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Floral morphology and pollen placement strategies of bat-pollinated flowers: a comparative analysis within a guild of chiropterophilous plants in a neotropical dry forest

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Abstract

Background Synchronopatric plant species, coexisting and flowering simultaneously, often engage in intense competition for pollen deposition on shared pollinators. This study focuses on the intricate dynamics of chiropterophilous flowers within the neotropical bat-pollination plant guild, specifically investigating the mechanical fit of reproductive structures to pollinating bats at the community level.

Methods Utilizing a diverse guild of bat-pollinated plant species from the Caatinga, the largest dry forest in the Neotropics, our research integrates various key components. Initially, we identified specific contact sites for floral reproductive structures on the bat's body, exploring diverse pollen placement strategies. Subsequent efforts involved characterizing floral traits within the guild and examining their associations with different pollen placement strategies. Precision in the contact of floral reproductive structures was estimated, and findings were integrated by associating pollen placement strategies and precision with the investment in pollen production.

Results We found that certain bat body parts, particularly the face and neck, were more frequently contacted by reproductive structures. The three identified categories of pollen placement strategies were evenly distributed among plant species, each linked to specific floral traits. Notably, the absence of oriented herkogamy prevailed in 70% of the species. Morphometric analyses unveiled significant variations in operative distances among species, emphasizing exceptional variability in certain outliers. While precision in pollen transfer was influenced by key factors, surprisingly, investment in pollen production did not differ among plants with distinct pollen placement strategies. Furthermore, it showed no correlation with fundamental accuracy.

Conclusions The subsequent exploration delves into the intricate associations between distinct floral characteristics and various pollen placement strategies, shaping the complex pollen landscape on bat bodies. This research provides valuable insights into the community-level dynamics of chiropterophilous flowers in the Caatinga Dry Forest, emphasizing the role of different pollen deposition strategies in facilitating the coexistence of multiple plant species within the chiropterophilous guild.

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Keywords Bat pollination, Chiropterophily, Floral morphology, Floral traits, Fundamental accuracy, Male–male competition, Nocturnal pollination, Pollen, Reproductive male fitness, Seasonally dry tropical forests

Background

Synchronopatric plant species (i.e., those occurring in the same area and flowering simultaneously) may share the same pollinators [1], subjecting them to intense competition for pollen deposition on their bodies [2]. This competition is characterized by a complex dynamic in which the pollen of these species is continuously added and removed by both conspecifics and other species [3]. This process can lead to pollen loss if it is frequently transferred to animals visiting sequentially flowers of different species which can result in different pollen interference effects [4]. Faced with this scenario, various strategies have evolved in flowers to avoid heterospecific pollen deposition while enhancing conspecific pollen deposition, among which mechanical fit between the flower and the pollinator stand out [5–11].

The manner in which pollen transfer occurs depends on the extent to which floral morphology and the reward offered by the plant manipulate and direct the movement of a potential vector in relation to the sexual organs of the flower [12]. The combination of these factors can generate a broad gradient of conditions, allowing for different strategies [8, 13]. At one extreme are flowers that scatter pollen diffusely onto pollinators, covering a substantial area of these animals' bodies without a precise location for pollen transfer. This process typically occurs in more generalist flowers, and its impact on pollen transfer effectiveness remains unclear [14–17]. At the other extreme, we find flowers with very specific pollen deposition sites on their pollinators' bodies, a process that generally occurs in flowers with significant phenotypic specialization [18] and is commonly associated with higher efficiency in pollen transfer [19].

A noteworthy example of investigation regarding pollen competition for space on pollinator bodies at the community level occurs in bat-pollinated flowers of Neotropics, driven by three primary reasons. Firstly, various interaction network studies have consistently demonstrated that bats specialized nectar-feeding in the Neotropics (ie. The subfamilies Glossophaginae and Lonchophyllinae – Phyllostomidae) are highly generalist in terms of the chiropterophilous plants they visit [20–24]. Also, bat pollinated species exhibit high morphological diversity and phenotypic traits expressed through floral morphophysiological features, such as pollen deposition at different locations on the pollinator's body [25, 26], thereby suggesting that there is some selective pressure to avoid morphological overlap among them. Finally, bats are recognized for their high efficiency in pollen transport through their fur, establishing a direct link between pollen export and import, thereby driving the selection of large quantities of pollen on flowers [19]. This is further intensified by a higher number of anthers per flower or a greater proportion of male flowers compared to bisexual flowers [27], as well as larger amounts of pollen grains than diurnal flowers [28].

Concerning this last aspect regarding pollen investment, it has been suggested that the high pollen production observed in chiropterophilous species may be associated with the pollinator's body surface area, given that bats have an extensive body surface, consequently favoring greater pollen deposition [29–31]. For a long time, the pollen/ovule (P/O) ratio has been proposed to reflect the likelihood of pollen grains reaching stigmas for the fertilization of available ovules [32–34], eventually being used as a predictor of reproductive systems [33]. However, some studies have demonstrated that pollinator dependence and pollination efficiency have a much greater influence on the evolution of the total number of pollen grains per flower than on the number of ovules. This requires caution when making inferences from the P/O ratio [35, 36]. Thus, approaches measuring pollen production across different species at the community level may allow for an understanding of other important factors in determining this attribute [37].

Studies involving pairs of congeneric model species have revealed functional aspects of how interspecific pollen flow mediated by bats occurs in Neotropical plant species [19]. Or even punctual sampling of pollen deposited on the bodies of nectar-feeding bats captured by nets [38, 39]. However, the processes operating at the community level, allowing for the coexistence of these diverse flower-pollinator mechanical adjustment strategies, are still poorly understood and limited to some excellent studies conducted in the Paleotropics with Pteropodidae bats [40–42].

In this work, we aim to investigate how the mechanical fit of reproductive structures of chiropterophilous flowers to pollinating bats (and their consequent pollen transference) occurs at the community level. To achieve this, we utilized a guild of bat-pollinated plant species from the Caatinga, which is the largest dry forest in the Neotropics and a hotspot for the occurrence of this pollination system [43]. Our comprehensive approach encompassed various key components. Firstly, we focused on identifying sites of contact for floral reproductive structures on the bat's body, exploring diverse pollen placement strategies. Subsequently, we delved into characterizing the floral traits of plants by assessing their prevalences within the guild and examining their connections to different pollen placement strategies employed. Further, we estimated the precision with which the contact of floral reproductive structures could occur. Finally, we integrated our findings by associating both pollen placement strategies and the precision of floral reproductive structures with the investment in pollen production.

For the above objectives, we integrated a thorough naturalistic observations and records of bat visits in the field along with morphological analysis of floral attributes. For the second objective, we used the fundamental inaccuracy index proposed by Armbruster [7]. This metric integrates different measures of phenotypic variation, considering the functional properties of flowers and analyzes how close a population is to its adaptive optimum, quantifying the precision of pollination [6, 7, 9, 44, 45] – see Materials and Methods for a more in-depth explanation). Finally, we quantified the pollen and ovule production of each species using classic protocols [34]. Our expectations were that different species within the chiropterophilous plant guild studied would exhibit diverse mechanical fit strategies to pollinating bats, allowing them to explore different parts of their body surface as pollen transfer sites. Additionally, we anticipated that this gradient of strategies would be related to fundamental inaccuracy, with flowers of more restrictive morphology being more precise. We also expected that less precise flowers would invest in larger quantities of pollen to ensure their reproductive efficiency.

Methods

Study area

We chose the Caatinga, considered the most diverse among dry tropical forests [46], due to its high frequency of chiropterophilous species, making it one of the most notable areas for such species globally [43, 47, 48]. In contrast to other forests where chiropterophilous species commonly occur in relatively low densities, the abundance of chiropterophilous species in the Caatinga facilitates the measurement of ecological processes at the population and community levels [49].

Fieldwork was conducted in the Catimbau National Park (PARNA Catimbau), located in the municipality of Buíque, State of Pernambuco (08°32′14"—08°35′12"S and 37°14′42"—37°15′02"W). The region has an average annual temperature of 25 °C and irregular rainfall, with an average annual precipitation of 700 mm. The dry season lasts for 6 to 8 months (August-February), with November being the driest month, and the rainy season concentrates from March to July, with May having the highest rainfall [50–52].

