

Network and parasitological analyses reveal latitudinal gradient in bats-ectoparasitic flies interactions across the Neotropic

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ABSTRACT

Ecological interactions between parasites and their hosts play a fundamental role in evolutionary processes. Selection pressures are exerted on parasites and their hosts, usually resulting in high levels of specificity. Such

is the case of ectoparasitic bat-flies, but how large-scale spatial gradients affect the dynamics of their interactions with their bat hosts is still unknown. In the present study, we investigated interaction patterns between bats and their ectoparasitic flies (Streblidae and Nycteribiidae), both presenting their peak of diversity in the Neotropical region, along a latitudinal gradient. Using network analyses and parasitic indices, grounded on the latitudinal diversity gradient theory, we evaluated how spatial gradients affect species interactions and parasitic indices at the macroscale level, predicting that interaction networks should become richer in species, leading to increases in network modularity, size, and specialization, and to a decrease in nestedness and connectance. We conducted a literature review, focusing on studies done in the Neotropical region, and data of our own authorship. We obtained a richness of 97 species of bats parasitized by 128 species of ectoparasitic flies, distributed into 57 interaction networks between latitudes 29°S and 19°N in the Neotropic. Network metrics and parasitic indices varied along the latitudinal gradient, with changes in richness of bats and their ectoparasitic flies and in the structure of their interactions; network specialization, modularity and connectance increase with latitude, while network size decreases with latitude. Regions closer to the equator had higher parasite loads. Our results show that interaction networks metrics present a latitudinal gradient and that such interactions, when observed at a local scale, hide variations that only become perceptible at larger scales. In this way, ectoparasites such as bat flies are not only influenced by the ecology and biology of their hosts, but by other environmental factors acting directly on their distribution and survival.

Key words: Biogeography, ecological networks, macroecology, parasitology.

INTRODUCTION

Ecosystems are composed of a wide diversity of ecological interactions at different trophic levels (Ricklefs & Relyea, 2016). Such interactions act on the dynamics and stability of the environments (Xing & Fayle, 2021), promoting biodiversity. The understanding of the time, space, and mode of these interactions is still incipient, despite being fundamental for the understanding of ecological patterns and processes and for the design of truly holistic conservation measures. In this context, ecological network analyses constitute a

fundamental tool for a comprehensive and integrated understanding of ecological systems (Delmas et al., 2019; Guimarães, 2020; Júnior et al., 2020), particularly the structural and functional dynamics of ecological interactions (Olesen et al., 2007; Vázquez et al., 2009). The interaction network approach has been known in the field of ecology since at least the 1940s, with the beginning of classic research on antagonistic relationships (Mello, 2010). However, only recently, has this tool become popular among ecologists (Dáttilo & Rico-Gray, 2018; Pellissier et al., 2018).

The relationship between parasites and their hosts deserves full attention in ecology (Gómez & Nichols, 2013). Indeed, biodiversity is driven and sustained by eco-evolutionary processes (Solé, 2022), in which parasites play a fundamental role. In 1973, the evolutionary biologist Leigh Van Valen formulated the Red Queen Hypothesis, where he postulated that interacting species in ecological networks are in constant co-evolution (Van Valen, 1973; Solé, 2022). Parasites are the commonest life form found on Earth (Poulin & Morand, 2000), and are in a perpetual arms race with their hosts (Papkou et al., 2016), making them key players in maintaining biological diversity. Parasites play fundamental roles at the individual level, acting on the host's immune response, at the population level, for example structuring host population density and affecting survival rates, and at the ecosystem level, acting for instance on nutrient cycles (Ebert et al., 2000; Gómez et al., 2012; Speer et al., 2020).

Among the parasitic systems found in mammals, bats (Chiroptera) and their ectoparasitic flies (Streblidae and Nycteribiidae), known as “bat-flies”, are a model system. This is due, on the one hand, to the diversity of biological and ecological characteristics of the Chiroptera, reflected in its taxonomic and ecomorphological variety, including body size, dietary and foraging strategies, roosting ecology, social systems, and colony size, as well as to the wide geographic distribution of the group (Patterson et al., 2007). On the other hand, the system is a model also because of the evolutionary characteristics of ectoparasites, particularly their high specificity, – about 70 to 90% of bat flies in the Neotropic parasitize a specific species or genus of bat (Marshall, 1982; Dick & Gettinger, 2005; Dick, 2007) –, adenotrophic viviparity and the

presence of winged, brachypterous, and wingless species (Marshall, 1982). Those diverse attributes found in the two groups contribute to different infestation patterns (Hiller et al., 2021).

