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Anther wall and pollen development in two species of *Rauvolfia* L. (Apocynaceae)

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Abstract

Although dioecy and gynodioecy are widely reported in Gentianales, they are rare in Apocynaceae, with reports for only three species. In these species, the pistillate flowers have empty anthers. To understand how this process occurs, it is important to gather information about the formation of anthers and pollen grains, but studies on the embryology of Apocynaceae are scarce and restricted. Here, we investigate anther wall and pollen grain development and the processes leading to pollen grain absence in *R. vomitoria*, a gynodioecious species. We compare it with *R. weddelliana*, a species with hermaphrodite flowers. To do so, the flowers of both species were collected at several developmental stages and processed using usual anatomical techniques. Mature pollen grains were analyzed via scanning electron microscopy. In both species, the anthers are tetrasporangiate, and the anther wall development is of the dicotyledoneous type. The tapetum is secretory, and the endothecium has fibrous thickenings. The tetrad of microspores is tetrahedral as a result of simultaneous cytokinesis. Each pollen grain is tricolpate, two celled, oblate, and smooth. Small perforations occur in the equatorial axis of the pollen only in *R. weddelliana*. Thick margins occur in the colpi of both species. Surprisingly, when the pistillate flowers of *R. vomitoria*, which came from the same population in which gynodioecy was reported, were analyzed, fertile anthers were found during this flower season. The only evidence of the sterility was the presence of some sterile microsporangia. Our results indicate that *R. vomitoria* may exhibit sexual lability.

Keywords Gynodioecy · Pollen grain · *Rauvolfia vomitoria* · *Rauvolfia weddelliana* · Rauvolfioideae

1 Introduction

Apocynaceae Juss. is positioned in Gentianales (asterids, lamiids) and include about 400 genera and 5000 species located in tropical and subtropical regions around the world (Stevens 2001 onwards; Endress 2004; APG IV 2016). Their flowers have gamopetalous corollas with epipetalous stamens, which are usually positioned above the style head.

The stamens present several stages of adnation to the style head in different genera, since those with free anthers until those adnate totally, forming a gynostegium. The pollen grains can present as monads, tetrads, or in pollinia (see Fallen 1986; Endress and Bruyns 2000; Rapini 2012). This variation makes the family especially interesting for studies of the evolution of floral characteristics and reproductive biology.

Anatomical studies of the floral structures of Apocynaceae have addressed the compartmentalization of functions in the style head, the presence and type of anther adnation (Schick 1980, 1982; Fallen 1986), floral ontogenesis with an emphasis on gynoecium development (Gomes 2006; Kotovski 2013), and secretory structures (Marasca 2008; Kotovski 2013). The studies related to reproductive biology have mainly identified plants with hermaphrodite flowers and mechanisms such as herkogamy, protandry (Herrera 1991; Albers and Van Der Maesen 1994; Lin and Bernardello 1999), protogyny

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(Sihag and Wadhwa 2011), and self-incompatibility (Waddington 1976; Haber 1984; Lopes and Machado 1999; Lipow and Wyatt 1999), which minimize or prevent self-pollination. In addition, there are reports of cryptic dioecy and gynodioecy in two species of *Rauvolfia* and one report of gynodioecy in one species of *Carissa*, the species with heterostylous flowers (Marloth 1932; Koch et al. 2002).

Although reports of dioecy and gynodioecy are scarce within the Apocynaceae, they are common for Gentianales, mainly for Rubiaceae (see Vinckier and Smets 2005; Souza et al. 2008; Li et al. 2010). Cryptic dioecy can also occur in this family, such as in *Mussaenda pubescens* Kunth, where even with well-formed anthers, long-styled flowers do not form pollen (Li et al. 2010). In this morph, the tapetal cells are vacuolated and disintegrated at the free microspore phase, leading to male sterility. These results show that embryological studies can help in understanding the structural aspects of dioecy. Moreover, embryological studies can help in understanding the evolution of characteristics within and among angiosperm families (Tobe 1989).

Regarding the Apocynaceae, there are few studies focused on embryology itself, and these are restricted to old manuscripts that are difficult to obtain. Moreover, they are controversial in relation to patterns of development (see Davis 1966). Maheswari Devi (1971) refers to the family as a heterogeneous group, pointing out the need for more studies to help understand the evolutionary patterns of embryological characteristics. As far as we know, there are no studies showing the processes that lead to male sterility for Apocynaceae.

Here, we analyze *Rauvolfia vomitoria* Afzel., a species that has individuals with hermaphrodite flowers and individuals with functionally pistillate flowers. The pistillate flowers are described as having all the floral whorls but empty anthers (Koch et al. 2002). Our objective is to identify the embryological characteristics related to pollen grain development and describe the structural processes that lead to male sterility in the functionally pistillate flowers of *R. vomitoria*. For this, we analyze the development of the anther wall layers, microsporogenesis and microgametogenesis in *R. vomitoria* and compare them with the same processes in *R. weddelliana* Müll. Arg., a species with hermaphrodite flowers.

2 Materials and methods

The flowers of *R. weddelliana* and distinct morphotypes (pistillate and hermaphrodite) of *R. vomitoria* were collected at several stages of development. The morphotypes of *R. vomitoria* were identified based on the flower characteristics described by Koch et al. (2002), and they were differentiated based on (1) the occlusion of the lower part of the corolla tube, which occurs only in perfect flowers, and (2) style-head morphology. The style head is spool-like in both morphotypes, with a membranaceus scraper structure around the base, like a cup or a skirt; a crown of hair on the top; and two small, short appendices in the apex. In the pistillate flower, however, the scraper structure around the base is shorter, and the sticky medium part is broader than in hermaphrodite flowers (Koch et al. 2002).

