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Plant anatomy: history and future directions

Occurrence of parietal and invasive tapetum in *Dyckia strehliana* (Bromeliaceae): first report for the family

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Abstract

Tapetal tissue plays essential roles in the formation of generative cells, as it is related to their nutrition and development in anthers. Among the few species of Bromeliaceae for which tapetal tissue has been describe, most have a secretory tapetum and one has an invasive tapetum. This study analyzed the developmental stages of the anthers of *Dyckia strehliana*, with emphasis on the tapetum, to identify variability in development and structure. Botanical material was collected on the banks of the Toropi River in the state of Rio Grande do Sul, Brazil, and processed using standard plant micro-techniques. During meiosis prophase, tapetal cells of some samples remained parietal while in the others, a proportional number, the invasion of the locular space begins, culminating in total invasion during the free microspore phase and without fusion of the protoplasts. Tapetal degeneration is complete before gametogenesis begins in both types. Thus, it is possible to describe two modes of development and tapetal structure, one parietal and one invasive, although the tapetum remains as a general secretory type in both cases. Thus, according to present work, the terms invasive and parietal are indicators of states of the secretory tapetum.

Key words: anther, Bromeliaceae, invasive tapetum, parietal tapetum, sporangium development.

Resumo

O tecido tapetal apresenta funções essenciais na formação de células generativas, pois está relacionado a nutrição e desenvolvimento destas nas anteras. Em Bromeliaceae, dentre as poucas espécies descritas, a maioria possui tapete secretor e uma espécie possui tapete invasivo. Neste estudo foram realizadas análises de fases do desenvolvimento de anteras com ênfase ao tapete em *Dyckia strehliana* com o objetivo de identificar variabilidade em desenvolvimento e estrutura. O material botânico foi coletado as margens do rio Toropi, Rio Grande do Sul, e processados por meio de microtécnica vegetal. Durante a prófase da meiose, as células tapetais de algumas amostras permaneceram parietais enquanto em outras, em número proporcional, inicia-se a invasão no espaço locular, culminando com invasão total durante a fase de micrósporos livres e sem fusão dos protoplastos. A degeneração tapetal está completa antes do início da gametogênese em ambos os tipos. Assim, é possível descrever dois modos de desenvolvimento e estrutura tapetal, um parietal e outro invasivo, embora o tapete permaneça como um tipo secretor geral em ambos os casos. Assim, de acordo com o presente trabalho, os termos invasivo e parietal são indicadores de estados do tapete secretor.

Palavras-chave: antera, Bromeliaceae, tapete invasivo, tapete parietal, desenvolvimento do esporângio.

Introduction

Bromeliaceae is considered a very diverse family within the order Poales (APG IV 2016), comprising approximately 3,000 species distributed in 78 genera, including the genus *Dyckia* (Gouda *et al.* 2021). Embryological aspects of some species of the genus have been studied, with emphasis on anthers (Sajo *et al.* 2005; Mendes *et al.* 2012). Tapetal tissue is recognized as secretory in Bromeliaceae (Pacini *et al.* 1985; Johri *et al.* 1992), although the plasmodial type is found in some genera, such as *Ananas* (Wunderlich 1954).

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For *Dyckia*, the tapetum has been described only for *Dyckia pseudococcinea*, and characterized as the secretory type (Mendes *et al.* 2012). Tapetal tissue has essential functions in the formation of generative cells (Bhandari 1984), making it also fundamental in the dispersion (Hesse 1980) and protection (Pacini 1997) of pollen grains.

