ORIGINAL ARTICLE

Changing the main course: strong bat visitation to the ornithophilous mistletoe *Psittacanthus robustus* (Loranthaceae) in a Neotropical savanna

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Abstract

The Neotropical genus *Psittacanthus* comprises mostly specialized ornithophilous mistletoes, with rare exceptions. *Psittacanthus robustus* is a common ornithophilous species from the South American savannas whose bright yellow flowers secrete copious diluted nectar. Due to a three-day-long anthesis and a short, non-restrictive floral tube, we suggest that the species also serves as a resource for flower-visiting bats. In a Cerrado area in central Brazil, we investigated the usage of the species by bats through systematic bat captures for pollen sampling, its nocturnal nectar secretion dynamics, mating system, and the relative dependence on diurnal and nocturnal pollinators for reproduction. Nine phyllostomid bat species visited *P. robustus*. Up to 50% of pollen samples from bats contained the species during peak flowering, equating or surpassing the prevalence of chiropterophilous species and representing roughly a third of the floral resources consumed by specialized nectarivores *Glossophaga soricina* and *Anoura caudifer*. Flowers actively produced nectar at night with volume and concentration values in the ideal ranges for bat consumption. Nectar is continuously secreted after sunset and accumulates in the absence of visitors. *Psittacanthus robustus* is self-compatible but seeds are set mostly by diurnal visitors. Nocturnal animals had a low and secondary contribution to plant fitness. This is the second report of bat pollination for the genus *Psittacanthus*, and the largest assemblage of bat visitors for the family Loranthaceae. Although ornithophilous, *P. robustus* is an important resource for bats in the Brazilian savanna, potentially representing a mixed or early transitional state toward bat pollination.

Abstract in Portuguese is available with online material.

KEYWORDS
bat pollination, Brazil, Cerrado, Loranthaceae, nectar dynamics, ornithophily, Phyllostomidae, pollination syndromes

1 | INTRODUCTION

Vertebrate-pollinated flowers have as recurrent characteristics a general robustness, enhanced visibility, and abundant nectar secretion, adaptations shaped to meet the energy needs and suit large pollinators such as birds, bats, and non-flying mammals (Dellinger et al., 2019; Muchhala, 2003; Sazima et al., 1999). This pattern is most remarkable among chiropterophilous (bat-pollinated) plants, whose pollinators require wider floral openings to insert their snouts and tongues to reach the resource, which typically comprises large volumes of nectar (Tschapka & Dressler, 2002). As a result, many bat-pollinated plants also attract a wider variety of floral visitors that may sometimes act as secondary pollinators, such as sphingid moths (Queiroz et al., 2021; Rocha et al., 2019), hummingbirds (Aguilar-Rodríguez et al., 2016; Muchhala et al., 2009), and non-volant mammals (Queiroz et al., 2016).

On the contrary, Neotropical ornithophilous or bird-pollinated species undergo a different trend, often presenting restrictive floral morphologies with long and tubular or hypocrateriform corollas that greatly restrict the access to the narrow-billed hummingbirds (Faegri & Pijl, 1979; Martén-Rodríguez et al., 2009; Thomson et al., 2000). Although these are generally reliable diagnostic traits of Neotropical hummingbird-pollinated species, we may find transitioning bird-pollinated species with a lower phenotypic specialization and a mixed dependence on birds and bats for pollination (Martén-Rodríguez et al., 2009; Sazima et al., 2004) as chiropterophilous species commonly originate from ornithophilous ancestors (Tripp & Manos, 2008). Recently, transitioned chiropterophilous species still relying, to a variable extent, on birds for pollination may also occur (Buzato et al., 1994; Freiberg, 2007).

Psittacanthus robustus (Loranthaceae) is an ornithophilous mistletoe found throughout South America and most commonly in the savannas of Brazil (Kuijt, 2009) that appears to show a lower degree of morphological specialization toward hummingbirds. The species has a bright yellow corolla that acquires a reddish-orange tint near senescence (Figure 1) and secretes a sweet and weak, sometimes absent scent, traits associated with ornithophily. Its diurnal nectar secretion and floral visitors (mostly hummingbirds) have also been thoroughly described by Guerra et al. (2014). However, these authors classified this species as a generalist based on its copious and highly diluted nectar, whose volume and concentration values fall in the range commonly observed for chiropterophilous species (Tschapka, 2004). Its flowers are also not remarkably restrictive in comparison with most of its congenerics, presenting very long and spreading filaments (Kuijt, 2009) and diverging petals that sometimes constitute a small tube at their base (Guerra et al., 2014), but often form no tube at all (Kuijt, 2009). Additionally, its three-day anthesis period suggests that both diurnal and nocturnal pollinators may visit its flowers during their lifespan, as has been observed in other vertebrate-pollinated plants with a 24 h or longer anthesis (Aguilar-Rodríguez et al., 2016; Sazima et al., 2004).