The samples were fixed in Karnovsky solution (Karnovsky 1965) for 48 h and stored in 70% ethanol. We analyzed 50 buds and flowers per morphotype and species. These were taken from three individuals of each *R. vomitoria* morphotype and five *R. weddelliana*. Fertile branches were incorporated into UEC and SORO herbaria [acronyms according to Thiers (continuously updated)] under the numbers UEC192563 (15°40′62″S, 55°83′38″W), UEC192564 (15°40′80″S, 55°82′82″W), and UEC192565 (15°40′69″S, 55°83′06″W) for the *R. weddelliana* and SORO4943 (22°86′97″S, 47°07′41″W) and SORO4939 (22°87′02″S 47°07′43″W) for the *R. vomitoria* with pistillate and hermaphrodite flowers, respectively. *Rauvolfia weddelliana* were collected in the Cerrado area inside the Chapada dos Guimarães National Park, Mato Grosso state, Brazil, and *R. vomitoria* were collected from the Collection of Aromatic and Medicinal Live Plants of the Center of Horticulture of the Agronomic Institute of Campinas (IAC), Campinas, São Paulo state, Brazil. These are the same individuals studied by Koch et al. (2002).

The floral buds and flowers were dissected to isolate the corolla with stamens, which were dehydrated in ethanol series and embedded with a Historesin Embedding Kit (Leica Microsystems) according to the protocol proposed by Paiva et al. (2011). The samples were sectioned at a thickness of 3–4 μm using a rotatory microtome (RM 2255, Leica), and the sections were stained with 0.05% toluidine blue (color index: 52040) in 0.1 M acetate buffer at pH 4.7 (O'Brien et al. 1964, modified) and counterstained with an aqueous solution of 0.02% ruthenium red (E.A.S. Paiva, personal communication). Chemical compound detection was performed for certain sections, which were submitted to the following dyes or reagents: ruthenium red (color index: 77800) to identify pectic compounds (Jensen 1962), phloroglucinol with hydrochloric acid to identify lignified cell walls (Sass 1951), Sudan IV (color index: 26105) to highlight the location of lipidic substances, Lugol's solution for detecting starch grains (Johansen 1940), ferric chloride with sodium carbonate to investigate the occurrence of phenolic substances (Johansen 1940), and an alcohol solution of 0.1% bromophenol blue (color index: N/A) and mercury chloride

10% to identify proteins (Mazia et al. 1953). The slides were mounted with Entellan (Merck) or distilled water, analyzed and recorded using a light microscope (BX53, Olympus). Additionally, differential interference contrast (DIC) was used to highlight the nuclei.

For the scanning electron microscopy, pre-anthesis bud flowers were fixed in Karnovsky solution (Karnovsky 1965) for 24 h, dehydrated in an ethanol series, dried with CO_2 using a critical point drier (CPD-030, Balzers), fixed on aluminum stubs, and coated with gold using a sputter coater (SCD-050, Balzers). The samples were analyzed in a scanning electron microscope (JSM 5800LV, Jeol).

We counted the number of stamens and fertile microsporangia in the two floral morphotypes of *R. vomitoria* and in flowers of *R. weddelliana*. We considered fertile microsporangia to be those that exhibited pollen grains and sterile microsporangia to be those absent of pollen. For this, 100 pre-anthesis flower buds from two individuals of each morphotype of *R. vomitoria* and 100 pre-anthesis flower buds for *R. weddelliana* were cut free hand and observed using a stereomicroscope (EZ4 HD, Leica).

3 Results

Anther wall development of hermaphrodite flowers of *R. weddelliana* and *R. vomitoria* – The stamen primordia of both species initiate as projections of floral meristem. In the early developmental stages, the anther is lobed in cross section (Fig. 1a). The outermost layer shows only anticlinal divisions and will give rise to the protoderm (Fig. 1b–e). The primary parietal layer (Fig. 1b) divides periclinally (Fig. 1c), giving rise to the secondary parietal layers (Fig. 1d). The outer secondary parietal layer divides again, originating the endothecium and the middle layer (Fig. 1e). The inner secondary parietal layer directly gives rise to the tapetum (Fig. 1e).

The anthers of the two species are tetrasporangiate (Fig. 1b, f). During the early stages of development, the wall is composed of protoderm, undifferentiated endothecium, one middle layer, a tapetum, and the sporogenic tissue (Fig. 1e). The protoderm includes cells with smaller vacuoles and apparent nuclei (Fig. 1e, g). The endothecium is one layered, and both the endothecium and middle layer have vacuolated cells, with thin cell walls and evident nuclei (Fig. 1e, g). The tapetum is secretory, one layered, and composed of isodiametric cells (Fig. 1e), which eventually become tangentially elongated cells (Fig. 1g). Tapetum cells contain cytoplasm with a dense appearance and one nucleus per cell (Fig. 1e, g), even in more advanced stages (Fig. 1h). In some regions, the tapetum and the middle layer can become two layered (Fig. 1g).

