Acta Botanica Brasilica - 34(1): 167-176. January-March 2020.
doi: 10.1590/0102-33062019abb0209

Flowering and pollination ecology of *Cleistocactus baumannii* (Cactaceae) in the Brazilian Chaco: pollinator dependence and floral larceny

Bruno Henrique dos Santos Ferreira[1, 2], **Camila Silveira Souza**[2, 3*], **André Luiz Silva Fachardo**[1],
Aline Conceição Gomes[1] and **Maria Rosângela Sigrist**[1, 4]

Received: June 19, 2019
Accepted: October 22, 2019

ABSTRACT

Cleistocactus baumannii is the only ornithophilous cactus species in the Brazilian Chaco. In addition, this species of Cactaceae invests heavily in flowering in the ecoregion. Such characteristics motivated us to evaluate the temporal availability of flowers in the context of its floral visitors. The reproductive system of *C. baumannii*, the number of individuals in flowering, flower abundance and the frequency and richness of floral visitors were evaluated and quantified. Nectar robbery was a frequent phenomenon in the studied population; therefore, we compared the pollen load deposited on the stigma of damaged (robbery) and undamaged flowers. In the Brazilian Chaco, *C. baumannii* is self-incompatible and has a continuous flowering pattern, providing floral resources throughout the year for nine species of floral visitors. One hummingbird species acted as a potential pollinator, and we consider *Xylocopa splendidula* to be a nectar robber. We found stigma of flowers damaged by nectar robbers to have lower pollen loads than those of undamaged flowers. This study highlights the importance of studying reproductive traits in different populations to understand changes in the reproductive success of plant species at different scales and possible causes, such as availability of floral visitors, incidence of robbers and flowering patterns.

Keywords: arid environments, *Chlorostilbon lucidus,* floral damage, nectar robbery, ornitophily, *Xylocopa splendidula*

Introduction

Cactaceae is one of the most diverse families in the Neotropics, with 1480 recognized species (Goettsch *et al.* 2015). This family represents some of the most conspicuous plants in the arid and semi-arid regions (Ortega-Baes *et al.* 2010) and an important floristic element of Caatinga and Chaco vegetation in Brazil (Pennington *et al.* 2000). Within the Cactaceae, bird flowers have been described for many species of cacti from different lineages, particularly in South America. In general, the taxonomic description of many of these species assumes that they have pollination systems specialised towards birds, hummingbirds, in particular (see Anderson 2001; Gorostiague & Ortega-Baes 2016).

Cleistocactus is cited as an example of extreme phenotypic specialisation to bird pollination (Anderson 2001). However, this specialization has previously only been described based on floral traits that suggest ornithophily (Rowley 1980;

---

[1] Programa de Pós-Graduação em Biologia Vegetal, Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, 79070-900, Campo Grande, MS, Brazil
[2] Programa de Pós-Graduação em Ecologia e Conservação, Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, 79070-900, Campo Grande, MS, Brazil
[3] Departamento de Botânica, Programa de Pós-Graduação em Botânica, Universidade Federal do Paraná, 81531-980, Curitiba, PR, Brazil
[4] Laboratório de Polinização, Reprodução e Fenologia de Plantas, Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, 79070-900, Campo Grande, MS, Brazil

* Corresponding author: souza.camila.bio@gmail.com

Scogin 1985; Rose & Barthlott 1994; Gomes & Araujo 2015). The first study to evaluate pollination ecology in *Cleistocactus* was developed in the Dry Chaco ecoregion and showed that *C. baumannii* was pollinated exclusively by hummingbirds, while *C. smaradigoflorus* was possibly pollinated by hummingbirds and bees (Gorostiague & Ortega-Baes 2016). It is interesting that the two species are closely related and that both are ornithophilous (Schlumpberger & Renner 2012). According to Gorostiague & Ortega-Baes (2016), many ornithophilous cacti may have generalized pollination systems (*e.g.* species of *Geohintonia*, *Mammillaria*, *Neolloydia*, *Pelecyphora* and *Pereskia*).

In Argentinean dry Chaco, *C. baumannii* flowers were visited by two hummingbird species, *Chlorostilbon lucidus* and *Colibri coruscans* (Gorostiague & Ortega-Baes 2016), while *C. baumannii* flowers in Brazilian humid Chaco were visited by one hummingbird and one bee species (Souza *et al.* 2017). In both Argentinean and Brazilian Chaco, just *Chlorostilbon lucidus* was considered a pollinator of *C. baumannii* (Gorostiague & Ortega-Baes 2016; Souza *et al.* 2017). In this sense, the extreme phenotypic floral specialization of *C. baumannii* does not impose limitations on its floral resources in Brazilian Chaco; instead, its nectar is available to bees and other floral visitors. Thus, the role of all floral visitors of *C. baumannii* needs be investigated because in a species with specialized floral morphology events of nectar robbing and theft may be recurrent (Souza *et al.* 2016).

Thieves are floral visitors that collect floral resources without pollinating the plant species, while nectar robbers collect floral resources in an illegitimate way, damaging the corolla through bites or pecks (see Inouye 1980). This damage may affect the behaviour of true pollinators and pollen flow distances (Irwin & Brody 1998; Maloof & Inouye 2000; Maloof 2001), reducing the fruit set of robbed plants, as well as the number of seeds per fruit and seed germination rate. Nectar robbers effectively cause selective pressure on plant reproductive fitness (Roubik *et al.* 1985), and in floral evolution, together or in opposition to pollinators (Zhang *et al.* 2014). However, nectar robbing may enhance cross-pollination by increasing the number of flowers the pollinators must visit. Consequently, this increases the traveling distances to obtain their daily energy, leading hummingbirds to visit flowers of different individuals (Irwin & Brody 1998; 2000; Maruyama *et al.* 2015).

In the Brazilian Chaco, a high frequency of visits comes from thieves and other non-pollinators (*e.g.*, florivores and robbers) (Souza *et al.* 2017), especially Cactaceae (Gomes *et al.* 2016; Ferreira *et al.* 2018). Most Cactaceae species in the Brazilian Chaco have long tubular flowers and high nectar production (Gomes & Araujo 2015; VGN Gomes *et al.* 2019), which are traits related to nectar robbing (Maruyama *et al.* 2015; Rojas-Nossa *et al.* 2016). This because some floral traits may be indicators of increased frequency of thievery, such as small and inconspicuous flowers, plants with many flowers and extended flowering (Rojas-Nossa *et al.* 2016; Souza *et al.* 2016). While nectar robbery is linked to flowers with long corolla (Maruyama *et al.* 2015).

