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THE FIRST MOLECULAR CHARACTERISATION OF *STEINERNEMA SILVATICUM* RECORDED IN POLAND AND ITS DIFFERENTIATION FROM *STEINERNEMA KRAUSSEI* USING RIBOSOMAL DNA (rDNA) SEQUENCES

PIERWSZA CHARAKTERYSTYKA MOLEKULARNA *STEINERNEMA SILVATICUM* WYKRYTEGO W POLSCE I JEGO ODRÓŻNIENIE OD *STEINERNEMA KRAUSSEI* NA PODSTAWIE SEKWENCJI DNA RYBOSOMALNEGO (rDNA)

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Streszczenie. Podczas badań nad występowaniem nicieni owadobójczych (EPN) z rodzin *Steinernematidae* oraz *Heterorhabditidae,* prowadzonych w północno-zachodniej Polsce, po raz pierwszy w Polsce zidentyfikowano gatunek *Steinernema silvaticum*. Identyfikacja gatunkowa nicieni, zwłaszcza odróżnienie od siebie tych najpodobniejszych, określanych mianem gatunków „siostrzanych", z wykorzystaniem metod morfometrycznych, jest pracochłonna i często problematyczna nawet dla doświadczonego badacza. Prezentowane badania są próbą odróżnienia *S. silvaticum* od *S. kraussei* przy zastosowaniu wyników sekwencjonowania rejonów rybosomalnego DNA (rDNA) – regionów ITS1 i LSU. Sekwencjonowane obszary miały długości odpowiednio 490 bp i 918 bp. Podobieństwo między izolatami *S. silvaticum* i *S. kraussei* w przypadku obydwu sekwencji było bardzo wysokie (98–99%). Dwa polimorficzne nukleotydy (SNP) w ITS1 i dwa w LSU pozwoliły na odróżnienie obu analizowanych gatunków.

Key words: internal transcribed spacer (ITS), large subunit of rDNA (LSU), *Nematoda*, *Steinernematidae.*
Słowa kluczowe: duża podjednostka rybosomalnego DNA (LSU), ITS, nicienie, *Steinernematidae.*

INTRODUCTION

Entomopathogenic nematodes (EPNs) of the family *Steinernematidae*, environmentally-friendly as well as relatively cost-effective and uncomplicated in terms of mass production, seem to be a desirable biological control agent of pests (Ehlers 2001, Shapiro-Ilan et al. 2006), alternative to other insecticides (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 also the occurrence of several insects in the soil can increase the efficiency of pest control with EPNs in a variety of agrocoenoses (Ehlers 2001).

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Many authors worldwide have confirmed that EPNs of the family *Steinernematidae* 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. New species, such as *Steinernema weiseri* (Mráček et al. 2003) or *Steinernema silvaticum* (Sturhan et al. 2005), have been 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 Liškova 1998) and Germany (Sturhan 1996, 1999), and numerous undetermined nematode isolates from eastern and northern Europe classified as the *glaseri*-group have been recognized as one species, *S. arenarium* (Sturhan and Mráček 2001).

Knowledge on the occurrence of entomopathogenic nematodes of the families *Steinernematidae* in Poland is fragmentary. In the literature, six species of EPNs of the family *Steinernematidae* have been reported from Poland. In our study, five species have been recorded in the study area, including *S. silvaticum* (Karbowska-Dzięgielewska 2013). Although *S. silvaticum* has not previously been noted in Poland, its natural occurrence has been confirmed in some European countries, i.e. Germany (Sturhan 1999), the Czech Republic (Mráček and Bečvář 2000, Sturhan and Mráček 2001, Mráček et al. 2005), England and the Netherlands (Hominick et al. 1995), Belgium and Sweden. Isolates of this species were previously determined as *Steinernema* sp. B (Sturhan 1996).

Identification of related species of nematode on the basis of morphological parameters is difficult and requires a lot of experience. Our purpose was to check the utility of molecular methods in the differentiation of *S. silvaticum* from other species of *Steinernematidae* – *S. kraussei*, which is one of the most similar species. Since we did not possess *S. kraussei* in our own collection, we used an isolate derived from Canada, kindly provided by Professor Tomalak.

Nematode species have most often been molecularly characterized 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). rDNA is a multi-copy, tandemly repeated array occurring in the nucleolar organizer region at either one or several chromosomal sites (Long and David 1980, after Nasmith et al. 1996). Within the rDNA cistron there 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. 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 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 the genera of several orders, e.g. *Steinernema* (Stock et al. 2001).