Studied species: guild of bat-pollinated plants

To depict the floral traits related to mechanical fit between flowers and bats, we included all the plant species occurring in the study area that are proven to be bat-pollinated, based on the list presented by Domingos-Melo et al. [43]. This allowed us to include a total of 20 species distributed among 16 genera from nine angiosperm families (Table 1). These species alternate their flowering periods throughout the year, ensuring a continuous presence of flowering plants, many of which have overlapping blooming periods (see details in Table 1). This dynamic creates a highly competitive environment among floral species for pollen transfer by bats, as all species exhibit coinciding anthesis periods. These periods begin in the late afternoon or early evening and last until early morning, with the majority of pollen being dispersed during the early hours of the night [53, 54]. For the selection of chiropterophilous plant species included in the fundamental inaccuracy, our inclusion criterion was a minimum population of 15 accessible individuals, enabling the measurement of intrapopulational phenotypic variation. The fundamental inaccuracy of each species was measured (Table 2), as well as pollen and ovule counts were carried out (Table 3). We randomly selected 20 individuals for each species, ensuring that populations of chiropterophilous plants were sampled in different locations within PARNA Catimbau (Vila do Catimbau, Pedra do Cachorro, Trilha das Torres, Alto das Torres, Pedra do Padre, Serrinha, and Alcobaça), with distances ranging from 2.0 km to 50.0 km. We ensured that all individuals were at least ten meters apart to guarantee they were not the same plant, considering that resprouting is a common behavior in the Caatinga [55].

Pollen deposition sites on bat bodies

While frugivorous bats may play a significant role as pollinators [21, 43], this study focused exclusively on the mechanical fit occurring between flowers and specialized nectar-feeding bats, namely those belonging to the subfamilies Glossophaginae and Lonchophyllinae (Phyllostomidae). In the study area, at least five bat species from these subfamilies have been recorded: *Anoura geoffroyi* Gray, 1838, *Glossophaga soricina* Pallas, 1766 (Glossophaginae), *Lonchophylla inexpectata* Moratelli and Dias, 2015, *L. mordax* Thomas, 1903, and *Xeronycteris vieirai* Gregorin and Ditchfield, 2005 (Lonchophyllinae) [58]. Despite having different nectar intake methods, Glossophagine and Lonchophylline bats visit flowers with

Table 1 Pollen placement strategies and floral morphological attributes of the guild of bat-pollinated plant species from PARNA Catimbau, Northeast Brazil

	Flowering period (Monthly records during the data collection supplemented with herbaria information from SpeciesLink.)	**Bats body part (h-head; f-face; n-neck; c-chest; b-belly; s-shoulder to back; w-wings; u-uropatagium)**	**Pollen placement (sensu [3])**	**Floral type (sensu [56])**	**Symmetry Vegetative whorl**	**Symmetry Reproductive whorl**	**Herkogamy (sensu [57])**	**Visit direction (Sternotribic; Nototribic; Frontal; All)**
Acanthaceae								
Harpochilus neesianus Mart. ex Nees	Throughout the year with a peak in October	s;u	Stamp	Gullet	Asymmetric	Zygomorphic	Approach	Nototribic
Bromeliaceae								
Dyckia spectabilis (Mart. ex Schult. & Schult.f.) Baker	August to November with a peak in September	h;f;n	Stroke	Pseudo-brush	Actinomorphic	Actinomorphic	Absent	Frontal
Cactaceae								
Pilosocereus catimbauensis N.P.Taylor & Albuq.-Lima	January to March with a peak in February	f	Stamp	Campanulate	Zygomorphic	Actinomorphic	Absent	Frontal
Pilosocereus catingicola (Gürke) Byles & Rowley	August to November with a peak in September	h;f;n	Stamp	Campanulate	Zygomorphic	Actinomorphic	Absent	Nototribic
Pilosocereus pachycladus F.Ritter	February to May with a peak in April	f	Stamp	Campanulate	Zygomorphic	Actinomorphic	Absent	Frontal
Xiquexique gounellei (F.A.C.Weber) Lavor & Calvente	February to May with a peak in April	f	Stamp	Tube	Zygomorphic	Actinomorphic	Absent	Frontal
Xiquexique tuberculatus (Werderm.) Lavor & Calvente	Throughout the year with a peak from October to January	f	Stamp	Tube	Zygomorphic	Actinomorphic	Absent	Frontal
Capparaceae								
Cynophalla flexuosa (L.) J.Presl	October to March with a peak in flowering between November and December	h;f;n;c	Diffuse	Brush	Actinomorphic	Actinomorphic	Absent	All
Neocalyptrocalyx longifolium (Mart.) Cornejo & Iltis	September to February with a peak in flowering between October and November	h;f;n;c	Diffuse	Brush	Actinomorphic	Actinomorphic	Reverse	All
Cleomaceae								
Tarenaya longicarpa Soares Neto & Roalson	Throughout the year with a peak from August to October	c;b;w	Diffuse	Flag	Zygomorphic	Actinomorphic	Absent	Sternotribic

Table 1 (continued)

	Flowering period (Monthly records during the data collection supplemented with herbaria information from SpeciesLink.)	**Bats body part (h-head; f-face; n-neck; c-chest; b-belly; s-shoulder to back; w-wings; u-uropatagium)**	**Pollen placement (sensu [3])**	**Floral type (sensu [56])**	**Symmetry Vegetative whorl**	**Symmetry Reproductive whorl**	**Herkogamy (sensu [57])**	**Visit direction (Sternotribic; Nototribic; Frontal; All)**
Convolvulaceae								
Ipomoea vespertilia D. Santos, G.C. Delgado-Junior & Buril	July to September with a peak in August	n;c	Stroke	Infundibuliform	Actinomorphic	Zygomorphic	Approach	Sternotribic
Fabaceae								
Bauhinia acuruana Moric	January to April with a peak in February and June to October with a peak in August	n;c	Stroke	Flag	Asymmetric	Zygomorphic	Approach	Sternotribic
Bauhinia pentandra (Bong.) D.Dietr	Throughout the year with a peak between November and February	c;b	Stroke	Flag	Asymmetric	Zygomorphic	Approach	Sternotribic
Calliandra aeschynomenoides Benth	June to September with a peak in July	h;n;c	Diffuse	Pseudo-brush	Actinomorphic	Actinomorphic	Absent	All
Hymenaea cangaceira R.B.Pinto, Mansano & A.M.G.Azevedo	December to February with a peak in January	n;c	Diffuse	Dish	Actinomorphic	Actinomorphic	Absent	All
Mimosa lewisii Barneby	Throughout the year with a peak from June to July	n;c	Stroke	Pseudo-brush	Actinomorphic	Actinomorphic	Absent	Sternotribic
Lythraceae								
Lafoensia glyptocarpa Koehne	June to September with a peak in July	c;b;w	Diffuse	Dish	Actinomorphic	Actinomorphic	Absent	Sternotribic
Malvaceae								
Ceiba glaziovii (Kuntze) K.Schum	July to September with a peak in August	h;f;n;c	Stroke	Dish	Actinomorphic	Zygomorphic	Approach	Frontal
Helicteres baruensis Jac	July to September with a peak in August	b	Stroke	Flag	Zygomorphic	Zygomorphic	Absent	Sternotribic
Pseudobombax marginatum (A.St.-Hil., Juss. & Cambess.) A.Robyns	June to September with a peak in July	h;f;n;c;b;s;w	Diffuse	Brush	Actinomorphic	Actinomorphic	Absent	All

Table 2 Pollination **i**naccuracy statistics of a sample from the guild of bat-pollinated plant species from PARNA Catimbau, Northeast Brazil. (Ordered from lowest to highest inaccuracy index)

