

Bats and hawkmoths form mixed modules with flowering plants in a nocturnal interaction network

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Abstract

Based on the conceptual framework of pollination syndromes, pollination networks should be composed of well-delimited subgroups formed by plants that diverge in floral phenotypes and are visited by taxonomically different pollinators. Nevertheless, floral traits are not always accurate in predicting floral visitors. For instance, flowers adapted to bat-pollination are larger and wider, enabling the exploitation by other nocturnal animals, such as hawkmoths. Thus, should an interaction network comprising bats and hawkmoths, the most important nocturnal pollinators in the tropics, be formed of mixed-taxon modules due to cross-syndrome interactions? Here, we analyzed such a network to test whether resource plants are shared between the two taxa, and how modules differ in terms of species morphologies. We sampled interactions through pollen grains collected from floral visitors in a Caatinga dry forest in northeastern Brazil. The network was modular yet interwoven by cross-syndrome interactions. Hawkmoths showed no restriction to visit the wider chiropterophilous flowers. Furthermore, bats represented a subset of a hawkmoth-dominated network, as they were restricted to chiropterophilous flowers due to constraints in accessing narrower sphingophilous flowers. As such, the bat-dominated module encompassed relatively wider flowers, but hawkmoths, especially long-tongued ones, were unrestricted by floral width or length. Thus, pollination of flowers with open architectures must be investigated with caution, as they are accessible to a wide array of floral visitors, which may result in mixed-pollination systems. Future research should continue to integrate different syndromes and pollinator groups in order to reach a better understanding of how pollination-related functions emerge from community-level interactions.

Abstract in Portuguese is available with online material.

KEYWORDS

Caatinga, chiropterophily, mixed-pollination, modularity, pollination syndromes, specialization, sphingophily

1 | INTRODUCTION

Plants with specialized phenotypes and highly derived floral traits tend to make ecologically specialized interactions and rely on a few effective pollinators (e.g., Johnson & Steiner, 1997; Manning & Goldblatt, 1997). However, adaptations to multiple guilds of pollinators that exert divergent selective pressures are far more common in nature than previously thought (Fenster et al., 2004). Thus, plants often show mixed-pollination systems, and many are ecological generalists (Armbruster, 2017).

Those mixed systems challenge the concept of pollination syndromes (*sensu* Faegri & Pijl, 1979), that is, the conceptual framework that predicts the most effective pollinator group of a plant based on its set of floral traits. Nevertheless, the concept of pollination syndromes is often seen as an effective framework, especially when considering that syndromes are not restricted to predicting only the most effective pollinator groups, but also secondary ones (Rosas-Guerrero et al., 2014).

Although this concept has met criticism regarding its usefulness to understand some systems (Ollerton et al., 2009), nocturnal pollination syndromes seem to be especially good predictors of real-world interactions (Muchhala & Jarrin, 2002). Flower-visiting bats (Chiroptera: Phyllostomidae) and hawkmoths (Lepidoptera: Sphingidae) are the two largest guilds of specialized nocturnal pollen vectors in the Neotropics (Borges et al., 2016). In addition, the pollination syndromes associated with them (chiropterophily and sphingophily, respectively) differ markedly in floral phenotype (Tripp & Manos, 2008). Sphingophilous flowers tend to be more restrictive than chiropterophilous flowers, and extreme cases of morphological specialization are commonly found among the former (Martins & Johnson, 2007; Nilsson, 1988). Long and narrow floral tubes matching the length of the hawkmoths' feeding apparatus are a convergent feature of sphingophilous species and greatly constrain the visitation by other pollinator guilds (Johnson et al., 2017). However, many species with open floral morphologies and exposed nectar are also pollinated by hawkmoths, which highlights that flowers pollinated by these insects do not always conform to a predictable architecture (Amorim et al., 2013; Koptur, 1983).

Open floral morphologies are a diagnostic trait of chiropterophilous plants, among which cup-, brush-, or bell-shaped flowers are the most common (Fleming et al., 2009). Although such floral types that conform to chiropterophily allow the interaction with larger pollinators such as bats, they also enable flowers to be exploited and pollinated by animals of other guilds, such as hummingbirds, bees, and hawkmoths (Amorim et al., 2013; Queiroz et al., 2016; Rocha et al., 2020). Exclusive dependence of flowers on bats, resulting in an exclusion of other pollinators through morphological restriction, does exist within the chiropterophilous syndrome but is rare (Muchhala, 2006; Muchhala & Thomson, 2009).

Due to the lack of morphological constraints of bat-pollinated plants, mainly those with open flowers, bats and hawkmoths likely share some food-plants. Ecologists have recently started to quantify

community-wide patterns of interaction in bat-plant (Sritongchuay et al., 2019; Stewart & Dudash, 2017) and hawkmoth-plant (Johnson et al., 2017; Sazatornil et al., 2016) pollination networks, but we still do not know the structure of the networks formed of both pollinator guilds together. To assess specialization in those mixed systems, we can use a network approach to gain insights on the structural characteristics of community-wide mutualistic interactions, such as modularity (Olesen et al., 2007). Modular networks are formed by subgroups of species that interact more among themselves than with the rest of the community (Dupont & Olesen, 2009). In pollination networks, modularity can reveal patterns of niche specialization between interacting species (Amorim, 2020; Maruyama et al., 2014; Phillips et al., 2020).

