1 **Natural history of two carpenter ants, *Camponotus renggeri* and *C. rufipes* (Hymenoptera:**
2 **Formicidae), in an Atlantic Forest reserve**

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15 Abstract

16 Widespread species face a wide variety of environmental challenges and their morphology,
 17 behavior, and natural history may change across their range. However, not rarely, natural history
 18 research is restricted to one or few locations. That is the case for *Camponotus renggeri* and *C.*
 19 *rufipes*. Both species occur across South America in different ecosystems, but most research on
 20 these species is restricted to the Brazilian savanna, known as Cerrado. Here, we describe the
 21 home range, nesting habits, and activity schedule of *C. renggeri* and *C. rufipes* in an Atlantic
 22 Forest reserve in SE Brazil. *C. renggeri* foraged exclusively during nighttime and *C. rufipes*
 23 remained active throughout the nycthemeron, but with little intensity during daylight hours. Most
 24 nests of both species were composed of dry straw, and home ranges varied from nearly 0.91 m²
 25 (*C. renggeri*) to 1.79 m² (*C. rufipes*). Foraging areas overlapped, especially in *C. renggeri*. Our
 26 field study reinforces the importance of natural history and what it adds to our knowledge on the
 27 ecology and behavior of *C. renggeri* and *C. rufipes* in Atlantic Forest.

The closely related carpenter ants *Camponotus renggeri* (Emery, 1894) and *C. rufipes* (Fabricius, 1775) have been extensively studied in terms of physiology (TAKAHASHI-DEL-BIANCO & al. 1998, GALIZIA & al. 1999), natural history (RONQUE & al. 2016, RONQUE & al. 2018) and genetics (AZEVEDO-SILVA & al. 2015, RONQUE & al. 2016, DE AGUIAR & al. 2017, AZEVEDO-SILVA & al. 2020). These species are morphologically similar, being distinguished mainly by the brightness of their tegument and by the color of their legs. Both species are commonly found on vegetation, where they feed on liquid resources from extrafloral nectaries and trophobionts (OLIVEIRA & FREITAS 2004, RONQUE & al. 2018). Despite similarities in distribution and interactions with plants, these species differ with respect to nest architecture, home ranges, and breeding systems. *C. renggeri* occupies nests built in dead tree trunks or underground, whereas *C. rufipes* may also construct nests out of dry straw (RONQUE & al. 2016, RONQUE & al. 2018, AZEVEDO-SILVA & al. 2020). Both species occur across South America in different ecosystems (JANICKI & al. 2016), but most research on these species is restricted to the Brazilian savanna, known as Cerrado.

The main goal of this study was to investigate the natural history of *C. renggeri* and *C. rufipes* in the Atlantic Forest. Specifically, we aimed to describe their home range, nesting habits, and activity schedule. For both species, our findings unveiled consistent differences in home ranges and nest architecture when compared to previous findings, although we observed similarities in natural history between populations from the Cerrado and Atlantic Forest. Our study is the first step towards a broader understanding of how the ecology and behavior of tropical ant species may vary across different ecosystems.

Fieldwork was carried out in an Atlantic Forest fragment surrounded by the sea and urban environment, at Xixová-Japuí State Park, São Vicente, São Paulo in Southeast Brazil (23°59'33.3" S 46°23'21.7" W). The Park is dominated by secondary ombrophilous forests, and some areas are at initial successional stage, with abundant herbaceous plants and low canopy cover (Figure 1A). The region has a typically hot and very humid climate, with mean annual temperature of 23.6 °C and mean annual precipitation of 2500 mm, being classified as tropical rainforest climate (*Af*) (PEEL & al. 2007). Observations were performed from February to April 2019 and from May to July 2019. The study area was divided into 10 plots (400 m² each), where

ants were searched for 20 minutes in each plot. We used sardine baits to find *C. renggeri* and *C. rufipes* nests. In each plot where we were able to find these species, from three to five nests of each were tagged and characterized for nest architecture, home range, and activity schedule.

