



# Unusual diversity of apomictic mechanisms in a species of *Miconia*, Melastomataceae

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## Abstract

Apomixis, the asexual formation of seeds, seems to be a reproductive alternative for many angiosperms, involving various pathways with different genetic and ecological consequences. It is common in some megadiverse tropical groups such as Melastomataceae, of which approximately 70% of the species studied so far in the tribe Miconieae are autonomous apomictics. Hence, *Miconia* appears to be a good model for the study of the embryological pathways associated with apomixis. In the present study, we analyzed the polyploid and autonomous apomictic *M. fallax* and compared its embryology to that of the diploid and sexual *M. pepericarpa*, both treelets species common in the Cerrado, the Neotropical savanna areas of Central Brazil. Ovule structure and basic megasporogenesis and megagametogenesis events were similar in both species. However, *M. fallax* showed exclusive features associated with apomixis: aposporous embryo sac development, with the parthenogenetic development of unreduced egg cells, autonomous endosperm formation, nucellar embryony and polyembryony. Moreover, both gametophytic and sporophytic apomixis occurred in parallel to the development of a sexual embryo sac, a rarely described condition, which probably confers a great reproductive flexibility to the species.

**Keywords** Adventitious embryony · Apospory · Autonomous apomixis · Megagametogenesis · Megasporogenesis · Polyembryony

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## Introduction

Despite the prevalence of sexual reproduction, apomixis—the asexual production of seeds—is an alternative that seems to have been important for the evolution and diversification of the angiosperms (Asker and Jerling 1992; Hojsgaard et al. 2014). In general, the apomictic process bypasses important steps of sexual reproduction, notably both meiosis and egg cell fertilization, and has been viewed as a temporal and spatial deregulation of the sexual developmental program (Koltunow 1993; Koltunow and Grossniklaus 2003; Hand and Koltunow 2014). Currently, apomixis is considered an important reproductive strategy often associated with geographical parthenogenesis and polyploidy (Carman 1997; Hörandl et al. 2008; Santos et al. 2012).

The seeds that result from the apomictic process can have embryos originating from a somatic cell of the ovule (sporophytic apomixis or adventitious embryony) or from an unfertilized egg cell of an unreduced embryo sac (gametophytic apomixis). In the latter case, the unreduced embryo sac originates from a megaspore mother cell in which meiosis was

suppressed or altered (diplospory), or from a nucellar cell called aposporous initial (apospory), by mitotic divisions in both cases (Asker and Jerling 1992; Crane 2001; Hand and Koltunow 2014).

More than one somatic cell in an ovule giving rise to embryos is a common phenomenon in adventitious embryony (Naumova 1993; Batygina and Vinogradova 2007). Moreover, the successful embryogenesis in an ovule with multiple embryo sacs, either aposporous embryo sacs or aposporous and sexual embryo sacs, can lead to polyembryony. Therefore, the sexual and asexual processes may occur in the same or in different seeds in a given apomictic species (Asker and Jerling 1992; Koltunow and Grossniklaus 2003; Batygina and Vinogradova 2007). Indeed, most plants are facultative apomictic. From an evolutionary perspective, the facultative apomixis seems to be advantageous by keeping both asexual and sexual reproductive modes (Koltunow and Grossniklaus 2003; Hojsgaard and Hörandl 2015). On the other hand, the apomixis is the only mode of reproduction by seeds in obligate apomictic species (Koltunow 1993; Richards 1997), which have been pointed out as exceptions in natural populations (Nogler 1984; Asker and Jerling 1992; Hojsgaard and Hörandl 2015).

Although apomixis usually bypasses sexual reproduction stages, not all apomicts are completely pollination independent. Most species are pseudogamous, which means that the nuclear fusion between the male gamete and the central cell is required for functional endosperm development. Pseudogamous apomicts seem to be prevalent among species with adventitious embryony or apospory. Pseudogamy is unnecessary only in autonomous apomicts, an apparently uncommon condition, prevalent among diplosporous apomicts (Asker and Jerling 1992; Koltunow and Grossniklaus 2003; Whittton et al. 2008).

Sporophytic and/or gametophytic apomixis has been reported for 78 flowering plant families and has arisen several times during the evolutionary history of angiosperms (Hojsgaard et al. 2014). Asteraceae, Poaceae and Rosaceae are historically cited as containing the majority of known apomictic genera (Carman 1997; Hojsgaard et al. 2014). However, the mostly tropical Melastomataceae has gained prominence due to their high frequency of autonomous apomictic species, particularly within the tribe Miconieae, which contains up to 70% of apomictic species (Renner 1989; Goldenberg and Shepherd 1998; Goldenberg and Varsassin 2001; Santos et al. 2012; Maia et al. 2016).

Studies concerning the cytological mechanisms of apomixis and their relation with sexuality within Miconieae have revealed important insights that are useful for developmental, taxonomic and ecological knowledge, but still involve a small percentage of species in this megadiverse group (Borges 1991; Cortez et al. 2012; Caetano et al. 2013a, b). Since studies with autonomous apomictic

species are concentrated mainly on herbaceous Asteraceae from temperate regions (Koltunow et al. 1998, 2000; Van Dijk 2003; Noyes 2007), members of the tropical and mostly woody Miconieae represent alternative models for studies of autonomous apomixis.

Despite their pollinator independence, some apomictic Miconieae species may produce viable pollen (Caetano et al. 2013b; Maia et al. 2016). Because these species receive visits from pollinators (Maia et al. 2016), we hypothesized that the sexual mechanism would co-occur with the apomictic one. Secondly, we hypothesized that pollination-independent species could produce an autonomous endosperm. In this study, we show similar sexual events of megasporogenesis and megagametogenesis in the apomictic and pollination-independent *M. fallax* DC. and its congener *M. pepericarpa* Mart. ex DC. Furthermore, *M. fallax* produces an autonomous endosperm, and the co-occurrence of apospory, adventitious embryony and sexuality, leads to development of polyembryonic seeds, showing a mixed reproductive strategy in this markedly diverse group.

## Materials and methods

### Plant materials

The two species of *Miconia* studied here are common shrubs/trees in the Cerrado, the Neotropical savanna region of Central Brazil, and have been previously studied for breeding system features by Goldenberg and Shepherd (1998). On the one hand, the pollination-independent, apomictic and polyploid *M. fallax* is a shrub species with a wide distribution from Peru, Venezuela and Guiana to southeastern Brazil. On the other hand, the diploid and sexual species *M. pepericarpa* is a Cerrado tree with a distribution restricted to some Brazilian states (Goldenberg and Shepherd 1998; Goldenberg 2009; Caetano et al. 2013b).

