



Corolla traits and their osmophores in three neotropical Malpighiaceae: the putative impacts on plant-pollinator interactions

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Abstract

This study presents the comparative, function-oriented analysis of petal structure and osmophore distribution in three Malpighiaceae species, complementing the established role of calyx elaiophores. Integrating light and electron microscopy with floral biology observations and visitor records, the authors document interspecific variation across core floral traits: anthesis timing; corolla senescence, color, and dimensions; petal micromorphology; and the spatial organization of scent-emitting areas. *Byrsonima coccolobifolia* possesses flat, textured petal surfaces without glands; scent is emitted from a lobed, striated basal outer region whose cells accumulate starch prior to anthesis, indicating metabolic provisioning. In contrast, *Banisteriopsis variabilis* and *Peixotoa reticulata* share rough petal surfaces but differ in epidermal cell shape—cone-shaped in *B. variabilis* versus rounded in *P. reticulata*. Both species display enlarged intercellular spaces and minute, vascularized scent glands along fimbriate petal margins that exhibit subcellular traits diagnostic of osmophores, supporting their role in volatile biosynthesis and release. Across the three focal taxa, the findings resolve two principal scent-emission strategies: a diffuse basal emission zone (*B. coccolobifolia*) versus discrete marginal osmophores (*B. variabilis*, *P. reticulata*). Functionally, petal surface architecture and mesophyll organization likely enhance visual signaling, while osmophores are central to attracting oil-collecting bees. Under an evolutionary framework, the evidence indicates ongoing specialization of floral tissues for scent production, with probable effects on pollinator attraction and specificity. The study thus refines our understanding of multimodal floral signaling in Malpighiaceae and suggests that diversification in petal anatomy and osmophore placement may shape plant–pollinator interactions and the evolution of pollination systems within the family.

Keywords Banisteriopsis · Byrsonima · Peixotoa · Corolla morphology · Pollinators · Scent glands

Introduction

Malpighiaceae, comprising approximately 1,300 species, is widespread and highly diverse in the New World tropics and subtropics, where 80% of the species are endemic (Anderson 1990). The species within Malpighiaceae family exhibit

significant variation in growth forms, fruit types (Anderson 1979), pollen grain morphology and chromosome number (Anderson 1990). However, among the species found in the Neotropical region, the flowers display a relatively uniform morphology, particularly regarding their attraction, orientation, and rewards for pollinators (Anderson 1979; Zhang et al. 2010; Davis and Anderson 2010; Possobom et al. 2015; Reposi et al. 2023; Gotelli et al. 2023). These flowers are bilaterally symmetrical and are positioned such that the posterior petal is erect and located at the back of the flower from a bee's perspective. This uniformity is attributed to their reliance on oil-collecting bees, especially from the tribes Centridini and Tapinotaspidini, which serve as their primary pollinators (Anderson 1979, 1990; Vogel 1990; Davis et al. 2014). Non-volatile oils are produced in specialized calyx glands known as elaiophores (Anderson 1990; Vogel 1990). Elaiophores are used as a diagnostic character

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for representatives of neotropical Malpighiaceae; some species may possess flowers that either have elaiophores or lack them entirely (Vogel 1974, 1990; Anderson 1979, 1990). Also, certain species have completely lost their elaiophores and may be pollinated by bees that collect pollen (see Cappellari et al. 2011).

In addition to calyx glands, other floral glands have been referenced in several studies examining the mechanisms of pollination and reproduction in plants from the Malpighiaceae family (Possobom et al. 2015; Sanches et al. 2023; Gotelli et al. 2023). Possobom et al. (2015) identified typical osmophores—finger-like glands located on the edges of frilly petals that help attract pollinators—*Diplopterys pubipetala* (A. Juss.) W. R. Anderson & C. Davis). Sanches et al. (2023) proposed that the petal glands in *L. floribunda* W. R. Anderson & C. Davis might act like colleter, but they also thought these glands could work as osmophores since their study only looked at fixed samples. Gotelli et al. (2023) did not report any glandular structures in the corolla of *Galphimia australis* Chodat but proposed that the entire petals might function as osmophores.

Osmophores or scent glands are located mainly on petals and other floral organs, such as sepals or stamens, or other specialized reproductive structures (Vogel 1990; Sazima et al. 1993; Gagliardi et al. 2016). Osmophores differ from other secretory structures in terms of their end products, deposition sites, and duration of secretion (Vogel 1990; Endress 1994; Effmert et al. 2006). They typically consist of an epidermis of specialized secretory cells and/or secretory parenchyma. They are concentrated in certain regions of the floral organs and can have different shapes, sizes and colours (Vogel 1990; Possobom et al. 2015; Possobom and Machado 2017, 2018; Gagliardi et al. 2016; Tölke et al. 2018; Macedo et al. 2023).

The presence of osmophores plays a crucial role in attracting visitors by utilizing chemical signals (Vogel 1990; Knudsen and Gershenzon 2020), since floral scents are recognized as one of the key factors influencing the interaction between flowers and their pollinators (Raguso 2004; Gervasi and Schiestl 2017). Even families like Malpighiaceae, among others, which reward pollinators with non-volatile oil through sepal elaiophores, floral volatiles appear to play a significant role in attracting oil-collecting bees (Steiner et al. 2011; Dötterl and Schöffler 2007; Schöffler et al. 2012, 2015). According to Schöffler et al. (2015) diacetin is a volatile compound widespread among unrelated oil plants, potentially serving as a private communication channel between these plants and oil-collecting bees. However, the presence of floral volatiles in Malpighiaceae remains a topic of debate. Some researchers argue that flowers are scentless (Teixeira and Machado 2000; Costa et al. 2006; Cappellari et al. 2011). Conversely, a strong floral fragrance has

been documented in four Malpighiaceae species, indicating it may have attracted a significant number of visiting bees (Baillon 1878 apud Vogel 1974). Also, there have been reports of scent flowers in twelve related Malpighiaceae species, which include four from *Banisteriopsis* C.B. Rob., three *Mascagnia* (D.C.) Bertero, two *Tetrapteryx* A. Juss., *Dicella bracteosa* Griseb., *Heteropteryx intermedia* Griseb., and *Stigmaphyllon lalandianum* Nied. (Sigrist and Sazima 2004); as well as in *D. pubipetala* (Possobom et al. 2015) and *D. floribunda* (Sanches et al. 2023).

Although calyx structure and elaiophores in Malpighiaceae are well documented (Cocucci et al. 1996; Castro et al. 2001; Seipold et al. 2004; Possobom et al. 2015; Araújo and Meira 2016; Possobom and Machado 2017, 2018), the corolla microstructure, its glands, and their impacts on plant–pollinator interactions remain insufficiently understood. Morphological and anatomical studies of petals and scent glands in the family are still too scarce to resolve these gaps. We therefore hypothesize that, in Malpighiaceae, interspecific variation in petal microstructure and in the spatial distribution of petal-associated scent glands (osmophores) represent adaptive differentiation that modulates pollinator attraction and specificity.

This study presents a detailed morphological and anatomical investigation of the corolla in Malpighiaceae, with emphasis on scent-producing tissues. We examined three sympatric, phylogenetically distant species from the Brazilian cerrado—*Byrsonima coccolobifolia* Kunth (byrsonimoids, basal clade), *Banisteriopsis variabilis* B. Gates, and *Peixotoa reticulata* Griseb (both stigmaphylloids, derived clade; Davis and Anderson 2010). The species share key floral traits, including diurnal anthesis, a symmetrical, pentamerous corolla, and a morphologically distinctive posterior petal; their sepals bear typical elaiophores (Possobom and Machado 2018). We combined field observations to document floral phenology and visitor assemblages with morphological analyses of petals and associated glands. We then evaluated how interspecific differences in petal architecture and glands distribution may influence plant–pollinator interactions.

Materials and methods

Study site, plant species and fieldwork

The study was performed with ten plants occurring in natural populations of *Banisteriopsis variabilis* (BOTU 28442–28448), *Byrsonima coccolobifolia* (BOTU 27630–27632) and *Peixotoa reticulata* (BOTU 28441) at a remnant from cerrado located in Pratânia municipality, São Paulo State, Brazil (22°48′ 48.8″ S and 48°44′ 33.7″ W).

As previously reported by Possobom and Machado (2018), the flowering period and intensity are variable between the studied species. *Banisteriopsis variabilis* plants flowered from February to June, *Byrsonima coccolobifolia* plants flowered from October to January, and a small number of *P. reticulata* plants bloomed annually (<50% of the labeled individuals) from June to September. The fruiting periods were from March to August (*B. variabilis*), from November to March (*B. coccolobifolia*), and from June to October (*P. reticulata*).

During the peak flowering of each species, we monitored approximately 15 individual plants from 6:00 AM to 6:00 PM, totaling 38 observation hours for *B. variabilis* (April–May), 20 h for *B. coccolobifolia* (November), and 15 h for *P. reticulata* (August). Because each species exhibits a distinct spatial distribution, observation time per individual ranged from 5 to 20 min, depending on whether plants were grouped or dispersed.

