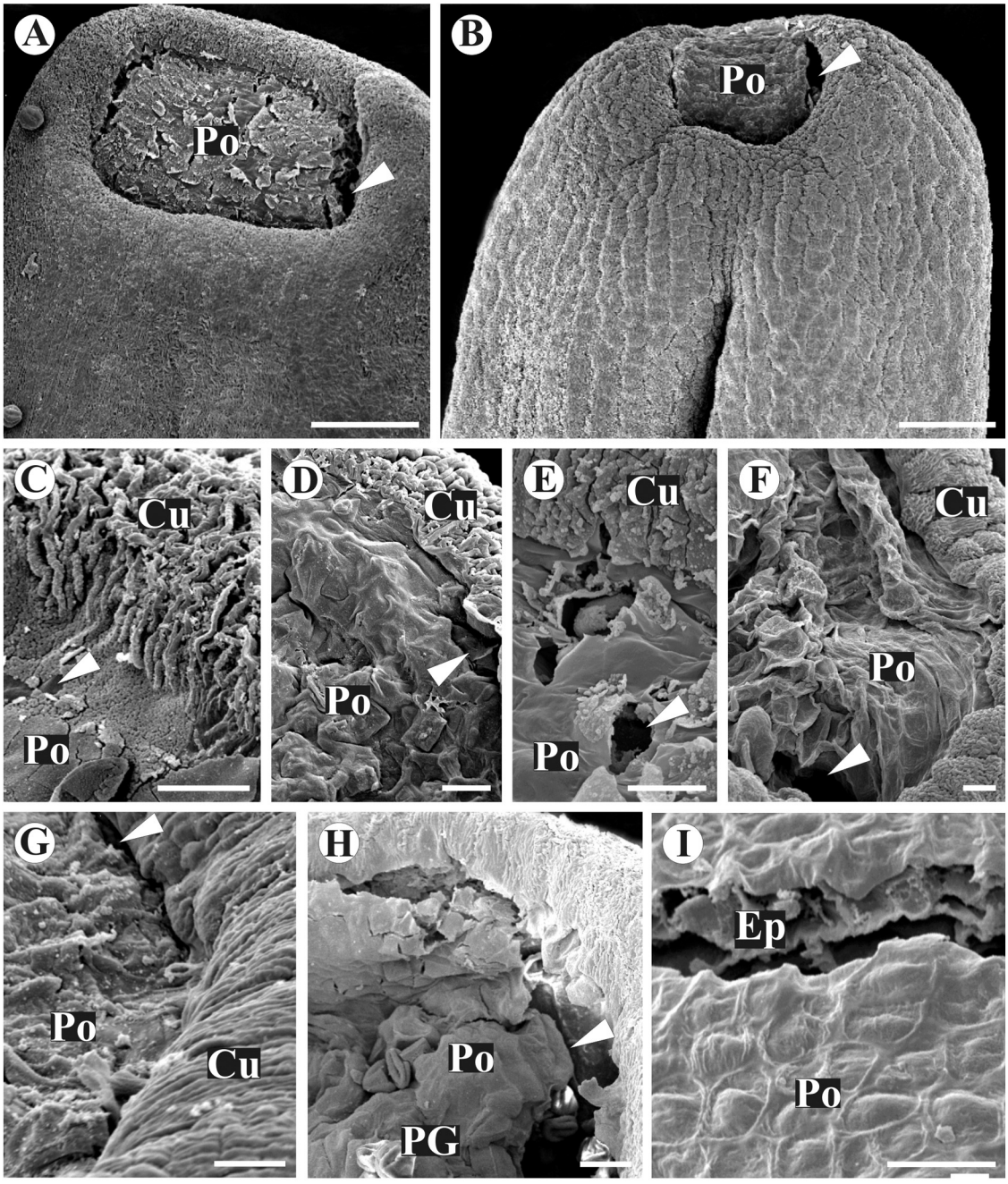

Fig. 5. Dehiscent mature anthers in *Miconia* under scanning electron microscopy. (A–I) Note that the pore opening starts from the epidermal cells located at the region between the portion with and without cuticle (arrowhead). Abbreviations: Cu = cuticle, Ep = epidermis, PG = pollen grain, Po = pore. (A, C, D) *M. fallax*. (B, E) *M. chamissois*. (F) *M. minutiflora*. (G) *M. pseudonervosa*. (H) *M. stenostachya*. (I) *Miconia paucidens*. Scale Bars: 100 μm in (A, B); 10 μm in (C–I).

a group with long anthers in which no structurally differentiated supporting tissue or cells were observed.