The samples were collected in Cerrado fragments of Itirapina, São Paulo State, Brazil (lat. 22°15'10"S, long. 47°49'22"W). Voucher specimens were deposited in the Herbarium of Universidade Estadual de Campinas (UEC), São Paulo State, Brazil, under the numbers 150450 (*M. fallax*) and 150451 (*M. pepericarpa*).

### Megasporogenesis, megagametogenesis, and endosperm and apomictic embryo origin

Megasporogenesis and megagametogenesis analyses for both species were carried out using different developmental stages from flower bud to flower anthesis. The origin of apomictic embryos was determined also using seeds of *M. fallax* at several developmental stages. Buds, flowers and fruits were collected from six individuals per species. Finally, the origin

and development of autonomous endosperm in *M. fallax* was determined from three individuals using seeds obtained from fruits of previously emasculated and bagged flower buds. All samples were fixed in 2% glutaraldehyde, 4% formaldehyde and 0.1 M phosphate buffer, pH 6.8 (McDowell and Trump 1976) for 24 h, and then dehydrated through a graded ethanol series, embedded in methacrylate resin (Historesin Leica), and sectioned with a tungsten carbide knife on a rotatory microtome (RM 2245, Leica Microsystems). Transverse and longitudinal serial sections, between 1 and 5  $\mu\text{m}$  thick, were stained with 0.05% toluidine blue (CI 52040) in citrate buffer, pH 4.8 (modified from O'Brien et al. 1964). Observations were made and photographs were obtained using an Olympus BX51 microscope equipped with an Olympus DP71 digital camera.

### Pollen tube growth

Stigmatic pollen deposition and pollen tube growth were analyzed as an indicator of sexual reproduction in *M. fallax*. Senescent flowers that had lost petals and stamens, but with an intact gynoecium, were fixed in formalin–acetic acid–ethanol (FAA) solution (1:1:18; v/v/v) (Johansen 1940). Styles were then cleared and softened in 7 N sodium hydroxide (NaOH) at room temperature for 30 min, washed with water and stained with water-soluble 0.1% aniline blue prepared in 0.1 N  $\text{K}_3\text{PO}_4$ . Observations were made, and photographs were taken under UV light (Martin 1959) using an Olympus BX51 microscope equipped with an Olympus DP71 digital camera.

### Polyembryony analysis

The presence and frequency of polyembryony in *M. fallax* were determined using 1200 seeds obtained from mature fruits from six individuals. The seeds were removed from the fruits, immediately immersed in 10% sodium hypochlorite ( $\text{NaClO}$ ) solution for 3 min, washed in distilled water and distributed inside plastic boxes with wet filter paper overlying cotton wool. The seed germination experiment was conducted under continuous white fluorescent light and controlled temperature of 28 °C. Germinated seeds were dissected under a stereomicroscope for both seedling counting and morphological analyses.

## Results

*Miconia fallax* and *M. pepericarpa* were similar in terms of ovule structure and some events of megasporogenesis and megagametogenesis as detailed below. Apart from these

shared conditions, we observed some cytological differences, related to the reproductive system.

### Ovule structure

We observed that the ovules are anatropous (*M. fallax*) or hemi-anatropous (*M. pepericarpa*), crassinucellate and bitegmic, with a “zigzag” micropyle (Fig. 1a–e). The outer integument is three-layered and the inner one is bilayered, both being non-vascularized (Fig. 1a–c). Periclinal and oblique cell divisions can occur in the micropylar region of the outer integument, making it thicker (Fig. 1d). The raphe has one vascular bundle extending from the funicle to the chalaza, where compact cells with a dense cytoplasm and prominent nucleus form a non-lignified hypostase (Fig. 1a, b, 3e). In *M. pepericarpa* the vascular bundle is surrounded by cells containing phenolic compounds (Fig. 1c).

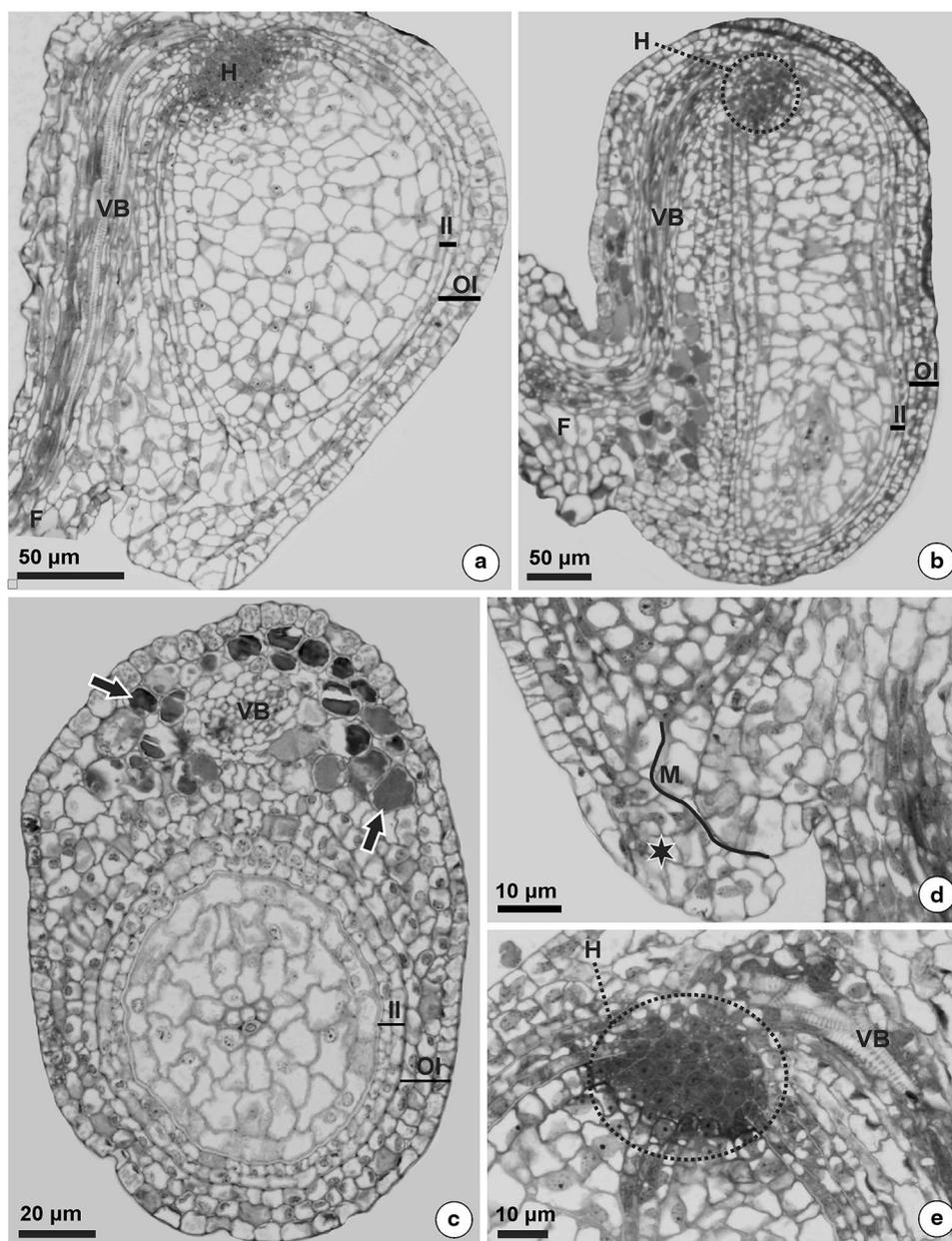
### Megasporogenesis and megagametogenesis