We gathered pre-anthesis buds and flowers from the first day to study their size and structure, while also recorded aspects of the floral events (anthesis, longevity, secretory activity, and changes in the floral morphology) and the floral visitors (frequency, behavior, and collected resources).

The visitors were categorized as potential pollinators (PP) when they visited many flowers on more than one individual plant and always contacted the reproductive parts of the flowers during the visits; occasional pollinators (OP) when they made sporadic visits and could occasionally contact the reproductive parts of the flowers during the visits; and robbers (R) when they did not contact reproductive structures during the visit. Visitors were also classified according to their behavior during the visit, foraging for oil, pollen, and/or androecium secretion.

The interactions recorded specifically between oil-collecting bees and the flowers of these same plants have already been reported by Possobom and Machado (2018); however, data on the frequency of these and other floral visitors will be presented here for the first time.

Most floral visitors were photographed, captured using glass containers or insect nets, examined for pollen, and sent for identification by a specialist on bees (Dr. A. J.C. Aguiar). Those insects that could not be captured were classified by the authors themselves in the field, generally as morphospecies based on their morphology and behavior during visits.

Floral scent assessment and neutral red assay

Fresh flowers from the three species were placed in closed glass containers to concentrate and evaluate scent emission through organoleptic assessment. To identify active cells and locate potential scent-producing areas on the flower petals, whole fresh flowers were immersed in a very diluted

red dye solution for 20 min (Vogel 1990; Dafni 1992). After this, the flowers were photographed.

Morphometric analysis

We obtained the corolla diameter from three flowers of each species using a digital pachymeter (Digimess). The posterior and lateral petals were imaged under a stereomicroscope (Leica M205C) coupled to a digital camera (Leica DFC 425). Measurements (length and width of the limb and claw; number and diameter of glandular structures, when present) were obtained from photographs using the LAS Leica application suite.

Microscopic examination

For surface examination, samples were fixed in 2.5% glutaraldehyde (0.1 M sodium phosphate buffer, pH 7.3), post-fixed in osmium tetroxide (0.5% in the same buffer), dehydrated in an aqueous ethanolic series, critical-point dried (CPD 020, Bal-Tec), mounted on a metallic support with adhesive, and sputtered coated with gold (10 nm) (MED 010, Balzers Union). The material was then examined with a scanning electron microscope (SEM) Quanta 200 (Fei Company, Gräfelfing, Germany) at 20 kV.

Pieces of petals were fixed in Karnovsky's solution (Karnovsky 1965), dehydrated through a graded aqueous ethanol series, and then embedded in glycol methacrylate historesin (Leica Microsystems, Nussloch/Heidelberg, Germany) for anatomical examination. Thin Sects. (4–6 µm) were cut using a semiautomatic microtome (Leica RM2245), stained with 0.05% toluidine blue, pH 4.5 (O'Brien et al. 1964), and mounted in synthetic resin (Entellan, Merck KGaA, Darmstadt, Germany). We examined the vascularization of the posterior and lateral petals by clearing samples according to Fuchs (1963) and mounting them in glycerin jelly under a coverslip.

For in situ detection of the main classes of substances, samples of petals were subjected to the following reagents: Lugol for starch grains, Sudan IV and Sudan Black for total lipids (Johansen 1940). Samples of the petal glands were also subjected to naphtol + dimethylparaphenylene-diamine (NADI) for terpenes (David and Carde 1964).

The sections were observed using a Leica DMR 500 (Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany) optical microscope equipped with a digital camera (Leica DFC).

For ultrastructural characterization of the glands (only in *Banisteriopsis variabilis* and *Peixotoa reticulata*), samples of the petals taken from preanthesis buds and newly opened flowers were fixed in 2.5% glutaraldehyde (0.1 M sodium phosphate buffer pH 7.3, overnight at +4 °C) and post-fixed

with 1% osmium tetroxide in the same buffer for 2 h at room temperature (Hayat 1989). After washing in distilled water, the material was dehydrated in aqueous acetone and embedded in Araldite resin (Araldite 502, Electron Microscopy Sciences, Hatfield, USA). Semi-thin (0.5 µm) and ultrathin (60 nm) sections were obtained using an ultramicrotome (Leica Reichert). The semi-thin sections were stained with 0.05% toluidine blue (O'Brien et al. 1964). The ultrathin sections were stained with a 2% uranyl acetate solution for 15 min and lead citrate for 15 min (Reynolds 1963) and then looked at and recorded using a Tecnai Spirit transmission electron microscope (TEM) at 60 kV from FEI Company in Germany.

Results

Floral visitors

A total of 224 visits were recorded, involving 25 species (including morphospecies) of insects, with bees being the main group of visitors (Fig. 1a–i; Table 1). Visitor diversity was highest in *Banisteriopsis variabilis* (16), followed by *Byrsonima coccolobifolia* (11) and *Peixotoa reticulata* (3). Four taxa were common to more than one plant species, and only one taxon was common to all the three species.

Flowers of *B. variabilis* and *B. coccolobifolia* attracted small (< 1 cm), medium (1–2 cm), and large (2–3 cm) visitors, while *P. reticulata* flowers attracted only small visitors (< 1 cm). The visitors, which could be robbers or potential/occasional pollinators, collect oil from the calyx glands, pollen, and secretions from glandular connectives or staminodes (Fig. a–i, Table 1). We observed no visitors foraging on the corolla parts.

Oil-collecting bees were responsible for approximately 90%, 50%, and 35% of visits to *B. variabilis*, *B. coccolobifolia*, and *P. reticulata*, respectively. They were likely pollinators in 82.6% of visits to *B. variabilis* and 24.6% of visits to *B. coccolobifolia*, while in *P. reticulata*, they only act as robbers. Furthermore, more than 40% of the visits to *B. coccolobifolia* were just to collect pollen, and about 60% of the visits to *P. reticulata* were for gathering the secretions from the androecium (Table 1).

Large and medium-sized bees belonging to the *Centris* and *Epicharis* were the most frequent potential pollinators in *B. coccolobifolia* (Fig. 1a) and *B. variabilis* (Fig. 1d–e), in addition to the small-sized *Monoeca* (Fig. 1f) in *B. variabilis*. These and other unidentified bees (Fig. 1b) landed on the flowers with their abdomens in contact with the reproductive structures, as previously reported by Possobom and Machado (2018). Sometimes, they held the claw or the basal part of the posterior petal blade with their mandibles and,

placing the first two pairs of legs in the space between the petals, reached the calyx glands. The collected bee specimens demonstrated pollen deposition in the ventral region of the body (Fig. 1f).

In *B. coccolobifolia*, in addition to the oil collection behavior, some bees, such as *Epicharis flava* and morphospecies 7 (Fig. 1b), also collected pollen, in the same visit, by vibrating the anthers, emitting a very characteristic sound (“buzz-pollination”) and releasing a visible cloud of pollen. Small bees, such as *Tetrapedia* sp. 1 (Fig. 1h) and *Tropidopedia flavolineata*, visited flowers at different developmental stages and acted exclusively as oil thieves, landing directly on the calyx (see Possobom and Machado 2018), sometimes with their mandibles holding the pedicel.

Other small bees, such as *Paratrigona* sp. (Fig. 1c, i), frequently landed on the calyx, on the petals, or directly on the androecium and walked to the stamens, where they collected pollen from the anthers (*B. coccolobifolia*), scraped the glandular connectives (*B. coccolobifolia* and *P. reticulata*), or the glandular staminodes (*P. reticulata*). Since *P. reticulata* individuals have indehiscent anthers (as previously reported by Possobom and Machado 2018), these bees could act as occasional pollinators only in *B. coccolobifolia*.

Large bumblebees representing the genus *Bombus* (Fig. 1g) were observed collecting pollen from *B. variabilis* and *B. coccolobifolia* flowers. They used their first pair of legs to collect and the other two pairs for support, moving from one flower to another in the same inflorescence, eventually coming in contact with the stamens and stigmas. Other small and medium-sized bees that also collected pollen were *Apis melifera* and *Trigonisca* sp. in *B. variabilis*, *Augochloropsis* sp., *Tetragonisca angustula* and *Xanthopedia* sp. in *B. coccolobifolia*.

The frequency of visits, behaviors exhibited during these visits, and the resources collected by floral visitors of the three Malpighiaceae species are summarized in Table 1.

Floral events and scents

All three species exhibited diurnal anthesis (Fig. 2a–i), with corolla senescence beginning two to three days later. *Banisteriopsis variabilis* exhibited a greater number of flowers per inflorescence and more inflorescences per plant compared to the other two species. Flowers near anthesis exhibited more erect pedicels and prominent claws on the posterior petals in *B. coccolobifolia* (Fig. 2a) and were characterized by corolla slackening in *B. variabilis* (Fig. 2d) and *P. reticulata* (Fig. 2f).