Pollenkitt and orbicules

It could be expected that poricidal anthers, which exhibit a small surface for pollen exposure, should contain features for facilitating pollen release and dispersal, such as the absence of a substance that adheres the pollen to the anther wall. Surprisingly, pollenkitt was observed covering the pollen grains in *Miconia* species (Cortez et al., 2014) and also in the poricidal Ericaceae (Pacini and Hesse, 2005). Therefore, pollenkitt must not be associated with anther dehiscence but rather with other roles for pollen dispersal such as protecting pollen against water loss, UV radiation, fungi/bacteria and hydrolysis and exocellular enzymes, conserving sporophytic proteins inside exine cavities and keeping pollen grains together during presentation and transport (Pacini and Hesse, 2005).

Orbicules also occur in *Miconia* species, even in sterile anthers in which the pollen grain wall is not properly formed (Cortez et al., 2012, 2014). They may facilitate pollen grain dispersal because they form a non-wettable surface inside the locule (Heslop-Harrison, 1968; Heslop-Harrison and Dickinson, 1969; Keijzer, 1987b). Such structures are commonly observed in anthers with secretory tapetum and have an important role in pollen wall structure (Bhandari, 1984).

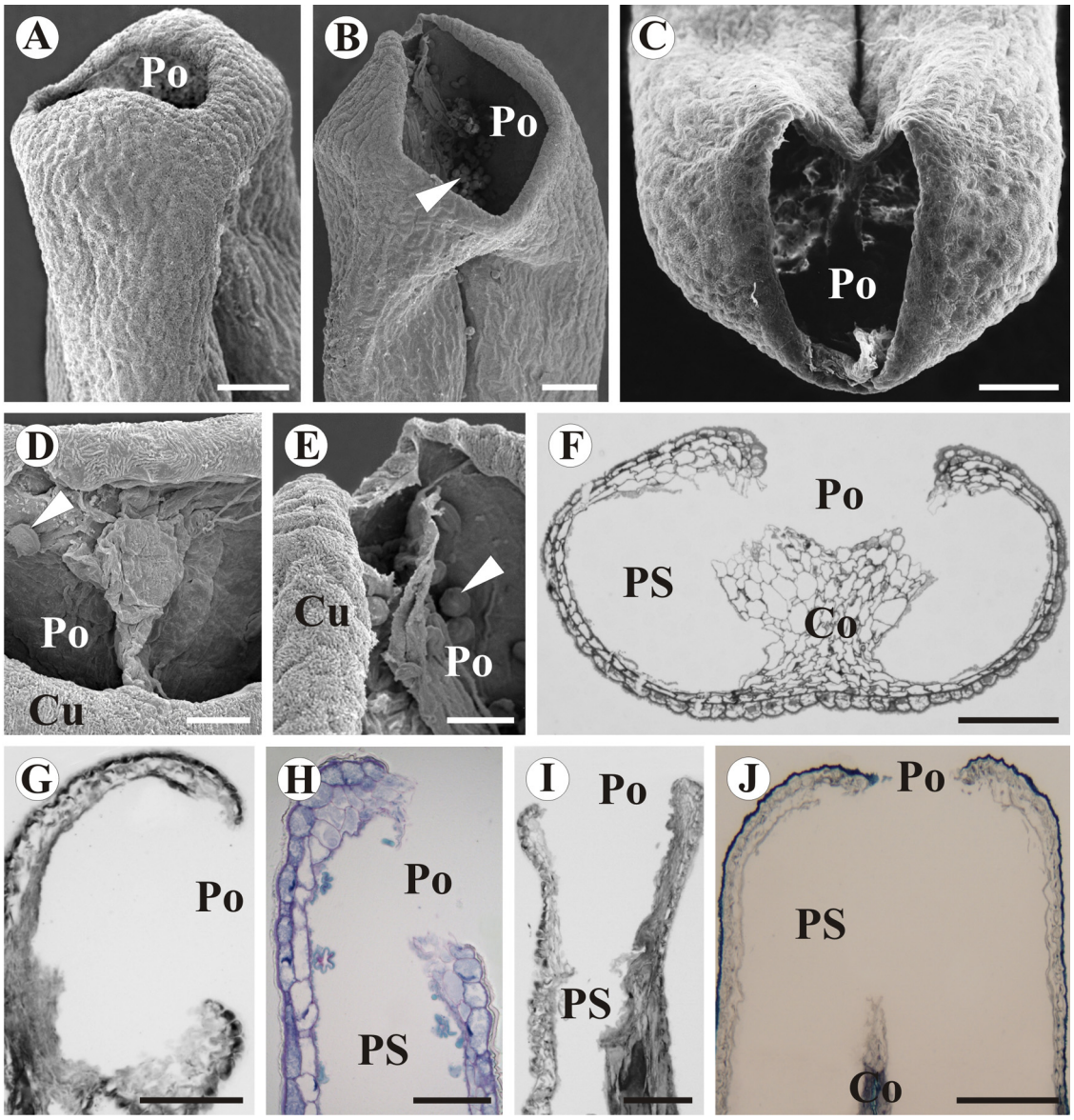

Fig. 6. Dehiscent mature anthers in *Miconia* after pollen release. (A–E) Anther apex with completely open pore (Po) under scanning electron microscopy. Note that the epidermal cells with cuticle (Cu) are kept intact even after pollen grains (PG, arrowhead) are released. (F) Anther apex transverse section. (G–J) Anther apex longitudinal sections under light microscopy. Note that at the pore region, the two pollen sacs (PS) converge in a single chamber where the pollen grains are released through the pore (Po). Abbreviation: Co = connective. (A, E) *Miconia chamissois*. (B) *M. minutiflora*. (C, F) *M. albicans*. (D, G) *M. stenostachya*. (H) *M. leucocarpa*. (I) *M. fallax*. Scale bars: 100 μm in (A–C); 30 μm in (D, E); 50 μm in (F–J).

