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Research Note

First report of an entomopathogenic nematode from continental Portugal

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Summary

In continental Portugal no information is available concerning the occurrence of entomopathogenic nematodes. During a survey in several different habitats from the southern regions of the country, several isolates where identified as *Steinernema feltiae*. This represents the first report of an entomopathogenic nematode genus for continental Portugal.

Key words: *Steinernema feltiae*; Portugal; sequence

Entomopathogenic nematodes (EPN) of the families Steinernematidae Chitwood & Chitwood, 1937 and Heterorhabditidae Poinar, 1976 are parasites of insects, and can be found under diverse ecological conditions, including cultivated fields, grasslands, deserts, forests and oceanic beaches (Hominick *et al.*, 1996). The need to find new alternatives that may reduce the use of harmful insecticides has promoted the exploitation of such organisms as biological agents. In the past decades, the recognition of EPN as successful biological agents to control important agricultural insect pests, has led to an increase in the research and use of these nematodes for this purpose. Additionally, extensive surveys for the detection and isolation of new useful species/strains of EPN, in distinct geographic areas were also undertaken (Gaugler, 2002).

In Portugal, the only region where studies of EPN have been made is in the Azores archipelago (Simões *et al.*, 1994; Medeiros *et al.*, 2000; Rosa & Simões, 2004). In these Atlantic islands, 1500 km west of Lisbon, several surveys have been undertaken, as part of a wide program to find endemic biological agents to control insect pests of pastures, such as the Japanese beetle, *Popillia japonica* (Newman) (Coleoptera: Scarabaeidae), and the armyworm, *Pseudaletia unipuncta* (Haworth) (Lepidoptera: Noctuidae) (Rosa *et al.*, 2000). Presently, the EPN species reported from the Azores archipelago correspond to *Steinernema carpocapsae* (Weiser, 1955) Wouts, Mráček, Gerdin & Bedding, 1982, *S. glaseri* (Steiner, 1929) Wouts, Mráček, Gerdin & Bedding, 1982 and *Heterorhabditis bacteriophora* Poinar, 1976. To date these species have been found only in the eastern and central groups of islands, the closest to the European and African continents (Rosa *et al.*, 2000). However, in continental Portugal there are no known reports of this group of nematodes.

In 2006 a national survey was initiated in order to study the presence and distribution of EPN in all continental territory of Portugal, especially in areas that are considered suitable habitats (e.g. sandy soils, cultivated fields, grasslands) for the presence of steinernematids and heterorhabditids. During a random survey in Alentejo (oak stands) and southern Tejo valley (rice paddies) regions, soil samples from different habitats were collected and assayed for the presence of EPN. At each sampling site several sub samples were taken, totalling approximately 2 L of soil from a depth between 3 and 20 cm, placed in a plastic bag and transported to the laboratory. In order to extract EPN from the soil, the *Galleria mellonella* L. (Lepidoptera: Pyralidae) trapping method (Bedding & Akhurst, 1975) was used. After soil homogenisation, a 1 L sub sample was placed in a plastic pot (12 cm diameter and 15 cm depth), with 20 last instar larvae of *G. mellonella*. The boxes were stored at 25 ºC, and after 6 – 12 days the dead *G. mellonella* larvae were collected and transferred to White traps (White, 1929). Harvested infective juveniles (IJs) were stored at 10 ºC in distilled water.

To confirm the identification of the nematodes harvested from the *G. mellonella* cadavers, a molecular characterization was conducted. DNA was extracted from many