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INSTITUTO DE CIÊNCIAS BIOLÓGICAS
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**FATORES ESTRUTURANTES, PADRÕES ESPAÇO-TEMPORAIS E NOVAS
INTERAÇÕES NA REDE ENTRE MORCEGOS VISITANTES FLORAIS E
PLANTAS DE UMA SAVANA NEOTROPICAL**

Ugo Mendes Diniz

Brasília

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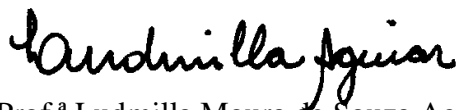
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RESUMO

A polinização por morcegos é um sistema tropical diverso e vital para o funcionamento de ecossistemas. Entretanto, a interação foi pouco estudada ao nível de comunidade e apenas recentemente tem-se tido o esforço de reconstruir suas redes de interação. Portanto, os fatores que estruturam as interações entre assembleias de plantas e morcegos visitantes florais são desconhecidos. O Cerrado, especificamente, destaca-se por ser subamostrado em termos de quiropterofilia. Por meio de uma amostragem zoocêntrica e ao longo de um ano fenológico, as interações entre morcegos visitantes florais e plantas foram amostradas em uma área de Cerrado no planalto central Brasileiro para reconstrução de sua rede mutualística. Foram analisados os papéis do acoplamento morfológico, sincronia fenológica e sobreposição espacial na estruturação da rede, assim como o efeito da abundância das espécies, ou neutralidade. Além disso, buscou-se descrever novas potenciais interações entre morcegos e plantas previamente não registradas. A rede mutualística entre plantas e morcegos visitantes florais apresentou uma estrutura pouco especializada e não aninhada, porém modular. Módulos foram associados a grupos funcionais de morcegos relacionados à filogenia, e separados por variáveis morfológicas. Entretanto, a sobreposição espaço temporal entre plantas e morcegos foi o principal preditor da estrutura da rede. Sub-redes temporais e espaciais revelaram sazonalidade no uso de plantas por morcegos e preferências intrínsecas pelo forrageamento em determinados tipos de vegetação, o que provavelmente é um reflexo da heterogeneidade espacial e da forte sazonalidade do Cerrado. Dentre as espécies consumidas por morcegos, a lorantácea ornitófila *Psittacanthus robustus* foi a segunda espécie mais visitada. Apesar de suas características ornitófilas, durante a estação chuvosa a espécie produziu ativamente néctar à noite e compôs até metade da dieta de morcegos visitantes florais. A magnitude dessa interação reforça o papel de morcegos como oportunistas ecológicos e sugere que *P. robustus* talvez seja um sistema em transição de síndrome. Este novo registro, juntamente com a visitação de outras plantas não-quirópterófilas, reforça que o Cerrado ainda é subamostrado em termos de interação morcego-plantas e ainda requer esforço para que se tenha conhecimento suficiente da riqueza do sistema e seu funcionamento no bioma.

Palavras-chave: Aninhamento, Cerrado, especialização, Loranthaceae, modularidade, morfologia, polinização por morcegos, quiropterofilia, redes ecológicas, savana neotropical, síndromes de polinização.

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INTRODUÇÃO GERAL

A polinização por morcegos é uma importante estratégia reprodutiva de angiospermas tropicais em termos de distribuição e ocorrência. Apesar de seu surgimento recente no Eoceno (Hu et al., 2008), mais de 250 gêneros de plantas pantropicais apresentam atualmente atributos florais adaptados à visita por morcegos nectarívoros, que são polinizadores relativamente grandes, noturnos e orientados por ecolocalização durante o forrageamento (Fleming et al., 2009). Estes atributos florais são bastante conspícuos no espectro morfofisiológico vegetal, e incluem antese noturna, corolas grandes com morfologias acusticamente conspícuas (campanuladas, cupuliformes ou em pincel), produção copiosa de néctar diluído e emissão de voláteis sulfatados (Faegri & Pijl, 2013, Willmer, 2011).

O Neotrópico abarca uma grande porção dos estudos acerca da síndrome devido à sua rica flora quiropterófila, a qual é especialmente representada por famílias como Cactaceae, Fabaceae e Malvaceae (Fleming et al., 2009). Nas florestas sazonalmente secas da Caatinga, por exemplo, plantas polinizadas por morcegos podem compor cerca de 13% da diversidade regional (Machado & Lopes, 2004). Em função disso, espécies quiropterófilas são a base da dieta para uma proporção relevante de espécies de morcegos no Brasil, não apenas para espécies especializadas das subfamílias Glossophaginae e Lonchophyllinae (Chiroptera: Phyllostomidae), as quais apresentam adaptações morfofisiológicas notáveis para uma dieta baseada em néctar e pólen (Datzman et al., 2010), mas igualmente para vários morcegos onívoros não especializados que utilizam recursos florais para complementação da dieta (e.g. Sazima et al., 1999). A diversidade de plantas quiropterófilas, portanto, é um elemento natural chave para manutenção de redes tróficas tropicais e apresentam valor conservacionista relevante.

As últimas décadas têm visto uma crescente literatura referente à quiropterofilia. Uma vasta quantidade de estudos de caso possibilitou a elucidação dos atributos florais relacionados à atração e interação com morcegos (e.g.; Aguiar-Rodriguez et al. 2019; Amorim et al., 2021; Buzato & Sazima, 1994; Machado et al., 1998; Martins et al., 2020), os quais estão relativamente solidificados. Seguindo ao nível de comunidade, vários estudos descreveram a importância relativa da síndrome em determinados ecossistemas (e.g. Oliveira & Gibbs, 2000; Machado & Lopes, 2004) e o uso de espécies vegetais por assembleias de morcegos nectarívoros (e.g. Muchhala & Potts, 2007; Sazima et al., 1999). Desse modo, tem-se conhecimento de certos padrões de partição de nicho dentro da guilda de morcegos visitantes florais. Por exemplo, flores quiropterófilas têm geralmente uma morfologia não restritiva, e não tendem a selecionar morcegos com base em caracteres morfológicos como tamanho do rosto ou língua (Fleming et al. 2009; Sperr et al., 2011). Ao mesmo tempo, morcegos morfológicamente mais especializados, os quais portam línguas e rostros mais longos, tendem a visitar uma maior diversidade de plantas em períodos de escassez de recurso, mantendo-se no nicho de visitantes florais (Gonzales-Terrazas, 2012, Tschapka, 2004). Portanto, ainda é incerto como as características morfológicas das espécies (e.g. comprimento da língua e rosto ou morfologia floral) estruturam a rede mutualística entre plantas e morcegos, assim como outras características intrínsecas, como fenologia ou preferências por determinados tipos de vegetação. Dentre os mais recentes avanços no estudo da quiropterofilia, destaca-se o estudo da interação ao nível de redes ecológicas com o objetivo de responder a essas perguntas.

Derivada da teoria de grafos aleatórios, a teoria de redes aplicadas à ecologia é um arcabouço teórico eficaz para descrever interações ao nível de comunidade, e possibilita a extração de inferências sobre relações de interdependência entre espécies e sobre a relação coevolutivos difusa entre grupos de espécies (Bascompte & Jordano, 2007). No

contexto da ecologia da polinização, a aplicação de conceitos da teoria de redes, como modularidade (Olesen et al., 2007), é capaz de identificar grupos funcionais relacionados às síndromes de polinização e determinar padrões de divergência de nichos por meio de atributos florais e de polinizadores (Phillips et al., 2020; Watts et al. 2016). No Neotrópico, alguns autores têm recentemente avançado o estudo das redes mutualísticas entre plantas e morcegos nectarívoros neotropicais. Mello et al. (2019) descreveram a meta-rede mutualística continental entre plantas e morcegos fitófagos (frugívoros e nectarívoros) na América Latina, reportando o papel de relações filogenéticas, ocorrência geográfica e variáveis morfológicas na estruturação de interações em diversas escalas. No Brasil, Queiroz et al. (2020) descreveram a rede mutualística noturna em uma área de Caatinga, a primeira rede ponderada contendo morcegos visitantes florais no Neotrópico, demonstrando uma alta sobreposição no uso de recurso florais por morcegos e destacando o papel chave da morfologia floral em possibilitar a interação com morcegos. Também na Caatinga, Cordero-Schmidt et al. (2021) descreveram em detalhe a rede entre morcegos nectarívoros (Glossophaginae e Lonchophyllinae) e plantas, reportando uma estrutura generalizada e estabilidade temporal.

Finalmente, a meta-rede de Oliveira et al. (2019), que sintetizou as interações de nectarivoria e frugivoria no Cerrado, possibilitou definir quais tipos de habitat devem ser focos de conservação destas interações no bioma, onde ca. 50% de vegetação nativa já foi alterada em função da expansão dos horizontes agrícolas e urbanos, e permanece sob perda crônica de biodiversidade (Strassburg *et al.* 2017). Apesar desta contribuição, o Cerrado ainda é um dos biomas brasileiros mais subamostrados em termos de interação entre plantas e morcegos polinizadores e carece de estudos que tenham amostrado a dieta de morcegos nectarívoros em toda uma comunidade. Apesar de conhecermos parte da quiropterofauna do bioma (Bernard et al. 2011), poucos autores de fato exploraram a

interação de morcegos com flores com profundidade. Os estudos tendem a focar em uma ou poucas espécies de morcegos ou plantas (Bobrowiec and Oliveira, 2012; Coelho and Marinho-Filho, 2002; Colevatti et al., 2010; Gibbs et al., 1999; Gribel and Hay, 1993; Martins et al. 2020). A estrutura das redes de polinização por morcegos está começando a ser desvendada principalmente na Caatinga (Cordero-Schmidt et al., 2021; Queiroz et al., 2020), e seus fatores estruturantes em larga escala (Mello et al., 2019). No entanto, as variáveis ecológicas que determinam as frequências de interações entre espécies ao nível local ainda são desconhecidas e o Cerrado permanece especialmente subamostrado no campo da quiropterofilia.

A identificação dos padrões e mecanismos associados ao processo de estruturação de redes de polinização é de forte interesse conservacionista, especialmente em ecossistemas sob regimes cíclico de seca e com forte heterogeneidade espacial. Nesses ecossistemas a manutenção da riqueza de plantas-recurso é um fator chave para assegurar a continuidade temporal dos sítios de alimentação de polinizadores e dos serviços ecossistêmicos associados (Schellhorn 2015). Os ambientes savânicos e sazonais do Cerrado, em especial, contêm as espécies nectarívoras especializadas *Lonchophylla dekeyseri* e *L. bokermanni*, duas representantes das poucas espécies de morcegos ameaçadas de extinção no Brasil (Aguiar 2016; Aguiar & Bernard 2016; Aguiar & Pereira 2019), o que intensifica a urgência por decifrar os padrões de interação de morcegos com espécies-recurso no escopo de comunidade.

Portanto, por meio de uma amostragem zoocêntrica intensiva ao longo de um ano em vegetação nativa e preservada do Cerrado no Planalto Central brasileiro, este trabalho se propôs a reconstruir a primeira rede mutualística ponderada entre morcegos visitantes florais e plantas em uma comunidade do bioma. Teve-se, como objetivo central, determinar quais as variáveis ecológicas responsáveis pela estruturação das interações

entre plantas e morcegos visitantes florais, com destaque para o papel da alta variabilidade ambiental (sazonalidade e heterogeneidade espacial) do Cerrado na seleção de recursos florais por morcegos ao longo do tempo e espaço. Com isso, foram objetivos deste trabalho, especificamente: (i) sistematizar o uso de recursos florais por morcegos visitantes florais (incluindo morcegos nectarívoros especializados e oportunistas) em uma região do Cerrado e descrever a estrutura de sua rede mutualística. (ii) Determinar quais os fatores estruturantes da rede de interações com base no paradigma nicho-neutralidade (Chávez-González *et al.* 2020, Vázquez *et al.* 2009). Ou seja, comparar o papel relativo da abundância das espécies em estruturar as interações (neutralidade, interações ocorrem ao acaso) e de mecanismos relacionados ao nicho das espécies, como acoplamento morfológico entre morcegos e flores e sobreposição espacial e temporal entre espécies. (iii) Descrever potenciais novas interações entre morcegos visitantes florais e plantas, desta forma contribuindo com o conhecimento acerca da síndrome no Cerrado.

Para abranger esses tópicos, esta dissertação encontra-se dividida em dois capítulos, formatados de acordo com periódicos internacionais de alta relevância para o tema. O primeiro capítulo buscou descrever a rede mutualística entre plantas e morcegos visitantes florais e identificar suas variáveis estruturantes, além de identificar potenciais padrões temporais e espaciais da rede derivados da sincronia fenológica entre espécies e preferências de morcegos por zonas de forrageamento, respectivamente. O segundo capítulo teve como foco a espécie ornitófila *Psittacanthus robustus* (Loranthaceae), para a qual não havia registro de visitação por morcegos, mas que se apresentou como um recurso chave na comunidade apesar de ter atributos florais fortemente relacionados à polinização por aves (Guerra *et al.*, 2014). O capítulo buscou investigar a dinâmica noturna de néctar da espécie, a relação com a taxa de visitação por animais noturnos e

compreender a contribuição relativa da visitação de animais diurnos e noturnos no seu sucesso reprodutivo.

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1 **CAPÍTULO I**

2 Manuscrito a ser submetido ao periódico *Functional Ecology*

3
4 **Morphology is a key axis of the flower-visiting niche of bats in a Neotropical**
5 **savanna, but spatiotemporal overlap with plants drives their interaction network**

6
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13
14 **ABSTRACT**

15 Bat pollination is a diverse and vital mutualistic system in tropical ecosystems.
16 Knowledge of the functional aspects of chiropterophily and the diversity of bat-pollinated
17 species is increasing, but the ecological variables that structure community-wide
18 interaction networks are still largely unknown. We reconstructed an interaction network
19 between phyllostomid bats of different feeding guilds and plants in the Brazilian Cerrado,
20 a biodiversity hotspot undersampled in terms of chiropterophilous interactions. We
21 collected data on species' abundance, morphologies, phenological match and spatial
22 overlap to assess if the network is assembled neutrally or by niche mechanisms through
23 a maximum likelihood approach. We found a generalized and moderately modular
24 network where bat rostrum length and body robustness explained individual bat degree
25 and determined module partitioning. Nectar bat modules comprised less robust species
26 with longer rostrums. Other modules included frugivores or insectivores only and

1 comprised plant species with wide flowers or flower-like inflorescences, several of which
2 were non-chiropterophilous and were not visited by specialized nectar bats. Wide-
3 flowered or short-tubes species, either chiropterophilous or not, were the most central for
4 the network, while nectar bats were less specialized than opportunistic frugivores as they
5 could access more species due to morphological specialization. However, the spatial and
6 temporal overlap between bats and plants, and not morphological fit, were the best
7 predictors of network structure. Specialized nectar bats were rarer in the dry season,
8 where nectarivory by frugivores in wide-flowered species increased. Frugivores were also
9 much more common inside the forest, creating a conspicuous sub-network with forest
10 plants, while nectar bats visited mostly savanna and forest-edge plants. Therefore, we
11 show that bat-flower interactions are not neutrally assembled despite entailing generalist
12 floral visitors, as phyllostomid bats and plants in the highly seasonal and heterogeneous
13 Cerrado show spatiotemporal trends which drive their interactions network. Floral and
14 bat morphology, in turn, dictate the formation of functional groups within the flower-
15 visiting niche that is associated with bats' phylogenetic affinity, but do not fine-tune
16 network structure. In addition, finding strong and novel interactions between bats and
17 non-chiropterophilous also suggested that the diversity of plants used by bats in the
18 Cerrado has not been sufficiently sampled.

19

20 **Keywords:** Bat pollination, Cerrado, Chiropterophily, Glossophaginae, pollination
21 syndrome, *Lonchophylla*, modularity, nectarivory, nestedness, network, specialization.

22

23

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25

1. INTRODUCTION

One of the main questions posed by community ecology is whether species interact according to observable niche mechanisms based on intrinsic species traits, or if networks are neutrally assembled, where interactions are determined by the relative abundance of species (Gravel et al., 2006; Krishna et al., 2008; Vázquez, 2005). Plant-pollinator interactions are a fertile ground to test these contrasting hypotheses regarding interaction drivers. The different adaptive pathways of flowering plants towards biotic pollen vectors have led to a myriad of pollination systems in nature with contrasting degrees of phenotypic specialization (Armbruster, 2017), which provide models to assess which ecological variables structure community-wide interactions along a specialization continuum (Vizentin-Bugoni et al., 2018). Studies have acknowledged that the relative abundance of species is often a good predictor of networks comprising generalized systems in communities with a low diversity of functional traits, such as temperate insect-pollination networks (Olinto and Fox, 2014; Vázquez et al., 2009).

On the other hand, niche mechanisms, which include morphological and spatiotemporal mismatches, either complement the role of abundance or are the main drivers in structuring networks of more derived pollination systems, mainly in tropical vertebrate or insect pollination networks where phenotypic specialization increases (Gonzalez and Loiselle, 2016; Sazatornil et al., 2016, Sonne et al., 2020). Unrealized interactions deriving from these ‘forbidden links’ (Vázquez et al., 2005) are often responsible for higher-level network patterns such as modules, i.e., subgroups of species within a network in stronger interactions with one another (Olesen et al., 2007). Modularity is a very informative network pattern as it can be associated with species traits to unveil diverging niches related to certain ecological mechanisms (Phillips et al., 2020). However, such association has seldom been performed (Maruyama et al., 2014), and the

1 interplay between neutrality and niche mechanisms in driving network structure are far
2 more explored in certain groups whose flowers present floral morphologies that
3 immediately suggest a correlation with pollinator mouthparts, such as the long and tubular
4 corollas of bird- (Biddick and Burns, 2018; Vizentin-Bugoni et al., 2014) and moth-
5 pollinated plants (Johnson et al., 2017; Sazatornil et al., 2016). Other vertebrate-
6 pollination syndromes, such as chiropterophily, comprise relatively large wide-flowered
7 species that tend not to selectively restrict nectar bats based on their morphology nor the
8 visitation by floral visitors outside the syndrome (Fleming et al., 2009, Queiroz et al.,
9 2020). At the same time, studies suggest that nectar bats have a relatively high overlap in
10 resource use and are not very selective towards their floral resources, especially when
11 they are abundant (Sperr et al., 2011, Tschapka et al., 2004), forming generalized
12 networks (Cordero-Schmidt et al., 2021). These patterns point to a possibly stronger role
13 of species abundances or other types of forbidden links other than morphology in
14 assembling their networks, such as spatiotemporal mismatches (Maruyama et al., 2014).

15 However, the functional study of chiropterophily at larger, community-wide
16 scales is still in its early stages despite the richness of tropical species within the
17 syndrome, whose more than 250 plant genera that it entails seconds only ornithophily
18 within the realm of vertebrate pollination syndromes (Fleming et al., 2009; Sekercioglu,
19 2006). Thus, the ecological variables that underlie network formation and module
20 partition in bat pollination systems have not been determined. There is little and
21 conflicting evidence, for instance, if differences in rostrum or tongue size, a trait related
22 to the specialization in floral resources (Solmsen, 1998), drives actual ecological
23 specialization and niche partitioning among sympatric bat assemblages (Sperr et al.,
24 2011; Henry and Stoner, 2011; Tschapka et al., 2004). Moreover, non-specialized and
25 opportunistic nectar-feeding phyllostomids, such as primarily frugivorous or omnivorous

1 bats, also act as floral visitors (Vieira and Carvalho-Okano, 1996; Gribel et al., 1999;
2 Silva and Peracchi, 1999). Nevertheless, they have been little explored in pollination
3 networks (Queiroz et al., 2020; Mello et al., 2019), impairing our understanding of the
4 functional groups within the chiropterophilous syndromes and how morphology and other
5 variables are associated with their emergence.

6 Therefore, we aimed at filling these gaps by reconstructing a thorough floral-
7 visitation network including all phyllostomid groups and addressing the different
8 ecological variables that potentially structure the network, focusing on species
9 morphologies, abundances, and spatiotemporal overlap. Our study was conducted in a
10 savanna of the Brazilian Cerrado, a biodiversity hotspot and habitat for at least six
11 specialized nectar bat species (Marinho-Filho et al., 2002). Although the Cerrado has
12 received considerable research effort on the ecology of pollination systems (e.g. Deus and
13 Oliveira, 2016; Ishara and Maimoni-Rodella, 2011; Oliveira et al., 2004; Oliveira and
14 Gibbs, 1999), bat-flower interactions have been studied in-depth by only a handful of
15 authors (Bobrowiec and Oliveira, 2012; Coelho and Marinho-Filho, 2002; Colevatti et
16 al., 2010; Gibbs et al., 1999; Gribel and Hay, 1993; Martins et al., 2020). Thus, our work
17 also aimed at building a solid database on the species used by bats in the region and their
18 relative importance in their diet, while also potentially uncovering novel interactions.

