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Species clarification of *Isaria* isolates used as biocontrol agents against *Diaphorina citri* (Hemiptera: Liviidae) in Mexico

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ABSTRACT

Entomopathogenic fungi belonging to the genus *Isaria* (Hypocreales: Cordycipitaceae) are promising candidates for microbial control of insect pests. Currently, the Mexican government is developing a biological control program based on extensive application of *Isaria* isolates against *Diaphorina citri* (Hemiptera: Liviidae), a vector of citrus huanglongbing disease. Previous research identified three promising *Isaria* isolates (CHE-CNRCB 303, 305, and 307; tentatively identified as *Isaria fumosorosea*) from Mexico. The goal of this work was to obtain a complete morphological and molecular characterization of these isolates. Comparative analysis of morphology established that the isolates showed similar characteristics to *Isaria javanica*. Multi-gene analysis confirmed the morphological identification by including the three isolates within the *I. javanica* clade. Additionally, this work demonstrated the misidentifications of three other *Isaria* isolates (CHE-CNRCB 310 and 324: *I. javanica*, formerly *I. fumosorosea*; CHE-CNRCB 393: *I. fumosorosea*, formerly *Isaria farinosa*), underlying the need for a full and correct characterization of an isolate before developing a biological control program. Finally, the inter-simple sequence repeat (ISSR) genotyping method revealed that the CHE-CNRCB 303, 305, and 307 isolates belong to three different genotypes. This result indicates that ISSR markers could be used as a tool to monitor their presence in field conditions.

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Introduction

Species within the genus *Isaria* (Hypocreales: Cordycipitaceae) are entomopathogenic fungi (EPF) with a widespread global distribution (Gams *et al.* 2005). The catalogue of the USDA-ARS Collection of Entomopathogenic Fungal Cultures (ARSEF)

contains more than 1000 *Isaria* strains from numerous countries in North, Central, and South America, Europe, Africa, Australia, and Asia. In addition, *Isaria* strains can infect different insect orders in all developmental stages, and are commonly isolated from soil (D'Alessandro *et al.* 2013). Originally, *Isaria* was considered a subsection within the

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