Therefore, in the present study, we aimed at unveiling the structure of an interaction network formed by plants, bats, and hawkmoths. Because most sphingophilous species present a more restrictive floral morphology than chiropterophilous species, we expected hawkmoths to exploit plants of both pollination syndromes, while bats should be constrained to chiropterophilous flowers. If this is true, first, the network should have a modular structure, with more modules including only sphingophilous plants and hawkmoths, and fewer mixed modules including chiropterophilous plants, bats, and hawkmoths. Second, if sphingophilous flowers restrict bat access through morphology, the gradient of morphological restriction imposed by flowers should be associated with module configuration. Thus, modules should differ in terms of floral tube characteristics and visitor tongue length, with hawkmoth-dominated modules containing longer and narrower flowers, and longer-tongued hawkmoths placed alongside longer-tubed flowers. Finally, sphingophilous plants should show higher species-level specialization (fewer mutualistic partners) than chiropterophilous plants, while the latter should be more generalist and stronger network connectors.

2 | MATERIALS AND METHODS

2.1 | Study area

Expeditions for the sampling of hawkmoths and bats were carried out in 2011 and 2012 in the Almas Farm (7°28'45" S, 36°54'18" W), a private protected area located in the Cariris Velhos region, Paraíba state, northeastern Brazil. This is an area of hiperxerophilous Caatinga, with an average annual rainfall of 674.22 mm concentrated in three to five months a year, usually from January to May, which results in a long dry period (Brazilian National Institute of Meteorology [INMET], 2020).

Almas Farm has 3,505 ha of bush-dominated, deciduous vegetation, with a predominance of succulent components (Andrade-Lima, 1981; Rodal & Sampaio, 2002). As already reported for other Caatinga areas (Machado & Lopes, 2004), the Almas Farm has a relatively high frequency of moth-pollinated (13%) and bat-pollinated species (11%) (Quirino & Machado, 2014).

2.2 | Plant-pollinator interactions

Interactions between plants and nocturnal floral visitors were assessed through pollen types found on the body of floral visitors. We sampled pollen loads on the bodies of bats and hawkmoths captured in the field, thus following a zoocentric approach (Jordano, 2016a; Jordano et al., 2009). Because we considered only these two most relevant pollinator groups in the region, other functional groups such as noctuid moths, nocturnal bees, and non-volant mammals were not sampled. Captures were carried out monthly from January 2011 to December 2012, from three to five nights per month, between 1800 hr and 0400 hr each night. Bats were captured with a 12 × 3-m mist net in the first 10 nights (1,000 mist-net hr) and with four 7 × 2.5-m mist nets in all subsequent nights (2,480 mist-net hr) (all nets with a 16 mm mesh size, 75/2 denier, Ecotone®). Nets were set at ground level.

Because the study site is composed mostly of open vegetation, the mist-nets were set atop rock outcrops, which contained denser patches of flowering plants, in order to increase capture rates. The patches often included both chiropterophilous and sphingophilous plants flowering simultaneously. All captured individuals of both specialized nectar-feeding bats (Phyllostomidae: subfamilies Glossophaginae and Lonchophyllinae) and opportunistic flower-visiting species were sampled for pollen. Pollen grains were collected from the external body of bats (head, abdomen, back, wings, and uropatagium) with cubes of stained glycerinated jelly, which were later mounted on microscope slides for identification through external morphology (Voigt et al., 2009). To reduce the risk of sample contamination, jelly cubes were always kept in isolated vials, and handling materials were sterilized between pollen samplings from different floral visitors. Following the sampling procedure, bats were marked with wing punches and then released. Recaptured individuals were not included in the sampling.

Hawkmoths were captured using light traps, which were also mounted atop rock outcrops during two new-moon nights each month as a means to increase trap attractiveness, adding up to 48 capture nights. One light trap was set up per night also from 18:00 hr to 04:00 hr, thus resulting in 480 hr of passive trapping. Hawkmoth and bat captures were carried out on consecutive nights and never simultaneously. After capture, each hawkmoth specimen was sacrificed and deposited individually in paper envelopes. Pollen grains were later collected from the external body and the stretched proboscis of hawkmoths using the same method as described above for bats.

All species of floral visitors and pollen types were identified to the lowest possible taxonomic level. Pollen types found on floral visitors were compared to a personal reference pollen collection composed of plants from the Almas Farm and surrounding area, as well as to the palynological collection at Feira de Santana State University, Bahia, Brazil. Each pollen type was classified as either "chiropterophilous" or "sphingophilous," based on the syndrome of the plant of origin. We used the classification of Quirino and Machado (2014), which listed the syndromes found in the study

area. The authors classified both sphingophilous and phalenophilous (pollination by noctuid moths) species as "phalenophilous," following the terminology used by Faegri and Pijl (1979), who included both groups in the same guild. Because we did not include noctuid moths in our sampling, these species found interacting with hawkmoths in the present work were referred to as "sphingophilous." Pollen loads could also contain pollen from plant species with syndromes other than our focal chiropterophilous or sphingophilous systems. These pollen types (e.g., diurnal species with anthesis at dusk or dawn) were grouped into the third category "other syndromes." Less than 10 grain of a pollen type present in a given sample were considered contamination, as well as pollen from anemophilous species (e.g., family Poaceae).

Because some night-blooming plant species from the study area start producing nectar in the late afternoon or end nectar production by early morning, individuals of diurnal hawkmoths may also be attracted to them during early or late anthesis. Therefore, additionally to captures by light traps, we also performed daytime observations (17:00 hr to 18:30 hr) to nocturnal flowers whose nectar dynamics spans beyond nighttime in order to complement the network with these potential diurnal interactions. Observations were made to the sphingophilous *Guettarda angelica* (Rubiaceae), 9 hr; *Aspidosperma pyrifolium* (Apocynaceae), 9 hr; *Amburana cearensis* (Fabaceae), 9 hr; and to the chiropterophilous *Ipomoea vespertilia* (Convolvulaceae), 12 hr; *Ceiba glaziovii* (Malvaceae), 12 hr; *Encholirium spectabile* (Bromeliaceae), 12 hr; *Helicteres baruensis* (Malvaceae), 9 hr. The following species were also observed early in the morning (05:30 hr to 07:00 hr): the chiropterophilous *Pseudobombax marginatum* (Malvaceae), 6 hr; and *Pilosocereus gounellei* (Cactaceae), 12 hr; and the sphingophilous *Cereus jamacaru* (Cactaceae), 12 hr.

