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Impact of *Hedychium coronarium* J. König (Zingiberaceae) on the assembly of spiders in the Parque Estadual das Fontes do Ipiranga, São Paulo State, Brazil¹

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Short title: Impact of *Hedychium coronarium* on the assembly of spiders

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ABSTRACT – (Impact of *Hedychium coronarium* J. König (Zingiberaceae) on the assembly of spiders in the Parque Estadual das Fontes do Ipiranga, São Paulo State, Brazil). The invasive *Hedychium coronarium* J. König (Zingiberaceae) is a competitive terrestrial herb. To understand the ecological relationships and verify whether *H. coronarium* impacts the mounting of spiders in the soil, 45 traps were installed, distributed in three treatments and three replicas. All spiders (292 individuals) collected between January and December 2018 were identified. The Intermediate Areas had the highest number of individuals collected and the highest diversity index, with 107 spiders collected (37% of the total), distributed in 14 species, followed by Dominated Areas with 97 individuals (33%) distributed in 12 species and Free Areas with 88 individuals (30%), distributed in 10 species. The analysis showed that the assembly of spiders in Parque Estadual das Fontes do Ipiranga does not present significant differences, these results may not be the result of chance and be related to *H. coronarium*. Furthermore, two families showed differences in their distribution.

Keywords: animal-plant interaction, biodiversity, spider fauna

RESUMO – (Impacto da *Hedychium coronarium* J. König (Zingiberaceae) sobre a assembleia de aranhas de solo do Parque Estadual das Fontes do Ipiranga, Estado de São Paulo, Brasil). A invasora *Hedychium coronarium* J. König (Zingiberaceae) é uma erva terrestre competitiva. Para entender como estão as relações ecológicas e verificar se o *H. coronarium* impacta a assembleia de aranhas no solo, foram instaladas 45 armadilhas, distribuídas em três tratamentos e três réplicas. Todas as aranhas (292 indivíduos) coletadas entre janeiro e dezembro de 2018 foram identificadas. As Áreas Intermediárias apresentaram o maior número de indivíduos coletados e o maior índice de diversidade, com 107 aranhas coletadas (37% do total), distribuídas em 14 espécies, seguidas por Áreas Dominadas com 97 indivíduos (33%) distribuídas em 12 espécies e Áreas Livres com 88 indivíduos (30%), distribuídas em 10 espécies. A análise mostrou que a montagem de aranhas no Parque Estadual das Fontes do Ipiranga não apresenta diferenças significativas, esses resultados podem não ser fruto do acaso e podem estar relacionados ao *H. coronarium*. Além disso, duas famílias apresentaram diferenças em sua distribuição.

Palavras-chave: araneofauna, biodiversidade, interação planta-animal

Introduction

Invasive alien species are those that, once introduced from other environments, adapt, start to reproduce and intensely proliferate. This can cause changes in ecological processes and harm native species. These biological invasions are the second biggest cause of biodiversity loss in the world (Mack *et al.* 2000, Latini *et al.* 2016). According to the Pombo *et al.* (2016) biological invasions produce changes and alterations in the ecological properties of the soil, in nutrient cycling, in water availability, in the trophic chains, the dynamic structure and distribution of populations and communities. Besides that, invasive alien species alter ecosystem functions, through changes in biomass distribution and decomposition rates (Helsen *et al.* 2018). Invasive alien species also interfere with evolutionary processes, with the local extinction of species and homogenization of ecosystems (Herrera *et al.* 2016).

Hedychium coronarium J. König (Zingiberaceae) known as the “White garland - lily”, is a monocot invasive alien of great prominence. It has herbaceous habit with competitive advantage over other species due to its rapid growing and its phytochemical properties (Castro 2013). It has aromatic inflorescence that attract nocturnal pollinators (*e. g.* moths) from January to March, *H. coronarium* has the dispersion of its seeds carried out by birds (Ferreira *et al.* 2016). It spreads asexually through rhizomes surpassing native vegetation (Santos *et al.* 2005).

