POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 35(1): 5–19, Y. 2020

SPECIES DIVERSITY AND MOLECULAR CHARACTERIZATION OF ENTOMOPATHOGENIC NEMATODES (HETERORHABDITIDAE, STEINERNEMATIDAE) ISOLATED FROM SOILS OF DIFFERENT ECOSYSTEMS IN NORTHERN POLAND

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K e y w o r d s: entomopathogenic nematodes, LSU, PCR-RFLP, Steinernematidae, Heterorhabditidae, natural occurrence, habitat preference, survey.

A b s t r a c t

Entomopathogenic nematodes (EPN) of the families Steinernematidae and Heterorhabditidae were isolated from 57 of 91 localities in northern Poland. Of 489 soil samples collected in the field, EPNs were recorded in 27 per cent of them. Steinernema species were more frequent than Heterorhabditis: five of the family Steinernematidae and two of the family Heterorhabditidae were identified. Nematodes *S. feltiae* was the most frequently recorded species in different ecosystems. There were two rare of entomopathogenic nematodes species to the Polish fauna: *S. silvaticum* (only in natural ecosystems) and *S. bicornutum* (only in agrocoenoses). It seems that the presence of a suitable host in the environment is the most important factor for EPNs. Simple PCR-RFLP system was used to differentiate eight EPN species.

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Introduction

Several qualities predispose nematodes of the families Steinernematidae and Heterorhabditidae as biological control agents of pests (EHLERS 2001, SHAPIRO-ILAN et al. 2006). Practical insect management with nematodes is widely used in North America, Europe and Japan (EHLERS 1996, 2001, KAYA et al. 2006, ANSARI et al. 2009). As safe to the environment and warm-blooded animals as well as relatively cost-effective and uncomplicated in mass production, entomopathogenic nematodes (EPNs) are competitive with other insecticides on the market (KAYA et al. 2006). Nematodes can be active in the soil over long periods of time and are highly resistant to adverse conditions in the surrounding environment (LEWIS et al. 2006). A wide range of potential hosts and the occurrence of several insects in the soil can increase the efficiency of pest control with EPNs in a variety of agrocoenoses (EHLERS 2001).

The infection potential of nematodes is high. Laboratory experiments show that nematodes of the family Steinernematidae can infect over 350 insect species of 13 orders (PETERS 1996) but each exhibits specific food preferences for insects occurring in the substrate. *Steinernema carpocapsae* is very attached to pests feeding in agrocoenoses, i.e. beetles of the families Elateridae and Scarabaeidae (ANSARI et al. 2003, 2009) and butterflies of the families Tortricidae and Noctuidae (BRUCK and WALTON 2007, CURTO et al. 2008) while *Steinernema feltiae* is recommended for use in covered crops to control Bibionid flies (Diptera: Bibionidae) (EHLERS 1996, KOWALSKA and KOMOSA 2006). Increased sensitivity to grub of beetles of the family Scarabaeidae (ANSARI et al. 2003) and larvae of Otiorhynchus beetles of the family Curculionidae (EHLERS 1996) is recorded in nematodes of the family Heterorhabditidae. Ecological preferences of local populations of these nematodes occurring naturally in different agro- and bioceonoses should be examined to use EPNs effectively in integrated crop protection programmes. Literature data show that the genetic pool of wild strains of appropriately selected nematodes can give rise to improved biological control (BURNELL and DOWDS 1996).

Many authors worldwide confirm that EPNs of the families Steinernematidae and Heterorhabditidae occur commonly and have been spreading (HOMINICK et al. 1996, HOMINICK 2002). As current European research into the biology and ecology of EPNs shows, these nematodes are rich in species and their environmental preferences vary. New species such as *Steinernema weiseri* (MRÁČEK et al. 2003) or *Steinernema silvaticum* (STURHAN et al. 2005) were discovered during extensive field investigations in the Czech Republic (MRÁČEK et al. 1999, MRÁČEK and BEČVÁŘ

2000, MRÁČEK et al. 2005, PŮŽA and MRÁČEK 2005), Slovakia (STURHAN and LIŠKOVÁ 1999) or Germany (STURHAN 1999) and numerous undetermined nematode isolates from eastern and northern Europe classified as the *glaseri*-group were recognized as one species, *S. arenarium* (STURHAN and MRÁČEK 2001).