Two types of tapetum are traditionally distinguished among angiosperms (Johri *et al.* 1992); the secretory type, in which the cells remain in their position of origin, and the amoeboidal type, in which the cells invade the loculus and form a "coenocytic plasmodium" (Bhandari 1984). A third type of tapetum, the invasive type, was observed in *Canna* (Cannaceae) (Tiwari & Gunning 1986) and is widely distributed among monocots (Furness & Rudall 1998). In this type, differentiation also includes invasion of the loculus, but there is no fusion of the protoplasts. The invasive type was considered intermediate between secretory and amoeboidal types by Furness & Rudall (2001) and as a variation of the amoeboidal tapetum by Shamrov *et al.* (2021). In addition, there are examples of species in Asteraceae that have two tapetal types (Ao *et al.* 2009; Yeung *et al.* 2011), including distinct cell types in the same loculus (Yeung *et al.* 2011). In this context, it is possible to understand the importance of studies in plant embryology, which continue to contribute with structural novelties about the tapetum.

Most studies of the tapetum in Bromeliaceae have been based on a few species, resulting in underrepresented generalization when considering the number of species both within the family and within *Dyckia* (Davis 1966; Johri *et al*. 1992; Furness & Rudall 1998; Sajo *et al.* 2005; Mendes *et al.* 2016). Added to this, little structural and developmental information has been described, resulting in the finding of secretory or amoeboidal types and/or the association of the tapetum with pollen sterility (Sajo *et al.* 2005; Mendes *et al.* 2016).

Many species of Bromeliaceae are endemic to the Brazilian state of Rio Grande do Sul (Forzza *et al.* 2020), such as *Dyckia strehliana* Büneker & R. Pontes, found on the banks of the Toropi River. It is a rheophytic species, which represents an unusual habitat for the genus, with individuals experiencing current and submersion during periods of river flood (Büneker *et al.* 2013). In addition, the area of occurrence of the species has experienced environmental degradation due to the construction of small hydroelectric plants at several points along the Toropi River, which represents an anthropic threat and means by which the process of extinction can be initiated (Büneker *et al.* 2013; Büneker & Witeck-Neto 2016).

Embryological studies on *D. strehliana*, among other species, have become a priority due to a combination of factors, such as endemism, place of occurrence, habitat and the growing threat from human action. During embryological analyses of *D. strehliana*, with emphasis on the anthers (Silva 2021), structural variations in tapetal tissue were observed, differing from the exclusively secretory pattern known for the tapetum of *Dyckia*, suggesting the need for greater attention to tapetal tissue. For other species, the anther tapetum structure, differing from the usual standards, ended up being the great motivator of studies, being indicated by the authors as "The most striking feature of microsporogenesis…" (Tiwari & Gunning 1986) or "The most novel finding of this study..." (Yeung *et al.* 2011). This brought the prospect of increasing knowledge about structural variability linked to embryology and tapetal tissue in *Dyckia*. Therefore, we analyzed different stages of tapetum development in *D. strehliana* with the objective of identifying and confirming the existence of variability and establishing bases for classification considering the patterns already determined in the current literature.

Material and Methods

Collection site and plant material

Dyckia strehliana is endemic to the banks of the Toropi River, between the municipalities of Júlio de Castilhos and Quevedos in the state of Rio Grande do Sul, where flower buds were collected in different stages of development and flowers in different stages of anthesis in the years 2017, 2018 and 2019 (Fig. 1a) (all at 29º23'26.9''S, 54º00'07.9'W). A voucher for the species was deposited in the Herbário do Centro de Ciências Naturais e Exatas (CCNE) of the Universidade Federal de Santa Maria (UFSM), with registration number SMDB 19350.

Collecting and flower fixation

To prevent mechanical damage, mainly perforations in the sporangia and consequent fixative access to the locular content, strict dissection standards were followed. Anthers were not removed from flowers for fixation. The removal of the flowers was carried out by means

of a transverse cut to the receptacle. The removed flowers were immediately immersed in fixative and subjected to negative pressure. Collected botanical material was fixed in the field with 3% glutaraldehyde in 0.1 M sodium phosphate buffer pH 7.2 (McDowell & Trump 1976; Gabriel 1982), while subjected to negative pressure by means of a plunger and manual action to facilitate fixative penetration of deeper tissues of floral organs.

