Article

Floral Specialization and Bat Pollination in Subtribe Cereinae (Cactaceae): A Morphological Approach

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Abstract: Cactaceae has many vertebrate-pollinated species, and in the subtribe Cereinae, several genera are indicated as being pollinated by bats. In this subtribe, we observed phenotypic specialization in floral morphological attributes associated with chiropterophily, allowing high precision in the determination of this pollination system. However, in loco pollination records of bats have been confirmed only in a few species. In this context, using a morphological approach, we studied the floral biology of 14 columnar cacti of Cereeae-Cereinae with emphasis on species with chiropterophilous attributes and confirmed the role of bats specialized in nectar consumption as pollinators of these taxa. The studied taxa have similarities in their floral *bauplan*, observed by analysing the overlap in the floral morphospace. The length and opening of the floral tube are important characteristics for pollen deposition. Approximately 156 visits by bats of the subfamilies Glossophaginae and Lonchophyllinae were observed among the studied taxa. Chiropterophily is the prevalent pollination system among Cereinae, and hereby, we verified this system in five of its genera. There is, however, much variation between diurnal and nocturnal systems within this subtribe, and variation among genera and within species of a given genus may reflect the evolutive pathways, this being worthy of future studies.

Keywords: Caatinga; chiropterophily; columnar cacti; floral morphology; Glossophaginae; Lonchophyllinae; nocturnal pollination

1. Introduction

Time of anthesis separates diurnal and nocturnal pollination systems [1]. Night pollination occurs in 30% of the families of Angiosperms [2], and, among the nocturnal systems, bats and sphingid moths are the main pollinators [3]. Bat-pollinated, or chiropterophilous, species appear in 28 orders and 67 families of Angiosperms, being the main pollination system of more than 520 species of tropical plants [4]. Pollinator bats are mostly specialised nectar consumers [4–6]; however, some species which are not typically nectarivorous may also promote pollination [6].

Bat-pollinated plants display particular characteristics, such as floral zygomorphy (which permits deposition of pollen on specific areas of the bat's body), relatively large and resilient flowers (which permit access and contact with the bat's body), and tubular or campanulate or sometimes brush-like, shape. Pale or white (sometimes greenish or dull) colours and emission of disagreeable smell of fermenting plant matter, rich in sulphurous compounds, are also frequently encountered, and dilute nectar is produced in large quantities [5–8].

Chiropterophily involves intense phenotype morphological specialization that allows for the high precision of the determination of this system in plant species [4,9,10]. Bats are

considered highly efficient pollinators, as they carry a large quantity of pollen and may transport the grains over long distances [4].

Cactaceae (Caryophyllales) comprise approximately 1.500 species [11,12] of chiefly succulent plants, many of which are confirmed as bat-pollinated [10,13–25], distributed almost exclusively on the American continent [11]. Cactaceae comprise approximately 128 genera and a vast array of growth forms, as well as wide variations in flower shape, size, and colour, interacting with diverse guilds of pollinators [26]. Regarding chiropterophily, there are at least 172 plant species distributed in 37 genera where bat/plant interactions are recorded [10,13,14,23].

Pollination in Cactaceae seems to have evolved from a diurnal system, with bee-pollinated species, to a nocturnal system as a derived lineage [27]. As well as nocturnal pollination, hummingbird pollination is also considered derived within the family [27]. While pollination by bats and sphingids is considered irreversible [28], some studies in Cactaceae have shown a reversal process to hummingbird pollination from bat pollination [4,27].

Despite presenting a common basic plan throughout the family, cactus flowers display strong variation in specialization of floral morphology, covering different pollination systems [26]. Bat-pollinated species have shorter flowers with thicker tissues compared with sphingophilous species, have resistant floral structures, are campanulate to tubular, emit strong smells, and secrete large quantities of nectar [7,10]. Such characteristics are found in columnar genera of Cactoideae placed in different tribes, such as Cereeae (*Pilosocereus*), Pachycereeae (*Carnegiea, Neobuxbaumia, Pachycereus, Stenocereus*), and Hylocereeae (*Weberocereus*), where bat pollination was confirmed [10,15–18,20–22,24,29].

The BCT clade (which includes the tribes Browningieae, Cereeae, and Trichocereeae) corresponds to many species of arborescent, columnar, and shrubby cacti with South American distribution [30]. Within clade BCT, Cereeae is the main tribe comprising eastern Brazilian cacti [31], at present referred to as subtribe Cereinae [32]. In Brazil, this group encompasses 136 species found in the six main biomes (Amazon Rainforest, Atlantic Rainforest, Caatinga, Cerrado, Pantanal, and Pampa), of which 108 are endemic to the country [33]. Relationships between Cereeae-Cereinae and other traditionally accepted subdivisions, such as Browningieae and Trichocereeae (Trichocereinae), have not yet been clearly established, and studies of the genera attempting to establish monophyletic groups have failed to naturally circumscribe them [30,32,34–36]. While subtribe Cereinae has been found to be monophyletic, the generic delimitation is still uncertain from the phylogenetic point of view [35,36], highlighting the need to use different techniques, such as targeting non-coding regions in order to attain better phylogenetic resolution [37].

Cereeae-Cereinae display varied growth forms, from tree-like, columnar (*Cereus, Pilosocereus*), unbranched, or basally branched columns (*Coleocephalocereus*) to globose, such as *Melocactus* and *Discocactus* [31,34]. Floral morphology is also varied, with several pollination systems being present throughout the species. The genus *Pilosocereus*, one of the largest in the subtribe, has flowers characteristically pollinated by bats [10,20], similar to what is found in *Cipocereus* and *Xiquexique* [10,24,25] as *Pilosocereus*. Considering that these genera represent a considerable part of the subtribe, only a few species have had bat pollination confirmed [10,16,24,25,38,39], the same being true for other genera of the subtribe that display floral attributes associated with chiropterophily.

Taking into consideration the need for floral biology studies in Cereinae, we investigated the floral biology of columnar species with emphasis on those that display floral attributes associated with bat pollination, from a morphological perspective. Considering the basic *bauplan* of Cactaceae floral morphology, our hypothesis is that, despite the existing variations in flower size and shape, chiropterophilous species of the subtribe occupy the same floral morphospace. Therefore, we predict that even if species have shape variation, the maintenance of the basic plan allows for the interaction with bats. We used 14 taxa of Cereinae from different Caatinga areas of Northeastern Brazil to test our hypothesis.