Identifying EPNs using the classical method requires considerable experience and is time-consuming. Molecular methods can be an efficient tool confirming isolates determined with the morphometric assessment. PCR-RFLP is an alternative to costly DNA sequencing for molecular identification of nematodes. It is less sensitive and more difficult to gather into databases than the DNA sequencing approach but it is uncomplicated, time-saving and easy to perform in a typical molecular biology laboratory. The identification procedure consists in amplification by PCR using appropriate primers, digesting products with several restriction endonucleases and comparing band patterns. The PCR-RFLP strategy has been used both in taxonomical and phylogenetic research into nematodes (REID et al. 1997, HOMINICK et al. 1997). A homogenous identification system of all known EPN species with the PCR-RFLP method has not been described to date due to the nature of the method, a large number of species and differences in their geographical distribution. At present a total of 75 species of the family Steinernematidae and 19 of the family Heterorhabditidae are listed in the NCBI database. Although numerous nematode sequences, including EPNs, are stored in GenBank, the genome structure of newly discovered species is not available. For instance, only one sequence of *S. silvaticum* is deposited in GenBank.

Nematode species were molecularly characterized most often by analyzing ribosomal DNA (rDNA), which has become a useful DNA region for classifying different eucaryotes at various taxonomical levels (HILLIS and DIXON 1991, after NASMITH et al. 1996). The rDNA is a multi-copy, tandemly repeated array occurring in the nucleolar organizer region at one or several chromosomal sites (LONG and DAWID 1980, after NASMITH et al. 1996). Within the rDNA cistron are coding and non-coding sequences that can be used to study various taxonomical levels, from within species populations to taxa at or above genera. The rDNA coding genes vary in evolutionary conservation from most-conserved 18S (SSU, small subunit), 5.8S to least-conserved 28S (LSU, large subunit).The spacer regions including ETS (external transcribed spacer), ITS (internal transcribed spacer) and IGS (intergenic spacer) are more variable than the gene regions and are generally used for analysis at or below the species level (BECKINGHAM 1982, after NASMITH et al. 1996)

The D2 and D3 expansion segments of the 28S rRNA are often sequenced in studies of nematode phylogenetics due to the availability of conserved primers amplifying DNA from many taxa, and the presence of phylogenetically informative sites (SUBBOTIN et al. 2007). The D2 and D3 segments are useful for analyzing relationships including higher taxonomical levels; examples include studies among orders of the phylum Nematoda (LITVAITIS et al. 2000), within the order Cephalobina (NADLER et al. 2006) as well as within genera of several orders, e.g. *Steinernema* (STOCK et al. 2001).

The aim of this study was to develop a simple and cost-effective system to identify EPNs naturally occurring in the fauna of Poland and Central Europe and to present their biodiversity (MRÁČEK et al. 2005), to establish sequences of the fragment within the 5'-end of the nuclear LSU rDNA that included the D2 and D3 domains for *S. silvaticum* and to compare it to the known sequences of other nematode species.

Materials and Methods

Field collection

Field studies were conducted in 2008–2013 in north-western Poland (Zachodniopomorskie Voivodeship), in a variety of ecosystems encompassing forest complexes, agrocoenoses (crop fields, green sites, orchards), coastal dunes and urbanized areas.

Nematode isolates were obtained from soil samples from 91 sites. Samples were collected using Egner's staff from 100 m^2 of the research area, three times per year, from spring to autumn. From a depth of up to 20 cm were collected 100 unit samples constituting the aggregate sample, with a total volume ca. 600 cm^3. The soil was transported to the laboratory in plastic perforated bags. Each soil sample was thoroughly mixed in the laboratory and placed in six 100 cm^3 plastic containers. The soil was successively wetted with water (5–15 ml H_2O) to obtain suitable moisture (70–80%).