The species can obstruct small streams and channels with its thick rhizomes (Lorenzi 2000, Amaral *et al.* 2008) and was considered the most damaging in Hawaii by forming extensive monospecific patches (Herrera *et al.* 2016). According to Correa (1984), *H. coronarium* can be used as an ornamental, medicinal, fungicide or cosmetic plant, and its textile fibers are used to produce cellulose paste (Kissmann & Groth 1991, Silva 2006, Martins *et al.* 2010, Carrara *et al.* 2012). In the study of Ferreira *et al.* (2016) a ban on the sale of this species in florists and nurseries is suggested as a means of control to prevent further invasions. Brazil's similar climate to Southeast Asia conditioned the growth of *H. coronarium* and its dispersion reached all Brazilian states, this information can be confirmed at virtual catalogs of Brazilian national herbariums (Flora do Brasil 2020). It is possible that its area of occurrence in the Brazilian territory was caused accidentally by transporting lots of commercial plants that arrived on ships or motivated for their use in landscaping (Primack & Rodrigues 2001, Ziller 2001, Petenon & Pivello 2008).

This species can negatively impact plant populations such as *Cucumis sativus* L. and animals like *Formicivora paludicola* Buzzetti *et al.* 2014 (Rodrigues & Lopes 2006, Del-Rio 2014). In addition to the rapid propagation of the species, the plant does not have a known effective predator. However, Castro (2013) presented the first herbivore record, inserting the *H. coronarium* in the food chain of *Hydrochoerus hydrochaeris* Linnaeus 1766.

Although several studies have been done on the use of this species and recent warnings about the impacts caused by this plant, studies related to arthropods, especially spiders, are scarce in the literature. Spiders are a megadiverse group with characteristics that make it possible to occupy different environments (World Spider Catalog 2021), they are grouped into guilds according to habitat and foraging (Uetz 1977, Cardoso *et al.* 2011), are known as efficient pest population controllers (Aguilar 1988, Flórez 2000) and can interact with invasive plants indirectly and any change in the spider assembly changes the arthropod community (Mgobozi *et al.* 2008). According Bultman & De-Witt (2008), the invasive alien species *Vinca minor* L. (Apocynaceae) influenced the mobility of native spiders, reducing their abundance.

In this study investigates the effect of *H. coronarium* on the assembly of soil spiders in the Parque Estadual das Fontes do Ipiranga (PEFI). It was expected that *H. coronarium* influences the abundance of spiders, because the species forms monospecific populations, as well as *V. minor*, cited above in the publication by Bultman & De-Witt (2008). The importance of the study about the diversity of spiders is even greater once considering that the area suffers from constant removal of land, the place is also a passage of vehicles and research groups, that is important to study the diversity of spiders in this region. The results expand the knowledge of ecological interactions between spiders and plants and reinforce the attention that we must have in relation to the introduction species in those conservation units.

Material and methods

Study area – The Parque Estadual das Fontes do Ipiranga (PEFI), located in the municipality of São Paulo, under the coordinates are 23°39'S e 46°37'W at an average altitude of 798 m (Melhem *et al.* 1981) and the predominant phytophysognomy is the Dense Ombrophilous Rainforest. The sampling units were selected near Lago das Garças,

bordering a dirt road that connects the Instituto de Botânica to the Zoológico de São Paulo where there is a wide occurrence of *H. coronarium* (figure 1).

Sample units - To compare the effect of *H. coronarium* on the assembly of soil spiders, was used a method similar to that of Robertson *et al.* (2011). However, in this study, only three treatments were performed, with three replicas each. The difference between this study and the one conducted by Robertson *et al.* (2011) is because in PEFI, the constant removal of invasive plants maintains few areas affected by *H. coronarium*. The study areas were chosen because they are close and hoping to eliminate the different influences from distant areas. In the protected area, it is the only environment that presents *H. coronarium* in a satisfactory amount for the three treatments. The three treatments include: (1) dominated areas (DA), where only *H. coronarium* covers the soil; (2) intermediate areas (IA), where *H. coronarium* covers 50% of the ground; (3) free areas (FA), where there is no occurrence of *H. coronarium*. Each sample unit has an area of 100 m² (figure 2 a-c).

