Zoological Studies **56:** 22 (2017)
doi:10.6620/ZS.2017.56-22

Overlap in Cave Usage and Period of Activity as Factors Structuring the Interactions between Bats and Ectoparasites

Roberth Fagundes[1,2,*], Yasmine Antonini[1], and Ludmilla MS Aguiar[3]

[1] *Instituto de Ciências Exatas e da Natureza, Universidade da Integração Internacional da Lusofonia Afro-Brasileira, Acarape, CE, 62785-000, Brazil*

[2] *Departamento de Biodiversidade, Evolução e Meio Ambiente, Universidade Federal de Ouro Preto, Ouro Preto, MG, 35400-000, Brazil*

[3] *Laboratório de Biologia e Conservação de Morcegos, Departamento de Zoologia, Universidade de Brasília, Campus Darcy Ribeiro, Brasília, DF, 70910-900, Brazil*

(Recieved 27 September 2016; Accepted 19 July 2017; Published 18 September 2017; Communicated by Sheng-Feng Shen)

Roberth Fagundes, Yasmine Antonini, and Ludmilla MS Aguiar (2017) Bats perform important ecosystem services such as pollination and seed dispersal. Bats are also hosts to obligate ectoparasites, which influence their behavior and, thus, compromise their ecosystem functions. Therefore, the study of the bat-ectoparasitic interaction network and its driven factors is basic for understanding variation in the ecosystem services provided by bats. In this study, we evaluated the structure of the network of interactions between bats and their ectoparasite arthropods, testing the roles of overlap in cave usage and period of activity as factors structuring the interactions. We conducted the study in caves within the Cerrado ecosystem near Brasília, Brazil. Our results show that the bat-ectoparasite network has a modular pattern and is highly specialized. The observed pattern was explained by the greater probability of transmission of ectoparasites among bats sharing the same cave during the same period of the year. Furthermore, our data showed that the rate of bat infestation by different ectoparasite species is related to the degree of exposure of bats according to their abundance and activity period, but not with the number of caves used to roost. Thus, we believe that the frequency of activity and encounters between bats, and therefore the rate of ectoparasite transmission, is an important regulatory mechanism of bat-ectoparasite networks. This ecological mechanism may facilitate the formation of specific interactions by spatial and temporal segregation and co-evolution of parasite species among groups of roosting bats.

Key words: Chiroptera, Streblidae, Cerrado, Interaction network, Caves, Brazil.

BACKGROUND

Bats are highly interactive mammals that can occupy several trophic levels (Gardner et al. 1977). Generally they feed on insects, fruits, leaves, flowers, pollen, nectar, blood and small vertebrates (Gardner et al. 1977; Lee and McCracken 2004; Thavry et al. 2017). Bats also provide important ecosystem services such as seed dispersal (Lobova et al. 2009), plant pollination (Fleming and Muchhala 2008; Thavrey et al. 2017) and population control by predation (Kalka et al. 2008). Moreover, bats are hosts to a variety of ectoparasites, such as fleas, bat bugs, mites and a group of obligate blood-feeding ectoparasitic flies (Patterson et al. 2007, 2008; Nowak-Chmura et al. 2012). These bat-ectoparasite interactions are considered highly specialized (Patterson et al. 2008; Aguiar and Antonini 2011), and involve ectoparasites of the families Streblidae and Nycteribiidae, which are highly diverse in tropical latitudes (Dittmar et al. 2006; Petersen et al. 2007). Nycteribiids are more diverse in the Eastern Hemisphere, whereas streblids are more diverse in the Western Hemisphere (Dick and Patterson 2006). Ectoparasites constitute a natural control

---

*Correspondence: Tel: +5531986689926. E-mail: roberthfagundes@gmail.com

of the population size of their hosts, and can compromise individual fitness and population growth when infection rates are high (Marshal 1982).

Parasitic interactions, such those between bats and ectoparasites, can be very complex, with a single parasitic species infecting one host species of one genus to several host species of many genera (Hofstede et al. 2004). At the community level, parasite and host species can form a complex network of interactions with highly specialized modular structure (Vazquez et al. 2005; Krasnov et al. 2012; Lima-Jr et al. 2012). Modular networks consist of species organized into subgroups (modules) of highly interconnected species with few interactions between groups (Lweinsohn et al. 2006). Network properties and patterns can provide insight into the dynamics of interactions and their evolution (Pariselle et al. 2011), thus several recent studies have investigated the structural mechanisms of modular networks (Graciolli and Carvalho 2001; Almeida-Neto et al. 2008; Pariselle et al. 2011). However, it is not clear which ecological mechanisms strengthen interactions within modules and drive co-evolution and specialization, and whether these mechanisms vary in importance across distinct types of interactions.

Modular networks can emerge from co-evolutionary convergence of traits that strengthen relationships (biological specialization) or prevent interactions (niche differentiation) (Lewinsohn et al. 2006). Modularity can also be influenced by temporal or spatial co-occurrence of species, which promote interactions, or segregation of species, which prevent interactions (Lewinsohn et al. 2006). In bat-ectoparasite interactions, the ectoparasites can actively disperse between bats and choose a specific host, which can lead to increased specificity of the interaction (Pariselle et al. 2011). However, ectoparasitic flies have limited dispersal abilities due to their small size and stunted wings (Aguiar and Antonini 2011), and depend on physical contact of their host with another bat to switch hosts (Patterson et al. 2008). Thus, the dispersion of these ectoparasites and the specificity of their interaction with bats could be related to the frequency of encounters among bats. Bat species frequently share caves and trees as fixed shelters to roost, allowing easy dispersion among visiting bats and by the ectoparasites that remain in the shelter (Marshal 1982; Graciolli and Carvalho 2001; Rachwald et al. 2016). Therefore, the degree of infestation by ectoparasites might be related to the frequency of exposure to infested bats and infested caves, and cross dispersion of ectoparasites might be higher between bat species sharing the same cave and roosting at the same time.

