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Orientador: Frederico de Siqueira Neves

Coorientador: Flávio de Carvalho Camarota

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Resumo

Os distintos tipos de interações mutualistas, como polinização, dispersão de sementes e mutualismos formiga-planta, vêm sendo examinados sob uma perspectiva de redes de interações. Dentre os aspectos abordados, destaca-se a “β-diversidade de interações”, métrica que mensura a dissimilaridade espaço-temporal de interações entre espécies. Estudos sobre as interações entre formigas e plantas apontam que a β-diversidade de interações entre comunidades aumenta com a distância geografia e que o rearranjo nas interações de espécies compartilhadas entre comunidades é o seu principal componente. As formigas, todavia, estão envolvidas com vários outros parceiros mutualistas, incluindo os insetos trofobiontes. Entretanto, apesar da relevância, pouco se conhece sobre como esse tipo de interação mutualística varia espacialmente. Nesse sentido, nós exploramos as dissimilaridades das interações mutualistas entre formigas e insetos trofobiontes em diferentes escalas espaciais e ao longo da distância geográfica em comunidades sob condições abióticas e bióticas similares. Avaliamos também a contribuição dos componentes ‘substituição de espécies’ e ‘rearranjo das interações’ da β-diversidade de interações. Nós verificamos que a β-diversidade de interações formiga-trofobionte é maior na escala regional e aumenta com a distância geográfica entre duas comunidades. O componente ‘substituição de espécies’ se mostrou mais importante para a β-diversidade de interações. Sugerimos que a fidelidade que resulta do comportamento de dominância de algumas espécies formigas sobre os trofobiontes que oferecem recursos mais qualificados levam a um baixo rearranjo de interações entre espécies compartilhadas por duas comunidades. No entanto, muitas espécies não são compartilhadas entre as comunidades, sobretudo com o aumento da distância entre elas. Isso se deve a presença de espécies de formigas subdominantes e trofobiontes menos agregadores, que geralmente são expelidos e apresentam alta rotatividade, gerando comunidades relativamente únicas no espaço já na escala local. Não obstante, esses processos parecem agir de forma secundária escala regional, impulsionados possivelmente por processos históricos, como dispersão limitada e especiação. Demos um importante primeiro passo para a compreensão do papel das escalas espaciais e da distância geográfica sobre os mutualismos de proteção. Mostramos que as interações formiga-trofobionte, ocorrendo em condições abióticas e bióticas semelhantes, mudaram com a distância geográfica e com a escala espacial, principalmente devido a troca na composição das espécies.

Palavras-chave: β-diversidade de interações, Dissimilaridade espacial, Formigas, Mutualismo, Trofobiose.

Abstract

The different types of mutualistic interactions, such as pollination, seed dispersal, and ant-plant mutualisms, have been examined from the perspective of interaction networks. Among the aspects addressed, the "β-diversity of interactions" stands out, which is a metric that measures the spatio-temporal dissimilarity of interactions between species. Studies on the interactions between ants and plants point out that the β-diversity of interactions between communities increases with geographic distance and that the rewiring in interactions of shared species between communities is its main component. Ants, however, are involved with several other mutualistic partners, including trophobiont insects. However, despite the relevance, little is known about how this type of mutualistic interaction varies spatially. In this sense, we explore the dissimilarities of mutualistic interactions between ants and trophobiont insects at different spatial scales and along with the geographical distance in communities under similar abiotic and biotic conditions. We also assessed the contribution of the 'species turnover' and 'interactions rewiring' components of β-diversity of interactions. We found that the β-diversity of ant-trophobiont interactions is greater at the regional scale and increases with the geographical distance between two communities. The 'species turnover' component proved to be more important for the β-diversity of interactions. We suggest that the fidelity that results from the dominant behavior of some ant species over the trophobionts that offer more qualified resources leads to a low rewiring of interactions between shared species by two communities. However, many species are not shared between communities, especially with the increasing distance between them. This is due to the presence of sub-dominant species of ants and less aggregating trophobionts, which are usually expelled and present high turnover, generating relatively unique communities in space already on the local scale. Nevertheless, these processes seem to act in a secondary way on a regional scale, potentially driven by historical processes, such as limited dispersion and speciation. We took an important first step towards understanding the role of spatial scales and geographic distance on protection mutualisms. We show that the ant-trophobiont interactions, occurring in similar abiotic and biotic conditions, changed with the geographical distance and the spatial scale, mainly due to the change in species composition.

Keywords: Ants, β-diversity of interactions, Mutualism, Spatial dissimilarity, Trophobiosis.

Sumário

Figuras e tabelas

Manuscrito elaborado nas normas da Nordic Society Oikos (adaptado para dissertação)

Geographical distance increases ant-trophobiont interaction dissimilarities by the turnover of species composition

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Introduction

Mutualistic interactions are widespread in nature, and not surprisingly, species mutual benefits and their evolutionary consequences have been extensively studied in ecological and evolutionary research (Rodriguez et al. 2017, Parmentier et al. 2020, Purificação et al. 2020). Studies of mutualisms between species are increasingly focusing on the understanding of mutualistic interaction properties of entire communities, rather than just a pair of few interacting species (Donatti et al. 2011, Maruyama et al. 2014, Lomáscolo et al. 2019). The ecological network approach is particularly useful to effectively assess and explore the mutualistic interaction structure of communities (de Andreazzi et al. 2020, Pizo et al. 2020, Peralta et al. 2020). In mutualistic networks, single species are represented as 'nodes' interconnected by 'links', which are the interactions (Bascompte 2009). Notably, such networks allow the visualization of interactions even in highly diverse communities, enabling the quantification and comparison of patterns across communities, including multiple spatial scales (Valdovinos 2019).

Advances in the knowledge of species-rich mutualistic networks are notable, especially in those involving pollination, seed dispersal, and ant-plant mutualisms (Dáttilo et al. 2013, Carstensen et al. 2014, Costa et al. 2018). A recent approach focuses on interaction dissimilarity in networks (β-diversity of interactions) across space and time (Poisot et al. 2012, 2015). This approach is extremely valuable to understand the role of spatial variation on networks, leading to the understanding of how changes in interacting species modulate the dynamics of ecological networks. The β-diversity of interactions has two additive components: species turnover and interactions rewiring (Poisot et al. 2012). When there is a prevalence of spatial species turnover, it implies that interactions are lost or gained as individuals of a species are present or absent in different locations. A stronger presence of interaction rewiring means that interactions are often spatially reassembled due to changes in the identity of the interactions between species. The study of β-diversity of interactions may provide key insights on the role of interactions on communities' assembly (Poisot et al. 2012, Carstensen et al. 2014, Luna et al. 2020). Local processes acting at an ecological time, such as niche adaptations, may depend on how the regional pool of species was shaped by evolutionary processes, such as species dispersion and geographic dynamics (Huston 1999, Suzuki and Economo 2021).