During microsporogenesis and microgametogenesis, the anther wall undergoes significant changes. The epidermal cells elongate tangentially and remain alive, and in some cases, their nuclei remain visible (Fig. 1i, j). The cells of the endothecium enlarge, acquire walls with fibrous thickening (Fig. 1i, j). The middle layer is crushed, so it is not present at the pollen dispersal stage. The tapetum begins to degenerate during the vacuolation of the microspore, and it remains completely degraded until anthesis (Fig. 1j).

Microsporogenesis of hermaphrodite flowers of *R. weddelliana* and *R. vomitoria* – Sporogenous cells are easily recognized by their large volume as compared to the cells from the parietal layers (Figs. 1e, g, 2a). The sporogenous cells initiate a callose deposit around them (Fig. 2a), isolating themselves from one another, except for certain cytoplasmic channels (Fig. 2b, c). The microspore mother cells (MMCs) undergo meiosis (Fig. 2c) with simultaneous cytokinesis (Fig. 2d), forming a tetrahedral tetrad of microspores (Fig. 2e, f). Synchronicity occurs in the meiotic divisions within the same microsporangium, but not within the same stamen or floral bud.

Microgametogenesis of hermaphrodite flowers of *R. weddelliana* and *R. vomitoria* – After microspore development, the callose is degraded, and the microspores become free. Exine is deposited in a thick layer around each microspore (Fig. 3a, b) and has quite an irregular outline (Fig. 3a, b). Then, the process of vacuolization begins, forcing the cytoplasm and nucleus to the periphery of the cell (Fig. 3c, d). By the end of vacuolization, the exine has developed a circular and uniform contour (Fig. 3d).

Asymmetric division occurs and gives rise to the vegetative cell, which is larger and vacuolated, and to the generative cell, which is smaller, peripheral, and has a lenticular contour (Fig. 3e). During this phase, the vacuole of the vegetative cell accumulates substances (Fig. 3e). After that, the cytoplasm becomes dense (Fig. 3f) via the accumulation of starch grains (Fig. 3g), and the vacuole decreases in size until it becomes invisible (Fig. 3f). The generative cell becomes rounded (Fig. 3f) and migrates into the vegetative cell's cytoplasm, coming close to its nucleus (Fig. 3h, i). The deposition of intine begins during the initial stages of microspore vacuolization (Fig. 3a), first in the colpi and then in other regions (Fig. 3a, e, f, h, i).

The mature pollen grain is tricolpate, oblate and has a smooth surface (Fig. 4a, b). The endoaperture is circular and located in a depression formed by the two thick margins of each colpus in *R. weddelliana* and in the hermaphrodite flowers of *R. vomitoria* (Fig. 4c, d). In the pistillate
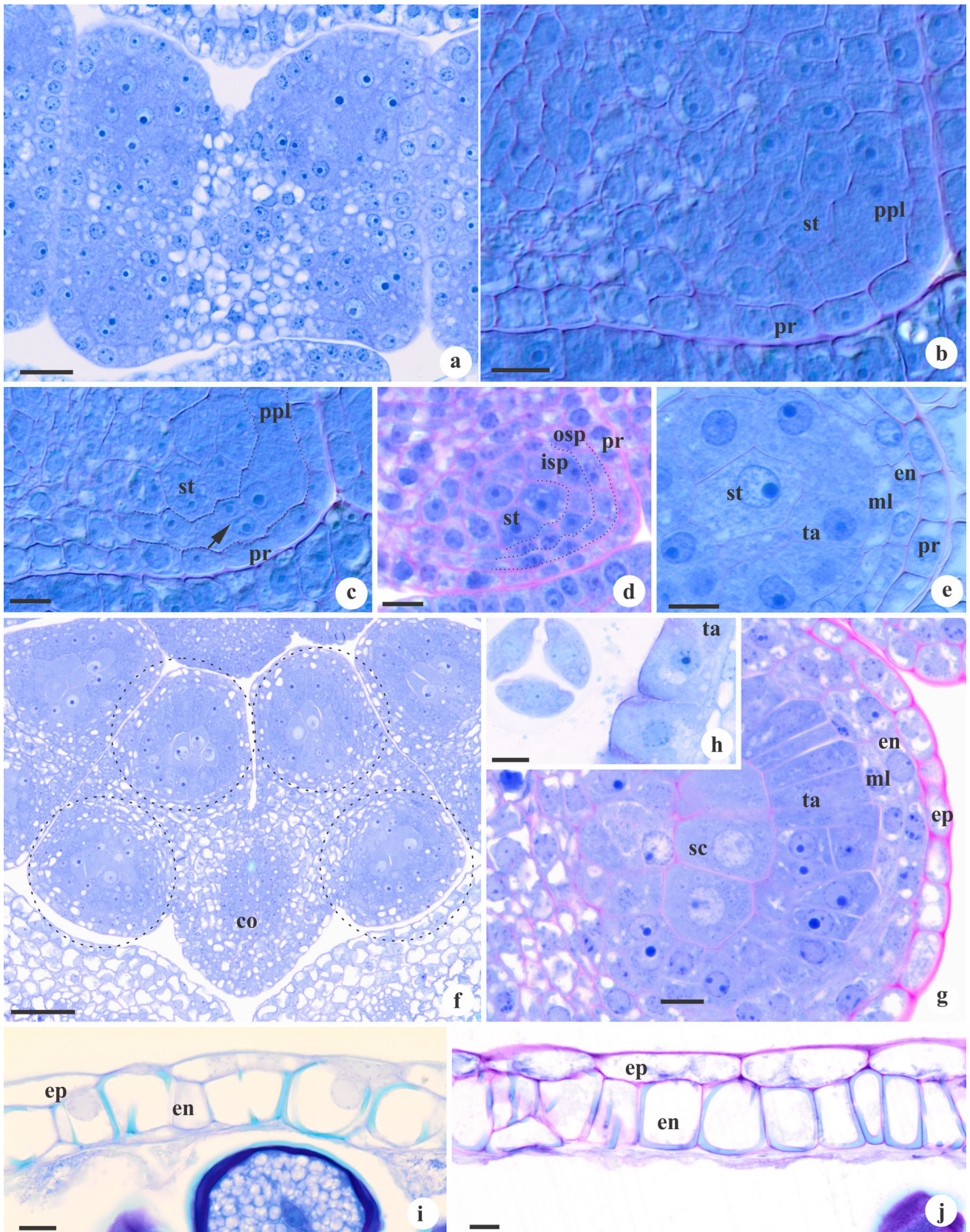
Fig. 1 Anther development in hermaphrodite flowers of *Rauvolfia vomitoria* Afzel. (**b–e**, **i**) and *Rauvolfia weddelliana* Müll. Arg. (**a**, **f–h**, **j**). Longitudinal sections (**i–j**). Transversal sections (**a–h**). **a** Early stage of anther, note the lobed shape. **b** Detail of microsporangium in early stages of development showing protoderm, primary parietal layer and sporogenic tissue, observation under differential interference contrast. **c** Primary parietal layer (delimited by the dotted lines) with cell division (arrow), observation under differential interference contrast. **d** Anther wall with the secondary parietal layers formed (delimited by the dotted lines). **e** Detail of anther wall with protoderm, endothecium, middle layer, tapetum and sporogenic tissue. **f** General view of anther showing four microsporangia (highlighted in dotted lines). **g** Anther wall, note the tapetum and middle layer with two layer in some points. **h** Detail of tapetum with 1-nucleated cells at the stage of tetrad of spores. **i–j** Parietal layers of anthers at the stage of dehiscence, note the presence of epidermis and endothecium. Scale bars = 10 μm (**b–e**, **g–j**); 20 μm (**a**); 50 μm (**f**). *co* connective, *en* endothecium, *ep* epidermis, *isp* inner secondary parietal layer, *ml* middle layer, *osp* outer secondary parietal layer, *ppl* primary parietal layer, *pr* protoderm, *sc* sporogenous cells, *st* sporogenic tissue, *ta* tapetum

