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Insights into the structure of plant-insect communities: Specialism and generalism in a regional set of non-pollinating fig wasp communities

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Abstract

Insects show a multitude of symbiotic interactions that may vary in degree of specialization and structure. Gall-inducing insects and their parasitoids are thought to be relatively specialized organisms, but despite their ecological importance, the organization and structure of the interactions they establish with their hosts has seldom been investigated in tropical communities. Non-pollinating fig wasps (NPFW) are particularly interesting organisms for the study of ecological networks because most species strictly develop their offspring within fig inflorescences, and show a multitude of life history strategies. They can be gall-makers, cleptoparasites or parasitoids of pollinating or of other non-pollinating fig wasps. Here we analysed a set of non-pollinating fig wasp communities associated with six species of *Ficus* section *Americanae* over a wide area. This allowed us to investigate patterns of specialization in a diverse community composed of monophagous and polyphagous species. We observed that most NPFW species were cleptoparasites and parasitoids, colonizing figs several days after oviposition by pollinators. Most species that occurred in more than one host were much more abundant in a single preferential host, suggesting specialization. The food web established between wasps and figs shows structural properties that are typical of specific antagonistic relationships, especially of endophagous insect networks. Two species that occurred in all available hosts were highly abundant in the network, suggesting that in some cases generalized species can be more competitive than strict specialists. The Neotropical and, to a lesser extent, Afrotropical NPFW communities seem to be more generalized than other NPFW communities. However, evidence of host sharing in the Old World is quite limited, since most studies have focused on particular taxonomic groups (genera) of wasps instead of sampling the whole NPFW community. Moreover, the lack of quantitative information in previous studies prevents us from detecting patterns of host preferences in polyphagous species.

1. Introduction

Interaction networks arise in a multitude of contexts such as social sciences (Burt, 1976; Friedkin and Johnsen, 1997), communication (Dorogovtsev and Mendes, 2003), molecular biology (Ma'ayan, 2011), and ecology (Bascompte and Jordano, 2006; Jordano, 1987; Memmott, 1999). Although network analysis is applied to different disciplines, organization levels and organisms, general patterns and unifying concepts can be used to describe their structure, such as centrality, connection intensity, interaction strength and modularity (Barrat et al., 2004; Brandes et al., 2003; Dunne et al., 2002; Olesen et al., 2007). In an ecological perspective, the characteristics of interaction networks have been investigated to detect emergent patterns in areas such as food webs, pollination ecology, seed dispersal and parasitic interactions. They allow addressing important questions such as vulnerability to extinction, ecological specialization, and pollination efficiency (Bascompte et al., 2006; Blüthgen et al., 2007; Memmott, 1999; Memmott et al., 2004; Taudiere et al., 2015). In the context of

plant-animal interactions, ecological network analysis contributes to the recognition of generalism and specialization patterns among species.

Such patterns may allow detecting the presence of underlying processes driving evolutionary diversification in symbiotic communities, concerning both antagonistic and mutualistic relationships (Forister and Feldman, 2011).

Plant-insect interaction networks vary in structure, yet some emerging patterns have been uncovered according to the type of interaction established. Mutualistic networks such as pollination and seed dispersal interactions tend to show nested structures that are cohesively organized around a central core of generalized interactions. This pattern has been hypothesized to arise from diffuse coevolution between plants and animals, and generates heterogeneous distributions of the number of interactions (Bascompte et al., 2003). On the other hand, specialized networks, organized around herbivorous insects and plants, or insects and their parasitoids, tend to show cohesive groups of animals associated with subsets of hosts, often called compartments or modules (Dormann and Strauss, 2014) which are generated by ecological or evolutionary trophic specialization (Olesen et al., 2007; Prado and Lewinsohn, 2004). Therefore, network structure may indicate the presence of underlying evolutionary mechanisms that generate the level of specialization/generalization observed within plant-insect communities.

The communities of micro-hymenoptera associated with fig trees (*Ficus*, Moraceae) may provide a powerful model to investigate processes driving the evolution of community structure. Indeed, these wasps include diversified sets of gall inducing wasps, cleptoparasites and parasitoids (Elias et al., 2012; Segar et al., 2013a). Further, most of these wasps breed exclusively within figs (the closed inflorescence of *Ficus*), and the adults of the different species emerge synchronously from their host fig. Therefore collecting whole communities is relatively easy. Furthermore, sampling the wasps emerging from all local *Ficus* species allows collection of the whole meta-community of fig wasps, and therefore they offer robust data for analysing the interconnections between the different communities using a network approach. Since each wasp utilizes a single fig flower ovary as oviposition site, and the cleptoparasites and parasitoids utilize a single larva to develop, it is straightforward to obtain a comparable quantitative measure of the degree of infestation for each species in each host. Therefore, we have a robust measure of the reproductive output of each wasp species in each host, and it is possible to obtain highly informative networks.

The relationship between fig trees and Agaonidae is a classic example of obligatory pollination mutualism. The fig is an urn-shaped inflorescence, which is lined inside by hundred(s) to thousands of uniovulate flowers depending on the *Ficus* species. Female pollinating wasps enter the fig by crawling through an aperture closed by bracts (the ostiole), oviposit in the ovaries of some pistillate flowers and pollinate others. The wasp larva develops instead of a would-be seed. Male offspring emerge into the fig cavity and mate with the females that are still enclosed in their galls. Then, female wasps emerge from their galls, leave the fig

and fly to another fig to oviposit and pollinate (Galil and Eisikowitch, 1969). Global phylogenetic analyses of *Ficus* and Agaonidae show that these groups have broadly radiated in parallel (Cook and Segar, 2010; Cruaud et al., 2012; Machado et al., 2005). Generally, each *Ficus* species is pollinated by one or a few species-specific agaonid wasps (for exceptions see e.g. Cornille et al., 2012).

Besides pollinators, fig inflorescences host species-rich chalcid wasp communities. Indeed, several unrelated lineages of chalcid wasps develop exclusively or mainly on figs (Bouček, 1993; Rasplus et al., 1998). They interfere with the reproductive success of both *Ficus* and pollinating wasps in different ways, as they differ widely in feeding habits (Elias et al., 2007; Peng et al., 2005). Some non-pollinating fig wasps (NPFW) induce galls and their offspring develop in pistillate flowers as pollinators do (Elias et al., 2012; Jansen-Gonzalez et al., 2014); in some cases, they may even induce galls in other fig tissues or organs (Beardsley and Rasplus, 2001; Bronstein, 1999; Ghara et al., 2014; Müller, 1886). Some phytophagous NPFWs (known as inquilines or cleptoparasites) feed on galls induced by other wasps and eliminate their larvae in the process (Joseph, 1958), while other groups are true parasitoids that feed directly on the larvae of phytophagous fig wasps (Tzeng et al., 2008). Finally, some wasps are facultative (Pereira et al., 2007) or obligatory seed eaters (Wang et al., 2014). Despite increasing interest in their biology and evolution (Cruaud et al., 2011a, 2011b; Elias et al., 2008), the communities of NPFW are poorly known (but see Compton and Hawkins, 1992; Compton and van Noort, 1992;

Kerdelhué et al., 2000; Segar et al., 2013b). Information on host specificity of NPFW is scarce and limited by sampling effort and lack of taxonomic resolution (Cook and Segar, 2010; Darwell and Cook, 2017). Thus, the level of host specificity in NPFW is subject to debate. In some lineages, host specificity is supported by evidence for parallel cladogenesis between *Ficus* and NPFWs (Jousselin et al., 2006). However, in some other studies, co-phylogenies suggest considerable host switching during their evolutionary history (Jiang et al., 2006). Datasets on Neotropical and African fig wasps show some NPFW species colonizing several hosts (Marussich and Machado, 2007; McLeish et al., 2012). These results suggest that host specificity and strict-sense co-evolution may not satisfactorily explain the diversification of NPFW and their host species.

