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Understanding male sterility in *Miconia* species (Melastomataceae): a morphological approach

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Abstract. Pollen abortion occurs in virtually all species and often does not prejudice reproductive success. However, large numbers of abnormal pollen grains are characteristic of some groups. Among them is *Miconia*, in which partial and complete male sterility is often related to apomixis. In this study, we compared the morphology of pollen grains over several developmental stages in *Miconia* species with different rates of male sterility. Our aim was to improve the knowledge of mechanisms that lead to male sterility in this ecologically important tropical group. Routine techniques for microscopy were used to examine anthers in several developmental stages collected from the apomictic species *Miconia albicans* and *M. stenostachya*. Both species are completely male sterile since even the pollen grains with apparently normal cytoplasm were not able to develop a pollen tube. Meiosis is a rare event in *M. albicans* anthers and happens in an irregular way in *M. stenostachya*, leading to the pollen abortion. *M. albicans* has more severe abnormalities than *M. stenostachya* since even the microspores and pollen grain walls were affected. Moreover, in *M. stenostachya*, most mitosis occurring during microgametogenesis was also abnormal, leading to the formation of bicellular pollen grains with two similar cells, in addition to the formation of pollen grains of different sizes. Notably, abnormalities in both species did not reach the production of Übisch bodies, suggesting little or no tapetum involvement in male sterility in these two species.

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Introduction

The reproductive success of the plants depends on the production of viable male and female gametes, as the new sporophyte originates from their fusion (Lersten 2004). However, some species can produce viable embryos even in the absence of viable pollen grains through a mechanism called apomixis (Koltunow *et al*. 1995). As apomixis is a useful mechanism for crop management, many studies have focused on economically important plants, such as several forages and *Citrus* species (Spillane *et al*. 2001), generally growing under controlled conditions. However, knowledge about the cytology of structures related to apomixis, the pollen grain and the embryo sac, and their ontogeny in natural populations of apomictic species is still scarce.

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

Structural and ultrastructural studies have revealed that male sterility in plants has multiple causes. The phenotypic manifestations of male sterility are diverse, including absence of stamens and anther dehiscence, abortion of pollen at any stage of its development, absence of anther dehiscence or defects in pollen tube formation (Budar and Pelletier 2001). Such pollen abnormalities may occur during ontogeny in virtually all angiosperm species, but most of them do not impede reproductive success (Lersten 2004). Some of the genes related to male sterility are now known, and phenotypic analysis of mutants has provided useful clues for understanding the events that lead to pollen abortion. Some of these gene mutations also affect other tissues in the anther, mainly the vascular tissue and the tapetum (Goldberg *et al*. 1993; Alves-Ferreira *et al*. 2007; Zhu *et al*. 2010).

Although structural and ultrastructural studies have provided useful information about male sterility in plants, they are scarce in Melastomataceae species. Therefore, the goal of this study was to investigate structural and ultrastructural features of pollen abortion in two apomictic species of *Miconia* that have low levels of pollen fertility (Goldenberg and Shepherd 1998). Specifically, we set out to understand the steps that give rise to

pollen abortion in these two apomictic species from natural populations. Observations were made over various stages of microsporogenesis and microgametogenesis. An understanding of the morphological consequences of irregularities during ontogeny of the pollen grains in natural populations that are characterised by low numbers of viable pollen grains, a phenomenon considered rare among angiosperms (Laser and Lersten 1972), is important for both structural and ecological studies. Moreover, understanding microspore development in different apomictic species will extend our knowledge of the important structural changes that can occur during pollen production.

Materials and methods

Field investigations were performed using two species, identified as apomictic by Goldenberg and Shepherd (1998), *Miconia albicans* (Sw.) Triana and *M. stenostachya* DC., growing in natural populations of cerrado in south-east Brazil from the municipalities of Itirapina (22°15′10′S and 47°49′22′W) and Campinas (22°54′20′S and 47°03′39′W), both in the state of São Paulo, from 2007 to 2010. The vouchers were deposited into the herbarium UEC (Universidade Estadual de Campinas, São Paulo State, Brazil) under the accession numbers 162376 and 162382, respectively.

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

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

Fresh pollen grains were submitted to a germination test using a protocol adapted from Santos and Mariath (1997) and successfully applied to *Miconia paucidens* DC., a sexual species with high pollen fertility (Goldenberg and Shepherd 1998), as a control. A germination medium containing 2% colourless gelatin, 20% sucrose, 0.01% boric acid and 0.05% calcium nitrate was used for optimal growth conditions. After incubation at room temperature (~25°C) for 3 h in the dark, pollen grains were examined under a light microscope to observe pollen tube formation.

Results

The development of *M. albicans* and *M. stenostachya* anthers proceeds normally until the microspore mother cell stage.

M. albicans

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

Although the tapetum degeneration was normal, the tapetal cell architecture remained at the free microspore stage for some time due to the wall that was formed by the fused orbicules (Fig. 2*a*, *b*). The mature anther wall, before dehiscence, was composed of the epidermis and the endothecium, as well as the orbicules that remained attached to the endothecium periclinal inner wall after the total tapetal cell degradation (Fig. 2*c*). Mature pollen sacs showed only abnormal structures, with several rates of degeneration in the cytoplasm and nucleus, along with anomalous size and shape, or total absence of cell contents (Fig. 2*c*). Most anthers were completely empty or only filled with an amorphous substance that was stained similar to orbicules and exine (Fig. 2*d*), with some having one or more obliterate pollen sacs (Fig. 2). Inside the pollen sacs, we found some abnormal microspores that remained in a dyad or tetrad arrangement due to the fusion of their walls (Fig. 2*f*–*j*). Protein, lipids and starch grains were not detected (negative reactions with xylidine de Ponceau, Sudan black B, PAS reagent and Lugol solution, respectively), as reserve substances inside the microspore cytoplasm. Very few bicellular pollen grains were observed, and even these were abnormal (Fig. 2*k*, *l*). In addition to the presence of some microspores and pollen grains with cell contents, the anomalous wall structure indicated that they were not properly formed (Fig. 2*f*–*l*).