	N (flowr/id)	Departure from optimum*	%TI	Trait imprecision (male function—OD)				Optimum (female function—OD)				Raw inaccuracy	Mean^2-scaled Inaccuracy
				Mean	Variance	CV	%TI	Mean	Variance	CV	%TI		
Harpochilus neesianus	30/15	7.02	21.64	58.80	66.22	13.84	29.12	65.80	111.98	16.08	49.20	227.42	0.066
Dyckia spectabilis	30/21	2.92	8.07	18.70	34.88	31.52	32.92	21.70	62.52	36.50	59.00	105.95	0.303
Pilosocereus pachycladus	36/36	0.39	0.30	27.50	16.53	14.79	32.50	27.10	34.18	21.58	67.20	50.86	0.067
Xiquexique tuberculatus	30/15	6.25	23.57	33.00	48.27	21.06	29.12	26.70	78.41	33.12	47.30	165.75	0.152
Neocalyptrocalyx longifolium	41/15	13.36	60.44	22.80	9.71	13.64	3.28	9.50	107.23	109.19	36.30	295.56	0.569
Tarenaya longicarpa	30/15	6.94	9.02	37.20	91.04	25.65	17.03	44.10	395.41	45.05	74.00	534.66	0.386
Bauhinia acuruana	20/15	8.91	46.69	25.10	19.64	17.65	11.54	34.00	71.11	24.78	41.80	170.23	0.270
Bauhinia pentandra	30/17	11.16	47.83	32.90	48.77	21.22	18.73	44.10	87.04	21.17	33.40	260.34	0.241
Calliandra aeschynomenoides	30/17	3.21	45.11	13.20	5.62	17.90	24.64	16.40	6.90	15.97	30.30	22.81	0.131
Hymenaea cangaceira	30/15	9.01	72.55	27.80	10.14	11.45	9.06	36.80	20.60	12.33	18.40	111.97	0.145
Mimosa lewisii	30/15	0.37	2.38	7.40	0.73	11.63	13.04	7.00	4.76	31.17	84.60	5.63	0.103

Table 3 Pollen and ovule production of a sample from the guild of bat-pollinated plant species from PARNA Catimbau, Northeast Brazil

	N (Bud/Ant.)	Anther per flower	Pollen per anther	Total pollen per flower	Ovules	P/O Ratio
Acanthaceae						
Harpochilus neesianus	10/2	2	13,688 ± 6267	27,375 ± 12,534	4	6844 ± 3133
Bromeliaceae						
Dyckia spectabilis	10/2	6	364,500 ± 133,178	2,187,000 ± 799,067	263 ± 74	8685 ± 2699
Cactaceae						
Pilosocereus catibauensis	5/10	615 ± 11	9833 ± 2009	6,051,250 ± 1,394,336	2050 ± 28	1771 ± 1685
Xiquexique tuberculatus	10/10	888 ± 7	13,763 ± 3302	12,225,650 ± 2,970,933	2617 ± 17	4669 ± 1119
Capparaceae						
Cynophalla flexuosa	10/15	66 ± 1	18,742 ± 1904	1,236,642 ± 123,379	81 ± 1	15,327 ± 1554
Neocalyptrocalyx longifolium	10/15	47	40,442 ± 1393	1,892,817 ± 71,797	38 ± 1	50,084 ± 1989
Cleomaceae						
Tarenaya longicarpa	10/3	6	208,375 ± 7968	1,250,250 ± 47,805	168 ± 1	7459 ± 271
Convolvulaceae						
Ipomoea vespertilia	10/5	5	1734 ± 29	8669 ± 147	4	2167 ± 37
Fabaceae						
Bauhinia acuruana	10/4	10	2858 ± 1277	28,584 ± 12,772	16 ± 6	1877 ± 847
Bauhinia pentandra	10/4	10	2723 ± 404	27,228 ± 4039	19 ± 1	1481 ± 267
Calliandra aeschynomenoides	10/5	19	4902 ± 220	93,138 ± 4179	18 ± 1	5174 ± 232
Hymenaea cangaceira	10/4	10	38,625 ± 10,541	386,250 ± 105,411	12 ± 2	33,671 ± 10,379
Mimosa lewisii	10/3	8	335,250 ± 4069	2,682,000 ± 32,554	10 ± 1	283,126 ± 16,564
Malvaceae						
Ceiba glaziovii	10/2	5	165,438 ± 50,178	827,188 ± 250,888	196 ± 18	4301 ± 1494

similar behavior [59] as they can perform hovering flights [57, 60, 61]. These bats are highly specialized for nectar collection, characterized by an elongated snout, reduced tooth size and number, and relatively long tongues compared to their body size [60, 62]. Moreover, the notable similarity in size and body structure of these bats makes them functionally comparable in their requirements for flower-pollinator mechanical fit [21, 63]. Here, we refer to the general bauplan of visiting bats for determine pollen deposition sites. In this regard, for classification purposes, the body of the bats was divided into eigth parts, in which contact with floral reproductive structures and pollen deposition was checked: i) face, ii), head iii) neck, iv) chest, v) belly, vi) wings, vii) back, and viii) uropatagium. Additionally, we classified the way in which contact with floral reproductive structures could occur into three categories proposed by Minnaar et al. [3]: i) stroke, ii) stamp, and iii) diffuse. Stroke pollen placement requires anthers to be dragged along a part of the pollinator's body in a consistent direction, leaving a trail of pollen. Stamp pollen placement refers to placing pollen where anthers are not dragged by the bodies of vectors but instead stamp the pollen onto the vectors' bodies in a single contact event. Diffuse pollen placement includes any mechanisms that place pollen over large, undefined areas of vectors.

We opted not to capture bats using mist nets to collect pollen from their bodies, once as observed in the field, the grains from different body parts became mixed when the bats struggled in the net, especially pollen deposited on their wings and ventral portions. Therefore, we resorted to focal observations and photographic records for this purpose. Given that nectar-feeding bats make visits of less than a second to the flower, the description of visit details in field focal observations was limited to items such as the bat's approach direction (frontal, from above, or below) and the manner of contact with the flower (hovering or grasping the flowers in flight).

Although bat floral visits are very quick, the specifics of the visit moment do not escape a good photographic camera lens. Therefore, the determination of pollen deposition sites on bat bodies was accomplished through photographs and videos taken with a Canon Rebel T3i camera + 70-300 mm f/4–5.6 DG Macro lens and a Sony HDR-PJ710 Handycam NightShot, respectively. The use of these images enabled the visualization of the contact location between reproductive structures and the bat's body at the exact moment of the visit and marks of recently deposited

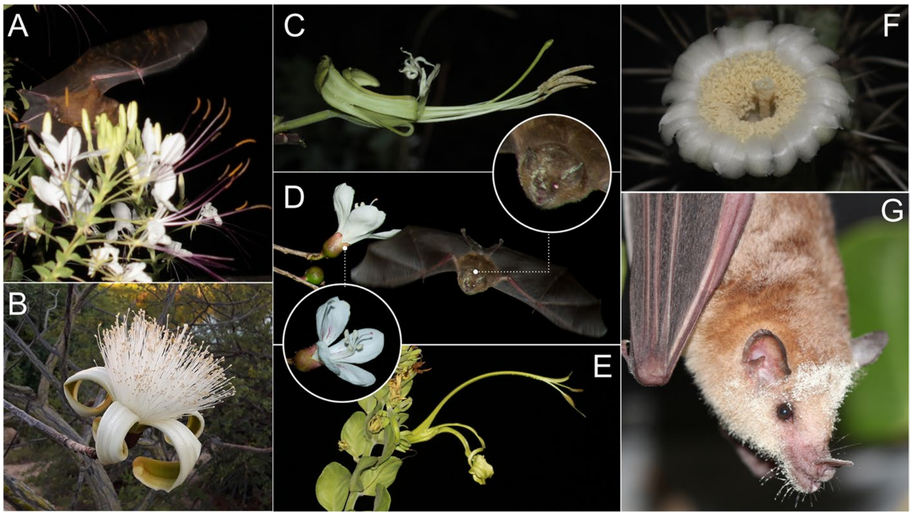

Fig. 1 Examples of pollen placement strategies observed among chiropterophilous flowers and their pollinating bats. **A** *Tarenaya longicarpa* (Cleomaceae) flowers, with distinct yellow pollen marks are evident diffuse pollen placement on the wings of the bats; **B** *Pseudobombax marginatum* (Malvaceae) have big brush type flowers promoting diffuse pollen placement into bats. **C** Flowers as *Bauhinia pentandra* (Fabaceae) and **D** *Ceiba gaziovii* (Malvaceae) deposit pollen through unidirectional strokes, respectively in sternotribic and frontal directions. Flowers of *Harpochilus neesianus* (Acanthaceae) (**E**) and *Xiquexique tuberculatus* (Cactaceae) (**F**-**G**) employ a stamp-like mechanism, depositing pollen on the bats' bodies, respectively on the uropatagium and facial regions. In photos **A** and **B**, the nectar-feeding bat *Glossophaga soricina* (Glossophaginae, Phyllostomidae) is shown, while photo C features *Lonchophylla* sp. (Lonchophyllinae, Phyllostomidae)

pollen on the bat's body (e.g., Fig. 1A, D). For each photograph in which a bat was in contact with a flower, we meticulously documented the specific points of contact between the bat and the flower's reproductive structures. Additionally, in photographs showing the bat immediately after leaving the flower, we carefully inspected the bat's body for pollen exhibiting the same coloration and characteristics as the flower visited. This examination aimed to identify probable deposition sites while at the same time ensuring that pollen grains from other species were not erroneously included. Only photographs that clearly displayed both the contact points and pollen deposition were considered in our analysis. Furthermore, we performed multiple independent evaluations of the photographs between authors to verify the accuracy of our observations.

