



Comparative analysis of the processes involved in the loss of elaiophores in two species of the christianelloid clade (Malpighiaceae)

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Abstract

Alicia anisopetala and *Callaeum psilophyllum* are two closely related species that belong to the christianelloid clade of the family Malpighiaceae. Both species are pollinated by oil-collecting bees and exhibit variations at specimen and population level in the number of elaiophores per flower. These floral glands that secrete non-volatile oils constitute an ancestral trait for the family. There is evidence that the observed variations in the number of elaiophores can be the result of processes of connation or reduction associated with differences in their vascularization. In order to identify which process occurs in each species, we conducted an anatomical study in natural populations of both species distributed along a wide range of their geographical distributions in Argentina. We collected flowers of different individuals, counted the number of elaiophores per flower, carried out exomorphological observations, and used conventional histological techniques to examine the vascularization of these glands. The floral anatomy of both species does not show any modifications in other whorls related to the fusion or reduction of elaiophores. Our results indicate that the process of loss of elaiophores in *A. anisopetala* is caused by incomplete connation and in *C. psilophyllum* by reduction, suggesting that the processes that lead to the loss of elaiophores in Malpighiaceae are homoplastic and would not reflect phylogenetic signals.

Keywords *Alicia* · *Callaeum* · Calyx · Floral glands · Vascularization

Introduction

Most Malpighiaceae species exhibit a fascinating evolutionary trait characterized by the presence of floral glands called elaiophores, which secrete non-volatile lipid as rewards to pollinators (Vogel 1974; Renner and Schaefer 2010). These glands play a crucial role in pollen transference since these species are associated with a particular syndrome of pollination with oil-collecting bees (Anderson 1979; Vogel 1990;

Renner and Schaefer 2010; Martins and Melo 2016; Torretta et al. 2017; Carneiro and Machado 2023).

This family is distributed in tropical and subtropical areas of the New and Old World, with most genera and species occurring in the Neotropic (Anderson 1990; Vogel 1990; Davis and Anderson 2010). The general morphology of the flowers of Neotropical Malpighiaceae species is conserved, especially in relation to attraction and reward to pollinators (floral conservatism, Anderson 1979). In most species, elaiophores are located in pairs on the abaxial face of the sepals (Anderson 1979; Vogel 1990). Although the ancestral characteristic of the family is the presence of 10 elaiophores in pairs in all five sepals (Souto and Oliveira 2013), the most frequent current condition is the presence of 8 elaiophores: four pairs on anterior-lateral and posterior-lateral sepals, while the anterior sepal usually lacks elaiophores (Aliscioni et al. 2022). However, in some Neotropical species, the number of elaiophores per flower is reduced [e.g., *Lophopterys floribunda* W.R. Anderson and C. Davis has a single gland on lateral sepal; (Sanches et al. 2023)] or is null [e.g., *Diplopterys lutea* (Griseb.) W.R. Anderson and C. Davis;

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(Sigrit and Sazima 2004)] or the glands are very reduced in size and considered as residuals [e.g., *Galphimia australis* Chodat; (Gotelli et al. 2023)]. There are also species (or specimens) with glandular (with elaiophores) and eglandular (without elaiophores) floral morphs [e.g., *Heteropterys intermedia* (A. Juss.) Griseb. (cited as *H. aceroides* Griseb, Sazima and Sazima 1989) and *Pterandra pyroidea* A. Juss. (Cappellari et al. 2011)]. In contrast, most Old World species have flowers with eglandular sepals and are pollinated by pollen or/and nectar-collecting bees (Vogel 1990; Zhang et al. 2016).

The loss of elaiophores is considered a derived condition in Malpighiaceae (Anderson 1990), and appears to have occurred at least 14 times, seven of them in the Neotropical region (Bonifácio et al. 2021). Previous studies suggest that these losses and the resulting variations in the number of elaiophores per flower may result from two processes: fusion or reduction of the glands. In the cases of fusion, connation and/or adnation processes may be involved, depending on the studied species (Souto and Oliveira 2013; Bonifácio et al. 2021). The process of fusion implies the union between epidermis, parenchyma, and vascular bundles from two organs shaping into a single organ (Puri 1951; Gifford and Foster 1989). If the organs belong to the same floral whorls, as for example sepal-sepal, the process is defined as connation. On the other hand, the process of reduction implies the loss of vascular bundles and organs, without fusion between them, sometimes with the gradual reduction on size of the organ and in other occasions with no gradualness (Puri 1951; Gifford and Foster 1989).

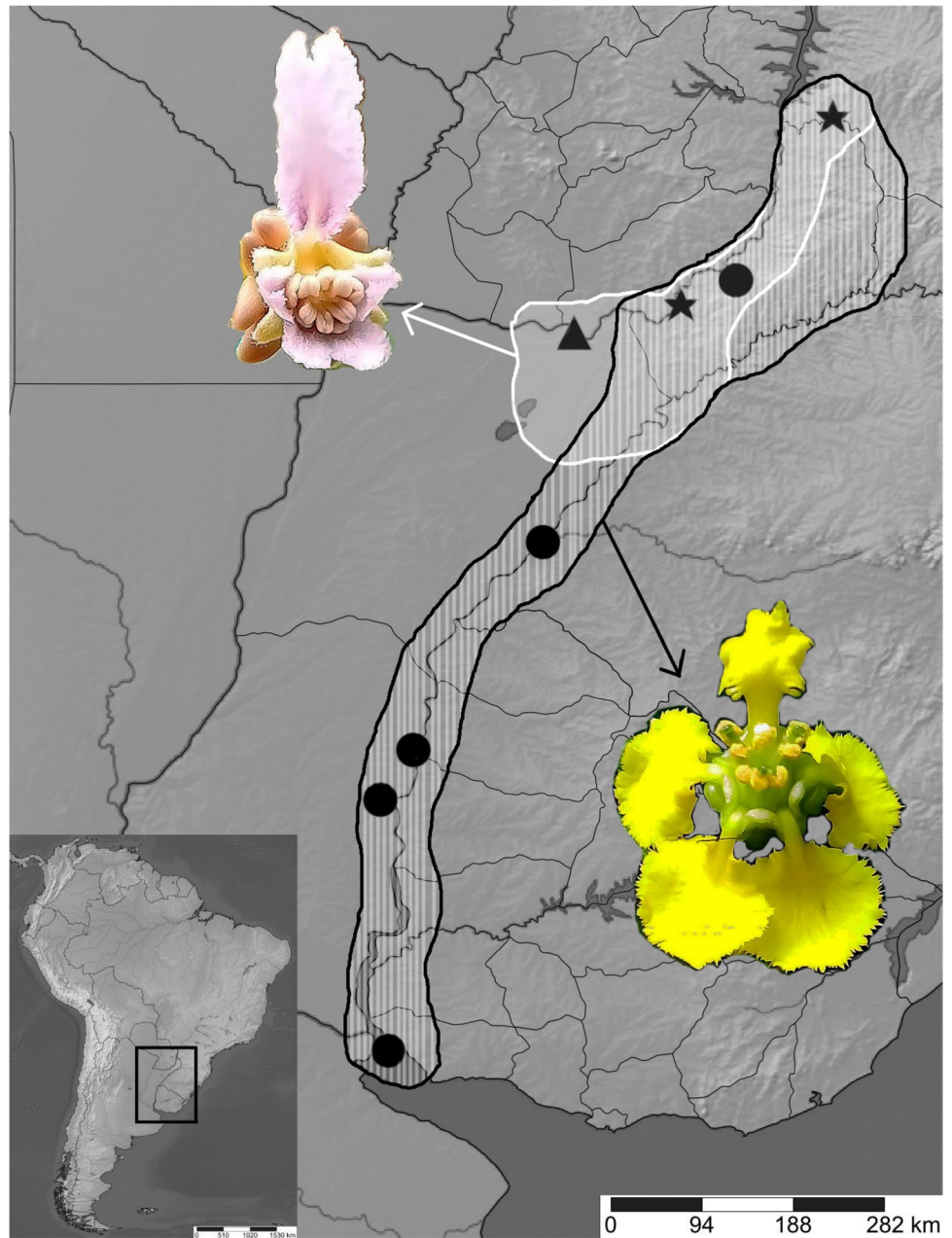
In plants, the vascularization is more conserved than external morphological characteristics and can provide insights into evolutionary steps preceding external changes (Puri 1951; Gifford and Foster 1989; Souto and Oliveira 2013). Comparative analyses on the vascularization of elaiophores were effective in elucidating which of these two processes (fusion or reduction) are related to variation in the number of these glands in flowers of Malpighiaceae species (Souto and Oliveira 2013; Bonifacio et al. 2021).