Flowers opened between 6 and 8 a.m. (or around 4 p.m.) in *B. coccolobifolia* (Fig. 2b), around 7 a.m. in *B. variabilis* (Fig. 2d), and at 6 a.m. in *P. reticulata* (Fig. 2g). Anthesis was characterized by the slow, centrifugal and nearly



Fig. 1 Floral events in *B. coccolobifolia* (a–c), *B. variabilis* (d, e) and *P. reticulata* (f–i). Pre anthesis buds (a, d, f). Note corolla loosening and claw (cl) prominence (a). Anthesis flowers (b, e, g, arrows). Note the centrifugal and almost synchronous movement of the petals. Flow-

ers with two or more days (a, c, d, e, h, i, asterisks). Note pedicels (pe) curvature in senescent flowers when compared with the newly opened flower (c); faded, dried or missing corollas (d–e); closed petals with a dry appearance. Bars=5 mm

Table 1 Frequency of the visits, behavior during the visits and resources collected by floral visitors of the three Malpighiaceae species

Taxa	Resource	Behavior	Relative Frequency (number of visits)		
			<i>B. coccolobifolia</i>	<i>B. variabilis</i>	<i>P. reticulata</i>
Hymenoptera					
Apinae					
Apini					
<i>Apis mellifera</i>	P	R		1.4% (2)	
<i>Bombus</i> sp.	P	R/OP	1.5% (1)	0.7% (1)	
<i>Paratrigona</i> sp. ^a	P/A	R/OP	24.6% (16)		
	A	R			64.3% (9)
<i>Tetragonisca angustula</i> ^a	P	R	1.5% (1)		
<i>Trigonisca</i> sp. ^a	P	R/OP		2.1% (3)	
Centridini					
<i>Centris analis</i> ^a	O	PP	7.7% (5)		
<i>Centris</i> sp. 1 ^a	O	PP		47.5% (69)	
<i>Centris</i> sp. 2 ^a	O	PP			
<i>Epicharis flava</i> ^a	O/P ^b	PP	16.9% (11)		
	O	PP		14.5% (21)	
Tapinotaspidini					
<i>Monoeca mourei</i> ^a	O	PP		16.5% (24)	
<i>Tropidopedia flavolineata</i> ^a	O	R			14.3% (2)
<i>Xanthopedia</i> sp. ^a	P	R/OP	6.2% (4)		
Tetrapediini					
<i>Tetrapedia</i> sp.01 ^a	O	R	15.4% (10)	5.5% (8)	21.4% (3)
<i>Tetrapedia</i> sp.02 ^a	I	OP		1.4% (2)	
Halictinae					
Augochlorini					
<i>Augochloropsis</i> sp. ^a	P	R/OP	9.2% (6)		
Unidentified bees					
Morphospecies 01	O	OP		2.1% (3)	
Morphospecies 02	O	PP		4.1% (6)	
Morphospecies 03	I	I		1.4% (2)	
Morphospecies 04	O	OP		0.7% (1)	
Morphospecies 05	O	R	4.6% (3)		
Morphospecies 06	O	R	1.5% (1)		
Morphospecies 07	O/P ^b	PP	10.8% (7)		
Unidentified insects					
Morphospecies 08	O	R		0.7% (1)	
Morphospecies 09	P	R		0.7% (1)	
Morphospecies 10	P/A	R		0.7% (1)	
Total			100% (65)	100% (145)	100% (14)

A – androecium secretion;
 I – inconclusive; O – oil; OP – occasional pollinator; P – pollen;
 PP – potential pollinator; R – robber. ^a floral visitor identified by specialist. ^b pollen collection through anther vibration

synchronized movement of the petals (Fig. 2b, d, g). After opening, the claws on the posterior petals maintained a vertical position, while on the other petals they assumed a reflexed (*B. coccolobifolia*) or horizontal (*B. variabilis* and *P. reticulata*) position in relation to the flower axis.

Fresh flowers had shiny petals (Fig. 2b-c, e, g) and gave off a strong, sweet smell like vanilla (*B. variabilis*) or a mild, slightly sweet smell (*B. coccolobifolia* and *P. reticulata*), which was hard for people to smell in *P. reticulata*. They showed an accumulation of secretion in the elaiophores of the sepals and clear droplets in the connectives, or secretory

staminodes. However, none of the species displayed any secretion on the surface of their petals under field environmental conditions. In *B. coccolobifolia*, flowers that were two or more days old, along with young shoots and fruits, had pedicels that curved at different angles toward the center of the flower cluster (Fig. 2c). In *B. variabilis*, the corolla of flowers gradually became opaque and lost coloration (Fig. 2e). In *P. reticulata*, the gradual centripetal bending of the petal claws caused the corolla to close in the late afternoon of the second day (Fig. 2h-i). In all three species, the corolla of senescent flowers gradually dried.

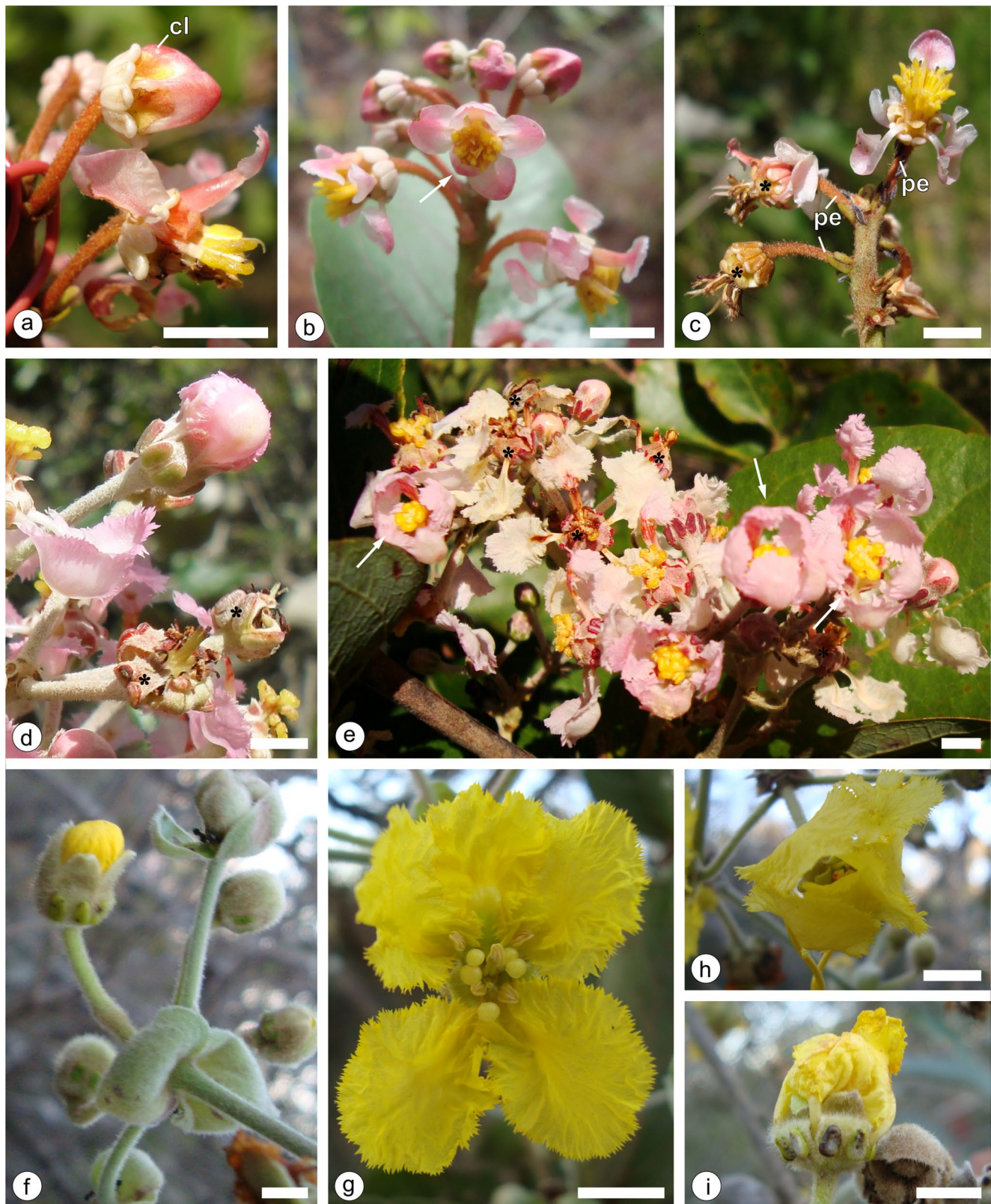


Fig. 2 Floral visitors of *B. coccolobifolia* (a–c), *B. variabilis* (d–h), and *P. reticulata* (i). *Centris analis* (a), Morphospecies 07 (b, photograph by Adachi, S.A), *Paratrigona* sp. (c, i), *Centris* sp. 1 (d), *Epi-charis flava* (e), *Monoeca mourei* (f), *Bombus* sp. (g), *Tetrapedia* sp. 1 (h). Oil-collecting bees contacted stamens and stigmas during the

visits (a, b, d, e); presented a large amount of pollen in the ventral region of the body (f); or landed directly on the calyx acting as robber (h). Pollen-collecting bees (c, i, g), eventually encountering stamens and stigmas. Scale bars :5 mm (a–e, g, h); 2 mm (f)