A study in the plant community in the Brazilian Chaco showed that plant species with highest abundance and longest flowering period presented the highest richness and/or frequency of floral visitors (Souza *et al.* 2017). Considering both number of flowers and flowering time, *C. baumannii* makes a heavy investment in flowering in the Brazilian Chaco (VGN Gomes *et al.* 2019). The flowering time of *C. baumannii* in the Brazilian Chaco can vary from five (Freitas *et al.* 2013) to eleven months (VGN Gomes *et al.* 2019). However, this phenophase is mainly concentrated in the rainy season (Freitas *et al.* 2013; VGN Gomes *et al.* 2019), a time with more richness and frequency of floral visitors in this area (Souza *et al.* 2017).

Flowering phenology (Freitas *et al.* 2013; VGN Gomes *et al.* 2019), floral morphology and pollination syndromes (Gomes & Araujo 2015), breeding system and pollination (Bianchi *et al.* 2000; Gorostiague & Ortega-Baes 2016; Souza *et al.* 2017) of *C. baumannii* have all been studied in different locations of the Gran Chaco domain. However, since the Gran Chaco is drier toward the west and more humid toward the east (*e.g.*, Brazilian Chaco) (Lewis 1991) and floral traits and pollinators can vary between populations in different locations and climate scenarios (Schlumpberger *et al.* 2009; Gorostiague *et al.* 2018; Rech *et al.* 2018), is important to investigate new populations in different environments (Morgan 2000). Here, we aim to determine if the reproductive biology of *C. baumannii* in the Brazilian Chaco differs from that in the Argentinean Chaco. We conducted a complete study of the flowering phenology, breeding system and pollination ecology of *C. baumannii* in a remnant of Brazilian Chaco vegetation (Thorn-Forest). For this, our study to investigate the availability of flowers for pollinators over the course of one year, the morphology and floral biology of this species, the dependence of *C. baumannii* on effective pollinators for fruit set and consequent reproductive success, and the total number of floral visitors and potential pollinators.

In addition, as the incidence of nectar robbers is frequent, we also investigated the variation in pollen deposition between damaged and undamaged flowers. Finally, we will discuss possible implications on the reproductive success of this species in the Brazilian Chaco. We expected that *C. baumannii* in the Brazilian Chaco: (i) would be a self-incompatible species and hummingbird dependent for fruit set, as occur in Argentinean Chaco (*e.g.* Bianchi *et al.* 2000); (ii) will have flowers explored by many floral visitors for being a key resource, where pollen deposition on stigma will be affected by nectar robbers, fact that can compromise its reproductive success. In conclusion, we expect that in periods with greater resource availability (number of flowers and individuals in flowering), the frequency and richness of floral visitors will also be higher.

Materials and methods

Study site

We collected data on flowering and floral visitors' occurrence from November/2009 to October/2010. Additional data on floral morphology and biology, breeding system, floral visitors and pollination were recorded from November/2015 to August/2016. The study was conducted in a remnant (21º42'04"S 57º53'06"W) of Chaco vegetation (Thorn-Forest) in Porto Murtinho, Mato Grosso do Sul (Carvalho & Sartori 2015). This site is characterized by a discontinuous canopy and predominance of spiny and microphyllous species (cf. Freitas *et al.* 2013; Souza *et al.* 2017). Diverse species of Cactaceae are endemic to this ecoregion, including *C. baumannii* (Ferreira *et al.* 2018; Gomes *et al.* 2018; VGN Gomes *et al.* 2019). Climate is hot and dry, with erratic rainfall throughout the year. The dry season is from April to September, with mean rainfall below 100 mm, and the rainy season is from November to February; March and October are considered transition months (Freitas *et al.* 2013). Average annual rainfall and temperature are 970.3 mm and 25 °C, respectively (Carvalho & Sartori 2015).

Cleistocactus baumannii

Cleistocactus baumannii (Lem.) Lem. is a columnar cactus (Pivatto *et al.* 2014), exhibiting small ascending cladodes with about 1.5 meters of branching, reaching 1.5m in height, or even more if supported by another plant (Mauseth & Plemons-Rodriguez 1998). Species has numerous flowers with bright orange-red colours (Lowry 2016). In the study area *C. baumannii* bloom more intensely in the rainy season (Freitas *et al.* 2013; VGN Gomes *et al.* 2019). Samples of *C. baumannii* were collected and deposited at the CGMS Herbarium of the Universidade Federal de Mato Grosso do Sul (CGMS 35477).

Morphology and floral biology

To describe the flower life events (*e.g.* longevity, presence of dichogamy) we marked floral buds (n = 20 flowers, two per plant) which were monitored throughout the floral anthesis. We describe the floral morphology from fresh and fixed flowers (n = 30 flowers from 14 individuals). We measured with a digital caliper the diameter and total length of the floral tube length and, anthers and stigmas length. In addition, we recorded qualitative floral traits, such as colour and presence of odour. We tested pollen viability with acetic carmine solution (Dafni 1992) from pre-anthesis floral buds fixed in 70 % FAA (n = 100, five per plant). Stigma receptivity was also assessed in situ from observations of stigmatic exudates presence across the floral anthesis of flowers marked randomly in different individuals (AC Gomes *et al.* 2019; VGN Gomes *et al.* 2019).

Nectar total volume was quantified in flowers previously bagged at the bud stage using microliter syringes of 200 μL (Hamilton, Reno, NV, USA) (n = 10 flowers of different individuals). Sugar concentration (% mass/mass of sucrose equivalents) was measured with a digital refractometer (n = 10 flowers from five individuals). To evaluate the nectar secretion pattern, we measured the volume and concentration of solutes in the accumulated nectar until 11 a.m. and again at 5 p.m.

Breeding system

We performed diverse reproductive treatments (Ferreira *et al.* 2018) (n = 12 flowers per treatment from different individuals) as follows: (1) natural pollination (control) – flowers were observed under natural conditions of pollination, without manipulation; (2) hand cross-pollination – flowers were emasculated and pollinated with exogenous pollen from other individuals; (3) "geitonogamy" – flowers were pollinated with pollen from other flowers of the same individual (Arroyo 1976); (4) spontaneous self-pollination – flowers were bagged the day before anthesis and observed until fruit set; (5) hand self-pollination – flowers were pollinated with endogenous pollen; (6) apomixis/agamospermy – floral buds were bagged the day before anthesis and then emasculated and bagged again. After approximately 40 days, we recorded the fruit set and then compared our results with those in the literature for other populations of *C. baumannii*. For a description of the breeding systems, we used such classic terminology as self-sterile and self-incompatible (Zapata & Arroyo 1978; Lloyd & Schoen 1992).

Phenology: flowering and occurrence of floral visitors

We carried out the flowering phenology and floral visitors sampling in 213 plants of *C. baumannii*. In relation the flowering, we quantified monthly the number of flowers (abundance) and number of flowering plants. Concomitantly, we sampled all floral visitors by means of focal observations from 6:00 a.m. to 6:00 p.m. totalling 96 hours of observation. During these observations, we sampled the frequency of each floral visitor (each contact of the animal with a flower), and these visits, when possible, were photographed.