The aim of this study was: 1) to establish sequences of the fragment within the 5'-end of the nuclear LSU rDNA that included the D2 and D3 domains and of the region ITS1 for *S. silvaticum* and *S. kraussei*, 2) to compare them to the known sequences of other nematode species, 3) to state if molecular analyses are a sufficient way to distinguish the studied species.

MATERIAL AND METHODS

Our material came from field studies conducted in 2004–2009 in north-western Poland (Karbowska-Dzięgielewska 2013). The molecular analysis was performed for DNA extracted from second generation females (50–100 adults pooled for each species) of *S. silvaticum* isolates (N30) from Poland and *S. kraussei* (N11) isolates from Canada with an A&A Biotechnology (Poland) ready-to-use set for universal genomic DNA isolation (Genomic Mini) according to the supplier's procedure. Two products of PCR amplification were sequenced: a fragment of the LSU rDNA gene and an ITS1 region.

The rDNA regions were amplified in PCR under standard conditions (detailed methodology upon request), in a Gene Amp® PCR System 9700 (Applied Biosystems), mostly using MBI Fermentas reagents and two pairs of primers: ITS1-forward ACGAGCCGAGTGATCCACCG (Cherry et al. 1997, after Adams et al. 1998) with ITS1-reverse TTGATTACGTCCCTGCCCTTT (Vrain et al. 1992, after Adams et al. 1998), and LSU-forward AGCGGAGGAAAAGAAACTAA (Nadler and Hudspeth 1998, after Stock et al. 2001) with LSU-reverse TCGGAAGGAACCAGCTACTA (Thomas et al. 1997, after Stock et al. 2001).

PCR products were separated by electrophoresis in 1.5% agarose gel with 1×TBE buffer, then isolated from gel and cloned with TOPO TA Cloning® (Invitrogen). Vector pCR®II-TOPO® was used. A GenomeLab DTCS – Quick Start Kit (Beckman Coulter) and standard M13 primers were used for PCR sequencing. Sequencing was performed in a Beckman Coulter CEQ 8000 Genetic Analysis System. Each DNA strain was sequenced in both directions and a consensus sequence was received using BioEdit software (Hall 1999). There were 3–5 clones of the two examined species sequenced in this way from both ITS1 and LSU products.

RESULTS AND DISCUSSION

The results of sequencing analysis performed for a fragment of the LSU rDNA gene and ITS1 region of the *S. silvaticum* isolate (N30) and the Canadian isolate of *S. kraussei* (N11) were deposited in the NCBI database. The GenBank accession numbers of LSU sequences are: KC631421-KC631425 for *S. kraussei* and KC631426-KC631428 for *S. silvaticum*. Sequences of ITS1 were placed under the numbers: KC631429-KC631431 for *S. kraussei* and KC631432-KC631434 for *S. silvaticum*.

Sequenced regions of ITS1 and LSU had lengths of 490 bp and 918 bp, respectively. A very high similarity was detected between the *S. silvaticum* isolate and the isolate of *S. kraussei*. This was 98–99% for the ITS1 sequence depending on the clone pair and the same regularity applied to the LSU region.

When compared to GenBank data, all the sequences of ITS1 (*S. silvaticum* and *S. kraussei*) exhibit a similar level of similarity (98–99%) to ITS1 (contained in gene sequences for 18S rRNA, ITS1, 5.8S rRNA, ITS2 and 28S RNA) of three strains of *S. kraussei*: AB243442.1 derived from Japan (Kuwata et al. 2006), AY230176.1 and AY230175.1 (Spiridonov et al. 2004) from UK and Germany, respectively.

An NCBI database search shows that the highest similarity of LSU sequences to the sequence of *S. kraussei* 28S ribosomal RNA gene, partial sequence exists between clones of isolate N30 and isolate N11 (GenBank: GU569053.1 – Lee and Stock 2010). The only nucleotide sequence assigned to *S. silvaticum* provided in GenBank is a large subunit ribosomal RNA gene, partial sequence (DQ399663.1). It was not one of the 100 most similar records to our isolates in a BLAST search and its alignment was most similar to the *S. feltiae* strain. Using this sequence as a comparison, the results show that all clone sequences were 98–99% similar on the inter- and intraspecific level as well.

Among ten nucleotide differences (single nucleotide polymorphism – SNP) in ITS1, there were only two common ones which discriminate the two analysed species (Table. 1).