Laboratory examinations
Isolation and multiplication of nematodes

Nematodes were isolated from the soil using the standard *Galleria mellonella* baiting insect method (BEDDING and AKHURST 1975). Morphological and morphometric features of infective-stage larvae (J_3) of these nematodes obtained with the WHITE method (1927) and adult individuals

of the second generation isolated from dead *Galleria* caterpillars by sectioning (NGUYEN and SMART 1997, HOMINICK et al. 1997, NGUYEN 2007a) were used to determine taxonomically individual nematode species.

At least two nematode isolates from different localities determined using morphological features were examined to establish PCR-RFLP profiles characteristic of individual species.

PCR-RFLP

Nucleic acid preparations were extracted from second generation females. For each species 50–100 adults were pooled and used. DNA was extracted with a ready to use set of *Kucharczyk* according to the supplier's procedure.

The rDNA regions were amplified by the PCR in 20 µl reaction. The following were added to each tube: 2 µl of 10 × PCR buffer, 2 µl of $MgCl_2$ (25 mM), 1.6 µl of dNTP mixture (2 mM each), 0.8 µl of forward primer (5 pM/1 µl), 0.8 µl of reverse primer (5 pM/1 µl), 0.2 µl Taq recombinant polymerase (5 U/1 µl), 0.8 µl of DNA (about 15 ng μl^{-1}) and 11.8 µl of distilled water. MBI Fermentas reagents were mostly used.

Three primer sets were used at the initial stage: ITS1-forward ACGAGCCGAGTGATCCACCG (CHERRY et al. 1997, after ADAMS et al. 1998) and ITS1-reverse TTGATTACGTCCCTGCCCTTT (VRAIN et al. 1992, after ADAMS et al. 1998), LSU-forward AGCGGAGGAAAAGAAACTAA (NADLER and HUDSPETH 1998, after STOCK et al. 2001) and LSU-reverse TCGGAAGGAACCAGCTACTA (THOMAS et al. 1997, after STOCK et al. 2001) as well as 18S-forward GCAAGTCTGGTGCCAGCAGC (FOUCHER and WILSON 2002) and 18S-reverse CCGTGTTGAGTCAAATTAAG (FOUCHER and WILSON 2002).

PCR reactions were carried out in a Gene Amp® PCR System 9700 (Applied Biosystems). PCR cycling parameters included denaturation at 95°C for 3 min, followed by 8 cycles of 95°C for 30 sec, 64°C→50°C (touchdown 2°C/1 cycle) for 60 sec, 72°C for 60 sec, followed by next 26 cycles of 95°C for 30 sec, 50°C for 60 sec, 72°C for 60 sec, followed by a postamplification extension at 72°C for 5 min.

After the PCR 5µl of the mixture, mixed with 9µl of distilled water with 1 µl of buffer and 0.5 µl enzyme (10 U μl^{-1}), was used to digest amplification products. Six endonucleases were initially used (AluI, HinfI, HhaI, HpyF3I, PvuII, RsaI). Incubation was conducted overnight in a laboratory incubator at 37ºC.

PCR and PCR-RFLP products were separated by electrophoresis in 1.5% agarose gel. 1· TBE buffer was used for gel preparation and electro-

phoresis. Electrophoresis was run at a constant voltage of 90 V for ca. 90 minutes. Agarose gel was stained with 0.5 μgml ethidium bromide for 5–10 minutes. Electrophoresis products were visualised using UVG:BOX Syngene (Biotech) and documented with GeneSnap7.02 (Synoptics Ltd). Amplification and digestion products were sized with a gene ruler 100 bp DNA Ladder Plus (MBI Fermentas) consisting of 14 fragments in the range 100–3000 bp.

