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Research Note

First record of *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae) in Slovenia

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Summary

In October 2007 we examined 80 soil samples from 16 different locations in the central part of Slovenia (the Notranjska region) and confirmed the presence of entomopathogenic nematodes only in two soil samples. This represents the first recorded instance of an entomopathogenic nematode in Slovenia. In sample B30 we confirmed the presence of *Steinernema feltiae* (Rhabditida: Steinernematidae) by means of a molecular technique. In Slovenia the application of entomopathogenic nematodes was hitherto possible only in laboratory experiments, while the Rules on Biological Plant Protection made the practical application of exotic organisms in the domestic environment entirely impossible. After the first record of the entomopathogenic nematode *S. feltiae* we expect the aformentioned agent to become an important alternative to insecticides in plant protection against pest insects.

Keywords: biological kontrol; entomopathogenic nematodes; first record; *Steinernema feltiae*

Introduction

Entomopathogenic nematodes (EPNs) from the families Steinernematidae and Heterorhabditidae are important insect pathogens. These soil organisms are mutually associated with bacteria from the genus *Photorhabdus* Boemare, Akhurst, and Mourant (genus *Heterorhabditis*) and bacteria from the genus *Xenorhabdus* Thomas and Poinar (genus *Steinernema*) (Burnell & Stock, 2000). After infection, the symbiotic bacteria are released into the insect hemocoel, causing septicemia and the death of the insect in 24 to 72 hours (Forst & Clarke, 2002).

In Slovenia we started the first research studies on EPNs in 2004. Due to the Rules on Biological Plant Protection, which do not allow the introduction of exotic organism into a natural ecosystem, all the original research was restricted primarily to laboratory experiments. In such experiments we tested the efficacy of commercial products which are based upon the active ingredient of entomopathogenic nematodes in order to control many species of pest insects: the Colorado potato beetle (*Leptinotarsa decemlineata* [Say]), the greenhouse whitefly (*Trialeurodes vaporariorum* [Westwood]), the western flower thrips (*Frankliniella occidentalis* [Pergande]) (Perme, 2005), the sawtoothed grain beetle (*Oryzaephilus surinamensis* [L.]), the granary weevil (*Sitophilus granarius* [L.]) (Trdan *et al*., 2006), and the flea beetle (*Phyllotreta* spp.) (Trdan *et al*., 2008). The results of these experiments confirmed the already known fact that - in optimal conditions - EPNs represent a very effective insect pest control agent.

Due to the broad spectrum of target hosts from the class Insecta, their application as a method of biological control of plants against pests is already very well known (Kaya & Gaugler, 1993). Because Slovenia would also like to join the countries around the world where application of EPNs is allowed also in the field, in the future we hope that our research on the aformentioned biological agents will lead to their common and widespread use on Slovenian territory. We will subsequently integrate the possible presence of EPN species in numerous field experiments in which we will study their efficacy in relation to some commercial products which are based on the active substance of entomopathogenic nematodes and other chemical products, whose number is decreasing each year. This is the reason there is an urgent necessity to find alternatives in the fight against problem pests.

Materials and methods

In October 2007 we examined 80 soil samples from 16 different locations in order to study the presence and distribution of EPNs, especially in areas that are considered suitable habitats (e.g. sandy soils, cultivated fields, grasslands, forests) for the presence of steinernematids and heterorhabditids (Table 1). The soil samples, five from

Table 1. Localities of EPN-positive soil samples

Sampling area	No. of soil samples	No. of samples with EPNs	% of samples with EPNs
Cultivated field	35	1	2.85
Grassland	20	1	5
Forest	15	0	0
Others	10	0	0
total	80	2	2.5

each sampling place, were taken in the Notranjska region of Slovenia, which is the south central part of the country. Each soil sample (approximately 1 kg) was a composite of 3 random subsamples taken at a depth of 3 – 15 cm in an area of 20 m^2. The samples were taken at least 100 m apart at each site. The samples were placed in polyethylene bags to prevent water loss and were kept in coolers (ca. 15 ºC) during transit to the laboratory. We used the "Galleria bait method", which is the most frequently used method of EPN detection in soil (Bedding & Akhurst, 1975). In the laboratory we placed 5 last instar larvae of the greater wax moth *Galleria mellonella* L. (Lepidoptera: Pyralidae) into perforated tubes for each soil sample. We stored the bags with soil samples at room temperature (20 ± 3 ºC). After 4 – 10 days, all insects were recovered, and parasitized cadavers were individually placed in a White trap (White, 1929) to allow the emergence of the infective-stage juveniles. The emerging nematodes were pooled for each sample and used to infect fresh *G. mellonella* larvae to produce nematodes for identification and establishment of cultures. Soil samples with negative nematode recovery were baited a second time to confirm the results of the first test. The harvested infective juveniles (IJs) were stored at 4 ºC in distilled water (Valadas *et al*., 2007).