Within the species-rich Neotropical genus *Psittacanthus*, *P. acinarius* (synonym of *P. corynocephalus*, Kuijt, 2009) is the sole known case of bat pollination in a group of about 120 species previously thought to be strictly ornithophilous (Araujo & Sazima, 2003; Fadini et al., 2018). However, as loranthaceous mistletoes are poorly studied in terms of pollination ecology (Arruda et al., 2012), exploring and understanding the dependence on nocturnal pollinators in ornithophilous species in the family is a significant step toward clarifying the evolution of vertebrate-pollination syndromes in this group, as well as potential mechanisms underlying the transition between ornithophily and chiropterophily. We thus aimed at (a) investigating the usage of *P. robustus* as a resource for bats through systematic capture expeditions in the Brazilian Cerrado to obtain pollen
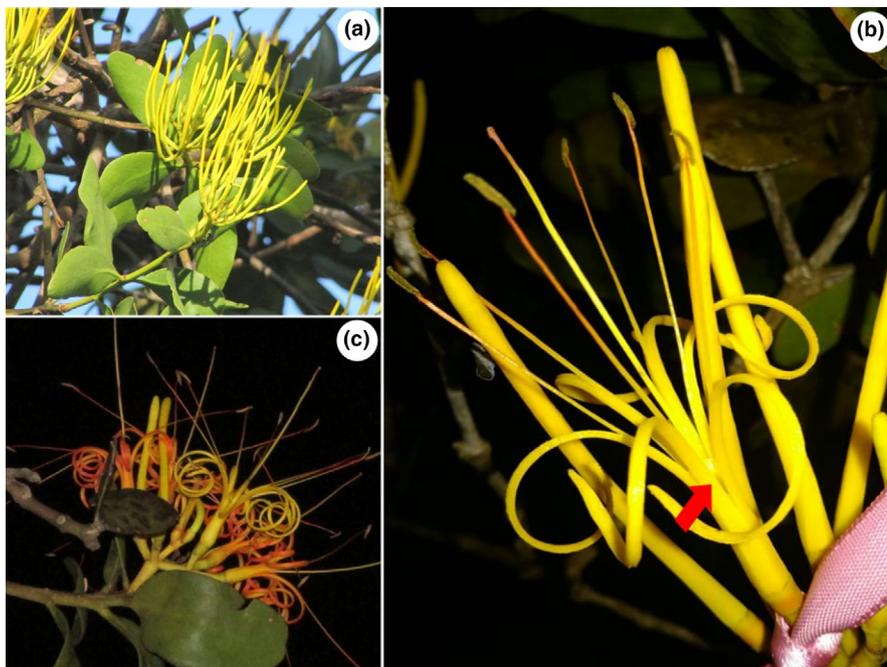

FIGURE 1 Dense inflorescences (a) and showy flowers (b and c) of the mistletoe *Psittacanthus robustus* (Loranthaceae) in central Brazil. The flowers have typical ornithophilous features, such as a tubular corolla and a bright yellow color (a and c) that changes to a reddish-orange as flowers approach senescence (c). The flowers secrete abundant and diluted nectar during the day, which sometimes accumulates between the base of the petals at early evening (b, red arrow)

samples; (b) assessing if *P. robustus* has active nocturnal nectar dynamics and if it correlates with the visiting frequency and behavior of bats and other nocturnal animals; and (c) defined the species' relative dependence on diurnal and nocturnal pollinators, as well as self-pollination, to set fruit.

2 | METHODS

2.1 | Study site and species

The study was conducted in the Brasília National Park (PNB), Federal District, Brazil (15°40'52" S, 47°59'17" W), located on the central Brazilian highlands and within the Cerrado biome. The PNB has 42.355 ha and encompasses large extensions of preserved primary Cerrado vegetation such as typical savanna formations dominated by a short, bushy plant community (cerrado *sensu stricto*), arboreal savannas (cerradão), and grasslands. The region has a tropical altitudinal climate (Cwa and Cwb climates according to the Köppen scale), with a warm rainy season from October to May and a cold mid-year dry season.

Psittacanthus robustus Mart. (Loranthaceae) is a hemiparasitic mistletoe species native to the savannas of South America and found parasitizing Vochysiaceae hosts (Monteiro et al., 1992). It is an abundant species in the PNB, occurring in higher densities in typical savanna formations (cerrado *sensu strict* and cerradão) and parasitizing *Vochysia thyrsoidea*, *Qualea multiflora*, and *Q. grandiflora* (Vochysiaceae) hosts. The flowering of *P. robustus* is long and occurs throughout the wet season, starting between October and November and ending by May (Guerra et al., 2014). All individuals in the same area flower in an approximately synchronous manner and expose their dense inflorescences containing several flower triads (Figure 1a), providing an abundant resource to floral visitors. Its corollas have a bright yellow coloration that progressively turn orange as anthesis progresses and have a short floral tube formed by the overlapping base of petals that normally spans from 0 cm to 3 cm (Guerra et al., 2014; Kuijt, 2009). Anthesis lasts for three days and starts in the early morning (05:00 h–07:00 h) of the first day, and anthers become dehiscent shortly after with most pollen removed within the first day (Guerra et al., 2014). Other aspects of floral biology were thoroughly explored by Guerra et al. (2014).

We carried out two distinct sampling protocols in the PNB. First, bat captures to sample pollen grains were performed monthly and covered the entire flowering period of the species, starting in October 2019 and continuing through the rainy season until February 2020. The remaining months of March, April, May, and June were sampled in 2021. This step was performed in eight fixed sampling sites spread throughout the park, selected randomly and not necessarily associated with *P. robustus* individuals (see the *Pollen sampling* section below). Secondly, procedures that focused on *P. robustus* individuals such as nectar dynamics protocols, observations of visitation frequencies, reproductive experiments, and exclusion trials were all performed in a fixed sub-population of 19 individuals

distributed along a 330 m cerrado *s.s.* edge in the PNB (15°37'27.0"S 48°01'16.5"W), between January and February 2021.