In this context, we analysed a set of NPFW communities associated with *Ficus* section *Americanae* in three areas of semi-deciduous forests in São Paulo state, Brazil. Large-scale sampling allowed us to thoroughly investigate patterns of specialization in a complex community composed of monophagous and polyphagous species with an array of life histories and a wide range of abundances in each *Ficus* host. As fig wasps have a delimited set of potential oviposition sites (one egg in each pistillate fig flower) and we measured the ovule occupancy rate of individuals belonging to each wasp species in the community in each host, our data allowed us to assess the ecological specialization of NPFW species with a quantitative network approach. Specifically, we aimed at answering the following questions: (1) how often

do the offspring of a NPFW species develop in more than one host; (2) are NPFW communities specialized; (3) do NPFW communities present a structured pattern; (4) even if wasp species use several hosts, is the species composition of the community associated with each of these hosts different. Furthermore, extra-Amazonian Brazil in general – and São Paulo state in particular – has suffered from huge deforestation. Such a situation may have impacted NPFW communities. A simple prediction is that the communities associated with the least common *Ficus* species may be strongly affected, showing species-poor communities and a lack or imbalance in some trophic levels, while the community associated with the most abundant *Ficus* species may be more typical.

2. Material and methods

2.1. Studied species

We analysed the NPFW communities associated with six *Ficus* species belonging to the section *Americanae* in three locations in São Paulo state, Brazil. *Ficus* sect. *Americanae* is the most diverse *Ficus* section in the Neotropical region, with more than 120 described species (Berg and Villavicencio, 2004). The *Ficus* species present locally included seven species of section *Americanae* and two species of section *Pharmacosycea*. We verified that the NPFW genera associated with *Ficus* section *Americanae* and with section *Pharmacosycea* were distinct. The communities associated with the later group were not investigated in this study. Figs belonging to section *Americanae* are monoecious, and the studied species vary in average fig diameter from ~1 to ~2.5 cm when mature (Table 1).

Ficus section *Americanae* is a clade that only diversified recently between the Oligocene and Miocene (~20–25 Ma, Cruaud et al., 2012; Machado et al., 2018). The group is monophyletic, but phylogenetic relationships within section *Americanae* are not well established (Cruaud et al., 2012; Machado et al., 2018). Species may grow as hemiepiphytes or as free standing trees (Berg and Villavicencio, 2004).

Table 1

Ficus section *Americanae* species sampled in this study, showing their characteristics (fig diameter and number of flowers per fig). Number of figs quantified and crops sampled in this study are also shown. Total of qualitative and quantitative samples is also divided by sample area (Gália/Ribeirão Preto/Teodoro Sampaio).

Host	Fig diameter (X ± SD cm)	No female flowers per fig (M ± SD)	No of figs	No of samples qualitative	No of samples quantitative
<i>F. citrifolia</i>	1.4 ± 0.2	550.4 ± 183.8	1024	58 (20/17/21)	51 (17/17/17)
<i>F. crocata</i>	2.3 ± 0.3	1460.1 ± 444.7	157	12 (1/0/11)	8 (0/0/8)
<i>F. eximia</i>	1.4 ± 0.2	642.4 ± 224.2	548	38 (13/13/12)	30 (10/10/10)
<i>F. luschnathiana</i>	0.9 ± 0.1	329.3 ± 115.8	100	6 (1/0/5)	5 (1/0/4)

<i>F. obtusifolia</i>	1.9 ± 0.3	874.5 ± 214.0	165	15 (11/3/1)	8 (4/3/1)
<i>F. trigona</i>	1.0 ± 0.2	270.2 ± 81.6	59	4 (2/2/0)	3 (2/1/0)

Despite some species in section *Americanae* presenting cauliflory, all species analysed in this study bear figs in the leaf axils or just below the leaves. Fig production within individual species varies in relation to growth form and size. For instance, *Ficus citrifolia* usually grows as a small hemiepiphyte and produces relatively small crops of a few thousand figs, whereas *F. luschnathiana* and *F. trigona* grow as hemiepiphytes or small trees and produce medium-sized crops. *Ficus obtusifolia*, *F. crocata* and *F. eximia* may be terrestrial or hemiepiphytic, usually have large tree crowns and produce larger crops.

Non-pollinating fig wasps occurring in the sampled fig trees include two genera belonging to the Agaonidae (*Sycophaginae*; *Anidarnes* Bouček and *Idarnes* Walker), one Eurytomidae genus (*Sycophila* Walker), three Pteromalidae genera (*Aepocerus* Mayr, *Ficicola* Heydon, and *Heterandrium* Mayr), and two genera belonging to the Torymidae (*Nannocerus* Mayr and *Physothorax* Mayr) (see: Bouček, 1993; Rasplus et al., 1998, for further systematic information).

2.2. Oviposition time and feeding regime

We considered these NPFW as belonging to three different ecological groups according to their timing of oviposition. *Anidarnes*, *Idarnes* group *incertus* and *Ficicola* are large gall inducers that colonize figs well before pollination and lay eggs in developing pistillate flowers or sometimes in the fig wall (Bronstein, 1999; Conchou et al., 2014; Müller, 1886). We will refer to these species as “prefloral colonizers”.

Idarnes species belonging to the *flavicollis* species group are also gall inducers, and oviposit in pistillate flowers at the same time as pollinators. We will refer to these species as “receptive phase colonizers” since they are seen on figs during the anthesis of the pistillate flowers (Elias et al., 2012). Their galls are almost the same size as pollinator galls and they may compete with them for oviposition sites.

Idarnes species belonging to the *carne* species group colonize figs after pollination – some species immediately after – and use galls induced by other wasps as oviposition sites, killing the host larva in the process (Elias et al., 2008; Pereira et al., 2007). Thus, they can be considered as cleptoparasites. Species belonging to the remaining wasp genera (*Heterandrium*, *Nannocerus*, *Physothorax* and *Sycophila*) also oviposit after pollination and are probably parasitoids or cleptoparasites of other fig wasps (Elias et al., 2008). We refer to these species as “interfloral colonizers”.

Observations on the biology of *Aepocerus* in French Guiana suggested that at least one species arrives at the same time as the pollinator, and may be a gall inducer (Conchou et al., 2014), however, we observed that a species associated with *F. citrifolia* in Ribeirão Preto

(Brazil) arrived well after pollination, suggesting that feeding regime variation is present within this genus; thus, they can be either gallers or cleptoparasites/parasitoids.

