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Behavior Manipulation of Crabronidae and Pompilidae (Hymenoptera) by the Entomopathogenic Fungus *Ophiocordyceps humbertii* (Ascomycota: Hypocreales) in an Amazonian Rainforest, Brazil

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RESUMO

Manipulação de comportamento de Crabronidae e Pompilidae (Hymenoptera) por fungo entomopatogênico *Ophiocordyceps humbertii* (Ascomycota: Hypocreales) em floresta amazônica, Brasil. Registramos e descrevemos a manipulação do parasitismo e do comportamento de três espécies diferentes de vespas aculeadas pelo fungo entomopatogênico *Ophiocordyceps humbertii*. Foram observados e coletados 30 espécimes parasitados, sendo cinco espécimes de *Larra* (Crabronidae), 21 de *Liris* (Crabronidae) e quatro de *Epysiron* (Pompilidae).
Palavras-chave: Fungo entomopatogênico, Insetos, Vespa aculeada, Vespa solitária, Vespa-zumbi.

ABSTRACT

The parasitism and behavior manipulation of three different species of aculeate wasps by the entomopathogenic fungus *Ophiocordyceps humbertii* was recorded and described. A total of 30 parasitized specimens were observed and collected, being five specimens of *Larra* (Crabronidae), 21 of *Liris* (Crabronidae), and four of *Epysiron* (Pompilidae).
Keywords: Aculeate wasps, Entomopathogenic fungi, Insects, Solitary wasp, Zombie-wasp.

Some parasitic organisms can manipulate the behavior of their hosts to maximize dispersal and complete their own life cycles (Moore, 2012; Poulin, 2010). Entomopathogenic fungi have been known to infect insect species of the orders Hymenoptera, Hemiptera, Coleoptera, Lepidoptera, Diptera, Megaloptera, Odonata, Orthoptera, and Blattaria (Araújo & Hughes, 2016). Depending on the host, the infection can also occur in different stages of development, like eggs, larvae, pupae, nymphs, and adults (Araújo & Hughes, 2016).

Amongst the behavior-manipulating entomopathogenic fungi, those of the genus *Ophiocordyceps* Petch, 1931 have been observed to infect the previously mentioned orders, particularly, ants

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(Hymenoptera) which are frequent hosts of one of the 20 entomopathogenic *Ophiocordyceps* species (Evans et al., 2011a, b; Hughes et al., 2011; Barbosa et al., 2015; Araújo & Hughes, 2016; Sobczak et al., 2017), like *Ophiocordyceps unilateralis* (Tul.) Petch 1931 clade, (sensu Araújo et al., 2018). In these cases, parasitized ants "controlled" by the fungus, leave their colonies in order to reach exposed leaves or branches to which they cling to with their legs and mandibles before death. This behavior favors the growth and dispersal of fungal spores (Andersen et al., 2009; Pontoppidan et al., 2009) and was described by Dawkins (1982) as a case of extended phenotype, since parasite genes are expressed in the phenotype of the host.

Parasitism of wasps by entomopathogenic fungi is poorly documented, especially in the Neotropical region. It has been noted that wasps can be infected with fungi by coming in contact with fungal spores in the air or even water droplets (Evans, 1989). Hughes et al. (2016) suggested the possibility of behavior manipulation of wasps by fungi but did not provide further details on how this can occur. Recently, Somavilla et al. (2019) reported fungal infections of 14 different species of social wasps (Vespidae: Polistinae) by the fungus *Ophiocordyceps humbertii* (C.P. 54 Robin; Sung et al., 2007) in the Amazon rainforest and Atlantic forest.

Though behavior manipulation by fungal infections on social Hymenoptera is relatively well-documented, no reports have been made so far about this kind of interaction involving solitary wasps. Here we report, for the first time, *O. humbertii* fungi infecting and manipulating the behavior different species of *Larra* Fabricius, 1793, *Liris* Fabricius, 1804 (Crabronidae) and *Epysiron* Schiodte, 1837 (Pompilidae).