M. stenostachya

Several meiotic irregularities were observed, including lagging chromosomes (Fig. 3*a*) at anaphase I and II. Many of the syncytia resulting from the completion of meiosis I and II had four nuclei of different sizes, more than four nuclei per syncytium (Fig. 3*b*) or showed evidence of nuclei fusion (Fig. 3*c*). In some cases, cytokinesis gave rise to dyads in addition to tetrads (Fig. 3*d*). The lagging chromosomes that did not migrate towards the two poles at anaphase I (unbalanced chromosome segregation) were enclosed in an extra nucleus at interkinesis, resulting in tetrads or polyads with one or more micronuclei (Fig. 4*a*–*e*). Callose wall deposition appeared to proceed normally and surrounded even the micronuclei (Fig. 4*a*–*d*). Some of the microspores of the tetrad showed more than one nucleus (Fig. 4*f*). Some tetrads exhibited microspores with several levels of cytoplasm and

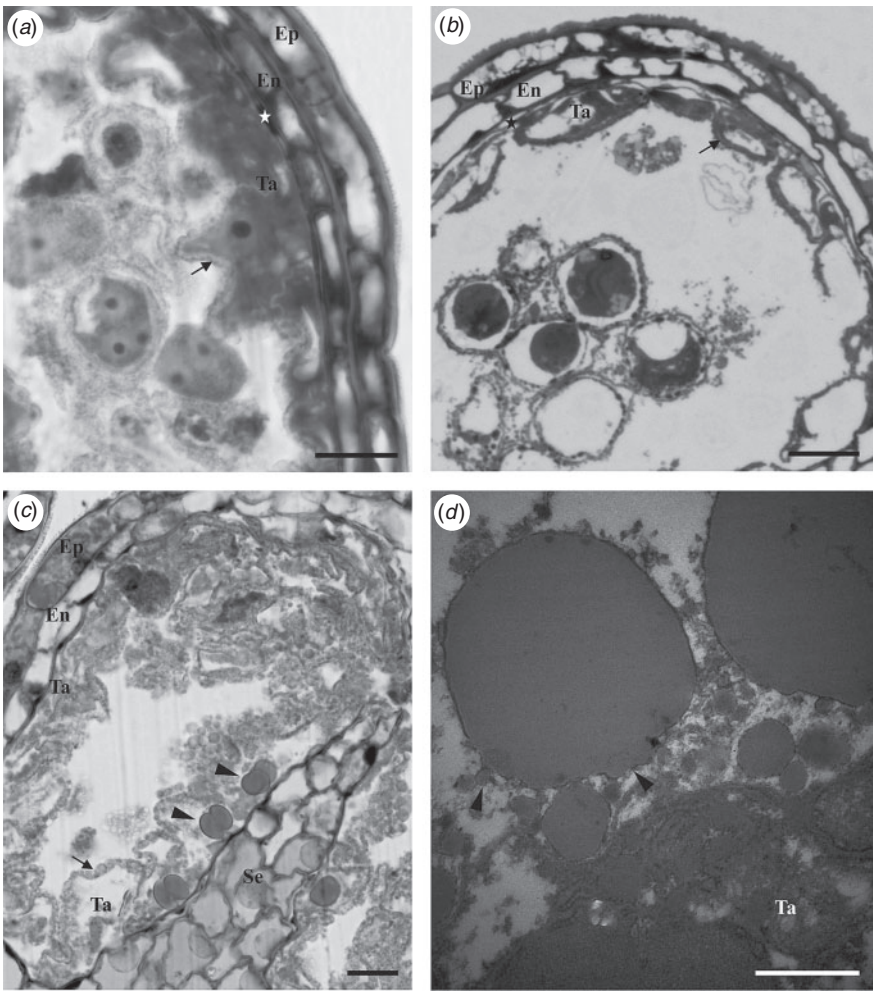

Fig. 1. Abnormalities in *Miconia albicans*. (*a*, *b*) Transverse section of the anther with abnormal microspores inside the loculus. The orbicules are aggregated over the tapetum (Ta) cell walls (arrows). (*c*) Bigger orbicules (arrowhead) and anomalous Ta wall (arrow). (*d*) Smaller orbicules aggregate to form the bigger ones (arrowhead) inside the Ta cytoplasm. En = endothecium, EP = epidermis, Se = septum, ★ = middle layer. Bars = 10 µm (*a*–*c*) and 1 µm (*d*).

nuclear disintegration (Fig. 4*g*) or cytoplasmic connections between adjacent microspores (Fig. 4*h*).