Table 2 Comparative morphology of the corolla of the three Malpighiaceae species

		<i>B. coccolobifolia</i>	<i>B. variabilis</i>	<i>P. reticulata</i>
Corolla	Diameter (n=3)	14.7±0.9 mm	27.4±1.9 mm	37.8±5.4 mm
	Color	Whitish pink	Pink (white to dark pink)	Yellow
	Limb morphology	Sagittate or hastate, slightly wavy margin	Orbicular to obovate, fimbriate margin	Orbicular to obovate, fimbriate margin
	Surface morphology	Flat, cells with striated or reticulate ornamentation	Papillate, conical cells	Papillate, globular cells
	Neutral red reaction	Base of the limb, close to the claw	Ends of fimbriae and small areas scattered in the limbs	Ends of fimbriae and small areas scattered in the limbs
Posterior petal	Claw (length X width)	Erect (3×0.8 mm)	Erect (4×1.5 mm)	Erect (4×1 mm)
	Limb (length X width)	Slightly concave or flat (2×4 mm)	Slightly concave or flat (7×6 mm)	Flat (8×9 mm)
	Glandular structures	Absent	Present	Present
	Glands location	-	Ends of fimbriae	Ends of fimbriae
	Glands number	-	55	90
Lateral petals	Glands diameter	-	Basal: 0.09–0.14 mm Lateral and apical: 0.04–0.08 mm	Basal: 0.13–0.18 mm Lateral and apical: 0.06–0.09 mm
	Claw (length X width)	Reflexed (3×0.6 mm)	Slightly reflexed (0.9×0.8 mm)	Erect (1×0.8 mm)
	Limb (length X width)	Concave (3×5 mm)	Concave (8×7 mm)	Slightly concave or flat (12×10 mm)
	Glandular structures	absent	present	present
	Glands location	-	Ends of fimbriae	Ends of fimbriae
	Glands number	-	65	130
	Glands diameter	-	Basal: 0.06–0.11 mm Lateral and apical: 0.03–0.07 mm	Basal: 0.03–0.1 mm Lateral and apical: 0.02–0.06 mm

Morphoanatomical characterization of the corollas and their osmophores

The corollas were zygomorphic and pentamerous and differed in size, color, and petal morphology (Table 2; Figs. 3, 4 and 5): whitish pink in *B. coccolobifolia* (Figs. 2a–c and 3a–b), predominantly pink in *B. variabilis* (Figs. 2d–e and 3c–d), and yellow in *P. reticulata* (Figs. 2f–i and 3e–f). Posterior petals were smaller than lateral ones, with flat to slightly concave limbs and erect claws (Table 2; Figs. 3a–f). Lateral petals had concave limbs and reflexed claws in *B. coccolobifolia* and *B. variabilis*, or erect claws in *P. reticulata*, and were typically thinner and shorter than posterior claws (Table 2; Figs. 3a–f). Margins were slightly wavy in *B. coccolobifolia* or fimbriate across species (Table 2; Figs. 3a–f).

In *B. coccolobifolia*, neutral red stained uniformly at the limb base near the claws (Fig. 3b; Table 2). In *B. variabilis* and *P. reticulata*, staining was positive at fimbrial tips with scattered small spots across all five petals (Fig. 3d, f; Table 2). Additional positive staining occurred in elaiophores and parts of the androecium and gynoecium, consistent with other secretory tissues or minor injuries.

SEM analysis showed that in *B. coccolobifolia*, petal surfaces were flat with a reticulate pattern near the claw (Fig. 4a–b) and striate across the limb (Fig. 4c); regions positive to neutral red were distinguished by lobed margins (2–6 lobes) and finer, multidirectional striations without cuticular breaks. In the other two species, petals were papillose, with conical cells in *B. variabilis* (Fig. 4h) and globose cells in *P. reticulata* (Fig. 4m). These exhibited bubble-like regions with intact or ruptured cuticles, or “withered” papillae—features likely corresponding to the small areas that reacted positively to neutral red.

No petal glands were found in *B. coccolobifolia*, whereas *B. variabilis* and *P. reticulata* possessed dilated fimbrial terminations forming tiny glands (Fig. 4d–g, i–l), most numerous and larger at the limb base, especially on the posterior petal (Table 2). In *B. variabilis*, glands were spatulate (posterior petal base, Fig. 4d–f) or finger-shaped (apex of the posterior and lateral petals, Fig. 4g), with mostly intact cuticle; fresh flowers (Fig. 4d–f) showed secretion-like microdroplets (Fig. 4f–g), and small cuticular pores (Fig. 4g). In *P. reticulata*, glands were globose; cuticles were intact in pre-anthesis buds (Fig. 4j) but exhibited apical splits (Fig. 4k) or pores (Fig. 4l) at the posterior petal base, exposing anticlinal walls where secretion likely accumulated prior to release.



Fig. 3 Corolla morphology and neutral red staining in *B. coccolobifolia* (a–b), *B. variabilis* (c–d), and *P. reticulata* (e–f). Newly opened flowers (a, c, e). Note the arrangement of posterior petals (pp) and lateral petals (lp). Neutral red reaction suggesting the odor-producing

areas (b, d, f). Note positive reaction on the claw (b), near the basal region of the limb, more conspicuous on posterior petal (b, arrows); or on the ends of fimbriae and on scattered regions of the limbs (d, f). Scale bars: 1 mm (a, b); 2 mm (c–f)

LM cross-sections revealed a uniseriate epidermis in all three species, sometimes underlain by collenchyma, frequent druse-containing idioblasts, and numerous vascular bundles (Fig. 5a–m). Vascular bundles from the claws of posterior and lateral petals were branched into all species. In *B. coccolobifolia*, bundle terminations did not reach petal margins or the putative odor-producing regions (Fig. 5a–c). In *B. variabilis* and *P. reticulata*, bundles extended to the margins, supplying each fimbria—and in some cases their associated glands (Fig. 5d–h, i–m).

In *B. coccolobifolia*, the claw and basal midrib region of the limb displayed cuboidal to elongated epidermal cells, larger abaxially, with very thick, sinuous outer periclinal walls under a thin cuticle; cytoplasm was peripheral and large vacuoles contained blue-green (toluidine blue) phenolic material (Fig. 5a–c). Subepidermally, both surfaces bore 1–4 layers of angular–lamellar collenchyma, plus parenchyma and phenolic idioblasts (Fig. 5a). The putative odor-producing area at the limb base comprised a slightly undulate uniseriate epidermis resembling the claw, with

some apparently depleted cells, over a non-vascularized parenchyma of 1–2 cell layers with large intercellular spaces (Fig. 5b); some samples showed lipophilic secretion (greenish with toluidine blue) released by these epidermal cells (Fig. 5b). The remaining limb exhibited cuboidal epidermal cells with thick periclinal walls and thin cuticle; the mesophyll had 1–4 layers of variably sized parenchyma with small intercellular spaces, plus vascular bundles (Fig. 5c).

In *B. variabilis* (Fig. 5d–h) and *P. reticulata* (Fig. 5i–m), the claw and basal midrib anatomy resembled *B. coccolobifolia*: an epidermis of elongated cells—sometimes papillary and smaller adaxially—with thick periclinal walls (especially abaxially) under a thin cuticle, and a subepidermal layer of angular collenchyma with milder thickenings plus vascularized parenchyma (Fig. 5d, g). The limb epidermis was papillary with conical cells in *B. variabilis* (Fig. 5e) and rounded cells in *P. reticulata* (Fig. 5j), both thin-walled with nuclei, reduced cytoplasm, and large vacuoles (particularly in *B. variabilis*). The mesophyll showed variable parenchyma cells with large intercellular spaces (aerenchyma