morphotype of *R. vomitoria*, the colpi have an inconspicuous margin. In *R. weddelliana,* perforations occur in the equatorial axis (Fig. 4d). The exine is thick (Fig. 4e, f). The intine contains pectic compound and is thick in the colpi and margins of colpi and the intine is thin between the colpi (Fig. 4e, f, h, i). Pollen grains are dispersed in a bicellular form (Fig. 4e, f). The vegetative cell cytoplasm is full of starch grains (Fig. 4g), and the nucleus occupies a central position (Fig. 4e, f). The generative cell is located near the vegetative cell's nucleus and has a fusiform contour and heavily stained cytoplasm and nucleus (Fig. 4f).

Anther wall, microsporogenesis and microgametogenesis of pistillate flowers of *R. vomitoria* – Anther wall development, microsporogenesis, and microgametogenesis in the pistillate flower morphotype of *R. vomitoria* occurs in the same way as described above. So, most flowers have anthers full of normal pollen grains. The only anomaly found was the non-formation of pollen grains in some microsporangia (Fig. 5a). About 19% of flowers had some microsporangia that were totally empty of pollen grains at maturity, and 73.7% of them had only one stamen with sterile microsporangia. That is, in our analyses, the pistillate flower morphotype was actually hermaphrodite because the anthers had normal pollen grains. In the hermaphrodite flower morphotype, malformed microsporangia were not found. We found no sterile microsporangia in *R. weddelliana*.