19

20 **2. METHODS**

21 *2.1 Study site*

22 This study was conducted in the Brasília National Park (PNB), Federal District,
23 Brazil (15°39'57" S; 47°59'38" W), a Protected Area within the city and national capital
24 Brasília. The Park has an area of 42.355 ha and is an important remnant of typical
25 vegetations found in the Cerrado of the central highlands of Brazil, which generally

1 comprises a mosaic of patches of gallery forests along rivers surrounded by a matrix of
2 bushy savanna (*Cerrado sensu stricto*), arboreal savanna, decidual dry forests and
3 grasslands (Silva et al., 2006). The climate in the region falls into the Cwa (1000 – 1200
4 m) and Cwb (above 1200 m) category in the Köppen scale, categorizing an altitudinal
5 tropical climate, with marked rainy (October to March) and dry (April to September)
6 seasons. The PNB may be regarded as a large ecological island due to the dense urban
7 and rural occupations surrounding it and low connectivity with other remnants.

8 The work was carried out in eight fixed sampling sites scattered evenly throughout
9 the PNB and separated by at least two kilometers from one another. The sites represented
10 the different types of vegetation within the savanna-forest gradient: four *Cerrado sensu*
11 *stricto* sites (typical savanna, containing a mostly bushy stratum with low stature trees);
12 two gallery forest edges sites (ca. 5 m from the forest limit, containing a transitional
13 community) and two gallery forest interior sites. We considered these vegetation types
14 the most appropriate foraging areas to sample interactions made by flower-visiting bats,
15 as most chiropterophilous plants are either bushes, trees or epiphytes, and rarely herbs
16 (Diniz et al., 2019).

17

18 *2.2 Bat and interaction samplings*

19 Bat-plant interactions were sampled monthly for one phenological year through a
20 zoocentric approach (Jordano, 2016), where interactions were assessed by pollen loads
21 collected from bat individuals. Monthly field campaigns to capture bats were carried out
22 from October 2019 to February 2020, and from August to September 2020. The
23 campaigns of March through July were conducted in 2021. Every month, we carried out
24 eight sampling nights during periods of low moonlight intensity, each associated to one
25 of the eight sites (except December and May, when five and seven sampling nights were

1 conducted, respectively). In each night, we set 10 mist nets (3 x 12 m, Ecotone®) at
2 ground level randomly within the site, which were opened at sunset and closed after six
3 hours. We accumulated a total sampling effort of 5520 mist net hours or 33120 m² of net
4 area.

5 All captured bats were sampled for pollen, irrespective of family or feeding guild.
6 We used glycerinated jelly cubes (ca. 4 x 4 x 4 mm) stained with 2% Safranin O (Voigt
7 et al., 2009) to collect pollen grains from the external body of bats (head, torso, wings
8 and uropatagium). Cubes were stored individually, and care was taken not to contaminate
9 samples with pollen from other individuals by sterilizing tools and use of disposable
10 gloves. Samples were later mounted on microscope slides for the identification of pollen
11 grain by external morphology through light microscopy. Palynomorphs were identified
12 to the lowest-possible taxonomical level using both specialized literature (e.g. Cassino
13 and Meyer, 2011; Salgado-Laboriau, 1973; Moreira et al., 2013) and a large personal
14 reference pollen collection from plants from the PNB (see the next section for details on
15 the collection of pollen from plants). Due to the occurrence of closely related plant species
16 with similar pollen morphologies in the samples, palynomorphs were sometimes
17 classified to the genus or family level, or grouped in entities representing two similar
18 species. Legitimate interactions with pollen types were only considered when 10 or more
19 pollen grains were found in a sample for small-grained species (less than 40 µm of polar
20 diameter) or 5 or more grains for large-grained species (40 µm or more).

21 After pollen collection, bats were identified using specialized keys (Dias et al.,
22 2016; Gardner et al., 2008), sexed and marked with banded necklaces. The following four
23 ecomorphological variables were measured for each individual: (i) forearm length and
24 (ii) body mass, used to calculate the body condition index (BCI, mass-forearm length
25 ratio) (Reynolds e Korine, 2009), a proxy of body robustness that is associated with the

1 ability of bats to hover and/or to land on plants to interact with flowers. We also calculated
2 (iii) the longest skull length (distance from the edge of the occipital region to the anterior
3 tip of the lower lip) and (iv) rostrum length (distance from the anterior tip of the eye to
4 the anterior tip of the lower lip), which were used to calculate the rostrum-skull ratio
5 (RSR), a proxy of morphological specialization to floral visitation, with bats with longer
6 rostra considered to be more specialized in nectar consumption (Tschapka, 2004). After
7 these measurements, bats were released. Vouchers of each species (a non-reproductive
8 male and female) were collected when possible and deposited in the Mammal Collection
9 of the University of Brasília. All permits to handle bats were granted.

10 To evaluate the sampling completeness of the bat community (Phyllostomidae
11 family only, which may act as floral visitors) and of the pollen types found on bats, we
12 employed the Chao1 asymptotic species richness estimator (Chao and Chiu, 2016). For
13 the estimation and plotting of the rarefaction curves, we used an individual-based
14 sampling effort, and sampling completeness was calculated as the percentage of bat or
15 pollen types found in the community in relation to the predicted by the estimator (Chacoff
16 et al., 2012).

17

18 *2.3 Assessment of the plant community*

19 In each of the eight sampling sites, we delimited either a 1000 x 10 m (Cerrado
20 *sensu stricto* and forest edge sites) or a 1000 x 5 m forest interior transection (tree density
21 in forest sites impaired visibility). We walked these transections monthly for one year
22 (January and February 2020, August to December 2020, and March to July 2021) to build
23 a floristic inventory of plants of interest and to estimate their monthly abundance of
24 flowering individuals. Plants of interest were defined as those already known to be

1 pollinated by bats and presenting chiropterophilous traits (*sensu* Faegri & Van Der Pijl,
2 2013, Willmer, 2011).

3 However, bats may also use plants that do not fit the chiropterophilous syndrome
4 (e.g., generalist flowers or plants from other syndromes with long antheses that include
5 nighttime) (Vieira and Carvalho-Okano, 1996; Queiroz et al., 2020). Therefore, we also
6 included in the monthly assessment any plant that could potentially be visited by bats,
7 whose flowers passes all the three following criteria:

8 1 – Nectar or pollen presented as the main reward to visitors. This criterium
9 excludes highly specialized insect-pollination system, such as oil-secreting
10 Malpighiaceae;

11 2 – Interaction surface of at least 1 cm. Interaction surface is here defined as the
12 area required by bats to interact with the flower, i.e., to insert their tongues and snouts
13 while hovering or to cling onto the inflorescence. This criterion excludes very small
14 generalist or insect-pollinated flowers where the visitation by bats is mechanically
15 unlikely and energetically unrewarding. The 1 cm threshold applies to the corolla
16 diameter in non-tubular actinomorphic flowers or to the diameter of the corolla opening
17 in tubular flowers. Exceptions were small and actinomorphic flowers aggregated in dense
18 inflorescences forming pseudanthia (“false flowers”) (e.g. genera *Calliandra*,
19 *Stryphnodendron*, *Lamanonia*). In these cases, the pseudanthium was considered the
20 pollination unit, and the 1 cm threshold was applied to inflorescence diameter.

21 3 – Reward must be promptly available for bats. This criterion excludes species
22 with selective morphological mechanisms, such as quill-shaped bee-pollinated flowers or
23 flowers with long and narrow calcars.

24 All flowering individuals of interest species found in the transections were
25 registered and had vegetative and reproductive organs collected for identification. A

1 variable number of flowers/inflorescences ($n = 5-18$, according to availability) were
2 collected per species for the morphometric analysis. For each species, we calculated floral
3 tube length (FTL), corresponding to the distance between the base of the corolla, calyx
4 or hypanthium (depending on the species) to its opening, and the corolla's outermost
5 diameter (COD), which refers to the interaction surface cited above and corresponds to
6 the diameter of the corolla opening (tubular flowers) or simply the corolla diameter (non-
7 tubular flowers). For pseudanthium-forming species, we measured the inflorescence
8 width. Pseudanthia and open flowers received a zero FTL value. Finally, we collected
9 reference pollen samples from all species by brushing the stained jelly cubes (see the
10 section above for details) against the anthers of open flowers, which were later mounted
11 on microscope slides and used to aid in the identification of pollen types found on bats.
12 Plant voucher were deposited in the Herbarium of the Botany Department, University of
13 Brasília.

14

15 *2.4 Data analysis*

16 **Network structure**

17 We used the interactions between bats and pollen types to build the weighted
18 adjacency matrix $i \times j$, with each cell corresponding to the number of individuals of bat
19 species i that had legitimate interactions with plant species or morphotype j . With this
20 matrix, we calculated three structural metrics to describe the network. First, nestedness,
21 using the weighted NODF metric (Almeida-Neto & Ulrich, 2011). Nested networks are
22 characterized by interaction asymmetries, where generalist core species interact with
23 gradually more specialized species, causing peripheral and ecologically specialized
24 partners to be only a subset of the pool with which generalists interact (González et al.,
25 2020). The index was normalized to vary from zero to one, with one representing a

1 perfectly nested network. Second, modularity, using the weighted Q_w metric calculated
2 by the DIRTLPAwb+ algorithm (Beckett, 2016). A modular network contains subgroups
3 of species in which interactions are stronger and more frequent than with species out of
4 these subgroups (Dupont & Olesen, 2009). In pollination networks, modules may reveal
5 ecological niches shared by functionally similar species (Amorim, 2020; Maruyama et
6 al., 2014). Q_w varies from zero to one, where one represents a perfectly modular network.
7 Third, complementary specialization through the H_2' metric (Blüthgen, 2010). This
8 metric measures how unique, on average, are the interactions made by specie in the
9 network, considering interaction weights. It varies from zero to one, where a value of one
10 indicates a specialized network where interactions perfectly complement each other
11 because species do not share partners.

12 We assessed the significance of structural metrics using a Monte Carlo procedure
13 based on random matrices. Employing the algorithm developed by Vázquez et al. (2009)
14 for pollination networks, we used or interaction matrix to build a set of 1000 random
15 matrices. The algorithm distributes interactions from the observed matrix randomly
16 among species, keeping network size and connectance (proportion of realized interactions
17 out of a total possible) constant, while allowing column and row sums to vary. The metrics
18 $wNODF$, wQ and H_2' were calculated for the 1000 matrices, generating a distribution for
19 each. The significance (p) of the metrics from the real interaction matrix was calculated
20 as number of times the random matrices had a metric value equal or higher than the
21 observed value, divided by 1000. Significance was considered $p \leq 0.05$.

22 Finally, with the objective of assessing whether bats from different feeding guilds
23 form one cohesive network or approach a compound network, we created two partial
24 networks from the observed matrix, one of which contained nectarivores only and their
25 interactions, and the other contained bats from other guilds (frugivores and insectivores)

1 and their interactions. These same three metrics were calculated, and the null model
2 procedure was repeated for each.

4 **Drivers of network structure**

5 The role of different ecological variables in structuring the network was
6 assessed using the approach of probability matrices developed by Vázquez et al. (2009).
7 This framework considers that an interaction matrix Y is a product of several probability
8 matrices of the same size as Y , with each matrix representing the probability of species
9 interacting based on an ecological mechanism. Thus, adapting the equation from Vázquez
10 et al. (2009) to our study system and objectives, we have:

$$11 \quad Y = f(A, M, P, S)$$

12 Where Y is the observed interaction matrix and a function of interaction
13 probability matrices based on species relative abundances (A), representing neutrality as
14 species interact by chance, and any of the following niche mechanisms: species
15 morphological fit (M), phenological overlap (P), representing temporal matching; and
16 spatial overlap (S), representing species spatial aggregation and foraging zones. We built
17 models containing each of these matrices in the following ways:

18 **Relative abundance (A):** matrix cells were the products of the relative abundances
19 of bat and plant species. Bats' relative abundances were determined through capture
20 frequencies (each species capture frequency divided by all captures) and plants' relative
21 abundances of flowering individuals were determined through transection frequencies
22 (each species' summed abundance in all transections and all months divided by the pooled
23 abundance of all species in the network). Cell values were normalized to sum one.

24 **Morphological fit (M):** cells were the probability of species interacting based on
25 morphological traits. Morphologically specialized bats are more likely to interact with

1 both open and tubed plants, while morphologically accessible flowers are more likely to
2 interact with all bats. For bats, we calculated the morphological specialization index
3 (MSI) as the ratio between RCR (rostrum-cranium ratio) and BCI (body condition index).
4 Higher values indicate a higher specialization to floral visitation due to a lower body
5 robustness and longer snout length. For plants, we used the corolla's outermost width
6 (COD) as a restriction variable, as it seems to be the most important variable in allowing
7 bat visitation (Queiroz et al., 2020). Most interest plants in the study did not have tubular
8 shapes or had very wide tubes, thus we did not include this variable to measure plant
9 restriction. Finally, each cell of this probability matrix was calculated as MSI multiplied
10 by COD. Higher cell values indicate better morphological fit and thus a higher probability
11 of interaction. Cell values were normalized so that the sum of all matrix elements would
12 result in one.

13 Phenological match (P): cells were the number of months that bat species and
14 flowering individuals of plants species co-occurred in the site. Cell values were
15 normalized to sum one.

16 Spatial overlap (S): cells were the number of individuals from a bat species
17 captured in sites where the plant species was registered in the transection, considering all
18 capture months. Cell values were normalized to sum one.

19 Because more than one ecological mechanism may be at play simultaneously in
20 driving network structure, we built an additional set of models resultant from the element-
21 wise multiplication of individual probability matrices. We produced all possible
22 combinations, resulting in the following models: MA, MS, MP, AS, AP, SP, MAS, MAP,
23 MPS, APS and MAPS, a total of 15 models plus a null mode in which all cells in the
24 matrix had the same probability value. All these compound matrices and the null model
25 were also normalized to sum one after multiplications.

1 To compare the fit of these probability models with the real data, we conducted a
2 maximum likelihood analysis (Vázquez et al., 2009). We calculated the likelihood of each
3 of these models in predicting the observed interaction matrix assuming a multinomial
4 distribution for the probability of interaction between species (Vázquez et al. 2009;
5 Vizentin-Bugoni et al., 2014). To compare model fit, we calculated the Akaike
6 Information Criterion (AIC) for each model and their variation in AIC (Δ AIC) in relation
7 to the best-fitting model. The number of species used in the probability matrices were
8 considered model parameters to penalize for model complexity. The simplest models (M,
9 A, P and S) had 28 parameters, while the most complex (MAPS) had 112 parameters,
10 resultant from matrix multiplications.

11 We repeated this procedure for the two partial networks containing either
12 nectarivore only or bats from other feeding guilds only to also assess the structuring
13 factors within distinct functional groups. To conduct these analyses, we excluded some
14 plant species from the network that could not have their interaction probabilities
15 measured, such as species that were found in pollen samples but not registered in the park,
16 or pollen types that could not be identified to the species level. Therefore, the interaction
17 networks Y and the two partial networks did not include these species.

18

19 **Exploring the individual factors**

20 *Morphology*

21 We assessed the effect of plant and bat morphological variables individually on
22 network structure and evaluated the existence of niche partition through complementary
23 morphologies. We followed the framework of Phillips et al. (2020) that correlates
24 network concepts (especially modularity) with the distribution of morphological variables
25 of pollinators to unveil patterns of niche divergence in pollination networks. Given the

1 most parsimonious module configuration suggested by the algorithm, we compared
2 modules in terms of the distribution of morphological variables of the bat (RCR and BCI)
3 and plant (FTL and COD) species that composed the module. We included in the analysis
4 measures from all bat individuals captured from the species composing the modules.
5 Differences between modules means were tested with one-way ANOVAs.

6 To test whether morphological traits from bats at the individual level affect their
7 role as floral visitors and fidelity to plant species, we measured the effect of bats'
8 individual MSI (morphological specialization index) on individual degree, i.e., the
9 number of pollen types found in each individual's sample through regression analysis.

10

11 *Temporal and spatial overlap*

12 To assess how phenological and spatial overlap between bats and plants structured
13 interactions and identify possible mismatches throughout the seasons or habitats, we
14 created the following sets of partial networks:

15 (i) Temporal networks. Four partial networks corresponding to bat captures and
16 interactions sampled during four distinct periods in the region characterized by
17 distinct assemblages of flowering plants: peak dry season (May – July), dry-
18 rainy transition (August – October), peak rainy season (November – January)
19 and rainy-dry transition (February – April).

20 (ii) Spatial networks. Three partial networks corresponding to bat captures and
21 interactions sampled in distinct vegetation types: *Cerrado sensu stricto*
22 (savanna), gallery forest edges and gallery forest interiors.

23 In addition, we measured two variables to translate the relative importance of
24 nectarivores and frugivores across seasons and habitats: the ratio of specialized nectar
25 bats captured concerning total captures in a given month or habitat type, and the

1 importance of frugivores, or the proportion of interactions with plants that were made by
2 frugivores concerning all interactions in a given month or habitat type. For the temporal
3 analysis, these two variables were measured for each month using capture sites as
4 replicates, and we used circular statistics to detect seasonal trends (Morellato et al., 2010).
5 We measured the mean degree of the Rho vector (r) and its length for each variable to
6 test for aggregation. The r vector varies from zero to one. Higher values indicate
7 aggregated data, suggesting a seasonal phenomenon. A Rayleigh test was performed for
8 each variable to test the significance of data aggregation. Because values were
9 proportions, they were multiplied by 100 before analysis

10 To compare the nectarivore ratio and nectarivory by frugivores across space, we
11 measured them for each habitat type (savanna, forest edge and forest interior) using
12 months as replicates and compared the means through one-way ANOVAs.

13

14 **Bat functional groups**

15 We calculated species-level network metrics to characterize each plant and visitor
16 species in terms of their importance to the network and specialization, and to compare
17 larger functional groups (specialized nectarivores *vs.* other guilds and chiropterophilous
18 plants *vs.* other syndromes). As a proxy for species importance, we used both their degree,
19 or the number of partners, a simple measure of species generality, and the weighted
20 closeness centrality index, which measures the number of shortest paths that cross a node
21 (i.e. a species) in the network and corrects for link weight. We employed the weighted
22 version of the index. Higher centrality values suggest species with a stronger effect on
23 network structure and dynamics (González et al., 2010). To measure species
24 specialization, we used the d' index, which measures a species' relative niche breadth

1 (Blüthgen 2010). It varies from zero to one, with values close to one suggesting a species
2 with unique interactions in comparison to the rest of the network.

3 To characterize and compare the functional groups regarding their pool of
4 ecological variables, we gathered species-level network indices (degree, closeness, and
5 specialization) and morphological variables (RCR and BCI for bats, FTL and COD for
6 plants) and ran a Principal Component Analysis to ordinate species and visualize
7 correspondences between variables. All variables were compared between specialized
8 nectarivores and other guilds, or between chiropterophilous plants and other syndromes,
9 through nonparametric Wilcoxon-Mann-Whitney tests.

10 11 **Software**

12 Analyses were performed in R 3.6.0 (R Core Team, 2020). Network metrics and
13 null models were generated with the *bipartite* package (Dormann et al., 2008), the
14 ordination and sampling completeness analyses were performed with the *vegan* package
15 (Oksanen et al., 2007), circular statistics were performed with the *circular* package (Lund
16 et al., 2017), and standard inferential statistics and generation of multinomial distributions
17 with the base *stats* package. The likelihood analysis was made by implementing the
18 function created by Vázquez et al. (2009). The software Gephi 0.9.2 (Bastian et al., 2009)
19 was used to draw the network.

20 21 **3. RESULTS**

22 *3.1 Bats and plant assemblages*

23 We captured a total of 386 bats from 23 species and three families (Tab. 1, Fig.
24 1). From this pool, 162 bats from 13 species belonging to Phyllostomidae and subfamilies
25 Carollinae, Glossophaginae, Lonchophyllinae, Micronycterinae and Stenodermatinae

1 were legitimate floral visitors. We recorded 302 interactions with 35 different pollen
 2 morphotypes, from which 18 were identified to the species level, two to the species group
 3 level (*Bauhinia* groups, containing species with similar flower and pollen morphologies)
 4 and four to the genus level (Tab. 2, Fig. 1). The remaining were identified either as wider
 5 taxonomical types or to the family level based on general morphological features. Two
 6 types remained unidentified. The species (the term ‘species’ will be used to refer to any
 7 of the 35 taxonomical entities) included both chiropterophilous and non-
 8 chiropterophilous species with a diverse range of floral morphologies and belonged to 16
 9 families with a strong representation of Fabaceae and Malvaceae.

10

11 **Table 1.** Bat species captured during 12 sampling months in the Brasília National Park, with absolute
 12 number of captured individuals (N), relative frequency (rN), the habitats in which they were reported (S –
 13 savanna, I – gallery forest interior, E – gallery forest edge), number and percentage of bats from each
 14 species that carried pollen, and the richness of plant species and/or pollen morphotypes found in their
 15 samples.

