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ARTICLE

Natural regeneration in Atlantic Forest Fragments: using ants (Hymenoptera: Formicidae) for monitoring a conservation unit

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Abstract. The Brazilian Atlantic Forest is considered one of the most threatened tropical forests in the world due to the extensive environmental impact it has endured throughout history. Only 12.4% of its original vegetation is estimated to remain. Even though reduced and highly fragmented, it houses enormous biodiversity, and its preservation is paramount to the maintenance of the country's fauna, flora, funga and microbiota. One of the most efficient measures adopted by public agencies aimed at protecting biodiversity has been the creation of conservation units. To evaluate the preservation state of protected areas, several environmental studies have been performed; species inventories are one among them. Ants are excellent bioindicators, for they are not only sensitive to environmental changes, but they also have a history of being used in impact assessment (*i.e.,* fragmentation). In this study we assessed the ant communities inhabiting the leaf litter in areas with different regeneration states at the RPPN Botujuru – Serra do Itapety (Mogi das Cruzes, São Paulo – Brazil). Mini-Winkler traps were used in the ant survey, and diversity analyses were performed. In total, we recorded 86 species of ants, with a highlight to *Camponotus cillae* Forel, 1912, a species that remained unrecorded for the state of São Paulo for over 100 years, and a possible new species of *Octostruma* Forel, 1912. Overall, the species found show that the areas are in the process of natural regeneration. Our data on RPPN Botujuru is unprecedented, and our species list has the potential of being used as an effective monitoring tool for this conservation unit.

Keywords. Species inventory; Biological Conservation; Richness, Environmental Reserve; Biodiversity.

INTRODUCTION

The Atlantic Forest is one of the most diverse biomes in Brazil, with records of over 20,000 species, around 6,000 of which are endemic to it (Marques & Grelle, 2021); it is considered one of the world *hotspots* of biodiversity (Rezende *et al.,* 2018; Hu *et al.,* 2021). Originally, it encompassed about 15% of the Brazilian territory, of which only 12.4% is now in existence (MMA, 2022). This biome is now severely fragmented and interspersed with urban areas (Baklanov *et al.,* 2018) and farmlands (Melo *et al.,* 2013), where *Eucalyptus* sp. and *Pinus* sp. (MacDicken, 2015), for instance, are cultivated. In face of the high endemism, and because it is under constant threat, this biome is considered an extreme priority in terms of conservation (Myers *et al.,* 2000; Carlucci *et al.,* 2021).

The creation of conservation units is an efficient practice to protect biodiversity and restore forest remnants of the Atlantic Forest biome (Sobral-Souza *et al.,* 2018; Lewis *et al.,* 2019). In Brazil, conservation units are ruled by the National System of Conservation Units (SNUC – Law 9.985/2000), and they have the conservation of species, habitats, and ecosystems as their main pillars (Brito, 2012; Medeiros *et al.,* 2011; Morini & Miranda, 2012). Several types of studies can be developed

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in conservation units (*e.g.,* biological inventories), which, in turn, are useful to evaluate the effectiveness of biodiversity protection (Le Saout *et al.,* 2013). Thus, they provide biological knowledge for several groups, especially of invertebrates, which are often neglected in biological inventories (Pinto *et al.,* 2017).

In Brazil, conservation units are organized into two categories: full protection units and sustainable use units (Brasil, 2000). Private Natural Heritage Reserves (RPPNs) belong to this last group, and they are conservation units that are private and perpetual. Brazil has around 1,500 RPPNs throughout its territory (Instituto Terra, 2020); the state of São Paulo counts with 99 of them (Secretaria de Infraestrutura e Meio Ambiente, 2019), most of which are located in the Atlantic Forest (Lima & Franco, 2014). Because they are private, RPPNS may be created by NGOs, companies, and mainly by large landowners (Wiedmann, 2001) who own fragments of native vegetation or potentially restorable areas (Bensusan, 2006), primarily rural. About 80% of the Atlantic Forest biome is located on private land (Ayala, 2010), which means the creation of RPPNs may play a key role in the conservation of biodiversity (Wilson *et al.,* 2010).

The need to identify and quantify the disturbances caused by human activities in varied types of environments has attracted the attention of researchers to the search for organisms that could interfere with the levels of integrity of the ecosystems (Couto *et al.,* 2010). One of the possible ways to monitor biodiversity change patterns is to employ species that are known bioindicators of environmental degradation (Gerhardt, 2002). In this sense, insects are considered excellent bioindicators, as they are widely distributed around the world, play key roles in ecosystems and are sensitive to environmental changes (Forister *et al.,* 2019).

Ants, in turn, reflect this diversity, representing 80% of animal biomass in some tropical ecosystems (Fittkau & Klinge, 1973). This group has approximately 16,000 valid species and subspecies, of which 1,748 occur in Brazil (Bolton, 2022); they possess highly variable lifestyles, diets, and behavior (Rosumek, 2017). Regarding ecological aspects, ants are considered great contributors to the maintenance of biodiversity, having an influence on the presence of other invertebrates (Vasconcelos *et al.,* 2000) and performing important ecosystem services (Viles *et al.,* 2021). Moreover, they are considered excellent witnesses to the environmental change due to their high sensitivity to disturbance. In addition, they are widespread, easy to sample, and are considerably abundant (Ribas *et al.,* 2012).