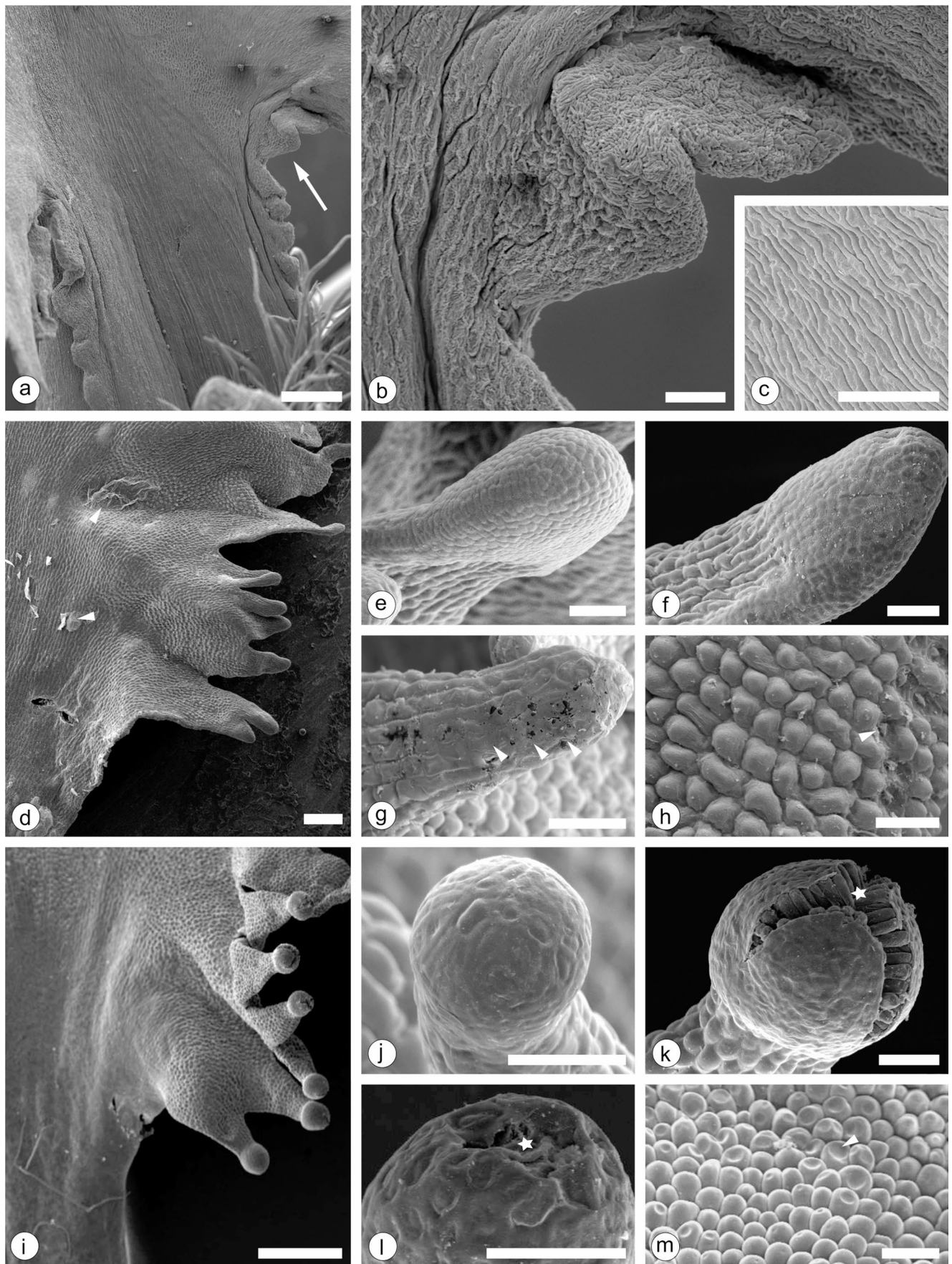


Fig. 4 Scanning electron micrographs of petals in *B. coccolobifolia* (a–c), *B. variabilis* (d–h), and *P. reticulata* (i–m). Posterior petals (a, d, i) show the claw (cl) and the basal part of the limb (li). Note numerous lobes (a, arrows), a petal surface with “bubbles” (d, arrowheads), and dilated terminations of each fimbriae (d, i). The surface of the basal part of the limb (b) shows reticulated ornamentation and thinner striations and intact cuticle on the osmophore. The striated surface of the posterior petal (c). Posterior (e) and lateral (j) petal glands are taken from buds. Note the slightly undulated surface with totally intact cuticle (e, j). Posterior petal glands taken from flowers (f, k). Note the spatulate shape and intact cuticle with microdroplets (f); the globose shape with an apical cuticle rupture (k, star). Lateral petal glands taken from flowers (g, l). Note the digitiform shape, almost intact cuticle with microdroplets and small pores (g, arrowheads); the apical cuticle rupture (l, star). Details of the papillated surfaces of the posterior petals (h, m). Note conical (h) or globose cells (m) and some “whitered” papillae (arrowheads). Scale bars: 250 μm (a, d, i); 50 μm (b–c, e–h, j–m)

tendency) and numerous vascular bundles (Fig. 5e, j); in *P. reticulata*, aerenchyma cells were smaller and girded by wider intercellular spaces.

In *B. variabilis* (Fig. 5g–h) and *P. reticulata* (Fig. 5k–m), petal glands comprised a uniseriate epidermis with a relatively thick cuticle over a central, xylem–phloem–vascularized parenchyma of elongated cells, sometimes with wide intercellular spaces (notably in *P. reticulata*), plus druse-containing idioblasts. At the base of posterior petals in *B. variabilis* (Fig. 5g–h) and across posterior petals in *P. reticulata* (Fig. 5k–l), glands exhibited columnar epidermal cells with dense cytoplasm and large nuclei. In lateral petals or at posterior apices (*B. variabilis*), glands were smaller, with cuboidal (sometimes more elongated in *P. reticulata*) epidermal cells, reduced cytoplasmic density, fewer parenchyma cells, and lacked vascularization (Fig. 5i, m). Post-anthesis in *P. reticulata*, some apical secretory cells showed senescence—sinuous walls and intensely stained cytoplasm—suggesting cessation of secretion.

Histochemistry revealed lipids in limb epidermal cells across all species, including the putative odor-producing regions of *B. coccolobifolia*. In *B. variabilis* and *P. reticulata*, gland epidermal cells contained terpenes and lipids; in *P. reticulata*, these also accumulated between anticlinal walls. Starch grains occurred in limb parenchyma—near the odor area in *B. coccolobifolia* and adjacent to vascular bundles in *B. variabilis* and *P. reticulata*—and were more abundant in pre-anthesis buds than in fresh flowers. No starch was detected within the petal glands of *B. variabilis* or *P. reticulata*.

Ultrastructural characterization of the petal glands

By TEM, petal glands of *B. variabilis* (Fig. 6a–m) and *P. reticulata* (Fig. 7a–j) were broadly similar, without notable differences between posterior and lateral petals. Secretory epidermal cells bore a thick cuticle (Figs. 6b–c and

7a–c); inner anticlinal and periclinal walls contained plasmodesmata linking epidermal cells and subtending cells (Figs. 6a–b and e and 7j). The cytoplasm was dense with a well-developed endomembrane system (Figs. 6a–c, e–f and h–i and 7a–b and e); nuclei were conspicuous with distinct nucleoli and euchromatin (Figs. 6b and 7a–b and i). The vacuole comprised either a single large vacuole or multiple small vacuoles with varied contents (Figs. 6a–b, j and l and 7f–g).

In both species, the cuticle presented two distinct regions: the proper cuticle and the cuticular layer (Figs. 6c and 7a and c). The cuticle proper was thin and uniformly electron-opaque, whereas the cuticular layer was electron-dense, had a reticulated appearance, and extended to the median lamella between the anticlinal walls of two adjacent cells, forming the cuticular flanges (Figs. 6b, d and g and 7a and c).

In *B. variabilis*, the cuticle was continuous with the outer periclinal walls along the full gland length in both pre-anthesis buds and fresh flowers (Fig. 6a–d). Fresh-flower samples often showed a swollen cuticular layer, likely from component loosening (Fig. 6d). Electron-dense material—presumed secretion residues—intermixed with cellulose microfibrils occurred in the flange region (Fig. 6d, g–h). The middle lamella between anticlinal walls was also loosened and contained electron-dense material (Fig. 6f–h).

In *P. reticulata*, the cuticle was only partially continuous with outer periclinal walls, chiefly in basal secretory cells (Fig. 7a–c). Apical cells showed pre-anthesis degradation of the cuticular layer, resulting in gaps with secretion in the flange region (Fig. 7b–d) or in a subcuticular space formed by cuticle detachment (Fig. 7a–b). The middle lamella dissolved, creating gaps between anticlinal walls that accumulated secretion (Fig. 7f). SEM indicated secretion release post-anthesis at the apical gland region via cuticle rupture (Fig. 4k–l). Periplasmic spaces of secretory epidermal cells were often enlarged (notably in *B. variabilis*) and contained membranous elements or electron-dense material (Fig. 6c, e, h).