Flowering descriptors (number of flowers (abundance) and number of flowering plants) were classified according to Newstrom *et al.* (1994). We used circular statistics to calculate the mean angle (or vector-μ) of each descriptor (phenophase), as well as its length (r). These analyses were performed using Oriana 2.0 software (Kovach 2004), applying Rayleigh's test (Z) to verify the probability level (p) (Zar 2010). The mean angle (μ) represents the average date of phenological activity, and r represents the degree synchronization (clustering) of the phenophases around the average date (Morellato *et al.* 2010). Higher value of r (> 0.5) correlates with greater aggregation of phenophase around the average date (possible seasonal or concentration phenophase) when Z values are significant ($p<0.05$).

We next verified the pattern of occurrence of floral visitors (richness and frequency) phenology. To accomplish this, we used the same circular statistics as those used for flowering data. We performed a simple linear regression to verify the relationship between richness and frequency of floral visitors (pollinators and non-pollinators, thieves and robbers) with the abundance and number of *C. baumannii* flowering plants using the vegan package (Oksanen *et al.* 2018) in R programming (R Development Core Team 2017).

Behavior of floral visitors

We performed focal observations of floral visitors during floral anthesis. Insects were collected with an entomological net and/or bottle with ethyl acetate. Later, specimens were mounted or placed in 70 % alcohol and sent to specialists for identification. Hummingbirds were pre-identified in the field with the help of an illustrated guide (Sigrist 2007), photographed, and then confirmed by specialists (see Souza *et al.* 2017). Insects collected were deposited in the Zoological Collection of the Universidade Federal de Mato Grosso do Sul (ZUFMS).

We classified floral visitors using the terminology developed to characterize floral larceny (Inouye 1980; Irwin *et al.* 2010) as follows: potential pollinators, non-pollinators, thieves, and robbers. This approach is pragmatic for revealing pollinators in a system (Jacobs *et al.* 2010), but requires more detailed testing to rank effectiveness (Gross & Mackay 1998; Gross *et al.* 2017). Visitors classified as potential pollinators were those that contacted both staminate (anthers) and pistilate (stigma) structures with pollen on the body, demonstrating the ability to transport pollen within and between flowers of different individuals. The latter is recognized as an important step in discerning pollinators from non-pollinators (Popic *et al.* 2013). Thieves were visitors observed collecting pollen and/or nectar without contact anthers or stigma (to nectar collect) and without damage the floral parts. Robbers were visitors observed damaging the flowers, as in chewing the corolla with the mouthparts, for example, to access the resource illegitimately without contacting the reproductive structures (primary robber). When the damage made by a primary robber was used for other floral visitor to obtain illegitimately the resource (*e.g.* nectar), the floral visitor was classified as secondary robber (Inouye 1980).

Effects of nectar robbing by counting pollen grains

Since *C. baumannii* received a high frequency of nectar robbers, we marked approximately 50 flowers for visitation throughout anthesis. On the next morning (~12h after end of the anthesis), we randomly collected 30 of these flowers, 15 flowers with signs of nectar robbing and 15 flowers with no signs. The collected flowers were stored in individual bottles containing 70 % FAA fixative and transported to the laboratory. In the laboratory, the stigmas of these flowers were mounted on slides and covered with laminula for analysis under optical microscopy. All *C. baumannii* pollen grains (checked with control material obtained from buds) that adhered to stigmatic tissue were quantified by scanning the slides, always moving it in the same direction. We performed a t-test (normal distribution) to compare differences in the number of pollen grains deposited on damaged and undamaged flowers using the vegan package (Oksanen *et al.* 2018) in R programming (R Development Core Team 2017).

Results

Morphology and floral biology

Flowers are hermaphrodite, diurnal, tubular, slightly curved and reddish (Fig. 1A), with floral tube extremities orange. In the human sense, *C. baumannii* flowers are showy, but not odoriferous. Floral tube measures an average of 48.19 (± 1.71) mm in length and 9.25 (± 0.61) mm in diameter. Androecium is polystemonous and heterodynamous with approximately 129 stamens, which form a staminal column with 49.76 (± 3.65) mm of length. Anthers have wine colour and are basifixed, rimose, and produce white pollen with high viability (89.06 ± 17.19 %). Gynoecium is syncarpous with a single style and multilobed stigma, which has 50.25 (± 3.68) mm in height. Stigma has with up to nine lobes but in the most flowers there is six (30.8 %). Ovary is inferior and presents around 505 (± 147) ovules. Nectar is produced in nectariferous tissue that lines the floral tube and is stored in the lower third of this tube.

Floral longevity of *C. baumannii* is approximately 48 hours. In pre-anthesis, it is possible to perceive a yellowish tone in the extremities of floral buds. This indicates that the perianth elements have already begun to move away. In the next morning (6:00 a.m.), the corolla and anthers are already open, but the stigmatic lobes are still leaning and not yet receptive. Thus, the flowers are functionally staminate at the beginning of anthesis. From 8:00 a.m. there is pollen in the anthers, and the stigmatic lobes are partially distended and receptive. By around 10:00 a.m., there is little pollen in the anthers, starting the “pistilate phase” of the flower until the next day. In the afternoon of the second day of anthesis, the stigmatic lobes begin to wilt, and the next morning, they are completely closed. Until end of the first morning (11:00 a.m.) of floral life, flowers produce and accumulate, on average, 59.8 (± 64.25) µL of nectar with 20.24% (± 4.9) of solute concentration. We did not find new accumulation of nectar at 5:00 p.m.