Daily activity schedule was described by the total number of workers entering and exiting nests during sessions of 30 min, every 2 h, over a period of 24 h. For home range estimation, 4 *C. renggeri* and 3 *C. rufipes* nests were observed for a total maximum of 16 h, encompassing their peak activity period. Workers were followed from nest entrance to the furthest point before returning to nest. These points were tagged and had their distance to the nest measured and relative geographical location determined with a compass. Home range was estimated using minimum convex polygons generated with the package *adehabitatHR* (CALENGE 2006) in R (R CORE TEAM. 2020). Due to low nest persistence, all observations of *C. renggeri* were carried out in the dry season, whereas observations of *C. rufipes* were performed during rainy season. During fieldwork, additional natural history records were made for both species using video, photographs, and notes (total of 32 h of observations for each species).

Nests were found in only 3 plots, and none of them had nests of both species. A total of 15 *C. renggeri* (12 nests in the same plot, one in a second plot and two nests opportunistically found outside of the plots) and 9 *C. rufipes* nests (all in the same plot) were identified and tagged. Of the total, 8 *C. renggeri* and 6 *C. rufipes* nests were unoccupied by the end of this research (July 2019), indicating low nest persistence (Figure 1B, C). Both species built their nests using mainly dry straw (*C. renggeri*: N = 15 nests; *C. rufipes*: N = 8 nests), but both could use additional materials, such as freshly fallen and dry leaves (Table 1). Many of these nests were suspended above ground, sustained by twigs (Figure 2). As for the activity schedule, the period of peak activity for both species was nocturnal, increasing after sunset (18 h) and decreasing before sunrise (05 h) (Figure 3). *C. rufipes* had a mean home range value of 1.79 m², and *C. renggeri* had a mean value of 0.91 m² (Table 2). Major workers of *C. renggeri* and *C. rufipes* were found engaging in aggressive behavior towards each other when crossing paths, resulting in the decapitation of *C. rufipes* (see Supporting information S1). These observations suggest that competition may occur between the two species.

Our findings in Atlantic Forest differ from those reported for other ecosystems, indicating that environment may play an important role in shaping *C. renggeri* and *C. rufipes* behavior. All but one *Camponotus rufipes* nests characterized in this study were made of dry straw, similarly to the observed in the Cerrado and in the Argentinian “Chaco” (WEIDENMÜLLER & al. 2009, RONQUE & al. 2018). Conversely, *C. renggeri* also presented this same nest architecture, differing from the nests in dead trunks reported for this species in Cerrado (RONQUE & al. 2018), whereas no nests of such type were found in the Atlantic Forest. It is known that vegetation may play a role in regulating availability of resources for nesting in ants (RONQUE & al. 2018). The Atlantic Forest fragment where this research was conducted is dominated by herbaceous plants, thus, dead trunks is not as abundant as leaf litter. This may explain the prevalence of nests made of dry straw over other nest architectures (SILVESTRE & al. 2003). The difference found for *C. renggeri* nests between different ecosystems indicates that this species is able to modify its nesting behavior in response to resource availability in different environments. Additionally, our findings expand the repertoire of nesting strategies reported for the *Camponotus* genus (PFEIFFER & LINSENMAIR 2000, SANTOS & al. 2005, TSCHINKEL 2005, YAMAMOTO & DEL-CLARO 2008, SANTOS & DEL-CLARO 2009).

Camponotus rufipes and *C. renggeri* presented smaller home ranges compared to other carpenter ants (PFEIFFER & LINSENMAIR 2000, YAMAMOTO & DEL-CLARO 2008, RONQUE & al. 2018). However, despite the impracticability of a direct comparison due to slight methodological differences, we observed much smaller values (Table 2) than previously findings reported for the same species (RONQUE & al. 2018). In the Cerrado, *C. rufipes* foraging area doubled during rainy season, whereas *C. renggeri* home range was not affected by different seasons (RONQUE & al. 2018). Given our observations occurred in different seasons for *C. rufipes* and *C. renggeri*, it is unclear whether the home range estimated for *C. rufipes* is indeed larger than *C. renggeri* in the Atlantic Forest or if it is an effect of seasonality. However, the differences reported between biomes may be related to many factors, such as temperature, humidity, colony size, competition, and food availability (HÖLLDOBLER 1987, BREED & al. 1990, GORDON 1995, MCGLYNN & al. 2003, RONQUE & al. 2018).

