



## ECOSYSTEMS

# The iconic cactus of the Caatinga dry forest, *Cereus jamacaru* (Cactaceae) has high sphingophily specialization and pollinator dependence

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**Abstract:** *Cereus jamacaru* is a cactus distributed in Northeastern Brazil, with high symbolic value to this region. However, the interaction, behavior and the role of pollinators remains poorly understood. Here, we investigate the reproductive biology, addressing the ecological significance of floral attributes, including details about floral signaling. The study was carried at three areas of the Caatinga, in 2015, 2017 and 2021. We analyzed the floral morphometry, volume and concentration of the nectar, and characterized the colour and scent of flowers. Additionally, we described the pollinator behavior and performed controlled pollination experiments. The 'Mandacaru' is self-incompatible, has nocturnal anthesis and the nectar is accumulated as droplets in a long hypanthial tube. The flowers have a reflective pattern with a dark outer surface and a white inner surface. (*E*)-nerolidol is the major component (87.4%) of its floral perfume. We registered the sphingid moth *Cocytius antaeus* visiting the flowers. The floral attributes, attractants and rewards drives to a sphingophily, and the pollination treatments showed the dependence to fruit set by *C. antaeus*, the pollinator registered. In this case, if the apparent lack of pollinator diversity encompasses its entire range, the loss of the hawkmoth could severely impact the reproductive success of the cactus.

**Key words:** Caatinga, floral scent, hawkmoth-pollination, "Mandacaru" cactus, self-incompatibility, spectral reflection.

## INTRODUCTION

The flowers of Cactaceae display a very conserved floral bauplan, since they are remarkably uniform in their structural organisation (Mandujano et al. 2010). They are inserted (actually inverted) into a specialized stem, comprising the 'pericarpel' (enclosing the ovary and carpels) surmounted by a hypanthial tube, which is sometimes very elongated (Gibson & Nobel 1986). Nonetheless, while this bauplan holds true for the vast majority of species, the flowers of Cactaceae present a great variety in their form and appearance

(Anderson 2001, Taylor & Zappi 2004, Hunt et al. 2006, 2013). This is because Cactaceae is a family that has undergone adaptive radiation processes, but despite this, is a group of plants that are strictly dependent on animals for pollination of their flowers (Vogel 1990). Thus, floral characteristics, such as flower position on the plant, perianth colours, shape and size, are extremely diverse and reflect the variety of pollination syndromes observed in this family (Rowley 1980, Pimienta-Barrios & del Castillo 2002, Mandujano et al. 2010).

In terms of morphology, the floral tube or hypanthium of Cactaceae varies greatly in length, ranging from short or almost non-existent (e.g. in *Opuntia*, *Rhipsalis*, *Tacinga*) to very long (e.g. in some *Cereus*, *Epiphyllum*, *Selenicereus*). This character is one of the key traits determining the interaction of cacti with different pollinator guilds (Schlumpberger et al. 2009). Another character that can indicate the level of specialization towards different pollinator guilds is floral symmetry, i.e. whether flowers are actinomorphic (e.g. in *Hatiora*, *Lepismium*, *Rhipsalis*) or zygomorphic (e.g. in *Cereus*, *Cleistocactus*, *Pilosocereus*, *Selenicereus*, *Schlumbergera*) (Mandujano et al. 2010). In addition to these morphological traits, another most obvious trait that may be related to pollinator systems in cacti is the timing of anthesis. In this case, taxa with diurnal anthesis are mainly pollinated by bees (Schlindwein & Wittmann 1997, Fachardo & Sigrist 2020, Arroyo-Pérez et al. 2021, Sousa et al. 2021) or hummingbirds (Locatelli & Machado 1999a, Leal et al. 2006, Gomes et al. 2014a, Gorostiague & Ortega-Baes 2016, Ferreira et al. 2020), whereas nocturnal taxa are more frequently pollinated by hawkmoths (Silva & Sazima 1995, Locatelli & Machado 1999b, Raguso et al. 2003a, Rojas-Sandoval & Meléndez-Ackerman 2009, Ferreira et al. 2018) and bats (Locatelli et al. 1997, Tschapka et al. 1999, Ibarra-Cerdena et al. 2005, Rocha et al. 2019).

The high diversity of cacti with nocturnal anthesis (e.g. *Cereus*, *Cipocereus*, *Pilosocereus*, *Weberocereus*, *Xiquexique*) may be related to physiological and environmental factors in habitat, which is called the 'arid nocturnal flowering and pollination hypothesis' (Borges et al. 2016). Plants that can undergo water stress, such as species from arid and semi-arid regions, preferentially flower at night as a strategy to reduce water loss (Borges et al. 2016). Among

Seasonally Dry Tropical Forests, the Brazilian Caatinga biome is known as the region with a higher proportion of nocturnal flowers (Machado & Lopes 2004, Quirino & Machado 2014, Queiroz et al. 2021), with estimates indicating that ca. 20% of the species of that region are pollinated by bats and sphingids (Machado & Lopes 2004).

The flowers of Cactaceae with nocturnal anthesis can be recognized by a series of characteristics, such as large to medium-sized flowers with white perianth, often somewhat zygomorphic, emitting a typical odour and nectar with comparatively lower sugar content (Rowley 1980, Pimienta-Barrios & del Castillo 2002). In the case of those pollinated by sphingids, the floral tube is especially long and narrow, the nectar accumulates at the base of this tube and is relatively abundant and diluted, while the pollen is moderately abundant and the floral scent is pleasant and sweet (Rowley 1980, Pimienta-Barrios & del Castillo 2002).

Floral signaling is an important component in the interaction between flowers and their pollinators (Varassin & Amaral-Neto 2014), and in the case of sphingophilous plants, studies have revealed a synergy between the visual appearance and scents of flowers leading to the success of floral visits (Raguso & Willis 2002, 2005). White tubular flowers (Johnson & Raguso 2016) and sweet floral scents (Knudsen & Tollsten 1993, Kaiser & Tollsten 1995, Miyake et al. 1998) are visual and olfactory cues shared by many species pollinated by hawkmoths (Manning & Snijman 2002, Raguso et al. 2003a, b, Schlumpberger & Raguso 2008, Balao et al. 2011, Johnson & Raguso 2016, Liu & Sun 2019, Albuquerque-Lima et al. 2020). Such patterns seem to be consistent in Cactaceae, although only a few studies have systematically described the patterns of both visual reflectance of flowers (colour) and floral scents (Kaiser & Tollsten 1995, Raguso et al. 2003a).

One particular genus of cacti that display many of the attributes described above for sphingophilous plants is *Cereus*, in which pollination by sphingids has been reported (Silva & Sazima 1995 = '*C. peruvianus*' [= *C. hildmannianus*], Locatelli & Machado 1999b = *C. fernambucensis*, *C. aethiops* = Egli & Giorgetta 2015). Among the species of the genus, one of the most widely distributed in Northeastern Brazil is *Cereus jamacaru* DC., popularly known as 'Mandacaru', an emblematic cactus of the Brazilian arid and semiarid region (Caatinga). This plant has great ethnobotanical significance (Lima-Nascimento et al. 2019, Silva et al. 2019) and is one of the better-known species of the genus, immortalized as a folkloric and cultural symbol in Brazilian music (Gonzaga 1953), soap opera (Ratton 1997) and literature (Cunha 1902).

In spite of being a famous symbol of the Brazilian Northeastern region, studies dealing with its natural history are scarce. The few studies performed so far have focused on the floral and fruit phenology of *C. jamacaru* in different areas of its extensive range in the Caatinga vegetation (Gomes et al. 2014b, Quirino & Machado 2014, Costa et al. 2020, Silva et al. 2020). Information on the interaction with pollinators and on the role of floral traits (e.g. colour, scents and staminal arrangement) in influencing the behavior of the pollinators during their floral visits remain little known. Given the importance of 'Mandacaru' for both natural and anthropogenic ecosystems, as well as the relevance of natural history studies (Travis 2020, Niet 2021), we aim to describe the floral and reproductive biology of this species, addressing the following questions: (1) What is the floral biology of the species and what are its floral visitors? (2) What is the reflection pattern of its flowers and what is the chemical composition of its floral scent bouquet? and (3) How is its reproductive success under natural conditions? Furthermore, we discuss the floral properties