Because our sampling involves very distinct groups, we analyzed sampling separately for bats and hawkmoths following Chacoff et al. (2012). We estimated rarefaction curves of pollen types found on bats and hawkmoths (nocturnal only) using an individual-based approach (Colwell et al., 2012), that is, by quantifying pollen types accumulated according to the total number of interactions recorded from captures visitor individuals. We used the nonparametric estimator of asymptotic species richness Chao1, which takes into account interaction abundances (Chao et al., 2009), to calculate the expected richness of pollen types for both curves. Sampling completeness was calculated as the percentage of the observed richness of pollen types in each curve in relation to the estimated asymptotic richness (Chacoff et al., 2012).

2.3 | Network structure

Data on the pollen types found on floral visitors were used to build a weighted incidence matrix of interactions ($A \times B$), in which rows correspond to floral visitors (i) and columns, to plants (j). Each matrix cell a_{ij} contained values of interaction frequencies, that is, the number of times a floral visitor species i was found carrying the pollen of a plant species j .

We did not consider the absolute number of pollen grains found on each individual floral visitor, but rather the presence or absence of pollen. Interaction frequencies, therefore, represented the number of individuals of floral visitor species i that carried pollen of plant species j . Weighted matrices are considered more informative than their binary counterparts for assessing local realized niches (Fründ et al., 2016), as links in the former are better proxies for reciprocal effects among species (Vázquez et al., 2005). Moreover, several weighted network metrics are less biased by sampling incompleteness than their binary versions (Vizentin-Bugoni et al., 2016).

We described the structure of the studied network using three network-level metrics. Complementary specialization (H_2') is a measure of niche divergence between species and varies between 0 and 1, where higher values of H_2' indicate higher specialization (Blüthgen, 2010). This measure is considered a good proxy for ecological specialization at the network level. Nestedness, assessed through the WNODF metric (Almeida-Neto & Ulrich, 2011), describes to which extent the interactions of species with fewer mutualistic partners form a subset of the interactions of species with more mutualistic partners. WNODF ranges from 0 (non-nested network) to 1 (perfectly nested network).

Weighted modularity (Q_w) assesses the extent to which species form subgroups with higher internal than external interaction density. This metric is a good proxy for ecological concepts such as guild and functional group. We used the Beckett weighted modularity maximizing algorithm DIRTLPAbw+ (Beckett, 2016), which computes faster, has more stable results, and is less sensitive to initial node labeling than previous algorithms used in the ecological literature (such as Guimerà, Barber, QuanBiMo, LPAb+, and LPAbw+). This algorithm assigns unique labels (modules) to each node from the smallest of the two species sets (higher or lower level) and then runs a sequence of steps of label propagation followed by module joining until the greatest value of modularity is achieved. The procedure is run multiple times with different label initializations. The values of weighted modularity (Q_w) range from 0 (no modular structure) to 1 (high density of heavier links within the modules). We made 100 iterations of the algorithm and reported the modular structure that resulted in the highest value of Q_w .

Finally, we also tested for a compound topology (sensu Lewinsohn et al., 2006) in the studied network. A compound network has a modular structure, but its modules show a different kind of internal structure, such as nestedness (see Pinheiro et al., 2019). We used a set of customized functions written in R to run this analysis (see Felix et al., 2017; Pinheiro et al., 2019, and the supplement). With these functions, we calculated nestedness in the entire network, between its modules, and within its modules. For this analysis, we used the WNODA metric, which is derived from WNODF, but focuses on decreasing marginal totals instead of decreasing fill, and allows analyzing completely filled matrices (Pinheiro et al., 2019). A compound network is expected to show higher nestedness within its modules than between its modules and in the entire network.

The significance of the network metrics H_2' , WNODF, WNODA, and Q_w was estimated through Monte Carlo procedures based on comparisons with randomized distributions generated with null models. We generated 1,000 randomized matrices based on the original weighted matrix using the algorithm proposed by Vázquez et al. (2007) for pollination networks, which assigns interactions randomly among pairs of species with the constraints that the matrix size and connectance remain the same as in the original matrix. The algorithm has an intermediate level of restriction as the randomized marginal totals vary from those of the original network (Dormann et al., 2019). For estimating the significance of WNODA at different network scales, we used the restricted null model (Pinheiro et al., 2019), which is an adaptation of the Vázquez null model for using with WNODA.

Significance (p) was estimated as the number of times the null model resulted in a network with a score equal or higher than the score measured in the original matrix, divided by the total number of randomizations (1,000). We report p -values exactly as calculated, and not as approximations. Network structure was considered to significantly deviate from the null model when $p \leq 0.05$.

2.4 | Species roles

To assess the relative importance of each node for the structure of the network, we calculated a set of species-level metrics. The centrality of a species was measured first as its normalized degree (nk), that is, the proportion of partners that a given species interacts with out of the total number of potential partners available in the network (Freeman, 1979). A species connected to a larger proportion of partners is assumed to be more influential in the structure and dynamics of its network (González et al., 2010). We also calculated betweenness centrality (BC), that is, the proportion of shortest paths that pass through a node (Freeman, 1977), which varies from 0 to 1. A species positioned between several pairs is assumed to contribute more to connecting different regions of the network (Mello et al., 2015). Finally, we quantified node specialization using Blüthgen's d' , which measures the specialization of a node to a set of other nodes and varies from 0 to 1 (Blüthgen, 2010). A more specialized node ($d' \rightarrow 1$) is assumed to represent a species that makes a set of interactions different from those made by other species of the same network (Mello et al., 2019).