Table 1. Differences between the sequences of the ITS1 rDNA region for *S. silvaticum* and *S. kraussei* – analysed in this study, together with the most similar from the GeneBank database
Tabela 1. Zróżnicowanie sekwencji regionu ITS1 rDNA między *S. silvaticum* i *S. kraussei* – uzyskanych w pracy i tych o największym podobieństwie, wyszukanych w bazie GeneBank

Taxon Takson	Nucleotide position Pozycja nukleotydowa									
	79	108	171	240	275	284	288	296	378	486
S.k. N11[1]	G	G	G	**T**	T	**T**	T	A	T	C
S.k.[2]	T	G	G	**T**	T	**T**	T	A	A	T
S.k.N.[3]	G	G	-	**T**	A	**T**	G	G	T	T
S.k.W.[4]	G	T	-	**T**	A	**T**	G	G	T	T
S.s. N30[5]	T	G	G	**C**	T	**C**	G	G	A	C

[1]*S. kraussei* – sequence consensus for KC631429 – KC631431.
[2]*S. kraussei* from Japan, AB243442.1.
[3]*S. kraussei* strain Nash from UK, AY230176.1.
[4]*S. kraussei* strain Westphalia from Germany, AY230175.1.
[5]*S. silvaticum* – sequence consensus for KC631432 – KC631434.
[1]*S. kraussei* – sekwencja konsensusowa dla KC631429 – KC631431.
[2]*S. kraussei* z Japonii, AB243442.1.
[3]*S. kraussei* szczep Nash from UK, AY230176.1.
[4]*S. kraussei* szczep Westphalia z Niemiec, AY230175.1.
[5]*S. silvaticum* – sekwencja konsensusowa dla KC631432 – KC631434.

In terms of the LSU region, two of thirteen SNPs allow a differentiation of *S. silvaticum* from *S. kraussei* strains (Table 2).

Regardless of the method (DNA parsimony, maximum likelihood, UPGMA – data not shown), three *S. silvaticum* sequences of ITS1 form a separate cluster, and the next two (European) or all three taxa of *S. kraussei* selected from the GenBank in a BLAST search group together with the cluster of *S. silvaticum* sequences. The relationship between the *S. silvaticum* isolate with the *S. kraussei* identified in the GenBank seems, therefore, to be stronger than with our model originating from Canada. Irrespective of the clustering method, our taxons of *S. silvaticum* were grouped together based on the LSU and, depending on the method used for tree construction, *S. silvaticum* from NCBI was out of all clusters or was joined with one of our *S. kraussei* strains (LN11k5).

Table 2. Differences between the sequences of the partial LSU rDNA region for *S. silvaticum* and *S. kraussei* – analysed in this study with the most similar from the GeneBank
Tabela 2. Zróżnicowanie sekwencji fragmentu regionu LSU rDNA między *S. silvaticum* i *S. kraussei* – uzyskanych w pracy i tych o największym podobieństwie, wyszukanych w bazie GeneBank

Taxon Takson	Nucleotide position Pozycja nukleotydowa												
	232	273	399	403	407	413	432	436	439	447	468	500	508
S.k. N11[1]	**T**	T	T	C	T	G	A	A	C	G	A	A	**C**
S.k. GU569053.1	**T**	T	T	C	T	G	A	A	C	G	A	A	**C**
S.s. N30[2] /*	**C/T**	T	C	A/C	T	A	T	C/A	A/C	G	A	A	**T/C**
S.s. DQ399663.1	**C**	A	T	C	C	G	A	A	C	A	G	C	**T**

[1]*S. kraussei* – sequence consensus for KC631421 – KC631425.
[2]*S. silvaticum* – sequence consensus for KC631426 – KC631428.
*intraspecific differences – nucleotide alternatively occurring in different clones.
[1]*S. kraussei* – sekwencja konsensusowa dla KC631421 – KC631425.
[2]*S. silvaticum* – sekwencja konsensusowa dla KC631426 – KC631428.
*różnice wewnątrzgatunkowe – różne nukleotydy w zależności od sekwencjonowanego klonu.

These results confirmed earlier phylogenetic maximum parsimony analysis of the alignments of *S. silvaticum* and other steinernematids with known rDNA sequences (ITS1+5.SS+ITS2) which defined *S. silvaticum* and *S. kraussei* as sister taxa (Nguyen 2007). However, the *S. silvaticum* isolate cannot be classified as any species other than *S. kraussei*, based on the recommendation that species can be differentiated using the sequence of the ITS region in *Steinernematidae* for the percentage of dissimilarity 5% or more (Nguyen 2007). Our observations suggest that ITS1 and LSU are not fully appropriate to discriminate species so closely related as *S. kraussei* and *S. silvaticum*.

Although DNA sequencing technologies are becoming widespread, the information on sequences of newly discovered species, such as *S. silvaticum*, available in databases is insufficient. Comparative molecular analyses of *S. silvaticum* are then difficult to implement using the available information. Our study represents a contribution to a deepening of knowledge in this area.