Results

We confirmed the presence of EPNs in 2.5 % of samples. Only 1 positive sample, B30 (taken on a chicory arable field near Cerknica (SW Slovenia, 45°48'N, 14°22'E, 572 m alt.) was identified at this time. The soil type of the positive B30 sample was loam. To confirm the identification of the isolated nematodes harvested from the larvae of the wax moth, a molecular characterization was conducted. Genomic DNA was extracted from individual nematodes and PCR was performed to multiply the ITS region using the primers TW81 and AB28, following Hominick *et al.* (1997). The PCR products were re-isolated from a 1 % TAE-buffered agarose gel using the E.Z.N.A. Gel Extraction Kit (Omega Bio-Tek, USA). The re-isolated sample was sequenced in the laboratory of the Agricultural Biotechnology Centre in Gödöllő, Hungary. The sequence was submitted to GenBank public database (Accesion Number: EU91485). The sample DNA sequence was compared to the sequences of the species *Steinernema* using a BLAST search at the National Centre for Biotechnology Information (NCBI) web site (www.ncbi.nlm.nih.gov). The sequences producing significant alignments and at least 99 % identity were derived from *Steinernema feltiae*: GenBank Accession No. DQ310469 and AF121050 (NGUYEN *et al*., 2001) (Fig.1). Bait larvae infected with B30 isolate displayed the grey-brown colouration and show typical morphological characters within the genus *Steinernema* Travassos, 1927 (Adams & Nguyen, 2002).

Discussion

Genetic studies proved that the nematode species is *Steinernema feltiae* Filipjev (1934). The ITS1-5.8S-ITS2 region, including the partial 18S and 28S rDNA genes (flanked by the above mentioned primers) of the B30 Slovenian isolate is 742bp long. BLAST searches (ALTSCHUL *et al*. 1990) in GenBank showed that the B30 Slovenian isolate has a high similarity (99%) with those sequences available for *S. feltiae* populations (e.g. accession numbers DQ310469 and AF121050). The sequence of other species from the *feltiae* group, namely *S. litorale*, was obtained from GenBank searches that exhibited a lesser degree of similarity with the Slovenian isolate and other *S. feltiae* populations (e.g. accession number AB243441) (Fig.1). The present study constitutes the first report of EPNs in Slovenia. *S. feltiae* was also recorded in Slovenia for the first time. *S. feltiae* has a wide distribution in temperate regions, being one of the most common species found in Europe, and in many other parts of the world (for a detailed EPN species distribution, see Hominick 2002).

We can place the mentioned species into the "*feltiae* group" of nematodes from the genus *Steinernema* (Nguyen, 2006); with regard to infective juveniles, it is known that they are between 1000 and 700 μm long. This nematode lives in symbiosis with the bacterium *Xenorhabdus bovienii* (Poinar, 1988). The nematode was first recorded in 1934, and its applied value in biological control of insect pests is well known (Ebssa, 2001). Some researchers have reported that *S. feltiae, S. intermedium* (or C1) and *S. affine* tend to appear on agricultural land (Sturhan, 1996). In Europe, the occurrence of *S. feltiae* has hitherto been confirmed in Austria, Belgium, Great Britain, the Czech Republic, Denmark (original), Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Poland, Slovakia, Spain, Sweden, Switzerland, Netherlands, Norway, Ukraine, Bulgaria, and Portugal (Hominick, 2002).