Free microspores were observed inside the anther loculus after callose wall disintegration. Some anthers had very dense contents inside the locule around the free microspores, and orbicules began to accumulate above the inner periclinal wall of the tapetum (Fig. 5*a*). Microspores with more than one nucleolus per nucleus (Fig. 5*b*, *c*), with more than one nucleus per cell or with cytoplasm degradation (Fig. 5*d*) were common. Some microspores of different sizes (Fig. 5*d*, *f*) or with anomalous exine staining (Fig. 5*e*) were also observed. Even the microspores with degenerating cytoplasm exhibited normal sporodermis, which were stratified in the intine and exine (Fig. 5*f*).

The mitotic division that gave rise to the vegetative and generative cells of bicellular pollen grains occurred synchronously with tapetum cell disintegration and was not always asymmetric; consequently, the formation of pollen grains with two cells of the same size was common (Fig. 6*a*, *b*). Even in the cases where mitosis was asymmetric, pollen grains were abnormal due to the presence of more than one nucleus inside the generative and/or vegetative cells (Fig. 6*a*). The vegetative and generative cells also showed ultrastructural abnormalities as disturbances in the organelles, the endomembrane system and the plasma membrane (Fig. 6*c*, *d*).

Prior to dehiscence, the mature anther wall was composed of the epidermis and the endothecium, as well as the orbicules that remained attached to the endothecium periclinal inner wall after complete tapetum degradation. Most cellular structures found inside the mature pollen sacs showed the following structural abnormalities: spherical or anomalous-shaped generative cells (Fig. 7*a*–*c*), degenerating cytoplasm (Fig. 7*d*), supernumerary nuclei inside the cytoplasm (Fig. 7*e*), cytoplasmic connections between adjacent structures (Fig. 7*f*), totally empty structures despite the presence of normal exine (Fig. 7*g*, *h*, *m*, *o*) and disturbed pollen wall morphology (Fig. 7*i*–*k*). Ultrastructural disturbances were also observed in the cytoplasm of both vegetative and generative cells of mature bicellular pollen grains (Fig. 7*m*–*o*).

Protein, lipids and starch grains were not detected (negative reactions with xylidine de Ponceau, Sudan black B, PAS reagent

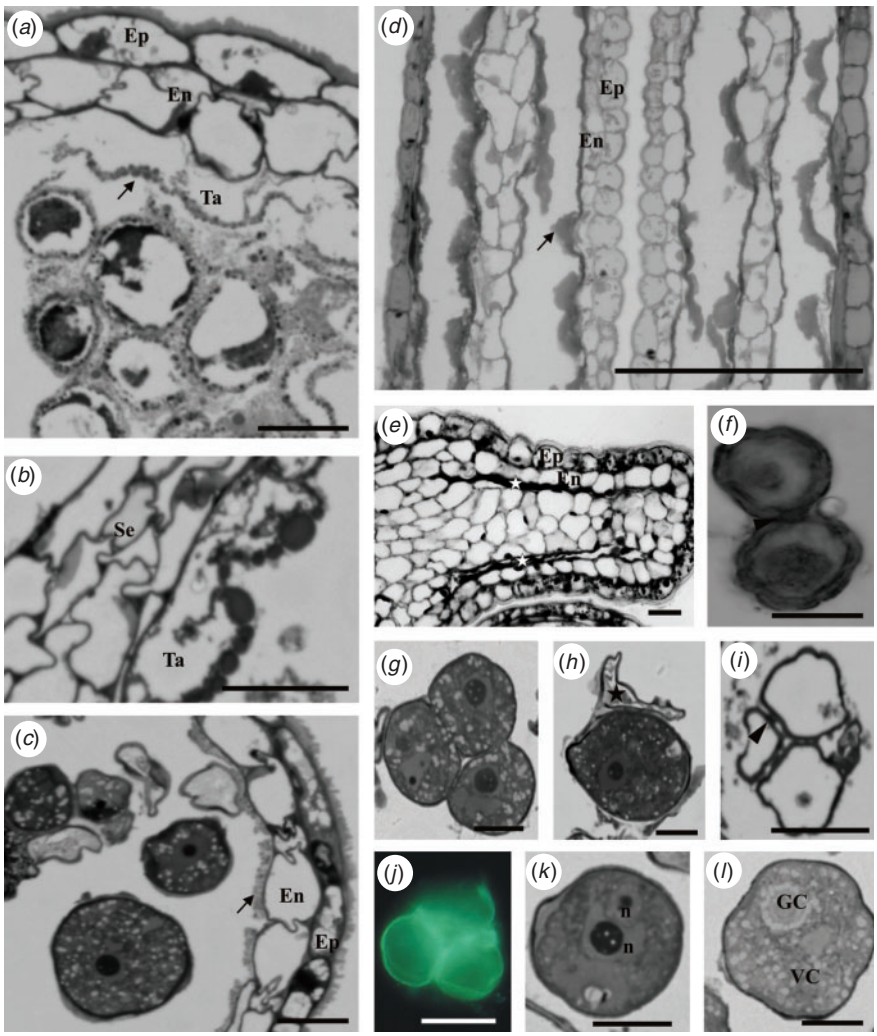

Fig. 2. Abnormalities in *Miconia albicans*. (*a*, *b*) Transverse section of an anther showing abnormal microspores and anomalous tapetum (Ta) wall (arrow). (*c*) The orbicules are aggregated over the endothecium (En) walls after total Ta degradation (arrow). (*d*) Longitudinal section of an empty mature anther showing anther wall composed of the epidermis (Ep) and endothecium (En). Note the presence of an amorphous substance inside the pollen sac (arrow). (*e*) Transverse section of a mature anther with obliterate pollen sacs (★). (*f–j*) Cell structures formed by exine fusion (arrowhead). Note the degenerated structures (★) and the exine under fluorescence in (*j*). (*k*) Cell structure with two nuclei (n). (*l*) Abnormal bicellular pollen grain with the vegetative cell (VC) and the generative cell (GC). Se = septum. Bars = 10 µm.

and Lugol solution, respectively) as reserve substances inside the microspore or pollen cytoplasm.