Despite the great importance of studies of the β-diversity of mutualistic interactions, there is still a paucity of empirical studies (Poisot et al. 2015, Burkle et al. 2016). The few studies assessing the β-diversity of interactions performed so far are based on ant-plant mutualistic interactions (CaraDonna et al. 2017) and arboreal-nesting ants and their host trees (Dáttilo and Vasconcelos 2019). These studies reached a similar conclusion that the β-diversity of interaction variation was mainly due to the rewiring of shared species associations (CaraDonna et al. 2017, Dáttilo and Vasconcelos 2019). A rewiring of species interactions is reasonable in the studied interactions, since either plant-pollinator and nesting ants and their hosting trees interactions are marked by the lack of fidelity of the involved partners (Petanidou et al. 2008, Alarcón et al. 2008, Camarota et al. 2015, 2016). Thus, there is no particular reason to expect an interaction to be kept at different spatial scales. However, it is well known that mutualistic interactions range from loose to obligatory interactions (Boucher et al. 1982). Therefore, there is a strong need to expand the studies of β-diversity of interaction networks to other mutualistic associations to better understand how ecological interactions change among communities. We argue that a more embracing approach is timely, as a better understanding of the spatial dissimilarities of ecological interactions is essential for basic knowledge, biodiversity maintenance, and conservation ecology (Burgos et al. 2007, Krishna et al. 2008).

Ants are involved in a plethora of mutualistic interactions with various partners, including plants and other insects (Rico-Gray and Oliveira 2007). Most studies of ant-related mutualism focus on ants and its associations with plants since they are ubiquitous in tropical ecosystems (Bronstein 1998), although the spatial variation of these interactions remains poorly evaluated. Among ant-plant mutualisms, there is a strong bias towards studies over protective interactions, such as ants and extrafloral nectaries (EFNs) bearing plants, in which ants offer protection in exchange for sugar-rich liquid food rewards (Del-Claro et al. 2018). Another kind of protective mutualism involves ants and trophobiont insects, in which ants protect sap-sucking hemipterans and harvest the honeydew from these insects (Delabie 2001). While interactions between ants and trophobiont insects are remarkably similar to the interactions between ants and EFNs (Rico-Gray and Oliveira 2007), there are contrasting differences that should be considered. The first difference regards resource distribution on a plant individual: while EFNs are scattered resources, spread in small portions all over the plant, the honeydew is produced by aggregated patches of insects, presenting a large and continuous flux of resources (Heil et al. 2000, Rico-Gray and Oliveira 2007). Second, honeydew is often more nutritionally valuable than extrafloral nectar, presenting higher concentrations of sugar and amino acids (Blüthgen and Fiedler 2004). The higher availability, stability and value of honeydew can ignite intense levels of interspecific competition among ants (Blüthgen and Fiedler 2004). Indeed, a recent comparison showed that ant-trophobiont networks have more compartmentalized and specialized interactions than those between ants and EFN-bearing plants (Fagundes et al. 2016). The differences in key properties even between similar mutualistic interactions indicate how challenging it is to infer general rules over the spatial variation of interacting communities. Therefore, incorporating studies of ant-trophobiont interactions would be essential to expand our knowledge of ant mutualisms, including their variation across multiple spatial scales.

To fulfill this gap, this study explored the dissimilarities in mutualistic interactions between trophobiont insects and their tending ants among spatial scales through geographic distance. We asked the following questions: (a) The interactions' dissimilarities between trophobiont insects and their tending ants change across geographical distance, considering distinct spatial scales? (b) Which component, species turnover or interaction rewiring, contributes the most to the spatial variation in β-diversity of interaction of the networks? We hypothesized that interactions between ants and trophobionts would be more dissimilar at a regional scale than at a local scale, with dissimilarities increasing across geographical distance. Hence, we expected that the β-diversity of interactions would increase with geographical distance and would be greater between communities located in different regions. Also, we expected that the contribution of the species turnover component for β-diversity would increase with geographical distance. The chances of species composition turnover increase with geographical distance, which plausibly enhance the gain or loss of interactions. We suggest interaction rewiring component to be secondary and constant along geographical distance due to the high fidelity and specialization of the partners involved in ant-trophobiont interactions (Blüthgen and Fiedler 2004, Fagundes et al. 2016).

Materials and Methods

Study area

We conducted this study on three regions located in Minas Gerais state, southwest of Brazil, during the rainy season (February and March of 2018). The northernmost area, considering our entire study area, is in Environmental Protection Area (EPA) Morro da Pedreira (19°17'S,43°53'W), in Santana do Riacho, the southernmost is in EPA São José (21°05'S,44°09'W), in São João Del Rei and the area between them is in the Natural Monument of Serra da Moeda (20°17'S,43°57'W), in Moeda. We will refer to them from now as MP, SJ, and MO, respectively. The climate in this region was classified as an altitudinal subtropical climate (Cwb) (Alvares et al. 2013), with mean temperature ranging from 17°C and 20°C and pluviometric indexes that exceed 1,300 mm per year. The areas are composed of *Campo rupestre* vegetation. It presents a continuum of herbaceous stratum and evergreen bushes. It emerges above 900 m of elevation. The *Campo rupestre* is considered an old climatically buffered infertile landscape (OCBIL) (Silveira et al. 2016, Morellato and Silveira 2018), presenting a very nutrient-impoverished and shallow Proterozoic quartzites soil (Oliveira et al. 2015) and high saturation of aluminum (Negreiros et al. 2012). All regions belong to the Biosphere Reserve of Serra do Espinhaço, recognized by UNESCO, which uplifted around 1.8 billion years ago.

Data collection

We selected three areas aligned in a north-south direction distant from each other at least 100 km. MP area is 200 km far from SJ and 100 km from MO, being SJ and MO distant 100 km from each other. Within each region, we selected three sites distant from each other at least 1 km, containing ten hostplants each. The hostplants are distant from each other at least 10 m (see Fig. 1).

