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Reproductive biology of columnar cacti: are bats the only protagonists in the pollination of *Pilosocereus*, a typical chiropterophilous genus?

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Abstract The Cactaceae display a wide array of pollination systems, with many different animals already confirmed as pollinators. *Pilosocereus* is one of the most conspicuous bat-pollinated genera characteristic of the tropical dry forests of Brazil, known as *Caatinga*. The role of bats, hawkmoths and bees as pollinators in natural populations was investigated for four *Pilosocereus* species (*P. catingicola*, *P. chrysostele*, *P. gounellei* and *P. pachycladus*). Earlier results obtained with *P. tuberculatus* were also included in the comparative discussion. These species depend mainly upon nocturnal visitors; however, *Xylocopa grisescens* bees also play a secondary role in the fruit set. Although chiropterophily is predominant in *Pilosocereus*, *P. gounellei* was visited and pollinated at night exclusively by sphingids. All species are described as presenting a chiropterophilous syndrome based on their nocturnal anthesis, strong and unpleasant flower scent, pale, white or cream inner perianth, compact and abundant flower parts, and large volume of nectar with low sugar concentration. Pollen is available and the stigma is receptive throughout anthesis. With the exception of *P. gounellei*, there was no fruit set from spontaneous or manual self-pollination, indicating that allogamy is the predominant reproductive system in all the species studied. These species thus rely on animals that function as efficient pollen vectors for their sexual reproduction. The *Pilosocereus* species present different levels of dependence on bats, from the participation of secondary pollinators to not being visited by bats at all. Dependence levels are associated with the species floral attributes, which, although subdued, can determine different pollination systems.

Keywords bat pollination · Caatinga · Cactaceae · chiropterophily · columnar cactus · hawkmoth pollination · mixed pollination systems · tropical dry forest

Introduction

Many pollination systems considered stereotypically specialized may have groups of secondary pollinators (Johnson and Steiner 2000; Fenster et al. 2004). This can be seen in the case of bat pollination, which is categorized in different levels of specialization (*sensu* Ollerton et al. 2007). Chiropterophily comprises intense

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phenotypic specialization, making it a highly predictable pollination syndrome (Fleming et al. 2009; Rosas-Guerrero et al. 2014). Functional observation of the effectiveness of bats as pollinators based on their morphological and behavioural characteristics (Muchhala and Thomson 2010) also leads to cases of ecological specialization (considering the number of pollinating species of bats; Muchhala 2006). Nevertheless, there are cases where species considered bat pollinated have mixed systems, usually involving other vertebrates or possibly insects, both diurnal and nocturnal (Muchhala et al. 2009; Queiroz et al. 2015, 2016).

The Cactaceae are renowned as a group where bat-pollination is common, with records for 172 species belonging to 37 genera (Vogel 1968; Dobat and Peikert-Holle 1985; Mutke et al. 2015). Many of these genera are exclusive from the northern, most expressive centre of diversity of the Cactaceae located in the deserts of Mexico and the United States (e.g. *Carnegiea*, *Neobuxbaumia*, *Pachycereus*, *Stenocereus*, *Weberocereus*), while others are endemic to Eastern Brazil (Taylor and Zappi 2004; Mandujano et al. 2010; Schlumpberger 2010; Hernández-Hernández et al. 2014), the third diversity centre for the family (e.g. *Coleocephalocereus*, *Espostoopsis*, *Facheiroa*, *Stephanocereus*). Flowers of these genera display attributes related to bat pollination, fitting within the chiropterophilous syndrome as described by Faegri and van der Pijl (1979), such as: night-time anthesis, pale or dull colours, often white, strong scent and vast amount of nectar with low sugar concentration.

Among numerous bat pollination records in the Cactaceae (Porsch 1939; Locatelli et al. 1997; Valiente-Banuet et al. 1996, 1997a, b, 2007; Rocha et al. 2007a,b; Munguía-Rosas et al. 2009; Martins et al. 2016), the majority are columnar and found within the subfamily Cactoideae. They are mostly concentrated in the tribe Pachycereeae and within the BCT clade that includes Trichocereeae alongside the formerly recognized the tribes Cereeae and Browningieae (Hernández-Hernández et al. 2011).

The Cactaceae are exclusively pollinated by animals, and besides bats we found records of many other groups (Porsch 1939; Grant and Grant 1979a, b, c; Grant et al. 1979; Hunt and Taylor 1990; Bustamante and Búrquez 2005; Mandujano et al. 2010; Schlumpberger 2010) such as bees (Alcorn et al. 1959; Fleming et al. 1994; Schlindwein and Wittmann 1995, 1997; Viana et al. 2001; Gorostiague and Ortega-Baes 2017; Martins and Freitas 2018), birds (Raw 1996; Locatelli and Machado 1999a; Aona et al. 2006; Nassar et al. 2006; Gorostiague and Ortega-Baes 2017), hawkmoths (Silva and Sazima 1995; Locatelli and Machado 1999b; Aona et al. 2006; Ortega-Baes et al. 2011; Ferreira et al. 2018) and lizards (Gomes et al. 2014). Additionally, studies including representatives of Pachycereeae revealed a geographic dichotomy where species found between Venezuela and Mexico have almost exclusively specialized bat pollination (Nassar et al. 1997; Valiente-Banuet 2002; Ibarra-Cerdeña et al. 2005), while the species found in extra-tropical deserts of North America display more generalist systems that involve diurnal animals (bees and birds) as well as bats in their pollination (Fleming et al. 1996, 2001; Valiente-Banuet 2002). On the other hand, despite being well documented (Fleming et al. 1996; Locatelli and Machado 1999a; Viana et al. 2001; Valiente-Banuet 2002; Soriano and Ruiz 2002; Aona et al. 2006; Rocha et al. 2007a,b; Munguía-Rosas et al. 2009), the role of secondary pollination in cactus species needs more detailed studies to be fully understood.

Pollination biology studies in *Pilosocereus* have revealed a bat-pollination trend and xenogamous reproductive system (Sosa and Soriano 1996; Locatelli et al. 1997; Nassar et al. 1997; Ruiz et al. 1997; Valiente-Banuet et al. 1997a, b; Rivera-Marchand and Ackerman 2006; Rocha et al. 2007a,b; Munguía-Rosas et al. 2009). Despite the obvious chiropterophilous attributes of its flowers and the effective bat-pollination recorded for some species, there are records of day-time visitors playing a role in 7% to 100% of fruit formation (Rivera-Marchand and Ackerman 2006; Rocha et al. 2007b; Munguía-Rosas et al. 2009).

In this context, we seek to throw light on the duality of specialization in bat pollination in contrast to the existence of groups of secondary diurnal and nocturnal pollinators. We also intend to test the level of predictability of chiropterophily associated with some species of the Cactaceae, a family that has a wide diversity of pollinator groups. Thus, we select four species of *Pilosocereus* (a genus commonly associated with pollination by bats) in order to answer the following questions: (1) What is the participation of bats in the pollination systems of *Pilosocereus*?; (2) How do bats differ in effectiveness against other pollinators?; (3) How do flower attributes link to different levels of bat dependence?; and (4) How does the reproductive system generate dependence on pollinators? From these questions, we expected to find that bats have a predominant role, being the most effective pollinators of the

Pilosocereus species. In addition, we expected different levels of association with bats to be related with floral attributes and xenogamy.