In this study, we aimed to investigate the topology and structuring mechanisms of networks of interactions between bat and ectoparasitic arthropods. We tested three main hypotheses: (1) the interaction network is highly specialized, with the formation of cohesive subgroups of species (modules), as expected for parasitic interactions; (2) the number of interactions involving a bat species is related to the number of caves they use to roost and the length of usage period; (3) bat similarities in cave usage reflects its similarities in parasitic interaction. Since dispersion of ectoparasites is limited to short distances among hosts, we predicted that bats that roost in the same caves during the same time of year would interact with a specific subgroup of ectoparasites, and form a module. Additionally, as alternative mechanisms, we tested if species abundance, body mass and gender of bats affected the network structure.

MATERIALS AND METHODS

Study area

The study took place in the Área de Proteçao Ambiental de Cafuringa (APAC; Cafuringa environmental protection area) in the metropolitan area of the city of Brasília in the Federal District of Brazil (15°30'-15°40'S and 47°50'-48°12'W). It is a protected area managed by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) and encompasses 46,000 ha with karst geomorphology and steep, dry slopes. The area is very important for the conservation of a huge portion of Cerrado (savanna) and Mesophytic Forest, which extends to the Amazon Basin. Bats and their ectoparasites were sampled in three caves separated by 10 km in interfluvial mesophytic forests. The caves are known as Gruta Fenda II (15°30'S 48°10'W), Gruta da Saúva (15°32'S 47°42'W), and Gruta Dois Irmãos (15°34'S 48°07'W). The climate of the area is considered to be tropical rainy (Aw) according to Köppen, with distinct seasons of dry winters and wet summers typical of the Cerrado biome (Graciolli and Carvalho 2001).

Sampling design

Bats were sampled for 12 consecutive months between May 2004 and April 2005 using two nylon mist-nets (6 × 3 m) placed 1.5 m apart in front of the entrances of the three study caves from 18:00 pm to 06:00 am for three nights per month (totaling 36 nights). Nets were checked continuously to avoid the accumulation of bats in the same net. Although there were usually one or two bats in the net at the same time, efforts were made to avoid contact between bats and the potential exchange of ectoparasites.

Each bat removed from the net was placed in its own cloth bag to prevent the ectoparasites from escaping. All ectoparasites were removed from the bats using forceps and were preserved in 70% ethanol. The number of parasite species per bat (interactions), and the number of individual ectoparasites (infestation) were quantified. One species of the genus *Hesperoctenes* was encountered, which we choose to include in the network analysis because of its functional equivalence to the ectoparasites and its uniqueness as an ectoparasite of *Molossops temminckii*. Bats were weighted (Pesola spring scale - 100 g) and measured. We classified females based on secondary sexual characteristics and the presence of a palpable foetus. All individuals were marked with numbered plastic rings to avoid counting the same individual twice. Bats were released after measurements had been completed.

Ectoparasites were identified to species using keys for Neotropical bat ectoparasites (see references in Aguiar and Antonini 2011), and confirmed by G. Graciolli. Voucher specimens of both bats and ectoparasites were deposited in the chiropteran collection of the Universidade de Brasília (CCUNB; more details in provided in Aguiar and Antonini 2011). Bats and ectoparasites were collected under a license provided by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio, N° 10624), which did not require approval by an ethics committee, but requires that standard ethical procedures are to be followed to avoid animal suffering.

Data analysis

For network analyses, we represented the interactions between ectoparasite species and bat species with an adjacency matrix *A*, where *aij* = the number individuals of bat species *i* parasitized by ectoparasite species *j*; the value was zero when no interactions took place. We calculated the average degree of interactions, which provides information regarding the mean number of interactions, connectance (Jordano 1987), which is the proportion of possible interactions actually performed and H2’ (Blüthgen et al. 2006), the index of interaction specialization. We compared those three variables between bat and ectoparasite species using t-test. We considered each species as a sample unit (*N* = 44). We use the “bipartite package” of R software to calculate H2’ and to plot the network. We tested whether groups of bat species are more associated with groups of ectoparasite species, as expected in a modular network, using the modularity index (*M*) based on simulated annealing (*SA*) calculated by MODULAR software. We estimated the significance of modularity using the Null Model II (*n* = 1000 randomizations for each network). For each positive modularity result, we classified bat species into sub-groups (modules) according to their shared interactions using MODULAR. We also determined nestedness of the network by calculating the NODF index using ANINHADO software, because modularity and nestedness pattern can cooccur in the same network.

We tested the effect of the number of caves used by a bat species (spatial overlap; predictor variable) and the period of usage (temporal overlap; predictor variable) on the number of interactions with ectoparasite species (interaction degree; response variable) and on the number of ectoparasites per bat (infection degree; response variable). We considered bat abundance as a covariate. We tested this relationship with General Linear Model (GLM) using software Past v.3.0. Bat species were considered as independent cases (*N* = 16). Although we know that bats that occupy the same cave are not necessarily in contact, we assume that there is a chance that they could exchange bat ectoparasites during their passage into and out of the cave (Dick 2007), and this probability may be enhanced when they forage at same period of the year. We also tested the effect of host gender, by comparing the response variable between females and males using *t*-test, and the effect of host weight, using GLM assuming normal distribution.

We tested the effect of overlap in cave usage and period of usage by bat species as factors driving modularity using the procedure by (Lima-Jr et al. 2012). We built a similarity matrix M, with elements m_{ij} = 1, if bat species ‘*i*’ and ‘*j*’ belong to the same module, and $m_{ij} = 0$ when a given paired interaction did not occur. We used this matrix as response variable. For the predictor variables, we built similarity matrices using Horn's similarity index of overlap (Horn 1966) calculated for 'spatial overlap', based on the co-occurrence of bat species in the same caves, and for 'temporal overlap', based on the co-occurrence of bat species active during the same period of the year. Alternatively, we tested the effect of bat abundance as neutral factor. For this, we built a matrix of similarity between bat species based on the number of individuals sampled using Bray-Curtis similarity index (Bray and Curtis 1957) for abundance data. We also tested the effect of body weight as additional factor structuring network modules. For this, we build a matrix of similarity between bat species based on average body weight using Bray-Curtis index. We also compared the difference between males and females in the number of interactions using t-test. We tested the correlation between modularity and the predictor factors by performing Mantel tests for matrix correlation including the sample effort as covariate matrix (Almeida-Neto et al. 2008). We illustrated the similarities between species using Non-Metric Dimensional Scaling (NMDS) for the similarity index. All similarity analyses and matrix correlations were performed with PAST 3.0 statistical software.