Although structural differences between *M. fallax* and *M. pepericarpa* were observed from the initial stages of megasporogenesis onwards, sexual events represented by megaspore mother cell differentiation, meiosis and megaspores formation, and embryo sac development, occurred in a similar way in both species (Figs. 2, 3).

In *M. fallax* and *M. pepericarpa*, the megaspore mother cell enlarged within the nucellus (Fig. 2a–c) and, through meiosis, gave rise sequentially to a dyad (Fig. 2d) and to a linear tetrad of megaspores (Fig. 2e, f). The functional megaspore was chalazal (Fig. 2e, f) and, after three rounds of mitotic divisions (Fig. 3a–c), originated an embryo sac of the *Polygonum*-type, with three antipodals, one binucleate central cell (Fig. 3d), two synergids (Fig. 3e) and one egg cell (Fig. 3e, f). In *M. pepericarpa*, fertilization was demonstrated by one pollen tube penetrating a synergid, with the subsequent formation of a zygote and a nuclear endosperm (Fig. 3g, h). Although direct evidence of fertilization was not detected for *M. fallax*, we observed pollen grains germinating on the stigmatic surface, and pollen tubes growing through the styles of senescent flowers (Fig. 3i, j).

For *M. fallax*, 69.05% of the 42 ovules observed had aposporous initial cells around the megaspore mother cell (Fig. 4a), megaspores (Fig. 4b, c) or developing embryo sac (Fig. 4d). The aposporous initial cells were similar to the functional megaspore, with a dense cytoplasm, conspicuous central nucleus, and one or two vacuoles (Fig. 4a–d). These aposporous initials gave rise to one to three aposporous embryo sacs, structurally similar to the *Polygonum*-type (Fig. 4e, f). Ovules in fresh open flowers

**Fig. 1** Ovule structure in sexual and apomictic species of *Miconia*. **a, b** Longitudinal sections of ovules in *M. fallax* and *M. pepericarpa*, respectively, demonstrating anatropous (**a**), hemi-anatropous (**b**) and bitegmic ovules, with a single vascular bundle and a hypostase. **c** Transverse section showing a three-layered outer integument and a bi-layered inner integument in *M. pepericarpa*. Arrows indicate phenolic-storing cells around the vascular bundle. **d** “Zigzag” micropyle in the *M. fallax* ovule in longitudinal section, also demonstrating periclinal divisions forming additional layers in the outer integument (asterisk). **e** Detail of hypostase cells in the *M. fallax* ovule. *F* funicle, *H* hypostase, *II* inner integument, *OI* outer integument, *M* micropyle, *VB* vascular bundle