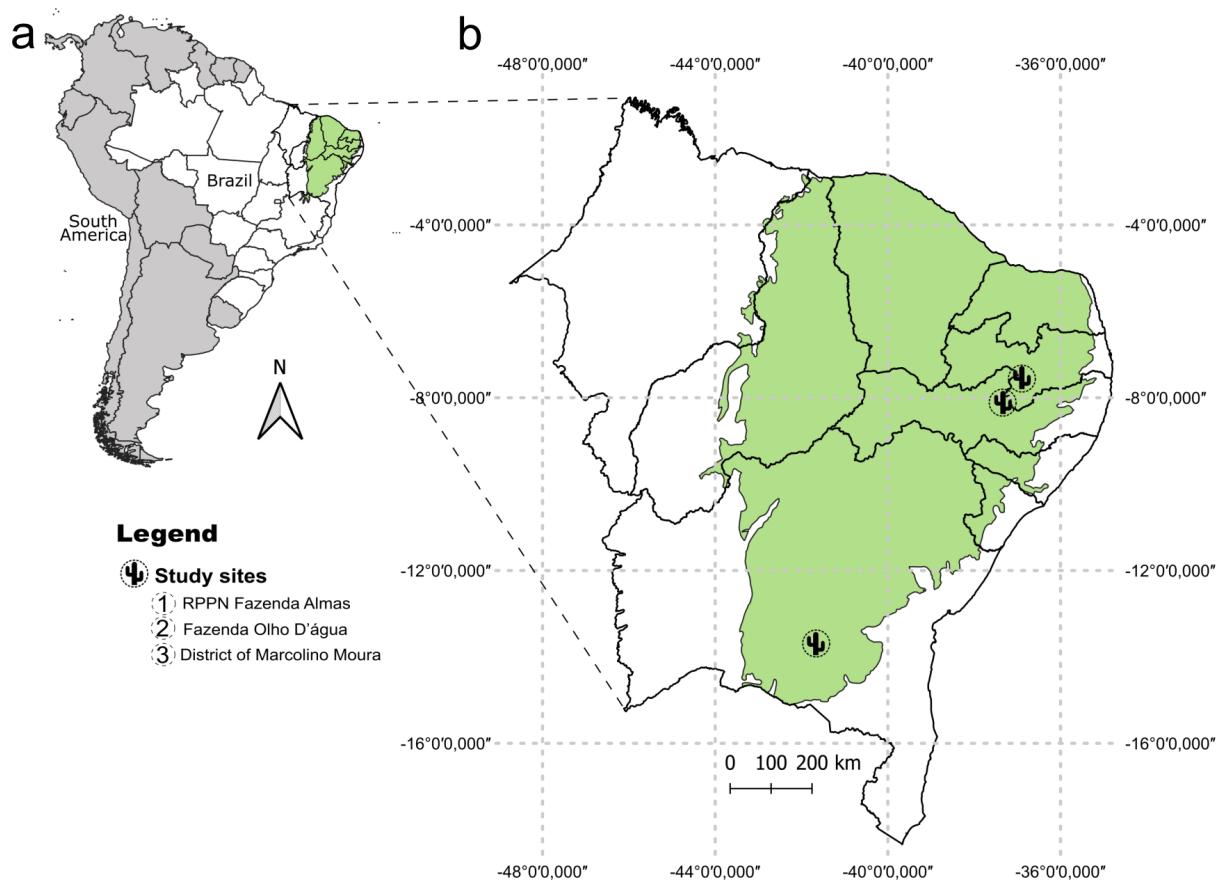
of its flower colour visual reflectiveness and the chemical composition of its scent to gather evidence suggestive of its floral syndrome related to the attractiveness to hawkmoths.

## MATERIALS AND METHODS

### Species studied

The genus *Cereus* Mill. comprises scandent, shrubby or tree-like cacti with a long life-cycle, growing on the soil or on rocks. It is widely distributed from the Caribbean to Argentina, with a centre of diversity in Brazil (Hunt et al. 2006, 2013), in which 16 native species are distributed in all biomes and eight are endemic (Zappi & Taylor 2020). *Cereus* is one of the most representative genera of Eastern Brazil (Taylor & Zappi 2004).

Among the columnar species of the genus, *C. jamacaru* can reach up to 12 m in height with a well-defined spiny trunk (Fig. 1a). The species is widely distributed, occurring throughout the Caatinga *sensu lato*, from the coast up to more than 1000 m a.s.l. (Taylor & Zappi 2004). It grows also in disturbed areas and is cultivated in gardens and farmyards for its use as a stock fence. *Cereus jamacaru* has nocturnal flowers with flowering and fruiting periods that are brief, stochastic and strongly seasonal. There seems to be a strong relationship between the end of the dry season and the start of the first rains, when mass flowering can be observed (Quirino & Machado 2014), though in wetter years it will flower repeatedly after the first rains. Fruits of 'Mandacaru' are highly appreciated by several bird species and also by humans (Lucena et al. 2013, Gomes et al. 2014b, Santos et al. 2019). A voucher has been deposited at the Herbarium of the Universidade de Brasília (UB), under the number Zappi 5100, with a duplicate at the Herbarium Geraldo Mariz of the Universidade Federal de Pernambuco (UFP).



**Figure 1.** Map indicating the study sites. (a) Map of South America, highlighting the geopolitical division of Brazil (white) and the Caatinga region (green). (b) Northeast region of Brazil, highlighting Caatinga area (green) and the three study sites.

### Study sites

The field work was carried out in natural populations at three areas of the Caatinga (Fig. 1), a biome typical of Northeastern Brazil, considered the largest continuous area of seasonally dry tropical forest of South America, also including irrigated pastures and plantations (Sampaio 1995, Silva et al. 2017). The rainfall patterns are irregular and a short-wet season (between 3–5 months) leads to a mean precipitation of around 500–750 mm year and a remarkable variation in rainfall patterns across years. Annual mean temperature is around 23–27°C and the whole area suffers from a water deficit during most of the year (Sampaio 1995, Silva et al. 2017).

Throughout several field campaigns, we found occasions of massive flowering of the species. We took these opportunities to collect miscellaneous floral and reproductive biology data (described below) concerning its natural history. Here these data are systematized and presented. The first population was visited in April 2015. It is located in a protected area known as the Reserva Particular do Patrimônio Natural (RPPN) Fazenda Almas (7°28'51"S, 36°54'40"W), in the municipalities of São José dos Cordeiros and Sumé, Paraíba state (hereafter FA), comprising an area of arboreal or shrubby Caatinga. The second population was visited during April 2017, at the Fazenda Olho D'água (FO) in the municipality of Sertânia (8°00'54.7"S

37°21'15.2"W), Pernambuco state, with remnants of open Caatinga mixed with large expanses of pastureland. The third population is located within the small farming community of the district of Marcolino Moura (MM), municipality of Rio de Contas (13°36'58.7" S, 41°41'02.2" W), Bahia state. This area comprised cattle pasture and small plantations, where *C. jamacaru* was one of a handful of remaining native species, and its specimens were also planted or allowed to grow as living fences. We used flowers for colour and scent analyses of the two first populations, but we performed the majority of the field observations and protocols, such as flowering events and pollinator frequency, reproductive system, flower morphometrics, and extra floral scent extractions in this last population, between January and March 2021.

### Floral biology and morphological measurements

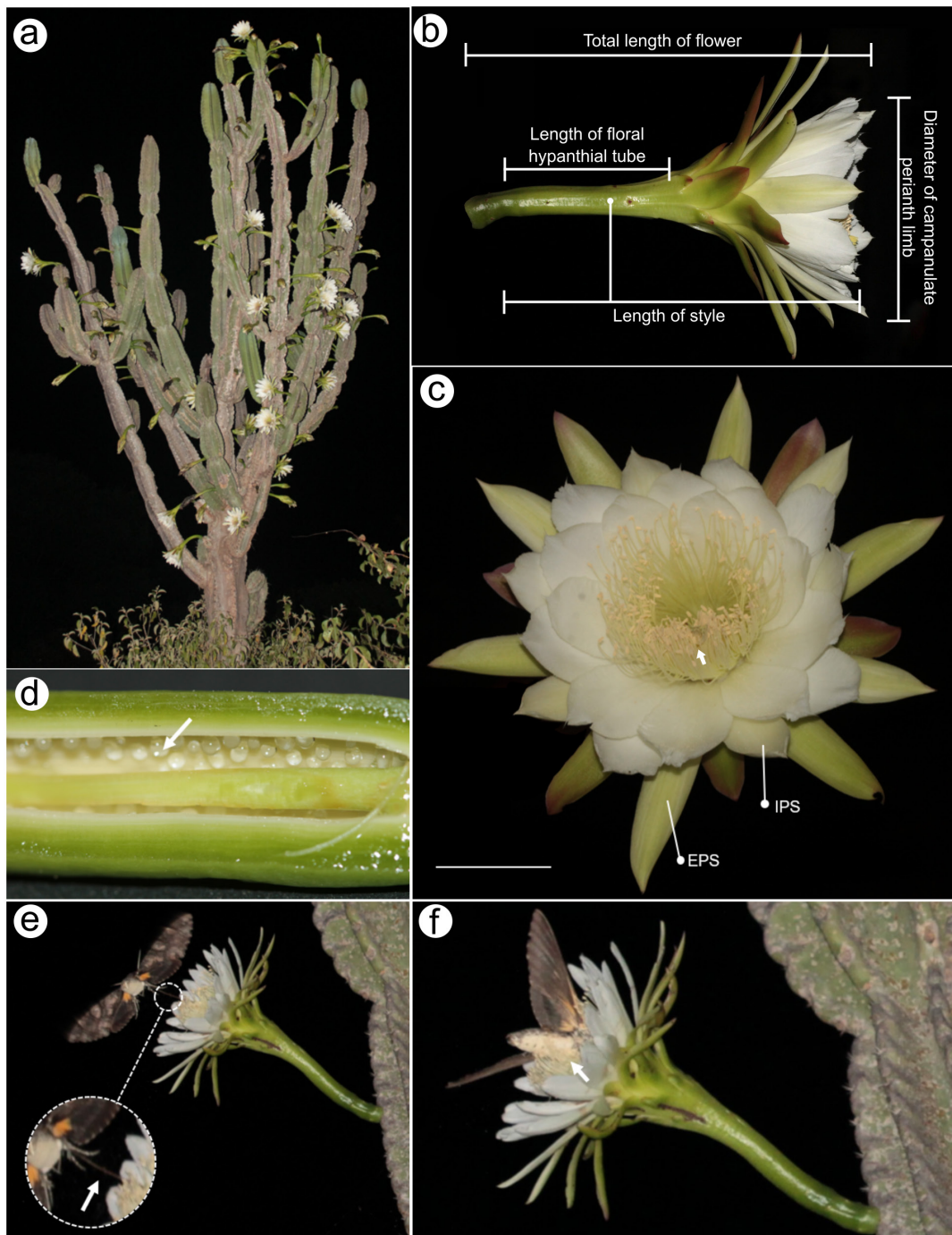
The number of open flowers per night was recorded during 12 non-consecutive nights in 15 individuals (from 15/January to 05/February). The time of anthesis was monitored in 15 different individuals. The floral morphology was analyzed from fresh material and images taken in the field. We measured the total length of flowers, the diameter of the perianth limb, the length of the floral (hypanthial) tube, and the length of the style (Fig. 2b). Measurements were made using a digital pachymeter (Starrett model 799, Massachusetts, USA) in 10 flowers of different individuals (one flower per plant). We counted both stamens and stigma-lobes. Qualitative determination of stigmatic receptivity was performed using the hydrogen peroxide test on flowers from different individuals (n = 10 flowers / 2 times in 5 individuals). Stigmas dipped in hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) were considered receptive when bubbles appeared on the stigmatic surface (Zeisler 1938). Measurements

were taken at the start of anthesis (around 6:30 pm) and after full anthesis (around 00:00 pm).

