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An arboreal ant that inhabits tunnels is recorded for the first time in the South region of Brazil

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Abstract

Crematogaster is a highly diverse ant genus found in most terrestrial ecosystems worldwide. Most *Crematogaster* species are arboreal, inhabiting various forest and open environments, and exhibit a wide range of behaviors. However, *Crematogaster stollii* is unique within this genus for constructing and living inside tunnel systems in the trees it occupies. Workers are never found on the tree trunk surfaces, instead, they always forage inside these tunnels. This species is primarily found in the Amazon and does not occur south of the Tropic of Capricorn. In our study, we documented this tunnel-building behavior for the first time in another but similar species (*Cr.* aff. *stolli*), in the South region of Brazil. We propose that this represents a new species awaiting formal description and provide data on the tree species where the ant occurs, its tunel-building behavior, its distribution on the trees, and the structures it uses as nests. We encourage myrmecologists to further explore the ant fauna of South Brazil, particularly agricultural fields containing associated natural forests. This region may still harbor undiscovered ant species and review surprising behaviors, such as the one we report here.

Palavras-chave: *Crematogaster*; Myrmecology; Structure; Tunnel-building behavior.

Formiga arboreal que habita túneis é registrada pela primeira vez na região Sul do Brasil

Resumo

Crematogaster é um gênero de formigas altamente diverso, distribuído na maioria dos ecossistemas terrestres ao redor do mundo. A maioria das espécies de *Crematogaster* é arborícola, habitando diversos ambientes florestais e abertos, e exibindo uma ampla gama de comportamentos. No entanto, *Crematogaster stollii* é única dentro deste gênero por construir e viver dentro de sistemas de túneis nas árvores que ocupa. As operárias nunca são encontradas nas superfícies dos troncos das árvores, em vez disso, elas sempre forrageiam dentro desses túneis. Esta espécie é encontrada principalmente na Amazônia e não ocorre ao sul do Trópico de Capricórnio. O presente estudo documenta esse comportamento de construção de túneis pela primeira vez em outra espécie semelhante (*Cr. aff. stolli*), na região Sul do Brasil. Propõe-se que isso represente uma nova espécie, aguardando descrição formal, e fornecemos dados sobre as espécies de árvores nas quais a formiga ocorre, seu comportamento de construção de túneis, sua distribuição nas árvores e as estruturas que usa como ninhos. Incentivamos os mirmecologistas a explorar mais a fauna de formigas do Sul do Brasil, especialmente em campos agrícolas com florestas naturais associadas. Esta região pode ainda abrigar espécies de formigas desconhecidas e comportamentos surpreendentes, como o que aqui relatado.

Palavras-chave: Comportamento de construção de tuneis; *Crematogaster*; Estrutura; Mirmecologia.

Introduction

Crematogaster (Formicidae: Myrmicinae) is a hiperdiverse and cosmopolitan ant genus (HÖLLDOBLER; WILSON, 1990; LONGINO, 2003). Most of the species are found in the tropics in countries from Central and South America, including Brazil (LONGINO, 2003). These ants are dominant in forest ecosystems, where many species nest and forage specifically on the canopy of high trees, and due to this habit, they are often regarded as arboreal ants (BUREN, 1958; LONGINO, 2003). Nonetheless, some *Crematogaster* species can also occur in temperate zones nesting on both, ground or rock surfaces (LONGINO, 2003).