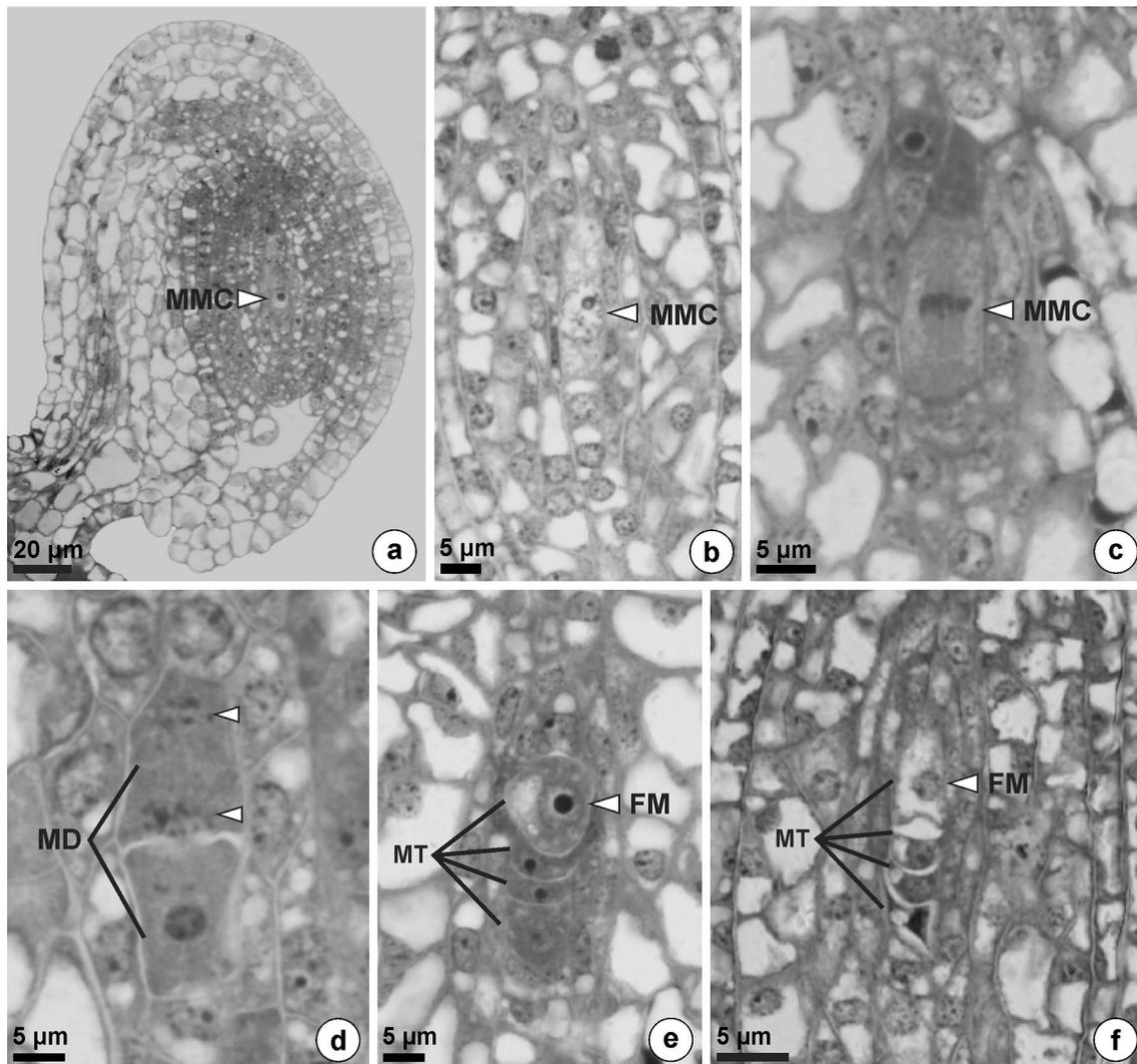


generally contained one mature embryo sac, while the others were under development.

### Embryo and endosperm origin in *Miconia fallax*

The embryos of *M. fallax* developed from each egg cell of one or more embryo sacs of the same ovule, as demonstrated by the embryo position inside the embryo sac (Fig. 4g–i). Moreover, in ovules from the same flower, supernumerary embryos could develop from nucellar cells that were positioned next to the hypostase (Fig. 4j) or around the embryo sac (Fig. 4k, l).

In seeds from immature fruits, the endosperm was at the nuclear developmental stage inside the embryo sac, including those obtained from emasculated flower buds (Fig. 4l, m). The presence of more than one embryo per seed confirmed that the species is polyembryonic (Fig. 4n), but it was not possible to distinguish between possibly zygotic and apomictic embryos. The events of sexual and apomictic reproduction in *M. fallax* can be viewed in the drawing of Fig. 5 in a summary form.



**Fig. 2** Megasporogenesis in sexual and apomictic species of *Miconia*. **a** Longitudinal section of a *M. fallax* ovule, showing a megaspore mother cell (MMC) in the nucellus. **b, c** Details of MMC in *M. pepericarpa* and *M. fallax*, respectively. **d** Megaspore dyad in *M. fallax*, in which the chalazal one goes through a second meiotic telophase

(arrowhead). **e, f** Megaspore tetrad in *M. fallax* and *M. pepericarpa*, respectively, with the functional chalazal megaspore. *FM* functional megaspore, *MD* megaspore dyad, *MMC* megaspore mother cell, *MT* megaspore tetrad

### Polyembryony in *Miconia fallax*

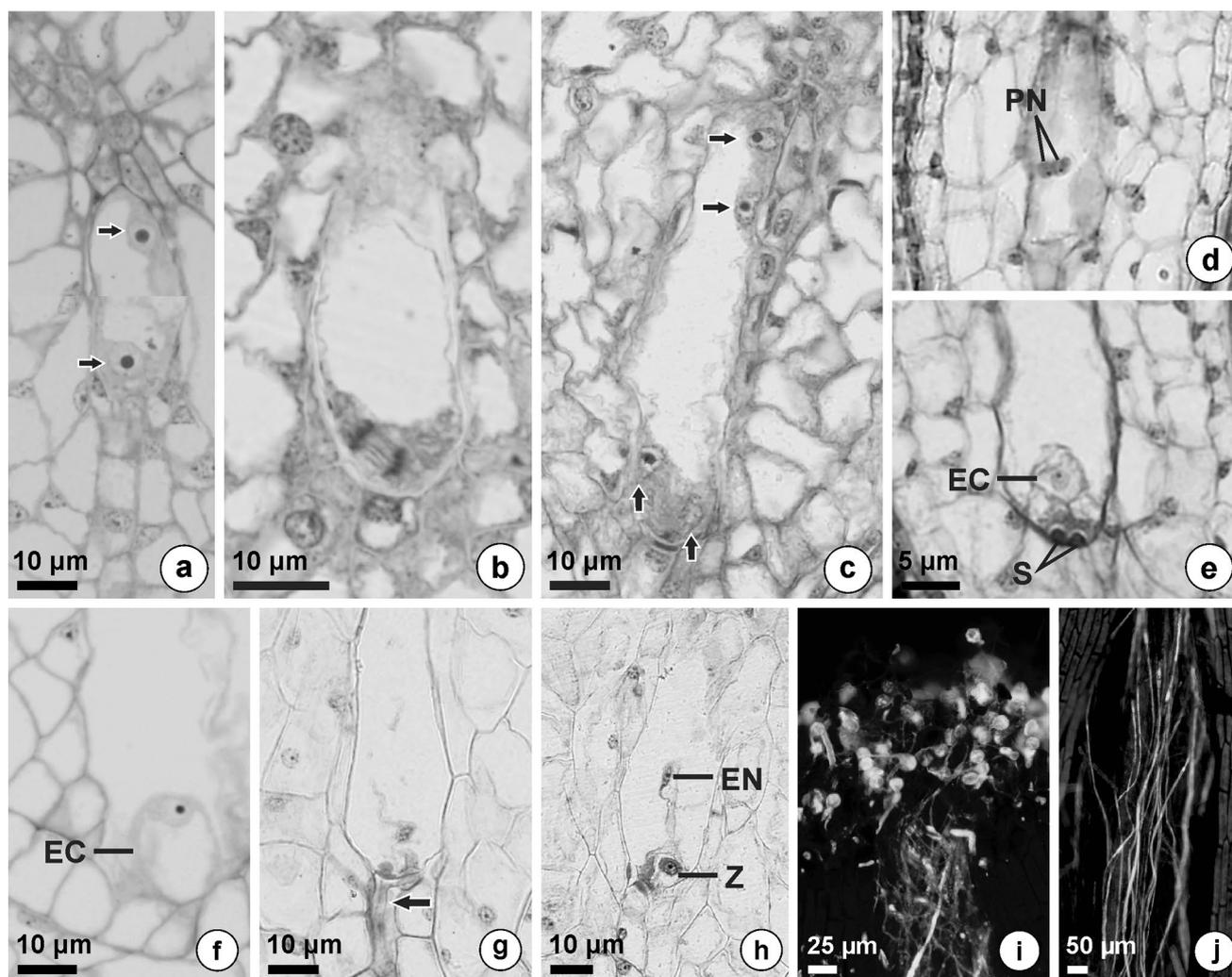
*Miconia fallax* seed germination occurred seven days after the beginning of the experiment. The mean percentage of germinated seeds was 67.4% ( $\pm 19.1$ ). Polyembryony was confirmed in the studied population, with a frequency of 34.2%. The maximum number of seedlings in a seed was four, with a mean of 1.4 ( $\pm 0.1$ ) seedlings per seed. Seeds containing one seedling were the most frequent case, corresponding to 66.1% of the germinated seeds, followed by those with two (28.49%), three (4.55%) and four seedlings (0.85%) (Figs. 6, 7a–h).