Seeing that the assessment of rDNA sequences may not be sufficiently informative to differentiate closely related species that are also highly diversified within species, additional analyses comprising other regions of the genome would have to be used. Nonetheless, it seems 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.

RECAPITULATION

The sequencing results for a fragment of the LSU rDNA gene and ITS1 region of the *S. silvaticum* isolate and the Canadian isolate of *S. kraussei* proved very high identity between species of interest (98–99%).

Sequenced regions of the lengths 490bp and 918bp for ITS1 and LSU, respectively, seemed to be insufficient to unreservedly differentiate *S. silvaticum* from *S. kraussei* because there were only 4 reliable SNPs among 23 (10 of ITS and 13 of LSU) which discriminate the two analysed species.

Molecular analysis of *Steinernematidae* should not replace morphological studies and will rather supplement them.

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REFERENCES

Adams B.J., Burnell A.M., Powers T.O. 1998. A phylogenetic analysis of *Heterorhabditis* (*Nemata*: *Rhabditidae*) based on internal transcribed spacer 1 DNA sequence data. J Nematol 30(1), 22–39

Ehlers R.U. 2001. Mass production of entomopathogenic nematodes for plant protection. Appl. Microbiol. Biot. 56, 623–633.

Hall T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids. Symp. Ser. 41, 95–98.

Hominick W.M. 2002. Biogeography. In: Gaugler R. Entomopathogenic Nematology, CABI Publishing, Wallingford, UK, ISBN 0851995675, 115–143.

Hominick W.M., Reid A.P., Bohan D.A., Briscoe B.R. 1996. Entomopathogenic nematodes – biodiversity, geographical distribution and the convention on biological diversity. Biocontrol. Sci. Techn. 6, 317–331.

Hominick W.M., Reid A.P., Briscoe B.R. 1995. Prevalence and habitat specificity of steinernematid and heterorhabditid nematodes isolated during soil surveys of the UK and the Netherlands. J. Helminthol. 69, 27–32.

Karbowska-Dzięgielewska M. 2013. Ekologiczne uwarunkowania występowania nicieni owadobójczych (*Steinernematidae, Heterorhabditidae*) w wybranych ekosystemach północno-zachodniej Polski [Environmental determinants of the occurrence of entomopathogenic nematodes (*Steinernematidae*, *Heterorhabditidae*) in selected ecosystems of the north-western Poland]. Rozprawa habilitacyjna. Szczecin, Wydaw. Uczelniane Zachodniopomorskiego Uniwersytetu Technologicznego ISBN 978--83-7663-142-4. [in Polish].

Kaya H.K., Aguillera M.M., Alumai A., Choo H.Y., de la Torre M., Fodor A., Ganguly S., Hazâr S., Lakatos T., Pye A., Wilson M., Yamanaka S., Yang H., Ehlers R.U. 2006. Status of entomopathogenic nematodes and their symbiotic bacteria from selected countries or regions of the world. Biol. Control 38, 134–155.

Kuwata R., Shigematsu M., Yoshiga T., Yoshida M., Kondo E. 2006. Phylogenetic analyses of Japanese steinernematid nematodes and their associating *Xenorhabdus bacteria*. Nihon Senchu Gakkai Shi 36, 75–85.

Lee M.M., Stock S.P. 2010. A multilocus approach to assessing co-evolutionary relationships between *Steinernema* spp. (*Nematoda: Steinernematidae*) and their bacterial symbionts Xenorhabdus spp. (gamma-Proteobacteria: Enterobacteriaceae). Syst. Parasitol. 77(1), 1–12.

Lewis E.E., Campbell J.F., Griffin C., Kaya H.K., Peters A. 2006. Behavioral ecology of entomopathogenic nematodes. Biol. Control 38, 66–79.

Litvaitis M.K., Bates J.W., Hope W.D., Moens T. 2000. Inferring a classification of the *Adenophorea* (*Nematoda*) from nucleotide sequences of the D3 expansion segment (26/28S rDNA). Can. J. Zool. 78, 911–922. doi:10.1139/cjz-78-6-911.

Mráček Z., Bečvář S., Kindlmann P. 1999. Survey of entomopathogenic nematodes from the families (*Nematoda: Rhabditida*) in the Czech Republic. Folia Parasit 46, 145–148.

Mráček Z., Bečvář S. 2000. Insect aggregations and entomopathogenic nematode occurrence. Nematology 2(3), 297–301.

Mráček Z., Sturhan D., Reid A.P. 2003. *Steinernema weiseri* n.sp. (*Rhabditida, Steinernematidae*), a new entomopathogenic nematode from Europe. Syst. Parasitol. 56, 37–47.