The volume (μL) and concentration (%) of the nectar were measured in flowers bagged in the field at pre-anthesis stage, and from such data, sugar mass (mg) was estimated according to Galetto & Bernardello (2005). Measurements were taken four hours after full anthesis (around 00:00 pm) of flowers (n= 10) in different individuals. Nectar was extracted from flowers using a graduated microsyringe (250 microlitres, Hamilton, Reno, Nevada, USA) and the sugar concentration (percentage of saccharose, mass / mass) was measured with a pocket refractometer (Master Refractometer 0-52%, Atago, Tokyo, Japan). We estimated the caloric content of nectar based on the equation of Scogin (1985) for Cactaceae species: Energy/ flower = concentration (% of solutes) × volume (ml) × 39, where 39 is the volume conversion unit that reflects calories per gram of sucrose.

### Colour measurements

The spectral reflection properties of *C. jamacaru* flowers were obtained for wavelengths between 300 and 700 nm using a spectrophotometer (JAZEL200, Ocean Optics, USA) (Peitsch et al. 1992). Measurements were done *in situ* on five flowers of two individuals. Given that the outermost and inner perianth-segments differ in colour, measurements were taken for both. External and internal perianth-segment colour measurements used one perianth-segment per flower and were taken on the inner face of the central part of the segment. During measurement, the probe was held at 45° to the target surfaces, to avoid glare. Polytetrafluoroethylene material WS'1 (Ocean Optics) was used as the white reference. The raw floral reflectance spectra were smoothed and visualized using the R package pavo (Maia et al. 2013).



**Figure 2.** Floral biology and pollination of *Cereus jamacaru* DC. (Cactaceae–Cereeae) in the Caatinga of NE Brazil. (a) *C. jamacaru* with open flowers. (b) Lateral view of a flower showing the measured flower traits. (c) Frontal view of flower of *C. jamacaru* showing the contrasting external (EPS) and internal (IPS) perianth-segments (the white arrow indicates evidence of the stigma lobes). Note also the stamen arrangement, the majority resting upon the lower side of the perianth. (d) Droplets of nectar (white arrow) within the floral tube of *C. jamacaru*. (e) Arrival of *Cocytius antaeus* (Drury, 1733) (Sphingidae) to flowers of *C. jamacaru*. The extended proboscis (white arrow) can be seen directed towards the floral tube. (f) Visit of *C. antaeus* to flower of *C. jamacaru*. The hawkmoth can be seen landing on the staminal platform (the white arrow indicates evidence of the stigma lobes). Note its ventral parts covered with pollen. White scale marker for B and C = 5 cm.

### Sampling and chemical characterization of floral volatile organic compounds

To obtain scent samples for thermal desorption (TD), floral volatile organic compounds (hereafter Floral VOCs) of *C. jamacaru* were collected through dynamic headspace methods (adapted from Dötterl et al. 2005), using flowers from populations FO ( $n = 2$ ) and MM ( $n = 3$ ). Individual flowers were bagged in a PET film oven bag (Bratschlauch, Melitta GmbH, Germany) for 15 min, starting at around 9:00 pm, when flowers were fully open. Afterwards, scented air was drawn for 30 min through a silicone tube by a membrane pump (Rietschle Thomas, Puchheim, Germany) at a constant flow rate of 200 mL min<sup>-1</sup>. The air enriched with floral VOCs inside the bags was trapped in a TD filter (length: 30 mm; inner diameter: 2.5 mm) containing adsorbent polymer (3 mg), consisting of a 1:1 mixture of Tenax TA (80/100 mesh, Supelco, USA) and Carboxen 100 (20/40 mesh, Supelco, USA). We collected five floral scent samples (one flower per plant) and, to detect environmental contaminants, negative controls (empty bags;  $n = 2$  / one for each population) were collected using the same aforementioned protocol. All TD samples were stored in 2 ml screw cap clear vials at -24°C until chemical analysis.

To identify the floral volatiles of *C. jamacaru*, TD samples were analyzed on a gas chromatograph coupled to a mass spectrometer (GC-MS; Agilent 7890A gas chromatograph, Agilent 5975C Series MSD mass spectrometer; Agilent Technologies, Palo Alto, CA, USA), equipped with a non-polar HP-5MS column (Agilent JandW; 30 m × 0.25 mm, i.d. 0.25 μm film thickness) and a thermal separation probe (TSP, Agilent Technologies). The TD samples were loaded into the TSP, which was then inserted into the modified GC injector. The injector worked at split mode (1:1) with a temperature of 250°C. GC oven temperature was set at 40°C for 2 min, then

increased at a rate of 4°C min<sup>-1</sup> until reaching final temperature of 230°C that was held steady for 5 minutes. Helium (He) carrier gas flow was maintained at a constant pressure of 7.0 psi. MS Source and quadrupole temperatures were set at 230 and 150°C, respectively. Mass spectra were taken at 70 eV (in electron ionization mode) with a scanning speed of 1.0 scan<sup>-s</sup> from  $m/z$  35–350.

For identification, a *n*-alkanes mixture (C<sub>8</sub>-C<sub>30</sub>) was injected and used to calculate the Retention Index (RI; Van den Dool & Kratz 1963) of each compound making up the floral scent bouquet. Compounds were then identified by comparing their mass spectra and retention indices with those of compounds available from mass spectral libraries (MassFinder 4, NIST11, Adams, and Wiley Registry™ 9th Edition), integrated to the software Agilent MSD Productivity ChemStation (Agilent Technologies, Palo Alto, USA). Confirmation of the identity of some of the compounds was obtained by comparison of both mass spectrum and GC retention index with those of authentic standards available in our compound collection. The peak areas on the chromatograms were integrated to obtain the total ion current signal, which was used to determine the relative percentages of each compound in relation to the sum of all compounds.

### Floral visitor observations

From late January to middle March 2021, pollinator observations were made during 10 non-consecutive nights at intervals in the period between 20:00 pm and 01:00 am, according to the flowering period and flower availability (totalling 36 hours of observation). Floral visitors were photographed using a camera (Canon EOS Rebel T3, Tokyo, Japan) with a macro lens (Canon EF 100mm, Tokyo, Japan), and a field tripod. Observations were made on five individuals and were carried out in one, sometimes two,

plants, depending on their proximity. For each plant all flowers within the visual field were monitored by two observers. A small red light that does not influence visits was used to assist the observation (Mitchell et al. 2015). This small light point was placed as far away as possible from the target plant and was especially useful to observe flowers higher up on the branches, which was the majority of flowers available for observation. Visit time, number of flowers visited per approaching visitor and duration of visits were recorded.

### Pollination and breeding system

The analysis of the reproductive system of *C. jamacaru* involved controlled pollination experiments (Radford et al. 1974). Thus, 40 flower-buds of 16 individuals were used for the different treatments, and the following procedures took place after the start of anthesis: (1) spontaneous self-pollination: floral buds were bagged before anthesis ( $n = 10$  flowers in 10 individuals) and maintained bagged until flower senescence without any manipulation; (2) hand self-pollination: flower-buds were bagged ( $n = 10$  flowers in 8 individuals), and, after anthesis and anther dehiscence, were pollinated by hand with their own pollen, bagged again and maintained in the bags until the following day; (3) hand cross-pollination: flower-buds in pre-anthesis were emasculated while their anthers were still closed and bagged ( $n = 10$  flowers in 9 individuals); afterwards, when the flowers were in full anthesis, they were pollinated with pollen from a different individual and bagged again; (4) natural pollination: flowers were marked and left exposed to flower visitors without any manipulation ( $n = 10$  flowers in 10 individuals). The distance of pollen donors used for the hand cross-pollination was of at least 10 metres, and the hand pollination took place between 8:00 pm and 10:00 pm. Fruiting was measured as the

number of flowers that set fruit per treatment, and seeds were counted for each fruit.

## RESULTS

### Floral biology

The first individuals to produce flower buds, in MM population, did so at the start of January 2021, with those flowers opening mid-January. This flowering period lasted until mid-March and the resulting fruits matured during April 2021. Some individuals produced more than 300 flowers during this period, each opening for a single night (Fig. 2a). The number of open flowers per individual on a given night ranged from three to 26. The flowers start opening synchronously at around 7:30 pm and were fully open by 8:30 pm and withered after sunrise at about 7:00 am of the following day.