A wide range of habits and behaviors is found in *Crematogaster* ants, and they can be involved in many well-studied interactions (HÖLLDOBLER; WILSON, 1990; LONGINO, 2003). Usually, *Crematogaster* colonies are densely populated, and, due to traits such as high aggressiveness, competition and predation, they can quickly dominate food sources and territories (LONGINO, 2003; RICHARD; FABRE; DEJEAN, 2001). Some species play roles in agriculture as natural enemies of crop pests such as the "ampola" of yerba-mate, *Gyropsylla spegazziniana* (DÍAZ, 1997), the brown marmorated stink bug *Halyomorpha halys* (CASTRACANI *et al.*, 2017; KAMIYAMA *et al.*, 2021), coffee pests such as the coffee berry borer *Hypothenemus hampei* and the coffee leaf miner *Leucopetera coffella* (REZENDE *et al.*, 2021); they can engage in interactions with extrafloral nectar producing plants usually protecting these in a type of mutualistic interaction (BLÜTHGEN *et al.*, 2000; DO NASCIMENTO; DEL-CLARO, 2010); they are found making decomposition of mammal carcasses (ADETIMEHIN *et al.*, 2024); and they are involved in a variety of interactions with other ant species, such as mirmicry, eavesdropping, social parasitism and mutualism (POWELL *et al.*, 2014; VANTAUX *et al.*, 2007). Furthermore, some few species possess distinct habits of living, using plant structures or even constructing elaborated nests for protection and/or avoid competition (HÖLLDOBLER; WILSON, 1990; LONGINO, 2003; PEETERS; WIWATWITAYA, 2014)

In contrast to the other species in the genus, *Crematogaster stollii* Forel 1885 stands out with respect to these habits of living. This ant species inhabits large trees and possess dense colonies of individuals, and it occurs both in large Amazon forests and open environments, such as trees along fences on the edges of roads and agricultural pastures (LONGINO, 2003). Their workers, however, are never found exposed foraging to the surface of trees it inhabits, instead, they carry out constructions of tunnel systems that extend from underground to reach up the tip of branches of large trees, so that the ants are only found inside these tunnels (LONGINO, 2003). Therefore, the foraging activity of the workers is restricted to the trails inside these tunnels.

With respect to the feeding habits, Crematogaster ants are considered as generalist predators or omnivores, depending on the species (HÖLLDOBLER; WILSON, 1990; LONGINO, 2003). But in case of tunnel inhabiting species, such as *Cr. stolli*, its feeding habit might be associated with predation and/or synphilia interactions with Homopterans and individuals from Ceoccoidea, which are very abundant inside the ant tunnels; or even cryptic resources provided by the plant (LONGINO, 2003). *Crematogaster stolli* was also found in mutualistic interaction with the invasive hibiscus cochineal *Maconellicocus hirsutus* (Green, 1908) (Hemiptera: Pseudococcidae) on cocoa, hibiscus and other ornamental plants (MARQUES *et al.*, 2018).

Crematogaster stollii occurs only in the Neotropics, with a distribution ranging from East to West of Central and South America, limited in the North by Guatemala and in the south by Bolivia and also occurring in Brazil (BOLTON, 1995; LONGINO, 2003). In Brazilian territory, however, this species has no distribution south to the Tropic of Capricorn, but in some Northeastern states (Bahia and Sergipe), the Midwest (Goiás and Mato Grosso) and mainly in the Amazonian region (Rondônia, Pará, Acre, Amazonas, Roraima, Tocantins and Amapá) (FOREL 1904, MANN 1916, KEMPF 1968, 1972, SOUSA-SOUTO *et al.* 2016, MARQUES *et al.* 2018, ANTWEB 2024). Therefore, to the best of our knowledge there is no occurrence of *Cr. stollii* in the South region of Brazil.

In the present paper we recorded for the first time this curious tunnel constructing behavior in another *Crematogaster* species, other than *Cr. stollii*, from the South region of Brazil. We provide data on the tree species used as substrate by *Cr.* aff. *stollii* ants, the behavior of constructing tunnels, as well as the description of structures functioning as nests for the ant. Therefore, our study provides new information on the habits and behaviors for this important hiperdiverse arboreal ant genus.

Materials and methods

The tunnels of *Cr.* aff. *stollii* were discovered by chance, during the scientific initiation project "*Ilex paraguariensis* (yerba-mate) and the associated ant fauna" conducted between 2018 and 2019. After completing one of the sampling events in a yerba-mate crop in São Mateus do Sul, located in the Southeast region of Paraná state, Brazil, we passed through a nearby fragment of native forest (25º 44' 30" S 50º 26'01" W). There, we observed tunnel constructions in several native trees, which immediately drew our attention (Fig. 2). We marked these locations, initiating the investigation that led to the findings presented in this paper. Although we did not measure humidity, the study site was near a floodplain, suggesting a relatively humid environment. The climate in this region is classified as subtropical humid, mesothermic, characterized by mild summers, well-distributed rainfall throughout the year, and frequent, severe frosts. Annual precipitation exceeds 1400 mm, with temperatures ranging from 13ºC and 21ºC, and annual average of 17ºC (VANHONI; MENDONÇA, 2008; ALVARES *et al*., 2013). The vegetal formation is Atlantic Forest, specifically Ombrophilous Mixed Forest (MAACK, 1981).