Data collection - Spiders were collected using pitfall traps. The pitfall traps were set up using a 2 – liter plastic pot. Each pot has an opening of 18 × 13 cm and is 12 cm deep (figure 2 d). They were filled with 500 ml of water mixed with neutral detergent and removed in 48 hours after installing them. In total 45 pitfall traps were collected each month for one year (five in each replica). They were positioned as follows: one of the pots in the center of the sample unit, another four at 1.5 m from each vertex towards the center (figure 3).

Data analysis - To check the sampling sufficiency and estimate the number of species potentially present, the following species richness estimators were used: Bootstrap, Jackknife 1, Jackknife 2, Chao 1, Chao 2, and ICE. Calculations were performed using the EstimateS 9.1 (Colwell 2013). The estimator curve was plotted together with the observed species curve. For this analysis, all treatments were counted together (each sample = total spiders collected monthly per treatment). It was verified whether there are significant differences ($p < 0.05$) between the number of collected individuals between treatments, using the Shapiro-wilk test, Levene test, ANOVA test, post-hoc Tukey HSD test, Kruskal-Wallis test, and post-hoc Dunn's, was using program R study (R Development Core Team 2009), but uninformative results were not discussed in this study. Through the program Past 2.1 (Hammer *et al.* 2001) was realized the Simpson Diversity t test (significant differences = < 0.05) and Cluster analysis was performed using the Bray-Curtis similarity index and the UPGMA clustering method.

Results

We collected 292 spiders belonging to 17 families. Only 35 individuals (12% of the total) are adults and represent 20 species belonging to 11 families. The six families represented only by young individuals were: Oxyopidae, Pholcidae, Sparassidae (four individuals each), Anyphaenidae (two), Philodromidae and Scytodidae (one individual each). Of the families that were sampled with adult individuals we have: Linyphiidae (nine adults), Corinnidae (eight), Theridiidae (seven), Salticidae (three), Idiopidae (two), Ctenidae, Nemesiidae, Pycnothelidae, Theridiosomatidae, Xenoctenidae and Zodariidae (all with only one adult).

The greatest richness was found in Theridiidae with five species, followed by Corinnidae and Linyphiidae, both with three species. Of the 20 species identified, only two were more abundant: *Ianduba varia* (Keyserling, 1891) with six individuals (17.14% of the total adults) and *Agyneta* sp. with five (14.28%). All other species had only one or two individuals each (table 1).

Although the species richness was small and the number of collected adult individuals was low, it was possible to make a Cluster analysis that indicated 82% similarity in the composition of families between the IA and DA treatments (figure 4). The IA treatment showed a greater number of species (14), followed by DA with nine and FA with four species (table 1), but the treatments not differentiated from each other.

The species richness estimators that came closest to the total of observed species were Bootstrap and Jackknife 1, they indicated 24 – 31 species potentially present (figure 5 a), this result was obtained when performed as a function of richness per treatment, where only Bootstrap was within the standard deviation in the three treatments (FA, IA, and DA), Jackknife 2 and Chao 1 were the least conservative, Jackknife 1 and Bootstrap were the most conservative. IA has the smallest difference between estimators (figure 5 c), DA and FA have the greatest difference between estimators (figure 5 b, d).

Considering the total number of collected individuals (youth and adults), the data have a normal distribution, and no difference was by the ANOVA test was detected (F-value = 0,414; P-value = 0,678) and the p-values in the Simpson Diversity *t* test were greater than 0.5. There are no significant differences in comparisons between treatments, corroborated by the Tukey HSD statistical test and the sampled data in each treatment are normally distributed according to the Shapiro - Wilk normality test (W-test) (table 2).

