Unveiling the trap-nesting bees and wasps’ fauna (Hymenoptera: Apocrita) and associated organisms of the Jardim Botânico do Rio de Janeiro, Brazil

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Abstract. Urban areas, as cities, are frequently overlooked as refuges for the native fauna. However, these places may support several species and house relevant biodiversity contributing to important ecosystemic functions. Wasps and bees (Hymenoptera: Apocrita) are important faunistic elements acting as predators/parasitoids and pollinators, respectively. Therefore, they must be surveyed and inventoried for conservation purposes, especially in cities located in the Atlantic Forest domain, a ravaged Brazilian biome. Accordingly, this study presents a species list of trap-nesting bees and wasps that occurs at the Jardim Botânico do Rio de Janeiro. The survey was conducted using the trap-nest method. Three types of trap-nests were offered totaling 1,038 traps: rubber hose, bamboo cane and plastic straw. The plastic straw traps were the most effective followed by the bamboo cane ones at attracting nesting wasps and bees. Between April/2017 and February/2019, 12 foundress species built nests: Tetrapedia curvitarsis (Apidae) (33 nests), Hylaeus sp. (Colletidae) (6), Auplopus cf. rufipes (Pompilidae) (5), Trypoxylon sp. (Crabronidae) (4), Pachodynerus nasidens (Vespidae) (3), Auplopus cf. brasiliensis (Pompilidae), Megachile benigna and Megachile sp. (Megachilidae), Euglossa pleosticta, Euglossa sp., and Eufriesea sp. (Apidae) and Penepodium sp. (Sphecidae) all with one nest. Also, four natural enemies were recorded: Chaenotetrastichus neotropicalis (Eulophidae), Gasteruption brachychaetum (Gasteruptiidae), Caenochrysis crotonis (Chrysididae) and Amobia sp. (Diptera: Sarcophagidae). Most bee nests were restrained to the matrix forest and nearby areas, whereas wasps built their nests predominantly in open areas. Regarding seasonality, bees tended to nest in the summer and early autumn influenced by mean temperature, in contrast of wasps that nested in late autumn, winter and spring, influenced by pluviosity. Besides many rare species, which suggests environmental disturbance, the Jardim Botânico do Rio de Janeiro showed a fairly diverse fauna and shows how forest fragment in cities may harbour important representatives of the native fauna.

Key-Words. Atlantic Forest; Insects; Parasitoid; Pollinator; Urban ecosystem.

INTRODUCTION

Habitat loss is a main problem throughout the world (Jacobson et al., 2019). Among the many affected biomes due to deforestation, mainly caused by urbanization processes, stands the Atlantic Forest with less than 30% of its natural cover (Rezende et al., 2018). Due to the colonization of Brazil during the 1500ys, the Atlantic forest was the first biome to suffer under the impact caused by the cities’ growth (Rezende et al., 2018). Because it is a hotspot of biodiversity (Myers et al., 2000), its fauna and flora are severely endangered by those impacts (Colombo & Joly, 2010). Furthermore, several ecosystemic functions are under disturbances due to forest loss such as water resources, thermic regulation of the environment, biological control of several organisms and pollination (Stangler et al., 2015), which directly affects human populations.

Hymenoptera, the order of insects comprehending bees, wasps and ants, is known for providing several ecosystemic functions as biological control by wasps acting as parasitoids and predators of insects and other invertebrate populations (O’Neill, 2001; Hanson & Gauld, 2006), and pollinators of flowering plants in the case of bees (Michener, 2007), that pollinate almost 85% of angiosperm species (Roubik, 1995). Due to those traits, Hymenoptera is an important group indicative of environmental quality (Staab et al., 2018).

Hymenoptera presents part of its species building their nests in pre-existing cavities (Batra, 1984; O’Neill, 2001). This behaviour appeared several times in the order, and consists basically on the adult female building nests concealed...
MATERIAL AND METHODS

The study area

Historically, the area where the JBRJ stands was built along a powder gun factory (Bediaga, 2007). During this period, the JBRJ was focused on research aiming the acclimation of exotic plants to the Brazil's tropical environment, mainly tea shrub crops of *Camellia sinensis* L. (Kuntz), besides other monocultures aiming exportation, and thus the area endured deforestation and introduc-

ion of alien species for many years.