Despite the presence of an apparently normal cytoplasm in some pollen grains, which were positively stained with acetic carmine, no pollen grain was able to develop a pollen tube in our *in vitro* experiments of pollen germination (Fig. 7*l*).

Discussion

Meiotic abnormalities

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

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

Some meiotic irregularities observed in *M. stenostachya* were also reported by Goldenberg and Shepherd (1998). The micronuclei observed in the tetrad cells are a consequence of lagging chromosomes, as cytokinesis and the last callose

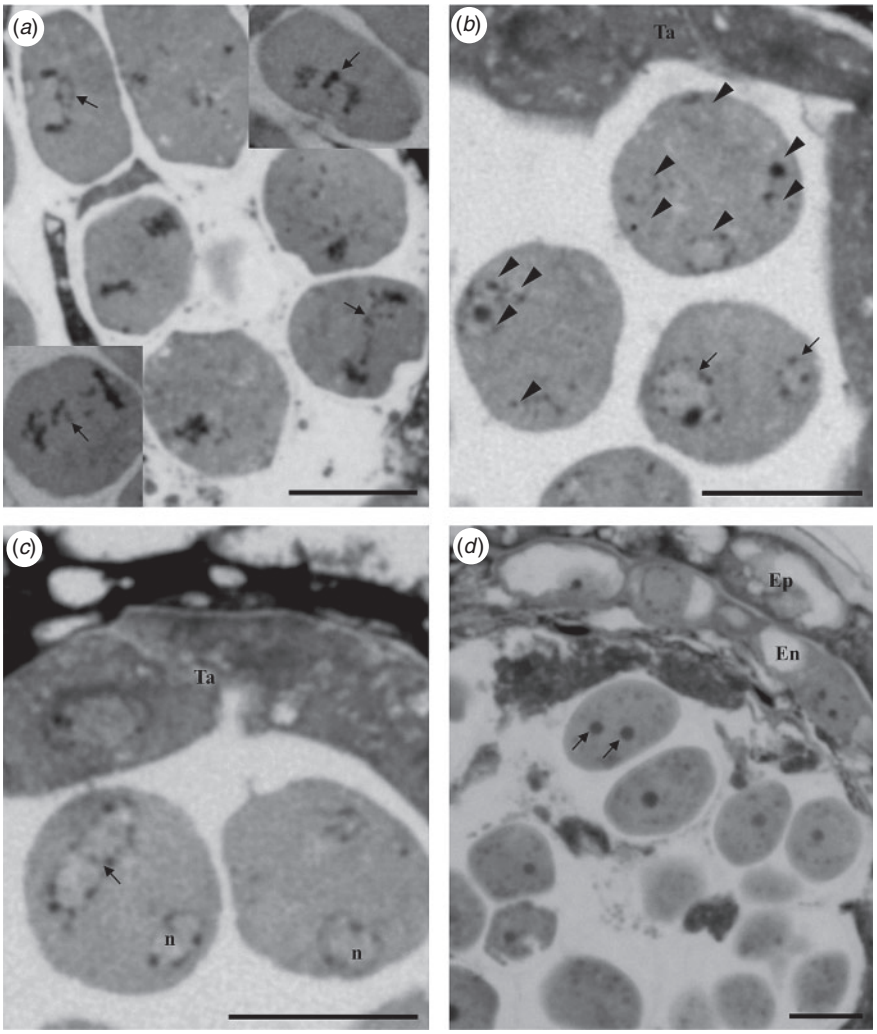

Fig. 3. Meiotic irregularities in *Miconia stenostachya*. (*a*) Lagging chromosomes (arrow) at anaphase. (*b*) Abnormal syncytia, with supernumerary (more than the usual four) nuclei (arrowhead), some of them different sizes (arrow). (*c*) Nuclear fusion (arrow) in syncytia. (*d*) Dyad formed after irregular cytokinesis. Note the presence of two nuclei (arrow) in the microspore. En = endothecium, EP = epidermis, n = nucleus, Ta = tapetum. Bars = 10 µm.

deposition around each microspore occurred even in the resulting small nuclei. Many studies of male sterility indicate that errors during meiosis are a frequent cause of male sterility in both natural and manipulated plants, for example *Eupatorium laevigatum* (Asteraceae, Bertasso-Borges and Coleman 2005), *Gagea lutea* (Liliaceae, Bohdanowicz *et al*. 2005), *Brachiaria jubata* (Poaceae, Risso-Pascotto *et al*. 2005) and *B. humidicola* (Poaceae, Calisto *et al.* 2008). The irregularities result in unbalanced microspores, which arrest in development. These errors may result from reduced cohesion of sister chromatids at metaphase I and metaphase II (Roeder 1997; Dawe 1998) or defects in the spindle apparatus (Jiang *et al*. 2009).