2.3. Data sampling and study locations

From September 2007 to December 2010 we collected samples within fragments of semi-deciduous Atlantic Forest and surrounding areas. Sampling locations encompassed the cities of Ribeirão Preto (21° 10'S, 47° 48'W), Gália (22° 24'S, 49° 42'W), Teodoro Sampaio (22° 26'S, 52° 18'W), and adjacencies. The three sites were located ca. 200–500 Km apart. A systematic survey of the abundance of the different fig species in the same locations provides density data within remaining forest patches for the different plant species (Coelho et al., 2014). We sampled all host species from section *Americanae* reported in Coelho et al. (2014) except *F. lagoensis*. Mature individuals of this host produced figs very sporadically and could not be sampled. Our sampling regime therefore covered the most common host species distributed throughout this biome, but excluded host species with a restricted distribution and species that are cultivated in urban areas but are probably introduced from other biomes.

For each reproductive episode (crop) of a tree, we collected about 20–50 figs just before ripening and transferred them to netting bags until the wasps emerged. Figs were opened after 24 h and further kept for 2–6 days; wasps were collected every 12–24 h. Wasps were killed by freezing and placed into 70% ethanol. Thereby we obtained qualitative information on species richness in each crop. For most crops, ca. 20 additional figs were kept individually in plastic flasks until the wasps emerged. We collected all emerged wasps from each fig, and unemerged wasps were removed from the galls. Thus, we could count numbers of wasps of each species in each fig. Because male wasps are often somewhat difficult to associate to females of the same species within a genus, we present numerical data for females only. We also quantified the number of female flowers, estimated by the summation of unused flowers, seeds, empty galls ('bladders'), and developed galls (i.e., the total number of wasps, since a single wasp emerge from each gall). The number of seeds, empty galls and unused flowers were estimated for the whole fig by counting these structures in one fourth of each fig in larger figs (i.e. *F. citrifolia*, *F. crocata*, *F. eximia* and *F. obtusifolia*). For smaller figs (i.e. *F. luschnathiana* and *F. trigona*) these structures were counted in the whole fig. The total number of flowers was used to standardize sampling effort as it yields a good measure of the fig size.

2.4. Species delimitation and voucher materials

Collected specimens were assigned to morphospecies based on their external morphology. Some specimens were card or point-mounted in order to facilitate identification and comparison. Our morphological identification method was validated by molecular investigation within *Idarnes* species, including 12 species and 49 samples (more details of our morphological analyses and molecular confirmation are given in supplementary data 1). Hence, at least in extra-Amazonian Brazil, it is possible to sort NPFW to species under a stereoscopic

microscope, allowing us to achieve large sample sizes. Transposition of the morphological technique used here to other continents should be tested. More importantly, we confirmed that the numerically most important NPFWs in our study system used several hosts, so that the most striking results presented below are based on morphological assignments confirmed by molecular data.

2.5. Multivariate qualitative analysis

We performed multivariate analyses to compare samples (crops) from different host fig species and different locations in relation to their associated wasps. We built a binary community data matrix where lines represented the samples and columns represented wasp species occurring in each sample; then we calculated the Jaccard dissimilarity indices for the data matrix and performed UPGMA hierarchical clustering (Legendre and Legendre, 1998). The cluster topology was analysed to evaluate whether samples clustered according to host species or according to locality. In addition, clustering effects of host species and locality were evaluated using distance-based permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001; McArdle and Anderson, 2001) on the Jaccard dissimilarity matrix, with 9999 resampling permutations. Since our sampling was unbalanced we also performed PERMANOVA analyses using just samples collected in *F. citrifolia* and *F. eximia* (see Table 1 for sampling effort). Analyses were performed using R 3.2.3 (R Core Team, 2017) packages *vegan* (Oksanen et al., 2017) and *ggdendro* (de Vries and Ripley, 2016).

2.6. Food web analyses

To measure linkage strength between wasp species and host plants we constructed a quantitative bipartite food web showing the abundance of NPFW species divided by the number of female fig flowers observed in each host. Plants and insects were ordered with a canonical correspondence analysis - CCA - to minimize interaction overlaps. We also plotted the two-mode projection as unweighted binary graphs in order to visually evaluate the centrality of each species in the network (Brandes et al., 2003; Martín González et al., 2010).

We estimated the network connectance to describe the number of interactions observed in the food web in relation to the maximum number of possible interactions in the matrix. Connectance varies from 0 (no interactions) to one (all species interact with each other). We measured the degree of specialization in the entire network using standardized two-dimensional Shannon entropy index (H_2). It standardizes H_2 between maximum and minimum H_2 entropy, ranging from 0 (extreme generalization) to 1 (extreme specialization) (Dormann et al., 2009).

In order to evaluate the structure of the community, we analysed nestedness and modularity indices (Bascompte and Jordano, 2006). Nestedness describes a food-web pattern in which specialist species interact with specific subsets of species that interact with the more generalist ones (Bascompte et al., 2003). The weighted NODF index describes the level of nestedness

within the community (Galeano et al., 2009) and varies between 0 (complete chaos) and 100 (completely nested).

Modules are aggregated groups of species that interact more with each other than with other species in the network. In order to test for modularity we used the QuanBiMo algorithm that identifies modules in weighted networks (Dormann and Strauss, 2014). The algorithm was allowed to run for 10^9 generations.

We compared the indices estimated for the food web with null models generated with the Patefield algorithm (Patefield, 1981), in which marginal totals are maintained constant, using 1000 replications. For H'_2 comparisons, null models were generated with the algorithm proposed by Vázquez et al. (2007) in which marginal sums and connectance are kept constant, using 1000 replications.

Network-level analyses were carried out for the complete network and for two subsets of species that have similar ecological niches: one comprising the galler species (large gall inducers and receptive-phase colonizers), and the other one comprising cleptoparasites/parasitoids. As the life history of *Aepocerus* spp. is dubious, we included these species only in the analyses with the complete network.

We used three species-level indices that describe host specialization (Dormann, 2011) to evaluate specificity of each species in the community. (1) The resource range is a qualitative index that estimates specificity based on the subset of resources exploited with a non-zero performance. It varies from zero, when a species uses all available hosts, to one, when a species occurs in a single host (Poisot et al., 2012). (2) The paired difference index (PDI) contrasts a species' strongest link with those over all remaining resources, and it was considered a robust index to differentiate specialism and generalism (Poisot et al., 2012). The index varies between 0 and 1; absolute values below 0.5 suggests generalism, while values above 0.5 suggests specialism (Poisot et al., 2012). (3) The weighted betweenness describes the centrality of a species in the network by its position between other nodes based on the weighted representation of the network (Butts, 2014). It varies from zero, indicating that the species is peripheral in the network, to one, indicating that the species is central in the network.

All analyses were performed using R version 3.2.3 (R Core Team, 2017) and the packages “vegan” (Oksanen et al., 2017), “bipartite” (Dormann, 2011; Dormann et al., 2008, 2009) and “sna” (Butts, 2014). The raw datasets used for community analyses are available in supplementary data 2.

3. Results

3.1. NPFW community structure and host utilization

We sampled 133 crops from six *Ficus* species and obtained quantitative data for 105 samples. A total of 111,555 NPFW specimens were identified to morphospecies (Table 1 and supplementary data 2). Fortytwo NPFW morphospecies belonging to eight genera were

recognized, and from those, 19 (45%) occurred in more than one host (Fig. 1, Fig. 2). *Idarnes* was the most species-rich genus observed in the fig species we sampled (Fig. 1).

