What’s in Your Head, Zombie? *Camponotus renggeri* Ants Parasitized by the Entomopathogenic Fungus *Ophiocordyceps camponoti-renggeri* in a Relic of Atlantic Forest in Caatinga Biome, Brazil

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ABSTRACT – Entomopathogenic fungi are widely known to parasitize and manipulate the behavior of various orders of arthropods. In this study, we recorded parasitism involving the entomopathogenic fungus *Ophiocordyceps camponoti-renggeri* and host ants of the species *Camponotus renggeri* in a relic of Atlantic Forest in Caatinga biome, in addition to characterizing the environmental and ecological aspects of the interaction. We found 210 infected ant workers, fixed predominantly on the margins of the abaxial face of leaves of the local vegetation. Most ants used simple phyllotaxis leaves for fixation and death, whose size varied widely. The parasitized ants died about 1.5 m above the ground. These results suggest that *Ophiocordyceps camponoti-renggeri* has high virulence and that the behavioral changes induced in its hosts favor their fitness.

Keywords: Biodiversity; formicidae; negative interaction; parasitism; pathogen.

O que Tem em Sua Cabeça, Zumbi? Formigas *Camponotus renggeri* Parasitadas pelo Fungo Entomopatogênico *Ophiocordyceps camponoti-renggeri* em um Relicto de Mata Atlântica no bioma Caatinga, Brasil

RESUMO – Fungos entomopatogênicos são amplamente conhecidos por parasitar e manipular o comportamento de várias ordens de artrópodes. Neste estudo, registramos o parasitismo envolvendo o fungo entomopatogênico *Ophiocordyceps camponoti-renggeri* e formigas hospedeiras da espécie *Camponotus renggeri* em um relícto de Mata Atlântica no bioma Caatinga, além de caracterizar os aspectos ambientais e ecológicos da interação. Encontramos 210 formigas operárias infectadas, fixadas predominantemente nas margens da face abaxial das folhas da vegetação local. A maioria das formigas usava folhas de filotaxia simples para fixação e morte, cujo tamanho variava amplamente. As formigas parasitadas morreram cerca de 1,5 m acima do solo. Esses resultados sugerem que *Ophiocordyceps camponoti-renggeri* possui alta virulência e que as mudanças comportamentais induzidas em seus hospedeiros favorecem sua aptidão.

Palavras-chave: Biodiversidade; formicidae; interação negativa; parasitismo; patógeno.

¿Qué Hay en Tu Cabeza, Zombi? Hormigas *Camponotus renggeri* Parasitadas por el Fongo Entomopatógeno *Ophiocordyceps camponoti-renggeri* en un Relicto de Floresta Atlântica en el bioma Caatinga, Brasil

RESUMEN – Los hongos entomopatógenos son ampliamente conocidos por parasitar y manipular el comportamiento de varios órdenes de artrópodos. En este estudio registramos el parasitismo que involucra al hongo entomopatógeno *Ophiocordyceps camponoti-renggeri* y hormigas hospederas
Introduction

Fungi represent the second largest group of eukaryotic organisms with 120,000 species currently accepted (Hawksworth & Lücking, 2017), representing 6.6% of the total estimated species in the world (Hawksworth, 2001). Because they are heterotrophic, fungi need to absorb organic compounds as a source of energy for their growth (Cooke & Swihpps, 1980). By reason of this biological characteristic, fungi evolve as saprophytes, feeding on dead organic matter, or symbionts, maintaining close associations with other living organisms (Maccheroni et al., 2004). In addition, they can act as parasites, being closely linked to the host, penetrating or degrading tissues to absorb essential substances for their development, causing the host’s death or disability (Delgado & Murcia, 2011; Araújo & Hughes, 2016). When they have this biological character, they are classified as entomopathogenic fungi (Mora et al., 2017).

About 700 species and 90 genera of entomopathogenic fungi have been described (Khachatourians & Qazi, 2008). These organisms are known to form parasitic relationships with different orders of arthropods (Shrestha et al., 2016; Sobczak et al., 2020), causing about 80% of the diseases that affect insects (Alves, 1998). In general, these interactions occur in the form of epizootics, causing the contamination of a significant number of ant individuals in tropical forests (Andersen et al., 2012). In some cases, the parasites adaptively manipulate the behavior of the hosts to increase their “fitness”, taking the host to places favorable to the efficient dispersion of their spores, thus increasing the transmission to new hosts (Araújo & Hughes, 2016).

The behavioral manipulation of hosts is considered a phenotypic extension of the parasitic organism (Dawkins, 1982), given that the atypical behavior developed in the host is not caused by the expression of its own genes, but by the genes belonging to the parasite (Hughes & Libersat, 2019). Thus, the parasite uses the host only as a vehicle for the dispersion and transmission of its genes, increasing its adaptive value (Dawkins, 1982; Hughes, 2013). According to Hughes et al. (2011), the behavioral alteration of “zombie” ants is triggered by the infection in the animal’s brain, which induces the host to seek mainly the leaves of the plants to fix their jaws and, later, to die (“death grip”).

