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RESEARCH ARTICLE

Pollen–pistil interaction in *Mauritia flexuosa*: structural and cytological aspects provide insight into the reproductive success of a dioecious palm with supra-annual flowering in a semiarid environment

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ABSTRACT

- The success of pollen–pistil interaction in *Mauritia flexuosa* (buriti), a palm adapted to the humid ecosystems, 'veredas', within the Cerrado, is influenced by intrinsic and environmental factors. Its supra-annual flowering, dioecy, and adverse climate conditions pose challenges for fertilization, therefore information on floral biology is essential. This study aimed to ascertain stigma receptivity, and elucidate structural, cytochemical, and ultrastructural aspects of the pollen–pistil relationship.
- Flowers were analysed at intervals post-anthesis (hpa) and post-pollination (hpp). A stigma receptivity test was performed using H_2O_2 solution. Pistil samples were processed for anatomical, histochemical, and electron microscopy evaluation.
- The stigma is wet and papillate type, with subepidermis containing sclerenchyma connected to vascular bundles. Stigma receptivity lasts around 36 hpa. The pollen tube penetrates the papilla at 2 hpp and develops in the symplast, towards the stylar canal. The papillae have loose cell walls that facilitate the secretion and contain a rich population of organelles, including large peroxisomes. Fertilization occurs 24 hpp, and during this period the stigma surface is free of pathogens.
- The vascular connection to the pistil surface favours the germination of pollen grains. The pistil has a strong protective system until fertilization occurs. The symplastic growth of the pollen tube in the stigma and the efficient secretory apparatus of the pistil contribute to rapid fertilization. These structural characteristics and secretion dynamics enhance reproduction of buriti, even with supra-annual flowering and in semiarid conditions.

INTRODUCTION

Reproductive success of angiosperms depends on the effective interaction between pollen and pistil following pollination (Lersten 2004). This process involves the complex relationship between two distinct generations, the gametophytic and the sporophytic, and is associated with a cascade of biochemical reactions, as well as structural and ultrastructural changes (Lersten 2004; Hiscock & Allen 2008; Serrano *et al.* 2008; Bosch & Wang 2020). These events predominantly occur on the stigma surface and along the stylar canal or transmitting tissue and are crucial for inducing pollen grain germination and development of the pollen tube towards the ovule (Kandasamy *et al.* 1994; Edlund *et al.* 2004; Serrano *et al.* 2008; Hafidh *et al.* 2016). Despite advances in knowledge over the past decades, many questions remain that require further in-depth studies, particularly concerning tropical tree species.

Changes occurring during the progamic phase (the period between pollination and fertilization) are modulated by fine adjustments on the surface of both the pollen and the pistil (Lersten 2004; Hafidh *et al.* 2016; Losada *et al.* 2017). Studies indicate that proteins can be found in the intine and pollen coat, whose role is to assist in recognition of the pollen grain by the stigma or are enzymes that act in degradation of the papillae cell walls, facilitating penetration of the pollen tube (Edlund *et al.* 2004; Lersten 2004; Bosch & Wang 2020). The pollen tube begins to elongate and develop through the apoplast or symplast, requiring nutrients produced by the pistil (Kandasamy *et al.* 1994; Edlund *et al.* 2004; Hafidh *et al.* 2016; Bosch & Wang 2020). The composition of pollen, the developmental pattern of the pollen tube, and the requirement for nutritive substances secreted by the pistil vary significantly between species, making histochemical and ultrastructural techniques essential for understanding this process and identifying peculiarities.

The Arecaceae family consists of around 2,700 species, with more than 240 genera, and has a predominantly tropical distribution (Dransfield *et al.* 2008; Lorenzi *et al.* 2010). Brazil has a significant representation of palm trees, with over 330 native species, many of which have high economic potential in addition to their ecological importance (Lorenzi *et al.* 2010). However, studies on floral biology and changes during the progamic phase are concentrated on a few species, notably those of greater economic interest, such as *Elaeis guineensis* Jacq. (Tandon *et al.* 2001), *Acrocomia aculeata* (Jacq.) Lodd. ex Mart. (Mazzottini-dos-Santos *et al.* 2015), *Cocos nucifera* L. (Hebbar *et al.* 2020), and *Butia capitata* (Mart.) Becc (Dias *et al.* 2022).

Mauritia flexuosa L. f. (buriti) has a wide distribution throughout South America and is the most abundant palm tree in Brazil (Lorenzi *et al.* 2010). This species exhibits particular evolutionary characteristics, with high genetic variability and historical demography, which has facilitated its occurrence in diverse environments, including the Cerrado, a biome marked by climatic seasonality (Virapongse *et al.* 2017; Melo *et al.* 2018). In this environment, the buriti occurs in the 'vereda' phytophysiognomy, which is a swampy ecosystem that is highly threatened. The buriti is considered a keystone species due to its various important roles in ecology and maintenance of the hydrological cycle (Lorenzi *et al.* 2010; Endress *et al.* 2013; Virapongse *et al.* 2017; Melo *et al.* 2018; Nunes *et al.* 2022). *Mauritia flexuosa* is a large, dioecious palm with a short flowering period found in the 'veredas' located in transition regions to a semiarid climate. It has supra-annual flowering and fruiting cycles (Ávila *et al.* 2023), highlighting the importance of studies on its pollination. Among papers on the reproductive biology of *M. flexuosa* (Rosa & Koptur 2013; Mendes *et al.* 2017; Ávila *et al.* 2023), there is no information regarding structural characteristics related to the progamic phase, making such data relevant for expanding knowledge on the reproductive biology of this iconic species and palms in general. Furthermore, anthropogenic actions have affected *M. flexuosa* populations in 'vereda' environments, causing a concerning reduction in the number of individuals (Nunes *et al.* 2022). Thus, expanding knowledge on the pollen–stigma relationship in this species will support conservation, domestication, and genetic improvement programs, as well as the preservation of its habitats.

The objective of this study was to evaluate aspects of the floral biology and understanding of the dynamics of the progamic phase of *M. flexuosa* by: (i) identifying the stigma receptivity period to characterize the floral cycle; and (ii) elucidating structural, cytochemical, and ultrastructural aspects of the pollen–pistil relationship.

MATERIAL AND METHODS

Botanical material and study area

The study was conducted in the Almescla 'vereda', located in the Environmental Protection Area of Rio Pandeiros (EPA Rio Pandeiros), municipality of Bonito de Minas, Minas Gerais, Brazil (15°22′50″S, 44°55′28″W). The 'veredas' are phytophysiognomies of the Cerrado, characterized by soil rich in organic matter and saturated with water, typically formed by a vegetation composed of herbaceous/grassy-like and arboreal-shrubby strata (Nunes *et al.* 2022). In northern Minas Gerais, there is a predominance of *M. flexuosa* (buriti) and *Mauritiella armata* (Mart.) Burret (xiriri) palms, which are considered keystone species (Ávila *et al.* 2022, 2023; Nunes *et al.* 2022). The climate of the region is classified as tropical wet and dry (Aw) according to Köppen, with well-defined dry and rainy seasons (Alvares *et al.* 2013).

The phenology of a natural population of *M. flexuosa* occurring in the 'vereda' was monitored and revealed that the flowering period occurs between October and February (Ávila *et al.* 2023). In the study area, ten adult individuals, five staminate and five pistillate, showing no symptoms of disease or predation, and with flowers in the pre-anthesis stage, were selected, and flowers collected in January and February 2023, during the peak of flower production (Ávila *et al.* 2022, 2023).

Stigma receptivity

Twenty branches of inflorescences containing pistillate flowers in the pre-anthesis stage were collected from random positions and placed in beakers with water. The samples were kept under ambient conditions and monitored for approximately 24 h until identification of floral anthesis (Losada *et al.* 2017). After this stage, the flowers in anthesis were identified, and some removed from the rachillae for evaluation at 2, 4, 8, 12, 24, 36, and 48 h post-anthesis (hpa), unpollinated. During all phases, from anthesis onwards, stigma receptivity was evaluated considering the following: (i) morphological changes, such as colour and moisture (Mazzottini-dos-Santos *et al.* 2015; Dias *et al.* 2022); (ii) peroxidase activity, indicated by the intensity of bubble formation on the stigma surface after the application of 6% hydrogen peroxide solution (H_2O_2) (Dafni & Maués 1998; Dias *et al.* 2022). Three flowers from each individual were tested in each period. Images were obtained using a digital camera attached to a stereomicroscope (Zeiss Lab AI/Axion Cam ICC 3, Jena, Germany).

Morphoanatomy and histochemistry of the pistil after pollination

Three branches of inflorescences were collected from staminate individuals during the same flowering period as the pistillate individuals; pollen grains were removed from flowers in anthesis using a hypodermic needle. The pistillate flowers in anthesis, identified in the branches that were kept in water, as described above, were manually pollinated and removed from the rachillae for evaluation at 2, 4, 8,12, 24 and 36 h post-pollination (hpp). Three flowers were tested in each period. The morphological changes observed on the stigma surface after contact with pollen were documented using a digital camera attached to a stereomicroscope (Zeiss Lab AI/Axion Cam ICC 3).

For anatomical evaluations, the stigmas, from each period, were fixed in Karnovsky's solution (Karnovsky 1965), for 24 h, dehydrated in an ethanol series, and included in 2-hydroxyethyl-methacrylate (Leica Microsystems, Heidelberg, Germany) (Paiva *et al.* 2011). Cross- and longitudinal-sections (5-μm thick) were obtained using a rotary microtome (HistoCore Autocut, Nussloch, Germany), stained with 0.05% toluidine blue, pH 4.7 (O'Brien *et al.* 1964, modified), and mounted on slides with acrylic resin (Itacril, Itaquaquecetuba, Brazil).