These species-level metrics were compared among syndrome categories, that is, chiropterophilous, sphingophilous, and other-syndrome plants by fitting generalized linear models (GLMs). We fitted a separate GLM for each metric, employing metric values as dependent variables and plant syndromes as the explanatory variable. Models were fitted with a quasibinomial error distribution and a logit link function and were validated by comparing them with null models with no explanatory variables. Significance was assessed by chi-squared tests. The significance of pairwise differences of metrics among syndromes was assessed through t tests after repeating

the GLMs once with each syndrome category as the baseline (intercept) for comparison. We did not compare groups of floral visitors, because there were many more hawkmoth species than bat species in the network, which would lead to an unbalanced design.

2.5 | Drivers of module partitioning

Given a modular structure, we assessed whether the species' morphologies would differ according to the modules assigned by the algorithm, and thus if visitor exclusion by morphological constraint was a relevant process in shaping the network's structure. We gathered data from the literature on the morphometry of structures related to floral restriction and to the access of pollinators to flowers.

For pollinators, we collected data on the length of feeding apparatus: proboscis length for hawkmoths (Johnson et al., 2017) and operational tongue length—palate length plus tongue extension beyond mouth—for bats (Winter & Helversen, 2003). Non-specialized nectarivorous bats, whose tongues do not extend significantly beyond the mouth, had only their palate length considered.

For plants, we collected data on floral tube length and diameter (see Table S1 for references and comments on the structures measured for each species). Whenever we could not find the data for a species in the literature, we extracted the measurements ourselves by compiling scaled photographic records of exsiccatae of the species uploaded on the Species Link data base (<http://www.splink.org.br/>) and using linear morphometric analysis in the ImageJ 1.8.0 software (Abramoff et al., 2004). In these cases, 30 flowers were used for measurement (see Table S1 for the examined material). Four species from the “other syndromes” category, whose pollen types that could not be identified to the lowest possible level, could not be included in this analysis.

We ran a GLM with a Gaussian error distribution for each morphological variable, in which the measurements (tube length, tube diameter, or tongue length) were set as the dependent variable, and module assignment as the explanatory variable. The significance of the models and pairwise differences among modules were assessed through the same procedure described above at the *Species roles* topic.

2.6 | Software

All analyses were run in R 4.0.2 (R Development Core Team, 2020). The computation of network metrics and the null model analysis were made using the package *bipartite* (Dormann et al., 2019), and the sampling completeness analysis was performed using the package *vegan* (Oksanen et al., 2013). The compound topology analysis was made using customized R functions (Felix et al., 2017; Mello et al., 2019; Pinheiro et al., 2019), which we made available in the supplement. The software Gephi 0.9.2 (Bastian et al., 2009) was used for graph drawing.

3 | RESULTS

3.1 | Plant-pollinator interactions

We analyzed the pollen samples of 117 bats of four species and 246 hawkmoths of 15 species and recorded 766 interactions with 24 plant species (see Tables S1 and S2 for a complete list of species). Out of the 15 hawkmoth species, *Aellopos* sp. (four individuals) was the only diurnal recorded visiting plants during dusk.

We recorded five plant species classified as sphingophilous and nine as chiropterophilous, based on diagnostic floral traits (see Figure 1 for examples). Eight additional pollen types that did not belong to either chiropterophilous or sphingophilous species were also found in at least one pollen sample and were included in the “other syndromes” category (Table S2). Two pollen types remained unidentified. Two species from the pollen pool were not studied by Quirino and Machado (2014): *Hippeastrum* aff. *elegans* (Amaryllidaceae) and *Cordia* aff. *rigida* (Rubiaceae). The latter has small and inconspicuous flowers and is reported to be visited by diurnal bees (Lopes et al., 2016) and was thus placed in the “other syndromes” category. The former does not have a formal register of its reproductive biology. Because of its robust, white flowers, which were visited mostly by bats (Figure 2), it was classified as “chiropterophilous.”

None of the pollen type rarefaction curves reached the estimated asymptotic species richness, although the observed richness of pollen types all fell into the standard error intervals of the estimated richness (Figure S1). Bat and hawkmoth samplings yielded, respectively, 92.9% and 99.1% of the expected asymptotic pollen richness.

The term *species*, when associated with plants, will be used henceforth to refer to each distinct pollen morphotype, and not necessarily to the lowest possible taxonomic level.

3.2 | Network structure

The network presented low to intermediate specialization ($H'_2 = 0.34$, $Z = 10.36$, $p = 0$), nestedness ($WNDOF = 0.34$, $Z = -3.01$, $p = 1.0$), and modularity ($Q_w = 0.37$, $Z = 15.29$, $p = 0$). In the compound topology analysis, nestedness within the modules was two times higher ($WNODA = 0.60$, $p = 0.48$) than between the modules ($WNODA = 0.30$, $p = 0.46$) and in the entire network ($WNODA = 0.38$, $p = 0.47$) (Figure S2). Therefore, the network shows specialization in the interactions, although not high. In addition, this specialization is organized mainly in subgroups. Finally, most of the network's nestedness comes from within its modules.