2.2 | Pollen sampling from bats

In each month of the rainy season, we captured bats for eight consecutive nights, each in a different sampling site within the PNB. The sites constituted a savanna-forest gradient, that is, four cerrado *s.s.* areas (15°44'18.2"S, 47°59'10.0"W; 15°40'49.1"S, 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 gallery forest borders (15°38'12.3"S, 47°56'11.7"W and 15°42'50.8"S, 48°03'37.5"W), and two gallery forest interiors (15°41'38.1"S, 47°58'12.0"W and 15°37'36.8"S, 48°01'04.9"W). The sites were separated from each other by at least two kilometers and homogeneously covered the entire area of the park. Each night we set 10 mist nets (3 × 12 m, Ecotone®, Poland) at ground level, placed randomly inside the capture sites from 18:00 h to 00:00 h, resulting in 4320 mist net hours.

All captured bats were identified according to Dias et al. (2016), had pollen samples collected from their external body, and were released afterward. We used glycerinated and stained gelatin cubes (Voigt et al., 2009) to collect pollen from the head, torso, wings, and uropatagium. Pollen samples were placed in individual vials for later mounting on slides for pollen identification through light microscopy. Any pollen type found in a sample numbering 5 or more grains was considered a legitimate interaction with a plant and was identified to the lowest-possible taxonomical level using specialized literature (Salgado-Labouriau, 1973) and a personal pollen collection from plants of the study site. Pollen types found in samples were classified as either coming from *P. robustus*, from chiropterophilous species, or from other syndromes (i.e., entomophilous, other ornithophilous) based on the traits cited by Faegri and Pijl (1979) and Proctor et al. (1996). In each capture site, a fixed 1000 × 10 m transect was set within the vegetation for the counting of flowering *P. robustus* individuals. Transects were also sampled monthly, simultaneously to bat captures.

We calculated the percentage of bat individuals from each species that carried pollen from *P. robustus* in relation to the total captures of that species and specificity (S), that is, among the individuals that interacted with *P. robustus*, the percentage of interactions that was represented by *P. robustus*. Higher specificity (S→1) indicates a higher usage of *P. robustus* by the bat species during its flowering period relative to other floral resources.

2.3 | Nectar dynamics

We assessed whether *P. robustus* actively produces nectar throughout the night (18:00 h–06:00 h) to reward pollinators; if it undergoes removal effect (enhanced secretion following nectar extraction); and if nectar is reabsorbed throughout the night in the absence of visitation (Ordano & Ornelas, 2004). We employed the protocols of Galleto and Bernardello (2005). A group of recently opened flowers

($N = 60$, 11 individuals) was marked and bagged at noon. To evaluate nocturnal nectar secretion, all flowers had any leftover nectar produced during the day removed at 18:00 h of the first anthesis day and were rebagged.

The 60 flowers were separated into groups of ten, and each group was assigned one of the six following accumulation treatments: (i) first removal at 19:00 h, five subsequent removals every two hours until 05:00 h; (ii) first removal at 21:00 h, four subsequent removals; (iii) first removal at 23:00 h, three subsequent removals; (iv) first removal at 01:00 h, two subsequent removals; (v) first removal at 03:00 h, one subsequent removal; (vi) a single removal at 05:00 h. In each removal, we measured nectar volume (microliters, 0–25 μl microsyringes, Hamilton®, Reno, USA), concentration in sugar equivalents (% mass/mass, 0–33% hand refractometer, Atago®, Tokyo, Japan), and mass of sugar equivalents [mg, formula of Galetto and Bernadello (2005)]. As secrete produce nectar until the afternoon of the third day (Guerra et al., 2014), this procedure was carried out in two consecutive nights to capture the entire lifespan of flowers. All flowers remained bagged when not being manipulated. Care was taken when extracting nectar from flowers not to damage the nectariferous disk. One flower from group (iii) was discarded due to damage caused by ants, resulting in a total of 59 treated flowers.

A different set of flowers ($N = 198$, 7 individuals) was left unbagged and separated into the same six time groups as described above (19:00 h to 05:00 h), each group containing a variable number of flowers ($N = 29–37$), according to availability. We sampled each group once for nectar volume, concentration, and sugar in one of these periods to assess the standing nectar crop available to nocturnal visitors throughout the night.

2.4 | Frequency of floral visitors

We used a 2.7 K Camcoder 2688 × 1520P video camera mounted on a 1.5 m tripod and equipped with an infrared sensor to record nocturnal floral visitors and quantify their visiting frequencies to flowers. For five days between January and February 2021, the camera was placed ca. 2 m from individuals of *P. robustus* ($N = 5$) facing branches containing several inflorescences (4–20 inflorescences, depending on the individual) from 19:00 h to 06:00 h. Filming was interrupted on several occasions due to rainfall and resumed immediately when possible. We recorded 47 h and 20 min in total.