The flowers of *C. jamacaru* (Fig. 2b) are large (Table I) with a mean total length of  $24.37 \pm 2.21$  cm. Its flowers present a morphological plan composed of two principal parts: the broadly campanulate perianth limb with a mean diameter of  $14.77 \pm 2.48$  cm (Fig. 2c) including the very numerous stamens; and the long, narrow floral (hypanthial) tube (Fig. 2b) subtending the perianth, with a mean length of  $14.73 \pm 0.93$  cm. These flowers display a slightly zygomorphic form due to the staminal arrangement, where the stamens are concentrated in the lower part of the horizontally held perianth limb by gravity (Fig. 2b). The external segments of the perianth are greenish tinged with reddish brown and fewer than the completely white, broader internal perianth-segments (Fig. 2c). The androecium has very much stamens ( $563 \pm 27$ ) of diverse lengths, disposed around the somewhat longer style, with a greater concentration beneath it, functioning as a landing platform for the pollinator (Fig. 2c). The gynoecium has a long style (mean  $14.53 \pm 0.31$  cm in length), terminated



**Table I. Measurements of the floral structures of *Cereus jamacaru*.**

Traits	Mean $\pm$ SD cm
Floral length	24.4 $\pm$ 2.2
Diameter of perianth	14.8 $\pm$ 2.5
Floral tube length	14.7 $\pm$ 0.9

by 12–17 stigma-lobes, which are receptive from the beginning of anthesis. The stigma-lobes are in contact with the numerous stamens and are receptive during the same period of dehiscence of the anthers.

The nectar is produced as droplets in the lower part of the floral tube (Fig. 2d), and throughout anthesis this nectar accumulates in the chamber at the base of the tube (above the ovary). The nectar has a mean sugar concentration of 24.5  $\pm$  4.1%, and its total production per flower was on average 198.6  $\pm$  92.4  $\mu$ l, with energy/flower (calorie/flower) of 1.897.623  $\pm$  147.747.

### Colour measurements

As already stated, the internal perianth-segments of the flower are white, while the external are greenish (Fig. 2c). The internal elements exhibit low ultraviolet reflection between lengths 360 and 400nm, but exhibit a reflection above 60% from 400nm to 700nm. The external elements present reflectance below 20%, with a peak between 500–600nm and a small peak near the red, close to 700nm (Fig. 3a).

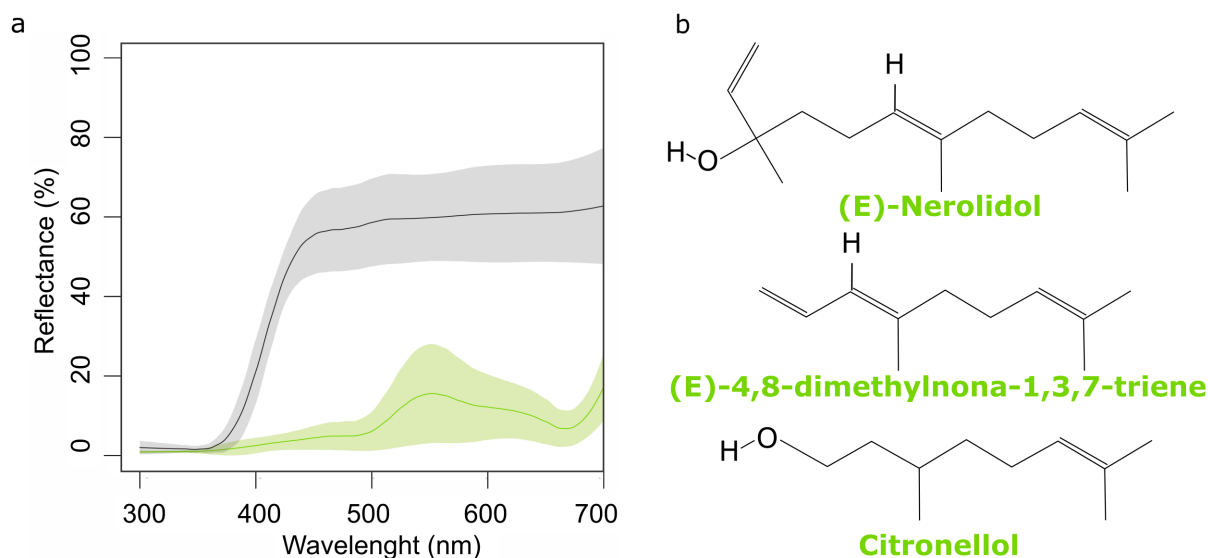
### Floral scent

A total of 23 volatile organic compounds was identified in the headspace samples of *C. jamacaru* flowers (Table II), of which 14 were sesquiterpenes and nine monoterpenes. The sesquiterpene (*E*)-nerolidol was the major component of the floral scent, accounting for about 87.4% of the total bouquet (Fig. 3b), followed by (*E*)-4,8-dimethylnona-1,3,7-triene

(5.18%). With the exception of citronellol (2.58%) and (*E,E*)-farnesal (1.46%), the other floral VOCs were found in relative amounts lower than <1% (Table II).

### Floral visitors and frequency

During our visual observations (36h), we recorded eight visits (0.22 visits/hour) to five different individuals of *C. jamacaru* made by hawkmoths (Sphingidae) (Fig. 2 e-f). Although its capture was not possible due to the height of the visited flowers, photographs allowed its identification as *Cocytius antaeus* (Drury, 1733), popularly known as the giant sphinx moth (Fig. 2 e-f). In the five individuals that received visits, the hawkmoths started approaching around 10:00 pm, and they visited consecutively several of the open flowers on each individual. During their visits, each hawkmoth approached and hovered in front of the flower before unrolling its proboscis and inserting it into the flower-tube (Fig. 2e) and then landed on the flower upon the mass of stamens (Fig. 2f). The hawkmoths landed in the perianth limb, composed of the inner perianth-segments, stamens and stigmas, and in this way, they contacted the reproductive structures of the flower (Fig. 2f). Pollen grains adhered to different parts of the hawkmoth's body, although the largest amount was deposited mainly on its ventral part; the ring formed by the numerous stamens also deposits pollen on the insect's back, as well as on the wings. When approaching the flower, the hawkmoth immediately contacts the stigma with its frontal portion, although much contact also occurs with the ventral portion of the insect's body (Fig. 2f). The visits generally lasted for about 30–50 seconds in each flower, and the hawkmoths spent about 3 minutes visiting the flowers of a given individual of *C. jamacaru* before flying to the next one. In addition to the giant sphinx, we observed other insects visiting the flowers before and



**Figure 3.** Visual and olfactory floral cues of *Cereus jamacaru* DC. (Cactaceae–Cereeae) in the Caatinga of NE Brazil. (a) Spectral reflectance colors of the inner and outer perianth-segment surfaces of *C. jamacaru*. The grey line indicates the surface of the inner segments, the green line the surface of the outer segments. The shaded areas are represented by  $\pm$  SD. (b) Chemical structure of the main components of the floral scent of *C. jamacaru*.

after anthesis, such as several Orthoptera, as well as stingless bees (*Trigona spinipes*). These insects did not contact reproductive parts and only damaged or consumed the floral tissues and pollen, without pollinating the flowers. Carpenter bees (*Xylocopa grisescens* Lepelletier, 1841) were also observed making some visits in the early morning hours. These bees penetrated deep into the flowers, all the way to the apex of the floral tube, presumably trying to access some of the nectar. Eventually these bees come into contact with the stigmas of the flowers.

### Breeding system

In the controlled pollination experiment, no fruit was set in the spontaneous and hand self-pollination treatments, demonstrating that *C. jamacaru* is self-incompatible and xenogamous (*sensu* Cruden 1977). Only two fruits (20%) were formed by natural pollination, while four (40%) were formed through hand cross-pollination. The fruits of hand cross-pollinated flowers set the highest number of seeds (Table III).

### DISCUSSION

Our study confirms hawkmoths as pollinators of *Cereus jamacaru*, a columnar cactus with floral characteristics, including the visual landing platform and scent, highly specialized for sphingid pollination. We discuss the role of the floral traits in the interaction with its specialized pollinator, and which factors are affecting the low reproductive success (in terms of fruit-production) in this iconic Caatinga tree cactus.

### Phenotypic specialization for hawkmoths in Cactaceae

Floral morphology and attributes, such as floral scent, as well as volume and nectar concentration of *C. jamacaru*, offer clear evidence of its specialization for hawkmoth pollination. *Cereus* and similarly *Arthrocereus*, *Echinopsis*, *Epiphyllum*, *Harrisia*, *Selenicereus* and *Strophocactus* are genera of Cactaceae with flowers highly specialized for pollination by sphingids (Rowley 1980, Pimienta-Barrios & del Castillo 2002). Vogel (1990) reports that c. 13% of

**Table II. Floral volatile organic compounds (VOCs) emitted by flowers of *Cereus jamacaru* (n=5).**

Compounds	Substance class	RI	n	Mean	SD
$\alpha$ -Pinene*	Monoterpene	932	2	0.21	0.31
Limonene*	Monoterpene	1030	2	0.10	0.16
(E)-4,8-Dimethylnona-1,3,7-triene*	Monoterpene	1118	5	5.18	3.04
Citronellal*	Monoterpene	1156	2	0.24	0.37
Citronellol*	Monoterpene	1233	2	2.58	5.09
cf. Isogeraniol	Monoterpene	1237	1	0.07	0.15
Neral*	Monoterpene	1245	1	0.02	0.05
Geraniol*	Monoterpene	1274	1	0.61	1.37
Citronellyl acetate	Monoterpene	1359	1	0.02	0.05
$\alpha$ -Copaene*	Sesquiterpene	1371	1	0.03	0.06
Cyperene	Sesquiterpene	1398	2	0.18	0.33
$\beta$ -Caryophyllene*	Sesquiterpene	1422	1	0.07	0.15
(E)- $\beta$ -Farnesene*	Sesquiterpene	1462	2	0.31	0.52
(Z,Z)- $\alpha$ -Farnesene	Sesquiterpene	1502	3	0.06	0.07
(Z)- $\alpha$ -bisabolene*	Sesquiterpene	1510	2	0.08	0.14
$\beta$ -Bisabolene*	Sesquiterpene	1516	3	0.72	0.99
$\delta$ -Cadinene	Sesquiterpene	1531	2	0.14	0.22
(E)- $\gamma$ -bisabolene	Sesquiterpene	1540	2	0.07	0.10
(Z)- $\alpha$ -bisabolene*	Sesquiterpene	1551	2	0.26	0.37
(E)-nerolidol*	Sesquiterpene	1572	5	87.37	6.89
(2E,6Z)-farnesal	Sesquiterpene	1725	1	0.21	0.46
(E,E)-farnesol*	Sesquiterpene	1732	1	0.02	0.03
(E,E)-farnesal*	Sesquiterpene	1752	1	1.46	3.26

**Note:** RI, retention index; n, number of samples in which compounds were recorded; Mean, relative percentage average; SD, standard deviation. \*Identification based on authentic standards.

cactus species are adapted to sphingophily, and other authors also report hawkmoth pollination in cacti (Haber & Frankie 1989, Barthlott et al. 1997, Raguso et al. 2003a, Rojas-Sandoval & Meléndez-Ackerman 2009, Ortega-Baes et al. 2011, Arzabe et al. 2018, Rocha et al. 2019).