After careful analysis, it was possible to determine the different pollen placement strategies for each plant species. Furthermore, as several of the chiropterophilous species included in this study have previously published studies on pollination biology, information regarding the mechanical fit between flowers and bats was also obtained from them (eg. [21, 43, 53, 54, 64–79].

Characterization of floral morphology

We considered various functional aspects in characterizing the flower-pollinator mechanical fit of the studied plant guild (Fig. 1). Thus, we described floral morphology through five characteristics that can summarize the main aspects important for the flower-pollinator adjustment. These are: i) floral type; ii) symmetry of vegetative whorls; iii) symmetry of reproductive whorls; iv) type of herkogamy; and v) orientation of reproductive structures. Next, we indicate how each of these characteristics was organized into different categories.

For the floral type, we used categories from classic literature on pollination biology (e.g., [56, 80]), considering general aspects of floral morphology, especially the access form to floral resources. We considered seven categories: i) brush (and pseudo-brush); ii) bell; iii) funnel/infundibuliform; iv) flag; v) dish; vi) throat; and vii) tubular.

Regarding symmetry, we based our classification on the work of Spencer and Kin [81], who categorized flowers into three groups: asymmetric (without symmetry), bilateral (zygomorphic – with a single symmetry plane), and radial (actinomorphic – with multiple symmetry planes). We decomposed symmetry into two components: the first related to vegetative whorls (calyx and corolla), mainly concerning attractiveness and the way the pollinator behaves during the visit,the second related to reproductive structures (androecium and gynoecium), determining the pollen transfer sites on the pollinator's body.

To understand the distribution of reproductive structures within the floral space, we classified chiropterophilous flowers adapting the classification proposed by Webb and Lloyd [13]. Considering the importance of plant-animal interaction, the authors categorized herkogamy into classes that distinguish floral types based on the position of anthers and stigmas in relation to the foraging trajectory of pollinators: i) Approach herkogamy—ApH, in which the stigma is positioned in front of or above the level of the anthers, constituting the pollinator's initial contact with the stigma; ii) Reverse herkogamy—ReH, when the stigma is positioned behind or below the level of the anthers, constituting the pollinator's initial contact with the anthers; iii) Absent herkogamy—AbH, when anthers and stigma are at the same level and make contact with the pollinator at the same moment during its approach, or when there is no clear determination of the approach form.

In concern the orientation of reproductive structures, we used four categories: i) frontal (when reproductive organs contact the pollinator in the exact direction of its approach without directing upwards or downwards); ii) sternotribic (reproductive organs curved upwards, resulting in pollen deposition on the ventral surface of the pollinator – Fig. 1C); iii) nototribic (reproductive organs curved downwards, promoting pollen deposition on the dorsal surface of the pollinator – Fig. 1E); and iv) all (when all directions of approach are possible).

Morphometry of operational distances of floral reproductive structures

The floral morphometry of each species was conducted using flowers in early anthesis and immediately after complete opening (sample sizes are indicated in Table 2). Morphometry was performed directly in the field in some species to ensure precise measurement of the operational distances of reproductive structures since the use of fixing agents could lead to morphological alterations (e.g., *Bauhinia acuruana*, *B. pentandra*, *Harpochilus neesianus*, *Hymenaea cangaceira*) or the loss of structures sensitive to handling (e.g., *Calliandra aeschynomenoides, Neocalyptrocalyx longifolium, Tarenaya longicarpa*) (Table 1). For species with a linear pistils/filaments and greater resilience to handling (*Dyckia spectabilis, Mimosa lewisii, Pilosocereus pachycladus* and *Xiquexique tuberculatus*) (Table 1), their flowers were immersed in a container with 70% alcohol, and their reproductive structures were measured in the laboratory.

In each species, we measured the operational distance of the reproductive structures, defined as the linear distance from the center of the anthers (male operational distance) or the center of the stigma (female operational distance) to a landmark representing the point of access to nectar. We established a landmark for each floral type based on observations of pollinator behavior. This included considerations such as the location where the bat accesses nectar, the position of reproductive structures, and their contact with the animal's body during flower visits. In open morphology flowers (with exposed nectar), operational distances were measured from the nectary to reproductive structures. In tubular or infundibuliform flowers, measurements were taken from the entrance of nectar chamber, as the limit of access to the bat's snout in these flowers occurs at the entrance of the nectar chamber, where the bat inserts part of its tongue to access the resource. Digital calipers (Mitutoyo Digimatic SR44) were employed for floral measurements.

Quantification of pollen production investment

To conduct pollen counting, we collected floral buds of each species in the pre-anthesis stage ($n = 5$ to 10) from different individuals and preserved them in plastic containers containing 70% alcohol. For flowers with a total pollen count exceeding 2000 grains, we performed an estimation using a Neubauer chamber following standard protocols [82]. The buds were dissected in a watch glass containing a solution of 1 ml lactic acid and glycerin in a 3:1 ratio. The content was thoroughly homogenized and subsequently deposited in the Neubauer chamber using a Pasteur pipette. Pollen grains visualization and counting were conducted under an optical microscope. For species with fewer than 2000 pollen grains per flower or with large pollen grains (> 100 μm), preventing their entry into the Neubauer chamber (e.g., *Bauhinia* spp. and *Ipomoea vespertilia*), we performed a direct count of the grains on a histological slide. In species with two levels of anthers, such as *Bauhinia* spp, we used two of the small anthers and two of the large ones. A drop of glycerine was used for the dissection of each anther, and after homogenizing the content, we completed the process with a coverslip.

The number of ovules of each ovary was counting on a Petri dish, under a stereomicroscope (4.0 × magnification). The pollen/ovule ratio (P/O) was obtained by multiplied the pollen grains per anther, by the number of anthers in the flower, and then divided by the number of ovules. To the andromonoecious species *Neocalyptrocalyx longifolium* and *Tarenaya longicarpa*, only bisexual flowers were considered.

Statistical analyses

We compared the frequencies of pollen placement strategies employed by the studied plant guild as well as the frequencies at which different bat body parts were explored as sites of pollen transport. To do it, we ran a chi-square homogeneity test using the 'stats' package in R. Additionally, we plotted the proportion of species exploring each body part, along with its 95% confidence interval, and compared it with the expected proportion to identify body parts with higher or lower proportions than expected. This analysis was performed with the 'Hmisc' package in the R software [83].

To compare the frequencies at which various categories in each morphological floral trait occur within the guild of bat-pollinated plants under study, we employed chi-square homogeneity tests using the 'stats' package in R [84]. Additionally, an Analysis of Similarity (ANOSIM) was conducted on the outlined morphological floral traits, followed by Non-Metric Multidimensional Scaling (NMDS) to explore the morphospace concerning floral morphologies. Initially, ANOSIM was utilized to statistically evaluate the significance of differences between sample groups, taking into account the types of pollen placement. Subsequently, NMDS visually represented dissimilarity between samples in a reduced-dimensional space. To perform both ANOSIM and NMDS, morphological categories were transformed into dummy variables, allowing for a quantitative representation of floral attributes. The analyses were executed using the 'vegan' package in the R programming environment [85].

To assess differences in male and female operational distance variations across studied species, a Linear Mixed Model (LMM) was employed. The Coefficient of Variation (CV) of operational distances was used as the response variable, while sex was the predictor variable, and species was treated as a random variable. This test was conducted using the 'nlme' package in the R software [86].