Linyphiidae was represented by 101 individuals, 47 (46.52%) of these collected in FA and 27 (26.74%) in each of the other areas, although the difference was not obtained in the ANOVA test (F-value = 1,274; P-value = 0,346). During the cold and dry period (April to September), Linyphiidae was collected mostly in FA and IA, the Shapiro-Wilk test showed normality of data in the distribution and no significant difference was obtained in the ANOVA test (F-value = 2,121; P-value = 0,201). While in the rainy period (October to March) it was collected mostly in DA (Figure 6), the Shapiro-Wilk test showed significant difference (W-test = 0,7500; P-value = 0,00000000000000002), but this difference it was not recovered in the Kruskal-Wallis test and in the Dunn post-hoc, prepared for the Bonferroni method.

Corinnidae was represented by 84 individuals, 32 (38.1%) of these collected in DA and 20 individuals (23.8%) collected in IA and FA, the ANOVA test not obtained difference (F-value = 0,98; P-value = 0,428). Both in the cold and dry period as in the hot and rainy period, the Corinnidae were more collected in DA and IA (figure 6 b), according to the ANOVA test (F-value = 0,208; P-value = 0,818) and in the post-hoc, the family has a normal distribution in the treatments. Only FA in cold and dry period, a family showing a significant difference (W-test = 0,7500; P-value = 0,00000000000000002), but this difference was not recovered in the Kruskal-Wallis test and in the Dunn's post-hoc equipped for the Bonferroni method.

Theridiidae was represented by 37 individuals, 16 (43%) of these collected in the DA, 13 (35%) collected in the IA and 8 (22%) collected in the FA. The DA showed a significant difference in the Shapiro - Wilk test (W-test = 0,7500; P-value = 0,000000000000000022), but this significant difference was not recovered in the Kruskal-Wallis test and in the Dunn's post-hoc, adjusted for the Bonferroni method. When compared to the time of year, the heat and humidity period did not show significant differences, but in the cold and dry period, DA and IA had no differences in the data and FA individuals were not collected from the family.

Xenoctenidae was represented by 30 individuals, 20 (67%) of these collected in the IA, 7 (23%) in the DA and 3 (10%) in the FA. The treatments showed a significant difference in the ANOVA test (F-value = 4,017; P-Value = 0.0781) and the Tukey HSD test obtained differences only between IA and FA (P-value = 0,0781). When compared to the time of year, the family showed differences only in the hot and humid period in the IA and FA and in the cold and dry period in the FA (W-test = 0,7500; P-value = 0,00000000000000002). Levene test using the mean as a basis, showing difference in the

homogeneity of variances only for period of heat and humidity (F-value = 5,0256; P-value = 0,05223), but when the median was used as a basis, this difference was not recovered (F-value = 0,4138; P-value = 0,6787). Data from the time of year did not show significant differences in the Kruskal-Wallis and Dunn's post-hoc, adjusted for the Bonferroni method.

Discussion

In this study an amount number of individuals was sampled, but most of them were young, making it impossible to identify at a specific level, the species richness observed is not associated with *H. coronarium*, unlike *V. minor* observed in Bultman's study & De-Witt (2008), but may be associated with the anthropic impact of the region due the study area is bordering a dirt road with constant movement of vehicles and a large flow of residents and employees who work in the PEFT's research areas and suffers from the constant removal of this invasive plant.

There is no widely used method for collecting spiders, so it is difficult to compare results. However, the collected data in the present research coincide with data from different locations. Linyphiidae and Theridiidae appear among the most abundant families in several studies (Lopes *et al.* 2008, Silva *et al.* 2014, Breitenbach *et al.* 2015). In the present study, Linyphiidae was collected more frequently in FA treatment.

The statistical results indicate that the treatments are similar in relation to the diversity of spiders. However, it was observed that the two most abundant families were collected mostly from different areas. Significant changes in temperature and humidity do not influence the distribution of the spider assembly (Maciel 2011). But leaf architecture can influence different guilds in different ways, even if it does not influence their prey (Diniz 2011).