RESULTS

We sampled 425 bats of 16 species interacting with 1,829 ectoparasites of 28 species (Tables 1, 2). Of the 425 sampled bats, 208 (49%) were females and 217 (51%) were males. Bats had 25.3 ± 20.8g (mean ± standard deviation), ranging from 6g (*Micronycteris minuta*) to 131 g (*Chrotopterus auritus*). Female bats had 24.1 ± 11.2 g while males had 26.8 ± 24.5 g. Bats had 2 ± 1.7 ectoparasites per individual, ranging from 1 to 12. Female bats had 1.8 ± 0.9 ectoparasites while male bats had 2.6 ± 1.7 ectoparasites per individual (*t*-test: $t = 1.4$; *d.f.* = 22; $p = 0.17$).

The bat-ectoparasite network presented 44 node species connected by 58 of 448 possible links, resulting in 13% of connectance. Bats presented 3.6 + 2.6 links per species while ectoparasites presented 2.1 ± 1.1 links per species ($t = 2.9$, *d.f.* = 42, $p < 0.001$). On average, female bats were linked to 3.6 ± 2.4 ectoparasite species while male bats were linked to 2.9 ± 1.9 ectoparasite species ($t = 0.8$, *d.f.* = 42, $p = 0.4$). The degree of specialization of the network ($H2'_{\text{observed}} = 3.82$) was higher than expected by chance ($H2'_{\text{random}} = 2.63$, $p < .0001$, Tables 1, 2). Bat species had a higher average degree of specialization (H2' = 0.8) compared to ectoparasite species (H2' = 0.65, *t*-test: $t = 2.5$, *d.f.* = 42, $p = 0.02$, Tables 1, 2). The network exhibited high modularity (Modularity = 0.61) and the index was higher than expected by chance (Null Model II: $p = 0.03$). Eight modules were identified (Fig. 1; Tables 1, 2). The network presented low nestedness (NODF = 12.96) and the pattern was not different than expected by chance (Null Model Ce: $p = 0.87$).

The number of caves used by bats to roost had a positive effect on the number of interactions between bats and ectoparasites (GLM: $R^2 = 0.7$; $t = 4.77$, $p = 0.001$; Fig. 2a), although it did not affect the number of parasites per bat (GLM: $R^2 = 0.08$; $t = -1.12$, $p = 0.3$). The length of the period of cave usage by bats did not predict the number of interactions in which bats were involved (GLM: $R^2 = 0.05$; $t = 0.33$, $p = 0.75$; Fig. 2b), although it positively affects the number of parasites per bat (GLM: $R^2 = 0.44$; $t = 3.32$, $p = 0.005$). The number of interactions with ectoparasites (GLM: $R^2 = 0.19$; $t = -0.36$, $p = 0.41$) and the number of ectoparasites per bat (GLM: $R^2 = 0.002$, $t = -0.2$, $p = 0.9$) did not increase with the abundance of bat species (Table 1). Bat weight did not affect the number of interactions with ectoparasite species (GLM: $R^2 = 0.03$; $t = 0.6$, $p = 0.5$) nor the number of ectoparasites per bat individual (GLM: $R^2 = 0.004$; $t = 0.27$, $p = 0.8$).

The modularity of the network was explained by the similarity between bat species within the modules regarding the use of caves for shelter (Mantel: $R^2 = 0.15$, $p = 0.04$; Figs. 1 and 3a) and the period of the year when they were found together in the same cave (Mantel: $R^2 = 0.13$, $p = 0.05$; Figs. 1 and 3b). There was no relationship between modularity and similarity between bat species according to their abundance (Mantel: $R^2 = 0.01$, $p = 0.53$) nor to their weight (Mantel: $R^2 = 0.5$, $p = 0.9$) (Tables 1, 2).

DISCUSSION

In this study, we confirmed the hypothesis that the network of interactions between a bat host and its ectoparasites is very specialized, composed by cohesive subgroups of highly interconnected