For assessing the accuracy of each chiropterophilous species studied, we utilized fundamental inaccuracy indices based on Armbruster et al. [7]. Adaptive accuracy provides a heuristic representation of how well a phenotype aligns with its expected optimum in a population, offering insights into the level of adaptation of that phenotype. Fundamental inaccuracy indices consider only floral morphology, disregarding the pollinator's effect on this adjustment. These indices are calculated from the means and variances of the phenotype of interest in a population, as well as its respective optimum. The formula used for inaccuracy was $i = (Mf—Mo)^2 + Vo + Vf$, where Mf is the mean of the phenotype in question, Mo is the mean of the optimum, Vo is the variance of this optimum, and Vf is the variance of the phenotype. For comparative purposes, it is crucial to scale the obtained inaccuracy values by dividing them by the square of the mean of the analyzed phenotype (i/Mf^2) [44, 45], 2009b, 2014ab). Concerning floral reproductive structures, the ideal pollen placement site on a pollinator should be related to the expected location of the pollinator with stigmas from other conspecific flowers [6]. Thus, for each species, we considered male operational distances as our phenotype of interest and female operational distances as our optimum (measured as described above) as reciprocal optima [6, 44, 87]. To assess the relative contribution of each component to the inaccuracy index, we conducted an LMM, where the percentage contributions to the accuracy index were the response variables, the components were the predictor variable, and species were treated as a random variable. This test was performed using the 'nlme' package in the R software. Finally, to determine if there were differences in the inaccuracy index among species with different pollen placement strategies, we conducted a Kruskal–Wallis rank sum test using the 'stats' package in R [84].

Regarding the investment in pollen production, we examined whether species with different pollen placement strategies differed in their total pollen production per flower and P/O ratio. For this, we conducted a Kruskal–Wallis rank sum test using the 'stats' package in R. We also investigated whether these attributes were related to the inaccuracy index of each species through Spearman's rank correlation, conducted with the 'stats' package in R.

Results

Pollen deposition sites on bat bodies

A great diversity of contact modes was observed between the reproductive floral structures of the studied plants and the bodies of bats (Fig. 1). These conditions resulted in a significant difference in the frequency at which bat body locations were used as pollen deposition sites ($\chi2 = 15.98$; df = 7; $p = 0.025$). The face and neck were the most common body parts to come into contact with the reproductive structures of flowers, each of these locations capable of transporting pollen from 55% of plants in the guild, a proportion higher than expected for the population as a whole (Fig. 2). The head, belly, wings, and back of the bats contacted reproductive structures in 35%, 25%, 15%, and 10% of the plants, respectively. The uropatagium was the least utilized site for pollen transport, falling below the expected for the population, as only one plant (5%) was recorded with reproductive structures contacting this area (Fig. 2).

Regarding the type of pollen placement, besides not detecting significative differences in the frequencies of strategies used ($\chi2 = 0.1$; df = 2; $p = 0.95$), there was practically no variation between the different categories, with frequencies ranging from 35%, in flowers with diffuse and stroke deposition, to 30% with flowers having stamp-type deposition.

Characterization of floral morphology

No significative differences were found in the frequencies at which floral types occurred among species ($\chi2 = 2.4$; df = 7; $p = 0.93$), although these frequencies varied widely.

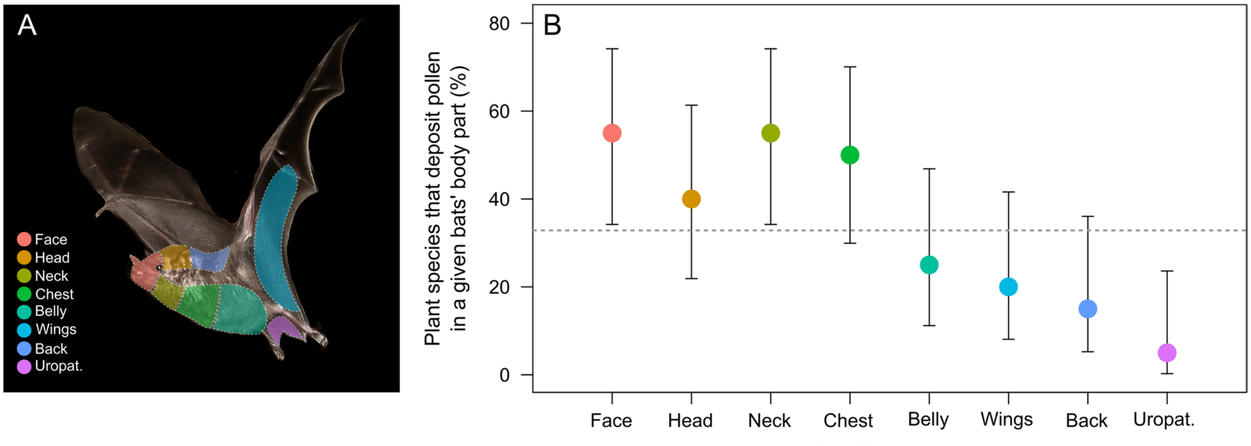

Fig. 2 **A** Illustration of the eight divisions of the bat's body used to assess contact with floral reproductive structures; the bat depicted is *Glossophaga soricina* (Glossophaginae, Phyllostomidae). **B** Dot plot depicting the proportion of plants within the chiropterophilous guild whose reproductive floral structures make contact with each specific body part of bats (whiskers denote the 95% confidence interval; dotted line denotes the proportion expected)

Brush floral types predominated, occurring in 30% of the analyzed species. These floral types are generally characterized by numerous long stamens and reduced perianth. We registered the flag floral type in 20% of the species. This type is characterized by upward-facing vegetative whorls acting as a flag directing the pollinator's visit, while reproductive structures are downward-facing. The campanulate, tube, and funnel-shaped floral types were represented by 15%, 10%, and 5% of the samples, respectively, with the majority of them being representatives of the Cactaceae family. All of these types are characterized by presenting their nectar at the end of a tubular-conical floral structure, differing in terms of narrowing and length. As a result, the bat needs to insert its tongue, snout, or head into the flower to access the resource. The dish-type, characterized by very open vegetative whorls and completely exposed nectar, occurred in 15% of the species. We found only a single species (*Harpochilus neesianus*) with the throat-type, characterized by a bilabiate flower with a tubular structure at the bottom, where the nectar is presented.

Regarding symmetry, no significative differences were detected in the frequencies of types among flowers, whether considering only vegetative structures ($\chi2 = 3.7$; df $= 2$; $p = 0.16$) or reproductive ones ($\chi2 = 3.2$; df $= 1$; $p = 0.07$). In this sense, 40% of the species had completely actinomorphic flowers in both vegetative and reproductive terms. Next, we found flowers with vegetative structures exhibiting bilateral symmetry and reproductive structures with actinomorphic symmetry, representing 30% of the sample. Flowers with asymmetrical vegetative whorls and reproductive structures with bilateral symmetry represented 15% of the studied guild. Only two species (10%) exhibited vegetative structures with actinomorphic symmetry combined with zygomorphy in reproductive organs. A single species (5%) was completely zygomorphic.

Relating to potential of flower to drive the bat approach, the only attribute with differences in frequencies between categories was herkogamy ($\chi2 = 13.3$; df $= 2$; $p = 0.001$), with 70% of the species with no herkogamy, followed by 25% with approach herkogamy (ApH), and a single species (5%) with reverse herkogamy (ReH), namely *N. longifolium*. In terms of the orientation in which pollen is deposited, no significant differences were detected between categories ($\chi2 = 5.5$; df $= 3$; $p = 0.14$), despite a wide variation in their frequencies (35% sternotribic, 30% frontal deposition, 25% without a specific orientation, and only 10% nototribic). The ANOSIM analysis revealed marked morphological differences between flowers employing different pollen placement strategies ($R = 0.414$; $p = 0.001$), as can be visualized in the NMDS (Fig. 3).