Prefloral colonizers usually occurred at low abundances, representing less than 2% of the total number of NPFW collected, but represented 21% of all sampled species (9 spp.). Receptive phase colonizers were less diversified (3 spp., 7% of the sampled species, Fig. 1), but usually occurred in higher abundances (supplementary data 3). They represented between 7% (*F. trigona*) and 82% (*F. luschnathiana*) of the female individuals of NPFW emerging from figs. Most species were interfloral colonizers (25 spp., 60% of the total number of NPFW, Fig. 1) and presented a wider range of abundances (supplementary data 3). Five species (12%) belonged to *Aepocerus*, which may be receptive phase or interfloral colonizers.

Most NPFW species associated with each *Ficus* species occurred in more than one host. About 11–33% of the species occurring in each host were strictly specific (Fig. 2). However, for 12 out of 19 species that occurred in multiple hosts, the relative abundance in their main host was > 75% (Fig. 3, supplementary data 2).

The network was clearly dominated by species of genus *Idarnes*, which represented 86%–99% of all female NPFW specimens in a particular host, except in *Ficus trigona* with only 59% of *Idarnes*. Among *Idarnes*, two species were particularly abundant: the gall-maker *I. flavicollis* (If06) and the cleptoparasite *Idarnes* sp. 9 (Ic09). Both of these species occurred in all host species and were also the most abundant NPFW in several hosts (Figs. 2 and 3A, supplementary data 3).

3.2. Similarity of the NPFW fauna among hosts and locations

Although some NPFW species used several hosts, we observed that the samples analysed clustered by *Ficus* species (Fig. 4; $R^2 = 0.50$ or 0.43 depending on whether all host species were included or not, Table 2). The clustering pattern due to the sampling location and to the interaction between host species and location were significant but these effects were comparatively weaker and accounted for much less of the deviance ($R^2 = 0.04$ or 0.07 Table 2) than the clustering by fig host. Interestingly there was no clear pattern of differentiation within host fig species among regions in the UPGMA grouping of samples. Some samples from *F. luschnathiana* and *F. trigona* did not cluster together due to the low proportion of strictly specific species (Figs. 2 and 4).

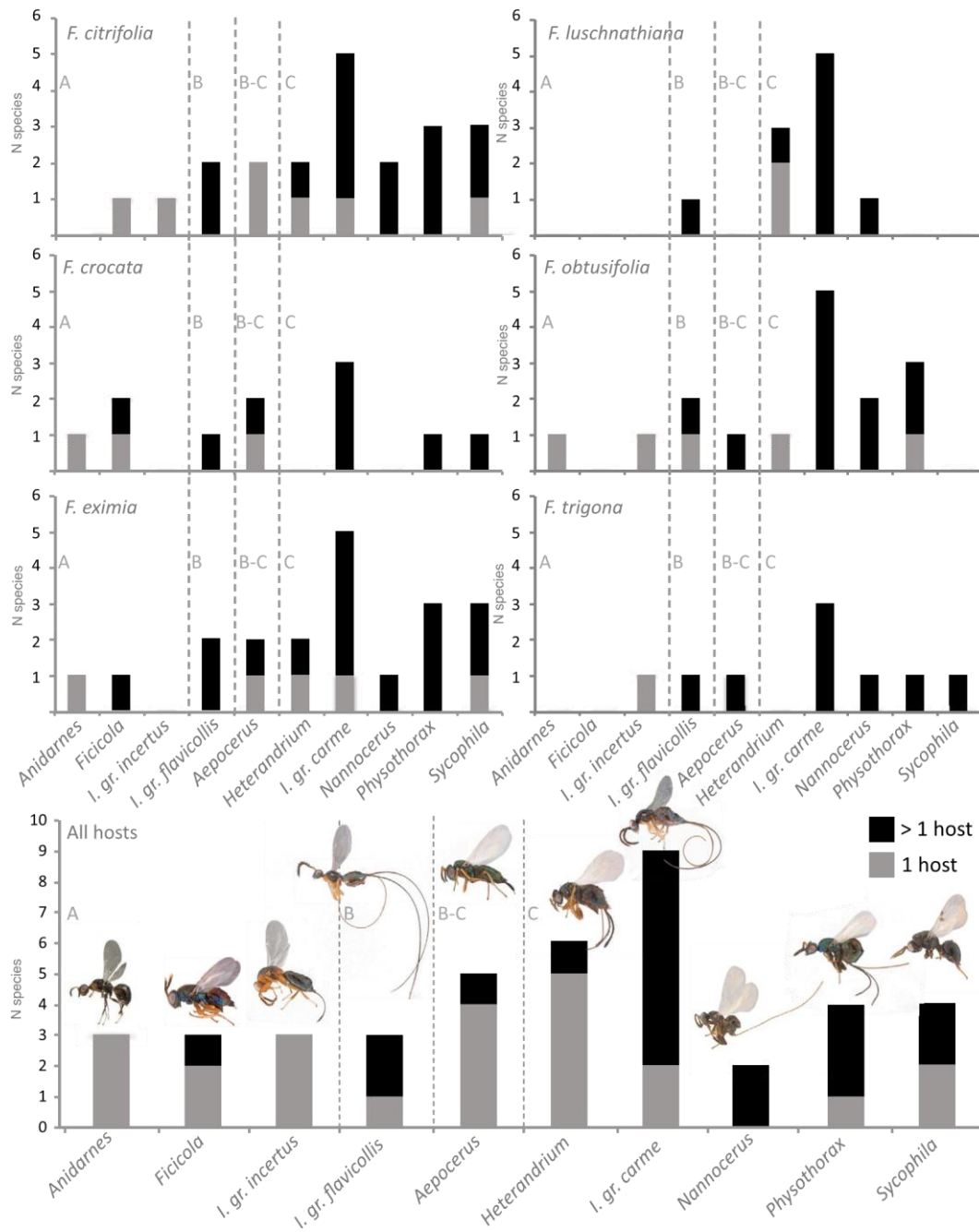


Fig. 1. Species richness for NPFW genera associated with sampled *Ficus* species. Bar colours indicate strictly specific (grey) and species that occurred in more than one host (black). Species are ordered by the fig developmental phase in which each wasp species arrive: A = Early gall inducers; B = receptive phase colonizers; B-C receptive or interfloral phase colonizer; and C = interfloral phase colonizers.

3.3. Specialization and food web structure

Quantitative food web analyses showed that the interaction between NPFW and *Ficus* sect. *Americanae* presented low connectance and high specialization H'_2 , compared with null models ($c = 0.33$; $H'_2 = 0.65$; Table 3). This pattern was also observed when investigating separately the gall-maker and cleptoparasite/parasitoid subsets (Table 3).

The weighted NODF was significantly lower than observed in null models ($wNODF = 22.7$, $P < 10^{-3}$; Table 3); hence the network was not nested. Indeed, the QuanBiMo algorithm indicated a modular structure in the network ($Q = 0.53$; $P < 0.01$), and mostly each host represented an independent module, except *F. crocata* and *F. obtusifolia* (Fig. 3B) that belonged to the same module. Analyses on the gall-maker and cleptoparasite/parasitoid subsets also showed the same modular structure (Table 3).