In sterile microsporangia of *R. vomitoria*, the anomaly apparently occurs in the vacuolation phase of the microspore, in which the exine and intine are formed but the vegetative cell degenerates (Fig. 5b, c). This process can also occur in some pollen grains that are inside normal microsporangia, side by side with normal pollen grains.
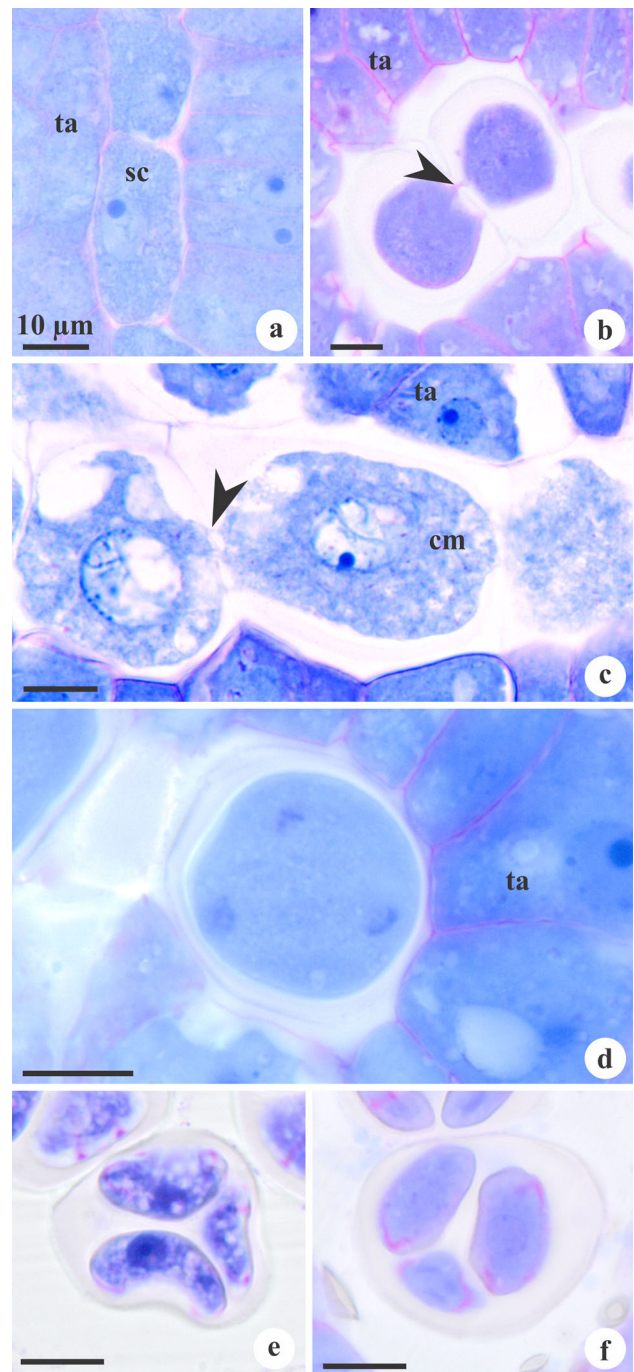

Fig. 2 Microsporogenesis in hermaphrodite flowers of *Rauvolfia vomitoria* Afzel. (**a**, **e**) and *Rauvolfia weddelliana* Müll. Arg. (**b–d**, **f**). Longitudinal sections (**a–c**). Transversal sections (**d–f**). **a** Sporogenous cells beginning to deposit callose. **b–c** Microspore mother cells with cytoplasmic channels (arrowhead). **d** Telophase II, note the absence of cell wall, characterizing the simultaneous type of cytokinesis. **e–f** Tetrahedral tetrads of microspores. Scale bars = 10 μm (**a–f**). *cm* microspore mother cell, *ta* tapetum *sc* sporogenous cell

While the anther continues its development, the pollen grains of the sterile microsporangium collapse, and after that, only the exine is visible (Fig. 5b, c).
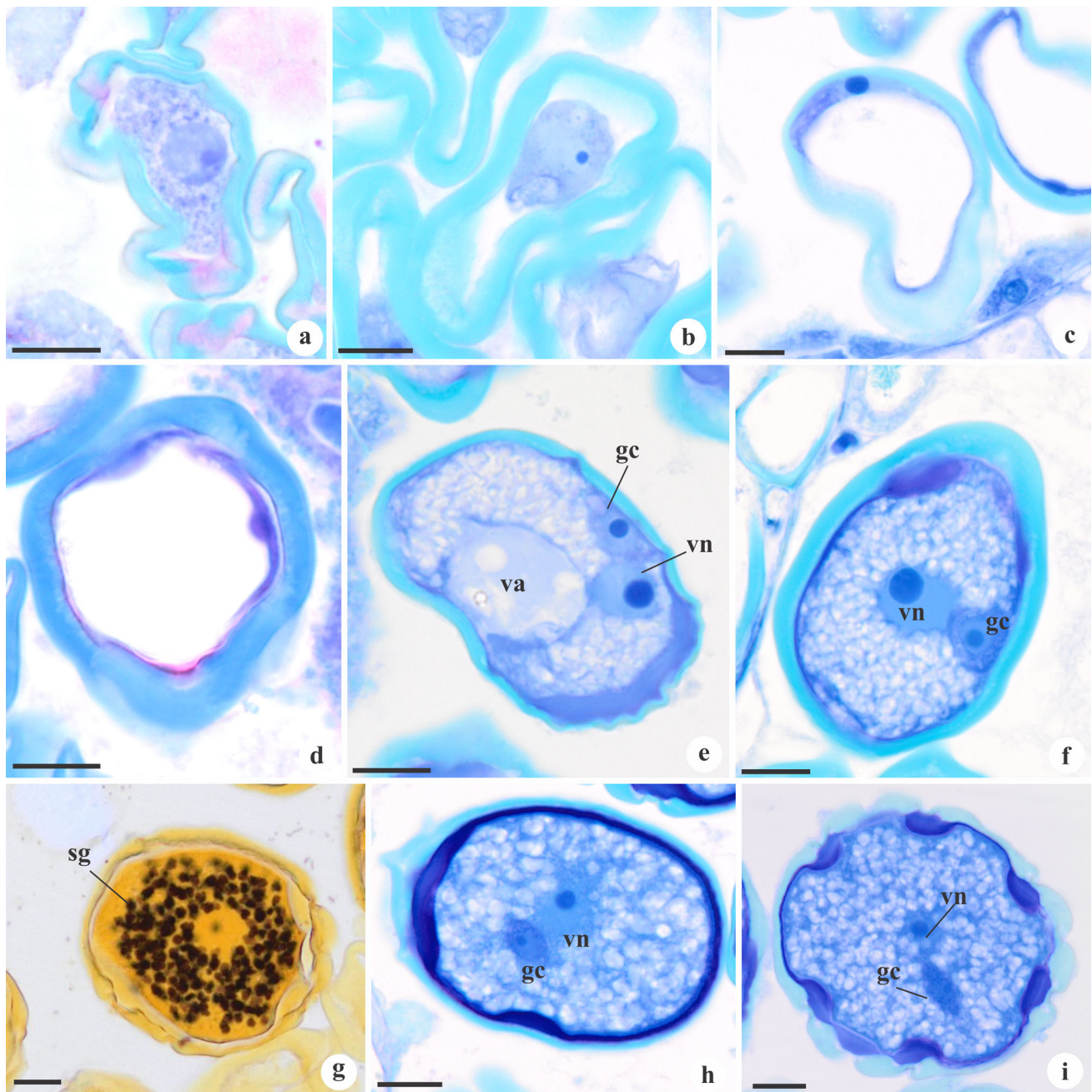
Fig. 3 Microgametogenesis in hermaphrodite flowers of *Rauvolfia vomitoria* Afzel. (**a**, **c–e**, **g**, **h**) and *Rauvolfia weddelliana* Müll. Arg. (**b**, **f**, **i**). Longitudinal sections. **a–b** Microspores with deposition of exine in irregular outline. **c** Microspores during the vacuolization process, note the nucleus in the periphery. **d** Microspores after vacuolization process, note the regular outline of exine. **e** Pollen grain with generative cell in the periphery and with lenticular shape. **f** Pollen grain with generative cell of rounded contour. **g** Pollen grain stained with Lugol's solution. **h–i** Generative cell close to the nucleus of vegetative cell. Scale bars = 10 μm (**a–i**). *gc* generative cell, *sg* starch grains, *va* vacuole, *vn* vegetative cell nucleus

