ORIGINAL ARTICLE

Structural diversity of elaiophores in Argentine species of Malpighiaceae: morphology, anatomy, and interaction with pollinators

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Abstract

Most Neotropical Malpighiaceae species are characterized by having zygomorphic flowers and oil glands in the sepals called elaiophores; these floral characteristics are associated with a particular pollination syndrome through oil-collecting bees. This work proposes a study about the structural characteristics of elaiophores in 18 species of Malpighiaceae present in Argentina. The main objectives are to describe the morphology and anatomy of the elaiophores, to detect variation in the number of glands, to compare similarities or differences in elaiophores of species belonging to different lineages, and to know about the potential pollinators and their association with the structural traits of the elaiophores. The morphology and the anatomy were studied using traditional methods of scanning electron and bright-field microscopes. Field trips were carried out to capture oil-collecting bee species on flowers, in different natural populations. Different measurements were taken in the flowers, elaiophores, and oil-collecting bees and were statistically analyzed. Although elaiophores showed a common pattern, some particularities in number, morphology, and anatomy were detected; few of these seem to be restricted to some groups of species phylogenetically related. As regards pollinators, a positive tendency was observed between the size of the flowers, elaiophores, and oil-collecting bees. However, the thickness of the cuticle presented a negative association with the size of the elaiophore and consequently with the floral diameter, which could be presumably related to the foraging behavior and/or the structure of oil-collecting apparatus of the bee species.

Keywords Anatomy · Elaiophore · Malpighiaceae · Oil-collecting bee · Pollinator

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Introduction

Malpighiaceae is a pantropical family, represented by approximately 1300 species which occur mainly in Neotropical region (Davis and Anderson 2010). Most Neotropical Malpighiaceae species are characterized by having zygomorphic flowers and oil glands in the sepals called elaiophores (Vogel 1974; Davis and Anderson 2010). Elaiophores are presents in approximately 90% of the species in the Neotropic (Anderson 1990; Vogel 1990), and this floral characteristic is associated with a particular pollination syndrome through oil-collecting bees (Anderson 1979; Vogel 1990). This syndrome, within the Neotropical species, is an adaptation to a group of pollinators composed by numerous species of the genera *Centris*, *Epicharis* (Apidae: Centridini) and *Monoeca* (Apidae: Tapinotaspidini) (Vogel 1974; 1990; Anderson 1990; Sigrist and Sazima 2004). The floral morphology of the Malpighiaceae allows the females of the oil-collecting bees to access the collection of oils while

efficiently pollinating the flowers (Anderson 1979; Vogel 1990).

The phylogeny of Malpighiaceae is well resolved, and several studies corroborates the monophyly of the family, and recovered many well-supported clades, with interrelationships well resulted in most cases (Cameron et al. 2001; Davis et al. 2001; 2002; Davis and Anderson 2010). In this evolutionary scheme, Davis et al. (2014) hypothesized that the floral morphology of the Malpighiaceae has maintained over tens of millions years via their pollinator interaction with oil-collecting bees. The very early acquisition of elaiophores in the history of the Neotropical Malpighiaceae matches with the origin of its pollinators showing the relevance of these glands for the evolution of this family (Renner and Schaefer 2010; Martins et al. 2015; Aguiar et al. 2020). However, Davis et al. (2014) demonstrated that species in nine clades of Malpighiaceae in Old and New World have lost elaiophores and acquired another pollination syndrome by pollen-collecting bees (Anderson 1979; Anderson and Corso 2007; Ren et al. 2013). Moreover, there are species with flowers of glandular and eglandular morphs (Sazima & Sazima 1989; Cappellari et al. 2011). This loss of oil-flower syndrome is considered a derived condition (Anderson 1990).

Vogel (1974) was the first to study the anatomy of the elaiophores in three species of Malpighiaceae. He theorized that elaiophores arise from nectaries, as a result of the adaptation to the pollinators (Vogel 1990). Later, several studies described the anatomical characteristics of the elaiophores in numerous species of Neotropical Malpighiaceae (Subramanian et al. 1990; Cocucci et al. 1996; Castro et al. 2001; Possobom et al. 2015; Araújo and Meira 2016; Possobom and Machado 2017; 2018). Although all these works find a similar anatomical pattern, significant variation has been recognized in some particularities of the epithelial cells, the cuticle thickness, and the release secretion of the elaiophores (Araújo and Meira 2016). Some of these differences were attributed to phylogenetic affinities between species. For example, Possobom and Machado (2018) noted that the elaiophores in species of the byrsonimoids clade differ from species of the stigmaphylloids clade in the attachment to the sepals and surface shape. Moreover, Davis et al. (2014) revealed that the presence of peltate gland showed significantly elevated rates of change in an evolutionary context.

On the other hand, there are few studies that relate the sizes of the flower and the elaiophores, the volume of the secretion, and the association with pollinators in Malpighiaceae. Carvalho et al. (2005) observed in *Stigmaphyllon paralias* A. Juss. that the size of the elaiophores and the quantity of oil produced were positively correlated. In a more recent work, Torretta et al. (2017) demonstrated in two other species of the same genus that the elaiophore total area decreases in relation to floral size along their latitudinal distribution; and particularly in *S. bonariense* (Hook. & Arn.) C. E. Anderson, the pollinator size appears to be related to the size of the floral glands.

Based on the current knowledge, elaiophores probably have some structural characters inherited from their ancestors and these glands acquired new characteristics due to the selection forces exerted by pollinators. However, which morphological and/or anatomical characteristics are more related to the ancestral inheritance and which suffered more selection pressure by the pollinators are still little understood. This work proposes a comparative study of the floral elaiophores and its relations with pollinators in 18 species of Malpighiaceae present in Argentina. The principal objectives are (1) to describe the morphology and anatomy of the elaiophores, (2) to detect variation in the number of glands, (3) to compare similarities and/or differences in elaiophores of species belonging to different lineages, and (4) to know about the potential pollinators and their association with the structural traits of the elaiophores.

Material and methods

Plant material

Numerous field trips were carried out during the years 2013–2017 around the Northeast, Northwest, and Center of Argentina to search natural populations in the context of the Flora Argentina project (Aliscioni and Torretta 2017). We focused our search on the NE, which is the region of Argentina with the greatest richness of Malpighiaceae (Aliscioni and Torretta 2017). Also, we looked for species in the other regions to cover a greater diversity and morphological variation of the family in Argentina. We located 18 species of 12 genera; Table 1 indicates the list of studied species, the clades to which they belong (Anderson et al. 2006; Davis and Anderson 2010), and the located populations and their geographical positions. The vouchers were deposited in the herbaria Gaspar Xuárez, Facultad de Agronomía, Universidad de Buenos Aires (BAA), and Instituto de Botánica Darwinion (SI).

Fresh flowers in anthesis ($n = 10$ to 50) from diverse individuals ($n = 2$ to 5) for each population were collected and fixed in formalin-acetic acid-alcohol mixture for 48 h and stored in 70% alcohol. In the field, one fresh flower of each sampled specimen was submerged in saturated alcoholic Sudan III solution to check the presence of lipids in the glands of the sepals.

In laboratory, from each fixed sample, we selected and observed 10 flowers in complete anthesis using a stereomicroscope. We registered the number of elaiophores per flower, and we took digital photographs with a camera incorporated in the stereomicroscope (with the same

Table 1 List of studied populations of species of Malpighiaceae with pollinators and its range of size (*M* medium; *L* large; *VL* very large). The species of Malpighiaceae are grouped following the phylogenies of Anderson et al. (2006) and Davis and Anderson (2010)

