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Research Note

Identification and intraspecific variability of *Steinernema feltiae* (Filipjev, 1934) isolates from different localities in Poland

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Summary

Presented study is part of a project aimed at identifying entomopathogenic nematode (EPN) species and analysing their distribution in various habitats of Poland. Here, an attempt was undertaken to determine intraspecific variability of nematodes of the species *Steinernema feltiae* isolated from seven different localities in central and southern Poland. Molecular characteristic and phylogenetic analysis was performed based on nucleotide sequences in the ITS region.
Research on the occurrence of EPNs in Poland have been conducted since the 1990s but there is still no data verified genetically, as well as data on the intraspecific variability of isolates *Steinernema feltiae.* This paper reports initial results of intraspecific variability *Steinernema feltiae* in Poland.
Keywords: entomopathogenic nematodes; isolates of *Steinernema feltiae*; ITS

Introduction

Entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditidae are worldwide widespread soil organisms. EPNs are obligatory lethal parasites of many insect species associated with soils. Therefore, they are commonly used in the biological plant protection (some species are produces on industrial scale). According to Nguyen and Hunt (2007) there are 60 species of the genus *Steinernema* but only 16 of the genus *Heterorhabditis*. One of the most common species in the world is *S. feltiae* (Filipjev,1934), which prefers climate of temperate zones. *S. feltiae* can be found in various habitats: in deciduous forests, meadows, orchards, gardens and croplands (Hominick, 1996; Mráček *et al*., 2005; Adams *et al*,. 2006). In Polish studies *S. feltiae* is reported as most often isolated species (Bednarek, 1990; Dzięgielewska, 2012).
Identification of entomopathogenic nematode species is traditionally based on morphological features but nowadays molecular methods using genetic markers are a more useful tool. In particular, identification of closely related nematode species with traditional method remains difficult and in many instances only the analysis of DNA sequences from species in question can provide an accurate identification (Liu & Berry, 1995). Internal transcribed spacer regions of the ribosomal DNA (rDNA) tandem repeat unit (ITS1 – 5.8S –ITS2) are required to species identification and in phylogenetic studies (Nguyen *et al*., 2001; Stock *et al.,* 2001; Hominick, 2002; Spiridonow *et al*., 2004). The ITS region gene sequences has also been used to study among- and intra-population variability of *Steinernema feltiae* and other Steinernematidis (Nguyen *et al*., 2001; Yoshida, 2003; Kuwata *et al*., 2006; Desta *et al*., 2011).
Research on the occurrence of EPNs in Poland have been conducted since the 1990s but there is still lack of genetically verified data from Poland. To our knowledge this is a first study of intraspecific variability based on ITS rDNA region of *Steinernema feltiae* in Poland. Research aimed at determine the intraspecific variability of strains *Steinernema feltiae* will continue and in the future, combined with their virulence.

Table 1. Sites of *S. feltiae* isolates

Isolate	Site characteristic
S feltiae KAT 13	field (*Miscantus giganteus* crop) (Silesia Region)
S. feltiae ZAG 4	deciduous forest, the Zagożdżonka River valley (Kozienicka Forest)
S. feltiae ZAG 11	deciduous forest, the Zagożdżonka River valley (Kozienicka Forest)
S. feltiae ZWO 4	meadow, the Zwoleńka River valley (Kozienicka Forest)
S. feltiae ZWO 21	meadow, the Zwoleńka River valley (Kozienicka Forest)
S. feltiae ZWO 23	meadow, the Zwoleńka River valley (Kozienicka Forest)
S. feltiae SIEW 1	field (wheat crop) (Silesia)

In this study we amplified and sequenced the ITS region to confirm determination of taxonomic status of seven Steinernematid isolates from different locations in Poland.

The aim of study was to identify intraspecific variability of these isolates and phylogenetic relationships among some *Steinernema* species living in Europe.

Material and Methods

Soil samples for the study on the occurrence of EPNs in Poland were collected in the years 2010 – 2011. In total, 167 soil samples were taken from 111 localities in Poland along north–south transect.