Morphometry of operative distances of floral reproductive structures

The operative distances of reproductive structures varied widely among different species in the studied guild, as subjected to morphometric analyses (Table 2). The longest structures were found in *Harpochilus neesianus*, reaching 65.8 ± 8.1 and 58.8 ± 10.6 mm in male and female functions, respectively. Conversely, the shortest occurred in *Mimosa lewisii* with 7.0 ± 0.8 and 7.4 ± 2.2 mm, respectively. The operative distances of other species were uniformly distributed between 10 and 45 mm. Regarding the variation in operative distances within each species,

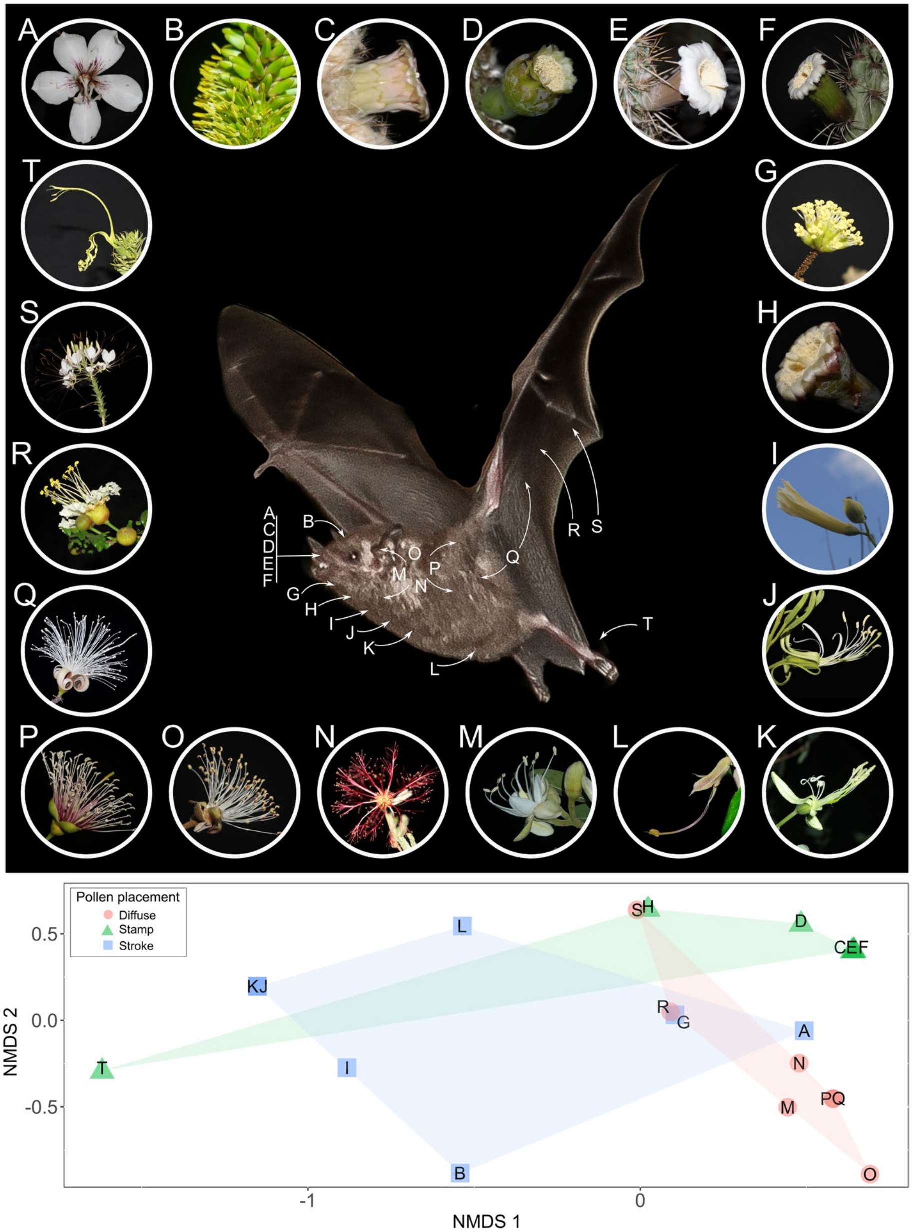

Fig. 3 Contact directions with bodies of pollinating bats generated by different floral morphologies of plants from chiropterophilous guild in PARNA Catimbau, Northeast Brazil. (Upper) Directions of approach (white arrows) of the reproductive structures of the flowers on the bodies of nectar-feeding bats during the floral visit, here exemplified in the *Glossophaga soricina* (Glossophaginae-Phyllostomidae) as an example. The labeled plant species include (ordered by the proximity of contact of structures on the bats' body): (**A**) *Ceiba glaziovii*, (**B**) *Dyckia spectabilis*, (**C**) *Pilosocereus catimbauensis*, (**D**) *P. pachycladus*, (**E**) *Xiquexique gounellei*, (**F**) *X. tuberculatus*, (**G**) *Mimosa lewisii*, (**H**) *Pilosocereus catingicola*, (**I**) *Ipomoea vespertilia*, (**J**) *Bauhinia acuruana*, (**K**) *B. pentandra*, (**L**) *Helicteres baruensis*, (**M**) *Hymenaea cangaceira*, (**N**) *Calliandra aeschynomenoides*, (**O**) *Neocalyptrocalyx longifolium*, (**P**) *Cynophalla flexuosa*, (**Q**) *Pseudobombax marginatum*, (**R**) *Lafoensia glyptocarpa*, (**S**) *Tarenaya longicarpa*, (**T**) *Harpochilus neesianus*. (Bottom) NMDS analysis (stress = 0.017) comparing different pollen deposition strategies based on floral morphological attributes

coefficients of variation (CV) ranged from approximately 10% to 45%, with no differences in CV between male and female operative distances of the studied species (numDf = 1; denDF = 10; F = 3.58; $p = 0.088$). A notable exception occurred in *N. longifolium*, in which the operative distance reached more than 100% CV.

Considering factors related to the precision in pollen transfer, we found that the components determining inaccuracy contributed differently to the determination of inaccuracy indices for species in the studied guild (numDf = 2; denDF = 20; F = 6.33; $p = 0.007$). In this regard, the main contributing factor to inaccuracy indices was the Variance of Optimum (interpreted here as female operative distances), while Departure from Optimum (difference between the means of male and female operative distances) and Trait Variance (interpreted here as male operative distances) played a secondary role (Fig. 4). There weren't significant differences in inaccuracy indices between species with different pollen deposition strategies (H = 3.48; df = 2, $p = 0.176$).

Quantification of pollen production investment

We found a wide variation in both pollen production investment and the number of ovules among species in the analyzed guild, ranging across four and three orders of magnitude, respectively. Cactaceae stood out with the highest values for both pollen per flower and ovules, with *Xiquexique tuberculatus* reaching a production exceeding ten million pollen grains per flower and 2617 ± 17 ovules. On the other hand, *Ipomoea vespertilia* did not surpass ten thousand pollen grains and had only four ovules. However, when analyzing the P/O ratio, we found a slightly narrower variation, within two orders of magnitude. At this point, *Mimosa lewisii* stands out with the highest P/O, 283,126 ± 16,564, while *Bauhinia pentandra,* 1481 ± 267, had the lowest.

Both the total amount of pollen per flower (H = 1.6838; df = 2; $p = 0.431$) and the P/O (H = 3.8171; df = 2; $p = 0.148$) varied among species regardless of their pollen placement strategies. Additionally, no relationship was found between the investment in pollen production and the accuracy of pollination mechanisms. The inaccuracy indices of species were not related to either the total pollen per flower ($\rho = 0.18$; $p = 0.632$) or their P/O ratios ($\rho = 0.02$; $p = 0.972$).

Discussion

Our investigation into the mechanical fit of reproductive structures in chiropterophilous flowers has yielded diverse results, offering valuable insights into the community-level dynamics. We found that certain bat body parts (i.e., face and neck) are much more frequently contacted by the reproductive structures of the flowers than others. Additionally, we observed that the three categories of pollen placement strategies are equally distributed among chiropterophilous plant species, and each one is associated with a specific set of floral traits. In the same vein, almost all categories present in each type of floral trait were evenly distributed throughout the guild, while the only attribute for which a prevalence was detected was the absence of oriented herkogamy, showed in 70% of the species. The morphometric analyses revealed expressive variations in operative distances among species, with certain outliers demonstrating exceptional variability. By estimate the potential precision of pollen transfer, the study identified key factors contributing to fundamental inaccuracy index, with the imprecision of the female function playing a more significant role across species. Surprisingly, the investment in pollen production did not differ among plants with distinct pollen placement strategies, nor was it correlated with the fundamental accuracy. In the discussion that follows, we comprehensively explain how distinct floral characteristics are intricately associated with various pollen placement strategies. We explore how these processes can shape the complex pollen landscape on bat bodies.

The absence of predominance of specific categories in almost all floral morphological traits analyzed suggests that the plant species within the guild integrate diverse floral traits in various combinations to exploit bat bodies as pollen transport sites in multiple ways.