From the footage, we quantified animal visiting frequencies. A visit was registered at any time an animal individual (a bat or a moth) interacted with an individual flower by hovering directly in front of it (bats and hawkmoths) or by clinging onto it (bats). If a visitor interacted with different flowers during the same bout, each interaction was counted as a distinct visit, but if the same visitor repeatedly interacted with one flower during the same bout, only one visit was counted. We considered as independent bouts every activity separated by 10 or more seconds of no activity. These intervals were counted independently for bats and hawkmoths. If more than one bat individual or moth individual was seen foraging simultaneously,

repeated interactions with the same flower were considered a single visit regardless of being delivered by one or more individuals. In order to correlate visitation frequency with nectar secretion, we separated visits according to time blocks corresponding to the six nectar sampling shifts: 19:00–20:59 h, 21:00 h–22:59 h, 23:00 h–00:59 h, 01:00–02:59 h, 03:00–04:59 h, and 05:00 h–06:00 h (dawn).

Additionally, we used a Canon SX500 IS camera attached to a 1.5 m tripod to photograph visitors' interaction with flowers to determine visitor identity and behavior during the interaction.

2.5 | Reproductive experiments

Psittacanthus robustus requires a pollen vector for most of its seed set output, either by xenogamous or geitonogamous pollen flow (Guerra et al., 2014). Thus, we performed selective visitor exclusion tests on individual flowers of *P. robustus* to assess the relative role of diurnal and nocturnal floral visitors on seed formation, as well as controlled pollination tests to describe the mating system of the study population. We marked and bagged 309 floral buds from 19 individuals, which were manipulated according to the following treatments upon the beginning of anthesis. Nocturnal exclusion test ($N = 44$): flowers were bagged during the night (18:00 h to 06:00 h) and left unbagged during the day (06:00 h to 18:00 h) throughout their three-day anthesis. Diurnal exclusion test ($N = 31$): flowers were bagged during the day (06:00 h to 18:00 h) and left unbagged during the night (18:00 h to 06:00 h) throughout the anthesis. Hand cross-pollination ($N = 31$): flowers had pollen from another individual brushed against their stigma and were left bagged until senescence. Hand self-pollination ($N = 31$): flowers had pollen from another flower of the same individual brushed against their stigma and left bagged until senescence (includes autogamy and geitonogamy). Autonomous self-pollination ($N = 41$): flowers were left bagged from anthesis until senescence without manipulation. Agamospermy ($N = 30$): flowers in the pre-anthesis state were emasculated and left bagged until senescence. Finally, 101 flowers were left unbagged and unmanipulated as a control group. Flowers from cross- and self-pollination, and agamospermy treatments were manipulated in the morning, following floral opening.

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

2.6 | Data analysis

Differences in total accumulated nectar and sugar mass by flower among the six accumulation treatments and differences in standing crop values for nectar volume, concentration, and sugar mass among the time groups, were assessed using one-way ANOVAs. For both

accumulation and standing crop measurements, only data for the first night of floral anthesis were used, as flower wilting during the second night greatly reduced sample size, and nectar volumes were negligible. Normality was checked for all response variables, and accumulation and standing crop volume and sugar mass values were log-transformed before analysis. Post hoc pairwise T-tests using Bonferroni corrections were employed to detect differences among time groups. Differences in bat and hawkmoths visitation frequencies among time groups were assessed using non-parametric Kruskal–Wallis tests.

We fit mixed-effects generalized linear models (GLMMs) with a binomial error distribution and *logit* link function to determine if fruit set success rates differed among reproductive experiment treatments. Success/fail results were set as the response variable, the reproductive treatment as the explanatory variable, and the inflorescence and individual into which flowers were nested were set as random effects. The analysis was performed in R studio 3.6.0 (R Development Core Team, 2019). GLMMs were performed with the package *lme4* (Bates et al., 2015).

3 | RESULTS

3.1 | Floral visitors

Between November and April, 240 bats of 20 species belonging to the families Phyllostomidae and Vespertilionidae were captured, among which 80 (33.3%) belonging to 11 species carried a total of 20 distinct pollen types. Out of the flower visitors, 34 (42.5%) bats belonging to four subfamilies carried pollen from *P. robustus* (Table 1). Other resources used by bats in the same period were

chiropterophilous plants from the families Fabaceae (*Bauhinia*, 3 spp.; *Hymenaea*, 2 spp. and *Inga edulis*), Caryocaraceae (*Caryocar brasiliense*), and Lythraceae (*Lafoensia pacari*); and plants from other syndromes of the families Asteraceae (tribe Vernoniae, 1 sp.), Convolvulaceae (*Merremia* sp.), Cunoniaceae (*Lamanonia ternata*), Fabaceae (Caesalpinoidae, 3 spp.), Myrtaceae (*Eucalyptus* sp.), and Smilacaceae (*Smilax* sp.). Three other types that remained unidentified and did not belong to chiropterophilous species from the site were placed in the "other syndromes" category.

The role of *P. robustus* as a resource for bats during its flowering period was variable. It was most prominent among specialized nectarivores, being present in 70% and 50% of samples from the glossophagines *Glossophaga soricina* and *Anoura caudifer*, respectively, representing about a third of the visited plant species of both bat species during the period (Table 1). It was also consumed, to a lesser extent, by the endangered nectarivore *Lonchophylla dekeyseri* and by several non-specialized floral visitors, such as frugivores and omnivores (Table 1).