Species	N	rN	Habitat type	Pollen carriers (%)	Pollen type richness	Network label
Molossidae						
<i>Molossops temminckii</i>	3	0.008	E	0 (0.0)	0	-
Mormoopidae						
<i>Pteronotus rubiginosus</i>	1	0.003	I	0 (0.0)	0	-
Phyllostomidae						
Carollinae						
<i>Carollia perspicillata</i>	62	0.161	S/E/I	23 (37.1)	12	Cper
Desmodontinae						
<i>Diphylla ecaudata</i>	1	0.003	S	0 (0.0)	0	-
Glossophaginae						
<i>Anoura caudifer</i>	24	0.062	S/E/I	23 (95.8)	14	Acau
<i>Anoura geoffroyi</i>	5	0.013	S/E/I	5 (100.0)	8	Ageo
<i>Glossophaga soricina</i>	43	0.111	S/E/I	41 (95.3)	14	Gsor
Lonchophyllinae						

<i>Lonchophylla dekeyseri</i>	22	0.057	S/E	19 (86.4)	8	Ldek
Micronycterinae						
<i>Micronycteris schmidtorum</i>	4	0.010	S	3 (75.0)	3	Msch
Stenodermatinae						
<i>Artibeus concolor</i>	1	0.003	I	1 (100.0)	1	Acon
<i>Artibeus lituratus</i>	79	0.205	S/E/I	14 (17.7)	7	Alit
<i>Artibeus planirostris</i>	24	0.062	S/E/I	11 (45.8)	5	Apla
<i>Dermanura cf. anderseni</i>	17	0.044	S/E/I	1 (5.9)	2	Dand
<i>Dermanura cinerea</i>	36	0.093	S/E/I	5 (13.9)	4	Dcin
<i>Platyrrhinus lineatus</i>	37	0.096	S/E/I	14 (37.8)	9	Plin
<i>Sturnira lilium</i>	13	0.034	S/E/I	2 (15.4)	2	Slil
<i>Sturnira tildae</i>	1	0.003	E	0 (0.0)	0	-
Vespertilionidae						
<i>Eptesicus diminutus</i>	1	0.003	S	0 (0.0)	0	-
<i>Eptesicus furinalis</i>	2	0.005	S	0 (0.0)	0	-
<i>Lasiurus blossevillii</i>	1	0.003	E	0 (0.0)	0	-
<i>Myotis diminutus</i>	1	0.003	E	0 (0.0)	0	-
<i>Myotis riparius</i>	2	0.005	E/I	0 (0.0)	0	-
<i>Myotis nigricans</i>	6	0.016	E/I	0 (0.0)	0	-
Total	386					

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From the plants found in pollen samples, 17 (two of which included more than one *Bauhinia* species) were found within the park. They had their abundance of flowering individuals measured, out of a total of 85 interest plant species from 29 families recorded (Supplementary Material, Tab. S1). Most chiropterophilous plants were found inside the site, except for the exotic forest tree *Ceiba pentandra* (Malvaceae) and the three native *Inga* species (Fabaceae) with mixed pollination involving nocturnal animals. These species, alongside the unidentified pollen types, were included in the main network but excluded from further analysis (except for the ordination results, where these species received an abundance value of one to represent rarity).

1 **Table 2.** Pollen morphotypes found on the body of bats throughout the twelve sampling months, identified to
2 the lowest-possible taxonomic level. Each type is followed by its frequency (N) and percentage in relation to
3 the total number of interactions, the sum flowering individuals recorded in the transections (n), the habitats in
4 which they were reported (CSS – Cerrado *sensu stricto*, GFI – gallery forest interior, GFE – gallery forest
5 edge), whether they are chiropterophilous (Chirop.) or not and floral shape. Dashes indicate that the species
6 was found in pollen samples but not in site (n, habitat type), or that chiropterophily or floral shape may not be
7 inferred by the taxonomical level achieved.

Pollen type	N (%)	n	Habitat type	Chirop.	Floral shape	Label
Acanthaceae						
<i>Justicia</i> sp.	1 (0.3)	-	-	No	-	Jusp
Amaryllidaceae						
<i>Hippeastrum glaucescens</i>	1 (0.3)	1	S	No	Infundibuliform	Hglau
Arecaecae						
<i>Syagrus</i> type	1 (0.3)	-	-	No	-	Asya
Asteraceae						
<i>Baccharis</i> type	1 (0.3)	-	-	No	-	Abacc
Vernoniae type	1 (0.3)	-	-	-	-	Asver
Caryocaraceae						
<i>Caryocar brasiliense</i>	46 (15.2)	95	S	Yes	Brush	Cbra
Combretaceae						
<i>Combretum fruticosum</i>	5 (1.7)	10	I	No	Pseudanthium	Cfru
Convolvulaceae						
<i>Ipomoeae procumbens</i>	1 (0.3)	1	S	No	Infundibuliform	Ipro
<i>Merremia tomentosa</i>	1 (0.3)	4	S	No	Infundibuliform	Mtom
Cunoniaceae						
<i>Lamanonia ternata</i>	23 (7.6)	30	E	No	Pseudanthium	Lter
Fabaceae						
<i>Bauhinia goyazensis</i> group ¹	25 (8.3)	46	S/E	Yes	Tubular	Bgoy
<i>Bauhinia holophylla</i>	11 (3.7)	4	S/E	Yes	Tubular	Bholo
<i>Bauhinia rufa</i> group ²	30 (9.9)	13	E	Yes	Tubular	Brufa
Caesalpinoidae sp. 1	3 (1.0)	-	-	-	-	Fcae1
Caesalpinoidae sp. 2	1 (0.3)	-	-	-	-	Fcae2
Caesalpinoidae sp. 3	1 (0.3)	-	-	-	-	Fcae3
<i>Hymenaea courbaril</i>	10 (3.3)	2	I	Yes	Cupuliform	Hcou
<i>Hymenaea stigonocarpa</i>	22 (7.3)	6	S	Yes	Cupuliform	Hsti
<i>Inga</i> aff. <i>laurina</i>	4 (1.3)	-	-	Yes	Pseudanthium	Ilau
<i>Inga edulis</i>	5 (1.7)	-	-	Yes	Tubular	Iedu
<i>Inga vera</i>	8 (2.6)	-	-	Yes	Tubular	Iver

<i>Mimosa</i> aff. <i>setosa</i>	1 (0.3)	26	S	No	Pseudanthium	Mset
Loranthaceae						
<i>Psittacanthus robustus</i>	35 (11.6)	61	S	No	Tubular	Prob
Lyrthraceae						
<i>Lafoensia pacari</i>	28 (9.3)	2	E	Yes	Campanulate	Lpac
Malvaceae						
<i>Ceiba pentandra</i>	13 (4.3)	-	I	Yes	Brush	Cpen
<i>Pseudobombax longiflorum</i>	6 (2.0)	21	E	Yes	Brush/urceolate ³	Plon
<i>Pseudobombax tomentosum</i>	4 (1.3)	4	S	Yes	Brush/urceolate ³	Ptom
Moraceae						
Moraceae type	2 (0.7)	-	-	-	-	Mora
Myrtaceae						
<i>Eucalyptus</i> sp.	3 (1.0)	-	-	Yes ⁴	Brush	Eusp
<i>Myrcia</i> sp.	1 (0.3)	31 ⁵	S	No	Brush	Myrsp
Myrtaceae type	1 (0.3)	-	-	-	-	Myrt
Sapotaceae						
<i>Pouteria</i> type	1 (0.3)	-	-	-	-	Spou
Smilacaceae						
<i>Smilax</i> sp.	1 (0.3)	-	-	No	Pseudanthium	Smil
Undetermined						
Undetermined 1	1 (0.3)	-	-	-	-	Indet1
Undetermined 2	1 (0.3)	-	-	-	-	Indet2

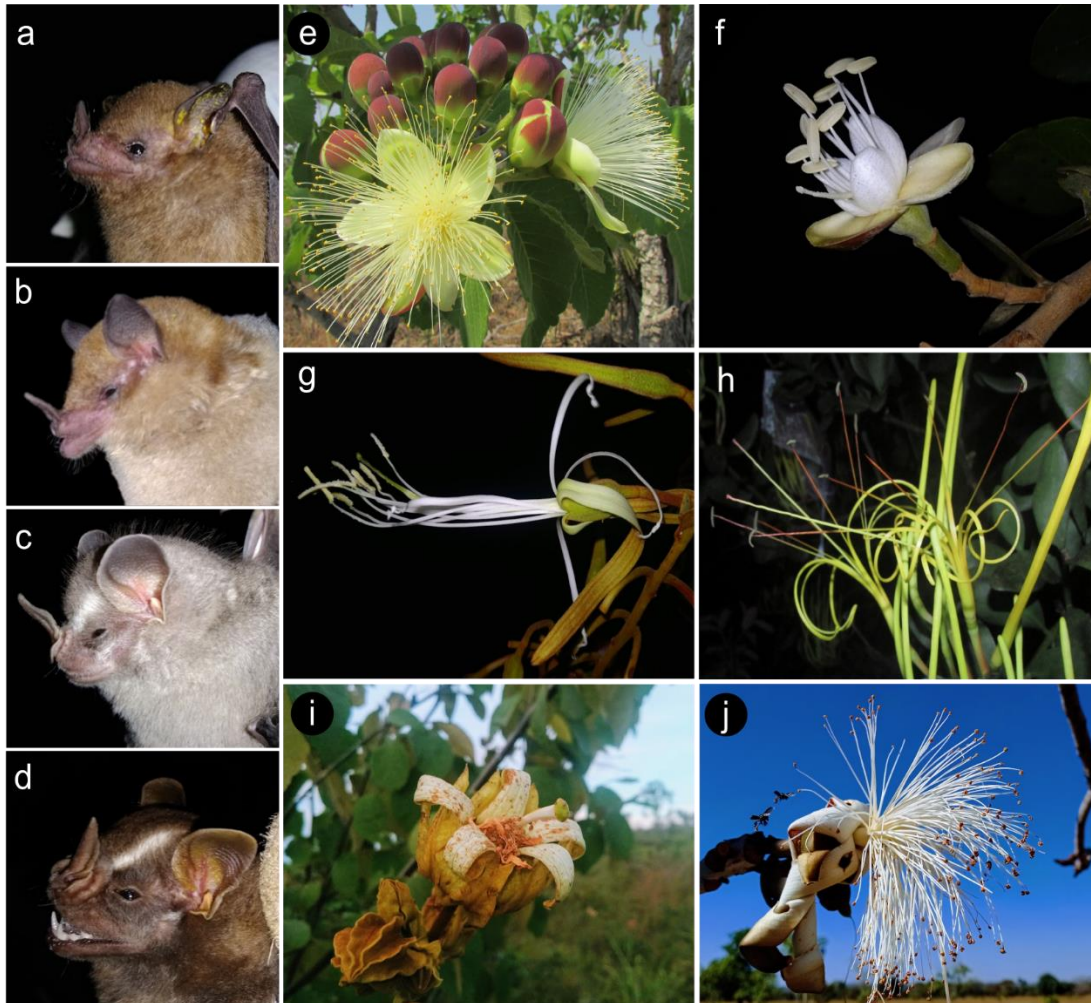
1 ¹ Includes the following species with similar flower and pollen morphology: *B. goyazensis* and *B. dumosa*;

2 ² Includes the following species with similar flower and pollen morphology: *B. rufa* and *B. longifoli*;

3 ³ Flowers have an overall brush-like appearance, but the nectar chamber is formed by the large urceolate calyx;

5 ⁴ Paleotropical chiropterophilous species introduced in the region for economic purposes and found around the PNB;

7 ⁵ Abundance is the sum of *M. eryocalyx* and *M. multifloral*, the two species found in the site with similar floral morphologies.

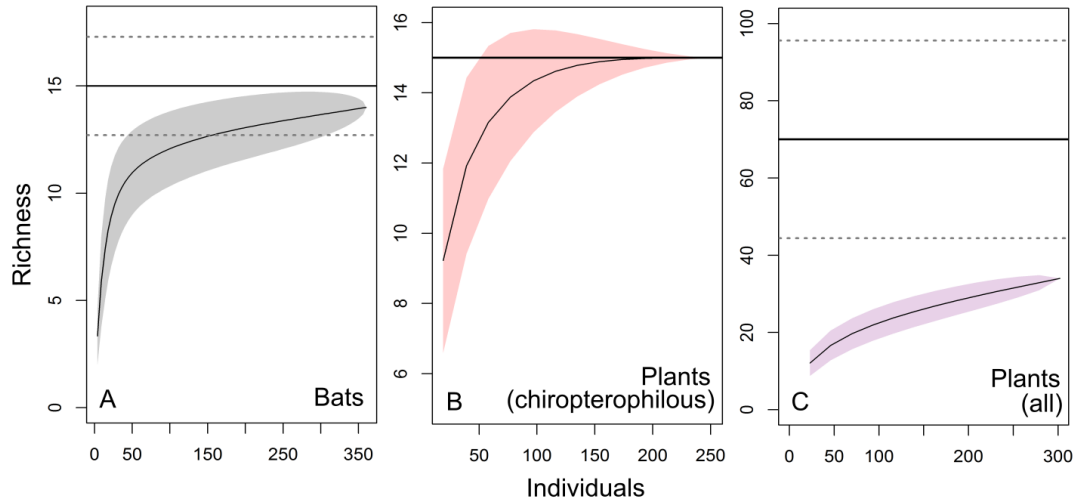


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3 **Figure 1.** A selection of typical flower-visiting bat species (a – d) and bat-visited plants in the PNB (e – j)
4 in the study site. **a** and **b** – Specialized nectarivores *Glossophaga soricina* and *Lonchophylla dekeyseri*,
5 respectively; **c** and **d** – opportunistic floral visitors *Dermanura cinerea* and *Artibeus lituratus*, respectively.
6 **e** – *Caryocar brasiliense* (Caryocaraceae); **f** – *Hymenaea stigonocarpa* (Fabaceae); **g** – *Bauhinia goyazensis*
7 (Fabaceae); **h** – *Psittacanthus robustus* (Loranthaceae); **i** – *Luehea grandiflora* (Malvaceae); **j** –
8 *Pseudobombax longiflorum* (Malvaceae).

9
10 The asymptotic species richness estimator revealed high sampling completeness
11 for phyllostomid bats (93.3% of bats species registered, or 14 out of 15 ± 2.29 SE), and a
12 complete sampling for chiropterophilous plants (15 out of 15 ± 0 registered in pollen
13 samples) (Fig. 2, A-B). However, when including the pollen types of non-
14 chiropterophilous plants in the sampling analysis, only 50% (35 out of 70 ± 25.59) of the
15 estimated community was sampled, resultant from the many singletons found

1 occasionally in samples (Fig. 2, C). Therefore, we henceforth consider the community
2 sufficiently sampled in terms of the core species assembling the network.

3



4

5 **Figure 2.** Rarefaction curves of bats species (A), pollen types from chiropterophilous plants (C) and pollen
6 types from all plants sampled in the study site. Shaded areas represent 95% confidence intervals, and solid
7 and dashed horizontal lines indicate the estimated asymptotic richness and its standard error, respectively.

8

9 3.2 Network structure

10 The interaction network between bats and plants was significantly and moderately
11 modular, with modules associated with feeding guilds (specialized nectarivores,
12 frugivores and insectivores), and was unspecialized, presenting significant yet low
13 interaction complementarity (Fig. 3, Tab. 3). A nested structure did not emerge in the
14 network and did not deviate from neutrality in the full or any of the partial networks,
15 although the nectarivore network was much more nested than the other networks. The
16 nectarivore network was much less modular and specialized. The partial network
17 containing other guilds behaved very similarly to the full network in all metrics,
18 presenting low nestedness, higher specialization and higher modularity.

19

1 **Table 3.** Structural metrics of the interaction network between flower-visiting bats and plants and of partial
 2 networks containing either specialized nectarivores only or species from other guilds only. Values in
 3 brackets are Z values. Bold Z values indicate a significance deviance from the null expectation ($p < 0.05$).

Metrics	Full network	Partial network (nectarivores)	Partial network (other guilds)
Size (i x j)	455	100	189
wNODF	0.206 (-3.150)	0.437 (-1.298)	0.176 (-2.179)
H ₂ '	0.370 (4.714)	0.245 (3.405)	0.357 (1.768)
Q _w	0.421 (8.793)	0.269 (5.303)	0.404 (2.863)

4

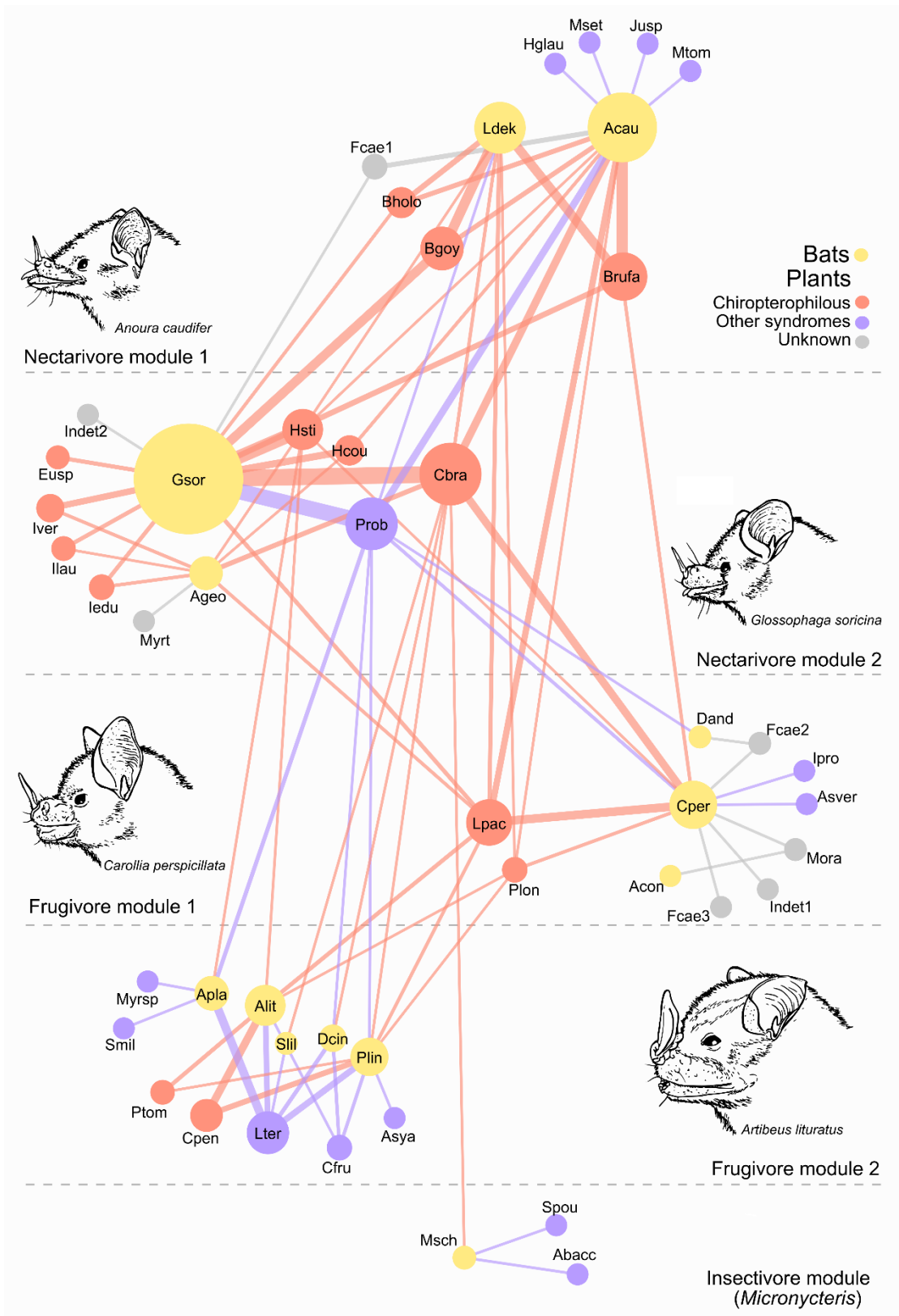
5 Module configuration was correlated with feeding guilds (Figure 4). The smaller
 6 and more morphologically specialized *Lonchophylla dekeyseri* and *Anoura caudifer* were
 7 placed in the same module alongside tubed plant species, especially *Bauhinia* spp. The
 8 larger *A. geoffroyi* and the basal Glossophaginae and less morphologically specialized
 9 *Glossophaga soricina* were placed in the same module with mostly open-flowered or
 10 short-tubed species such as *Caryocar brasiliense*, *Hymenaea* spp, and the ornithophilous
 11 *Psittacanthus robustus*. Two modules were formed solely by frugivores and the last by
 12 the insectivorous *Micronycteris schimdtorum*, who only interacted once with *C.*
 13 *brasiliense* and with two non-chiropterophilous species. The frugivore modules were
 14 dominated either by robust, open-flowered, or wide-tubed species such as *Ceiba*
 15 *pentandra*, *Lafoensia pacari*, *Pseudobombax longiflorum* and *P. tomentosum*, or by non-
 16 chiropterophilous and pseudanthia-forming species such as *Lamanonia ternata* and
 17 *Combretum fruticosum*.

18

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1

2 **Figure 3.** Interaction network between flower-visiting phyllostomid bats and plants in a savanna of central
 3 Brazil. Nodes represent species and lines, pairwise interactions. Line width corresponds to interaction
 4 frequency and node size to the sum of a species' interactions. Plants are divided as chiropterophilous, non-

1 chiropterophilous, or unknown system. Modules in the network are divided by dashed lines and
 2 accompanied by a schematic illustration of the most important bat species in the module. Species codes and
 3 its associated species are found in Tables 1 and 2.