Clade	Species	Population	Georeference	Accumulated period of observation (hours)	Legitimate visitors	Visitor rank size
Hiraeoids	*Hiraea fagifolia* (DC.) A. Juss	Misiones, P.N. Iguazú	S 25° 40' 57" W 54° 26' 58"	2	*Epicharis* sp. 1	L
Malpighioids	*Mascagnia divaricata* (Kunth) Nied	Salta, Embarcación	S 23° 12' 37" W 64° 5' 50"	2	-	-
		Misiones, P.N. Iguazú	S 25° 41' 33" W 54° 26' 52"	5	*Centris (Hemisiella) tarsata*; *Monoeca armata*	M; M
		Misiones, Guaraní	S 27° 13' 43" W 54° 1' 13"	2	*Centris (Centris) varia*	L
		Misiones, San Ignacio	S 27° 16' 44" W 55° 33' 11"	3	-	-
		Misiones, Alem	S 27° 45' 45" W 55° 13' 57"	3	-	-
Tetrapteroids (christianelloids)	*Callaeum psilophyllum* (A. Juss.) D.M. Johnson	Bs. As., Isla Martín García	S 34° 10' 56" W 58° 14' 56"	8	*Centris (Hemisiella) trigonoides*; *Centris (Trachina) fuscata*; *Centris (Hemisiella) tarsata*	M; L; M
Tetrapteroids (carolus)	*Dicella nucifera* Chodat	Misiones, P.P. Teyú Cuaré	S 27° 15' 53" W 55° 32"	4	*Epicharis* sp. 2	L
		Misiones, Candelaria	S 27° 26' 57" W 55° 44' 55"	2	-	-
		Misiones, Guaraní	S 27° 31' 56" W 55° 11' 51"	2	-	-
		Misiones, Alem	S 27° 45' 27" W 55° 15' 51"	2	-	-
	Tricomaria usillo Hook. & Arn	La Rioja, Talampaya	S 29° 53' 26" W 67° 51' 12"	2	*Centris (Paracentris) brethesi*	M
		Catamarca, Fiambalá	S 27° 41' 21" W 67° 37' 36"	2	*Centris (Paracentris) brethesi*	M
Tetrapteroids (heteropterys)	*Heteropterys argyrophaea* A. Juss	Misiones, Apóstoles	S 27° 43' 2" W 55° 29' 23"	2	-	-
		Corrientes, Santo Tomé	S 28° 33' 37" W 56° 1' 36"	3	-	-
		Corrientes, Yapeyú	S 29° 28' 4" W 56° 48' 33"	4	*Monoeca pulchella*	M
	Heteropterys dumetorum (Griseb.) Nied	Jujuy, Calilegua	S 23° 29' 40" W 65° 2' 53"	2.5	-	-
		Salta, Embarcación	S 24° 46' 59" W 65° 24' 37"	2	-	-
		Salta, Cayafate	S 25° 10' 16" W 65° 29' 43"	2	-	-
	Heteropterys glabra Hook. & Arn	Misiones, Candelaria	S 27° 28' 47" W 55° 29' 0"	4	-	-
		Formosa, San Francisco de Laishi, Reserva El Bagual	S 26° 10' 55" W 58° 56' 32"	6	*Centris (Hemisiella) trigonoides*; *Centris (Hemisiella) tarsata*	M; M
		Bs. As., San Antonio de Areco	S 34° 14' 37" W 59° 28' 25"	2	-	-
	Heteropterys hypericifolia A. Juss	Formosa, San Francisco de Laishi, Reserva El Bagual	S 26° 10' 47" W 58° 56' 31"	6	*Centris (Hemisiella) tarsata*; *Centris (Hemisiella) nigriventris*; *Centris (Hemisiella) trigonoides*	M; M; M
	Heteropterys intermedia (A. Juss.) Griseb	Misiones, Montecarlo	S 26° 29' 21" W 54° 40' 14"	3	*Centris (Hemisiella) tarsata*; *Monoeca pulchella*; *Monoeca armata*	M; M; M
		Misiones, San Martín	S 26° 48' 39" W 54° 55' 53"	2	-	-
		Misiones, P.P. Teyú Cuaré	S 27° 15' 52" W 55° 32' 7"	5	*Monoeca armata*; *Monoeca pulchella*	M; M
		Misiones, San Ignacio	S 27° 16' 39" W 55° 34' 20"	2	-	-
		Misiones, Guaraní	S 27° 32' 27" W 55° 12' 54"	2	*Monoeca armata*	M
		Misiones, Alem	S 27° 38' 37" W 55° 28' 24"	2	-	-
	Heteropterys syringifolia Griseb	Misiones, Alem	S 27° 46' 7" W 55° 15' 7"	2	-	-
Tetrapteroids (niedenzuella)	*Niedenzuella sericea* (A. Juss.) W.R. Anderson	Misiones, San Ignacio	S 27° 15' 52" W 55° 32' 7"	2	-	-
		Misiones, P.P. Teyú Cuaré	S 27° 16' 39" W 55° 33' 13"	3	-	-
Tristellateioids	*Heladena multiflora* (Hook. & Arn.) Nied	Corrientes, Yapeyú	S 29° 28' 4" W 56° 48' 33"	2	-	-

Table 1 (continued)

Clade	Species	Population	Georeference	Accumulated period of observation (hours)	Legitimate visitors	Visitor rank size
Stigmaphylloids (aspicarpoids)	*Aspicarpa pulchella* (Griseb.) O'Donell & Lourteig	Misiones, Candelaria	S 27° 26′ 57″ W 55° 44′ 55″	2	-	-
	Janusia guaranitica (A. St-Hil.) A. Juss	Salta, Calilegua	S 23° 12′ 60″ W 64° 5′ 60″	2	*Centris (Hemisiella) tarsata*	M
		Formosa, San Francisco de Laishi, Reserva El Bagual	S 23° 12′ 60″ W 64° 5′ 60″	4	*Centris (Paracentris) tricolor*	M
		Misiones, P.P. Teyú Cuaré	S 27° 16′ 27″ W 55° 34′ 16″	3	-	-
		Misiones, Bonpland	S 27° 28′ 7″ W 55° 29′ 22″	2	-	-
		Misiones, Alem	S 27° 38′ 21″ W 55° 29′ 42″	2	-	-
		Corrientes, Santo Tomé	S 28° 5′ 26″ W 55° 41′ 52″	2	-	-
		Entre Ríos, La Paz	S 30° 45′ 48″ W 59° 39′ 18″	2	-	-
		Córdoba, S. Marcos Sierra	S 30° 46′ 60″ W 64° 38′ 60″	1	-	-

Table 1 (continued)

Clade	Species	Population	Georeference	Accumulated period of observation (hours)	Legitimate visitors	Visitor rank size
Stigmaphylloids	*Banisteriopsis muricata* (Cav.) Cuatrec	Misiones, P.P. Teyú Cuaré	S 27° 15′ 38″ W 55° 33′ 26″	6	*Epicharis (Triepicharis) analis*	VL
					Epicharis (Hoplepicharis) affinis	VL
					Centris (Heterocentris) bicornuta	M
					Centris (Trachina) similis	L
					Centris (Hemisiella) tarsata	M
					Centris (Centris) varia	L
	Stigmaphyllon bonariense (Hook. & Arn.) C. E. Anderson	Misiones, Iguazú	S 25° 40′ 40″ W 54° 27′ 8″	5	*Centris (Hemisiella) tarsata*	M
					Centris (Hemisiella) trigonoides	M
					Epicharis sp. 3	VL
		Misiones, San Ignacio	S 27° 17′ 20″ W 55° 33′ 59″	5	*Centris (Hemisiella) tarsata*	M
					Centris (Hemisiella) trigonoides	M
					Centris (Centris) varia	L
		Corrientes, Corrientes	S 27° 32′ 2″ W 58° 47′ 59″	10	*Centris (Centris) flavifrons*	VL
					Centris (Hemisiella) tarsata	M
					Centris (Hemisiella) trigonoides	M
					Centris (Centris) varia	L
		Corrientes, Yapeyú	S 29° 28′ 1″ W 56° 48′ 31″	5	*Centris (Melacentris) obsoleta*	VL
					Centris (Hemisiella) trigonoides	M
		Entre Ríos, Concordia	S 31° 22′ 14″ W 57° 59′ 41″	4	*Centris (Centris) flavifrons*	VL
					Centris (Trachina) fuscata	L
					Centris (Hemisiella) trigonoides	M
		Entre Ríos, Colón	S 32° 14′ 9″ W 58° 7′ 30″	2	*Centris (Centris) flavifrons*	VL
					Centris (Trachina) fuscata	L
		Entre Ríos, Ibicuy	S 33° 54′ 0″ W 58° 52′ 47″	2	*Centris (Hemisiella) trigonoides*	M
		Bs. As., Zárate	S 34° 5′ 55″ W 59° 0′ 9″	2	*Centris (Trachina) fuscata*	L
					Centris (Hemisiella) trigonoides	M
		Bs. As., Otamendi	S 34° 11′ 1″ W 58° 15′ 2″	2	*Centris (Trachina) fuscata*	L
		Bs As., Isla Martín García	S 34° 12′ 19″ W 58° 53′ 9″	10	*Centris (Centris) flavifrons*	VL
					Centris (Hemisiella) tarsata	M
					Centris (Hemisiella) trigonoides	M
					Centris (Panacentris) tricolor	M
		Ciudad Autónoma de Bs. As	S 34° 35′ 27″ W 58° 28′ 46″	4	*Centris (Trachina) fuscata*	L
					Centris (Hemisiella) trigonoides	M
	Stigmaphyllon jatrophifolium A. Juss	Misiones, San Ignacio	S 27° 17′ 17″ W 55° 32′ 47″	2	*Centris (Hemisiella) trigonoides*	M
		Misiones, Bonpland	S 27° 30′ 1″ W 55° 30′ 3″	2	–	–
		Misiones, Apóstoles	S 28° 0′ 27″ W 55° 36′ 39″	2	*Centris (Xanthemisia) bicolor*	M
					Centris (Hemisiella) tarsata	M
		Corrientes, Yapeyú	S 29° 27′ 56″ W 56° 48′ 30″	4	*Centris (Trachina) fuscata*	L
					Centris (Melacentris) obsoleta	VL
					Centris (Hemisiella) tarsata	M
		Entre Ríos, Concordia	S 31° 22′ 14″ W 57° 59′ 41″	3	*Centris (Hemisiella) trigonoides*	M
					Centris (Hemisiella) tarsata	M
		Entre Ríos, Colón	S 32° 13′ 1″ W 58° 11′ 11″	2	*Centris (Hemisiella) trigonoides*	M

magnification to ensure that photographs were comparable). For each species, flowers with atypical morphologies (eglandular or unusual number of elaiophores) were replaced to standardize the elaiophore measurements. The photographs were used to measure the diameters of the flowers (as proxy of floral size), and the maximum length, maximum width, and thickness of the elaiophores were measured in the first sepal to the right of the flag petals in frontal view for each flower, following the methods used in Torretta et al. (2017). The measurements were taken with Motic Images Plus 2.0 ML software associated to Axio Vs40 V 4.8.2.0 (Carl Zeiss). The measured elaiophores were hand-transversal sectioned and mounted in temporary slides to measure the thickness of the cuticle, as well as the length and width of the secretory cells of the epithelium using the mentioned program, but observing the sample in a bright-field microscope. These measurements were taken from hand cuts with the objective that all the measurements made (external and internal) correspond to the same elaiophores and to avoid that the values of some structures could be modified by conventional histology techniques.

SEM and LM observations

The micromorphology and the anatomy of the elaiophores of all studied species ($n = 18$) were observed and described using scanning electron and bright-field microscopes. For scanning electron microscopy (SEM), complete elaiophores were dehydrated and subjected to critical-point drying using liquid CO_2. The material was then sputter-coated with gold and examined using a Philips XL 30 TMP microscope at an accelerating voltage of 80 kV. For the anatomical observations in bright-field microscope (LM), transverse and longitudinal sections of the elaiophores of 18 species studied were obtained, dehydrating the material in an ethanol series, transferred to xylene, embedded in paraffin (58 °C), and sectioned at a thickness of 7–9 μm on a rotary microtome (Leitz Wetzlar) using conventional methods. Histological samples were stained with Safranin-Fast Green and mounted in Canada balsam (Zarlavsky 2014).