Four modules were identified in the network (Figure 2). All bat species belonged to a single module, along with most plant species deemed as chiropterophilous. The only exceptions were *Bauhinia cheilantha* and *Pilosocereus gounellei*, which were visited to a similar extent by both bats and hawkmoths. These two plant species were assigned to one of the other three modules, all of which encompassed hawkmoths only. Two



FIGURE 1 An overview of some species and interactions recorded in a Caatinga dry forest in northeastern Brazil. Chiropterophilous plants: *Encholirium spectabile* receiving visits from the specialized nectar-feeding bat *Glossophaga soricina* (a) and the unspecialized *Phyllostomus discolor* (b); *Pseudobombax marginatum* (c) and *Pilosocereus gounellei* (d) interacting with the specialized *Lonchophylla mordax*; *Ipomoea vespertilia* receiving a visit from *G. soricina* (e) and being approached by the hawkmoth *Agrilus cingulata* (f); (g) frontal view of the bell-shaped flower of *Pilosocereus chrysostele*. Sphingophilous plants: (h) *Tocoyena formosa* visited by *Manduca rustica*; (i) lateral view of the long, tubular flower of *Cereus jamacaru*

hawkmoth species, *Protambulyx strigilis* and *Eumorpha obscura*, were also placed in the module dominated by bats and chiropterophilous species.

3.3 | Species-level metrics

Specialization varied significantly among plant species with different pollination syndromes ($\chi^2 = 0.86$, $df = 19$, $p < 0.0001$). Sphingophilous plant species ($d' = 0.44 \pm 0.07$) were more specialized in their floral visitors than chiropterophilous ($d' = 0.24 \pm 0.09$) (estimate = 0.93, $t = 3.86$, $p < 0.005$) and other-syndrome species ($d' = 0.21 \pm 0.09$) (estimate = 1.08, $t = 4.25$, $p < 0.0005$). Nevertheless, the latter two did not differ from one another ($p = 0.56$; Figure 3a).

Plant species with different syndromes also differed significantly from one another in terms of normalized degree ($\chi^2 = 1.51$, $df = 19$, $p < 0.001$). Nevertheless, this difference was detected only

between other-syndrome species ($nk = 0.14 \pm 0.09$) and chiropterophilous species ($nk = 0.39 \pm 0.21$; estimate = 1.37, $t = 3.26$, $p < 0.01$). Sphingophilous species ($nk = 0.29 \pm 0.09$) did not differ from the other groups ($p > 0.05$ for both; Figure 3b).

Finally, pollination syndromes did not explain the performance of a plant species as a network connector ($\chi^2 = 0.957$, $df = 19$, $p = 0.76$; Figure 3c). However, the chiropterophilous plants *Pilosocereus gounellei* and *Bauhinia cheilantha* and the diurnal plant *Croton* sp. stood out for reaching the highest betweenness centrality values in the network ($BC = 0.11$).

3.4 | Module assignment and species morphologies

Two out of the three morphological variables assessed explained part of the module assignment detected for plant and pollinator