In this study, we assessed the composition of the ant fauna in a recently implemented RPPN, which consists almost entirely of *Eucalyptus* with the understory displaying varying levels of complexity. We intend to show the record of species and the trophic guilds, as the natural regeneration process of the native vegetation occurs. Furthermore, we intend to provide the first taxonomic inventory of the ant fauna of this conservation unit, so it can be used in future monitoring projects.

MATERIAL AND METHODS

Study site

The study was performed at RPPN Botujuru, situated in the municipality of Mogi das Cruzes (SP, Brazil). Established in 2015, RPPN Botujuru was created to protect one of the most important Atlantic Forest fragments in the state of São Paulo (Fig. 1), Serra do Itapety

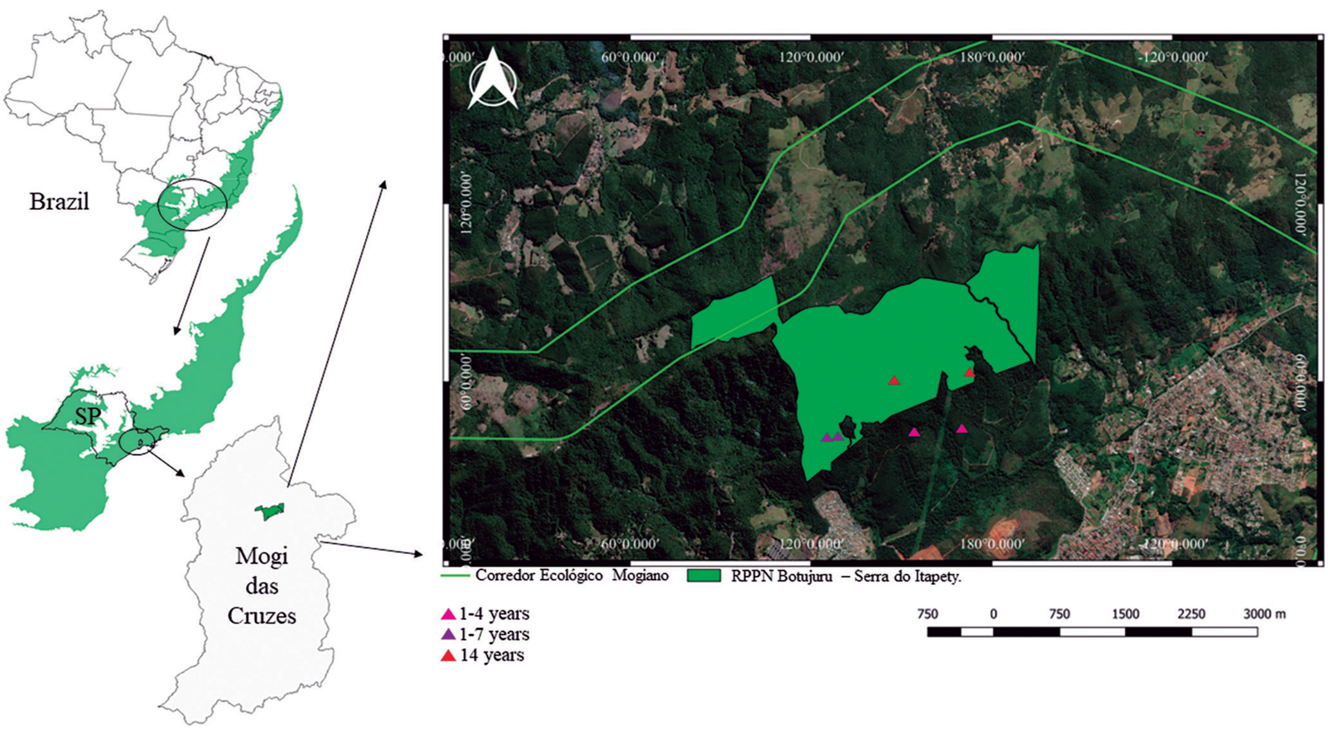

Figure 1. Botujuru Private Natural Heritage Preserve location and the respective collecting sites.

Table 1. Geographic coordinates for the areas collected.

Age of areas (years)	Geographic coordinates
1-4	23°28′58″S 46°09′30″W
1-4	23°28′59″S 46°09′49″W
7-12	23°29′01″S4 46°10′20″W
7-12	23°29′02″S 46°10′24″W
14	23°28′ 41″S 46°09′57″W
14	23°28′38″S 46°09′27″W

(Ecofuturo, 2016), being part of a buffer zone of a recently created ecological corridor known as Corredor Ecológico Mogiano (Mogi das Cruzes, 2020). The RPPN was kept for a long time as a *Eucalyptus* sp. and *Pinus* sp. management area, but it was inactivated, and now it has areas that have not been managed in over 20 years. *Eucalyptus* plantations correspond to 75% of the total area of the RPPN (Ecofuturo, 2016), which means approximately 328 ha.

The phytophysiognomy is classified as Dense Ombrophilous Forest (Colombo & Joly, 2010), with altitudes varying from 600 m to 850 m above sea level. The climate is defined as a Humid Tropical Forest, with the annual precipitation being higher than 2,000 mm, and there is no distinctive seasonality. The rainy season occurs from late September to late March, and the dry season occurs from late June to late September (CPTEC/INPE, 2022).