Therefore, studying the floral vascularization could help to understand the evolutionary changes that lead to the variation in the number of elaiophores in Malpighiaceae species (Souto and Oliveira 2013; Bonifácio et al. 2021). Souto and Oliveira (2013) analyzed the floral vasculature in three Malpighiaceae of species that belong to different phylogenetic clades: *Janusia mediterranea* (Vell.) W.R. Anderson, *Mascagnia cordifolia* (A. Juss.) Griseb., and *Tetrapteryx chamaecerasifolia* A. Juss. These authors observed variations in the process of the loss of elaiophores in the anterior sepal: connation in *M. cordifolia* flowers and reduction in those of *J. mediterranea* and *T. chamaecerasifolia*. On the other hand, based on specimens from herbaria, Bonifácio et al. (2021) studied three species

belonging to the acmantheroid clade which is a basal lineage of the family; this clade comprises three genera with variations in the number of elaiophores. These authors studied one species of each genus: *Acmanthera latifolia* (A. Juss.) Griseb. (three specimens with 10-glandular flowers), *Coleostachys genipifolia* A. Juss. (three specimens with eglandular flowers), and *Pterandra pyroidea* A. Juss. (three specimens with 8-glandular flowers and three specimens with eglandular flowers). Bonifácio et al. (2021) propose two different hypotheses to explain eglandular sepals: reduction in some flowers of *P. pyroidea* and connation between adjacent elaiophores from lateral sepals in *C. genipifolia*. However, for this last species, the successive processes that led to the complete loss of elaiophores are difficult to interpret. Despite many studies focused on the anatomy and/or morphology of elaiophores (Vogel 1974; Mamede 1993; Cocucci et al. 1996; Castro et al. 2001; Possobom 2008; Possobom et al. 2015; Araujo and Meira 2016; Possobom and Machado 2017; Possobom and Machado 2017; Arévalo-Rodrigues et al. 2020; Aliscioni et al. 2022), few have considered their variations at specimen and population level (Sazima and Sazima 1989; Carvalho et al. 2005; Bonifácio et al. 2021; Aliscioni et al. 2022; Gotelli et al. 2023), and only two studies focused on floral vascularization (Souto and Oliveira 2013; Bonifácio et al. 2021).

The Malpighiaceae family represents a highly supported monophyletic group (Davis and Anderson 2010), with well-supported major clades, except the tetrapteroid, stigmaphyllid, and malpighiod clades that still need resolution of the internal relations among genera that compose them (Davis and Anderson 2010). *Alicia* W.R. Anderson and *Callaeum* Small are two sister genera of the christianelloid clade included in the major tetrapteroid clade, according to phylogeny proposed by Davis and Anderson (2010). *Alicia anisopetala* (A. Juss.) W.R. Anderson and *Callaeum psilophyllum* (A. Juss.) D.M. Johnson are the unique two species of this clade present in Argentina. The flowers of these species exhibit marked morphological differences, especially in the corolla size and petal morphologies (Fig. 1): in *A. anisopetala*, flowers are small (1.2–1.5 cm) and the posterior petal is much larger than the four lateral ones (Anderson 2006) and in *C. psilophyllum*, flowers are big (2.5–2.8 cm), and the posterior petal is smaller than the four lateral ones (Johnson 1986). In relation to the number of elaiophores, variations were reported among flowers of the same plant in *C. psilophyllum* (Aliscioni et al. 2022) but there are no reports for *A. anisopetala*. These variations raise questions about the underlying mechanisms that govern the development of elaiophores in these plants. Based on this, the present study proposes a comparative analysis of floral morphology and vascularization of elaiophores in *A. anisopetala* and *C. psilophyllum* in natural populations of Argentina at

Fig. 1 Distribution of the populations of the two species in Argentina, white area for *Alicia anisopetala* and black area for *Callaeum psilophyllum* (two arrows to a photograph of each correspond flower). The squares represent the places where *A. anisopetala* was found, the circles represent the places where *C. psilophyllum* was found, and the stars where both species coexisted



specimen and population level to explain which processes are responsible for the loss of these oil-rewarding glands.

Materials and methods

Study species and material collection

Alicia anisopetala and *Callaeum psilophyllum* are both woody vines. *Alicia anisopetala* has a wide distribution spanning from Peru, Bolivia, Brazil, Paraguay to Argentina. Its flowers present pink petals, and are grouped in pseudoracemes. On the other hand, *C. psilophyllum* is distributed

across Brazil, Bolivia, Paraguay, Uruguay, and Argentina. Its inflorescences are umbels or short racemes composed of 4–6 flowers with lemon-yellow petals. In Argentina, the geographic distributions of these two species are partially overlapped, *A. anisopetala* is restricted to Misiones province and northeast of Corrientes province, while *C. psilophyllum* is distributed from Misiones, Corrientes, Entre Ríos to the northeast of Buenos Aires provinces (Fig. 1). It was also registered in Jujuy and Salta provinces, in the northwest of Argentina.

Field trips were carried out between 2018 and 2023, during flowering time of *A. anisopetala* and *C. psilophyllum* (from December to March) across the entire geographical

distributions in Argentina (Fig. 1) at the provinces of Buenos Aires, Entre Ríos, Corrientes, and Misiones to locate natural populations and collect flowers of each specimen found. Sampling was carried out during consecutive years, but not all sites were visited on each. Visits to each located population consisted of 1–2 days of data collection.

Flowers in pre-anthesis ($n = 10$ to 50), anthesis ($n = 10$ to 50), and post-anthesis ($n = 10$ to 50) from each specimen were fixed in FAA (formalin-acetic acid-alcohol mixture). In the field, to confirm that the glands on each flower were elaiophores, fresh flowers for some specimen were submerged in an aqueous solution of 0.1% neutral red and Sudan III for 1–8 h to locate metabolically active zones and the presence of lipids, respectively (Zarlavsky 2014).