Stamen arrangement is an important characteristic in flowers of Cactaceae with

hummingbird pollination syndrome (e.g. in species of *Cleistocactus*, *Denmoza*, *Disocactus*, *Matucana*, *Nopalea*, *Oreocereus* and *Schlumbergera*), since they are pressed against the upper side of the floral tube and perianth limb, ensuring pollen deposition on the top and back of the bird's head (Gorostiague & Ortega-Baes 2016, Ferreira et al. 2020). Similarly, it is possible to identify a level of specialization in the arrangement of the stamens in sphingophilous species, where their organization has a function in relation to the landing platform used by the insect (Netz & Renner 2017), which also can help pollinators against predation, since they can land and diminish conspicuous movement (Wasserthal 1993, 1997). In the case of Cactaceae, many sphingophilous genera display such a stamen arrangement (e.g. *Arthrocereus*, *Echinopsis*, *Epiphyllum*, *Harrisia*, *Selenicereus* and *Strophocactus*) and field observations on other species of *Cereus* confirm this pollinator behavior (Silva & Sazima 1995, Egli & Giorgetta 2015), which was likewise observed in the present study of *C. jamacaru*.

In sphingophilous plants the scents and visual signals are fundamental to the interaction and orientation of the hawkmoths and studies have confirmed that these two forms of attraction act together to guarantee the behavior of the insects and their precision in the search for flowers (Raguso & Willis 2002, 2005). The scent tracks are considered the primary long-distance attraction for the hawkmoths, aiding their orientation towards the flowers (Andersson et al. 2002, Goodrich & Raguso 2009). The floral odour of *C. jamacaru* is dominated by (E)-nerolidol, (E)-4,8-dimethylnona-1,3,7-triene, citronellol and (E,E)-farnesal, which account for more than 96% of the total composition of the floral scent. All these compounds are in accordance with that expected for flowers pollinated by hawkmoths (Knudsen & Tollsten 1993, Kaiser & Tollsten 1995,

**Table III. Number of fruit and seeds of *Cereus jamacaru* formed in the pollination experiments.**

Treatments	Fruits	Seeds
Cross-pollination (n=10)	1°	1269
	2°	1614
	3°	5120
	4°	3527
Control (Natural pollination) (n=10)	1°	1010
	2°	1422

Miyake et al. 1998, Manning & Snijman 2002, Raguso et al. 2003a, b, Schlumpberger & Raguso 2008, Balao et al. 2011, Johnson & Raguso 2016, Albuquerque-Lima et al. 2020).

(*E*)-nerolidol is the major compound of the floral scent bouquet of *C. jamacaru*. It is one of the most common (and usually the major) compound of floral scents of sphingophilous plants (Knudsen & Tollsten 1993, Kaiser & Tollsten 1995, Manning & Snijman 2002, Raguso et al. 2003b, Balao et al. 2011, Albuquerque-Lima et al. 2020), including different species of nocturnal cacti (Schlumpberger & Raguso 2008, Lemaitre et al. 2014). Although experimental proof of its behavioral meaning is still missing, studies have showed that this compound trigger strong antennal responses in different hawkmoth species, such as *Hyles livornica* (Balao et al. 2011), *Manduca sexta* (Fraser et al. 2003), *Sphinx perelegans* (Raguso & Light 1998). This strongly suggests that (*E*)-nerolidol is involved in the signaling of sphingophilous flowers to their pollinators. Future experimental studies testing this compound are needed and might confirm its key role in attracting several hawkmoths species.

Whilst scent is the means to attract the insects at long distances from the flower, the visual clues are important for the positioning of the hawkmoths upon arrival at the flower and to induce the proboscis extending behavior

(Balkenius & Dacke 2010). In the case of *C. jamacaru* the reflective pattern of its flowers, with a white interior and dark external perianth-segments, matches that of other flowers of sphingophilous species (Raguso et al. 2003a, Johnson & Raguso 2016, Liu & Sun 2019). The sphingids display excellent visual abilities (Raguso & Willis 2002, 2005, Balkenius et al. 2006) and the white perianth gives a strong contrast with the dark background. Indeed, there is evidence that these hawkmoths respond positively to white objects and lighter markings (Kugler 1971, Ippolito et al. 2004, Johnson & Raguso 2016). In the case of *C. jamacaru* the white internal perianth-segments contrast strongly with the dark background of the plant's stems, which match the outer segments in colour. Upon arrival, the hawkmoths are already extending their proboscis towards their target, the white flower interior triggering this behavior.

Besides the above-mentioned combination of scent and visual attractants, the nectar produced by *C. jamacaru* matches that expected for sphingophilous flowers (Scogin 1985) and encountered in other species of *Cereus* (Locatelli & Machado 1999b). In many Cactaceae, nectar secretion occurs along the inner face of the floral tube (Nassar et al. 1997). The flowers of the genus *Cereus* have a long floral tube and the production of nectar in drops in *C. jamacaru* may be a result of this tube stretching, which makes it difficult for the drops to accumulate at the end of the floral tube. Nectar droplets have also been reported for '*C. peruvianus*' [= *C. hildmannianus*] (Silva & Sazima 1995).

Compared with other *Cereus* species, the volume of nectar produced per night by *C. jamacaru* is amongst the largest (Silva & Sazima 1995, Locatelli & Machado 1999b), though its concentration resembles that of other species (Scogin 1985, Locatelli & Machado 1999b). The mean energy resource (calories/flower) offered

by the nectar of *C. jamacaru* is higher than the values established for most sphingophilous species ( $93 \pm 107$  cal) by Scogin (1985). However, included in his study were other species of *Cereus*, such as *C. forbesii* [=/~ *C. hankeanus*] and *C. vargasianus*, and also *Selenicereus spinulosus*, which likewise displayed high energy values, corresponding with the findings of our study. Whilst the sphingids use the flowers of *C. jamacaru* as a landing platform, thereby conserving their energy, they need to travel long distances and hover before being able to access the nectar. This requires great expenditure of energy and thus it is possible that the nectar of *C. jamacaru* is offering compensation compatible with the needs of its visitors.

### **Pollinator dependence and low reproductive success in sphingophilous Cactaceae**

The giant sphinx moth, *Cocytius antaeus* was the floral visitor that proved to be an effective pollinator. This species, with its long proboscis, is involved in the pollination of other *Cereus* species (Locatelli & Machado 1999b), as well as other Cactaceae (Rocha et al. 2019). Besides these, this hawkmoth is known as a pollinator of other plants with long floral tubes, compatible with its long tongue (Haber & Frankie 1989, Oliveira et al. 2004, Houlihan et al. 2019, Queiroz et al. 2021). Queiroz et al. (2021), while studying an interaction network in a diverse group of nocturnal-flowered Caatinga plants, observed that some species apparently displaying the sphingophilous syndrome (e.g. *Pilosocereus gounellei* [= *Xiquexique gounellei*]) are in fact generalists, being visited by hawkmoths and bats. In the case of *C. jamacaru*, these authors considered it as a sphingid specialist since they only observed visitation by hawkmoths (*Agrius cinclata*, *Eumorpha analis*, *Isognathus allamandae*, *Manduca rustica* and *M. sexta*). Likewise, in a seasonal semideciduous Atlantic

Forest fragment in São Lourenço da Mata, Pernambuco, northeastern Brazil, L.M. Primo (unpublished data) studying a community of nocturnal species showed that *C. jamacaru* was only visited by the long-tongued hawkmoth *M. sexta*.

The close relationship between *Cereus jamacaru* and hawkmoths may be related to the interaction between their floral morphology and the tongue length of their pollinator. Previous studies have established that sphingophilous plants can be divided into the two systems corresponding with short-tubed flowers pollinated by short-tongued hawkmoths and those with long floral tubes pollinated by long-tongued species (Anderson et al. 2010, Johnson & Raguso 2016, Sazatornil et al. 2016, Johnson et al. 2017). *Cereus jamacaru* clearly belongs to the second system, its elongated flower being strongly adapted to pollination by long-tongued hawkmoths.