Eucalyptus plantations with different ages and understory regeneration were selected for the sampling; the areas in which they occurred have the following ages: 1-4 years (no understory), located in the buffer zone of RPPN Botujuru – Serra do Itapety; 7-12 years (with an understory composed of shrubby vegetation), and 14 years (with an understory displaying both shrubby and arboreal vegetation). For each type of plantation, we collected in two different areas. The geographic coordinates of the sampled areas are located in Table 1.

Ant survey

The collections were conducted in March and April 2018. In each area (n = 6), we delimited two linear transects of 200 m each, where eight parcels of 1 m^2 were distributed, each 10 m apart from the other. The leaf litter present in these parcels was then collected, sieved, and put into mini-Winkler extractors (Agosti *et al.,* 2000), where they remained for 48 hours. A total of 96 samples were collected, being 16 per area.

The ant species were initially classified and identified into subfamilies and genera (Baccaro *et al.,* 2015; Camacho *et al.,* 2022), and in morphospecies/species through the comparison with the specimens from the reference collection of the Alto Tietê Myrmecology Laboratory (LAMAT-UMC) (Suguituru *et al.,* 2015; Souza-Campana *et al.,* 2020) and with identification keys (Fernández, 2003; Wilson, 2003; Ortiz-Sepulveda *et al.,* 2019; Camacho *et al.,* 2020). The vouchers were deposited at the regional ant reference collection of Alto Tietê (Universidade de Mogi das Cruzes). The collections were approved by the Environment Ministry/System of Authorization and Information on Biodiversity – MMA/SISBIO) under the license 34037-1.

Data analysis

To assess the regeneration in the areas, the relative frequency was calculated based on the number of occurrences of each species (presence and absence) (Gotelli & Colwell, 2001). The ant communities were evaluated using the Shannon-Wiener (H′) diversity and Pielou equitability indices, as well as the similarity test (ANOSIM) adopting the significance index $p < 0.05$. Similarity was represented using the non-metric multidimensional scale (NMDS) test (Past software version 3.14, Hammer *et al.,* 2001) using the Bray-Curtis Similarity Index of species occurrence values. Trophic guilds were classified according to Delabie *et al.* (2000).

RESULTS

In total, we collected eight families distributed in 38 genera and 86 species and morphospecies (Table 2). The Shannon diversity index varied between H′ = 3,40 (no understory) and H′ = 3,57 (with understory) (Table 2). Myrmicinae was the most frequent subfamily in all sampled areas (68.5%), followed by Ponerinae (11.4%) and Formicinae (10.8%). Among the Myrmicinae genera, *Pheidole* Westwood, 1839 and *Solenopsis* Westwood, 1840 were the richest and most frequent, being present in all of the sampled areas in great abundance. For the species, *Pheidole sospes* Forel, 1908 (7,6%) was the most frequent, followed by *Strumigenys denticulata* Mayr, 1887 (5.7%). Ponerinae was widely represented by species of *Hypoponera* Santschi, 1938, such as *Hypoponera* sp.4 (5.3%), *Hypoponera* sp.6 (2.3%) and *H. foreli* (Mayr, 1887) (0.3%), and *Odontomachus* Latreille, 1804, with *O. affinis* Guérin-Méneville, 1844 (0.2%) and *O. chelifer* (Latreille, 1802) (0.1%). Among the Formicinae, the genus *Brachymyrmex* Mayr, 1868 was recorded in all of the areas sampled with high occurrence levels, being represented chiefly by *B. admotus* Mayr, 1887 (5.15%) and *B. heeri* Forel, 1874 (2.11%). Dolichoderinae, Dorylinae, Ectatomminae, Procerattinae, and Pseudomyrmecinae were represented by three or fewer species.

Thirteen species (15.11%) were found only in areas whose ages are estimated at 1-4 years, for example, three species of *Camponotus* Mayr, 1861 and five species of *Pheidole* Westwood, 1839. For the areas between 7-12 years, 11 species (12.79%) were exclusive; examples are *Anochetus altisquamis* Mayr, 1887, *O. chelifer,* and *O. meinerti* Forel, 1905. The oldest areas (14 years) recorded nine exclusive species (10.46%) such as *Acanthognathus ocellatus* Mayr, 1887, *Brachymyrmex patagonicus* Mayr, 1868, and *Mycetophylax strigatus* (Mayr, 1887). Twenty-six species (30.23%) were present in all areas.

In total, nine trophic guilds were recorded (Fig. 2). The litter omnivore and scavenger guild dominated in every

Table 2. Frequency of occurrence (%) of the species recorded according to the age of the area.