2. Material and Methods

2.1. Study Sites

Fieldwork was carried out between September 2019 and January 2023 in natural populations occurring in fragments of Caatinga in the states of Bahia and Pernambuco, Northeastern Brazil (Figure 1; Table 1). Caatinga is considered the largest and most continuous seasonally dry tropical forest of South America [40,41]. Rainfall is irregular, and the rainy season is short, lasting from three to five months, and yearly precipitation ranges between 500 and 750 mm. The mean temperature in the region varies between 23 and 27 °C [41].
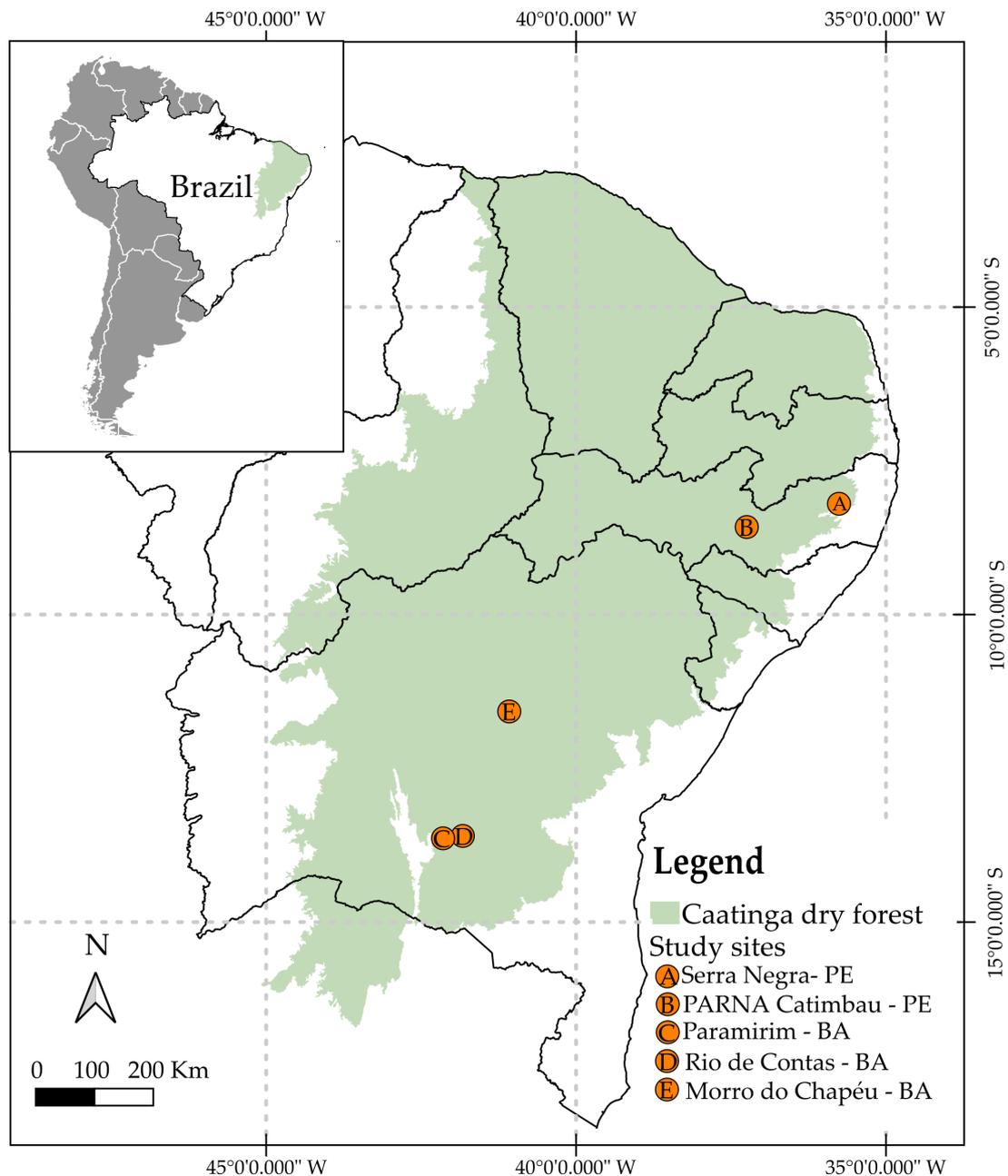

Figure 1. Map indicating the sites of the Cereinae taxa studied. The Caatinga area within Northeastern Brazil is highlighted in green, and the five study sites are represented by orange circles. Note: States of study areas, PE: Pernambuco, BA: Bahia.

Table 1. List of taxa of Cereinae (Cactaceae) studied, study sites, coordinates, number of plants and flowers studied, and hours of observation of floral visitors in the field.

Cereeae Taxa	Study Sites *	Coordinates	Number of Plants	Number of Flowers	Hours of Observation
Coleocephalocereus goebelianus (Vaupel) Buining	C	13°40′23″ S 42°04′03″ W	7	7	8
Leocereus bahiensis Britton & Rose	D	13°35′54″ S 41°49′43″ W	3	8	8
Micranthocereus purpureus (Gürke) F.Ritter	D	13°35′54″ S 41°49′43″ W	4	4	5
Pilosocereus catimbauensis (N.P.Taylor & Albuq.-Lima) N.P.Taylor & Albuq.-Lima	B	8°34′34″ S 37°14′49″ W	6	8	4
P. glaucochrous (Werderm.) Byles & G.D.Rowley	E	11°32′47″ S 41°10′34″ W	3	5	4
P. pachycladus F.Ritter subsp. *pachycladus*	D	13°35′54″ S 41°49′43″ W	3	6	4
P. catingicola subsp. *salvadorensis* (Werderm.) Zappi	B	8°34′34″ S 37°14′49″ W	6	7	5
P. pentaedrophorus (Cels) Byles & G.D.Rowley subsp. *pentaedrophorus*	A	8°11′53″ S 35°45′34″ W	3	5	4
P. pentaedrophorus subsp. *robustus* Zappi	C	13°40′23″ S 42°04′03″ W	3	6	4
Stephanocereus leucostele (Gürke) A.Berger	C	13°40′23″ S 42°04′03″ W	4	5	5
S. luetzelburgii (Vaupel) N.P.Taylor & Eggli	D	13°35′54″ S 41°49′43″ W	3	7	6
Xiquexique gounellei (F.A.C.Weber) Lavor & Calvente	C	13°40′23″ S 42°04′03″ W	10	15	8
X. ×*heptagonus* N.P.Taylor & Albuq.-Lima.	B	8°34′34″ S 37°14′49″ W	6	15	12
X. tuberculatus (Werderm.) Lavor & Calvente	B	8°34′34″ S 37°14′49″ W	10	15	16

Notes: * Name of the locations: A: Parque Ecológico de Serra Negra; B: PARNA Catimbau; C: Livramento de Nossa Senhora and Paramirim; D: Rio de Contas; and E: Morro do Chapéu.

The five study sites (Figure 1) and the number of taxa studied per site are featured in Table 1. Area A is the Parque Ecológico de Serra Negra in the municipality of Bezerros, Pernambuco (Figure 1, Table 1). This Park includes granitic outcrops and higher altitude *brejo* forest in a Caatinga matrix [42]. Area B is the Parque Nacional do Catimbau (PARNA Catimbau) in the municipalities of Buíque, Tupanatinga, and Ibimirim, Pernambuco. The PARNA Catimbau is dominated by arenitic rocks inserted in a vegetation mosaic with different physiognomies of Caatinga [43]. Area C constitutes private farmlands in the municipalities of Livramento de Nossa Senhora and Paramirim, Bahia, mostly of scrubby Caatinga alongside pastures and small plantations, where the cacti represent the few remaining native species. Area D is located in the south of the Chapada Diamantina, municipality of Rio de Contas, Bahia, in *campo rupestre* associated with quartzitic outcrops and mostly above 1000 m a.s.l. [44]. Area E is at the northern limit of the Chapada Diamantina, municipality of Morro do Chapéu, including *campo rupestre* and its ecotone with Caatinga known as *carrasco* [45].