Microtechnique

Samples were removed from the fixative and immersed in 0.1M sodium phosphate buffer pH 7.2 (Gabriel 1982). The samples were then, sequentially, placed in two series of washes in distilled water for 20 minutes, subjected to Extran 5% detergent for seven days, and dehydrated in ethanol series (10, 30, 50, 70, 90 and 99%) with 20 minutes in each stage (O'Brien & McCully 1981). The material was then subjected to a pre-infiltration solution, based on 2-hydroxyethyl methacrylate (HEMA) and 99% ethyl alcohol at a 1:1 ratio, for 24 hours and then to infiltration with pure HEMA for 12–24 hours. The material was embedded in HEMA (Gerrits & Smid 1983), with each block containing only the six anthers of the same flower. Transverse and longitudinal sections of the samples, varying from 0.5 to 3 µm in thickness, were made using a Thermo Scientific Finess ME+ rotary microtome and then stained with Toluidine Blue pH 4.4 (Feder & O'Brien 1968). A total of 60 anthers, ten flowers from eight different individuals were analyzed.

Analysis

Periodic Acid-Schiff reactive (PAS) was used for the detection of total polysaccharides (O'Brien

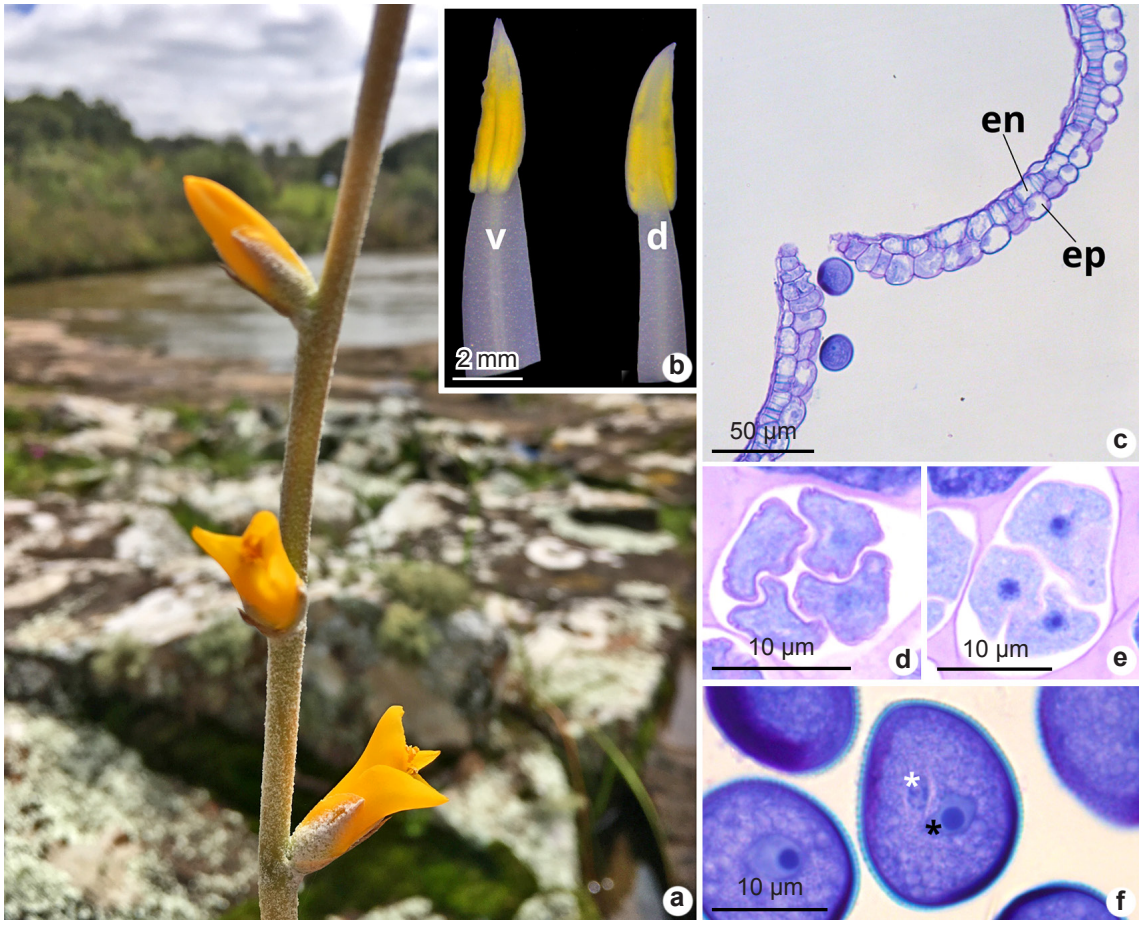

Figure 1 – a-f. General aspects of *Dyckia strehliana* – a. inflorescence; b. anthers in ventral (v) and dorsal (d) view; c. mature anther wall; d. tetrad with isobilateral organization; e. decussate tetrad; f. bicellular pollen grain, vegetative nucleus (black asterisk) and cell generative (white asterisk). (en = endothecium; ep = epidermis).

& McCully 1981); Ruthenium Red for the detection of pectins (Johansen 1940); Calcofluor White for cellulose (O'Brien & McCully 1981); Neutral Red for pollenkitt (Kirk 1970); and Propidium Iodide for nucleus (Crompton *et al.* 1992). Histological slides were analyzed using a Leica DM2000 microscope with a DFC 295 digital image capture system, and a Zeiss AxioImager A2 fluorescence microscope equipped with a Zeiss MCr digital image capture system.

Results

General aspects

The anther of *D. strehliana* (Fig. 1b) at maturity presents only epidermis and endothecium in the sporangia (Fig. 1c). Microsporogenesis is of the successive type with isobilateral and decussate tetrads (Figs. 1d-e). Pollen grains are released bicellularly (Fig. 1f).

Tapetum