More than half of the seeds with two seedlings (50.4%) and all seeds with three or four seedlings exhibited seedlings

that were markedly different in size and morphology (Fig. 7f–h). The smaller seedlings often had poorly developed and morphologically irregular cotyledons (Fig. 7g, h). In some seeds (2.9%) the cotyledons were oriented toward the micropylar region (Fig. 7b). These inverted seedlings were originated from monoembryonic as well as polyembryonic seeds.

### Discussion

Although the ovule structure and basic megasporogenesis and megagametogenesis events were similar for *M. fallax* and *M. pepericarpa*, in the apomictic *M. fallax*, we observed



**Fig. 3** Megagametogenesis in sexual and apomictic species of *Miconia*. **a** *Miconia fallax*: binucleate (arrows) embryo sac. **b** Binucleate embryo sac with the micropylar nucleus at mitotic anaphase. **c** Tetranucleate (arrows) embryo sac. **d, e** Details of the mature embryo sac showing polar nuclei (**d**), synergids and egg cell (**e**) in *M. pepericarpa*. **f** Detail of the mature embryo sac showing an egg cell in

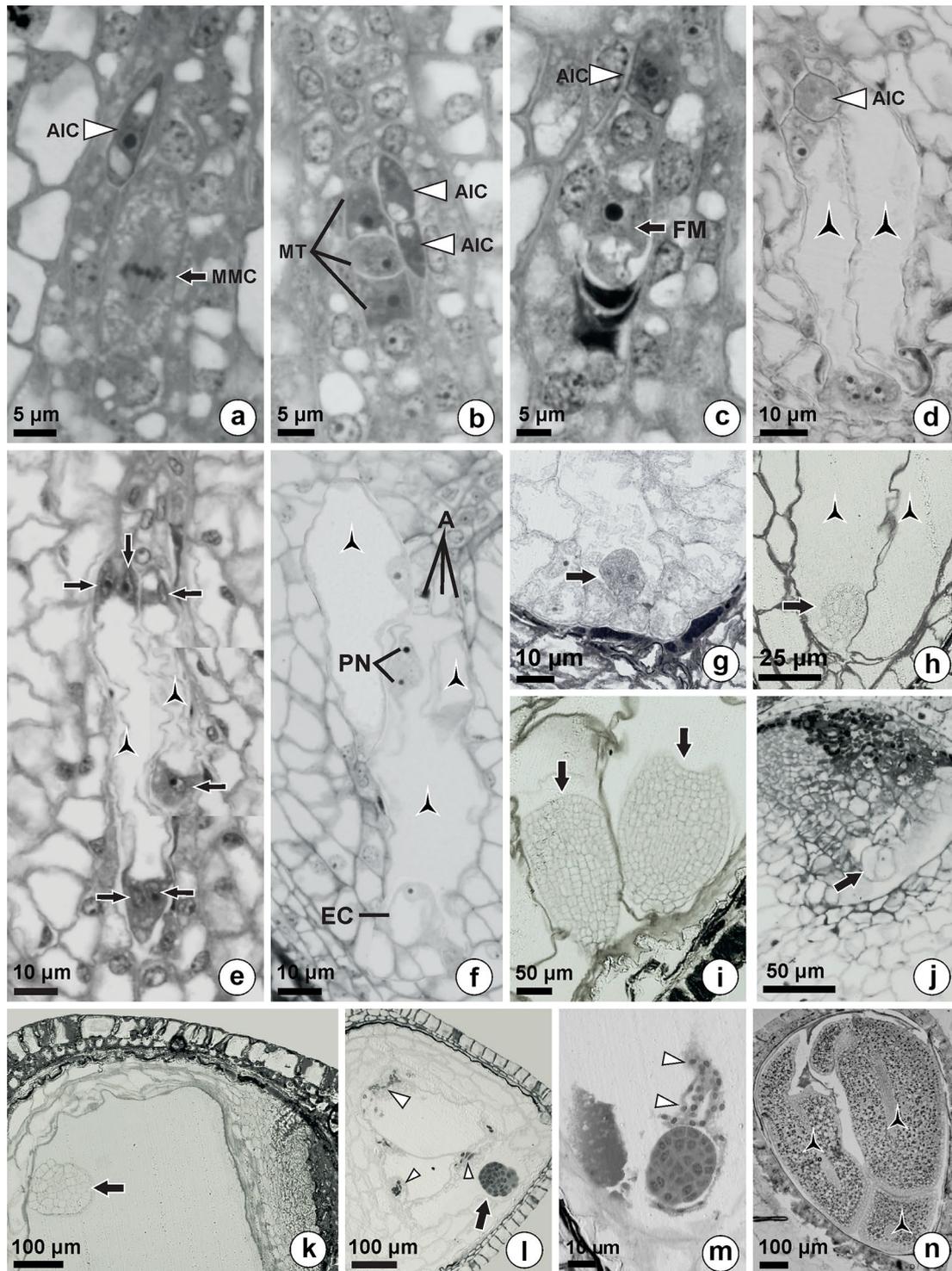
*M. fallax*. **g** Pollen tube reaching the embryo sac (arrow) in *M. pepericarpa*. **h** Detail of zygote and an endosperm nucleus in *M. pepericarpa*. **i, j** Pollen grains germinating on the stigma surface (**i**) and pollen tubes growing in style (**j**) of *M. fallax*. EC egg cell, EN endosperm nucleus, PN polar nuclei, S synergid, Z zygote

aposporous embryo sacs development, with the later parthenogenetic development of unreduced egg cells, autonomous endosperm formation and nucellar embryony. Our results indicate that this species is autonomous apomictic, with both gametophytic (apospory) and sporophytic apomixis. Moreover, we suggest that *M. fallax* is a facultatively apomictic species, exhibiting a mixed reproductive system in which the apomixis occurs in parallel to sexuality.

