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Chrysophyllum marginatum (Sapotaceae): Generalist pollination and cryptic gynomonoecious

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Abstract

Chrysophyllum (Chrysophylloideae, Chrysophylleae) is the second largest genus in the Sapotaceae. Studies of pollination ecology in this genus are non-existent, although there are records of entomophily for this family. Considering the lack of detailed studies on pollination ecology and sexual systems in *Chrysophyllum* species, we investigate the floral morphology and biology and floral visitors of *Chrysophyllum marginatum* to verify which sexual system is present in the studied population and whether flowers of this species are visited and pollinated by different insect groups. The population of *C. marginatum* has weak and cryptic gynomonoecy because the plants produce a low percentage of functionally pistillate flowers (4.2%) and these flowers appear to be perfect flowers (hermaphrode). Flowers of *C. marginatum* are phenotypically, ecologically and functionally generalist because: (a) they are actinomorphic, open and not restrictive in terms of access to floral resources; (b) they are visited by 26 species of insects that are potential pollinators; and (c) among these species several groups can be effective pollinators, mainly bees and flies, according to the most effective pollinator principle. We consider bees and flies to be the main pollinator group of *C. marginatum*, due to their high visitation rate, richness and intrafloral visiting behavior, and because they especially forage among plant individuals and are able to promote xenogamy. Nectaries were found in the ovary base and osmophores in the petal margins, as floral attractants. For *Chrysophyllum*, this is the first record of gynomonoecy and for the family this is considered the second record. *Chrysophyllum marginatum* has generalist and entomophilous pollination, as recorded in other Sapotaceae Neotropical species.

KEYWORDS
bee, entomophily, fly, nectary, sexual system

1 | INTRODUCTION

Plant–pollinator interactions span the spectrum from obligate specialists (a single species of pollinator services for one plant species) to facultative generalists, in which flowers may be adequately pollinated by a taxonomically broad range of pollinators (Ollerton, Killick, Lamborn, Watts, & Whiston, 2007). The level of specialization or generalization in pollination systems depends, for example, on the integration between the floral morphology and biology, and the morphology, behavior (intrafloral and foraging mode) and habit of floral visitors (Olesen, Dupont, Ehlers, & Hansen, 2007). In this respect, floral attributes (morphological, physiological and ecological) are relevant because flowers with a more restrictive morphology and/or a more elaborate function (e.g., flag, gullet) (sensu Faegri & van der Pijl, 1979) could be more specialized for pollinators by restricting or preventing access to their floral resources (e.g., nectar, pollen and oil). The opposite occurs with more "generalist" flowers, such as those that have radial symmetry, are open (e.g., dish- to bowl-shaped and/or inconspicuous) and have accessible nectar and pollen (Olesen et al., 2007; Ollerton et al., 2007).

However, flowers with a generalist phenotype (i.e., morpho-functional adaptations exhibited by a flower) can be ecologically (i.e., pollinator richness, with which the plants/species interact) and/or "functionally" (i.e., diversity of the pollinator of a plant at a higher taxonomic level or functional group, e.g., "bee pollinated" or "fly pollinated") (sensu Ollerton et al., 2007) specialist, and vice versa. For example, open bowl, actinomorphic, "generalist" flowers of *Rhipsalis neves-armondii* (Cactaceae) present cryptic floral specialization because they are pollinated by a single pollination functional group (bees) (Martins & Freitas, 2018). The same is recorded for the andromonoecious species *Angelica sylvestris* (Apiaceae), the flowers of which are pollinated by muscoid and syrphid flies, although they are visited by over 70 species of insects (Niemirski & Zych, 2011). Nevertheless, generalist pollination (by different functional groups) can be advantageous in habitats subjected to a disturbance (e.g., urban, anthropized), either seasonal or heterogeneous, in which pollinator fauna may be uncertain or depleted (Gómez & Zamorra, 2006). Thus, in these ecosystems, compared with specialist pollination, generalist pollination would be favored or selected, especially in small, inconspicuous open flowers, which are less restrictive in terms of access to their floral resources and contact with anther(s) and stigma(s) (Olesen et al., 2007).

Chrysophyllum (Chrysophylloideae, Chrysophylleae) is the second largest genus in the Sapotaceae family and includes 71 known species, mostly distributed in the Neotropics (43 spp.) (Pennington, 1990). In Brazil, 31 species have been recorded, with 14 endemics. Most species are registered in the Amazon Forest (20 spp.), but some species of the genus are registered in the Atlantic Forest (14 spp.), Caatinga (2 spp.) and Cerrado (5 spp.) (Sossai & Alves-Araújo, 2017). Flowers of the *Chrysophyllum* species, as in most Sapotaceae, are generally small, inconspicuous and cream/yellowish or greenish (Pennington, 1990) but with a variation in the corolla shape (Faria et al., 2017), which can be more or less open or restrictive, permitting anthers, stigma and floral resources (nectar) to be accessible/exposed to floral visitors/potential pollinators or not.

As far as we are aware, studies of pollination ecology in this genus are non-existent or are not available. However, according to Pennington (2004), the majority of Sapotaceae species are certainly entomophilous. Indeed, in the Brazilian Caatinga and restinga, Sapotaceae species that have flowers with diurnal (*Manilkara subsericea*, *Sideroxylon obtusifolium*) or nocturnal anthesis (*Pouteria venosa*) present generalist pollination by bees, butterflies, flies and/or wasps (diurnal anthesis) (Gomes, Pinheiro, Lima, & Santiago-Fernandes, 2010; Kiill, Martins, & Silva, 2014) or beetles and flies (nocturnal anthesis) (Gomes & Pinheiro, 2007). Recently, Lassen, Nielsen, Lompo, Dupont, and Kjær (2018) verified that although different small insect species can pollinate *Vitellaria paradoxa* flowers, a higher percentage of fertilization occurs after pollination by the exotic honeybee *Apis mellifera*. Nevertheless, on the island of Mauritius, honeybees are less efficient pollinators of the two *Sideroxylon* species (*S. cinereum*, *S. puberulum*) than white-eye birds (*Zosterops borbonicus mauritianus*, *Z. chloronothos*) (Hansen, Olesen, & Jones, 2002). Additionally, in *Chrysophyllum*, little is known about the anatomy of the nectary and osmophores, the floral secretory structures responsible for the production of nectar and odor, respectively, which are directly involved in the flower–pollinator interaction (Teixeira, Marinho, & Paulino, 2014).