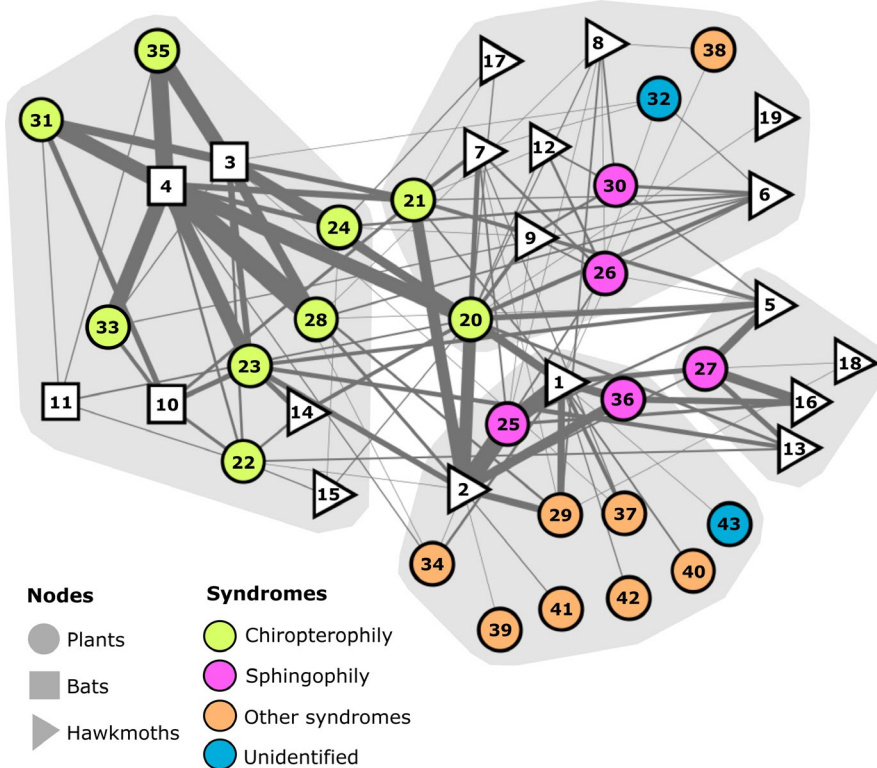


FIGURE 2 The nocturnal plant-pollinator interaction network in a Tropical Dry Forest of northeastern Brazil. Pollinator nodes are drawn in white, while plant nodes are colored according to pollination syndromes. Link width is proportional to interaction frequency. Gray polygons around groups of nodes represent the interaction modules identified with the Beckett modularity detection algorithm. Pollinators: 1. *Callionima grisescens*; 2. *Erinnyis ello*; 3. *Lonchophylla mordax*; 4. *Glossophaga soricina*; 5. *Isognathus allamandae*; 6. *Agrius cingulata*; 7. *Eumorpha vitis*; 8. *Eumorpha analis*; 9. *Manduca rustica*; 10. *Phyllostomus discolor*; 11. *Artibeus planirostris*; 12. *Manduca sexta*; 13. *Xylophanes tersa*; 14. *Protambulyx strigilis*; 15. *Erinnyis obscura*; 16. *Aellopos* sp.; 17. *Cocytius antaeus*; 18. *Callionima parse*; 19. *Pseudosphinx tetrio*. Plants: 20. *Pilosocereus gounellei*; 21. *Bauhinia cheilantha*; 22. *Ceiba glaziovii*; 23. *Encholirium spectabile*; 24. *Ipomoea vespertilia*; 25. *Guettarda angelica*; 26. *Tocoyena formosa*; 27. *Amburana cearensis*; 28. *Helicteres baruensis*; 29. *Croton* sp.; 30. *Cereus jamacaru*; 31. *Pseudobombax marginatum*; 32. Indet.1; 33. *Hippeastrum* aff. *elegans*; 34. *Piptadenia stipulacea*; 35. *Pilosocereus chrysostele*; 36. *Aspidosperma pyrifolium*; 37. *Combretum* sp.; 38. *Anadenanthera colubrina*; 39. *Cordia* aff. *rigida*; 40. *Allophylus* sp.; 41. *Poinciana* sp.; 42. *Schinopsis brasiliensis*; 43. Indet.2

species in the network. The length of the feeding apparatuses of pollinators varied significantly among modules ($\chi^2 = 11,162.0$, $df = 3$, $p < 0.0001$), with one hawkmoth module being the only containing visitors with longer apparatuses than the other modules ($p < 0.05$ for all comparisons; Figure 4). It encompassed the hawkmoths with the longest proboscis in the network: *Agrius cingulata*, *Manduca rustica*, *M. sexta*, and *Cocytius antaeus* (>95 mm length).

Floral tube diameter also differed among modules ($\chi^2 = 530.96$, $df = 3$, $p < 0.005$), but only between the bat-dominated module, which contained chiropterophilous species only, and a hawkmoth-dominated module that contained small sphingophilous flowers (i.e., *Guettarda angelica*, *Aspidosperma pyrifolium*) and inconspicuous flowers from other syndromes (estimate = 12.32, $t = 1.87$, $p < 0.01$). There were no other significant pairwise differences in floral diameter ($p > 0.05$ for all). Finally, floral tube length did not differ among modules ($\chi^2 = 17,772.0$, $df = 3$, $p = 0.06$), yet all long-tubed flowers were placed alongside long-proboscis hawkmoths (Figure 4).

4 | DISCUSSION

In the present study, we show that pollination syndromes predict only part of the structure of a nocturnal interaction network formed by bats and hawkmoths. On the one hand, the morphologically restrict sphingophilous plants excluded bats and formed modules that contained only hawkmoths. On the other hand, hawkmoths had no constraint in visiting most chiropterophilous plants, which showed, on average, lower interaction specialization toward the visitors in the network than the sphingophilous plants.

Thus, although bats interacted strongly with chiropterophilous plants, the only module that contained bats also included hawkmoths, making bat-plant interactions a subset in a network dominated by hawkmoths. This may be caused by a marked difference in species richness between those taxa. Hawkmoths presented almost four times more species and over twice captured individuals than bats. Although this difference could be interpreted as a consequence of the higher abundance of hawkmoths in nature compared to bats, the different methods employed to sample visitors (passive for bats

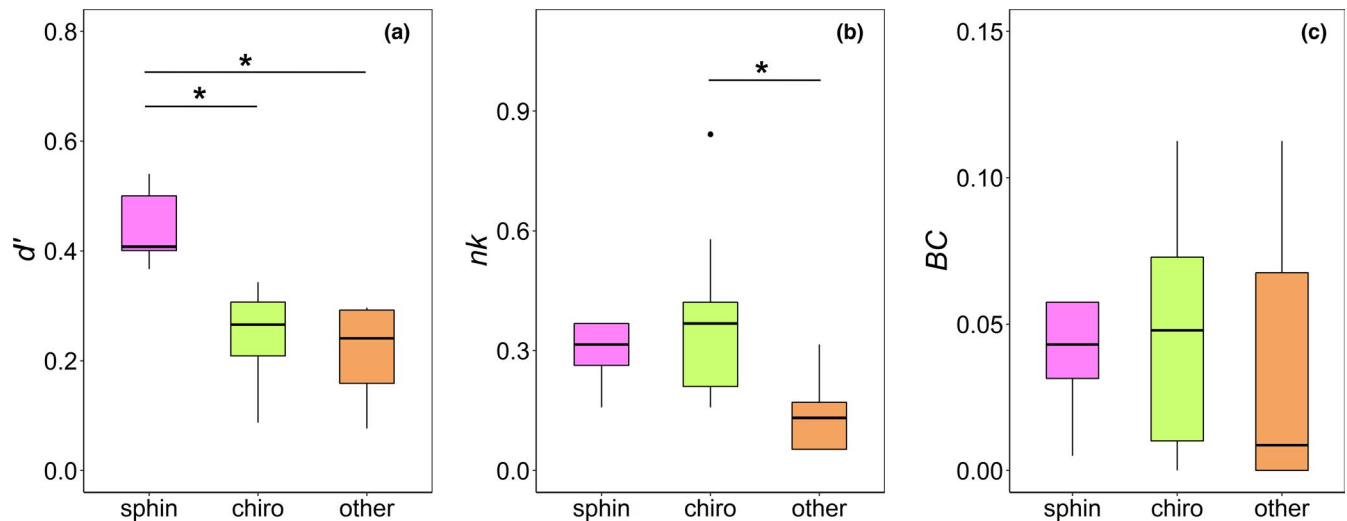


FIGURE 3 Distribution of species-level metrics for plants belonging to different pollination systems in the nocturnal pollination network. *sphin*: sphingophilous species; *chiro*: chiropterophilous species; *other*: species with other pollination syndromes. nk : normalized degree; d' : Blüthgen's specialization index; BC: betweenness centrality. Asterisks represent significant differences between groups (GLM)

