THREE SPECIES OF ENTOMOPATHOGENIC NEMATODES OF THE FAMILY STEINERNEMATIDAE (NEMATODA: RHABDITIDA) NEW TO CONTINENTAL PORTUGAL

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Summary. A survey was conducted to determine the species of entomopathogenic nematodes occurring in continental Portugal. Nematodes were recovered from soil samples collected from Alentejo (South) and central Portugal from 2006 to 2009. Nematode isolates were identified based on morphology and sequence analysis. Phylogenetic analysis was based on sequences of partial 28S (D2D3 domain), internal transcribed spacer (ITS) and cytochrome C oxidase subunit I gene (*COI*). Nematode isolates 59F, 15G, 20F and 2B were characterized in detail. In the collected samples, nematodes from the genera *Steinernema* and *Heterorhabditis* were identified, namely *Heterorhabditis bacteriophora* (1.9%), *Steinernema feltiae* (11.5%), *S. kraussei* (0.32%), *S. intermedium* (0.32%) and *Steinernema* sp. (0.63%) *glaseri*-group. Isolate 2B shows morphological characteristics identical to *S. intermedium*, which is a member of the *affine/intermedium*-group and is characterized by the presence of strongly curved and robust spicules with a distinct rostrum in the male, and dorsal tail depression in third-stage infective junveniles (IJ). However, the phylogenies based on the three molecular markers revealed that isolate 2B is more closely related to *S. affine* than to *S. intermedium*. Based on morphological observations, isolate 20F was identified as *S. kraussei*, which is a member of the *kraussei/feltiae*-group, characterized by IJs with a straight body of medium length (mean = 700-950 µm), lateral field mostly with eight ridges, rather broad, flatly rounded and continuous cephalic region, excretory pore at mid-pharynx level; males with mucronate tail, yellowish spicules ca. 50 µm long and wide manubria; females with short conoid tail with pointed non-mucronate tip. Based on morphology and sequence analysis, isolates 59F and 15G were considered conspecific and identified as a species belonging to the *glaseri*-group. More detailed studies are necessary to decide whether these isolates represent a new species.

Key words: *Steinernema intermedium, S. kraussei, Steinernema* sp. *glaseri*-group, cytochrome oxidase c, ribosomal sequences, phylogeny.

Entomopathogenic nematodes (EPNs) of the families Steinernematidae Chitwood and Chitwood, 1937 and Heterorhabditidae Poinar, 1976 are lethal parasites of insects, being widely distributed in soils worldwide (Hominick *et al*., 1996). These nematodes have been known since the XVIIth century and include more than 30 families (Kaya and Stock, 1997). Hunt (2007) reported 55 valid species of the genus *Steinernema* Travassos, 1927 and eleven species of the genus *Heterorhabditis* (Hunt, 2007). However, after 2007 many more entomopathogenic nematode species have been described, mainly from Africa and Asia (Khatri-Chhetri *et al*., 2011).

Nematodes of the families Steinernematidae and Heterorhabditidae are potential candidates to be used as bio-control agents due to their capacity for controlling insect pests worldwide (Kaya and Stock, 1997; Bedding, 1998; Shapiro-Ilan *et al*., 2002). Classified as parasitoids and pathogens, they have many attributes that give them the ability to be used as bio-pesticides: they can be found under diverse ecological conditions including cultivated fields, forests, grasslands, deserts and ocean beaches (Hominick *et al.,* 1996). The third-stage infective juveniles (IJ) of these families are non-feeding, free living stages in the soil where they can survive for extended periods. They are safe to non-target organisms and to the environment, can be mass produced, formulated and easily applied as bio-pesticides (Georgis and Kaya, 1998), being compatible with some insecticides (Chen *et al*., 2003), and have a broad host range of insect pests in a variety of habitats (Kaya and Gaugler, 1993; Gaugler, 2002).

Insects play an important role in agricultural production and have acquired resistance to insecticides during recent decades (Ahmad *et al*., 2003) following the increase usage of pesticides. Entomopathogenic nematodes represent an alternative to insecticides, the identification of new species in different countries and ecosystems being of major importance in pursuing this objective. These nematodes have a complex life cycle inside the insect host. When infective juveniles locate a potential host in the soil, they move towards it and penetrate the insect body, via natural openings or areas of thin cuticle. Once inside the body cavity, they release their symbiotic bacteria (belonging to the genera *Xenorhabdus* and *Photorhabdus* for steinernematids and heterorhabditids, respectively), which multiply rapidly, causing insect death within 48 hours by septicemia (Kaya and Koppenhofer, 1999). IJs feed on liquefying

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