In relation to the sexual system, hermaphroditism predominates in *Chrysophyllum* neotropical species (67.4%), followed by dioecy (27.9%) and monoecy (4.7%) (Pennington, 1990). Among the five dioecious neotropical *Chrysophyllum* species, Pennington (1990) mentions the possibility of the occurrence of bisexual flowers, one of which may be functionally male. Within Chrysophylleae, both monoecious and dioecious conditions are known, but dioecy seems to predominate (Pennington, 2004).

Considering the lack of detailed studies of pollination ecology and sexual systems in *Chrysophyllum* species, here, we investigate the floral morphology and biology and floral visitors of *C. marginatum* (Hook. & Arn.) Radlk. in the Reserva Particular de Patrimônio Natural of the Universidade Federal de Mato Grosso do Sul (RPPN/UFMS) and evaluate the frequency and behavior of its floral visitors to verify: (a) which sexual system is used by the studied *C. marginatum* population, (b) whether floral morphology restricts access to floral resources to floral visitors; and (c) whether the flowers of this species are visited and pollinated by different groups of insects (i.e., whether *C. marginatum* has entomophilous and generalist pollination). We expect that our *C. marginatum* population has:

(a) a hermaphrodite sexual system, as occurs in all species in the section to which it belongs (*Chrysophyllum* section) (Pennington, 1990); and (b) generalist and entomophilous, as recorded for most species of Sapotaceae with small and inconspicuous flowers.

2 | MATERIALS AND METHODS

2.1 | Study site and species

We conducted this study in April 2019 on *Chrysophyllum marginatum* plants occurring in the riparian forest edge ($n = 4$ plants) and "cerradão" remnant ($n = 10$) in the RPPN/UFMS (20°27′S and 54°37′W, 530 m), Campo Grande, Mato Grosso do Sul, Brazil. The details of the climate and vegetation of the study area are provided in Oliveira and Sigrist (2008).

Chrysophyllum marginatum is a semideciduous large shrub or small tree approximately 2–10 m in height and is widely distributed in South America, with records in Argentina, Bolivia, Brazil, Paraguay and Uruguay (Pennington, 1990). In Brazil, *C. marginatum* occurs in the midwest, south and southeast (except Espírito Santo) regions and Bahia and Sergipe states, as well as in the Cerrado (*lato* sensu), as "carrasco" vegetation, semideciduous forest or riparian or gallery forest (Carneiro, Alves-Araújo, & Faria, 2020). The inflorescence is cauliflorous axillary fascicles with pediculate flowers, and in the study area, the species flowers from February to May (Jennifer Elaine Maier, pers. com.). The fruit is a berry with a dark pericarp and zoochory dispersal (Pennington, 1990). The voucher specimen of the plant species was collected and deposited in the CGMS Herbarium (F.M. Leme n° 136 e 137).

2.2 | Sexual system

To study the sexual system (i.e., gender expression and its occurrence at the intrafloral, individual and population levels) (sensu Cardoso et al., 2018), we randomly selected and collected flowering branches (secondary or tertiary) of *C. marginatum* from the first 10 plants of the species occurring on a pre-existing trail in a cerradão remnant. In the laboratory, we removed the fascicles from the branches (Figure 1a) and fixed them in 70% formalin-aceto-alcohol (FAA) (Johansen, 1940). Later, under a stereomicroscope, we quantified stamen and ovule numbers and verified the presence of pollen in the anthers in all pre-anthesis buds of the fascicles. We compared the frequency of the floral phenotypes (perfect, functionally pistillate) in the population by the *t*-test using the PAST program (Hammer, Harper, & Ryan, 2001) with a significance level of 5%.

2.3 | Floral morphology, anatomy and biology

We studied the floral anatomy, morphology and biology in situ and/or in the laboratory from fresh and fixed material in either 70% alcohol or FAA. In the laboratory, we measured the flowers ($n = 32$, eight per plant) using a digital caliper: floral diameter and length, corolla tube length, anthers and stigma height. Other floral attributes, such as the presence of an odor, dichogamy and flower longevity, were reported through direct observations in the field on marked flowers ($n = 11$) on pre-anthesis. We tested the pollen viability using acetocarmine (Dafni, 1992) and stigma receptivity through changes in coloration (clear to dark) and turgidity using a magnifying glass (Lupenbrille). We detected osmophores in 20 flowers by the neutral red test (Dafni, 1992).

For the floral anatomical study, flowers and pre-anthesis flowers were dehydrated in an ethanol series of up to 95%, embedded in methacrylate (Historesin-Leica) and sectioned in both the transverse and longitudinal planes (3–5 μm thick) using a rotary microtome Leica RM 2145 (Wetzlar, Germany). Serial sections were stained with 0.05% toluidine blue in phosphate buffer (pH 5.8) (O'Brien, Feder, & McCully, 1964), mounted on a slide using water and observed under a light microscope. For histochemical analyses, the flower sections embedded in methacrylate were investigated using the following reagents: Sudan Black B and Sudan IV for total lipids (Pearse, 1985), Lugol for starch (Johansen, 1940), Bromophenol Blue for proteins (Mazia, Brewer, & Alfert, 1953), Toluidine Blue for the detection of phenolic compounds (O'Brien et al., 1964) and period acid-Schiff (PAS) for the detection of neutral polysaccharides (Jensen, 1962). Photomicrographs were obtained using a Leica DFC 495 digital camera coupled to a Leica DM 5500 B light microscope. For surface analysis, flowers were dissected under a stereomicroscope, dehydrated in an ethanol series, critical point dried in a Quorum Technologies CPD 7501 instrument (East Sussex, UK), mounted on metal supports on carbon adhesive tape, and sputtered with gold in a Denton Vacuum Desk III instrument (Moorestown, Nova Jersey, EUA). Electron micrographs were obtained with a JeolJSM-6380LV scanning electron microscope (SEM, Tokyo, Japan) at 15kv (adapted from Leme & Scremin-Dias, 2014; Leme, Schönenberger, Staedler, &Teixeira, 2020).