Assemblages of pollinators

To study the assemblages of potential pollinators of the species of Malpighiaceae, we captured oil-collecting bee species on flowers, in different natural populations, and in different days and times (2–6 days per population, between 8:00 and 19:00 h). We considered as potential legitimate pollinator those species that we observed contacting the reproductive structures during foraging behavior. Later, in the laboratory, the bee species captured were identified and assigned to three size groups depending on their intertegular spans (Cane 1987): medium, intertegular spans < 4 mm;

large, 4–6 mm; and very large, > 6 mm (Torretta et al. 2017). Measurements were taken using a micrometer to the nearest 0.1 mm, under a stereomicroscope in the laboratory, and we calculated an average pollinator size for each species of bee. The collected bees were deposited in the Entomological Collection of the General Botany Unit, Facultad de Agronomía, Universidad de Buenos Aires, Argentina (FAUBA), and the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN).

Statistical analysis

We first calculated the mean for values of floral diameter (cm), length, width, and thickness of the elaiophore (mm), thickness of the cuticle (μm), and length and width of the epithelial cells (μm) of flowers ($n = 10$) for each species (Table 2). For those species represented by several populations, we average the values obtained for each population studied. It is worth mentioning that two species with a high number of populations studied, the flower size did not vary significantly throughout the distribution for *S. bonariense* ($n = 11$ populations) and it did for *S. jatrophifolium* A. Juss. ($n = 6$ populations) (Torretta et al. 2017). However, the size of the flowers in all populations can be considered large. On the contrary, in both species, the total area of elaiophores per flower decreased inversely with latitude (Torretta et al. 2017). Correlation between these calculated variables was tested by Spearman's rank correlation using the "rcorr" function from the "Hmisc" package (Harrell 2018) in the statistical program R-3.4.3 (R Core Team 2017). Spearman's rank correlation coefficient (rs) is equal to Pearson correlation coefficient but based on ranked variable values (Quinn and Keough 2002).

Results

Variation in the number of elaiophores

The glands in the sepals of all studied specimens reacted positively with Sudan III solution confirming the presence of lipids; so the glands correspond to elaiophores. The most frequent condition is eight elaiophores per flower, the glands are placed in pairs on the abaxial surface of the anterior-lateral and posterior-lateral sepals, and the anterior sepal usually lacks elaiophores (Fig. 1a–b). However, we detected some species with individuals with flowers with variable number of elaiophores; even though eight glands is common, flowers with 7, 9, and 10 well-developed glands were identified in *Banisteriopsis muricata* (Cav.) Cuatrec. (population Provincial Park Teyú Cuaré, Misiones), *Callaeum psilophyllum* (A. Juss.) D.M. Johnson (population Martín García Island, Buenos Aires, Fig. 1e), *Janusia guaranitica*

Table 2 Mean values for floral diameter and morphological and anatomical characteristics of the elaiophore (length of the elaiophore, width of the elaiophore, thickness of the elaiophore, thickness of the cuticle, length of the epithelial cells, width of the epithelial cells) for the 18 studied species of Malpighiaceae

Species	Floral diameter (cm)	Length of the elaiophore (mm)	Width of the elaiophore (mm)	Thickness of the elaiophore (mm)	Thickness of the cuticle (μm)	Length of the epithelial cells (μm)	Width of the epithelial cells (μm)
Aspicarpa pulchella	2.25	1.40	0.80	0.30	3.50	50.00	7.75
Banisteriopsis muricata	1.55	2.35	0.75	0.15	3.50	55.00	7.50
Callaeum psilophyllum	1.65	1.60	1.30	0.70	6.25	105.00	7.00
Dicella nucifera	1.85	2.60	0.80	0.85	5.50	47.50	7.75
Heladena multiflora	1.10	1.55	1.25	0.40	5.25	60.00	10.00
Heteropterys argyrophaea	0.95	1.25	0.65	0.65	6.00	65.00	7.00
Heteropterys glabra	1.60	1.70	1.10	0.45	7.50	77.50	8.50
Heteropterys hypericifolia	0.90	1.45	0.70	0.25	7.50	65.00	7.00
Heteropterys intermedia	0.90	1.30	0.45	0.25	7.00	80.00	7.00
Hiraea fagifolia	1.90	1.75	1.10	0.55	3.25	65.00	7.25
Janusia guaranitica	1.40	1.60	0.50	0.30	4.50	105.00	9.50
Mascagnia divaricata	1.40	1.90	1.05	0.90	4.75	37.50	8.00
Stigmaphyllon bonariense	2.55	2.75	1.45	0.75	4.50	97.50	7.50
Stigmaphyllon jatrophifolium	2.40	2.35	1.45	0.55	3.50	32.50	6.50
Tricomaria usillo	1.40	1.55	0.60	0.25	6.00	31.00	7.50
Heteropterys dumetorum	1.65	1.25	0.85	0.25	9.00	55.00	7.00
Heteropterys syringifolia	1.30	1.45	0.55	0.40	6.75	55.00	7.00
Niedenzuella sericea	1.40	2.75	1.05	0.85	8.50	60.00	8.50

(A. St.-Hil.) A. Juss. (population Embarcación, Salta), *Heteropterys argyrophaea* A. Juss. (population Apóstoles, Misiones), *H. glabra* Hook. & Arn. (population Candelaria, Misiones), *H. syringifolia* Griseb. (population Leandro N. Alem, Misiones), *Hiraea fagifolia* (DC.) A. Juss. (population National Park Iguazú), and *Stigmaphyllon bonariense*

Fig. 1 Variation in the number of elaiophores. **a–b** Flowers with elaiophores without stain (left) and stained with Sudan III (right); **a** *Heteropterys glabra*, the stained flower (right) with 7 elaiophores. **b** *Stigmaphyllon bonariense*. **c–d** Flowers with eglandular morph; **c** *Hiraea fagifolia*, **d** *Heteropterys intermedia*. **e** Flower with atypical number of elaiophores in *Callaeum psilophyllum*. References: pp posterior petal (flag petal), as anterior sepal (eglandular sepal). *Scale bars* **a, c** 3.5 mm, **b** 5 mm, **d** 1 mm, **e** 2.5 mm
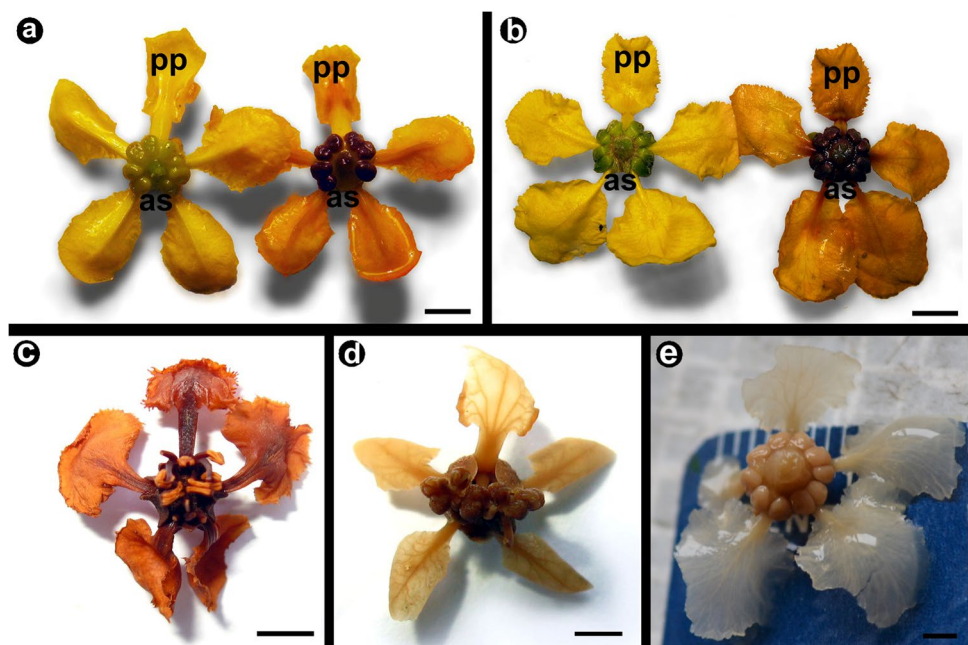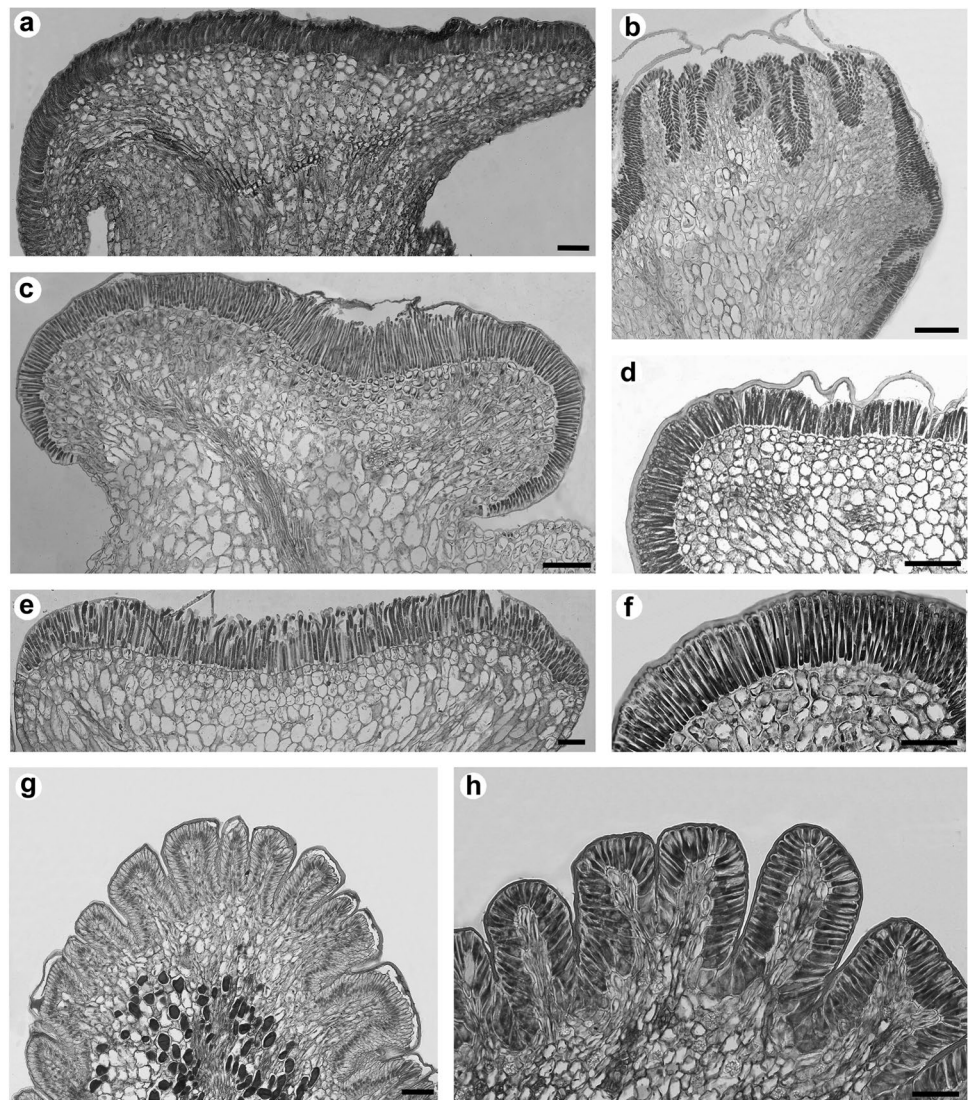
Fig. 2 Bright-field microscope images, longitudinal sections of elaiophores. **a** *Heladena multiflora*, **b** *Tricomaria usillo*, **c** *Callaeum psilophyllum*, **d** *Heteropterys syringifolia*, **e** *Janusia guaranitica*, **f** *Heteropterys hypericifolia*, **g** *Dicella nucifera*, **h** *Stigmaphyllon jatrophifolium*. *Scale bars* **a–c, e–h** 50 μm, **d** 20 μm

