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Structure and evolution of polysporangiate anthers in Melastomataceae

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ABSTRACT

Polysporangiate anthers, i.e., anthers bearing more than four sporangia, are an unusual condition that has been reported in several angiosperm families, including Melastomataceae. The structure of anthers in Melastomataceae and taxa from its sister lineage was investigated in 302 species, which were categorized as polysporangiate or non-polysporangiate based on original and literature data. Additionally, the evolutionary history of the anther type in the family was assessed by stochastic character mapping. The polysporangiate anthers of Melastomataceae exhibit two corrugated thecae with transverse septa of parenchymatous tissue partitioning the four pollen sacs into several small locules, this being the most common type of sporangia multiplication among angiosperms. Despite the fact that non-polysporangiate anthers are the most common character-state observed and have been reconstructed in most ancestral nodes of the phylogeny, a relatively large number of transitions to polysporangiate anthers were estimated in our analyses. One event was detected in *Microlicia*. Most species with polysporangiate anthers are the most common condition among angiosperms, dithecal polysporangiate anthers are the most common condition among angiosperms, dithecal polysporangiate anthers are the most common condition among angiosperms, dithecal polysporangiate anthers occur in at least 74 taxa of Melastomataceae, in a unique association with poricidal dehiscence.

1. Introduction

The stamen is the pollen-bearing organ of flowers (Endress, 1996). It frequently consists of a filament and an anther, which is the site of microsporogenesis and pollen grain development (Scott et al., 2004). The tetrasporangiate arrangement of the anther is relatively uniform within angiosperms, with four microsporangia, or pollen sacs, organized pairwise into two thecae (Endress, 2011). However, two pollen sacs commonly appear in transverse view at anthesis due to confluence of the paired locules (Endress, 1996). Despite the stability of this morphological feature, another type of anther regarding the number of sporangia, polysporangiate or multisporangiate anthers (= with more than four sporangia), has been reported in less than 10 % of angiosperm species belonging to at least 24 flowering families (Endress and Stumpf, 1990; Tsou and Johnson, 2003).

Polysporangiate anthers exhibit an athecal organization when the anther subdivision into two thecae is not apparent (Endress and Stumpf, 1990; Amaral et al., 2017). However, these anthers frequently retain a dithecal organization with transverse and sometimes also longitudinal septa of sterile tissue partitioning the four pollen sacs into numerous sporangia (Endress and Stumpf, 1990; Tsou and Johnson, 2003). The septa that divide the loci are lined with tapetal cells that may include some parenchymal tissue (*"parenchymatous septum"*) or not (*"tapetal septum"*) (*sensu* Tobe and Raven, 1986). Polysporangiate anthers are frequently associated with dehiscence through longitudinal slits (Endress and Stumpf, 1990), but in Melastomataceae they are found

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Fig. 1. Light microscopy of longitudinal sections of polysporangiate anthers of Melastomataceae species. A, Meriania tomentosa. B, Miconia aliquantula. C, Miconia alternidomatia. D-E, Miconia cercophora. White arrows indicate the septa between adjacent sporangia and black arrows indicate druse crystals in septa, anther wall, and connective cells.

along with poricidal dehiscence (Baumgratz et al., 1996; Lima et al., 2019).

Melastomataceae is a megadiverse family of Myrtales, comprising *c*. 5.100 species, most of them exhibiting anthers with poricidal dehiscence (Renner, 1989; MELNet, 2007). Its members have predominantly dithecal and tetrasporangiate anthers that become bilocular at the final stages of development (Tobe and Raven, 1983a; Renner, 1993; Medeiros and Roos, 1996; Cortez et al., 2015). Polysporangiate anthers have been reported in species of *Microlicia* (Microlicieae) (Baumgratz et al., 1996; Lima et al., 2019) and *Meriania* (Merianieae) (Mendoza-Cifuentes and Fernández-Alonso, 2010; but see the discussion). Among other groups in the family, this condition is still unknown.

Although androecium morphology has historically provided useful taxonomic characters for Melastomataceae (Triana, 1872; Jacques-

Félix, 1981; Renner, 1993; Clausing and Renner, 2001; Goldenberg et al., 2003, 2008; Mendoza-Cifuentes and Fernández-Alonso, 2010; Michelangeli et al., 2011, 2013), its anatomical features are still largely undescribed for most species. Little is known about the actual distribution of polysporangiate anthers across the family, and how many times such feature has evolved in this clade remains unclear. Thus, the objective of the present study was to provide an extensive account of the structure of polysporangiate anthers across Melastomataceae and to reconstruct the evolutionary history of this condition in the phylogeny of the family.