Material and methods

Study area and species

Field work was carried out in two areas of the Caatinga, a semiarid biome exclusive to northeastern Brazil. The Catimbau National Park (PARNA Catimbau) in Pernambuco State (8°32′14″ – 8°35′12″ S and 37°14′42″ – 37°15′02″ W), a locality dominated by arenitic rock outcrops and a vegetation mosaic formed mainly by shrubby, semi-deciduous, xerophytic Caatinga (Rodal et al. 1998; Andrade et al. 2004) with 25°C mean annual temperature and 1,095.9 mm yearly rainfall (SUDENE 1990), and Dona Soledade Farm (DSF) in the State of Paraíba (07°20′30″ – 7°20′85″ S and 36°18′06″ – 36°18′ 31″ W), comprising Caatinga vegetation with mean annual temperature of 25°C and 330 mm yearly rainfall concentrated between April and June (Governo da Paraíba 2007). Fieldwork in both areas was carried out between September 2003 and December 2005, with focal observations totaling 680 h.

Pilosocereus is amongst the largest genera of columnar Cactaceae from subfamily Cactoideae, with 40 species found between Mexico and Paraguay (Hunt et al. 2006). There are 29 species recorded for Brazil, 26 of which are endemic (BFG 2015), distributed in five out of six biomes (Caatinga, Cerrado, Atlantic Rainforest, Pantanal and Amazon Rainforest), mostly in open vegetation types associated with various types of rock outcrop (Zappi 1994; Taylor and Zappi 2002, 2004).

The four species of *Pilosocereus* studied: (1) *P. catingicola* (Gürke) Byles & G.D. Rowley subsp. *salvadorensis* (Werderm.) Zappi (from here on referred to only as *P. catingicola*), (2) *P. chrysostele* (Vaupel) Byles & G.D Rowley, (3) *P. gounellei* (F.A.C. Weber) Byles & G.D. Rowley Subsp. *gounellei* (from here only *P. gounellei*) and (4) *P. pachycladus* F. Ritter subsp. *pernambucoensis* (F. Ritter) Zappi (from here on only *P. pachycladus*), are endemic to the Caatinga, excepting *P. catingicola*, with wider distribution spanning over two Brazilian Biomes (Caatinga and Atlantic Rainforest). The four species flower continuously throughout the year, but there are differences in their flowering peaks: *P. catingicola* (flowering peak from February to May), *P. chrysostele* (March–June), *P. gounellei* (alternating peaks in the months of January, March and June) and *P. pachycladus* (January–August) (Rocha 2007a). The individuals used formed discontinual groups, distant from each other between 500 and 5,000 m. Therefore, our choice to carry out experiments and observations was based on the availability and access to the flowers in each field day for along the populations of their respective species: *Pilosocereus catingicola* (*n* = 32, PARNA Catimbau); *P. pachycladus* (*n* = 31, Dona Soledade Farm) both tree-like; *P. chrysostele* (*n* = 36, PARNA Catimbau) erect, shrubby, but not branched above the base; and *Pilosocereus gounellei* (*n* = 30, Dona Soledade Farm), shrubby and spreading.

Identification of botanical material was based on species keys, descriptions and observations from specialized literature (Zappi 1994; Taylor and Zappi 2004). The voucher specimens were herborized and deposited at the herbaria of the Universidade Federal de Pernambuco (UFP) and Universidade Estadual de Santa Cruz (HUESC) under the following numbers: *P. catingicola* (E.A. Rocha 1596, 1597), *P. chrysostele* (E.A. Rocha 1490, 1514, 1534), *P. gounellei* (E.A. Rocha 1177, 1486, 1520) and *P. pachycladus* (E.A. Rocha 1178, 1487, 1521).

Participation of bats in *Pilosocereus* pollination systems

Description of pollinator behaviour

In order to compare the role of bats in the pollination system of *Pilosocereus* species we recorded their visiting behavior, as well as that for any other floral visitors. The records were carried out in the peak of flowering by continuous naturalistic observations (Lehner 1979) in focal individuals of the four species of *Pilosocereus* throughout anthesis, starting at dusk (18:00~18:30 h) and lasting until midday of the following day (10 nights/day totalling 170 h of observation by species). These observations were complemented by the analysis of photographs taken during the visits. From these techniques we differentiate nectar and pollen robbers from pollinators and determinate the respective pollen deposition areas in their bodies.

To identify species of animal visitors we utilized different methods: bats – for four nights, the individuals were captured with mist nets placed between the observed plants from 17:00 to 5:00 h and verified the existence of pollen in the bats body parts that contact the ring of anthers present in the respective flowers (determined via photographs, see previous paragraph). These animals were conditioned in alcohol to morphological analyses and the species were determined by specialists, Drs. Deoclécio Queiroz Guerra (UFPE) and Ivan Sazima (UNICAMP) (voucher specimens were incorporated into the Coleção de Mamíferos da Universidade Federal de Pernambuco – Astúa and Guerra 2008); hummingbirds – recorded by photography and identified using their plumage patterns; insects – actively captured with nets and kept dry (insect voucher specimens were deposited at the Laboratório de Biologia Floral e Reprodutiva da Universidade Federal de Pernambuco).

Pollination network

We organized data of the four *Pilosocereus* species and their visitors in an adjacency matrix P × A, with cactus species listed as rows (P) and species of visitors listed as columns (A) (Bascompte 2007). From then we represented the network system across a graph, where the nodes represented visited plants and animals and the edges are their interactions. We provided a measure of connectance (the proportion of possible edges that are actually associated with nodes) and we also calculated the degree centrality (number of edges connected to a given node, which is a type of local centrality) for each species of *Pilosocereus* (Lau et al. 2017). For each visitor group we calculated the average degree centrality considering the different species. We used Pajek 3.12 software (Batagelj and Mrvar 1998) to build the graph and extract the connectance.

Comparison of effectiveness between pollinators

The putative pollinators had their effectiveness analysed in the four species studied, through selective exposure to the different visitors. This involved exposing flowers of each species from different individuals to visits exclusively by a group of pollinators. For this, flowers in pre-anthesis were isolated with individual semi-permeable paper bags. After opening, in some moment of the anthesis the bag was removed, and the flower exposed to a visit by a specific pollinator while we monitored different visitors (from 18:30 to 3:30 h – bats or hawkmoths; from 4:00 to 9:00 h – *Xylocopa grisescens* or hummingbirds). After this exposure the flower was again bagged to avoid further visits from any pollinators. Thus we have four selective pollination experiments: (i) bats (*n* = 45 flowers for each species: *P. catingicola*, *P. chrysostele*, *P. pachycladus*), (ii) Hawkmoths (*n* = 45 flowers in *P. gounellei* and *n* = 15 flowers for *P. catingicola and P. pachycladus*), (iii) *Xylocopa grisescens* (*n* = 15 flowers for each specie: *P. catingicola, P. gounellei*, *P. pachycladus*) and (iv) Hummingbirds (*n* = 6 flowers for each specie *P. catingicola, P. gounellei*, *P. pachycladus*). Finally, fruit and seed set in these flowers were monitored after 25–40 days.

Additionally, the putative negative influence of *Trigona spinipes* bees in fruit set was considered. All four selective pollination experiments (involving bats, hawkmoths, *Xylocopa grisescens* and hummingbirds) were done comparatively for all four species of *Pilosocereus* using two different flower groups: (i) exposed and (ii) not exposed to active foraging by *Trigona spinipes* in the morning after anthesis.

The effectiveness of these groups was compared using chi-square tests, considering equivalent proportions of fruit formation as expected values.

Comparison between floral traits of different levels of bat dependence

Morphology/anthesis/nectar availability

Data regarding number of open flowers per night (peak of flowering), flower measurements, viability of pollen grains, colour and scent, anthesis time, sequence and duration, as well as nectar availability, volume and concentration were recorded following Dafni et al. (2005).

Stigma receptivity was tested using potassium permanganate 0.25% (Robinsohn 1924) and hydrogen peroxide 5% (Dafni et al. 2005), observing the stigma reaction through a magnifying lens (Zeisler 1938; Galen and Plowright 1987).