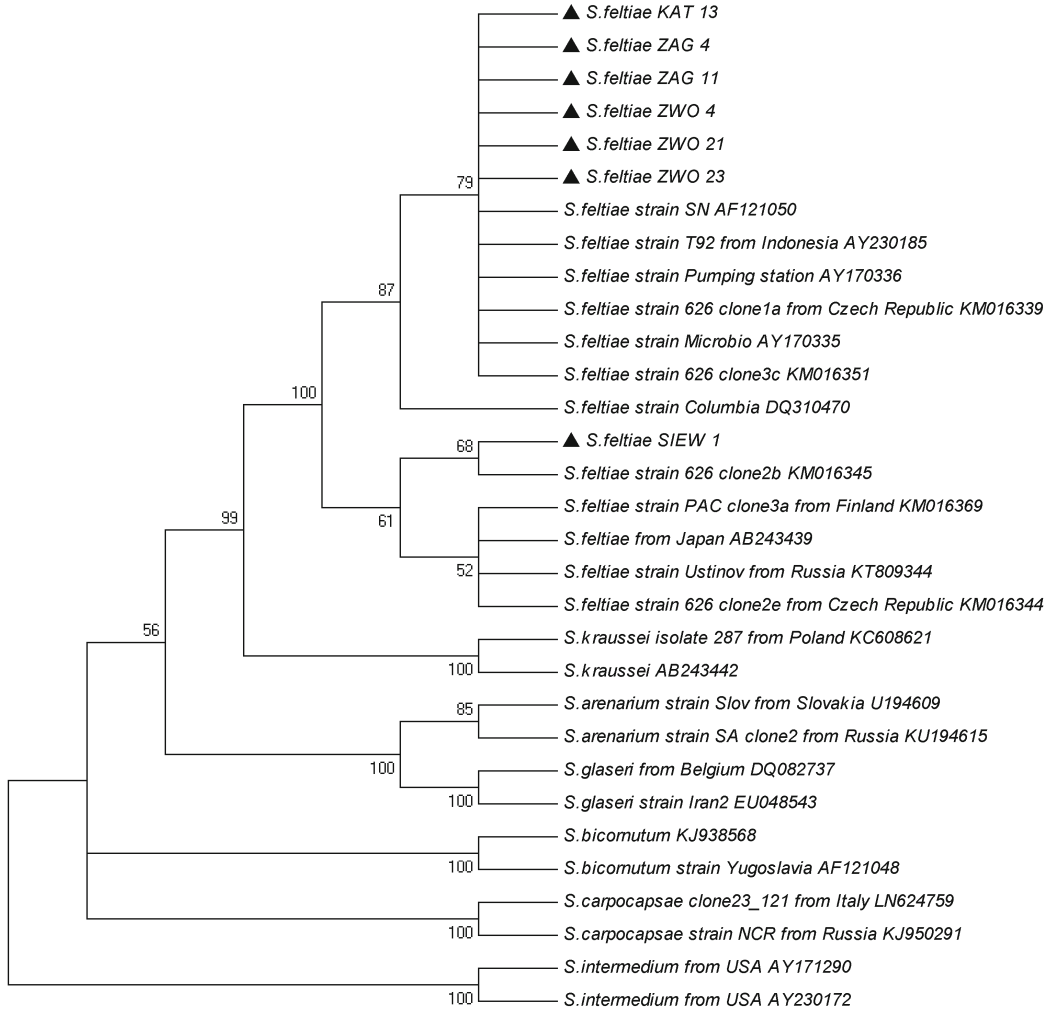

Fig. 1. Phylogenetic relationship among Steinernematid species based on the sequences of the ITS region determined by the NJ method. Numbers on branches more than 50% indicated the percentage of 10000 bootstrap replicates. Sequences obtained in this study are marked with black triangle

In the laboratory, EPNs were isolated with the trap method using live bait (larvae of *Galleria mellonella* L. Lepidoptera: Pyralidae) (Bedding & Akhurst, 1975). Each soil sample was mixed and divided among 5 containers of a volume of 250 cm^3. Five larvae of *G. mellonella* were placed in each container which was then placed in an incubator at 20 °C. The first control was performed after 5 days by replacing dead insects by alive ones. This procedure was repeated every two days until the twentieth day of experiment. White's traps (White, 1927) were used to collect invasive larvae (IJs) of nematodes. Traps were placed in an incubator at 25 °C. After two weeks, invasive larvae were obtained from dead insects, pipetted to bottles for tissue culture and placed in a water. Bottles were preserved in a refrigerator at 4 °C. IJs larvae obtained from White's traps were then used to infect the next larvae of *G. mellonella* to maintain the culture and to identify species and strains.
Nematodes were identified using morphological criteria (Poinar, 1990; Adams & Nguyen, 2002). Isolated nematodes classified as *S. feltiae* (Table 1) were analysed by molecular methods to confirm species affiliation and their intraspecific variability.
DNA of EPNs isolate was extracted from 100 – 1000 individuals of the invasive juveniles (IJS) by phenol-chloroform method and precipitated with ethanol as described by Tumialis *et al.* (2014).The ITS 1-5.8S – ITS 2 region of rDNA was amplified with PCR method using 18s and 26s primers as described by Vrain *et al*. (1992). All PCR reaction consisted of an initial denaturation step of 3 min. at 95 °C, followed by 35 cycles at 95 °C for 30 s, at 50 °C for 30s and at 72 °C for 60s with final extension step of 5 min at 72 °C. Amplification products were purified by ethanol precipitation and directly sequenced with the BidDye Terminator Cycle sequencing Ready Reaction Kit v 3.1 (Life Technologies).