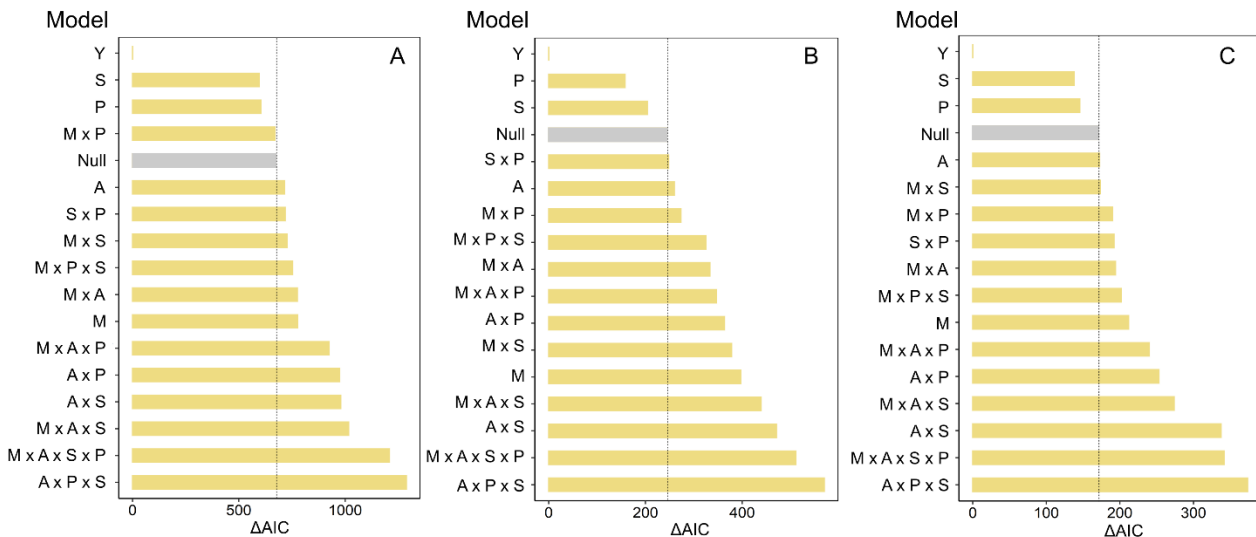
4

5 3.3 Drivers of network structure

6 Model likelihood

7 Out of the 15 models based on interaction probability matrices and their
 8 combinations, spatial and temporal overlap alone were the two best predictors of network
 9 structure across all networks (full, nectarivore only, and other guilds only) (Fig. 4). All
 10 other models either approached or performed worse than the null model. Morphology and
 11 its combinations with other factors were consistently ranked among the least-fitting
 12 models, as well as the combinations including species relative abundances (Fig. 4). More
 13 complex models comprising three or four variables were also generally weaker.

14



--

16 **Figure 4.** The likelihood of different models based on interaction probability matrices of explained the
 17 observed interaction matrix, expressed in their variation in the Akaike Information Criterion in relation to
 18 the best fitting model (the interaction matrix itself). Models are organized from best fit (top) to worst fit
 19 (bottom). A – full network; B – nectarivores only; C – other guilds only. Model label: Y – observed matrix,

1 M – morphology; A – relative abundance; S – Spatial overlap; P – phenological overlap; Null – benchmark
2 null model.

3

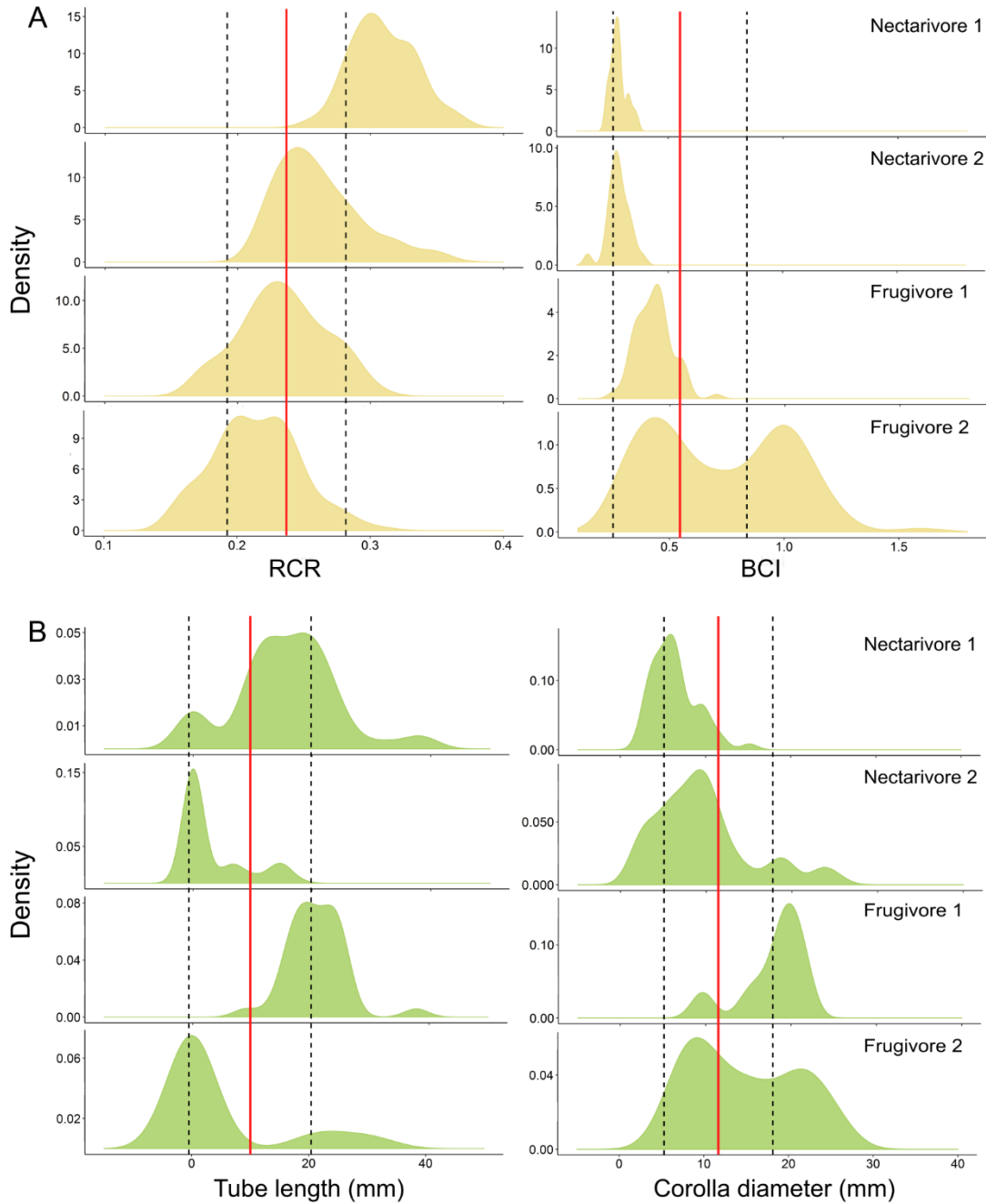
4 **The effect of species' morphologies**

5 All species morphological variables varied significantly across modules (Figure
6 6). Bat rostrum-cranium ratio was significantly higher in the nectarivore module 1 (*A.*
7 *caudifer* and *L. dekeyseri*) and successively lower in the other nectarivore module (*A.*
8 *geoffroyi* and *G. soricina*) and frugivore modules ($F_{3,360} = 123.00$, $p < 0.0001$) with all
9 pairwise comparison at $p < 0.001$. (Fig. 5, A). Body condition index was similar in both
10 nectarivore modules ($p = 0.99$), but much lower than the frugivore modules ($F_{3,358} =$
11 89.72 , $p < 0.0001$, all other pairwise comparisons with $p < 0.05$). Frugivore modules also
12 differed ($p < 0.001$), mainly due to the disproportionately large-bodied *Artibeus* species
13 inserted in the frugivore module 2 (Fig. 5, A).

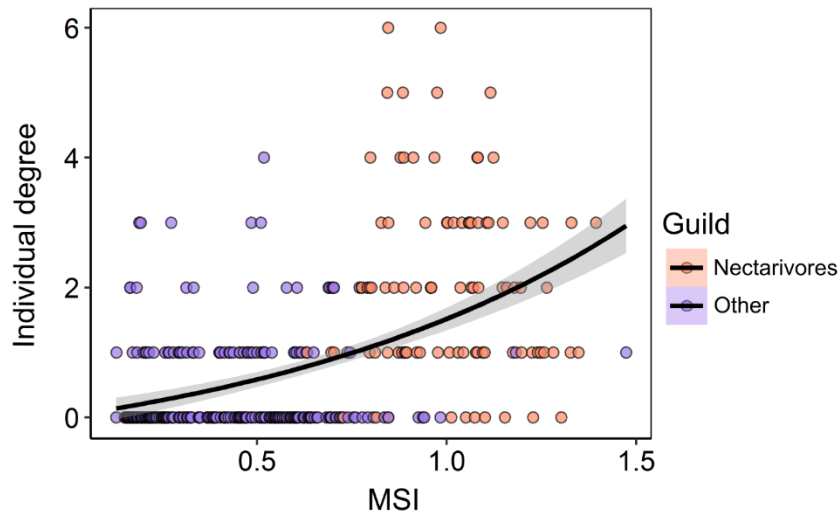
14 Modules also varied in terms of floral tube length ($F_{3,204} = 57.68$, $p < 0.0001$) and
15 diameter of the corolla/floral tube opening ($F_{3,202} = 48.17$, $p < 0.0001$) (Fig. 5, B).
16 Nectarivore module 2 had mostly open-flowered species, alongside frugivore module 2
17 ($p = 0.58$, but $p < 0.05$ for all other pairwise comparisons), contrasting with the longer-
18 tubed flowers in the nectarivore module 1 and frugivore module 1. These latter two,
19 however, contrasted sharply in terms of corolla opening, with nectarivore module 1
20 presenting the most restrictive flowers, and both frugivore modules having either wide-
21 tubed flowers (e.g., *Lafoensia pacari*, *Pseudobombax* spp.) or open flowers organized in
22 pseudanthia (e.g., *Combretum fruticosum*, *Lamanonia ternata*). All modules differed in
23 terms of corolla opening ($p < 0.005$ for all pairwise comparisons).

24 At the bat individual level, the individual degree increased exponentially in
25 relation to their morphological specialization index (exponential regression: $r^2 = 0.19$;

1 $F_{1,366} = 89.34; p < 0.0001$) (Fig. 6). Morphologically unspecialized bats (i.e., more robust
 2 bodies and shorter rostrums) were much less likely to visit more than one plant species or
 3 to act as floral visitors at all.



4
 5 **Figure 5.** Density distribution of morphological variables from bats (A, RCR – rostrum-cranium ratio –
 6 and BCI – body condition index) and plants (B, floral tube length and corolla diameter) according to the
 7 assigned modules. Module names correspond to those in Figure 1. Solid red line indicates the mean of
 8 each variable for all species pooled and dashed lines indicate standard deviation



1 **Figure 6.** Exponential effect of effect of individual morphological specialization of bats (MSI) on their
 2 individual degree (number of simultaneous partners found in pollen samples). Individuals are colored by
 3 guild (nectarivores or others). The shaded grey area around the trend line indicates the 95% confidence
 4 interval.
 5

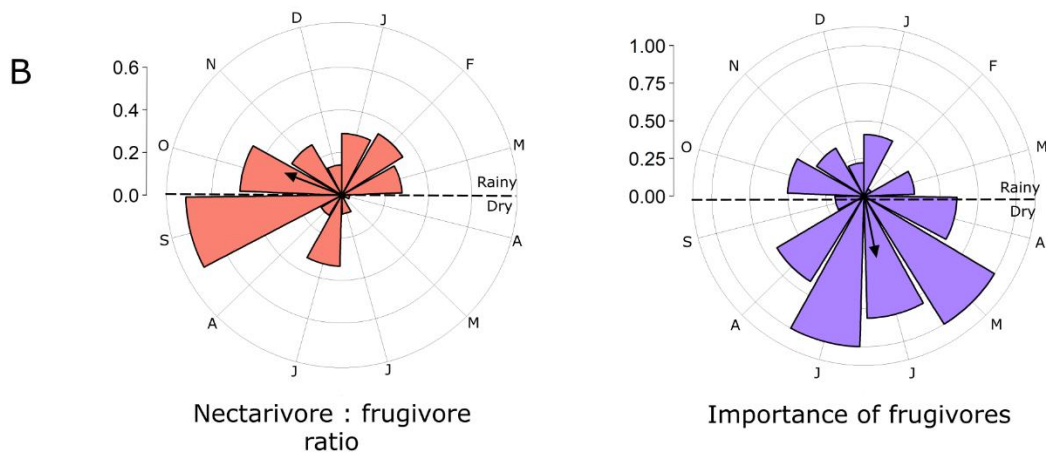
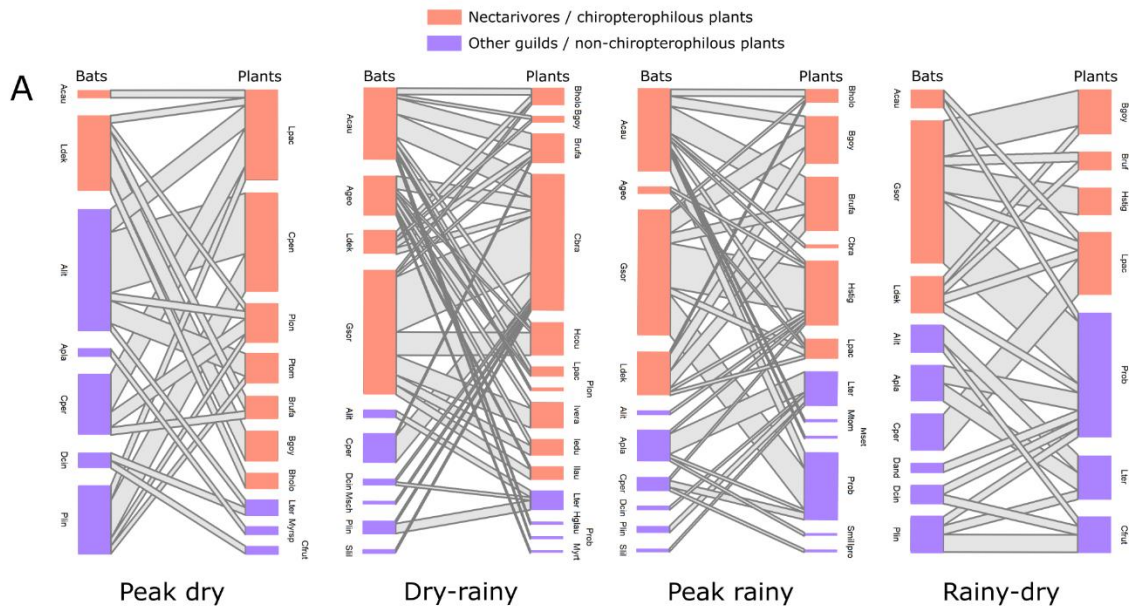
7 **The effect of spatiotemporal overlap**

8 The temporal and spatial partial networks varied considerably among themselves,
 9 as well as the participation of nectarivores and frugivores across space and time.
 10 Nectarivores were much more frequent in the network and had the highest diversities in
 11 the dry-rainy transition and in the peak of rainy season, where they interacted with the
 12 central species *Caryocar brasiliense*, *Bauhinia* spp., *Hymenaea stigonocarpa* and
 13 *Psittacanthus robustus* (Fig. 7, A). Nectarivore-frugivore ratio was significantly
 14 aggregate ($r = 0.31$, $p < 0.001$), with a peak in the dry-rainy transition (October, mean
 15 degree = $276.11^\circ \pm 86.7^\circ$) (Fig. 7, B), simultaneously with the explosive flowering of *C.*
 16 *brasiliense*. The vector was shortened due to a slight peak in the rainy season during the
 17 flowering of *P. robustus* (Fig. 7, B), which caused a bimodal aggregation.

18 Nectarivore ratios decreased steeply in the dry season, while the participation of
 19 frugivores in the network increased (Fig. 7, A). The peak dry network had the lowest

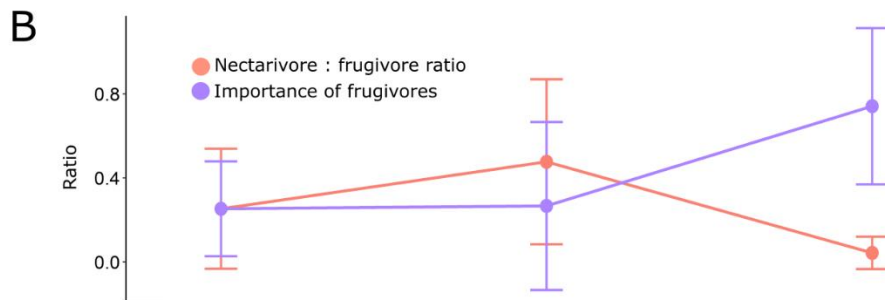
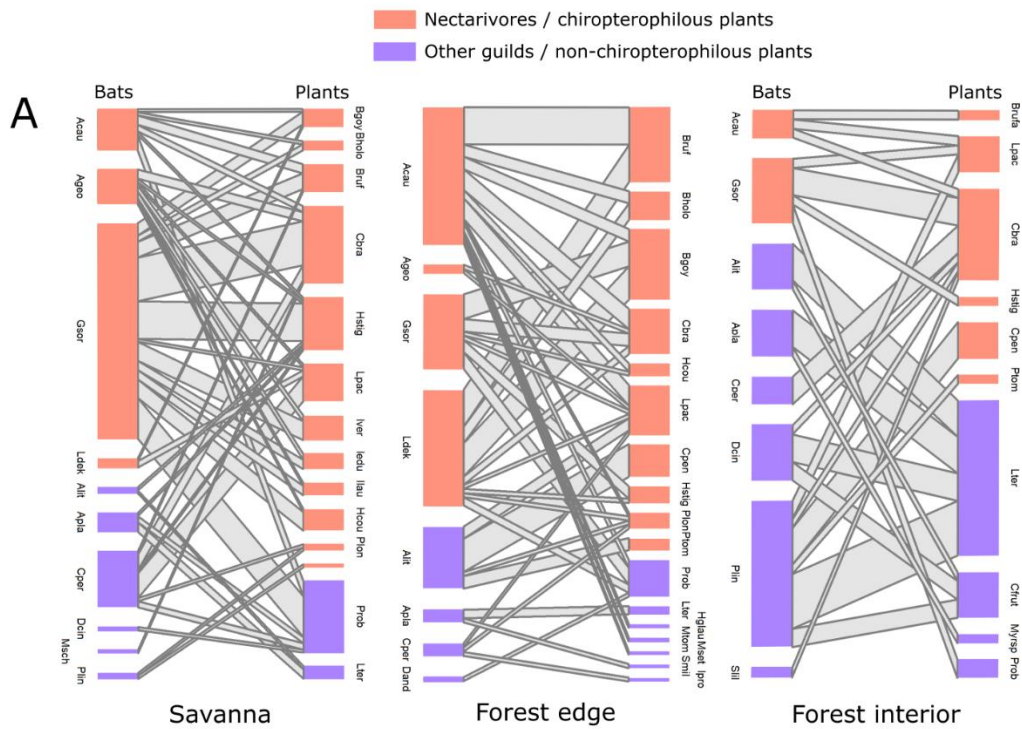
1 nectarivore diversity and was the only one that did not include the abundant and generalist
2 nectarivore *Glossophaga soricina*. It saw an increase in the importance of frugivores like
3 *Artibeus planirostris* and *Platyrrhinus lineatus* visiting the wide-flowered *Ceiba*
4 *pentandra*, *Pseudobombax* spp. and *Lafoensia pacari*, and it coincided with the peak in
5 importance of frugivores as floral visitors (June, mean degree = $157.58^\circ \pm 85.19^\circ$). The
6 importance of frugivores was also aggregate ($r = 0.33$, $p < 0.001$), with a low vector value
7 due to a small increase during the beginning and the peak of the rainy season as they also
8 visited *C. brasiliense* and *P. robustus* (Fig. 7, B).

9 The partial network also varied strongly across the three vegetation types in which
10 interactions were samples. The savanna and forest edge networks were similar, contain
11 more interactions between nectarivores and chiropterophilous plants (Fig. 8, A). The
12 nectarivores from module 1, *Lonchophylla dekeyseri* and *Anoura caudifer*, were more
13 frequent visitors in the forest edge, alongside *Bauhinia* spp., which typically occur in
14 these transitions. Meanwhile, the savanna network was dominated by *G. soricina*, which
15 visited mainly savanna plants (e.g., *C. brasiliense*, *P. robustus*, *H. stigonocarpa*). Inside
16 forests, on the other hand, nectar bats were much rarer, and the interactions sampled from
17 them in these environments corresponded to savanna or edge plants and never plants
18 found inside forests only. Nectarivore-frugivore ratios were similar between savanna and
19 forest edge, but much smaller inside forests ($F_{2,33}=6.42$, $p < 0.005$) (Fig. 8, B). On the
20 other hand, frugivores dominated the forest interior network and interacted mostly with
21 forest plants (*L. ternata*, *C. pentandra*, *C. fruticosum*) and visited savanna plants
22 sporadically (Fig. 8, A). Frugivore importance was low and similar in savanna and forest
23 border sites, but significantly higher within forests ($F_{2,33}=4.36$, $p < 0.05$) (Fig. 8, B).