	Age of areas (years)			Trophic guild
	1-4	7-12	14	
Dolichoderinae				
Linepithema neotropicum Wild, 2007	0.86	2.12	2.09	Omnivore
Tapinoma melanocephalum (Fabricius, 1793)	—	—	0.84	Omnivore
Dorylinae				
Labidus praedator (Smith, 1858)	0.43	0.35	—	Army ants
Neocerapachys splendens (Borgmeier, 1957)	—	0.71	0.42	Specialist litter predator
Ectatomminae				
Heteroponera dentinodis (Mayr, 1887)	3.43	2.83	2.93	Litter omnivore and scavenger
Holcoponera striatula Mayr, 1884	4.29	1.77	1.67	Litter generalist predator
Typhlomyrmex reichenspergeri (Santschi, 1929)	0.43	—	—	Litter generalist predator
Formicinae				
Brachymyrmex admotus Mayr, 1887	5.15	7.07	2.93	Omnivore
Brachymyrmex heeri Forel, 1874	3.43	1.41	1.67	Omnivore
Brachymyrmex patagonicus Mayr, 1868	—	—	0.42	Omnivore
Camponotus cillae Forel, 1912	1.29	—	0.42	Omnivore
Camponotus rufipes (Fabricius, 1775)	0.43	—	—	Omnivore
Camponotus senex (Smith, 1858)	2.15	—	—	Omnivore
Camponotus sp.1	0.43	0.35	—	Omnivore
Camponotus sp.10	—	—	0.84	Omnivore
Camponotus textor Forel, 1899	0.43	—	—	Omnivore
Myrmelachista arthuri Forel, 1903	—	0.35	—	Omnivore arboreal-nesting dominant
Myrmelachista nodigera Mayr, 1887	1.72	—	—	Omnivore arboreal-nesting dominant
Nylanderia sp.1	1.72	0.71	1.26	Omnivore
Myrmicinae				
Acanthognathus ocellatus Mayr, 1887	—	—	0.42	Specialist litter predator
Acanthognathus rudis Brown & Kempf, 1969	3.43	3.18	2.51	Specialist litter predator
Acromyrmex niger (Smith, 1858)	—	—	0.42	Soil or litter nesting fungu-grower
Apterostigma sp.2	0.86	0.71	0.42	Soil or litter nesting fungu-grower
Basiceros disciger (Mayr, 1887)	—	0.71	—	Litter omnivore and scavenger
Carebara sp.1	1.29	3.89	4.18	Omnivore
Crematogaster curvispinosa Mayr, 1862	—	1.06	0.84	Omnivore arboreal-nesting dominant
Cyphomyrmex rimosus (Spinola, 1851)	3.86	2.12	4.18	Soil or litter nesting fungu-grower
Hylomyrma reitteri (Mayr, 1887)	5.15	2.83	4.18	Specialist litter predator
Lachnomyrmex victori Feitosa & Brandão, 2008	—	0.71	—	Litter omnivore and scavenger
Mycetarotes senticosus Kempf, 1960	—	—	0.84	Soil or litter nesting fungu-grower
Mycetophylax strigatus (Mayr, 1887)	—	—	0.42	Soil or litter nesting fungu-grower
Octostruma sp.1	—	0.35	0.84	Specialist litter predator
Octostruma rugifera (Mayr, 1887)	0.43	1.06	0.84	Specialist litter predator
Octostruma stenognatha Brown & Kempf, 1960	—	0.71	0.84	Specialist litter predator
Oxyepoecus myops Albuquerque & Brandão, 2009	—	0.71	0.42	Omnivore
Pheidole aff. *dione*	—	0.35	—	Litter omnivore and scavenger
Pheidole gertrudae Forel, 1886	0.43	0.71	0.84	Litter omnivore and scavenger
Pheidole sarcina Forel, 1912	—	7.07	5.02	Litter omnivore and scavenger
Pheidole sigillata Wilson, 2003	0.43	2.47	2.09	Litter omnivore and scavenger
Pheidole sospes Forel, 1908	7.30	8.83	6.69	Litter omnivore and scavenger
Pheidole sp.8	2.15	—	0.84	Litter omnivore and scavenger
Pheidole sp.12	0.43	0.71	—	Litter omnivore and scavenger
Pheidole sp.15	0.43	—	—	Litter omnivore and scavenger
Pheidole sp.16	0.43	0.71	—	Litter omnivore and scavenger
Pheidole sp.17	—	0.35	—	Litter omnivore and scavenger
Pheidole sp.20	—	0.35	0.42	Litter omnivore and scavenger
Pheidole sp.21	0.43	—	—	Litter omnivore and scavenger
Pheidole sp.23	—	—	0.42	Litter omnivore and scavenger
Pheidole sp.24	0.86	—	—	Litter omnivore and scavenger
Pheidole sp.29	0.43	—	—	Litter omnivore and scavenger
Pheidole sp.32	0.43	—	—	Litter omnivore and scavenger
Pheidole sp.34	—	0.35	—	Litter omnivore and scavenger
Pheidole sp.36	—	—	0.84	Litter omnivore and scavenger