For histochemical analyses, tests were conducted with periodic acid and Schiff's reagent (PAS) (Feder & O'Brien 1968) to identify

neutral polysaccharides; with xylidine-ponceau (Vidal 1970), for proteins; with Sudan black (Pearse 1972), for lipids; with choriphosphine under UV light (Weis *et al.* 1988), for acidic polysaccharides; with aniline blue under UV light (Smith & McCully 1978), for callose; with toluidine blue, pH 4.7 (O'Brien *et al.* 1964, modified; Ribeiro & Leitão 2019), whose metachromasia allowed identification of mucilages and phenolic compounds. All sections were evaluated, and the images were obtained using a photomicroscope (Zeiss Lab AI/Axion Cam ICC 3).

Micromorphology and ultrastructure

For the characterization of structural changes on the stigma surface, after pollination and pollen tube development, the same phases described for the morphoanatomical study were considered. Stigmas were excised from three pistils, at each time point, fixed in Karnovsky's solution (Karnovsky 1965) and dehydrated in an ethanol series. The samples were dried to critical point in CO_2, mounted on aluminium stubs, and coated with a 10 nm layer of gold using a metallizer (Bal-Tec-MD20, Leica Microsystems, Heidelberg, Germany) (Robards 1978). The material was examined, and the images obtained using a scanning electron microscope (Quanta 200, FEI Company, Eindhoven, Netherlands) at 12–20 kV.

For the ultrastructural evaluation, 2 mm^2 fragments of the stigma and the stylar canal, from three flowers at the same development stages described earlier, were fixed in Karnovsky's solution (Karnovsky 1965), post-fixed in 1% osmium tetroxide in 0.1 M phosphate buffer, pH 7.2, and infiltrated with Araldite resin (Roland 1978). Ultrathin sections were contrasted with uranyl acetate (Watson 1958) and lead citrate (Reynolds 1963) and examined using a transmission electron microscope (Tecnai G2-12-Spirit, Philips/FEI Company, Eindhoven, Netherlands) at 80 kV.

RESULTS

Morphology

The inflorescences of female plants are branched and the pistillate flowers are borne along the rachillae, which correspond to second-order branches (Fig. 1A). The flowers are globular, sessile, trimerous, gamosepalous and gamopetalous. They have green bracts at the base, greenish-yellow sepals, and the petals are orange and free at the apical portion (Fig. 1A,B). The androecium is infertile, comprising six staminodes, and the gynoecium is syncarpous tricarpellary, with three pale yellow stigmatic lobes (Fig. 1C). During anthesis the stigma is wet (Fig. 1C) and receptive, as indicated by the activity of peroxidases (Fig. 1D). After pollination, the exudation increases copiously, facilitating the adhesion, hydration, and germination of the pollen grains, and the surface of the stigma darkens (Fig. 1E,F,I,J). In unpollinated flowers, the receptivity remains high until 36 hpa (Fig. 1G,H,K); and is completely lost by 48 hpa (Fig. 1L).

Micromorphology

The stigma of the pistillate flower has three lobes, which are free from each other in the apical portion, short and very close, forming splits on the surface that merge to create the central stylar canal (Fig. 2A,B); each lobe has conspicuous depressions (Fig. 2A,D,G,J). The epidermis of the stigma consists of elongated papillae and multicellular glandular trichomes, which, during anthesis, secrete a viscous substance by rupturing the cuticle (Fig. 2B,C). After 24 h of anthesis the stigma surface shows increased secretion accumulation, covering the papillae (Fig. 2D–F). The contact with pollen grains induces pronounced exudation, which accumulates especially in the region of the stylar canal, where there is a higher concentration of developing pollen tubes (Fig. 2G–K). After 36 h of pollination, fungal hyphae are noticeable amidst the germinated pollen grains (Fig. 2L).

Anatomy

In the pistillate flower, sepals are adnate to the petals, and staminodes are adnate to the petals. The pistil has emergences in the shape of scales forming the ovary wall; it is tricarpellary, trilocular, with one ovule per locule (Fig. 3A). The stigma is three-lobed and vascularized (Fig. 3B). The style is short, richly vascularized, and includes the *compitum* region, where it forms the central stylar canal (Fig. 3A,C). The stigma and style possess a subepidermal layer with conspicuous sclerenchyma, containing elongated and lignified cells that accumulate phenolic compounds (Fig. 3A–F). During anthesis, the epidermis of the stigma has multicellular trichomes with thickened and lignified walls (Fig. 3D) and bicellular glandular trichomes (Fig. 3E), associated with the papillae (Fig. 3F), both secreting phenolic compounds and mucilage. The stylar canal is sinuous along the ovary (Fig. 3C,G), whose epidermis presents papillose cells with thickened outer periclinal faces and composed of pecto-cellulosic material. The cytosol is intensely stained and nuclei are conspicuous (Fig. 3H). These features are similar in the epidermis of the locule and the apical portion of the ovule (Fig. 3I,J). The vascular bundles contain large tracheal elements that are close to the epidermis of the stigma and stylar canal. The sclerenchyma cells have conspicuous pits connecting the tracheal elements to the epidermis (Fig. 4A,B,D,E). After 2 h of pollination the stigma papillae exhibit intense mucilage secretion, which is notable on the cell wall and in the periplasmic space (Fig. 4B,C). During this phase, newly-germinated pollen grains are observed on the surface of the stigma (Fig. 4C), but the cells of the stylar canal retain the same characteristics observed during anthesis. After 4 and 12 h of pollination, conspicuous vacuolation is observed in the papillae of the stylar canal, and there is accumulation of extracellular mucilage, where numerous pollen tubes develop on the surface (Fig. 4E,F). On the surface of the stigma, the pollen tubes penetrate the papillae, which, after 12 h, continue to exhibit mucilage secretory activity (Fig. 4G,H).

Histochemistry

During anthesis, protein synthesis occurs in the cytosol of the papillae, with release to the surface of the stigma (Fig. 5A). There is a larger accumulation of proteins in the papillae of the stylar canal (Fig. 5B). At this phase, secretion of neutral polysaccharides is conspicuous on the surface of the stigma (Fig. 5C). In the epidermis of the stylar canal, this compound is strongly marked in the outer periclinal face of the wall and in numerous small vacuoles dispersed in the cytosol (Fig. 5D). After 12 h of pollination, the epidermis of the stigma and the stylar canal maintain secretory activity—there is an accumulation of proteins in the cytosol (Fig. 5E) and in intercellular

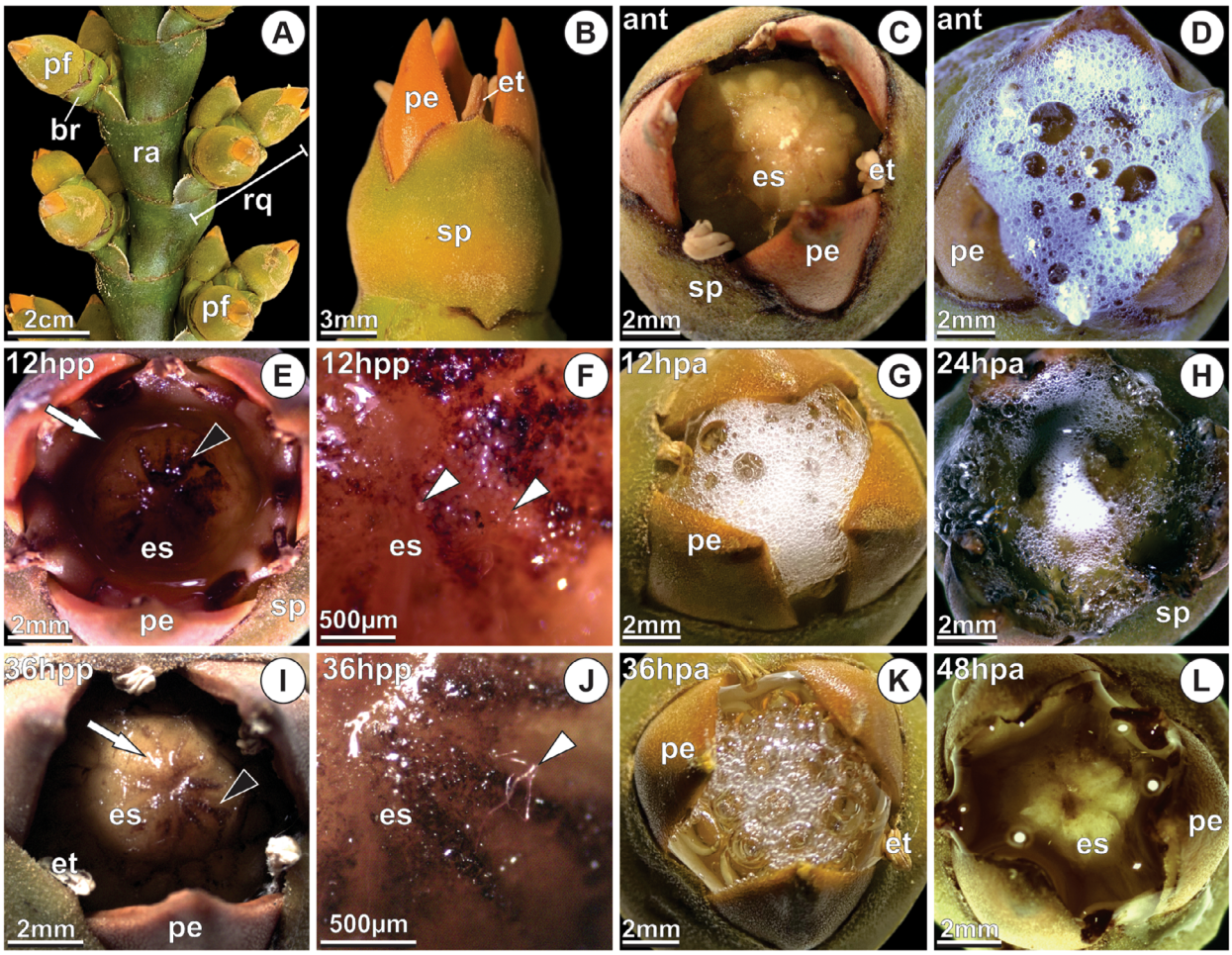

Fig. 1. Morphology of the pistillate flower of *Mauritia flexuosa* during the receptivity and pollination period. (A) Rachilla with pistillate flowers. (B) Pistillate flower showing sepals, petals, and staminodes. (C, D) Pistillate flower in anthesis displaying wet and receptive stigma. (E) Stigma with mucilaginous secretion 12 hpp (white arrow) and stigma darkening (black arrowhead). (F) Pollen grains adhered to the stigma surface (white arrowheads). (G, H) Receptive stigma at 12 and 24 hpa, respectively, unpollinated. (I, J) Stigma 36 hpp, highlighting mucilaginous substance (white arrow), surface darkening, and presence of pollen tubes (white arrowhead). (K, L) Receptive and non-receptive stigma at 36 and 48 hpa, respectively, unpollinated. ant, anthesis; br, bracteoles; es, stigma; et, staminode; hpa, hours post-anthesis; hpp, hours post-pollination; pf, pistillate flower; pe, petal; ra, branch rachilla; rq, rachilla; sp, sepal.