### Ovule structure

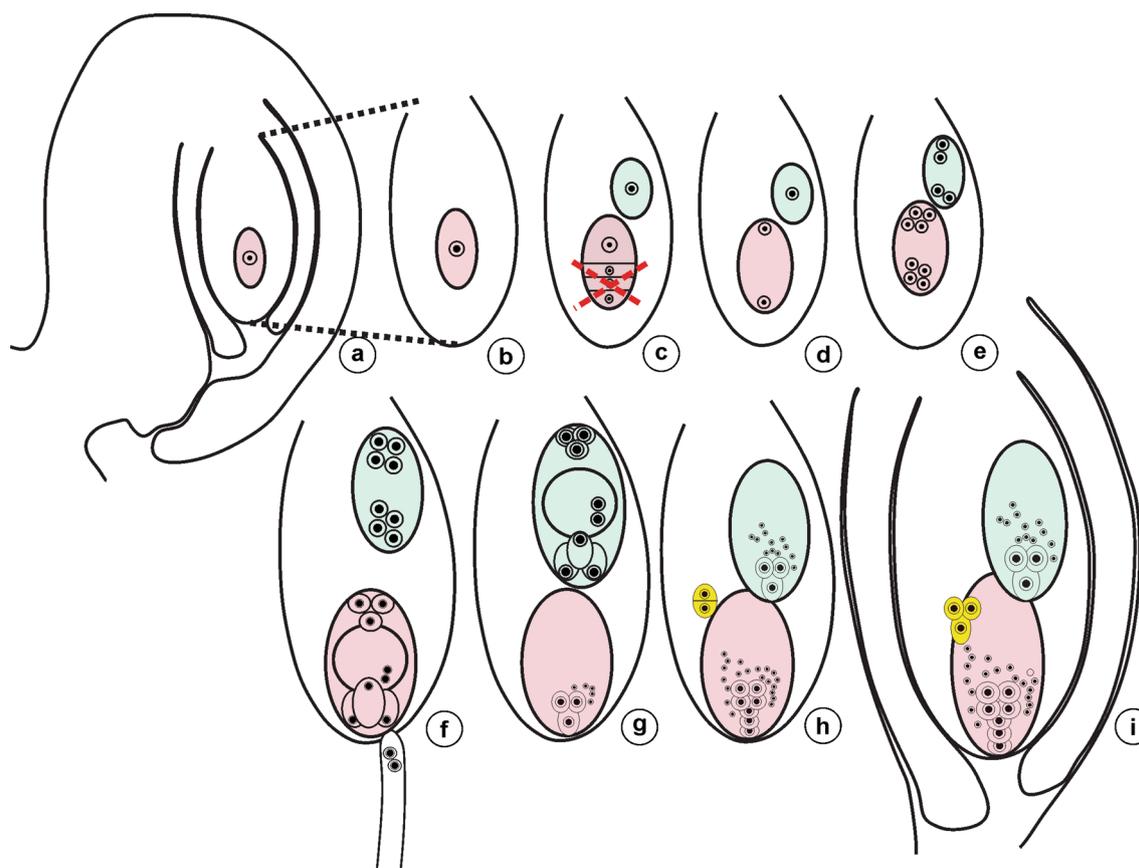
The structural characteristics of the *M. fallax* and *M. pepericarpa* ovules were similar. The crassinucellate ovule with two not vascularized integuments, the inner one two-layered,

and the “zigzag” micropyle are stable conditions within Melastomataceae (Tobe and Raven 1983; Medeiros and Morretes 1996; Cortez and Carmello-Guerreiro 2008; Caetano et al. 2013a; Ribeiro et al. 2015; Caetano et al. 2017). However, the outer integument thickness and the ovule curvature are variable characters within the family (Tobe and Raven 1983; Medeiros and Morretes 1996; Cortez and Carmello-Guerreiro 2008; Caetano et al. 2013a; Ribeiro et al. 2015; Caetano et al. 2017). Since embryological characters are recognized due to their systematic importance (Davis 1966; Tobe 1989; Johri et al. 1992), and the outer integument thickness has been useful for clade delimitation within Melastomataceae (Caetano et al. 2017), the systematic significance of ovule curvature is yet to be tested for the group.



**Fig. 4** Megagametogenesis and embryogenesis in the apomictic species *Miconia fallax*. **a–d** Aposporous initial cells, indicated by arrowheads, differentiate above the megaspore mother cell (arrow) (**a**), megaspore tetrad (**b**), functional megaspore (arrow) (**c**) and developing embryo sacs (triangles) (**d**). **e** Ovule with a tetranucleate and binucleate embryo sacs (triangles). Arrows indicate the embryo sacs nuclei. **f** Ovule with a mature and two developing embryo sacs (triangles). **g** Developing proembryo, indicated by an arrow, within an embryo

sac. **h, i** Developing embryos (apomictic and/or sexual), indicated by arrows and probably originated from egg cells, within embryo sacs (triangles). **j** Adventitious proembryo, indicated by an arrow. **k–m** Adventitious embryos, indicated by arrows (**k, l**) and autonomous endosperm development, indicated by arrows head (**l, m**). **n** Polyembryonic seed with three visible embryos (triangles). **A** Antipodals, **AIC** aposporous initial cell, **EC** egg cell, **FM** functional megaspore, **MMC** megaspore mother cell, **MT** megaspore tetrad, **PN** polar nuclei



**Fig. 5** Events of sexual and apomictic reproduction in the ovule of *Miconia fallax*. Cells and structures involved in the sexual processes are shown in red, while those associated with apomixis are displayed in blue and yellow. **a, b** Differentiated megaspore mother cells (red) in the nucellus. **c** Megaspore tetrad (red) and aposporous initial cell (blue). **d** Binucleate embryo sac (red) and aposporous initial cell

(blue). **e** Octanucleate (red) and tetranucleate (blue) embryo sacs. **f** Pollen tube reaching the mature embryo sac (red) and octanucleate embryo sac (blue). **g** Zygotic proembryo and endosperm (red) and mature embryo sac (blue). **h, i** Zygotic embryo and endosperm (red), aposporous proembryo and autonomous endosperm (blue) and adventitious proembryos (yellow)