Although there seems to be a close relationship between *C. jamacaru* and long-tongued sphingids species, some studies have shown the interaction of bats with *C. jamacaru* flowers in both Caatinga and urban areas (personal observations, L. M. Primo, unpublished data, Cordero-Schmidt et al. 2021). Among the species interacting with 'Mandacaru', the bat *Glossophaga soricina* shows opportunistic behavior (Lemke 1985) and performs hovering visits to consume pollen of *C. jamacaru* flowers. However, the effect of visits by these bats in these areas has not been measured, and further observations are needed to measure the role of bats in the reproductive success of *C. jamacaru*. In the case of carpenter bees observed visiting the flowers in the early hours of the morning, at the end of anthesis, although they landed in different ways on the stamens and stigmas of the flowers before accessing the floral tube, in the days following anthesis no fruit was developed,

indicating that they do not be effective pollen vectors for this species.

We have not recorded herkogamy or dichogamy, i.e. spatial and temporal separations, between male and female functions in the flower, which could generate pollen self-deposition (Roldán & Ashworth 2018). However, the self-incompatibility of *C. jamacaru* demonstrated by our study shows that this cactus depends on sphingids as pollen vectors for the production of fruits and seeds. Egli & Giorgetta (2015) likewise report self-incompatibility in cultivated plants of *C. jamacaru* and *C. validus*. In the investigated populations (MM) we observed that fruit set of *C. jamacaru* was low and even lower in naturally than in manually pollinated flowers, though the fruits of both contained large numbers of seeds. Low fruit set has been reported in other Cactaceae pollinated by sphingids (Walter 2010). Hawkmoth pollination has been studied for various genera of Cactaceae (Silva & Sazima 1995, Barthlott et al. 1997, Locatelli & Machado 1999b, Raguso et al. 2003a, Rocha et al. 2007, Rojas-Sandoval & Meléndez-Ackerman 2009, Ferreira et al. 2018, Rocha et al. 2019) and although these studies have demonstrated that hawkmoths are pollen vectors for these taxa, a question that arose in some cases is the apparently low frequency of their visits (Schlumpberger & Raguso 2008, Ferreira et al. 2018), which can suggest a shortage of pollinators and/or transported pollen (Suzán et al. 1994). Small quantities of pollen and/or pollinators are believed to be a factor responsible for low pollen flow and consequently low reproductive success in some species of Angiosperms that require hawkmoths with long proboscis for effective pollination (Amorim et al. 2014).

Diverse factors could explain the low reproductive success in plants, such as questions connected with climate and weather, limited availability of pollen and/or pollinators, high

competition for the latter, anthropogenic effects and predation of flowers and fruits (McIntosh 2002). In *C. jamacaru*, different intrinsic factors could be influencing the formation of fruits. Their low reproductive success could be due to few pollinators, in this case hawkmoths, which during our field observations were rare with only a single species visiting its flowers, and to the loss of pollen as the insect visits flowers on the same individual cactus. The sphingids showed a behavior of visiting all the open flowers on a single individual, perhaps losing pollen meanwhile and failing to successfully pollinate it due to the species' demonstrable self-incompatibility. However, the large number of seeds per fruit in *C. jamacaru* (c. 1000–>3000), and its efficient system of dispersal by birds (Personal observations; Gomes et al. 2014b, Santos et al. 2019) may in part explain its extensive range and abundance in many Caatinga sites.

It is worth noting that part of the study was carried out in an area marked by severe anthropogenic effects, a reality found for many of the distribution sites of *C. jamacaru*, as well as for other species of the family (Zappi & Taylor 2011). Cactaceae is among the three most threatened plant groups (Goettsch et al. 2015) and particularly in the case of Brazil, which has a large number of endemic species, few are inserted in protected areas (Amaral et al. 2022, Carvalho et al. 2022). Furthermore, a concern highlighted by the present study is the low diversity and frequency of pollinators for *C. jamacaru* at the studied populations. If this proves to be the case throughout its range, the loss of the pollinating hawkmoth species could severely impact the reproductive success of this cactus.

## Acknowledgments

This paper is part of the PhD Thesis of SAL in the Post-Graduate Program in Plant Biology of the Universidade Federal de Pernambuco. To Lidyane Aona and Dr. Vanessa Gomes for the reading and suggestions in a first version, still as a chapter of the PhD thesis of SAL. To Roberto Lima for the logistical support at RPPN Fazenda Almas. This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) – Brazil (Research grants to ICM 311021/2014-0, 310508/2019-3 and Universal 459485/2014, 437424/2018-9 and to DCZ 304178/2021-7); by Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE) – Brazil (APQ-0808-2.03/16 to ICM and APQ-0226-2.03/21 to ADM); and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) – Brazil, with a post-graduate scholarship to SAL (88882.347730 / 2019-01) and Financing Code 001.

## REFERENCES

- ALBUQUERQUE-LIMA S, DOMINGOS-MELO A, NADIA TCL, BEZERRA ELS, NAVARRO DMAF, MILET-PINHEIRO P & MACHADO IC. 2020. An explosion of perfume: Mass flowering and sphingophily in the Caatinga dry region in Brazil. *Plant Spec Biol* 35: 243-255.
- AMARAL DT, BONATELLI IA, ROMEIRO-BRITO M, MORAES EM & FRANCO FF. 2022. Spatial patterns of evolutionary diversity in Cactaceae show low ecological representation within protected areas. *Biol Conserv* 273: 109677.
- AMORIM FW, WYATT GE & SAZIMA M. 2014. Low abundance of long-tongued pollinators leads to pollen limitation in four specialized hawkmoth-pollinated plants in the Atlantic Rain forest, Brazil. *Naturwissenschaften* 101: 893-905.
- ANDERSON B, ALEXANDERSSON R & JOHNSON SD. 2010. Evolution and coexistence of pollination ecotypes in african *Gladiolus* (Iridaceae): pollinator driven floral divergence. *Evolution* 64: 960-972.
- ANDERSON EF. 2001. The cactus family. Portland: Timber Press, 777 p.
- ANDERSSON S, NILSSON LA, GROTH I & BERGSTRÖM G. 2002. Floral scents in butterfly-pollinated plants: possible convergence in chemical composition. *Bot J Linn Soc* 140: 129-153.
- ARROYO-PÉREZ E, JIMÉNEZ-SIERRA CL, ZAVALA HURTADO JA & FLORES J. 2021. Shared pollinators and sequential flowering phenologies in two sympatric cactus species. *Plant Ecol Evol* 154: 28-38.
- ARZABE A, AGUIRRE LF & BALDELOMAR M. 2018. Pollination system of two endemic Bolivian cacti: *Harrisia tetraacantha* and *Neoraimondia herzogiana*. *Bradleya* 36: 178-188.
- BALAO F, HERRERA J, TALAVERA S & DÖTTERL S. 2011. Spatial and temporal patterns of floral scent emission in *Dianthus inoxianus* and electroantennographic responses of its hawkmoth pollinator. *Phytochemistry* 72: 601-609.
- BALKENIUS A & DACKE M. 2010. Flight behavior of the hawkmoth *Manduca sexta* towards unimodal and multimodal targets. *J Exp Bio* 213: 3741-3747.
- BALKENIUS A, ROSÉN W & KELBER A. 2006. The relative importance of olfaction and vision in a diurnal and a nocturnal hawkmoth. *J Comp Physiol A* 192: 431-437.
- BARTHLOTT W, POREMBSKI S, KLUGE M, HOPKE J & SCHMIDT L. 1997. *Selenicereus wittii* (Cactaceae): An epiphyte adapted to Amazonian Igapó inundation forests. *Plant Syst Evol* 206: 175-185.
- BORGES RM, SOMANATHAN H & KELBER A. 2016. Patterns and processes in nocturnal and crepuscular pollination services. *Q Rev Bio* 91: 389-418.
- CARVALHO CE, MENEZES MO, ARAÚJO FS & SFAIR JC. 2022. High endemism of cacti remains unprotected in the Caatinga. *Biodivers Conserv* 31: 1217-1228.
- CORDERO-SCHMIDT E, MARUYAMA PK, VARGAS-MENA JC, OLIVEIRA PP, SANTOS FAR, MEDELLÍN RA, RODRIGUEZ-HERRERA B & VENTICINQUE EM. 2021. Bat-flower interaction networks in Caatinga reveal generalized associations and temporal stability. *Biotropica* 53: 1546-1557.
- COSTA PM DE A, SOUZA VC, COSTA VS, BARROS ES & OLIVEIRA ISS. 2020. Fenofases reprodutivas em uma população de mandacaru (*Cereus jamacaru*) e facheiro (*Pilosocereus pachycladus* subsp. *pernambucensis*) (Cactaceae). *Braz Jour Dev* 6: 30536-30545.
- CUNHA E. 1902. Os sertões: campanha de Canudos. Rio de Janeiro: Laemmert & C. Editores, 632 p.
- DÖTTERL S, WOLFE LM & JÜRGENS A. 2005. Qualitative and quantitative analyses of flower scent in *Silene latifolia*. *Phytochemistry* 66: 203-213.
- EGGLI U & GIORGETTA M. 2015. Flowering phenology and observations on the pollination biology of South American Cacti. 2. *Cereus aethiops*. *Haseltonia* 21: 19-40.
- FACHARDO ALS & SIGRIST MR. 2020. Pre-zygotic reproductive isolation between two synchronopatric *Opuntia* (Cactaceae) species in the Brazilian Chaco. *Plant Biol* 22: 487-493.