spaces (Fig. 5F). During this phase, synthesis of polysaccharides is conspicuous in the stigma papillae, and these compounds are also present in the pollen grains and developing pollen tubes (Fig. 5G). In the papillae of the stylar canal, there is accumulation of polysaccharides in the cytosol (Fig. 5H). After 36 h of pollination, there is a high concentration of proteins on the surface of the stigma and in the stylar canal, associated with the start of vacuolation (Fig. 5I,J), as also observed with the secretion of polysaccharides (Fig. 5K,L).

The pollen grains begin to germinate at ca. 2 hpp (Fig. 6A) and penetrate the papillae of the stigma, continuing to grow towards the stylar canal (Fig. 6B). During pollen tube development, irregularly deposited callose plugs can be observed (Fig. 6C). After 24 h of pollination, the pollen tubes reach the ovule (Fig. 6D).

Ultrastructure

During anthesis, the papillae of the stigma are covered with a thin cuticle, which expands with the accumulation of substances in the subcuticular space (Fig. 7A). The cell wall is slightly electron-dense, with notable adhesion of secreted compounds, in a granulocrine approach, to the periplasmic space (Fig. 7A,B). The protoplast is dense and rich in organelles. Polarization of the epidermal cells is evident, with a conspicuous nucleus in the basal portion, and organelles and vesicles originating from the dictyosomes, and endoplasmic reticulum concentrate in the apical portion (Fig. 7B). The peroxisomes are large, containing electron-dense crystalloid inclusions, and are especially prevalent neighbouring the nucleus (Fig. 7B). The cytosol contains numerous mitochondria with well-developed cristae, dictyosomes, ribosomes, and vesicles (Fig. 7C). The endoplasmic reticulum is increased and associated with ribosomes, being predominant at the periphery of the cells (Fig. 7D); there are small vacuoles dispersed in the cytosol (Fig. 7E).

The papillae of the stylar canal have a thicker and more electron-dense external periclinal cell wall, with inclusions of substances with a granular appearance (Fig. 7F). There is strong protoplast retraction, with an accumulation of

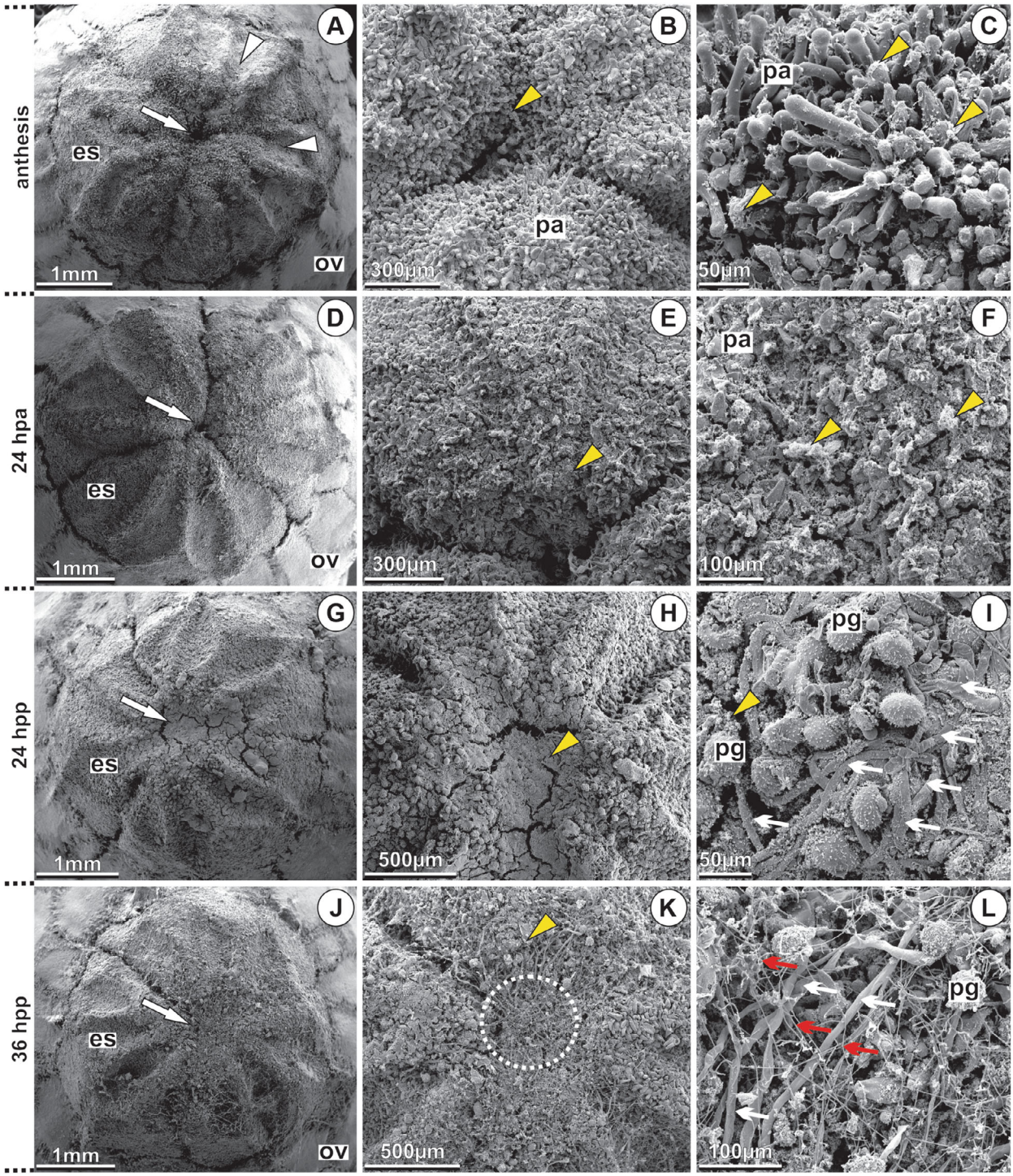

Fig. 2. Micromorphology of the stigma of *Mauritia flexuosa* during pollination. (A) Trilobed stigma with depressions on the surface (white arrowheads) and central stylar canal (white arrow). (B, C) Papillose epidermal cells on the stigma surface with secretion (yellow arrowheads). (D, F) Stigma with increased mucilaginous secretion released by epidermal cells (yellow arrowheads). (G, H) Stylar canal (white arrow) occluded by surface secretion (yellow arrowhead) and germinated pollen grains. (I) Stigma surface with numerous germinated pollen grains, with emphasis on developed pollen tubes (white arrows). (J, K) Pollen tubes concentrated in the stylar canal region (dotted circle) interspersed with accumulated secretions (yellow arrowhead). (L) Proliferation of fungal hyphae (red arrows) near pollen tubes (white arrows). es, stigma; hpa, hours post-anthesis; hpp, hours post-pollination; ov, ovary; pa, papilla; pg, pollen grains.

substances in the periplasmic space, originating from the fusion of vesicles with the plasma membrane (Fig. 7G,H). The protoplast is dense and contains vacuoles with granular substances, autophagic vacuoles, abundant mitochondria, dictyosomes, and endoplasmic reticulum (Fig. 7I,J). At the periphery of the cells, the endoplasmic reticulum is