	Age of areas (years)			Trophic guild
	1-4	7-12	14	
Pheidole sp.38	—	—	0.42	Litter omnivore and scavenger
Pheidole subarmata Mayr, 1884	0.86	0.35	—	Litter omnivore and scavenger
Pheidole triconstricta Forel, 1886	0.43	0.35	0.42	Litter omnivore and scavenger
Rogeria sp.1	—	0.35	0.42	Litter omnivore and scavenger
Solenopsis sp.2	2.15	5.65	6.69	Litter omnivore and scavenger
Solenopsis sp.3	7.30	1.77	4.18	Litter omnivore and scavenger
Solenopsis sp.4	4.72	1.41	1.26	Litter omnivore and scavenger
Solenopsis sp.7	—	0.71	1.67	Litter omnivore and scavenger
Solenopsis sp.8	—	3.18	2.51	Litter omnivore and scavenger
Strumigenys cosmostela Kempf, 1975	0.86	1.06	0.84	Specialist litter predator
Strumigenys crassicornis Mayr, 1887	—	1.06	2.09	Specialist litter predator
Strumigenys denticulata Mayr, 1887	6.44	5.30	5.44	Specialist litter predator
Strumigenys eggersi Emery, 1890	0.43	0.71	—	Specialist litter predator
Strumigenys elongata Roger, 1863	1.72	1.41	—	Specialist litter predator
Strumigenys louisianae Roger, 1863	—	1.41	0.84	Specialist litter predator
Wasmannia affinis Santschi, 1929	7.30	2.83	5.86	Omnivore
Ponerinae				
Anochetus altisquamis Mayr, 1887	—	0.71	—	Litter generalist predator
Hypoponera foreli (Mayr, 1887)	—	0.71	0.42	Litter generalist predator
Hypoponera sp.3	—	2.12	0.84	Litter generalist predator
Hypoponera sp.4	0.86	6.71	7.11	Litter generalist predator
Hypoponera sp.6	7.73	—	—	Litter generalist predator
Hypoponera sp.9	0.86	—	—	Litter generalist predator
Neoponera crenata (Roger, 1861)	0.43	—	—	Soil cryptic predator
Odontomachus affinis Guérin-Méneville, 1844	0.43	—	0.42	Generalist predator
Odontomachus chelifer (Latreille, 1802)	—	0.35	—	Generalist predator
Odontomachus meinerti Forel, 1905	—	0.35	—	Generalist predator
Pachycondyla harpax (Fabricius, 1804)	0.86	1.41	2.09	Soil cryptic predator
Rasopone ferruginea (Smith, 1858)	—	0.35	—	Soil cryptic predator
Proceratiinae				
Discothyrea sexarticulata Borgmeier, 1954	0.43	2.12	2.09	Specialist litter predator
Pseudomyrmecinae				
Pseudomyrmex phyllophilus (Smith, 1858)	1.29	—	0.42	Omnivore arboreal-nesting dominant
Partial abundance (área)	9.139	3352	6.382	
Full abundance	18.873			
Richness	86			
Shannon's Diversity (H')	3.40	3.57	3.57	
Pielou's Equitability	0.883	0.873	0.895	

one of the areas studied, with 279 records, being widely represented by species of the genera *Pheidole* and *Solenopsis*. This guild was followed by omnivore (161) and specialist litter predator (157) guilds. The less common guilds were army ants (2) and generalist predators (4), represented by *Labidus praedator* (Smith, 1858) and species of the genus *Odontomachus,* respectively. None of the guilds were exclusive to any of the areas sampled.

The ant communities of the areas with understory (7-12 years and 14 years) formed a different group ($p < 0.05$) from those of the younger areas (1-4 years) (Fig. 3).

DISCUSSION

In this work, we are reporting on the ant communities in an area of Atlantic Forest that is part of a RPPN, which is situated in a buffer zone of an important ecological corridor in the state of São Paulo, called Corredor Ecológico Mogiano. Our results show that the number of species is similar to those of Pacheco *et al.* (2009) and Suguituru *et al.* (2011) in a secondary forest, located in the same region of our study, and using the same sampling methods used here. On the other hand, when we compare it with the richness of ants of Parque Natural Municipal Francisco Affonso de Mello (108 species; Suguituru *et al.,* 2013), a conservation unit adjacent to the RPPN, the richness recorded by our study is lower, and the species are more generalists.

Forest loss tends to reduce the occurrence of environmentally specialized species, favoring the presence of those with more generalist habits (Martins *et al.,* 2011). This pattern corroborated our results, given that areas without an understory showed a larger number of generalist guilds, while in those with a developed understory the specialist guilds were more numerous (Fig. 2). The older areas, those with understory, must have been positively influenced by the recovering native vegetation at RPPN Botujuru, specifically with the offer of more foraging and nesting resources (Aguiar *et al.,* 2022).

Neocerapachys splendens (Borgmeier, 1957) and *Discothyrea sexarticulata* Borgmeier, 1954, specialist species, showed low frequency in the areas without an understory (0.43% and 0%, respectively), while those with regenerated understory have recorded a higher occurrence of both species (0.71% and 2.12% for areas with 7-12 years, and 0.42% and 2.09% for those with 14 years, respectively). The understory regeneration brings more complexity to the environment because it offers more food sources, foraging sites, and, mainly, microclimates that can contribute to a higher diversity of species (Kaspari, 1996; Campos *et al.,* 2003; Cramer & Willig, 2005; Laurance *et al.,* 2006). Consequently, the regeneration influences positively the restructuring of ant communities, added to the high resilience observed in Atlantic Forest fragments (Cheung *et al.,* 2010), the area can become similar to areas of native vegetation in terms of species