Fig. 2. Light microscopy of longitudinal sections of polysporangiate anthers of Melastomataceae species. A, Miconia donaeana. B, Miconia mayeta. C-D, Miconia stenopetala. White arrows indicate the septa between adjacent sporangia and black arrows indicate druse crystals in septa, anther wall and connective cells.

2. Materials and methods

2.1. Categorization of the anther type and structure of polysporangiate anthers

Anthers of 145 species from different lineages of Melastomataceae were anatomically investigated and categorized according to sporangia number as non-polysporangiate (two or four pollen sacs) or polysporangiate (more than four pollen sacs) (Supplementary material). Additionally, data for 148 other species of Melastomataceae and nine taxa from its sister lineage, a clade consisting of the families Crypteroniaceae, Alzateaceae and Penaeaceae (Conti et al., 2002), were obtained from literature reports (Supplementary material), totalizing 302 species.

The number of sporangia was obtained by anatomical studies of predehiscent mature anthers cut into longitudinal sections. Anthers were either obtained from fresh material fixed in FAA (50 % ethanol, 37 % formaldehyde and glacial acetic acid, 18:1:1, v/v) (Johansen, 1940) or from rehydrated herbarium specimens (Smith and Smith, 1942) (Supplementary material). Anthers were dehydrated in an ethanol series,



Fig. 3. Longitudinal view of polysporangiate anthers of Melastomataceae species. A, Scanning electron microscopy of *Microlicia euphorbioides*. B-C, Light microscopy of *Microlicia fasciculata*. D, Light microscopy of *Microlicia polystemma*. White arrows indicate the septa between adjacent sporangia.

embedded in historesin (Leica Microsystems and Heraeus Kulzer GmbH, Germany) and longitudinally sectioned with an AO Spencer 820 (GMI Inc. Minnesota, US) or RM 2245 (Leica, Germany) rotary microtome with a steel blade. Five-µm thick sections were adhered to glass slides and stained with 0.05 % toluidine blue in 0.01 M phosphate buffer, pH 6.8 (O'Brien et al., 1964). Observations were carried out and digital images were obtained using a DM 5000 light microscope (Leica Microsystems, Heidelberg, Germany) coupled with a DFC 390 digital camera (Leica Microsystems, Heidelberg, Germany) or with a Zeiss Axioplan compound microscope equipped with a Nikon DXM1200C digital camera.

A scanning electron microscopy analysis was performed using polysporangiate anthers of *Microlicia euphorbioides*, in order to illustrate the micromorphology of this anther type in the family. Anthers were dehydrated in an ethanol series, critical point dried using liquid CO_2 on a CPD030 (Bal-Tec AG, Balzers, Liechtenstein) apparatus, adhered to metal stubs using carbon adhesive tape, and coated with gold with a SCD050 (Bal-Tec AG, Balzers, Liechtenstein) sputter coater. Observations were carried out and digital images were obtained with a JSM-6610LV (JEOL, Japan) scanning electron microscope at 15 kV.

2.2. Phylogenetic hypothesis

The most recent and comprehensive phylogenetic hypothesis for Melastomataceae available in the literature (Reginato et al., 2020) was used to base our analyses of ancestral character estimation. Briefly, this phylogeny included all sequences of Melastomataceae and its sister CAP clade (Crypteroniaceae + Alzateaceae + Penaeaceae; Conti et al., 1997; Berger et al., 2016) available in GenBank, covering 1684 species (ca. 33 % of accepted species). Sequences were filtered and individual loci aligned and concatenated. Final alignment included 10 plastome markers (*accD-psaI, atpB-rbcL, atpF-atpH, matK, ndhF, psbA-trnH, psbK-psbI, rbcL, rpl16, trnL*, and *trnS-trnG*), two nuclear ribosomal spacers (nrETS and nrITS), and one low copy nuclear marker (*waxy*).



