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Research Note

Steinernema kraussei (Steiner, 1923) (Rhabditida: Steinernematidae) – the first record from Poland

D. TUMIALIS[1], R. GROMADKA[2], I. SKRZECZ[3], E. PEZOWICZ[1], A. MAZURKIEWICZ[1], E. POPOWSKA - NOWAK[4]

[1]Faculty of Animals Sciences, Department of Animal Environment Biology, Warsaw University of Life Sciences – SGGW, Ciszewskiego 8 Street, 02 – 786 Warsaw, Poland, E-mail: *dorota_tumialis@sggw.pl*, *elzbieta_pezowicz@sggw.pl*, *anna_mazurkiewicz@sggw.pl*; [2]Institute of Biochemistry and Biophysics, PAS, Pawińskiego 5a Street, 02-106 Warsaw, Poland, E-mail: *robert@ibb.waw.pl*; [3]Department of Forest Protection, Forest Research Institute, Braci Leśnej 3 Street, Sękocin Stary, 05-090 Raszyn, Poland, E-mail: *I.Skrzecz@ibles.waw.pl*; [4]Cardinal Stefan Wyszynski University, Faculty of Biology and Environmental Sciences, Wóycickiego1/3 Street, 01 – 938 Warsaw, Poland, E-mail: *eponak@poczta.onet.pl*

Summary

This study reports the first record of *Steinernema kraussei* from Poland. The nematode was isolated from coniferous woodlands in 4 localities in central Poland. Preliminary identification of the species was done based on morphometric measurements. To confirm nematode species of the genus *Steinernema* the result was supported by the description of the ITS region.

Keywords: entomopathogenic nematodes; *Steinernema kraussei*; localities in Poland

Introduction

Entomopathogenic nematodes (EPNs) from the families *Heterorhabditidae* and *Steinernematidae* are widespread lethal parasites of many insect species. They are a cosmopolitan group being found all over the world except Antarctica (Poinar, 1990; Hominick, 2002). EPNs have a broad specificity in host selection. Noteworthy, however, *Steinernematidae* and *Heterorhabditidae* show different preferences in the selection of insect hosts: *Steinernematidae* prefer insects of the orders *Diptera*, *Hymenoptera* and *Orthoptera*, while *Heterorhabditidae* – insects of the order *Coleoptera* (Peters, 1996). They are known to play an important part in the regulation of soil food webs (Spiridonov *et al*., 2007). Nematodes of both families are also widely used in biological plant protection and are now produced in a mass scale.

Steinernematidae are more numerous than *Heterorhabditidae*. According to Nguen and Hunt (2007) 60 species are described of the genus *Steinernema* but only 16 of *Heterorhabditis*. Moreover, new species from various regions of the world are still described like e.g. *Steinernema brazilense* (Nguyen *et al*., 2010) or *Steinernema ichnusae* (Tarasco *et al.,* 2008).

Identification of closely related species of the nematodes remains difficult, especially if diagnostic characters are environmentally influenced or overlap. In many instances, analysis of the DNA sequences from species in question can provide an accurate identification (Liu & Berry, 1995). Hence, actual distribution of many species needs verification and confirmation by genetic methods, more so, that data on the occurrence of these nematodes are relatively old. The latter is also true for the area of Poland.

S. kraussei is a Holarctic species. It was first isolated from the Geggen Mountains in Westphalia, Germany (Steiner, 1923) and then from Czechoslovakia by Mráček (1977) who published re-description of this species in 1994 (Mráček, 1994). In the 1990s the species was noted in other European countries: in the Netherlands, Great Britain (Hominick *et al*., 1995), Switzerland (Steiner, 1996) and Spain (Garcia del Pino & Palomo, 1996). The species was also found in North America (Stock *et al*., 1999).

S. kraussei is isolated from slightly acidic soils (Steiner, 1996; Adams *et al.,* 2006), often in areas overgrown by coniferous forests (Sturhan, 1995, Mráček *et al*., 2005). The species has not yet been reported from Poland, though its occurrence should have been expected taking into account its general distribution and presence in the neighbouring countries.

Experimental procedures

Soil samples were collected since April till November of the years 2010 – 2011 from various habitats (forests, fields, meadows, abandoned lands) spread along a north-south belt across Poland.