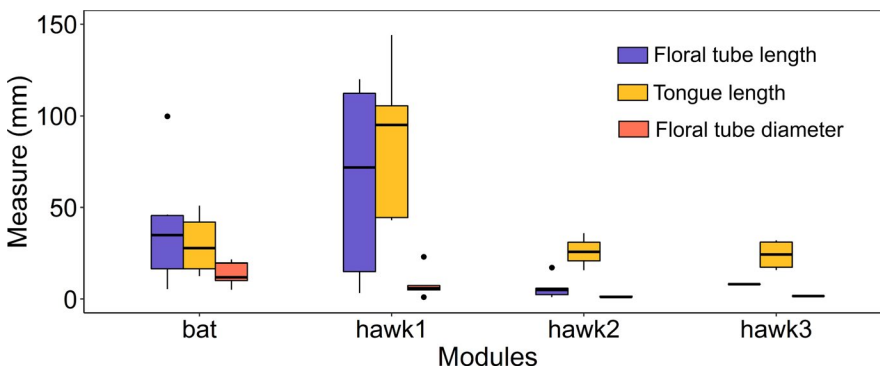


FIGURE 4 Distribution of morphological trait measurements for plants (floral tube length and diameter) and floral visitors (tongue length) composing the nocturnal network, classified by module assignment. Modules are divided into a bat-dominated module (*bat*) and three hawkmoth-dominated modules (*hawk1*, *hawk2*, and *hawk3*)

and active for hawkmoths) may be responsible for their contrasting richness. Notwithstanding, even though bats would require a larger additional sampling effort to reach the expected pollen type richness compared to hawkmoths, as suggested by the rarefaction curves, both groups reached a sufficiently high percentage of sampling completeness by falling into the confidence intervals of the asymptotical richness estimator.

This dominance of a visitor group has been observed in single-guild nocturnal pollination networks. Long-tubed flowers exclude short-proboscis hawkmoths while long-proboscis hawkmoths can forage on a wider variety of partners (Moré et al., 2007). Similarly, old-world opportunistic flower-visiting bats share only a subset of the partners of specialized nectarivorous bats (Sritongchuay et al., 2019). Our results also support that the modularity resultant from a gradient of morphological restriction, which is recurrent in plant-pollinator systems (Dalsgaard et al., 2009; Jordano, 2016b), will often emerge also in systems comprising phylogenetically distant pollinators (e.g., Danieli-Silva et al., 2012).

In the studied network, floral width appears to pose a significant yet weak gradient of morphological restriction, with hawkmoth-dominated modules encompassing relatively narrower

flowers. This seems enough to block bat access, while the inverse for chiropterophilous flowers allows interactions with both taxa. Moreover, our results corroborate the wider potential niche of long-tongued hawkmoths (Johnson et al., 2017), whose module presented significantly longer feeding apparatuses coupled with a large variation in floral tube length, spanning from those of the long- and narrow-tubed *C. jamaru* and *T. formosa* (specialists), to those of the wide chiropterophilous *B. cheilantha* and *P. gounellei* (generalists). Such variation was likely a key factor in homogenizing floral tube lengths among modules.

Although the morphological gradients of flowers were not as conspicuous as expected, the incidence of several unrealized interactions derived from morphological matching was likely responsible for the observed low specialization, modularity, and nestedness, creating non-inclusive sets of interactions (Almeida-Neto et al., 2007). Generalist bat species (i.e., higher degrees—*L. mordax*, *G. soricina*) do not interact with any of the narrow and generalist sphingophilous plants due to their clear morphological restriction. Similarly, the specialist, long-tubed sphingophilous plants (such as *T. formosa* and *C. jamaru*) interact more frequently with long-tongued hawkmoths, a matching also observed by Sazatornil et al. (2016)

and possibly a result of the energetical constraint of larger hawkmoths that require the more abundant nectar of long-tubed flowers (Johnson et al., 2017).

All those constraints and trait-matching processes might explain the structure of the studied network. We found evidence that it has a compound topology (sensu Pinheiro et al., 2019). In other words, it has a modular structure, but its modules seem to be internally nested. In fact, the network's nestedness comes mostly from within than between the modules. Compound topologies have also been observed in other networks with high taxonomic diversity (Felix et al., 2017), composed of different kinds of interactions (Mello et al., 2019), or that contain mixed taxonomic groups (Genrich et al., 2017). As the studied network does also harbor mixed taxa, its compound topology might have emerged as a result of the constraints faced by taxonomically distant pollinators when using the resources offered by different plant families and genera. Once a constraint is resolved and the pollinator gets associated with a module, its interactions within this module become more flexible, which allows nested associations to emerge.