Fig. 4. Morphology of flowers and polysporangiate anthers of Melastomataceae species. A-B, Meriania tomentosa. C-D, Miconia alternidomatia. E-F, Miconia donaeana. (A-B by S. Moreno; C-D by F.A. Michelangeli and E-F by A.S. Dellinger).

Phylogenetic inference was performed with maximum likelihood (ML) estimation plus 1000 rapid bootstraps (–f a) using the GTR + G model in RAxML 8.2.10 (Stamatakis, 2014). The ML tree was time-scaled using secondary calibrations with penalized likelihood (for calibration details see Reginato et al., 2020) implemented in treePL 1.0 (Smith and O'Meara, 2012). The original phylogenetic tree was pruned to match the anther type data set with the drop.tip function of the R Package ape 5.3 (Paradis et al., 2004). The pruned tree included 152 terminals.

2.3. Ancestral character estimation

The evolutionary analysis of the anther type in Melastomataceae was evaluated by stochastic character mapping (Huelsenbeck et al., 2003; Bollback, 2006), implemented in the R package phytools 0.7–20 (Revell, 2012). Given the restricted size of the data set, the simplest model of character evolution was employed ("ER" – Equal rates). A

total of 1000 stochastic maps were generated and summarized using the make.simmap and describe.simmap functions (Revell, 2012). The results were summarized and plotted on the phylogeny using the R package ape 5.3 (Paradis et al., 2004).

3. Results

3.1. Structure of the polysporangiate anthers of Melastomataceae

Polysporangiate anthers were observed in 10 of 145 newly studied species: *Meriania tomentosa* (Merianieae, Fig. 1A), *Miconia aliquantula* (Fig. 1B), *M. alternidomatia*, (Fig. 1C), *M. cercophora* (Fig. 1D, E), *M. donaeana* (Fig. 2A), *M. mayeta* (Fig. 2B) and *M. stenopetala* (Fig. 2C, D) (Miconieae), and *Microlicia euphorbioides* (Fig. 3A), *M. fasciculata* (Fig. 3B, C), and *M. polystemma* (Fig. 3D) (Microlicieae).

In the species with polysporangiate anthers there is a typical



Fig. 5. Polysporangiate anthers in *Microlicia* species. A-B, Flower and anther morphology in *Microlicia noblickii*. C-D, Scanning electron microscopy of *Microlicia euphorbioides*. (A-B by F.A. Michelangeli).

dithecal organization, as illustrated in *Meriania tomentosa* (Fig. 4A, B), *Miconia alternidomatia* (Fig. 4C, D), *M. donaeana* (Fig. 4E, F), *Microlicia noblickiii* (Fig. 5A, B) and *M. euphorbioides* (Fig. 5C, D). This dithecal condition is also found in tetrasporangiate anthers of *Meriania urceolata* (Fig. 6A), *Miconia albicans* (Fig. 6B), *M. octona* (Fig. 6C) and *Microlicia crenulata* (Fig. 6D), which are species related to the polysporangiate taxa.

In polysporangiate anthers the sporangia are subdivided into several small locules by transverse septa consisting of parenchyma cells (Figs. 1A–E, 2 A–D, 3 A–D). *Miconia alternidomatia* (Fig. 1C), *M. cercophora* (Fig. 1D, E) and *M. mayeta* (Fig. 2B) exhibit a markedly corrugated thecal surface, in which the anther wall curves deeply toward the locule, delimiting part of the small sporangium. Druse crystals may occur in the cells of the septa (Figs. 1D, E, 2 A, C), anther wall (Figs. 1D, E, 2 B, C), and connective tissue (Figs. 1E, 2 C). Cellular degeneration occurs in both transverse and intersporangial septa (Fig. 1C–E, 2 A–D), although vestiges remain visible.

3.2. Occurrence and evolution of polysporangiate anthers in Melastomataceae

Most of the 302 species categorized according to sporangia number exhibit non-polysporangiate anthers (original data and literature reports, Supplementary material). Polysporangiate anthers occur in 74 taxa within the genera *Meriania*, *Miconia* and *Microlicia* (Table 1).