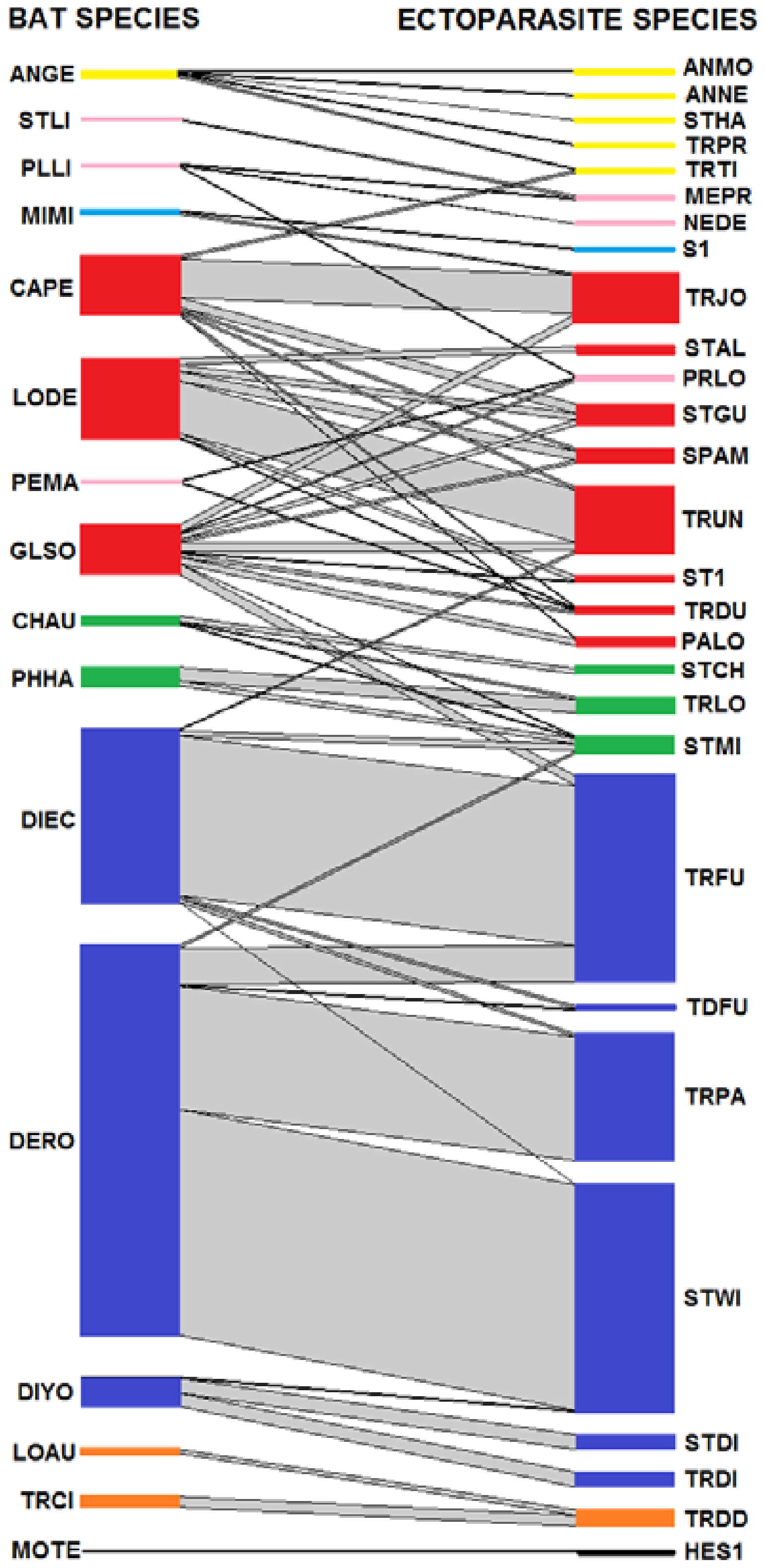

Fig. 1. Network of interaction between bats and ectoparasites. Within each network, the rectangles represent species of bats (left) or ectoparasites (right) and the lines represent the interactions. The width of the rectangle represents the abundance of the species and the width of the lines represents the percentage of interactions between a given pair of species. Species are arranged to represent modularity. Species with rectangles marked with a continuous line on the side belongs to the same module. Species labels are in tables 1 and 2.

Table 1. List of bat species studied and network descriptors. Module column show the number representing the species sub-group. H2’ column show the specialization index

Species name	Family	Code	Module	Caves used to roost	Months roosting in caves	Local abundance	Interactions with ectoparasite species	H2'
Anoura geoffroyi	Phyllostomidae	ANGE	1	2	1	3.0	5	0.95
Peropteryx macrotis	Emballonuridae	PEMA	2	1	1	1.0	2	0.66
Platyrrhinus lineatus	Phyllostomidae	PLLI	2	2	2	3.0	3	0.87
Sturnira lilium	Phyllostomidae	STLI	2	1	2	1.0	1	0.87
Micronycteris minuta	Phyllostomidae	MIMI	3	1	2	1.0	2	0.67
Carollia perspicillata	Phyllostomidae	CAPE	4	3	7	33.0	7	0.77
Glossophaga soricina	Phyllostomidae	GLSO	4	3	3	28.0	10	0.37
Lonchophylla dekeyseri	Phyllostomidae	LODE	4	3	7	35.0	6	0.75
Chrotopterus auritus	Phyllostomidae	CHAU	5	2	3	4.0	3	0.78
Phyllostomus hastatus	Phyllostomidae	PHHA	5	1	6	12.0	2	0.88
Desmodus rotundus	Phyllostomidae	DERO	6	3	11	171.0	5	0.75
Diaemus youngi	Phyllostomidae	DIYO	6	1	12	9.0	3	0.74
Diphylla ecaudata	Phyllostomidae	DIEC	6	3	8	102.0	6	0.95
Lonchorhina aurita	Phyllostomidae	LOAU	7	2	4	3.0	1	0.85
Trachops cirrhosus	Phyllostomidae	TRCI	7	1	8	7.0	1	0.91
Molossops temminckii	Molossidae	MOTE	8	1	1	1.0	1	1.0

Table 2. List of ectoparasite species studied and network descriptors. Module column show the number representing the species sub-group. H2' column show the specialization index

Species name	Family	Code	Module	Interactions	H2'
Anastrebla caudiferae	Streblidae	ANNE	1	1	0.7
Anastrebla modestini	Streblidae	ANMO	1	1	0.7
Strebla harderi	Streblidae	STHA	1	1	0.7
Trichobius tiptoni	Streblidae	TRTI	1	1	0.55
Trichobius propinquus	Streblidae	TRPR	1	2	0.7
Neotrichobius delicatus	Hippoboscidae	NEDE	2	1	0.73
Megistopoda proxima	Streblidae	MEPR	2	2	0.91
Paratrichobius longicrus	Streblidae	PRLO	2	3	0.63
Morphospecies 1	-	S1	3	1	0.88
Strebla altmani	Streblidae	STAL	4	1	0.46
Trichobius dugesii	Streblidae	TRDU	4	1	0.39
Paraeuctenodes longipes	Streblidae	PALO	4	2	0.5
Strebla cf. *altmani*	Streblidae	ST1	4	2	0.36
Trichobius uniformis	Streblidae	TRUN	4	2	0.67
Speiseria ambigua	Hippoboscidae	SPAM	4	3	0.38
Strebla guajiro	Streblidae	STGU	4	3	0.41
Trichobius joblingi	Streblidae	TRJO	4	3	0.77
Strebla chrotopteri	Streblidae	STCH	5	1	0.88
Trichobius longipes	Streblidae	TRLO	5	3	0.87
Strebla mirabilis	Streblidae	STMI	5	5	0.28
Strebla diaemi	Streblidae	STDI	6	1	0.82
Trichobius diaemi	Streblidae	TRDI	6	2	0.82
Trichobius parasiticus	Streblidae	TRPA	6	2	0.36
Strebla wiedmani	Streblidae	STWI	6	3	0.56
Trichobius furmani	Streblidae	TRFU	6	4	0.6
Morphospecies 2	-	S2	6	2	0.6
Trichobius d. dugesioides	Streblidae	TRDD	7	2	1
Hesperoctenes sp.	Polycteenidae	HES1	8	1	1