In order to collect the ants, we gently removed the upper parts of the cartoon tunnels where the trails containing the ants were present and captured 15 workers of *Cr.* aff. *stollii* by hand, using a tweezer. We then analyzed the ants under the light of a stereomicroscope in the Ecology lab in the State University of Paraná (UNESPAR), campus União da Vitória. The morphology traits and comparisons were based on the key of Crematogaster species and images contained in Longino (2003). We used a Zeiss Stereo DiscoveryV20 steromicroscope attached to a Zeiss Axiocam 305 color video camera in the *Laboratório de Sistemática e Biologia de Formigas – Universidade Federal do Paraná*, Curitiba, Brazil to obtain high-resolution images of the ant workers. The photos were combined through image stacking using the software Combine ZP 1.0 to improve their quality.

We further recorded the tunnel constructions by taking measures of height and width with the use of a 0, 2 mm precision manual caliper. Furthermore, we sampled botanic material from the trees where the ant tunnels were found, in order to identify their species. The plant identification was gently carried out by Rogério Krupek with material from the herbarium of the UNESPAR, campus União da Vitória.

Results

The workers of *Cr.* aff. *stollii* we found in our site of study are between 2.0 up to 2.2 mm length, they are light brown in tonality, with the gaster slightly darker than the rest of the body and the legs are relatively clearer whit shades ranging from brown until yellow, similar to the mesosoma color (Fig. 1 A). The head is wide, with the posterior edge emarginated and erect setae on the face, in numbers up to 20; the antennal scapes are short so that they do not reach the head occiput (Fig. 1 B). In the Mesosoma, there are long and filiform bristles (Fig. 1. A and C). A focus on the propodeal spiracle reveals it is not larger than the base of the propodeal spine (Fig. 1. C).

We recorded the ant and their tunnel constructions in four arboreal species typical of Mixed Ombrophilous Forest: *Cinamodendron dinisii* Schwacke (Canelaceae), *Podocarpus lambertii* Klotzsch ex Endl. (Podocarpaceae), *Sebastiania commersoniana* (Baill.) L.B. Sm. & Downs (Euphorbiaceae) and *Nectandria* sp. (Lauraceae). The *Cr.* aff *stollii* ant tunnel constructions were 1.0 (+-0.2) cm wide and 0, 85 (+-0.2) cm high in mean, in the different plant species where they were recorded. The tunnel distributions along the tree trunk were similar among the tree species it occurs: the tunnels start at ground level and extend along one side of the main trunk surface towards the canopy of each plant (Fig. 2). In most cases, it is impossible to visualize where the tunnels start or end; they appear to enter the bark or even the trunk and they subsequently emerge again to the surface, becoming visible. There may be more than one tunnel over the main trunk of each tree and, as the trunk branches upwards and spread, the tunnels accompany some branches, sometimes it appears to be a gallery of tunnels. We also recorded a tunnel connection formed between two adjacent trees (*Cr. dinisii* and *Nectandria* sp.) at the point where the branches intersected. The communication of the ant tunnels, between these two trees indicates that the size of the colonies in this ant species can be very large (Fig. 2).