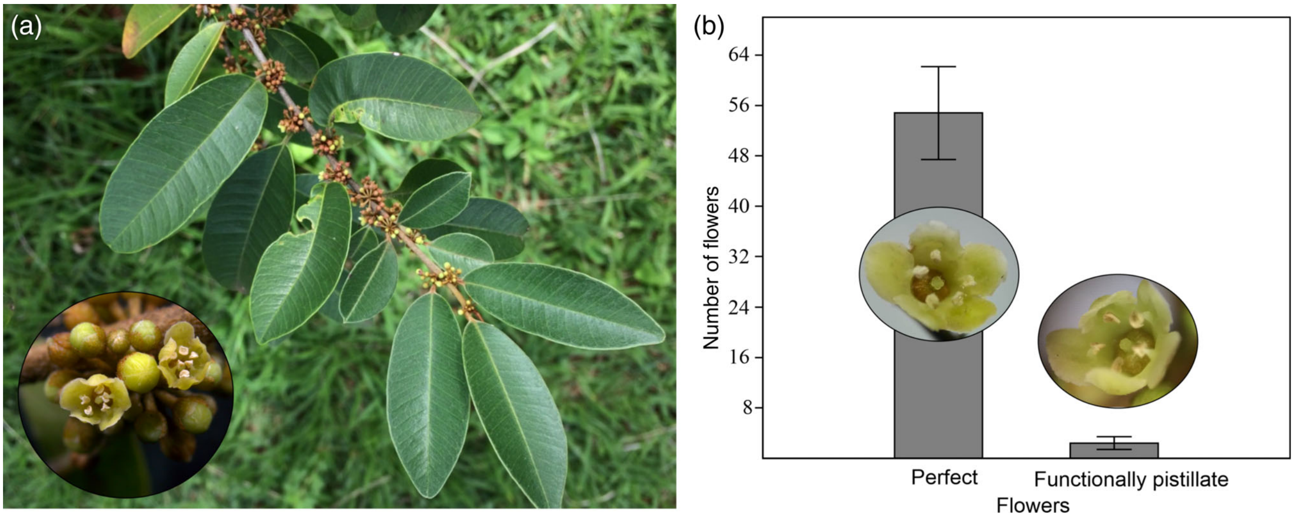

FIGURE 1 *Chrysophyllum marginatum*. (a) Inflorescences (fascicles) on plant and details. (b) Average number (and standard deviation) of perfect and functionally pistillate flowers in 10 plants in the "cerradão" remnant, Campo Grande, Mato Grosso do Sul, Brazil

2.4 | Floral visitors and pollinators

We sampled diurnal *C. marginatum* floral visitors on plants ($n = 4$) from the riparian forest edge over 2 days, from 08:00 a.m. to 12:30 p.m. (first day) and 12:30–18:00 p.m. (second day) (15 min per plant every hour), for a total of approximately 10 h of observations. In the field, we recorded the number of flowers visited, the visit number (defined as the interval in which the flowers of the focal plant were continuously visited by a specific floral visitor) and, when possible, the reward collected (nectar, pollen) and visit behavior, which was recorded through notes, photographs and/or videos. For each floral visitor, we calculated the visitation rate (VR) (modified from Herrera, 1989) as follows: VR = number of visits × number of flowers visited × number of plants visited/total observation time (10 h). We classified floral visitors as pollinators if they made contact with the anthers and stigma during visits. Insects with a body size $\leq$5.0 mm were considered thieves because they generally did not contact the anthers/stigma. When possible, we collected floral visitor specimens with nets and/or waterproof bags, which were kept in plastic vials. Later, floral visitors were identified and deposited at the Zoological Collection of Universidade Federal de Mato Grosso do Sul (ZUFMS). We used one-way ANOVA ($p \leq 0.05$) (Zar, 2010) (INSTAT version 5.3) to assess differences in the flower visitation rate among bees, flies and other floral visitors (ants, butterflies, flower bug/hemipteran, wasps). Additionally, we constructed a network of interactions between individuals of *C. marginatum* and their floral visitors, to better visualize potential pollinators. The graph was drawn in Pajek 5.09 (Mrvar & Batagelj, 2016) using the Kamada-Kawai – separate components method, in which floral visitors or individuals of *C. marginatum* with a larger number of interactions are drawn closer to the center of the network.

3 | RESULTS

3.1 | Sexual system

Among the 10 plants sampled, six had functionally pistillate flowers (Figure 1b); that is, the *C. marginatum* population was gynomonoecious. However, most of the analyzed pre-anthesis buds were hermaphrodite (perfect flowers) ($n = 548$; 95.8%), with a lower percentage of functionally pistillate flowers ($n = 24$; 4.2%) (paired *t*-test 7.088, $p = 5.74\text{E-}05$) (Figure 1b) and a ratio of perfect: functionally pistillate flowers of ~23:1.

3.2 | Floral morphology, anatomy and biology

In the *C. marginatum* fascicles, we recorded up to 15 buds, of which one to three opened per day (Figure 1a). The flowers were small (mean ± standard deviation = 1.49 ± 0.05 mm and 1.9 ± 0.16 mm for the diameter and length, respectively), inconspicuous, white or cream, actinomorphic, open, pentamerous, epipetalous, nectariferous, odoriferous and diurnal (Figures 1a,b and 2a). The sepals were greenish and covered by brownish peltate trichomes (Figure 2b). The corolla was campanulate, with free lobes and a basal tube (Figure 1b), with a length of 1.17 (± 0.11) mm. The corolla basal tube had an inner papillous epidermis (Figure 2i,k), and the free lobes had common epidermis