Pollen viability was verified in grains stained with carmine acetic 2% for 15 flowers from different individuals (Radford et al. 1974). The pollen/ovule ratio (P/0) was estimated through pollen grain counts using a Newbauer chamber (Moura et al. 1987). Pollen estimates were made in 15 pre-anthesis flower-buds collected from different individuals and preserved in alcohol (70%), for which grains from 10 stamens per flower-bud were removed and prepared separately, according to the methodology described by Lloyd (1972). All ovules were removed from the same bud and manually counted.

Volume and concentration of nectar sugar was measured using graduated micro-syringes with 25- and 50-µl capacity and Atago N1 pocket refractometer (0 to 32%) in 10 previously bagged flowers of different individuals for each species. Two-hourly measurements were made for each flower throughout anthesis (19:00–9:00 h).

Floral distinction between species

We used species characteristics to perform a principal component analysis (PCA) using the program *Fitopac* 2.0 (Shepherd 2009) in order to verify which attributes best distinguish the species among the different levels of association with bats.

Fruit set and reproductive system

Spontaneous and manual self-pollination experiments were carried out in the four studied species of *Pilosocereus* to analyse the reproductive system by bagging 45 flower-buds in pre-anthesis per species/treatment with semi-permeable paper bags. Manual self-pollination was performed in newly opened flowers that were bagged again, whereas for spontaneous self-pollination there was no manipulation. For both experiments the bags were removed 24 h after the end of the anthesis, and flowers were marked and followed to observe putative fruit set. The control test (natural pollination) was carried out by simply marking the flowers with adhesive tape and following them for putative fruit set.

Fruit formation between treatments and species was compared using chi-square tests, considering equivalent proportions of fruit set as expected values.

Results

Participation of bats in *Pilosocereus* pollination systems

Description of pollinator behaviour

During this study, day and night floral visitors were observed in *P. catingicola*, *P. chrysostele*, *P. gounellei* and *P. pachycladus* (Fig. 1a, b, d, g and j), including bats, hawkmoths, beetles, bees, ants and hummingbirds.

Even though they were not quantified, we observed dozens of visits by bats at night to *P. catingicola*, *P. chrysostele* and *P. pachycladus* (Fig. 1c, e, f and l). The bat's behaviour was similar for *P. catingicola* and *P. pachycladus*: both practically crash into the flower, introducing their whole head and part of their upper body within the flower tube. However, for *P. chrysostele*, in which the shorter flower-tube is totally immersed in the pseudo-cephalium and the bristles and spines are closer to the visitor, the bats have to hover in front of the flowers or sometimes hang on from the outside of the flower-tube, introducing only part of their head into the flower and stretching their tongue through a gap between filaments and style in order to reach the nectar. During their visits, the bats gather large quantity of pollen on their snout, neck and forehead generally making contact with the stigma with the lower part of their body. Concomitantly, in the captured bats (*Artibeus jamaicensis*, *A. lituratus*, *Glossophaga soricina*, *Lonchophylla mordax* and *Platyrrhinus recifinus*) we found spots of white pollen that coincide with their body contact records.

On the other hand, only nocturnal visits of hawkmoths (*Agrius cingulatus*, *Cocytius antaeus*, *Erinnyis alope*, *E. ello ello*, *E. swairsoni*, *Eumorpha fasciatus* and *Manduca rustica rustica*) were observed in *P. gounellei* (Fig. 1h, i). Some of these hawkmoths also visited eventually *P. catingicola* and *P. pachycladus* (Fig. 1k). Hawkmoth visits were not observed only in *P. chrysostele*. All species of hawkmoth contact the stamens and stigma of the studied cacti with their abdomen when they entered the flower to collect nectar.

In the morning after anthesis, *P. catingicola*, *P. chrysostele*, *P. gounellei* and *P. pachycladus* were generally visited by several bee species. The first one was *Xylocopa grisescens*, penetrating almost completely into the flower-tube to collect nectar, making contact with the stigma with its dorsal body and being the only bee considered as a pollinator. Following that, individuals of *Trigona spinipes*, presenting always varied behaviour, visit the flowers, moving around the anthers and stigma to collect pollen or reaching inside of the flower to gain access to the nectar. In this last case the bees need to break through the barrier formed by the lower filaments of the group of stamens that surround the style and make it difficult to reach the nectar chamber. During the process *T. spinipes* often damages the flowers by perforating the flower-tube or destroying the filaments, styles and

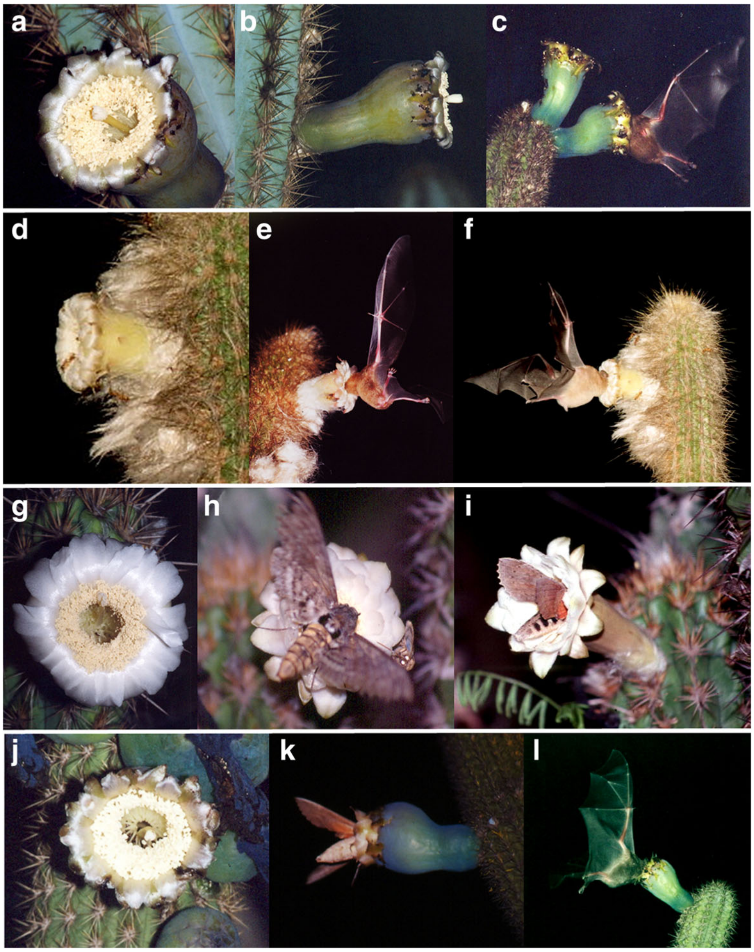

Fig. 1 Flowers and nocturnal pollinators – *Pilosocereus catingicola*: **a**, **b** – Flower frontal and side view, and **c** – visit of *Lonchophyla mordax* (Glossophaginae). *P. chrysostele*: **d** – Flower frontal view, **e** – visits of *Lonchophyla mordax* and **f** – *Glossophaga soricina* (Glossophaginae). *P. gounellei*: **g** – Flower frontal view, **h** – visit of *Cocytius antaeus* and **i** – of *Erynnys ello ello* (Sphingidae). *P. pachycladus*: **j** – Flower frontal view, and visits of **k** – *E. ello ello* (Sphingidae) and **l** – *G. soricina* (Glossophaginae)

stigma to open a route to reach the nectar. As well as those two bee species, individuals of *Apis mellifera* were observed in all four studied *Pilosocereus* species, behaving similarly to *Trigona spinipes*. However, *A. mellifera* did not cause damage to the floral structures of these *Pilosocereus* species.