Exomorphological description and variations at specimen and population level of number of elaiophores per flower

In the laboratory, the fixed material was meticulously examined under a magnifying glass to determine the number of elaiophores per flower. This approach allowed us to assess the elaiophore morphologies and variations in the number of elaiophores per flower in species at population level and to observe if there is a variation at specimen level. To determine the variations in both species, we counted the number of elaiophores from 10 flowers of each revealed specimen. Then, we calculated the percentages of the different elaiophore numbers per flower for each species at specimen and population level.

Elaiophore vascularization

To study the vascularization of the elaiophores, five fixed flowers from each population of each species with variable numbers of elaiophores were dehydrated in an ascending ethanol series, transferred to xylene, and embedded in paraffin (58 °C). Then with a rotating microtome, longitudinal and transverse 7- μ m-thick seriated sections were performed. Finally, histological samples were stained with Safranin-Fast Green and mounted in Canada balsam (Zarlavsky 2014) for observation under a Motic bright-field microscope. Photomicrographs were taken using Motic images plus. Diagrams were made from selected photomicrographs with “PhotoStudio6” software.

Results

We located and evaluated 11 specimens for *Alicia anisopetala* and 25 specimens for *Callaeum psilophyllum*. The studied specimens/populations of *A. anisopetala* were located in Iguazú National Park (Dept. Iguazú), Teyú Cuaré

Provincial Park, and Natural Reserve Osununú (Dept. San Ignacio), province of Misiones and Bahía Carayá (Dept. Ituzaingó), province of Corrientes (Fig. 1). The specimens/populations of *C. psilophyllum* were located in San Ignacio (Dept. San Ignacio), provincial route 223 and Cuña-Pirú river (Dept. Libertador General San Martín) and Iguazú National Park (Dept. Iguazú), province of Misiones, Yapeyú (Dept. San Martín), province of Corrientes, El Palmar National Park (Dept. Colón) and San Carlos Park, Concordia, (Dept. Concordia) province of Entre Ríos, and the multipurpose Martín García Natural Reserve Island (Dept. San Isidro) province of Buenos Aires (Fig. 1, Table 1).

Exomorphological description and variations at specimen and population level of elaiophores

All found specimens of *Alicia anisopetala* and *Callaeum psilophyllum* have flowers with glands that reacted positively with Sudan III solution (for the presence of lipids) and with neutral red (signs of metabolic activity), confirming their classification as elaiophores. No specimen presents eglanular flowers.

In both species, the oil glands are typically arranged in pairs on the abaxial face of the sepals. However, variability in the number of elaiophores was registered. The number of glands per flower in both species displays light differences between species and variation at population level, with different percentages of flowers with variable numbers of elaiophores among populations (Table 1, Figs. 2a–f and 3a–f).

The flowers of *Alicia anisopetala* display eight (81.9%), nine (12.2%), or ten (5.9%) exomorphologically recognized elaiophores (Table 1, Fig. 2a–c). These variations are the result of the degree of (or the lack of) fusion between some pairs of elaiophores. There are flowers that externally seem to present complete fusion of two pairs of elaiophores, in which each pair comprises one elaiophore from the anterior sepal and the other ones from the lateral one (Fig. 3a, resulting in 8 elaiophores flower), or complete fusion of one pair and the partial unification or approximation of other pair of elaiophores (Fig. 3b, resulting in 9 elaiophores flower), and even flowers without fusion of elaiophores (Fig. 3c, 10 elaiophores flowers).

The flowers of *Callaeum psilophyllum* mainly exhibit eight elaiophores (83.3%), but show high variation in the number of oil glands: we observed flowers with six (0.3%), seven (4.7%), nine (3.3%), and ten (8.4%) elaiophores (Fig. 2d–f) (Table 1). However, in El Palmar National Park population, the most common configuration was ten elaiophores per flower (two on each sepal). This means that there are flowers in which both elaiophores on the anterior sepal are absent (Fig. 3d), flowers in which only one elaiophore on the anterior sepal disappears completely (Fig. 3e), flowers

Table 1 Variation in the number of elaiophores expressed in percentage for each population

Species	Province	Population	Geographical coordinates	# of specimens	Percentage of the number of elaiophores per flower for each population				
					6	7	8	9	10
<i>Alicia anisopetala</i>	Corrientes	Ituzaingó, Bahía Carayá	27°31'35.1"S 56°31'46.0"W	3			80.0	16.7	3.3
	Misiones	Iguazú National Park	25°41'14.2"S 54°26'53.4"W	1			80.0	10.0	10.0
		San Ignacio	27°15'41.4"S 55°32'33.1"W	7			85.7	10.0	4.3
TOTAL				11			81.9	12.2	5.9
<i>Callaeum psilophyllum</i>	Buenos Aires	Martín García Natural Reserve Island	34°10'51.5"S 58°15'21.8"W	3		6.7	93.3		
	Entre Ríos	El Palmar National Park	31°52'04.2"S 58°12'36.2"W	10	2.0	3.0	34.0	12.0	49.0
		Concordia, San Carlos Park	31°22'16.1"S 57°59'52.6"W	2		10.0	90.0		
	Corrientes	Yapeyú	29°28'41.4"S 56°49'13.3"W	5			96.0	4.0	
	Misiones	Iguazú National Park	25°41'14.2"S 54°26'53.4"W	1			100.0		
		Route 223 and Cuña-Pirú river	27°02'29.2"S 55°01'54.8"W	1		10.0	90.0		
		San Ignacio	27°16'43.7"S 55°34'43.2"W	3		3.3	80.0	6.7	10.0
TOTAL				25	0.3	4.7	83.3	3.3	8.4

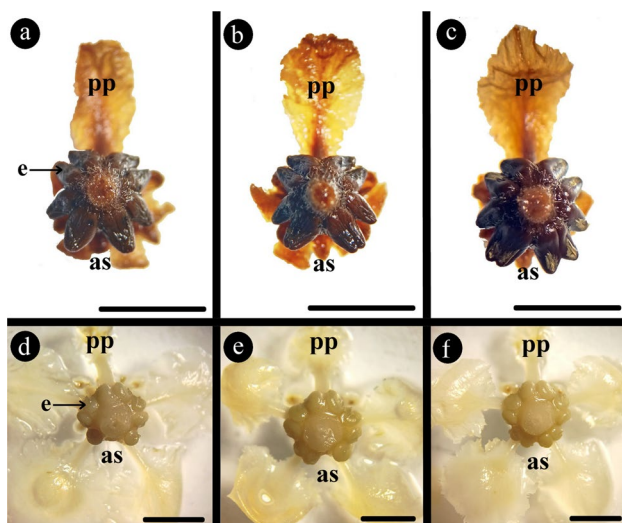


Fig. 2 Elaiophores variation. **a–c** Flowers of *Alicia anisopetala*. **d–f** Flowers of *Callaeum psilophyllum*. **a, d** Flowers with typical number of elaiophores (8 elaiophores). **b, e** Flowers with nine oil glands. **c, f** Flower with the ancestral characteristic of ten elaiophores. **as**, anterior sepal (usually eglandular); **e**, elaiophore; **pp**, posterior petal (flag petal). Scale bars: **a–f** 1 cm

with all elaiophores (Fig. 3f), and a few flowers that also lost one or both elaiophores of the anterior-lateral sepals.