2.2. Studied Taxa

Floral morphology and bat pollination were studied in 12 species, one heterotypic subspecies, and one nothospecies (Table 1). All taxa that were included belong to Cereeae-Cereinae [35], with distributions in Eastern Brazil [31] (Figure 2). Considering that genetic studies to investigate the circumscription of the tribe are ongoing, we adopted the classification provided by Taylor and Zappi [31], modified after Nyffeler and Eggli [32] and Lendel [35], totalling 15 genera (*Arrojadoa* Britton & Rose, *Brasilicereus* Backeb., *Cereus*

Mill, *Cipocereus* Ritter, *Coleocephalocereus* Backeb., *Discocactus* Pfeiff., *Espostoopsis* Buxb., *Facheiroa* Britton & Rose, *Leocereus* Britton & Rose, *Melocactus* Link & Otto, *Micranthocereus* Backeb., *Pilosocereus* Byles & Rowley, *Stephanocereus* A.Berger, *Stetsonia* Britton & Rose, and *Xiquexique* Lavor, Calvente & Versieux), of which we studied six genera (Table 1; Figure 2). Taxa identification was confirmed by NPT and DCZ.
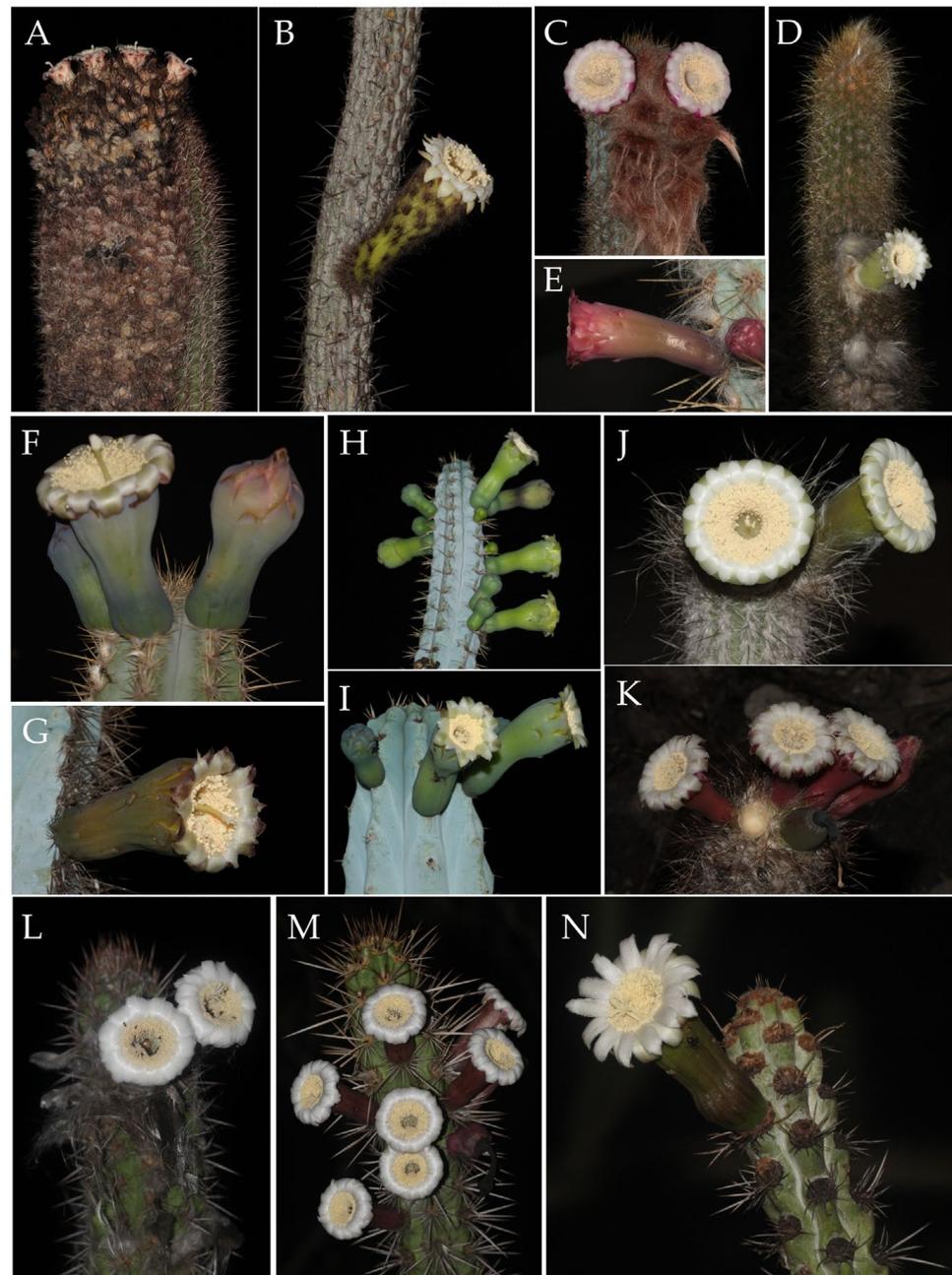

Figure 2. Cereinae species studied displaying floral attributes associated with bat pollination. (**A**) *Coleocephalocereus goebelianus*, (**B**) *Leocereus bahiensis*, (**C**) *Micranthocereus purpureus*, (**D**) *Pilosocereus catimbauensis*, (**E**) *P. glaucochrous*, (**F**) *P. catingicola*, (**G**) *P. pachycladus* subsp. *pachycladus*, (**H**) *P. pentaedrophorus* subsp. *pentaedrophorus*, (**I**) *P. pentaedrophorus* subsp. *robustus*, (**J**) *Stephanocereus leucostele*, (**K**) *S. luetzelburgii*, (**L**) *Xiquexique gounellei*, (**M**) *X.* ×*heptagonus*, and (**N**) *X. tuberculatus*.

2.3. Floral Biology

The number of open flowers per night as well as the time of anthesis were recorded through focal observations in individuals of the studied taxa [46]. Observations were

carried out on non-consecutive days, depending on the flower availability of each taxon. Anthesis was observed in 1 to 3 flowers per taxon (one flower per individual). Anther dehiscence was reported through direct observation, and stigma receptivity was tested using hydrogen peroxide 3% [46], observing stigmatic reaction through bubble formation, indicating the stigma is receptive.

Standing crop nectar measurements were taken in flowers of different individuals (Table 1) (n = one flower per individual) soon after full anthesis of the flower. Nectar volume was measured using a graduated microsyringe (250 microlitres, Hamilton, Reno, Nevada, EUA), and sugar concentration (percentage of saccharose, mass/mass) was measured with a pocket refractometer (Master Refratometer 0–52%, Atago, Tokyo, Japan) [47]. The nectar calorie content was estimated using the Scogin [48] equation for Cactaceae species: Energy/flower = concentration (% sugar) × (nectar volume in ml) × 39, with 39 being the conversion unit of volume that reflects calories per gram of saccharose.