Small ants (*Cephalotus* spp., *Dorymirmex* spp. and *Pseudomirmex* spp.) and beetles (*Ciclocephala cenata* and *Ciclocephala* sp.) were also observed visiting flowers of all studied *Pilosocereus* species. The beetles were sighted occasionally at the start of the evening remaining inside the flower until it closed. The ants are attracted by the nectar at the start of the anthesis and may remain in the flower until its closure. Both ants and beetles may occasionally touch sexual organs of *P. catingicola*, *P. chrysostele*, *P. gounellei* and *P. pachycladus*, but because of the limited dispersion and the low amount of pollen they transported were not considered pollinators.

The hummingbirds *Chlorostilbon aureoventris*, *Heliomaster squamosus* and *Anopetia gounellei* visited flowers of three out of the four *Pilosocereus* species, excepting *P. chrysostele.* The birds introduced their beak and head within the flower-tube in order to reach the nectar and eventually make contact between their neck and thorax with anthers and stigmas. But as the stigma was by then dislodged towards the lower part of the flower, and the anthers had low pollen availability, the role of hummingbirds as pollinators did not become evident.

Pollination network

The network generated from the records of the floral visitors in the studied species of *Pilosocereus* (Fig. 2) showed a very integrated network with no compartments or modules and a connectance of 0.67.

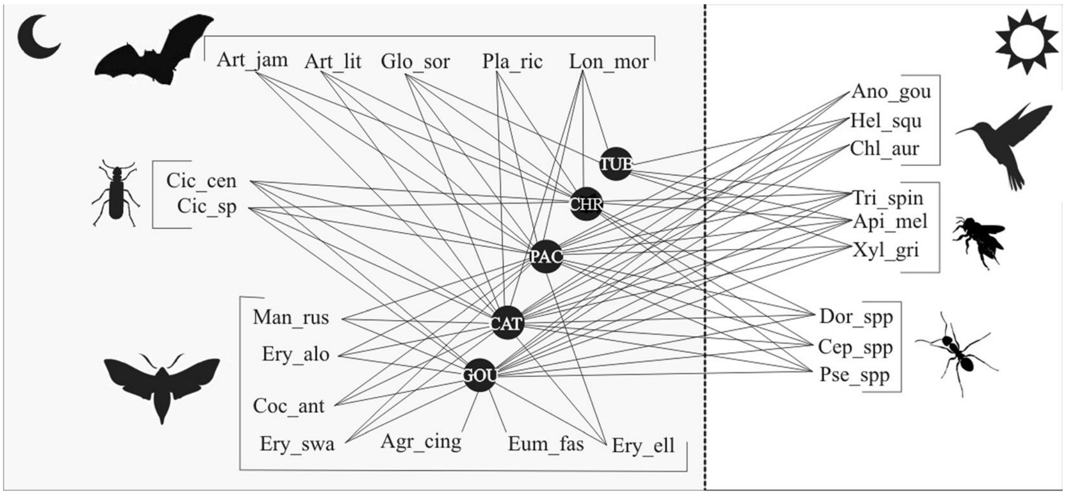

Fig. 2 Network of nocturnal and diurnal floral visitors of five *Pilosocereus* species in two caatinga areas in northeastern Brazil. Data ond *P. tuberculatus* were obtained from Rocha et al. (2007b). **Bats**: Art_jam – *Artibeus jamaicensis*, Art_lit – *Artibeus lituratus*, Glo_sor – *Glossophaga soricina*, Pla_ric – *Platyrrhinus ricifinus* Lon_ mor – *Lonchophylla mordax*, **beetles**: Cic-cen – *Ciclocephala cenata*, Cic_sp – *Ciclocephala* sp; **moths**: Man_rus – *Manduca rustica rustica*, Ery_alo – *Erynnis alope*, Coc_ant – *Cocytius antaeus*, Ery_ swa – *Erynnis swairsoni*, Agr_cing – *Agrius cingulatus*, Eum_fas – *Eumorpha fasciatus*, Er_ell – *Erynnis ello ello*; **hummingbirds**: Ano_gou – *Anopetia gounellei*, Hel_squ – *Heliomaster squamosus*, Chl_aur – *Chlorostilbon aureoventris*; **bees**: Tri_spin – *Trigona spinipes*, Apo_mel – *Apis mellifera*, Xyl_gri – *Xylocopa grisescens*; **ants**: Dor_spp – *Dorymirmex* spp, Cep_spp – *Cephalothes* spp, Pse_spp – *Pseudomirmex* spp; **plants**: TUB – *Pilosocereus tuberculatus*, CHR – *Pilosocereus chrysostele*, PAC – *Pilosocereus pachycladus*, CAT – *Pilosocereus catingicola*, GOU – *Pilosocereus gounellei*

In relation to bat dependence, it is possible to separate the *Pilosocereus* species in three different cases. The first one is *P. chrysostele* and *P. tuberculatus*, which had only bats as nocturnal pollinators and showed the lowest degree centrality, of respectively 12 edges (four them are bats species) and six edges (two them are bats species); the second case is *P. catingicola* and *P. pachycladus* with a mixed system involving bats and sphingids, showing the highest degree centrality, both with 21 edges (five them are bats and five them are sphingids). Finally, the isolated case of *P. gounellei*, the unique species that presented only sphingids as nocturnal pollinators, showing an intermediate degree centrality of 18 edges (seven them are sphingids).

The average degree centrality of effective pollinators was similar between bats and sphingids, 3.4 ± 0.6 and 2.4 ± 1.0 respectively ($U = 7.5$; $P = 0.104$). The only pollinating bee, *Xylocopa grisescens* presented four edges, not interacting with *P. chrysostele.*

Comparison of effectiveness between pollinators

Bats were equally efficient in all the cactus species they visited, as were the hawkmoths (Table 1). In the mixed species system of *P. catingicola* and *P. pachycladus*, bats and hawkmoths showed similar efficiency. On other hand, the role of *Xylocopa grisescens* is significantly smaller and equal in all species when compared with any of the other two nocturnal systems. We also confirm the non-performance of hummingbirds as pollinators, since there was no fruit set as a result of their visits. The floral damage caused by *Trigona* bees affected the fruit production only in two cases, namely the pollination of *P. gounellei* by hawkmoths and *P. chrysostele* by bats.

Comparison between floral traits of different levels of bat dependence

Morphology

The flowers in all four species are slightly zygomorphic and most often are found distally on the branches and turned towards the outside of the plant (Fig. 1). The pericarpel, flower-tube and outer perianth segments are fleshy and vary from pinkish-green to brown or pale-green, while the inner perianth segments are white and delicate, reflexed at anthesis; the pericarpel is subglobose (Table 2), the flower-tube is straight, slightly curved, narrow or campanulate varying between species (Fig. 3). The polystemonous androecium has hundreds

Table 1 Selective pollination and fruit set in four study species of *Pilosocereus* (Cactaceae) in the Caatinga (Numbers parentheses represent the additional effect of florivory by the bee *Trigona spinipes*)

Species	Bats Flower/Fruit [%]	Hawkmoths Flower/Fruit [%]	*Xylocopa grisescens* Flower/Fruit [%]	Hummingbirds Flower/Fruit [%]
P. catingicola	45/41 (91%)^{Aa}	15/12 (80%)^{Aa}	15/4 (26.7%)^{Ba}	10/0 (0%)
	45/33 (73.3%)	15/7 (42.7%)	15/1 (6.7%)	10/0 (0%)
P. chrysostele	45/42 (95.6%)^{a}	–	–	–
	45/17 (40%)*	–	–	–
P. gounellei	–	45/43 (96%)^{Aa}	15/7 (46.7%)^{Ba}	6/0 (0%)
	–	45/33 (73.3%)*	15/3 (20%)	6/0 (0%)
P. pachycladus	45/42 (95.6%)^{Aa}	15/13 (86.7%)^{Aa}	15/5 (33.3%)^{Ba}	10/0 (0%)
	45/37 (82.2%)	15/9 (60%)	15/2 (13.3%)	10/0 (0%)