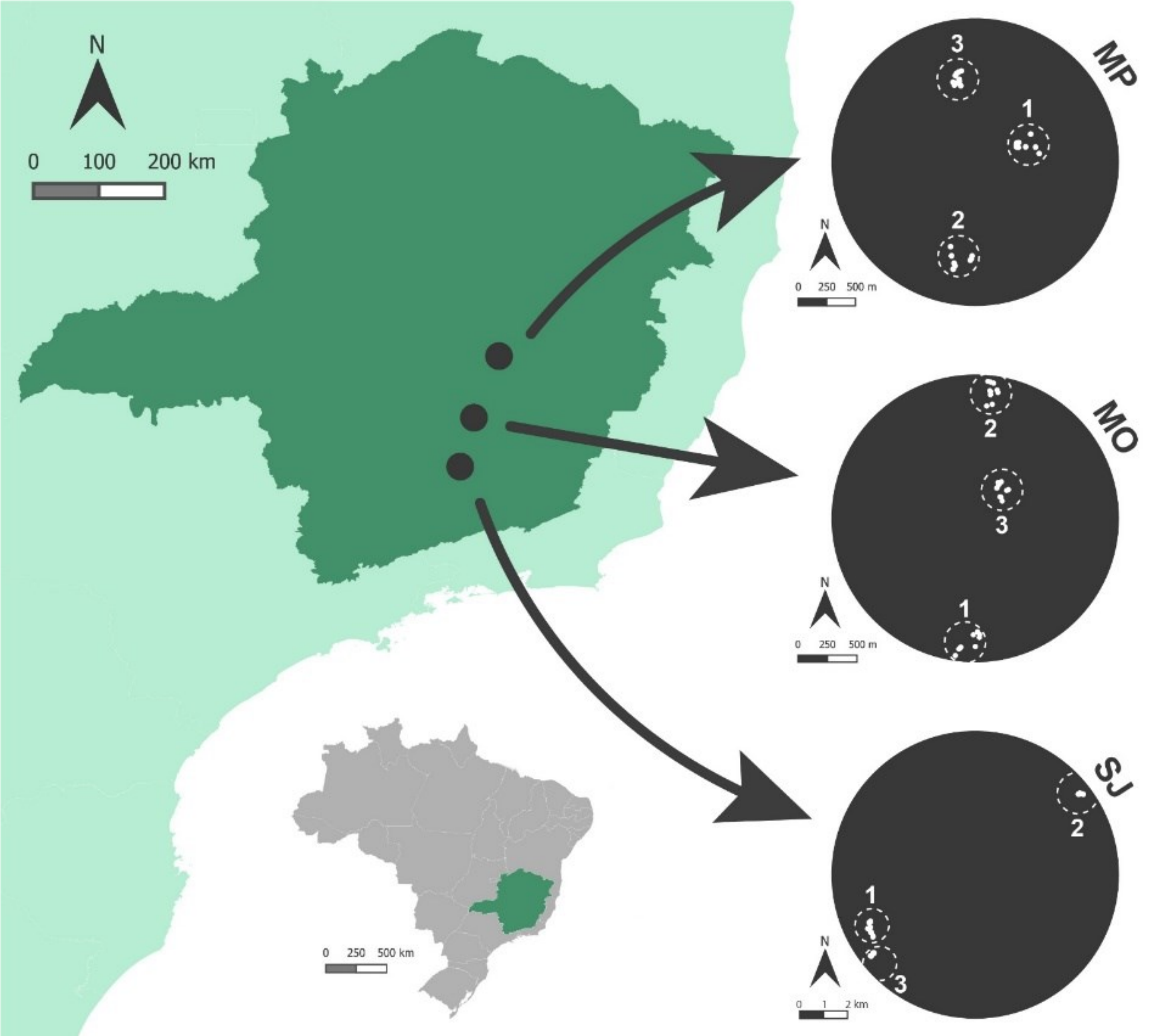

Figure 1 – Schematic map showing the geographical locations of sampling sites in Minas Gerais state. Each dark gray circle represents a region, whereas each white dashed circle represents a local community. White dots are each tree sampled. MP = Environmental Protection Area Morro da Pedreira; MO = Natural Monument of Serra da Moeda; and SJ = Environmental Protection Area São José.

We have only sampled in areas with the same vegetation structure, the *campo rupestre*. Although presenting the same vegetation structure, the sampled areas are isolated rocky outcrops within the Espinhaço mountain range. We also standardized the elevation wherein we collected our data (between 1,000 and 1,300m a.s.l. and the plant system where the interactions occurred. We selected the mistletoe *Psittacanthus robustus* Mart. (Loranthaceae) as hostplant for interactions occurrence, always associated with their main host tree *Vochysia thyrsoidea* (Vochysiaceae). *P. robustus* perform photosynthesis and depends physiologically on its host just for water/soil nutrients acquisition. Due to its poor stomatal control, it presents a high transpiration rate, maintaining a high flux of sap within its vessels (Barão 2015). This species occurs parasitizing its primary host tree, *V. thyrsoidea* Pohl. (Vochysiaceae) over the southern Espinhaço range (Teodoro et al. 2010, 2013, Guerra et al. 2018). Thus, we collected data just in *P. robustus* parasitizing *V. thyrsoidea*. We used the beating technique and an entomological umbrella to collect the insects (Campos et al. 2006, Neves et al. 2010). We choose arbitrarily three *P. robustus* branches to apply the beating technique. This technique consists of knocking each branch 10 times. All insects that fall into the umbrella are conducted to a funnel-like path and stored in subjacent plastic bags Samples were taken to the lab, where ants and trophobiont insects were sorted and identified. We used our field observations (5 min in each plant before applying the beating technique) and the review by Delabie (2001) to classify insects as trophobionts and determine where interactions happened. We sampled each plant once, between 8 a.m. - 11 a.m. and between 14 p.m. - 17 p.m.

Data processing

We considered each sampling site as independent communities to build each matrix A, where Aij = number of times in which the ant species j was found in association with the trophobiont species i within the site (Fagundes et al. 2016). To avoid overestimating both ants and trophobionts, we counted only how many times species of both trophic levels coexist in the same hostplant, in a total of 10 hostplants per site. We, thus, summed the number of times the species coexist to reach interaction frequencies. In other words, ant-trophobiont interaction frequency between two species would vary from 0 to 10 in each network.

To answer our questions, we calculated the β-diversity of interactions (βwn) and its components spatial turnover of species composition (βst) and the interaction rewiring (βos) among networks of all sampling sites. Here, we used presence/absence of interactions to clearly distinguish 'species turnover' from 'no species turnover'. The values were obtained using the R package bipartite function betalinkr_multi, configured with "commondenom" partitioning method (Novotny 2009, Legendre 2014). Thus, we built three matrices with values of: (a) βwn (b) βst, and (c) βos between all networks, following a framework proposed by Poisot et al (2012). There was a total of 36 pairwise values in each matrix. The sum of both βst and βos results is the total interaction dissimilarity, or βwn (Poisot et al. 2012). We built a geographical distance matrix between each ant-trophobiont network using the haversine formula to find the real distances between two points in a spherical world (not flat) using latitude and longitude values. We considered as a local scale the comparisons between networks within each site. As they are approximately 1 km from each other, it is possible to have a dispersion of the organisms in an ecological time (Huston 1999, Oliver et al. 2008, Chave 2013, Belmaker et al. 2015). The regional scale was considered when a pair of compared networks were situated in different sites (*e.g.* a network at MP versus a network at MO or a network at SJ).