Flowers employing diffuse pollen placement predominantly exhibited a brush-type morphology with actinomorphic symmetry. The flowers with this floral type were characterized by easily accessible nectar, occasionally fully exposed, and numerous stamens. These characteristics enable resource access from any direction, with pollen being deposited on various parts of bat bodies simultaneously (eg. *Calliandra aeschynomenoides, Cynophalla flexuosa, Neocalyptrocalyx longifolium, Pseudobombax marginatum*). Exceptionally, diffuse pollen deposition also occurred in other floral types, but in these cases, it seemed to be a feature conditioned by the considerable length of reproductive structures. An example is the flag-type of *Tarenaya longicarpa*, in which long stamens deposit pollen diffusely, mainly on bat wings.

(See figure on next page.)

Fig. 4 **A** Ridgeline plot showing the observed frequencies of male and female operational distances of different species used in the calculation of the inaccuracy index (ordered from shortest to longest). **B** Boxplot comparing the different relative contributions of the components of the inaccuracy index across plants from the chiropterophilous guild (species: a- *Mimosa lewisii*, b- *Calliandra aeschynomenoides*, c- *Neocalyptrocalyx longifolium*, d- *Dyckia spectabilis*, e- *Pilosocereus pachycladus*, f- *Bauhinia acuruana*, g- *Xiquexique tuberculatus*, h- *Hymenaea cangaceira*, i- *Bauhinia pentandra*, j- *Tarenaya longicarpa*, k- *Harpochilus neesianus*; boxplot elements: black horizontal line – median; ends of the box – upper and lower quartiles: extremes of line – interquartile range from median)

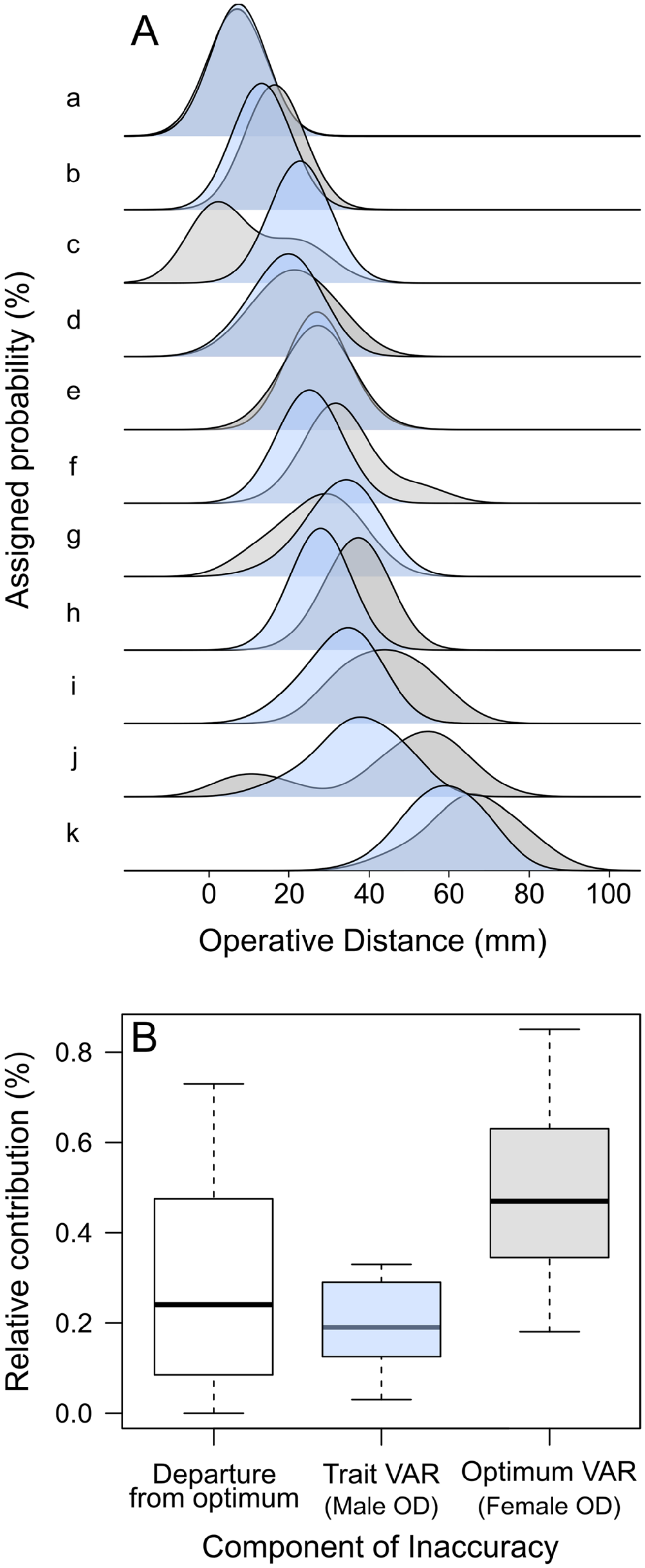

Fig. 4 (See legend on previous page.)

Despite this species offering nectar in completely exposed drops [70], the flag petals appear to guide bat visits [88]. Similarly, dish-type flowers of *Hymenaea cangaceira* and *Lafoensia glyptocarpa* have fully exposed nectar [70] and long stamens radiating from the flower center. Regarding the fundamental inaccuracy index, species with a diffuse pollen placement strategy, specifically *Tarenaya longicarpa* (0.386) *and N. longifolium* (0.569), displayed the high values. However, other species employing the same strategy, such as *Calliandra aeschinomenoides* (0.131) and *Hymenaea cangaceira* (0.145), exhibited intermediate values. This partially agree our initial expectations. These conditions partially support our expectations that in diffuse pollination, there is no need for a precise correspondence between the lengths of male and female floral structures.

Among flowers with stroke-type pollen placement, those with nototribic pollen deposition stand out, encompassing various floral types such as flag, funnel-shaped, and dish. In each of these floral types, nectar is confined to the base of the floral tube, forcing bats to access it in a specific direction, whether in flag-type flowers [67], funnel-shaped flowers [75], or dish-type flowers [70]. Consequently, the flowers of all these species rub their anthers against parts of the bat, such as the bat's neck, chest, or belly, as it moves toward the nectar, leaving a trail of pollen. An uncommon case of stroke-type pollen placement in our sample was *Mimosa lewisii*, which, despite having a brush-type morphology and completely exposed nectar drops, exhibit stroke pollen deposition due to the short length of the reproductive structures [79]. Flowers employing the stroke-type pollen placement strategy showed intermediate inaccuracy values, including species such as *Mimosa lewisii* (0.103), *Bauhinia pentandra* (0.241), *B. acuruana* (0.270), and *Dyckia spectabilis* (0.303).

Most flowers exhibiting a stamp-type pollen placement are found in tubular or campanulate-shaped cactus flowers with frontal pollen deposition on bat faces. In Cactaceae species, a curtain of anthers surrounds the interior of the floral tube, and by inserting their head into this tube, bats receive a pollen stamp on their face [64, 76, 89]. Notably, the throat-type of *Harpochilus neesianus* [78] deviates from this pattern, utilizing a lever mechanism that pushes reproductive structures against the uropatagium of visiting bat. Species employing the stamp-type pollen placement strategy, namely *Harpochilus neesianus* (0.066) and *Pilosocereus pachycladus* (0.067), exhibited the lowest inaccuracy values, indicating the highest floral precision among the species in this study. In this sense, pollen deposition in a specific region of the pollinator's body could be a way for the plant to ensure that particular area of the animal belongs to it, considering the stick characteristics of bat fur [29]. In contrast, *Xiquexique tuberculatus*, utilizing the same strategy, demonstrated intermediate values.

Regarding the differences in the contribution of male and female functions to the precision of pollination systems in chiropterophilous flowers, we initially expected the male function to contribute more to inaccuracies due to asymmetry between male and female functions [23, 27, 90]. Surprisingly, our results demonstrated the opposite. One possible explanation is that the male function involves spreading pollen across the animal's body, and part of the variation in male function is influenced by the bat's brief interaction time with the plant [76, 91, 92], during which it remains still for a fleeting moment in the flower. In contrast, the female function is limited to the act of contacting the stigma. Consequently, the stigma operational distance could experience greater variability across a plant population as it attempts to make contact with various areas of the bat's body, considering that the stigmatic surface area is much smaller than the bat's body surface area covered with pollen [29].