In the early stages of development, the tapetum cells maintain cellular structure with the meristematic characteristics of the parent cells, unlike the other microsporangium cells. They grow together with the archesporial tissue, although less markedly, developing no vacuoles or only diminutive ones, and by their isodiametric or radially elongated contour, they differ from other sporangial tissues, in a stage prior to meiosis (Fig. 2a). At this stage, stratification sites are formed, with two and three strata alternating among sites, with only one cell layer in the tapetal tissue being common (Fig. 2a).

Important changes initiate in the tapetum cells beginning in prophase of meiosis. The boundary between archesporial and tapetal tissues has accentuated irregularity due to the increase of stratification and greater elongation of tapetal cells and projection of tapetal cells between meiocytes (Fig. 2b). The absence of tapetal cells in some places, which allows direct contact of meiotic cells with cells of the inner middle layer and the intersporangial septum, also occurs (Fig. 2c). As for the cells, the beginning of nuclear fusions, vacuolation, acytokinetic mitoses (Fig. 2b) and loss of the cellulosic portion of the cell wall (Fig. 2d) can be observed, in addition to an increase in cell volume. It should be noted that, in general, the cells remained binuclear (Fig. 2b-c,e-f).

Still in the prophase of meiosis, meiocytes were identified amid tapetal cells (Fig. 2e). During the tetrad of the microspore phase, what appears to be a significant change in the structure and chemical composition of the walls initiates (Fig. 3a-b). The cells remained parietal in position in 60% of the analyzed anthers (Fig. 3c), while there was invasion of the locular space in the other 40% (Figs. 2b,f; 3b,d). During meiosis, substitutions of position of tapetal cells by meiocytes (Fig. 2c) or invasion of the locular space by tapetal cells (Fig. 2e-f) were observed. Total invasion by tapetal cells, during the free microspore phase, was also observed, which remained independent and totally deformed (Fig. 3d). In the first stages of locule invasion, the tapetal nuclei maintain a similar structure while parietal, in general aspects, to the other cells of the anther (Fig. 3e-f). Both tapetum types were found in different flowers on the same plant.