Understanding the patterns of these associations at the biogeographic scale is a fundamental ecological question, as different features along environmental gradients shape the richness and structure of interactions between species. Especially for bats and their ectoparasitic flies, attributes such as roosting ecology and colony size can be factors structuring these interactions, as roost are crucial for the reproductive success and survival of these bat flies (Urbieto et al., 2022). Unavoidably, bay occupancy is dependent on adequate roost availability in the surrounding environment (Guimarães & Ferreira, 2014), so a macroscale look may reveal modulations in these interactions along the latitudinal gradient.

A trend towards increasing richness and parasite loads for bat flies towards the equator is to be expected, due to the availability of many, and particularly large, caves in the tropical region (Ladle et al., 2012; Vargas-Menos et al., 2020; Barros et al., 2021; Tanalgo et al., 2022). These cavities, when used by bats are known as “bat caves” (Ito et al., 2022). Among these, there are “hot caves”, which are certain underground chambers presenting consistently high temperatures generated by the presence of very large bat colonies, together with specific speleological characteristics (Ladle et al., 2012; Ito et al., 2022). Several bats roosting there have geographic ranges restricted to the tropical region, and are found exclusively or predominantly in these “hot caves”, as is the case of the genus *Pteronotus* in the Neotropic (Ladle et al., 2012; Tanalgo et al., 2022; Ito et al., 2022). The combination of these factors should promote greater diversity of bat flies, fitting the general latitudinal diversity gradient pattern. The latitudinal gradient in species richness, with an increasing number of species towards the equator, is one of the most accepted patterns in ecology (Rohde, 1992; Mittelbach et al., 2007), and is more clearly observed at larger scales than on smaller scales (Mittelbach et al., 2007). In contrast, recent large-scale studies on latitudinal gradients in biotic interactions have shown mixed results, not supporting the idea that specialization is always higher in the tropics (Molles & Ollerton, 2016). Studying such wide-scale associations and comparing interaction networks in different environments along the latitudinal gradient may help us recognize the environmental factors structuring those interactions (Pellissier et

al., 2018; Xing & Fayle, 2021), and how those networks are shaped concomitantly with changes in the richness of species of both parasites and hosts.

The Neotropical region, extending from Central Mexico and the Caribbean Islands to southern South America (Dinerstein et al., 2017), is characterized by its incredible biodiversity (Dinerstein et al., 2017). Here bats present their peak of taxonomic and eco-morphological diversity (Aguirre, 2002; Sampaio et al., 2003). Indeed, at the local level, we may find up to 117 bat species, with most environments harboring over 50 species per 25km² (Fischer et al., 2018; Delgado-Jaramillo et al., 2020). Furthermore, particularly in neotropical rainforests, bats account for about 40% of the mammal species present (Emmons & Feer, 1997; Aguirre, 2002; Sampaio et al., 2003). With no surprise, this is reflected in the richness and diversity of their ectoparasitic flies, which is positively correlated with that of their hosts (Dick & Gettinger, 2005).

Here, we hypothesize that the patterns of interactions between bats and their ectoparasitic flies are shaped by the latitudinal diversity gradient. Specifically, we expect a modification of the structure of the interaction networks between bats and their ectoparasitic flies throughout the Neotropic. As we approach the tropics interaction networks should become richer in species, following the typical latitudinal diversity gradient; as a result, this should lead to an increase in network modularity, size, and specialization, and a decrease in nestedness and connectance. Secondly, we expect greater richness and ectoparasitic load at lower latitudes. This should reflect the greater diversity of bats and the presence of larger colonies at lower latitudes in the Neotropical region (De Oliveira et al., 2018), supported by an apparent high availability of hot caves (Ladle et al., 2012) as reflected by recent findings in northeastern Brazil (e.g. Vargas-Mena et al., 2018).