(population cultivated in Ciudad Autónoma de Buenos Aires). Moreover, we observed some species with flowers with elaiophores (glandular morph) and flowers without elaiophores (eglandular morph) in the same individuals. This characteristic was detected in *Aspicarpa pulchella* (Griseb.) O´Donell & Lourteig (population Candelaria, Misiones), *Hiraea fagifolia* (population National Park Iguazú, Misiones, Fig. 1c), and *Heteropterys intermedia* (A. Juss.) Griseb. (population San Ignacio, Misiones, Fig. 1d). Contrarily, *Tricomaria usillo* Hook. & Arn. is distinguished because all analyzed specimens invariably presented flowers with 10 elaiophores; thus, all sepals are glandular in this species. However, in some flowers of this species, the elaiophores of the anterior sepal are frequently very close or partially fused to the correspondent neighboring elaiophores of the adjacent sepals, and the flower seems to have eight glands (Fig. 4b).

Morphological and anatomical characteristic of the elaiophores

The oil gland of all studied species shows a common pattern. The elaiophores are ovate, rounded or narrow ovate in outline, 1.0–3.0-mm long, 0.4–1.6-mm wide, sessile to subsessile, with the exception of *Heladena multiflora* (Hook. & Arn.) Nied. which presents peltate glands (Fig. 3a–b). The secretory epithelium is formed by uniseriate and columnar epidermal cells, 25–110-μm long, 5–11-μm wide, with big nucleus, dense cytoplasm, and small vacuoles; the cuticle varies between 3- and 9-μm thick. Usually blisters formed by distention of the cuticle could be observed (Fig. 2b–e). In most of the samples, where the cuticle was broken or detached, it was observed that the columnar epidermal cells are not fused together laterally, giving the secretory tissue a trichomatous papilose appearance (Fig. 2c–e).

Fig. 3 Scanning-electron micro-scope images. **a–b** *Heladena multiflora*, **a** complete calix with eight peltate glands and in left inferior angle a detail of a elaiophore with small broken cuticular blister (arrow), **b** pair of elaiophores corresponding to the left and right posterior lateral sepals. **c** Elaiophores of *Heteropterys argyrophaea*. **d** Elaiophores of *Heteropterys dumetorum*, the arrows indicate the secretion after the cuticle is broken. **e** Elaiophores of *Heteropterys hypericifolia*. **f** Elaiophores of *Heteropterys syringifolia*. *Scale bars* **a** 1000 μm, **b–f** 500 μm
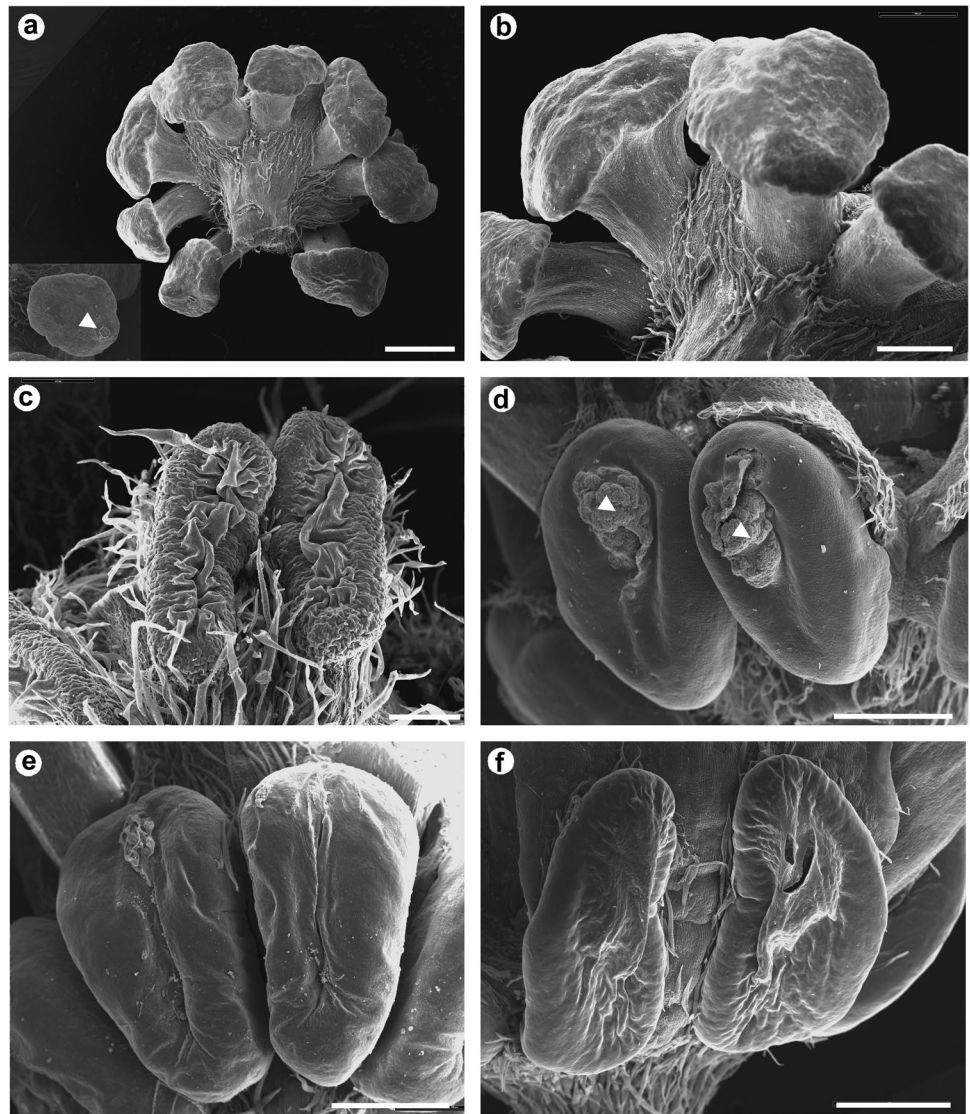

Under the epidermal cells, parenchymatic cells are commonly isodiametric, and in most species, two regions can be distinguished: subsecretory parenchyma directly below the epithelium and internal parenchyma. Subsecretory parenchyma comprises 1–4 rows of cells, compactly arranged, without intercellular spaces, with dense peripheral cytoplasm, evident nuclei, abundant plastids, and a large central vacuole, while the internal parenchyma has larger cells of translucent cytoplasm, small intercellular spaces, druses, and tannin deposits (Fig. 2f–g) are frequently present in this area that is vascularized by xylem and phloem (Fig. 2a–c, g–h).

Although the general pattern is common to all elaiophores, considering the clades of the studied species, some differences can be observed. The tristellateioids clade, represented here by *Heladena multiflora,* is the only studied species to present peltate elaiophores, with a peduncle 0.5–0.7-mm high, and smooth to irregular surface, a small blister in the middle to distal zone of the elaiophore (Figs. 2a and

3a–b). The elaiophores of *Hiraea fagifolia*, which belongs to the hiraeoids clade, are characterized by smooth surface, slightly concave, with blisters usually formed in the middle to distal zone.