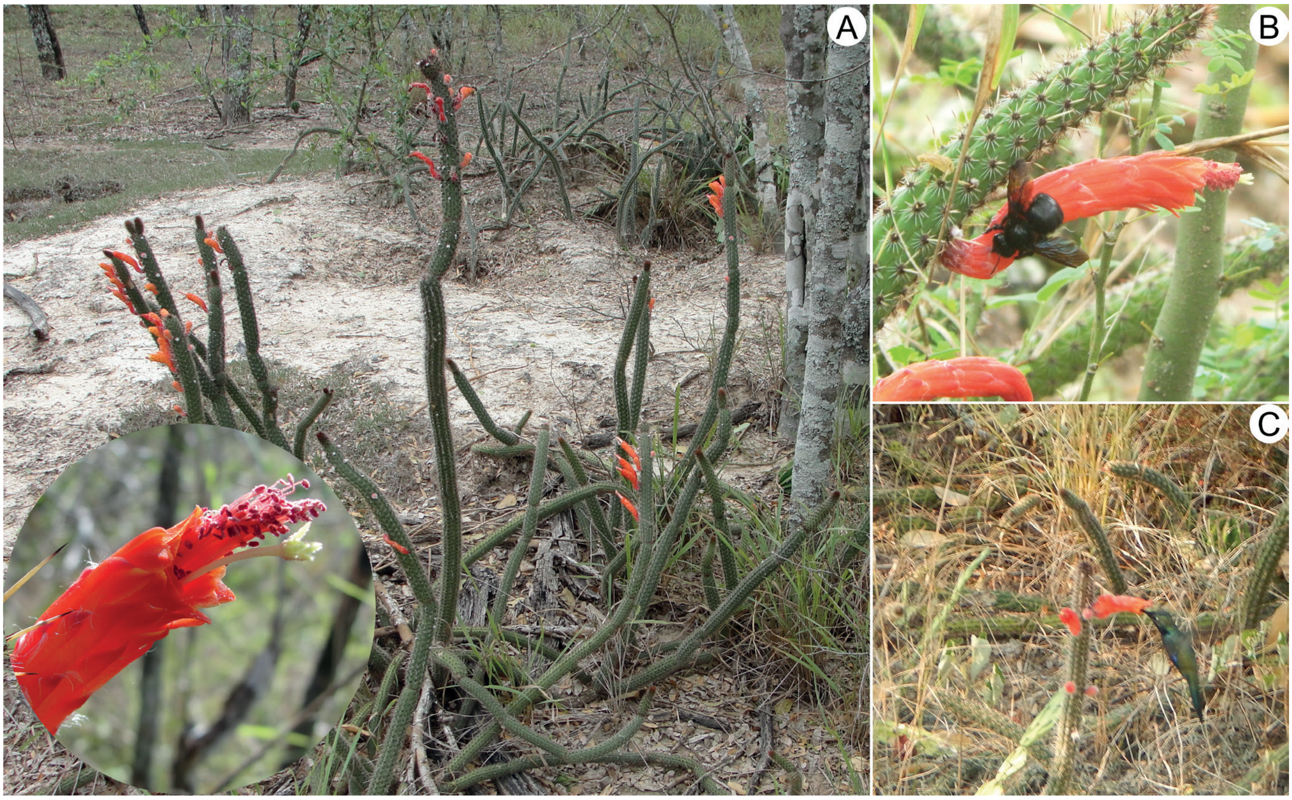

Figure 1. A. *Cleistocactus baumannii*: plants and flowers in Brazilian Chaco. **B.** Bee *Xylocopa splendidula* (nectar robber) and **C.** The hummingbird *Chlorostilbon lucidus* (potential pollinator) collecting nectar in *C. baumannii* flowers.

Breeding system: does *Cleistocactus baumannii* need pollinators?

In all studied populations, *C. baumannii* fruit set only by hand cross- and natural pollinations (Tab. 1). In Brazilian population, fruit set by natural pollination was greater than cross-pollination. However, cross-pollination fruit set in Brazil was lower compared to Argentinean populations, but similar for natural pollination (Tab. 1). In all populations, *C. baumannii* is self-sterile and self-incompatible and therefore, totally pollinator dependent.

Phenology: more flowers and flowering plants lead to greater richness and frequency of floral visitors in *Cleistocactus baumannii*

In the Brazilian Chaco, *C. baumannii* flowered for 12 months with mean dates in March (number of plants in flowering) and April (number of flowers) in the transitional season (from rainy to dry season) (Tab. 2, Fig. 2). Thus, *C. baumannii* flowering pattern is continuous (sensu Newstrom *et al*. 1994) and seasonal (*r* > 0.5; *p* <0.0001). The same was recorded for floral visitors (pollinators, non-pollinators)(r> 0.6; p <0.0001), except for richness of the pollinators (non-seasonal) (r = 0.3, p = 0.2) and the pattern of non-pollinators (extended, nine months), which were not sampled in part of the dry season (June-August). Moreover, all descriptors of the floral visitors (richness, frequency) presented mean dates in December. (Tab. 2, Fig. 2). Both richness (r2 = 0.87, p <0.0001) and frequency (r2 = 0.93, p <0.0001) of non-pollinators and richness (r2 = 0.47, p <0.05) 0.0001) and frequency of pollinators (r2 = 0.54, p <0.05) were highly correlated with the number of flowers in *C. baumannii*. The same was found for the number of flowering plants in that the richness (r2 = 0.54, p <0.05) and frequency (r2 = 0.35, p <0.05) of pollinators and richness (r2 = 0.68, p <0.0001) and frequency (r2 = 0.80; p <0.0001) of non-pollinators were also highly correlated with the number of *C. baumannii* individuals in bloom.

Are the floral visitors of *Cleistocactus baumannii* all potential pollinators? Effects of damage by nectar robbers on stigma pollen deposition

Cleistocactus baumannii flowers were visited by bees (n = 5 spp.), ants (n = 2 spp.), butterfly (*Pyrisitia* sp.) and hummingbird (*Chlorostilbon lucidus*) (Fig. 1C) (Tab. 3), which collected nectar (seven spp.) and/or pollen (bees: *Apis mellifera*, *Megachile* sp., *Xylocopa splendula*). *Xylocopa splendula* (Fig. 1B), *C. lucidus* and *A. mellifera* were the most frequent floral visitors and occurred in most sampling months (Tab. 3). To collect nectar, *C. lucidus* hovers in front of the flower, introduces the beak into the floral tube and collects nectar legitimately, contacting anthers and stigma

Table 1. Breeding system of different natural populations of *Cleistocactus baumannii* in Gran Chaco (South America).

Population			Treatments					Control	Reference
Country	Location	Vegetation Type	Spontaneous self-pollination	Hand self-pollination	Hand cross-pollination	"Geitonogamy"	Agamospermy	Natural pollination	
Brazil	Porto Murtinho, Mato Grosso do Sul	Chaco Thorn-Forest	0	0	16.66 %	0	0	25 %	Our study
Argentina	La Bodeguita, Salta	Dry Chaco	0	0	100 %	-	-	28 %	Gorostiague & Ortega-Baes 2016
Argentina	Las Gamas, Santa Fé	Chaco Woodland	-	0	88.9 %	-	-	61.9 %	Bianchi *et al.* 2000
Mean			0	0	68.52 %	0	0	38.3 %	

Table 2. Duration, number of observations throughout the year (N), mean vector (μ) and mean date, mean vector standard deviation (SD), mean vector length (r) and Rayleigh (Z) test of reproductive phenophases of *Cleistocactus baumannii*, pollinators and non-pollinators in a remnant of Chaco vegetation (Thorn Forest), Mato Grosso do Sul, Brazil.

Data	N	Mean vector SD	μ (mean date)	r	Z	p
Number of plants in flowering	213	56.568°	86.291°(29-Mar)	0.51	55.452	0
Flowering –number of flowers	1532	55.467°	90.464°(02-Apr)	0.61	578.2	0
Pollinators/events - Richness	16	88.203°	356.932°(27-Dec)	0.306	1.496	0.2
Pollinators/events- frequency	284	46.552°	343.42°(14-Dec)	0.719	146.767	0
Non-pollinators/events-Richness	39	67.172°	345°(16-Dec)	0.5	9.86	0
Non-pollinators/events-frequency	422	58.102°	335.621°(06-Dec)	0.6	210.6	0

Table 3. Frequency (visits number), visit behaviour and occurrence of the floral visitors recorded on flowers of *Cleistocactus baumannii*in a remnant of Brazilian Chaco vegetation.

