



First record of *Ophiocordyceps dipterigena* (Ascomycota: Hypocreales: Ophiocordycipitaceae) infecting adults of *Melanagromyza sojae* (Diptera: Agromyzidae) in Brazil

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ABSTRACT: This note is the first report on the infection of adult stage of *Melanagromyza sojae* Zehntner, 1900 (Diptera: Agromyzidae) by the entomopathogenic fungus *Ophiocordyceps dipterigena* (Hypocreales: Ophiocordycipitaceae) (Berk. & Broome) G. H. Sung, J.M. Sung, Hywel-Jones & Spatafora, in subtropical Brazil. Entomopathogenic fungi, which attack dipteran insects, are of great scientific and economic importance mainly due to their potential as biological control agents of insect pests. Our findings bring new perspectives on the geographical distribution and host range of *Ophiocordyceps dipterigena*. We emphasize the need of further studies and research on new biological control agents of agricultural pests such as the soybean stem miner fly.

Key words: biological control, entomopathogenic fungi, natural infection, stem miner fly, soybean pests.

Primeiro registro de *Ophiocordyceps dipterigena* (Ascomycota: Hypocreales: Ophiocordycipitaceae) infectando adultos de *Melanagromyza sojae* (Diptera: Agromyzidae) no Brasil

RESUMO: Esta nota é o primeiro registro da infecção no estágio adulto de *Melanagromyza sojae* Zehntner, 1900 (Diptera: Agromyzidae) pelo fungo entomopatogênico *Ophiocordyceps dipterigena* (Hypocreales: Ophiocordycipitaceae) (Berk & Broome) G. H. Sung, J.M. Sung, Hywel-Jones & Spatafora, no Brasil subtropical. Fungos entomopatogênicos, que atacam dípteros, são de importância científica e econômica principalmente devido ao potencial como agentes de controle biológico de pragas de insetos. Este trabalho traz novas perspectivas sobre a distribuição geográfica e variação de hospedeiro de *Ophiocordyceps dipterigena*. Destacamos a necessidade de ampliar o conhecimento sobre novos agentes de controle biológico de pragas agrícolas como a mosca minadora do caule da soja.

Palavras-chave: controle biológico, fungos entomopatogênicos, infecção natural, mosca minadora do caule, pragas da soja.

Entomopathogenic fungi infect many insect orders as well as arachnids in order to complete part of their development and the dispersion of sexual spores (EVANS, 1982). The fungal families *Clavicipitaceae*, *Cordycipitaceae* and *Ophiocordycipitaceae* (Euscomycetes: Hypocreales) include some of the most important and widespread group of entomopathogenic fungi (ARAÚJO & HUGHES, 2016). As an ecological group, these pathogens have broad geographical and host range and are of great ecological and economic importance, due to their potential as biological control agents and also as a source of medicinal products (FARIA & WRAIGHT, 2007; SHRESTHA et al., 2010; SOSA-GÓMEZ et al., 2010). The genus *Ophiocordyceps*

Petch, 1931 (Hypocreales: *Ophiocordycipitaceae*) is a widely distributed group of fungi with approximately 200 described species (CROUS et al., 2004) infecting a wide range of insects from the following orders Coleoptera, Blattaria, Dermaptera, Diptera, Hymenoptera, Hemiptera, Isoptera, Lepidoptera, Mantodea, Orthoptera and Odonata (EVANS, 1982; SUNG et al., 2007; SOSA-GÓMEZ et al., 2010; ARAÚJO & HUGHES, 2016). However, despite the potential of this fungal group as natural control agents of insect populations, only few entomopathogenic species have been studied in detail. For instance, *Ophiocordyceps unilateralis* Tulasne & Tulasne, 1865 core clade *sensu* (ARAÚJO et al., 2018) that infects Camponotini ants and *Ophiocordyceps*

sinensis (Berk, 1843) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora, which infects lepidopteran larvae, have been the most studied species (EVANS & SAMSON, 1984; EVANS et al., 2011; ZHANG et al., 2012; SOBCZAK et al., 2017). Although, other complex of species are still under studied, as it is the case of pathogens of flies (order Diptera).

Dipteran families including *Asilidae*, *Calliphoridae*, *Muscidae* and *Tabanidae* have also been found parasitized by entomopathogenic fungi (EVANS, 1982; WATSON et al., 1993; STEENBERG et al., 2001; LASTRA et al., 2006; ARAÚJO & HUGHES, 2016). Despite this, there are few reports in the literature of dipteran insects infected by *Ophiocordyceps* and little is known about these specific associations (EVANS, 1982; BARBOSA et al., 2016). The family Agromyzidae (Diptera), also called leaf miner flies, comprises a large group of exclusively phytophagous flies. The larval stage parasitizes a wide variety of plant species and infest economically important plants (SPENCER, 1973; BENAVENTE-CORAI et al., 2005). *Melanagromyza sojae*, also known as the soybean stem-miner, which attacks several species of *Fabaceae*, has a wide geographic range (Africa, India, Asia and Oceania) and has been considered an important pest of soybean crops (WANG, 1979; TALEKAR, 1989). This species has also been reported in South America, increasing concerns about its potentially negative effects on soybean crops in Brazil (LINK et al., 2009; HIROSE & MOSCARDI, 2012). In Brazil, agromyzid flies infesting soybean fields were first reported by GASSEN & SCHNEIDER (1985) in the state of Rio Grande do Sul. LINK et al. (2009) recorded *M. sojae* from five localities in the municipality of São Francisco de Assis, and ARNEMANN et al. (2016) identified *M. sojae* from both Rio Grande do Sul and Santa Catarina states through molecular characterization. Recently, SALGADO-NETO et al. (2017a) collected specimens of *M. sojae* which were parasitized by parasitoid wasps from different genera within *Pteromalidae* (Hymenoptera) in the localities of Tupanciretã and Cruz Alta, Rio Grande do Sul State. These findings have brought valuable information about some natural control agents of *M. sojae* that could be used in biological control programs. Global spread and new records of *M. sojae* in soybean fields from South America (GUEDES et al., 2017) highlight the need for further studies on the ecology and management of such a potentially invasive pest. In the present study we aimed to identify the fungus parasitizing adults of *M. sojae* in subtropical Brazil, increasing the information about