Elaiophore vascularization

Flowers of *Alicia anisopetala* show a central eustele at the base of the receptacle with little secondary growth and the outlines of the calyx glands (Figs. 4a and 5a). A median

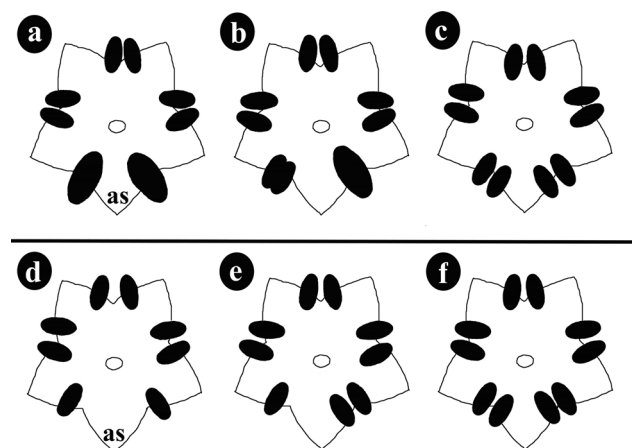


Fig. 3 Diagram of oil calyx glands. **a–c** Variation in the number of elaiophores of *Alicia anisopetala*. **d–f** Variation in the number of elaiophores of *Callaeum psilophyllum*. Calyx with ten (**a, d**), nine (**b, e**), and eight (**c, f**) elaiophores. **as**, anterior sepal

vascular trace and two laterals are emitted from the eustele to each sepal. Then, each lateral one innervates one gland. Five central petal traces are observed but there is no sepal-petal complex. A vascular trace is emitted to each stamen and traces towards the ovary are also found. One central trace and two lateral traces are emitted per carpel. In the apex of the ovary, three bundles are individualized to each carpel (Fig. 4a–c). A genuinely eglandular sepal is not present. However, elaiophores next to the anterior sepal may not be individually distinguished by external morphology. In such cases, the anterior sepal possesses a central vascular bundle that bifurcates into the two adjacent elaiophores

Fig. 4 Vascularization of the flower in *Alicia anisopetala*, longitudinal (a, b) and transvers sections (c). **a** Diagram of a flower showing vascular traces of the different whorls. **b** Picture showing vascular traces from the central vascular bundle towards the different whorls. **c** Vascular traces of the elaiophores (e), sepals (s), petals (p), base of the androecium (a), and gynoecium (g). Grey: central vascular bundle and gynoecium vascular traces. Green: vascular traces of sepals and elaiophores. Purple: vascular bundle of the petals. Yellow: Vascular bundle of stamens. Scale bars: **a** 2.5 cm; **b** 1 cm; **c** 430 μ m

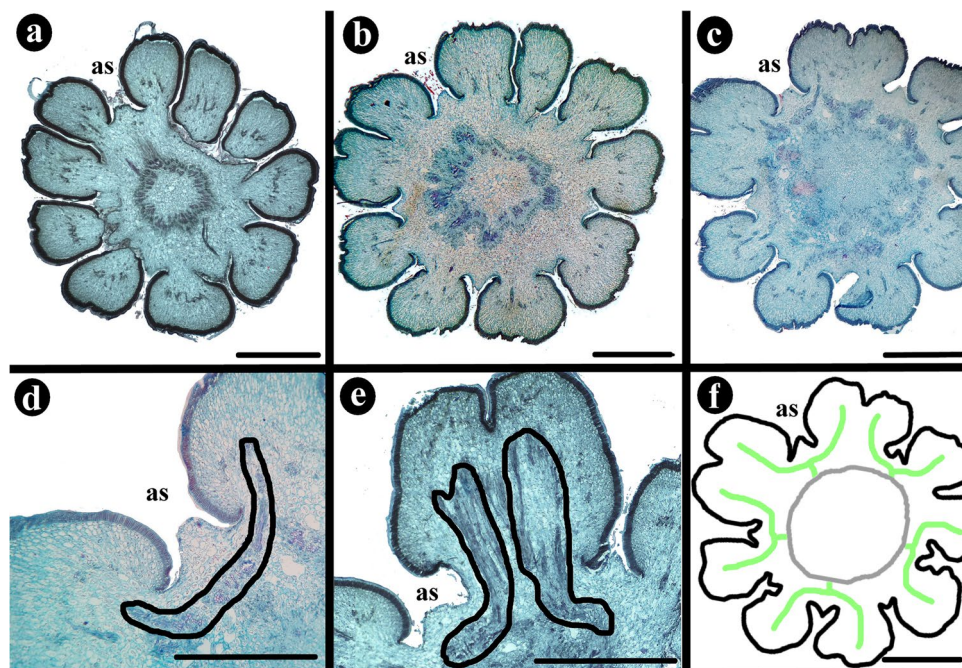
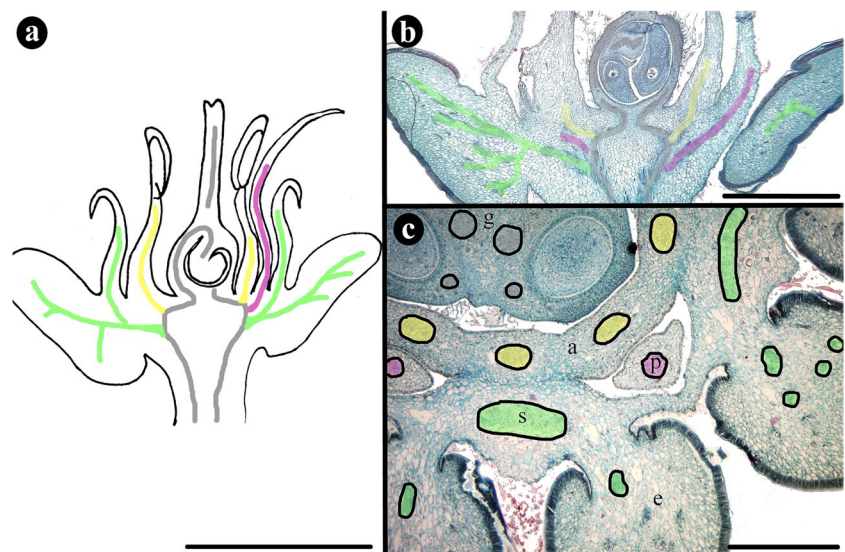


Fig. 5 Vascularization of the elaiophores in *Alicia anisopetala*. Note the position of the anterior sepal (as) (a–e) Cross sections of pictures with bright-field microscope. **a** General aspect of a flower with ten exomorphologically distinct elaiophores. **b** General aspect of a flower with nine exomorphologically distinct elaiophores. **c** General aspect of a flower with eight exomorphologically distinct elaiophores. **d** Detail of the vascularization of elaiophores adjacent to the anterior

sepal in a flower with ten elaiophores. **e** Detail of the vascularization of the elaiophore adjacent to the anterior sepal in a flower with nine exomorphologically distinct glands, a vascular trace can be observed for each elaiophore. **f** Diagram of the vascular traces towards sepals and glands in flowers with eight exomorphologically distinct glands. Scale bars: **a–c**, **f** 900 μ m; **d**, **e** 600 μ m

independently of the degree of fusion (Fig. 5a–f). In flowers that exomorphologically present eight, nine, or 10 elaiophores, 10 glands can always be anatomically distinguished at some proximal point (Fig. 5a–c). This is due to the different grades of fusion between the elaiophores of

the anterior sepal and the adjacent lateral sepal. This means that by slicing the same flower, we can identify the ten elaiophores at the base, while at the top of the calyx, the four elaiophores furthest from the flag petal may present different degree of fusion, forming what seems two large elaiophores.