We studied species in four clades into the tetrapteriods clade. The elaiophores in *Heteropterys* Kunth that belongs to heteropterys clade (tetrapteriods clade) are relatively similar in the six studied species, generally with smooth, or irregular surface in *H. argyrophaea*, with a central longitudinal depression or fold where the blisters are frequently formed (Figs. 2d, f and 3c–f). In the carolus clade (tetrapteroids clade), mature elaiophores present striate surface. They are very notorious in *Dicella nucifera* Chodat with the folds being radiantly oriented to the distal zone (Figs. 2g and 4a). In *Tricomaria usillo*, they present a striate surface, their folds being less notorious and located more parallel (Figs. 2b and 4b). The elaiophores of *Callaeum psilophyllum* in the christianelloids clade (tetrapteroids clade) present

Fig. 4 Scanning-electron microscope images. **a** Elaiophores of *Dicella nucifera* with striate surface. **b** Complete calix of *Tricomaria usillo* with ten glands, arrow indicate two contiguous elaiophores of the anterior and the anterior-lateral sepals partially fused on the bases of the glands. **c** Elaiophores of *Janusia guaranitica*. **d** Elaiophores of *Aspicarpa pulchella*. **e** Elaiophore of *Stigmaphyllon bonariense* with striate surface. **f** Elaiophore of *Stigmaphyllon jatrophifolium* with striate surface. *Scale bars* **a** 1000 μm, **b–f** 500 μm
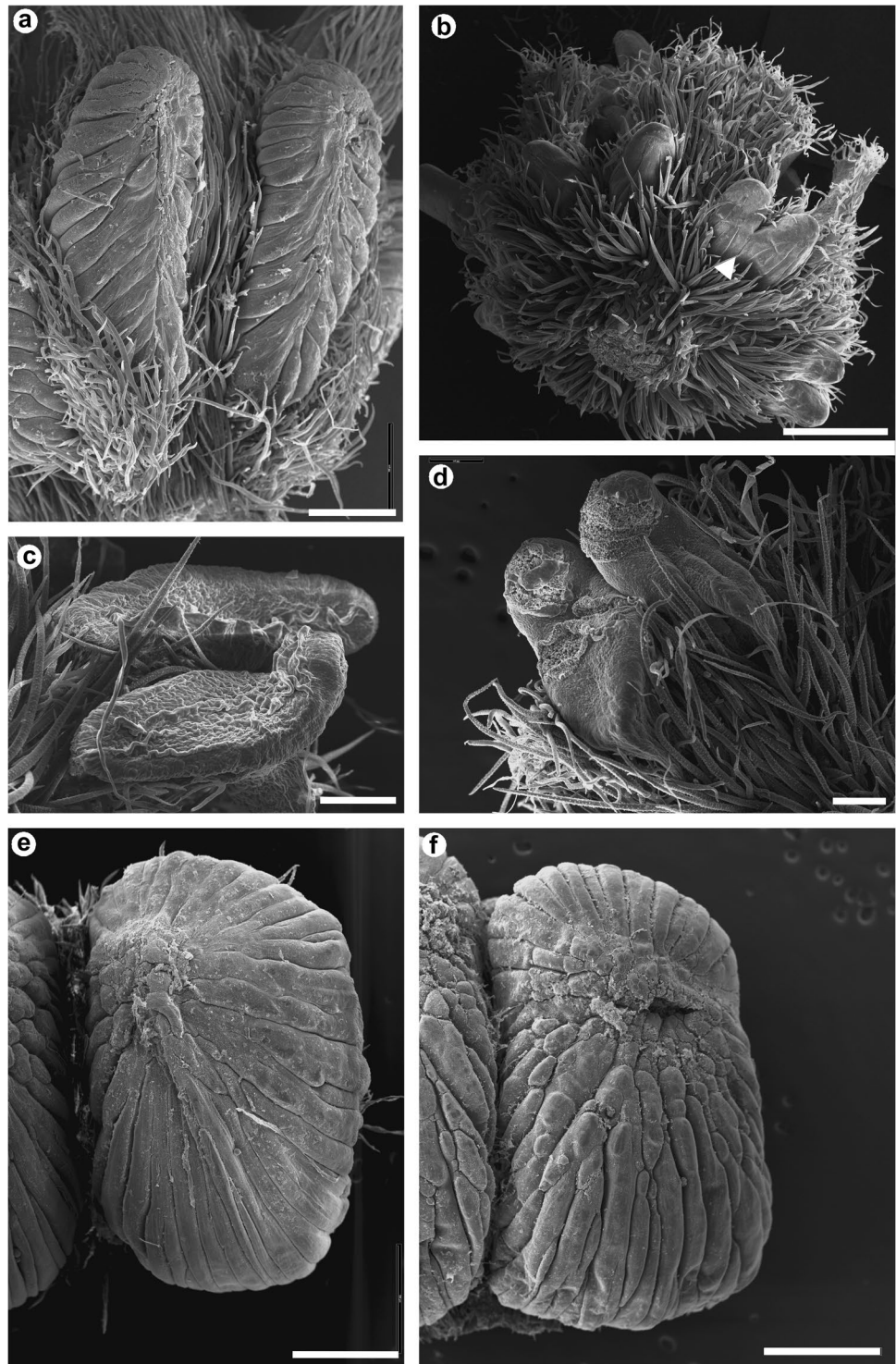
smooth surface with blisters usually formed in the middle zone (Fig. 2c). The surface of the elaiophores in *Niedenzuella sericea* (A. Juss.) W.R. Anderson in the niedenzuella clade (tetrapteroids clade) is also smooth, but with a slight concavity in longitudinal direction in which the blister is usually formed.

In the unresolved stigmaphylloids clade, there is high morphological variability: *Janusia guaranitica* (Figs. 2e and 4c) and *Aspicarpa pulchella* (Fig. 4d) that belong to the aspicarpoids clade have elaiophores with smooth surface, with blisters usually in distal and middle zone; while the glands of *Banisteriopsis muricata*, *Stigmaphyllon*

Table 3 Spearman's rank correlation coefficients (r_s) among floral diameter and morphological and anatomical characteristics of the elaiophore (length of the elaiophore, width of the elaiophore, thickness of the elaiophore, thickness of the cuticle, length of the epithelial cells, width of the epithelial cells) for the 18 studied species of Malpighiaceae. (*) Asterisks indicate significant correlations ($p < 0.05$)

	Floral diameter (cm)	Length of the elaiophore (mm)	Width of the elaiophore (mm)	Thickness of the elaiophore (mm)	Thickness of the cuticle (µm)	Length of the epithelial cells (µm)
Length of the elaiophore (mm)	0.50*					
Width of the elaiophore (mm)	0.64*	0.53*				
Thickness of the elaiophore (mm)	0.31	0.55*	0.54*			
Thickness of the cuticle (µm)	−0.49*	−0.35	−0.23	-0.09		
Length of the epithelial cells (µm)	−0.16	−0.08	0.02	0.02	0.18	
Width of the epithelial cells (µm)	−0.01	0.36	0.08	0.20	−0.13	0.04

bonariense (Fig. 4e), and *S. jatrophifolium* (Figs. 2h and 4f) have striate surface, folds located radially, and blisters in the central zone.

Finally, the elaiophores of *Mascagnia divaricata* (Kunth) Nied., of the malpighioids clade, are variable in size among the sepals, being the largest those that are next to the flag petal, the surface is smooth, and the location of the blister is variable.

Visits of pollinators and correlation of the characters of elaiophores

We collected potential pollinators in 13 species of studied Malpighiaceae. The different populations were visited by 12 species of *Centris*, five species of *Epicharis* (Centridini), and two species of *Monoeca* (Tapinotaspidini) (Table 1). Despite the efforts in observation, no potential pollinators were observed in *Heteropterys dumetorum* (Griseb.) Nied., *H. syringifolia*, *Niedenzuella sericea*, *Heladena multiflora*, and *Aspicarpa pulchella*. In relation to body size of the pollinators, nine species were classified as medium, five as large. and five as very large species (Table 1).

We found a significant positive correlations between the floral diameter and length of the elaiophore (rs = 0.50, *n* = 18, *P* < 0.05), and with the width of the elaiophore (rs = 0.64, *n* = 18, *P* < 0.05) (Table 3). On the contrary, there was a significant negative correlation with thickness of the cuticle (rs = −0.49, *n* = 18, *P* < 0.05) (Table 3). There were also positive correlations between the length of the elaiophore and the width of the elaiophore (rs = 0.53, *n* = 18, *P* < 0.05) and with the thickness of the elaiophore (rs = 0.55, *n* = 15, *P* < 0.05) (Table 3). Finally, we found a significant positive correlation between the width of the elaiophore and the thickness of the elaiophore (rs = 0.54, *n* = 15, *P* < 0.05) (Table 3).

The results indicate that the medium-size oil-collecting bees visited species of Malpighiaceae independently of diameter of the flowers and size of the elaiophores. However, large and very large bees tend to visit species with flowers from medium to large size and with larger elaiophores (Fig. 5). The results also indicate a potential association between the thickness of the cuticle of the elaiophore, the floral diameter, and the visitors' genera (Fig. 6). Individuals of *Monoeca* tend to visit species with smaller flowers but elaiophores with a thick cuticle (Fig. 6). On the other hand, individuals of *Epicharis* visited bigger flowers, with elaiophores having thinner cuticle. Individuals of *Centris* visited flowers independently of their size (Fig. 6).

Discussion

We studied 18 species and only four of them (*Heteropterys dumetorum, H. hypericifolia* A. Juss*., Niedenzuella sericea, Mascagnia divaricata*) showed in all sampled flowers, the typical generalized pattern associates with the floral elaiophores (absence of eglandular morphs, constant number of glands, sessile to subsessile glands, smooth to irregular surface). The remaining species were variable and some of these characters exhibited some kinds of discrepancies. Two of these differences are persistent at inter-specific level, such as the presence of peltate elaiophores (Fig. 7, purple triangles) and surface with folds (Fig. 7, blue squares). Other differences, however, such as variations in the number of elaiophores (Fig. 7, red diamonds) and the presence of eglandular flowers (Fig. 7, green circles), were inconsistent within the same individual and among populations of a species.