2.4. Floral Traits and Morphometry

To evaluate the floral morphology of each taxon, we collected flowers in the field and placed them in 70% alcohol. Flowers of each taxon were collected during the same reproductive event, depending on the availability of flowers during the nights of observation. Measurements were carried out in the laboratory, using one flower per plant of different individuals (Table 1) with a digital caliper (Starrett model 799, Athol, MA, USA). Seventeen floral traits were measured, including length and width of the flower, diameter of flower opening, length and width of the pericarpel, and length of flower tube (Table 2). Those quantitative floral attributes were selected using the identification literature [31,49].

Table 2. Quantitative floral morphological characteristics measured of flowers of Cereinae (Cereeae-Cactaceae) species pollinated by bats.

Characteristics
1. Flower length (FL)
2. Flower width (FW)
3. Flower tube opening diameter (OP)
4. Pericarpel length (PL)
5. Pericarpel width (PW)
6. Length of the floral tube (LFT)
7. Width of the widest portion of the floral tube (WFT)
8. Length of the external elements of the perianth (LEP)
9. Width of the external elements of the perianth (WEP)
10. Length of the internal elements of the perianth (LIP)
11. Width of the internal elements of the perianth (WIP)
12. Length of the nectar chamber (LCN)
13. Width of the nectar chamber (WCN)
14. Style length (SL)
15. Style width (SW)
16. Length of the stigma lobes (LSL)
17. Width of the stigma lobes (WSL)

The floral dimensions of the taxa were grouped in a two-dimensional dispersion diagram with 95% confidence ellipses by principal components analysis (PCA) from a variance–covariance matrix between the groups. We examined the normality of the data using a Shapiro–Wilk test. Once certain that the data presented a non-normal distribution, we used the Kruskal–Wallis test (significance level = $p < 0.05$) to investigate whether the taxa presented significantly different means for their attributes. Following this procedure, we conducted a *post-hoc* Dunn test (level of significance = $p < 0.001$) to determine whether the taxa have significantly different mean values for each one of the floral traits. All analyses were carried out using Rstudio v3.6.2 software [50].

2.5. Floral Visitors

Direct observations of flowers in anthesis were conducted to record floral visitors of the studied taxa. Observations were carried out for each taxon in 1–2 individuals per census, totalling 89 h (Table 1). Each taxon was observed from 17:30 h to 22:00 h, and photographs were taken with a camera equipped with a macro lens (Canon EOS Rebel T3; Canon EF 100 mm, Tokyo, Japan) and a field tripod.

3. Results

3.1. Floral Biology

The flowers of the studied taxa appear generally in the distal part of the branches, with the exception of *Leocereus bahiensis* (Figure 2B), where flowers may appear from the middle of the stem. Six taxa had flowers borne in a sunken lateral cephalium (e.g., *Coleocephalocereus goebelianus*, Figure 2A) or a pseudocephalium (*Pilosocereus catimbauensis*, Figure 2D). For eight taxa, the flowers appear from floriferous areoles, lacking cephalium or pseudocephalium structures (*P. pachycladus*, Figure 2G and *X. tuberculatus*, Figure 2N) (Table 3).

The anthesis of most studied taxa started at dusk, between 17:00 and 18:00, remaining open throughout the night until the following morning. The only exception was *Leocereus bahiensis*, with flowers opening after 12:00 and remaining open for the afternoon and into the night, until the next morning. The flowers of all studied taxa open only once and last for a single night. During the observations, the number of flowers open per individual in a single night varied between one, as in *L. bahiensis* (Figure 2C), up to seven open flowers, as seen in *Xiquexique ×heptagonus* (Table 3). Some of the taxa present scent during the opening of the flower, and this intensifies as the opening is complete. Flowers varied regarding the presence or absence of scent and in the type of odour. In *C. goebelianus* (Figure 2A), scent was not perceptible; in *Micranthocereus purpureus* and *S. luetzelburgii* (Figure 2K), the odour was disagreeable but not intense, whereas *P. pachycladus* (Figure 2G) and *S. leucostele* (Figure 2J) had strong, pungent scents resembling garlic or rotten cabbage. In all taxa, anther dehiscence and stigmatic receptivity occurred during the start of anthesis.

Flowers are actinomorphic to slightly zygomorphic (Figure 2, Table 3). The flower tube is straight to slightly curved, narrow, infundibuliform or campanulate, varying among studied taxa (Figure 3). The colour of the exterior of the flowers (flower tube and outer perianth segments) colour varies widely (Figure 2) from magenta and reddish in *M. purpureus* and *S. luetzelburgii*, olive green in *P. pachycladus* and *X. tuberculatus*, and bluish in *P. catingicola* (Table 3). All taxa have white inner perianth segments (Figure 2).

3.2. Quantification of Floral Traits

The analysis of principal components (PCA) did not clearly separate the taxa, which occupy a partly overlapping morphospace (Figure 3). The PCA coefficient explains 75.2% of the variation in the two first axes, with Axis 1 and 2 being responsible for, respectively, 66.19% and 9.01% of the variation. The main attributes correlated with Axis 1 were floral diameter (FW), diameter of the opening of the tube (OP), flower length (FL), and length of style (SL), while for Axis 2, it was the length of stigma lobes (LSL) (Table 4).

Taxa varied in flower size (FL = flower length × FW = flower diameter), with the smallest flowers belonging to *C. goebelianus* (FL = 39.49 mm, FW = 22.61 mm) (Figure 2A) and the largest in *P. catingicola* (FL = 90.38 mm, FW = 72.73 mm) (Figure 2F). Mean values of the floral attributes are presented in Table A1. Taking into account the mean flower size of all taxa (FL = 60.67 mm, FW = 36.79 mm), the PCA showed little overlap of the floral morphospace among taxa that have shorter, narrower flowers (*C. goebelianus*) and others with longer and more open flowers (*X. gounellei*), occupying opposite sides of the morphospace (Figure 4).

Table 3. Floral biology and attributes of Cereinae (Cactaceae) taxa studied.

Cereinae Taxa	Habit *	Cephalium	Floral Form	Symmetry	External Colour	Internal Colour	No. of Flowers **
Coleocephalocereusgoebelianus	sg	pre	tub	act	Pale beige	White	2–4
Leocereusbahiensis	ere/ss	abs	tub	zyg	Green	White	1–3
Micranthocereuspurpureus	cbb	pre	cam	act	Magenta	White	1–2
Pilosocereuscatimbauensis	sg/cbb	pre	cam	zyg	Olive green	White	1–2
P. glaucochrous	tr	abs	tuc	zyg	Magenta	White	1–3
P. pachycladus subsp. *pachycladus*	tr	abs	cam	zyg	Olive green	White	1–3
P. catingicola subsp. *salvadorensis*	tr	abs	cam	zyg	Blue	White	1–5
P. pentaedrophorus subsp. *pentaedrophorus*	tr	abs	cam	zyg	Green	White	1–3
P. pentaedrophorus subsp. *robustus*	tr	abs	cam	zyg	Green	White	1–3
Stephanocereusleucostele	cab	pre	tuc	act	Green	White	1–7
S. luetzelburgii	bot	pre	cam	act	Reddish	White	1–4
Xiquexiquegounellei	can	abs	tub	zyg	Beige	White	1–5
X. ×heptagonus	can	abs	tub	zyg	Dark magenta	White	1–7
X. tuberculatus	can	abs	tub	zyg	Olive green	White	1–5

