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A natural fungal infection of a sylvatic cockroach with *Metarhizium blattodeae* sp. nov., a member of the *M. flavoviride* species complex

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ABSTRACT

A wild, forest-dwelling cockroach from the subfamily *Ectobiidae* (order *Blattodea*) in a nature reserve in Cavalcante, in the state of Goiás, Brazil, was found to be infected by a new, genetically distinct species in the *Metarhizium flavoviride* species complex that we describe here as *Metarhizium blattodeae*. The status of this fungus as a new species is supported by both multigenic sequence comparisons and protein profiles generated by MALDI-TOF (matrix-assisted laser desorption/ionization time-of-flight) mass spectrometry. This is one of the first reports of a naturally occurring fungal pathogen affecting any sylvatic (forest-dwelling) cockroach from any part of the world. *M. blattodeae* caused up to 96 % mortality of *Periplaneta americana* nymphs (a serious peridomestic cockroach species) after 10 d.

Introduction

Hypocrealean entomopathogenic fungi have long been studied as candidates for the microbial control of synanthropic cockroach pests. The cosmopolitan German and American cockroaches, *Blattella germanica* and *Periplaneta americana* (*Blattodea*), respectively, are among the most troublesome of household insect pests and are well known for both their

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hardiness and their ability to evade diverse control measures. Fungal activity against these insects has been shown with the important and widely used fungal biocontrol agents *Beauveria bassiana* and *Metarhizium anisopliae* (Mohan *et al.* 1999; Quesada-Moraga *et al.* 2004; Abedi & Dayer 2005; Lopes & Alves 2011; Hubner-Campos *et al.* 2013; Gutierrez *et al.* 2015). Knowledge about the potential of these fungi for cockroach control is based mainly on assays done under laboratory conditions with fungal strains originating from other host insects or substrates. Studies on cockroaches as target hosts have often emphasized the combined effects of fungus and synthetic insecticides (Kaakeh *et al.* 1996, 1997; Zurek *et al.* 2002). One commercialized mycoinsecticide based on *M. anisopliae* was registered with the US Environmental Protection Agency (Registration #70644-8) for cockroach control (Faria & Wraight 2007), but this registration has since been cancelled, and the manufacturer, EcoScience Corporation, is no longer in business. In Guatemala, however, a *Metarhizium*-based product (Zero QK-S 0,4 DP) has been sold by Agricola El Sol for use against domestic cockroach pests for many years.

More than 4000 cockroach species are known worldwide, but most have no importance as pests and mostly live far from human habitations (Bell *et al.* 2007; Beccaloni 2014). There are surprisingly few reports of natural fatal infections of any cockroach species, synanthropic or not, caused by any fungus that is clearly pathogenic for these insects, and these reports generally suggest that only very few cockroach individuals were negatively affected by these fungi. Although Roth & Willis (1960) compiled a surprisingly long list of fungi and yeasts from cockroaches, most of these fungi are primarily saprobic or facultative pathogens, and many were reported only from gut contents or feces. The cockroach fungi noted by Roth & Willis (1960) that cause diseases of insects include ectoparasitic ascomycetes (*Herpomyces* species: *Laboulbeniales*) and the cordycipitoid fungi now classified as *Ophiocordyceps amazonica* and *Ophiocordyceps blattae* (*Hypocreales*: *Ophiocordycipitaceae*), as well as a few poorly supported reports of *Metarhizium*.

Archbold *et al.* (1987) and Appel *et al.* (1987) studied an entomopathogenic yeast affecting the German cockroach, *B. germanica*. One of the authors (RAH) is aware of an unpublished finding in the early 1980's of an entomophthoralean fungus, probably *Batkoa* species based on the globose shape of the conidia, affecting cockroaches in a grain field near the Rothamsted Experimental Station in the United Kingdom. *Hymenostilbe ventricosa* was described from forest-dwelling cockroach nymphs in Thailand (Hywel-Jones 1995), and Cummings (2009) reported finding *B. bassiana* affecting single cockroaches at two different locations within New Zealand.

The cockroach from which this new fungus was isolated was collected as part of routine survey of vegetation and soil surfaces adjacent to aquatic sites where the primary intention of the field work was to find fungal pathogens of mosquitoes and other dipteran vectors of serious human and animal diseases. These vegetation surveys were undertaken to help to expand the comparatively poor understanding of the biota of Brazilian fungal entomopathogens (Sosa-Gómez *et al.* 2010). The scarcity of information about such fungi, especially in nonagricultural sites, is primarily a result of the lack of collecting efforts to find such fungi.

The present study reports a natural infection by *Metarhizium blattodeae* sp. nov. affecting a sylvatic cockroach species collected in Central Brazil. The status of this new species is supported by both gene sequence and mass spectrometric data about the proteins on its conidial surfaces, and we characterize the morphology and confirm the pathogenicity of this new fungus against *P. americana* nymphs under laboratory conditions.

Material and methods

Field location, collecting and initial processing of material

The survey for entomopathogenic fungi in which this new fungus was found was done during the rainy season (February 2015) in a tropical secondary gallery forest in the privately owned and operated Bacupari Reserve, close to the city of Cavalcante in northern Goiás state, Central Brazil. Leaves in the vegetation up to 2 m high, as well as plant remains on the soil, were checked for mycotized arthropod cadavers. Material with dead specimens was carefully removed, transferred to paper bags and placed in a polystyrene cooler at 20 °C (Benjamin *et al.* 2004) for later examination and processing in a field laboratory.

Dead arthropods and their fungal pathogens were examined microscopically for their taxonomically significant morphological characteristics, and conidia were subsequently transferred to quarter-strength Sabouraud dextrose agar + yeast extract (SDAY/4: 2.5 g L^{-1} peptone, 10 g L^{-1} dextrose, 2.5 g L^{-1} yeast extract, 20 g L^{-1} agar) amended with chloramphenicol (0.05 %) in 60 × 15 mm Petri dishes. Dishes were sealed with parafilm and incubated at 25 ± 3 °C and natural photophase. The development of fungi and of any contaminants was checked daily, and contaminants were removed or clean transfers of uncontaminated fungal growth from the presumptive pathogen were made to fresh medium. Once a pure culture was established, all further transfers were onto SDAY/4 without additional antibiotics.

The fungus reported here was prepared for long-term preservation in the IPTSP Laboratory of Invertebrate Pathology as IP 414 following protocols from Humber (2012b), and was also co-deposited in the USDA Collection of Entomopathogenic Fungi (Ithaca, NY) as ARSEF 12850.

Morphological evaluations

An infected cockroach was identified morphologically based on keys in Costa Lima (1938). Conidial inoculum from this fungus was grown at 25 ± 1 °C, 75 ± 10 % relative humidity (RH) and a 12 h photophase on Sabouraud dextrose agar for 14 d (Bischoff *et al.* 2009). The fungus was investigated based on morphological characteristics, and semi-permanent slide mounts were prepared in lactophenol-cotton blue according to Humber (2012a). Fungal microstructures were examined by brightfield or phase contrast microscopy (Leica DMLS 020-518.500), measured microscopically (Nova 180i-T; Toupview) and documented with a digital camera (UCMOS01300KPA). Photomontages of multiple focal planes for the conidia and conidiophores were prepared using Helicon Focus Pro software (www.heliconsoft.com). Measurements were based on