Following this first recorded instance of entomopathogenic nematodes in Slovenia (*Steinernema feltiae*), we expect that the use of these biological agents against insect pests will become an important alternative to insecticides. Since the first record of only one species (*S. feltiae*) does not generalize the application of all species of entomopathogenic nematodes which are employed in programmes for the biological control of pests (Laznik *et al*., 2008) in the field, we will continue with such type of research also in the near future. At the moment, only the species *S. feltiae* has the status of native species and as a consequence of this also the possibility of outdoor application in Slovenia (MAFF, 2008). In the first phase we will include domestic strains of entomopathogenic nematodes in numerous field experiments in which we hope to test their efficacy to control

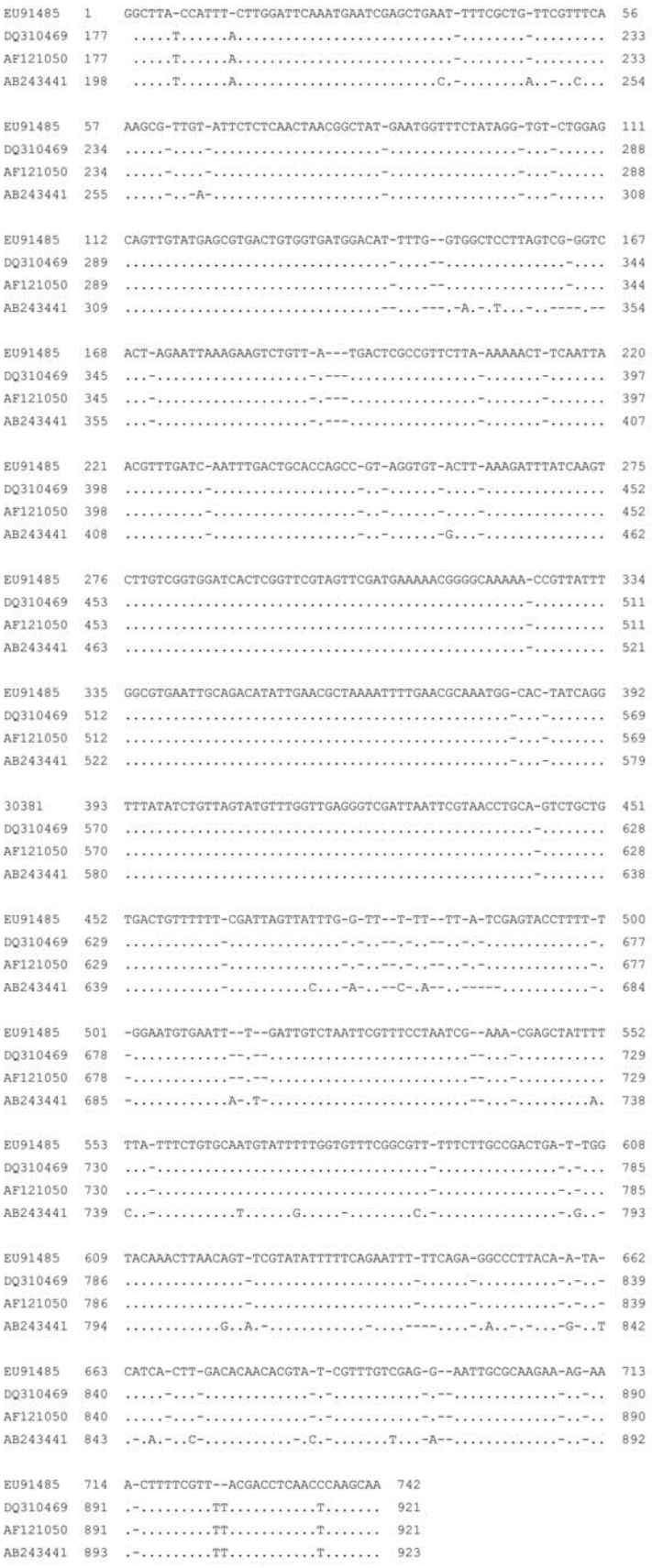

Fig.1 Multiple sequence alignment of the ITS rDNA region (including partial fragments of the 18S and 28S rDNA genes) of 4 *Steinernema* species. The code EU91485 corresponds to the Slovenian isolate of *Steinernema feltiae* (B30). Codes DQ310469 and AF121050 are *Steinernema feltiae* strains from Russia and USA. Code AB243441 corresponds to a *Steinernema litorale* strain from Japan.

pests which are not easy to control with insecticides due to their massive occurrence in the harvesting period and against pests which are resistant to insecticides, etc.

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