Notes: * Habit: sg = Single columnar, ere = Erect, cbb = Columnar branched at base, cab = Columnar solitary or branched above base, tr = Tree-like, and bot = Bottle-shaped; Cephalium: pre = Present and abs = Absent; Floral form: tub = Tubular, cam = Campanulate, and tuc = Tube curved; Symmetry: act = Actinomorphic and zyg = Zygomorphic; ** No. flowers: variation of the smallest and largest number of open flowers observed in each individual per night.
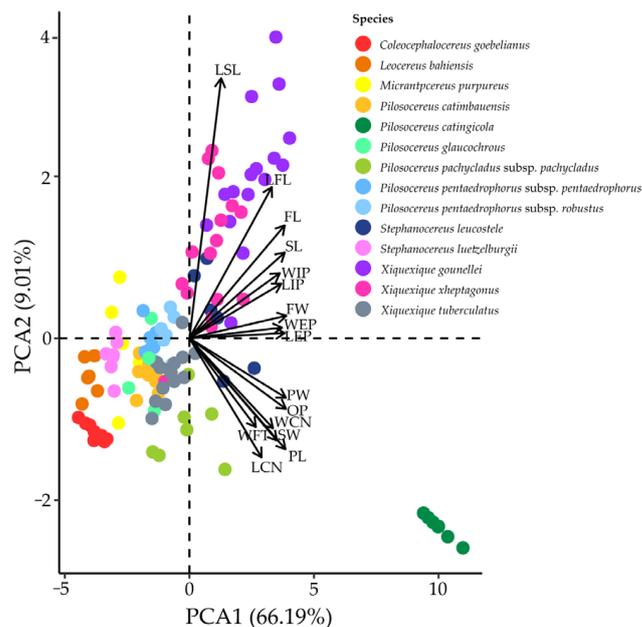
Figure 3. Principal Component Analysis (PCA) related to floral morphological characteristics of taxa from Cereinae for floral attributes associated with bat pollination. The abbreviations for each of the 17 measured variables of floral morphology are presented in Table 2.

Table 4. Centered correlation of traits of Cereinae with floral attributes associated with bat pollination and axes of principal component analysis (PCA; standardized eigenvectors 1.0).

Traits *	Axis 1	Axis 2
FL	0.81	-
FW	0.84	0
OP	0.83	0.04
PL	0.68	0.1
PW	0.83	0.03
LFT	0.61	0.18
WFT	0.81	0.11
LEP	0.8	0
WEP	0.77	0
LIP	0.75	0.02
WIP	0.73	0.04
LCN	0.46	0.13
WCN	0.63	0.06
SL	0.81	0.06
SW	0.39	0.07
LSL	0.09	0.55
WSL	0.4	0.02

Note: * The abbreviations for each of the 17 measured variables of floral morphology are presented in Table 2.

The result of the Kruskal–Wallis test made it evident that there are significant differences in flower attributes of all studied taxa (Table 5). The paired comparisons performed by the Dunn test show that taxa varied significantly in flower length (FL), diameter of opening (FW), diameter of flower tube (WFT), width of the external perianth segments (WEP), length of the inner perianth segments (LIP), and width of the inner perianth segments (WIP) (Table 4). Regarding the other traits, the taxa had greater similarity and overlap (Table 5). *Pilosocereus catingicola* was the taxon with the largest difference in floral morphology compared with the other taxa, followed by *C. goebelianus*, *X. gounellei*, and *L. bahiensis* (Table 5).
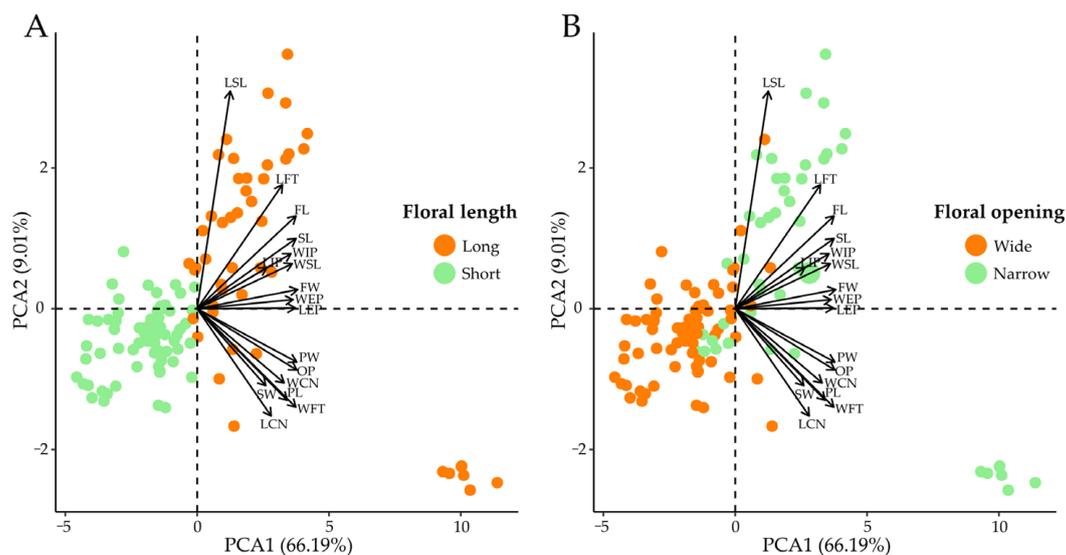
Figure 4. Principal Component Analysis (PCA) results related to (**A**) floral length and (**B**) floral width of Cereinae taxa for floral attributes associated with bat pollination.

Table 5. Results of the Kruskal–Wallis and Dunn tests of the measured characteristics of the flowers of Cereinae (Cactaceae) taxa pollinated by bats.

Traits *	Kruskal–Wallis		Dunn Test **					
FL	$H_{(13)} = 96.81$	$p < 0.001$	goe-cag	goe-gou	goe-hep	cag-lue	lue-gou	
FW	$H_{(13)} = 96.76$	$p < 0.001$	goe-cag	goe-gou	bah-cag	bah-gou		
OP	$H_{(13)} = 76.84$	$p < 0.001$	goe-cag	bah-cag				
PL	$H_{(13)} = 76.16$	$p < 0.001$	goe-cag	cag-lue				
PW	$H_{(13)} = 80.23$	$p < 0.001$	cag-lue					
LFT	$H_{(13)} = 89.91$	$p < 0.001$	goe-gou	cag-lue	lue-gou			
WFT	$H_{(13)} = 91.42$	$p < 0.001$	goe-cag	bah-cag	cag-lue	cag-tub		
LEP	$H_{(13)} = 73.47$	$p < 0.001$	bah-cag					
WEP	$H_{(13)} = 81.1$	$p < 0.001$	goe-cag	goe-gou	bah-cag	bah-gou	cat-cag	
LIP	$H_{(13)} = 99.93$	$p < 0.001$	bah-cag	bah-gou	cat-cag	cat-gou		
WIP	$H_{(13)} = 87.13$	$p < 0.001$	goe-cag	goe-gou	bah-cag	bah-gou	cat-cag	cat-gou
LCN	$H_{(13)} = 72.18$	$p < 0.001$	pur-cag					
WCN	$H_{(13)} = 49.54$	$p < 0.001$	bah-cag					
SL	$H_{(13)} = 85.46$	$p < 0.001$	goe-cag	goe-gou	cag-lue	lue-gou		
SW	$H_{(13)} = 64.02$	$p < 0.001$	cag-lue					
LSL	$H_{(13)} = 76.42$	$p < 0.001$	goe-gou	goe-hep				
WSL	$H_{(13)} = 55.99$	$p < 0.001$	cat-cag					