MATERIALS AND METHODS

Data collection

The present study was based on scientific publications on bats and the ectoparasitic bat-flies of the Streblidae and Nycteribiidae (Supplementary Data 1), as well as on our own data collected between 2016 and 2022 at several sites in the southern Brazilian Atlantic Forest. We conducted a bibliometric search for articles

indexed in the databases: Scopus, Web of Science, ScienceDirect and Scielo, using the keywords “bats” or “Chiroptera”, and “ectoparasite” or “Nycteribiidae” or “Streblidae”, in English. We added the retrieved manuscripts to Mendeley, in which we excluded duplicates (articles indexed in more than one database). After compiling the articles, we selected those including studies done in the Neotropical region, containing information on species richness and abundance for ectoparasites and bats, and latitude data. For those manuscripts where the geographic location was not given in detail, we extracted approximated geographic coordinates using Google Earth. For manuscripts with more than one sampling site, we separated them into different datasets. The data used in the research comprised articles published from 1999 to 2021.

Structuring of interaction networks

For each dataset we built weighted matrices, based on the frequency of occurrence of the interactions between bat-flies and their bat hosts. For each matrix, we plotted the interaction networks, calculating the following indices: i) specialization (H2): depicts the level of exclusivity of the ecological interactions, measuring niche complementarity between species, with values varying from 0 to 1, from the least to the most specialized the network (Blüthgen, 2010); ii) modularity: evaluates the way in which nodes are partitioned into separate subsets within the network as a whole, using the “Beckett” metric (Beckett, 2016; Mello et al., 2016); iii) nestedness (NODF metric): evaluates how closely a matrix comes to being perfectly nested, i.e. the overlap of more specialist species with more generalist species, varying from 0 to 100, respectively from non-nested to fully nested networks (Almeida-Neto et al., 2008); iv) connectance: evaluates the proportion between the interactions found in the network and all the possible interactions (Mello et al., 2016); and v) network size: the number of vertices found in the network (Mello et al., 2016). To calculate the indices and generate the networks, we used the *vegan*, *bipartite*, and *network* packages (Dormann, 2009; Oksanen et al., 2018; Butts, 2020) in the R environment.

Parasitic indices

In parasitology, for each parasite sample, usually, the sampling units are the individual hosts (Reiczigel et al., 2019). Thus, the presence of parasites at the individual or population level creates patterns that may be described by specific parasitic indices describing aspects of the parasitic infections (Reiczigel et al., 2019). Here, for each set of data, i.e., for each sampling location, we used two of the mainly used parasitological indices: i) general prevalence of infestation (P): the proportion of infected hosts in relation to the total set of hosts sampled; and ii) mean infestation intensity (MI): the arithmetic mean of parasites found in a set of infected hosts, excluding parasite-free hosts. The two indices were calculated using the Quantitative Parasitology 1.0.15 environment (QPWeb, Reiczigel et al., 2019).

Evaluating the influence of latitude on network structure, richness, and parasitic indices

To analyze whether there was a difference in the structure of the interaction networks along the latitudinal gradient, we used Generalized Linear Models (GLM). We used the structural network metrics (specialization, modularity, nestedness, connectance, and network size) as response variables and latitude as the predictor variable. We also used network size as a predictor of specialization, modularity, nestedness and connectance, to remove the effect it might have on these structural network metrics. Indeed, some metrics might be sensitive to changes in network size; for example, nestedness tends to increase with larger networks, (Nielsen & Bascompte, 2007). We used multiple regression in the GLM models. For models with response variables in the form of proportions, we used the Beta distribution function, and for count response variables, the Poisson distribution function. We performed residual analysis to evaluate the error distribution. Generalized Linear Models were also used to evaluate whether ectoparasites richness and parasitic indices varied along the latitudinal gradient. After performing all the analyses, we verified the existence of spatial autocorrelation in the residuals. All analyzes were performed in the R environment (R Development Core Team, 2018), using the packages *glmmTMB*, *ncf*, *visreg* and *ggplot2* (Nychka et al., 2002; Wickham, 2016; Brooks et al., 2017; Breheny & Burchett, 2017).