Vascularization is observed in the anterior sepal independently of the number of elaiophores exomorphologically recognized (Fig. 5d–f); the anterior sepal has a central vascular trace that bifurcates into the glands (Fig. 5d). Therefore, the elaiophore formed by the partial fusion of two adjacent elaiophores is innervated by two central vascular bundles of the anterior sepal and the one next to it (Fig. 5e–f). This does not vary between populations.

Flowers of *Callaeum psilophyllum* show at the base of the receptacle the floral vasculature is organized as a central eustele. The receptacle increases rapidly in diameter and glands become defined with independent vascular traces (Figs. 6a and 7a). A bundle from the central eustele branches towards the sepal and it emits two lateral traces that innervate the glands. Further above, the same bundle from the central eustele branches again to innervate the petal. It continues to branch and for each stamen one vascular bundle is found simultaneously with the individualization of the three intercarpellary complexes, each with one central bundle and two lateral ones (Fig. 6a–g). Flowers with different number of elaiophores present differences on the vascularization (Fig. 7a–i). Flowers with ten elaiophores present the anterior sepal with their central vascular bundle which innervates both glands (Fig. 7a, d, g). Transverse sections of flowers with nine elaiophores show a different pattern. In this case, the anterior sepal presents only one gland (Fig. 7b, e). This

sepal presents a central vascular bundle which only branches towards the persistent gland but not towards the absent one (Fig. 7e, h). Furthermore, flowers with eight elaiophores present the anterior sepal eglandular (Fig. 7c). The vascular bundle supplying the eglandular sepal does not emit traces or leaves rest of them towards the absent glands (Fig. 7f, i).

As consequence of the fusion or reduction of elaiophores, we did not observed any modifications in the anatomy of the flowers of both species in other whorls related.

Discussion

The flowers of *Alicia anisopetala* and *Callaeum psilophyllum* exhibit variations in the number of elaiophores, being the presence of eight elaiophores the more common condition and these variations were observed at specimen and population level. Our results agree with those reported for other species of Malpighiaceae (Gates 1982; Sazima and Sazima 1989; Castro et al. 2001; Carvalho et al. 2005; Costa et al. 2006; Cappellari et al. 2011; Possobom et al. 2015; Possobom and Machado 2017; Bonifácio et al. 2021; Aliscioni et al. 2022).

In the description of genus *Alicia*, Anderson (2006) reported flowers with eight elaiophores for its species, but our observations showed that exomorphologically this

Fig. 6 Vascularization of flowers in *Callaeum psilophyllum*, longitudinal (a–e) and transvers sections (f–g). **a** Diagram of a flower showing vascular traces of the different whorls. **b** Picture showing vascular traces from the central vascular bundle towards the different whorls. **c** Vascular traces of the ovary. **d** Vascular traces of the sepal and elaiophore. **e** Vascular traces of the elaiophore, sepal, petal, and stamen. **f** Vascular traces of the sepals (s), petals (p), base of the androecium (a), and gynoecium (g). **g** Transvers section showing elaiophores (e), vascular traces of the sepals (s), petals (p), and base of the androecium (a). Grey: central vascular bundle and gynoecium vascular traces. Green: vascular traces of sepals and elaiophores. Purple: vascular bundle of the petals. Yellow: Vascular bundle of stamens. Scale bars: **a** 2 cm; **b, c, f, g** 430 μ m; **d, e** 130 μ m

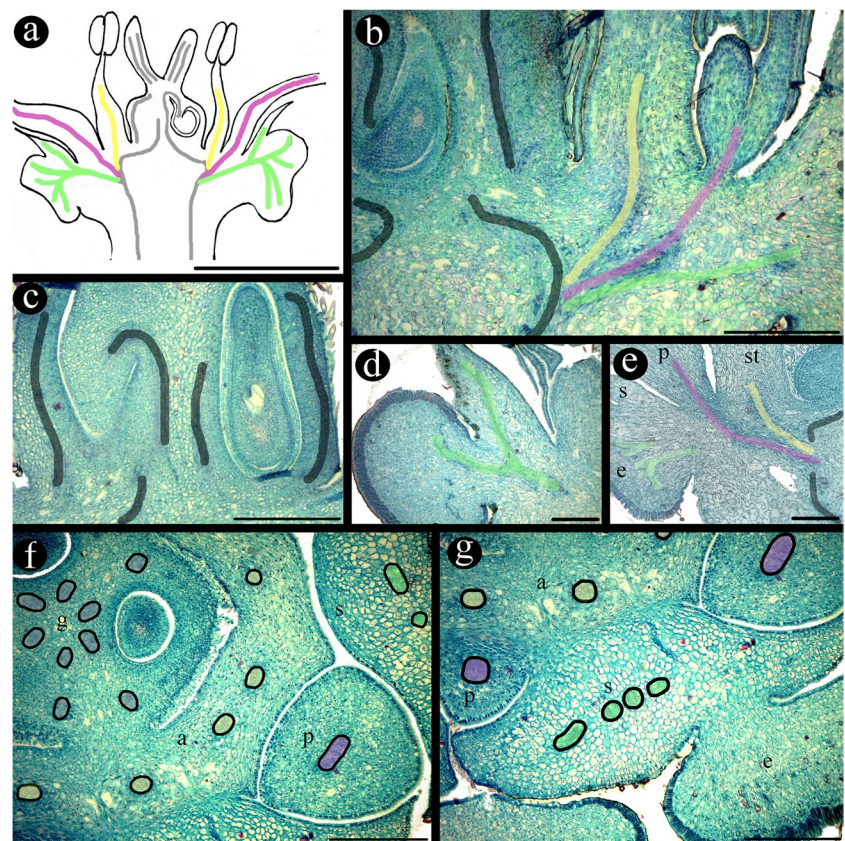
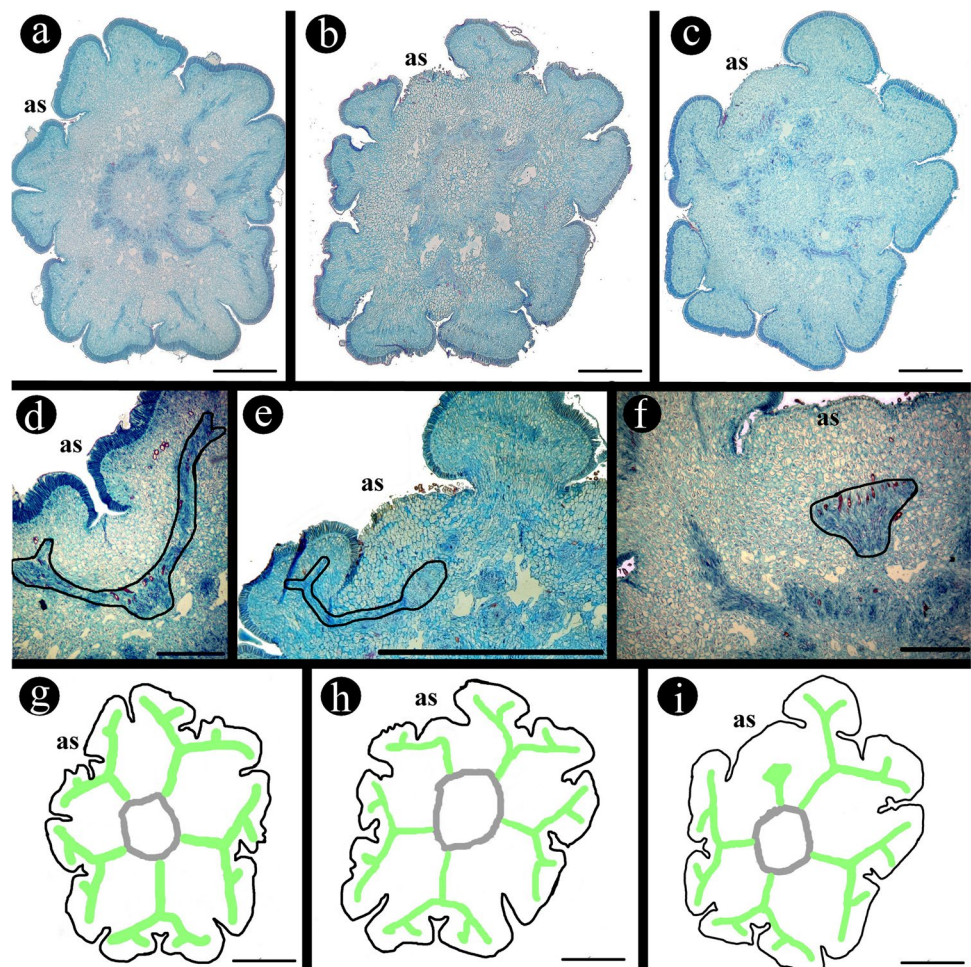