Beginning in the free microspores stage, the tapetal cells launch into degeneration. However, there is no moment of rupture and the consequent leakage of contents, although at the beginning of gametogenesis several samples showed material with a positive reaction for polysaccharides suspended in locular fluid (Fig. 3g). In the mature anther, tapetal cell remnants are found in the form of pollenkitt (Fig. 3h).

Discussion

The characteristic of locule invasion in a representative percentage of flowers defines the tapetal tissue in *D. strehliana* as demonstrated. This combination of character states is, so far, unprecedented in *Dyckia* and Bromeliaceae. Although microsporangia structure in *D. strehliana* is similar to that described for other species of the genus (Sajo *et al.* 2005; Mendes *et al.* 2012), it is possible to suggest that this species is an exception to the description of Pacini *et al.* (1985), because, according to the authors, invasive tapetal cells would be correlated with reduced locular spaces and reduced microspore numbers.

Apparently, the initiation of meiosis is a common moment for the invasion of tapetal cells into the loculus to begin, in a gradual process, thereby establishing themselves. This characteristic, when it occurs, is accentuated in the free microspores phase, when the tapetal cells, in the process of degeneration, are in suspension in the locular fluid together with the microspores. Although it is difficult to precisely define the exact moment of loss of cell functionality according to the methodology used in the present study, we suggest, at least in an initial period, that the tapetal cells themselves are functional due to their nuclei