4 Discussion

Non-formation of pollen grains – Although we selected the pistillate and hermaphrodite morphotypes according to Koch et al. (2002), we did not find flowers with all their anthers empty. Thus, we did not find completely pistillate individuals, because 81% of the flowers termed pistillate

had stamens whose microsporangia were well formed and full of pollen grains. In addition, in flowers with microsporangia without pollen grains, this characteristic appeared in only one or two anthers.

It is noteworthy that these results were obtained in the same population of *R. vomitoria* in which Koch et al. (2002) reported gynodioecy and sterile pistillate flowers.
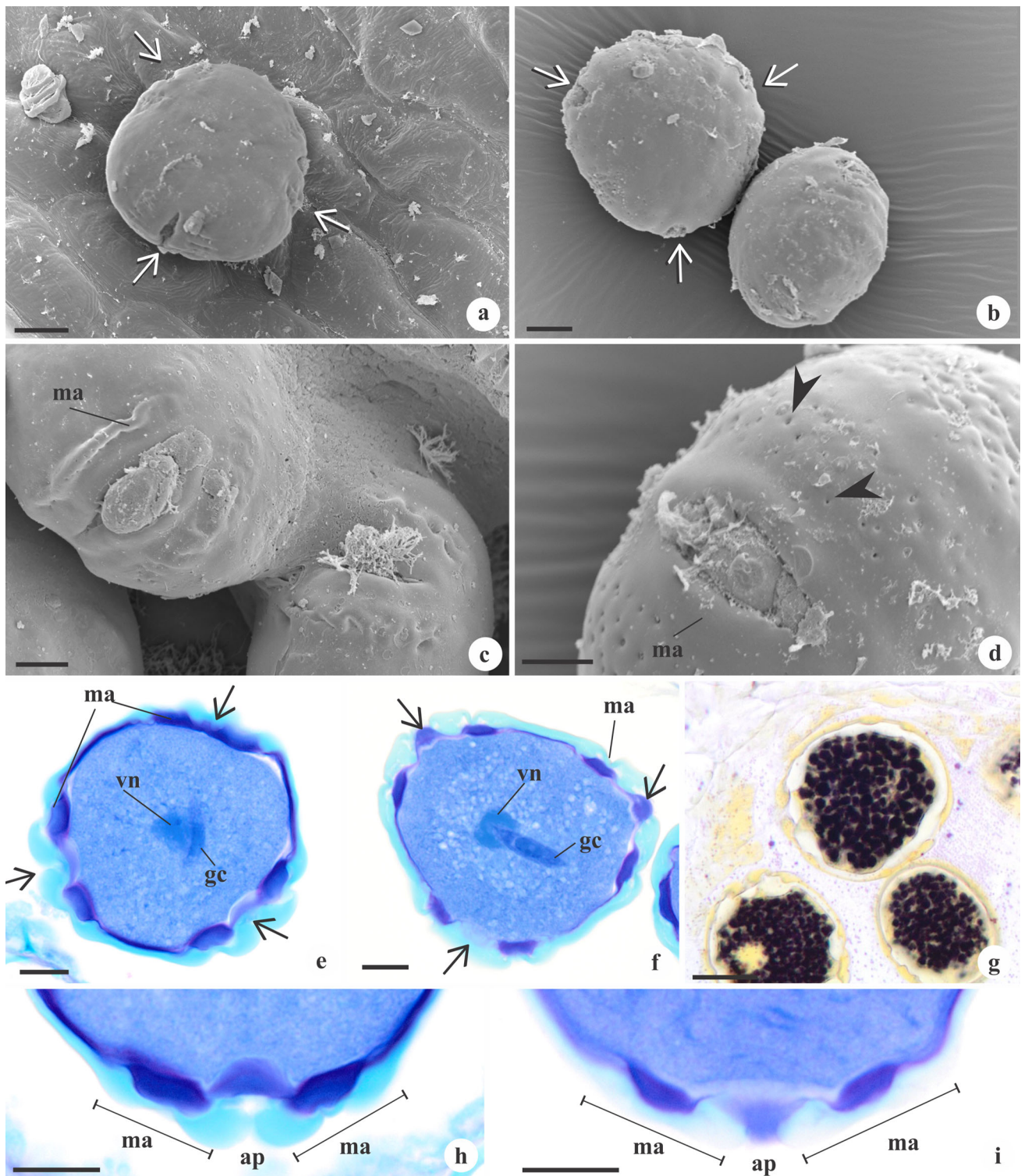
Fig. 4 Mature pollen grains in hermaphrodite flowers of *Rauvolfia vomitoria* Afzel. (**a**, **c**, **e**, **g**, **h**) and *Rauvolfia weddelliana* Müll. Arg. (**b**, **d**, **f**, **i**). SEM images (**a–d**) and transverse sections (**e–i**). **a** General view of pollen grain in polar view, note the three colpi (arrow). **b** General view of a pollen grain in polar view and another in equatorial view, note the three colpi (arrow). **c**, **d** Details of colpi showing its thick margins, see the small perforations in equatorial axis of *R. weddelliana* (d, arrowhead). **e**, **f** Two-celled pollen grains. **g** Pollen grain full of starch grain, stained with Lugol's solution. **h**, **i** Details of colpi showing the thick margin and aperture. Scale bars = 5 μm (**c**, **d**); 10 μm (**a**, **b**, **e**, **f**, **h**, **i**); 20 μm (**g**). *ap* aperture of colpus, *gc* generative cell, *ma* margin of colpus, *sg* starch grains, *vn* vegetative cell nucleus