The species-level indices show that despite some species presenting a broad host range (Fig. 5), they are mostly specific when quantitative data was analysed ($PDI > 0.73$ for all species). In fact, 85% of the species were very specialized ($PDI > 0.9$), and only three of them showed a comparatively high centrality in the network (weighted betweenness > 0.1). Species arriving before fig receptivity were mostly highly specialized, while species arriving at receptive phase and onwards showed both slightly generalized species and extreme specialists (Fig. 5). The PDI was significantly higher than expected by the null models even for the most generalized species, except for *Sycophila* sp. 3 (Table 4), due to the low number of individuals sampled ($N = 10$ individuals).

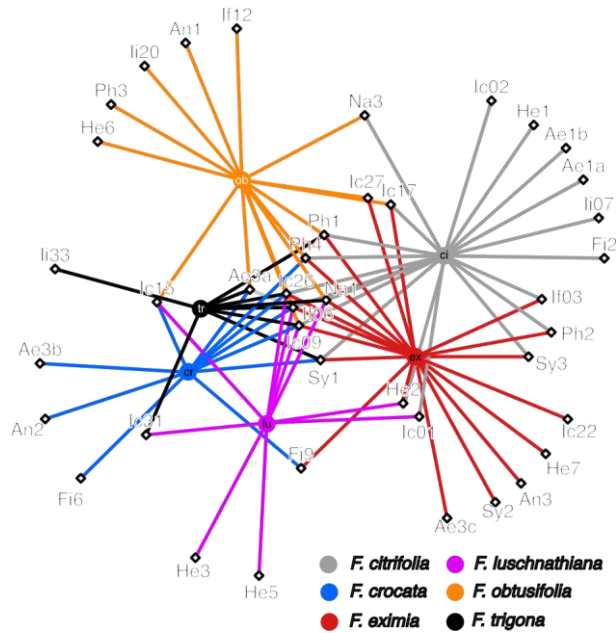


Fig. 2. Two-mode projection network of NPFW occurring in *Ficus* species sampled (unweighted). Abbreviations for hosts: ci = *F. citrifolia*; cr = *F. crocata*; ex = *F. eximia*; lu = *F. luschnathiana*; ob = *F. obtusifolia*; tr = *F. trigona*. Abbreviations for wasp genera and species groups: Ae = *Aepocerus*; An = *Anidarnes*; Fi = *Ficicola*; He = *Heterandrium*; Ic = *Idarnes* group carne; If = *Idarnes* group flavicollis; Li = *Idarnes* group incertus; Na = *Nannocerus*; Ph = *Physothorax*; Sy = *Sycophila*.

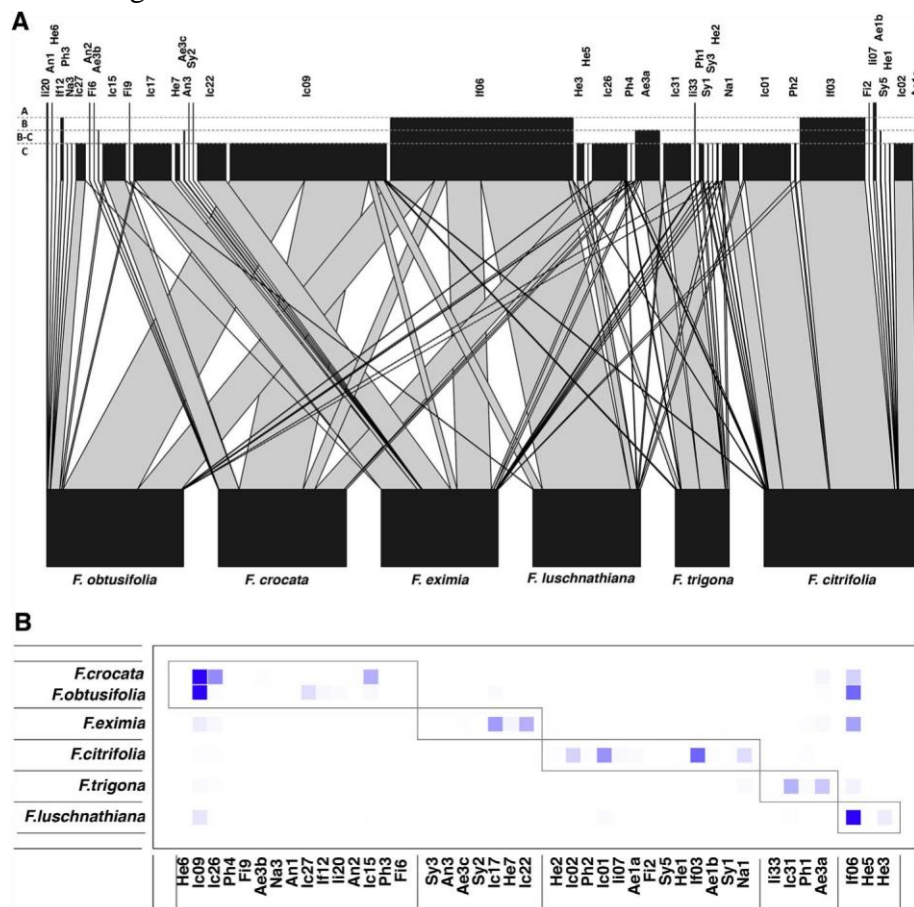
4. Discussion

In this study we described the community structure and patterns of specialism/generalism in NPFW associated with *Ficus* section Americanae. This is the first study providing a large quantitative dataset on all the NPFW emerging from a regional community of *Ficus* species. Several species were not strict specialists and emerged from at least two host species. Nevertheless, a majority of NPFW species were specialists and emerged from a single *Ficus* host. Some species, despite occurring in more than one host, showed higher abundance in a particular host species, which may indicate a strong host preference or a more efficient reproduction in this host. A few NPFW were produced in similar numbers by two hosts, and the two most abundant species, a receptive phase colonizer (*Idarnes flavicollis* – If06) and a cleptoparasite (*Idarnes* group carne sp. 09 – Ic09) occurred in all hosts.

We observed that the different host species showed variation in the composition of their associated NPFW community. The community of NPFW associated with *F. citrifolia* (the most abundant host species in all habitats - Coelho et al., 2014) followed the classical pattern as understood from other regions of the world, with an assemblage of mostly specialist species, including typical proportions of the different functional groups (Segar et al., 2013b). The NPFW

community associated with *F. luschnathiana* was the only community dominated by a single species, the generalist gall-maker *Idarnes flavicollis* (If06), representing more than 80% of the emerging NPFW individuals. *Idarnes flavicollis* emerged in much larger numbers from several other fig species (supplementary material 2). We propose that *F. luschnathiana* is present at such low densities (Coelho et al., 2014) that it can only sustain a very limited and generalist complement of NPFW fauna, leaving much empty space (non-utilized flowers). Moreover, a lower abundance of pollinators and gall inducers may contribute to a reduced fauna of cleptoparasites and parasitoids.

Fig. 3. A) Bipartite network of NPFW and their hosts. Line widths represent the relative abundance of each species in each host (number of specimens/number of flowers analysed). Box heights in the higher level represent the phase in which each wasp species arrive: A = Early gall inducers; B = receptive phase colonizers; B-C receptive or interfloral phase colonizer; and C = interfloral phase colonizers. B) Quantitative module analysis of the network generated by the QuanBiMo algorithm. Color intensity represents the relative abundance of each species (columns) in their fig hosts (rows). Rectangles indicate modules. Abbreviations for genera follow Fig. 2.