Notes: Taxa: goe = *Coleocephalocereus goebelianus*; bah = *Leocereus bahiensis*; pur = *Micranthocereus purpureus*; cat = *Pilosocereus catimbauensis*; cag = *P. catingicola* subsp. *salvadorensis*; lue = *S. luetzelburgii*; gou = *Xiquexique gounellei*; hep = *X.* ×*heptagonus*, and tub = *X. tuberculatus*. * The abbreviations for each of the 17 measured variables of floral morphology are presented in Table 2. ** This table features only taxa where significance level = p < 0.001.

3.3. Nectar Standing Crop and Floral Visitors

Nectar was available in the flowers throughout the observation period in the 14 studied taxa. It was produced in large volume and had low sugar concentration. The nectar produced was accumulated in the nectar chamber, located at the base of the flower tube. Volume measurements varied from 132.5 ± 50 (*C. goebelianus*) to 1331.25 ± 486.25 μL (*P. catingicola*) (Table 6). The mean concentration of sugars in the nectar varied between 17.5 ± 0.25 and $20 \pm 4.5\%$ (Table 6). The energy provided by the nectar of these taxa varied between 90.09 and 908.40 calories/gram (Table 6).

Table 6. Volume, concentration, and energy of standing crop nectar of Cereinae (Cactaceae) taxa pollinated by bats. Number of visits by bats to flowers of each taxa.

Cereinae Taxa	Nectar Volume (μL)	Nectar Concentration (%)	Energy (Calories/Grams)	Number of Bat Visits	Ratio Number of Visits/Hour
Coleocephalocereusgoebelianus	132 ± 50	17.5 ± 0.25	90.09	24	3 h
Leocereusbahiensis	175 ± 33	20 ± 1.7	136.5	29	3.6 h
Micranthocereuspurpureus	212.5 ± 53	19 ± 1.4	157.09	3	0.6 h
Pilosocereuscatimbauensis	339.5 ± 50	20 ± 0.5	264.42	20	5 h
Pilosocereusglaucochrous	250 ± 60	21 ± 0.9	204.75	3	0.75 h
P. pachycladus subsp. *pachycladus*	688.30 ± 207	20 ± 1.3	536.64	4	1 h
P. catingicola subsp. *salvadorensis*	1331.25 ± 486.25	20 ± 4.5	908.40	8	1.6 h
P. pentaedrophorus subsp. *pentaedrophorus*	240 ± 26.7	21 ± 0.6	196.56	7	1.75 h
P. pentaedrophorus subsp. *robustus*	228 ± 55.5	20 ± 0.9	177.84	19	4.75 h
Stephanocereusleucostele	388.125 ± 146	19 ± 0.9	265.3	13	2.6 h
S. luetzelburgii	202.85 ± 44.70	19.20 ± 1.02	151.25	1	0.16 h
Xiquexiquegounellei	365 ± 94 *	23 ± 11 *	327.40	7	0.87 h
X. ×heptagonus	256 ± 28.24	21.9 ± 4.8	218.6	22	1.46 h
X. tuberculatus	213.3 *	12.3 *	102.17	25	1.66 h

Note: * Data from Rocha et al., 2007; 2020.

Bats belonging to subfamilies Glossophaginae and/or Lonchophyllinae were observed visiting all studied taxa (Figure 5). Visits started when flowers were completely open, at approximately 18:00–18:30 h. The behaviour of the bats was similar for all studied taxa where visits were observed, with bats hovering in front of the flowers and inserting the head in the flower tube aiming to reach the nectar chamber (Figure 5). Visits were brief, lasting less than a second. During the observation, the gap between visits varied between 5 and 60 min. The total number of visits also varied (Table 6), with taxa receiving many visits along the observation period, such as *C. goebelianus* (Figure 5A), *Leocereus bahiensis* (5B), *S. leucostele* (Figure 5J), and *X. ×heptagonus* (Figure 5M), while *S. luetzelburgii* (Figure 5K) received a single bat visit during the observation period. However, taking into account the rate of visits per hour, *P. pentaedrophorus* subsp. *robustus* (Figure 5I) was the taxon with the highest frequency of visits, followed by *C. goebelianus* and *S. leucostele* (Table 6). The bats received large quantities of pollen per visit, and the grains were deposited on the face (Figure 5A–C,G,H,M), neck (Figure 5C–E,J), and abdomen (Figure 5D,J). Bats also frequently contacted the stigma with these pollen-covered body parts.