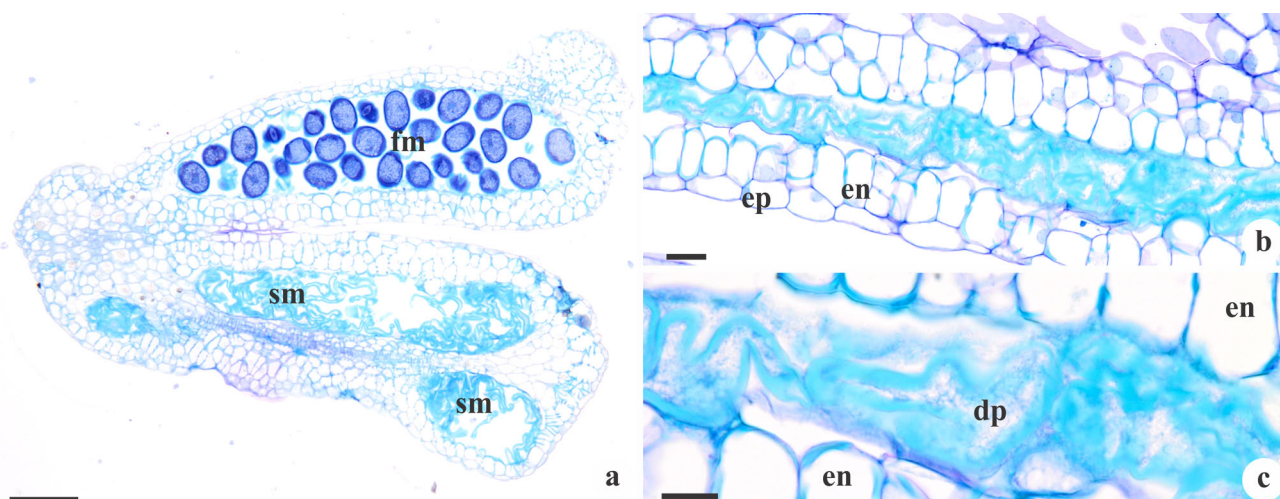
Fig. 5 Anthers of pistillate morphotype flower of *Rauvolfia vomitoria* Afzel. Longitudinal sections. **a** General view showing two sterile microsporangia and one microsporangium with pollen grains. **b** Detail of sterile microsporangia. **c** Pollen grains in degeneration, note the absence of cytoplasm and nucleus. Scale bars = 10 μm (**c**); 20 μm (**b**); 100 μm (**a**). *dp* degenerating pollen grain, *en* endothecium, *ep* epidermis, *fm* fertile microsporangium, *sm* sterile microsporangium

This population likely has some level of sexual lability, expressing different morphotypes over time in the same individual, as discussed in Korpelainen (1998). This was suggested for *R. sellowii* also because Koch et al. (2002) found some pollen in the anthers of a few pistillate flowers.

Gynodioecy is found in 275 angiosperm genera (Renner 2014), a number that may be an underestimate because gynodioecy can be cryptic. While gynodioecy seems to be reasonably common, Freeman et al. (1997) state that the presence of sexual lability in gynodioecious species is considered non-adaptative and rare. A survey conducted by Varga and Kytöviita (2016) found that sexual lability had been recorded in only 22 gynodioecious species. According to these authors, the presence of sexual lability may be underestimated because its detection requires the analysis of the same individual over time. Thus, the possibility of sexual lability in *R. vomitoria* is important record for the understanding of Apocynaceae reproductive systems, and new research focusing on this may elucidate how labile sexual expression is in this species or in other species of the genus.

