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SHORT COMMUNICATION

Record of *Ophiocordyceps unilateralis sensu lato*, the zombie-ant fungus, parasitizing *Camponotus* in an urban fragment of Atlantic Rainforest in southeastern Brazil

Bruno Corrêa Barbosa[a]*, Vitor Ribeiro Halfeld[b], João Paulo Machado de Araújo[c], Tatiane Tagliatti Maciel[a] & Fábio Prezoto[a]

[a]*Laboratório de Ecologia Comportamental e Bioacústica (LABEC), Universidade Federal de Juiz de Fora, Juiz de Fora, Minas Gerais, Brazil;* [b]*Laboratório de Protozoologia, Universidade Federal de Juiz de Fora, Juiz de Fora, Minas Gerais, Brazil;* [c]*Department of Biology, Penn State University, University Park, State College, PA, USA*

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Ophiocordyceps is a fungal pathogen of ants of the tribe Camponotini. It is called zombie fungus, since it changes the host behavior, causing them to die in an exposed position, typically clinging onto and biting into the adaxial surface of shrub leaves. This study aimed to describe the occurrence of parasitic associations between *Ophiocordyceps* and ants of the genus *Camponotus* in an urban fragment of Atlantic Rainforest in southeastern Brazil and to measure the rate of hyperparasitism in *Ophiocordyceps* by other fungi in the same location. We found 57 individuals of four species of ants and three species of fungus. The age categories of fungi were equally distributed, and rate of hyperparasitism was 17.5% (n = 10). The sampled area was recognized as an important site of *Ophiocordyceps* occurrence.

Keywords: fungal diversity; hypocreales; host specificity; parasite manipulation

Introduction

The genus *Ophiocordyceps* Petch contains several species of fungi specialized to infect and kill their ant hosts (Evans et al. 2011a). Some species, such as *O. unilateralis s.l.*, are capable of manipulating the host's behavior, causing it to abandon the colony and act to its own detriment, to enhance the parasite's fitness (Andersen et al. 2009). This behavior, in which genes of the parasite are expressed in the host's phenotype, may be classified as an extended phenotype (Dawkins 1982).

Ophiocordyceps unilateralis s.l. infecting ants within the tribe Camponotini is common, especially in tropical forests. The host dies in exposed locations, clinging to the midrib of leaves by its mandibles, often followed by the growth of hyphae (Evans & Samson 1984). The rise of compact hyphae from the host's dorsal pronotum forming conspicuous stromata (stalks) is characteristic of this group of fungi. Ascomata (fertile parts) develop on these stromata and produce the sexual spores that are ejected and spread out after they reach maturity (Kepler et al. 2011; Evans et al. 2011b).

This study describes the occurrence of parasitic associations between *Ophiocordyceps* fungi and ants of the genus *Camponotus* in an urban fragment of Atlantic Rainforest in southeastern Brazil, and quantifies the rate of hyperparasitism in *Ophiocordyceps* by other fungi at the same location.

Materials and methods

The botanical garden of Federal University of Juiz de Fora is located in the urban area of Juiz de Fora, in southeastern Brazil (21°43′28″ S, 43°16′47″ W; elevation 510–820 m). The area (84 ha) consists of a secondary fragment of Atlantic Rainforest, with characteristics of a seasonal semi-deciduous forest.

Collections of parasitized insects took place monthly between April and July of 2013 and June 2014 with the use of active search techniques. Infected ants were collected to estimate abundance and frequency of hyperparasitism in the ant populations. They were then categorized into four groups, according to the stage of development of the infection: fresh, immature, mature and hyperparasitized (Andersen et al. 2012). The fresh stage represents the initial phase of fungal development in the ant, which means that the host had been recently killed (about 5 days before). The immature stage refers to specimens lacking ascomata, but presenting stromata. Those considered mature were in either of two conditions: ready to eject the spores, or found dry and/or damaged by natural causes. We considered fungi found with parasites to be hyperparasitized; this normally occurs on old fungi and makes them sterile (Andersen et al. 2012).

Ant identification was based on the identification key proposed by Fernández (2003) and complemented by