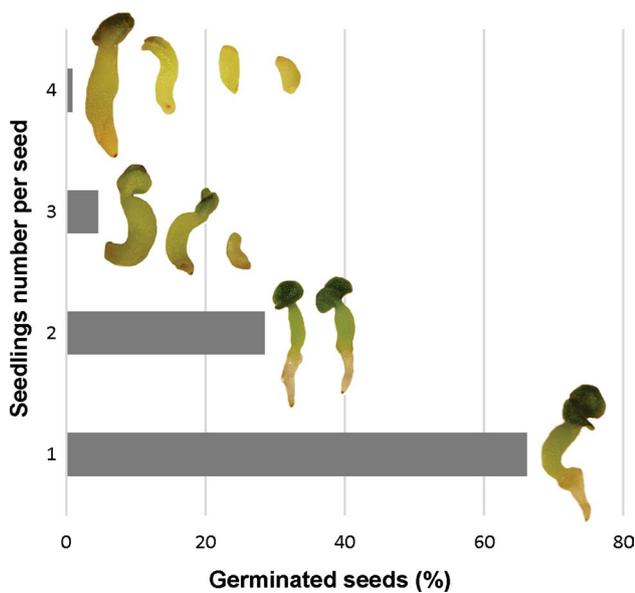
### Megasporogenesis, megagametogenesis and the structural basis of apomixis

*Miconia fallax* and *M. pepericarpa* exhibited a similar sexual development process, indicating that apomixis does not alter completely the phylogenetically conservative events of megasporogenesis and megagametogenesis in the apomictic *M. fallax* species. The linear megaspore tetrad formation, with the functional chalazal megaspore, and *Polygonum*-type embryo sac are conditions that seem to be stable within Melastomataceae (Tobe and Raven 1983; Johri et al. 1992; Medeiros and Morretes 1996; Caetano et al. 2013a) and possibly common among sexual and facultative apomictic species.

Despite these similarities, *M. fallax* exhibited embryological differences related to apomixis. The aposporous initial cells (AICs) started to develop concomitantly with the megasporogenesis and were identified by their position, as well as by structural features (see Naumova and Willemse

1995; Koltunow et al. 1998; Naumova and Vielle-Calzada 2001; Guan et al. 2006; Galla et al. 2011). Apparently, a correct positioning of AICs in the ovule is important for the development of the aposporous embryo sac, probably due to the expression of particular factors restricted to a specific area in the ovule (Koltunow et al. 1998; Galla et al. 2011). Moreover, a cellular polarization resulting from vacuole development seems to mark the transition of the aposporous initial cells to the aposporous embryo sac (Naumova 1997; Naumova and Vielle-Calzada 2001).

The differentiation and development of more than one AIC gave origin to multiple embryo sacs in a single ovule of *M. fallax*. In fact, several AICs may develop within the nucellar tissue, giving rise to ovules with supernumerary embryo sacs (Koltunow et al. 1998; Albertini et al. 2001; Koltunow and Grossniklaus 2003; Guan et al. 2006; Yao et al. 2007). This indicates that in aposporic species, all nucellar cells carry the genetic information needed to develop into embryo sacs, even if only a few cells, adjacent



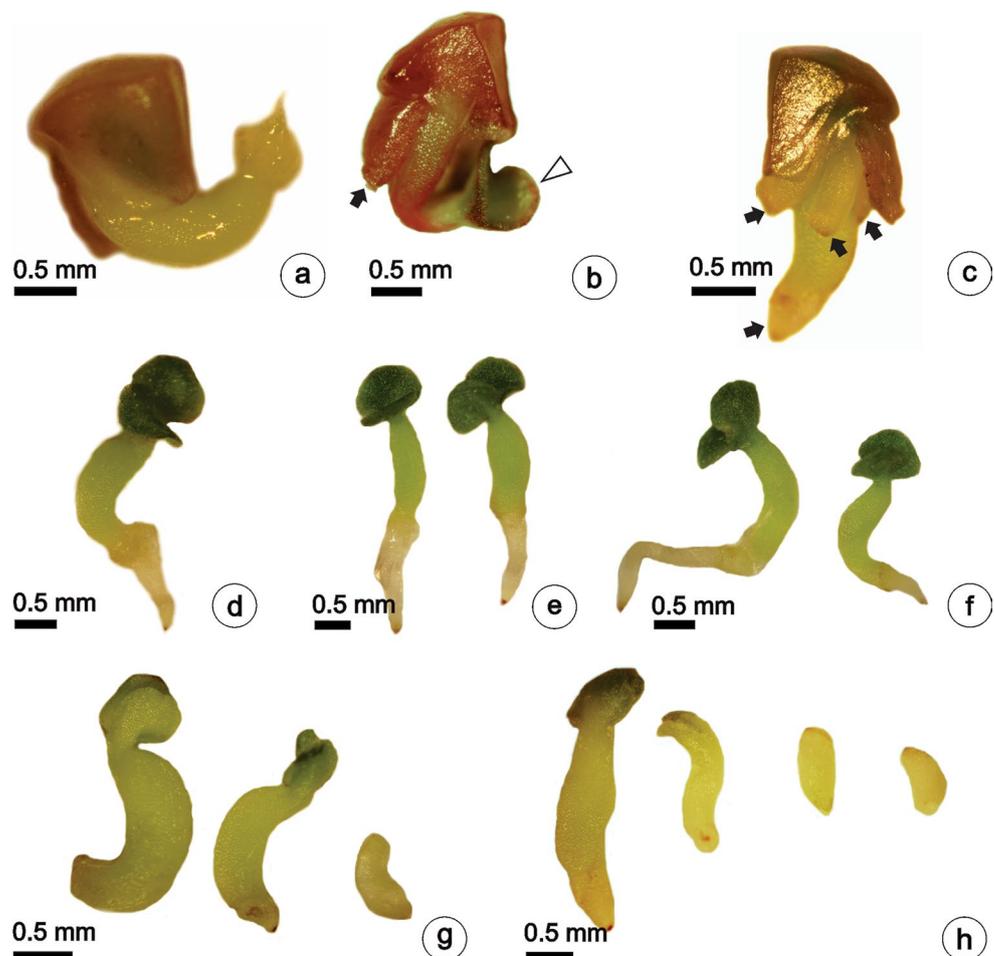
**Fig. 6** Percentage of germinated seeds with one, two, three or four seedlings in the apomictic species *Miconia fallax*

to the embryo sac and/or megaspores, actually do so (Nau-mova 2008). On the other hand, the occurrence in a single ovule of both aposporous and sexual embryo sacs, as observed in *M. fallax*, does not appear to be a common event (Nogler 1984; Alves et al. 2001). The apospory frequently leads to the abortion of the sexual process (Koltunow et al. 1998; Wen et al. 1998; Tucker et al. 2003; Yao et al. 2007), suppressing this event through changes in gene expression (Tucker et al. 2003), which did not appear to occur in the species studied here.

