

## Occurrence of entomopathogenic nematodes in Polish soils

### Ocorrência de nematoides entomopatogênicos em solos da Polônia

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#### – NOTE –

#### ABSTRACT

Entomopathogenic nematodes (*Steinernematidae* and *Heterorhabditidae*) were studied in Polish soils according to the: season, habitat and altitude. In total, 167 soil samples were taken from 111 localities in Poland along north-south transect. Entomopathogenic nematodes were isolated from 53 soil samples (31.7%). Nematodes species were identified genetically from 16 samples, and in all other 37 samples nematodes were determined to the genus level. Three species of the genus *Steinernema* (*Steinernema kraussei*, *S. feltiae*, *S. carpocapsae*) and one of the genus *Heterorhabditis* (*Heterorhabditis megidis*) were recorded. *Steinernema feltiae* was the most common species. There was not a significant association between the occurrence of nematodes and season or altitude. The type of habitat significantly ( $P < 0.05$ ) affected the occurrence of nematodes. Nematodes were most frequent in samples collected from coniferous forests (20 findings).

**Key words:** distribution, *Steinernema*, *Heterorhabditis*, Poland, season, habitat.

#### RESUMO

Os nematoides entomopatogênicos (*Steinernematidae* e *Heterorhabditidae*) foram estudados em solo polonês de acordo com estação, habitat e altitude. Foram coletadas um total de 167 amostras de solo em 111 localidades diferentes ao longo da trajetória Norte-Sul da Polônia. Os nematoides entomopatogênicos foram extraídos em 53 das amostras de solo (31,7%). As espécies de nematoides foram geneticamente identificadas a partir de 16 amostras, as outras 37 amostras foram usadas para determinar o gênero. Três espécies do gênero *Steinernema* (*Steinernema kraussei*, *S. feltiae*, *S. carpocapsae*) e uma espécie do gênero *Heterorhabditis* (*Heterorhabditis megidis*) foram identificadas. A espécie mais comum foi a *S. feltiae*. Não foi encontrada uma relação significativa entre a presença de nematoides com a estação do ano e a altitude. O tipo de habitat afetou significativamente

( $P < 0,05$ ) a ocorrência de nematoides. Os nematoides foram encontrados mais frequentemente em amostras recolhidas em florestas de coníferas (20 no total).

**Palavras-chave:** distribuição, *Steinernema*, *Heterorhabditis*, Polônia, estação, habitat.

Entomopathogenic nematodes (EPNs) are cosmopolitan organisms living on all continents except Antarctica. One of the most common species in the world is *Steinernema feltiae* (Filipjev), which prefers temperate zones. Less frequent but also of global range is *S. carpocapsae* (Weiser). *Steinernema feltiae* and *S. affine* (Bovien) are most often isolated species from among Steinernematidae family in Europe. Other European species include *S. carpocapsae* (isolated in Portugal, Slovakia, Germany, Italy, Spain and Switzerland), *S. glaseri* (Peters) (Portugal and Spain), *S. kraussei* (Steiner) (Belgium, Czech Republic, Slovakia, Germany, Switzerland and Great Britain), *S. intermedium* (Poinar) (Czech Republic, Slovakia, Germany and Switzerland), *S. bicornutum* Tallosi, Peters & Ehlers (Slovakia, Czech Republic, Germany and Switzed), *S. arenarium* (Artyukhovsky) (southern Italy). From *Heterorhabditidae* family, *Heterorhabditis bacteriophora* (Poinar) and *H. megidis* Poinar, Jackson & Klein belong to the species of world range and occur frequently (HOMINICK, 2002). However, nematodes of the family *Heterorhabditidae* are rarely

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isolated in Europe (HOMINICK, 2002) and some have a limited range. Research on the occurrence of EPNs in Poland has been conducted since the 1990s. So far, the presence of the following species: *S. feltiae*, *S. affine*, *S. bicornutum*, *S. carpocapsae*, *S. arenarium*, *S. kraussei*, *H. megidis* and *H. bacteriophora* (BEDNAREK, 1990; DZIĘGIELEWSKA & KIEPAS-KOKOT, 2004; DZIĘGIELEWSKA, 2012; TUMIALIS et al., 2014) has been established. Missing genetically verified data from Poland and the dispersion of such information in only local journals justifies the need for this publication.