Nowadays, the JBRJ (22°58′14″S, 43°13′18″W) comprehends 137 hectares, from which 54 are known as arboretum, with about 8,000 native and exotic species from several Brazilian biomes, as well as from other parts of the world. This part of the park has an intense movement of visitors and the JBRJ staff. The remaining 83 hectares are contiguous with the Parque Nacional da Tijuca and the Parque da Cidade (Rangel & Neiva, 2013). The area is covered by a Dense Ombrophilous Forest (Freitas & Carrijo, 2016) inserted at the urban area of the Rio de Janeiro city (Fig. 1). Its climate is classified as Am following the Köppen Climate Classification System, with mean annual rainfall of 1,278 mm and mean annual tempera-

ture of 23.2°C. The Figure 2 displays mean temperatura and rainfall taken during the months the study was performed. Climatic data was obtained from the Jardim Botânico Station under the responsibility of the Sistema Alerta/Geo-Rio. This climatic station records temperature and pluviosity data each 15 minutes during the whole month. The coldest months were May to August and the hottest ones were December to February. Regarding the rainfall, the rainiest months were April, June, November and February.

Field methods and data collection

The study was conducted from April/2017 to February/2019. For sampling bees, the trap-nest method was employed (Krombein, 1967). Traps made of bamboo cane internodes and rubber hose with of 22 cm and 15 cm in length, respectively, were set (375 of each material). In each sampled point, 15 traps of bamboo cane with the diameter ranging from 0.4 to 2.4 cm and 15 nests with three diameter classes were placed in one plastic bottle with its neck and bottom taken off for ease water flow from away of the traps when raining. Additionally, 15 traps made of rubber hose, encompassing three diamete-

classes, 1.27 cm, 0.95 cm and 0.79 cm, were also set in plastic bottles. Both types of traps are displayed in Fig. 3. These traps were similar to those used by (Oliveira & Gonçalves, 2017) and hang in three branches with nylon ropes 3 m from the ground in shady places. The bamboo cane traps and rubber hose traps were placed together in 25 points, each of them considered one sample unit (Fig. 3A). These points stood apart from one another by 125 m approximately, distributed in five transects with five points each, spanning from the forest matrix to the
arboretum, nearby the Jardim Botânico Avenue. Trap-nests made of plastic straws were offered as well, set in wooden boxes in three points (T2P1, T3P3 and T5P5, Fig. 3B), for assessing the bees that use small diameters along the matrix forest to the arboretum (Fig. 1). Each of these three points with 96 traps with 0.5 cm of diameter. A total of 1,038 cavities was offered for the bees and wasps in this study.

Traps were inspected weekly and finished nests taken bi-weekly to the Laboratório de Hymenoptera at the Museu Nacional do Rio de Janeiro (HYMN) for analysis. In order to maintain the number of nests constant in the field, collected nests were replaced by empty ones with the same characteristics. In the laboratory, the nest entrances were closed with organza fabric fasten with rubber strings to hinder emerged individuals to flee and eventual parasites to attack nearby nests. Some nests were open some days after collected for architecture study. The nests were analysed under the stereomicroscope, photographed and sized.

Specimens emerged were sexed, pinned and labelled and the species identification was made by using the following keys: Mitchell (1930), Dreisbach (1963), LaSalle (1994), Menke & Fernández (1996), Willink & Roig-Alsina (1998), Hanson & Gauld (2006), Michener (2007), Nemésio (2009), and Marinho et al. (2019). Additionally, experts confirmed the identifications as stated in the acknowledgement section.

**Data Analysis**

For data analysis purposes, the matrix forest is considered the transect 1 and 2, encompassing points in and near the forest matrix; whereas transects 3, 4 and 5 is considered the arboretum. The occupancy rate of trap-nests was obtained dividing the number of collected nests by the number of nests offered multiplied by 100. The data normality was evaluated using the Shapiro-Wilk test. For assessing the influencing of temperature and pluviosity on nesting activity, the Spearman correlation test was performed. These analyses were carried out in the R language (R Core Team, 2019) through RStudio version 1.1.463.
Abundance data was displayed using a Whittaker plot (ranking-abundance graph) regarding all the periods that the traps were maintained in the field. The jackknife 1 species estimator was used to estimate the number of species in the area based on species presence-absence data, and the ACE species estimator was used for estimating the number of species based on their abundance (Magurran, 2013). Each nest was considered as one individual for purpose of analysis. The graphs were made using the ggplot2 package in the R platform (Wickham, 2016).