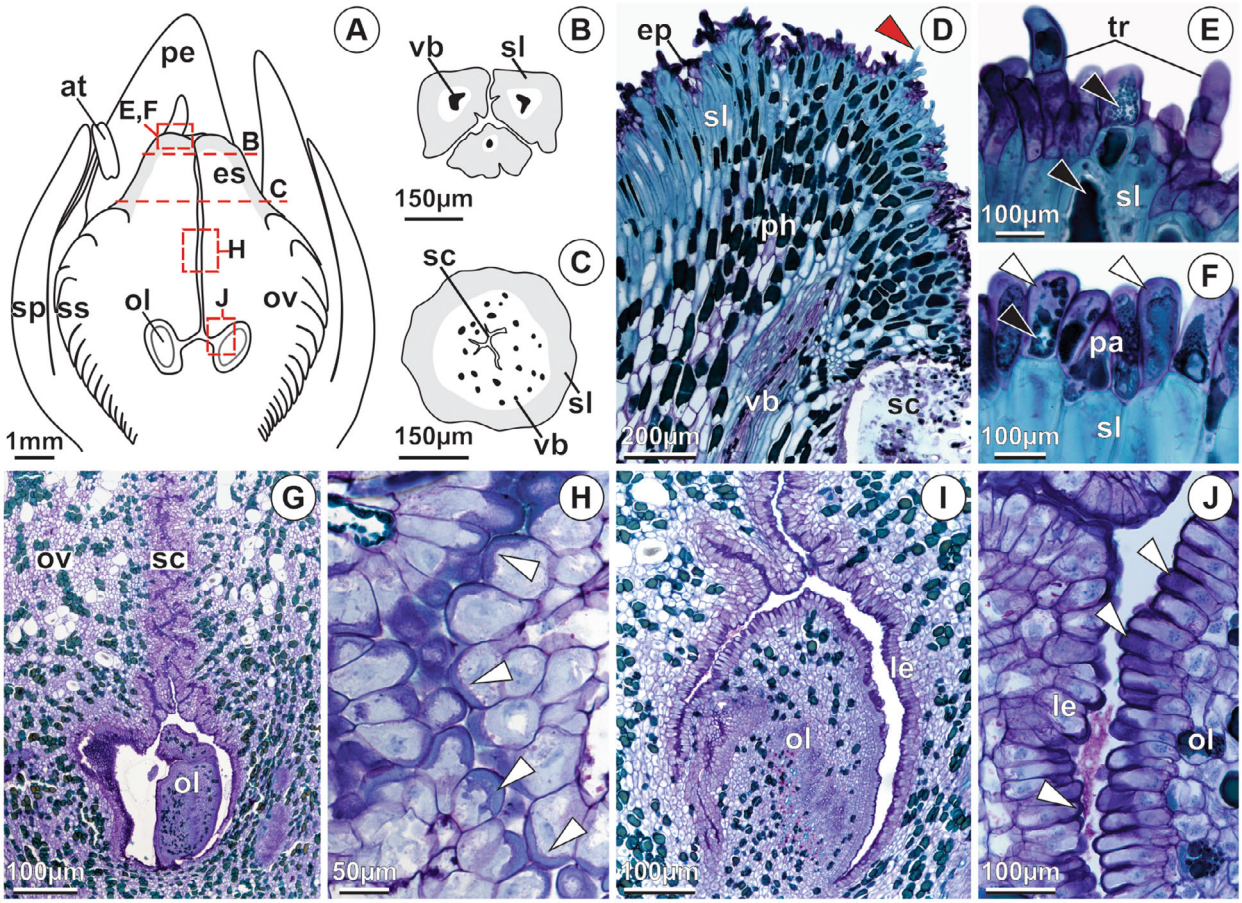

Fig. 3. Anatomy of the pistillate flower of *Mauritia flexuosa* during anthesis. (A) Longitudinal section of the pistillate flower, showing adnation between sepals and petals and between petals and staminode; ovary with scaly wall. (B) Apical portion of the pistil in cross-section, indicating the trilobed stigma and (C) *compitum* region in the style, forming the stylar canal. (D) Stigma epidermis with lignified wall trichomes (red arrowhead), (E) multicellular glandular trichomes, and (F) papillae. (D–F) Abundant phenolic compounds in the stigma epidermis and subepidermis, (G) in the ovarian mesophyll, and (I) in the ovule. (H, J) Epidermis of the stylar canal, locular epidermis, and apical portion of the ovule are papillose, with thickened periclinal outer cell walls composed of pecto-cellulosic material (H, J). at, anther; ep, epidermis; es, stigma; le, locular epidermis; ol, ovule; ov, ovary; pa, papillae; pe, petal; ph, phenolics; sc, stylar canal; sl, sclerenchyma; sp, sepal; ss, scaly; tr, trichome; vb, vascular bundle.

proliferated, with large cisternae, and is associated with ribosomes. The dictyosomes have well-developed cisternae and produce numerous vesicles (Fig. 7J). The peroxisomes are conspicuous and contain crystalloid inclusions (Fig. 7K).

After 12 h of pollination, the papillae retain a dense protoplast and active secretion, in a granulocrine pattern, with accumulation of substances in the periplasmic space (Fig. 8A). The pollen tubes penetrate the papillae cell wall and develop inside the symplast, where vacuolation is pronounced (Fig. 8B,C). The pollen tubes possess a thin and electron-lucent cell wall, and the protoplast is dense and rich in organelles (Fig. 8D,E). Numerous vesicles, dictyosomes, endoplasmic reticulum, mitochondria, and the nucleus of the vegetative cell are observed in the pollen tubes inside papillae, indicating recent penetration (Fig. 8D,E). Lipids droplets and small vesicles are dispersed in the cytosol, and there is an accumulation of substances at the periphery of the pollen tube (Fig. 8E). The papillae of the stylar canal have a thick outer periclinal cell wall that is slightly electron-dense, with notable inclusions of secreted content in a granulocrine manner in the periplasmic space; a similar substance is accumulated in the intercellular spaces (Fig. 8F,G). The protoplast is dense, rich in mitochondria, peroxisomes, rough endoplasmic reticulum, and dictyosome vesicles (Fig. 8H).

DISCUSSION

Mauritia flexuosa is a palm tree native to the Amazon that has expanded its range to the seasonal environments of the Cerrado biome, where it exhibits supra-annual reproductive phenology. The non-seasonal flowering and pollination under dry conditions pose challenges for the reproduction of this species. New characteristics for Arecaceae are reported in this study, such as the stigma surface protection system and the rapid and copious secretory process associated with vascularization (which advances pollen grain germination and pollen tube development). The speed of fertilization and the stigma surface protection system are factors indicating a strong relationship with the environment, contributing to the reproductive success of the species in this semiarid region.

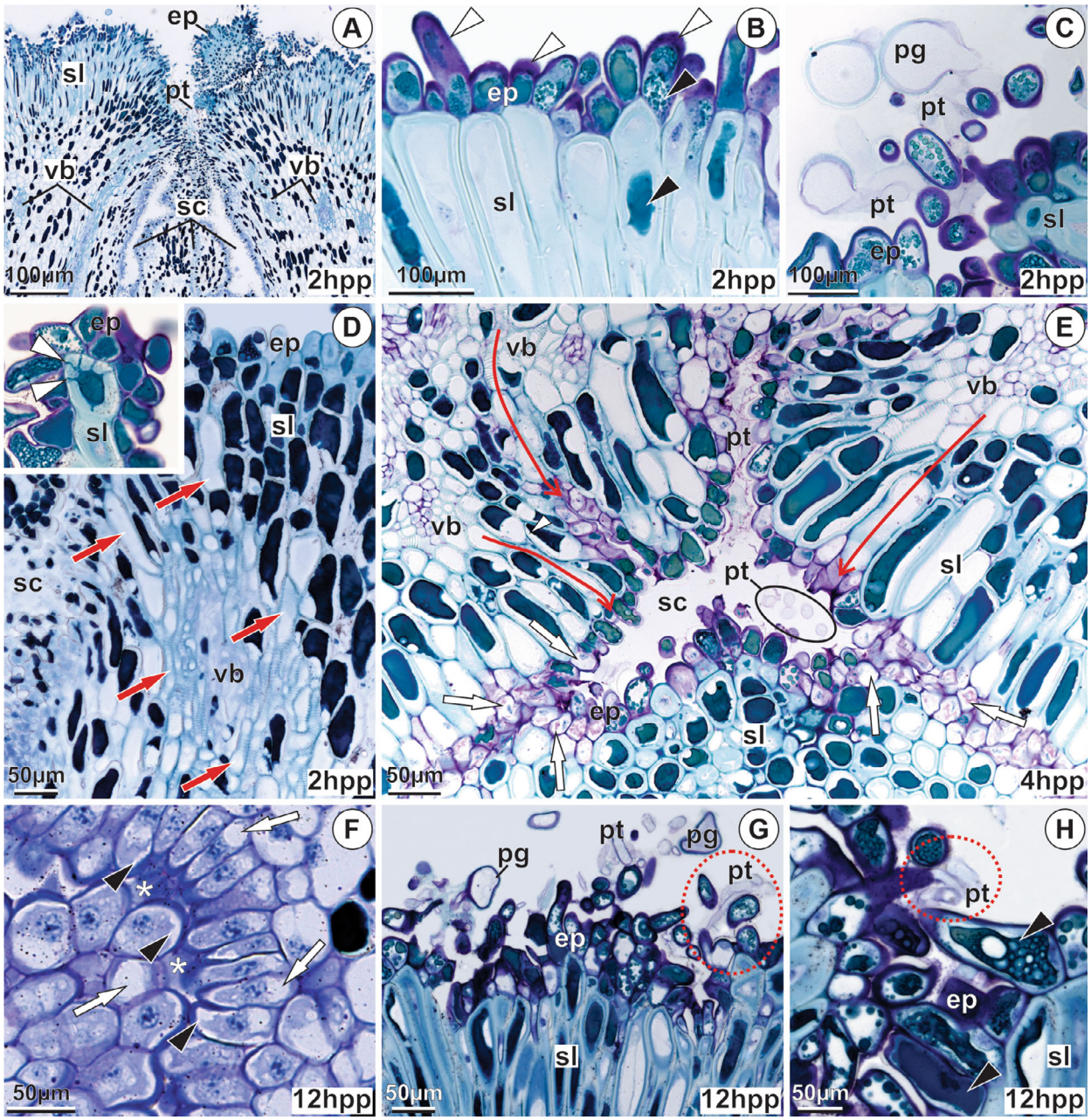

Fig. 4. Anatomy of the pistillate flower of *Mauritia flexuosa* during pollination. (A–D; F–H) longitudinal sections; (E) cross-section. (A) Vascularized stigma, with bundles near the surface. (B, C) Mucilage-secreting papillae (white arrowheads) and accumulation of phenolic compounds (black arrowheads). (C) Germinated pollen grains 2 hpp. (D, E) Stylar canal, 2 and 4 hpp, with large tracheal elements reaching the surface of the stylar canal (red arrows), where developing pollen tubes and vacuolation of epidermal cells (white arrows) can be observed. Note presence of pits (white arrowheads) in the sclerenchyma (red arrows indicate direction of water flow). (F) Vacuolation, 12 hpp, of the papillae of the stylar canal (white arrows) and accumulation of substances in periplasmic (black arrowheads) and extracellular (asterisks) spaces. (G, H) Surface of the stigma with pollen tubes among the papillae (red circle). ep, epidermis; sc, stylar canal; sl, sclerenchyma; sp, sepal; pg, pollen grain; pt, pollen tube; vb, vascular bundle.