1

2 **Figure 7.** Temporal trends of the interaction network between flower-visiting bats and plants in the Brasília
 3 National Park. A – Partial networks from the different time periods during one seasonal year. Species are
 4 separated as within chiropterophily (nectarivores and chiropterophilous plants) and outside chiropterophily
 5 (frugivores and non-chiropterophilous plants). Species labels are found in Table 1. B – Temporal variation
 6 in the ratio between nectarivores and frugivores (left) and the proportion of frugivores that visited plants.
 7 (right). Arrows indicate the angle of the mean vector.



1

2 **Figure 8.** Spatial trends of the interaction network between flower-visiting bats and plants in the Brasília
 3 National. A – Partial networks from the different vegetation types in which bats were captured. Species are
 4 separated as within chiropterophily (nectarivores and chiropterophilous plants) and outside chiropterophily
 5 (frugivores and non-chiropterophilous plants). Species labels are found in Table 1. B – Variation in the
 6 ratio between nectarivores and frugivores and in the importance of frugivores as floral visitors across the
 7 different vegetation types.

8

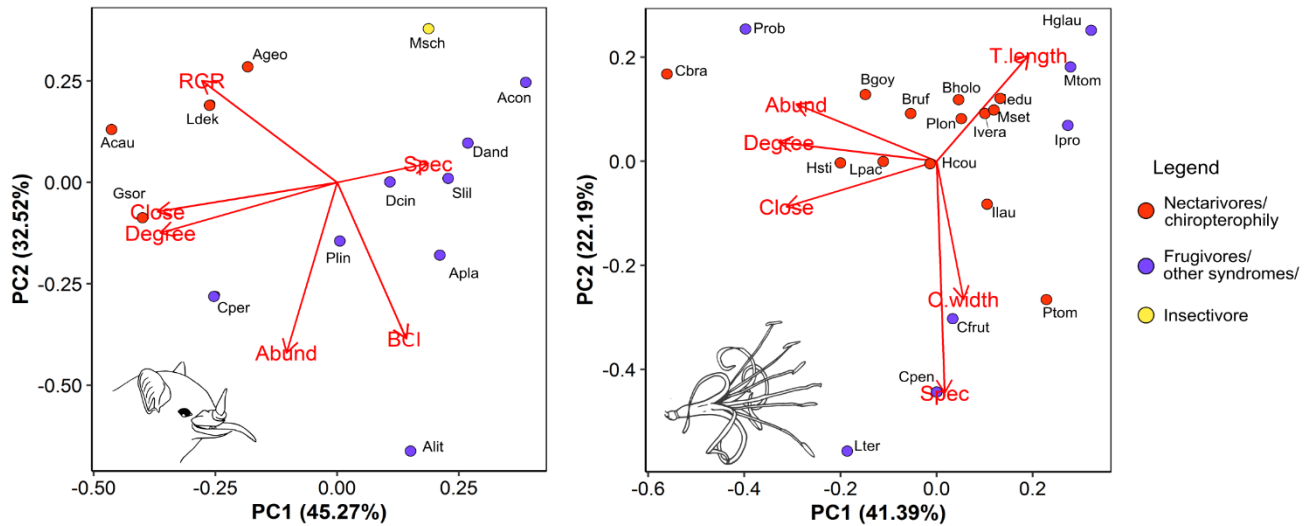
9 **Species traits**

10 Specialized nectar bats form a clear distinct functional group in relation to other
 11 bat guilds, being significantly longer snouted and having less robust bodies, which lead
 12 to a lower ecologically specialized in terms of usage of floral resources and higher

1 centrality (Tab. 4, Fig. 9). BCI was negatively related to degree and centrality and slightly
 2 related to specialization. Relative abundance did not vary significantly among groups,
 3 and alongside RCR, was slightly positively related to degree and centrality. Among bats
 4 from other guilds, the frugivorous *Carollia perspicillata* had the highest RCR and was
 5 the most abundant and therefore closer to other bats species in terms of degree and
 6 centrality (Fig. 9). There were no differences, however, between chiropterophilous plants
 7 and non-chiropterophilous plants in any of the ecological variables (Tab. 4, Fig. 9). Long-
 8 tubed and wide-flowered species occurred in both groups. Abundance was the closest
 9 dictator of centrality and species degree, opposing tube length. Abundant, short-
 10 tubed/open-flowered and central species included the chiropterophilous *C. brasiliense*
 11 and the ornithophilous *P. robustus*. The non-chiropterophilous and generalist *L. ternata*
 12 was both very specialized and central to the network, due to strong but exclusive
 13 interactions with frugivores.

14 **Table 4.** Comparisons of species-level network metrics and ecological variables between nectarivores and
 15 bats from other guilds, and between chiropterophilous plants from other syndromes.

Variables	Nectarivores / Chiropterophilous	Other guilds / Non- chiropterophilous	Z (p)
Bats			
RCR	0.29 ± 0.03	0.22 ± 0.02	2.62 (0.009)
BCI	0.28 ± 0.02	0.51 ± 0.24	-2.16 (0.031)
Degree	11.00 ± 3.46	5.00 ± 3.67	2.16 (0.030)
Specialization	0.32 ± 0.04	0.45 ± 0.21	-2.01 (0.044)
Closeness centrality	0.06 ± 0.01	0.02 ± 0.01	2.62 (0.009)
Relative abundance	0.06 ± 0.04	0.08 ± 0.07	-0.08 (0.938)
Plants			
Tube length (mm)	11.36 ± 10.01	11.18 ± 11.73	0.00 (1.000)
Corolla diameter (mm)	10.91 ± 6.32	11.98 ± 4.47	-0.24 (0.812)
Degree	3.92 ± 2.31	2.00 ± 2.73	1.41 (0.159)
Specialization	0.24 ± 0.14	0.29 ± 0.20	0.20 (0.842)
Closeness centrality	0.02 ± 0.01	0.01 ± 0.01	1.71 (0.08)
Relative abundance	0.05 ± 0.08	0.03 ± 0.07	-0.99 (0.319)



2
 3 **Figure 9.** Principal Component Analysis organizing bat (left) and plant (right) species according to their
 4 species-specific network indexes and ecological variable. Each Principal Component is accompanied by
 5 the percentage of variation in the data explained by them. Species are colored by guild (nectarivores,
 6 frugivores, insectivore) and syndrome (chiropterophilous, others). Loadings are represented by red arrows.
 7 Loading label: Abund – Relative abundance; BCI – Body condition index; Close – weighted closeness
 8 centrality; Degree – degree; RGR – Rostrum-cranium ratio; Spec – specialization (d'). Labels for species
 9 are found in Tables 1 and 2.

11 5. DISCUSSION

12 We reconstructed the weighted interaction network between plants and flower-
 13 visiting bats in a savanna of central Brazil and determined that interactions are not
 14 neutrally structured, but rather driven by the spatiotemporal overlap between species.
 15 This is the third report of a weighted network for Neotropical flower-visiting bats
 16 (Cordero et al., 2021; Queiroz et al., 2020), the first such description for the Cerrado,
 17 building up on the binary meta-network of Oliveira et al. (2019), and the first to explicitly
 18 assess the role of network drivers at a local scale. The generalized nature of the network
 19 agrees with recent findings suggesting that specialized nectar bats are not ecological
 20 specialists (Cordero-Schmidt et al., 2021, Queiroz et al., 2020), and the low performance

1 of morphology in the likelihood analysis in comparison to other structuring factors
2 confirms that bat-pollinated plants are generally not selectively restrictive towards nectar
3 bats based on snout length (Gonzalez-Terrazas et al., 2012, Sperr et al., 2011).

4 Morphology, in turn, seems to define distinct flower-visiting niches and determine
5 more general patterns in the network, as suggested by the module configuration. Smaller,
6 hovering and long-snouted bats were frequent flower visitors and formed a conspicuous
7 group with mostly chiropterophilous plants, assembling an internally nested sub network.
8 Both wide-flowered (e.g. *C. brasiliense*) and tubular-flowered plants (e.g. *Bauhinia* spp.)
9 were present in this subnetwork, many of which have delicate and single flowers as
10 pollination units (*Bauhinia* spp., *Inga* spp.) and require hovering visits (Tschapka and
11 Dressler, 2002). Frugivores and insectivores, on the other hand, were more specialized
12 nodes and bats had to resort to wider flowers (e.g. *C. Pseudobombax* spp.), flowers in
13 large inflorescences (e.g. *C. brasiliense*, *C. pentandra*) or pseudanthium-forming
14 inflorescences (*Combretum fruticosum*, *Lamanonia ternata*) to which they could perform
15 clinging visits (Fleming et al., 2009; Gibbs et al., 1999). Their subnetwork was less nested
16 and more specialized, probably because these animals do not use nectar or pollen as main
17 resource and are only opportunistic floral visitors due to their non-specialized
18 morphology (Clairmont et al., 2014).

19 Bat morphology in our study system was much related to bat phylogeny, dividing
20 mainly nectarivores from frugivores, and its role in structuring modules was similar to
21 the phylogenetic signal in the continent-wide multilayer network studied by Mello et al.
22 (2019). The authors found that phylogeny and geographic constraints were responsible
23 for the assemblage of frugivory and nectarivory modules by bats in South America, and
24 that modules were internally nested and driven other variables, such as intrinsic species
25 traits or co-occurrence. Similarly, but at a local scale, we show that the emergence of the

1 two subnetworks is correlated to bat functional groups distinctive morphologies and
2 associated to phylogenetic groups, while pairwise interactions within the subnetworks
3 were finetuned by other variables, such as plant phenology and spatial overlap between
4 species. Therefore, bat snout length and body condition should be seen as a proxy for
5 morphological specialization towards floral resources, as suggested by several authors
6 (Freeman, 1995, Tschapka et al., 2008; Winter and von Helversen, 2003). However,
7 differences in these characteristics among specialized nectarivores do not seem to be
8 necessarily related to a floral restriction gradient as widely reported for the mouthparts of
9 other pollinators such as hawkmoths (Johnson et al., 2017, Sazatornil et al., 2016) and
10 birds (Vizentin-Bugoni et al., 2014) although exceptions may occur (Muchhala and
11 Thompson, 2009). Instead, bat morphology dictates which types of food items floral
12 visitors may use as other types of resources in times of food shortage. Frugivores are both
13 morphologically unspecialized nectar consumers and highly opportunistic floral visitors,
14 consuming nectar or pollen only in times of fruit shortage, which corresponds to the dry
15 season in the Cerrado (Amaral et al., 2016; Batalha and Martins, 2004). This temporal
16 trend explains their increased importance as floral visitors during this period, which also
17 coincides with the explosive flowering of the forest tree *Ceiba pentandra* (Gribel et al.,
18 1999), who underwent intense consumption by frugivores and surprisingly no visitation
19 by nectarivores, which has been reported in forests from higher latitudes with different
20 seasonal patterns (Lobo et al., 2005). *Carollia perspicillata*, which is deemed an
21 unspecialized frugivores due to its cranial features (Santana and Dumont, 2009), had
22 longer rostrum length values than other frugivores and intermediate body condition,
23 which is likely why it maintained a steady role as a floral visitor throughout the year.

24 Regarding the nectarivore guild, less morphologically specialized bats such as *G.*
25 *soricina* and *A. geoffroyi* may feed on insects and fruits in periods of flower scarcity

1 (Zortéa, 2003), while bats with longer and more delicate snouts are less prone to consume
2 harder food items due to weaker bite forces (Santana et al., 2012). This may explain why
3 the longer-snouted *L. dekeyseri* and *A. caudifer*, which were inserted in the same module,
4 were always present in the network across all seasons, consuming less abundant plants
5 such as *Bauhinia* spp., *L. pacari* and *Pseudobombax longiflorum* during the dry season,
6 while the other nectarivores may have increased their usage of other food items during
7 this period. The dry season in the Cerrado is generally recognized as a period of higher
8 nectar availability and consumption by bats like *L. dekeyseri* (Coelho and Marinho-Filho
9 et al., 2002) while the rainy season poses flower shortage. We suggest, in fact, that the
10 dry season is a key period for the most morphologically specialized nectar bats in the
11 region, which partition their niche with less specialized bats and successfully forage on
12 less abundant plants with low energy density, as reported for other Neotropical
13 communities (Tschapka 2004, Sperr et al., 2011). Meanwhile, the dry-rainy transition and
14 peak rainy season were key periods for less specialized nectarivores as it entails the
15 flowering central and abundant species such as *C. brasiliense* and *P. robustus*, species
16 with copious and accessible nectar that are more profitable for shorter-snout bats
17 (Gonzalez-Terrazas et al., 2012). By March, when the flowering of *P. robustus* starts to
18 wane, the bats *G. soricina* and *A. geoffroyi* decreased in activity, a trend also reported for
19 these two species in a neighboring Cerrado site (Zortéa and Alho, 2008).

20 The very central role of the ornithophilous *P. robustus* in the network was
21 surprising but corroborates with the expectation that nectar bats are ecological generalists
22 and exploit whatever profitable resource is available even if it does not fit the expected
23 syndrome, which has also been reported for hummingbirds (Chmel et al., 2021;
24 Maruyama et al., 2013). The ornithophilous *P. robustus* was an especially good contender
25 as a resource for bats due to its long and cornucopia-like flowering (many flowers

1 produced daily throughout a long period) (Gentry, 1974) during the wet season, long
2 anthesis producing copious nectar, a high density in the study site and a more accessible
3 nectar chamber (Guerra et al., 2014). This previously interaction with *P. robustus* is a key
4 finding due to its importance to bats in the region, which seconds only that of *C.*
5 *brasiliense*, and may even represent an example of transitioning system between
6 ornithophily and chiropterophily (Diniz et al., *in prep*).

7 Regarding the spatial component, we showed that certain bat species have intrinsic
8 preferences of foraging areas, leading to differing spatial networks. Nectar bats were far
9 more common in open savanna areas and forest edges, where chiropterophilous plants
10 were more diverse and abundant. Within these specialized nectarivores, an examination
11 of partial spatial networks also provides insight on their module configuration.
12 *Lonchophylla dekeyseri* and *A. caudifer* were much more detectable in forest edges, and
13 plants in their module (*Bauhinia* spp.) were more common in these areas. Bats found
14 more often in savannas, such as *G. soricina* and *A. geoffroyi*, interacted more with
15 savanna species (*C. brasiliense*, *H. stigonocarpa*, *P. robustus*). The same was observed
16 for frugivores, as the module comprising *Carollia perspicillata*, found more often in
17 savanna and edge sites, included the savanna/edge species *L. pacari*, and even the *C.*
18 *perspicillata* bats found inside forests interacted with savanna plants only. Forest-
19 dwelling plants were the most often food items consumed by other frugivores, which
20 forage mostly inside forests (Oliveira et al., 2019).

21 The discrepancy in capture rates between nectarivore and frugivores in our study
22 site was surprising as nectar bats such as *Anoura* spp. are commonly found in
23 ombrophilous forests (Oprea et al., 2009, Sazima et al., 1999). The Cerrado, however, is
24 a unique vegetation mosaic with patches of dense gallery forests along rivers surrounded
25 by the bushy savannas or grasslands (Silva et al., 2006). These patches are associated with

1 an increased frugivore activity in comparison to the surrounding matrix due to a higher
2 density of chiropterocoric plants (Avila-Cabadilla et al., 2021), which explains their
3 preference towards this vegetation and dominance in the forest network, with a
4 consequent exclusion of nectar bats likely due to competition. Competition pressure
5 would explain why nectar bats that remained in the network during the dry season
6 abstained from visiting the forest tree *C. pentandra*, which has a very high energy density
7 (Gribel et al., 1999) and has been reported to be pollinated by both frugivores nectarivores
8 in the Amazon (Gribel et al. 1999, Lobo et al., 2005). Therefore, the unique temporal and
9 spatial patterns of the network may be fruit of the highly seasonal and heterogeneous
10 nature of the South American savannas and could differ in communities from more stable
11 environments. As such, we need more evidence on the structuring factors on bat-flower
12 networks in regions comprising other latitudes and vegetation types to build a fuller
13 picture of how interactions are assembled along geographical gradients (Mello et al.,
14 2019).

15

16 **CONCLUSION**

17 We have shown that species morphology is an important axis of the bat pollination
18 niche and define the formation of functional groups that appear to have a phylogenetic
19 signal, but phenology and spatial overlap are stronger predictors of pairwise interactions.
20 We also build on the previous and scarce literature (Bobrowiec and Oliveira, 2012;
21 Coelho and Marinho-Filho, 2002; Colevatti et al., 2010; Gibbs et al., 1999; Gribel and
22 Hay, 1993; Martins et al., 2020) to greatly advance the knowledge of bat-plant
23 interactions in the Cerrado, both by showing intrinsic preferences of bats to certain
24 species and floral types and that fruit bats are important components of the network and
25 should be consistently sampled for interaction in future works.

1 By revealing previously unrecorded interactions with non-chiropterophilous
2 plants, we also highlight that bat-plant interactions in the biome are far from sufficiently
3 sampled. Next steps to better understand this system include incorporating pollination
4 efficiency into networks to quantify the roles of these different functional bat groups with
5 their contribution to plant fitness. Assessing how bat-flower networks are assemble in
6 other environments with different seasonal regimes and spatial configurations is also key
7 in understanding how network structuring factors vary across space, and whether there
8 are universal predicting factors of network structure. Finally, even our best models
9 (spatial and temporal overlap) left a considerable amount of unexplained variations in the
10 observed matrix which suggest that other factors not evaluated by us may also be at play
11 in structuring interactions. Other intrinsic plant traits that affect bat foraging should be
12 analyzed in future works on bat-flower networks, such as nectar secretion dynamics
13 (Bobrowiec and Oliveira, 2012), energy density (Tschapka, 2004) and floral volatile
14 composition (Gonzalez-Terrazas et al., 2016).

15

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23 Author contribution: U.M.D. and L.M.S.A. conceived the work and methodology.
24 U.M.D. collected and curated, and analyzed the data. U.M.D. and L.M.S.A. wrote the
25 final version of the manuscript.

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1 **SUPPLEMENTARY MATERIAL**

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3 **Table S1.** All plants registered in the transections that were classified as potential resources for bats,
 4 according to the criteria specified in the methods. Habitat type: CSS – Cerrado *sensu stricto*, GFI – gallery
 5 forest interior, GFE – gallery forest edge), their pollination syndrome and floral shape.