Statistical analysis

To test the β-diversity of interactions *versus* geographical distance correlation on pairwise networks, we performed Mantel tests. We fitted a separated model for the independent variables βwn, βst, and βos using geographical distance as the predictor variable. Mantel test is a permutation test for similarity of two matrices, here the total β-diversity of interactions and its components between pairs of sites as a function of geographical distance (Smouse et al. 1986). We used "mantel.test" R function, which permutes the rows and columns of the second matrix in a random way and compares the permuted distribution with the Z-statistic of observed data.

To test if the β-diversity of interactions and its components change depending on the sampling scale, we fitted general linear models (GLM's) (Nelder and Wedderburn 1972). The total β-diversity of interactions and its components were set as response variables and the scale (Local and Regional) as the explanatory variable. We adjusted the models' residual distributions for the Gaussian family and evaluated the dispersion parameters using graphical analysis through Q-Q plot and dividing the models' residual deviance by the degrees of freedom.

We complementarily built a Non-metric multidimensional scaling (NMDS) (Kenkel and Orloci 1986) and analyzed with an Analysis of similarities (ANOSIM) (Clarke 1993) to test whether there is a significant difference in the ants and trophobiont species composition among study sites and regions. All analysis were conducted in R Core Team version 3.6.2 (2019).

Results

We recorded 189 interactions between 22 ant species and 11 trophobiont species (Fig. 2). In local networks, we recorded from 12 to 45 interactions (mean ± SE = 20.11± 3.38) comprising from three to 11 ant species (6.22 ± 0.83) and from three to eight trophobiont species (4.78 ± 0.52) (Fig. 3). The overall β-diversity of interactions on pairs of communities was high, varying from 0.48 to 1. The high β-diversity of interactions was mainly due to a spatial turnover of species composition (βst) component, which varied from 0.32 to 1, whilst the interaction rewiring (βos), showed to be secondary, varying from 0 to 0.37 (Table 1).

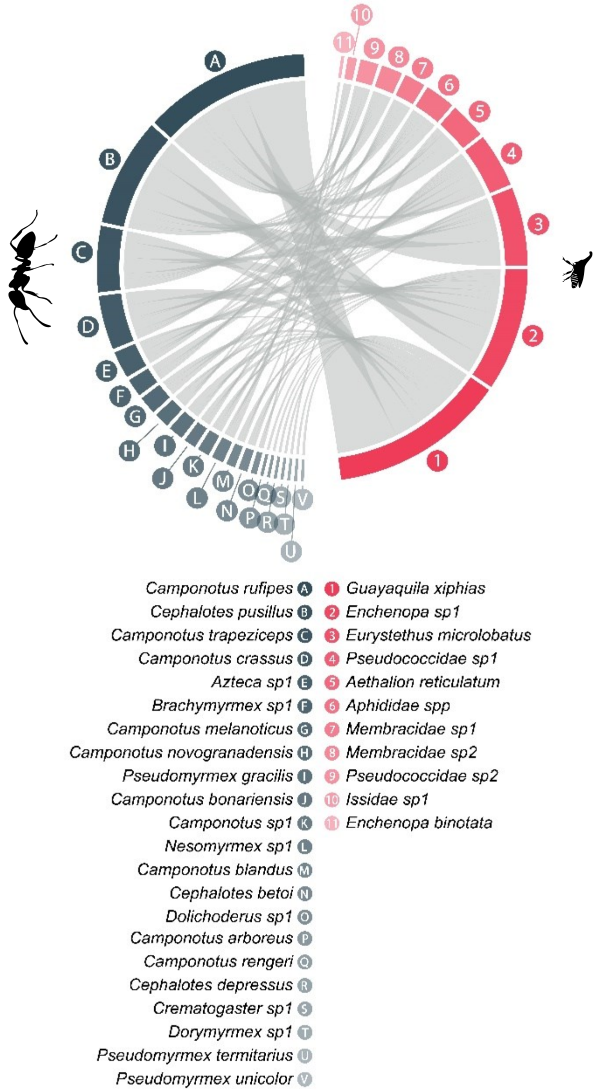

Figure 2 – Representation of all ant (dark blue) and trophobiont (red) species interactions in this study (a *Metaweb*). Concentric bars represent one species, and its width represents the sum of that species interactions. Gray links represent the interactions between ants and trophobionts species.

Figure 3 – Ant-trophobiont local networks. Acronyms represent each of the studied regions: Environmental Protection Area Morro da Pedreira (MP), EPA São José (SJ), and Natural Monument of Serra da Moeda (MO). The numbers from 1 to 3 represent the local sampled sites.

Table 1 – Mean value and standard error found for *Metaweb*, Local, and Regional scale β-diversity of interactions (βwn) and its components, Spatial species turnover (βst) and Interaction rewiring (βos). Values were rounded for 2 decimal places.

	Metaweb		**Local Scale**		**Regional scale**	
Variable	Mean	SE	Mean	SE	Mean	SE
βwn	0.83	0.02	0.75	0.07	0.85	0.02
βst	0.71	0.02	0.62	0.06	0.74	0.02
βos	0.12	0.02	0.12	0.03	0.12	0.02

We found a positive correlation between the geographical distance between two networks and the β-diversity of interactions (Fig. 4A, z = 3278.551, p = 0.01). We observed the same for the spatial species turnover component (Fig. 5A, z = 2807.116, p = 0.04), but not for the interaction rewiring component (Fig. 5A– Table 2), which indicates that the dissimilarities in ant-trophobiont interactions happened mostly due to changes in species composition and not due to rewiring of interactions between shared species. Both ant and trophobiont local communities are compositionally more similar to each other than between other regional communities (Supplementary materials – Fig. 1).

The β-diversity of interactions was greater between networks located in different regions (regional scale) (0.85 ± 0.02) than between those located within the same region (local scale) (0.75 ± 0.07) (Fig. 4B, Table 2, $F_{(1,34)}$ = 5.40, p = 0.03), although both values still high. We also found a greater spatial species turnover component of β-diversity of interactions between networks located in different regions (0.74 ± 0.02) than between those located within the same region (0.62 ± 0.06) (Fig. 5B, $F_{(1,34)}$ = 5.33 , p = 0.03). There was no difference in interaction rewiring among spatial scales (Fig. 5B, Table 2).