Results and Discussion

Entomopathogenic nematodes were isolated from 53 out of 167 analysed soil samples. The presence of *S. feltiae* was recorded in 11 samples.
DNA was isolated from 7 out of 11 samples. The ITS region of the rDNA was successfully amplified by PCR reaction and sequenced. To indicate the taxonomic position of the nematode, the ITS sequences of studied isolates were compared with the existing data available in GenBank (www.ncbi.nlm.nih.gov). The BLASTN search in GenBank revealed that Polish isolates had a high similarity (99 – 100 %) with those sequences for *Steinernema feltiae*. Sequences of other *Steinernema* species exhibited a lower degree of similarity with the isolates obtained during this study. The ITS sequences of seven studied isolates were aligned using GenDoc with ITS sequences of different strains of *Steinernema feltiae* and six species of *Steinernema* occurring in Europe, which have been deposited in GenBank. The aligned sequence data were analyzed by the Neighbour-Joining method using MEGA (Tamura *et al.* 2011). Phylogenetic analyses of ITS rDNA sequences placed isolates from Poland (ZWO23, ZWO21, ZAG11, ZAG4, KAT13, SIEW1) in a clade with other isolates/strains of *Steinernema feltiae* (Fig 1).

Table 2. Pairwise similarity of studied isolates (samples)

Isolate	**Identities**						
	ZAG4	ZAG11	ZWO4	ZWO21	ZWO23	KAT13	SIEW1
ZAG4	-	980/980 (100 %)	980/980 (100 %)	980/980 (100 %)	980/980 (100 %)	980/980 (100 %)	904/919 (98 %)
ZAG11	0/980 (0 %)	-	980/980 (100 %)	980/980 (100 %)	980/980 (100 %)	980/980 (100 %)	904/919 (98 %)
ZWO4	0/980 (0 %)	0/980 (0 %)	-	980/980 (100 %)	980/980 (100 %)	980/980 (100 %)	904/919 (98 %)
ZWO21	0/980 (0 %)	0/980 (0 %)	0/980 (0 %)	-	980/980 (100 %)	980/980 (100 %)	904/919 (98 %)
ZWO23	0/980 (0 %)	0/980 (0 %)	0/980 (0 %)	0/980 (0 %)	-	980/980 (100 %)	904/919 (98 %)
KAT13	0/980 (0 %)	0/980 (0 %)	0/980 (0 %)	0/980 (0 %)	0/980 (0 %)	-	904/919 (98 %)
SIEW1	12/919 (1.3 %)	12/919 (1.3 %)	12/919 (1.3 %)	12/919 (1.3 %)	12/919 (1.3 %)	12/919 (1.3 %)	-
	Gaps						

Five Polish isolates (ZWO23, ZWO21, ZAG11, ZAG4) from Mazovian Region (Kozienicka Forest) and one isolate (KAT13) from Silesia Region clustered together with *S. feltiae* from Czech Republic (Rudolfov), Indonesia and other locations and showed sequence identities between 99 % and 100 %. Isolate SIEW1 from Silesia Region was closer to the isolate from Czech Republic (Rudolfov) (99 % identities) than any other from Poland. The analyzed sequences derived from different locations (Poland, Europe and other places in the world) did not show any relationship with regard to their geographical relationship. Similarity of studied isolates is shown in Table 2.

The intraspecific variability of ITS rDNA region in *S. feltiae* (11 isolates) reported by Spiridonov *et al*. (2004) was in the range of 0 – 2.4 %. The intraspecific variability of ITS sequences for European isolates obtained from Izhersk (Russia), United Kingdom, Merelbeke (Belgium), Czech Republic and San Bernardino (Switzerland) ranged from 0 – 1.6 % and reaching up to 2.4 % between the British (A2) and the Armenian isolates. Desta *et al.* (2011) studying the ITS rDNA region in four isolates (SCM, SNGD, SNC, Ssp60) of *S. feltiae* found lower intraspecific variability (0.2 – 0.9 %). In this study intraspecific variability of examined ITS region varied between 0 and 2 %, which was at similar level when compared with European isolates reportered by Spiridonov *et al*. (2004).

Obtained results confirm species affiliation of studied nematodes and show high similarity among the isolates.

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