The flowering of *P. robustus* started in November and peaked in March, decreasing quickly after that until its end in May (Figure 2). During these months, bat usage of the species increased accordingly, reaching its maximum in February and March when *P. robustus* represented half of all resources consumed by bats individuals combined, equaling and surpassing chiropterophilous plants, respectively (Figure 2a).

We recorded 65 bats visits from specialized nectarivores (Glossophaginae and Lonchophyllinae) to *P. robustus* flowers on the footage. When visiting flowers, bats hovered quickly (ca. 1s) and very close to the flowers to insert the snout between the petals and touched both the long and herkogamous reproductive whorls with various parts of their bodies, but especially the abdomen and lower

TABLE 1 Nocturnal floral visitors of *Psittacanthus robustus* sampled systematically through mist nets in the Brasília National Park (bats) or observed visiting plants directly (hawkmoths)

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

Note: The number of individuals captured (N), percentage of *P. robustus* visitors, and specificity (S, the proportion of visitations represented by *P. robustus*) apply only to bats caught in mist nets during the systematic sampling from November to April.
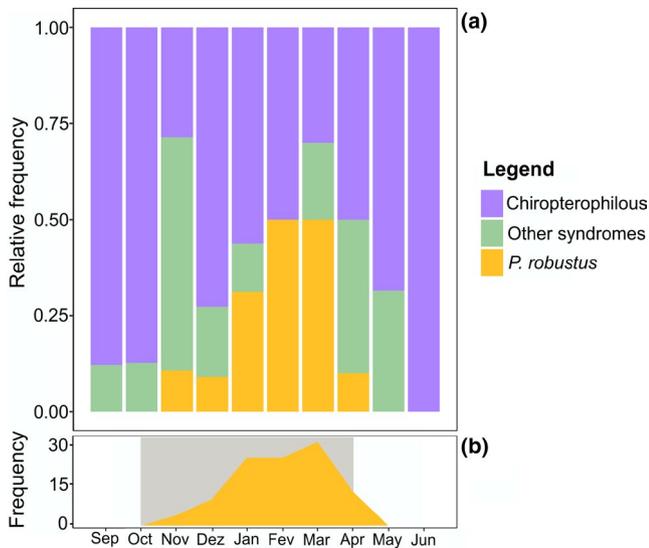
FIGURE 2 Relative frequency of plants used as a resource by flower-visiting bats in each month based on pollen loads recorded from captured individuals (a) and the sum of sighting frequencies of flowering *Psittacanthus robustus* individuals at the fixed transects (b) from the end of the dry season, through the rainy season (shaded gray area), and up to the beginning of the dry season. In (a), resource plants are grouped as typical chiropterophilous plants (i.e., floral traits related to bat pollination and visitation by bats already registered in the literature), plants from other pollination syndromes, and *P. robustus* individually

side of wings (Figure 3b). Although we focused on flower-visiting bats, hawkmoths were also recorded visiting plants frequently throughout the night in footage and photographs. A total of 142 hawkmoth visits were recorded, and the hawkmoth *Agrius cingulata* was the only species identified in photographs and footage (Figure 3). Hawkmoths behaved unpredictably while visiting flowers, hovering from a distance and insert the proboscis between the petals to feed and not touching reproductive parts (Figure 3c) on roughly half of visits, or behaving similarly to bats by hovering very close to flowers and touching anthers and stigma with their bodies (Figure 3d). Small Noctuid moths also visited flowers on several occasions, but they never touched anthers or stigmas and acted as nectar robbers.

3.2 | Nectar accumulation

On the first night of anthesis, flowers of *P. robustus* produced large and diluted volumes of nectar during the first hours after sunset and continued to produce smaller volumes steadily until sunrise, even after successive removals (Table 2). Although nectar volumes and sugar mass decreased after the first removals in all groups, sugar mass stabilized afterward, while sugar concentration remained roughly constant throughout the entire night and early morning. Accumulation groups did not differ significantly in terms of total accumulated nectar volumes ($F_{1,57} = 0.20$, $p = .66$) or sugar mass ($F_{1,57} =$
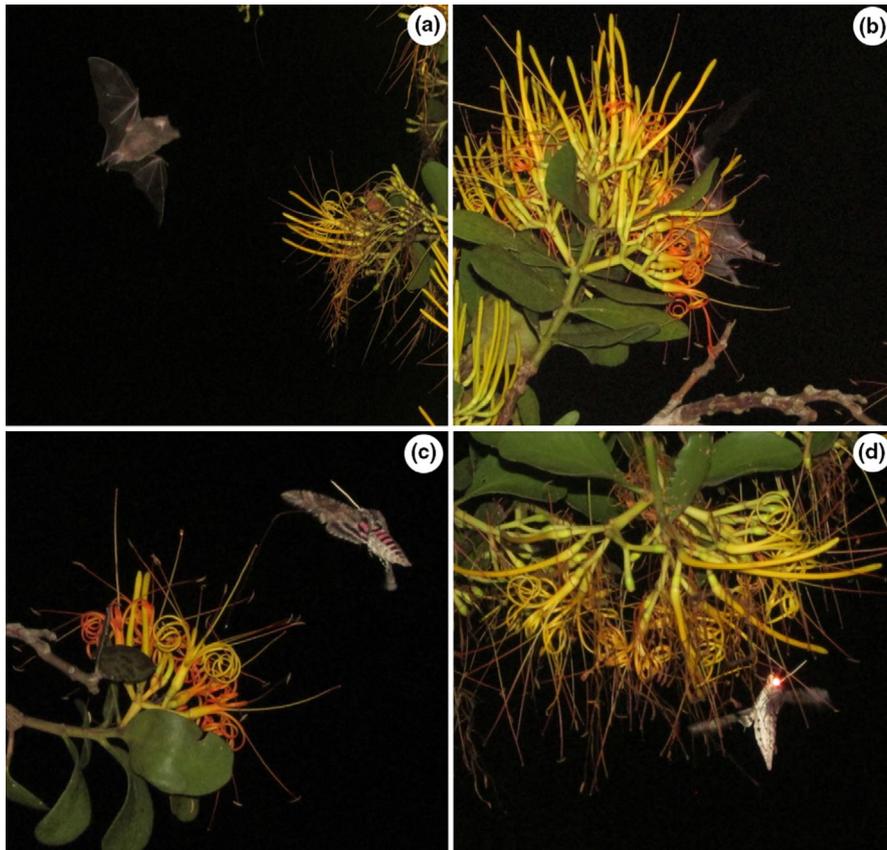