Specimens were observed and collected in saplings of *Cocos nucifera* Linnaeus (Arecaceae), in an open area at Rio Preto da Eva (2°44'24.8"S 59°41'28.7"W, 35 m a.s.l.), Amazonas State, Brazil (Figure 1a). Parasitized individuals were actively searched for, collected, and placed in microtubes with silica for desiccation. These samples were then taken to the Hymenoptera Laboratory of the Instituto Nacional de Pesquisas da Amazônia (INPA) in Manaus, Amazonas State, Brazil. Crabronidae and Pompilidae specimens were identified with the keys proposed by Menke & Fernandez (1996) and Fernandez et al. (2018) respectively. The fungi species was identified based on morphological characters, such as fruiting bodies as well as host association. Voucher wasp specimens, and consequently the parasite fungi, were both deposited in the Invertebrate Collection of INPA (curator M.L. Oliveira). Field photographs were taken using a smartphone Samsung Galaxy S8 whilst specimens were photographed in the lab using a Leica MC170 HD digital camera attached to a Leica M165C stereomicroscope. These photographs were then stacked and combined using Leica Application Suite V4.11.

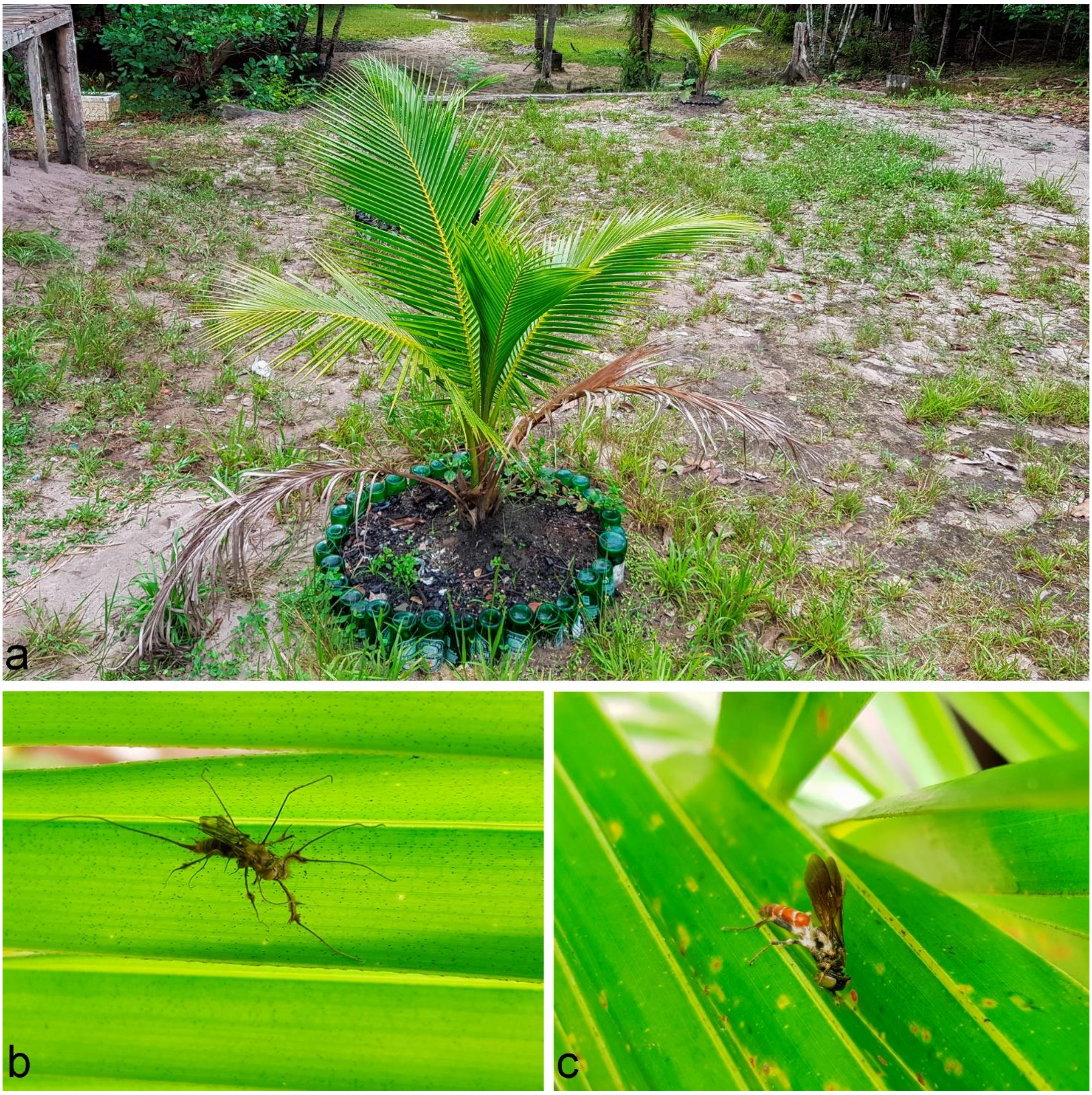

Figure 1: (a) *Cocos nucifera* Linnaeus (Arecaceae), in an open area at Rio Preto da Eva, Amazonas, Brazil. (b) Specimen of *Larra* Fabricius, 1793 (Crabronidae) *in situ*. (c) Specimen of *Liris* Fabricius, 1804 (Crabronidae) *in situ*.

Thirty wasp specimens parasitized by *O. humbertii* were collected: four specimens of *Larra* (Figures 1b, 2a) (Crabronidae), 21 of *Liris* (Figures 1c, 2b) (Crabronidae), and five *Epysiron* (Figure 2c) (Pompilidae: Pompilini). Every specimen collected was found with its mandibles and/or claws firmly grasping the edges of coconut leaves along the vegetation of a mand-made clearing in an Amazon rainforest fragment. The parasitized wasps were found approximately 0.80 m above the forest floor and, in all cases, their wings were unfolded to the sides of the body or longitudinally raised above the metasoma. The legs were always flexed and in some cases the mycelia emerged from several sutures throughout the body, adhering to the leaf surface and likely assisting with the fixation of the host on the substrate.