Espécie	Habitat type	Syndrome	Bat visitation	Floral shape
Acanthaceae				
<i>Justicia clivalis</i>	GFI	Ornithophily	No	Tubular
<i>Justicia irwinii</i>	GFI	Entomophily	No	Campanulate
<i>Ruellia incompta</i>	CSS	Entomophily	No	Infundibuliform
Alstroemeriaceae				
<i>Alstroemeria gardneri</i>	CSS	Ornithophily	No	Infundibuliform
<i>Alstroemeria stenopetala</i>	GFE	Sphingophily	No	Campanulate
Amaryllidaceae				
<i>Hippeastrum glaucescens</i>	CSS	Ornithophily	Yes	Infundibuliform
Apocynaceae				
<i>Mandevilla illustris</i>	CSS	Entomophily	No	Infundibuliform
<i>Mandevilla pohliana</i>	CSS	Entomophily	No	Infundibuliform
<i>Odontadenia lutea</i>	CSS	Entomophily	No	Infundibuliform
Arecaceae				
<i>Syagrus comosa</i>	CSS	Entomophily	No	Cupuliform
Bignoniaceae				
<i>Adenocalymma pedunculatum</i>	CSS	Entomophily	No	Infundibuliform
<i>Amphilophium elongatum</i>	GFE, GFI	Entomophily	No	Infundibuliform
<i>Anemopaegma acutifolium</i>	CSS	Entomophily	No	Infundibuliform
<i>Anemopaegma arvense</i>	CSS	Entomophily	No	Infundibuliform
<i>Arrabidaea sceptrum</i>	CSS	Entomophily	No	Infundibuliform
<i>Bignonia cf. corymbosa</i>	GFI	Entomophily	No	Infundibuliform
<i>Fridericia cf. florida</i>	GFI	Entomophily	No	Infundibuliform
<i>Fridericia platyphylla</i>	GFE	Entomophily	No	Infundibuliform
<i>Handroanthus chrysothrichus</i>	CSS	Entomophily	No	Infundibuliform
<i>Jacaranda caroba</i>	GFE	Entomophily	No	Infundibuliform
<i>Jacaranda ulei</i>	CSS	Entomophily	No	Infundibuliform
<i>Jacaranda</i> sp.	GFI	Entomophily	No	Infundibuliform
Calophyllaceae				
<i>Kielmeyera abdita</i>	CSS	Entomophily	No	Rotate
<i>Kielmeyera coriaceae</i>	CSS, FGE	Entomophily	No	Rotate
<i>Kielmeyera pumila</i>	CSS	Entomophily	No	Rotate

<i>Kielmeyera variabilis</i>	CSS	Entomophily	No	Rotate
Caryocaraceae				
<i>Caryocar brasiliense</i>	CSS	Chiropterophily	Yes	Brush
Celastraceae				
<i>Plenckia populnea</i>	GFE	Entomophily	No	Pseudanthium
Chrysobalanaceae				
<i>Couepia grandiflora</i>	CSS	Entomophily	No	Brush
Combretaceae				
<i>Combretum fruticosum</i>	GFI	Ornithophily	Yes	Brush
Convolvulaceae				
<i>Ipomoea sp.</i>	CSS	Entomophily	No	Infundibuliform
<i>Ipomoea procumbens</i>	CSS	Entomophily	Yes	Infundibuliform
<i>Jacquemontia velutina</i>	CSS	Entomophily	No	Infundibuliform
<i>Merremia digitata</i> var. <i>elongata</i>	CSS	Entomophily	No	Infundibuliform
<i>Merremia tomentosa</i>	CSS	Entomophily	Yes	Infundibuliform
Cunoniaceae				
<i>Lamanonia ternata</i>	GFE	Entomophily	Yes	Pseudanthium
Erythroxylaceae				
<i>Erythroxylum tortuosum</i>	CSS	Entomophily	No	Pseudanthium
Fabaceae				
<i>Bauhinia dumosa</i>	GFE	Chiropterophily	Yes	Tubular
<i>Bauhinia goyazensis</i>	CSS, GFE	Chiropterophily	Yes	Tubular
<i>Bauhinia holophylla</i>	CSS	Chiropterophily	Yes	Tubular
<i>Bauhinia longifolia</i>	GFE	Chiropterophily	Yes	Tubular
<i>Bauhinia rufa</i>	GFE	Chiropterophily	Yes	Tubular
<i>Calliandra dysantha</i>	CSS, GFE	Ornithophily	No	Pseudanthium
<i>Hymenaea courbaril</i>	GFI	Chiropterophily	Yes	Cupuliform
<i>Hymenaea stigonocarpa</i>	CSS, GFE	Chiropterophily	Yes	Cupuliform
<i>Leptolobium dasycarpum</i>	GFE	Entomophily	No	Pseudanthium
<i>Mimosa clausseii</i>	CSS, GFE	Entomophily	No	Pseudanthium
<i>Mimosa regnelli</i>	CSS, GFE	Entomophily	No	Pseudanthium
<i>Mimosa setosa</i>	CSS, GFE	Entomophily	Yes	Pseudanthium
<i>Mimosa somnians</i>	CSS, GFE	Entomophily	No	Pseudanthium
<i>Stryphnodendron adstringens</i>	CSS	Entomophily	No	Pseudanthium
<i>Tachigali vulgaris</i>	CSS	Entomophily	No	Pseudanthium
Gentianaceae				
<i>Calolisianthus speciosus</i>	CSS	Entomophily	No	Campanulate
<i>Sinningia elatior</i>	GFE	Ornithophily	No	Tubular

Iridaceae

<i>Gladiolus verdickii</i>	CSS	Entomophily	No	Cupuliform
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Lamiaceae

<i>Hypenia macrantha</i>	CSS	Ornithophily	No	Tubular
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<i>Amasonia hirta</i>	CSS	Entomophily	No	Tubular
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<i>Rhabdocaulon denudatum</i>	GFE	Entomophily	No	Tubular
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Loranthaceae

<i>Psittacanthus robustus</i>	CSS	Ornithophily	Yes	Tubular
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Lythraceae

<i>Diplusodon</i> spp.	CSS, GFE	Entomophily	No	Rotate
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<i>Diplusodon virgatus</i>	CSS, GFE	Entomophily	No	Rotate
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<i>Lafoensia pacari</i>	GFE	Chiropterophily	Yes	Campanulate
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Malvaceae

<i>Eriotheca pubescens</i>	CSS, GFE	Entomophily	No	Brush
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<i>Luehea divaricata</i>	GFI	Entomophily	No	Brush
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<i>Luehea grandiflora</i>	CSS, GFE	Chiropterophily	No	Cupuliform
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<i>Pavonia rosa-campestris</i>	CSS, GFE	Entomophily	No	Rotate
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<i>Peltaea polymorpha</i>	CSS, GFE	Entomophily	No	Rotate
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<i>Pseudobombax longiflorum</i>	GFE	Chiropterophily	Yes	Brush/urceolate
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<i>Pseudobombax tomentosum</i>	CSS	Chiropterophily	Yes	Brush/urceolate
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Myrtaceae

<i>Campomanesia adamantium</i>	CSS	Entomophily	No(?)	Brush
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<i>Campomanesia</i> cf. <i>aurea</i>	CSS	Entomophily	No(?)	Brush
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<i>Campomanesia pubescens</i>	CSS	Entomophily	No(?)	Brush
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<i>Campomanesia sessiliflora</i>	CSS	Entomophily	No(?)	Brush
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<i>Myrcia ericalyx</i>	CSS	Entomophily	Yes	Brush
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<i>Myrcia</i> cf. <i>multiflora</i>	CSS, GFE	Entomophily	Yes	Brush
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<i>Psidium</i> sp.	CSS	Entomophily		Brush
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Orobanchaceae

<i>Esterhazyia splendida</i>	CSS	Ornithophily	No	Tubular
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Proteaceae

<i>Roupala montana</i>	CSS, GFE	Entomophily	No	Pseudanthium
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Rubiaceae

<i>Ferdinandusa speciosa</i>	GFE	Ornithophily	No	Tubular
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Rutaceae

<i>Spiranthera odoratissima</i>	CSS	Entomophily	No	Tubular
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Styracaceae

<i>Styrax ferruginea</i>	CSS	Entomophily	No	Rotate
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Velloziaceae

<i>Vellozia squamata</i>	CSS	Entomophily	No	Infundibuliform
Vochysiaceae				
<i>Qualea grandiflora</i>	CSS	Entomophily	No	Calcarate
<i>Qualea multiflora</i>	CSS	Entomophily	No	Calcarate

1

2 **CAPÍTULO II**

3 Manuscrito submetido ao periódico *Annals of Botany*

4
5 Original Article

6
7 **Nocturnal nectar secretion by the ornithophilous mistletoe *Psittacanthus robustus***
8 **(Loranthaceae) triggers a strong dependence of bats in a Neotropical savanna**

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17 Running title: Nocturnal nectar dynamics and bat pollination of *Psittacanthus robustus*

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27 **ABSTRACT**

28 • Background and Aims: The Neotropical genus *Psittacanthus* comprises mostly
29 specialized ornithophilous mistletoes, with rare exceptions. *Psittacanthus robustus* is a
30 common ornithophilous species from the South American savannas whose flowers
31 secrete copious diluted nectar. Due to their long anthesis, we suggest that flowers actively
32 secrete nectar at night, serving as resource for flower-visiting bats. We investigated the
33 importance of this species as a resource for bats, their role in its reproduction and assessed
34 the species' nocturnal secretion dynamics.

35 • Methods: The work was conducted within the Cerrado biome in central Brazil.
36 We performed systematic mist net captures to sample pollen grains from bats during the
37 flowering period of *Psittacanthus robustus*, measured nectar accumulation and standing
38 crop, and performed visitor exclusion and hand pollination tests to define the species'
39 mating system and dependence on different animal groups for reproduction.

40 • Key Results: Nine phyllostomid bat species fed on *Psittacanthus robustus*, mainly
41 specialized nectarivores. As much as 50% of pollen samples from bats contained the
42 species during peak flowering, surpassing the prevalence of chiropterophilous species and
43 representing roughly a third of the diet of nectarivores. Flowers actively produce nectar
44 at night with volumes and concentrations falling into the ideal range for bats. Nectar is
45 most abundant after sunset and is continuously secreted after successive removals,
46 accumulating in the absence of visitors. *Psittacanthus robustus* is self-compatible but
47 seeds are set mostly by diurnal visitors. Nocturnal animals did not have a strong
48 contribution to seed set and acted as complementary pollinators.

49 • Conclusions: This is the third report of bat-pollination for the genus *Psittacanthus*,
50 and largest assemblage of bat visitors for the family Loranthaceae. Although generally
51 considered ornithophilous, *Psittacanthus robustus* secretes nectar at night and is a key

52 resource for bats in the Brazilian savanna, representing a potential early transitional or
53 mixed-dependency state involving bat pollination in a mostly ornithophilous genus.

54

55 **Keywords:** Bat pollination, Cerrado, chiropterophily, Loranthaceae, *Glossophaga*
56 *soricina*, mating system, nectarivory, nectar dynamics, ornithophily, Phyllostomidae,
57 *Psittacanthus*, pollination syndromes.

58

59 **1. INTRODUCTION**

60 Flowers pollinated by vertebrates have as recurrent characteristics a general
61 robustness, enhanced visibility, and abundant nectar secretion, adaptations shaped to meet
62 the energy needs and suit large pollinators such as birds, bats, and non-flying mammals
63 (Sazima *et al.*, 1994; Muchhala 2003; Dellinger *et al.*, 2019). This pattern is most
64 remarkable among chiropterophilous (bat-pollinated) plants, whose pollinators require
65 wider floral openings to insert their snouts and tongues to reach the resource, which
66 typically comprises large volumes of sucrose and hexose-rich nectar (Tschapka and
67 Dressler, 2002; Flemming *et al.*, 2009). Hence many bat-pollinated flowers present a
68 combination of abundant and accessible resources that attract a wider variety of floral
69 visitors that may sometimes act as secondary pollinators, such as sphingid moths (Gribel
70 and Hay, 1993; Rocha *et al.*, 2019; Queiroz *et al.*, 2021), hummingbirds (Muchhala *et al.*,
71 2009; Aguilar-Rodriguez *et al.*, 2016) and non-volant mammals (Queiroz *et al.*, 2016).

72 On the other hand, Neotropical ornithophilous or bird-pollinated species undergo
73 a different trend, often presenting restrictive floral morphologies with long and tubular or
74 hypocrateriform corollas with narrow openings that greatly restrict the access to the
75 narrow-billed hummingbirds (Faegri and Pijl, 2013; Thomson *et al.*, 2000; Martén-
76 Rodríguez *et al.*, 2009). Although these are ultimately reliable diagnostic traits of

77 Neotropical hummingbird-pollinated species (but not necessarily predictors of
78 hummingbird visitation, see Maruyama *et al.*, 2013), we should expect to find
79 transitioning bird-pollinated species with a lower phenotypic specialization and a mixed
80 dependence on birds and bats for pollination (Sazima *et al.*, 1994; Martén-Rodríguez *et*
81 *al.*, 2009) since a common trend among chiropterophilous species is to derive from
82 ornithophilous ancestors (Tripp and Manos, 2008). Recently-transitioned
83 chiropterophilous species still relying, to a variable extent, on birds for pollination may
84 also occur (Buzato *et al.*, 1994; Freiberg, 2007).

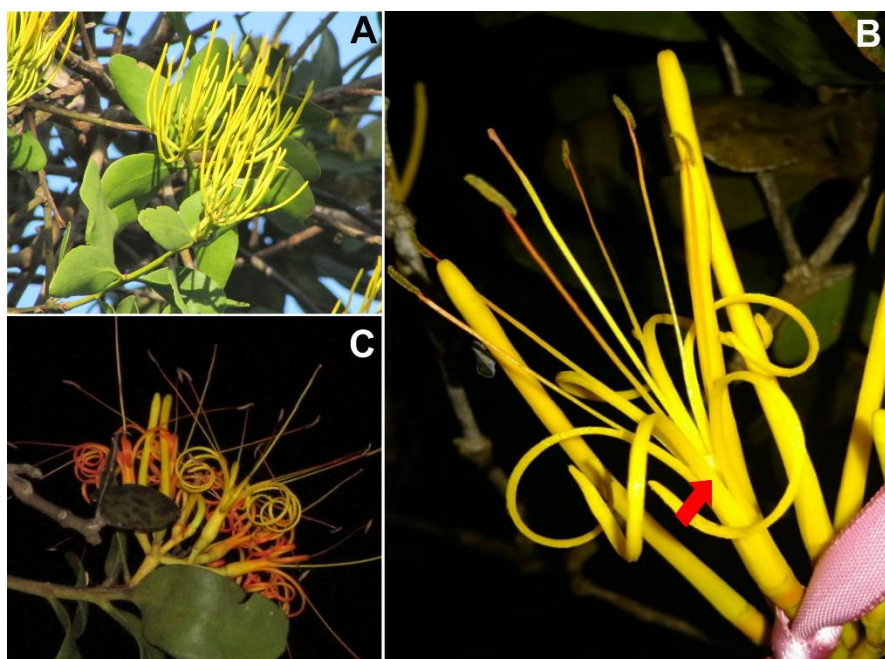
85 *Psittacanthus robustus* (Loranthaceae) is a mistletoe found throughout South
86 America and most commonly in the savannas of Brazil (Kuijt, 2009), and seems to fit
87 within this spectrum. The species has ornithophilous features that do not suggest the
88 interaction with bats, such as a bright yellow to orange tubular corolla (Figure 1) and
89 diurnal nectar secretion, and its diurnal visitors (mostly hummingbirds) have been
90 thoroughly described (Guerra *et al.*, 2014). However, Guerra *et al.* (2014) have classified
91 this species as a generalist based on its copious and highly diluted nectar, whose volume
92 and concentration values fall in the range expected for chiropterophilous species (e.g.
93 Tschapka, 2004). Although neither nectar secretion during the night nor visitation by
94 nocturnal animals has been investigated for the species, large volumes of nectar can be
95 frequently registered accumulated at night inside the flowers' short tube (Figure 1, C),
96 appearing as a promptly available resource for bats. Furthermore, its three-day anthesis
97 period also suggests that both diurnal and nocturnal pollinators visit its flowers during
98 their lifespan, as has been observed in other vertebrate-pollinated plants with a 24h or
99 longer anthesis (e.g. Sazima *et al.*, 1994; Aguilar-Rodríguez *et al.*, 2016).

100 Within the species-rich Neotropical genus *Psittacanthus*, *P. acinarius* and *P.*
101 *corynocephalus* are the few known cases of bat-pollination in a group of about 120

102 species previously thought to be strictly ornithophilous (Araújo and Sazima, 2003; Fadini
103 *et al.*, 2018). However, loranthaceous mistletoes are poorly studied in terms of pollination
104 ecology (Arruda *et al.*, 2012). Therefore, exploring and understanding the dependence on
105 nocturnal pollinators in ornithophilous species in the family is a significant step towards
106 clarifying the evolution of vertebrate-pollination syndromes in this group, as well as
107 potential mechanisms underlying the transition between ornithophily and chiropterophily.
108 Here, we investigated the usage of *P. robustus* as a resource for bats through systematic
109 capture expeditions in the Brazilian Cerrado. We also assessed the nocturnal nectar
110 dynamic of *P. robustus* to find out if the species actively secrete nectar during the night
111 and whether nectar secretion correlates with the visiting frequency and behavior of bats
112 and other nocturnal animals. Finally, we measured and compared the contribution of
113 diurnal and nocturnal pollinators to fruit set through visitor exclusion trials to quantify
114 the species' dependence on both groups for reproduction.

115

116



117

118 **Figure 1.** The dense inflorescences (A) and showy flowers (B and C) of the mistletoe *Psittacanthus*
119 *robustus* (Loranthaceae) in central Brazil. The flowers have typical ornithophilous features, such as a corolla
120 tube and a bright yellow color (A and B) that changes to a reddish-orange as flowers approach senescence
121 (C). The flowers secrete abundant and diluted nectar during the day, which accumulates inside the tube at
122 night in the absence of visitors (B, red arrow).

123

124 **2. MATERIALS AND METHODS**

125

126 *2.1 Study site and species*

127

128 The study was conducted in the Brasília National Park (PNB), Federal District, Brazil
129 (15°40'52.0"S, 47°59'17.4"W), located on the central Brazilian highlands, within the
130 Cerrado biome and located in the national capital Brasília. The PNB has 42,355 ha and
131 encompasses large extensions of preserved primary Cerrado vegetation such as typical
132 savanna formations dominated by a shorter, bushy plant community (Cerrado *sensu*
133 *stricto*), arboreal savannas (Cerradão), and grasslands. The region has a tropical
134 altitudinal climate (Cwa and Cwb climates according to the Köppen scale), with a warm
135 rainy season from October to May and a cold mid-year dry season.

136 *Psittacanthus robustus* Mart. (Loranthaceae) is a hemiparasitic mistletoe species
137 native to and most commonly found in the savannas of South America parasitizing
138 Vochysiaceae hosts (Monteiro *et al.*, 1992). It is an abundant species in the PNB and
139 occurs in higher densities in typical savanna formations (Cerrado *sensu stricto* and
140 Cerradão). *Psittacanthus robustus* was seen within the sampling site parasitizing
141 *Vochysia thyrsoidea*, *Qualea multiflora* and *Q. grandiflora* (Vochysiaceae) hosts. The
142 flowering of *P. robustus* is long and occurs throughout the wet season, starting between
143 October and November and ending by May. During flowering, all individuals in the same

144 area flower in an approximately synchronous manner and expose their dense
145 inflorescences containing several flower triads (Figure 1, A), providing an abundant
146 resource to floral visitors. Here we do not discuss the basic floral biology of *P. robustus*
147 as it has been thoroughly explored by Guerra *et al.* (2014).

148 We carried out two distinct sampling steps in the PNB. First, bat captures to
149 sample pollen grains were performed monthly and covered the entire flowering period of
150 the species, starting in October 2019 and continuing through the rainy season until
151 February 2020. The remaining months of March, April, May, and June were sampled in
152 2021. This step was performed in eight fixed sampling sites spread throughout the park,
153 selected randomly and not necessarily associated with *P. robustus* individuals (see the
154 *Pollen sampling* section below). Secondly, procedures that focused on *P. robustus*
155 individuals such as nectar dynamics protocols, observations of visitation frequencies,
156 controlled pollination, and exclusion trials were all performed in a fixed sub-population
157 of 19 individuals distributed along a 330 m Cerrado *s.s.* edge in the PNB (15°37'27.0"S
158 48°01'16.5"W), between January and February 2021.

159

160 2.2 Pollen sampling from bats

161 We performed monthly bat capture expeditions in the PNB from the end of one
162 dry season through the beginning of the next, totaling nine months (October – June), in
163 order to comprise the entire flowering period of *P. robustus*. Each month, we captured
164 bats for eight consecutive nights, each in a different sampling site within the PNB. The
165 sites corresponded to four Cerrado *s.s.* areas (15°44'18.2"S, 47°59'10.0"W; 15°40'49.1"S,
166 48°04'07.9"W; 15°39'05.3"S, 48°00'06.7"W and 15°41'59.5"S 47°59'52.2"W), two
167 gallery forest interiors (15°41'38.1"S, 47°58'12.0"W and 15°37'36.8"S 48°01'04.9"W),
168 and two gallery forest borders (15°38'12.3"S, 47°56'11.7"W and 15°42'50.8"S,

169 48°03'37.5"W). The sites were separated from each other by at least two kilometers and
170 homogeneously covered the entire area of the park. Each night we set 10 mist nets (3 x
171 12 m, Ecotone®, Poland) at ground level, placed randomly inside the capture sites from
172 1800h to 0000h, resulting in 4320 mist net hours.

173 We collected pollen samples from all captured bats. We used glycerinated and
174 stained gelatin cubes (Voigt *et al.*, 2009) to collect pollen from their entire external body
175 (head, torso, wings, and uropatagium). Pollen samples were placed in individual vials for
176 later mounting on slides for pollen identification through light microscopy. Bats were
177 identified using specialized keys (Gardner *et al.*, 2008; Dias *et al.*, 2016), sexed, weighed,
178 and were released. We also collected pollen samples directly from the anthers of open
179 flowers of several flowering plants found in the park, among which *P. robustus*, using the
180 same method described above to create a reference slide collection. Only pollen samples
181 from bats that contained five or more grains of *P. robustus* were considered legitimate
182 interactions. In each of the eight capture sites, a fixed 1000 x 10 m transect was set within
183 the vegetation to assess the frequency of flowering *P. robustus* individuals. Transects
184 were also sampled monthly from October to June.

185 For the bat species caught in mist nets that carried *P. robustus* pollen, we
186 calculated the percentage of bat individuals from each species that carried pollen from *P.*
187 *robustus* in relation to the total captures of that specie, and specificity (S), i.e. within the
188 individuals that interacted with *P. robustus*, the percentage of their diet that was
189 represented by *P. robustus*. Higher specificity ($S \rightarrow 1$), indicates a higher dependence of
190 *P. robustus* by the bat species during the flowering period.

191

192 *2.3 Nectar dynamics*

193 We investigated the nocturnal nectar dynamics of *P. robustus* to assess if the
194 species actively produces nectar throughout the night to reward pollinators (henceforth,
195 the term “night” will be used to refer to the period between 1800h and 0600h); if the
196 nectar suits the requirements of bats; if it undergoes removal effect (enhanced secretion
197 following nectar extraction); and if nectar is reabsorbed throughout the night in the
198 absence of visitation (Ordano and Ornelas, 2004). We employed the protocols of Galleto
199 and Bernardello (2005) and Queiroz *et al.* (2016). A group of recently opened flowers (n
200 = 60, 11 individuals) was marked and bagged. At 1800h of the first anthesis day, all
201 flowers had any leftover nectar produced during the day removed to simulate the start of
202 anthesis and were rebagged.

