## *Steinernema kraussei* (Steiner, 1923) (Rhabditida: Steinernematidae)

## – the first record from Poland

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**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 bra­- .....*

*zilense* (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.

At each sampling site soil samples were taken from an area of approximately 100 m2 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 cm3 each. Then, 6 larvae of *Galleria mellonella* were placed in every pot. Pots were placed in an incubator at 20 oC. 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 oC for c. 2 weeks until obtaining the invasive larvae from dead larvae of *G. mellonella*. Nematode larvae were stored at 4 oC.

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.

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 l Tris buffer (10mM, pH = 7.4) and the tubes were frozen at ‑80 oC for 30 minutes. Then, the nematodes were incubated at 65 oC for 2h in 100 l of lysis buffer ((100 mM Tris-HCl pH = 7.4; 100 mM NaCl; 50 mM EDTA; 1 % SDS; 1 % -merkap­toethanol; 200 μg/ml proteinase K). DNA was extracted with phenol (saturated 10mM Tris-HCl solution pH = 7.4) and then chloroform and precipitated with 2.5 volume of ethanol. DNA for PCR was resuspended in 50 l 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 °C for 3 minutes followed by 35 cycles at 95 °C for 30 seconds, at 50 °C for 30 seconds, and at 72 °C for 60 seconds. The last step was carried out at 72 °C 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’‑ATCGGAGTCGCTTTGAGTGACGG‑3’) and r‑nema (5’‑GACACCGGCGGTTGGACGAA-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

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.

Table 3. Distribution of blast hits

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

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 intra-specific 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.

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** | | |  |

Sites of *S. kraussei* were situated in 2 forest districts in Central Poland in the habitats named “fresh coniferous forest” according to the forest typology and *Peucedano-Pinetum* and *Leucobryo-Pinetum* in phytosociological terminology.

*S. kraussei* was found at the following localities:

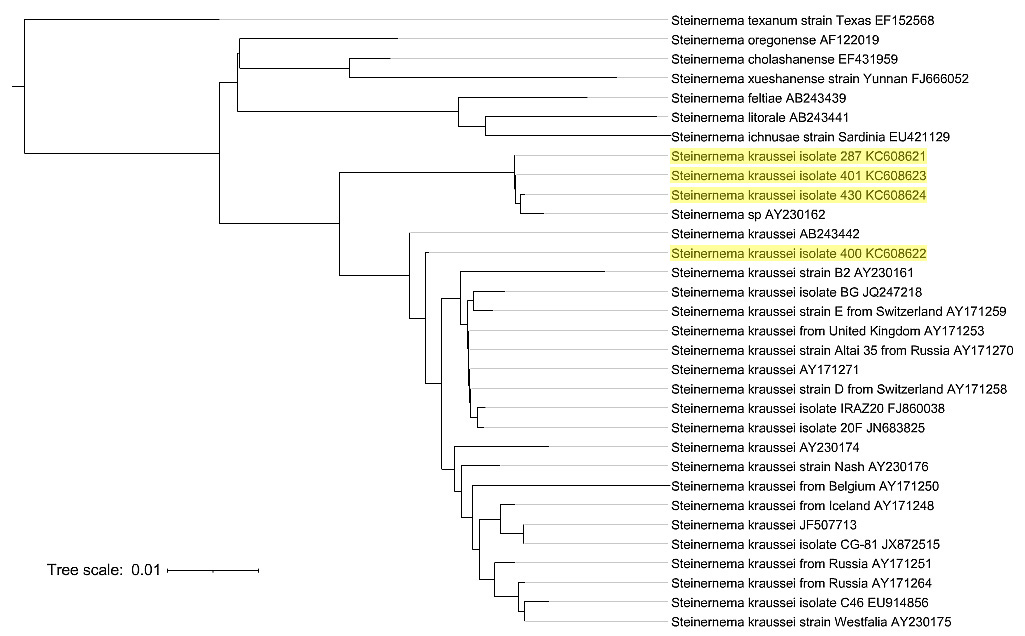


Fig. 1. Distance tree of blast result. Database set was nucleotide collection (nr/nt) from NCBI (The National Center for Biotechnology ). Tree was done using neighbor joining method (Saitouand Nei, 1987) with max sequences difference 0.1

Samples no. 1 and 2: Forest Division Mińsk Mazowiecki, Forest Region Dobre, forest compartment 400 and 401 (geographic coordinates: N52°19’ 14.448”, E21°40’ 42.1896”).

Sample no. 3: Forest Division Mińsk Mazowiecki, Forest Region Siennica, forest compartment 430 (N52°34’ 19.4628”, E20°47’ 46.6944”).

Sample no. 4: Forest Division Celestynów, Forest Region Torfy, forest compartment 287 (N52°3’ 49.2444”, E21°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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