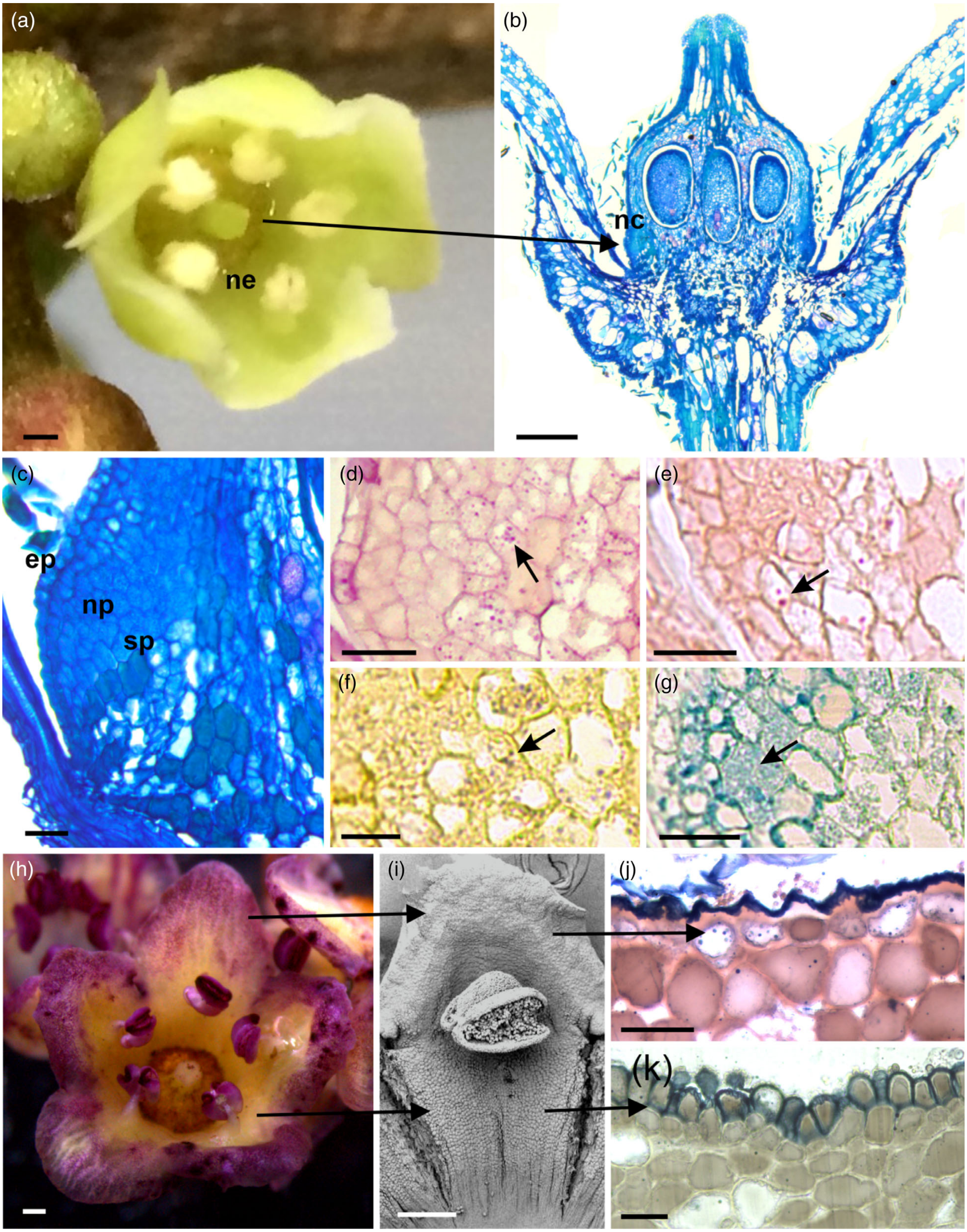

FIGURE 2 Floral secretory structures of *Chrysophyllum marginatum*. (a) Flower with nectar (ne). (b) Longitudinal section of the flower; note the nectary at the ovary base (nc, arrow). (c) Nectary structure; note the epidermis (ep), nectary parenchyma (np) and subnectary parenchyma (sp). (d) Nectary with a positive reaction for neutral polysaccharides (stain: PAS), (e) positive reaction for lipids (stain: Sudan IV), (f) positive reaction for starch grains (stain: Lugol) and (g) positive reaction for proteins (stain: Bromophenol blue). (h) Fresh flower with the osmophore stained with neutral red on the free corolla lobes. (i) Petal with the stamen on SEM (scanning electron microscope); note the difference in the epidermis surface. (j) Cross-section of the petal free lobe; note the positive reaction for lipids in epidermal cells (stain: Sudan black). (k) Cross-section of the basal corolla tube (stain: Sudan black). Bars: (a, b) 250 μm; (c–g) 25 μm; (h, i) 250 μm; (j, k) 25 μm

TABLE 1 Size (body length), visitation rate, floral reward collected and pollination performance/stigma–anther contact rate of the floral visitors of *Chrysophyllum marginatum* in urban riparian forest, Campo Grande, Mato Grosso do Sul, Brazil. Visitation rate = visit number × number of plants visited × number of flowers visited/total observation time

Floral visitor (species number)	Size/body length (mm)	Visitation rate	Floral reward collected	Stigma–anther contact rate (%)
BEES (9)		4,692.2		
Apis melifera L.	11.6	1,917.6	Nectar	100
Augochloropsis sp. 1	6.1	39.6	Pollen	100
Augochloropsis sp. 2	6.33	0.2	Pollen	100
Exomalopsis sp.	8.4	2,564.8	Nectar, pollen	100
Paratrigona cf. *lineata* (Lepeletier, 1836)	5.3	0.2	Pollen	100
Pseudagapostemon sp.	6.4	12	Pollen	100
Tetragonisca cf. *angustula* Latreille	5.2	1.1	Pollen	100
Trigona spinipes (Fabricius, 1793)	5.6	131.2	Pollen	100
Halictidae sp.	6.7	25.5	Nectar, pollen	100
FLIES (15)		1,222.8		
Chrysomya sp.	13.3	467.2	Nectar	100
Eristalis spp.	9.7	541.2	Nectar	100
Lucilia sp.	7.5	1.4	Nectar	100
Ocyptamus sp. 1	11.3	5.2	Nectar	100
Ocyptamus sp. 2	7.2	19.5	Nectar	100
Acalyptratae1	2.3	9.6	Pollen	0
Acalyptratae 2	3.0	0.8	Pollen	0
Diptera 1	8.5	0.4	Pollen	100
Diptera 2	6.3	171.6	Nectar, pollen	100
Diptera 3	-	0.8	Nectar	100
Diptera 4	-	4.6	Pollen	100
Diptera 5	-	0.1	?	100
Diptera 6	-	0.1	?	100
Diptera 7	3.6	0.2	Nectar	0
Diptera 8	9.3	0.1	?	100
WASPS (4)		3.4		
Agelaia sp.	11.6	1.8	Nectar	100
Polybia paulista (Ihering, 1896)	9.3	0.4	?	100
Vespidae 1	-	0.6	Nectar	100
Vespidae 2	-	2.4	?	100
ANTS (3)		62.6		
Camponotus sp.	4.6 ± 0.1	50.4	*	0
Formicidae 1	3.0	12	*	0
Formicidae 2	2.9	0.2	*	0
BUTTERFLIES (2)		0.2		
Lepidoptera 1	11.1	0.1	Nectar	0
Lepidoptera 2	-	0.1	Nectar	0
HEMIPTERAN (1)		5.2		
Carpocoris sp.	10.9	5.2	Nectar	100