Floral visitors	Visits number	Floral visitor main behaviour	Months number (occurrence)
HYMENOPTERA			
Bees			
Apis mellifera (Linnaeus, 1758)	105	Pollen thief	8 (Nov-May/Oct)
Xylocopa splendidula Lepeletier, 1841	269	Primary nectar robber, Pollen thief	8 (Nov-May/Oct)
Ceratina (Rhysoceratina) prox. *Volintans* (Schrottky, 1907)	22	Secondary nectar robber	7 (Nov-Mar/Sep-Oct)
Tetragonisca angustula (Schwarz, 1938)	6	Secondary nectar robber	3 (Dec/Sep-Oct)
Megachile sp.	6	Pollen thief	3 (Nov-Jan)
Ants			
Camponotus cf. *senex* (Smith 1858)	7	Secondary nectar robber	4 (Nov-Jan/Oct)
Crematogaster sp.	3	Secondary nectar robber	2 (Nov/Jan)
LEPIDOPTERA (butterfly)			
Pyrisitia sp.	4	Nectar thief	2 (Nov/Jan)
TROCHILIDAE (hummingbird)			
Chlorostilbon lucidus (Shaw, 1812)	251	Potential Pollinator	12 (all months)

with the upper portion of the beak and front of the head (Fig. 1C). Its visits last about two seconds, and then it flies to another plant, visiting usually only one flower per plant. Therefore, *C. lucidus* is a potential pollinator of *C. baumannii*.

Apis mellifera, *Megachile* sp. and *X. splendula* to collect pollen land on the anthers and gather pollen with the first and second pairs of legs, then transferring it to the third pair, contacting the anthers and stigma with the ventral portion of the body and legs, but acting mainly as pollen thieves (see discussion). Besides, in all visits, *X. splendidula* collected nectar as follow: the bee lands on the perianth and walks to the base of the floral tube where it pierced the tube to rob nectar (see Video S1 in supplementary material). After a few seconds the bee goes to another flower usually from the same plant where it performs similar behaviour, being considered as primary nectar robber. The hole left by *X. splendidula* was used by the bees *Ceratina* (*Rhysoceratina*) prox. *volintans* and *Tetragonisca angustula* and ants to access the floral nectar, acting as secondary nectar robbers. *Pyrisitia* sp. was considered as nectar thief because it does not contact anthers and stigma to collect nectar.

By the end of floral anthesis, we recorded less pollen grains adhered to the stigmatic surface of flowers damaged by primary nectar robbing (*X. splendula*) than non-damaged flowers. We found higher pollen deposition on stigmas of undamaged flower (825 ± 215; t = 3.45; df = 28; $p < 0.0001$) in relation to damaged flowers by nectar robbing (*X. splendula*) (542 ± 236; Fig. 3).

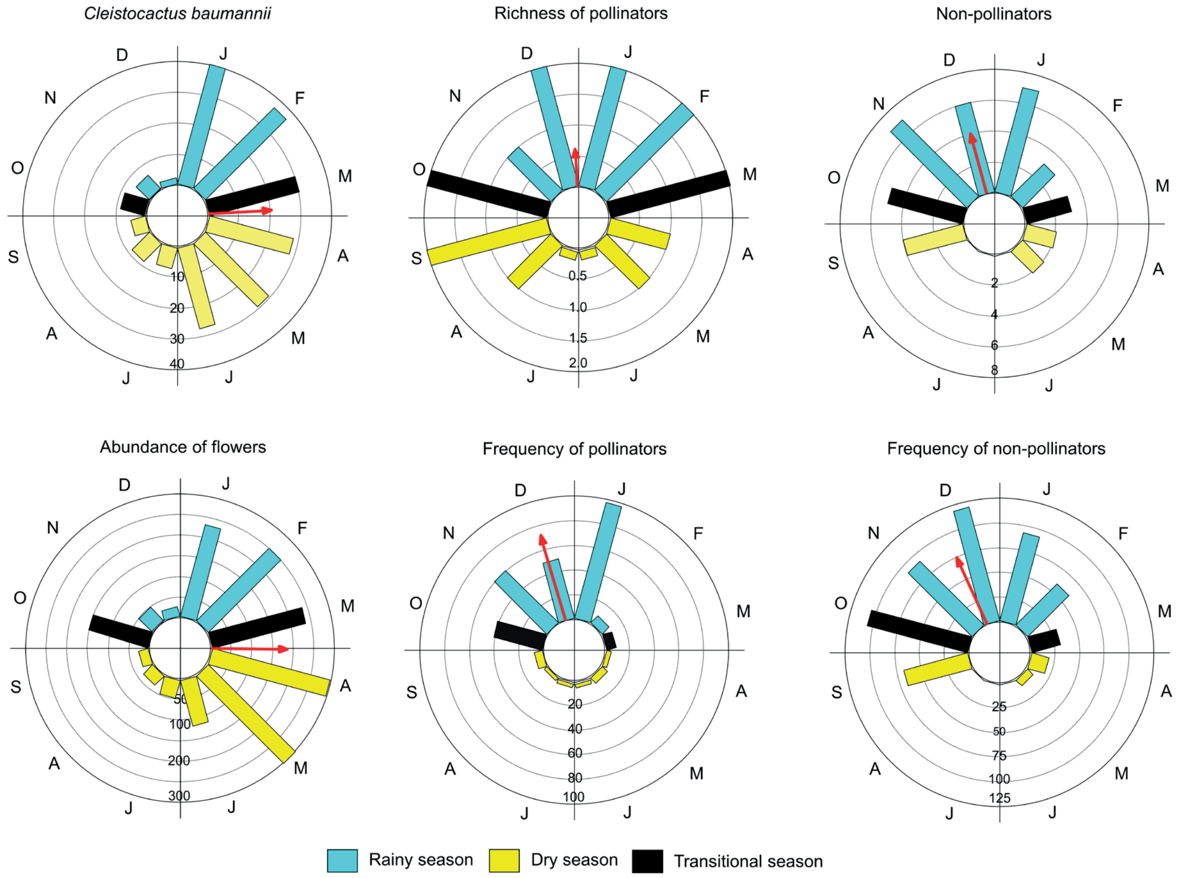

Figure 2. Circular histograms of flowering and floral visitors of *Cleistocactus baumannii* across the year in the Brazilian Chaco. Letters around the circle indicate the months and numbers inside the histograms indicate the number of species/individuals in the respective month. Vectors indicating the length and direction of the mean are in red.