Cloning and sequencing

PCR products were separated in 1% agarose gel. 1×TAE buffer was used. The gel and buffer contained 1 mM l^{-1} guanosine. PCR products were isolated from agarose gel with TOPO TA Cloning® (Invitrogen). Vector pCR®II-TOPO® was used. *E. coli* competence was induced chemically according to the procedure of Invitrogen. LB Agar medium (A&A Biotechnology) and LB-Medium (Carl Roth) were used. GenomeLab DTCS – Quick Start Kit (Beckman Coulter) and M13 primer were used for PCR sequencing. PCR products were treated with Agencourt CleanSEQ® Magnetic Beads (Beckman Coulter). Sequencing was performed in a Beckman Coulter CEQ 8000 Genetic Analysis System. DNA was sequenced in both directions and consensus sequence was received using BioEdit (HALL 1999).

Results

Entomopathogenic nematode fauna

Entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae were isolated from 57 of 91 localities, that is 63% of the total number of sites. Of 489 soil samples collected in the field, EPNs were recorded in 131 (27% of the total number of samples) – Table 1. Altogether seven species of EPN were identified: five of the family Steinernematidae and two of the family Heterorhabditidae (Table 1).

Nematodes of the family Steinernematidae recorded in 100 samples (over 76% of the total number of samples containing nematodes) were represented by five species (Table 1). *Steinernema feltiae* (Filipiev): the most frequently recorded species in northern Poland in different ecosystems, recorded in over 47% nematode-containing samples, *Steinernema affine* (Bovien) present in over 14% of nematode-containing samples, often co-occurring with *S. feltiae* in soil, especially in city parks. Its recorded both in open and forested sites. *Steinernema silvaticum* (Sturhan, Spiridonov & Mrácek) relatively rarely occurring in the study area (above 5% of nem-

atode-containing samples), especially in urban forestations and in forest complexes. *Steinernema bicornutum* (Tallosi, Peters & Ehlers) was one of rare species recorded in Poland, too; isolated from orchards and urban green areas (2% of species identified). *Steinernema carpocapsae* (Weiser): the least frequently recorded species in the study area (0.8% of nematode-containing samples).

Nematodes of the family Heterorhabditidae isolated from 35 samples (27% of the total number of nematode-containing samples) were represented by two species (Table 1):

Table 1

The occurrence of entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae in northern Poland

Ecosystem	Total number of samples	Number of samples with nematode [%]	Number of samples with more than one nematode species	Number of samples with:							
				Steinernema feltiae	*Steinernema silvaticum*	*Steinernema affine*	*Steinernema carpocapsae*	*Steinernema bicornutum*	*Heterorhabditis bacteriophora*	*Heterorhabditis megidis*	*Steinernema* spp.
Urbincoenoses:											
urban and industrial areas	121	34 (28)	4	15	2	5	0	1	0	15	0
Agrocoenoses:											
orchards	19	12 (63)	0	3	0	1	0	2	0	6	0
crop fields	48	17 (35)	0	12	0	0	1	0	0	5	0
green sites (pastures, meadows)	24	6 (25)	0	0	0	2	0	0	4	0	0
Biocoenoses:											
forests	165	50 (30)	0	27	5	7	0	0	0	0	11
coastal dunes	112	11 (10)	0	5	0	0	0	0	1	4	1
Total	**489**	**131**	**4**	**62**	**7**	**15**	**1**	**3**	**5**	**30**	**12**

Heterorhabditis megidis (Poinar, Jackson & Klein): the most frequently recorded species (23% of nematode-containing samples) beside *S. feltiae*, recorded both in open areas and partly open areas (dunes, lawns, rose bushes) as well as in forestations (orchards, city parks),

Heterorhabditis bacteriophora (Poinar): the least frequently recorded species of the family Heterorhabditidae (3.8% nematode-containing samples) – Table 1; known only from open areas (meadows, dunes).