Different superscripted capital letters in the same line show statistics differences between different pollinators by plant species ($P < 0.05$)

Different superscripted lower case letters in the same column show statistics differences between different plant species by pollinators ($P < 0.05$)

*Statistics differences between fruit set in the absence and presence of *Trigona spinipes* (number in box) by pollinator by plant species ($P < 0.05$)

of stamens and is organized in two groups, the innermost (protecting the nectar chamber) with stout longer filaments that are turned towards the style, and the outer group, more abundant, with more slender, shorter and straight filaments. In all species studied, both groups of stamens release vast quantities of pollen with high viability (around 97%). The gynoecium has hundreds of ovules with parietal placentation, the style is long (same length of flower- tube or slightly longer) with lobed stigma, obtuse at apex, and exserted slightly above the anthers. Nectar production in the species under study is limited to the tissues above the ovary, and nectar accumulates in a bulging nectariferous chamber at the base of the flower-tube.

Anthesis

During the flowering peak (according to Rocha 2007a,b, see MM), the number of open flowers per night varied from two to 15 flowers per individual / night between the studied species (Table 2). All species have flowers lasting for a single night. Anthesis for all species studied started at dusk, between 17:00 and 18:00 h, with gradual spreading of the perianth segments. Anthesis is asynchronous, prolonged for an hour, and flowers are totally open between 18:30 and 19:30 h, remaining attractive to visitors until around 9:00 h in the following morning. At the beginning of the anthesis a faint scent of

Table 2 Main differences between the four study species of *Pilosocereus* (Cactaceae) in the Caatinga.

Traits	*P. catingicola*	*P. chrysostele*	*P. gounellei*	*P. pachycladus*
Open flowers by night	5 ± 4^{a}	3 ± 1^{a}	12 ± 5^{a}	5 ± 4^{a}
Flower length [cm]	6.78 ± 0.36^{a}	4.51 ± 0.27^{a}	7.18 ± 0.71^{a}	6.74 ± 0.39^{a}
Tube opening [cm]	2.73 ± 0.19^{a}	2.16 ± 0.16^{a}	2.31 ± 0.31^{a}	2.7 ± 0.2^{a}
Flower shape	Campanula/funnel	Tube	Tube	Campanula/funnel
Number of pollen grains per anther	1,388 ± 91^{a}	1,389 ± 99^{a}	1,234 ± 103^{a}	1,299 ± 104^{a}
Number of ovules	7,562 ± 702^{a}	3,812 ± 322^{b}	6,254 ± 739^{a}	7,518 ± 683^{a}
Ovary shape	Elongate	Depressed	Elongate	Elongate
Total nectar volume [µl]	1,596 ± 159^{a}	755 ± 268^{b}	365 ± 94^{b}	1,643 ± 501^{a}
Nectar concentration [%]	15 ± 6^{a}	19 ± 7^{a}	23 ± 11^{a}	14 ± 4^{a}

Different superscripted letters in the same line show statistics differences between different plant species by trait ($P < 0.05$)

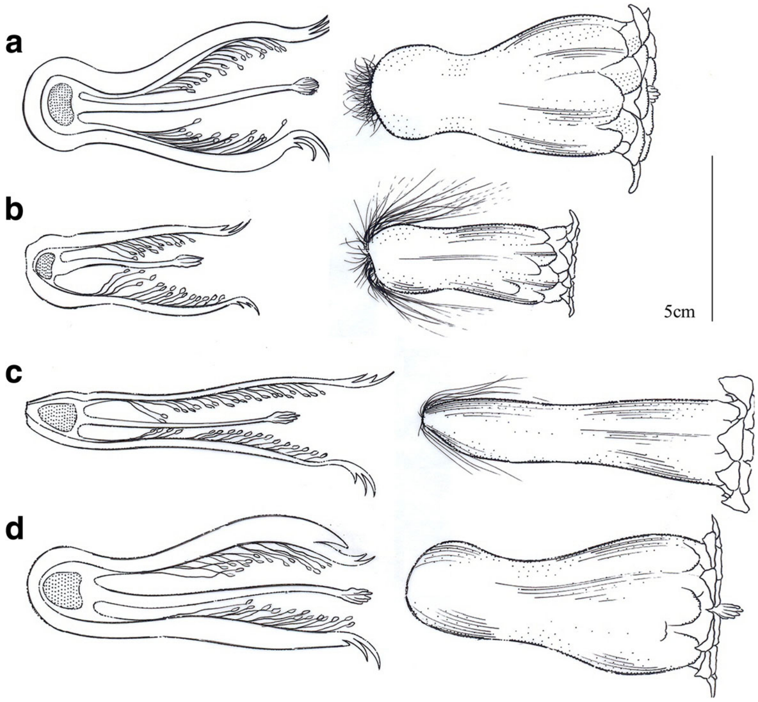

Fig. 3 Side view of the flowers of *Pilosocereus* (Cactaceae) studied; **a** – *P. catingicola*, **b** – *P. chrysostele*, **c** – *P. gounellei* and **d** – *P. pachycladus*

rotten cabbage is noticed, and this diminishes gradually after full opening, especially in *P. gounellei*. Anther dehiscence initiates shortly after the flowers open (slight protandry). Stigmatic receptivity occurs from flower opening until the end of anthesis. During the last 4–5 h of anthesis the style relaxes and moves downward. At this stage, the stigma lobes that were grouped together at the beginning of anthesis, open and spread out, easing the deposition and adherence of pollen from other flowers to the receptive part of the stigma-lobes.

Nectar availability

The nectar availability is similar between the studied species. Nectar is available almost throughout the whole period when the flowers remain open, but the quantity is higher in the first four hours following anthesis (19:00–23:00 h). After this initial period, the production diminishes (23:00–1:00 h), with an abrupt fall from 1:00 h in the morning, after which it continues to taper gradually until it ceases between 5:00 and 9:00 h. In general, all species produced large quantities of nectar with low sugar concentration, but with variations between them (Table 2).

Floral distinction between species

The PCA showed a separation between the three types of systems identified in *Pilosocereus* (Fig. 4). Both axis 1 and 2 presented good power of explanation of around 40%. The main attributes correlated with axis 1 were ovule number and tube opening, while for axis 2, the number of open flowers and nectar concentration were more important (Table 3).

The flowers pollinated only by bats at night are relatively shorter and show less open tubes, with smaller number of ovules and nectar volume; however, nectar concentration is intermediate. The species found to have mixed systems, in turn, have more pollen grains and higher volume of nectar, which is less concentrated. Finally, *P. gounellei*, the only species found to have hawkmoths as the unique nocturnal pollinators, is distinguished from the others by having many open flowers per night, smaller quantities of a more concentrated nectar and smaller amounts of pollen grains (Table 2).