203 The 60 flowers were separated into groups of ten, and each group was assigned
204 one of the six following accumulation treatments: (i) first removal at 1900h, five
205 subsequent removals every two hours until 0500h; (ii) first removal at 2100h, four
206 subsequent removals; (iii) first removal at 2300h, three subsequent removals; (iv) first
207 removal at 0100h, two subsequent removals; (v) first removal at 0300h, one subsequent
208 removal; (vi) a single removal at 0500h. In each removal, we measured nectar volume
209 (microliters, 0-25 μ L microsyringes, Hamilton[®], Reno, USA), concentration in sugar
210 equivalents (% mass/mass, 0-33% hand refractometer, Atago[®], Tokyo, Japan), and mass
211 of sugar equivalents [mg, using volume and concentration values in the formula of Galletto
212 and Bernadello (2005)]. Flowers lasted for three days and produced nectar until the
213 afternoon of the third day (Guerra *et al.*, 2014); hence this procedure was carried out in
214 two consecutive nights to capture the entire lifespan of flowers. By 1900h of the third
215 day, most flowers had wilted and none produced nectar. All flowers remained bagged
216 when not being manipulated. One flower from group iii was discarded due to damage
217 caused by ants, resulting in a total of 59 flowers.

218 A different set of flowers ($n = 198$, 7 individuals) was left unbagged and separated
219 into the same time groups as described above (1900h, 2100h, 2300h, 0100h, 0300h, and
220 0500h), each group containing a variable number of flowers ($n = 29 - 37$), according to
221 availability. We sampled each group once for nectar volume, concentration, and sugar in
222 one of these periods to assess the standing nectar crop available to nocturnal visitors
223 throughout the night and early morning.

224

225 *2.3 Frequency of floral visitors*

226 We used a 2.7 K Camcorder 2688X1520P video camera mounted on a 1.5m tripod
227 and equipped with an infrared sensor to record nocturnal floral visitors and quantify their
228 visiting frequencies to flowers. For five days, the camera was placed ca. 2 m from
229 individuals of *P. robustus* ($n = 5$) facing branches containing several inflorescences (4 –
230 20 inflorescences, depending on the individual) from 1900h to 0600h. Filming was
231 interrupted on several occasions due to rainfall and resumed immediately when possible.
232 We recorded 47h and 20 min in total.

233 From the footage, we quantified animal visiting frequencies. A visit was registered
234 at any time an animal individual (a bat or a moth) interacted with an individual flower by
235 hovering directly in front of it (specialized nectarivorous bats and hawkmoths) or by
236 clinging onto the flower (non-specialized bats). If a visitor interacted with different
237 flowers during the same bout, each interaction was counted as a distinct visit, but if the
238 same visitor repeatedly interacted with one flower during the same bout, only one visit
239 was counted. We considered as independent bouts every activity separated by 10 or more
240 seconds of no activity. These intervals were counted independently for bats and
241 hawkmoths. On the rare occasion in which more than one bat individual or moth
242 individual was seen foraging simultaneously, we were unable to separate the visits of

243 different individuals and did not distinguish them (i.e. repeated interactions with the same
244 flower were considered a single visits regardless of being delivered by one or more
245 individuals). In order to correlate visitation frequency with nectar secretion, we separated
246 visits according to time blocks corresponding to the six nectar sampling shifts: 1900-
247 2059h, 2100h-2259h, 2300h-0059h, 0100-0259h, 0300-0459h and 0500h-0600h (dawn).

248 Additionally, we used a Canon SX500 IS camera attached to a 1.5 m tripod to
249 photograph visitors' interaction with flowers to determine visitor identity and behavior
250 during the interaction. This was done non-systematically between nectar sampling shifts
251 and other activities whenever we had additional time. Hawkmoths that appeared on
252 footage and photographs were identified using specialized literature (Camargo *et al.*
253 2018)

254

255 *2.4 Fitness quantification*

256 *Psittacanthus robustus* requires a pollen vector for most of its seed set output,
257 either by xenogamous or geitonogamous pollen flow (Guerra *et al.*, 2014). Thus, we
258 performed selective visitor exclusion tests on individual flowers of *P. robustus* to assess
259 the relative role of diurnal and nocturnal floral visitors on seed formation, as well as
260 controlled pollination tests to describe the mating system of the study population. We
261 marked 309 flowers from 19 individuals, which were manipulated according to the
262 following treatments. Nocturnal exclusion test (n = 44): newly opened flowers were
263 bagged during the night (1800h to 0600h) and left unbagged during the day (0600h to
264 1800h) throughout their three-day anthesis. Diurnal exclusion test (n = 31): newly opened
265 flowers were bagged during the day (0600h to 1800h) and left unbagged during the night
266 (1800h to 0600h) throughout the anthesis. Hand cross-pollination (n = 31): newly opened
267 flowers had pollen from another individual brushed against their stigma and were left

268 bagged until senescence. Hand self-pollination (n = 31): newly opened flowers had pollen
269 from another flower of the same individual brushed against their stigma and left bagged
270 until senescence. Autonomous self-pollination (n = 41): flowers were left bagged from
271 anthesis until senescence without manipulation. Agamospermy (n = 30): flowers in the
272 pre-anthesis state were emasculated and left bagged until senescence. Finally, 101 flowers
273 were left unbagged and unmanipulated as a control group. All flowers manipulated were
274 bagged individually before anthesis during the bud stage to avoid pollen contamination
275 from other flowers, with the exception for the hand self-pollination treatment, where more
276 than one flower was sometimes isolated with the same bag.

277 Fertilized flowers of *P. robustus* develop into monospermic drupes; thus, the
278 fitness of each treatment group was calculated as the ratio of flowers that succeeded in
279 developing into seeded fruits (Guerra *et al.*, 2014). The fruit set was assessed three months
280 after the tests were performed (April 2021). The Self Compatibility Index (SCI) was
281 calculated as the fruit set after hand self-pollination divided by the fruit set after hand
282 cross-pollination (Lloyd and Schoen, 1992).

283

284 2.5 Data analysis

285 Differences in total accumulated nectar and sugar mass by flower among the six
286 accumulation treatments and differences in standing crop values for nectar volume,
287 concentration, and sugar mass among the time groups, were assessed using one-way
288 ANOVAs. For both accumulation and standing crop measurements, only data for the first
289 night of floral anthesis were used, as flower wilting during the second night greatly
290 reduced sample size, and the remaining flowers yielded negligible nectar quantities.
291 Normality was checked for all response variables, and accumulation and standing crop
292 volume and sugar mass values were log-transformed before analysis. Post-hoc pairwise

293 T-tests using Bonferroni corrections were employed to detect differences among time
294 groups. Differences in bat and hawkmoths visitation frequencies among time groups were
295 assessed using Kruskal-Wallis tests (post-hoc tests were not applicable).

296 We fit mixed-effect generalized linear models (GLMMs) with a binomial error
297 distribution and *logit* link function to determine if fruit set success rates differed among
298 manipulation treatments and the control group. Success/fail results were set as the
299 response variable, the six treatments plus control as the explanatory variable, and the
300 inflorescence and individual into which flowers were nested were set as random effects.
301 The analysis was performed in R studio 3.6.0 (R Development Core Team, 2020).
302 GLMMs were performed with the package *lme4* (Bates *et al.*, 2015).

303

304 **3. RESULTS**

305 *3.1 Floral visitors*

306 Between November and April, 240 bats of 20 species belonging to the families
307 Phyllostomidae and Vespertilionidae were captured, from which 80 (33.3%) from 11
308 species carried pollen from 22 plant species. Out of the flower visitors, 34 (42.5%) bats
309 belonging to the family Phyllostomidae and subfamilies Glossophaginae,
310 Lonchophyllinae, Carollinae and Stenodermatinae carried pollen from *P. robustus* (Table
311 1). Other resources used by bats in the period included the chiropterophilous *Lafoensia*
312 *pacari* (Lythraceae), *Hymenaea* spp., *Bauhinia* spp. (Fabaceae) and the generalist
313 *Lamanonia ternata* (Cunoniaceae). The role of *P. robustus* in the diet of bats during its
314 flowering period was variable. It was most prominent among specialized nectarivores,
315 being present in 70% and 50% of samples from the glossophagines *Glossophaga soricina*
316 and *Anoura caudifer*, respectively, representing about a third of the diet of both species

317 during the period (Table 1). *Psittacanthus robustus* was also used, to a lesser extent, by
318 the endangered nectarivore *Lonchophylla dekeyseri* and by several non-specialized floral
319 visitors, such as frugivores and omnivores of the subfamilies Carrollinae and
320 Stenodermatinae (Table 1).

321 Although we focused on assessing the role of bats as floral visitors of *P. robustus*,
322 hawkmoths were also seen visiting plants frequently throughout the night during direct
323 observations. Therefore, we report here the hawkmoths sighted and their visitation
324 frequencies to flowers from the footage. However, we point out that the diversity of
325 hawkmoth visitors is most likely underrepresented compared to bats since hawkmoths did
326 not undergo systematic sampling. The flowering of *P. robustus* started in November and
327 grew steadily in intensity until January and February, peaking in March and decreasing
328 quickly after that until its end in May (Figure 2). During these months, bat dependence
329 on the species increased accordingly, reaching its maximum in February and March when
330 *P. robustus* represented half of all resources consumed by bats, equaling and surpassing
331 chiropterophilous plants, respectively (Figure 2, A).

332 We recorded 65 bats visit and 142 hawkmoth visits on the footage, and several
333 more on still photographs. While visiting flowers, bats were always legitimate pollinators,
334 hovering very close to the flowers to insert the snout in the tube and touching the long
335 and herkogamous reproductive whorls with various parts of their bodies, but especially
336 the abdomen and lower side of wings (Figure 3, B). Hawkmoths, on the other hand,
337 behaved unpredictably while visiting flowers. On about half of visits, hawkmoths would
338 hover from a distance and insert the proboscis into the tube to feed and not touch
339 reproductive parts (Figure 3, C). The other type of visit was similar to those performed
340 by bats, where hawkmoths hovered very close to flowers or landed on them and touch
341 anthers and stigma with their bodies (Figure 3, D). Small Noctuid moths also visited

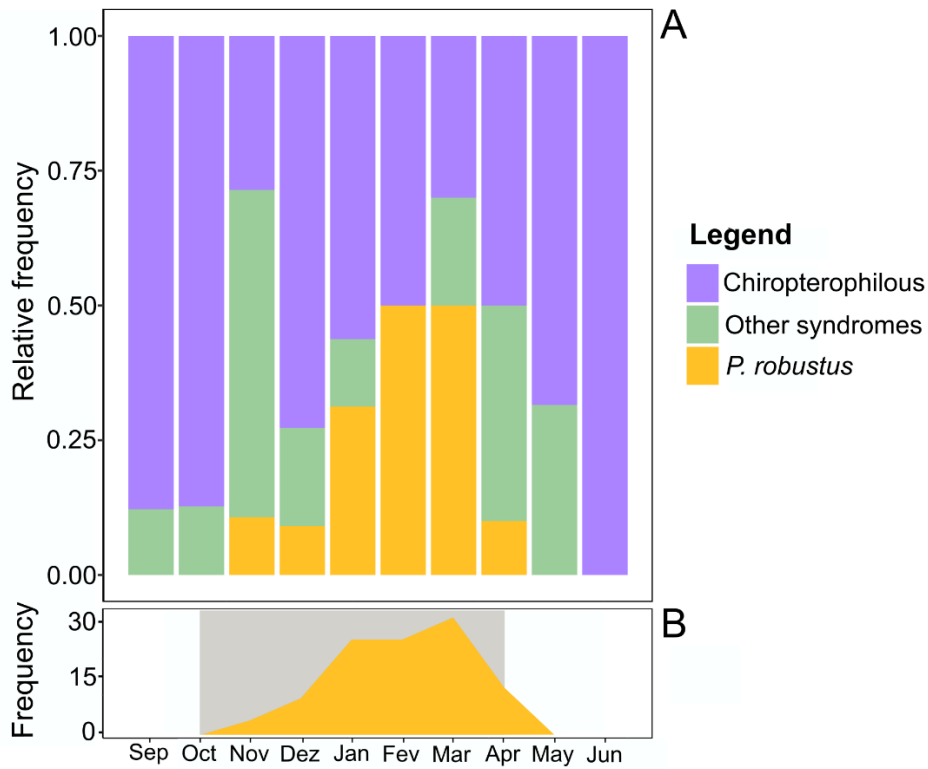
342 flowers on several occasions, but they never touched anthers or stigmas and acted as
 343 nectar robbers.

344

345 **Table 1.** Nocturnal floral visitors of *Psittacanthus robustus* sampled systematically through mist nets in
 346 the Brasília National Park (bats) and observed visiting plants directly (bats and hawkmoths). The number
 347 of individuals captured (N), *P. robustus* visitors, and specificity (S) apply only to bats caught in mist nets
 348 during the systematic sampling from November through April.

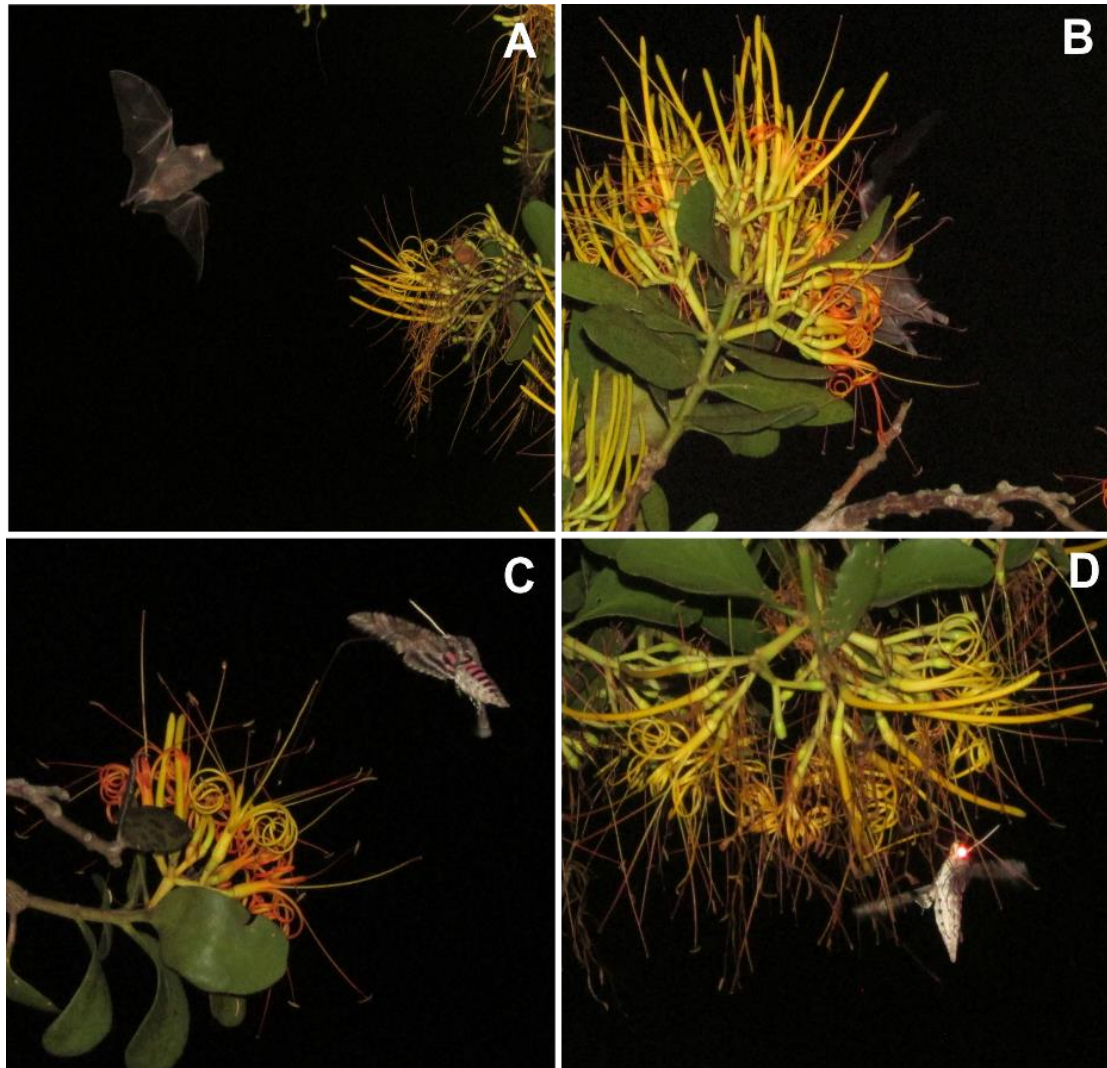
Species	Method of record	N	<i>P. robustus</i> visitors (%)	S
Chiroptera (Phyllostomidae)				
Carollinae				
<i>Carollia perspicillata</i> Linnaeus	Mist net	40	1 (2.5)	0.08
Glossophaginae				
<i>Anoura caudifer</i> Geoffroy	Mist net	14	7 (50.0)	0.28
<i>Glossophaga soricina</i> Pallas	Mist net	27	19 (70.4)	0.36
Lonchophyllinae				
<i>Lonchophylla dekeyseri</i> Taddei, Vizotto & Sazima	Mist net	12	1 (8.3)	0.08
Stenodermatinae				
<i>Artibeus planirostris</i> Spix	Mist net	19	3 (15.8)	0.21
<i>Dermanura cinerea</i> Gervais	Mist net	35	2 (5.7)	0.50
<i>Platyrrhinus lineatus</i> Geoffroy	Mist net	17	1 (5.9)	0.25
Lepidoptera (Sphingidae)				
<i>Agrius cingulata</i> Fabricius	Photographs	-	-	-
<i>Protambulyx eurycles</i> Herrich-Schäffer,	Capture*	-	-	-

349 * An individual was seen visiting the plant and was captured with a hand net, identified and released.



350

351 **Figure 2.** Relative frequency of plants used as a resource by flower-visiting bats in each month based on
 352 the pollen types sampled (A) and the sum of sighting frequencies of flowering *Psittacanthus robustus*
 353 individuals at the fixed transects (B) from the end of the dry season, through the rainy season (shaded grey
 354 area), and up to the beginning of the dry season. In A, resource plants are grouped as typical
 355 chiropterophilous plants (i.e. floral traits related to bat pollination and visitation by bats already registered
 356 in the literature), plants from other pollination syndromes, and *P. robustus* individually.



357

358 **Figure 3.** Some nocturnal visitors of the ornitophilous mistletoe *Psittacanthus robustus*. Glossophagine
 359 bats, approaching (A) and pollinating flowers (B), and the hawkmoth *Agrius cingulata* feeding from afar
 360 (C) and pollinating (D) flowers.

361

362 3.2 Nectar accumulation

363 On the first night of anthesis, flowers of *P. robustus* produced large and diluted
 364 volumes of nectar during the first hours after the simulated beginning of anthesis at sunset.
 365 The flowers continued to produce smaller volumes steadily until sunrise, even after
 366 successive removals (Table 2). Although nectar volumes and sugar mass decreased after
 367 the first removals in all groups, sugar mass stabilized after that, while sugar concentration

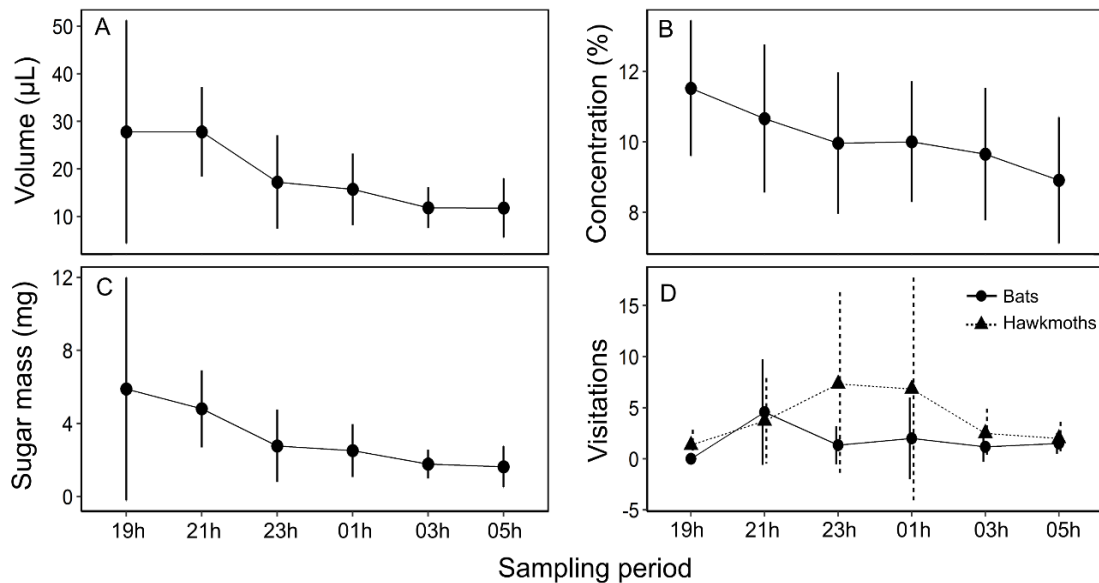
368 remained roughly constant throughout the entire night and early morning. Accumulation
369 groups did not differ significantly in terms of total accumulated nectar volumes ($F_{1,57} =$
370 $0.20, p = 0.66$) or sugar mass ($F_{1,57} = 2.92, p = 0.09$) per flower. Nectar accumulation still
371 occurred in a few flowers on the second night of anthesis, but nearly all flowers were
372 either empty or had already wilted by midnight (Table 2).