Molecular examinations

The combination of one primer set (LSU-forward and LSU-reverse) and two endonucleases (HinfI, RsaI), best differentiating the species, was selected based on initial samples with three different primer sets and six restriction enzymes. All eight species of the genera Heterorhabditidae and Steinernematidae were distinguished using this set. A band ca. 950 bp in length was the amplification product in the presence of the primer sets.

The sum of the PCR-RFLP fragments for different species varied (620–1440 bp) and did not always equal the length of the initial product (Figure 1, Table 2). It was smaller in some cases and some digestion products may have been so similar in length that they could not be differentiated on the electrophoretic image display. These fragments possibly include products of digestion with HinfI of the 320 bp LSU amplicon of *S. carpocapsae*. Similarly long fragments obtained with RsaI were recorded for *S. affine* (2 × 200 bp), *S. bicornutum* (2 × 120 bp), *S. feltiae* and *S. kraussei* (2 × 200 bp). If all fragments for the species are of this length, a total length of 920–940 bp would be obtained, which is similar to the initial amplicon.

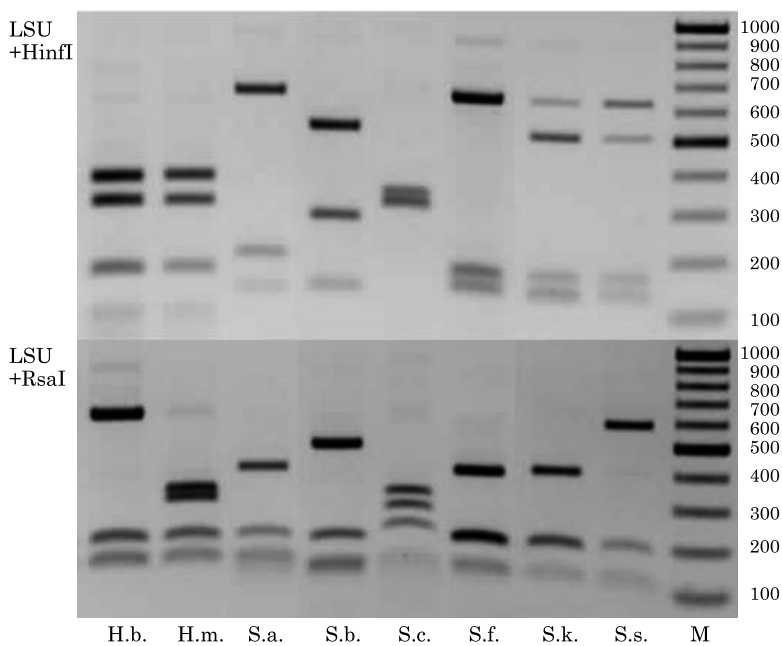

Fig. 1. Images of electrophoretic separation of fragments obtained by digesting LSU-region amplification products using HinfI and RsaI enzymes, characteristic of *Heterorhabditis bacteriofora* (H.b.), *H. megidis* (H.m.), *Steinernema affine* (S.a.), *S. bicornutum* (S.b.), *S. carpocapsae* (S.c.), *S. feltiae* (S.f.), *S. kraussei* (S.k.), *S. silvaticum* (S.s.). M – DNA length marker

Table 2

Fragment lengths (bp) obtained by digesting LSU-region amplification products using HinfI and RsaI enzymes, characteristic of *Heterorhabditis bacteriofora* (H.b.), *H. megidis* (H.m.), *Steinernema affine* (S.a.), *S. bicornutum* (S.b.), *S. carpocapsae* (S.c.), *S. feltiae* (S.f.), *S. kraussei* (S.k.). Fragments probably commigrating in gel are marked in bold

Specification	H.b.	H.m.	S.a.	S.b.	S.c.	S.f.	S.k.	S.s.
HinfI	380	380	630	520	**320**	630	630	630
	300	300	200	280	300	180	500	500
	180	180	140	140	–	140	180	180
	80	80	–	–	–	–	**130**	**130**
Total	940	940	970	940	620	950	1440	1440
RsaI	620	320	400	500	320	400	400	620
	200	300	**200**	200	280	**200**	**200**	200
	140	200	140	**120**	220	120	120	140
	–	140	–	–	140	–	–	–
Total	960	960	740	820	960	720	720	960