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

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

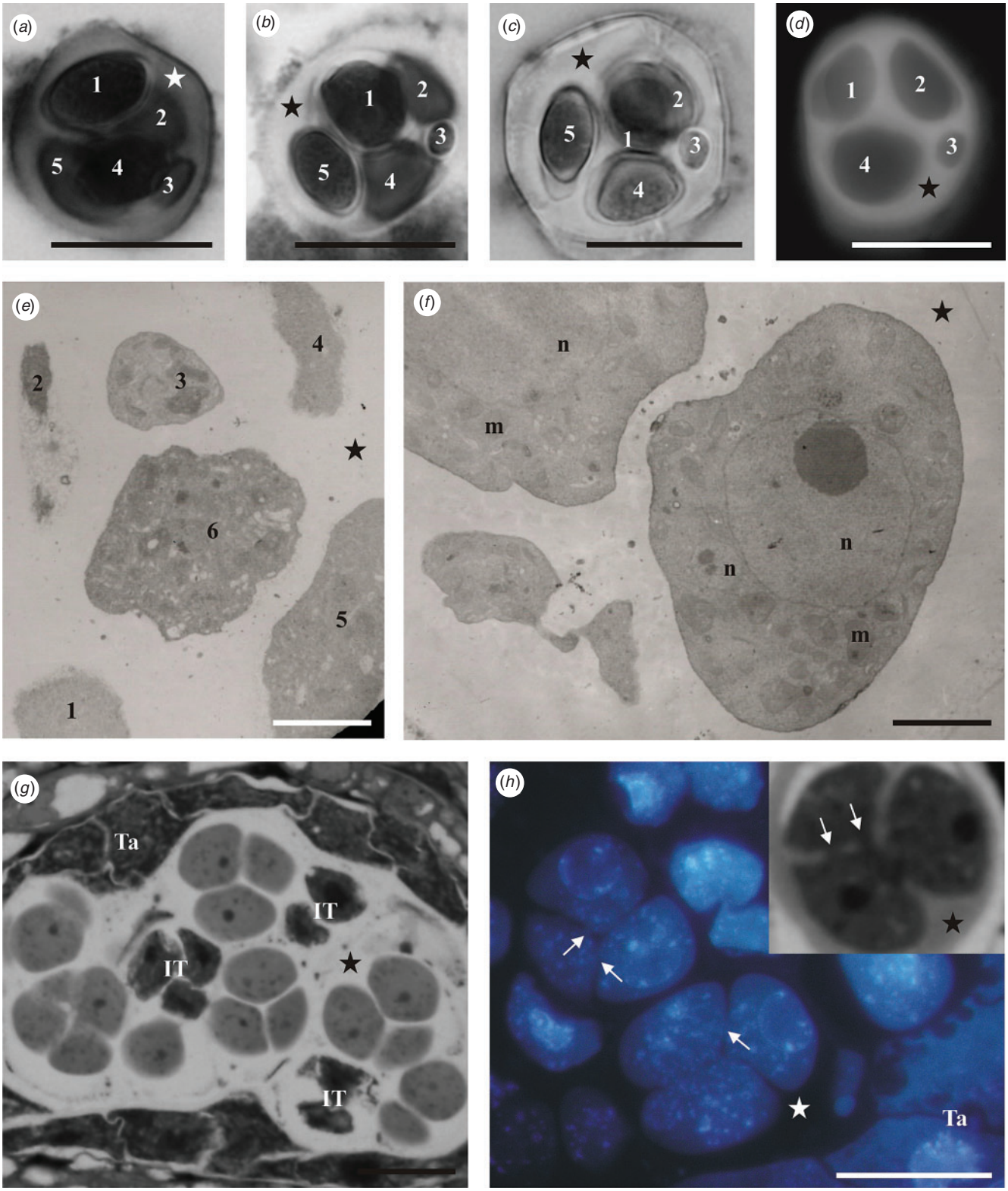

Fig. 4. Microspore tetrads in *Miconia stenostachya*. (*a*–*e*) Abnormal tetrads with micronuclei (3). Note the fluorescence and aniline blue in (*d*). (*f*) Mitochondria (m) and nuclei (n) in the microspore of a tetrad. (*g*, *h*) Transverse sections of the anther with both intact and degenerating (DT) tetrads. Some tetrads show cytoplasmic connections among adjacent microspores (arrow). Note the tetrads under DAPI staining in (*h*). Ta = tapetum, ★ = callose wall. Bars = 10 µm (*a*–*d*, *g*, *h*) and 0.9 µm (*e*, *f*).