Variation in the number of elaiophores

Our observations revealed that, although the most frequent condition is that the flowers have 8 glands, variation can be present within a plant; we find a certain variability in the number of elaiophores and the presence of glandular and eglandular flowers in a same individual. Eight species showed few flowers within the same plants with 7, 9, and 10 well-developed elaiophores (*Banisteriopsis muricata, Callaeum psilophyllum, Heteropterys argyrophaeae, H. glabra,*
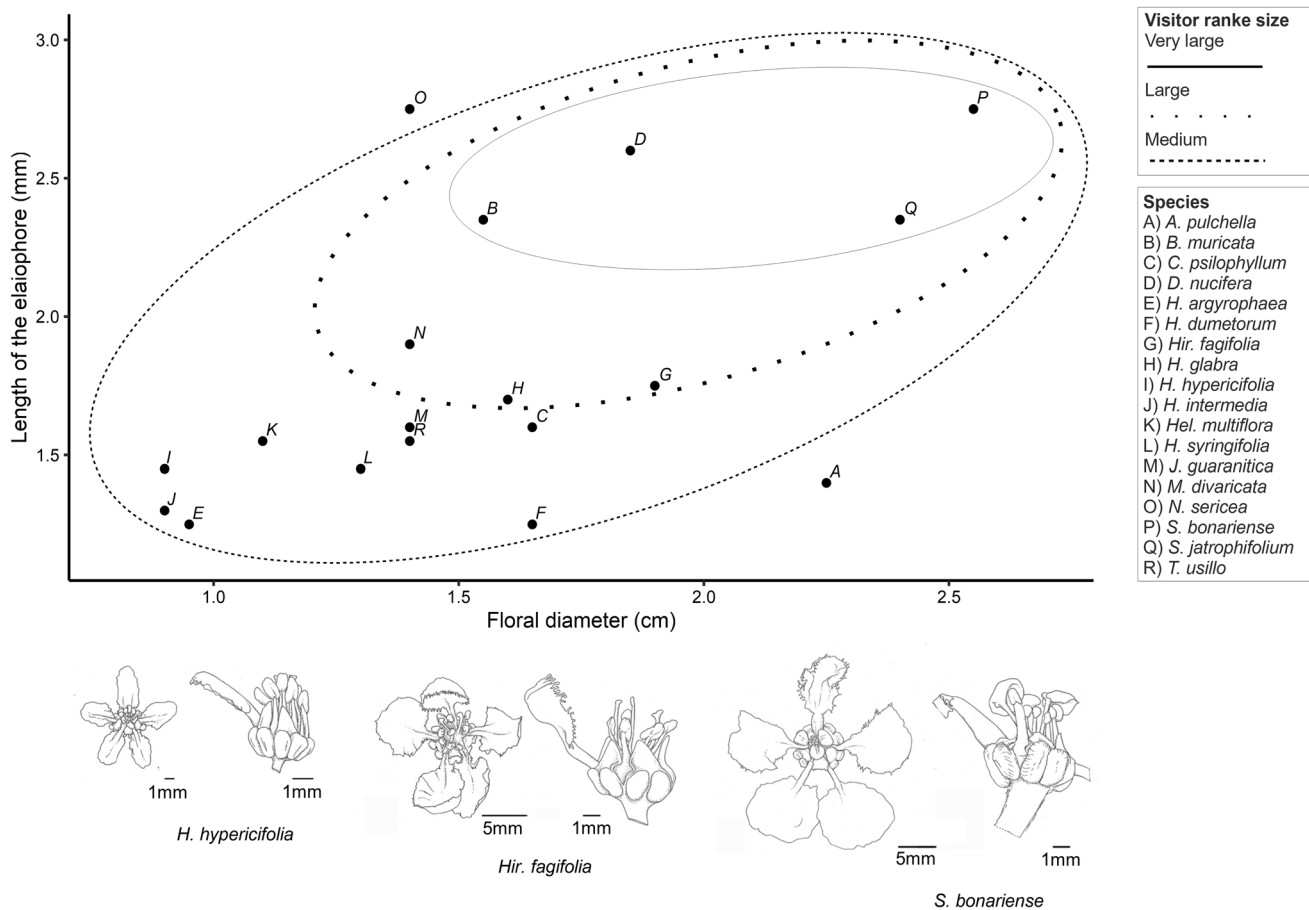
Fig. 5 Association between floral diameter (drawings of three species are included as examples of different flower sizes), length of the elaiophore and oil-collecting bees size. According to their intertegular spans, bees were categorized as medium, large, and very large

visitors and Malpighiaceae species were grouped in the scatter plot as shown by general pattern. Correlation between length of the elaiophore and oil-collecting bees is significant (rs = 0.5; $P < 0.05$)

H. syringifolia, Hiraea fagifolia, Janusia guaranitica, Stigmaphyllon bonariense); and three species presented flowers with or without elaiophores in a same plant (*Aspicarpa pulchella, Hiraea fagifolia, Heteropterys intermedia*). These conditions were present in several linages and seem to be frequent in Malpighiaceae (Fig. 7), however in our study was not constant and varied among the populations analyzed in each of the species.

Anderson (1990) points out that the presence of eglandular morphs in oil-rewarding species of Neotropical Malpighiaceae is a condition that can be found at every taxonomic-level individual within populations and populations within species. Sazima and Sazima (1989) reported eglandular morphs in *Heteropterys aceroides* Griseb. (currently *H. intermedia*) and *Banisteriopsis muricata* in Brazil., and demonstrated that eglandular morphs attract oil-gathering bees by deceit representing a kind of automimicry. Because oil production is an energetically valuable resource, automimicry would increase the attractiveness of pollinators at a lower cost. However, this hypothesis would seem to be valid

for species with explosive flowering and/or inflorescences with a large number of flowers (as for example *H. intermedia*). Our results in *Aspicarpa pulchella*, a subshrub erect with few inflorescences uniflorous (Aliscioni and Torretta 2017), could suggest resource limitation. Other species that were mentioned having populations/individuals with both floral morphs are *Byrsonima sericea* DC. (Teixeira and Machado 2000), *Stigmaphyllon paralias* (Carvalho et al. 2005), and *Pterandra pyroidea* A. Juss. (Cappellari et al. 2011). These last authors indicated that *P. pyroidea* is undergoing a transition in pollination syndrome, from a specialized (oil-rewarding flower) towards a generalized (pollen-rewarding flower) pollination system, which would seem to be a favorable condition to increase the range of pollination.

The majority of the Neotropical species of Malpighiaceae have eight glands in the flowers because four sepals have two elaiophores and the anterior sepal lacks glands, and probably the absence of glands in the anterior sepal arose very early in the family. However, some atypical cases were observed in eight-glandular species, such as some flowers with 7, 9,
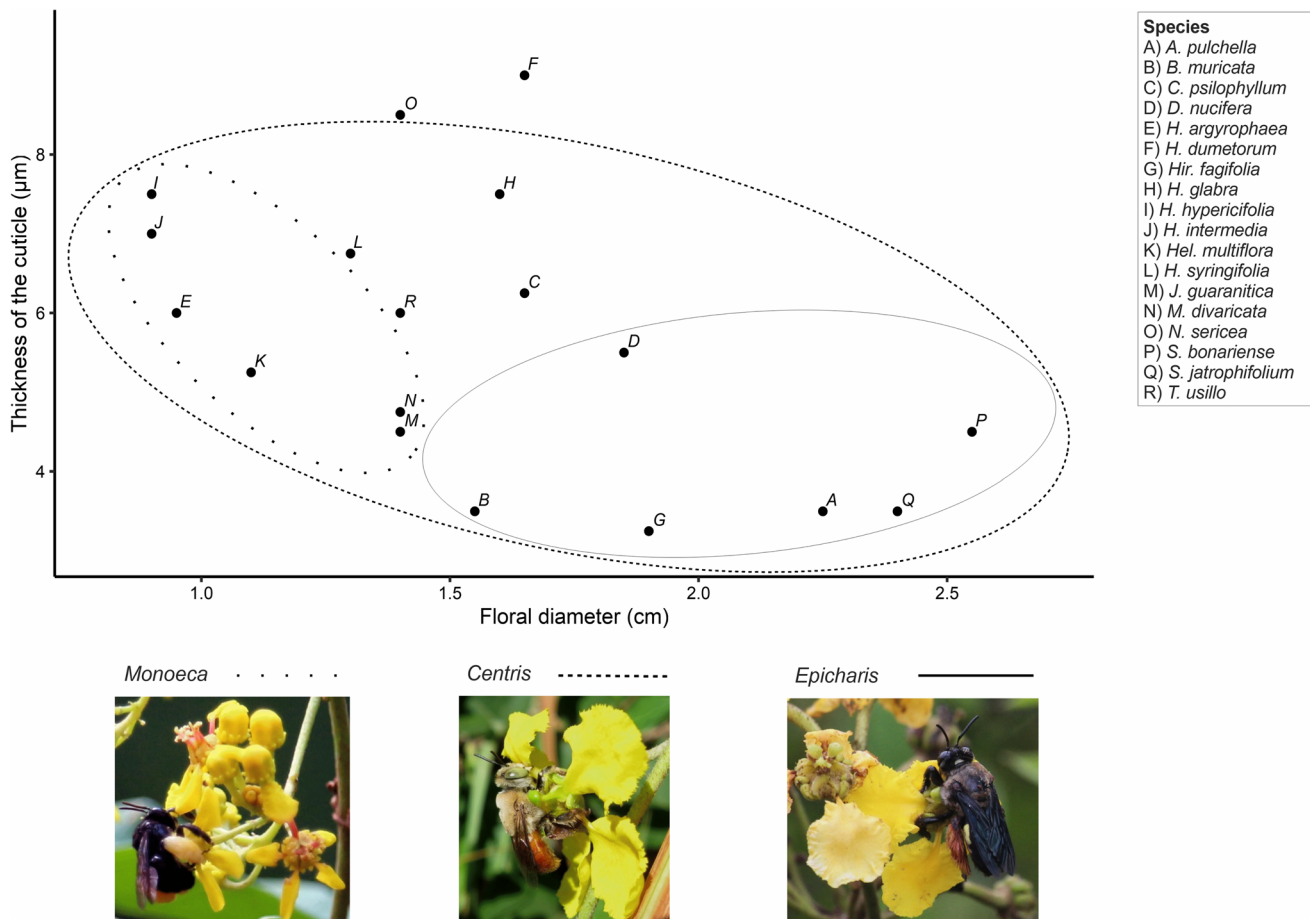
Fig. 6 Association between floral diameter, thickness of the cuticle and oil-collecting bees´ genera. Legitimate visitors included species of *Monoeca*, *Centris*, and *Epicharis* and Malpighiaceae species were grouped in the scatter plot as shown by general pattern. Correlation between floral diameter and thickness of the cuticle is significant (rs = 0.49, $P < 0.05$)

