Manipulação do comportamento de vespas (Hymenoptera: Vespidae) pelo fungo entomopatogênico *Ophiocordyceps humbertii* em Mata Atlântica no Ceará, Brazil

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ABSTRACT: Behavioral manipulation of hosts by parasites is important to increase the parasite fitness. Some species within the genus *Ophiocordyceps*, notably pathogens of ants, induce changes in the host's behavior, leading the host to die outside the colony and locking its mandibles on the edge of leaves. This study was conducted in Massif of Baturité, and for the first time, we describe the interaction between fungi and wasps in Northeastern Brazil. We found 24 specimens, eight of each species of wasp, all infected with *Ophiocordyceps humbertii*. The wasps were *Agelaia pallipes*, *Apoica flavissima*, and *Polybia chrysothorax*. All the hosts were found with their mandibles biting along the edges of leaves.

KEY-WORD: Entomopathogenic fungi, Insects, parasite manipulation, social wasps, zombie-wasp.

INTRODUCTION

Some parasitic organisms can manipulate the behavior of their hosts to maximize their dispersal and to complete their life cycles (Moore, 2012; Poulin, 2010). Entomopathogenic fungi can infect insect species across 20 orders, occurring in all stages of development, i.e., eggs, larvae, pupae, nymphs, and adults (Araújo and Hughes, 2016).

Some entomopathogenic fungi, are capable of manipulating the host's behavior aiming to increase dispersion. For example, species in the fungal genus *Ophiocordyceps* are known to infect a wide variety of hosts (Araújo and Hughes, 2016). These fungi have been observed on 11 insect orders. Particularly, ants (Hymenoptera) in the tribe Camponotini are frequently parasitized by one of 26 entomopathogenic fungi (Evans et al., 2011a, b; Hughes et al., 2011; Araújo and Hughes, 20016; Sobczak et al., 2017). In these cases, the parasitized ants leave their colonies, and before the ant dies, the fungus induces the ant to cling to an abaxial leaf surface with its mandibles. This favors the growth and dispersal of fungal spores (Andersen et al., 2009; Pontoppidan et al. 2009). This behavior was described by Dawkins (1982) as a case of extended phenotype, because parasite genes are expressed in the phenotype of the host.

The parasitism of wasps by entomopathogenic fungi is poorly described, mostly in Neotropical regions. Moreover, it is known that wasps can get infected with fungi when they come into contact with fungal spores present in the air or even in water droplets (Evans, 1989). Hughes et al. (2016) suggested that wasps can be manipulated by fungi, but they do not provide further details on how this can occur. Here we report *O. humbertii* infecting three species of social wasps across three different genera.

MATERIALS AND METHODS

The data were collected in the Massif of Baturité region, in the trails of São Luiz, Pacoti municipality (4° 180 40″ S, 38° 580 05″ W, altitude of 840 m), located in the Ceará state, Brazil. This area features a predominance of the mountainous semi-deciduous tropical forest, with a mean annual temperature of 20.8°C and mean annual precipitation of 1221 mm (Araújo et al., 2007) (Fig. 1A, B). Massif of Baturité is located within the Atlantic forest biome, a biodiversity hotspot. Most studies on fungi and their arthropod hosts are concentrated in the North and Southeast of the Brazil. Recently, Sobczak et al. (2017) reported *O. unilateralis* parasitizing the ant *Camponotus atriceps* in Massif of Baturité, and suggested that this area is promising for the study of the interaction between arthropods and entomopathogenic fungi. The wasps were found parasitized along a 22 km transect in Mata do Purgatório (Fig. 1B). We sampled between January 2016 and March 2018. We actively searched for specimens, and all parasitized individuals were collected and placed in Eppendorf tubes with silica gel to drying. Samples were taken to the laboratory of Ecology and Evolution of Universidade de Integração Internacional da Lusofonia Afro Brasileira. (UNILAB). To identify the social wasps, we used the keys proposed by Richards (1978), and we compared specimens to previously identified material. The identification of fungi was performed by João Paulo Machado de Araújo from Pennsylvania State University based on morphological characters, such as fruiting bodies and identification of the hosts. We deposited the voucher specimens in the Invertebrate Collection of the Instituto Nacional de Pesquisas da Amazônia, Manaus (INPA; curator ML Oliveira), and fungal samples were deposited in the Herbarium of UNILAB (curator JCMSM Sobczak).