Linyphiidae are generally associated with litter, building sheet – shaped webs close to the ground, and are commonly found to pitfall traps (Ferro 2008, Ubick *et al.* 2005). Linyphiidae was the most abundant family in other works (Ferro 2008, Silva *et al.* 2014, Breitenbach *et al.* 2015). Spiders of this family prefer the interior of the forest according to Gonçalves-Souza (2008), corroborating with the obtained data in this study. The greater number of individuals collected in DA in the hot and humid seasons of the year, may be associated with the search for climate protection provided by the leaf density

of *H. coronarium*, which provides a cooler microenvironment, but further studies are needed to test this hypothesis.

Corinnidae are, in general, larger than the Linyphiidae and have a glossy black color, which leaves them visibly more exposed to predators. It is possible that these spiders avoid FA to seek refuge in environments with greater leaf density that provide greater protection against predators, as pointed out by Dinniz (2011). Another influencing factor is the preference of this family for moist environments (Patucci 2018) and *H. coronarium* can accumulate a small amount of water in some parts of the plant.

Spider families can be divided into five behavioral guilds according to Diniz (2011). The collections of this study indicated that the most frequent families belong to the Builders of Three –dimensional Web (TDW) represented by Linyphiidae and Theridiidae and to the Night Corridors (NC) represented by Corinnidae and Xenoctenidae. The spiders of the TDW guild have a greater interest in denser leaf architecture and preference for plants with larger spaces between the leaves (Diniz, 2011), the statistical data found for Linyphiidae corroborate the preference for open areas. The data obtained for Theridiidae need further studies to understand its dynamics. While NC guild spiders prefer environments with complex structure, probably due to the protection against predators and parasites offered by the plant architecture (Diniz 2011) and the higher occurrence of NC guild spiders found in AD, corroborates the literature.

Conclusion

This research indicated that *Hedychium coronarium* does not directly impact the population dynamics of soil spider assemblages. Despite the significant differences, not having been recovered in post-hoc, larger studies may point to impacts when studying groups of lesser behavioral complexity, such as guilds, families, and species. Weaver spiders have a preference for IA, however, Linyphiidae has different preferences influenced according to the periods of the year. Runner spiders, on the other hand, showed preference for DA throughout the year. Studies on this type of ecological relationship in preserved sites are necessary to complement the information available here, since there are invasions of *H. coronarium* in many conservation units in Brazil and the understanding of relationships like these can help in the protection of species, mainly predatory arthropods such as spiders that are important controllers of insect populations.

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Author Contributions

Giovanni Pupin: Contribution to data collection; contribution to data analysis and interpretation; contribution to critical revision; contribution to manuscript preparation.

Zedenil Mendes: Contribution in the concept and design of the study; contribution to data analysis and interpretation; contribution to critical revision, adding intellectual content; contribution to manuscript preparation.

Conflicts of interest

There is no conflict of interest.

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Table 1. Number of adult individuals collected by species of spider in three selected treatments near Parque Estadual das Fontes do Ipiranga, São Paulo, São Paulo State, Brazil. DA: dominated areas; IA: intermediate areas; FA: free areas.

Families / Species	DA	IA	FA
Corinnidae			
<i>Corinna</i> sp.1		1	
<i>Corinna</i> sp.2		1	
<i>Ianduba varia</i> (Keyserling, 1891)	5	1	
Ctenidae			
<i>Ctenus ornatus</i> (Keyserling, 1877)	1		
Idiopidae			
<i>Idiops camelus</i> (Mello-Leitão, 1937)		1	1
Linyphiidae			
<i>Agyneta</i> sp.		2	3
<i>Dubiaranea</i> sp.	1	1	
<i>Sphecozone</i> sp.	1	1	
Nemesiidae			
<i>Rachias</i> sp.	1		
Pycnothelidae			
<i>Acanthogonatus</i> sp.		1	
Salticidae			
<i>Euophrys</i> sp.	1	1	
<i>Thiodina</i> sp.	1		
Theridiidae			
<i>Chrysso</i> sp.	2		
<i>Cryptachaea</i> sp.			1
<i>Dipoena pumicata</i> (Keyserling, 1886)		1	
<i>Euryopsis</i> sp.	1	1	
<i>Theridion</i> sp.			1
Theridiosomatidae			
<i>Theridiosoma</i> sp.		1	
Xenoctenidae			
<i>Odo</i> sp.		1	
Zodariidae			
<i>Tenedos garoa</i> Candiani, Bonaldo & Brescovit, 2008		1	
TOTAL	14	15	6