species. We also confirmed the hypothesis that the number of cave used to roost increased the number of ectoparasite species associated with a bat (parasitic interactions), although the extension of their monthly activity in those caves determinates the amount of ectoparasites individuals carried by the bats (parasitic load). This results indicates that visiting more caves increase contact with different parasite species while remaining in caves for longer times enhance the accumulation of parasites. Overlap in cave usage and in roosting period also explained that network modularity, that is, bat species sharing caves at the same period of the year interacted with a specific subgroup of ectoparasites. This finding corroborates a previous study (Patterson et al. 2009) that demonstrated that bats roosting in caves (permanent, enclosed structures) are more likely to be infested by more species of

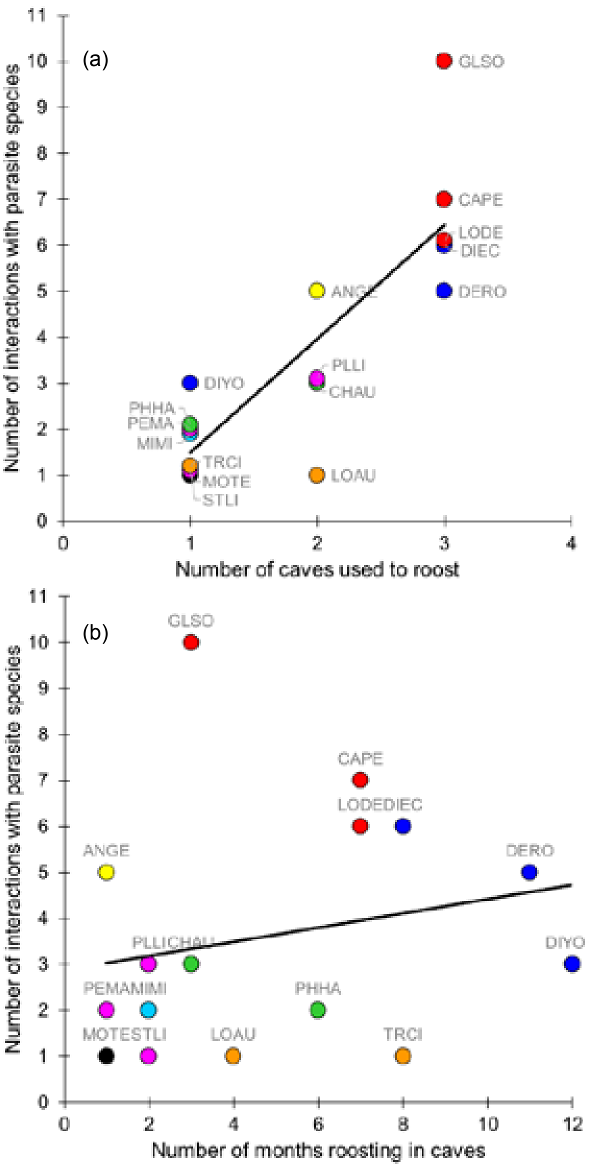

Fig. 2. Relations between the number of interactions that bat species performed with ectoparasite species; the number of caves used to roost (a), and the number of months that bats were roosting in caves (b). Note that only cave usage had a significant effect on interactions. Species labels are in table 1.

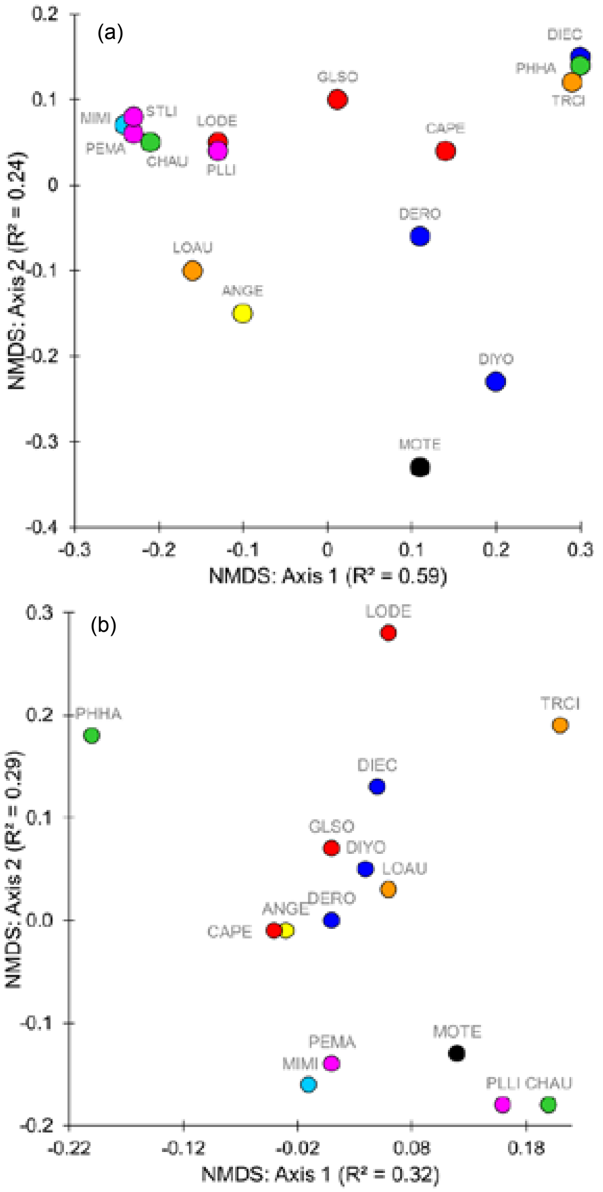

Fig. 3. Non-metrical dimensional scaling analysis showing the similarities between bat species according to (a) the sharing of caves used to roost based on Horn' index, and (b) the temporal overlap in use of caves to roost based on Horn' index. Each circle represents a species of bat, and the labels indicate the module to which the species belongs.

ectoparasites and carry heavier parasite loads. The abundance, weight and gender of bat species also did not affect the number of interactions, corroborating the effect of parasite exchange among bats roosting in the same caves. In summary, our results show that the frequency and specialization of bat-ectoparasite interactions is structured not only by the frequency of encounters between bats and ectoparasites, but also by the exchange of parasites among co-roosting bat vectors, thus creating the specific interactions of each module in the network.