RESULTS

Eight specimens of *Agelaia pallipes* Olivier 1792 (Fig. 1C), eight of *Apoica flavissima* van der Vecht 1972 (Fig. 1D), and eight of *Polybia chrysothorax* Lichtenstein 1796 (Fig. 1E) were found parasitized by *O. humbertii*. In all cases, the wasps were found with their mandibles biting along to the edges of leaves, along the vegetation within the dense forest. The parasitized wasps were found approximately 1.6 m above the forest floor, and, in all cases, their wings were spread or lifted upwards and their legs were also attached them to the substrate. We also observed that in some cases the fungal mycelia burst out from the host sutures, creeping on the leaf surface.

DISCUSSION

We documented, for the first time, wasps being parasitized, and their behavior manipulated by an entomopathogenic fungus in Northeastern Brazil. The behavioral manipulation of parasitized wasps allows the fungus to place the host in a microenvironment, which is optimal for fungal development and further sporulation. Loreto et al. (2014) have shown that in the case of *O. unilateralis*, if the host-fungus is removed form the leaf and placed on the forest floor, the fungus cannot develop. According to Tanada and Kaya (1993), the most favorable temperatures for the development of mycological infections fall between 20°C and 30°C, and high humidity (above 90%) is usually required for spore germination, which is why this type of association is common in tropical rainforests.

These three species are all swarm-founding wasps and have large biomass indices for social wasps in the Neotropics. *Agelaia* and *Polybia* the largest colony size (Carpenter and Marques, 2001). *Apoica* is a group with a few species and is characterized by its nocturnal foraging (Pickett and Wenzel, 2007). All species are widely distributed and are usually collected in the forest understory or in transition areas (Somavilla et al., 2014)

Infection by *Ophiocordyceps humbertii* occurred exclusively in the adult stage, possibly when wasps where foraging, where some females remain in the colony, doing maintenance (Carpenter and Marques, 2001). Another indication that the infection occurred during foraging, is that all the wasps were located only in the understory, which is usually occupied by social wasps when foraging (Somavilla et al., 2014). In this study, all three species exhibit the same biting behavior. First, the infected wasp sought a leaf, with no apparent preference for a particular plant species. The wasps then used their mandibles to attach to the edge of the leaf, which allowed for ideal conditions for growth of the fungus. After the wasps died, the fungus continued to develop, and the fruiting body (ascoma) burst out from the wasp joints, escaping from the inner part through the sutures (i.e. mouth, leg joints and stinger). We observed that the fungus took over the wasp body, between the membranes of the sclerites and antennal joints

It is estimated that entomopathogenic fungal diversity might be exceptionally high, and most of these species still need to be described, as it is the case of *O. unilateralis* complex (Evans et al., 2011b, Araújo et al. 2018). We also increase the known host-use breadth and geographic range of *O. humbertii* with records from the Northeast Atlantic forest. Our results indicate that different strategies of behavioral manipulation are employed by these fungi in order to place the host at a specific location that optimizes fungal development and dispersion. For example, the fungus induce the wasp to clinging the leaf and move away from the nest

The Massif of Baturité is highly relevant to studies involving entomopathogenic fungi and their hosts, and certainly will provide wich advancements in the discussions about the evolutionary relationships and existing ecological between fungi and their hosts.