Table 2. P-values of the Simpson Diversity t test, Tukey HSD test and the normality test (W-test) for the assemblages of soil spiders in three treatments close to Garças lake in the Parque Estadual das Fontes do Ipiranga, São Paulo, São Paulo State, Brazil. DA: dominated areas; IA: intermediate areas; FA: free areas.

<i>t test</i>		Tukey HSD		<i>W test</i>	
Compared areas	P value	Compared areas	P value	Areas	P value
DA and IA	0,71	DA and IA	0,90	FA	0,37
DA and FA	0,69	DA and FA	0,88	IA	0,15
FA and IA	0,35	FA and IA	0,65	DA	0,28

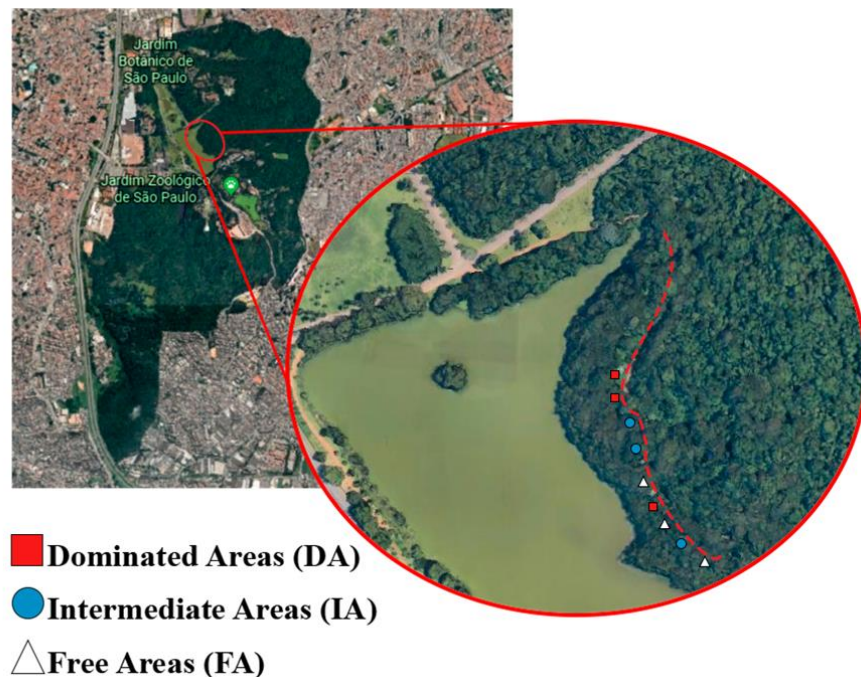


Figure 1. Aerial view of the Parque Estadual das Fontes do Ipiranga and the trail (Dashed Black) near the Garças lake (detail), São Paulo State, Brazil. Adapted from Google Earth (2019).



Figure 2. General aspect of the sampling units of the three treatments performed on the trail near the Parque Estadual das Fontes do Ipiranga, São Paulo, São Paulo State, Brazil. a: area dominates for *Hedychium coronarium*. b: intermediate area. c: free area. d: view of the fall trap.