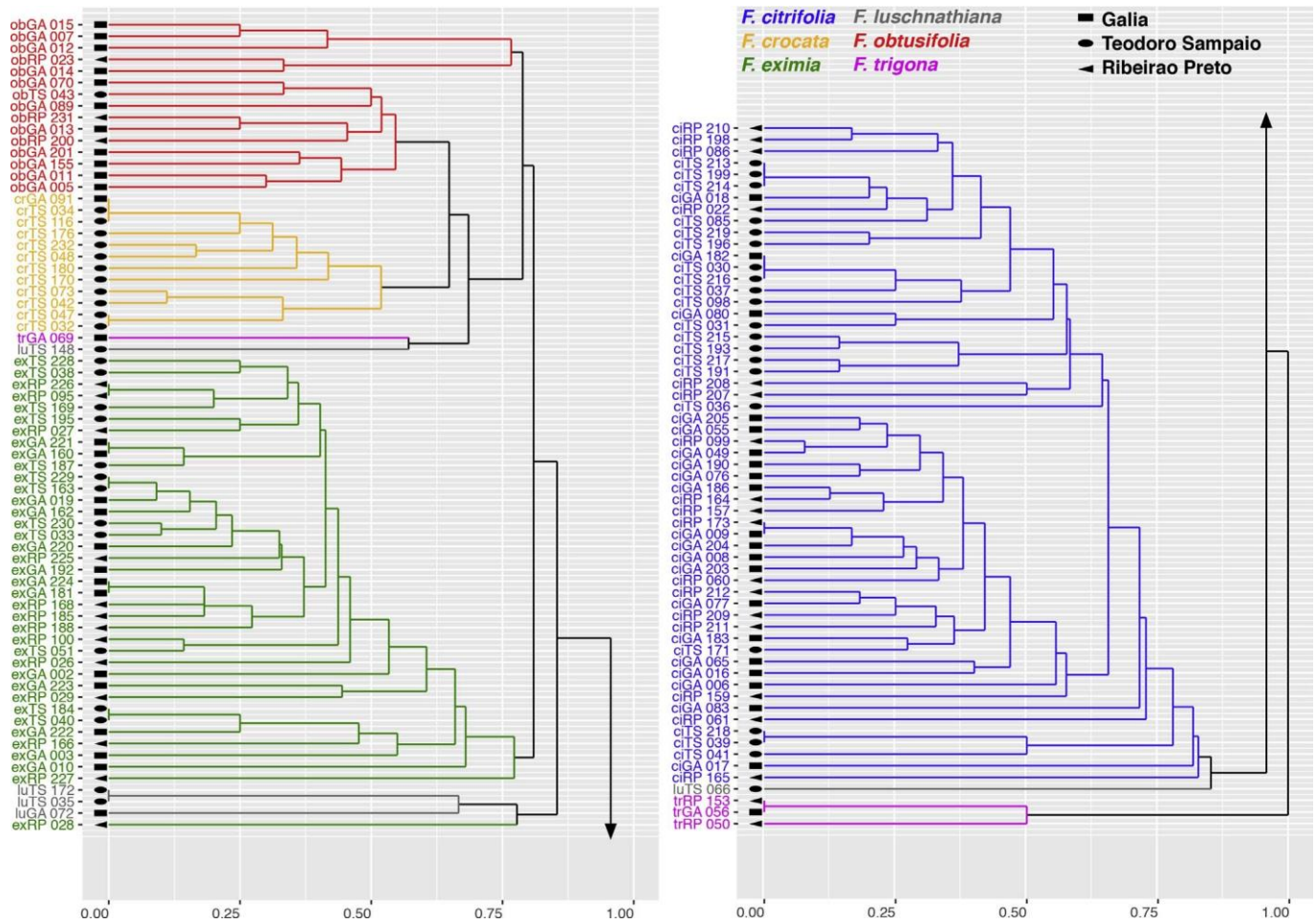


Fig. 4. Cluster analysis (UPGMA) for Jaccard similarity index of NPFW species present in each crop. Labels include host species (ci = *F. citrifolia*, cr = *F. crocata* ex = *F. eximia*, lu = *F. luschnathiana*, ob = *F. obtusifolia* and tr = *F. trigona*) and sampling subset (GA = Gália, RP = Ribeirão Preto and TS = Teodoro Sampaio)

Table 2 PERMANOVA analysis on the Jaccard distance from the presence/absence matrix of NPFW species in each sample (crop).

	Df	F Model	R2	Pr
All hosts				P =
Host	5	28.45	0.50	0.0001
Area	2	5.63	0.04	P =
				0.0001
Host:Area	7	1.81	0.05	P =
				0.0007
Residuals	116		0.41	
F. eximia and ifolia				P =
F. citr	1	81.12	0.43	0.0001

Host				
Area	2	6.20	0.07	P = 0.0001
Host:Area	2	2.72	0.03	P = 0.0105
Residuals	89		0.47	

This interpretation is supported by total lack of the highly specific early gallers in *F. luschnathiana* while all other figs hosted at least one species. *Ficus trigona* presents a somewhat more balanced community, with two abundant NPFW species (one of which was a specialist) and the presence of an early galler. Nevertheless, the proportion of NPFW comparatively to pollinators was very low suggesting that limited colonization by non-specialist NPFW resulted in reduced overall parasitism. *Ficus obtusifolia* presented a diversified community of NPFW with two early galler species. However, the community was dominated by two generalist species representing 87% of all NPFW. *Ficus eximia* and *F. crocata* presented more balanced communities with three abundant species (> 10% of the NPFW) out of which only one was a generalist (Fig. 3A). *Ficus eximia* and *F. crocata* were the largest and tallest trees that we sampled (Berg and Villavicencio, 2004). They usually reach large sizes, consequently producing large crops. We may suggest that large size compensated for low density in allowing the survival of a diversified community of

Network indices, standard scores, and p-values calculated for the complete network and for the gall-maker and parasites (cleptoparasite + parasitoid) subsets. *Aepocerus* species were excluded from the analysis of gall-maker and parasitic subsets since information on their natural history is inconclusive (see topic 2.2).

Table 3

Indices	Whole Network			Gall-maker			Parasites		
	value	z	p-value	value	z	p-value	value	z	p-value
Connectance	0.33	-34.71	< 0.001	0.26	-15.58	< 0.001	0.37	-31.68	< 0.001
H' ₂	0.65	5.36	< 0.001	0.89	2.60	< 0.001	0.74	6.87	< 0.001
Modularity (Q)	0.53	89.74	< 0.01	0.34	123.41	< 0.01	0.53	254.06	< 0.01
wNODF	22.70	-13.11	< 0.001	17.30	-14.32	< 0.001	25.80	-9.81	< 0.001