The network of interactions between bats and their ectoparasites exhibited a specialized structure composed of modules with different sizes and degrees of specialization. We found eight cohesive sub-groups of species of bats that interact with specific subsets of species of ectoparasites. Modularity has been found in other parasitic networks involving fish and metazoan parasites of tropical floodplains of Brazil (Lima-Jr et al. 2012; Bellay et al. 2013), lizards and endoparasites in the semiarid region known as Brazilian Caatinga (Brito et al. 2014). In contrast, host-ectoparasite networks may also exhibit nested structure, as has been found with bats (Patterson et al. 2009), fishes (Lima-Jr et al. 2012) and other vertebrates and their respective ectoparasites (see meta-analysis in Brito et al. 2014). However, we found low nestedness, with a degree not different than expected by chance. Patterson et al. (2009) found that for bats, nestedness appears to be most developed in host-parasite systems with long-term infestations that accumulate over time, and less developed in short-term infestations. We found that most ectoparasites in bats are ectoparasitic flies, which are short-term parasites, which explain the low nestedness observed. In parasitic networks, nestedness is related to generalism and opportunism (Bellay et al. 2013), while modularity is related to specialization and co-evolution of traits (Krasnov et al. 2012), which corroborates the high degree of specialization observed. However, modularity and nestedness are the extremes of a gradient of interaction patterns (Olesen et al. 2007), and may be dependent on environment context, species composition and ecological scale (Newman 2006; Barber 2007; Mucha et al. 2010).

Overlap in cave usage had a strong effect on the accumulation of interactions and modularity of bat-ectoparasite network, while bat abundance, weight, gender had no effect. This indicates that bat-ectoparasite interaction can be structured by parasite dispersion frequency among co-existing bats more than specific matches of traits between host and parasite through phylogenetic relatedness, nor even sexual differences and body size. Three modules were supported by similarities in cave usage: *S. lilium*, *P. lineatus* and *P. macrotis*; *C. perspicillata*, *G. soricina* and *L. dekeyseri*; and *D. ecaudata*, *D. rotundus* and *D. youngi.* For these modules, overlap in cave usage was higher for bat species within than between modules. Two modules were not fully supported by similarities in cave usage. Both *C. auritus* and *P. hastatus*; *L. aurita* and *T. cirrhosus*. *C. auritus* and *P. hastatus* were interacting with the same exclusive parasite species *D. youngi*, which was very common in the caves where they roosted. *L. aurita* and *T. cirrhosis* were interacting with the exclusive specialist parasite *T. dugesioides*. These types of specialized interactions do not depend on parasite dispersion but on matching of coevolved traits (Stang et al. 2006; Krasnov et al. 2012). The single-species modules of *A. geoffroyi* and *M. temminckii* were also explained by the overlap in cave usage, but in this case the species were rare and restricted to one cave that was rarely used by other species. To the contrary, *M. minuta* was also rare and restricted to one cave, but occurred in a cave used by several other bat species, and so its module could not be explained by cave usage. We encourage future studies to evaluate the genetic similarities among ectoparasites from the same species living in different bat species sharing the same caves and the same bat species but from different caves to evaluate the importance of overlap in cave usage to the dynamic of ectoparasite populations.

Temporal overlap in cave-roosting periods also explained the pattern of modularity for interactions between bats and ectoparasites. Temporal overlap, like spatial overlap in cave usage, may increase the probability of the exchange of parasites between co-occurring species. Additionally, parasites with temporal variation in their activity and reproduction may require specific hosts. The phenological cycling of ectoparasite reproduction may match the period of high roosting activity of the bat host to increase the infection success. It is important to note that temporal overlap does not explain modularity of all species within some module. For example, in the *D. ecaudata*, *D. rotundus* and *D. youngi* module, the first two species coexisted in the same caves throughout the same period of the year, but the third species only co-existed with the first two between October and February. The same was observed for the module of *C. perspicillata*, *G. soricina* and *L. aurita*, in which the third species co-existed with the other two between September and December while the other species co-existed throughout the year. In these cases, cave sharing was more important than overlap of roosting period. Moreover, temporal overlap explained single-species modules better that spatial overlap, because those bat species used the same caves in which bat species from other modules roosted but did not encounter these species by roosting in different periods of the year.

Phylogenetic relatedness among bat species may also affect network modularity. Bats were taxonomically more similar within modules than between modules. Similarities in bat phenotypes due to conservatism of common traits act as filters to guilds of parasite species with similar preferences (Lima-Jr et al. 2012; Wiens et al. 2013). In mammal-flea interactions, phylogenetic relatedness between hosts was found to be an important driver of interaction specialization and modularity (Krasnov et al. 2012; Lima-Jr et al. 2012). On the other hand, parasites within a module are distantly related, as observed in the present study, and may reflect convergence of traits or dispersion of ectoparasites between co-occurring species (Lima-Jr et al. 2012). The success of bat-ectoparasite interactions depends on the quality of the blood of bats and their anti-parasite defenses, and on parasite ability to successfully disperse and colonize the host (Galbe and Oliver-Jr 1992; Krasnov et al. 2012). So, closely related bats might be similar in these traits and therefore attract a similar guild of parasites, thereby creating modules. In our study, phylogeny relatedness was important explaining two modules of insectivorous bat species of the families Emballonuridae and Molossidae, and one module of three blood-sucking bat species of the family Phyllostomidae. The other five modules had Phyllostomidae bats, but they were not closely related. It is important to note that highly specialized modules did not contain closely related bats, which may indicate convergence in traits related to parasite-defense among bat species or traits related to host-attack among ectoparasite species (Krasnov et al. 2012). This influence of phylogenetic relatedness on modularity has also been observed for fruit-eating bats (Mello et al. 2011), food webs (Rezende et al. 2009) and mutualistic networks (Donatti et al. 2011).