Stochastic mapping reconstructed most nodes in the phylogeny as non-polysporangiate, including the ancestral node of Melastomataceae (Fig. 7). The number of transitions from non-polysporangiate to polysporangiate anthers was estimated to be eight, while a single reversal in one lineage of *Microlicia* (see below for further details) from polysporangiate to non-polysporangiate was recovered. Up to now, polysporangiate anthers have been found in three different tribes within Melastomataceae. One event was recovered in *Meriania tomentosa* of the tribe Merianieae and four events were recovered in Miconieae: in *Miconia aliquantula*; in the common ancestor of *M. cercophora* + *M. donaeana*; in *M. stenopetala*; and in the common ancestor of *M. alternidomatia* + *M. mayeta*. In Microlicieae, the results are ambiguous; in one scenario polysporangiate anthers have evolved twice: in a clade with *Microlicia minima* + *M. confertiflora* + *M. amblyseapala* and in *M. graveolens*, with a reversal in *M. confertiflora*; while the other scenario predicts three independent transitions to polysporangiate (in *M. minima*, *M. amblysepala*, and *M. graveolens*).

4. Discussion

4.1. Structure of polysporangiate anthers of Melastomataceae

Polysporangiate anthers are an unusual condition in angiosperms (Endress and Stumpf, 1990) but occur in at least 74 species of Melastomataceae belonging to *Meriania*, *Miconia* and *Microlicia*. These species exhibit a typical dithecal organization, corroborating the general scenario suggested for angiosperms (Endress, 2011). The athecal form of polysporangiate anthers has been associated with specialized pollination syndromes (Endress and Stumpf, 1990), such as in some resin flowers of *Clusia* (Amaral et al., 2017). In contrast, a dithecal condition is less restricted and may be related to different types of pollination systems.

In addition, the thecal surface of polysporangiate anthers of Melastomataceae seems to be commonly corrugated. This association has been reported in *Microlicia* (Almeda and Pacifico, 2018; Pacifico



Fig. 6. Morphology of flowers and non-polysporangiate anthers of Melastomataceae species. A, Meriania urceolata. B, Miconia albicans. C, Miconia octona. D, Microlicia crenulata. (A, C-D by F.A. Michelangeli; B by P.A.Cortez).

and Almeda, 2018; Lima et al., 2019) and it was suggested for *Meriania* (Mendoza-Cifuentes and Fernández-Alonso, 2010). Although the corrugated thecal surface has been associated with the polysporangiate condition, such association should be considered with caution since the two states are not always observed together. For example, *Huberia* (Baumgratz, 2004; Bochorny et al., 2019), species of *Graffenrieda* (Goldenberg and Meirelles, 2011; Michelangeli and Ulloa Ulloa, 2013), and some species of *Miconia* (Martins et al., 2009b) may exhibit

stamens with corrugated thecae but with tetrasporangiate anthers.

The presence of multiple sporangia cannot be associated with other anther characteristics that are diverse in Melastomataceae, with different groups exhibiting dimorphic or isomorphic anthers, large basal extensions of the connective (or not), dorsal and ventral appendages (or both or neither) or a rostrate, attenuated or broad apex (Renner, 1989, 1993). This same morphological diversity is present across the species with polysporangiate anthers. For example, dorsal appendages are

Table 1

List of species with polysporangiate anthers in the Melastomataceae. In the "Reference" column, newly generated records are indicated by "This study", while literature records are indicated by their original publication. A list including all studied taxa is available in Supplementary material. *presumably polysporangiate anthers, since it was assumed due to the presence of a corrugated thecal surface, and not direct observation.

Tribe	Species	Reference
Merianieae	Meriania boliviensis Cogn.*	Mendoza-Cifuentes and Fernández-Alonso, 2010
	Meriania vargasii Wurdack*	Mendoza-Cifuentes and Fernández-Alonso, 2010
	Meriania weberbaueri J.F.Macbr.*	Mendoza-Cifuentes and Fernández-Alonso, 2010
Miconieae	Miconia aliquantula Wurdack	This study
	Miconia alternidomatia Michelang.	This study
	Miconia cercophora Wurdack	This study
	Miconia donaeana Naudin	This study
	Miconia mayeta (D.Don.) Michelang.	This study
	Miconia stenopetala Griseb.	This study
	Microlicia abairana R.Romero & Woodgyer	Romero and Woodgyer, 2018
		(continued on next page)

Table 1 (continued)