Anthers – The divisions in the anther primordium that ultimately lead to the parietal layers in *R. weddelliana* and *R. vomitoria* are of the dicotyledonous type described by Davis (1966). According to her, this pattern is found in a large fraction of current eudicots, with this type of formation being recorded for 43 angiosperm families, among them the Asclepiadaceae, which are currently located within the Apocynaceae as a subfamily of the Asclepiadoideae (Simões et al. 2010; Endress et al. 2014).

Among the Apocynaceae, the endothecium is fibrous (Johri et al. 1992) and may range from weakly developed

in *Cerbera odollam* Gaertn. and *Vallaris solanacea* (Roth) Kuntze (as *Vallaris heynei* Spreng., Rau 1940) to having five to six layers in *Voacanga foetida* (Blume) Rolfe (Maheswari Devi 1971). In the studied species of *Rauvolfia*, the endothecium is one layered and well developed with fibrous thickenings.

In the studied species of *Rauvolfia*, only one middle layer was observed. In some regions, two middle layers can be observed. In fact, the presence of two middles layers in some regions is a common characteristic (Johri et al. 1992), and the number of middle layers in *R. vomitoria* e *R. weddelliana* is in accordance with the literature regarding the Gentianales order, with one exception being the Asclepiadoideae subfamily of Apocynaceae, whose members have four to five middle layers (Johri et al. 1992).

The tapetum is a much-conserved characteristic within the Gentianales order, being mostly of the secretory type (Johri et al. 1992), as found in the *Rauvolfia* species studied. Even though, some variation is found. For example, there are records of ameboid tapetums among the Gentianaceae and Loganiaceae. The tapetum is an important layer because of its nutritive function during pollen development (Davis 1966; Pacini 2010). Due to its nutritional importance, the malformation or early degeneration of the tapetum will cause pollen sterility (Laser and Lersten 1972), as has already been reported for several angiosperm species (Shugaeva 1979; Sajo et al. 2005; Li et al. 2010; Mendes et al. 2016). In *Trachelospermum lucidum* (D. Don) K. Schum. (as *Trachelospermum fragrans* Hook. F.), another Apocynaceae, the failure of the tapetum to provide nutrition for the microspores was probably implicated in the disorganization of microspores (Sud 1984), as seen here for some microsporangia of *R. vomitoria*. In our study, the

tapetum had the same anatomical characteristics in fertile and sterile microsporangium, so the male sterility of some microsporangia should not be related to the tapetum.

Microsporogenesis – In the Apocynaceae and most Gentianales, microsporogenesis mainly follows the simultaneous pattern of cytokinesis (Johri et al. 1992), as observed in both studied species, but variations occur within the family. Cytokinesis of a successive type has been reported in *Apocynum, Holarrhena,* and *Plumeria* (Lattoo 1974; Chauhan 1979). In the Asclepiadoideae, successive cytokinesis was reported for the genera *Caralluma, Daemia, Cynanchoideae* (Maheswari Devi 1964) and *Oxystelma* (Johri et al. 1992). In some species, both types are present, as reported for *Catharanthus pusillus* (Murr.) G. Don (Bhasin 1971) and *Rauvolfia canescens* L. (Meyer 1938).

Pollen grains – The tetrad of microspores may present only one shape, as in the species studied here and in *Trachelospermum lucidum* (D. Don) K. Schum. (as *Trachelospermum fragrans* Hook. f.), in which it is tetrahedral (Sud 1984). Alternately, several formats can occur in the same species, as in *Holarrhena pubescens* Wall. ex G. Don (as *Holarrhena antidysenterica* (L.) Wall. ex A. DC.—Lattoo 1974). The pollen grains also vary and are found in two-celled (species of this work; Bhasin 1971; Lattoo 1974; Johri et al. 1992) and three-celled forms (Sud 1984).

In *Rauvolfia,* pollen grains represent an important diagnostic characteristic because they are described as having thickenings on both sides of the colpi (Pichon 1948; Pasha and Roy 1980), a characteristic not found in the closely related genera. Our data confirm this information for the two species analyzed. In addition, we found a difference in the surfaces of the pollen grains, with perforations occurring only along the equatorial axis of *R. weddelliana.*

Above all, our results confirm those obtained regarding Apocynaceae and even Gentianales and described in the classic books of embryology (Davis 1966; Johri et al. 1992). We did not find differences in microsporogenesis and microgametogenesis between the two species of *Rauvolfia.* However, it is important to note that few genera of Apocynaceae have been studied so far and that the confirmation of patterns in the family depends on additional studies investigating species and genera that have not yet been analyzed. The differences in the surfaces of the pollen grains, with the presence of perforations along equatorial axis of *R. weddelliana* and the confirmation of thickenings on both sides of colpi, show the importance of pollen characteristics in identifying taxonomically useful characteristics for the delimitation of genera or species. Further

studies can determine whether these characteristics occur in other species of *Rauvolfia* and whether they truly have taxonomic importance. We did not find anthers without pollen grains, so we could not characterize the development of empty anthers in the pistillate flowers of *R. vomitoria.* We believe that the gynodioecy reported in this species may be labile and vary over time, which can only be confirmed with additional studies.

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