Hummingbird visits at dusk by *Phaethornis pretrei* just at the beginning of the anthesis were recorded for *Micranthocereus purpureus*, *Stephanocereus luetzelburgii*, and *Pilosocereus pachycladus* in Area D, in the *campo rupestre* environment. Although the flowers of *Leocereus bahiensis* open in the early afternoon, visits by hummingbirds or other guilds of diurnal pollinators were not observed.
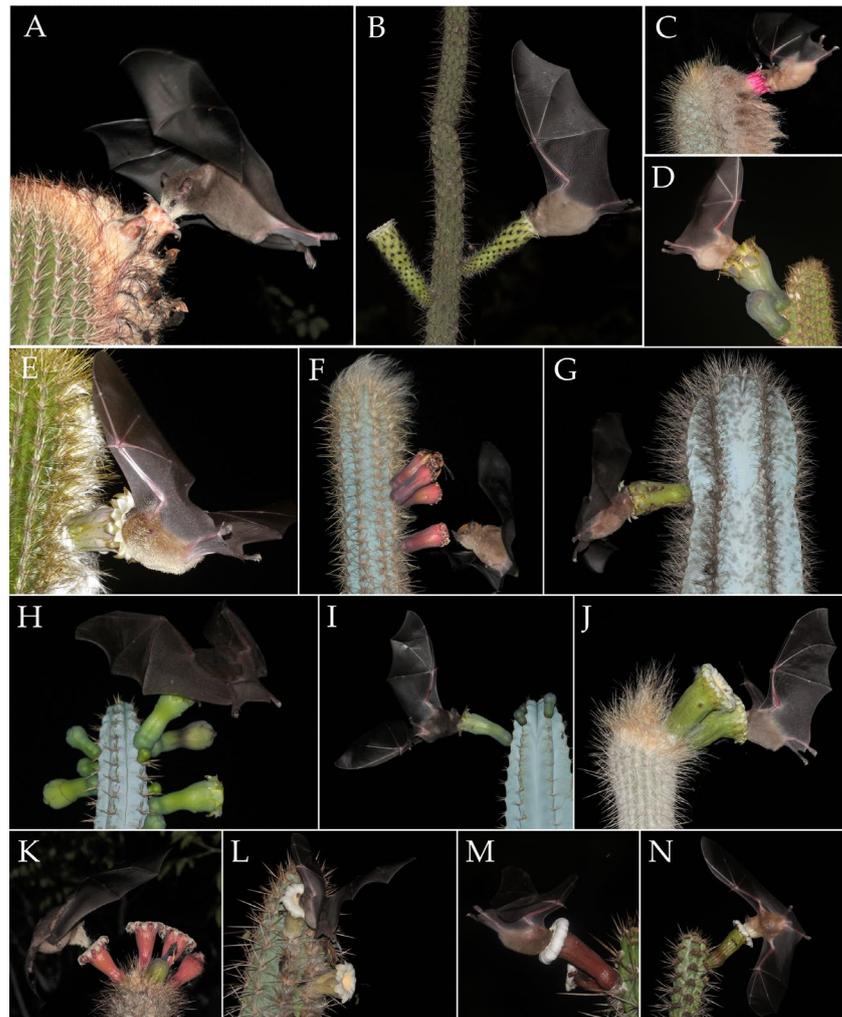
Figure 5. Nectarivorous bats visiting the taxa of Cereinae studied. (**A**) *Coleocephalocereus goebelianus*, (**B**) *Leocereus bahiensis*, (**C**) *Micranthocereus purpureus*, (**D**) *Pilosocereus catingicola*, (**E**) *P. catimbauensis*, (**F**) *P. glaucochrous*, (**G**) *P. pachycladus*, (**H**) *P. pentaedrophorus* subsp. *pentaedrophorus*, (**I**) *P. pentae-drophorus* subsp. *robustus*, (**J**) *Stephanocereus leucostele*, (**K**) *S. luetzelburgii*, (**L**) *Xiquexique gounellei*, (**M**) *X. ×heptagonus*, and (**N**) *X. tuberculatus*.

4. Discussion

In the present study, we show a synthesis of original data regarding the floral biology of diverse genera/species of Cereeae-Cereinae (Cactaceae-Cactoideae) that display characteristics of the bat pollination syndrome, some of them being new in situ records for bat pollination. On the basis of our observations, we describe the floral biology and its attributes in addition to confirming the interactions of bats specialised in the consumption of nectar of 12 species, one heterotypic subspecies, and one natural hybrid. Below, we discuss the factors that identify the role of bats that are fundamental to pollination success in diverse genera and taxa of the subtribe and compare their floral biology in relation to other taxa of Cactaceae.

4.1. Floral Biology and Variation in Floral Morphology among Chiropterophilous Taxa of the Subtribe Cereinae

The flowers of the Cereinae studied display many characteristics in common associated with bat pollination, such as stout, resilient floral structures capable of resisting the vigorous visits of bats seeking nectar; nocturnal anthesis; disagreeable floral scents (disagreeable to the human nose); dull-coloured outer perianth segments/bract scales

contrasting with much paler to white inner segments; nectar chamber protected by stamen filaments basally incurved towards the style; and high volumes of nectar [7,14,29,34,49,51]. These characteristics are corroborated by studies of other genera of Cactaceae, such as those involving *Cipocereus* (Cereinae); *Carnegiea, Neobuxbaumia, Pachycereus, Stenocereus* (Pachycereeae); and *Weberocereus* (Hylocereeae), all of which have similar floral morphology and are likewise pollinated by bats [7,13,14,17–19,21,24,25,29,39,52,53].

As a whole, the availability of the open flowers throughout the night, timing of the start of anthesis, stigma receptivity, and release of pollen from the anthers is similar amongst the taxa studied here and coincide with those of other cactus species pollinated by bats [10,16,20]. Even though some species can present variations in the timing of anthesis [54,55] as well as characteristics suggesting transitions towards other types of pollinator [10,31], in general, the species of Cactaceae display floral specialisations consistent with the distinct guilds of pollinators and in accord with their pollination syndromes [1,26]. Some of the taxa studied herein were visited by hummingbirds during the early stages of anthesis, suggesting that these taxa may display a duality or a transitional situation between dusk and nocturnal flowering pollinators [39]. It is interesting that the bat visits recorded in two of these taxa were few when compared with the other taxa studied (a single visit for *Stephanocereus luetzelburgii* and three for *Micranthocereus purpureus*), and that the odour of their flowers was not as overpowering as found for some of the other taxa. Specifically, in the case of *Micranthocereus purpureus*, the outer tube and perianth segments are bright magenta, a colour often associated with hummingbird pollination [26].

In addition to similarities in relation to their floral biology, the flowers of the family Cactaceae display a basic *bauplan* shared amongst its species [56]. In the case of the Cereinae studied, our results demonstrate that, while there is a degree of diversity in the size and morphology of flowers, in general, there is overlapping of the morphospace and a shared overall structural plan.

The chief characteristic separating the flowers of Cereinae is size, whether longer or shorter, broader or narrower. In species pollinated by bats, measurements of floral length and breadth are key characteristics for determining the chiropterophilous syndrome as opposed to other pollination systems [57,58].

In species with floral similarities which share the same pollinators (see below), there is a requirement for mechanisms that maintain reproductive isolation via reproductive barriers [59,60]. Mechanical isolation is the mechanism linked to floral morphology [61–63]. In bat-pollinated plant communities, the tendency is for species to be differentiated by the place upon the bat on which pollen is deposited, in avoidance of heterogeneous pollen admixtures [64,65]. In the case of Cactaceae, bat-pollinated species have flowers presenting a mass of stamens and densely grouped anthers so that at each visit, many pollen grains are deposited on the body of the bat [10,66]. Taking into consideration the morphological similarities, it is reasonable to think that flower size and especially the diameter of the access to the flower are fundamental to where the pollen is deposited on the bat [58]. In the case of the taxa studied, we observed that flowers with a broad opening, such as *Pilosocereus catingicola*, deposit pollen on the face, neck, and thorax, whereas in narrower opening flowers, such as in *Coleocephalocereus goebelianus*, the pollen is deposited only on the bat's face.

In spite of the short or long, narrow or broad floral extremes, the taxa studied here present close similarities and overlap in part of their morphospace, which could mean that pollen is deposited in the same parts of the pollinator's body, resulting in pollen admixtures. With the sharing of the same guild of pollinator (in this case, bats), the mixing of different pollen is likely among sympatric species, introducing the possibility of hybridisation [67,68]. In Cactaceae, the occurrence of natural hybrids in not uncommon and has been reported in various genera of Cereeae-Cereinae [31,69–71], as is seen here in the example of *Xiquexique* ×*heptagonus*, where we believe contact between sympatric populations of *X. gounellei* and *X. tuberculatus* effected by moths and bats has caused this hybridisation event [68].

4.2. Participation of Bats in the Pollination Systems of Cereinae

Our observations confirm the role of bats as pollinators of all the taxa studied. It is estimated that 53 species of bats are involved with the pollination of innumerable flowering plant species [72]. In the Neotropics, bats have a close association with cacti [14] and have been recognised as "cactophilic" [51]. As noted in other studies, the bats display a common behaviour of rapid, hovering visits, in general, following predetermined routes between the plants visited [73].