Tribe	Species	Reference
Microlicieae	Microlicia acuminata Naudin	Flora do Brasil, 2020
	Microlicia amblysepala Ule	Flora do Brasil, 2020
	Microlicia aurea Wurdack	Baumgratz et al., 1996
	Microlicia aureoglandulosa Woodgyer & R.Romero	Romero and Woodgyer, 2018
	Microlicia baccharoides Mart. ex Naudin	Pataro et al., 2017
	Microlicia bahiensis Markgr.	Almeda and Pacifico, 2018
	Microlicia balsamifera (DC). Mart.	Baumgratz et al., 1996
	Microlicia blanchetiana (Naudin) Cogn.	Flora do Brasil, 2020
	Microlicia chausantha Wurdack	Baumgratz et al. 1996
	Microlicia colombiana Humberto Mend. & R. Romero	Mendoza-Cifuentes et al. 2019
	Microlicia comparilis Wurdack	Pataro et al 2017
	Microlicia contasensis Woodgver & Zappi	Woodgver and Zappi, 2009
	Microlicia crispa Woodgyer & R.Romero	Romero and Woodgyer, 2018
	Microlicia cuneata Naudin	Flora do Brasil, 2020
	Microlicia curticalycina R.Romero & Woodgyer	Romero and Woodgyer, 2018
	Microlicia euphorbioides Mart.	Lima et al., 2019; This study
	Microlicia fasciculata Mart. ex Naudin	Baumgratz et al., 1996; This study
	Microlicia flavovirens Woodgyer & Zappi	Woodgyer and Zappi, 2009
	Microlicia ganevii Woodgyer & R.Romero	Romero and Woodgyer, 2018
	Microlicia graveolens DC.	Lima et al., 2019
	Microlicia helvola (Spreng.) Triana	Lima et al., 2019
	Microlicia hirridula Naudia	Pataro et al., 2013
	Microlicia nispiaula Naudin Microlicia incignic Schltdl	Martins et al., 2009a
	Microlicia intercalicina Dataro & P. Domoro	Pataro et al. 2012
	Microlicia isostemon Wurdack	Baumgratz et al. 1996
	Microlicia latifolia D O Diniz-Neres & M J Silva	Diniz and Silva 2018
	Microlicia leucopetala Wurdack	Pataro et al., 2017
	Microlicia longirostrata R.Romero, Fontelas & Versiane	Romero et al., 2019
	Microlicia longisepala Wurdack	Pataro et al., 2017
	Microlicia luetzelburgii Markgr.	Baumgratz et al., 1996
	Microlicia lutea Markgr.	Baumgratz et al., 1996
	Microlicia macedoi L.B.Sm. & Wurdack	Flora do Brasil, 2020
	Microlicia macropetala Pataro & R.Romero	Pataro et al., 2013
	Microlicia maximowicziana Cogn.	Romero and Woodgyer, 2011
	Microlicia minima Markgr.	Baumgratz et al., 1996
	Microlicia monitola Wurdack	Baumgratz et al., 1996
	Microlicia neglecta Cogn	Pataro et al. 2017
	Microlicia noblickii (Wurdack) A B Martins & Almeda	Almeda and Martins 2001
	Microlicia nodotricha Almeda & R.Pacifico	Almeda and Pacifico, 2018
	Microlicia ordinata (Wurdack) Almeda & A.B.Martins	Almeda and Martins, 2001
	Microlicia oxyanthera Naudin	Koschnitzke and Martins, 2007
	Microlicia parvula (Markgraf) Koschnitzke & A.B.Martins	Baumgratz et al., 1996
	Microlicia pilosissima Cogn.	Flora do Brasil, 2020
	Microlicia pinheiroi Wurdack	Pataro et al., 2017
	Microlicia polystemma Naudin	This study
	Microlicia pulchra Pataro & R.Romero	Pataro et al., 2013
	Microlicia ramosa Pilger	Versiane et al., 2016
	Microlicia reicharatiana Cogn.	Rodrigues, 2005
	Microlicia retundifolia Illo	Almeda and Martins 2012
	Microlicia setosa (Spreng) DC	Flora do Brasil 2020
	Microlicia sincorensis (DC.) Mart	Baumgratz et al 1996
	Microlicia stenodonoides D.O.Diniz-Neres & M.J.Silva	Diniz-Neres and Silva, 2017
	Microlicia subaequalis Wurdack	Baumgratz et al., 1996
	Microlicia subalata Wurdack	Baumgratz et al., 1996
	Microlicia subsetosa DC.	Baumgratz et al., 1996
	Microlicia sulfurea Hoehne	Flora do Brasil, 2020
	Microlicia taxifolia Naudin	Flora do Brasil, 2020
	Microlicia torrendii Brade	Pataro et al., 2017
	Microlicia wurdackiana Almeda & A.B.Martins	Almeda and Martins, 2012