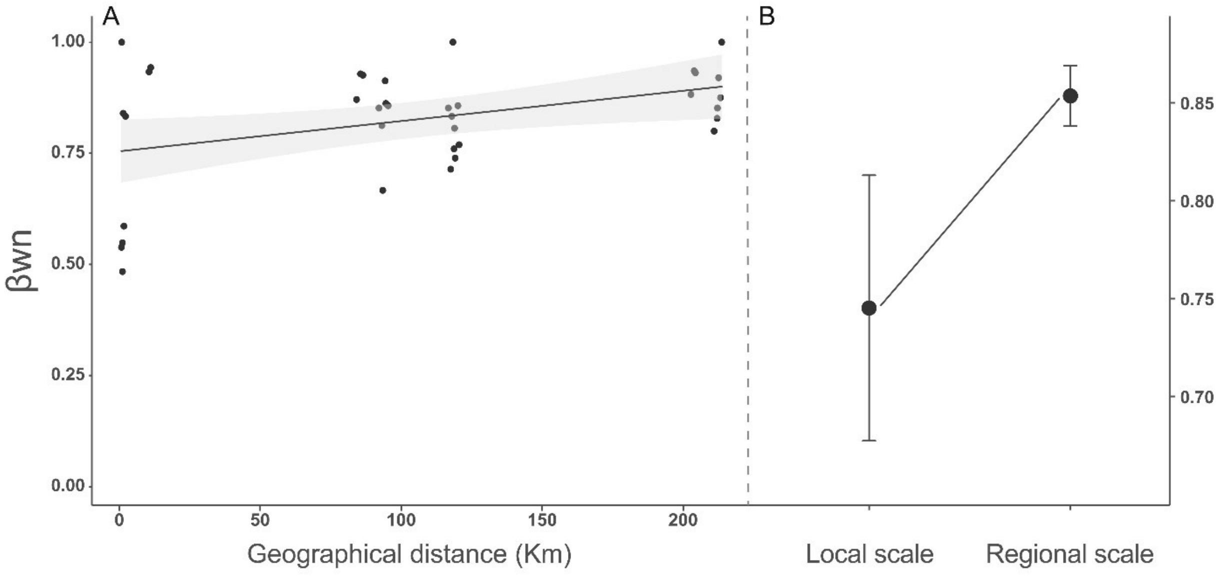

Figure 4- (A) Effect of geographical distance on β-diversity of interactions (βwn). Each black dot represents the βwn between a pair of networks. The light grey shadow around the line represents the confidence interval of 95%. Notice that there are more dots around 100 km than around 1km and 200 km due to comparisons between local networks located in the two most distant regions with those present in the most central region. (B) The βwn values dispersion depending on their spatial scale class (local or regional) were represented by boxplots. The dark gray lines in boxplots represent the means. Asterisks signalize significant results.

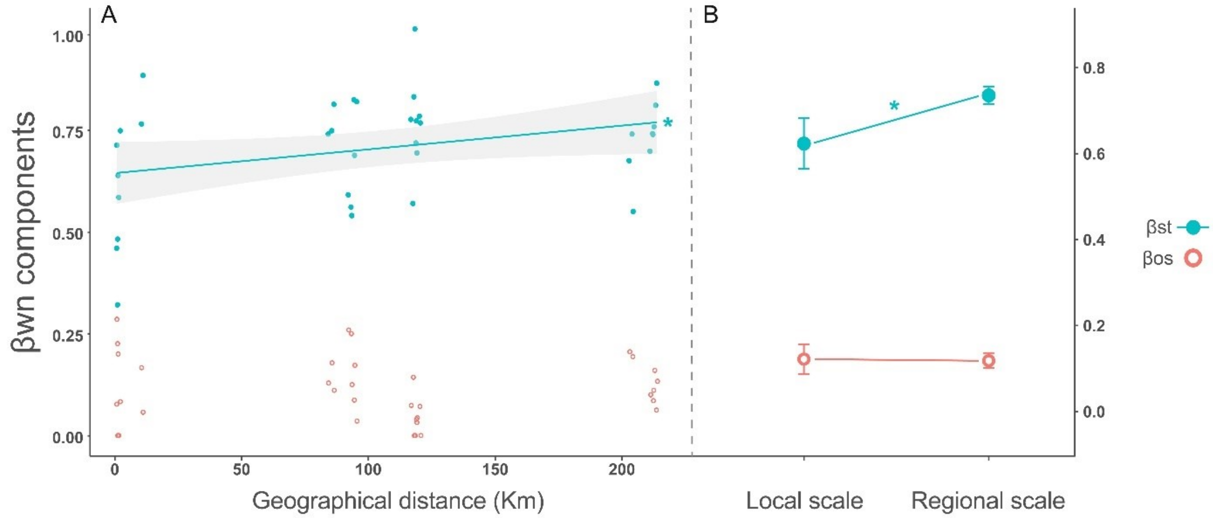

Figure 5- (A) Effect of geographical distance on the components of β-diversity of interactions. The components are spatial species turnover (βst) (ciano) and Interaction rewiring (βos) (salmon). Each ciano dot represents the βst between a pair of networks. Each salmon dot represents the βos between a pair of networks. The light grey shadows around the line represent confidence intervals of 95%. (B) The βst and βos values dispersion depending on their spatial scale class (local or regional) were represented by boxplots. The dark gray lines in boxplots represent the means. Asterisks signalize significant results.

Table 2 – Statistical values for models adjusted with geographical distance (left) and spatial scale (right) predictor variables. Response variables correspond to total β-diversity of interactions (βwn) and its components Spatial species turnover (βst) and Interaction rewiring (βos). Significative p-values are represented in bold. Values were rounded for 2 decimal places.

	~ Geographical distance		**~ Spatial scales**	
Variable	Z	p-value	F(df)	p-value
βwn	3278.55	**0.01**	5.40(1,34)	**0.03**
βst	2807.12	**0.04**	5.33(1,34)	**0.03**
βos	471.43	0.30	0.01(1,34)	0.91

Discussion

In the present study, we found that the dissimilarities of interactions between trophobiont insects and their tending ants change from local to regional scale and that this change was consistently higher with increasing geographical distance. Importantly, we found that the species turnover was the main component to the β-diversity of interactions, consistently increasing with geographical distance. Interaction rewiring had only limited importance in determining the β-diversity of interactions and remained constant across distance. We found high values of β-diversity of interactions, and these values were high even between those communities located near each other. Thus, from 1 km until 200 km of distance, the interaction dissimilarities in ant-trophobiont associations continuously increased. Ant-trophobiont interactions revealed a complex spatial structure and some discrepancies when compared to other mutualisms involving ants.