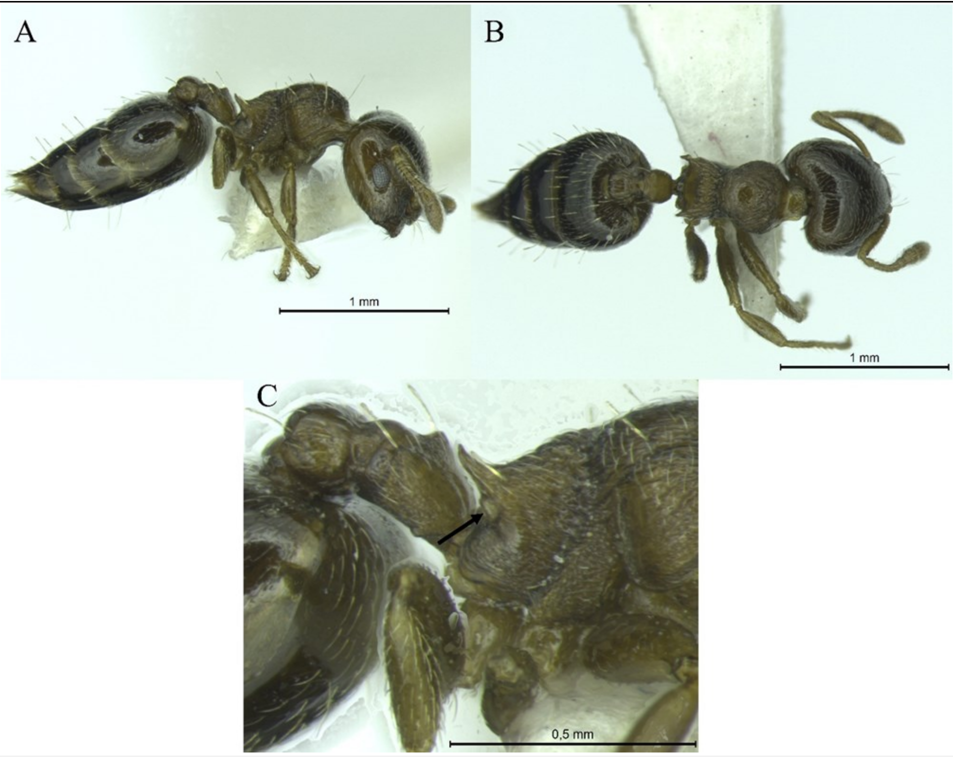

Figure 1. *C.* aff. *stollii* worker collected in the Southeastern region of Paraná (BR); (25º 44' 30" S 50º 26' 01" W), Brazil in April 2019. Details from a (a) side view, (b) dorsal view and (c) metasoma regions, focusing on the propodeal spiracle (black setae). Pictures by the author.

Figure 2. Scheme of tunnels and nest structures of *Crematogaster* aff. *stollii* recorded in the Southeastern region of Paraná (BR) (25º 44' 30" S 50º 26' 01" W), April 2019. Shown is the (a) *Cinnamodendron dinisii* bark ring used as refuge and nests by *Crematogaster stollii* activity; (b) cartoon tunnels constructed by the ant workers along the trunk; and (c) juveniles (eggs and perhaps larvae, white in color) inner to the ring barks of *Cinnamodendron dinisii*. Pictures by the author.

In trees of *C. dinisii*, a dilation of the bark tissues forms a type of ring in the trunk, which interrupt the ant tunnels. By carefully removing parts of this structure, it is possible to observe *Cr.* aff *stollii* juveniles (eggs and perhaps larvae, white in color, Figure 2, C). The closer to the soil, the thicker these rings become in the trunk, reaching up to 25 cm height and a thickness beyond the circumference of 5 cm. The width of these structures is quite variable, some have 36 cm, 40 cm, 45 cm, while others reach the entire circumference of the trunk. The tunnel that emerges after passing through this ring, always part from the opposite side of its arrival. Further, we recorded tunnels of *Cr.* aff. *stollii* in a tree of *P. lambertii*. We observed an agglomerate of workers, eggs and winged individuals, by lifting a thick crust formed in the trunk by the accumulation of bark and associated mosses. In all over the cases, we only observed active workers foraging when inside the tunnels.