Caveats about data gathering

Collections was intended to occur during April/2017 to August/2018. However, the dread accident that occurred to the Museu Nacional on September 2nd, 2018 (Zamudio et al., 2018) caused the loss of all specimens and nests collected at that point. The traps, therefore, were maintained in the field to try recovering the vouchers of this study between October/2018 and February/2019. Table 1 summarise these two periods. There was no inspection of the traps in September/2018.

Data on mortality of the nesting species will be presented at the extent it was observed until the data that the museum suffered the accident. Most nests had bees and wasps yet-to-be emerged, which constrained the possibility to observe individuals diapausing from those dead by natural conditions. Despite the material loss, a huge effort was made to narrow down as much as possible the identification of the species presented, and the authors acknowledge the experts that helped us in this endeavour in the pertinent section.

RESULTS

Species composition, natural enemies and nest distribution across the area

From the 1,038 cavities offered in the JBRJ, 58 nests of bees (Anthophila) and wasps (Apoidea and Vespoidea) were collected, with an occupancy rate of 0.05%. These nests were built by 12 foundress species: seven of bees, comprehending the families Apidae, Colletidae and Megachilidae with 44 nests built; and five wasps’ species, represented by the families Pompilidae, Vespidae, Sphecidae and Crabronidae with 14 nests built (Tables 1-2 and Fig. 4).

Overall, 77 specimens emerged until September/2018 (Table 1) from 69 brood cells. In the first period (April/2017 to August/2018), considering the free-living adult Hymenoptera, 18 bees’ specimens and 16 wasp’s specimens spawned from the nests, and 43 specimens of brood parasites. Fifteen nests of T. curvitarsis Friese (Apidae), and the nests of Euglossa sp. (Apidae), Eufriesea sp. (Apidae) and Hylaeus (Colletidae) were lost in the fire, with bees still in the brood cells. Only one female of M. benigna Mitchell (Megachilidae) emerged from the nest with three brood cells. The cell in the middle were empty, but with the full load of pollen and nectar. The outermost cell had a larva in the first instar, but it died dried, probably for the unsuitable reclosure of its...
cell in the laboratory. Two immatures in pre-pupa stage of *A. cf. rufipes* (Banks) were lost in the fire, as well as the nests of wasps collected until August/2018.

After the Museu Nacional’s accident, 16 more nests were collected between October/2018 to February/2019 (Table 1), and from those, 11 individuals emerged from 53 brood cells. Only two bees, and seven wasps spawned from the nests collected in that last period. Additionally, two brood parasites emerged (Tables 1 and 2). Most cells were attacked by fungi or the specimens were dead for unknown causes hindering the spawn of adults.

During the period of April/2017 to August/2018, *Chaenotetrastichus neotropicalis* Marinho, Costa & Vivallo (Chalcidoidea: Eulophidae) attacked two cells of *Auplopus cf. brasiliensis* (Dreisbach) (Pompilidae), but only the specimens of one cell were able to emerge, all of them females; one *Gasteruption brachychaetum* Schrottky wasp (Evanioidea: Gasteruptiidae) attacked two cells of *Hylaeus* sp. (Colletidae). After the fire, during October/2018 to February/2019, *Caenochrysis crotonis* (Ducke) (Chalcidoidea: Chrysididae) attacked one cell of *Auplopus cf. rufipes*; and the fly species *Amobia* sp.
(Diptera: Sarcophagidae) that attacked three cells of *Hylaeus* sp., where four puparia were later encountered in the nest, but only one fly emerged. These information are summarised in Table 2.

The most abundant bee species was *T. curvitarsis*, and the most abundant wasp species was *A. cf. rufipes* (Fig. 5).

The number of species estimated for the JBRJ by the estimators Jackknife 1 and ACE was 19.65 and 23.39 (Fig. 6), respectively, which means that the number of trap-nesting species sampled in the area was between 64.13% and 76.33% of the total pool. Most species recorded were represented by rare species in the area (i.e., built only one nest). Spatially, most nests were collected in the transects in the forest matrix and nearby (Fig. 7). *Tetrapedia curvitarsis* and *Hylaeus* sp. built nests exclusively in the matrix forest, whereas wasps’ nests were built predominantly in the open areas of the arboretum (Table 1).