or 10 elaiophores in this work, or some species with small or vestigial glands in the anterior sepal as was noted by Gates (1982). Based on anatomical observations, Souto and Oliveira (2014) demonstrated that the absence of gland in the anterior sepal can be produced by two different processes; some species loss the glands of the anterior sepals by an evolutionary process of reduction inherited from a common ancestor or by connation caused because the lateral vascular bundles of the anterior sepal being shared with the adjacent lateral sepals. The first case would explain the presence of irregular numbers of elaiophores in typical eight glands species and the presence of vestigial glands in the anterior sepal. On the other hand, the initiation of connation process could be present in some examined specimens of *Tricomaria usillo*. This xerophitic endemic species of Argentina is distinguished from the rest of the studied species for having always ten elaiophores, so all sepals are glandular. However, in some flowers, the two elaiophores of the anterior sepal are close to the respective adjacent anterior-lateral sepals. And in few cases, the anterior glands are partially fused to the

gland of the contiguous sepal, similar than was described in *Mascagnia cordifolia* (A. Juss.) Griseb. (Souto and Oliveira 2014). The presence of ten glands is verified as an ancestral condition in Malpighiaceae (Souto and Oliveira 2014). Our observations would indicate that *Tricomaria usillo*, an isolated geographically species, could have retained some ancestral characteristics as having all glandular sepals, but it could be beginning an initial process of connation directed towards the loss of elaiophores in the anterior sepal, a condition commonly derived in the Neotropical species.

Morphological and anatomical characteristic of the elaiophores

Regarding the internal structure of the elaiophores, our results coincide with the general description reported for other species of Malpighiaceae (Vogel 1974; Subramanian et al. 1990; Cocucci et al. 1996; Castro et al. 2001; Possobom et al. 2015; Araújo and Meira 2016; Possobom and Machado 2017, 2018). The epithelial cells of the elaiophores

Fig. 7 Schematic representation of species studied positioned in an abbreviated phylogeny postulated by Anderson et al. (2006) and Davis and Anderson (2010). References: green circle, presence of eglandular flowers; purple triangle, peltate elaiophores; red diamond, flowers with atypical number of elaiophores (7, 9, 10) in species eightglands; blue square, elaiophores with surface with folds. Species without symbols: typical pattern of floral elaiophores (absence of eglandular flower, sessile to subsessile elaiophores, constant number of elaiophores, with smooth to irregular surface)
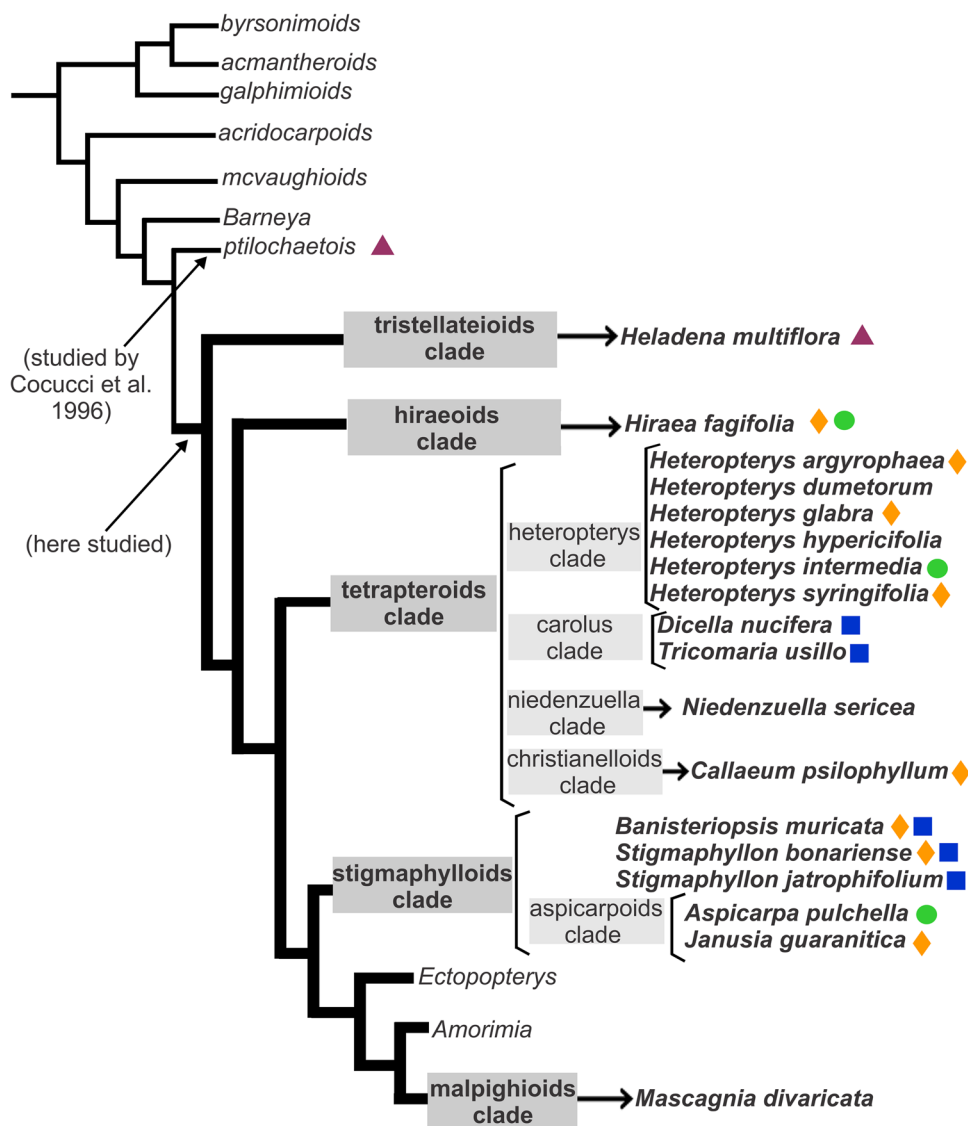
showed the typical characteristics of a secretory cell, very active metabolically, with big nucleus, small vacuoles, and a dense intensely colored cytoplasm. In some cases, its function seems to be complemented by the subsecretory parenchymal tissue adjacent to the epidermis. In some of the species analyzed, no such distinction was observed; however, it is estimated that it could be due to variations in the state of gland secretion. Likewise, vascular bundles with xylem and phloem were observed in the internal parenchyma, which is also important for the provision of nutrients necessary for secretion (Evert 2006). The presence of druses and tannin deposits were a frequent characteristic in the analyzed elaiophores, common in cells of the most internal parenchymal zone. Such cellular contents could be associated with some protection mechanism by deterring the attack of herbivores or pathogens and maintaining the glands in optimal condition for the pollinators (Cote and Gibernau 2012).

The analyzed elaiophores here are from sessile to subsessile; Possobom and Machado (2018) described two types of glands for those elaiophores that are not notoriously peltate; one type called subsessile, with convex surface, and marginally inserted, and a second type called shortly peltate, with concave surface, and more centrally inserted in the sepal. These authors point out that the second type may be more efficient in the use of energy since the glandular epithelium is restricted only to the upper surface. However, in the present study, we could not establish a precise boundary between these two different types.

Heladena multiflora (tristellateioids clade) was the only analyzed species which has truly peltate elaiophores. In these elaiophores, the distance between the secretory surface of the gland and the insertion site in the sepal may be related to the interaction between the pollinator and the

gland and should have ecological impact (Araújo and Meira 2016). However, Simpson (1989) studied the peltate elaiophores of *Dinemandra* A. Juss. and *Dinemagonum* A. Juss. and indicated that the behavior of *Centris* in these two species is basically similar to that observed in most tropical Malpighiaceae, with non-peltate elaiophores. Unfortunately, we could not observe any potential pollinators in *Heladena multiflora* to test these contrasting hypotheses. These species have small flowers and the pedicels of the elaiophores locate the glandular surfaces in such a way as to facilitate the stereotyped foraging behavior of oil-collecting bees (Simpson 1989; Cocucci et al. 1996).

Peltate elaiophores are present in the species *Heladena multiflora* and its sister genus *Henleophytum* H. Karst. of the clade tristellateioids, and in *Dinemagonum gayanum* A. Juss. and *Dimenandra ericoides* A. Juss. (Cocucci et al. 1996) of the clade ptilochaetoids. Mapping these characters in the phylogeny of the family (Davis and Anderson 2010) is probably that the peltate elaiophores could have a common phylogenetic origin in an ancestral lineage that included the ptilochaetoids clade, was conserved in the branch that includes *Heladena multiflora* and its sister genus *Henleophytum*, but was lost in other lineages of both clades (Fig. 7). However, the pelted glands of *Dinemagonum* and *Dimenandra* are basally connate, forming pairs between glands on adjacent sepals (Simpson 1989; Cocucci et al. 1996), which differs from those of *Heladena multiflora* (Fig. 3a–b) and *Henleophytum*. Also, the number of elaiophores for flower varies between these clades, six in species of ptilochaetoids clade and eight in those in tristellateioids clade. Therefore, these differences suggest a possible independent origin of these glands in each clade.