- 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 Biol* 20: 101-112.
- FERREIRA BHS, SOUZA CS, FACHARDO ALS, GOMES AC & SIGRIST MR. 2020. Flowering and pollination ecology of *Cleistocactus baumannii* (Cactaceae) in the Brazilian Chaco: pollinator dependence and floral larceny. *Acta Bot Bras* 34: 167-176.
- FRASER AM, MECHABER WL & HILDEBRAND JG. 2003. Electroantennographic and Behavioral Responses of the Sphinx Moth *Manduca sexta* to Host Plant Headspace Volatiles. *J Chem Ecol* 29: 1813-1833.
- GALETTO L & BERNARDELLO G. 2005. Rewards in flowers: nectar. In: DAFNI A, KEVAN PG & HUSBANDS BC (Eds), *Practical Pollination Biology*. Cambridge: Enviroquest, p. 264-313.
- GIBSON AC & NOBEL PS. 1986. *The cactus primer*. Cambridge: Harvard University Press, 286 p.
- GOETTSCHE B ET AL. 2015 High proportion of cactus species threatened with extinction. *Nature Plants* 110: 1-7.
- GOMES VGN, QUIRINO Z & ARAUJO H. 2014b. Frugivory and seed dispersal by birds in *Cereus jamacaru* DC. ssp. *jamacaru* (Cactaceae) in the Caatinga of Northeastern Brazil. *Braz J Biol* 74: 32-40.
- GOMES VGN, QUIRINO ZGM & MACHADO IC. 2014a. Pollination and seed dispersal of *Melocactus ernestii* Vaupel subsp. *ernestii* (Cactaceae) by lizards: an example of double mutualism. *Plant Biol* 16: 315-322.
- GONZAGA L. 1953. Xote das meninas. In: Gonzaga L, Dantas J. Album: O Xote das Meninas / 13 de dezembro. Rio de Janeiro (RJ): RCA Victor.
- GOODRICH KR & RAGUSO RA. 2009. The olfactory component of floral display in *Asimina* and *Deeringothamnus* (Annonaceae). *New Phytol* 183: 457-469.
- GOROSTIAGUE P & ORTEGA-BAES P. 2016. How specialised is bird pollination in the Cactaceae? *Plant Biol* 18: 63-72.
- HABER WA & FRANKIE GW. 1989. A Tropical Hawkmoth Community: Costa Rican Dry Forest Sphingidae. *Biotropica* 21: 155-172.
- HOULIHAN PR, STONE M, CLEM SE, OWEN M & EMMEL TC. 2019. Pollination ecology of the ghost orchid (*Dendrophylax lindenii*): A first description with new hypotheses for Darwin's orchids. *Sci Rep* 9: 12850.
- HUNT D, TAYLOR NP & CHARLES G. 2006. *The new cactus lexicon 1, Text*. Milborne Port: Dh publications, 373 p.
- HUNT D, TAYLOR NP & CHARLES G. 2013. *The new cactus lexicon, Illustrations*. Milborne Port: Dh publications, 527 p.
- IBARRA-CERDENA CN, INIGUEZ-DAVALOS LI & SANCHEZ-CORDERO V. 2005. Pollination ecology of *Stenocereus queretaroensis* (Cactaceae), a chiropterophilous columnar cactus, in a tropical dry forest of Mexico. *Am J Bot* 92: 503-509.
- IPPOLITO A, FERNANDES GW & HOLTSFORD TP. 2004. Pollinator preferences for *Nicotiana alata*, *N. forgetiana*, and their F<sub>1</sub> hybrids. *Evolution* 58: 2634-2644.
- JOHNSON SD & RAGUSO RA. 2016. The long-tongued hawkmoth pollinator niche for native and invasive plants in Africa. *Ann Bot* 117: 25-36.
- JOHNSON SD, MORÉ M, AMORIM FW, HABER WA, FRANKIE GW, STANLEY DA, COCUCCI AA & RAGUSO RA. 2017. The long and the short of it: a global analysis of hawkmoth pollination niches and interaction networks. *Funct Ecol* 31: 101-115.
- KAISER R & TOLLSTEN L. 1995. An introduction to the scent of cacti. *Flavour Fragr J* 10: 153-164.
- KNUDSEN JT & TOLLSTEN L. 1993. Trends in floral scent chemistry in pollination syndromes: floral scent composition in moth-pollinated taxa. *Bot J Linn Soc* 113: 263-284.
- KUGLER H. 1971. Zur Bestäubung großblumiger *Datura*-Arten. *Flora* 160: 511-517.
- LEAL FC, LOPES AV & MACHADO IC. 2006. Polinização por beija-flores em uma área de caatinga no Município de Floresta, Pernambuco, Nordeste do Brasil. *Braz J Bot* 29: 379-389.
- LEMAITRE AB, PINTO CF & NIEMEYER HM. 2014. Generalized pollination system: Are floral traits adapted to different pollinators? *Arthropod-Plant Inte* 8: 261-272.
- LEMKE TO. 1985. Pollen Carrying by the Nectar-Feeding Bat *Glossophaga soricina* in a Suburban Environment. *Biotropica* 17: 107-111.
- LIMA-NASCIMENTO AM, BENTO-SILVA JS, LUCENA CM & LUCENA RFP. 2019. Ethnobotany of native cacti in the northeast region of Brazil: can traditional use influence availability? *Acta Bot Bras* 33: 350-359.
- LIU C-Q & SUN H. 2019. Pollination in *Lilium sargentiae* (Liliaceae) and the first confirmation of long-tongued hawkmoths as a pollinator niche in Asia: Hawkmoth pollination in *Lilium sargentiae*. *J Syst Evol* 57: 81-88.
- LOCATELLI E & MACHADO ICS. 1999a. Comparative study of the floral biology in two ornithophilous species of Cactaceae: *Melocactus zehntneri* and *Opuntia palmadora*. *Bradleya* 17: 75-85.



- LOCATELLI E & MACHADO ICS. 1999b. Floral biology of *Cereus fernambucensis*: a sphingophilous cactus of restinga. *Bradleya* 17: 86-94.
- LOCATELLI E, MACHADO IC & MEDEIROS P. 1997. Floral Biology and Bat Pollination in *Pilosocereus catingicola* (Cactaceae) in Northeastern Brazil. *Bradleya* 15: 28-34.
- LUCENA CM ET AL. 2013. Use and knowledge of Cactaceae in Northeastern Brazil. *J Ethnobiol Ethnomed* 9: 1-11.
- MACHADO IC & LOPES AV. 2004. Floral traits and pollination systems in the Caatinga, a Brazilian Tropical Dry Forest. *Ann Bot* 94: 365-376.
- MAIA R, ELIASON CM, BITTON P-P, DOUCET SM & SHAWKEY MD. 2013. pavo: an R package for the analysis, visualization and organization of spectral data. *Methods Ecol Evol* 4: 906-913.
- MANDUJANO MC, CARRILLO-ANGELES IG, MARTÍNEZ-PERALTA C & GOLUBOV J. 2010. Reproductive biology of Cactaceae. In: RAMAWAT KG (Ed), *Desert plants - biology and biotechnology*. Berlin: Springer, p. 197-230
- MANNING JC & SNIJMAN D. 2002. Hawkmoth-pollination in *Crinum variable* (Amaryllidaceae) and the biogeography of sphingophily in southern African Amaryllidaceae. *S Afr J Bot* 68: 212-216.
- MCINTOSH ME. 2002. Plant size, breeding system, and limits to reproductive success in two sister species of *Ferocactus* (Cactaceae). *Plant Ecol* 162: 273-288.
- MITCHELL TC, DÖTTERL S & SCHAEFER H. 2015. Hawk-moth pollination and elaborate petals in Cucurbitaceae: The case of the Caribbean endemic *Linnaeosicyos amara*. *Flora* 216: 50-56.
- MIYAKE T, YAMAOKA R & YAHARA T. 1998. Floral scents of hawkmoth-pollinated flowers in Japan. *J Plant Res* 111: 199-205.
- NASSAR JM, RAMÍREZ N & LINARES O. 1997. Comparative pollination biology of Venezuelan columnar cacti and the role of nectar-feeding bats in their sexual reproduction. *Am J Bot* 84: 918-927.
- NETZ C & RENNER SS. 2017. Long-spurred *Angraecum* orchids and long-tongued sphingid moths on Madagascar: a time frame for Darwin's predicted *Xanthopan/Angraecum* coevolution. *Biol J Linn Soc* 122: 469-478.
- NIET T. 2021. Paucity of natural history data impedes phylogenetic analyses of pollinator-driven evolution. *New Phytol* 229: 1201-1205.
- OLIVEIRA PE, GIBBS PE & BARBOSA AA. 2004. Moth pollination of woody species in the Cerrados of Central Brazil: a case of so much owed to so few? *Plant Syst Evol* 245: 41-54.
- ORTEGA-BAES P, SARAVIA M, SÜHRING S, GODÍNEZ-ALVAREZ H & ZAMAR M. 2011. Reproductive biology of *Echinopsis terscheckii* (Cactaceae): the role of nocturnal and diurnal pollinators. *Plant Biol* 13: 33-40.
- PEITSCH D, FIETZ A, HERTEL H, DE SOUZA J, VENTURA DF & MENZEL R. 1992. The spectral input systems of hymenopteran insects and their receptor-based colour vision. *J Comp Physiol A* 170: 23-40.
- PIMIENTA-BARRIOS E & DEL CASTILLO R. 2002. Reproductive biology. In: PARK N (Ed), *Cacti. Biology and uses*. Berkeley: University of California Press, p. 75-90.
- QUEIROZ JA, DINIZ UM, VÁZQUEZ DP, QUIRINO ZM, SANTOS FAR, MELLO MAR & MACHADO IC. 2021. Bats and hawkmoths form mixed modules with flowering plants in a nocturnal interaction network. *Biotropica* 53: 596-607.
- QUIRINO ZGM & MACHADO IC. 2014. Pollination syndromes in a Caatinga plant community in northeastern Brazil: seasonal availability of floral resources in different plant growth habits. *Braz J Biol* 74: 62-71.
- RADFORD AE, DICKINSON WC, MASSEY JR & BELL CR. 1974. *Vascular plant systematics*. New York: Harper and Row, 891 p.
- RAGUSO RA & LIGHT DM. 1998. Electroantennogram responses of male *Sphinx perelegans* hawkmoths to floral and "green-leaf volatiles." *Entomol Exp Appl* 86: 287-293.
- RAGUSO RA & WILLIS MA. 2002. Synergy between visual and olfactory cues in nectar feeding by naïve hawkmoths, *Manduca sexta*. *Anim Behav* 64: 685-695.
- RAGUSO RA & WILLIS MA. 2005. Synergy between visual and olfactory cues in nectar feeding by wild hawkmoths, *Manduca sexta*. *Anim Behav* 69: 407-418.
- RAGUSO ROBERT A, HENZEL C, BUCHMANN SL & NABHAN GP. 2003a. Trumpet Flowers of the Sonoran Desert: Floral Biology of *Peniocereus* Cacti and Sacred *Datura*. In *J Plant Sc* 164: 877-892.
- RAGUSO ROBERT A, LEVIN RA, FOOSE SE, HOLMBERG MW & MCDADE LA. 2003b. Fragrance chemistry, nocturnal rhythms and pollination "syndromes" in *Nicotiana*. *Phytochemistry* 63: 265-284.
- RATTON CA. 1997. *Mandacaru, Telenovela*. Rio de Janeiro (RJ): Rede Manchete.
- ROCHA EA, DOMINGOS-MELO A, ZAPPI DC & MACHADO IC. 2019. Reproductive biology of columnar cacti: are bats the only protagonists in the pollination of *Pilosocereus*, a typical chiropterophilous genus? *Folia Geobot* 54: 239-256.