Fig. 7 Vascularization of elaiophores in *Callaeum psilophyllum*. Note the position of the anterior sepal (as) (a–f) Cross sections of pictures with bright-field microscope. **a** General aspect of a flower with ten elaiophores. **b** General aspect of a flower with nine elaiophores. **c** General aspect of a flower with eight elaiophores. **d** Detail of the vascularization of elaiophores adjacent to the anterior sepal in a flower with ten elaiophores. **e** Detail of the vascularization of the only elaiophore adjacent to the anterior sepal in a flower with nine glands. **f** Detail of the central vascular bundle of the anterior eglanular sepal of a flower with eight elaiophores. **g–i** Diagram of the vascular traces towards sepals and glands in flowers with (g) ten elaiophores, (h) nine elaiophores, and (i) eight elaiophores. Scale bars: a–c, g–i 90 μ m; d, f 30 μ m; e 110 μ m



number varies from eight to ten at specimen and population level. Moreover, our anatomical observations revealed that ten elaiophores are always present since they can be individualized at some point. This means that the number of elaiophores per flower that are exomorphologically observed in this species respond to a different degree of fusion between some elaiophores, which is never totally complete. Given that the fusion occurs between organs of the same floral whorl, this species exhibits an incomplete connation process. According to Puri (1951), the fusion of any two organs begins with the epidermis, followed by the parenchyma, and finally the vascular tissues. Therefore, vascular traces are expected to be found in this species, where elaiophores are undergoing a connation process. Our anatomical observations support the presence of vascular traces of ten elaiophores in flowers of *A. anisopetala*.

In the taxonomic revision of genus *Callaeum*, Johnson (1986) described flowers with eight elaiophores for all its species, but our observations, in concordance with Aliscioni et al. (2022), show variable number of elaiophores per flower at specimen and population level (from six to ten). Souto and Oliveira (2013) described the way

to distinguish the loss mechanism through the possible remains of vascular traces. According to our anatomical observations, the process of loss of these oil glands in the anterior sepal (and some gland/s in lateral sepals) is reduction due to the absence of vascular traces in the eglanular sepals. Moreover, we did not find elaiophores of smaller size (data not reported), which might suggest that these glands never developed.

Our results show that calyx vasculature is variable between studied species. This agrees with those reported by Bonifacio et al. (2021), who observed that the calyx vasculature of three species from acmantheroid clade is complex and is not uniform between genera, species, and floral morphotypes (glandular vs. eglanular). On the contrary, this does not agree with results of Souto and Oliveira (2013), who suggested that the information of the number of elaiophores per flower and their variations could be relevant to a better resolution of the Malpighiaceae phylogeny. Although there is little evidence, it would seem that the processes that lead to the loss of elaiophores are homoplastic and would not reflect phylogenetic signals. An increased number of species studied in these aspects would give a more precise answer.

This study suggests that it may be insufficient relying solely on exomorphological observations to comprehend the intricate processes influencing external morphology and it underscores the necessity to extend beyond the analysis of a single population or specimen. We believe that a comprehensive understanding requires the examination of diverse populations, incorporating variability into both exomorphological and anatomical analyses. This holistic approach is necessary given the diversity in the number of elaiophores and the potential existence of populations lacking discernible exomorphological variations.

Both studied species exhibit different loss processes that result in diverse number of elaiophores per flower. These variations can be influenced by genetic factors, hormonal regulation, environmental conditions, and/or selection pressures on their phenotypic expression associated to the mutualistic relationship with oil bees (Sazima and Sazima 1989; Cappellari et al. 2011; Souto and Oliveira 2013; Torretta et al. 2017; Bonifácio et al. 2021; Aliscioni et al. 2022). Other morphological characteristics were explored in relation to potential factors underlying such diversity. Torretta et al. (2017) examined elaiophore size variation in two *Stigmaphyllon* species along the latitudinal gradient of the plants' distributions, suggesting that different selection pressures influenced phenotypic appearance, associated with pollinator size in one species and climate conditions in the other ones.

Similarly to some flowers of *Alicia anisopetala*, the flowers of *Lophopterys floribunda* present a single, large gland on each glandular lateral sepal (the anterior sepal is eglandular) that could result from the fusion of two elaiophores within the same sepal (Sanches et al. 2023). These authors quoted "Although no morphoanatomical evidence of fusion has been observed, the single large sepalar gland suggests a fusion event of two small glands, as observed in *Acridocarpus* (Guesdon et al. 2019)" suggesting complete fusion among elaiophore pairs of each glandular sepals (Sanches et al. 2023) and they propose that presence of large elaiophores could be a strategy to enhance visibility for pollinator and to maximize oil resources performance, allowing more secretion to be collectible by pollinating bees. However, it seems that the loss of elaiophores by a fusion process does not significantly affect the total oil production per flower as was demonstrated for *Stigmaphyllon paralis* A. Juss (Carvalho et al. 2005). Our findings further indicate that, for certain species such as *Alicia anisopetala* and potentially for *S. paralis*, these fusions do not necessarily result in diminished oil output. One large elaiophore would produce the same amount of oil as two smaller ones, since the first is the result of the fusion of the two elaiophores. A possible explanation for such adaptation could be that the proximity of glands in adjacent sepals, followed by their fusion, enables these "large elaiophores" to be strategically positioned

in sepal areas that are more accessible to oil-collecting bees, allowing easier foraging floral oil (Sanches et al. 2023).

On the other hand, *Callaeum psilophyllum* flowers exhibited the general pattern of most Neotropical Malpighiaceae species with four 2-glandular sepals and the eglandular anterior sepal, due to a reduction process. However, in this species, we observed a high variation in the number of elaiophores per flower. A possible explanation for this species is that a reduction of elaiophores per flower might imply fewer resources invested in the production of floral oil, since this floral reward is expensive for plants to produce (Buchmann 1987). Numerous species of Malpighiaceae exhibit variability in the number of elaiophores and the presence of glandular and eglandular flowers in a same individual or among individuals (Sazima and Sazima 1989; Aliscioni et al. 2022; Castro et al. 2022; Queiroz et al. 2023). Sazima and Sazima (1989) demonstrated that in individuals with glandular and eglandular flowers, the latter attract and deceive oil-gathering bees representing automimicry. We did not observe eglandular flowers in *C. psilophyllum* in any of the populations studied; therefore, automimicry cannot be considered for this species.