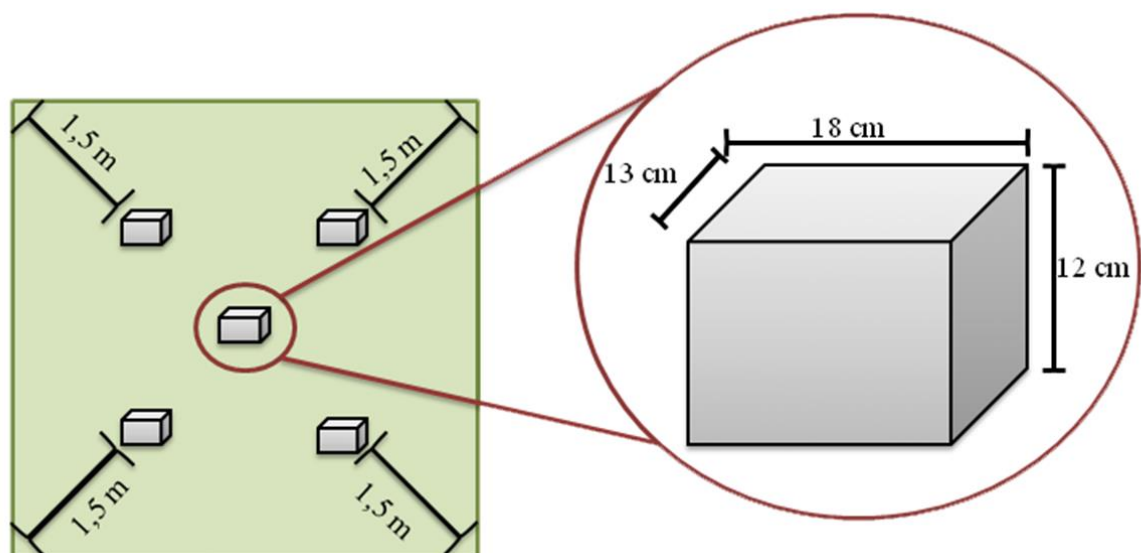


Figure 3. Scheme of the distribution of the fall traps within a sample unit (on the left) and their dimensions (on the right).

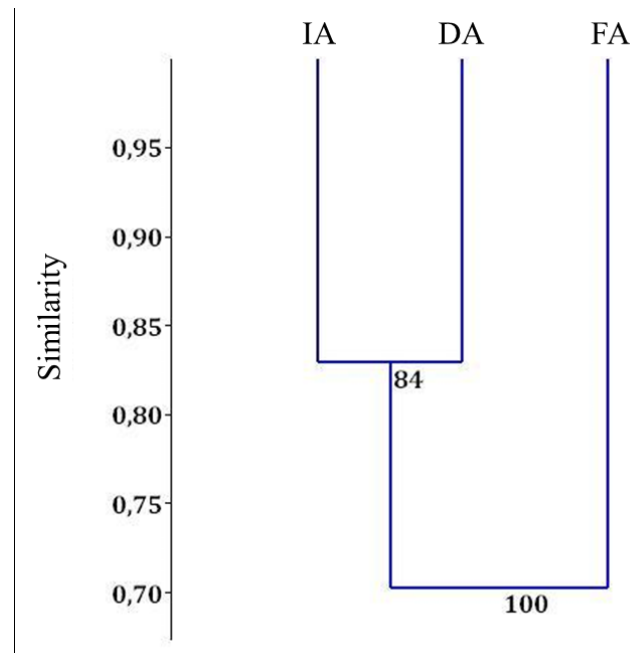


Figure 4. Cluster graph indicating the similarity between the assemblies of soil spiders, considering the abundance of adult and young spiders collected in three selected treatments close to the Parque Estadual das Fontes do Ipiranga, São Paulo, São Paulo State, Brazil. DA: dominated areas. IA: intermediate areas. FA: free areas. (Phenetic coefficient = 0,9493).