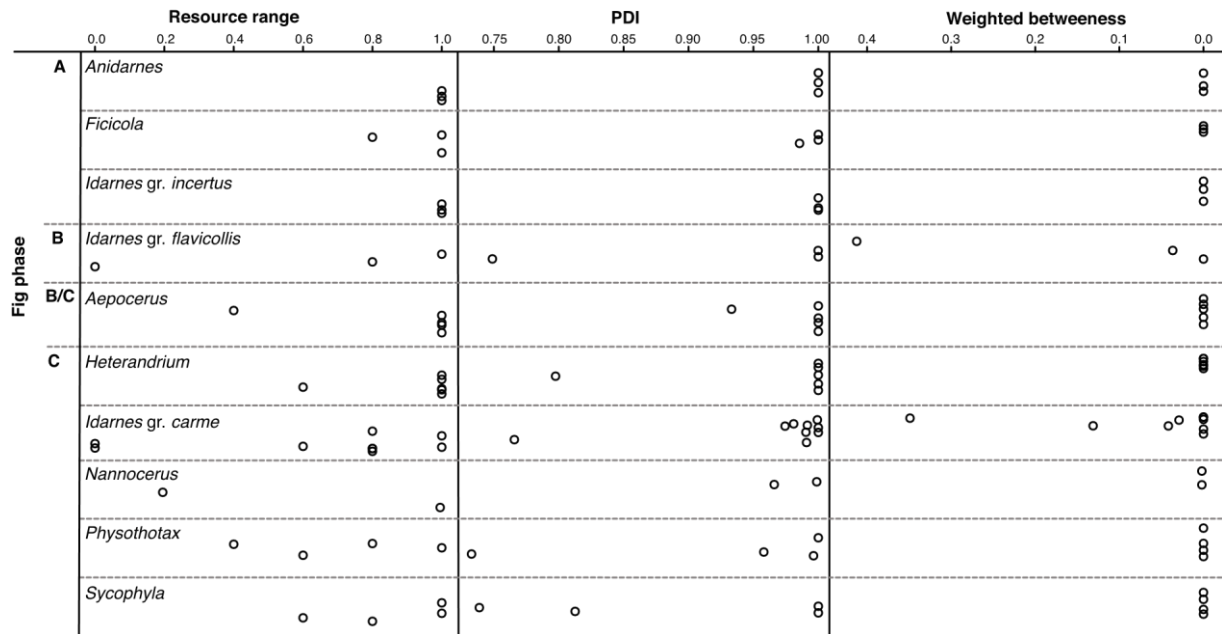


Fig. 5. Species-level indices calculated for NPFW species, sorted by genera and colonization phase. A = Early gall inducers; B = receptive phase colonizers; B-C receptive or interfloral phase colonizer; and C = interfloral phase colonizers.

Table 4 Paired difference index comparison with null models. Ae = Aepocerus; He = Heterandrium; Ic = Idarnes group carne; If = Idarnes group flavicollis; Na = Nannocerus; Ph = Physothorax; Sy = Sycophila.

Species	PDI	z-score	P-value
Ae3a	0.93	8.80	< 0.001
He2	0.8	3.45	< 0.001
Ic09	0.77	26.4	< 0.001
Ic26	0.97	20.41	< 0.001
If06	0.75	21.36	< 0.001
Ph1	0.73	4.09	< 0.001
Sy1	0.74	1.80	< 0.003
Sy3	0.81	0.32	=0.3
Na1	0.97	15.62	< 0.001

NPFW. Indeed, while there is no published general overview of the diversity of NPFW communities, gynodioecious figs, which are often small in size, present simpler communities than the larger monoecious species. Hence we may suggest as a working hypothesis that aspects of a *Ficus* species density and size may play a role in determining the structure and specificity of their NPFW community. We may wonder how much local conditions – notably variation in local density of the different *Ficus* species – affect the composition of NPFW communities and patterns of host sharing.

This study presents data from three different locations spanning 500 km, a quite large distance, but quite limited at the scale of Brazil. The local context is one of extreme recent habitat loss through deforestation (Coelho et al., 2014). We may therefore wonder whether the composition of NPFW communities and especially the presence of generalist wasp species is a product of recent events or whether it has to be explained by processes occurring over larger time scales. At the end of the 19th century Fritz Müller collected fig wasps on what he thought was a set of six distinct *Ficus* species belonging to section *Americanae* in Blumenau (Santa Catarina state, south of São Paulo state). According to Müller, *Idarnes flavicollis* was collected from five of these host species (Müller and Möller, 1915 p. 1073). We therefore conclude that *I. flavicollis* was already using several hosts over 100 years ago, before major deforestation. Hence the particularity of the NPFW communities associated with *Ficus* section *Americanae* in São Paulo state predates major human perturbation. Our collections from Amazonian Brazil and Costa Rica suggest higher NPFW specialization (Farache, Cruaud & Rasplus, unpublished data). Therefore, the situation in Southern extra-Amazonian Brazil could be unusual and may be explained by 1) recent population expansions due to Pleistocene climatic oscillations (< 2.6 Ma; Cohen et al., 2013; Francoso et al., 2016); 2) particular conditions associated with the region such as a globally relatively dry climate and the effect of oscillations of the regional climate or 3) the recent diversification of section *Americanae* (Cruaud et al., 2012). The fauna of NPFW associated with section *Americanae* is unique due to the overwhelming predominance of the subfamily Sycophaginae (59%–99% of NPFW in a host belonging to genus *Idarnes*). This is probably best explained by the recent diversification of this section. The presence of an *Idarnes* species in Dominican amber indicates that the Sycophaginae are associated with *Ficus* section *Americanae* for at least 15–20 Ma (Farache et al., 2016).

In terms of species richness, we observed a high number of parasitic species (cleptoparasites and parasitoids) compared to gall inducers (prefloral and receptive-phase colonizers). In terms of wasp abundance, interfloral colonizers were numerous, but receptive-phase colonizers were also highly frequent and sometimes outnumbered other species groups in the community. Prefloral colonizers were always observed in low abundance. They probably have their oviposition capacity constrained by flower development, as pistillate flowers are not completely developed at this phase (Basso-Alves et al., 2014). Therefore, they may use mechanisms of gall induction that differ from the one shown by receptive-phase colonizers. Wasps colonizing *Ficus*

section Americanae at prefloral phase have relatively thicker and shorter ovipositors. They induce large galls that may occupy a considerable volume of the fig lumen, diminishing the space for the development of seeds and of other wasps. The induction of large galls is usually correlated to small brood sizes (Bronstein, 1999). Receptive-phase colonizers arrive concomitantly with pollinators, and compete with them for oviposition sites (Cook et al., 1997; Elias et al., 2012). Species that oviposit during the receptive phase might use chemical cues similar to those attracting pollinators to their host fig species, as experimentally demonstrated for *F. racemosa* (Proffit et al., 2007). Since the fig receptive phase only lasts for a few days, and oviposition and gall-inducing mechanisms in receptive flowers are complex (Elias et al., 2012), this feeding habit may be constraining and restricted to a few groups. *Idarnes flavicollis* is to our knowledge the first documented case of a galler at receptivity developing in large numbers in several host figs. The emission of species-specific volatile compounds peaks at fig receptivity, allowing recognition of the host fig by their specific pollinators. As *I. flavicollis* develops in large numbers in several host figs, we may propose that the differences among species in receptive fig odours is a trait that allows pollinating wasp to locate their specific host, but it is in no way a constraint that would prevent a wasp from using several hosts. Some volatile compounds in the fig scent blend are common among different fig species (Hossaert-McKey et al., 2016) and *Idarnes flavicollis* might use them to identify its hosts. Interfloral phase is relatively much longer than the receptive phase (about 30–40 days), thus the temporal window to locate trees is probably less constraining for species colonizing figs during the interfloral phase. We suggest that this larger time frame for oviposition may open opportunities for diversification of species and of feeding regime. Indeed, interfloral colonizers can be cleptoparasites or parasitoids (Cook and Rasplus, 2003; Kjellberg et al., 2005), yet some interfloral colonizers show high plasticity in their feeding regime and a few species can even use seeds as a larval resource (Pereira et al., 2007; Wang et al., 2014).

