

**First report of the occurrence of *Ophiocordyceps melolonthae* (Ascomycota: Hypocreales: Ophiocordycipitaceae) in larvae of *Diloboderus abderus* Sturm (Coleoptera: Melolonthidae) in Brazil**

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SALGADO-NETO, G., VALMORBIDA, I., GUEDES, J.V.C., BLUME, E. **First report of the occurrence of *Ophiocordyceps melolonthae* (Ascomycota: Hypocreales: Ophiocordycipitaceae) in larvae of *Diloboderus abderus* Sturm (Coleoptera: Melolonthidae) in Brazil.** Biota Neotropica. 15(2): e20140108. <http://dx.doi.org/10.1590/1676-06032015010814>

**Abstract:** This note is the first report on the infection of *Diloboderus abderus* Sturm (Coleoptera: Melolonthidae) larvae by the fungus *Ophiocordyceps melolonthae* (Hypocreales: Ophiocordycipitaceae) in subtropical Brazil. Identification was made possible by extraction and sequencing of the fungal DNA that was covering the larvae's mouthparts, prothorax, cuticle, and digestive tract (alimentary canal). Amplification, sequencing and comparison of the ITS region of the ribosomal DNA with voucher sequences of GenBank were performed and were 95% similar to *Ophiocordyceps melolonthae*. The fungus is an entomopathogen which attacks Melolonthidae larvae, having scientific and economic importance because of the need for increased knowledge on its distribution and on alternatives for biological control of white grubs.

**Keywords:** Biological control, dissemination, entomopathogen, entomopathogenic fungi, natural infection.

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**Resumo:** Esta nota é o primeiro registro da infecção de larvas de *Diloboderus abderus* Sturm pelo fungo *Ophiocordyceps melolonthae* na região subtropical do Brasil. A identificação foi possível graças à extração e sequenciamento do DNA do fungo que cobria o aparelho bucal, protórax, cutícula e aparelho digestivo (canal alimentar) das larvas. Amplificação, sequenciamento e comparação da região ITS com seqüências voucher do GenBank foram realizados, mostrando 95% de similaridade com *Ophiocordyceps melolonthae*. O fungo é um entomopatôgeno que ataca larvas Melolonthidae, tendo importância científica e econômica devido à necessidade de aumento do conhecimento sobre sua distribuição e de alternativas de controle biológico de corós.

**Palavras-chave:** Controle biológico, disseminação, entomopatôgeno, fungos entomopatogênicos, infecção natural.

Entomopathogenic fungi have broad host range (DeFaria & Wraight 2007), geographical range and potential to control white grubs. For these reasons, it is important to collect, purify and conserve germplasm of the wide variety of species as they may be used in a selection of strains to perform a biological control program. The conservation of strains in reference collections should be priority for some genotypes that may be lost due to local environmental changes (Hernandez-Velazquez et al. 2011).

In Brazil, previous records have shown that natural epizooties caused by the fungus *Cordyceps unilateralis* (Euascomycetes: Hypocreales) were found in adults of the ants *Camponotus* sp. and *Atta cephalotes* (Hymenoptera:

Formicidae) in the Amazon Forest (Andrade 1980). Recently, in Minas Gerais, *Ophiocordyceps unilateralis* (Hypocreales: Clavicipitaceae) was discovered as a specific fungal pathogen of the ant species *Camponotus rufipes*, *C. balzani*, *C. melanoticus* and *C. novogranadensis* (Formicidae: Camponotini) (Evans et al. 2011). Moreover, epizooties caused by fungi in the "Planalto Region" of the state of Rio Grande do Sul have been the main cause of white grubs collapse in wheat. The fungi *Metarhizium anisopliae* (Metchnikoff) Sorokin, *Beauveria bassiana* (Bálsamo) Vuillemin and *Cordyceps* sp. are the most common fungi found infecting Melolonthidae larvae (Gassen 1992, Salvadori 2000, Salvadori & Pereira 2006).



**Figure 1.** *Ophiocordyceps melolonthae* infecting *Diloboderus abderus* larva.  
Foto: Salgado-Neto, 2014.

The *Cordyceps* genus was established as an ascomycete, fungal pathogen of arthropods bearing the ascospore producing structures on stromata arising from the host cadaver. *Cordyceps unilateralis*, originally characterizing species with non-fragmenting ascospores, was reorganized in the genus *Ophiocordyceps* (Hypocreales: Ophiocordycipitaceae), which currently comprises around 160 species (Sung et al. 2007, Evans et al. 2011). These parasites infect many different insects with a wide ecological range. The orders infected are Coleoptera, Blattaria, Dermaptera, Diptera, Hymenoptera, Hemiptera, Isoptera, Lepidoptera, Mantodea, Orthoptera and Odonata (Evans et al. 2011, Araújo & Hughes, 2014).

Here, we report the infection of *Ophiocordyceps melolonthae* in the third instar larvae of the white grub *Diloboderus abderus* in subtropical Brazil. Beside increasing the information about its geographical distribution, our finding is also important to biological control due to the increase of white grubs occurrence in cultivate and uncultivated fields in Brazil.

Trenches (50 cm long x 25 cm wide x 30 cm deep) were opened in a native grassland and 35 samples spaced 64 m apart were taken in approximately 15 hectares, at the district of Umbú, Rosário do Sul – Rio Grande do Sul, Brazil (30°35'S and 54°46'W). Both in 2011 and 2012, larvae of *D. abderus* with soil were collected individually in 60 mL plastic containers and transported to the Laboratory of Integrated Pest Management of Universidade Federal de Santa Maria (UFSM) to confirm the white grub species. The larvae presented a whitish cover and a horn like structure attached to it (Figures 1 and 2). In the Laboratory of Phytopathology of UFSM the larvae were superficially disinfected and rinsed in sterile distilled water.