RESULTS

From the literature search, we obtained 49 research papers containing the necessary information for the analyses, from which we generated 57 interaction networks. The datasets were distributed from southern Mexico to southern Brazil, between latitudes 29°S and 19°N (Figure 1 and Supplementary Data 1).



Figure 1. Sampling locations for the 48 studies retrieved in the bibliometric search, from which we generated 57 interaction networks between bats and ectoparasitic bat-flies.

184 We obtained data for 97 bat species, distributed in 43 genera and seven families, and 128 species of bat-
185 flies, 111 species and 20 genera of the Streblidae, and 17 species of the Nycteribiidae, all from the genus *Basil*
186 (Figure 2 - Supplementary Data 2). The bat species with the highest parasite richness was *Glossophaga soricina*
187 (Pallas, 1766), with 28 species of ectoparasites, followed by *Carollia perspicillata* (Linnaeus, 1758), with 22
188 species of bat-flies. Of all the bat species parasitized in this study, 29 were parasitized by solely one species of bat-
189 fly.

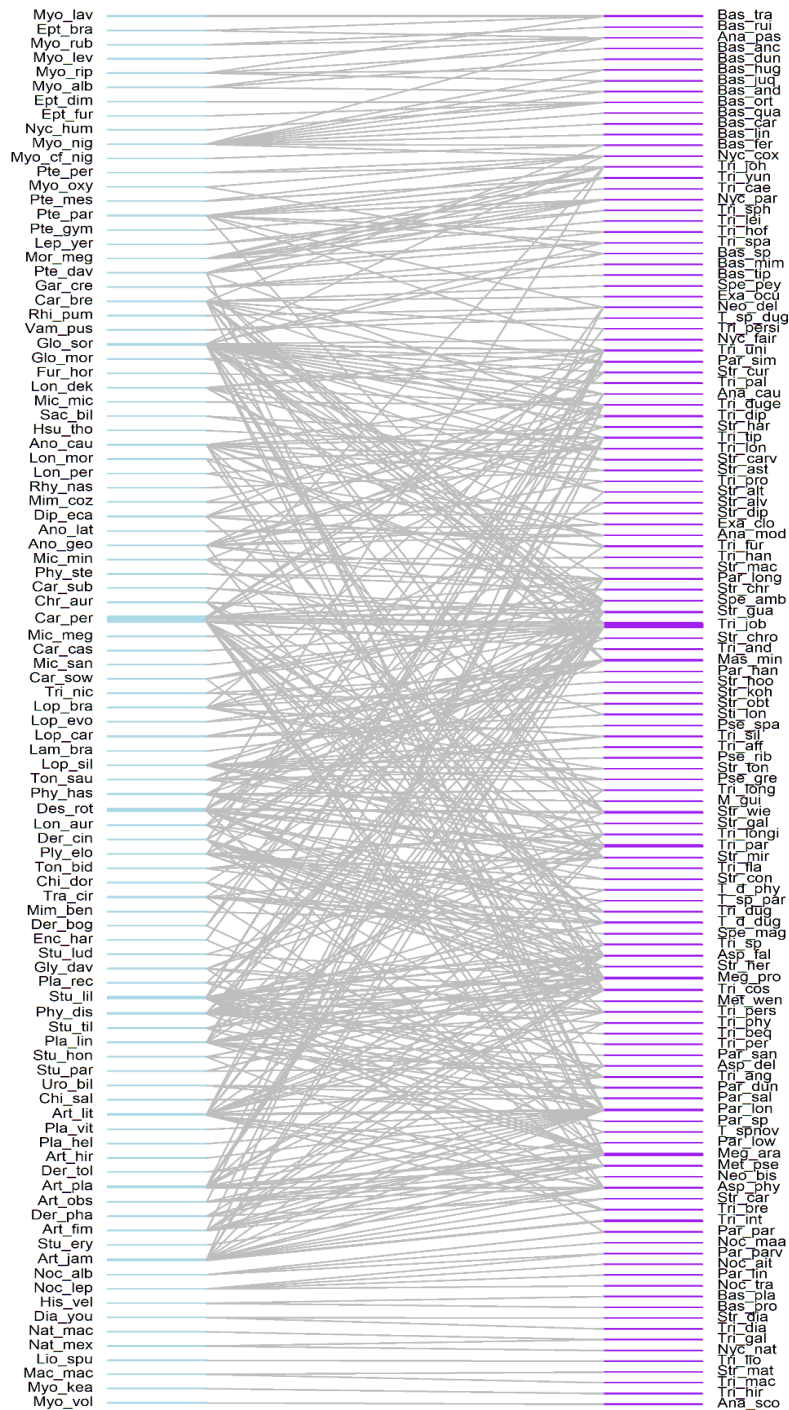


Figure 2. Interaction network with unified data for the entire Neotropical Region, between bats (Chiroptera) and their ectoparasitic flies (Streblidae and Nycteribiidae). Scientific names of both groups in code (Supplementary Data 3).