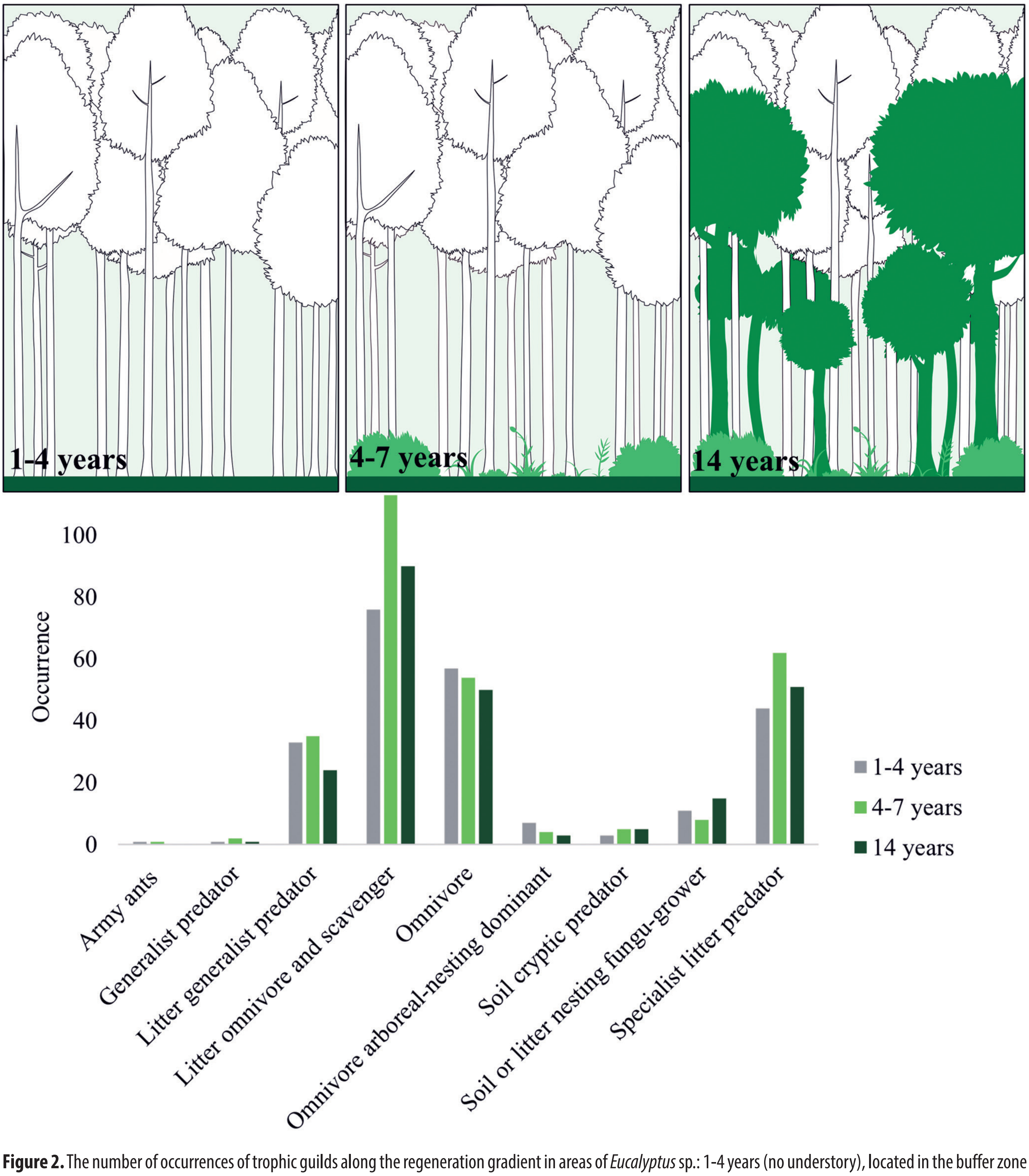

Figure 2. The number of occurrences of trophic guilds along the regeneration gradient in areas of *Eucalyptus* sp.: 1-4 years (no understory), located in the buffer zone of RPPN Botujuru – Serra do Itapety; 7-12 years (with an understory composed of shrubby vegetation), and 14 years (with an understory displaying both shrubby and arboreal vegetation).

richness (Mentone *et al.,* 2011; Martello *et al.,* 2018). This type of regeneration is called passive, and allows the successional stages to occur naturally, and in this way, organisms such as ants can establish themselves in areas such as old *Eucalyptus* plantations, without the need to perform cuts (Benayas *et al.,* 2008).

The species and guilds sampled are grouped mainly in Myrmicinae, which is bound to this subfamily's high plasticity in terms of ecological niches they display (Hölldobler & Wilson, 1990). Examples of this are *Pheidole* and *Solenopsis* which occur in high frequency in *Eucalyptus* plantations (Barbosa & Fernandes, 2003; Fonseca & Diehl, 2004) and are generalists, which confers them advantages when compared to other guilds. We highlight *P. sospes,* which is abundant in varied ecosystems (Pereira *et al.,* 2007); *Solenopsis* spp. are dominant in the occupation of the leaf litter and soil in disturbed environments (Majer & Delabie, 1999).

Species of other subfamilies, such as *Anochetus altisquamis, Odontomachus* spp. and *Hypoponera* spp. are key constituents of the leaf litter regarding invertebrate predation (Brandão, 1999; Brandão *et al.,* 2015; Fernandes *et al.,* 2021). We still emphasize *B. admotus,* an omnivorous species dependent on the stratum moisture (Brandão *et al.,* 2009; Silva & Brandão, 2010), widely recorded in the three areas sampled.

Ectatomminae, Procerattinae, Pseudomyrmecinae, Dolichoderinae, and Dorylinae were undersampled in this study. Ectatomminae was especially represented by *Holcoponera striatula* (Mayr, 1884), a widespread species in the Neotropical region (Rosumek, 2017). Dorylinae was represented by *L. praedator* (Smith, 1858), characterized by seasonal nests with large numbers of workers, which are blind and predate on other social insects, such as other ants and termites (Borowiec, 2016). Another important record is of *N. splendens,* species scarcely sampled in biodiversity studies (Franco *et al.,* 2019; Wazema *et al.,* 2019) and classified as specialist predators of other social insects (Palacio, 2019).