Almost half flowers of the individuals of the El Palmar National Park population bear 10 elaiophores. The high abundance of individuals in this population could allow the pollen transference among them generating a population with low genetic variability (Sazan et al. 2014; Avalos et al. 2021). On the other hand, this population also exhibits the major variation in the number of elaiophores per flower. This could also be explained by the large number of individuals studied. Possibly, if in other populations the number of individuals found had been greater, we would also have found this variability in the number of elaiophores per flower.

To sum up, variability in the number of elaiophores is a recurrent characteristic in several Neotropical species of Malpighiaceae (Sazima and Sazima 1989; Teixeira and Machado 2000; Aliscioni et al. 2022; Castro et al. 2022; Queiroz et al. 2023). Despite this variability, pollinators in most cases are oil-collecting bees (Reposi et al. 2023), as in our focal species: flowers of *Alicia anisopetala* are legitimately visited by *Centris bicolor* Lepeletier, *C. collaris* Lepeletier, *C. discolor* Smith, *C. mocsaryi* Friese, *C. trigonoides* Lepeletier, *Epicharis affinis* Smith and one undetermined species of *Monoeca* (Reposi and Torretta pers. obs.; Sigrist and Sazima 2004), while flowers of *Callaeum psilophyllum* are legitimately visited by *Centris flavifrons* (Fabricius) (Fig. 8), *C. proxima* Friese, *C. tarsata* Smith, and *C. trigonoides* (Aliscioni et al. 2018; Torretta et al. 2022; Reposi and Torretta pers. obs.). Moreover, we observed females of oil-collecting bees of genera *Paratrapedia s.l.* and *Tetrapedia* illegitimately foraging for oil in flowers of both species (Aliscioni et al. 2022, Torretta et al. 2022; Reposi and Torretta pers. obs.). However,



Fig. 8 *Centris flavifrons* visiting flowers of *Callaeum psilophyllum*. The black arrow indicates the elaiophores of the flower

shifts in the composition of the pollinator pool have been observed in other Neotropical species with eglandular flowers. This is due to the absence of floral oil as reward to pollinators and are accompanied by certain floral morphological changes, such as the presence of big anthers, high amount of orbicules that could allow a rapid release of pollen from the anthers, and absence of stigmatic cuticle, as in the case of *Galphimia australis* (Gotelli et al. 2023). Other particular case is reported for *Pterandra pyroidea*, one species with populations with plants with oil-secreting (glandular) flowers, plants with non-oil-secreting (eglandular) flowers, or a mix of both plant types (Cappellari et al. 2011). In this species, eglandular flowers do not act as mimics of their oil-producing conspecifics to attract pollinators, and both floral morphs are visited mainly by pollen-collecting bumblebees (*Bombus* spp.).

Conclusion

This study examined processes involved in the exomorphological loss of elaiophores in two species of the christianelloid clade. Our results demonstrated that in both species, the responsible processes were different: incomplete connation of elaiophores in *Alicia anisopetala* flowers and reduction of elaiophores in *Callaeum psilophyllum* flowers. We also highlighted that these strategies should be comprehensively examined, considering both external morphology and internal anatomy. Finally, due to the observed differences between these two closely related species, we suggest that the processes that lead to the loss of elaiophores are homoplastic and would not reflect phylogenetic signals.

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Author contributions SDR, MMG, and JPT conducted the research. SDR wrote the manuscript. MMG, MRN, SSA, and JPT reviewed and completed the information. SDR and MRN prepared, processed, and observed the preparations. All authors read and approved the final manuscript.

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Data availability The data sets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

References

- Aliscioni SS, Gotelli MM, Torretta JP (2018) Structure of the stigma and style of *Callaeum psilophyllum* (Malpighiaceae) and its relation with potential pollinators. *Protoplasma* 255:1433–1442. <https://doi.org/10.1007/s00709-018-1245-x>
- Aliscioni SS, Gomiz NE, Agüero JI, Torretta JP (2022) Structural diversity of elaiophores in Argentine species of Malpighiaceae: morphology, anatomy, and interaction with pollinators. *Protoplasma* 259:789–807. <https://doi.org/10.1007/s00709-021-01699-x>
- Anderson WR (1990) The origin of the Malpighiaceae, the evidence from morphology. *Mem NY Bot Gard* 64:210–224
- Anderson WR (2006) Eight segregates from the Neotropical genus *Mascagnia* (Malpighiaceae). *Novon* 16:168–204. [https://doi.org/10.3417/1055-3177\(2006\)16\[168:ESFTNG\]2.0.CO;2](https://doi.org/10.3417/1055-3177(2006)16[168:ESFTNG]2.0.CO;2)
- Anderson WR (1979) Floral conservatism in Neotropical Malpighiaceae. *Biotropica* 11:219–223. <https://doi.org/10.2307/2388042>
- Araújo JS, Meira RMSA (2016) Comparative anatomy of calyx and foliar glands of *Banisteriopsis* CB Rob. (Malpighiaceae). *Acta Botanica Brasílica* 30:112–123
- Arévalo-Rodríguez G, de Almeida RF, Cardoso-Gustavson P (2020) Anatomy of staminal glands in the Stigmaphyllon clade sheds light into new morphotypes of elaiophores and osmophores in Malpighiaceae. *Pl Syst Evol* 306:1–9. <https://doi.org/10.1007/s00606-020-01680-w>
- Avalos AA, Marrero HJ, Ferrucci MS, Torretta JP (2021) Stigmas arrangement, reproductive system, and maternal reproductive success in two species of *Stigmaphyllon* (Malpighiaceae): does pollinator size matter? *Plant Ecol* 222(11):1263–1279
- Bonifácio SKV, de Almeida RF, Amorim AMA, Oliveira DMT (2021) Floral synorganization in acmantheroid clade suggests