The zombification induced by fungi of the *Ophiocordyceps unilateralis* complex (Ophiocordycipitaceae: Hypocreales) in ants of the *Camponotus* genera (Formicidae: Formicinae) is well documented in several tropical forests around the world (Loreto et al., 2014; Araújo et al., 2015, 2018), highlighting the wide geographical distribution of this interaction. Given the climatic and environmental conditions, tropical forests are the most favorable region for the occurrence of parasitism in ants caused by entomopathogenic fungi (Evans, 1974; Araújo et al., 2018). In the Brazilian Amazon, for example, natural epizootics have been recorded in adult individuals of *Camponotus* sp. caused by fungi *O. unilateralis* complex (Andrade, 1980; Andriolli et al., 2019). In the state of Ceará, remnants of the Atlantic Forest are areas with high potential for carrying out studies that demonstrate the importance and ecological and evolutionary significance of parasitic interactions between different groups of organisms (Sobczak et al., 2017a, 2017b, 2020).

Although many recent discoveries have been published, little is known about the distribution of species of entomopathogenic fungi and the ecology of their associations with arthropod hosts, mainly...
with ants. This fact is more aggravating considering that Camponotus spp. is one of the most species-rich genus with 1,524 species currently described with pandemic distribution (Janicki et al., 2016; Antweb, 2020; Antmaps, 2020), with diverse and well distributed workers by forest strata and dominant species that can change the regional diversity and ecosystem dynamic (Vicente & Izzo, 2021; Silva-Viana et al., 2021; Vicente et al., 2016). Among the Camponotus species, Camponotus renggeri Emery, 1894 has the known distribution from Panamá to Uruguay (Brandão, 1991; Basset et al., 2012). Thus, the aim of this study was to know and characterize the ecological and environmental aspects of parasitism involving the entomopathogenic fungus Ophiocordyceps camponoti-renggeri Araujo et al., 2015 and the host ant Camponotus renggeri Emery, 1894, as well as expanding the area of occurrence of this interaction.

Material and Methods

Study area

The study was conducted on the Purgatório trail (4° 13.355’S, 38° 53.732’W), located in the humid forest of the municipality of Pacoti, Ceará state, Brazil. With 400 m of extension, the trail is inserted in the area of Maciço de Baturité, a Conservation Unit of the state of Ceará that covers 32,690 hectares distributed in 13 municipalities of the region (Nascimento et al., 2010). The Serra de Baturité (4° 4’30”S and 38° 52’39.15 W) represents the main geographic reference of the Maciço de Baturité, being characterized as a type of humid enclave within the semiarid region, with average annual temperature and precipitation of 20.8° C and 1,221 mm, respectively (PDITS, 2008).

Due to its geographical location and altitude varying from 600-1100 m, the Serra da Baturité contains in its highest areas a complex and diversified vegetation that acts as an ecological refuge for many species of animals, in addition to offering essential conditions for the supply of different hydrographic basins in the region (PDITS, 2008). These humid forests contain remnants of the forest complex of the Atlantic Forest and the Amazon Forest. Because of this, they have great biodiversity and high rates of endemism (Rebouças et al., 2021; Moro et al., 2015).

Results

Two exploratory field expeditions were carried out in February 2019 and January 2020, both during rainy season, in order to register and collect specimens of Camponotus ants parasitized by entomopathogenic fungus. In the first expedition, a visual search for infected ants was carried out along the trail. All ants that had a death grip on the vegetation were collected and placed in sterile microtubes using tweezers. The samples were taken to the Laboratory of Ecology and Evolution of University of International Integration of Afro-Brazilian Lusophony (UNILAB), where photos of the specimens and the division of the stage of development in which the fungi were found were taken. At this time, the stage of development of the parasitic fungus was categorized into four phases: Fresh, Stroma, Mature and Hyperparasitized (Andersen et al., 2012). Subsequently, the samples were sent for taxonomic identification at the Laboratório de Mirmecologia – Centro de Pesquisas do Cacau (CEPEC-CEPLAC), Bahia state, Brazil, to the taxonomist Jacques Hubert Charles Delabie.
In the subsequent expedition, 73 infected ants were found. Of the total number of parasitized individuals, the majority (79.45%, n = 58) were found in leaves of trees. Infected ants were also found in climbing vegetation (12.33%, n = 9), in young trees (6.85%, n = 5) and in a monocot species (1.37%, n = 1). The dead individuals of parasitized ants were invariably found on the leaves of the plants (100%, n = 73), often located on the abaxial face of this vegetative organ (97.26%, n = 71) especially close to the leaf apex (Fig. 1A). A single specimen was found on the adaxial side (1.37%) and another on the recombinant leaf rake. All individuals located on the abaxial and adaxial faces of the leaves were adhered by the mandibles to the margins or to the central rib of the leaf. There was a higher frequency of individuals adhering to the margins (79.45%, n = 58) than to the central ribs of the leaves (19.18%, n = 14).