191 The specialization index (H2) was high throughout the Neotropic, ranging from 0.45 to 1.00, with the
192 vast majority (83%) above 0.80. H2 responded positively to latitude, i.e, as latitude increases, networks tended to
193 be more specialized ($\beta=2.298$; $p=0.025$; $R^2=0.089$ – Figure 3). Modularity ranged from 0.28 to 0.88, responding
194 positively to the size of the network ($\beta=4.860$; $p<0.001$; $R^2=0.296$ – Figure 3). Nestedness and connectance were
195 generally low, ranging from 0.00 to 23.34 and 0.05 to 0.33, respectively. Connectance responded negatively to the
196 size of the network ($\beta=-8.889$; $p<0.001$; $R^2=0.677$ – Figure 3). Network size ranged from 8 to 67, also responding
197 negatively to latitude ($\beta=-7.253$; $p<0.001$; $R^2=0.154$ – Figure 4a).

198 The richness of ectoparasites responded positively to the richness of their hosts ($F=30.21$; $p<0.001$;
199 $R^2=53.27$ – Figure 5a), which indirectly influenced the opposite response for latitude (Figure 5b). Prevalence
200 ranged from 20.12 to 84.30%, but did not respond to latitude, despite the mean infestation intensity has shown a
201 negative relation with latitude ($F=11.84$; $p=0.001$; $R^2=0.224$), ranging from 0.92 to 6.12 (Figure 4b).