Soil samples were collected in spring (April to June), summer (July to September) and autumn (September to November) in 2010 and 2011. In total, 167 soil samples were taken from 111 localities in Poland along north-south transect. Sites of sample collection were selected to reflect the spectrum of diversity of habitats and climate in Poland. Soil samples were taken from various places like forests, fields, meadows and barren lands. From each site, 5 soil samples were taken with the Egner probe to a depth of 15-25cm of a combined mass of 1kg soil per site. In the laboratory, EPNs were isolated with the trap method using live bait (larvae of *Galleria mellonella* L., Lepidoptera: *Pyralidae*) (BEDDING & AKHURST, 1975). White's traps (WHITE, 1927) were used to collect infective juveniles (IJs) of nematodes. Traps were placed in an incubator at 25°C. After two weeks, IJs were obtained from dead insects, pipetted to bottles for tissue culture and placed in water. Bottles were preserved in a refrigerator at 4°C. Nematodes were identified using morphological criteria (ADAMS & NGUYEN, 2002). Infective juveniles from each sample were used for genetic identification. The identification of species was based on conserved regions 18S and 26S. DNA of each nematode species was extracted from few (20-30) individuals. In the PCR reactions, one set of primers was used: 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 rDNA tandem unit from *Caenorhabditis elegans* (GenBank accession number X03680). 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). Complete sequences of the ITS1-5.8S-ITS2 region of the rDNA cistron (977bp) were obtained for 16 samples of nematodes. Sequencing quality and

counting assembly were checked using Pregap4 and Gap4 programmes (STADEN, 1996). All alignments were verified manually. The obtained results were statistically processed using STATISTICA 8.0 and PQStat 1.4. Pearson's chi square test was used to verify the relationship between nematode occurrence, season, habitat type and altitude. For small and unequal groups the Fisher's exact test was used.

Entomopathogenic nematodes were isolated from 53 out of 167 analysed soil samples (31.7%). Most of them were nematodes from the genus *Steinernema*, which were isolated from 48 (90.6%) samples. Nematodes of the genus *Heterorhabditis* were isolated from 4 (7.5%) samples, and one sample (1.9%) contained representatives of both families. Nematode species were identified genetically from 16 samples. The presence of *S. feltiae* was recorded in 11 samples, *S. kraussei* was found in 4 samples, *S. carpocapsae* in one and two samples contained *H. megidis*. Three species – *S. feltiae*, *S. carpocapsae* and *H. megidis* - were reported together in one sample. In the remaining 37 samples, nematodes were determined to the genus level. From among 22 samples collected in spring, 21 contained nematodes of the genus *Steinernema* with *S. feltiae* found in 7 samples and *S. kraussei* in one. In summer, nematodes of the genus *Steinernema* were isolated from 9 samples (*S. feltiae* in 1 sample and *S. carpocapsae* in 1 sample) and those from the genus *Heterorhabditis* were reported in two samples (*H. megidis* in one sample). Out of 21 samples taken in autumn, entomopathogenic nematodes from the *Steinernematidae* family were isolated from 19 samples (*S. feltiae* in three samples, *S. kraussei* in one sample) and *Heterorhabditidae* nematodes from two samples (*H. megidis* in one sample). The performed studies did not show statistically significant seasonal differences in the presence of nematodes ( $P > 0.05$ ). Entomopathogenic nematodes were isolated from all six types of habitats (Figure 1). The type of habitat significantly ( $P < 0.05$ ) affected the occurrence of nematodes. Nematodes were most frequent (20 findings) in samples collected from coniferous forests (moist mixed coniferous forest on clayey-sandy soil and fresh coniferous forest on sandy soil), followed by those in fields (16 findings) and the least frequent in meadows (two findings). In total, 31 samples with nematodes were reported in forest habitats. Out of this, four contained *S. feltiae* and 4 - *S. kraussei*. From meadows and barren lands, nematodes were isolated in a total of six samples; one sample showed the presence of *S. feltiae*. In fields, nematodes of the family Steinernematidae were found in 12 samples - six of them contained