Discussion

In the studied population, *C. baumannii* has continuous flowering pattern (all year), differing slightly to reported for Cactaceae in some arid or semi-arid ecosystems, such as deserts (McIntosh 2002), savannas (Fonseca *et al.* 2008) and thorn-forests (Ortíz *et al.* 2010), including in Brazilian Chaco (Gomes *et al.* 2016; Ferreira *et al.* 2018; VGN Gomes *et al.* 2019), which the predominant pattern was extended (*sensu* Newstrom *et al.* 1994). According to VGN Gomes *et al.* (2019) this fact demonstrates the importance of cacti as resources for animals throughout the year, mainly in dry environments.

Cleistocactus baumannii flowers are source of pollen and nectar for anthophilous fauna. In the Brazilian Chaco, *C. baumannii* nectar is more voluminous (average = 59.8 µl) than that recorded in Argentina (average = 24.9 µl), but it is more diluted (average = 20.24 %) in relation Dry Chaco population (average = 64.5 %) (Gorostiague & Ortega-Baes 2016). According to Tamm & Gass (1986), it is important to investigate the combined effects of nectar volume and concentration on the rate and preference of energy intake of possible pollinators. This was done

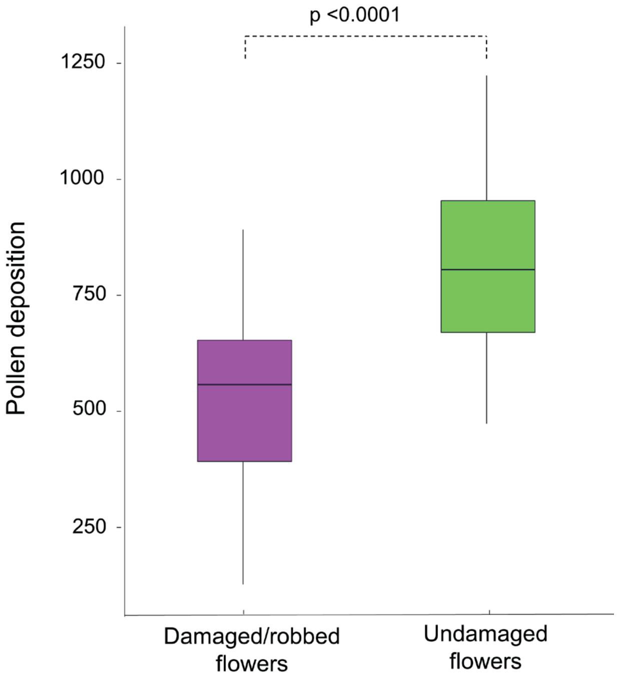

Figure 3. Pollen deposition on stigma of damaged and undamaged flowers of *Cleistocactus baumannii* in the Brazilian Chaco.

by Scogin (1985) who then defended *C. baumannii* is ornithophilous.

Indeed, diverse floral traits of *C. baumannii* suggest pollination by hummingbirds (ornithophily) such as diurnal anthesis, “long” tubular flower, reddish color and copious nectar production. Although the predictive validity of pollination syndromes remains controversial, some studies provide evidence that floral traits may be linked to the main pollinator group (Armbruster *et al.* 2011; Danieli-Silva *et al.* 2012; Rosas-Guerrero *et al.* 2014). In fact, *C. baummanni* is ornithophily, as found here (see below) and in several previous studies (Scogin 1985; Gomes & Araujo 2015; Gorostiague & Ortega-Baes 2016). This means that hummingbirds are the main functional group exerting selective pressure on the floral traits of these species, even though these traits do not exclude other floral visitors and potentially fewer effective pollinators. According to Fenster *et al.* (2004), associate effectiveness and functional groups of pollinators with floral design clearly contributes to the understanding of the relevance of pollination syndromes.

Effective pollinators are extremely important for *C. baumannii* in relation to the reproductive system of the species. Although anthers and stigma are arranged at about the same level (no herkogamy), we did not register fruit set after spontaneous self-pollination. In addition, certain temporal separation of reproductive structures occurs in flowers, as pollen is released before the stigma receptivity (partial protandry). This probably helps to reduce self-pollination and clogging of stigma with “inadequade” pollen, as the species has self-incompatibility (Ross 1981; Bianchi *et al.* 2000; Gorostiague & Ortega-Baes 2016). *Cleistocactus baumannii* does not fruit by “geitonogamy”, a process genetically similar to self-pollination (Arroyo 1976). Thus, the population studied maintains self-incompatibility, an important mechanism to prevent inbreeding (Godoy *et al.* 2018). However, the fruit set of *C. baumannii* in the Brazilian Chaco by natural conditions and mainly by hand cross-pollination was very low compared to other populations in Argentina (Bianchi *et al.* 2000; Gorostiague & Ortega-Baes 2016).

We registered only 25 % fruit set under natural conditions. This low value may be related to the frequency of illegitimate visitors (Inouye 1980), especially nectar robbers (Irwin *et al.* 2001; Bergamo & Sazima 2018). Among the nine species of floral visitors that we observed, only one species - the hummingbird *C. lucidus* - act as potencial pollinator; the other species (mainly bees) acted as non-pollinators, especially as nectar robber (see below), probably due to the long tube corolla, which restricts legitimate access to nectar. According to Gorostiague & Ortega-Baes (2016), ornithophilous characteristics do not restrict other pollinators (other than birds) that could use the resources offered by the flower. We show that *C. baumannii* and anthophilous fauna are related based on richness and frequency of floral visitors, pollinators or not, positive related with the flowering of this species. Therefore, the diversity of the anthophilous fauna in the studied chaquenian vegetation probably are sensitive to, or dependent on, the availability of floral resources of species as *C. baummanni* (Souza *et al.* 2017).

Bees visit the flowers of *C. baumannii* to collect pollen and nectar. The most frequent floral visitor, *X. splendidula*, while collecting pollen, contacts *C. baumannii*’s reproductive structures. However, after collecting pollen, *X. splendidula* collects nectar illegitimately as a robber. In this case, this bee species could be both pollinator and robber of flowers of the same species (Navarro 2000). However, this bee species would not be as effective pollinator considering the reproductive system of *C. baumannii* and its visiting pattern and behaviour. *Xylocopa splendidula* visited all flowers of each individual, potentially promoting mainly self-pollination and “geitonogamy” (Video S1 in supplementary material). We found that flowers with apparent signs of nectar robbery (damage) has lower deposition of pollen on their stigmas. Nectar robbers, such as *X. splendidula*, can decrease the reproductive success of *C. baumannii* in two ways: by reduce the number of flowers visited by effective pollinators (hummingbirds), and/or by clogging the stigma with pollen from the same individual (see Goulson *et al.* 1998).