Table 1. The list of analysed and comparative species or isolates

Species	Location (strain)	GenBank accession number	Reference of material
Steinernema kraussei	Japan (Hokkaido, Hamatonbetsu)	AB243442	Kuwata et al., 2006
Steinernema sp.2	UK (isolate B3, site 249)	AY230162	Spiridonov *et al*., 2004
Steinernema cholashanense	China (Sichuan)	EF43195	Nguyen *et al*., 2008
Steinernema kraussei	Poland (isolate no. 287)	KC608621	this study
Steinernema kraussei	Poland (isolate no. 400)	KC608622	this study
Steinernema kraussei	Poland (isolate no. 401)	KC608623	this study
Steinernema kraussei	Poland (isolate no.430)	KC608624	this study

For the purpose of this study the Polish isolates were named with numbers of forest compartment in which they were isolated.

At each sampling site soil samples were taken from an area of approximately 100 m^2 to a depth of 10 – 25 cm in four repetitions. Then the samples were mixed to obtain c. 1 kg of homogenous sample. In total 138 samples were collected. In the lab, EPNs were isolated with the method of soil traps with live bait. Each sample was distributed among 6 pots of a volume of 250 cm^3 each. Then, 6 larvae of *Galleria mellonella* were placed in every pot. Pots were placed in an incubator at 20 °C. After 5 days the first control was performed, dead insects were removed and replaced by live ones (Bedding & Akhurst, 1975). This procedure was repeated every two days until the twentieth day of experiment. Dead larvae of *G. mellonella* were placed in modified White nematode traps (White, 1929). The traps were kept in an incubator at 25 °C for c. 2 weeks until obtaining the invasive larvae from dead larvae of *G. mellonella*. Nematode larvae were stored at 4 °C.

Nematodes were identified using morphological criteria (Poinar, 1990; Adams & Nguyen, 2002). Part of larvae from each sample were used for genetic identification. The list of studied species and isolates is presented in Table 1.

DNA extraction

DNA of each nematode species was extracted from a few (100 – 1000) individuals. The nematodes were rinsed in Ringer solution, resuspended in 50 µl Tris buffer (10 mM, pH = 7.4) and the tubes were frozen at -80 °C for 30 minutes. Then, the nematodes were incubated at 65 °C for 2 h in 100 µl of lysis buffer (100 mM Tris-HCl pH = 7.4; 100 mM NaCl; 50 mM EDTA; 1 % SDS; 1 % β-merkaptoethanol; 200 µg/ml proteinase K). DNA was extracted with phenol (saturated 10 mM Tris-HCl solution pH = 7.4) and then chloroform and precipitated with 2.5 volume of ethanol. DNA for PCR was resuspended in 50 µl of 10 mM Tris-HCl, pH = 7.4.

PCR amplification and sequencing

One set of primers was used in the PCR reactions: 18S (5'-TTGATTACGTCCCTGCCCTTT-3') and 26S (5'-TTTCACTCGCCGTTACTAAGG-3') as described by Vrain *et al.,* (1992), corresponding to nucleotide position 2503 – 2523 and 3774 – 3794, respectively, of the sequence of the rDNA tandem unit from *Caenorhabditis elegans* (GenBank accession number X03680). All PCR reactions were conducted with the cycling profile: 1 cycle at 95 ºC for 3 minutes followed by 35 cycles at 95 ºC for 30 seconds, at 50 ºC for 30 seconds, and at 72 ºC for 60 seconds. The last step was carried out at 72 ºC for 5 minutes. PCR products were purified by ethanol precipitation and used for direct sequencing with the BigDyeTerminator Cycle Sequencing Ready Reaction Kit v. 3.1. (Life Technologies). The primers used in this step were the 18S and 26S described above and two internal primers that were synthesized for this study: f-nema (5'-ATCGGAGTC GCTTTGAGTGACGG-3') and r-nema (5'-GACACCG GCGGTTGGACGAA-3'). The f-nema and r-nema primers were designed with the Primer3 program v.0.4.0 (http://frodo.wi.mit.edu/). Complete sequences of the ITS1-5.8S-ITS2 region of the rDNA cistron (977bp) were obtained for four samples of nematodes. Sequencing quality and counting assembly were using Pregap4 and Gap4 programs (Staden, 1996). All alignments were verified manually.