Most early gall-maker species observed in this study were host specific, a feature consistent with observations in systematic studies of gall-inducing Neotropical NPFW clades (Farache et al., 2013, 2017). For the other groups, variation in specificity was high, and species ranged from strictly specific to generalist, without a clear taxonomic pattern. Probably a multitude of factors may influence the host range in NPFW. For instance, network analyses suggested that both gall-maker and parasitic subsets are specialized. Further taxonomic and evolutionary studies within each lineage are needed in order to understand host specialization patterns in fig wasps.

Although fig wasp species using more than one host were frequent, 55% of the NPFW species occurred in only one *Ficus* species. Overall, host-plant specificity tends to be higher in concealed herbivorous guilds than in externally feeding insects. Some studies reported 50% or more specialist species in leaf-chewing, sap-sucking or seed-eating insect communities (Diniz and Morais, 1997; Novotny and Basset, 2005; Odegaard, 2000; Thomas, 1990), and therefore host specialization may rise in antagonistic relationships. Indeed, gall-inducing insects – as endophytic organisms – seem to be highly host-specific, yet sampling difficulties and lack of

quantitative data hinder the understanding of their community structure (Bourg and Hanson, 2014; Carneiro et al., 2009).

Non-pollinating fig wasp species occurring in more than one host species were observed in previous studies, yet analyses on the whole community of NPFW are lacking for most biogeographic regions. Our observations on the Neotropical fauna associated with *Ficus* section *Americanae* are congruent with previous molecular studies in the same *Ficus* section (Marussich and Machado, 2007), in which ~23% of the species occurred in more than one host. In the Afrotropical region, species occurring in several hosts were observed in interfloral colonizers (McLeish et al., 2010). Studies in a NPFW genus (*Philotrypesis*) in Asia show that they may be mostly host specific, with the exception of two species that are associated with the hosts *F. microcarpa* and *F. benjamina* (Zhou et al., 2012). *Sycophaga* is distributed through the Old World and most species seem to be host specific (Weiblen and Bush, 2002) but an internal receptive phase colonizer, *Sycophaga silvestrii*, seem to occur in two hosts in Africa (Kerdelhué et al., 2000). For both *Philotrypesis* and *Sycophaga*, the documented cases of occurrence in multiple hosts probably indicate marginal use of an alternative host by a specialist wasp. Thus, the NPFW seem to be less specific in the Neotropical region than in the Afrotropical region, and most specific in the remaining tropical Old World communities. Comparisons between biogeographic regions are still limited by (1) the lack of quantitative data and (2) the lack of sampling of the whole community.

The NPFW-*Ficus* network was highly un-nested, and instead significantly modular. This structure may be expected for specific antagonistic relationships such as endophytic insects - host plants and host parasitoid interactions. Such structure indicates that the community subset associated with each host species poorly interact with other groups, indicating specialization (Dormann and Strauss, 2014). Although some species (interfloral colonizers especially) showed a wide host range, they tended to show asymmetric ovule occupancy rates in different hosts, which indicate preference or differential development success according to host. Moreover, cluster analyses showed that samples from the same host fig were more similar to each other irrespective to their geographic locations. This pattern indicates that although NPFW species are shared among hosts, the faunal composition is idiosyncratic for each host, suggesting specificity. Studies on host choices by insects show that multiple factors govern the evolution of host specificity. Allelochemicals, feeding habits, nutritional chemistry and plant morphology are major determinants of host preference and specificity (Thompson and Pellmyr, 1991). According to the current knowledge, host preference in NPFW seems to reflect the selection imposed by the broad morphological and physiological diversity of fig trees. *Ficus* species vary greatly in fig size, fig wall thickness, number and size of pistillate flowers, and chemical scent cues (Proffitt et al., 2007). Due to the constraints imposed by these plant traits, NPFWs may oviposit preferentially in a fig tree to which they are better adapted. Indeed, NPFW species exhibit variable ovipositor lengths and structures, which correlate both with trophic ecology and phenotypic characteristics of the figs, such as wall thickness (Elias et al., 2017; Farache et

al., 2016; Ghara et al., 2011; Zhen et al., 2005). Thus, such morphological variation may be related to host specialization.

Ecological specialization theory suggests that specialists should outperform generalist species due to the presence of specific adaptations that allow them to exploit a particular host. Generalist species are adapted to develop in a suite of hosts, but may not present a combination of characteristics that allow them to exploit all species they use efficiently – “the jack of all trades is a master of none” (Forister et al., 2012; Futuyma and Moreno, 1988). However, this does not appear to be the case for the gall-maker *Idarnes flavicollis* and the cleptoparasite *Idarnes* sp. 9. Both species occurred in all fig species available, and were the most abundant species in some of their host plants. Therefore, we observed that, at least in this Neotropical NPFW communities, more generalized species may have a higher ovule occupancy rate in some hosts than strict specialists. This observation may be related to the scarcity of the resources fig wasps utilize. The low abundances of fig trees in extra-Amazonian Neotropical forests (Coelho et al., 2014) coupled with the asynchrony of fig fructification result in both spatial and temporal constraints for NPFW to find their hosts at the right stage of development. In this context, generalist species may easily find suitable figs to lay eggs, which may explain the high abundances of *I. flavicollis* and *I. sp. 9*.

5. Conclusion

Specialization in natural communities and guilds has rarely been quantified due to the difficulty to generate informative networks and to obtain representative samples of whole communities (Ballantyne et al., 2015; Forister et al., 2012). The NPFW associated with *Ficus* show diverse life history traits and represent a suitable and informative model to quantify specialization since it is possible to obtain a quantitative measure of the relative ovule occupancy rate of each species in the whole community. We observed that despite the occurrence of wasps that are not restricted to a single host species, the community of NPFW associated with *Ficus* section *Americanae* shows a specialized structure. This study opens a new perspective in our understanding of fig wasp communities. Further analyses in other biogeographic regions will allow a better understanding of the evolution of host specificity in symbiotic insect-plant relationships.

Morphological identification of fig wasps at species level can be challenging (especially for pollinators), and the lack of a taxonomic background may hinder the understanding of fig wasp communities.

Nevertheless, while sampling a local community, morphological identification can be achieved, and the sampling of cryptic species may be relatively unlikely. Indeed, Darwell and Cook (2017) have shown that, in Australia, cryptic NPFW species are largely allopatric. Our molecular analyses in *Idarnes* discussed in supplementary material show that when the same morphospecies was found on multiple hosts, it represented the same biological species. Yet a thorough molecular sampling would be necessary to identify cryptic species, especially if they

are rare. Further, care should be taken to avoid considering coding NUMTS or heteroplasms as cryptic species (Cruaud et al., 2017).

In this paper we described how Neotropical NPFW communities are structured and their intricate patterns of specialization and generalism. We suggest that these patterns may vary over continents, and even within the neotropics. Therefore, we encourage further quantitative sampling in other biomes and biogeographic regions in order to test this conjecture.

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