Cacti are amongst the more important elements of Neotropical vegetation, principally in forested drylands [74], amongst these the Caatinga [31,75]. In these environments, cacti have a fundamental role in maintaining populations of nectar-feeding bats [10,20,66]. In the case of the Caatinga, the Cereeae-Cereinae is the principal group of cacti present in these seasonally dry forests [31]. Here, we confirm the strong relationship between the cacti studied and their bat visitors, so it is likely that these taxa are important resources for sustaining bat populations. The nectar offered is secreted in large volume and low concentration of sugars, as is to be expected for the chiropterophilous syndrome [10,16,20,76]. Likewise, this energy resource is in accord with that established by Scogin [48].

As well as bat visits, we have recorded hummingbird visits in some of the taxa (see above). As well as bright, contrasting colours typically associated with hummingbird pollination and the relative length of the tubular flowers, allowing bird access to floral resources, the characters of the nectar may also be a factor that permits the visit of other guilds to the species, as occurs between the species *Ipomoea marcellia* and *I. vespertilia* (Convolvulaceae), where in both species, bats and hummingbirds act as pollinators [77,78]. Scogin [48] shows that some Cactaceae species had no significant difference in the sugar concentration among moth [79], bat, and hummingbird flowers, with overlapping concentrations in flowers visited by different pollinators. Therefore, such overlap in this character may favour the pollination of the species through sharing pollinators [10,20,76,80,81].

Considering the conservation of cacti and bats, we know that Eastern Brazil, unfortunately, is considered a hotspot for threatened cacti [82], being one of the three geographical areas where cacti are endangered by habitat destruction caused mostly by local farming and agroindustry and mining. The species studied, however, are not considered threatened, except from possibly the narrow endemic *Pilosocereus catimbauensis* from PARNA Catimbau, a protected area that has not yet been fully established. Curiously, while studying pollination guilds involved in the pollination of *P. catimbauensis* and another four species of columnar cacti in this location [10], we have established that the other species rely on a combination of bats and moths as effective pollinators, while *P. catimbauensis* was solely reliant on five bat species, suggesting a narrower dependency of the cactus upon the presence of bats. The resources offered by all studied cactus species to nectarivorous bats are fundamental to maintaining bat populations in the Caatinga, as these are often on offer during the dry season and enable the pollinator to feed between flowering booms of angiosperm species that are more water-dependent than cacti. Unfortunately, we currently lack precise information regarding the identity of the bat species involved in the pollination of the studied species. However, it is known that habitat destruction and degradation have been found to have an effect on present and future bat populations in the region [83].

5. Final Considerations

Chiropterophily is, as a whole, the most common pollination system among Cereinae, and this study has verified this system in five genera within the subtribe, and here, we highlight new records for bat pollination. The prevalence of bat pollination may be related to the growth form of Cactoideae, where the columnar habit favours the interaction with bats [56]. Even if for some genera, there is specialization towards one pollination system, for others, there is variation between diurnal and nocturnal systems, demonstrating that the pollination systems in the subtribe Cereinae vary among genera and within the species of a given genus, reflecting different evolutive pathways. Future studies in progress combining phylogenetic and floral biology data may reveal how these pathways evolved within the

family, considering that the pollinators were determinant for floral diversification and may explain the wide pollination system variation within the group. These studies will answer important questions regarding the evolution of night pollination systems in Cactaceae.

Appendix A

Table A1. Mean values of the measured (mm) characteristics of the flowers of the species of the subtribe Cereinae (Cereeae-Cactaceae) pollinated by bats.

Traits *	GOE	BAH	PUR	CAT	CAG	GLAU	PAC	PPE	PPR	LEU	LUE	GOU	HEP	TUB
FL	39.5	47.9	42.57	53.03	90.38	51.92	55.85	55.12	56.22	70.62	43.05	78.23	70.74	56.37
FW	22.61	16.01	30.6	29.35	72.73	24.58	30.77	24.17	25.15	36.59	26.39	49.29	40.43	32.86
OP	11.16	9.12	13.2	15.5	38.29	13.2	19.65	12.82	13.59	17.51	12.94	19.72	16.42	15.42
PL	4.3	8.86	6.83	7.92	21.75	10.81	8.88	10.64	10.78	11.47	6.92	8.96	9.54	8.9
PW	6.44	7.7	10.08	15.18	28.44	13.59	13.32	13.07	13.47	15.61	8.81	14.95	14.52	14.35
LFT	30.47	33.4	26.9	35.82	54.56	35.38	40.85	38.18	39.75	54.48	29.55	54.05	48.6	38.58
WFT	14.54	13.33	17.33	20.38	46.83	18.71	24.52	18.51	19.12	25.33	16.1	21.88	20.95	18.06
LEP	9.58	7	8.52	9.38	24.29	9.6	10.86	9.41	9.68	11.81	8.95	15.69	12.84	9.31
WEP	4.46	2.2	7.67	5.57	17.54	6.92	8.3	6.77	7.22	6.88	7.24	11.16	8.59	7.67
LIP	9.96	6.66	12.13	7.59	25.65	7.8	11.08	7.64	8.02	16.05	10.79	18.7	15.46	12.54
WIP	4.95	3.08	7.76	4.52	14.95	6.18	7.27	6.16	6.5	7.4	6.66	12.04	7.76	6.54
LCN	8.65	11.6	4.66	9.06	20.29	12.66	12.32	12.12	12.7	13.75	7.6	9.35	10.89	9.16
WCN	7.54	6.36	9.06	8.45	14.65	7.98	8.18	7.62	8.4	8.49	7.69	9.41	8.84	9.48
SL	31.62	38.46	32.82	39.27	68.56	35.02	41.06	38.89	40.52	47.98	30.61	56.19	47.83	42.76
SW	1.46	0.98	0.95	1.67	2.38	1.27	2.18	1.26	1.7	1.52	0.91	1.63	1.59	1.64
LSL	2.57	4.85	5.86	6.8	5.51	7.98	5.14	7.59	8.58	6.54	5.41	9.37	8.26	5.41
WSL	0.49	0.63	0.53	0.46	0.98	0.64	0.68	0.64	0.63	0.51	0.61	0.81	0.73	0.75

Notes: Species: GOE = *Coleocephalocereus goebelianus*; BAH = *Leocereus bahiensis*; PUR = *Micranthocereus purpureus*; CAT = *Pilosocereus catimbauensis*; CAG = *P. catingicola* subsp. *salvadorensis*; GLAU = *P. glaucochrous*; PAC = *P. pachycladus* subsp. *pachycladus*; PPE = *P. pentaedrophorus* subsp. *pentaedrophorus*; PPR = *P. pentaedrophorus* subsp. *robustus*; LEU = *Stephanocereus leucostele*; LUE = *S. luetzelburguii*; GOU = *Xiquexique gounellei*; HEP = *X. ×heptagonus*, and TUB = *X. tuberculatus*. * The abbreviations for each of the 17 measured variables of floral morphology are presented in Table 2.

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