Secretory epidermal cytoplasm displayed features of high metabolic activity, including abundant free ribosomes/polyribosomes, extensive ER, numerous mitochondria, plastids, lipid droplets, and multivesicular bodies (Figs. 6a–k and 7a–i). In *P. reticulata*, senescent and active cells co-occurred (Fig. 7b). Plastids were numerous, large, polymorphic, and electron-dense; in *B. variabilis* they contained tubules and small lipid droplets (Fig. 6b, h), whereas in *P. reticulata* they held dark dense particles (Fig. 7a, d–e). Mitochondria were elongated with dense matrix and well-developed cristae, often grouped near the plasma membrane, plastids, or lipid droplets (Figs. 6a–i and 7e–g). ER (SER short cisternae; RER long narrow tubules) was pervasive and frequently

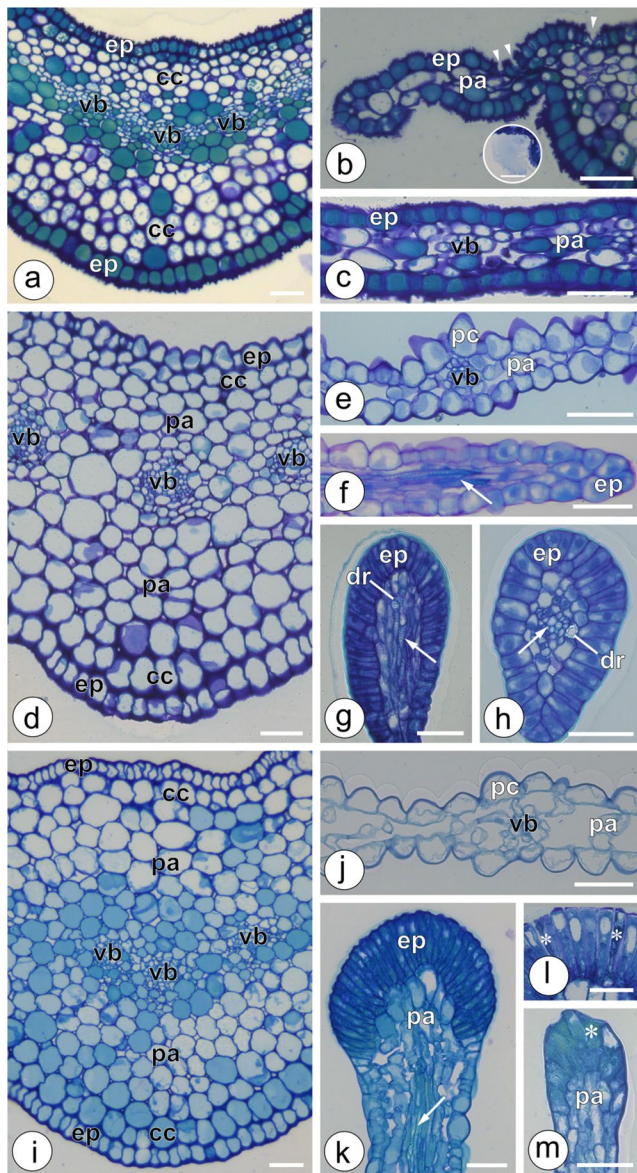


Fig. 5 Light micrographs of petal sections stained with toluidine blue in *B. coccolobifolia* (a–c), *B. variabilis* (d–h) and *P. reticulata* (i–m). Cross sections of posterior petals showing the structure of the claws (a, d, i), odor-producing region (b), limbs (c, e, j), or odor-producing glands (h). Longitudinal sections of posterior (g, k, l) or lateral (f, m) petals showing the structure of the odor-producing glands. Note the epidermis (ep), collenchymatous cells (cc), parenchyma (p.a.), vascular bundles (vb), and drusen (dr); striated cell wall and fine cuticle of the epidermal cells (a–c); depleted epidermal cells (arrowheads) and detail of the lipophilic secretion in the white circle (b); papiliform epidermal cells (pc), and parenchyma cells with large intercellular spaces (e, j); differences between the secretory epidermis and parenchyma of the posterior petal glands (g–h, k, l) and lateral petal glands (f, m); cells with senescence signs (l, m - asterisks). Scale bars: 50 μm

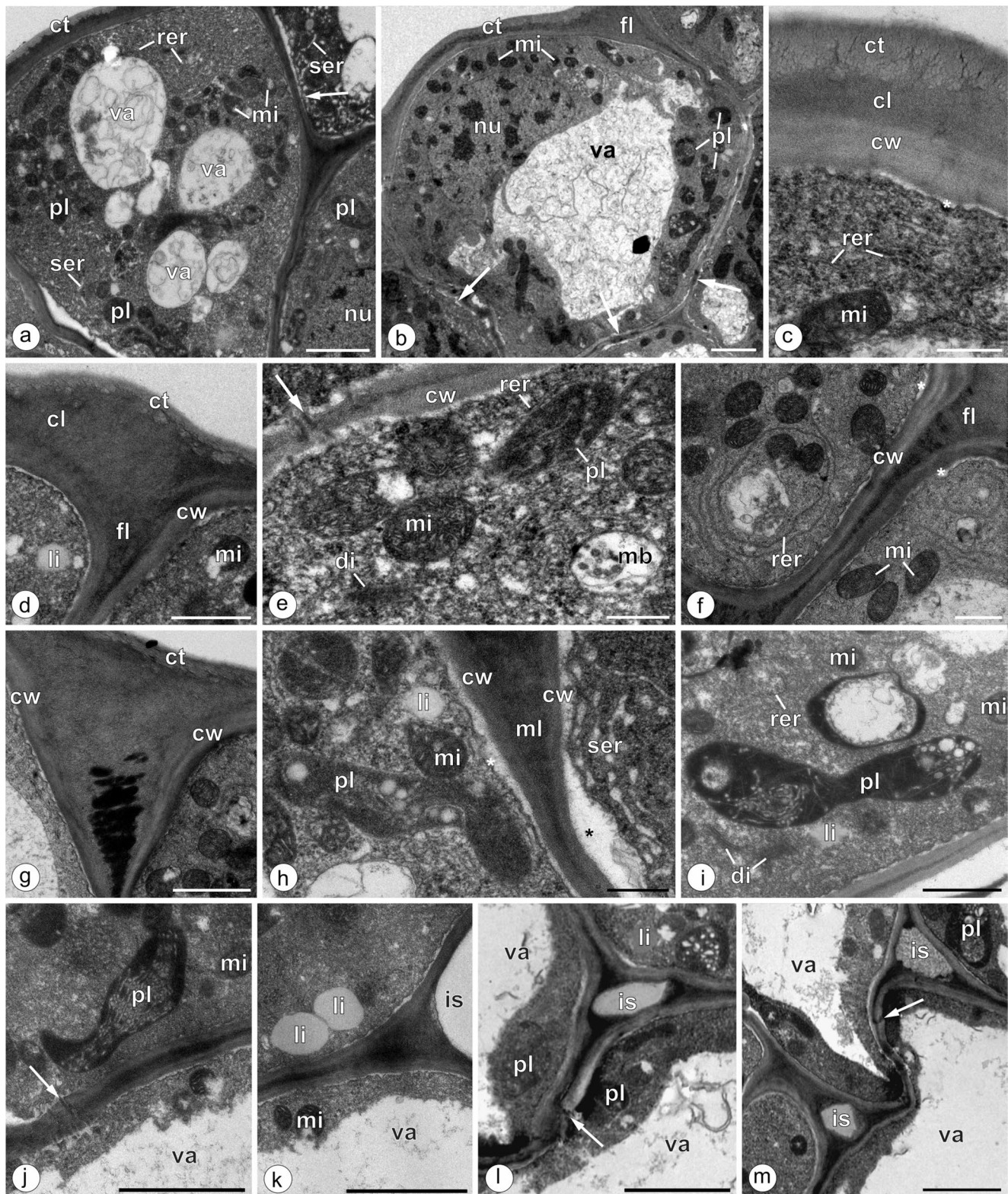
Fig. 6 Transmission electron micrographs of the posterior (b, c, f) and lateral (a, d, e, i) petal glands taken from buds (a, c, d, e) or flowers (b, f, i) of *B. variabilis*. General views of the secretory epidermal cells (a–b). Details of the secretory cell cuticle (c), cell walls (c–h), and cytoplasm (c–i). Note the thick cuticle continuous with the outer periclinal cell walls (cw) and formed by the proper cuticle (ct) and the cuticular layer (cl); the swelling of the cuticular layer and of the middle lamella, where electron-dense material was observed, especially in the cuticular flanges (fl.) region; enlarged periplasmic spaces (*) containing membranous structures; the abundant and organelle-rich cytoplasm with free ribosomes, polyribosomes, numerous mitochondria (mi), numerous plastids (pl), and extensive endoplasmic reticulum (er) represented by short cisternae (ser) or narrow tubular elements (rer), in addition to dictyosomes (di), multivesicular bodies (mb), and lipid droplets (li); the voluminous nuclei (nu), vacuoles (va) with membranous content, and plasmodesmata connecting adjacent cells (arrows). Parenchyma cells and the basal part of secretory epidermal cells (j–m). Note the thin walls, large intercellular spaces (is) containing lipid inclusions (li), plasmodesmata (arrows), reduced and organelle-rich cytoplasm, vacuoles (va) containing membranous structures, and nuclei (nu); numerous mitochondria (mi), and plastids (pl). Scale bars: 2 μm (a, b); 500 nm (c–e, g–m); 1 μm (f)

adjacent to the plasma membrane and around organelles (Figs. 6a–i and 7e). Dictyosomes were scarce (Figs. 6i and 7e–f). Lipid droplets were abundant—especially in fresh flowers—near the plasma membrane, dispersed in the cytoplasm, or inside vacuoles (Figs. 6h and 7b, d–f and f). Vacuoles were numerous/small in pre-anthesis, filled with granular and membranous material, and in *P. reticulata* also contained lipid and phenolic substances (Figs. 6a–b and i and 7b and e–g). Multivesicular bodies with dense vesicles occurred in secretory cells (Fig. 6e).

Subepidermal parenchyma cells (Figs. 6j–m and 7h–j) had thin, plasmodesmata-linked walls; conspicuous nuclei; peripheral, organelle-rich cytoplasm; and large central vacuoles with fibrillar material (Fig. 6j–m), additionally containing phenolics in *P. reticulata* (Figs. 7h–j). Their cytoplasm featured SER, RER, mitochondria, and plastids like epidermal ones, with electron-opaque lipid inclusions. In *B. variabilis*, intercellular spaces were filled with electron-opaque, lipidic material (Fig. 6l–m).