FIGURE 3 Some nocturnal visitors of the ornithophilous mistletoe *Psittacanthus robustus*. Glossophagine bats, approaching (a) and pollinating flowers (b), and the hawkmoth *Agrius cingulata* feeding from afar (c) and approaching (d) flowers

2.92, $p = .09$) per flower. Nectar accumulation still occurred in a few flowers on the second night of anthesis, but most flowers from all groups were either empty or had already wilted by midnight (Table 2).

3.3 | Nectar standing crop and visitation frequency

Standing crop values of nectar volume, concentration, and sugar mass varied significantly among time blocks of the first anthesis night (Figure 4a–c). Nectar volume averaged 19.03 ± 13.91 μl (mean $\pm$ SD, $N = 198$ measurements) throughout the night and was higher just after sunset ($F_{5,192} = 15.9$, $p = 4.00e{-}13$), with similar means at 19:00 h and 21:00 h. Nectar volumes after 21:00 h were lower in all subsequent time blocks ($p < .05$ in all comparisons with 19:00 h), and started stabilized from midnight (Figure 4a). Nectar concentration averaged $10.15 \pm 2.01\%$ and was higher just after sunset ($F_{5,192} = 7.6$, $p = 1.6e{-}06$), also being stable between 19:00 h and 21:00 h ($p = .9$), but more diluted from 23:00 h until dawn when compared to 19:00 h ($p < .05$ for all comparisons) (Figure 4b). Sugar mass averaged at 3.31 ± 3.37 mg and had a trend similar to nectar volume, with variable but higher values after sunset and a peak at 21:00 h ($F_{5,192} = 16.79$, $p = 9.5e{-}14$), with all subsequent time blocks presenting increasingly lower sugar masses (all comparisons with 19:00 h at $p < .05$). Bat and hawkmoths visitation frequencies, however, were overall low [pooled time blocks: $\bar{x} = 1.86 \pm 3.15$ (bats) and $\bar{x} = 4.01 \pm 6.19$ (hawkmoths)] and did not change significantly throughout the night (bats: $\chi^2 = 7.2$, df = 5, $p = .21$; hawkmoths: $\chi^2 = 2.3$, df = 5, $p = .81$), with slight peaks of bat visitation at 21:00 h and of hawkmoth visitation between 23:00 h and 01:00 h (Figure 4d).

3.4 | Exclusion trials and mating system

Most seeds in *P. robustus* were set through xenogamy, but the species has a moderate level of self-compatibility and is also able to self-pollinate autonomously and to set seeds without pollen deposition, securing a constant marginal fruit set (Table 3). The natural fruit set was low and comparable to the diurnal exclusion, agamospermy, hand self-pollination, and spontaneous self-pollination. The success ratio of the nocturnal exclusion treatment was high and similar to that of hand cross-pollination (Table 3). The sum of success ratios of diurnal and nocturnal exclusion treatments approaches the ratio observed from hand cross-pollination. None of the treatments yielded a large success rate (maximum of 0.55 from hand cross-pollination).

4 | DISCUSSION

4.1 | An important resource for flower-vising bats

Flowers of *P. robustus*, although presenting ornithophilous features and contrasting with its typically chiropterophilous sister species *P. acinarius*, are widely used by specialized Glossophaginae

nectarivores during the peak of the rainy season in central Brazil. The consumption of *P. robustus* by bats can even surpass local chiropterophilous flowers in terms of relative usage of floral resources. This is the second empirical observation of bat visitation, and potentially pollination, in a species of the genus (Araujo & Sazima, 2003; Fadini et al., 2018; Kuijt & Hansen, 2015). The richness of bat species vising *P. robustus* was also unexpectedly high, and it corresponds to the largest assemblage of bat visitors reported for the family Loranthaceae.