### Seasonality of nesting activity

The main period of bee nesting was between January and April, when *Tetrapedia* and *Hylaeus* concentrated their activity. *Megachile benigna* had its nest collected in October/2017, whereas the Euglossini species had their nests collected in May/2018. Bees seemed to repeat the pattern in 2019, when nests were collected in January and February (Fig. 4).

On the other hand, wasps had two seasons of nesting, comprehended between April/2017 to July/2017 and between December/2017 to February/2018 (Fig. 4). *Auplopus cf. rufipes* nested during April, May and September in 2017 and in November/2018. The other *Auplopus* species collected, *A. cf. brasiliensis*, nested only in July/2017. *Penepodium* sp. nested in December/2017, *Trypoxylon* sp. in January/2018, and *P. nasidens* in February/2019.

Regarding the climatic factors assessed, the trap-nesting assemblage activity had a positive correlation with temperature, according to the Spearman correlation test ($R = 0.6, p = 0.0023$), but not with rainfall ($R = 0.2, p = 0.36$). When taking bees and wasps nesting data apart, nesting activity was positively correlated with temperature for bees ($R = 0.69, p = 3e-04$), but not for the wasps ($R = 0.22, p = 0.32$); on the other hand, wasps’ activity was positively correlated with rainfall ($R = 0.46, p = 0.025$), but not bees’ activity ($R = 0.11, p = 0.63$).

### Nest substrate

The type of trap-nest most used by the nesters was those made with plastic straw due to *Tetrapedia curvitarsis* and *Hylaeus* sp. that built their nests exclusively in those traps. The remained species nested in the bamboo cane traps, and only one species – *Auplopus cf. brasiliensis* – used the traps made of hose.
Nest intruders

Besides the trap-nesting Hymenoptera, undesirable nesters (those that lodged into the traps, but were neither solitary free-living Hymenoptera, nor any species associated with it) also occupied the traps set in field: many species of ants, which comprised the major source of nest occupancy, hindering bees and wasps for building their nests; several insects of the order Orthoptera and Isoptera also took shelter, impeding the Hymenoptera cavity nesters to use the traps; other Arthropods, such as spiders and myriapods; as well as small vertebrates like frogs and lizards. All traps occupied by these organisms were only bamboo cane traps.

DISCUSSION

This study is the first conducted in the State of Rio de Janeiro using the trap-nest method in an urban area. Overall, few studies were carried out in the State: Moure (1943, 1944, 1958) provided records of some bee species from the Parque Nacional do Itatiaia, most of them Halictid bee species; and some inventories were performed focusing on the Euglossini fauna (Apidae) (Tonhasca-Jr. et al., 2002; Aguiar & Gaglianone, 2012). Concerning wasps, only the Sphecidae family has records (Buys, 2009), but the other families registered in this inventory have no data in the literature for the State of Rio de Janeiro.

The scant knowledge of Hymenoptera records in the state of Rio de Janeiro allowed the filling of gaps on some species distribution. Tetrapedia curvaturas and Megachile benigna are considered new records for the state (Moure, 2012; Moure et al., 2012a). Gasteruption brachychaetum, although not a common species in trap-nest surveys, were recorded by Macedo et al., (2012) in the campus of the Universidade de São Paulo, in the São Paulo city, Brazil, and its broad distribution (Argentina to Mexico) is summarised in Macedo (2011). The present record is the first since 1935 in the JBRJ (Macedo, 2011) and the second host association with the genus Hylaeus in the Neotropics. Amobia sp. was another new record of host-cleptoparasite interaction concerning Hylaeus, since there is no study registering Amobia flies emerging from its nests (Spofford et al., 2012). Species of Amobia are known to parasitize several species belonging to the families Crabronidae, Sphecidae and Vespidae (Rocha-Filho et al., 2019; Spofford et al., 2012), and records of this fly species exist only for Halictidae and Megachilidae species (Moradeshaghi & Bohart, 1968; Spofford et al., 2012).