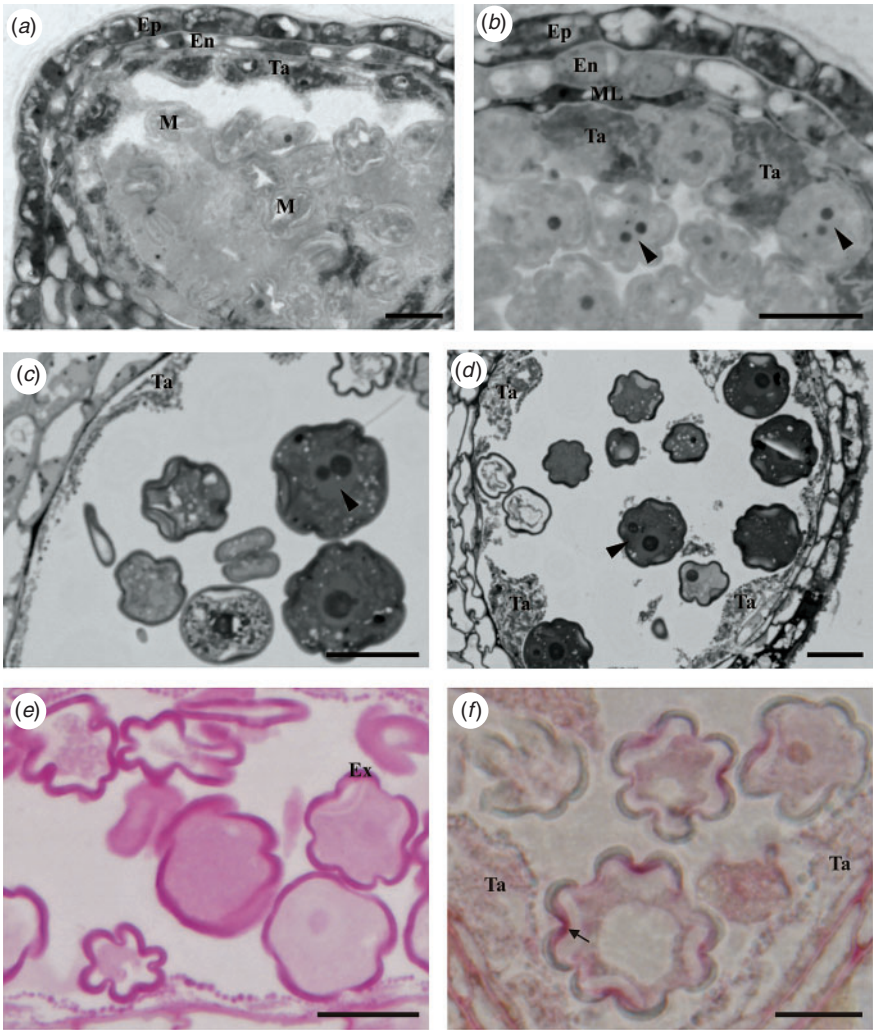

Fig. 5. Free microspores in the anther of *Miconia stenostachya*. Transverse sections. (*a*) Abnormal microspores (M) surrounded by dense locular fluid. (*b–d*) Microspore with more than one nucleolus (arrowhead). Note the presence of microspores of different sizes. (*e*) Unusual positive reaction for PAS in the exine (Ex). (*f*) Unusual number of apertures in one large microspore. Note the positive reaction with Ruthenium red in the intine (arrow). En = endothecium, EP = epidermis, ML = middle layer, Ta = tapetum. Bars = 10 µm.

(or seeds with viable embryos) along with no viable pollen grain for fertilisation process may indicate the absence of meiosis during megasporogenesis, as the formation of a diploid embryo sac is a requirement for the production of diploid embryos.

Mitotic abnormalities

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

Microspore and pollen wall abnormalities

Unlike *M. stenostachya*, in which abnormalities are restricted to the cytoplasm, the abnormal pollen wall structures observed in *M. albicans* indicate a more severe type of male sterility. Beyond the inner polysaccharide intine, the pollen wall normally has a thick outer sculptured exine that is composed mainly of sporopollenin (Scott 1994). In some male sterile species, pollen wall abnormalities may be related to precocious callose wall degradation (Worall *et al.* 1992; Tsuchiya *et al.* 1995), abnormal primexine formation (Ariizumi *et al.* 2005, 2008) or defective sporopollenin synthesis or polymerisation (Ariizumi *et al.* 2003). In *Actinidia deliciosa* (Actinidiaceae), the sexine and nexine are abnormal or absent (Biasi *et al.* 2001), and in *Arabidopsis thaliana* (Brassicaceae) abnormal exine ornamentation is due to post-meiotic defects (Taylor *et al.*