A few factors may explain such high exploitation by bats of this otherwise characteristic ornithophilous species. First, floral morphology plays a major role in allowing bats to access the nectar, considering that corolla opening greatly determines whether bats may access or not the nectar of a given species (Queiroz et al., 2021). Contrasting with most specialized ornithophilous flowers and many other species in the genus, the divergent petals of *P. robustus* do not form a clear floral tube (Kuijt., 2009). Less restrictive corollas are deemed necessary in separating mixed bat-hummingbird pollination systems from specialized ornithophilous ones (Aguilar-Rodríguez et al., 2016; Martén-Rodriguez et al., 2009), and its spreading and extremely long filaments, opposed to short and fasciculate androeciums seen in many species in the genus (Kuijt, 2009), suggest some adaptation to larger pollen vectors.

Secondly, despite its steady-state flowering (*sensu* Gentry, 1974) that yields few open flowers per night over for several months (Guerra et al., 2014), the daily flowering of *P. robustus* probably results in high energy density values per area unit due to its high density in the site. At the same time, the abundance of flowering chiropterophilous plants in the region tend to decrease in the rainy season (Coelho & Marinho-Filho, 2002), and the high availability of *P. robustus* in this period, coupled with the long lifespan of flowers and non-enclosed nectar should be enough to draw bats toward it. Such interaction is not surprising, since nectarivorous bats are highly opportunistic and adaptable to resource availability pulses, exploiting plants belonging to generalized pollination systems in periods of nectar shortage (Amorim et al., 2012; Muchhala et al., 2009; Vieira & Carvalho-Okano, 1996).

4.2 | The role of nectar dynamics

Psittacanthus robustus revealed active nectar secretion from sunset through dawn. Therefore, the nocturnal standing nectar crop is not simply leftover from diurnal secretion and accumulates in the absence of nocturnal visitation. Allied to its accessible morphology, this secretion pattern and nectar traits corroborate with the existence of a generalized system in *P. robustus* (Guerra et al., 2014). Regarding nectar quality and quantity, sugar concentration in *P. robustus* falls into the ideal range for consumption by bats and is even lower than values reported for the chiropterophilous *Psittacanthus acinarius* (mean 15.2 μl at 16.5% during the first hours of anthesis) (Fadini et al., 2018), falling within the expected range of ca. 10% to 20% concentration for Neotropical chiropterophilous species (Sanmartin-Gajardo & Sazima, 2005; Sazima et al., 1999; Tschapka, 2004).

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 Methods section for details on removal groups)

Groups		19–20 h	21–22 h	23–00 h	01–02 h	03–04 h	05–06 h	Total	19–20 h	21–22 h	23–00 h	01–02 h	03–04 h	05–06 h	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							–	–

Note: Variables measured were nectar volume (µl), sugar concentration (%), and mass of sugar equivalents (mg). Dashed cells indicate that values could not be measured. Means are followed by the standard deviation (SD). Values without SD are single measurements. Volume and sugar mass totals of each night are written in bold type.

FIGURE 4 Measurements of nectar standing crop: volume (a), sugar concentration (b), and sugar mass (c), as well as bat and hawkmoth visitation frequencies (d), throughout the first anthesis night of *Psittacanthus robustus*. Vertical bars indicate standard deviations
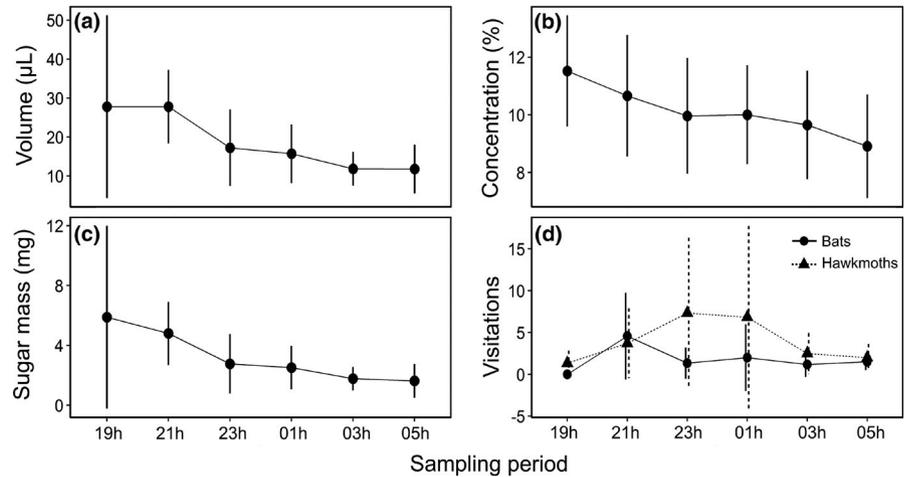

TABLE 3 Fruit set and success ratios from the total number of treated flowers (*n*) from different manipulation treatments in *Psittacantthus robustus* and self-compatibility index

Treatment	Individuals	n	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

Note: Letters beside success ratio values indicate significantly different groups (*p* < .05, as determined by the GLMM).