*Corresponding author. Email: barbosa.bc@outlook.com

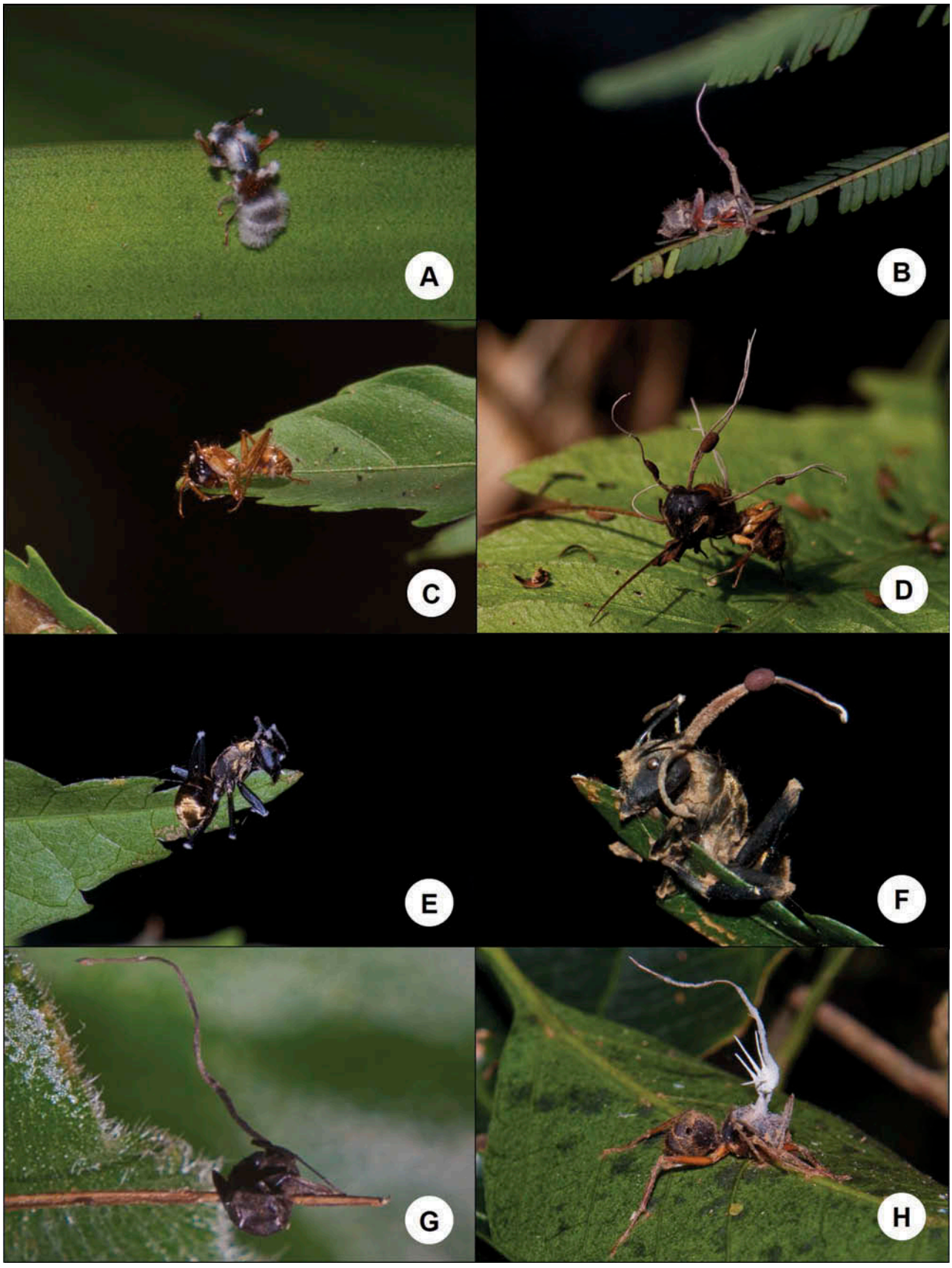

Figure 1. Ants infected by *Ophiocordyceps* and their stages of development found in the botanical garden of the Federal University of Juiz de Fora, Brazil. *C. rufipes* parasitized by *O. camponoti-rufipedis*: A, fresh; and B, mature. *C. balzani* parasitized by *O. camponoti-balzani*: C, fresh; and D, mature. *C. sericeiventris* parasitized by *O. unilateralis*: E, fresh; and F, mature. G, *C. crassus* parasitized by *O. unilateralis* (mature). H, *C. rufipes* hyperparasitized.

Professor Harry Evans, CABI. Fungi were also identified by Prof. Evans based on morphological analysis of fruiting bodies and identification of the hosts present.

Results

During the study, 57 individuals belonging to four species within the genus *Camponotus* were found infected by three species of *Ophiocordyceps: O. camponoti-rufipedis* Evans et al., 2011a parasitizing *C. rufipes* (Fabricius, 1775) (Figure 1A, B); *O. camponoti-balzani* Evans et al., 2011a parasitizing *C. balzani* Emery, 1894 (Figure 1C, D); and *O. unilateralis* (Tul.) Petch, parasitizing *C. sericeiventris* Mayr, 1861 (Figure 1E, F) and *C. crassus* Mayr, 1862 (Figure 1G).

Following our categorization, 15 were classified as fresh, 15 as immature and 17 as mature. The rate of hyperparasitism in these populations was approximately 17% (n = 10) (Figure 1H).

Discussion

Hyperparasitic fungi can play a crucial role in the control of *Ophiocordyceps*–host interactions, when successfully established in the population (Morozov et al. 2007; Andersen et al. 2012). As suggested by Andersen et al. (2012), since these hyperparasites are responsible for the sterilization of these entomopathogenic fungi, they might mitigate the impact of *Ophiocordyceps* on *Camponotus* populations. However, in contrast to their study, performed in another area of Atlantic Rainforest, where 55% of the *Ophiocordyceps* were hyperparasitized (Andersen et al. (2012), the much lower proportions of hyperparasitism observed by us suggest a relatively weak influence on the entomopathogenic fungi.

Among the Formicidae, the genus *Camponotus* is considered the second most diverse of the Neotropical region (Fernández 2003). The *Camponotus* species found in the present study are described as opportunists due to their ability to feed on nectaries, living or dead prey and even Hemiptera and Lepidoptera exudates (Schilman & Roces 2005; Yamamoto & Del-Claro 2008), being able to easily adapt to urban environments (Fernandes et al. 2014). Such adaptability increases the *Camponotus* individuals' susceptibility to infection by *Ophiocordyceps*.

Evans et al. (2011b) noticed the high diversity of entomopathogenic fungi in tropical regions, estimating that dozens or even hundreds of species are yet to be described. Associations of the ants *C. rufipes, C. balzani* and *C. sericeiventris* with *Ophiocordyceps* have been predominantly observed in protected areas with remnant native vegetation (Andrade 1980; Evans et al. 2011b; Andersen et al. 2012). The record of *O. unilateralis, O. camponoti-rufipedis* and *O. camponoti-balzani* in the botanical garden of Juiz de Fora Federal University highlights the importance of this area as a possible reservoir of undocumented species and further studies may contribute to the elucidation of taxonomic, phylogenetic and ecological issues involving this relationship.

ORCID

Bruno Corrêa Barbosa http://orcid.org/0000-0003-2421-6239

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