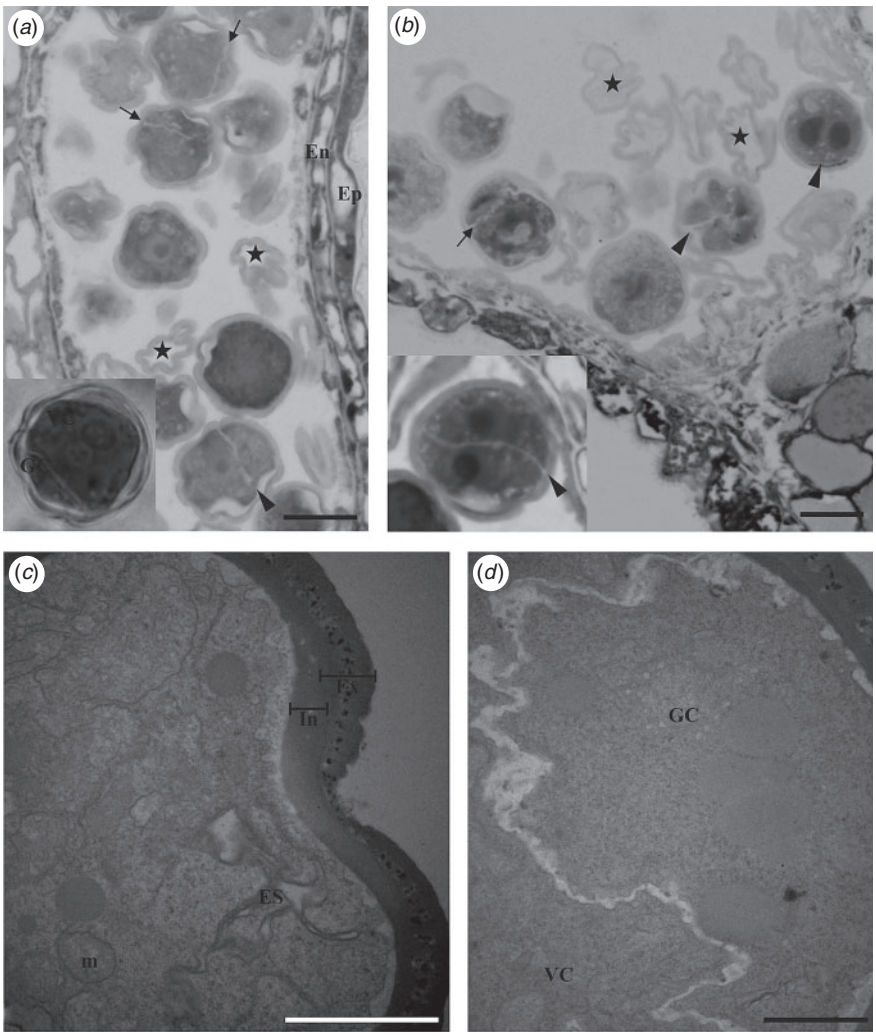

Fig. 6. Microgametogenesis in *Miconia stenostachya*. (*a*, *b*) Transverse section of anther showing abnormal asymmetric (arrow) and symmetric (arrowhead) mitosis, along with empty structures (★). (*c*, *d*) Abnormal pollen grains with normally structured exine (Ex) and intine (In). En = endothecium, EP = epidermis, ES = endomembrane system, GC = generative cell, VC = vegetative cell, m = mitochondria. Bars = 10 µm (*a*, *b*) and 3 µm (*c*, *d*).

1998). Because of the rarity of normal tetrads in *M. albicans*, we were not able to identify which kind of abnormality was responsible for abnormal pollen wall formation, but we can exclude the defective sporopollenin synthesis since orbicules were present. The primexine forms around microspores, between the callose wall and the microspore plasma membrane, at the tetrad stage (Owen and Makaroff 1995) as a scaffold for sporopollenin deposition. Regardless of the causes of abnormal primexine formation, we can consider it as a strong candidate responsible for abnormal pollen wall formation in *M. albicans*.

Hybridisation and polyploidisation

The relationship between the genomes of the parental species has great influence on the determination of chromosome pairing and recombination processes and, thus, on the extent of meiotic irregularities and gamete viability (de Jong *et al*. 1993). Indeed, somatic hybrids are generally less fertile, which is a major problem for sexual reproduction (Pijnacker *et al*. 1992). Meiotic irregularities may be related to hybridisation and have important evolutionary consequences. The chromosome counts for *M. albicans* showed different numbers ($2n = 34$ and $2n = 48$), perhaps because the samples were from different regions (Goldenberg and Shepherd 1998). The number obtained for *M. stenostachya* ($n = \sim 26$) suggests that this species may be triploid in the studied population. Goldenberg and Shepherd (1998) also reported a strong indication of hybrid origin for this species based on observation of meiotic irregularities. In the same areas where *M. stenostachya* and *M. albicans* were collected, other *Miconia* species are present, including *M. fallax*, *M. rubiginosa* and *M. leucocarpa*. Interestingly, *M. fallax* is also apomictic and probably diploid ($n = \sim 17$) according to Goldenberg and Shepherd (1998) or polyploid ($2n > 60$) according to A. P. S. Caetano (unpubl. data), and its vegetative and reproductive structures are very similar to those of *M. stenostachya*. Therefore, if *M. stenostachya* is truly a hybrid in origin, *M. fallax* is a strong candidate for one of its