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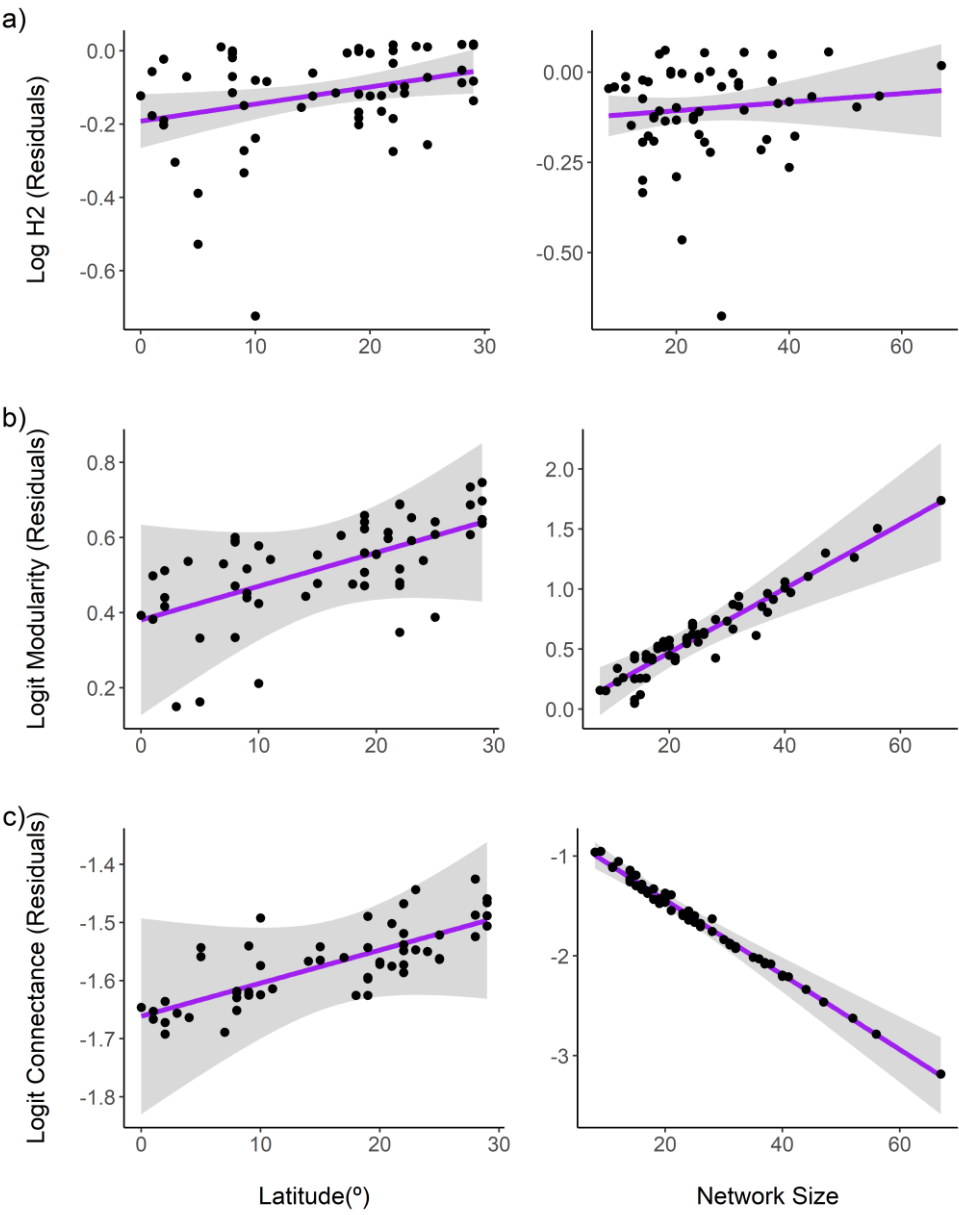


Figure 3. Variation of a) specialization (H2), b) modularity, and c) connectance of networks between bats and their ectoparasitic flies in the Neotropical region in response to latitude and network size.

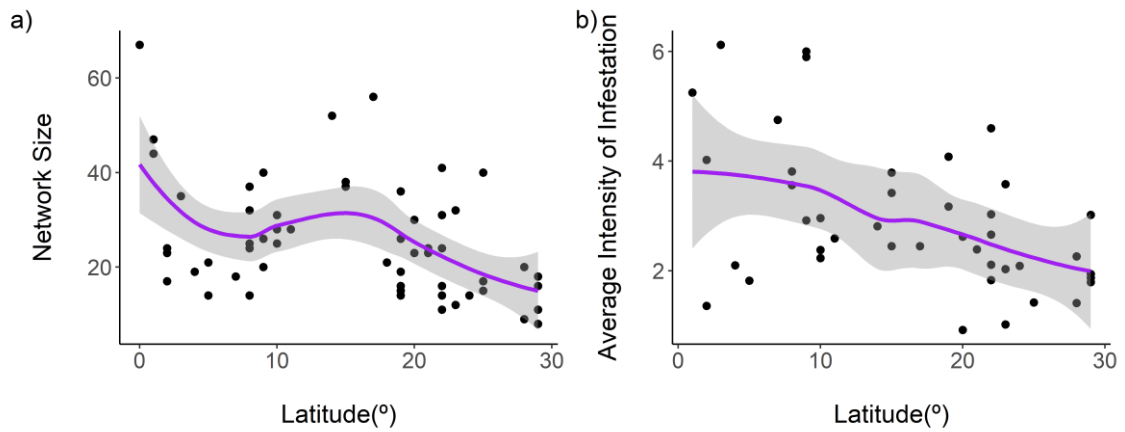


Figure 4. Variation in the size of the networks between bats and their ectoparasitic flies with latitude (a) and variation in the average intensity of infestation with latitude (b), in the Neotropical region.