Fruit set and reproductive system

In all studied species the fruit is baccate, depressed-globose, dehiscent through an irregular central slit,

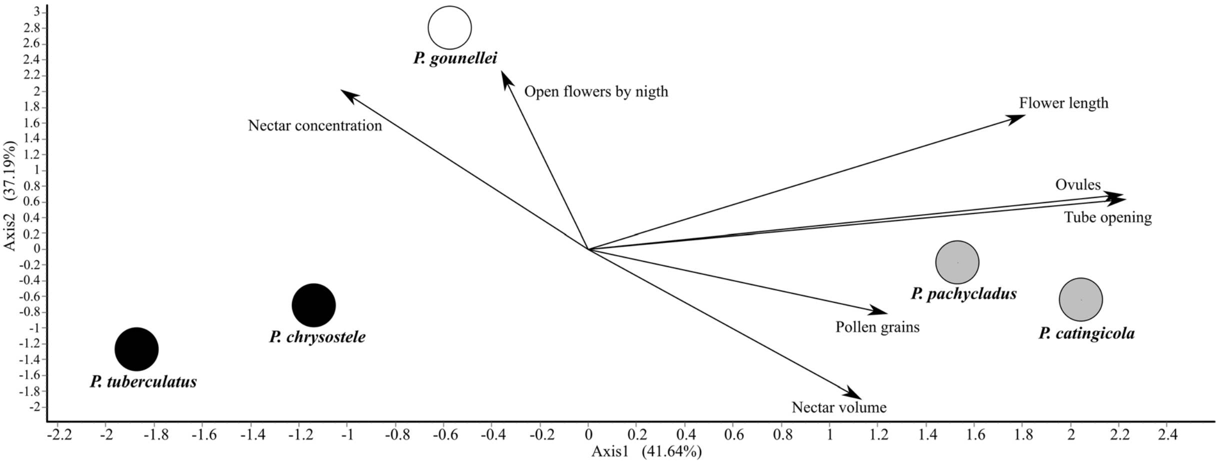

Fig. 4 Principal component analysis (PCA) based on floral traits. Each circle represents a *Pilosocereus* species whose colours indicate the pollination systems: black – only bats as night pollinators; grey – bats plus participation of hawkmoths; white – without bats as night pollinators, only hawkmoths. (*data from Rocha et al. 2007)

pericarp green to purplish when ripe, with sweet, solid, magenta funicular pulp bearing numbers ranging between approximately 2,600 and 8,500 black or dark brown seeds. Fruits reach full maturity within 25–40 days.

Tests to evaluate the reproductive system have shown that *P. catingicola*, *P. chrysostele* and *P. pachycladus* are self-incompatible, while neither spontaneous nor manual self-pollination formed fruits. None of the four species is agamospermic or autogamous and only *P. gounellei* is self-compatible. All species have the same reproductive success in the natural formation of fruits and although they are different in relation to the number of seeds produced, the proportion of seed set in relation to the number of ovules is also the same (Table 4).

Table 3 Centred correlation of traits of *Pilosocereus* (Cactaceae) species and axes of principal component analysis (PCA; standardized eigenvectors 1.0)

Traits	Axis1	Axis2
Flower length	0.4370	0.4101
Nectar concentration	−0.2473	0.4873
Nectar volume	0.2727	−0.4581
Open flowers by night	−0.0867	0.5426
Ovules	0.5352	0.1673
Pollen grains	0.2993	−0.1966
Tube opening	0.5385	0.1533

Discussion

Our results show a central role for bats in *Pilosocereus* pollination systems. We considered secondary pollinators and reproductive systems and found variation from a total dependence on bats to a partial dependence, with a single species where bats did not act as pollinators. Floral attributes that differentiate the flowers between these different levels of dependence of bats are morphological, nectar abundance and concentration and number of flowers open during the same night. We discuss below the factors that determine the central role of bats as pollinators in these species, as well as comparing their floral biology in relation to other groups of the Cactaceae.

Role of bats and other animals in *Pilosocereus* pollination

The similarity of *P. catingicola* and *P. pachycladus* flowers is not restricted to their morphologic traits, but it is also reflected by their visitors and pollinators. Both species receive nocturnal visits by bats (*Glossophaga soricina* and *Lonchophylla mordax*) and hawkmoths (*Cocytius antaeus*, *Erinnyis alope*, *E. ello ello*, *E. swairsoni* and *Manduca rustica rustica*). According to Baker (1961), hawkmoths are susceptible to environmental and climate factors, with oscillating numbers that may mean that they may become rare in an area, resulting in a large number of not visited flowers. Moreover, Duarte-Junior and

Table 4 Results of selective pollination experiments (n = 45 flowers/treatments/species) on *Pilosocereus* species (Cactaceae) in the Caatinga.

Treatment	*P. catingicola*	*P. chrysostele*	*P. gounellei*	*P. pachycladus*
Spontaneous self-pollination – fruit set	0	0	0	0
Manual self-pollination – fruit set	0	0	6 (13%)	0
Natural pollination – fruit set	33 (73%)^a	18 (40%)^a	33 (73%)^a	19 (42%)^a
*Natural pollination – seed set**	6,314 ± 1.051^a (83%)^a	3,159 ± 439^b (83%)^a	5,438 ± 1.173^{ab} (87%)^a	6,100 ± 1.357^a (81%)^a

Different superscripted letters in the same line show statistics differences between different plant species by treatment ($P < 0.05$)

*Proportions to seed formation calculated from ovule median per species

Schlindwein (2005) and Primo et al. (2013) have recorded highly variable richness and abundance of hawkmoths in the Caatinga linked to the rainfall regime, where an extreme dry season with leafless vegetation regulates the life cycle of these insects, which may be absent for many months of the year. Similar trends were recorded for dry semideciduous forests by Primo et al. (2013). Harber and Frankie (1989) consider that hawkmoth visits to chiropterophilous flowers are more frequent than bat visits to sphingophilous flowers due to the restrictive characters found in the latter, such as their long, narrow tube.

It is probable that bat visits in *P. gounellei* were not favoured because of some subtle differences in its floral traits, such as more modest nectar production (Table 1) and narrower and longer flower-tube when compared to other *Pilosocereus* species (Fig. 2). Access to its flowers was also a possible factor, as these are positioned near the ground or near other branches from the same plant and from others, at lower heights than was observed in *P. catingicola* and *P. pachycladus* (field observations). In fact, their orientation is also different, with the upper part of the flower coming forward in relation to the lower part, a flower shape so unexpected that botanical artist Margaret Mee has drawn them upside down when composing a plate for this species. (Andrade-Lima 1989). Exclusive sphingophily is a rare occurrence within Brazilian *Pilosocereus*, as bats and hawkmoths seem to be associated with resource share in this and other cactus genera, as can be seen in *Micranthocereus purpureus* (Aona et al. 2006), *P. catingicola* (Locatelli et al. 1997) and *P. pachycladus* (present study).

It is interesting to compare the sphingophily detected in *P. gounellei* with former studies carried out with sympatric, bat-pollinated *Pilosocereus tuberculatus* (Rocha et al. 2007a,b), both members of a distinct subgenus, *Pilosocereus* subgenus *Gounellea* (Zappi 1994). It is possible to attribute their pollinators with a barrier role which might be interrupting the gene-flow between these closely related species and ensuring their speciation (Ferreira et al. 2018).

Glossophaga soricina and *Lonchophylla mordax* were the only pollinators of *P. chrysostele*. In fact, several authors (Alvarez and González 1970; Lemke 1984; Valiente-Banuet et al. 1997a, b; Simmons and Wetterer 2002) consider *G. soricina* the main pollen vector of several chiropterophilous cacti, as this bat has a well known role as a cactophilous opportunistic bat (i.e. this species or some of its populations make use of cactus resources when available, even though their survival does not depend exclusively upon them).

Many bat species are known to visit cactus species, and some, such as *Lonchophylla mordax*, have been diagnosed as cactophilous species with poorly known behaviour (Aona et al. 2006). From now on, this bat species may be considered an effective cactophilous species, as it also visits *P. chrysostele*, *P. catingicola*, *P. pachycladus* and *P. tuberculatus* (Rocha et al. 2007a,b), displaying an important role as pollen vector of chiropterophilous cacti throughout this park.