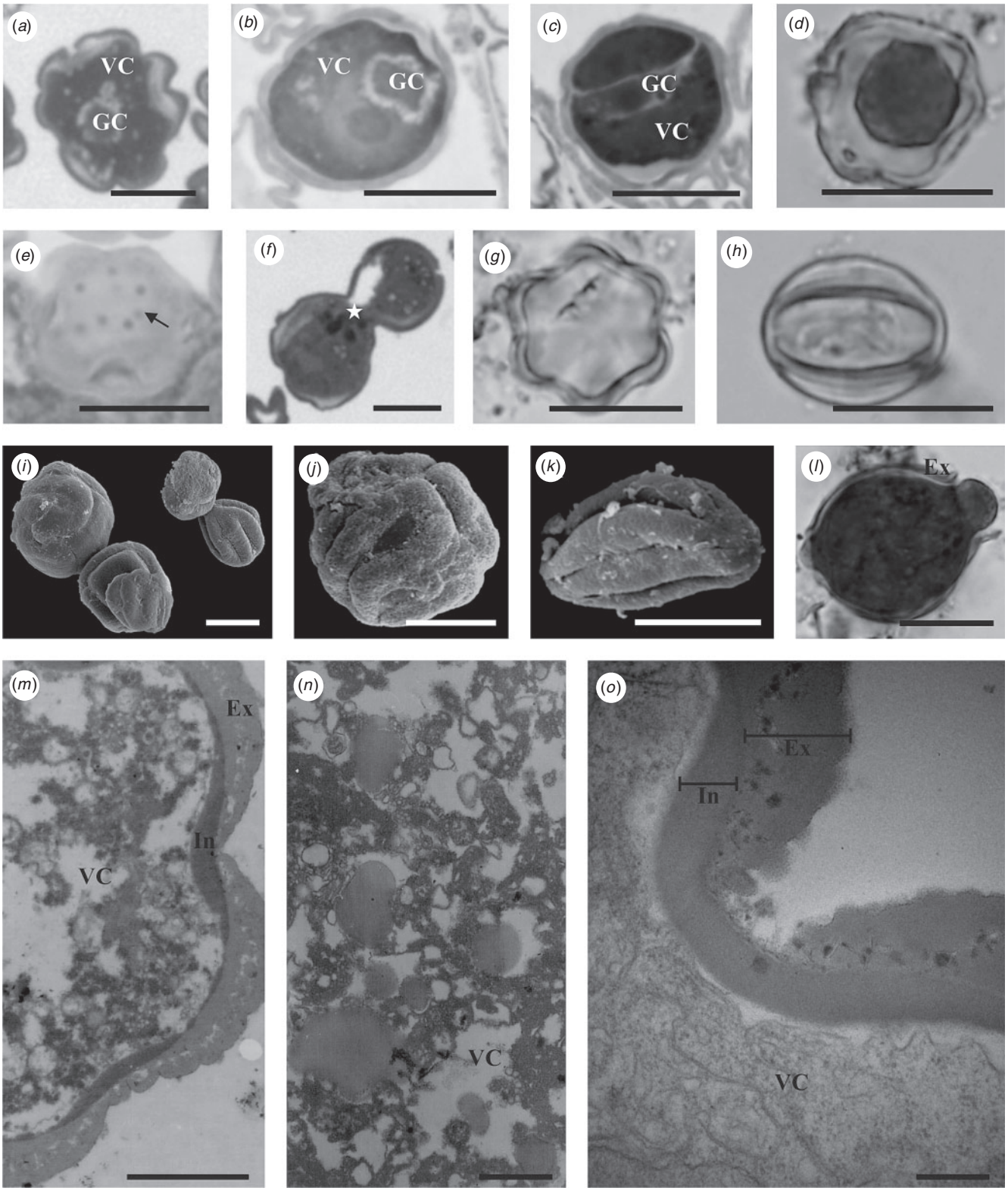

Fig. 7. Abnormal cell structures found in mature anthers of *Miconia stenostachya*. (*a–c*) Abnormal pollen grains with vegetative cells (VC) and generative cells (GC). (*d*) Cellular structure with degenerating cytoplasm. (*e*) Cell structure with supernumerary nuclei (arrow). (*f*) Cytoplasmic connection (★) between two adjacent bicellular pollen grains. (*g*, *h*) Empty cell structure with normal exine and apertures. (*i–k*) Abnormal pollen grains. (*l*) Pollen grain with stained cytoplasm and normal exine (Ex) that is unviable due to its inability to form the pollen tube. (*m–o*) Abnormal pollen grains with degenerating cytoplasm but pollen wall normally structured in exine (Ex) and intine (In). Bars = 10 µm (*a–l*), 1.7 µm (*m*), 0.5 µm (*n*) and 1 µm (*o*).

sister clones (R. Goldenberg, pers. comm.). Another interesting fact is that *M. fallax* pollen grains, unlike those of *M. stenostachya*, were able to germinate when manually deposited at the stigma surface of *M. albicans*, although in an abnormal way (P. A. Cortez, pers. obs.). This is not surprising because *M. albicans*, *M. fallax* and *M. stenostachya* are phylogenetically related species (R. Goldenberg, pers. comm.).

Polyploidisation is considered a major evolutionary force in plants, being of fundamental importance to angiosperm diversification (Soltis *et al*. 2004, 2009). As a result, the application of species concepts has become problematic (Soltis *et al*. 2007). Otto and Whitton (2000) suggest that polyploidisation may be the single most common mechanism for sympatric speciation in plants. Molecular studies of polyploid formation indicate that intragenomic rearrangement and altered gene regulatory relationships can contribute to evolutionary flexibility (Soltis *et al*. 2009). In addition to the absence of meiosis and fertilisation, this may explain why apomictic groups are so diverse, as observed in *Miconia*. Despite advances in the understanding of polyploidy, the consequences of genetic, physiologic and ecological changes for polyploid plants in natural populations are essentially unknown (Soltis *et al*. 2004).

Conclusion

The present study shows that morphological tools can shed light on apomictic reproduction in wild plants. The inability of the apparently viable pollen grains of *M. albicans* and *M. stenostachya* to germinate using germination tests conducted *in vitro* with thousands of pollen grains, along with the lack or low number of pollen grains naturally deposited over the stigmatic surface of these species, strongly suggest that sexual reproduction is not important in these species. Moreover, our data indicate that although the pollen grain cytoplasm maintains its affinity for cytological staining, the apparatus that allows the successful growth of the pollen tube does not necessarily do so, and so even stained pollen grains may be nonviable, which means that both *M. albicans* and *M. stenostachya* are completely male sterile. This conclusion is very significant, as some authors have questioned whether obligate apomixis exists (Asker 1979). The pollen grain abnormalities in the apomictic *Miconia* species studied to date are a consequence of meiotic irregularities, which may affect even the early meiotic steps, as observed in *M. albicans*, also leading to the pollen wall abnormalities. As observed in the ontogenetic studies conducted in our laboratory with other apomictic and sexual *Miconia* species, the pollen grain abnormalities are not related with the anther wall since anther wall layers develop in a normal way (P. A. Cortez, S. M. Carmello-Guerreiro, S. P. Teixeira, unpubl. data). The ecological significance of this mode of reproduction among Melastomataceae is unclear, but we note that the wide distribution of the apomictic species in Brazil indicates the evolutionary success of this type of asexual reproduction.

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