Results and discussion

Nematodes from the family *Steinernematidae* and *Heterorhabditidae* were isolated from 54 out of 138 samples (36.23 %). The nematodes in 18 samples (33.33 %) were identified to the species, and those in the remaining 36 samples (66.66 %) to the family level.

Table 2. Measurements (in µm) of the infective-stage juveniles of *Steinernema kraussei*

Character	Mean	Range
Total length	918.03	809.6 – 1048.8
Greatest width	34.7	31.2 – 38.4
EP	62.8	56.4 – 67.2
PhB	133.7	122.4 – 144
Tail length	77.3	69.6 – 86.4
Ratio a	26.94	
Ratio b	6.87	
Ratio c	11.88	
Ratio d	0.47	
Ratio e	0.81	

EP – distance from head to excretory pore; PhB – distance from head to pharynx base; ratio a – length divided by width; ratio b – length divided by PhB; ratio c – length divided by tail; ratio d – EP divided by PhB; ratio e – EP divided by tail length

Table 3. Distribution of blast hits

No. isolate/ hit	Acc. number	977 bp query cover	Identity %
***S. kraussei* (isolate no. 287)**			
Steinernema sp.	AY230162	100 %	99
Steinernema kraussei	AB243442	100 %	97
Steinernema cholashanense	EF431959	100 %	95
***S. kraussei* (isolate no. 400)**			
Steinernema kraussei	AB243442	100 %	99
Steinernema sp.	AY230162	100 %	96
Steinernema cholashanense	EF431959	100 %	96
***S. kraussei* (isolate no. 401)**			
Steinernema sp.	AY230162	100 %	99
Steinernema kraussei	AB243442	100 %	97
Steinernema cholashanense	EF431959	100 %	95
***S. kraussei* (isolate no. 430)**			
Steinernema sp.	AY230162	100 %	99
Steinernema kraussei	AB243442	100 %	97
Steinernema cholashanense	EF431959	100 %	95

Nematodes of the family *Steinernematidae* were most frequent and contributed in 92.59 % to the number of isolated species. *S. feltiae* was the dominating species (11 samples), *S. carpocapse* was found in 1sample. Only one isolated species - *Heterorhabditis megidis* (2 samples) - represented the family *Heterorhabditidae*. *S. kraussei* was found in 4 samples (from April and October). Results of morphometric measurements used to identify the species are listed in Table 2.

To confirm the determination of this species, DNA was isolated from 4 samples. The PCR reactions successfully amplified part of the ribosomal DNA. The aligned sequences included the entire amplified and sequenced PCR product (173 bases of the 18S flanking region, the entire ITS1-5.8S-ITS2, and 66 bases of the 28S flanking region). Comparison of each obtained sequence with nucleotide database (at NCBI) showed about 99 – 97 % sequence identity with the consensus sequence for *Steinernema kraussei* partial rDNA sequence (Acc. No. AB243442). The main hits of blast (Basic Local Alignment Search Tool, Altschul SF *et. al., 1990*) are shown in Table 3. The similarity in DNA sequences for the closely related *Steinernema* species is less than 95 % (Nguyen *et al.,* 2001). The divergence of intraspecific region in the clade of *S. kraussei* is between 2.4 and 2.8 % (Spiridonov *et al.,* 2004). The figure 1 shows the distance tree of sequences other isolates of *Steinernema kraussei* and a few other *Steinernema sp.* producing significant alignments. The obtained results strongly confirm species affiliation of investigated nematodes. Similarity of samples is shown in Table 4.