Few studies have detected resource sharing between bats and hawkmoths (Locatelli et al. 1997; Tschapka et al. 1999; Aona et al. 2006; Munguía-Rosas et al. 2009). The role of diurnal visitors to nocturnal anthesis cacti is well known and indicates their importance as secondary or minor pollinators (Alcorn et al. 1959; Valiente-Banuet et al. 1997a, b; Locatelli et al. 1997; Locatelli and Machado 1999b; Viana et al. 2001; Aona et al. 2006; Rocha et al. 2007a,b; Munguía-Rosas et al. 2009; Walter 2010; Martins et al. 2016).

Alcorn et al. (1959) and Walter (2010) consider that some species of cacti maintaining flowers open during the night until the following morning and producing nectar in two turns are linked to the presence of diurnal visitors, mainly bees, that act as efficient pollen vectors, contributing to fruit formation, similarly to nocturnal visitors. This differs from the present work since the daytime pollinators were less efficient than the nocturnal ones. We found a similar situation for *P. catingicola*, *P. gounellei* and *P. pachycladus*, but the only diurnal pollinator, the bee *Xylocopa grisescens* was not as efficient as the nocturnal pollinators. Rocha et al. (2007b) study of *P. tuberculatus* have shown that, while the species was pollinated by *Glossophaga soricina* and *Lonchophylla mordax* bats and by *Xylocopa grisescens* bees, the bats were the more efficient pollen vectors. In *P. chrysostele* the dependency on bats is more complete, as these two species of bat were the only visitors recorded for this plant.

Colaço et al. (2006) observed that flower part destruction in *Melocactus glaucescens* and *M. paucispinus* by *Trigona spinipes* can compromise the pollination and fertilization in these species, as many of the flowers observed had their stigma and the majority of the stamens destroyed. Such damage is often observed in other plant species (Renner 1983; Malerbo-Souza et al. 2002; Boiça Jr et al. 2004) and in other Cactaceae found in the Caatinga, for instance *Cereus jamacaru*, *C. albicaulis*, *Harrisia adscendens*, *Pilosocereus catingicola*, *P. gounellei*, *P. pachycladus*, *P. pentaedrophorus*, *P. piauhyensis*, *Tacinga inamoena* and *T. palmadora* (Rocha pers. obs.).

The overlap in the use of floral resources by diurnal (*Xylocopa grisescens*) and nocturnal (*G. soricina*, *L. mordax* or hawkmoths) visitors may favour the success of *P. catingicola*, and *P. pachycladus* and reflect in their large distribution ranges. In the two latter species, the absence of one or both species of bats may be compensated by the presence of others or even by the bee.

Finally, it is important to consider the possible reasons why other floral visitors do not act as pollinators. Regarding beetles, although they are recurrently found in Cactaceae flowers in the Caatinga, their function as pollinators is uncertain. If on the one hand their pollen transport efficiency is unknown, their role as a consumer of floral parts (including reproductive parts) is evident, so studies focused specifically on this interaction are needed (Domingos-Melo 2015). In relation to ants, flowers that can use their pollination services have very restricted characteristics, such as small, light flowers with few ovules and small plants (Domingos-Melo et al. 2017), which clearly are not features observed in *Pilosocereus* species. In the case of hummingbirds, we have an atypical situation, considering that many Cactaceae are pollinated by these animals (Raw 1996; Locatelli and Machado 1999a; Aona et al. 2006; Nassar et al. 2006), and there are known occurrences of mixed systems involving hummingbirds and bats (Queiroz et al. 2015; Muchhala et al. 2009). It is possible that the strong morphological adjustment of bat plants by producing flowers with floral tubes that are wide enough do no longer allow the contact of hummingbirds with the reproductive structures. In *P. gounellei* flower-tube length may also be an issue as a population in Bahia, near Morro do Chapéu, had the majority of its flowers split from the side by nectar-robbing hummingbirds (Zappi pers. comm.).

Floral biology in Cactaceae bat pollination

The flowers of the four studied *Pilosocereus* species display many attributes commonly associated to chiropterophily, such as presence of thick, resistant flower parts (perianth segments, stamens, style and stigma) that are able to withstand bat visits seeking nectar; nocturnal anthesis; unpleasant scent that resembles rotten cabbage; dull outer flower colours contrasting with the inner elements that are paler, usually white; bulging nectar chamber protected by innermost filaments that are bent towards the style; large quantities of nectar (Porsch 1939; Faegri and van der Pijl 1979; Dobat and Peikert-Holle 1985; Zappi 1989, 1994; Simmons and Wetterer 2002). Species of several genera of Cactaceae, such as *Carnegiea*, *Cipocereus*, *Coleocephalocereus*, *Espostoopsis*, *Facheiroa*, *Neobuxbaumia*, *Pachycereus*, *Stenocereus*, *Stephanocereus* and *Weberocereus*, have similar morphology and are also pollinated by bats (Porsch 1939; Alcorn et al. 1959; Vogel 1968; Faegri and van der Pijl 1979; Dobat and Peikert-Holle 1985; Sosa and Soriano 1996; Tschapka et al. 1999; Valiente-Banuet et al. 1997a, 1997b; Valiente-Banuet et al. 2007; Rocha et al. 2007a,b; Rego et al. 2012; Martins et al. 2016).

However, even in cases where the pollination syndrome is very clear, such as chiropterophily (Muchhala and Jarrin 2002), the pollinator agent may differ or vary (Waser 1983; Herrera 1995; Siqueira Filho and Machado 2001; Willmer 2011). For instance, some chiropterophilous cacti have mixed strategies that attract different visitors, such as hawkmoths, bees and hummingbirds (Valiente-Banuet et al. 1996, 1997b; Fleming et al. 2001; Molina-Freaner et al. 2004; Aona et al. 2006). Some cactus flowers have transitional characteristics whereby both bats and hummingbirds are attracted, such as *Cipocereus minensis* and *Cipocereus pleurocarpus* (Taylor and Zappi 2008), *Micranthocereus purpureus* and *Pilosocereus coerulescens* (Zappi pers. comm.). According to Valiente-Banuet et al. (1997a), diurnal and nocturnal pollinator share was also observed for Mexican *Pachycereus weberi* and *Pilosocereus chrysacanthus*, both visited by night by bats and by hummingbirds and bees during the day.

The flowers of the four *Pilosocereus* species analysed last for a shorter period than flowers of certain species as *Carnegia gigantea* and *Trichocereus pasacana* (McGregor et al. 1962; Viana et al. 2001), where the flowers remain open for up to 72 h. However, they are similar to the ones of *Neobuxbaumia macrocephala*, *N. mezcalaensis* and *N. tetetzo*, with flowers lasting between 13 and 15 h (Valiente-Banuet et al. 1996, 1997b). The synchrony between flower anthesis and anther dehiscence was also recorded for studies of *Pilosocereus moritzianus*, *Subpilocereus horrispinus* and *S. repandus* (Nassar et al. 1997), *Pilosocereus catingicola subsp. salvadorensis* (Locatelli et al. 1997), *Trichocereus pasacana* (Viana et al. 2001) and *Pilosocereus tuberculatus* (Rocha et al. 2007). Duration of stigma receptivity of *P. catingicola*, *P. chrysostele*, *P. gounellei* and *P. pachycladus* matches results found in other studies with chiropterophilous cacti (Locatelli et al. 1997; Valient-Banuet et al. 1997b; Viana et al. 2001; Rocha et al. 2007b).

The low number of open flowers per night is one of the characteristics associated with trapliner pollinator behaviour (Feinsinger and Colwell 1978), representing also a strategy adopted by some species of bats in arid regions, establishing daily routes and sometimes migrating to places where there is more abundant availability of resources on offer (Sosa and Soriano 1992; Petit 1997; Ruiz et al. 1997; Valiente-Banuet 2002; Rocha et al. 2007).