Note: (–) floral visitor not collected but photographed; (*) no rewards collected; (?) the visitor collects rewards, but it was not possible to identify which one.

cells that were secretory (Figure 2i,j). Free corolla lobes reacted positively for osmophores with red neutral (Figure 2h), and its cells had drops of lipids when stained with Sudan black (Figure 2j). The corolla basal tube formed a cup that contained nectar (Figure 2a) secreted by a nectary present at the ovary base (Figure 2b). The

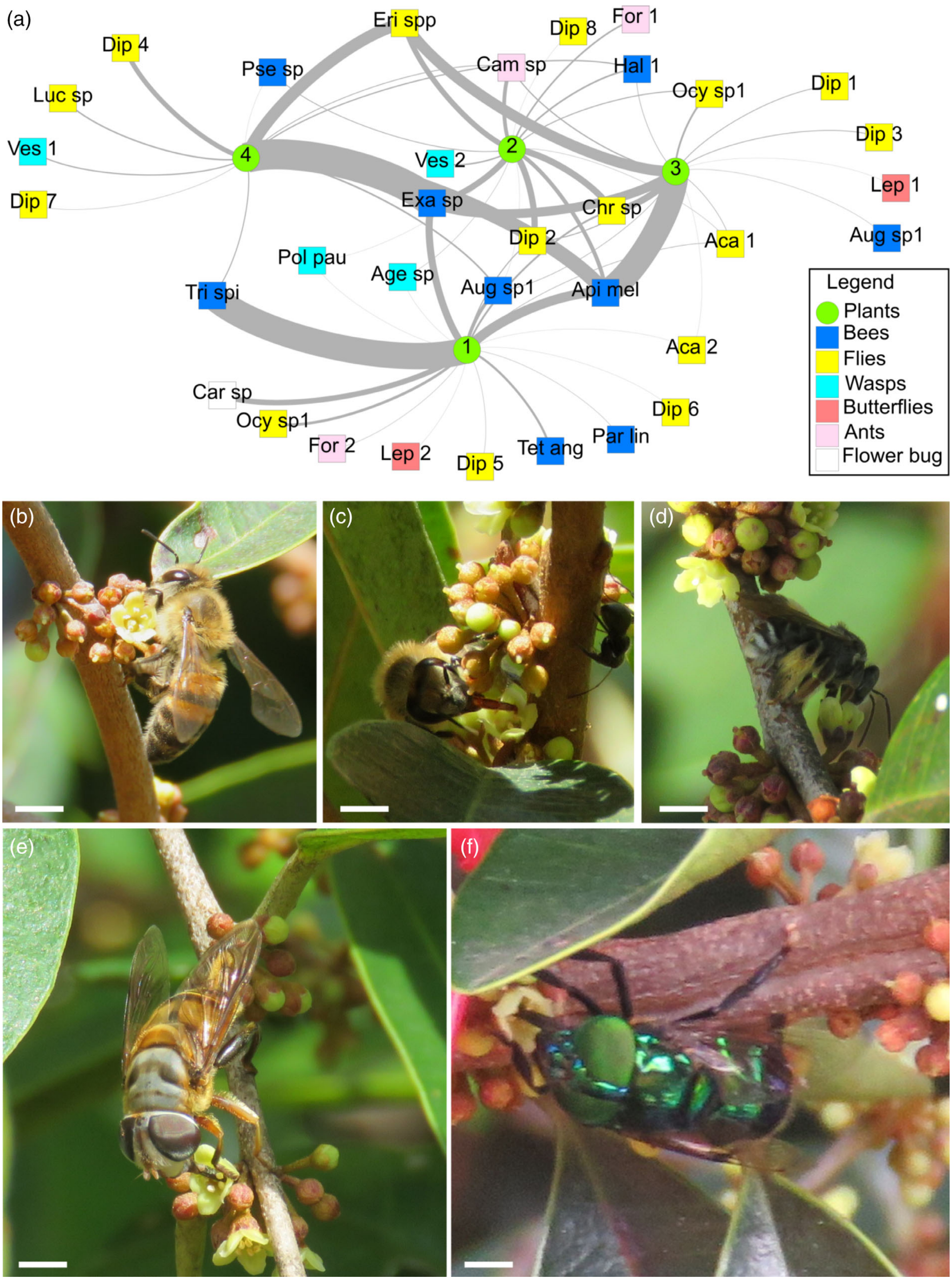

FIGURE 3 Legend on next page.

flowers had five (82.7%), or rarely three (0.7%), four (3.0%) or six (13.6%), stamens, which were epipetalous, isodinamous and opposite the corolla lobes (Figure 1b) and 1.5 (± 0.04) mm in height. The anthers were basifixed and rimose and produced white pollen with pollenkitt (~ oil drops), and had relatively low viability (65.3 ± 27.6%). The gynoecious was syncarpous, with five (93.2%), six (5.8%) or four carpels (1.0%), with one ovule per locule. The ovary was also covered by brown trichomes (Figure 1b). The stigma was large, papillous, five or four lobed and positioned below the stamens (1.05 ± 0.04 mm height), showing reverse herkogamy (Figure 1b). Nectar was produced in small quantities by the nectary, which is located at the base of the floral tube, around the base of the ovary (Figure 2a,b). The nectary was composed of an epidermis with nectar pores, a nectary and subnectary parenchyma (Figure 2c); no vascular bundles were observed, and only the regular vascular system of organs was present. The nectary and subnectary parenchyma had neutral polysaccharides (Figure 2d), lipids (Figure 2e), starch grains (Figure 2f) and proteins (Figure 2g). Starch grains and neutral polysaccharides were found in greater quantities in the subnectary parenchyma.