Subsequently, some were frozen and others were dissected and separated into the following parts: mouthparts, prothorax, cuticle, and digestive tract (alimentary canal), which were then placed in Eppendorf tubes containing 100 mL of 0.85% saline solution.

Afterwards, the parts were added to Petri dishes with PDA (Potato Dextrose Agar) media and incubated in a growth chamber with a temperature of 25.6 °C and a photoperiod of 12h for seven days. Since there was no growth on PDA, frozen specimens with the whitish structure resembling a stroma of *Ophiocordyceps* spp. (Evans et al. 1999) were sent to the Biological Institut of São Paulo for molecular identification.

Extraction of the isolated DNA was performed according to the method employing the reagent C.T.A.B. (cetyltrimethylammonium bromide) described by Doyle & Doyle (1987). The stroma was triturated in micro tubes with the aid of a plastic pistil and the extracted genomic DNA was subjected to polymerase chain reaction (PCR) for amplification of the ITS region (Internal Transcribed Spacer) located between the genes encoding the 18S and 28S ribosomal RNAs. The primers for the ITS region were ITS1 (5'-TCCGTAGGTGAACCTGGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990). The amplified products were purified by precipitation with polyethylene glycol (Schmitz & Riesner 2006), subjected to sequencing by the chain termination reaction method employing the reagent Big Dye 3.1 (Applied Biosystems) and analyzed by automated capillary sequencer 3500 L (Applied Biosystems). The sequence obtained was deposited (GenBank access code KR082313) and compared to voucher sequences present in the GenBank of the



**Figure 2.** *Ophiocordyceps melolonthae* infecting *Diloboderus abderus* larva.  
Foto: Salgado-Neto, 2014.

National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov>), using the Blastn program (Altschul et al. 1990). The ITS sequenced is the following: AACCGCGGCGCCCGCCGGAGGACCCACGACTCTCT-TCCGCCCCAGCGGCATCTTCCGGGTGAGCCATCAC-GAATGAGTCAAACTTCAACAACGGATCTCTTGGT-TCTGGCATCGATGAAGAACGCAGCGAAATGCGATAA-GTAATGTGAATTGCAGAATTCAGTGAATCATCGAAT-CTTTGAACGCACATTGCGCCCCGCCAGCACTCTGGCG-GGCATGCCTGTCCGAGCGTCATTTACGCCCTCGAGC-CCCCCGGGGCTCGGCCTTGGGGCCCGCCCGCCG-CGCGCCGCCCCCAAATCCAGTGGCGACCCCGCCG-GGCCTCCCCTGCGCAGTAGCACACGCCTCGCACCG-GAGCCCCGGCGGCGGTCTCGCCGTGAAACGCGAC-CAGCCTCCAGGAGAGTTGACCTCGGATCAGGTAGG-GGTACCCGCTGAACTTAAGCATATCAATAAGCGGAG-GAA. The fungus was identified as *Ophiocordyceps melolonthae* (Spatafora et al. 2007), being the probable cause of larval death.

The species *Ophiocordyceps melolonthae* (= *Cordyceps melolonthae*) were studied in the United States of America (Mains 1958) and Mexico (Pérez-Silva 1977, Guzmán et al. 2001). Evans et al. (1999) showed the fungus being parasitic to *Cochliotus melolonthoides* (Gerst.), a Scarabaeidae from Tanzania, and on a melolonthid larva buried in Amazonian Ecuador forest soil that agrees well with the specimens studied here. Lloyd (1920) described subspecies of *C. melolonthae* from Brazil as “growing from the head of some larva” with subcylindric or globose stromata, about 20 mm long. From the macromorphology of stromata present in 10 collected larvae it was determined that the specimens collected represented a species of *C. melolonthae*. This fungus is considered one of the strongest pathogens to scarabaeid insects, and there have been many attempts to use it as an agent for biological control of Melolonthidae larvae (Mains 1958, Ferron 1981, Sung et al. 2007).

Morphological investigations are necessary to characterize these teleomorphs and to determine whether they are synonyms as previously described *Cordyceps* species, such as *C. staphylinidicola* (Kobayasi & Shimizu 1982), *C. sulfurea* (Kobayasi & Shimizu 1983) and *C. scarabaeicola* (Kobayasi & Shimizu 1976). Progress in methods for in vitro fruiting of *Cordyceps* species with *Beauveria* anamorphs are promising for developmental studies of *Beauveria* and its *Cordyceps* teleomorphs through integrated phylogenetics, developmental and mating studies (Sung et al. 2006, Lee et al. 2010, Rehner et al. 2011).

The natural occurrence in Melolonthidae larvae suggests that this fungus may play an important role in the control of white grubs and it must encourage more extensive studies on the possibility of utilizing this fungus in biological control programs.

## Acknowledgments

We thank Dr. Stephen A. Rehner, Systematic Mycology and Microbiology Laboratory/USDA-ARS, Dr. Ludwig H. Pfenning, Departamento de Fitopatologia/UFLA and Dr. Ricardo Harakava (Instituto Biológico de São Paulo), Laboratório de Bioquímica Fitopatológica, for their assistance in fungi identification.

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Received 16/07/2014

Revised 11/05/2015

Accepted 8/06/2015