The variation found in sugar nectar concentration fits within data found by Locatelli et al. (1997) for *Pilosocereus catingicola* in a seaside forest (*Restinga*) in the Brazilian state of Paraíba; Molina-Freaner et al. (2004) for *Pachycereus pecten-aboriginum* in México and by Aona et al. (2006) in *Micranthocereus purpureus*, all pollinated by nocturnal and diurnal visitors, while it is higher than what was found by Machado et al. (1998), Tschapka et al. (1999) and Machado and Vogel (2004) in chiropterophilous flowers in other plant families.

According to Scogin (1985), variation of nectar in some Cactaceae species did not present significant difference in sugar concentration between hawkmoth, bat and hummingbird flowers, and there are overlaps between the concentrations found for different floral visitors. Some authors (Locatelli and Machado 1999b; Aona et al. 2006; Rocha et al. 2007a,b) consider that such overlaps probably favour pollination of cactus species through sharing of pollinators.

The proportion and composition of nectar sugars may determine the guild of floral visitors received by a plant species (Baker and Baker 1982, 1983). According to Baker and Baker (1983) and Baker et al. (1998), bats have preference for hexose rich nectar, while hawkmoths tend to visit flowers with nectar rich in sucrose or other disaccharides. The explanation for exclusive bat visits in *P. chrysostele* and by hawkmoths in *P. gounellei* may reside in the future analysis of the chemical composition of *Pilosocereus* species nectar.

Pollen is an important resource for the diet of Glossophaginae bats during their visits to cactus species (Sosa and Soriano 1992; Valiente-Banuet et al. 1996; Petit and Freeman 1997; Ruiz et al. 1997). It was recorded for *Pilosocereus tuberculatus*, also at the Parque Nacional do Catimbau (Rocha et al. 2007a,b). It is possible that this behaviour is also present in the three studied species of *Pilosocereus*, due to the vast amounts of pollen produced by each flower, and the presence of pollen deposited on the bodies of other three bat species (*Artibeus jamaicensis*, *A. lituratus* and *Platyrrhinus ricifinus*) captured near the focal plants, that had been seen feeding so far only on cactus fruits in the area.

Reproductive system and dependence of pollinators

The Cactaceae have several mechanisms that favour allogamic reproduction, such as androdioecy (Valiente-Banuet et al. 1997a,b), gynodioecy (Parfitt 1985; Hoffman 1992) and trioecy (Fleming et al. 1994); however, a large proportion of the species, such as *P. catingicola*, *P. chrysostele, P. gounellei* and *P. pachycladus* are hermaphrodite (Silva and Sazima 1995; Schlindwein and Wittmann 1995, 1997; Raw 1996; Valiente-Banuet et al. 1996; Locatelli et al. 1997; Locatelli and Machado 1999a,b; Tschapka et al. 1999; Molina-Freaner et al. 2004; Aona et al. 2006; Valiente-Banuet et al. 2007). According to Viana et al.

(2001), the lack of fruit initiation in self-pollinated flowers of *Trichocereus pasacana* indicates that fruit set in this species depends on the initial fertilization of the ovules by pollen from cross-fertilization, and this seems to be true also for the reproductive system of *P. catingicola*, *P. chrysostele, P. gounellei* and *P. pachycladus*. As well as these mechanisms, dichogamy was observed in nocturnal flowers of *Carnegiea gigantea* (Alcorn et al. 1959), *C. jamacaru* (Porsch 1939) and *C. peruvianus* [= *Cereus hildmannianus*] (Silva and Sazima 1995), where anther dehiscence occurs still while inside the flower-bud, before the stigma becomes receptive (protandry).

The absence of fruit set in manual and spontaneous self-pollination suggests that *P. catingicola*, *P. chrysostele* and *P. pachycladus* are predominantly allogamous, in agreement with other results obtained for the family (Valiente-Banuet et al. 1996, 1997a, b; Locatelli et al. 1997; Viana et al. 2001; Colaço et al. 2006; Rocha et al. 2007b), making it clear that these species of *Pilosocereus* are totally dependent on pollen vectors for sexual reproduction. Despite the fact that *P. gounellei* has around 13.3% fruit formation from manual self-pollination, it is possible to consider the species as predominantly allogamous as it depends mainly upon cross pollination performed by hawkmoths.

To summarize, nocturnal pollinators such as bats are fundamental for the reproductive success of *P. catingicola*, *P. chrysostele* and *P. pachycladus* and hawkmoths for *P. gounellei*. Regarding the diurnal visitors, early morning visits by *Xylocopa grisescens* may contribute as alternative pollinators for all species, excepting *P. chrysostele*, increasing together with the nocturnal pollinators, the rate of fruit set and the gene flow, in line with the findings by Rocha et al. (2007b) for *P. tuberculatus*. *Xylocopa grisescens* could be particularly important in places with absence of or rarity of nocturnal pollinators.

The exclusive dependency of columnar cacti upon bat pollination reported for Venezuelan species (Nassar et al. 1997) is similar to the results obtained by this study for *P. chrysostele*, where only bats visited and pollinated this species. The results of selective pollination in absence and presence of *Trigona spinipes* show that the bee affected more specialized taxa such as *P. chrysostele* (solely pollinated by bats) and *P. gounellei* (exclusively pollinated by hawkmoths). More generalist taxa, such as *P. pachycladus* and *P. catingicola*, visited both by bats and hawkmoths, did not show significant difference in fruit set either in the presence or absence of the bee, suggesting that *Trigona spinipes* could not affect the reproductive success of more generalist species. In turn, the significant decrease in fruit set when *Trigona spinipes* is present indicates that this insect has a negative effect on *P. chrysostele* and *P. gounellei* that may affect future prospects of re-population of these species at the study areas.

Comparisons of the pollinator efficiency amongst the studied species of *Pilosocereus* have shown a significant difference in fruit setting for all *Pilosocereus*, under study between bats and *Xylocopa grisescens* bees or between hawkmoths and *X. grisescens* bees. The relatively high number of fruits formed in natural pollination for *P. pachycladus* may be a consequence of its habitat share with a single other species of the genus, *P. gounellei*, that does not receive bat visits. Furthermore, *X. grisescens* contributes to an increase of the pollen flow of *P. pachycladus*, as the pollinator share during different periods (night and day) increases the possibility of fruit set. It is possible that the changes in the position and accessibility of the stigma lobes late in the anthesis may reflect an adaptation to increase pollen deposit by the daytime visitors. It is important to mention that, during selective flower exposition, flowers visited exclusively by *X. grisescens* (during 4:30–9:00 h) had a high quantity of pollen. These were bagged during the nocturnal part of the anthesis, this being rather different from what was observed under natural conditions, where, because of intense bat activity, smaller quantities of pollen remain available early in the morning.

According to Valiente-Banuet (2002 and references therein), Mexican columnar cacti have two patterns regarding their pollination system. One more generalist towards their northern limit of distribution makes these species less vulnerable to environmental disturbances, while the other, more specialized and found towards the centre of the species distribution, coincides with the area where these would suffer more in cases of habitat disturbance. This may be attributed to climate, that tropical in the centre-south portion of their distribution in comparison to more temperate, harsher climates found towards the north. It seems that the *Pilosocereus* species studied also have two pollination patterns, independently from their geographic location, with *P. chrysostele* as a more specialized species pollinated only by bats, and *P. catingicola*, *P. gounellei* and *P. pachycladus* with more general, mixed pollination systems, similar to *P. tuberculatus* (Rocha et al. 2007b). Meanwhile, overlap in the floral resources used by *Glossophaga soricina* and *Lonchophylla mordax* (and possibly by other bat species) may favour *P. catingicola*, *P. chrysostele* and *P. pachycladus*, as the absence of one of the pollinators may be compensated by the presence of the other.

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