Stigma receptivity and floral cycle

The stigma receptivity period of *M. flexuosa* is short, lasting about 36 h after floral anthesis, and the stigma surface has attributes that enhance its competence for pollination and rapid pollen grain germination. The depressions observed on the stigma, along with the epidermis with papillae and trichomes, increase the contact surface for pollen capture and hydration. The pale coloration and moisture on the stigma surface are morphological characteristics indicative of receptivity, as confirmed by peroxidase testing and in situ pollen germination. After pollination, the stigma darkens and there is a significant increase in exudate. Similar morphological features indicative of receptivity have been observed in the stigmas of the palm trees *A. aculeata* (Mazzottini-dos-Santos *et al.* 2015) and *B. capitata* (Dias *et al.* 2022), although the surface of this

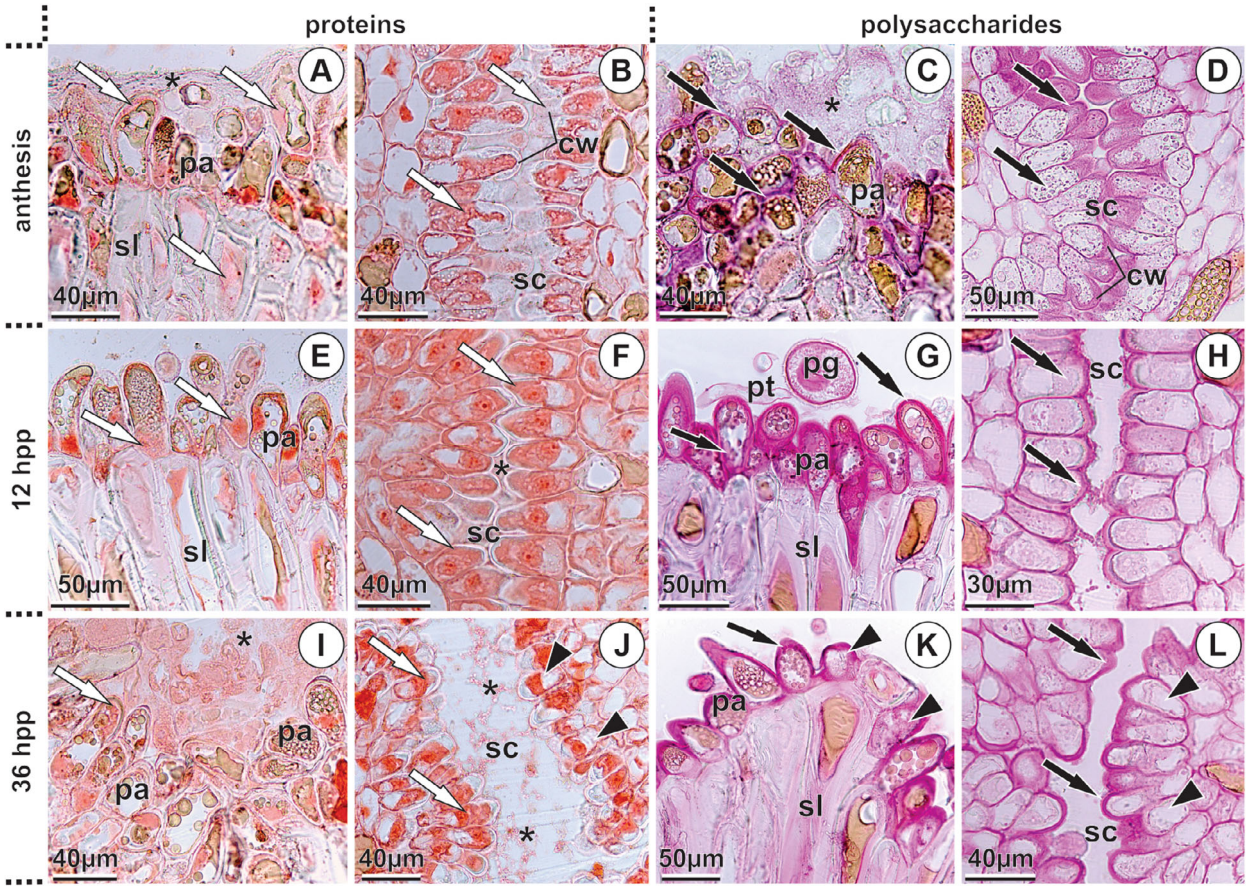

Fig. 5. Histochemistry of the pistillate flower of *Mauritia flexuosa* during pollination. (A, B, E, F, I, J) proteins stained red (white arrows) with xylidine-ponceau. (C, D, G, H, K, L) neutral polysaccharides stained magenta (black arrows) with periodic acid and Schiff's reagent. (A) Anthesis: proteins in the stigma papillae, in the sclerenchyma, and on the surface (asterisk). (B) Proteins in the cytosol and cell wall in the stylar canal. (C) Polysaccharides in cell walls, cytosol, and surface (asterisk) of the stigma papillae and (D) in the stylar canal. (E) 12 hpp: proteins in the stigma and (F) in the stylar canal, especially in the extracellular space (asterisk). (G, H) Greater accumulation of polysaccharides in the cell wall and cytosol; note polysaccharides in the pollen grain and pollen tube. (I, J) 36 hpp: Notable accumulation of proteins in the extracellular space (asterisks), associated with vacuolation (black arrowheads). (K, L) Reduced presence of polysaccharides and conspicuous vacuolation (black arrowheads). cw, cell wall; sc, stylar canal; pa, papillae; pg, pollen grain; pt, pollen tube; sl, sclerenchyma.

structure in these species presents longitudinal furrows or ventral grooves on the lobes. Although Tandon *et al.* (2001) considered this characteristic particular to *E. guineensis*, it appears to be common in Arecaceae, resulting from the partial fusion of the carpels (Uhl & Moore 1971; Reis *et al.* 2023).

The morpho-anatomical characteristics (ventral slits and formation of the compitum) of the *M. flexuosa* gynoecium indicate that it has a plicate origin (in which each carpel resembles a leaf with a folded margin, where ovules attach – see Reyes-Olalde *et al.* 2023). At the base of the gynoecium, there is complete carpel fusion (synascidiate), and in the apical portion, a single synplicate stylar canal forms. Although an ontogenetic study of pistillate flowers in *M. flexuosa* has been conducted (Reis *et al.* 2023), this classification of the gynoecium was not included, and some developmental stages were not considered, highlighting the need for further studies. Floral ontogeny studies in Arecaceae are scarce, but this carpel development pattern has been described for some species, such as *Geonoma interrupta* (Stauffer *et al.* 2002) and *Syagrus inajai* (Genovese-Marcomini *et al.* 2013). Carpel fusion is of evolutionary significance, as it is essential for forming other structures critical to reproduction, such as the pollen tube transmitting tissue, which may form from inner layers of the carpel leaf (Reyes-Olalde *et al.* 2023).

The stigma of *M. flexuosa* is wet and secretes hydrophilic compounds through papillae and trichomes. Furthermore, it has conspicuous vascularization, with tracheal elements that irrigate the surface of the structure, playing an important role in maintaining moisture (Fig. 9). Although the vascularization of the pistil, reaching the apex of the style, has been described for *M. flexuosa* (Reis *et al.* 2023), there was, until now, no record of its connection to the stigma surface or stylar canal, nor was the correlation with secretion in the stigma established, making this the first report on these aspects. In a study on the reproductive biology of buriti, conducted in the Amazon, the stigma was described as dry, and staminate and pistillate flowers lasted up to 5 days, with pollination predominantly carried out by wind (Rosa & Koptur 2013). Our results, obtained in semiarid conditions, are divergent, which could indicate phenotypic plasticity of the species. Considering the short viability period of flowers in the inflorescences and the dioecy of the species (Ávila *et al.* 2023), we believe that the

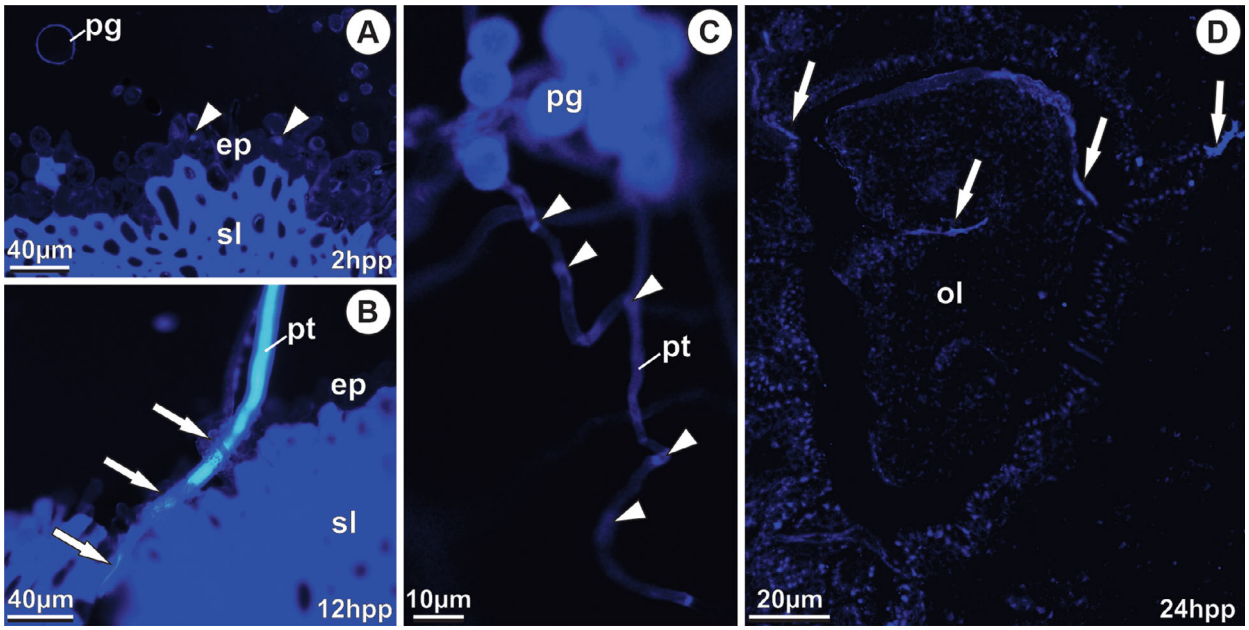

Fig. 6. Histochemistry of the pistillate flower of *Mauritia flexuosa* after pollination. (A) Initiation of pollen grain germination on the stigma surface (arrowheads) 2 hpp. (B) Pollen tube among papillae in the epidermis of the stigma (arrows) 12 hpp. (C) Callose plugs along the pollen tube (arrowheads). (D) Pollen tubes reach the ovule (arrows) 24 hpp. ep, epidermis; hpp, hours post-pollination; ol, ovule; pg, pollen grain; pt, pollen tube; sl, sclerenchyma.