Chrysophyllum marginatum flowers open before 07:00 a.m. and last for approximately 2 days. In pre-anthesis flowers, there is no nectar production, the anthers are usually open (88.9% of the pre-anthesis flowers) and the stigma may be receptive (41.7%) or not (58.3%). During anthesis, the flowers emit a slightly unpleasant, idiopathic odor (sensu Vogel, 1983), similar (perhaps) to urine, which is perceivable at a long distance and is produced by osmophores, which occur on the edge of the corolla lobes and anthers (Figure 2h–k).

3.3 | Floral visitors and pollinators

Thirty-four insect species visited *C. marginatum* flowers belonging to six groups, ants (three spp.), bees (nine spp.), butterflies (two spp.), flies (15 spp.), flower bugs (hemipteran) (one sp.) and wasps (four spp.), that collected pollen and/or nectar (except ants) (Table 1, Figure 3). Bees and flies visited a greater number of flowers than the other visitors and visited all plants (Figure 3a), and they also had a higher visitation rate (VR) in relation to the other floral visitor groups ($VR_{bees} = 521 \pm 989$; $VR_{flies} = 82 \pm 178$; $VR_{other\ floral\ visitors} = 7 \pm 16$) (ANOVA, $p = 0.077$) (Table 1). Two species of bees (*Apis mellifera* and *Exomalopsis* sp.) and some fly species (*Eristalis* spp. and *Chrysomya* sp.) (Figure 3b–f) recorded the highest visitation rate (Table 1). Bees land on a fascicle and go to a flower, where they collect nectar with the proboscis (Figure 3b–d) or pollen with the anterior and median legs. After 1 to 3 s, bees leave the flower and visit another flower of the fascicle or a flower of another nearby fascicle, usually walking and, more rarely, in flight, where they perform a similar behavior. Flies and wasps perform similar visiting behaviors, except that flies collect pollen with the mouthpiece and take a longer time per flower than wasps. When visiting flowers, "larger" bees, flies, flower bugs and wasps (≥ 5 mm; 26 spp.) usually make contact with anthers and stigma with the mouthpiece and/or the first pairs of legs (Figure 3b–f); that is, there is a high probability of stigma–anther contact (Table 1). Ants generally patrol the inflorescences/flowers, moving between them without collecting any floral resources. Butterflies collect floral nectar without making contact with anthers and stigma.

4 | DISCUSSION

The population of *C. marginatum* studied has cryptic gynomonoecy because the functionally pistillate (female) flowers appear to be perfect (hermaphroditic) flowers (Mayer & Charlesworth, 1991) and it is difficult to differentiate them without detailed morphological observations (Mendez & Munzinger, 2010). In fact, we recorded the occurrence of functional pistillate flowers when we carried out pollen viability tests on pre-anthesis buds and verified the absence of pollen in the anthers of these flowers. In Sapotaceae, unisexual

FIGURE 3 (a) Interactions network (Kamada-Kawai) between *Chrysophyllum marginatum* plants ($n = 4$) (circles) and their floral visitors ($n = 34$ spp.) (squares) from the campus of the Universidade Federal de Mato Grosso do Sul (MS), Campo Grande, MS, Brazil. The line thickness indicates the number of flowers visited. Bees *Apis mellifera* (b, c) and *Exomalopsis* sp. (d), and flies *Eristalis* sp. (e) and *Chrysomia* sp. (f), taking nectar from flowers of *C. marginatum*. Bars: 2.0 mm. Legend to network floral visitors: Aca 1 = Acalyptratae 1, Aca 2 = Acalyptratae 2, age sp = *Agelaia* sp., Apimel = *Apismelifera*, Augo sp1 = *Augochloropsis* sp. 1, Augo sp2 = *Augochloropsis* sp. 2, cam sp = *Camponotus* sp., car sp = *Carpocoris* sp., Chrsp = *Chrysomya* sp., dip 1 = Diptera 1, dip 2 = Diptera 2, dip 3 = Diptera 3, dip 4 = Diptera 4, dip 5 = Diptera 5, dip 6 = Diptera 6, dip 7 = Diptera 7, dip 8 = Diptera 8, Eri sp. =*Eristalis* spp., Exo sp = *Exomalopsis* sp., for 1 = Formicidae 1, for 2 = Formicidae 2, Hal sp = Halictidae sp., Lep 1 = Lepidoptera 1, Lep 2 = Lepidoptera 2, Luc sp = *Lucilia* sp., Ocy sp1 = *Ocyptamus* sp. 1, Ocy sp2 = *Ocyptamus* sp. 2, par lin = *Paratrigona* cf. *lineata*, pol Pau = *Polybiapaulista*, Psesp = *Pseudagapostemon* sp., Tet ang = *Tetragonisca* cf. *angustula*, tri spi = *Trigona spinipes*, Ves 1 = Vespidae 1, Ves 2 = Vespidae 2 flowers are more common in Chrysophylleae, in which dioecy predominates and the simplest form of sexual dimorphism involves the loss of anthers in functional pistillate flowers, with the filaments remaining (Pennington, 2004). However, in two monoecious neotropical species of *Chrysophyllum* (*C. colombianum*, *C. cuneifolium*), anthers are absent in pistillate flowers (Pennington, 1990) and sexual dimorphism occurs in unisexual flowers of the dioecious species *Chrysophyllum sparsiflorum*: pistillate (female) flowers have a tubular corolla and their staminate (male) flowers are cup-shaped (Faria et al., 2017).

Gynomonoecy is a rare sexual system in angiosperms (except in Asteraceae) and has been reported in 23 families, 570 genera and 2.8–4.7% of flowering plant species (Mamut & Tan, 2014; Mamut, Xiong, Tan, & Huang, 2014; Mendez & Munzinger, 2010). This sexual system has evolved several times in flowering plants and is considered to be an intermediate evolutionary step in the pathway to monoecy (Mamut et al., 2014; Mendez & Munzinger, 2010). In Sapotaceae, gynomonoecy was recorded for the first time in *Planchonella* species (*P. endlicheri*, *P. laetevirens* and *P. latihila*), with cryptic gynomonoecy in two species (*P. laetevirens* and *P. latihila*), because the corolla length is shorter in pistillate compared to perfect flowers in *P. endlicheri* (Mendez & Munzinger, 2010). Therefore, this is the second record of gynomonoecy in the family and the first for *Chrysophyllum*. Here, *C. marginatum* may be characterized as a weakly gynomonoecious species because the plants produce a low percentage of pistillate flowers (4.2%) (sensu Miller & Diggle, 2003). In general, the presence of the two types of flowers within populations is determined by genetic and/or environmental factors (e.g., nutrition, humidity, light and temperature) (Mamut & Tan, 2014; Mamut, Xiong, Tan, & Huang, 2017).