While the dissimilarity of ant-trophobiont interactions was already high at local scales, it increased with geographical distance mainly by the turnover of species composition. This means the farther a community is from another the higher is their interaction dissimilarities due to species turnover via increment or loss. Thus, the species A that interacts with the species B at a site tends to lose its partner or gain an interaction partner C, or both, at another site. The high turnover of species leads to an increase in β-diversity of interactions, as different species interactions are found when we scale up the distance between the biological communities. The increase in species composition dissimilarity with geographical distance has also been found in studies involving the taxonomic β-diversity of distinct taxa, including dung beetles, bees, wasp and ants (da Silva and

Hernández 2014, Perillo et al. 2017, Castro et al. 2020). We found a similar pattern, but now focused on the interactions β-diversity. However, our results are contrasting with a prior study over interactions between ants and different species of trees (Dáttilo and Vasconcelos 2019). The former study found only a small contribution of the species turnover component and a large contribution of interactions rewiring, even between communities spatially far from each other (Dáttilo and Vasconcelos 2019). The high rewiring of species interactions means that when species co-occur in different sites, they tend to interact in one, but not in another. Neutral abundance-based processes (*i.e.* species are ecologically equivalent), and the opportunistic and facultative nature of ant plant interactions, are potential explanations for the large change in species interaction's identity from a site to another. However, in stronger mutualistic interactions, such as those involving ants and trophobionts, there is often intense competition between ant species for honeydew-producing hemipteran aggregations (Blüthgen & Fiedler 2004), which implies some fidelity between partners when they co-occur and interact via trophobiosis. Niche-based processes (*i.e.* processes acting in species n-dimensional niche, determining its realization under certain conditions) are more likely to play a significant role at a local scale, but slightly reflects in historical processes at a regional scale since they imply some specialization level between partners acquired over time.

We found high values of β-diversity of ant-trophobiont interactions by species compositional turnover, increasing with geographical distance already at a local scale, and this may be mainly related to the preference and dominant behavior of the ant species with their partners. First, the higher the aggregation of a trophobiont, the higher the amount of produced honeydew. Second, different trophobiont species may produce honeydew with higher nutritional values than others (Blüthgen & Fiedler 2004). There were eleven species of honeydew-producing hemipterans in the studied area, and these species may present differences in their attraction to protective ants. Despite we have commonly found more aggregate trophobiont species, such as *Guayquila xiphias, Eurystethus microlobatus and Aethalion reticulatum*, occurring broadly within regions, but not always between regions, some fewer aggregate species were poorly shared between sites. Therefore, those trophobiont species with larger aggregations and more nutritious honeydew would be tended by more ant species or by numerically dominant ants. Guerra et al. (2011) found that larger aggregations of the hemipteran *Eurystetus microlobatus* were visited by more ant individuals, including the overly aggressive and dominant *Camponotus rufipes*. The less aggregate unprotected trophobionts are usually expelled or consumed by predators, presenting a high turnover across sites (or hostplants). Furthermore, the ant-trophobiont interactions may have modular networks (Costa et al. 2016), and presented low niche overlap, reflecting a high ant interspecific segregation with dominant competitors monopolizing the best resources (Costa et al. 2016, Fagundes et al. 2016). Those ant species with an efficient recruitment system and more aggressive behavior tend to dominate resources and expel other dominant and subdominant ant species (Parr & Gibb, 2010). However, these aggressive species often allow the presence of submissive ants (Flores-Flores et al. 2018), and this coexistence is often enhanced by niche differentiation (Houadria et al. 2015, Camarota et al. 2016). Unlike the more aggregated dominant ants, the submissive ant species are often scattered in the landscape, which allows the assembly of unique communities of interactive species in space. Ultimately, the presence of different species of submissive ants increases the total β-diversity of interactions along with geographical distance by changing species composition.

Another factor that may influence the high spatial turnover of ant-trophobiont interactions increasing with geographical distance already at local scales is the specific nature of trophobiont insects and its association with the hostplant physiology and distribution pattern. There is a high abundance of the mistletoe *Psittacanthus robustus* in the studied areas (Guerra et al. 2018). This tree species performs a fundamental role in shaping dissimilar communities of herbivore insects (de Freitas and Rossi 2015), and this happens to be true over space. *P. robustus* has a limited number of scattered tree hosts (Guerra et al. 2018), which in turn may limit the species local movement of both ants and herbivores, which would favor spatially modular insect communities. *P. robustus* has poor stomatal control, which increases the sap flow and its attractivity to sap-sucking insects, favoring their permanence on the plant (Barão 2015). Additionally, the sap-sucking hemipterans tend to choose plants with high quality of sap, and once they find a particular plant, they usually stay on it throughout its life (Delabie 2001). The protection by their ant partners leads trophobiont insects to reinforce their habit of remain on the same tree, where they can occupy different regions of the tree depending on their specific habits (Teodoro et al. 2010, Guerra et al. 2011). Thus, trophobiont scattered distribution due to *P.robustus* distribution and attractiveness may contribute with a part of ant-trophobiont spatial dissimilarities of interactions.

We found that sites within a region (local scale) present lower interaction dissimilarity between them when compared to the interaction dissimilarity between locations situated in distinct regions. While spatial species turnover is higher between communities located in distinct regions than those located within the same region, interaction rewiring remains low in both local and regional scales. Here, we have chosen three protected areas situated in mountain ridges distant from each other by at least 100 km. The land situated between them has been used in different ways (*e.g.* agriculture, urban ventures, livestock, and mining) (Sonter et al. 2014). Within each one, we have selected three locations approximately 1 km from each other, being all of them at the same elevation, presenting similar abiotic conditions, and the same vegetation type. We also sampled the interactions on the same hostplant (*P. robustus*), which was parasitizing the same tree species (*Vochysia thyrsoidea*). Thus, our sampling design enabled us to discuss the importance of species dispersion and the role of distant regions in interaction processes involving ants and trophobiont insects. Ants and trophobionts are widespread in nature, but here we showed an aggregated distribution over space, with a high composition dissimilarity between sites at a regional scale.

The mechanisms driving species distribution may be slightly different at local and regional scales. At the local scale, dominance behavior by ants (*i.e.* niche adaptations) and trophobiont occurrence in a scattered hub plant are the principal drivers to location dissimilarities in species interactions (Parr 2008). Despite these above-cited mechanisms seeming to also act at the regional scale, historical contingency processes, such as dispersal limitation over time (*i.e.* neutral distribution) and speciation are usually consider as the most important drivers to insects (Kemp et al. 2017) at larger spatial scales, and consequently may be close to the truth to ant-trophobiont interaction dissimilarities (Ricklefs 1987, Huston 1999, Srivastavaa 1999). Indeed, trophobiosis relationship involving ants is very ancient, dating from Miocene (15-20 Ma). Many species have differentiated and disperse since then worldwide, while trophobiosis kept been found in different regions, suggesting that these interactions have evolved independently many times (Johnson et al. 2001). However, we must be careful to tackle historical aspects. In this study we shed light on the magnitude of interactions dissimilarities across geographical distance and scales, controlling for some abiotic and biotic conditions, which allow us to discuss some ecological aspects, but not to precisely infer about historical processes. We did not consider phylogenetic information, which is crucial to fully understand the role of communities' assembly over long periods of time.