wet stigma enhances the possibility of rapid capture, adhesion, hydration, and germination of pollen, thereby facilitating successful fertilization under dry conditions. Additionally, field observations (submitted manuscript) indicate that pollination is more effective through interactions with insects, particularly bees.

The maintenance of moisture on the stigma surface can be considered crucial for the progamic phase, such that environmental changes can impact seed formation. In *C. nucifera*, it has been evidenced that high temperatures significantly reduce stigma surface secretion and receptivity. However, the artificial maintenance of high moisture around inflorescences may favour extension of the progamic phase (Hebbar *et al.* 2020).

The buriti palm originates in the Amazon, a humid environment, but it shows significant demographic variability, with a large distribution in Brazil and occurrences in the Cerrado (Melo *et al.* 2018). The transition across different environments may have shaped changes in phenological, morphophysiological, and pollination ecology. However, environmental shifts, particularly reduced water availability in 'veredas' attached, with increasing temperatures, can impact the species' reproductive cycle, necessitating further research in this direction.

Structural, cytochemical, and ultrastructural aspects of the pollen–stigma relationship

In *M. flexuosa*, the composition and organization of the stigma papillae cell wall enables penetration of the pollen tube into the stigma and its nutrition throughout development on the surface of the stylar canal. The secretion of polysaccharides on the stigma surface occurs in a granulocrine approach; these compounds cross the cell wall, which has a loosely organized and pectic composition, and accumulate in the subcuticular space, being released upon cuticle rupture (Fig. 9). The presence of a cuticle is not commonly described for wet stigmas and has been linked to facilitating pollen tube penetration (Hiscock & Allen 2008). However, the thin cuticle on buriti palm papillae did not limit the secretion process, nor pollen tube development in the symplast, possibly due to enzyme synthesis, as evidenced by the numerous polyribosomes and conspicuous protein synthesis throughout the pistil epidermis. A similar pattern has been observed in *E. guineensis* (Tandon *et al.* 2001).

In the stigma, protein synthesis and release on the surface may also be related to pollen grain recognition through interaction with proteins present in the pollen coat (Kandasamy *et al.* 1994; Edlund *et al.* 2004; Goring 2017). In the stylar canal, compounds accumulated in the periplasmic space were gradually incorporated into the cell wall for subsequent release on the surface, seemingly thickening it. A similar pattern was observed in *Citrus limon* (Rutaceae), where the papillae of the stylar canal have two distinct layers in the outer periclinal cell wall. The superficial layer has a less electron-dense wall with an irregular organization pattern, giving the wall a looser appearance, facilitating pollen tube nutrition, which grow on its surface. The inner layer was granular, electron-dense, and heterogeneous, as a result of incorporated secretions (Ciampolini *et al.* 1981). In *Arabidopsis*, the papillose and secretory structure of the stylar canal was linked to pollen tube growth and nutrition, involving biochemical signalling exchanges between the two structures (Kandasamy *et al.* 1994). In *E. guineensis*, a distinct pattern was observed, with straighter cell walls in the apical region of the pistil, becoming papillose only near the ovules, whose function has not been clearly elucidated (Tandon *et al.* 2001). Protein and polysaccharide synthesis is intense in the papillae of the stigma, stylar canal, locule epidermis, and ovule of the buriti, with secretion onto the surface. The proteins secreted by the stigma may function in pollen grain recognition, similar to those occurring in the exine

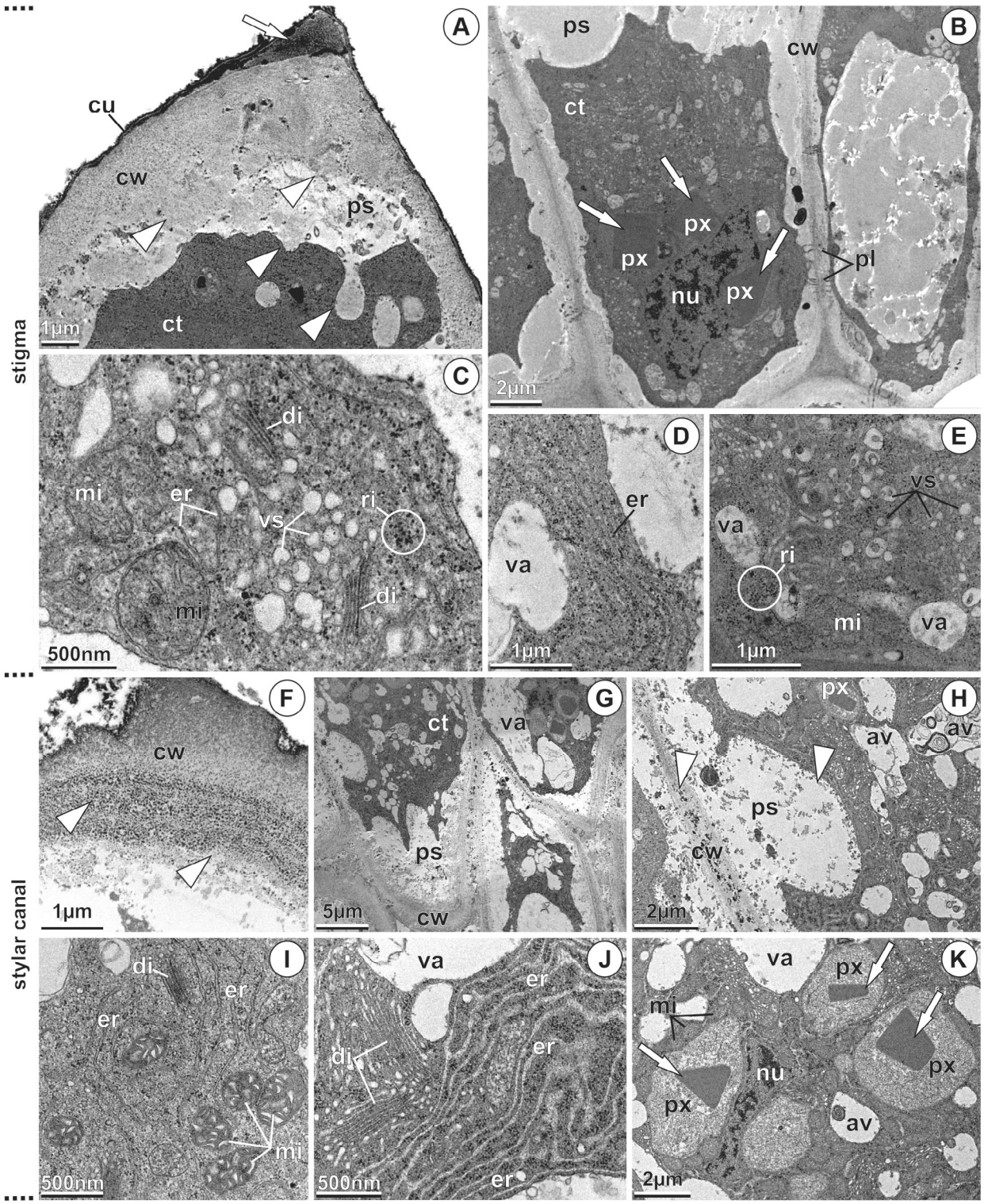

Fig. 7. Ultrastructure of the stigma and style canal of the pistillate flower of *Mauritia flexuosa* during anthesis. (A) Stigma cell containing secretion in the subcuticular space (white arrow) and periplasmic space (white arrowheads). (B) Polarized protoplast with basal nucleus associated with peroxisomes. (C) Numerous mitochondria, ribosomes, and dictyosomes. (D) Endoplasmic reticulum at the cell periphery, and (E) numerous vesicles and vacuoles in the cytosol. (F–H) Style canal cell with accumulation of granular material in the periplasmic space and cell wall (white arrowheads). (I, J) Abundant mitochondria, dictyosomes, endoplasmic reticulum, and (K) peroxisomes. av, autophagic vacuole; ct, cytosol; cu, cuticle; cw, cell wall; di, dictyosomes; er, endoplasmic reticulum; nu, nucleus; mi, mitochondria; pl, plasmodesmata; ps, periplasmic space; px, peroxisomes; ri, ribosomes; va, vacuole; vs, vesicles.