- ROCHA EA, MACHADO IC & ZAPPI DC. 2007. Floral biology of *Pilosocereus tuberculatus* (Werderm.) Byles and Rowley: a bat pollinated cactus endemic from the "Caatinga" in northeastern Brazil. *Bradleya* 25: 129-144.
- ROJAS-SANDOVAL J & MELÉNDEZ-ACKERMAN E. 2009. Pollination biology of *Harrisia portoricensis* (Cactaceae), an endangered Caribbean species. *Am J Bot* 96: 2270-2278.
- ROLDÁN JS & ASHWORTH L. 2018. Disentangling the role of herkogamy, dichogamy and pollinators in plant reproductive assurance. *Plant Ecol Divers* 11: 383-392.
- ROWLEY G. 1980. Pollination syndromes and Cactus taxonomy. *Cactus Succ J G Brit* 42: 95-98.
- SAMPAIO EVSB. 1995. Overview of the Brazilian Caatinga. In: BULLOCK SH, MOONEY HA & MEDINA E (Eds), *Seasonally Dry Tropical Forests*. Cambridge: Cambridge University Press, p. 35-63.
- SANTOS LDN, PEREIRA IMS, RIBEIRO JR & LAS-CASAS FMG. 2019. Frugivory by birds in four species of Cactaceae in the Caatinga, a dry forest in Brazil. *Iheringia Ser Zool* 109: e2019034.
- SAZATORNIL FD, MORÉ M, BENITEZ-VIEYRA S, COCUCCI AA, KITCHING IJ, SCHLUMBERGER BO, OLIVEIRA PE, SAZIMA M & AMORIM FW. 2016. Beyond neutral and forbidden links: morphological matches and the assembly of mutualistic hawkmoth-plant networks. *J Anim Ecol* 85: 1586-1594.
- SCHLINDWEIN C & WITTMANN D. 1997. Stamen movements in flowers of *Opuntia* (Cactaceae) favour oligolectic pollinators. *Plant Syst Evol* 204(3): 179-193.
- SCHLUMBERGER 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. *Ann Bot* 103: 1489-1500.
- SCHLUMBERGER BO & RAGUSO RA. 2008. Geographic variation in floral scent of *Echinopsis ancistrophora* (Cactaceae); evidence for constraints on hawkmoth attraction. *Oikos* 117: 801-814.
- SCOGIN R. 1985. Nectar constituents of the Cactaceae. *Southwest Nat* 30(1): 77-82.
- SILVA ÉEM, PAIXÃO VHF, TORQUATO JL, LUNARDI DG & LUNARDI VO. 2020. Fruiting phenology and consumption of zoochory fruits by wild vertebrates in a seasonally dry tropical forest in the Brazilian Caatinga. *Acta Oecol* 105: 103553.
- SILVA JMC, LEAL IR & TABARELLI M. 2017. Caatinga: the largest tropical dry forest region in South America. Springer, 487 p.
- SILVA LFCR, VALLE LS, NASCIMENTO ARC & MEDEIROS MFT. 2019. *Cereus jamacaru* DC. (Cactaceae): From 17<sup>th</sup> century naturalists to modern day scientific and technological prospecting. *Acta Bot Bras* 33: 191-197.
- SILVA WR & SAZIMA M. 1995. Hawkmoth pollination in *Cereus peruvianus*, a columnar cactus from southeastern Brazil. *Flora* 190: 339-343.
- SOUSA MP, VEROLA CF, RIBEIRO-SILVA S, ZAPPI DC, SOUZA EH, MORAES EM, TAYLOR NP & AONA LYS. 2021. Pollen-feeding bees in *Uebelmannia pectinifera* subsp. *pectinifera* - reproductive biology of an endemic cactus from the *campo rupestre* of eastern Brazil. *Nord J Bot* 39: njb.02924.
- SUZÁN H, NABHAN GP & PATTEN DT. 1994. Nurse Plant and floral biology of a rare night-blooming *Cereus*, *Peniocereus striatus* (Brandege) F. Buxbaum. *Conserv Biol* 8: 461-470.
- TAYLOR N & ZAPPI D. 2004. *Cacti of Eastern Brazil*. Kew: Royal Botanic Gardens, 512 p.
- TRAVIS J. 2020. Where is natural history in ecological, evolutionary, and behavioral science? *Am Nat* 196: 1-8.
- TSCHAPKA M, HELVERSEN O & BARTHLOTT W. 1999. Bat pollination of *Weberocereus tunilla*, an epiphytic rain forest cactus with functional flagelliflory. *Plant Biol* 1: 554-559.
- VAN DEN DOOL H & KRATZ PD. 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J Chromatogr A* 11: 463-471.
- VARASSIN IG & AMARAL-NETO LP. 2014. Atrativos. In: RECH A, AGOSTINI K, OLIVEIRA PE & MACHADO IC (Eds), *Biologia da Polinização*. Rio de Janeiro (RJ): Editora Projeto Cultural, 151-168 p.
- VOGEL S. 1990. Radiación adaptativa del síndrome floral en las familias neotropicales. *B Acad N Cien Córdoba* 59: 5-30.
- WALTER HE. 2010. Floral biology of *Echinopsis chiloensis* ssp. *chiloensis* (Cactaceae): Evidence for a mixed pollination syndrome. *Flora* 205: 757-763.
- WASSERTHAL LT. 1993. Swing-hovering combined with long tongue in hawkmoths, an antipredator adaptation during flower visits. In: BARTHLOTT W, NAUMANN CM, SCHMIDT-LOSKE K & SCHUCHMANN KL (Eds), *Animal-Plant Interactions in Tropical Environments*. Bonn: Zoologisches Forschungsinstitut und Museum Alexander Koenig, p. 77-87.
- WASSERTHAL LT. 1997. The Pollinators of the Malagasy Star Orchids *Angraecum sesquipedale*, *A. sororium* and *A. compactum* and the Evolution of Extremely Long Spurs by Pollinator Shift. *Bot Acta* 110: 343-359.

ZAPPI D & TAYLOR NP. 2011 Status de Conservação. In: SILVA SR, ZAPPI D, TAYLOR NP & MACHADO M (Eds), Plano de ação nacional para a conservação das Cactáceas. Série Espécies Ameaçadas n° 24. Brasília: Instituto Chico Mendes de Conservação da Biodiversidade, p. 57-63.

ZAPPI D & TAYLOR NP. 2020. Cactaceae in Flora do Brazil 2020. Jardim Botânico do Rio de Janeiro. Available in: <<http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB1434>>. Accessed: 20 Jan 2022.

ZEISLER M. 1938. Über die Abgrenzung der eigentlichen Narbenfläche mit Hilfe von Reaktionen. *Beih Z Bot Zent* 58: 308-318.

#### How to cite

ALBUQUERQUE-LIMA S, DOMINGOS-MELO A, MILET-PINHEIRO P, NAVARRO DMAF, TAYLOR NP, ZAPPI DC & MACHADO IC. 2023. The iconic cactus of the Caatinga dry forest, *Cereus jamacaru* (Cactaceae) has high sphingophily specialization and pollinator dependence. *An Acad Bras Cienc* 95: e20220460. DOI 10.1590/0001-3765202320220460.

*Manuscript received on May 24, 2022;  
accepted for publication on December 5, 2022*

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#### Author contributions

SAL, DCZ and ICM conceptualized the study and designed the methodology. SAL, NPT and DCZ collected the data under the supervision of ICM. ADM collected and analyzed the floral reflectance data. PMP performed data analysis of floral scents analysis with contributions from DMAFN. SAL wrote the first draft of the manuscript with contributions from ADM, PMP, NPT, DCZ and ICM. NPT is a native speaker and checked the English language. SAL led the writing of the final version of the manuscript, with contributions of ICM. All authors contributed critically to all versions and gave final approval for publication.