Despite the advances in the understanding of network properties of mutualistic interactions (Krishna et al. 2008, Vázquez et al. 2009, Dáttilo and Vasconcelos 2019), there is still a dearth of studies focused on the spatiotemporal variation of these interactions (Poisot et al. 2015, Burkle et al. 2016, Costa et al. 2016). Importantly, the patterns observed from the few studies performed so far must be interpreted with caution, and each case should be considered with care before generalizing. Here, we provide an important first step towards the understanding of the role of spatial scales and geographical distance over ant protective mutualisms. Summarizing, we showed that ant-trophobiont interaction dissimilarities, happening in similar abiotic and biotic conditions, increased with geographical distance and was greater at the regional scale, mainly due to species composition turnover. However, we must have in mind that interaction dissimilarities between ant-trophobiont networks were high already at a short geographical distance, but with low rewiring. It gives us a clue that these interactions reflect a certain specialization and fidelity of partners when they co-occur, in a system where they rarely co-occur, forming relatively unique interactions over space. Importantly, those dissimilarities at different spatial scales are potentially explained by different processes.

Perspectives

We hope to encourage future studies to be made to explore how β-diversity of mutualistic interactions relates to communities' assembly processes over time. We believe that studies approaching interactions at the ecological time may provide insights about how dissimilarities are established (or not) over space. Furthermore, a step forward must be taken to fully understand how interaction dissimilarities work at the regional scale. Group's phylogenies have much to say about in this sense, as they can provide data on the historical processes behind the interactions.

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Supporting information

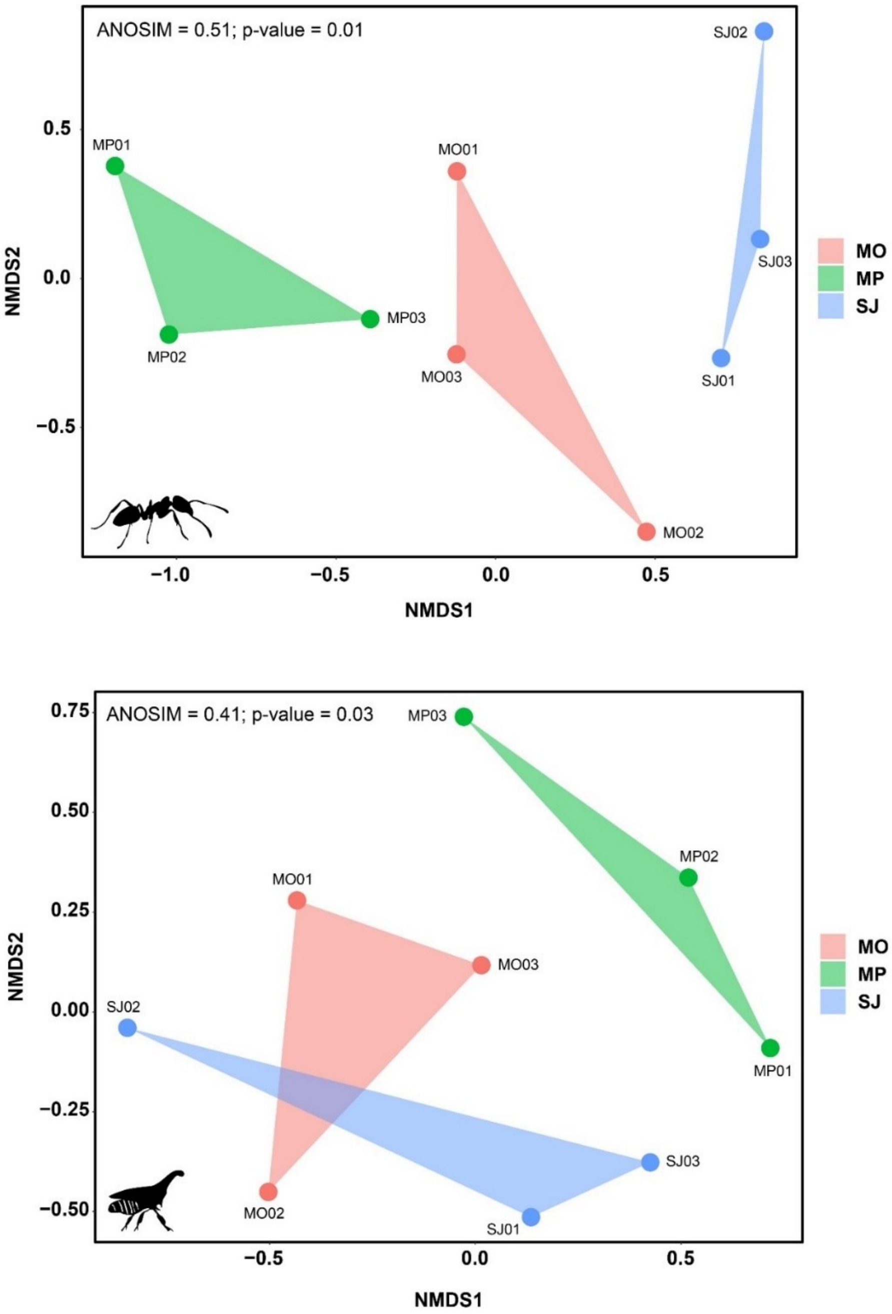

Figure 1 – Ants and trophobionts communities species composition represented in two axes with Non-metric multidimensional scaling (NMDS). Points represent local communities, whilst colors represent geographic regions. Pink points and shades represent Natural Monument of Serra da Moeda (MO), green represents Environmental Protection Area Morro da Pedreira (MP) and blue represents Environmental

Table 1- Ant occurrences in sample sites situated within the sampled regions. This species table also includes ants that were not interacting with trophobiont insect. MP = Environmental Protection Area Morro da Pedreira; MO = Natural Monument of Serra da Moeda, and SJ = Environmental Protection Area São José.