Possobom and Machado (2017) studied the elaiophores of *Banisteriopsis variabilis* B. Gates and described the secretory surfaces with folds or convoluted. Araújo and Meira (2016) mentioned that this character is widely represented in species of the genus *Banisteriopsis* C.B. Rob and Vogel (1974) in *Stigmaphyllon littorale* A. Juss. (currently *S. bonariense*). The presence of elaiophores with surface with folds was here observed in *Dicella nucifera* and *Tricomaria usillo* (carolus clade into tetrapteroids) and *Banisteriopsis muricata, Stigmaphyllon bonariense*, and *S. jatrophifolium* (stigmaphylloids clade). Our results for *S. bonariense* agree with those reported by Vogel (1974). Thus, the presence of this kind of glands was previously known for some representatives of the stigmaphylloids clade, and after our observations, some species of the carolus clade would also be added (Fig. 7). This attribute was interpreted as increasing the secretory surface and the location where the exudate is accumulated, making available a larger amount of oil to pollinators (Araújo and Meira 2016).

Visits of pollinators and correlation of the characters of elaiophores

Concerning pollinators, we observed that species of medium-sized bees visited small to large flowers, while large and very large-sized bees prefer species of Malpighiaceae with big flowers (Fig. 5); the bigger bees probably access more resource in these flowers because the glands are also bigger. The medium-sized bees required development periods shorter than large and very large bees (Michener 2007), therefore emerged earlier in the flowering season than large and very large species. This allows that these bees used all Malpighiaceae species available. Possibly, with the appearance of the large and very large species, and their preferential use of the largest flowers, the medium-sized bees are displaced to species of Malpighiaceae with smaller flowers. Additionally, in Argentina, large and very large-sized bees are less abundant and diverse than medium bees, such as *Centris trigonoides* and *C. tarsata* which exhibited an ample geographic range (Roig-Alsina 2000; Torretta et al. 2017) and interact with more variable flower size. On the other hand, the thickness of the cuticle of the elaiophore presented a negative association with the floral diameter.

In the present work, some species of *Epicharis*, a genus with large to very large bees, were observed collecting floral oil on *Hiraea fagifolia, Banisteriopsis muricata, Dicella nucifera*, and *Stigmaphyllon bonariense* that have flowers of relatively medium to large size (floral diameter 1.2–2.7 cm). The genus *Epicharis* have conspicuous basitarsal combs formed by single rows of large, erect, apically flattened, expanded, and curved setae on both the fore- and mid-basitarsi (Neff and Simpson 1981). This particular arrangement allows the maintenance of a continuous rigid scraping edge while providing sufficient flexibility to allow the comb to conform the irregular surfaces of the oil glands (Neff and Simpson 1981). The cuticle of the elaiophores in the flowers of *Hiraea fagifolia, Banisteriopsis muricata, Dicella nucifera*, and *Stigmaphyllon bonariense* resulted moderate to thinner than other studied species, and additionally the three last named species have elaiophores with striate surface. Thus, the flexibility of the comb may adapt better to an irregular surface, although they would make less pressure to break the cuticle. More observations are necessary to ratify this idea, since the genus *Epicharis* collects floral oils exclusively in Malpighiaceae (Martins et al. 2015; Martins and Melo 2016) and was reported by other authors on different species of *Banisteriopsis, Byrsonima* Rich. ex Kunth, *Heteropterys, Mascagnia* (Bertero ex DC.) Bertero, *Peixotoa* A. Juss., *Stigmaphyllon* A. Juss., among others (Vogel 1974; Gottsberger 1986; Albuquerque 1989; Albuquerque and Rêgo 1989; Sazima and Sazima 1989; Barros 1992; Pedro 1994; Rebêlo 1995; Albuquerque and Mendonça

1996; Gaglianone 2000; 2003; Teixeira and Machado 2000; Neves and Viana 2001; Gimenes et al. 2002).

We identified two species of *Monoeca*, a genus with medium size bee, as legitimate floral visitors on flowers of *Heteropterys intermedia*, *H. argyrophaea*, and *Mascagnia divaricata*, all species with small to medium flowers (floral diameter 0.8–1.5 cm). These results would indicate that these oil-collecting species principally use small/medium flowers of species of Malpighiaceae. We could also notice that these species have elaiophores with a thick cuticle (4.5–7.5 μm). Neff and Simpson (1981) described the basitarsal combs of *Monoeca* as being similar in shape and position to those of the Centridini species, but a distinctive feature is that the posterior setae of the ventral surface show a degree of enlargement that appears to be unique in this genus. Although these bees would visit small/medium flowers, they would have particularly large setae to break thick cuticles in the elaiophores. There are previous studies on floral records and pollen loads on *Monoeca* that corroborate association with Malpighiaceae (Gottsberger, 1986; Cunha and Blochtein, 2003; Sigrist and Sazima, 2004; Rozen et al. 2006; da Rocha-Filho and Melo 2011), particularly with the genus *Heteropterys* (Torretta and Roig Alsina 2016); however, relations with the morphology of elaiophore were not mentioned. *Monoeca*, similarly to *Epicharis*, forage exclusively Malpighiaceae species flowers for oil (Aguiar et al. 2020).

There are numerous works that mention species of *Centris* collecting floral oils on diverse genus of Malpighiaceae, as *Banisteriopsis, Byrsonima, Dimenandra, Dinemagonum, Heteropterys, Janusia* A. Juss.*, Lophanthera* A. Juss.*, Macvaughia* W.R. Anderson*, Malpighia* L.*, Mascagnia, Peixotoa, Stigmaphyllon, Tricomaria* (Vogel 1974; Raw 1979; Simpson and Neff 1983; Simpson 1989; Aguiar 2003; Gaglianone 2003; Aliscioni et al. 2019). However, the species of *Centris* does not collect oils exclusively on the Malpighiaceae family (Vogel 1988; Roig Alsina 2000; Vivallo 2013; Martins et al. 2015; Martins and Melo 2016; Vivallo 2020), and bees of this genus can visit both epithelial and trichomatic elaiophores. Some species of *Centris* have adapted to the collection of oils in other families such as Calceolariaceae and Plantaginaceae (Vogel 1974; Vogel and Machado 1991; Machado 2002; Sersic 2004; Cosacov et al. 2012; Tadey 2012; Martins et al. 2013; Giannini et al. 2013). The versatile capacity of the bee of the genus *Centris* to collect oil in epithelial and trichomatous elaiophores is also seen, in some way, within the Malpighiaceae. We observed in some cases, once the cuticle is broken, that the elaiophores have a very similar appearance to a trichomatous gland. Possobom and Machado (2018) explained in *Banisteriopsis variabilis* and *Peixotoa reticulata* Griseb. that elongated and narrow cells of the epithelium become papillose by dissolution of the middle lamella, contributing to an increase in secretory surface, and show similarity to trichomatous elaiophores.

Contrasting to the constancy of comb structure in species of *Epicharis* and *Monoeca*, the species of *Centris* are more variable in this aspect; the most distinctive feature of the fore-basitarsal is the presence of a series of giant spatulate setae inserted on the posterior portion of the flattened ventral surface of the basitarsus, which differ in number and enlargement depending of the species. Neff and Simpson (1981) point out that this variation appears to be related to the range of oil flowers utilized by a particular species because the apices of at least some of the giant setae are expanded and overlap in different way. Vogel (1974) called this combination of characters a "hood organ" that would probably permit a greater range of scraping motions, but this structure can be absent or little developed in some species of *Centris*.

Neff and Simpson (1981) showed that species such as *Centris trigonoides* and *C. tricolor* presents big apices of the posterior giant spatulate setae that also allows the formation of scraping surface advantageous in trichomatous elaiophores (as are presented for example in Iridaceae). Evidently, *Centris* has much more diversity of its oil-collecting structures, which makes this genus more flexible in terms of the species that can visit and collect oils. In this work, we cannot discern a tendency in the relationship between the characteristics of elaiophore and oil-collecting structures in the legs of the species of *Centris*. However, our data show some hints that the size of the flower and consequently of the elaiophore are somewhat related to the size of the species of *Centris*; for example, a medium species as *Centris tarsata* was observed in small, medium, and large flowers of Malpighiaceae, but the very large species of *Centris* as *C. obsoleta* and *C. flavifrons* were observed in flowers of *Stigmaphyllon bonariense* and *S. jatrophifolium*, which have large flowers. On the other hand, Vogel (1988) and later Giannini et al. (2013) mentioned that bees of different sizes visit the same flowers of Malpighiaceae, and reciprocally the same species of *Centris* can be found as a floral visitor in different species of plants. For this reason, these authors infer that there is no specificity in the relationships between *Centris* species and Malpighiaceae. Probably, although the bees of the genus *Centris* can forage on flowers of various sizes, in some conditions there is preference such as the one we detected in this work. However, it is expected that this predisposition can be modified or influenced under different ecological aspects such as competition between species, availability of resources, and environmental conditions.

Conclusions

We consider that some characteristics present in the elaiophores of Malpighiaceae appear to be constant among related species, which presumably were inherited from their

ancestors. Examples are the presence of peltate glands in species of the tristellateioids and ptilochaetoids clades (one or two possible origins) and the presence of elaiophores with striate surface in some species of the stigmaphylloids clade and carolus clade belong to the tetrapteroids clade. Nonetheless, it is evident that Malpighiaceae have the capacity to generate variants in the elaiophores. Although some of them are inconsistent or fluctuate, they may be an adaptation to improve the interactions with pollinators. Our study analyzes 18 of the 46 species (approx. 40%) present in Argentina, but it represents a limited diversity of this family in the Neotropics; therefore, speculating in an evolutionary context seems risky. Based on our observations, we believe that this combination between inherited characters together with morpho-anatomical innovations in the oil glands will surely expand pollination strategies and explain the success and wide distribution of the Neotropical Malpighiaceae. We hope that this work stimulates the comparative study of elaiophores among species in clades little or not yet studied, and/or species with restricted distributions and can shed some light into the evolutionary patterns of these oil gland in this diverse and exciting family.

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