Regarding the Euglossini, Euglossa pleisticta was a common species in the inventories performed using scent baits in the Rio de Janeiro State (Rebêlo & Moure, 1995; Tonhasca-Jr. et al., 2002; Ramalho et al., 2009), a common method to lure males of this tribe. However, it is not common in trap-nest surveys, even those using bamboo canes, which offer a suitable place for the nest architecture of these species. The other unidentified species of Euglossa was sorted different from E. pleisticta due to nest architecture, since its nest were lost in the museum’s fire. One species of Eufriesea were also collected, and the female observed building the nest, and judging from the golden reflexes in the metasoma, the female might be E. mussitans, a species recorded for Rio de Janeiro State (Rebêlo, 2001; Moure et al., 2012b), but the loss of material hindered emergence and identification.

Concerning the wasps species collected, only underground-nesting Penepodium species was previously recorded in the Rio de Janeiro State (Buys, 2009), and as long as some accept trap-nests (Garcia & Adis, 1993), probably it is a new record of species for the State. Pachodynerus nasidens is a vespid with a large distribution in Brazil and some records are made in the Atlantic Forest (Nascimento & Garófalo, 2014; Rocha-Filho et al., 2017). Although the species of Auplopus collected in this study were not fully sorted out, even at genus level, interesting data were gathered. The species Caenochrysis crotonis usually are associated with Trypoxylon nests (Perioto & Lara, 2018) and seems to have a broad distribution in the Neotropics with occurrences recorded in Peru and Brazil (Belém – Pará, Amazonian domain) (Anteparra et al., 2012; Obrecht & Huber, 1993; Rasmussen & Asenjo, 2009; Santos et al., 2017). They act as endo or cleptoparasites (there are not precise observations and the behaviour of this species could not be recorded here) and this is the first record of Caenochrysis crotonis as parasitoid of Auplopus nests, as well as, the first record of this species for the Atlantic Forest domain. Also, the study conducted in the area discovered a new species for Science, the parasitoid Chaeotetrestichus neotropicalis (Marinho et al., 2019), which shows the importance of inventories in urban areas and how these areas may support still unknown hymenopteran fauna. Studies with higher number of nests collected recorded several natural enemies of pre-existing cavity nesters (Gazola & Garófalo, 2009; Rocha-Filho et al., 2017), and such cleptoparasites species are indicative of environmental quality (Sheffield et al., 2013), since their populations are the first to be affected for any habitat disturbance.

The faunal composition observed in the JBRJ was similar to other studies conducted in Atlantic Forest fragments using the trap-nest method, such as those of Alves-dos-Santos (2003), Gazola & Garófalo (2009), Gaglianone et al. (2011) and Rocha-Filho et al. (2017). These localities present a similar faunal composition with species of Tetrapedia, Hylaeus, Megachile, Euglossini, Pachodynerus and Auplopus. Considering all the extent of the Atlantic Forest biome, some inventories performed in its domain display different faunistic elements and dynamics. For example, in this study, a fairly number of species were collected, but most of them presented low abundance. The study of Flores et al. (2017), for instance, found more diversity of wasps than of bees using trap-nests, and Guimarães-Brasil et al. (2020) found a different species composition considering only bees. Regarding the species collected only in studies performed in the Southeast region of Brazil, such as Loyola & Martins (2006), Gazola & Garófalo (2009), Nascimento & Garófalo (2014) and Rocha-Filho et al. (2017), at family level, the
inventories present similarities but, at species level, there are differences on the composition of the trap-nesting community structure. It is interesting to observe that a common element in the inventories, the bee species of the genus *Centris* Fabricius, was not recorded in the traps set in the field. Perhaps, it was due the great number of ground-nesting Centridini that occurs in the arboretum and nearby areas of the JBRJ as *Epicharis* Klug and other ground-nesting *Centris* species, displacing the smaller trap-nesting bees of this genus.

The trap-nesting community had high dominance of the bee *T. curvitaris* evidenced by the rank-abundance graph. This pattern combined with several *singletons* is indicative of habitat disturbance (Magurran, 2013). It may explain the low number of species collected compared with the number of species predicted by the estimators. Also, the type of traps used probably influenced the dynamic of species occupancy. Tetrapedia and *Hylaeus* species nested only in traps made of straws, and this is possible linked with their nest architecture, since most nests built by these species depends on the very cavity walls where they are inserted. Because they are the smallest free-living Hymenoptera species sampled in the area using the trap-nests, they were restricted to the forest matrix (transect 1) because small bees have small flight autonomy (Gathmann & Tscharntke, 2002), so that nesting only occur were resources abound.