Although the amount of pollen of the species was not explained by the type of pollen deposition strategy or fundamental accuracy, it is important to note that all species exhibited high values of pollen grains per flower and P/O ratio. The high pollen production exhibited by chiropterophilous species could be a potential source of selective pressure, as some nectar-feeding bats (Phyllostomidae-Glossophaginae) include pollen grains in their diet [93]. Additionally, this could be a contrivance used to compensate for the diffuse pollination that some species perform,by spreading pollen across the bat's body, they can ensure that a small part is removed by conspecific stigmas. In *Calliandra aeschynomenoides* and *Mimosa lewisii* the contribution to pollen production may be even more intense, as the pollen grains of these species are grouped in poliads and tetrads, respectively. According to Cruden et al. [33, 94], the pollen-ovule ratio of species with grouped pollen grains tends to be low; however, in this study, this was confirmed only for the number of ovules.

The chiropterophilous species studied here employ various strategies for mechanical adjustment to bats, which can result in a complex pollen landscape on the bodies of these animals. Some species with similar floral morphology manage to avoid phenological overlap, while others prevent morphological overlap through the differential pollen deposition on the pollinator's body. Sympatric plant species with similar floral morphology, sharing the same flowering period, and depositing pollen in the same region of a pollinator's body, may lose pollen grains whenever the vector touches the reproductive organs of its competitor [95, 96]. However, divergence in floral morphology can mitigate this competition by placing pollen in different regions of the pollinator's body [26, 67, 97]. Below, we explore how each of these processes may be occurring in the species within the studied guild.

One of the most explored areas for pollen deposition on the bodies of bats is the face. This is largely due to several species of cacti, in which the bats' behavior of inserting their heads into the ring of anthers at the entrance to the flower causes pollen to be deposited on their faces [64, 76, 89, 98]. Given the potential for competition among these species, different strategies arise in these plants, such as enhancing mechanical fit, increasing pollen production, or avoiding phenological overlap. For example, *Pilosocereus pachycladus* exhibited the least deviation from the optimal pollination point among the Cactaceae species in this study, indicating higher floral precision. In contrast, *Xiquexique tuberculatus* showed greater variation in the mean operational distances of male and female functions but invested more in pollen production than *P. pachycladus*. In both species, the male function contributed more to floral precision. Despite *Xiquexique tuberculatus* flowering year-round, the species avoids overlap with *P. pachycladus* by differing in peak flowering periods. Furthermore, stamp pollen deposition in Cactaceae could be a strategy to compensate for the low floral display exhibited by these species, as they produce few flowers per day [76, 99], possibly experiencing more intense negative effects of pollen loss to other species [100].

Another set of locations on the body of bats where intense competition for pollen deposition space appears to occur is the continuous area of the neck, chest, and belly. Plants that come into contact with these areas appear to differ significantly in the length of their reproductive structures. A clear example is that *Bauhinia* species bloom in part simultaneously and besides sharing similar floral morphology, they have common pollinators, the bats *Glossophaga soricina* and *Lonchophylla mordax* [67]. It is possible that the female function of *B. pentandra* (larger than *B. acuruana*) has evolved to touch more areas of the bat's body, avoiding morphological pollen overlap with its congeners, given the species' overlap during peak flowering, and both species' reproductive structures touching the ventral region of the bat's body [67]. Even more dramatic is the example of *Helicteres baruensis*, which, despite having nototribic pollen deposition, has an androgynophore so long that it reaches the terminal parts of the bat's belly, where other plants can hardly reach [71].

Furthermore, regarding plants with precise nototribic pollen deposition, other strategies may involve ensure more effective and abundant pollen deposition than other species. A possibility is intensifying the mechanical fit by causing the bat to hold itself even more firmly against the flowers. Tubular or funnel-like flowers [18], as *Ipomoea vespertilia*, secrete and store nectar at the base of the long floral tubes [74]. In this scenario, longer floral tubes extend the duration of bat visits, intensifying the mechanical fit and precision between the flower and the pollinator [19, 101]. In other plants, the arboreal habit combined with explosive flowering can confer a significant advantage by increasing the amount of pollen involved in the competition for space on the bat's body. *Hymenaea cangaceira* flowers do not restrict access to floral resources, its floral morphology allows the bat to insert its head into the flower and touch the stigma [68–70]. The species has an arboreal habit, sequential flowering, and each individual remains in bloom for two to four weeks, exposing thousands of flowers per night to attract pollinators [70]. These conditions can make it difficult for other plant species to compete with the large quantities of pollen that can be deposited over many sequential visits in *H. cangaceira*.

Plants with diffuse pollen deposition reach various parts of the bats' bodies, including the wings and back, which are rarely contacted by other plants with more precise deposition strategies, such as stamp and stroke. However, flowers that deposit pollen diffusely may also compete for space on the bodies of bats with plants with pollen deposition in specific locations. An example is the overlap in the means of the female function between *Bauhinia pentandra* and *Tarenaya longicarpa* suggests that these species may be competing for space on the pollinator's body, as they share a common pollinator, the bat *Glossophaga soricina* [88]. *Tarenaya longicarpa* exhibits diffuse pollen deposition, as anthers place pollen in various areas of the bat's body. In contrast, *B. pentandra* exhibits higher floral precision than *Tarenaya longicarpa* is consistent with expectations for flowers with fused floral parts. This adjustment is more easily observed in flowers that promote pollen deposition in a specific region of the pollinator's body, such as those with tubular morphology [101].

Another important point to note is the existence of sexual systems beyond hermaphroditic flowers in plants with diffuse pollen deposition. For instance, *Neocalyptrocalyx longifolium* has late-acting self-incompatibility to prevent self-fertilization [102] and, therefore, invests significantly in pollen production and dispersion to ensure reproduction due to its high dependence on bats for pollination. Additionally, the species is andromonoecious [102], and although it invests in many flowers to attract pollinators, only some of them will be available for pollination, as in others, the female organs are non-functional. *Tarenaya longicarpa* has a high dependence on pollinators for reproduction [103]; however, the species is polygamodioecious, having male, female, and bisexual flowers on the same individual [88], which likely contributed to the high inaccuracy in the female function.

Finally, one last area of the bat's body that deserves to be highlighted here is the uropatagium. *Harpochilus neesianus* illustrates a fascinating example of how exclusive mechanical adaptations can benefit plants in their pollination strategies. This species has evolved a unique system in which the bat deposits and removes pollen on a specific part of its body—the uropatagium membrane [54, 78]. This exclusivity allows *H. neesianus* to flower throughout the year, potentially competing with many other plant species. However, its ability to secure pollen in this private area gives it a significant advantage. According to Armbruster et al. [8], this specialized adaptation can enhance the plant's fitness, especially in situations with high visitation rates and uncertain pollen delivery [3, 104, 105]. Thus, *H. neesianus* stands out as a species apt to minimize competition for pollen with other plants, highlighting the importance of precise mechanical adjustments in evolutionary success. This condition may also have facilitated the development of a negative removal effect on nectar production (as described for *H. neesianus*), a strategy that manipulates bat behavior to encourage more visits across different individuals in the population rather than returning to the same flowers [54].

Conclusion

In conclusion, this study elucidates diverse pollination strategies employed by chiropterophilous plants, underscoring their adaptability to optimize reproductive success. The mechanical alignment between floral structures and bat pollinators emerges as a critical determinant of efficient pollination in these ecosystems. The findings contribute valuable insights to the field of floral ecology, shedding light on the intricate relationships between plants and their pollinators. Notably, the study emphasizes the importance of conserving specialized bat pollinators, highlighting their role in maintaining biodiversity and facilitating successful plant reproduction.

The observed variations in reproductive strategies among species accentuate the plasticity and efficacy of pollination mechanisms in response to specific ecological contexts. While interspecific competition for pollinator space is evident, morphological adaptations mitigate potential pollen interference, emphasizing the significance of divergent floral traits. As global ecosystems face challenges, understanding and preserving these specialized interactions become paramount [106, 107]. Conservation efforts targeting both chiropterophilous plants and their bat pollinators are essential for sustaining the delicate balance of these ecosystems. Ultimately, this study contributes not only to scientific knowledge but also advocates for the broader recognition of the ecological importance of bat-mediated pollination in maintaining the resilience and biodiversity of natural habitats.

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Authors' contributions
CASP conducted data collection, fieldwork, and wrote the initial draft. ICM secured funding, provided oversight, and conducted reviews. ADM conceptualized the study, conducted data collection, fieldwork, formal analysis, figure generation, secured funding, conducted final text review, and supervised the project. All authors have reviewed and approved the final manuscript.

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Availability of data and materials
The datasets used in the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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