present in *Meriania* (Fig. 4A, B) but absent in *Miconia* (Figs. 4C–F, 6 B, C), whereas in *Microlicia* appendages are present but ventral (Fig. 6D). Similarly, species of *Microlicia* have rostrate anthers with a pedoconnective and are usually dimorphic, while the polysporangiate species *Miconia* and *Meriania* lack these characters.

The formation of polysporangiate anthers due to sporangia separation by transverse septa, as found in Melastomataceae, is the simplest and most common type of sporangia multiplication (Endress and Stumpf, 1990). The parenchymatous nature of the transverse septa in mature polysporangiate anthers allows us to classify it as a parenchymatous septum (*sensu* Tobe and Raven, 1986) as previously described in *Microlicia* (Lima et al., 2019), in other Myrtales (Tobe and Raven, 1984b; Tobe and Raven, 1986) and, even, in other angiosperm taxa (Tsou and Johnson, 2003; González and Rudall, 2010). Parenchymatous septa persist during pollen development until anther dehiscence but are already in a degradation process at the final stages of anther development (Tobe and Raven, 1986; Tsou and Johnson, 2003; Lima et al., 2019), as observed in the present study. On the other hand,



Fig. 7. Stochastic mapping of anther type (polysporangiate *x* non-polysporangiate) onto the resulting phylogenetic tree of Melastomataceae from the maximum likelihood estimation. Red corresponds to the non-polysporangiate status, and blue to the polysporangiate status (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

tapetal septa composed only of tapetal cells that degenerate during pollen grain development are totally absent in mature anthers (Tobe and Raven, 1986; Tsou and Johnson, 2003). Tapetal septa have not been observed in Melastomataceae, although they have been described in Onagraceae, another member of the Myrtales (Tobe and Raven, 1986).

4.2. Evolution of polysporangiate anthers in Melastomataceae

Despite the fact that non-polysporangiate anthers are the most common character-state observed in Melastomataceae and have been reconstructed in most ancestral nodes of the phylogeny, a relatively high number of transitions to polysporangiate anthers were estimated in our analyses. However, the actual number of times this feature evolved in the family is surely higher, since our sampling is still sparse especially when coupled with phylogenetic information.

At least one species in the tribe Merianieae, Meriania tomentosa, is polysporangiate, but polysporangiate anthers may occur in other species of the genus. Mendoza-Cifuentes and Fernández-Alonso (2010) hypothesized that M. boliviensis, M. vargasii and M. weberbaueri exhibit this condition, presumably due to the presence of a corrugated thecal surface, however, as discussed in the previous item, this is not infallible. In Miconia, polysporangiate anthers have arisen at least four times, in a total of six species. Most species with polysporangiate anthers belong to Microlicia in which, thus far, 64 out of 128 species categorized have been found to present this character-state (Table 1), representing approximately 41 % of the genus (Flora do Brasil, 2020). Although ambiguous, our analyses indicated that one reversal from polysporangiate to tetrasporangiate might have occurred within Microlicia. The scarcity of molecular data for this genus restricted the number of species sampled in our analyses. However, the high frequency of species with polysporangiate anthers in Microlicia indicates that this specialized structure may be more labile in this clade and probably plays an important role in the floral evolution of this genus.

This phylogenetic lability of polysporangiate anthers in Melastomataceae indicates a low phylogenetic signal, as expected for adaptive traits (Blomberg et al., 2003). Indeed, different roles have been proposed for polysporangiate anthers in the family, including pressures from both pollination and developmental processes. Baumgratz et al. (1996) suggested that the polysporangiate anthers of buzz-pollinated flowers might have an adaptive value. Such hypothesis is based in the idea that the septa modify the pattern of pollen grain release, partitioning the grains along subsequent visits (Baumgratz et al., 1996). However, it is unclear whether the degenerating septal cells, as observed in mature anthers, could provide physical support that maintains the anther functionally compartmentalized (Tsou and Johnson, 2003; Lima et al., 2019), and if indeed they have an effect on pollen dosage per pollinator visit.