distribution of natural biological control agents of the soybean stem miner fly.

Melanagromyza sojae adults infected with fungus were collected from off-season soybean plants in 2017. Twenty samples spaced 10 meters apart were taken in two soybean fields located in the district of Arroio Grande (29°40'S and 53°44'W), municipality of Santa Maria, Rio Grande do Sul state, Brazil. Flies and fungus stroma were collected in a 60mL container and transported to the laboratory of Agropan for species identification. The fungus identity was confirmed through morphological analysis following FREIRE (2015). Despite the fly's advanced state of decomposition, its identification was possible through molecular characterization according to the methodology followed by ARNEMANN et al. (2016). Genomic DNA was extracted from the fly's body by using the ReliaPrep™ gDNA Tissue Miniprep System kit (Promega). Amplification of the mitochondrial cytochrome oxidase I gene (COI) was performed by using the primers COI-F: 5'-GATTTTTTGGKCA YCCMGAAG-3' and COI-R: 5'-CRAATACRGCTCCTATWGATAAWAC-3' and the 28S rRNA gene amplification by using the primers D2A: 5'-ACAAGTACCGTGAGGGAAAGTTG-3' and D3B: 5'-TCGGAAGGAACCAGCTACTA-3' (DE LEY et al., 1999). The amplified products were purified by precipitation with polyethylene glycol (SCHMITZ & RIESNER, 2006), subject to sequencing by the chain termination method using the BigDye 3.1 sequencing kit (Applied Biosystems) and analyzed by automated capillary sequencer 3500L (Applied Biosystems). Sequences obtained were then compared to voucher sequences available on the GenBank of the National Center for Biotechnology Information (NCBI) using the Blastn program <<http://www.ncbi.nlm.nih.gov>>. Results confirmed that the infected flies belonged to *M. sojae*. Four female flies were reported on soybean plant leaves and presented two globular yellow structures protruding from their head that corresponded to the stroma of *O. dipterigena*.

The first record of Diptera infected by *O. dipterigena* in Brazil was reported by SOBESTIANSKY (2005) from Nova Petrópolis in the Rio Grande do Sul State; although, in this case, the host species identification was not possible. Later, FREIRE (2015) collected specimens belonging to *O. dipterigena* complex parasitizing flies of the family Muscidae, Asilidae and Micropezidae in the states of Santa Catarina, Paraná and Amazonas, however whether these specimens are in fact *O. dipterigena* or an unidentified *Ophiocordyceps* species still remains to be elucidated. BARBOSA et al. (2016)

reported flies from the family *Muscidae* infected by *O. dipterigena* in the Minas Gerais state, Brazil. This is the first record in Rio Grande do Sul of the natural action of *O. dipterigena* parasitizing adults of *M. sojae* (*Agromyzidae*). Furthermore, this is the first time an adult Agromyzid fly is reported as host of *O. dipterigena* in south Brazil.

Ophiocordyceps dipterigena is a natural control agent of Agromyzid flies; and therefore may be of great interest in biological control programs of agricultural pests. To date, most biocontrol products are obtained from few fungi species, none of which have been developed from fungi belonging to Ophiocordyceps. The potential of Ophiocordyceps as a biological control agent of crop pests has been previously explored by SALGADO-NETO et al. (2017b) who tested the pathogenicity of *Ophiocordyceps melolonthae* (Louis René Tulasne and Charles Tulasne) G.H. Sung, J.M. Sung, Hywel-Jones and Spatafora, 2007 (Hypocreales: *Ophiocordycipitaceae*) against white grub pests (Coleoptera: *Melolonthidae*). Despite this, no previous study attempted to test the natural action of Ophiocordyceps fungus against soybean pests. Furthermore, biocontrol agents based of fungi have been used mainly against Coleoptera, Hemiptera and Lepidoptera, but few target dipteran insects (FARIA & WRIGHT, 2007). Our finding indicated that *O. dipterigena* might be considered as a potential candidate for the biological control of Agromyzid flies, opening new possibilities for the use of entomopathogenic fungi in biological control programs. Further studies are encouraged to test the effectivity of *O. dipterigena* in reducing the host population, therefore, its potential as a biological control agent of the soybean stem miner.

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DECLARATION OF CONFLICT OF INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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