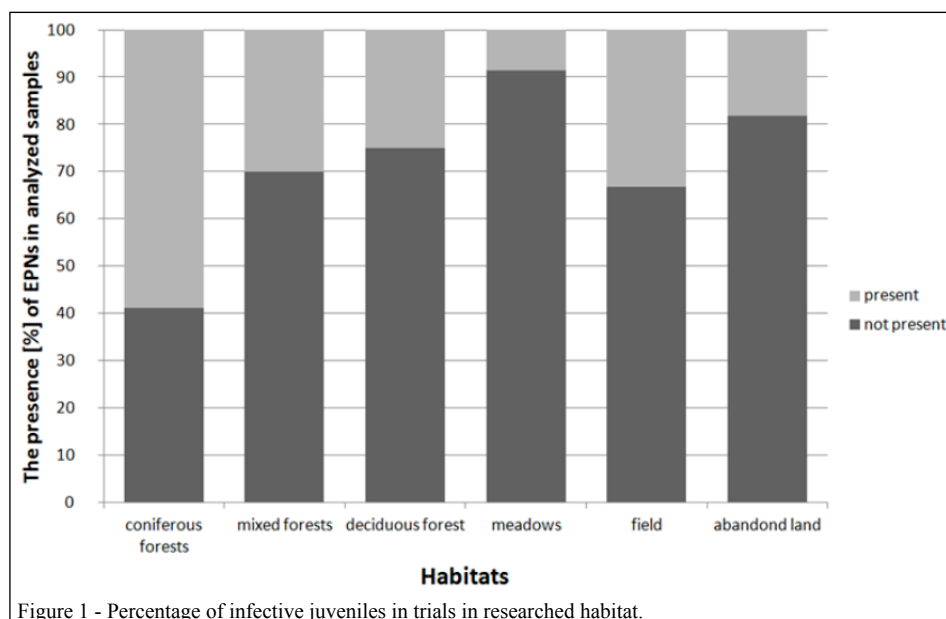


Figure 1 - Percentage of infective juveniles in trials in researched habitat.

*S. feltiae* and one *S. carpocapsae*. Nematodes of the family *Heterorhabditidae* were noted only in four samples from fields; two of them contained *H. megidis*. *Steinernema feltiae* appeared as the dominating species (11 samples = 20.4%) among all the isolated EPNs. It was most often isolated in spring (7 samples) from the fields. *Steinernema kraussei* constituted 7.5% (4 samples) of all isolated species. In three out of four samples, *S. kraussei* was isolated from forests in spring and in one forest sample collected in autumn. *Heterorhabditis megidis* constituted 3.8% (2 samples) of all isolated species. In both cases, its presence was recorded in field samples; once in summer and once in autumn. The presence of *S. carpocapsae* was reported in only one sample (1.9%) collected in summer from the field. No statistically significant effect ( $P > 0.05$ ) of the altitude on the occurrence of EPNs was reported.

Summarizing, the following species of EPNs has been recorded in Poland: *S. feltiae*, *S. affine*, *S. bicornutum*, *S. carpocapsae*, *S. arenarium*, *S. kraussei*, *H. megidis* and *H. bacteriophora* (DZIĘGIELEWSKA & KIEPAS-KOKOT, 2004; DZIĘGIELEWSKA, 2012; TUMIALIS et al., 2014). In this research from among 167 studied samples, nematodes were isolated from 53 samples (31.7%). This result is similar to those obtained by DZIĘGIELEWSKA & KIEPAS-KOKOT (2004) who isolated EPNs in 38 out of 97 samples (39%) and by DZIĘGIELEWSKA (2012) who analysed 156 samples and found EPNs in 43 of them (27.6%). The nematodes from *Steinernematidae*, which constituted 92.5% of the records, were more frequently found in

the presented study. *Heterorhabditidae* family was represented by only one species (*H. megidis*) found in two samples (3.8%), which confirmed rarity of *Heterorhabditidae* nematodes in Europe (HOMINICK, 2002). Similar proportions were reported in studies by MRÁČEK (2005), DZIĘGIELEWSKA (2012) and STURHAN & LIŠKOVÁ (1999). In this study, three species of the genus *Steinernema* and one species of *Heterorhabditis* were reported. In the present study, the dominating species *S. feltiae* was reported in 11 samples (20.6%). The observations made by BEDNAREK (1990) in agroecosystem in different regions of Poland showed the presence of *S. feltiae* in 75.5% of samples with EPNs. DZIĘGIELEWSKA & KIEPAS-KOKOT (2004) found 63% share of *S. feltiae* in the assemblages of entomopathogenic nematodes dwelling in industrial areas, while in the studies carried out in orchards, its share was smaller (27%), but the species was still dominating (DZIĘGIELEWSKA, 2012). In performed studies, *S. kraussei* was reported in four samples (7.5%), similarly to Slovakia (STURHAN & LIŠKOVÁ, 1999). Nematodes were isolated in all seasons but no significant seasonal differences were found in the presence of nematodes. Similarly MRÁČEK (2005) and GARCIA DEL PINO (1996) did not find the relationship between the occurrence of EPNs and seasons. MRÁČEK (2005) collected the samples during the whole year, whereas Garcia Del Pino (1996) isolated nematodes in spring and autumn. In contrast, the season affects the number of individuals. MRÁČEK (1982) stated that there are two peaks of nematode abundance: in July and November. This author assumed that the high abundance in July

resulted in the occurrence of favourable ecological conditions for nematodes. In contrast, the high abundance in autumn is associated with an increase of the number of soil hosts. The only species present in all seasons was *S. feltiae*. In studies by MRÁČEK (2005) two species - *S. feltiae* and *S. kraussei* - were isolated the year round. Most nematodes were found in samples isolated from coniferous forest soils. The same results were obtained in Slovakia, Germany and Czech Republic (STURHAN & LIŠKOVÁ, 1999; MRÁČEK et al., 2005). When analysing the list of species and their distribution in neighbouring countries, one may expect more species than determined so far in Poland.

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