373

374 *3.3 Nectar standing crop and visitation frequency*

375 Standing crop values of nectar volume, concentration and sugar mass varied
376 significantly among time blocks of the first anthesis night (Figure 4 A-C). Nectar volume
377 averaged at $19.03 \pm 13.91 \mu\text{L}$ (mean \pm s.d., $n = 198$ measurements) throughout the night
378 and was higher just after sunset ($F_{5,192} = 11.4, p = 1.18\text{e-}09$), with means at 1900h and
379 2100h similar, and nectar volumes after 2100h lower in all subsequent time blocks ($p <$
380 0.05 in all comparisons with 2100h). Volumes just after sunset were highly variable, but
381 still significantly higher than the hour preceding dawn (0300h and 0500h, $p < 0.05$ for
382 both comparisons), when nectar stabilized at low volumes (Fig 4 A). Nectar concentration
383 averaged at $10.15 \pm 2.01 \%$ and nectar was most concentrated just after sunset ($F_{5,192} =$
384 $7.6, \text{d.f.} = 192, p = 1.6\text{e-}06$), also being stable between 1900h and 2100h ($p = 0.9$), but
385 more diluted from 2300h until dawn when compared to 1900h ($p < 0.05$ for all
386 comparisons) (Figure 4 B). Sugar mass averaged at $3.31 \pm 3.37 \text{ mg}$ and had a trend similar
387 to nectar volume, with variable but mostly high values after sunset and a peak at 2100h
388 ($F_{5,192} = 13.6, \text{d.f.} = 192, p = 2.5\text{e-}11$), with all subsequent time blocks presenting
389 increasingly lower sugar masses (all comparisons with 2100h at $p < 0.05$). Bat and
390 hawkmoths visitation frequencies, however, were overall low throughout the night
391 [pooled time blocks: $\bar{x} = 1.86 \pm 3.15$ (bats) and $\bar{x} = 4.01 \pm 6.19$ (hawkmoths)] and
392 unpredictable (bats: $\chi^2 = 7.2, \text{d.f.} = 5, p = 0.21$; hawkmoths: $\chi^2 = 2.3, \text{d.f.} = 5, p = 0.81$)

393 with a slight peaks of bat visitation at 2100h and of hawkmoth visitation between 2300h
394 and 0100h (Figure 4 D).



395

396 **Figure 4.** Measurements of standing crop nectar volume (A), sugar concentration (B) and sugar mass (C),
397 as well as bat and hawkmoth visitation frequencies (D) throughout the first anthesis night of *Psittacanthus*
398 *robustus*. Verticals bars indicate standard deviations.

399

400 3.4 Exclusion trials and mating system

401 Most seeds in *P. robustus* were set through xenogamy, but the species has a
402 moderate level of self-compatibility (SCI = 0.55) and is also able to self-pollinate
403 autonomously and to set seeds without pollen deposition, securing a constant marginal
404 fruit set (Table 2). The natural fruit set was low and comparable to the diurnal exclusion,
405 agamospermy, hand self-pollination and spontaneous self-pollination. Conversely, the
406 success ratio of the nocturnal exclusion treatment was high and similar to that of hand
407 cross-pollination (Table 2). The sum of success ratios of diurnal and nocturnal exclusion
408 treatments approaches the ratio observed from hand cross-pollination. None of the
409 treatments, however, yielded a large success rate (maximum of 0.55 success rate in hand
410 cross-pollination)

- 1 **Table 2.** Results of nocturnal nectar accumulation trials from 60 flowers of *P. robustus* throughout the two first anthesis nights and according to the six removal groups (see the
2 Methods section for details on removal groups). Variables measured were nectar volume (μL), sugar concentration (%) and mass of sugar equivalents (mg). Dashed cells indicate
3 that values could not be measured. Values are followed by the standard deviation (s.d.). Values without s.d.n are single measurements

Groups	Night 1							Night 2							
	19-20h	21-22h	23-00h	01-02h	03-04h	05-06h	Total	19-20h	21-22h	23-00h	01-02h	03-04h	05-06h	Total	
i	μL	15.3 ± 10.89	13.5 ± 10.3	8.8 ± 5.1	8.0 ± 5.9	4.2 ± 3.9	5.3 ± 4.8	55.1 ± 21.7	0.9 ± 2.8	0.6 ± 1.7	0.6 ± 1.8	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 1.6	2.5 ± 7.9
	mg	2.9 ± 2.1	2.5 ± 1.9	1.4 ± 0.7	1.6 ± 1.1	0.8 ± 0.7	1.1 ± 1.0	9.3 ± 8.5	1.5	1.0	1.1	0	0	-	0.4 ± 1.1
	%	11.01 ± 2.1	9.9 ± 1.4	9.3 ± 2.1	9.7 ± 1.5	9.7 ± 1.8	9.3 ± 2.1		10.8	11	11.9	-	-	-	
ii	μL		15.3 ± 13.8	6.1 ± 5.3	4.7 ± 4.5	3.0 ± 3.3	2.3 ± 2.8	36.0 ± 26.9		0.5 ± 1.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 1.2
	mg		2.9 ± 2.6	1.4 ± 0.9	0.9 ± 0.7	0.8 ± 0.4	0.6 ± 0.4	5.3 ± 4.9		0.2	0	0	0	0	0.02 ± 0.1
	%		9.5 ± 2.1	9 ± 1.4	7.6 ± 2.4	8.6 ± 1.5	7.9 ± 1.3			4.5	-	-	-	-	
iii	μL			34.7 ± 26.7	8.1 ± 10.2	9.8 ± 9.9	4.8 ± 7.2	63.7 ± 44.9			1.5 ± 4.8	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.6	1.8 ± 4.8
	mg			6.3 ± 5.6	1.1 ± 2.0	1.7 ± 1.6	1.5 ± 1.6	9.3 ± 9.1			3.0	0	0	0.3	0.3 ± 1.0
	%			9.7 ± 2.8	10.4 ± 1.7	8.6 ± 2.7	9.8 ± 2.3				12	-	-	10.8	
iv	μL				22.4 ± 15.6	6.1 ± 5.9	4.0 ± 3.1	35.0 ± 19.6				0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	mg				3.6 ± 3.3	1.4 ± 1.5	0.9 ± 0.5	5.2 ± 5.0				0	0	0	0
	%				8.6 ± 2.7	8.8 ± 3.1	9.0 ± 2.7					-	-	-	.
v	μL					34.8 ± 22.6	5.2 ± 3.7	44.3 ± 21.8					0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	mg					5.2 ± 3.9	0.7 ± 0.4	5.3 ± 4.3					0	0	0
	%					8.6 ± 1.6	7.5 ± 1.8						-	-	
vi	μL						36.4 ± 15.3	36.4 ± 15.2						0.1 ± 0.2	0.1 ± 0.2
	mg						4.6 ± 2.5	4.6 ± 2.5						-	-
	%						8.5 ± 3.0							-	

5 **Table 3.** Fruit set and resultant success ratio from the total number of treated flowers (*n*) from different
6 manipulation treatments in *Psittacanthus robustus*, and self-compatibility index. Letters beside success
7 ratio values indicate significantly different groups ($p < 0.05$, as determined by the GLMM)

Treatment	Individuals	<i>n</i>	Fruit/seed set *	Success ratio
Exclusion of nocturnal visitors	6	44	18	0.41 ^a
Exclusion of diurnal visitors	9	31	5	0.16 ^{b,c}
Hand cross-pollination	7	31	17	0.55 ^a
Hand self-pollination	6	31	10	0.32 ^{a,b}
Spontaneous self-pollination	10	41	11	0.27 ^{a,b,c}
Agamospermy	5	30	4	0.13 ^c
Unmanipulated control	12	101	27	0.27 ^{b,c}
Total		309		
Self-Compatibility Index				0.59

8 * Fruits are monospermic drupes

9

10 **4. DISCUSSION**

11

12 *4.1 A key resource for flower-visiting bats*

13 The present work reports the largest assemblage of bat visitors for the family
14 Loranthaceae, the first thorough description of nocturnal nectar dynamics in
15 *Psittacanthus*, and the third empirical observation of bat pollination in the genus (Araújo
16 and Sazima, 2003; Kuijt and Hansen, 2015; Fadini *et al.*, 2018). The flowers of *P.*
17 *robustus*, although ornithophilous in morphological features, contrasting with its typically
18 chiropterophilous sister taxa *P. acinarius* and *P. corynocephalus*, are widely used by
19 specialized Glossophaginae nectarivores during the peak of the rainy season in central
20 Brazil. They can even surpass local chiropterophilous flowers in terms of relative

21 contribution to their diet. A few factors may explain such high exploitation of this
22 otherwise characteristic ornithophilous species.

23 First, floral morphology is likely playing a main role in allowing bats to access
24 the resource, as corolla opening seems to be among the most relevant morphological
25 variables in determining whether bats may access or not the nectar of a given species
26 (Queiroz *et al.*, 2021). The floral tube in *P. robustus* is not too long (2 – 3 cm, Guerra *et*
27 *al.* 2014) nor very constricted as seen in most specialized ornithophilous flowers, and has
28 a relatively wide opening which allows the insertion of the bat's snout and tongue. Less
29 restrictive corollas are deemed necessary in separating mixed bat-hummingbird
30 pollination systems from specialized ornithophilous ones (Sazima *et al.*, 1994; Martén-
31 Rodríguez *et al.*, 2009; Aguilar-Rodríguez *et al.*, 2016).

32 Secondly, *P. robustus* is a large and robust plant species that produces hundreds
33 of flowers throughout its reproductive phenophase and is usually found in high densities
34 in the study site, with often clumped individuals. Despite its steady-state flowering (*sensu*
35 Gentry, 1974) that yields few open flowers per night per individual for several months
36 during the rainy season (Guerra *et al.*, 2014), its daily flowering probably results in high
37 energy density values per area unit. At the same time, the diversity and abundance of
38 chiropterophilous flowering plants in the region tend to decrease in the rainy season
39 (Coelho and Marinho-Filho, 2002), as the abundant keystone species *Caryocar*
40 *brasiliense* (Caryocaraceae) ends its flowering by October – November (Gribel and Hay,
41 1993) and bats rely mostly on less abundant species such as *Bauhinia* spp., *Hymenaea*
42 *stigonocarpa* (Fabaceae) and *Lafoensia pacari* (Lythraceae) (UM. Diniz and LMS
43 Aguiar, UnB, Brasília, Brazil, unpub. res.). The high availability of *P. robustus* in this
44 period, coupled with the long lifespan of flowers and non-enclosed nectar should create
45 an attraction strong enough to draw bats towards it. Such interaction is not surprising,

46 since nectarivorous bats are highly opportunistic and adaptable to resource availability
47 pulses, shifting to a more insect- or fruit-centered diet during times of nectar shortage
48 (Heithaus *et al.*, 1975; Tschapka and Dressler, 2002; Soto-Centeno *et al.*, 2006; Amaral
49 *et al.* 2019), but also often exploiting plants belonging to generalized pollination systems
50 (Vieira and Carvalho-Okano, 1996; Muchhala *et al.*, 2009; Amorim *et al.*, 2012), which
51 *P. robustus* seems to show.

52

53 4.2 The role of nectar dynamics

54 The final factor, nectar secretion, corroborates with the existence of a generalized
55 system in *P. robustus*. Although pollinated by various nectarivorous birds whose
56 visitation frequency is linked to nectar secretion (Guerra *et al.*, 2014), flowers are actively
57 replenished with nectar on the first night after successive removal, and sugars are not
58 reabsorbed. Therefore, the nocturnal standing nectar crop is not necessarily a leftover or
59 byproduct from diurnal secretion and accumulates in the absence of nocturnal visitation.
60 Regarding nectar quality and quantity, the sugar concentration in *P. robustus* is within
61 the ideal range for consumption by bats. Concentration was similar but slightly lower than
62 reported for the two chiropterophilous *Psittacanthus corynocephalus* (mean 16.55%,
63 Araújo and Sazima, 2003) and *P. acinarius* (mean 16.5%, Fadini *et al.*, 2018), and nectar
64 volume produced after sunset is comparable to that of *P. acinarius*. Compared to other
65 Neotropical chiropterophilous species, sugar concentration falls into the expected range
66 of ca. 10 to 20% concentration (Sazima *et al.*, 1999; Tschapka, 2004; Sanmartin-Gajardo
67 and Sazima, 2005).

68 Nectar volumes, however, are not necessarily optimal for bats. Values
69 accumulated during the first night were lower than most chiropterophilous species, which
70 usually accumulate from 100 to ca. 4000 μL (Sazima *et al.*, 1999; Slauson, 2000;

71 Bobrowiec and Oliveira, 2012), depending on the species resource offering strategy
72 (Tschapka, 2004). Accumulated values are considerably lower when compared to the first
73 anthesis morning and afternoon. They are similar to the first measures of the second
74 anthesis morning (Guerra *et al.*, 2014), suggesting that most of the species' resources are
75 allocated to attract birds right after anthesis start and decrease steadily as flowers
76 approach senescence. Such strategy is similar to the “fail-safe” mechanism described for
77 several ornithophilous or mixed-pollination systems, where flowers are adapted to bird-
78 pollination but may resort to bats given a lack of diurnal pollen deposition to guarantee
79 optimal seed set (Martén-Rodríguez *et al.*, 2009; Wolf and Stilles, 1989). The exact
80 mechanism often occurs in mixed systems with long nectar secretion periods in which
81 bats act as primary pollinators, and hummingbirds take on the “assurance” role (Aguilar-
82 Rodríguez *et al.*, 2016; Queiroz *et al.*, 2016). The nocturnal standing crop values shown
83 by us, although low, approach some species with mixed-dependence on hummingbirds
84 and bats such as *Siphocampylus sulfureus* (Campanulaceae) (Sazima *et al.*, 1994) and
85 exemplify the trade-off to balance fitness and the energy expenditure required to attract
86 both groups.

87 Following the calculations of Gribel and Hay (1993), and the same sugar
88 requirements of specialized nectar bats used of ca. 3.32 g of sugar a night and average
89 consumption of 17.5 μL per visit (Helvesen and Reyer, 1984), bats would require about
90 40 individuals of *P. robustus* to meet their daily energetic requirements [considering a
91 mean concentration of 10.15%, the mean accumulated volume of 55.1 μL per flower in
92 constant secretion rate and an average of 10 flowers per individual per night, Guerra *et*
93 *al.* (2014)]. This is not a large figure for the area (the study site alone consisting of a 300
94 m road contained >25 individuals in succession) and may aid bats in meeting their needs
95 during the rainy season, especially as this number may be even lower considering that

96 bats may complement their diets with other items, as pointed out by Gribel and Hay
97 (1993).

98

99 *4.3 Seed set and pollination system*

100 Despite its modest nocturnal nectar offering, the confirmation of active secretion
101 by *P. robustus* and its various interactions with bats and hawkmoths allows us to extend
102 its keystone species status (Arruda *et al.*, 2012) beyond the bird fauna of Central Brazil
103 and towards bats, and potentially to hawkmoths and other insects. The dependences
104 between plants and pollinators, however, appear to be very asymmetrical, as the high
105 usage of *P. robustus* by bats is counterbalanced by their relatively low contribution to
106 seed set. Most of *P. robustus* seeds were still set by their primary diurnal pollinators,
107 whose contribution to fitness approached the hand cross-pollination seed set. Seed set by
108 nocturnal pollinators was comparable with hand self-pollination, autonomous self-
109 pollination, agamospermy and, curiously, natural fruit set rates. Guerra *et al.*, (2014)
110 observed a slightly higher value of natural seed set (40%) in flowers exposed to
111 pollinators (they did not evaluate the differential role of nocturnal and diurnal animals),
112 which was also lower than hand cross-pollination. They also observed a much higher self-
113 compatibility index (0.91).

114 A possible explanation for this scenario is that bats and hawkmoths are acting as
115 vectors of endogenous pollen and not as long-distance cross pollinators, thus reducing
116 fitness due to inbreeding depression caused by a local medium self-compatibility index.
117 The observed behavior of hawkmoths is good evidence for this prediction, as they visit
118 several flowers from the same individual in succession. Such behavior was rare in the
119 bats seen in the footage. However, due to the clumped nature of *P. robustus* individuals
120 in the area and the low availability of chiropterophilous flowering plants, we should

121 expect bats to overexploit the same and neighboring individuals and to restrict their
122 movement until the resource in a given patch is depleted, resulting in low cross-
123 pollination rates. Although trap-lining pollinators and thus generally regarded as great
124 out-crossers (Fleming et al. 2009), bats may become territorial and move less between
125 individuals of plant species in disturbed habitats where resources are scarce (Diniz *et al.*,
126 2019; Lemke, 1985) or in species with high population densities and clumped
127 distributions such as *Caryocar brasiliense* (Caryocaraceae) in the Cerrado, which showed
128 short-distance pollen dispersal and low rates of cross-pollination (Collevatti *et al.*, 2010).
129 Regarding the low natural fruit set, flowers that remained enclosed during the night and
130 open during the day in the nocturnal exclusion treatment may have accumulated enough
131 night-secreted nectar to be more attractive to effective hummingbirds on the second
132 anthesis day, which could explain why this treatment yielded a higher seed set than the
133 uncontrolled group. Nonetheless, this matter still requires formal experimentation.

134 Despite the minor role of nocturnal animals in seed set, the species' less
135 specialized morphology and general nectar traits point to a generalized pollination system
136 with a stable secondary dependence on bats or perhaps in some stage in the transition
137 towards chiropterophily. Studies suggest that *P. robustus* is closely related and may share
138 a monophyletic clade with the chiropterophilous *P. acinarius* (Ortiz-Rodriguez *et al.*,
139 2018), which indicates some phylogenetic signal in these exceptional pollination systems
140 within the group and possibly a common ancestor that evolved away from ornithophily.
141 Fadini *et al.* (2018) comment on a possibly monophyletic group formed by *P. acinarius*,
142 *P. robustus*, and the bee-pollinated *P. eucalyptifolius*, stating that the clade might be more
143 recent than the other bird-pollinated *Psittacanthus*. Thus, the discovery of bat-pollination
144 in *P. robustus*, aside from acknowledging it as a novel and central resource for
145 Neotropical nectar bats, also sheds some light on the evolution of chiropterophilous or

146 generalized pollination systems in an overwhelmingly ornithophilous genus and calls for
147 more investigations of nocturnal visitors in wide-flowered ornithophilous species in the
148 group.

149

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162

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CONSIDERAÇÕES FINAIS

Este trabalho definiu a rede de interações entre morcegos visitantes florais e plantas em uma área de Cerrado, revelando uma rede moderadamente especializada, com módulos definidos, e permeada por mecanismos de nicho. Enquanto o acoplamento morfológico entre flores e visitantes, uma variável chave na estruturação de redes de polinização, definiu principalmente os grupos funcionais de polinizadores da rede, a alta variabilidade ambiental do Cerrado foi a melhor determinante da estrutura fina a rede. Morcegos nectarívoros especializados formam grupos funcionais distintos, com aqueles com rostros mais longos persistindo ao longo de todo o ano e interagindo especialmente com espécies menos abundantes ocorrentes em borda de mata e savana. Simultaneamente, morcegos nectarívoros com rostros mais curtos foram associadas à floração de algumas espécies chave ocorrentes em áreas de savana típica, como a quiropterófila *Caryocar brasiliense*, a qual floresce no fim da seca, e da ornitófila *Psittacanthus robustus* (Loranthaceae), com floração longa durante a estação chuvosa.

Outras espécies não quiropterófilas utilizadas frequentemente por morcegos incluíram *Lamanonia ternata* (Cunoniaceae) e *Combretum fruticosum* (Combretaceae), estas por morcegos frugívoros oportunistas em ambientes de mata. De fato, enclaves de matas de galeria na matriz de savana, locais com alta densidade de frugívoros, tiveram uma dominância deste grupo funcional no nicho de visitação floral, principalmente na seca, uma estação com baixa disponibilidade de frutos. A utilização intensa de espécie não quiropterófilas como recurso por morcegos com diferentes graus de especialização em consumo de néctar, além de nos sugerir cautela ao considerar síndromes de polinização como preditores de visitantes florais, reforça o papel de morcegos visitantes florais como generalistas ecológicos e de visitantes florais oportunistas. Essa plasticidade parece ser especialmente aparente em ambientes altamente sazonais com pulsos de disponibilidade de recursos ao longo do tempo e espaço, como o Cerrado. É imprescindível a identificação dos fatores estruturantes de interações morcego-planta em outros tipos de ambientes com variações no grau de heterogeneidade para solidificação do papel relativo de variáveis de nicho na estruturação de redes mutualísticas, e da compreensão do que leva morcegos visitantes florais a selecionar recursos em comunidades ricas.