Mráček Z., Bečvář S., Kindlmann P., Jersáková J. 2005. Habitat preference for entomopathogenic nematodes, their insect hosts and new faunistic records for the Czech Republic. Biol. Control 34, 27–37.

Nadler S.A., Bolotin E., Stock P. 2006. Phylogenetic relationships of *Steinernema Travassos*, 1927 (*Nematoda: Cephalobina: Steinernematidae*) based on nuclear, mitochondrial and morphological data. Syst. Parasitol. 63, 161–181.

Nasmith C.G., Speranzini D., Jeng R., Hubbes M. 1996. RFLP analysis of PCR amplified ITS and 26S ribosomal RNA genes of selected entomopathogenic nematodes (*Steinernematidae, Heterorhabditidae*) J. Nematol 28(1), 15–25.

Nguyen K.B. 2007. Methodology, morphology and identification. In: Nguyen KB, Hunt DJ (Eds). Entomopathogenic nematode: Systematics, Phylogeny and Bacterial symbionts. Nematology Monographs and Perspectives. Vol. 5. Brill Leiden-Boston, 59–119.

Půža V., Mráček Z. 2005. Seasonal dynamics of entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* as a response to abiotic factors and abundance of insect hosts. J. Invertebr. Pathol. 89, 116–122.

Shapiro-Ilan D.I., Gouge D.H., Piggott S.J., Patterson Fife J. 2006. Application technology and environmental considerations for the use of entomopathogenic nematodes in biological control. Biol. Control 38, 124–133.

Spiridonov S.E., Reid A.P., Podrucka K., Subbotin S.A., Moens M. 2004. Phylogenetic relationships within the genus *Steinernema* (*Nematoda: Rhabditida*) as inferred from analyses of sequences of ITS1-5.8S-ITS2 region of the rDNA and morphological features. Nematology 6(4), 547–566.

Stock S.P., Campbell J.F., Nadler S.A. 2001. Phylogeny of *Steinernema Travassos*, 1927 (*Cephalobina: Steinernematidae*) inferred from ribosomal DNA sequences and morphological characters. J. Parasitol. 87(4), 877–889.

Sturhan D. 1996. Studies on the natural occurrence and distribution of entomopathogenic nematodes. Russ J. Nematol 4, 98.

Sturhan D. 1999. Prevalence and habitat specificity of entomopathogenic nematodes in Germany. Proceeding of a workshop held at Todi, Perugia, Italy, 16 to 29 May, 1995. Luxembourg: European Commission, COST 819, 123–132.

Sturhan D., Lišková M. 1998. Studies on the natural occurrence of entomopathogenic nematodes in the Slovak Republic. Nematologica 44(5), 586.

Sturhan D., Mráček Z. 2001. Records of the *Steinernema glaseri* groups (*Nematoda: Rhabditida*) in Europe, with notes on the morphological characteristics of this species group. Abstract, SIP 34th Annual Meeting, Noordwijkerhout, The Netherlands, August 25–30, 2001, 52–53.

Sturhan D., Spiridonov S.E., Mráček Z. 2005. *Steinernema silvaticum* sp. N. (*Rhabditida: Steinernematidae*), a new entomopathocenic nematode from Europe. Nematology 7(2), 227–241.

Subbotin S.A., Sturhan D., Vovlas N., Castillo P., Tanyi Tambee J., Moens M., Baldwin J.G. 2007. Application of the secondary structure model of rRNA for phylogeny: D2–D3 expansion segments of the LSU gene of plant-parasitic nematodes from the family *Hoplolaimidae Filipjev*, 1934. Mol. Phylogenet. Evol. 443, 881–890.

Abstract. During faunistic studies conducted in north-western Poland on the entomopathogenic nematodes (EPN) of the families *Steinernematidae* and *Heterorhabditidae*, species *Steinernema silvaticum* was identified for the first time in Poland. Nematode species identification, using morphometric methods, is laborious, even for an experienced researcher. *S. silvaticum* and *S. kraussei* are defined as "sister taxa" and it is difficult to distinguish these two species. The study is an attempt to differentiate *S. silvaticum* from *S. kraussei* using sequencing results of the rDNA – ITS1 and LSU regions. Sequenced regions of ITS1 and LSU had lengths of 490bp and 918bp, respectively. A very high similarity between the *S. silvaticum* isolate and the isolate of *S. kraussei* was detected in the case of both sequences (98–99%). There were only two nucleotide differences in ITS1 and two in LSU region, which discriminate the two analysed species.