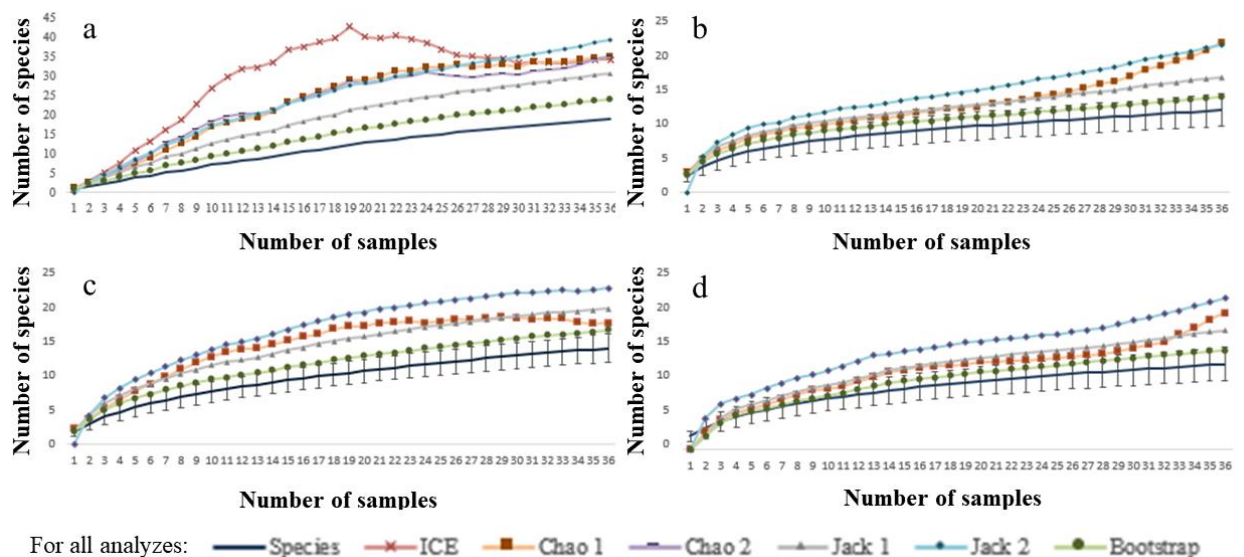


Figure 5. Curve of observed species and richness estimators of soil spider species collected in Parque Estadual das Fontes do Ipiranga, São Paulo, São Paulo State, Brazil. a: all treatments. b: Dominated area. c: Intermediate area. d: Free area.

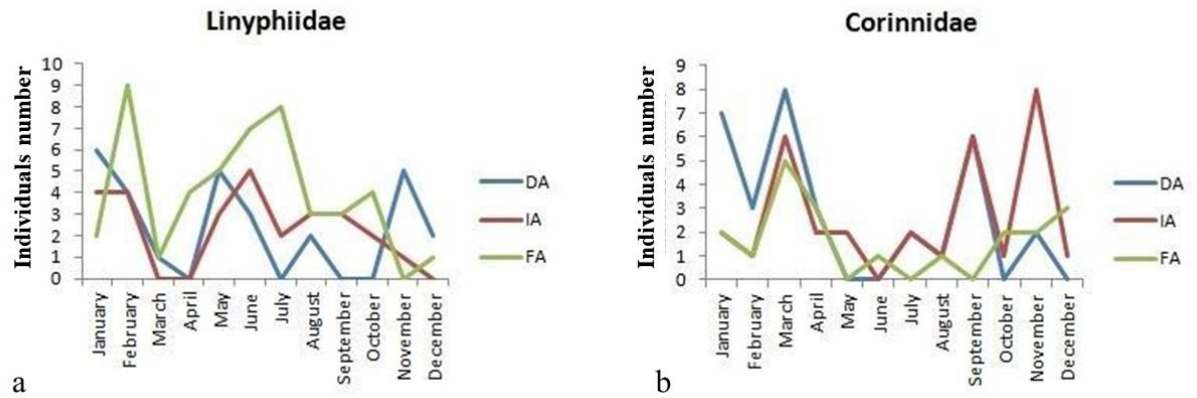


Figure 6. Number of individuals collected in three treatments close to Parque Estadual das Fontes do Ipiranga, São Paulo, São Paulo State, Brazil. a: Number of individuals in the Linyphiidae family. b: Number of individuals in the Corinnidae family. DA: Dominated Areas. IA: Intermediate Areas. FA: Free Areas.

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