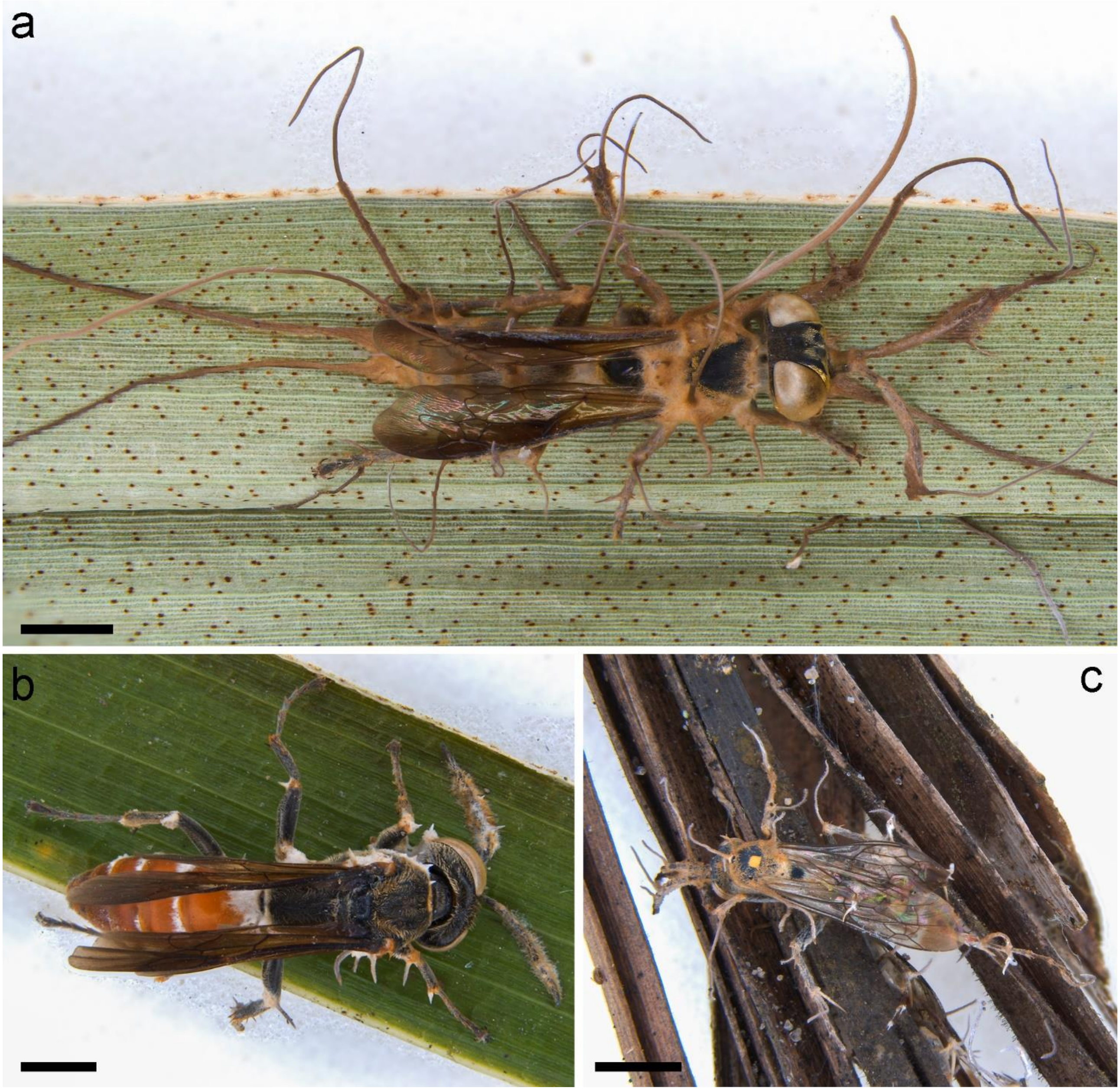

Figure 2. (a) Specimen of *Larra* Fabricius, 1793 (Crabronidae). (b) Specimen of *Liris* Fabricius, 1804 (Crabronidae). (c) Specimen of E*pysiron* Schiodte, 1837 (Pompilidae) all in the fruiting-bodies phase. Scale 2 mm.

Given the conditions in which the wasps were found, we assume that the infected specimens sought a leaf to which they clung using their mandibles and/or claws in a position which offered the ideal conditions for fungal development. Upon the death of the host, the fungus continued to develop, with the fruiting body (ascoma) and mycelia bursting out from joints, sutures, inter-sclerite membranes, stinger, and mouthparts of the insect and, as previously mentioned, different sutures throughout the body.

This kind of behavioral manipulation allows the fungi to mature in a relatively stable environment with constant temperature and humidity. According to Tanada & Kaya (1993), the most favorable temperatures for the development of mycological infections lies between 20°C and 30°C, while high humidity (above 90%) is usually required for spore germination. Given these conditions, this type of association is very common in tropical rainforests.

In specimens of *Larra*, *Liris*, and *Epysiron* the fungus acted similarly as it does in ants (Evans, 1982; Evans & Samson, 1982, 1984), with foraging wasps being infected, and the vegetative mycelium developing and consequently replacing the living tissue of the host. The mycelium subsequently reaches the nervous system, causing the wasp to either fly or climb up the vegetation, biting the axial surface or tip of exposed leaves or small branches before dying.

We recorded for the first time an instance of *O. humbertii* fungus manipulating solitary wasps in order to complete its life cycle. The behavior inducted by *O. humbertii* in these solitary Hymenoptera is very similar to that of other species within the *O. unilateralis* clade (sensu Araújo et al., 2018) which manipulate ants and the records previously made of *O. humbertii* manipulating social wasps (Vespidae: Polistinae) (Somavilla et al., 2019). Further studies might elucidate what seems to be a group of cryptic species within *O. humbertii* as well as how such characters evolved along with the evolutionary history of host and parasite.

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