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First report of the genus *Heterorhabditis* (Nematoda: Heterorhabditidae) from continental Portugal

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Summary

Until recently, the only entomopathogenic nematode (EPN) species reported from continental Portugal, was of the genus *Steinernema.* Following a national survey of EPNs in continental Portugal, several natural and managed habitats have been surveyed in the southern part of the country. From 57 soil samples collected using the *Galleria mellonella* trapping method, three samples yielded EPN. Morphological characterization and sequence analysis of the ITS regions of ribosomal DNA allowed the identification of EPN isolates as *Heterorhabditis bacteriophora*, representing the first report of this genus for continental Portugal.

Key words: *Heterorhabditis bacteriophora*; entomopathogenic nematode; Portugal

Introduction

The recognition of entomopathogenic nematodes (EPN) as successful biological agents in controlling important insect pests has promoted current knowledge on species biodiversity and their distribution within distinct geographical areas. These species belong to the families Steinernematidae Chitwood & Chitwood, 1937 and Heterorhabditidae Poinar, 1976. Although obligatory insect parasites, these nematodes are capable of exploiting a wide range of soil habitats, such as cultivated fields, grasslands, dry areas, forests or ocean beaches and because of that they are widely distributed throughout the world (Hominick, 2002).

The presence and distribution of EPNs in continental Portugal is poorly known. The first report of an EPN species was made recently (Valadas *et al.*, 2007), with the detection of *Steinernema feltiae* (Filipjev, 1934) Wouts, Mráček, Gerdin & Bedding, 1982 from the Alentejo and Tejo valley regions. Following the initial surveyed habitats (oak stands and rice paddies), other natural and managed habitats have been selected and surveyed in Alentejo, and extended to some areas of the Algarve.

Material and methods

Soil sampling and nematode isolation

A total of 57 soil samples were collected from different types of natural and cultivated habitats distributed within five different habitats (Table 1). Each composite sample (3 – 4 subsamples), with a final volume of 2L of soil, was collected at a depth of 0 – 20 cm and randomly taken from an area of 200m^2. In order to obtain EPN from the soil, the *Galleria mellonella* L. (Lepidoptera: Pyralidae) trapping method (Bedding & Akhurst, 1975) was used. After mixing the soil, a 1L subsample was placed in a plastic pot (12 cm diameter and 15 cm depth), with 20 last instar larvae of *G. mellonella*. The pots were stored at 25 ºC, and after 6 – 12 days, the dead *G. mellonella* larvae were collected and transferred to White traps (White, 1929). Every 3 – 4 days, dead larvae were removed from the soil. Harvested infective juveniles (IJ's) were stored in an incubator at 10 ºC in distilled water.

Molecular characterization

For morphological observations, several specimens of each isolate were collected from dead *G. mellonella*, mounted in agar 2 % with 0.01 % sodium azide temporary slides, and observed with an Olympus BX-51 light microscope. For molecular analyses, a single adult pregnant female of each isolate was used to extract genomic DNA using the JETQUICK Tissue DNA Spin Kit (GENOMED). PCR reactions were performed to amplify the complete ITS (Internal Transcribed Spacer)-rDNA region, using the forward primer TW81 (5´-GTTTCCGTAGGTGAACC TGC-3´) and the reverse primer AB28 (5´-ATATGCTTA AGTTCAGCGGGT-3´) (Joyce *et al.,* 1994). A 50 µL PCR reaction was prepared with 20 µL of extracted DNA, 2,5U