As for the size of the leaves used for ant’s fixation, there was a large variation in size (4.3 cm to 30.4 cm between the apex and the junction with the petiole), with an average size of 14.1 cm. Most ants died on simple phyllotaxis leaves (98.63%, n = 72), with a single individual being observed on a recomposed leaf. Regarding the height at which the parasitized ants died, a considerable variation was observed (76 cm to 2.18 m above the ground), with an average height of 1.5 m. Zombie ants were found between 1 to 4 leaves per tree, with an average of 1.55 leaves with zombie ant per host tree.

**Discussion**

The relatively large number of parasitized ants reported in this study reveals that the collection area constitutes a climatically favorable environment for the establishment of parasitic associations between the parasitic fungus *Ophiocordyceps camponoti-renggeri* and hosts *Camponotus renggeri*. Given that the relatively constant conditions of high humidity and temperature in tropical forests are ideal prerequisites for fungal infection (Evans, 1974; Andriolli et al., 2019), the Serra de Baturité has ideal conditions for the establishment of parasitic relationships of the fungus-ant type given its climate and high rates of parasitism in other taxa (PDITS, 2008; Sobczak et al., 2017b, 2019, 2020).

The most infected ant workers (69.35%) had recently been killed (fresh), had stroma (stroma) or were parasitized by other fungi (hyperparasitized), while only 42 specimens (30.66%) contained sexually fruiting mature bodies, capable of

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**Figure 1** – The death grip of *Camponotus renggeri* on the leaf’s abaxial face (A) and approximate view of the host ant infected by *Ophiocordyceps camponoti-renggeri* with mycelium and fruiting body of the parasite. Source: Emily O. Fonseca.
producing spores and effectively dispersing them. This pattern of higher proportions of fresh dead ants coincides precisely with the region’s rainy season, a pattern that is seen for parasitized *Camponotus atriceps* and *C. bispinosus* that were more abundant in periods with greater air humidity (Cardoso-Neto et al., 2019). This shows that, although the number of parasitic ants was high, most of these individuals were in a stage of life whose risk of infection for the rest of the colony was low. Similar results were obtained by Andersen et al. (2012) in a study on the interaction dynamics between Ophiocordyceps and its host ants. Other studies have found that, due to hyperparasitism, the fruiting bodies of Ophiocordyceps fungi are unable to release their spores, thereby reducing the impacts caused on Camponotus populations (Andersen et al., 2012; Araújo et al., 2018). Because ants contribute about 25% of animal biomass in tropical forests (Schultz, 2000), entomopathogenic fungi occupied an ecological niche little explored by other living beings. Thus, it is observed that hyperparasitic fungi perform the population control of Ophiocordyceps and guarantee the permanence of Camponotus colonies in the tropical forests of the world (Morozov et al., 2007; Andersen et al., 2012; Araújo & Hughes, 2016).

The frequent presence of dead ants fixed on the leaf margins close to the apex was also observed by Araújo et al. (2015) in a study that described three new species of specific Camponotus hosts on the fungus Ophiocordyceps in the Brazilian Amazon. This result, however, differs from those demonstrated by Sobczak et al. (2017) and Andersen et al. (2009) in which the authors observed a higher frequency of individuals adhering to the central ribs in relation to those fixed to the margins. The adaptation of death in the vicinity of the leaf apex can be explained by the dew drops formed on these tips throughout the year, thus enabling the continuous and consistent development of fungi (Araújo et al., 2015). It is also noteworthy that the choice of the host plant ant is not selective, but based mainly on environmental factors of temperature and humidity favorable to the development, close to the soil, of the parasitic fungus (Andersen et al., 2009; Andriolli et al., 2019). The strategic position of death of the parasitized ant (upside down) facilitates the release and deposition of fungal spores on the forest floor, which subsequently infect other ants that pass through (Araújo et al., 2015).

The observed frequency of ants parasitized at heights above the ground ranging from 76 cm to 2.18 m (mean = 1.5 m) is similar to other observations made in the locality (Sobczak et al., 2017). In contrast, in other studies of Ophiocordyceps parasitized ants *Colobopsis leonardi* (Emery, 1889) was recorded very close to the ground, at an average height of 25.20 cm (Andersen et al., 2009). The authors suggest that lower heights provide more favorable conditions for the development of the parasite. This fact can be seen in a study made in Amazon Forest that demonstrate that Camponotus (Myrmotherix) atriceps (Smith, 1858) parasitized by Ophiocordyceps camponoti-atricipis can be found in higher areas in raised environments than at the forest edge, where light is available from lower layers (Andriolli et al., 2019). The preference for higher heights, however, may occur due to the type and structures provided by the plant species used for the death grip (Sobczak et al., 2017).

*Camponotus* is a frequent and diverse genus in neotropical regions, with many key species in processes and interactions, many of which are still unknown or with unknown distribution and life history (Santos-Silva et al., 2016, Vicente et al., 2016, 2018, 2019; Dattilo et al., 2020; Jory & Feitosa, 2020). In addition, the diversity of parasites that can lead to this behavior and the frequency with which it happens is still superficially studied.

**Conclusion**

This study brings essential information about the natural history of the fungus, as well as the dynamics of the fungus. Knowing aspects of the life history of the organisms involved in the relationship can help to understand the dynamics of parasitism and can contribute with biological control programs.

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