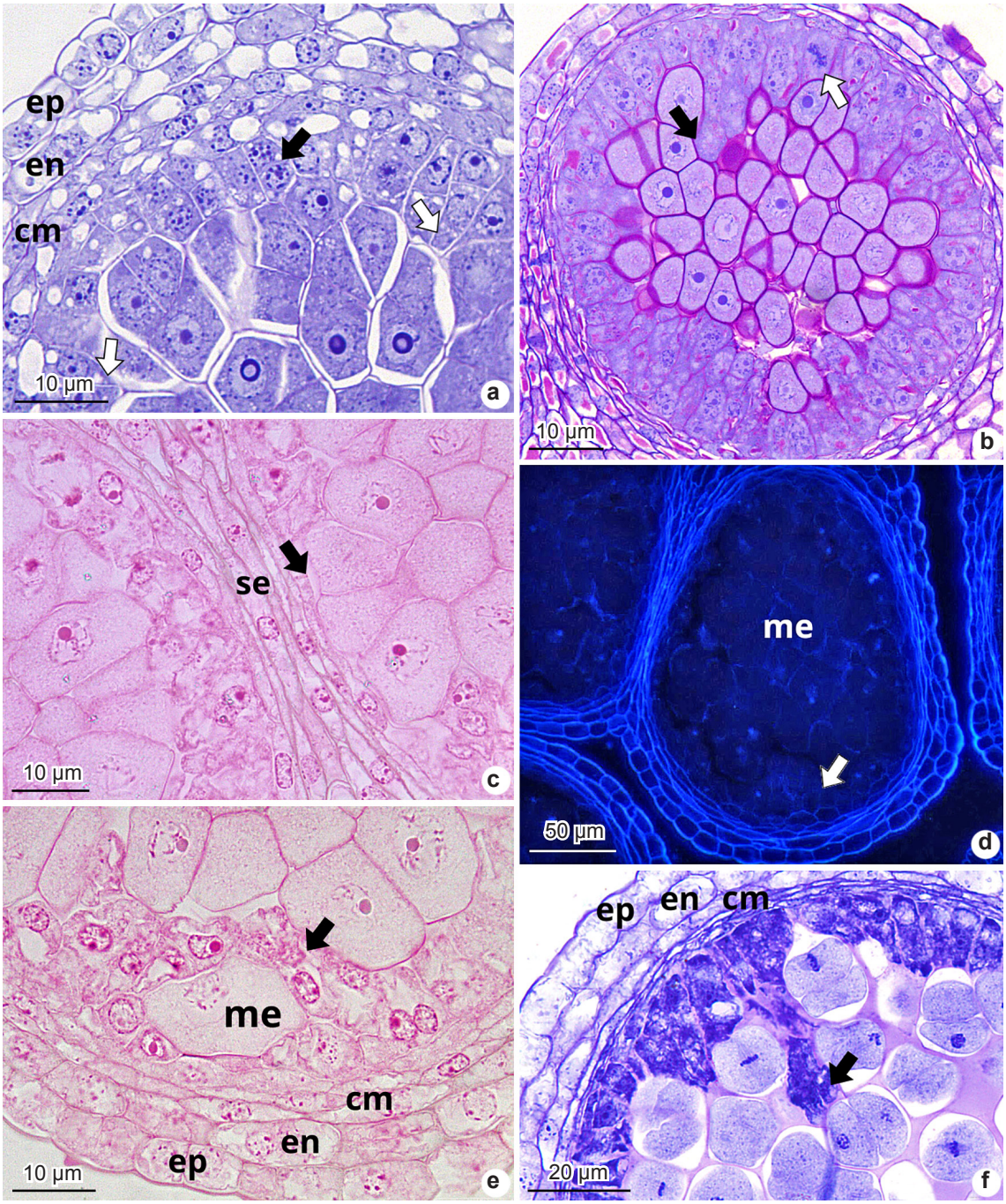

Figure 2 – a-f. Different stages of the tapetum – a. stratified region (arrow), elongated cells towards archesporial tissue (arrowhead); b. intrusive cells (arrows) and in acytokinetic divisions (arrowhead); c. absence of tapetum in the septum region (arrow), stained with Ruthenium Red; d. tapetum cells (arrow), without signal of the cellulose on the walls, stained with Calcofluor white and under fluorescence; e. meiocyte engulfed by tapetal cells (arrow), stained with Ruthenium Red; f. tapetal cells intrusion into the loculus. (ep = epidermis; en = endothecium; cm = middle layer; se = septum; me = meiocyte).