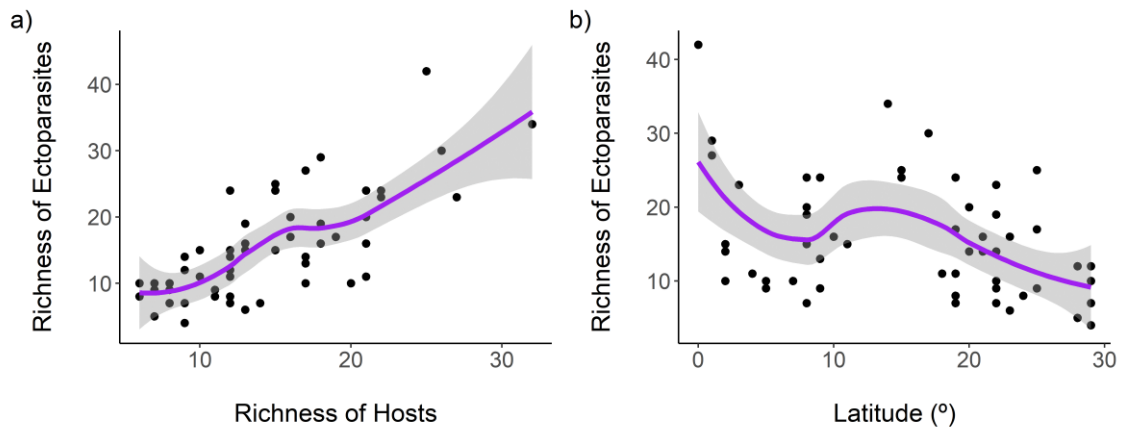


Figure 5. Variation in ectoparasite richness in relation to host richness (a) and latitudinal gradient (b), along the Neotropical region

DISCUSSION

Here we present the first evaluation of the structuring of the interaction networks between bats and their ectoparasitic flies, as well as of parasitic indices, at a macroscale along the Neotropical region. Although the number of studies on interactions between bats and their ectoparasitic flies has increased in recent years, the vast majority are still carried out at local or regional scales (e.g. Fagundes et al., 2017; Salinas-Ramos et al., 2018; Hiller et al., 2020; Trujillo-Pahua & Ibáñez-Bernal, 2020; Tlapaya-Romero et al., 2021; Biz et al., 2021). Fewer

221 studies have provided data gathered on larger scales (but see Eriksson et al., 2019; Saldaña- Vázquez et al., 2019;
222 Júnior et al., 2020).

223 Interaction patterns at the local scale (Fagundes et al., 2017; Durán et al., 2019; Hiller et al., 2021) may
224 hide variations in the relationship between species, but when observed at a larger scale may reveal modulations, as
225 we detected here. Knowing that parasites are highly specific and that their distribution is limited not only by the
226 presence of their hosts but also by other environmental variables (Marshall, 1982), the latitudinal variation in the
227 structuring of the ecological networks was expected. Indeed, not only the type of interaction affects network
228 structure, but also those environmental factors of which latitude is only a proxy, such as temperature and humidity
229 (Taplaya-Romero et al., 2021), as they vary on large scales, but remain reasonably stable on smaller scales (Alvares
230 et al., 2013).

231 The latitudinal diversity gradient postulates that the closer to the tropics, the greater the biological
232 diversity (Allen & Gillooly, 2006). This general rule has exceptions in some groups (mostly known in some plant
233 and bird families) but is marked in bats and their ectoparasitic flies (Ramos Pereira & Palmeirim, 2013; Durán et
234 al., 2017; Biz et al., 2021). Indeed, we detected an indirect effect of latitude on network metrics essentially because
235 latitude influences network size. Sites with greater species richness should harbor a greater number of ecological
236 interactions, with the consequent increase in the size of the networks and, consequently, larger networks, i.e, those
237 with a higher number of species, should be found at lower latitudes. Despite the increase in species richness toward
238 the tropics, specialization remained high in lower latitudes, though slightly (but significantly) greater in higher
239 latitudes. Such suggests that the traditionally known pattern in ecology regarding the latitudinal gradient in
240 specialization – species tend to be more specialized in the tropics – is not as a rule, and the results can be mixed
241 (Moles & Ollerton, 2016). Meta-analysis studies have concluded that there is not much evidence for a general
242 effect of latitude on niche breadth, and that biotic interactions are not always more specialized at lower latitudes
243 (Vázquez & Stevens, 2004). This pattern in specialization possibly also results from the extremely high specificity
244 (Marshall, 1982) shown by bat flies of the Streblidae and Nycteribiidae. In those families 87% of the species are
245 monoxenic, parasitizing a single bat species (Dick, 2007), and a few are found on congener hosts or on a second,