Discussion

Our study is the first to identify another Crematogaster species (*Cr.* aff. *stolii*) that construct and lives inside tunnel systems in the tree species it inhabits (LONGINO, 2003). This behavior was otherwise only recorded for *Cr. stollii* ants that occurs in the Amazonian region, and we now found it in another similar species in the South of Brazil. Despite sharing this habit, there are significant morphological differences among *Cr.* aff. *stollii* and *Cr. stolli*. They both possess all characters of crinosa complex of species: broad head with emarginate posterior border, short scapes, and erect setae on the face (LONGINO, 2003). However, the propodeal spiracle in *Cr.* aff. *stollii* is not larger than the base of the propodeal spines (Fig. 1. C), as it is in *Cr. stollii* (LONGINO, 2003). Further, the size of the *Cr.* aff. *stollii* workers is sharply smaller (2.0 - 2.2 mm) than those found on *Cr. stolli* (ca. 5.0 – 5.1 mm) (LONGINO, 2003). Therefore, we suggest *Cr.* aff. *stollii* is a new species waiting for a description, and we encourage taxonomists to accomplish this task in the future.

We provide a description of the tunnels and their distribution on the tree trunks occupied by *Cr.* aff. *stollii*. The tunnel connections we found between large and adjacent trees suggest that *Cr.* aff. *stollii* colonies may be very large possessing many individuals and multiple queens, as it is for *Cr. stollii* (LONGINO, 2003). This tunnel making behavior deserves further investigation but, living inside tunnels may be a strategy associated to protection from natural enemies and/or avoid competition (BRONSTEIN; ALARCON; GEBER, 2006; HEIL; MCKEY, 2003; JANZEN, 1966). Devoting more studies in this regard is important since many ant species possess specialized mutualistic interactions with plants, mainly defending them from herbivorous attacks (JANZEN, 1966).

Some ants from Myrmelachysta and Azteca genera also possess this habit of constructing and living inside tunnels in trees and, as it is for *Cr. stollii* in the Amazonia, their food resources come directly or indirectly, entirely from the plants they occupy (HÖLLDOBLER; WILSON, 1990; LONGINO, 2003). Based on the habit, we suggest *Cr.* aff. *stollii* benefit from similar food resources present inside the tunnels, either directly provided by the plant (cryptic resources) or not (predation and/or sinphilia interactions with hemipterans inhabiting the tunnels). Nonetheless, the presence of a smaller propodeal spiracle compared to *Cr. stollii* that suggests that *Cr.* aff. *stollii* might go for preys or other food items out of the tunnels. A smaller spiracle could avoid the loss of water by evaporation and thus dehydration will not be a problem in this case (HÖLLDOBLER; WILSON, 1990; LONGINO, 2003).

Despite Longino (2003) claimed he never found a place in the complex colony of *Cr. stollii* he could claim to be a central nest, structures functioning as nests were identified in trees from *C. dinisii* in our study for *Cr.* aff. *stollii*. Tree species possessing structures, spaces, thick dilated barks, easily removable fibers and organic material are all plant traits perhaps associated with the occurrence of these tunnel constructing ants, allowing them to establish their colonies. The *Cr. stollii* ants use plant fibers to construct theirs tunnels and sometimes incorporate capsules of dead worker heads to reinforce the structure of these tunnels (LONGINO, 2003). We suggest something similar to *Cr.* aff. *stolli* based on the observations of their tunnels and the plant species it inhabits.

Final considerations

We recorded colonies of *C.* aff. *stollii* in four arboreal species which are commonly associated to yerba mate (*Ilex paraguariensis*) crops in the South region of Brazil (DANIEL, 2009). We encourage mirmecologists to explore more the ant fauna from the South of Brazil, emphasizing agricultural fields containing associated natural forests (GERHARDT, 2013). During our research in yerba mate agroecosystems, we revealed hundreds of ant species (IASCZCZAKI *et al*., 2024, unpublish.) with some of them new to the Science (IASCZCZAKI; HOLDEFER, 2021) or with new status of distribution (IASCZCZAKI *et al*., 2021). Yerba mate agroecosystems are frequently consorted to the native forest and it may function as a refuge for the biodiversity of ants (JUNQUEIRA; DIEHL; DIEHL-FLEIG, 2001) because most of the surrounding of these areas are intensively cultivated monoculture systems. In conclusion, the region of study and its components may harbor still not explored ant species, as well as intriguing and unexpected behaviors, such as the one we reported in our study.

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