In *C. marginatum*, gynomonoecy may favor female function and can be advantageous in reducing self-pollination within (autogamy) and between flowers of the same plant (geitonogamy), which in this species is favored due to: (a) the small flowers gathered in congested inflorescences, (b) the proximity between inflorescences, (c) the foraging behavior of pollinators and (d) the small amount of nectar, which can cause faster intrafloral visits. In fact, several hypotheses have been proposed and accepted to explain the evolution and maintenance of gynomonoecious species, such as reducing autogamy and geitonogamy, increasing cross-pollination (allogamy) and preventing pollen–pistil interference (Mamut et al., 2017). The last two hypotheses may also be considered for *C. marginatum*. Additionally, in perfect flowers, partial protandry (in 58.3% of flowers) and reverse herkogamy may also restrict pollen–stigma interference. Nevertheless, if there are no self-incompatibility (SI) mechanisms in this species, gynomonoecy may decrease only the chances of self-pollination in its strictest sense (pollen of a given flower deposited onto its own stigma), but it does not exclude the possibility of geitonogamy, which is genetically like self-pollination (Arroyo, 1976; Lenzi & Paggi, 2020; Maciel, Cardoso, & Oliveira, 2020). In this sense, pollen transfer among flowers of the same individual, and consequently geitonogamous selfing, may possibly be promoted by secondary pollinators that are less effective than the main pollinator group (see Stebbins, 1970). Secondary pollinators, although less effective, are important co-pollinators, which can increase the fruit set or even ensure reproduction when there is a deficit of specialized pollinators, and may promote more generalized pollination systems (Ferreira, Gomes, Souza, Fabri, & Sigrist, 2018; Fleming, Sahley, Holland, Nason, & Hamrick, 2001; Holland & Fleming, 2002; Sato & Kato, 2017).

Flowers of *C. marginatum* are phenotypically, ecologically and functionally generalist (sensu Ollerton et al., 2007) because: (a) they are actinomorphic, open and not restrictive in terms of access to their floral resources (primarily nectar, secondarily pollen); (b) they are visited by 26 species of potential insect pollinators; and (c) among these, several groups may be effective pollinators, mainly bees and flies, according the Most Effective Pollinator Principle (sensu Stebbins, 1970). In effect, the floral attributes of *C. marginatum* – small and open flowers, grouped in congested inflorescences, with pale color, exposed anthers/stigma and accessible nectar/pollen, and odorous and nectariferous – are in agreement with the pollination system "small diverse insects" (previously called generalist insects), which includes a varied assemblage of relatively small insects, such as bees, beetles, flies, wasps and butterflies (sensu Bawa, Bullock, Perry, Coville, & Grayum, 1985). This pollination system is very common in woody flora of the Costa Rican tropical rainforest (Bawa et al., 1985) and Brazilian Cerrado vegetation (Oliveira & Gibbs, 2000, 2002 and references; Martins & Batalha, 2006), and in species with floral attributes similar to those of *C. marginatum* (see Moreira & Freitas, 2020). In the same way, generalist pollination by bees, beetles, butterflies, flies and/or wasps is common in Sapotaceae (Gomes & Pinheiro, 2007; Gomes et al., 2010; Kiill et al., 2014; Lassen et al., 2018).

In this study, we consider bees to be the main pollinator group of *C. marginatum*, followed by flies, due to the high visitation rate (4,692.2 and 1,222.8, respectively), intrafloral visiting behavior, foraging among plants and/or richness. According to the Most Effective Pollinator Principle, a flower's characteristics will be molded by those pollinators that visit it most frequently and effectively in the region where it is, but this does not mean that the flower is pollinated exclusively by this vector (sensu Stebbins, 1970). Pollination by the Hymenoptera (mainly bees) and/or Diptera orders is common in Sapotaceae species (e.g., *Manilkara subsericea*, *Pouteria venosa*, *Sideroxylon obtusifolium* and *Vitellaria paradoxa*) (Gomes & Pinheiro, 2007; Gomes et al., 2010; Kiill et al., 2014; Lassen et al., 2018) and other families with floral attributes similar to neotropical Sapotaceae, such as Erythroxylaceae, Flacourtiaceae (e.g., Bawa et al., 1985; Oliveira & Gibbs, 2000) and Rhamnaceae (e.g., Medan & Arce, 1999 and references). In Panama, *Chrysophyllum cainito* seems to be pollinated mainly by species of *Tetragonisca* bees (Gonzálvez, Chen, & Rodríguez-Gironés, 2015; Parker et al., 2010). Bees are the most common pollinators, whereas flies are the second most common pollinators (Larson, Kevan, & Inouye, 2001). In general, bees are the main pollinators in Brazilian vegetation types because they directly depend on floral resources for the survival of both adults and broods (Souza, Aoki, Alcantara, Laroca, Sazima, Pott, & Sigrist, 2017). Flies have been mentioned as pollinators or regular visitors of approximately 555 species of plants and pollinators of over 100 cultivated plants, such as onion, cashew and mango (Kearns, 2001; Ssymank, Kearns, Pape, & Thompson, 2011). In addition, despite the "apparent" short sampling time of floral visitors in this study (10 h), our data were similar to those recorded in a study of daytime antophilic fauna in one of the study areas ("Cerradão") during 2008, in which bees (e.g., *Apis mellifera*, *Augochloropsis* aff. *euphrosyne*, *Paratrigona lineata*, *Scaptotrigona postica* and *Trigona spinipes*) and flies (e.g., *Hemilucillia*, several species of Syrphidae and Sarcophagidae) were the main floral visitors and potential pollinators (Jennifer E. Maier, pers. com., unpublished data).