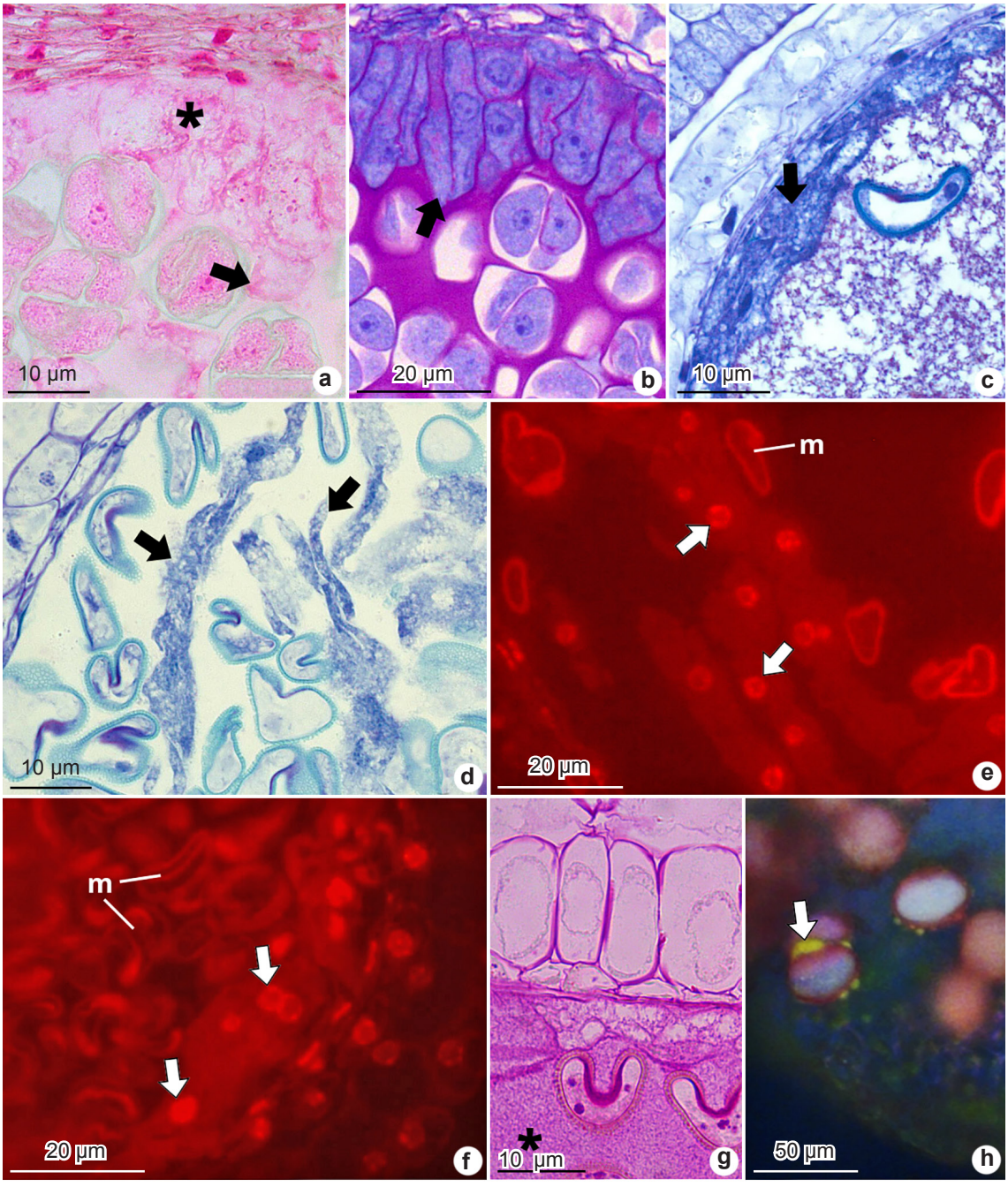

Figure 3 – a-h. Tapetum degradation – a. tapetal cells without middle lamella (asterisk) invading the loculus (arrow), evidenced by Ruthenium Red; b. tapetal cells elongated towards the loculus and without cell walls (arrow); c. tapetal cells in the parietal position (arrow); d. invasion of the loculus by the tapetal cells (arrows); e. nucleus (arrow) of invasive tapetum cells, using propidium iodide in fluorescence; f. nucleus (arrow) of parietal tapetum cells, using propidium iodide in fluorescence; g. polysaccharides in the locular fluid (asterisk), evidenced by PAS reagent; h. pollen grain with pollenkitt (arrow), stained with Neutral Red reagent and under fluorescence. (m = microspore).

structure. The cell degradation is accentuated and, therefore, it is not believed that there is any secretory activity at the end of sporogenesis and, especially, after the first mitosis, which marks the beginning of gametogenesis. This period in which degeneration occurs is an important aspect that typifies the tapetum as secretory (Pacini *et al.* 1985).

The coexistence of two types of tapetum on the same plant is unprecedented in Bromeliaceae. This condition has been reported for some species of Asteraceae. Distinct tapetum types were observed in different flowers of *Aster subulatus* Michx. (Ao *et al.* 2009), while in the same locule of *Carthamus tinctorius* L. In *C. tinctorius*, depending on the different cell fates, both pollenkitt and trifine were identified, with pollenkitt produced by parietal cells and trifine by invasive cells (Yeung *et al.* 2011).