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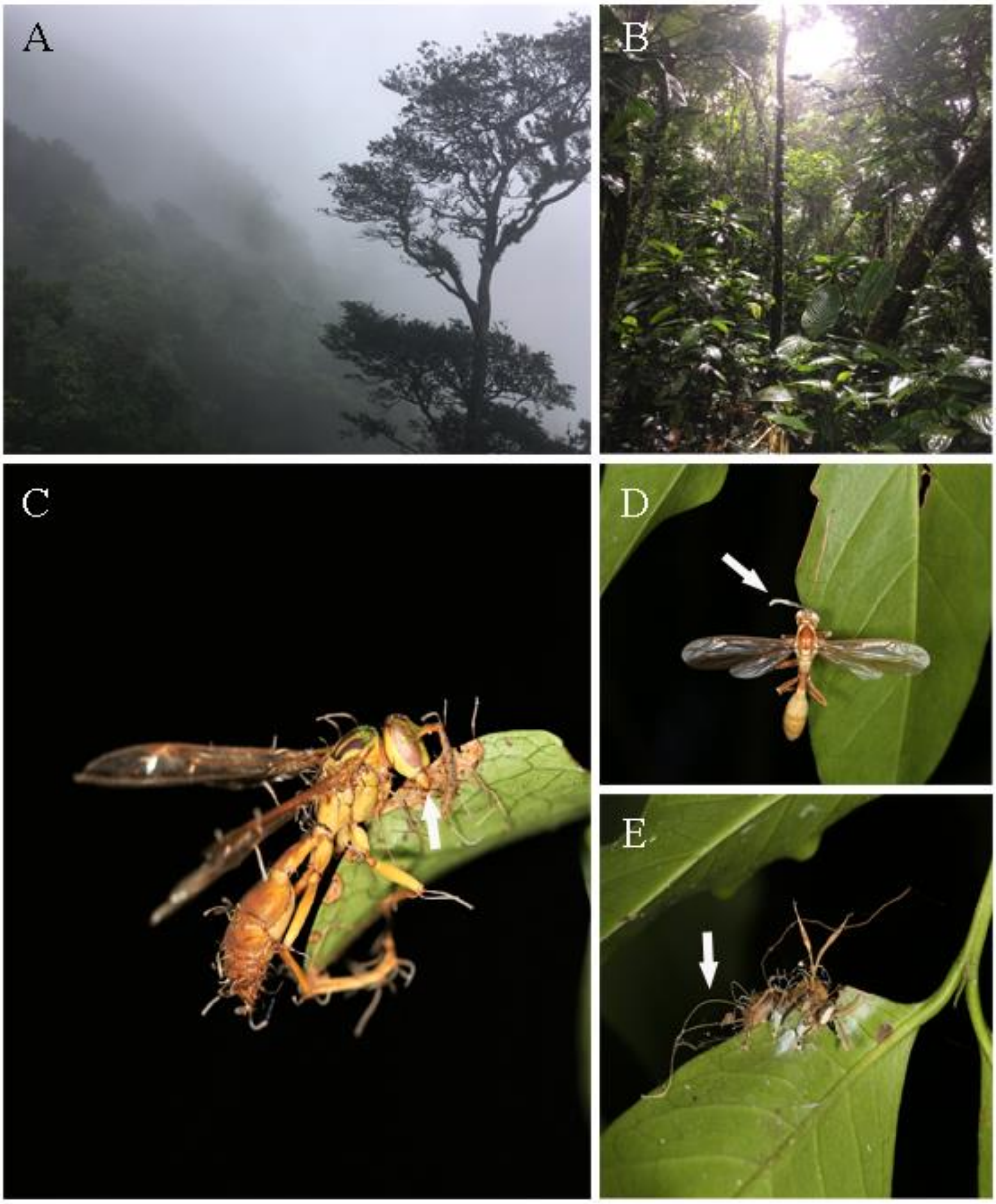

Fig. 1. A) Overview of mountainous semi-deciduous tropical forest; b) internal view of the area where the wasps were collected; C) *Agelaia pallipes*; D) *Apoica flavissima*; E) *Polybia chrysothorax*