Here, bees of *Exomalopsis* sp. (VR = 2,564.8) and *Apis mellifera* (VR = 1917.6) (both Apidae) and flies of *Eristalis* spp. (Syrphidae) (VR = 541.2) and *Chrysomya* sp. (Calliphoridae) (VR = 467.2) were the main pollinators of *C. marginatum* in the studied population based on the visitation rate (including the number of visits, and plants and flowers visited) and stigma–anther contact rate (100%). The high visitation rate of these bees may be attributed to the characteristics of their visit behavior, that is, rapid foraging, which covers large portions of the flowering branches, and appearing to move more frequently among trees than most other insect species, resulting in more flowers visited, probably leading to cross-pollination; however, this needs to be tested. *Apis mellifera* is an exotic social bee and generalist for food resources and is widely known as a pollinator of Sapotaceae species for which there are pollination data (e.g., *Manika subsericea*, *Sideroxylon cinereum*, *S. obtusifolium*, *S. puberulum* and *Vitellaria paradoxa*) (Gomes et al., 2010; Kiill et al., 2014; Lassen et al., 2018). However, attention should be paid to the high visitation rate of the native and solitary bee *Exomalopsis* sp., whose genus is rarely recorded as interacting with Sapotaceae flowers, except for *Manilka amazonica* and *M. huberi*, which are pollinated by native bees of several genera, including *Exomalopsis*, and by Syrphidae flies, such as *Eristalis* and *Ornidia* species (Maués, 2001, 2007). On the other hand, polliniferous flowers of *Argania spinosa* (Sapotaceae) have a typical strong fragrance and attract many insects, particularly flies of the Calliphoridae family, mainly *Chrysomya* species (Nerd, Irijimovich, & Mizrahi, 1998). In general, Syrphidae flies (also *Lucilli*a spp.) visit flowers that provide easy access to their pollen and nectar. On the other hand, Calliphoridae species (also *Ocyptamus* spp.) are often sampled in flowers with an unpleasant odor (e.g., feces, urine and fungi) because they have a preference for garbage and similar resources (Souza-Silva, Fontenelle, & Martins, 2001). *Chrysophyllum marginatum* flowers have these attributes, justifying their being visited and pollinated by species of both families. The other floral visitors, with sizes ≥5 mm (n = 22 spp.), may be considered occasional pollinators due to the lower visitation rate but together present a relatively high visitation rate (VR = 424).

Very small insects (< 5 mm) and butterflies are considered nectar and/or pollen thieves of *C. marginatum* because their anther–stigma contact seems less probable because the animal body size is very small and the proboscis is long and slender, respectively. The occurrence of pollinating or thieving depends on the adjustment between the morphologies (e.g., body size and length and strength of mouthparts) of the floral visitors and the visited pollination unit (e.g., flower or inflorescence), as well as the intrafloral behaviors of the visitors (Souza et al., 2017) that can allow anther–stigma contact. Ants only "patrol" on branches, and their role in interacting with *C. marginatum* plants needs to be investigated. The presence and abundance of ants among the flowers may influence the behavior and frequency of visits by other floral visitors, including potential pollinators (Souza, Baronio, Weirich, Oliveira, Ferreira, Arruda, & Aoki, 2020). So, although it was not demonstrated that ants were interfering in *C. marginatum* pollination (this study), the information recorded here is important for future research on plant–insect interactions.

Nectaries and osmophores were the secretory structures found in flowers of *C. marginatum* and are associated with the biotic pollination (Fahn, 1979; Endress, 1994). In Sapotaceae, nectaries have been reported in a few studies that provide a morphological description (Pennington, 2004; Hawthorne, 2014);

osmophores are also described (Gomes et al., 2010; Terra-Araujo, Faria, Ribeiro, & Swenson, 2012), or only odor is recorded (Gama, Barbosa, & Oliveira, 2011).

In *C. marginatum*, the nectary is similar morphologically to that described for Neotropical Sapotaceae species, formed by a small ring-shaped disk around the ovary base within opened flowers with radial symmetry (Pennington, 2004; Hawthorne, 2014). Histochemical analysis shows that the nectar composition is variable in *C. marginatum* (e.g., proteins, lipids, neutral polysaccharides and starch grains); this variation occurs in species visited by a diversity of generalist pollinators (Tölke, Galetto, Machado, Lacchia, & Carmello-Guerreiro, 2015; Tölke, Bachelier, Lima, Galetto, Demarco, & Carmello-Guerreiro, 2018) and also in *C. marginatum*.

Osmophores in *C. marginatum* occur in the petal margin, which differs from Sapotaceae *Micropholis guyanensis*, which has osmophores located in specific points of the petals (Terra-Araujo et al., 2012). Thus, *C. marginatum* has a greater area of secretor tissues of scents than *Micropholis guyanensis*, which may be related to the "strong" intensity of odor in *C. marginatum* flowers, which is perceivable at a long distance. This attribute may favor the attraction of a great diversity of floral visitors/pollinators to *C. marginatum*, but studies are needed to confirm this. In *C. marginatum*, the osmophores are diffuse and structurally like neighboring cells, which makes them difficult to identify; thus, they have been poorly studied (Marinho, Souza, Barros, & Teixeira, 2014). Inside the floral tube, epidermal cells have a papillate surface that does not react to osmophores when stained with neutral red; it is likely that the papillate cells are related to nectar resorption (Stpiczyñska, 2003; Stpiczyñska & Nepi, 2006) by other floral parts than the nectary itself, as found in *Linaria vulgaris* (L.) Mill., Scrophulariaceae (Nepi, Pacini, Nencini, Collavoli, & Franchi, 2003). Additionally, in *C. marginatum*, the osmophore and nectary have lipids in the cells; thus, both secretory structures may emit an odor (Baudino, Caissard, Bergougnoux, Jullien, Magnard, Scalliet, Cook, & Hugueney, 2007; Tölke, Capelli, Pastori, Alencar, Cole, & Demarco, 2019), which may increase the attractiveness of the flower.

5 | CONCLUSIONS

To our knowledge, this is the first study of the pollination ecology of the *Chrysophyllum* genus, whose population has weak and cryptic gynomonoecy, which can favor female function and can be advantageous in reducing self-pollination within (autogamy) and between flowers of the same plant (geitonogamy). As expected, due to its floral attributes (e.g., actinomorphic and open flowers, grouped in congested inflorescences, with pale color, exposed anthers/stigma, accessible nectar/pollen, odorous, nectariferous), *C. marginatum* has generalist and entomophilous pollination (26 spp. of insects), mainly by bees and flies, which are generally the main groups of floral visitors in seasonal vegetation (Souza et al., 2017). The nectaries and osmophores of *C. marginatum*are are anatomically characterized in this study for the first time, with unprecedented information that needs to be further studied via ultrastructure analysis.

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