Since our results demonstrated the capacity of reduced embryo sac formation and pollination, and pollen viability was about 29% (Caetano et al. 2013b), we suggest that *M. fallax* is a facultative apomictic species. It has been proposed that since the apomictic representatives of Melastomataceae with relatively high pollen viability attract pollinators, they might produce seeds through both apomictic and sexual events (Maia et al. 2016). In fact, the majority of the apomictic plants retain the ability to reproduce sexually, and some degree of sexuality has been associated with the three kinds of apomictic mechanisms (Bicknell et al. 2003; Martins and Oliveira 2003; Aliyu et al. 2010; Sartor et al. 2011; Noyes and Givens 2013). Apparently, the ratio

**Fig. 7** Seedling emergence and morphology in the apomictic species *Miconia fallax*.

**a–c** Seedling emergence from monoembryonic (**a**) and polyembryonic (**b**, **c**) seeds. Arrows indicate embryos. In figure **b** it is possible to see one embryo with the cotyledons oriented to the micropylar region (arrow-head) and other embryo with a root apex oriented to the micropylar region (arrow). **d** Seedling from a monoembryonic seed. **e** Morphologically similar seedlings from a polyembryonic seed. **f–h** Morphologically different seedlings from seeds with two, three and four embryos, respectively



between sexual and asexual reproduction in some apomictic species may vary under different environmental conditions, since different stress situations alter the proportion of sexual embryo sacs in facultative apomictic *Eragrostis curvula* (Rodrigo et al. 2017). The importance of sexuality in facultative apomictics is the possibility of maintenance of some genetic diversity within the species, conferring adaptability to this taxon (Mazzucato et al. 1996; Richards 1997; Bicknell and Koltunow 2004). Moreover, sexuality could avoid genomic decay and extinction in facultative apomictic taxa (Hojsgaard and Hörandl 2015).

The formation of an embryo by parthenogenesis of the egg cell of an aposporous embryo sac in *M. fallax* allows classifying this species as having gametophytic apomixis of the apospory type. At the same time, *M. fallax* also exhibits sporophytic apomixis, demonstrated by the development of the adventitious nucellar embryos. The presence of two apomictic mechanisms in a single Miconieae species corroborates the idea that sporophytic and gametophytic apomixis can both occur in parallel (Naumova 1993; Koltunow and Grossniklaus 2003; Naumova 2008; Yao et al. 2007). Furthermore, the occurrence of diplospory in *M. albicans* (Caetano et al. 2013a) makes the genus a repository of all types of apomictic pathways described to date and suggests that their regulation may not be that different (Rutishauser 1982).

*Miconia fallax* also exhibited an endosperm formation without fertilization. In the apomictic species with autonomous endosperm development such as *M. fallax* (this study), *M. albicans* (Caetano et al. 2013a), *Hieracium* (Koltunow et al. 1998) and *Taraxacum* (Cooper and Brink 1949), there is a tolerance of unbalanced maternal versus paternal genome contributions (m:p), because there is no paternal contribution to endosperm formation. Strategies that aim to tolerate the unbalance in the m:p ratio, or ensure the 2m:1p ratio, have been interpreted as prerequisites for apomixis maintenance (Grimanelli et al. 1997; Quarin 1999; Koltunow and Grossniklaus 2003) and could explain, among other factors, the frequency of this reproductive system in Melastomataceae.

### Polyembryony in *Miconia fallax*

The development of aposporous and adventitious apomictic embryos, associated or not with the zygotic ones, led to polyembryony in *M. fallax*. On the other hand, monoembryony was reported in *M. pepericarpa* and other sexual species of Melastomataceae (Mendes-Rodrigues and Oliveira 2012). The association between apomixis and polyembryony has been observed for different Melastomataceae species (Mendes-Rodrigues and Oliveira 2012), but multiple embryos can originate from suspensor cells (Subramanian

1944), indicating that polyembryony is not always related to apomixis in this group.

The frequency of polyembryonic seeds in Melastomataceae varies from 0.18 to 34.2% and depends on both the species and population analyzed (Mendes-Rodrigues and Oliveira 2012). Although up to five embryos may occur in a single Melastomataceae seed, the mean number of embryos per seed is always close to one (Mendes-Rodrigues and Oliveira 2012). A tendency to a reduction of embryo size and to an increase in the seedling mortality ratio has been suggested for seeds with a larger number of embryos (Ladd and Cappuccino 2005; Mendes-Rodrigues et al. 2011, 2012), indicating a selective pressure to reduce the number of embryos per seed. It is possible that the seed size, particularly regarding the small seeds of Melastomataceae, restricts the proper development of multiple embryos. This possibility is supported by the different sizes and morphological anomalies among seedlings from polyembryonic seeds observed here for *M. fallax*, for other Melastomataceae (Mendes-Rodrigues and Oliveira 2012) and for other polyembryonic species (Koltunow et al. 1998; Mendes-da-Glória et al. 2001; Costa et al. 2004; Bittencourt and Semir 2005; Mendes-Rodrigues et al. 2005, 2012).

Despite these possible size restrictions, some advantages have been attributed to polyembryony such as the increased chance of occurrence of at least one established seedling per seed (Ladd and Cappuccino 2005; Hotchkiss et al. 2008) and the increased fitness of individuals growing in patches (Allee effect) (Cappuccino 2004). In this respect, it is suggested that polyembryony, apparently a frequent processes in Miconieae, can favor both the reproductive success and frequency of this tribe in the Neotropics (Mendes-Rodrigues and Oliveira 2012).

### Conclusion

The polyploid *M. fallax* showed some exclusive features associated with apomixis, such as aposporous initial cell differentiation, more than one embryo sac in a single ovule, embryo development by apospory and adventitious embryony, autonomous endosperm formation and polyembryony. But it retains the mechanisms for reduced embryo sac production and sexual reproduction observed in the studied population. These distinct mechanisms provide great reproductive flexibility to the species, associating independence of pollinators with eventual sexual embryo production, bringing together gene variability and reproductive assurance. In addition, the occurrence of diplosporous apomixis elsewhere in the genus makes the *Miconia* a repository of all apomixis pathways recorded to date. It has been proposed that apomixis may enhance the diversification rates of some plant groups, and the occurrence of this diversity of reproductive

alternatives in the *Miconia* may explain, among other factors, its enormous species richness.

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