Discussion

This study provides a functional comparison of petal microstructure and osmophores in three Malpighiaceae species and shows species-specific secretory architectures that likely modulate scent storage and release. Although all three share diurnal anthesis, sepals with oil-producing elaiophores, and a symmetrical, pentamerous corolla with distinct posterior



petals, each species exhibits unique petal microstructure and secretory organization. Ultrastructural and histochemical evidence supports predominantly lipophilic secretions and a combination of symplastic and apoplastic transport.

Osmophores recur basally near the petal claw, likely serving as odor guides that align bee handling with sepal oil glands. Together, these differences in petal microstructure, scent-producing tissues, and aspects of floral biology and

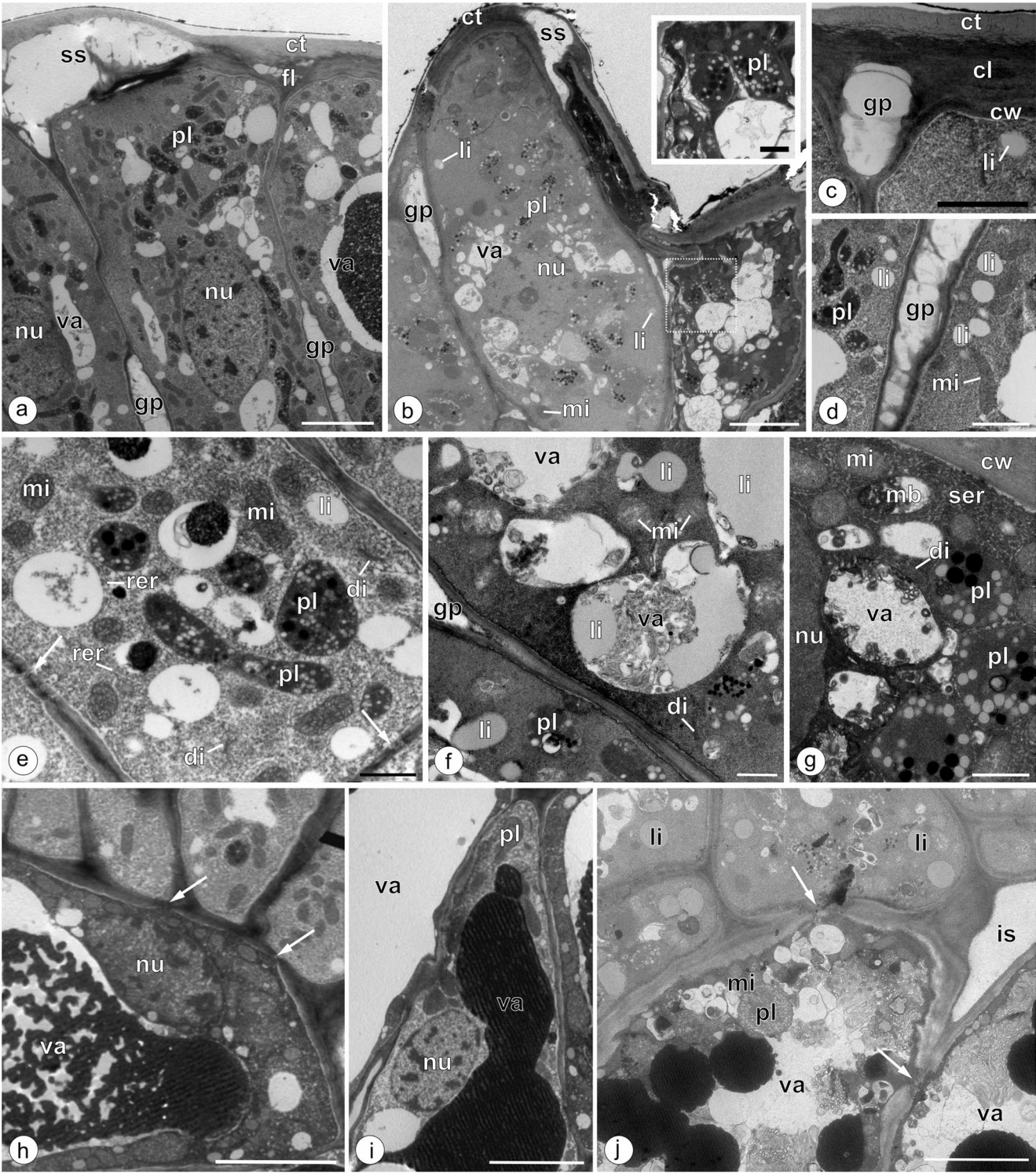


Fig. 7 Transmission electron micrographs of the posterior (a, c–f, h–i) and lateral (b, g) petal glands taken from buds (a, c–e, f, h) or flowers (b, f–g, i) of the *P. reticulata*. General views of the secretory epidermal cells (a, b). Note subcuticular space (ss), sites of secretion accumulation in the region of the flanges (fl.) and gaps (gp) between the anticlinal walls; senescent cells with electron-dense cytoplasm (detail). Details of the secretory cells cuticle (ct), cell walls (c–g), and cytoplasm (c–g). Note the thick cuticle (ct), partially continuous with the outer periclinal cell walls (cw) and formed by the *sensu stricto* cuticle (cu) and the cuticular layer (cl); the abundant and organelle-rich cytoplasm with free ribosomes, polyribosomes, numerous mitochondria (mi), plastids (pl) with lipid globules, extensive endoplasmic reticulum (er) represented by short cisternae (ser) or narrow tubular elements (rer), in addition to dictyosomes (di) and multivesicular bodies (mb); numerous lipid droplets (li) dispersed throughout the cytoplasm, near the plasma membrane (li*), or being released by vesicles (li*); the voluminous nuclei (nu), vacuoles (va) with membranous, phenolic or lipidic content and plasmodesmata connecting adjacent cells (arrows). Parenchyma cells and basal part of secretory epidermal cells (h–j). Note thin walls, plasmodesmata (arrows), reduced and organelle-rich cytoplasm, vacuoles (va) containing membranous structures or phenolic substances, and nuclei (nu); numerous mitochondria (mi), and plastids (pl). Scale bars: 5 μ m (a–b, h–j); 2 μ m (c–d); 1 μ m (b–detail, e–g)

visitor interactions underscore functional divergence among species.

Linking floral visitors, phenological dynamics, floral scents, and petal morphology

P. reticulata flowers sparsely with few simultaneous blooms, unlike the synchronized, floriferous *B. variabilis* and *B. coccolobifolia* (Possobom and Machado 2018). Lower visitor diversity in *P. reticulata* likely reflects shorter flowering period, smaller floral display, and brief flower longevity (closing by day two), all of which reduce detectability and reward predictability. Visual signals may also vary across floral phases; newly opened flowers appear more conspicuous due to petal iridescence, vivid colors, and the posture of posterior and lateral petals. Larger, synchronous displays generally increase attractiveness and visitation probability (Ohashi and Yahara 2004; Bauer et al. 2017). Consistently, greater flower abundance and longer blooming correlate with higher bee diversity, as shown for *Byrsonima umbellata* versus *B. rotunda* (Mendes et al. 2011).

Each species shows distinct senescence signals that culminate in corolla desiccation and abscission (Gegear and Laverty 2004): pedicel curvature in *B. coccolobifolia*, petal dulling/fading in *B. variabilis*, and corolla closure in *P. reticulata*. Such signals likely guide pollinators toward rewarding flowers and away from depleted ones, reinforcing signal-based foraging hypotheses (Melo et al. 2018). In *B. variabilis*, posterior petal color shifts from yellow to orange/red with age; bees collecting pollen prefer yellow-backed flowers, which signal higher pollen availability, whereas oil-foraging visits occur indiscriminately (Melo et

al. 2018). This pattern suggests that bees use different cues for different rewards at the same flowers.

Petal surface microstructure closely relates to pollinator type and shows evolutionary lability (Alcorn et al. 2012; Bäuer et al. 2017; Kraaij and van der Kooi 2020; Ojeda et al. 2016; Macedo et al. 2023). Mesophyll architecture modulates absorption, reflection, and refraction, enhancing saturation and brightness and thus attractiveness (Kay et al. 1981; van der Kooi et al. 2016; Kooi et al. 2019). In these species, tactile cues from epidermal textures likely play a minor role because bees recognize flowers before landing and perform stereotyped oil collection. Papilliform epidermal cells on petal limbs, as in *B. variabilis* and *P. reticulata*, associate with darker, velvety colors, whereas flatter cells in *B. coccolobifolia* relate to higher gloss. Mesophyll with abundant intercellular spaces forms aerenchyma that reflects UV and visible light, potentially amplifying signals (Kevan and Lane 1985). Color changes during floral aging in Malpighiaceae further guide visitors by indicating floral phase or resource status (Anderson 1979; Costa et al. 2006; Bezerra et al. 2009; Mendes et al. 2011; Melo et al. 2018).