Sites of *S. kraussei* were situated in 2 forest districts in Central Poland in the habitats named “fresh coniferous forest”

Table 4. Pairwise similarity of studied samples, query length 977 bp

No. isolate	*S.kraussei* (isolate no. 287)	*S.kraussei* (isolate no. 400)	*S.kraussei* (isolate no. 401)	*S.kraussei* (isolate no. 430)
			Identities	
S.kraussei (isolate no. 287)	--	947/979 (97 %)	977/977 (100 %)	977/977 (100 %)
S.kraussei (isolate no. 400)	4/979 (0 %)	--	947/979 (97 %)	946/979 (97 %)
S.kraussei (isolate no. 401)	0/977 (0 %)	4/979 (0 %)	--	976/977 (99 %)
S.kraussei (isolate no. 430)	0/977 (0 %)	4/979 (0 %)	0/977 (0 %)	--
			Gaps	

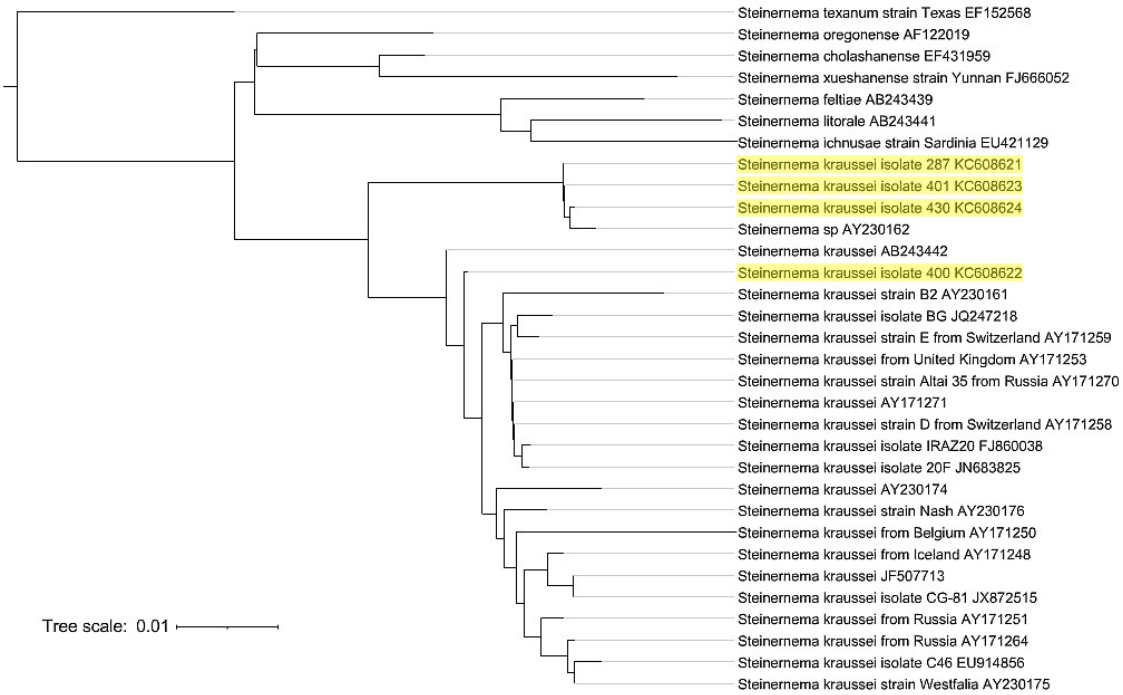

Fig. 1. Distance tree of blast result. Database set was nucleotide collection (nr/nt) from NCBI (The National Center for Biotechnology). Tree was done using neighbor joining method (Saitou & Nei, 1987) with max sequences difference 0.1

according to the forest typology and *Peucedano-Pinetum* and *Leucobryo-Pinetum* in phytosociological terminology.

S. kraussei was found at the following localities:

Samples no. 1 and 2: Forest Division Mińsk Mazowiecki, Forest Region Dobre, forest compartment 400 and 401 (geographic coordinates: N52º 19' 14.448", E21º 40' 42.1896").

Sample no. 3: Forest Division Mińsk Mazowiecki, Forest Region Siennica, forest compartment 430 (N52º 34' 19.4628", E20º 47' 46.6944").

Sample no. 4: Forest Division Celestynów, Forest Region Torfy, forest compartment 287 (N52º 3' 49.2444", E21º 25' 20.3232").

The study confirmed that *S. kraussei* is a typically forest species (Hominick, 2002), that prefers coniferous stands. Even in such sites, the species is not common since it was found in only 4 out of 36 samples collected from such habitats.

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