Regarding the traps made of hose, that were not attractive due to its almost non-occupancy, possible because the material and the supports used for set them in field were not suitable for nesting of wasps and bees due to the luminosity. Bamboo canes, on the other hand, are usually used in inventories (Maclvor, 2017) and usually well-accepted. However, in tropical and subtropical regions they often are attacked by fungi (Morato & Matins, 2006; Staab et al., 2018), which was the case in this study. Additionally, ants and other animals were much more prevalent in the traps than the Hymenoptera aimed to survey, and even the inspections carried out biweekly were not enough for augmenting the trap-nest occupancy. Few studies report the occupancy of traps by undesirable nesters (Barthélémy, 2012), even considering that trap-nests are sufficient attractive for other animals, such as ants (Cobb et al., 2006), to subsidize studies with such organisms.

Regardless of the substrates offered, the absence of bees (especially the small-sized ones) and wasps in the arboretum may be due to lack of one or more resources necessary for nesting (*e.g.*, nesting substrate, prey for wasps and pollen for bees, etc.). All these factors summed up, mirrored the occupancy rate in this study that was one of the lowest compared with another trap-nest surveys performed in Atlantic Forest fragments (Loyola & Martins, 2006, 2008; Krug & Alves-dos-Santos, 2008; Oliveira & Gonçalves, 2017).

Other restraining feature of the JBRJ concerning bees, is that most plant species are exotic and represented for one or few individuals, and which hinder the foraging of native species for pollen locally. This was, probably, the main reason the small-sized bee species were not found in the arboretum. Merging the exotic flora with native plants have the potential to help the bee fauna to establish and thrive (Schlindwein, 2004), so that Botanical Gardens in urban contexts, such as the JBRJ, are important actors at the conservation of species. On the other hand, all wasp species collected in the JBRJ used mud to build their nests, as well as water to moist and give it form for their nests and cells partitions. Both materials abound in the arboretum, besides the preys collected by them (cockroaches, spiders and insect larvae). Similar differences were observed by Matos et al. (2016) studying the faunistic composition of trap-nesting wasps and bees in an agroecological system. Those authors suggested that, in fact, habitat requirements of bees and wasps are different.

The climatic factors assessed in this study influenced differently bees and wasps in the JBRJ. Away from tropics, bees have their activity highly impelled by temperature (Roubik, 1992), such as observed in other studies in Brazil (Alves-dos-Santos, 2003; Gazola & Garófalo, 2009; Oliveira & Gonçalves, 2017) and the bee activity at the JBRJ corroborated this pattern. Wasps, on the other hand, had their activity influenced by rainfall. Although many nests were not collected, the pattern of *A. cf. ruípes* seemed to be similar to the pattern observed by Loyola & Martins (2006) for another *Auplopus* species, *A. militaris* (Lynch-Arribalzaga). That spread phenology across the year may be linked to prey collecting to feed their immature, as long as it is known that pompilid species hunt species accordingly with their habit, and not specifically by prey species (Rodriguez et al., 2016). The Vespidae, Crabronidae and Sphecidae species sampled in the JBRJ also nested in the same months repeating the pattern observed in other studies carried out in Atlantic Forest fragments (Loyola & Martins, 2006; Nascimento & Garófalo, 2014). Considering it, the rainfall might create suitable microhabitats favouring the presence of insect larvae and spiders, the food source of the immatures of the wasps caught here.

**CONCLUSION**

The community of trap-nesting bees and wasps in the JBRJ is characterised by high dominance of two species: *T. curvitaris* and *Hylaeus* sp. Although the JBRJ trap-nesting Hymenoptera community seemed to be under disturbance, it harbours many species, including high-trophic level ones, such as the parasites, which is indicative for great potential for conservation, even in an urban context. Therefore, we strongly suggest that the use of trap-nest hotels may positively influence the number of nests in the area, as well as, the increase of ruderal and native plants in the arboretum.

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