Beyond visual guidance, Malpighiaceae petals provide mechanical support: many oil-collecting bees grasp the posterior petal claw with their mandibles (Possobom and Machado 2017). The claw's anatomy supports this behavior; outer cells with thickened, collenchyma-like walls provide firmness while permitting flexible petal movement, facilitating stable handling during oil collection.

Except for *B. variabilis*, which emits a strong scent, *B. coccolobifolia* and *P. reticulata* show mild scents, more noticeable after headspace concentration. Human perception does not capture all volatiles and differs from insect perception, so organoleptic assessment is subjective, especially for “no scent” claims (Faegri and van der Pijl 1979; Endress 1994). Floral scent composition varies among species and can also converge across unrelated taxa (Dobson 2006; Knudsen et al. 2006). Therefore, Malpighiaceae flowers likely differ in volatile concentration and/or composition regardless of phylogeny. Targeted chemical and behavioral assays can clarify how scent profiles integrate with this specialized pollination system.

In *B. coccolobifolia*, scent localizes in a limb region with distinct micromorphology yet composed of epidermal cells like the rest of the limb. In *Galphimia australis* (galphimiods; Davis and Anderson 2010), petals lack complex secretory structures, and a single epidermal layer acts as a secretory surface over the whole petal, with connective tissues also contributing (Gotelli et al. 2023). This pattern supports Vogel's (1990) view that a single-layer epidermal osmophore can represent a transition toward diffuse emission across the entire petal. By contrast, the other two species show more complex petal glands on the fimbriate

margins, interpreted as typical osmophores, together with epidermal cell groups indicative of additional diffuse odor emission.

Petal glands with similar anatomy occur in several Malpighiaceae as *Heteropterys chrysophylla* Kunth (Vogel 1974), *Dinemandra ericoides* (A) Juss. (Cocucci et al. 1996), *Diplopterys pubipetala* (Possobom et al. 2015), and *Lophopterys floribunda* (Sanches et al. 2023). Vogel (1974) and Lobreau-Callen (1989) link such glands to lipid production, and Possobom et al. (2015) first associate them with volatile emission. Sanches et al. (2023) hypothesize a colleter function in *L. floribunda* for protection against desiccation. In contrast, our results for *(B) variabilis* and *P. reticulata* indicate lipophilic secretions released only after anthesis, which argues against a colleter role and supports an osmophore-related function.

Flowers emit temporally and spatially patterned scents, and bees can discriminate hundreds of floral odors that guide efficient foraging (Schoonhoven et al. 2005). In all three species, osmophores occur at or concentrate toward the base of the limb—particularly on posterior petals—being most localized in *B. coccolobifolia* and more concentrated basally in *B. variabilis* and *P. reticulata*. This narrowed basal region functionally positions visitors to access sepal oil glands in Neotropical Malpighiaceae (Anderson 1979; Zhang et al. 2010). Enhanced scent concentration at the limb base likely acts as odor guides sensu Vogel (1990), analogous to nectar guides, directing bees to the oil rewards.

The role of scent is consistent with the need for legitimate visits: oil-collecting bees access the reward only when they place the two anterior leg pairs within the spaces near the petal claws. Our observations confirm the labelled handling of oil bees. Across Malpighiaceae with petal glands, these glands concentrate near the claw at the petal base (Vogel 1974; Cocucci et al. 1996; Possobom et al. 2015; Guesdon et al. 2018, 2019; Sanches et al. 2023). This recurrent placement likely guides bees to the correct position, acting as odor guides as suggested by Possobom et al. (2015).

Despite having active osmophores and elaiophores, *P. reticulata* receives no legitimate visits in our observations. Pollinators can learn odor–reward associations (Dafni 1992), but they may also associate certain scents with low or absent rewards, reducing visitation. *Peixotoa reticulata* does not offer pollen—critical for oil-collecting bees—and its floral oils likely deplete rapidly due to spontaneous release (Possobom and Machado 2018). These factors together likely undermine reward predictability and discourage consistent visitation.

Neutral red patterns align with localized active zones on the corolla and support spatial specialization of odor release, a common strategy in pollinator attraction (Galetto

et al. 1997; Pansarin et al. 2009). Together, the corolla's morphofunctional diversity acts as a fine-tuning mechanism that shapes visitor behavior and specificity within Malpighiaceae.

Cytological evidence confirms osmophore function

Our ultrastructural data consistently indicate that the petal region of *B. coccolobifolia*, which exhibits micromorphology distinct from the rest of the limb, functions as an osmophore. Volatile emission entails high energetic costs, typically with starch present before scent release and depleted afterward (Vogel 1990; Silva 1990/1992; Effmert et al. 2006). In *B. coccolobifolia*, tissues adjacent to the non-vascularized osmophore are starch-rich before anthesis and starch-depleted after flowering, conforming to this osmophore pattern. In contrast, petal glands on the fimbriate margins in *B. variabilis* and *P. reticulata* lack starch, as also reported for *Cyphomandra/Cyphomandropsis* spp., *Cyclopogon elatus*, and *Grobya amherstiae* (Cocucci 1996; Wiemer et al. 2009; Pansarin et al. 2009). Combined with petal gland vascularization, these observations suggest that *B. variabilis* and *P. reticulata* osmophores mobilize energetic substrates directly from the phloem rather than local starch reserves.

Emission routes further corroborate osmophore activity. Osmophores commonly release scent as it is produced (Vogel 1990; Silva 1990/1992), with volatiles diffusing through cuticular microchannels or pores, accumulating subcuticularly and being discharged by cuticle rupture, or occasionally exiting via stomata (Pridgeon and Stern 1983; Stern et al. 1987; Sazima et al. 1993; Cocucci 1996; Plachno et al. 2010; Kowalkowska et al. 2015; Macedo et al. 2023). SEM indicates species-specific pathways: in *B. coccolobifolia*, secretion crosses the cuticle; in *B. variabilis*, it traverses the cuticle or exits through minute pores; and in *P. reticulata*, a predetermined cuticular rupture after anthesis releases the secretion.

Subcellular evidence reinforces the osmophore interpretation, particularly in the petal-gland species *B. variabilis* and *P. reticulata*. Ultrastructural traits—dense cytoplasm; plastids with starch grains and/or lipid globules; numerous mitochondria; abundant SER; scattered lipid droplets; and relatively few dictyosomes—indicate a predominantly lipophilic metabolic route for volatile biosynthesis, consistent with scent glands in other taxa (Pridgeon and Stern 1983; Curry 1987; Stern et al. 1987; Silva 1990/1992; Curry et al. 1991; Effmert et al. 2006; Melo et al. 2010; Plachno et al. 2010; Adachi and Machado 2020). Plastids containing lipid globules and dark inclusions (likely phenolics) are common in osmophores of other species that also exhibit terpenes

in their fragrance composition (Kowalkowska et al. 2012; Plachno et al. 2016). In our material, terpenes and lipids were histochemically detected in the marginal petal glands.

Differences in cuticle continuity and rupture patterns align with distinct emission strategies reported for osmophores (Vogel 1990; Davies et al. 2003). In *B. variabilis*, secretion crosses the plasmalemma, accumulates between cell-wall microfibrils and the cuticular stratum, and exits via fine cuticular canals; pore formation in older glands likely reflects repeated, pressure-driven discharges (Pridgeon and Stern 1983; Plachno et al. 2010; Kowalkowska et al. 2015, 2018; Paiva 2016). In *P. reticulata*, secretion accumulates intercellularly—especially beneath the outer epidermis and in central epidermal cells—and is released upon rupture of the outer cuticle. Consequently, *B. variabilis* likely emits scents continuously, whereas *P. reticulata* releases them in pulses or a single burst, consistent with their floral longevities (≈ 3 days vs. 1 day). Intercellular accumulation in *B. variabilis* indicates apoplastic transport, and abundant plasmodesmata in both species supports symplastic transport supplying precursors and energy to secretory cells (Fahn 1979).

Conclusion

This study reveals species-specific architectures of petal microstructure and osmophores across three Malpighiaceae, indicating distinct modes of scent storage and release.

Together, the energetic context, emission architecture, and ultrastructural signatures provide convergent evidence that these petal glands are osmophores, with species-specific structural configurations underpinning distinct volatile-release dynamics.

These morpho-functional differences likely modulate detectability, reward predictability, and foraging behavior and—together with shared traits in *B. variabilis* and *P. reticulata*—point to an evolutionary trajectory toward greater specialization in corolla design and scent emission. Targeted chemical profiling and behavioral assays are now needed to link scent composition to visitor specificity and to inform conservation strategies for this keystone tropical lineage.

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Author contributions Silvia Rodrigues Machado and Clivia Carolina Fiorilo Possobom conceived and designed the research. Clivia Carolina Fiorilo Possobom performed field observations, sampling, and laboratory work. Clivia Carolina Fiorilo Possobom and Silvia Rodrigues Machado conducted data analyses, wrote the original draft, and reviewed and edited the manuscript.

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Data availability All data generated or analyzed in this study are included in this published article.

Declarations

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