In comparison with other ornithophilous mistletoes, the volume accumulated by *P. robustus* flowers in one night is similar or higher than the accumulated during the entire lifespan of flowers of *P. schideanus* (Ramírez & Ornelas, 2010), *P. calyculatus* (Azpeitia & Lara, 2003) and *P. auriculatus* (Pérez-Crespo et al., 2016). Nectar concentration is also significantly lower in *P. robustus*. Despite these large volumes, night-accumulated nectar in *P. robustus* is still considerably lower when compared to the accumulated in the first anthesis morning and afternoon and approach the first measures of the second anthesis morning (ca. 58 μL, Guerra et al., 2014), suggesting that most of the species' resources are allocated to attract birds right after the start of the anthesis. Such strategy is similar to the "fail-safe" mechanism described for several ornithophilous or mixed-pollination systems, where flowers resort to bats given a lack of diurnal pollen deposition (Martén-Rodríguez et al., 2009; Wolf & Stiles, 1989). The nocturnal standing crop values shown by us, in fact, approach some species with mixed dependence on hummingbirds and bats such as *Siphocampylus sulfureus* (Campanulaceae) (Sazima et al., 2004) and exemplify the trade-off to balance fitness and the energy expenditure required to attract both groups.

4.3 | Bird and bat pollination

The interaction between *P. robustus* and nocturnal visitors, however, appears to be counterbalanced by a lower fruit set resultant from the diurnal exclusion treatment. Most of *P. robustus* seeds were set by diurnal pollinators, whose contribution to fitness approached the hand cross-pollination seed set. Diurnal exclusion treatments resulted in a low fruit output similar to those of self-pollination and agamospermy. Therefore, it appears that *P. robustus* is self-compatible (also showed by Guerra et al., 2014) and can maintain a basal fruit output either autonomously or by pollinator-mediated self-pollination, with increments to fitness provided mostly by diurnal pollinators.

Guerra et al. (2014) showed that most pollen is removed from anthers of *P. robustus* during the first anthesis day (morning /afternoon), which suggests that intensive pollination activity occurs early in the anthesis by hummingbirds. They also speculate that excessive visitation may damage or remove pollen from the stigma and lower plant fitness, which would potentially explain the low fruit set of flowers exposed to both groups. Therefore, nocturnal visitors could potentially add to overall fitness in *P. robustus* populations given the scenario of a limitation of hummingbirds for pollen flow, resembling

the fail-safe mechanism (Martén-Rodríguez et al., 2009), but, otherwise, an antagonistic role of bats and hawkmoths as nectar robbers and sources of stigma damage cannot be discarded. However, we suggest further exploration of the role of bats as pollinators and outcrossers of *P. robustus*. Bats are considered excellent pollen vectors due to their traplining behavior and general lack of territoriality resultant from an efficient spatial memory (Sazima et al., 1999; Winter & Stich, 2005), their high mobility (Dick et al., 2008; Heithaus et al., 1975), and ability to respond to long distance olfactory cues (Gonzalez-Terrazas et al., 2016). Thus, bats might enhance pollen flow between isolated individuals and contribute significantly to population genetic diversity. We also highlight that our reproductive results are very preliminary, as we are unable to isolate the effect of bats on fruit set from that of hawkmoths or from selfing rates, and future studies should thus include measurements of pollen removal and deposition.

Despite the preliminary minor role of nocturnal animals in seed set, the species' morphological and nectar traits suggest a generalized pollination system that could profit from an opportunistic use of flowers by bats, or perhaps still transitioning toward chiropterophily. The latter hypothesis requires more in-depth reproductive experiments, but recent studies suggest that *P. robustus* may share a monophyletic clade with the chiropterophilous *P. acinarius* (Ortiz-Rodriguez et al., 2018). Fadini et al. (2018) comment on a possibly monophyletic group formed *by P. acinarius*, *P. robustus*, and the bee-pollinated *P. eucalyptifolius*, stating that the clade might be more recent than the other bird-pollinated *Psittacanthus*. These exceptional pollination systems in the genus, therefore, could have originated from an ancestor that departed from the typical ornithophilous syndrome. Thus, the discovery of bat visitation to *P. robustus* also sheds some light on the evolution of non-ornithophilous or generalized species in the genus.

Finally, we underline the importance of including the night period and usage of night vision cameras to assess potential nocturnal visitors and their behaviors in species regarded as typically pollinated or dispersed by diurnal animals. Mistletoes, for instance, have had key aspects of their reproductive ecology updated, such as the establishment of non-mistletoe-specialist frugivores in dispersing seeds to suitable hosts (Watson & Rawsthorne, 2013), which also includes nocturnal mammals (Amico et al., 2017), or the occurrence non-hummingbird pollination in *Psittacanthus* (Fadini et al., 2018). These pollination and dispersal paradigms should continue to be revisited with more comprehensive sampling designs. Species with several-day antheses, nectar replenishment, and less restrictive floral tubes, such as *P. calyculatus* (Azpeitia & Lara, 2006) and *P. schiedeanus* (Ramírez & Ornelas, 2010), for example, are good contenders for such type of sampling and would perhaps reveal a richer assemblage of visitors that includes nocturnal pollinators.

CONFLICT OF INTEREST

The corresponding author confirms on behalf of all authors that there have been no involvements that might raise the question of bias in the work reported or in the conclusions, implications, or opinions stated.

AUTHOR CONTRIBUTIONS

UMD involved in conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, and writing (original draft and final manuscript). NLSF involved in data curation, investigation, methodology. LMSA involved in conceptualization, project administration, funding acquisition, supervision, and writing (review and editing).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in the Dryad Digital Repository: https://doi.org/10.5061/dryad.1c59zw3ws (Diniz et al., 2022).

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