Local abundance of bat species did not contribute to the formation of specialized interactions and modularity. Only the module formed by *C. perspicillata* (*N* = 33 bats), *G. soricina* (28) and *L dekeyseri* (*N* = 35) reflected similarity in species abundance, comprising the most parasitized bat species with a combined total of 12 parasite species. This module also had more interactions than the module formed by the most abundant species, *D. ecaudata*, *D. rotundus* and *D. youngi*. Other modules encompass species with low similarities in abundance (see Table 1 for details). These contrasting results probably reflect that abundance is not a good predictor for modularity, although relative abundance is an important factor in structuring nested networks (Krishna et al. 2008). It is expected that abundant species should interact more than rare species. Nevertheless, we observed rare species with several interactions, such as *A. geoffroyi* which accumulated five non-specialized ectoparasite interactions, while the most abundant bat, *D. rotundus*, had the same number of interactions, with one of them being highly frequent. Therefore, abundance did not affect interaction frequency, but can be an important driver of specialization (Krasnov et al. 2012), because very abundant host species exhibited more specialized interactions (Krasnov et al. 2012). The bat-ectoparasite network seems to be structured by ecological factors, such as those studied here, rather than neutral factors such as relative abundance. The inability of abundance to explain properties of biological networks has been found elsewhere, such as for mutualistic ant-plant networks (Dáttilo et al. 2014).

CONCLUSIONS

Our study showed that the network of interactions between bats and ectoparasites is highly specialized and is comprised of modules with highly interconnected species. This modularity indicates that highly interactive species, as well as specialists, do not share the same parasite species, and so cannot interact with virtually all parasites encountered. There is a threshold number of parasitic interactions that a bat cannot exceed by simply increasing the number of caves visited. In fact, our results indicate that modularity may be caused by the frequency of encounters between bat species. In other words, bat species share more parasites when they meet each other more frequently. Therefore, for hosts to become exposed to specific parasites, it is necessary that they encounter other vectors at proximities close enough such that bat ectoparasites can disperse. This ecological mechanism of exchanging parasites by sharing roosts, associated with specialized characters of co-evolved pairs of species, creates successful interactions that are the main structural factors of networks of interactions between bats and their ectoparasitic ectoparasites. We encourage future studies to pursue the difficult task of study the dispersion of parasites between hosts to a better understanding of the importance of cave sharing and overlap in foraging period as structuring mechanisms of bat-ectopasite networks.

Acknowledgments: We thank the MMA/FNMA for the financial support related to this work. We thank the two anonymous reviewers of PloS One and the two anonymous reviewers of Zoological Studies for their constructive criticism. Yasmine Antonini received a scholarship from CNPq and Roberth Fagundes from CAPES. We also thank Gustavo Graciolli for taxonomic identification of the species of ectoparasitic bat ectoparasites.

REFERENCES

Aguiar LMS, Antonini Y. 2011. Descriptive ecology of bat ectoparasites (Diptera: Hippoboscoidea) associated with vampire bats (Chiroptera: Phyllostomidae) in the cerrado of Central Brazil. Mem Inst Oswaldo Cruz **106:**170-176.

Almeida-Neto M, Guimarães P, Guimarães Jr. PR, Loyola RD, Ulrich W. 2008. A consistent metric for nestedness analysis in ecological systems: reconciling concept and measurement. Oikos **117:**1227-1239.

Barber MJ. 2007. Modularity and community detection in bipartite networks. Physical Review E **76:**e066102.

Bellay S, Oliveira EF, Almeida-Neto M, Lima Junior DP, Takemoto RM, Luque JL. 2013. Developmental stage of parasites influences the structure of fish-parasite networks. PLoS One **8:**e75710.

Blüthgen N, Menzel F, Blüthgen N. 2006. Measuring specialization in species interaction networks. Ecology **6:**1-12.

Bray JR, Curtis JY. 1957. An ordination of the upland forest communities of Southern Wisconsin. Ecol Monog **27:**325-349.

Brito SV, Corso G, Almeida AM, Ferreira FS, Almeida WO, Anjos LA, Mesquita DO, Vasconcellos A. 2014. Phylogeny and micro-habitats utilized by lizards determine the composition of their endoparasites in the semiarid Caatinga of Northeast Brazil. Parasitol Res **113:**3963-3972.

Dáttilo W, Marquitti F, Guimarães PR, Izzo TJ. 2014. The structure of ant-plant ecological networks: Is abundance enough? Ecology **95:**475-485.

Dick CW, Patterson BD. 2006. Bat ectoparasites: obligate ectoparasites of bats. *In*: Morand S, Krasnov BR, Poulin R (eds) Micromammals and macroparasites, 1st edn. Springer, Japan

Dick CW. 2007. High host specificity of obligate ectoparasites. Ecological Entomology **32:**446-450.

Dittmar K, Porter ML, Murray S, Whiting MF. 2006. Molecular phylogenetic analysis of nycteribiid and streblid bat flies (Diptera: Brachycera, Calyptratae): implications for host associations and phylogeographic origins. Mol Phylogenet Evol **38:**155-170.

Donatti CI, Guimaraes PR, Galetti M, Pizo MA, Marquitti F, Dirzo R. 2011. Analysis of a hyper-diverse seed dispersal network: modularity and underlying mechanisms. Ecol Lett **14:**773-781.

Fleming TH, Muchhala N. 2008. Nectar-feeding bird and bat niches in two worlds: pantropical comparisons of vertebrate pollination systems. J Biogeogr **35:**764-780.

Galbe J, Oliver-Jr JH. 1992. Immune response of lizards and rodents to larval Ixodes scapularis (Acari; Ixodidae). J Med Entomol **29:**774-783.