Conclusions

We concluded that hummingbirds appear to be more efficient at promoting cross-pollination in *C. baumannii*, since they only visited one flower per plant. The increased deposition of pollen on stigma of undamaged flowers evidences the efficiency of *C. lucidus* in the pollination service, while other visitors would be less effective, decreased the reproductive success of *C. baumannii*. In fact, the occasional contribution of nectar robbers to pollination of *C. baumannii* did not show any positive effects in our study. The production of nectar, a resource collected by hummingbirds, occurs only during the onset of anthesis. This indicates that legitimate visitors with a few quick visits can deposit more pollen on the stigma of *C. baumannii*, than visitors that are pollen collectors and nectar robbers. Finally, this study highlights the importance of studying reproductive traits in different populations to understand changes in the reproductive success of plant species at different scales and possible causes, such as availability of floral visitors, incidence of robbers and flowering patterns.

Acknowledgements

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) – Finance Code 001 and financial support to CSS (88887.318827/2019-00); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financing the fieldwork via Casadinho II Project. Mr. S. Oliveira for permission to access Fazenda Retiro Conceição; Mr. N. Cintra, his family and employees, for lodging and permission to access Fazenda Anahí. J.R. Fabri for his help in the field and two anonymous reviewers for suggestions that improved the manuscript quality.

References

Anderson EF. 2001.The cactus family. Portland, Timber Press.

Armbruster WS, Gong YB, Huang SQ. 2011. Are pollination "syndromes" predictive? Asian *Dalechampia* fit neotropical models. The American Naturalist 178: 135-143.

Arroyo MTK. 1976. Geitonogamy in animal pollinated tropical angiosperms. A stimulus for the evolution of self-incompatibility. Taxon 25: 543-548.

Bergamo PJ, Sazima M. 2018. Differential outcomes of nectar robbing on the reproductive success of a melittophilous and an ornithophilous species. International Journal of Plant Sciences 179: 192-197.

Bianchi MB, Gibbs PE, Prado DE, Vesprini JL. 2000. Studies on the breeding systems of understorey species of a Chaco woodland in NE Argentina. Flora 195: 339-348.

Carvalho FS, Sartori ÂL. 2015. Reproductive phenology and seed dispersal syndromes of woody species in the Brazilian Chaco. Journal of Vegetation Science 26: 302-311.

Dafni A. 1992. Pollination ecology: a practical approach. Oxford, Oxford University Press.

Danieli-Silva A, Souza JMT, Donatti AJ, *et al*. 2012. Do pollination syndromes cause modularity and predict interactions in a pollination network in tropical high-altitude grasslands? Oikos 121: 35-43.

Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD. 2004. Pollination syndromes and floral specialization. Annual Review of Ecology, Evolution, and Systematics 35: 375-403.

Ferreira BHS, Gomes AC, Souza CS, Fabri JR, Sigrist MR. 2018. Pollination and reproductive system of synchronopatric species of Cactaceae (Cactoideae) subject to interspecific flow of pollen: an example of ecological adaptation in the Brazilian Chaco. Plant Biology 20: 101-112.

Fonseca RBS, Funch LS, Borba EL. 2008. Reproductive phenology of *Melocactus* (Cactaceae) species from Chapada Diamantina, Bahia, Brazil. Revista Brasileira de Botânica 31: 237-244.

Freitas T, Souza C, Aoki C, Arakaki L, Stefanello T, Sartori ÂL, Sigrist MR. 2013. Flora of Brazilian humid Chaco: composition and reproductive phenology. Check List 9: 973-979.

Godoy FMDR, Lenzi M, Ferreira BHS, Silva LV, Zanella CM, Paggi GM. 2018. High genetic diversity and moderate genetic structure in the self-incompatible, clonal *Bromelia hieronymi* (Bromeliaceae). Botanical Journal of the Linnean Society 187: 672-688.

Goettsch B, Hilton-Taylor C, Cruz-Piñón G, *et al.* 2015. High proportion of cactus species threatened with extinction. Nature Plants 1: 1-7.

Gomes AC, Ferreira BHS, Souza CS, *et al*. 2019. Adaptive response of extreme epiphyte *Tillandsia* species (Bromeliaceae) is demonstrated by different sexual reproduction strategies in the Brazilian Chaco. Botanical Journal of the Linnean Society (in press). doi: 10.1093/botlinnean/boz104

Gomes VGN, Araujo A. 2015. Cacti species from the Brazilian Chaco: floral and fruit traits. Gaia Scientia 9: 1-8.

Gomes VGN, Fava WS, Zappi D, Araujo AC. 2018. An illustrated field guide as a tool for conservation of Cacti Species in the Brazilian Chaco. Cactus and Succulent Journal 90: 201-203.

Gomes VGN, Koroiva R, Araujo AC. 2016. Vertebrate florivory on the short-columnar cactus *Echinopsis rhodotricha* K. Schum. in the Brazilian Chaco. Plant Ecology 217: 1481-1487.

Gomes VGN, Valiente-Banuet A, Araujo AC. 2019. Reproductive phenology of cacti species in the Brazilian Chaco. Journal of Arid Environments 161: 85-93.

Gorostiague P, Ortega-Baes P. 2016. How specialised is bird pollination in the Cactaceae? Plant Biology 18: 63-72.

Gorostiague P, Sajama J, Ortega-Baes P. 2018. Will climate change cause spatial mismatch between plants and their pollinators? A test using Andean cactus species. Biological Conservation 226: 247-255.

Goulson D, Stout JC, Hawson SA, Allen JA. 1998. Floral display size in comfrey, *Symphytum officinale* L. (Boraginaceae): relationships with visitation by three bumblebee species and subsequent seed set. Oecologia 113: 502-508.

Gross CL, Mackay D. 1998. Honeybees reduce fitness in the pioneer shrub *Melastoma affine* (Melastomataceae). Biological Conservation 86: 169-178.

Gross CL, Whitehead JD, Souza C, Mackay D. 2017. Unsuccessful introduced biocontrol agents can act as pollinators of invasive weeds: Bitou Bush (*Chrysanthemoides monilifera* ssp. *rotundata*) as an example. Ecology and Evolution 7: 8643-8656.

Inouye DW. 1980. The terminology of floral larceny. Ecology 61: 1251-1253.

Irwin RE, Brody AK. 1998. Nectar robbing in *Ipomopsis aggregata*: effects on pollinator behavior and plant fitness. Oecologia 116: 519-527.

Irwin RE, Brody AK. 2000. Consequences of nectar robbing for realized male function in a hummingbird-pollinated plant. Ecology 81: 2637-2643.

Irwin RE, Brody AK, Waser NM. 2001. The impact of floral larceny on individuals, populations, and communities. Oecologia 129: 161-168.

Irwin RE, Bronstein JL, Manson JS, Richardson L. 2010. Nectar robbing: ecological and evolutionary perspectives. Annual Review of Ecology, Evolution, and Systematics 41: 271-292.