An atypical electrophoretic image was also displayed for *S. kraussei* and *S. silvaticum*. The total length of LSU fragments formed after cleaving with HinfI was greater than the length of the undigested amplification fragment. Two products of the same or near the same length are probably produced by the PCR but they differ by the sequence in one of the sites recognized by RsaI. Cleaving with one of them produces fragments 630, 180, 130 bp in length while the second one consists of fragments 500, 180, 130, 130 bp in length.

Discussion

The knowledge on the occurrence of entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae in Poland is fragmentary and rarely describes the nematode/environment correlation. Sites discussed in the literature include selected agrocoenoses (meadows and arable fields) of central and southern Poland (Bednarek 1990, Jaworska and Dudek 1992, Jaworska et al. 1997, Matuska and Kamionek 2008, Ropek and Nicia 2008, Tumialis et. al. 2016), forest complexes in southern and north-eastern Poland (Kamionek et al. 1995) and urban and industrialized areas in central Poland (Pezowicz 2002, Tomalak 2005). Eight species of EPNs have been reported from Poland in the literature: six species of the family Steinernematidae and two species of the family Heterorhabditidae. *Steinernema feltiae* (Bednarek 1990, Jaworska and

DUDEK 1992, DZIĘGIELEWSKA and KIEPAS-KOKOT 2004, DZIĘGIELEWSKA 2012, TOMALAK 2005, ROPEK and NICIA 2008, TUMIALIS et. al. 2016) and *S. carpocapsae* (BEDNAREK 1990, KAMIONEK et al. 1995, JAWORSKA et al. 1997, PEZOWICZ et al. 2008, TUMIALIS et. al. 2016) are the most frequently recorded species of the family Steinernematidae. Nemtodes *S. affine*, *S. bicornutum* and *S. kraussei* are noted less frequently in the Polish fauna (DZIĘGIELEWSKA 2012, TOMALAK 2005, TUMIALIS et. al. 2014). The occurrence of *S. glaseri* should be confirmed and verified (KAMIONEK et al. 1995). A new species of the *glaseri*-group, *S. arenarium* (TOMALAK 2003, SKRZYPEK et al. 2011), and *S. kraussei* (TUMIALIS et al. 2014) have been reported recently. *S. arenarium* is thought to be a European equivalent to *S. glaseri*, a species widespread in North America, especially the United States (MRÁČEK et al. 2005). *Heterorhabditis megidis* and *H. bacteriophora* are the two species of the family Heterorhabditidae identified in Poland (JAWORSKA et al. 1997, DZIĘGIELEWSKA 2012, TOMALAK 2005, MATUSKA and KAMIONEK 2008, TUMIALIS et. al. 2016).

Six of the nematode species above mentioned have now been confirmed from north-western Poland. Only *S. arenarium*, *S. glaseri* and *S. kraussei* have not been isolated from the area to date. Altogether seven species of the families Steinernematidae and Heterorhabditidae have been recorded in the study area, including *S. silvaticum*. The total number of EPNs identified in Poland after 2000, including six species of the genus *Steinernema* and two of the genus *Heterorhabditis*, is slightly lower than that in other countries of central Europe. Eleven nematode species have been recorded in the Czech Republic, including eight of the genus *Steinernema* and two of the genus *Heterorhabditis* (MRÁČEK et al. 2005). A total of 13 species have been noted in Germany, including ten species of the genus *Steinernema* (STURHAN 1999). Nine species have been identified in Slovakia: eight of the genus *Steinernema* and one of the genus *Heterorhabditis* (STURHAN and LIŠKOVÁ 1999). Ten nematode species have been isolated in northern Europe (England): eight of the genus *Steinernema* and two of the genus *Heterorhabditis* (HOMINIK et al. 1995).