Gardner AL, Jones JKJ, Carter DC. 1977. Feeding Habits. In: Baker RJ, Jones JKJ, Carter DC (eds) Biology of Bats of the New World - Family Phyllostomidae, 1st edn. Texas Tech University Press, Austin.

Graciolli G, Carvalho CJBD. 2001. Moscas ectoparasitas (Diptera, Hippoboscoidea, Nycteribiidae) de morcegos (Mammalia, Chiroptera) do Estado do Paraná, Brasil. I. Basilia, taxonomia e chave pictórica para as espécies. Rev Bras Zool **18:**33-49.

Horn HS. 1966. Measurement of overlap in comparative ecological studies. The Am Nat **100:**419-424.

Hofstede HM, Fenton MB, Whitaker JJO. 2004. Host and host-site specificity of bat ectoparasites (Diptera: Streblidae and Nycteribiidae) on Neotropical bats (Chiroptera). Can J of Zool **82:**616-626.

Jordano P. 1987. Patterns of mutualistic interactions in pollination and seed dispersal - connectance, dependence asymmetries, and coevolution.The Am Nat **129:**657-677.

Kalka MB, Smith AR, Kalko EK. 2008. Bats limit arthropods and herbivory in a tropical forest. Science **320:**71-71.

Krasnov BR, Fortuna MA, Mouillot D, Khokhlova IS, Shenbrot GI, Poulin R. 2012. Phylogenetic signal in module composition and species connectivity in compartmentalized host-parasite networks. The Am Nat **179:**501-511.

Krishna A, Guimaraes-Jr PR, Jordano P, Bascompte J. 2008. A neutral-niche theory of nestedness in mutualistic networks. Oikos **117:**1609-1618.

Lewinsohn TM, Prado PI, Jordano P, Bascompte J. 2006. Structure in plant animal interaction assemblages. Oikos **113:**174-184.

Lee Y, McCracken GF. 2004. Flight activity and food habits of three species of myotis bats (chiroptera: vespertilionidae) in sympatry. Zool Stud **43:**589-597.

Lima-Jr DP, Giacomini HC, Takemoto RM, Agostinho AA, Bini LM. 2012. Patterns of interactions of a large fish-parasite network in a tropical floodplain. J Anim Ecol **81:**905-913.

Lobova TA, Geiselman CK, Mori SA. 2009. Seed dispersal by bats in the Neotropics. New York Botanical Garden Press, New York.

Marshall AJ. 1982. Ecology of Insects Ectoparasitic on Bats. *In*: Kunz TH (ed) Ecology of Bats 1st edn. Boston University, Boston.

Mello MAR, Marquitti FMD, Guimaraes-Jr PR, Kalko EKV, Jordano P, Aguiar MAM. 2011. The modularity of seed dispersal: differences in structure and robustness between bat-and bird-fruit networks. Oecologia **167:**131-140.

Mucha PJ, Richardson T, Macon K, Porter MA, Onnela JP. 2010. Community structure in time-dependent, multiscale, and multiplex networks. Science **328:**876-878.

Newman ME. 2006. Modularity and community structure in networks. Proc Natl Acad Sci USA **103:**8577-8582.

Nowak-Chmura M, Siuda K, Wegner Z, Piksa K. 2012. Species diversity of ticks (Acari: Ixodida) on migrnat birds on the Baltic Sea Cost of Poland. Zool Stud **51:**1411-1417.

Olesen JM, Bascompte J, Dupont YL, Jordano P. 2007. The modularity of pollination networks. Proc Natl Acad Sci USA **104:**19891-19896.

Pariselle A, Boeger WA, Snoeks J, Bilong CFB, Morand S, Vanhove MPM. 2011. The Monogenean parasite fauna of Cichlids: a potential tool for host biogeography. Int J of Evol Biology **13:**1-16.

Patterson BD, Dick CW, Dittmar K. 2007. Roosting habits of bats affect their parasitism by bat flies (Diptera: Streblidae). Journal of Tropical Ecology **23:**177-189.

Patterson BD, Dick CW, Dittmar K. 2008. Parasitism by bat ectoparasites (Diptera: Streblidae) on neotropical bats: effects of host body size, distribution, and abundance. Parasitol Res **103:**1091-1100.

Patterson BD, Dick CW, Dittman K. 2009. Nested distribution of bat flies (Diptera: Streblidae) on Neotropical bats: artifact and specifity in host parasite studies. Ecography **32:**481-487.

Petersen FT, Meier R, Kutty SN, Wiegmann BM. 2007. The phylogeny and evolution of host choice in the Hippoboscoidea (Diptera) as reconstructed using four molecular markers. Mol Phylogenet Evol **45:**111-122.

Rachwald A, Bradford T, Borowski Z, Racey PA. 2016. Habitat preferences of soprano pipistrelle *Pipistrellus pygmaeus* (Leach, 1825) and common pipistrelle *Pipistrellus pipistrellus* (Schreber, 1774) in two different woodlands in north east Scotland. Zool Stud **55:**22. doi:10.6620/ZS.2016.55-22.

Rezende EL, Albert EM, Fortuna MA, Bascompte J. 2009. Compartments in a marine food web associated with phylogeny, body mass, and habitat structure. Ecol Lett **12:**779-788.

Stang M, Klinkhamer PGL, Meijden E. 2006. Size constraints and flower abundance determine the number of interactions in a plant-flower visitor web. Oikos **112:**111-121.

Thavry H, Cappelle J, Bumrungsri S, Thona L, Furey NM. 2017. The diet of the cave nectar bat (*Eonycteris spelaea* Dobson) suggests it pollinates economically and ecologically significant plants in southern Cambodia. Zool Stud **56:**17. doi:10.6620/ZS.2017.56-17.

Wiens JJ, Ackerly DD, Allen AP, Anacker BL, Buckley LB, Cornell HV. 2013. Niche conservatism as an emerging principle in ecology and conservation biology. Ecol Lett **13:**1310-1324.

Vazquez DP, Poulin R, Krasnov BR, Shenbrot GI. 2005. Species abundance and the distribution of specialization in host-parasite interaction networks. J Anim Ecol **74:**946-955.