246 non-primary, host (Dick, 2007). At lower latitudes, there is overlap in the geographic distribution of host species
247 of oligoxenous and polyxenous flies, which associate with congener and confamilial hosts (Graciolli & Dick, 2004;
248 Dick, 2007; Taylor & Tuttle, 2019), while at higher latitudes there is a lower richness of hosts (Ramos Pereira &
249 Palmeirim, 2013), making the interactions between ectoparasites and bats somewhat more restricted, that is,
250 specialized.

251 The behavioral and biological attributes of bats and bat-flies may influence the intensity of parasitic loads.
252 Bat species aggregating in colonies with many individuals in more stable roosts tend to harbor a greater number
253 and richness of ectoparasites (Patterson et al., 2007). The best explanation for this pattern is related to the
254 reproductive biology of bat flies. The two families of bat-flies present adenotrophic viviparity (Hagan, 1951), in
255 which the female must leave the host to deposit a single third instar larva on the substrate of the roost (Marshall,
256 1982). Subsequently, this larva takes around 28 days to hatch into an adult fly, and then search for a potential host
257 (Marshall, 1970). Thus, ectoparasite survival increases the longer the hosts stay in the roosts. Still, bat roosting
258 ecology is extremely varied (Kunz & Lumsden, 2003), from species consistently forming colonies of thousands of
259 individuals in permanent roosts, particularly caves, to species that roost alone or form small groups of a few
260 individuals in ephemeral shelters, such as foliage (Kunz & Lumsden, 2003). Other species of bats show significant
261 plasticity in the choice of roosts and in the solitary-gregarious gradient, shaping roost occupancy patterns according
262 to roost and food availability in a given area (Kunz & Lumsden, 2003).

263 The ecological diversity of their hosts, associated with environmental changes, including in the landscape
264 or climate, may lead to observable changes in the reproductive success of ectoparasitic bat-flies (Reckardt & Kerth,
265 2007; Tlapaya-Romero et al., 2021). Indeed, higher temperatures result in shorter incubation times for other groups
266 of bat ectoparasites, increasing reproduction rates (Tlapaya-Romero et al., 2021). The higher infestation intensity
267 we found at lower latitudes may be explained by higher average temperatures along the year and by the more
268 predictable regional climates overall. Larger, more stable, and permanent roosts are possibly also more available
269 here (Guimarães & Ferreira, 2014; Cecav, 2020), and these can harbor higher host richness (Barros et al., 2021).
270 In tropical regions, the formation of bat colonies with thousands of individuals is notable, especially in those

271 species using underground roosts obligatorily or frequently. In the Neotropic, only *Tadarida brasiliensis* presents
272 records of large colonies beyond the tropical region (Vargas-Mena et al., 2018; Boero et al., 2020). De Oliveira
273 (2018) observed greater number of individuals in the Brazilian savanna, when compared to the Atlantic Forest,
274 which extends to higher latitudes. Considering that the epidemiological modeling theory postulates that the
275 transmission of parasites between hosts is influenced by population density (Poulin, 2004), the larger the host
276 colony, the greater the probability of infection. A corollary of this theory is that parasite abundance will respond
277 positively to host density (Arneberg et al., 1998). In conclusion, environmental changes along the latitudinal
278 gradient will directly influence host abundance and, subsequently, also the parasitic infestation patterns.

279 Interaction networks indirectly respond to latitude in a pattern that is only perceptible on a large scale, a
280 finding revealing the need for further macroecological studies to understand the factors behind the structure of
281 those ecological interactions. Future studies on interaction patterns between bats and their ectoparasitic flies should
282 consider the roosting ecology of the hosts and the latitudinal variation of environmental variables such as
283 landscape, climate, and roost permanence and availability.

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