Some groups overcome those constraints though. For instance, the ability of hawkmoths of exploiting plants belonging to different pollination syndromes is consistent with the evidence that these animals play the role of ecological generalists in their communities, especially long-tongued ones, and reinforces their role as connectors of nocturnal, and perhaps even diurnal, pollination systems (Haber & Frankie, 1989; Nilsson et al., 1987). The exploitation by hawkmoths of flowers with less restrictive shapes reached even species belonging to other syndromes beyond chiropterophily, especially by small-tongued hawkmoths (e.g., Amorim, 2020). Much like many chiropterophilous plants, species such as *Anadenanthera colubrina*, and those from the genera *Combretum* and *Croton* exhibit lush brush-like inflorescences. Based on studies with other species of those genera, these are probably insect-pollinated but have anthesis that spans beyond daytime (e.g., Ekeke & Agbagwa, 2015; Freitas et al., 2001), which could have enabled the visitation by hawkmoths and also sporadically by bats. These species, however, were mostly marginal in the network, receiving few visitors. This was expected since diurnal visitors were not the target of our work. These species would likely have more central roles if sampled in a different context.

Our results also suggest that hawkmoths are important components of mixed-pollination in night-blooming plants. In plants with brush-like flowers and generalized pollination systems, hawkmoths can be, along with bats, among the most effective pollinators (Cruz-Neto et al., 2015). Moreover, as revealed by our results, hawkmoths potentially act as relevant secondary pollinators to species regarded as chiropterophilous, or perhaps even as effective as bats (Rocha et al., 2020). Here, we bring further evidence that chiropterophilous plants with open floral architectures are especially prone to present multiple groups of floral visitors and mixed systems (Queiroz et al., 2016). Further works on chiropterophilous species should, therefore, always consider coupling visitation data with pollinator efficiency (e.g., Santiago-Hernández et al., 2019).

We reported certain species whose syndromes were not good predictors of pollinator attraction and visitation. The species *Bauhinia cheilantha*, classified as chiropterophilous (Quirino & Machado, 2014), has a potential mixed-pollination system as it interacted with bats and hawkmoths with similar intensities. The same occurs for the hypergeneralist *Pilosocereus gounellei*, whose syndrome is a contentious subject as different authors have reported different pollinator activity. Although deemed as chiropterophilous and evidence of bat-pollination being found for it in one region (Cordero-Schmidt et al., 2017), only visitation by hawkmoths has been reported for it in another Caatinga area (Rocha et al., 2020). The same dichotomy was observed for the chiropterophilous *Lafoensia pacari* (Lythraceae), whose large cup-like flowers were visited by hawkmoths only in a survey in Costa Rica (Haber & Frankie, 1989) and exclusively by bats in the Brazilian Cerrado (Sazima & Sazima, 1975). These contrasting visitor assemblages are certainly a result of local pollinator assemblages, but we also suggest that these species do not fall into simple syndrome categories. *Pilosocereus gounellei*, specifically, seems to act as a keystone species for nocturnal communities of floral visitors in the Caatinga. Its year-round flowering phase (Quirino & Machado, 2014), and consequently the overlap with the phenophases of both pollinator groups, may have also been a key factor in homogenizing its interaction frequencies among guilds.

We also highlight a few unexpected interactions between plants and hawkmoths found in the study site. The species *Tocoyena formosa*, despite its very long and narrow tube (ca. 110 mm, Figure 1H), interacted with three short-tongued hawkmoths (30–45 mm proboscis). While the possibility of sample contamination cannot be excluded, the short-tongued hawkmoths could in fact be attracted by these flowers' display or odor and try to feed on it, in which case they could in fact act as effective pollinators even though they do not consume nectar. Conversely, given if nectar accumulates inside the floral tube of *T. formosa*, short-tongued species could profit from a portion of the resource. A similar case was reported for the short-tongued hawkmoth *Pachylia ficus* (40 mm proboscis length), a legitimate pollinator of the long-spurred orchid *Dendrophylax lindenii* (at least 120 mm spur length) in North America (Houlihan et al., 2019). The short-tongued *P. ficus* possibly consumes a smaller quantity of accumulated nectar in comparison with *Cocytius antaeus*, the long-tongued pollinator of *D. lindenii* with a matching proboscis length. Given the uncertainty surrounding these interactions with *T. formosa*, we suggest in-depth investigations of these morphological mismatches in future works.

Finally, we underline that we do not assume that hawkmoths pollinated the chiropterophilous plants that they visited, as we did not measure the efficiency of floral visitors. Our conclusions are based on pollinator attraction and visitation to flowers, which can but not necessarily result in effective pollination. Hawkmoths may frequently visit large brush- or cup-shaped flowers, that is, typical chiropterophilous flowers, without touching their reproductive parts or just contacting their anthers, thus not acting as effective pollinators of these plants (e.g., Domingos-Melo et al., 2019; Machado et al., 2006). Visitation frequency was taken as a measure

of the intensity of a visitor's dependence on a given plant resource, but it is not a good surrogate for assessing plant reliance on floral visitors for pollination. Careful observations of pollen transfer must be carried out before classifying floral visitors as pollinators, as pollination networks tend to shift in structure after the exclusion of inefficient pollinators (Santiago-Hernández et al., 2019). Reliable data on the species' natural history are thus necessary to avoid making biased conclusions about the structure and dynamics of mutualistic networks.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The proper authorization to capture, handle, and process animals was issued by the Chico Mendes Biodiversity Conservation Institute—ICMBio (SISBIO no. 25582).

AUTHOR CONTRIBUTIONS

JAQ, ICM, and ZMQ conceptualized the study and designed the methodology. ICM acquired the funding. JAQ carried out data curation and investigation under the supervision of ICM and ZMQ. FARS identified the pollen types. UMD, JAQ, DPV, and MARM analyzed the data. JAQ wrote the original draft of the manuscript, and UMD wrote and reviewed the final version with the contributions of JAQ, ICM, DPV, and MARM.

DATA AVAILABILITY STATEMENT

The processed data that support the findings of this study, as well as the scripts used to analyze those data and produce the figures, are available on GitHub (<https://doi.org/10.5281/zenodo.4131207>).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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