Discothyrea sexarticulata is not frequent in leaf litter samplings, probably because its colonies count with few individuals (Katayama, 2013) and minute workers (Brandão *et al.,* 2009). Moreover, it is a species associated with native vegetation (Wazema *et al.,* 2020) which has a highly specialized feeding habit (Brown, 1957), for they depend on the eggs of small arthropods like spiders and millipedes (Dejean & Dejean, 1998). Thus, the presence of this species may be explained by the regeneration of the understory and the increase in resources it brings with it. Data on the occurrence of *D. sexarticulata* are still incipient in *Eucalyptus* plantations (see Suguituru *et al.,* 2011 and Mentone *et al.,* 2011); thus, our work adds new records for Alto Tietê.

Our results show that the older areas were the most diverse, which again suggests the influence of the understory (Grimbacher & Hughes, 2002; Underwood & Fisher, 2006; Chu *et al.,* 2019), especially in the records of specialist species. Areas with ages estimated to be between 1-4 years presented the lowest values for all indexes, which is because the underbrush, composed mainly of grasses, and the management of the soil do not allow the formation of leaf litter. These events cause disturbance in the edaphic fauna, leading to significant distress in the community (Martello *et al.,* 2018). Thus, a less complex environmental structure with limited resources negatively influences the establishment of ant species (Martello *et al.,* 2018). Species of Myrmicinae were the ones that occurred the most and, among the recorded guilds, litter omnivore and scavenger were present in all areas, for their genera encompass several species classified as dominant (Delabie *et al.,* 2000). The records of species like *N. splendens* e *D. sexarticulata* show that the

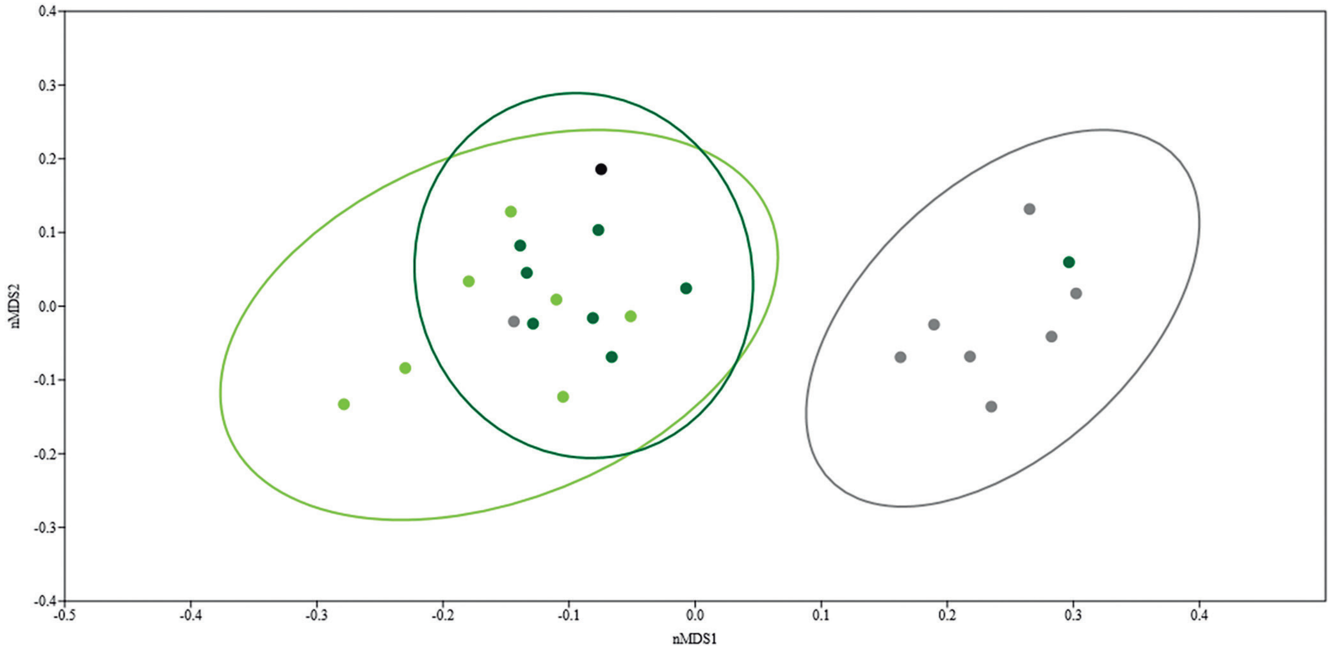

Figure 3. Non-metric multidimensional scaling (NMDS) Bray-Curtis type for the areas with 1-4 years (grey), 7-12 years (light green), and 14 years (dark green). (Anosim = 0,0001).