S. silvaticum was recorded in soil samples collected in urban forestations with *Quercus robur* L. and *Quercus petraea* Mattuschka Liebl. and from large compact forest complexes (Peucedano-Pinetum and Querco roboris-Pinetum) where *Pinus silvestris* L. dominates in the tree stand. It was recorded in sites of mass occurrence of herbivorous Hymenoptera of the families *Diprionidae* and Cynipidae and the butterfly *Bupalus piniastris* L. of the family Geometridae. Although natural hosts of *S. silvaticum* have not been identified to date (STURHAN et al. 2005), it seems highly probable that these insect species can potentially be their hosts, especially Diprion

species (Hymenoptera) or geometers (Lepidoptera) which burrow in the soil to winter during the developmental cycle.

The natural occurrence of *S. silvaticum* nematodes has been relatively recently recorded in Poland (DZIĘGIELEWSKA et al. 2015, LIS et al. 2018). Earlier its has been confirmed in some European countries, i.e. Germany (STURHAN 1999), the Czech Republic (MRÁČEK and BEČVÁŘ 2000, STURHAN and MRÁČEK 2000, MRÁČEK et al. 2005), England and the Netherlands (HOMINIK et al. 1995), Belgium and Sweden STURHAN et al. (2005). Nematodes *S. silvaticum* seems to be attached to forestations and forest communities rather than to open areas. This is also suggested in studies of STURHAN (1999, 2005) and MRÁČEK et al. (2005). Studies using rDNA sequences (ITS1 + 5.SS + ITS2) define *S. silvaticum* and *S. kraussei* nematodes as sister taxa (NGUYEN 2007b). Similarly, the sequencing results for a fragment of the LSU rDNA gene and ITS1 region of the *S. silvaticum* isolate and *S. kraussei* isolate proved very high identity between both of species (DZIĘGIELEWSKA et al. 2015).

The earlier phylogenetic maximum parsimony analysis of alignments of *S. silvaticum* and other Steinernematids with known rDNA sequences (ITS1 + 5.SS + ITS2) consistently placed this species together with *S. feltiae, S. jollieti*, *S. kraussei*, *S. oregonense* and *S. weiseri* (NGUYEN 2007b). Also, the phylogeny of both nuclear and mitochondrial genes indicated close relationships of the Polish *S. silvaticum* isolates with *S. kraussei*, *S. oregonense* and *S. cholashanense* (LIS et al. 2018).

It is expected that further entomopathogenic nematode species new to the fauna of Poland will be recorded. This is supported by the zoogeographical range of the occurrence of nematodes of the family Steinernematidae in Europe. Species likely to occur in Poland, especially of the genus *Steinernema*, have been isolated from the soil in Poland's neighbours such as the Czech Republic, Germany or Slovakia (MRÁČEK et al. 2005, STURHAN 1999, STURHAN and LIŠKOVÁ 1999, STURHAN et al. 2005). These include *Steinernema intermedium* or *S. weiseri* (MRÁČEK et al. 2003, BAZMAN et al. 2008).

It seems, however, that genetic testing will not replace morphological examinations even if more data becomes available and advances in genetic research are made, and will rather serve to support and supplement them. Although DNA sequencing technologies are becoming widespread, PCR-RFLP is still a simple and efficient diagnostic method in research into EPNs.

Our survey of entomopathogenic nematodes in north-western Poland contributes and extends information concerning these nematodes in different habitants. The occurrence of entomopathogenic nematodes will corre-

spond to the occurrence of their insect hosts in the environment. Will also be addicted from abiotic factors specific to the type of habitat (e.g. soil texture, soil pH, soil temperature, soil moisture). Understanding the biotic resources in different ecosystems, including the species diversity of local entomopathogenic nematode populations, will allow more effective use of these beneficial organisms in biological protection against various important plant pests.

Accepted for print 21.02.2020

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