The predominant pollination system in Melastomataceae is quite specialized. Although some exceptions occur in nectar-producing flowers visited by vertebrates, flies, and wasps (Varassin et al., 2008; Santos et al., 2010; Kriebel and Zumbado, 2014), or in some oil-flowers presumably pollinated by oil-collecting bees (Buchmann and Buchmann, 1981; Buchmann, 1987), most species exhibit pollen flowers that are predominantly buzz-pollinated (Renner, 1989). In this case, the resource explored by the pollinators is the pollen, which is obviously stored in the dithecal, poricidal anthers. Our results demonstrated that changes from tetrasporangiate to polysporangiate anthers may occur without change in pollinators, as observed in Microlicieae and Miconia, where the polysporangiate species and their close relatives are likely buzz-pollinated. On the other hand, the occurrence of polysporangiate anthers in the hummingbird/bat pollinated species Meriania tomentosa (Dellinger et al., 2019) indicates that this condition is not exclusive of buzz-pollinated flowers in the family. Current data do not allow us to conclude whether polysporangiate anther evolved after or before the transition to vertebrate pollination. Expanded sampling of focal groups would also be a promising avenue for future studies on the relationship of anther features and pollinators.

On the other hand, some studies have claimed that polysporangiate anthers might be an adaptation in order to overcome developmental constraints. It has been suggested for Melastomataceae (Lima et al., 2019) and other angiosperm families (Tobe and Raven, 1986; Tsou and Johnson, 2003) that polysporangiate anthers provide more direct contact between pollen grains and tapetal cells during stamen growth, assuring an ideal nutritional condition for pollen grain development. In this respect, polysporangiate anthers would be expected to be more common in larger anthers, but this correlation is not always found (Endress and Stumpf, 1990). For example, Melastomataceae contain many species with large and non-polysporangiate anthers (such as the 10 mm long ones in *Pleroma urvilleanum* (as *Tibouchina urvilleana* in Martins et al., 2009b), and species with small and subdivided anthers (such as *Miconia stenopetala* with anthers 1.5–1.8 mm long; Wurdack, 1984). The relationship between subdivided sporangia and high nutritional efficiency has been shown for some species of Annonaceae which produce large compound pollen units that require a high nutritional rate (Tsou and Johnson, 2003). However, this does not seem to be the case for Melastomataceae, since their pollen grains are released in small monads $(10-25 \,\mu\text{m})$ (Patel et al., 1984; Chantaranothai, 1997; Cortez et al., 2015; Kriebel et al., 2017).

5. Conclusion

Tetrasporangiate anthers are an evolutionarily conserved condition found in the vast majority of angiosperms. However, we showed here that dithecal polysporangiate anthers with parenchymatous septa have evolved independently within Melastomataceae in the genera Meriania, Miconia and Microlicia (but much more frequently in the latter), with an apparent similar morphology. Although we have presented here the most comprehensive analyses of this character-state for the family, the actual number of times this feature evolved in the family is probably higher. The phylogenetic lability of this condition revealed by our analyses suggests that it may be an adaptive trait, whose functionality has been debated but not yet clarified. Understanding the structure and evolutionary history of deviations from the stable stamen form provides new insights into the importance of specialized structures such as polysporangiate anthers, representing the first step towards clarifying their role in the biology of some lineages. Comparative phylogenetic studies, including data on floral morphology, phenology, pollen and reproductive biology of some particular lineages in which polysporangiate anthers are a common feature (e.g. Microlicia), could further improve our understanding of the evolution of this character and its adaptive significance.

CRediT authorship contribution statement

Ana Paula Souza Caetano: Conceptualization, Methodology, Investigation, Writing - original draft, Project administration. Marcelo Reginato: Methodology, Formal analysis, Writing - original draft. Renato Goldenberg: Conceptualization, Visualization, Writing - review & editing. Priscila Andressa Cortez: Conceptualization, Investigation. João Paulo Basso-Alves: Data curation, Investigation. Fabián A. Michelangeli: Conceptualization, Resources, Writing - review & editing. Sandra Maria Carmello-Guerreiro: Resources, Funding acquisition. Simone Pádua Teixeira: Resources, Supervision, Project administration, Funding acquisition, Writing - review & editing.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ppees.2020.125556.

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