According to Shamrov *et al.* (2021), the tapetum types differ by the time of cell fusion, by the contact of the tapetum cytoplasm with the external surface of the pollen grain, and by the longevity in the locule. According to Pacini *et al.* (1985), tapetum type is linked to parameters such as locule diameter, pollen shape and/or dry/wet habitats; however, regardless of the type, this tissue is related to the same activity, secreting substances involved in the development of pollen grains (Pacini 1997). According to Pacini *et al.* (1985), the tapetum can present a series of modifications, all directed towards a more active provisioning of nutrients to the microspore. According to the authors, a direct contact between tapetum cells and all the microspores requires a highly efficient diffusion of nutrients to the internal locular parts to ensure uniform nutrition to the microspores. The present study observed many microspores per locule in *D. strehliana*, with the innermost ones not having direct contact with the tapetum. Possible solutions to this problem, according to Pacini *et al.* (1985), would be linked to the mobility of microspores or tapetum cells invading the loculus, thus guaranteeing and maintaining intimate contact. Furthermore, we believe that the early separation of meiocytes, observed in *D. strehliana*, would allow the diffusion of nutrients to deeper regions in the tissue. That is, it would also appear to be a strategy for nutrient diffusion. This feature is observed in other Bromeliaceae (Sajo *et al.* 2005; Oliveira *et al.* 2015). At the same time that such a hypothesis would reinforce the mechanisms that facilitate the diffusion of nutrients, signals and stimulants to development, it could also facilitate the intrusion of tapetal cells between the meiocytes. However, it does not occur in all species and, in the case of the present study, did not occur in all analyzed anthers of *D. strehliana*.

In Bromeliaceae, Sajo *et al.* (2005) found secretory tapeta with a tendency to invade the loculus, which the authors characterized as an intermediate type. Furness & Rudall (2001, 2006), on the other hand, indicated such a tapetum as non-syncytial invasive. We partially agree with the classifications provided by the respective authors. There is a general aspect regarding tapetal types that should be considered, in our opinion: whether there is maintenance of individualization of cells in the tissue or not. In cases where, regardless of cytological structural transformation, the cellular unit is maintained until cell death, we have the secretory type and its respective subdivisions. This context is presented by Shamrov *et al.* (2021) and can be applied to the current study. Kamelina (2002) approached this view with the term "cellular". The term 'cellular' seems better to us than 'secretory', but it should not be confused with 'cellular' sensu Kamelina (2002), which seems to represent a less comprehensive description. We understand that from a structural perspective, as a tapetal cell, the maintenance of the structural unit is fundamental, meaning the preservation of the plasma membrane, cytoplasm, and nucleus, regardless of external changes, such as cell wall modifications or changes in position.

Thus, we suggest that the tapetum of *D. strehliana* be considered as a general secretory type, as expected for *Dyckia*, while remaining parietal in some flowers and invasive in others. We believe that two conditions allow the invasion of the loculus by tapetal cells, these being the formation of locular spaces since prophase of meiosis and the change in the chemical composition of the wall of the tapetal cells, since the structure of the wall apparently changes in preparation for and during tapetum secretory activity. These conditions could help, in part, with the invasive features, although the loss of connection between tapetal cells is more important in this regard in explaining the mutual intrusion of tapetal cells and meiocytes. It is essential to point out that both the formation of locular spaces and the change of parietal structure in tapetal cells always occur, regardless of invasion of the locular space or not. However, the loss of connection between the cells cannot be, under the conditions of the present study, affirmed to occur in cases where the tapetum

remains parietal. Thus, in an attempt to maintain the bases of the classification presented by Pacini *et al.* (1985), some of the flowers, therefore, would have microsporangia whose tapetal cells would initially have persistent cell walls, although with altered chemical composition, and would remain around the loculus. In the other flowers, the tapetal cells, although with persistent cell walls with altered chemical composition, would invade the locular space, being in this second condition the more drastic alteration of cellular forms. It is important to emphasize that although this is an important record, it is not possible to consider it as the unique character for the species. Therefore, we await further analysis and the expansion of studies to include other populations of the species.

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Data availability statement

In accordance with Open Science communication practices, the authors inform that all data are available within the manuscript.

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