Jacobs JH, Clark SJ, Denholm I, Goulson D, Stoate C, Osborne JL. 2010. Pollinator effectiveness and fruit set in common ivy, *Hedera helix* (Araliaceae). Arthropod-Plant Interactions 4: 19-28.

Kovach WL. 2004. Oriana for Windows, version 2.0. Pentraeth, Kovach Computer Services.

Lewis JP. 1991. Three levels of floristical variation in the forests of Chaco, Argentina. Journal of Vegetation Science 2: 125-130.

Lloyd DG, Schoen DJ. 1992. Self-and cross-fertilization in plants. I. Functional dimensions. International Journal of Plant Sciences 153: 358-369.

Lowry M. 2016. A synopsis of the genus *Cleistocactus* Lemaire (Cactaceae). Bradleya 34: 148-186.

Maloof JE. 2001. The effects of a bumble bee nectar robber on plant reproductive success and pollinator behavior. American Journal of Botany 88: 1960-1965.

Maloof JE, Inouye DW. 2000. Are nectar robbers cheaters or mutualists? Ecology 81: 2651-2661.

Maruyama PK, VizentinBugoni J, Dalsgaard Bo, Sazima I, Sazima M. 2015. Nectar robbery by a hermit hummingbird: association to floral phenotype and its influence on flowers and network structure. Oecologia 178: 783-793.

Mauseth JD, Plemons-Rodriguez BJ. 1998. Evolution of extreme xeromorphic characters in wood: a study of nine evolutionary lines in Cactaceae. American Journal of Botany 85: 209-218.

McIntosh ME. 2002. Flowering phenology and reproductive output in two sister species of *Ferocactus* (Cactaceae). Plant Ecology 159: 1-13.

Morellato LPC, Alberti LF, Hudson IL. 2010. Applications of circular statistics in plant phenology: a case studies approach. In: Hudson IL, Keatley MR. (eds.) Phenological research. Dordrecht, Springer. p. 339-359.

Morgan MT. 2000. Evolution of interactions between plants and their pollinators. Plant Species Biology 15: 249-259.

Navarro N. 2000. Pollination ecology of *Anthyllis vulneraria* subsp. *vulgaris* (Fabaceae): nectar robbers as pollinators. American Journal of Botany 87: 980-985.

Newstrom LE, Frankie GW, Baker HG. 1994. A new classification for plant phenology based on flowering patterns in lowland tropical rain forest trees at La Selva, Costa Rica. Biotropica 26: 141-159.

Oksanen AJ, Blanchet FG, Friendly M, *et al.* 2018. Vegan: Community Ecology Package. R Packag. version 2.5-1. https://CRAN.R-project.org/package=vegan

Ortega-Baes P, Sühring S, Sajama J, *et al*. 2010. Diversity and conservation in the cactus family. In: Ramawat K. (ed.) Desert plants. Berlin/ Heidelberg, Springer. p. 157-173.

Ortíz F, Stoner KE, Perez-Negron E, Casas A. 2010. Pollination biology of *Myrtillocactus schenckii* (Cactaceae) in wild and managed populations of the Tehuacán Valley, México. Journal of Arid Environments 74: 897-904.

Pennington RT, Prado DE, Pendry CA. 2000. Neotropical seasonally dry forests and Quaternary vegetation changes. Journal of Biogeography 27: 261-273.

Pivatto MS, Funes G, Ferreras AE, Gurvich DE. 2014. Seed mass, germination and seedling traits for some central Argentinian cacti. Seed Science Research 24: 71-77.

Popic TJ, Wardle GM, Davila YC. 2013. Flower-visitor networks only partially predict the function of pollen transport by bees. Austral Ecology 38:76-86.

R Development Core Team. 2017. R: A language and environment for statistical computing. Vienna, R Foundation for Statistical Computing. https://www.r-project.org/

Rech AR, Jorge LR, Ollerton J, Sazima M. 2018. Pollinator availability, mating system and variation in flower morphology in a tropical savanna tree. Acta Botanica Brasilica 32: 462-472.

Rojas-Nossa SV, Sánches JM, Navarro L. 2016. Nectar robbing: a common phenomenon mainly determined by accessibility constraints, nectar volume and density of energy rewards. Oikos 125: 1044-1055.

Rosas-Guerrero V, Aguilar R, Martén-Rodríguez S, *et al*. 2014. A quantitative review of pollination syndromes: do floral traits predict effective pollinators? Ecology Letters 17: 388-400.

Rose MJ, Barthlott W. 1994. Coloured pollen in Cactaceae: a mimetic adaptation to hummingbird-pollination? Botanica Acta 107: 402-406.

Ross R. 1981. Chromosome counts, cytology, and reproduction in the Cactaceae. American Journal of Botany 68: 463-470.

Roubik DW, Holbrook NM, German Parra V. 1985. Roles of nectar robbers in reproduction of the tropical treelet *Quassia amara* (Simaroubaceae). Oecologia 66: 161-167.

Rowley G. 1980. Pollination syndromes and cactus taxonomy. The Cactus and Succulent Journal of Great Britain 42: 95-98.

Schlumpberger BO, Cocucci AA, Moré M, Sérsic AN, Raguso RA. 2009. Extreme variation in floral characters and its consequences for pollinator attraction among populations of an Andean cactus. Annals of Botany 103: 1489-1500.

Schlumpberger BO, Renner SS. 2012. Molecular phylogenetics of *Echinopsis* (Cactaceae): Polyphyly at all levels and convergent evolution of pollination modes and growth forms. American Journal of Botany 99: 1335-1349.

Scogin R. 1985. Nectar constituents of the Cactaceae. Southwest Naturalist 30: 77-82.

Sigrist T. 2007. Guia de campo—Aves do Brasil oriental. São Paulo, Avis Brasilis.

Souza CS, Aoki C, Alcantara DMC, *et al*. 2017. Diurnal anthophilous fauna in Brazilian Chaco vegetation: phenology and interaction with flora. Brazilian Journal of Botany 40: 203-213.

Souza CS, Aoki C, Ribas A, Pott A, Sigrist MR. 2016. Floral traits as potential indicators of pollination *vs*. theft. Rodriguésia 67: 309-320.

Tamm S, Gass CL. 1986. Energy intake rates and nectar concentration preferences by hummingbirds. Oecologia 70: 20-23.

Zapata TR, Arroyo MTK. 1978. Plant reproductive ecology of a secondary deciduous tropical forest in Venezuela. Biotropica 10: 221-230.

Zar JH. 2010. Biostatistical analysis. 5th. edn. Upper Saddle River, Pearson Prentice-Hall.

Zhang YW, Zhao JM, Inouye DW. 2014. Nectar thieves influence reproductive fitness by altering behaviour of nectar robbers and legitimate pollinators in *Corydalis ambigua* (Fumariaceae). Journal of Ecology 102: 229-237.