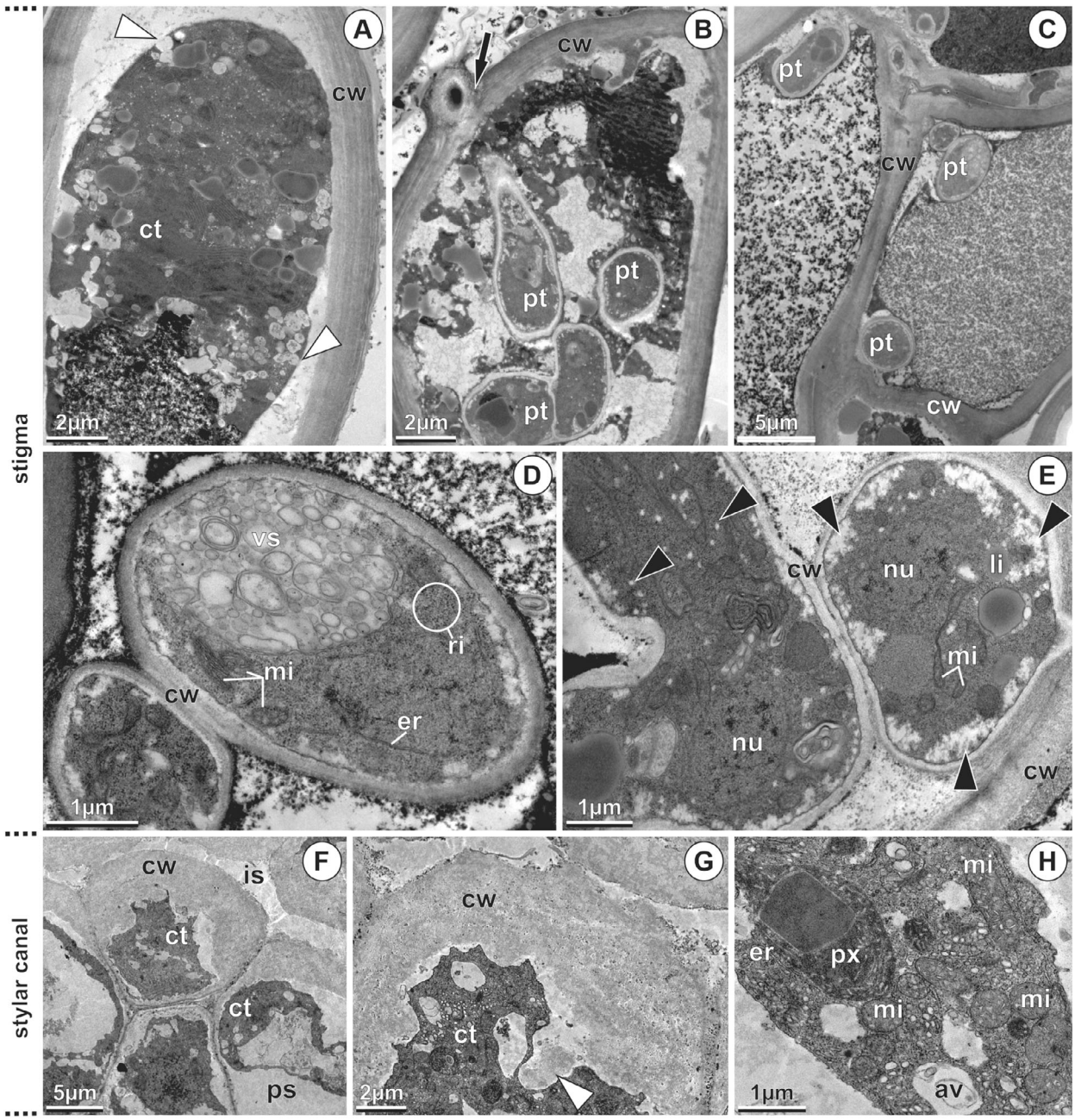

Fig. 8. Ultrastructure of the stigma and style canal of the pistillate flower of *Mauritia flexuosa* 12 hpp. (A) Stigma papilla secreting substances into the periplasmic space (white arrowheads). (B, C) Pollen tubes penetrating the cell wall (black arrow) and in the symplast. (D, E) Apical portion of pollen tubes at the cell periphery with numerous vesicles and organelles; accumulation of substances at the tube periphery due to fusion of small vesicles (black arrowheads). (F, G) Papillae of the style canal with a thick external periclinal cell wall and inclusion of substances from the periplasmic space. (H) Protoplast with numerous mitochondria, endoplasmic reticulum, and peroxisomes. av, autophagic vacuole; ct, cytosol; cw, cell wall; er, endoplasmic reticulum; is, intercellular space; li, lipid; mi, mitochondria; nu, nucleus; pt, pollen tube; px, peroxisome; ri, ribosomes; va, vacuole.

and pollen coat, as observed in other species (Lersten 2004; Serrano *et al.* 2008; Bosch & Wang 2020). Furthermore, the secretion from ovule papillae may indicate the functional role of the megagametophyte in attracting the pollen tube when it needs to exit the stylar canal (Hafidh *et al.* 2016).

The compitum region, formed by the partial fusion of carpels near the ovules (Reis *et al.* 2023), has also been described in other palm species, potentially acting as a nectary (Uhl & Moore 1971). In *Olea europaea* L., accumulation of lipids and sugars in the phloem of the style has been linked to the transport function of these substances near the pollen tube transmitting tissue (Serrano *et al.* 2008). However, in the present study, it was observed that the tracheal elements in the stigma are larger in diameter compared to the phloem and extend to the surface of the structure. Release of water dilutes the secretion of polysaccharides and other compounds, producing a

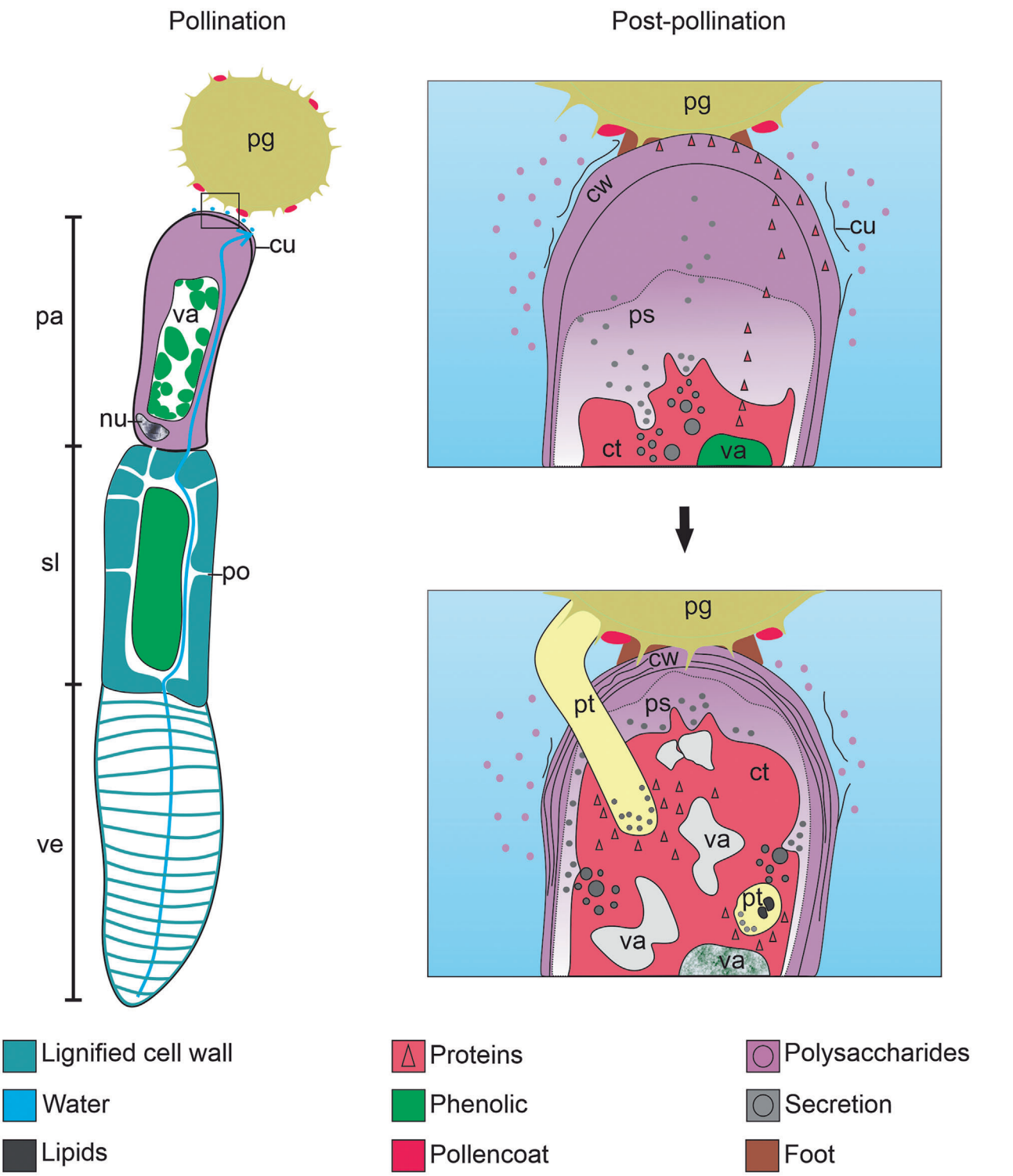

Fig. 9. Schematic representation of the secretion dynamics in the pistil of *Mauritia flexuosa* after pollination. The stigma is wet due to the synchronized secretion of hydrophilic compounds by the papillae and the release of water from vascular bundles connected to the surface. The blue line indicates water flow from the tracheal elements through the pits of the sclerenchyma and papilla. After pollination, water exudation is intensified, which facilitates pollen grain adhesion and foot formation. This leads to rupture of the cuticle, loosening of the cell wall, and penetration of the pollen tube into the symplast, followed by papilla vacuolation. ct, cytosol; cu, cuticle; cw, cell wall; nu, nucleus; pa, papilla; pg, pollen grain; po, pit; pt, pollen tube; ps, periplasmic space; sl, sclerenchyma; va, vacuole; ve, vessel element.

hydrophilic, sugar-rich exudate. This process was fundamental for pollen adhesion, germination, and pollen tube development, which occurred rapidly, 2 h after pollination. Despite conspicuous sclerenchyma in the subepidermal layers of the stigma, the pits are numerous and voluminous, facilitating rapid water transit to the surface (Fig. 9). Pistil vascularization is a common feature in flowers, with the number of vascular bundles varying between species, and there is no direct connection to the pistil secretory surface (Tandon *et al.* 2001; Serrano *et al.* 2008; Mazzottini-dos-Santos *et al.* 2015; Losada *et al.* 2017; Reis *et al.* 2023). The rapid pollen germination and pollen tube development occur similarly in *E. guineensis* (Tandon *et al.* 2001). Increased water on the stigma surface after pollen contact is evident in many species (Tandon *et al.* 2001; Lersten 2004; McInnis *et al.* 2006; Losada *et al.* 2017), often associated primarily with vesicular secretion from stigma papillae (Bosch & Wang 2020).