The roles of orbicules and pollenkitt in *Miconia* pollen release, dispersal and deposition on the stigma deserve more accurate studies.

Endothecium

A typical fibrous endothecium is not found in the clade formed by Alzateaceae, Crypteroniaceae, Melastomataceae and Penaeaceae, but it is found in Olisbeoideae and *Pternandra* (Melastomataceae) (APG III, 2009; Clausing and Renner, 2001; Conti et al., 1997). The present study demonstrates that in *Miconia* species, the anther dehiscence mechanism is independent of any type of fibrous cells functioning as a mechanical layer. The endothecium is a specific anther subdermal layer in which cells are commonly characterized by some type of wall thickening, often lignified (Bandhari, 1984), and it is usually related to the longitudinal dehiscence mechanism. Even in those species with no endothecium, some type of mechanical tissue, i.e., tissue with thickened cell walls, is related to dehiscence, as observed for the poricidal anthers of *Senna* (Marazzi et al., 2007) and some *Solanum* (García et al., 2008) species. The fibrous endothecium is the mechanical layer exerting opposite forces that open the anther and expose the pollen grains to pollinators (Keijzer, 1987a; Keijzer et al., 1996; Matsui et al., 1999). In *Miconia* species, the only mechanical structure leading to the anther aperture is the cuticle, which is responsible for producing the forces that lead to pore epidermis breakage. If this mechanism occurs in all Melastomataeae, it will be an interesting point for ecological and evolutionary discussions and depends on further studies, with broader sampling, considering that this group comprises approximately 5000 species (Penneys, 2014).

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