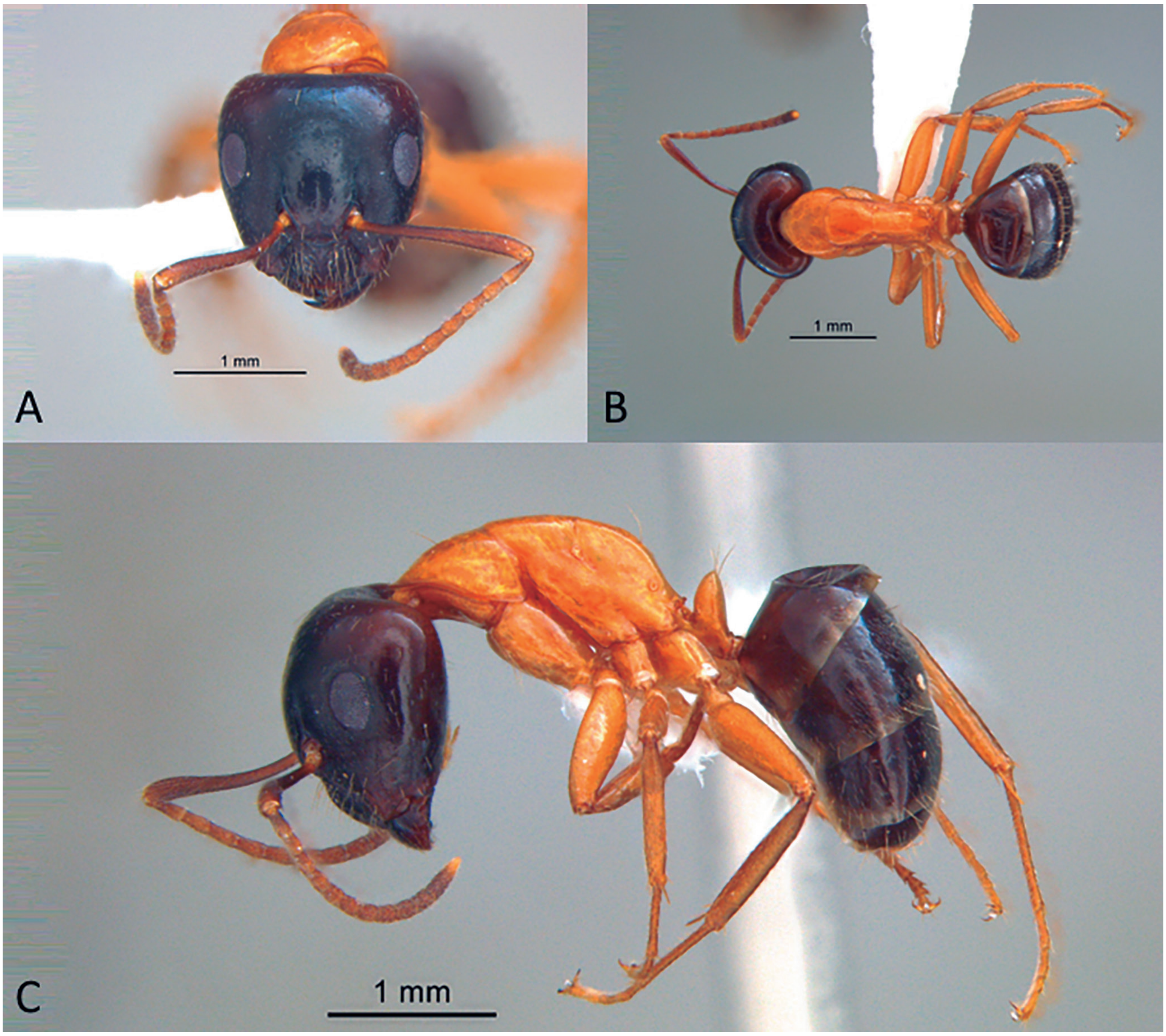

Figure 4. *Camponotus cillae* recorded for the RPPN Botujuru: (A) front view; (B) dorsal view; (C) side view.

regeneration of the older areas provides resources to those species with more specialized habits.

We underscore the presence of two species, a possible new species of *Octostruma* Forel, 1912, recorded in the areas with an understory, and *Camponotus cillae* Forel, 1912 (Fig. 4) recorded in Brazil only by the type series in the state of São Paulo (Botucatu) by Forel in 1912 (Forel, 1912). Data on their biology is scarce in the literature. This new record highlights the importance of new studies on biodiversity to our comprehension of the distribution of species (Janicki *et al.,* 2016).

In addition, our results show the potential of RPPN Botujuru; the occurrence of *C. cillae* and the possible species of *Octostruma* in the Botujuru RPPN emphasizes the importance of conservation units for threatened biomes. Fragments of Atlantic Forest found in conservation units are important for the preservation of biodiversity, acting as refuge areas for species that suffer from anthropogenic pressures such as deforestation and urban growth (Gardner *et al.,* 2009; Pardini *et al.,* 2009; Lima *et al.,* 2020), providing resources, even for those rarely collected. Another important factor is the potential of conservation units to discover new species, mainly invertebrates (Liu *et al.,* 2022), a group that lacks inventory data.

They also emphasize the need to advance in the identification of recorded morphospecies, increase sampling efforts, use other collection techniques to better understand the diversity of ants and other faunal and floristic groups in the RPPN. Moreover, these results also underscore the need for the creation of measures aiming at preserving the area, given that the urbanization process has been intense in the vicinity of the conservation unit.

CONCLUSION

The diversity of ants evaluated in this first work, carried out with only one collection campaign, indicates that the natural regeneration process is having positive effects in abandoned areas. The fragments that compose the RPPN Botujuru may prove to be very representative of the Alto Tietê region regarding the conservation of *the*

Atlantic Forest. The species list generated by our study and their classification in trophic guilds provide data that can contribute to the monitoring of the ant diversity of these conservation units in the long term.

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