Region	**Site**	**Ant Genus**	**Ant Morphospecies**	**Ant abundance**
MP	**MP01**	*Azteca*	*Azteca sp1*	1
		Camponotus	*Camponotus rufipes*	209
		Camponotus	*Camponotus sp1*	3
		Cephalotes	*Cephalotes pusillus*	6
		Dorymyrmex	*Dorymyrmex sp1*	1
	MP02	*Camponotus*	*Camponotus melanoticus*	6
		Camponotus	*Camponotus rufipes*	115
		Camponotus	*Camponotus trapeziceps*	1
		Cephalotes	*Cephalotes pusillus*	21
	MP03	*Azteca*	*Azteca sp1*	1044
		Camponotus	*Camponotus crassus*	3
		Camponotus	*Camponotus rufipes*	20
		Camponotus	*Camponotus trapeziceps*	4
		Cephalotes	*Cephalotes pusillus*	6
MO	**MO01**	*Camponotus*	*Camponotus arboreus*	2
		Camponotus	*Camponotus blandus*	2
		Camponotus	*Camponotus crassus*	12
		Camponotus	*Camponotus novogranadensis*	1
		Camponotus	*Camponotus sp1*	8
		Camponotus	*Camponotus trapeziceps*	12
		Cephalotes	*Cephalotes pusillus*	16
		Crematogaster	*Crematogaster sp2*	1
		Pseudomyrmex	*Pseudomyrmex sp1*	1
	MO02	*Brachymyrmex*	*Brachymyrmex sp1*	59
		Camponotus	*Camponotus crassus*	4
		Camponotus	*Camponotus trapeziceps*	1
		Cephalotes	*Cephalotes depressus*	2
		Cephalotes	*Cephalotes pusillus*	3
	MO03	*Camponotus*	*Camponotus bonariensis*	1
		Camponotus	*Camponotus crassus*	11
		Camponotus	*Camponotus rufipes*	15
		Camponotus	*Camponotus trapeziceps*	2
		Cephalotes	*Cephalotes pusillus*	1

SJ	**SJ01**	*Brachymyrmex*	*Brachymyrmex sp1*	40
		Camponotus	*Camponotus bonariensis*	1
		Camponotus	*Camponotus crassus*	3
		Camponotus	*Camponotus novogranadensis*	2
		Camponotus	*Camponotus rufipes*	30
		Camponotus	*Camponotus trapeziceps*	4
		Cephalotes	*Cephalotes betoi*	1
		Cephalotes	*Cephalotes depressus*	3
		Cephalotes	*Cephalotes pusillus*	1
		Crematogaster	*Crematogaster sp1*	4
		Pseudomyrmex	*Pseudomyrmex gracilis*	2
	SJ02	*Atta*	*Atta sp1*	1
		Azteca	*Azteca sp1*	1
		Camponotus	*Camponotus blandus*	2
		Camponotus	*Camponotus crassus*	1
		Camponotus	*Camponotus rufipes*	6
		Camponotus	*Camponotus trapeziceps*	4
		Cephalotes	*Cephalotes betoi*	5
		Cephalotes	*Cephalotes pusillus*	6
		Dolichoderus	*Dolichoderus sp1*	2
		Gnamptogenys	*Gnamptogenys sulcata*	1
		Nesomyrmex	*Nesomyrmex sp1*	4
		Pseudomyrmex	*Pseudomyrmex termitarius*	2
	SJ03	*Camponotus*	*Camponotus crassus*	42
		Camponotus	*Camponotus novogranadensis*	11
		Camponotus	*Camponotus rengeri*	1
		Camponotus	*Camponotus rufipes*	57
		Camponotus	*Camponotus trapeziceps*	6
		Cephalotes	*Cephalotes betoi*	1
		Cephalotes	*Cephalotes depressus*	1
		Cephalotes	*Cephalotes pusillus*	1
		Crematogaster	*Crematogaster sp1*	4
		Gnamptogenys	*Gnamptogenys sulcata*	1
		Pseudomyrmex	*Pseudomyrmex gracilis*	8
		Pseudomyrmex	*Pseudomyrmex termitarius*	1
		Pseudomyrmex	*Pseudomyrmex unicolor*	1

Table 2- Trophobiont insect occurrences in sample sites situated within the sampled regions. MP = Environmental Protection Area Morro da Pedreira; MO = Natural Monument of Serra da Moeda, and SJ = Environmental Protection Area São José.

Region	**Site**	**Trophobiont Family**	**Trophobiont Morphospecies**	**Trophobionts on 10 trees**
MP	**MP01**	Aethalionidae	*Aethalion reticulatum*	277
		Pseudococcidae	*Pseudococcidae sp2*	189
		Membracidae	*Enchenopa sp1*	2
		Pentatomidae	*Eurystethus microlobatus*	7
		Membracidae	*Guayaquila xiphias*	607
	MP02	Aethalionidae	*Aethalion reticulatum*	1
		Membracidae	*Enchenopa sp1*	20
		Pentatomidae	*Eurystethus microlobatus*	457
		Membracidae	*Guayaquila xiphias*	45
		Membracidae	*Membracidae sp1*	5
		Membracidae	*Membracidae sp2*	2
	MP03	Pseudococcidae	*Pseudococcidae sp2*	217
		Membracidae	*Enchenopa sp1*	19
		Pentatomidae	*Eurystethus microlobatus*	50
MO	**MO01**	Aphididae	*Aphididae spp*	23
		Pseudococcidae	*Pseudococcidae sp1*	36
		Membracidae	*Enchenopa sp1*	52
		Membracidae	*Guayaquila xiphias*	2
	MO02	Aethalionidae	*Aethalion reticulatum*	1
		Aphididae	*Aphididae spp*	5
		Pseudococcidae	*Pseudococcidae sp1*	770
		Membracidae	*Guayaquila xiphias*	1
		Membracidae	*Membracidae sp1*	3
		Membracidae	*Membracidae sp2*	2
	MO03	Aphididae	*Aphididae spp*	15
		Membracidae	*Enchenopa sp1*	24
		Membracidae	*Guayaquila xiphias*	49
		Membracidae	*Membracidae sp1*	4
SJ	**SJ01**	Pseudococcidae	*Pseudococcidae sp1*	19
		Pseudococcidae	*Pseudococcidae sp2*	1
		Membracidae	*Enchenopa sp1*	2
		Membracidae	*Guayaquila xiphias*	22
	SJ02	Aphididae	*Aphididae spp*	4
		Pseudococcidae	*Pseudococcidae sp1*	12

		Pseudococcidae	*Pseudococcidae sp2*	1
		Membracidae	*Enchenopa binotata*	2
		Membracidae	*Enchenopa sp1*	3
		Pentatomidae	*Eurystethus microlobatus*	33
		Issidae	*Issidae sp1*	2
		Membracidae	*Membracidae sp2*	4
	SJ03	Pseudococcidae	*Pseudococcidae sp1*	14
		Membracidae	*Enchenopa sp1*	1
		Membracidae	*Guayaquila xiphias*	68