Beyond seasonal changes in foraging, daily cycles may also influence a colony’s activity. We

showed that *C. renggeri* foraged exclusively during night, whereas *C. rufipes* remained active throughout the nycthemeron, with little activity during the day. Similar patterns were reported for this species in Cerrado, with *C. renggeri* activity being negatively affected by temperature and positively by humidity, whereas *C. rufipes* was not affected by these environmental conditions (RONQUE & al. 2018). Indeed, nocturnal behavior is common among tropical ant species, presumably because it allows them to avoid high temperatures and low humidity during the day (PFEIFFER & LINSSENMAIR 2000, RONQUE & al. 2018). Additionally, temporal segregation can be a result of competition and resource sharing, particularly in exudate-feeding *Camponotus* species (DEL-CLARO & OLIVEIRA 1999, OLIVEIRA & al. 1999, RONQUE & al. 2018), even though most species can combine exudate with animal prey in their diets (OLIVEIRA & FREITAS 2004, RONQUE & al. 2018). These mechanisms could explain not only the difference in daily activity, but also the observed spatial partition between *C. renggeri* and *C. rufipes*. Accordingly, we recorded an aggressive encounter of major workers of *C. renggeri* and *C. rufipes*, which further suggest an antagonistic relationship between these species.

This work focused on the description of natural history traits of *C. renggeri* and *C. rufipes* in the Atlantic Forest, an ecosystem where the ecology of these species is poorly explored. We consistently showed that the behavior of ants may vary across biomes, shedding new light on natural history of *Camponotus* species in tropical ecosystems. We explored which differences in the behavior of species may emerge in different environments and showed how ant behavior may vary across ecosystem. Identifying and understanding such variation is crucial to elaborate more complex and robust questions regarding ant ecology. Finally, we expect that our work encourages further investigation on ant natural history, which would be important to increase our knowledge on tropical systems.

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Figures and tables captions

Table 1: Nest architecture of *Camponotus renggeri* and *Camponotus rufipes* in an Atlantic Forest Reserve, southeastern Brazil.

Nest architecture	No. of nests	
	<i>Camponotus renggeri</i> (N = 15)	<i>Camponotus rufipes</i> (N = 9)
Dry straw	2	4
Dry straw and dry leaves	10	4
Dry straw and fresh leaves	3	0
Underground	0	1

Table 2: Home ranges of *Camponotus renggeri* and *Camponotus rufipes* in an Atlantic Forest Reserve, southeastern Brazil.

Species	N° of nests	Season	Mean n° ± SD of foragers sampled	Mean area ± SD of home ranges (m²)	Minimum and maximum sizes of home ranges (m²)
<i>Camponotus renggeri</i>	4	Dry/cold	12.5 ± 3.84	0.91 ± 1.10	0.13 – 2.81
<i>Camponotus rufipes</i>	3	Rainy/hot	27.67 ± 8.38	1.79 ± 0.79	1 – 2.87

Figure 1: (A) General view of the Atlantic Forest reserve physiognomy in which the study was carried out. Distribution map of (B) *Camponotus renggeri* and (C) *C. rufipes* nests. Dots represent tagged nests, darker circles around them represent the mean foraging range for each species in the Atlantic Forest; lighter circles represent the standard deviation. Stars indicate nests observed in the research. Orange represents nests which were unoccupied by the end of the research, and green represents occupied nests by the end of the research. The larger dotted lines indicate trails of the at Xixová-Japuí State Park (PEXJ), in Southeast Brazil. The smaller dotted line represents a transportation route between nests.

280 **Figure 2:** *Camponotus renggeri* dry straw nest found in an Atlantic Forest Reserve, southeastern
281 Brazil. Note the presence of whole leaves and intertwining branches in the architecture.

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283

284 **Figure 3:** Daily activity of *Camponotus renggeri* (N = 4) and *C. rufipes* (N = 3) colonies in an
 285 Atlantic Forest reserve, southeastern Brazil. Daily activity was calculated by the number of ants
 286 leaving/entering the nests (Mean $\pm$ SE). Air temperature and humidity for each day were
 287 collected in the Brazil's National Institute for Space Research (INPE) database (Mean), and

288 humidity data was missing from some hours of the day. The arrows indicate sunrise (white) and
289 sunset (black). N/A stands for not available.

290 **Supporting information S1:** Aggressive encounter between major workers of *C. renggeri* and
291 *C. rufipes*, resulting in the decapitation of *C. Rufipes*.