The protoplast of stigma papillae contains numerous and voluminous peroxisomes with conspicuous crystals, associated with many mitochondria and polysomes, which are indeed involved in the synthesis of reactive oxygen species (ROS) and enzymes. The synthesis of ROS, especially H_2O_2, as well as peroxidases and esterases by stigma papillae, are described in many angiosperm species and may play crucial roles in pollen recognition, signalling cascades for pollen germination and tube development, and protection of the stigma from pathogens (McInnis *et al.* 2006; Hiscock & Allen 2008; Losada *et al.* 2017).

Tests for peroxidase detection using H_2O_2 solution on the stigma surface are commonly employed, as revealed by bubble formation (Dafni & Maués 1998; Dias *et al.* 2022). Additionally, histochemical tests have been utilized to detect the presence of H_2O_2 on the receptive stigma surface of various angiosperm species (McInnis *et al.* 2006). The peroxisomes are organelles involved in the synthesis of catalase, an important antioxidant that can neutralize ROS by releasing water and oxygen. This process is linked to stigma receptivity indication and can be compromised at elevated environmental temperatures (Chen *et al.* 2021). Although the wet stigma of buriti secretes polysaccharide-rich substances, creating a favourable environment for fungal growth, contamination only occurred when the receptivity period began to decline, typically 36 hpp and after fertilization had been completed. Studies indicate that the synthesis of compounds with protective effects, especially H_2O_2 in the stigma, is relevant for inhibiting pathogen contamination (McInnis *et al.* 2006; Hiscock & Allen 2008; Losada *et al.* 2017) until fertilization occurs. This protective function in buriti may have been facilitated by the high presence of peroxisomes in the stigma and stylar canal. Moreover, the presence of sclerenchyma and the accumulation of phenolic compounds in the stigma, as observed in this species, may also play an important role in its protection.

In *M. flexuosa*, the pollen tube invades the stigma papilla and develops in the symplast until it reaches the stylar canal, where it continues its development on the surface. Within approximately 24 hpp, the pollen tube reaches the ovule, and fertilization occurs. The pectic structure and composition of the papillae cell wall facilitate the transit of secreted substances and the penetration of the pollen tube. Secretory papillae are found along the entire stylar canal, locule epidermis, and on the ovule surface. In *E. guineensis*, however, the pattern is different, with a smoother proximal region of the stylar canal and papillae only near the ovule, although the role of this structure remains unclear (Tandon *et al.* 2001). Nevertheless, secretion of mucilage, polysaccharides, proteins, along with vacuolation, indicates that the entire stylar canal modulates pollen tube growth, which develops on the surface of the papillae (Losada *et al.* 2017). Studies indicate that for the pollen tube to penetrate the papilla cell, it is necessary to trigger the expansion and loosening of the outer periclinal cell wall of the papilla (Goring 2017; Losada *et al.* 2017; Bosch & Wang 2020), like what is observed in *M. flexuosa* (Fig. 9). This process undoubtedly requires strong enzymatic activity at the interaction site, and such enzymes are supplied by the stigma and are involved in compatibility recognition (Bosch & Wang 2020).

The pattern of pollen tube growth can vary, developing either in the apoplast, through the cell wall matrix (Bosch & Wang 2020), or in the symplast (Losada *et al.* 2017). In both cases, the interaction between the sporophyte and the gametophyte during this phase is complex and involves signalling from both structures (stigma and pollen tube). We observed numerous vesicles, dictyosomes, and endoplasmic reticulum in the apical portion of the pollen tube, which has a thin cell wall and loose organization pattern. These features indicate that the pollen tube is also secretory, potentially synthesizing enzymes that degrade the papilla cell wall, thus facilitating penetration. However, nutritive substances, such as polysaccharides and amino acids, are also absorbed by the pollen tube (Tandon *et al.* 2001; Lersten 2004), thus explaining the conspicuous invaginations of the membrane.

Ecological aspects of floral biology in 'veredas' of the semiarid region

Structural features of the pistil and secretion dynamics during the progamic phase observed here can be associated with pollination ecology to ensure seed production in *M. flexuosa* within a semiarid environment. The rapid recognition of pollen and abundant secretion, with water release on the stigma upon contact with it, may compensate for the short receptivity period, thereby ensuring greater adhesion and germination of pollen grains. The stigma receptivity time varies greatly among species and may be related to environmental factors, dichogamy patterns, and pollinator activity (Lersten 2004; Losada *et al.* 2017). A study suggests that in species occurring in environments with milder temperatures or strong temporal separation due to dichogamy, the stigma can remain receptive for a longer period, increasing the probability of successful pollination (Losada *et al.* 2017). However, in tropical and subtropical species, stigma receptivity periods tend to be short, making timing for pollination crucial, particularly when carried out by biological agents (McInnis *et al.* 2006; Losada *et al.* 2017). Information regarding stigma receptivity is scarce for Areacaceae and non-existent for *M. flexuosa*. In *A. aculeata* (Mazzottini-dos-Santos *et al.* 2015) and *B. capitata* (Dias *et al.* 2022), both species from the Cerrado, the stigma can remain receptive for 3 and 7 days, respectively.

Contemplating the short duration of flower maintenance and viability within the inflorescence in *M. flexuosa*, the speed of pollination and fertilization is crucial to prevent ovule degeneration. Thus, we believe that the effectiveness of these processes is strongly related to interactions with biological

agents. A phenological study conducted in the 'veredas' of the Cerrado showed that this species exhibits supra-annual flowering (Ávila *et al.* 2023). Furthermore, the synchronization of flowering and ecological interactions in this environment revealed that rapid pollination is associated with insect visits, especially bees (submitted manuscript), unlike anemophily, as described by Rosa & Koptur (2013) in the Amazonian environment. In addition, many visits by bees and beetles were observed on the male and female flowers of *M. flexuosa* in the study area. Stingless bees were the most common group of visiting bees, with numerous individuals visiting many flowers, thus ensuring pollen flow for a prolonged period. Furthermore, *M. flexuosa* pollen is frequently found on the bodies of pollinators, indicating that stingless bees and beetles are potential pollinators (submitted manuscript) and that this pollen is the primary floral resource offered in staminate flowers.

Moreover, other structural aspects in pistillate flowers, such as terpenoid secretion in petals and staminodes emitting odour during anthesis (submitted manuscript), and production of microspores in the staminode as a potential secondary floral resource (Reis *et al.* 2023), indicate insect pollination. Odour during anthesis has also been observed in flowers of *E. guineensis* (Tandon *et al.* 2001) and *A. aculeata* (Mazzottini-dos-Santos *et al.* 2015), with the latter related to the presence of osmophores, also to insect visits. In the absence of nectar, odour can be the primary floral attractant, enhancing reproductive success in tropical environments, particularly through interactions with biological agents.

The stigma receptivity period of *M. flexuosa* is short, lasting approximately 36 h. The stigma has papillae and trichomes that secrete polysaccharides and proteins responsible for pollen recognition, adhesion, and germination, which occurs rapidly, within about 2 h. The vascularization of the pistil reaches the stigma and stylar canal surface, increasing moisture on the surface, thereby contributing to dilution of compounds and rapid hydration, and pollen germination. Stigma papillae have a pectic cell wall with a loose organization, facilitating substance transport and pollen tube penetration. The pollen tube grows on the surface of the stylar canal, which has a thicker outer periclinal cell wall related to the inclusion of secreted compounds for pollen tube nutrition and guidance, reaching the ovule within 24 h.

Our data indicate that the pistil exhibits significant structural characteristics during the progamic phase, ensuring the reproductive success of *M. flexuosa*, an iconic palm of the semi-arid 'veredas'. The stigma protection system is efficient, with an epidermis that secretes phenolic compounds and well-developed sclerenchyma in the subepidermal layers. The vascularization of the pistil, together with secretion from the stigma papillae and trichomes of the stylar canal, plays a crucial role in creating a microenvironment conducive to pollen grain adhesion and germination, facilitating rapid fertilization of the ovules. Maintaining a wet stigma is a relevant factor for the viability of the progamic phase. Cytological aspects of the pistil's inner epidermis and the pollen tube indicate a strong interaction between them, promoting pollen tube growth within the symplast (stigma) and along the surface (stylar canal), where it receives nutrition and signalling until reaching the ovules. Given the notable plasticity of this species' reproductive cycle in relation to its diverse environmental conditions, there is great potential for further studies on this interaction.

AUTHOR CONTRIBUTIONS

HCMS conceived and designed the study. LMR, PPF, IFPA, CSS and YRFN provided logistic and development support. HCMS and LMR conducted the first draft of the manuscript. HCMS, PPF, IFPA, CSS, YRFN, and LMR read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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