- hypotheses to explain elaiophore suppression in Malpighiaceae. *Flora* 281:151870. <https://doi.org/10.1016/j.flora.2021.151870>
- Buchmann SL (1987) The ecology of oil flowers and their bees. *Annu Rev Ecol Syst* 18(1):343–369
- Cappellari SC, Haleem MA, Marsaioli AJ, Tidon R, Simpson BB (2011) *Pterandra pyroidea*: a case of pollination shift within Neotropical Malpighiaceae. *Ann Bot* 107:1323–1334. <https://doi.org/10.1093/aob/mcr084>
- Carneiro LT, Machado IC (2023) Oil flowers and related oil-collecting bees: a 50-year timeline of knowledge and future directions. *Arthropod-Plant Interact* 17:543–562. <https://doi.org/10.1007/s11829-023-10000-1>
- Carvalho PDD, Borba EL, Lucchese AM (2005) Variation in the number of glands and oil production in flowers of *Stigmaphyllon paralias* A. Juss. (Malpighiaceae). *Acta Bot Brasil* 19:209–214. <https://doi.org/10.1590/S0102-33062005000200002>
- Castro MA, Vega AS, Múlgura ME (2001) Structure and ultrastructure of leaf and calyx glands in *Galphimia brasiliensis* (Malpighiaceae). *Am J Bot* 88:1935–1944. <https://doi.org/10.2307/3558420>
- Castro JB, Machado G, Singer RB (2022) Müllerian mimicry between oil-producing orchids and Malpighiaceae? An old hypothesis finally tested. *Sci Nat* 109:3. <https://doi.org/10.1007/s00114-021-01771-9>
- Cocucci AA, Holgado AM, Anton AM (1996) Morphology and anatomy of the stalked elaiophores of *Dinemandra ericoides*, an endemic Malpighiaceae of the Atacama desert, Chile. *Darwiniana* 34
- Costa CBN, Costa JAS, Ramalho M (2006) Biologia reprodutiva de espécies simpátricas de Malpighiaceae em dunas costeiras da Bahia, Brasil. *Rev Bras Bot* 29:103–114. <https://doi.org/10.1590/S0100-84042006000100010>
- Davis CC, Anderson WR (2010) A complete generic phylogeny of Malpighiaceae inferred from nucleotide sequence data and morphology. *Am J Bot* 97:2031–2048. <https://doi.org/10.3732/ajb.1000146>
- de Queiroz LP, Costa JAS, Costa CBN (2023) Did *Moldehawera* flowers evolve through mimicry with oil-producing Malpighiaceae?. *BioRxiv* 2023–06. <https://doi.org/10.1101/2023.06.15.545073>
- Gates B (1982) *Banisteriopsis*, *Diplopterys* (Malpighiaceae). *Flora Neotrop* 30:1–237
- Gifford EM, Foster AS (1989) Morphology and evolution of vascular plants, 3rd ed. W.H. Freeman and Company, San Francisco
- Gotelli M, Aliscioni S, Kuo PT, Torretta JP (2023) Are the floral morphology and anatomy of *Galphimia australis*, an atypical neotropical Malpighiaceae, associated to a new pollination syndrome? *Protoplasma* 260:1047–1062. <https://doi.org/10.1007/s00709-022-01829-z>
- Guesdon IR, Amorim AM, Meira RMSA (2019) Functional role and evolutionary contributions of floral gland morphoanatomy in the Paleotropical genus *Acridocarpus* (Malpighiaceae). *PLoS ONE* 14:e0222561. <https://doi.org/10.1371/journal.pone.0222561>
- Johnson DM (1986) Revision of the Neotropical genus *Callaeum* (Malpighiaceae). *Syst Bot* 335–353
- Mamede MCH (1993) Estudo comparativo de fores casmógamas, cleistógamas e de frutos de *Camarea afnis* St.-Hil. (Malpighiaceae). *Acta Bot Brasil* 7:21–31. <https://doi.org/10.1590/S0102-33061993000100002>
- Martins AC, Melo GA (2016) The New World oil collecting bees *Centris* and *Epicharis* (Hymenoptera, Apidae): molecular phylogeny and biogeographic history. *Zool Scr* 45:22–33. <https://doi.org/10.1111/zsc.12133>
- Possobom CCF (2008) Estrutura e função das glândulas florais e dos nectários foliculares em *Diplopterys Pubipetala* (A. JUSS.) WR Anderson & C. Cav. Davis (Malpighiaceae)
- Possobom CCF, Machado SR (2017) Elaiophores: their taxonomic distribution, morphology and functions. *Acta Bot Brasil* 31:503–524. <https://doi.org/10.1590/0102-33062017abb0088>
- Possobom CCF, Guimarães E, Machado SR (2015) Structure and secretion mechanisms of floral glands in *Diplopterys pubipetala* (Malpighiaceae), a Neotropical species. *Flora* 211:26–39. <https://doi.org/10.1016/j.flora.2015.01.002>
- Puri V (1951) The role of floral anatomy in the solution of morphological problems. *Bot Rev* 17:471–553. <https://doi.org/10.1007/BF02882536>
- Renner SS, Schaefer H (2010) The evolution and loss of oil-offering flowers: new insights from dated phylogenies for angiosperms and bees. *Philos Trans R Soc Lond B Biol Sci* 365:423–435. <https://doi.org/10.1098/rstb.2009.0229>
- Reposi SD, Avalos AA, Gotelli MM, Aliscioni SS, Torretta JP (2023) Reproductive biology of Malpighiaceae: how much do we know? *Plant Syst Evol* 309:25. <https://doi.org/10.1007/s00606-023-01863-1>
- Sanches MM, Guesdon IR, Alves Meira RMS (2023) Diversity and functional roles of floral glands in Malpighiaceae: insights in *Lophopterys floribunda* WR Anderson and C. Davis *Protoplasma* 260:1555–1567. <https://doi.org/10.1007/s00709-023-01871-5>
- Sazan MS, Bezerra ADM, Freitas BM (2014) Oil collecting bees and *Byrsonima cydoniifolia* A. Juss. (Malpighiaceae) interactions: the prevalence of long-distance cross pollination driving reproductive success. *An Acad Bras Ciênc* 86:347–358
- Sazima M, Sazima I (1989) Oil-gathering bees visit flowers of eglandular morphs of the oil-producing Malpighiaceae. *Bot Acta* 102:106111. <https://doi.org/10.1111/j.1438-8677.1989.tb00073.x>
- Sigrist MR, Sazima M (2004) Pollination and reproductive biology of twelve species of Neotropical Malpighiaceae: stigma morphology and its implications for the breeding system. *Ann Bot* 94:33–41. <https://doi.org/10.1093/aob/mch108>
- Souto LS, Oliveira DMT (2013) Evaluation of the floral vasculature of the *Janusia*, *Mascagnia* and *Tetrapteryx* species as a tool to explain the decrease of floral organs in Malpighiaceae. *Flora* 208:351–359. <https://doi.org/10.1016/j.flora.2013.05.002>
- Teixeira LAG, Machado IC (2000) Sistema de polinização e reprodução de *Byrsonima sericea* DC (Malpighiaceae). *Act Bot Bra* 14:347–357. <https://doi.org/10.1590/S0102-33062000000300011>
- Torretta JP, Aliscioni SS, González Arzac A, Avalos AA (2017) Is the variation of floral elaiophore size in two species of *Stigmaphyllon* (Malpighiaceae) dependent on interaction with pollinators? *Plant Ecol Divers* 10:403–418. <https://doi.org/10.1080/17550874.2018.1434567>
- Torretta JP, Aliscioni SS, Marrero HJ, Avalos AA (2022) Oil flowers of Malpighiaceae and oil-collecting bees: loyalty and robbery in a highly specialized system. *Apidologie* 53:30. <https://doi.org/10.1007/s13592-022-00945-2>
- Vogel S (1974) Ölblumen und ölsammelnde Bienen. *Abhandlungen Akademie Wissenschaften Mathematisch-Naturwissenschaften Klasse. Trop Subtrop Pflanzenwelt* 7:283–547
- Vogel S (1990) History of the Malpighiaceae in the light of pollination ecology. *Mem NY Bot Gard* 55:130–142
- Zarlavsky GE (2014) *Histología Vegetal: técnicas simples y complejas*. Sociedad Argentina de Botánica, Ciudad Autónoma de Buenos Aires
- Zhang W, Kramer EM, Davis CC (2016) Differential expression of CYC2 genes and the elaboration of floral morphologies in *Hiptage*, an Old World genus of Malpighiaceae. *Int J Pl Sci* 177:551–558. <https://doi.org/10.1086/687225>

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