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Habitat preference for entomopathogenic nematodes, their insect hosts and new faunistic records for the Czech Republic

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Abstract

Steinernematids and heterorhabditids are widespread soil nematodes. The dependence of their distribution on habitat type, soil texture, soil pH, and altitude has been studied in some detail, while much less is known about how their occurrence depends on the abundance and habitat preference of their insect hosts. Here we surveyed the entomopathogenic nematode fauna of the Czech Republic and evaluated the impact of ecosystem type, habitat, soil, season, altitude, and insect host species on their prevalence. We also examined the effect of temperature on their isolation rates in the laboratory. Nine species of the genus *Steinernema* (*S. kraussei*, *S. feltiae*, *S. affine*, *S. carpocapsae*, *S. intermedium*, *S. arenarium*, *S. bicornutum*, *S. weiseri*, and *S. silvaticum*) and two of the genus *Heterorhabditis* (*H. bacteriophora* and *H. megidis*) were recorded for the Czech Republic. Nematodes occurred in all ecosystems and habitats tested. They were more abundant in tree habitats and light soils and in sites with abundant suitable insect hosts; seasonality and altitude had no significant impact on their occurrence. At two laboratory temperatures (15 and 22 °C) different numbers of isolates were obtained from the *Galleria* bait traps. Abundance of entomopathogenic nematodes in soil samples varied considerably and there were at most five baiting replicates (in habitats with many suitable insect hosts).

Keywords: Entomopathogenic nematodes; Steinernematidae; Heterorhabditidae; *Steinernema kraussei*; *S. feltiae*; *S. affine*; *S. carpocapsae*; *S. intermedium*; *S. arenarium*; *S. bicornutum*; *S. weiseri*; *S. silvaticum*; *Heterorhabditis bacteriophora*; *H. megidis*

1. Introduction

Entomopathogenic nematodes (EPNs) from the families Heterorhabditidae and Steinernematidae are widespread, recorded from all continents excluding Antarctica (Hominick et al., 1996; Hominick, 2002). Some species are endemic to islands (e.g., *Steinernema cubanum* Mráček, Hernandez & Boemare, and *Steinernema puertoricensis* Roman & Figueroa), whereas some species seem to be ubiquitous [e.g., *Steinernema carpocapsae* (Weiser), *Steinernema feltiae* (Filipjev), *Heterorhabditis bacteriophora* Poinar)]. They are soil organisms, adapted to most climatic conditions in hot, temperate, and cold zones, distributed from lowlands to high alpine altitudes (Steiner, 1996). They parasitize insect developmental stages living in soil and some humid cryptic microhabitats, e.g., bark crevices or trunk tunnels.

The dependence of the distribution of these nematodes on factors like habitat type, soil texture, soil pH, and altitude has been studied in some detail (e.g., Hominick and Briscoe, 1990a,b; Yoshida et al., 1998). Individual species of EPNs differ in their habitat preference. For example, *S. feltiae* and *Steinernema affine* (Bovien) are more common in open habitats, such as fields and meadows (Sturhan and Lišková, 1999), whereas *Steinernema*

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kraussei is mostly found in woodlands (Mráček et al., 1999; Sturhan, 1995). Type of soil also seems to be important. EPNs are more prevalent in light (especially sandy or organic) soils (e.g., Miduturi et al., 1996). Decrease in soil pH may reduce the host-finding ability and prevalence of *S. kraussei* (Steiner) (Fisher and Führer, 1990). Steiner (1996) reported selection in the EPN pH preference, which was found relatively low for *S. kraussei*, avoiding pH extremes for *Steinernema intermedium* (Poinar), and *S. feltiae* and close to neutral for *S. affine*. Soil texture plays an important role in the EPNs' dispersal (Georgis and Poinar, 1983) and persistence (Kung et al., 1990). Surprisingly, much less is known about the impact of prevalence, abundance, and habitat preference of their insect hosts, which may significantly affect the occurrence of EPNs.

Mráček et al. (1999) published limited results on survey, but in this paper we posed different questions with a substantially extended dataset. We report further findings of a survey, which allows some questions to be asked regarding species occurrence in the Czech territory. For example, how do ecosystems and habitat preference (close-forested as opposed to open habitats), and additional questions how soil type (texture, organic content), season, and altitude affect their occurrence. In addition, what is the impact of insect hosts on EPN prevalence? Does the laboratory baiting method influence the success of nematode isolation? What is the approximate EPN abundance in soil samples?

2. Materials and methods

2.1. Field collection

During the years 1996–1998, we collected 587 soil samples (each at an approximate volume of 3 dm^3) from the territory of the Czech Republic. In each of these localities, one or more sampling sites (different habitats) were chosen at random or targeted if any high occurrence of insects or outbreaks was recorded. Each sampling site was characterized by the type of ecosystem, habitat, soil, latitude, longitude, and altitude. We selected habitats, in which only one potential insect host species was dominant, such as the web-spinning sawfly in spruce monocultures or group of target pests, such as a noctuid and geometrid moth complex in orchards. Thus, habitats with dense shrub undergrowth, which usually has a diverse and abundant insect fauna that may significantly influence the occurrence of nematodes, were eliminated from our sampling. The presence of EPNs in each sampling site was evaluated by baiting soil samples with wax moth larvae (Bedding and Akhurst, 1975; Mráček, 1980).

We sampled from March to December, but less frequently in winter. Each soil sample was obtained by using an iron core (3.6 cm diameter, 20 cm depth, approximate volume 200 cm^3) five times at each sampling site (to get a constant volume of soil) and transported to the laboratory in a plastic bag. The texture of soils was estimated by feel as the sand/silt and silt/clay with a low or high organic content http://ltp-www.gsfc.nasa.gov/globe/tbf/txtbyfel.htm.

2.2. Laboratory tests

For the laboratory tests each soil sample was mixed, divided into two subsamples, and a part of these set in the "*Galleria* trap" (Mráček, 1980) with 5 *Galleria* per trap, which were kept at 15 and 22 °C, respectively. The later instar of the greater wax moth, *Galleria mellonella* (L.), was used. To prevent the *Galleria* larvae from escaping, they were placed in a small steel mesh pocket situated in the center of a petri dish (15 cm diameter). Mortality of *G. mellonella* was assessed after 5 days. Dead *Galleria* larvae were divided into two batches; one was dissected to obtain adult nematodes of the second generation (more accurate identification) and the other one was cultured at a laboratory temperature on a water trap to obtain infective juveniles (IJs). Both adults and IJs were fixed in 4% formalin and stored for identification.

We assessed the abundance of nematodes in 27 randomly chosen positive sites by the number of adult nematodes recovered from the soil samples by baiting. In each "*Galleria* trap," all larvae (dead and alive) were replaced by a new batch of five living larvae. This was repeated as long as at least one *Galleria* larva died. The number of repeat baits, dead larvae, and adult nematodes in all cadavers was counted.

2.3. Statistical analysis

Statistical significance of differences between percentages of recovered nematodes was tested by means of the log-likelihood ratio for contingency tables (G test—Zar, 1984). The multivariate data were analyzed by means of the linear method of direct gradient analysis (RDA, Redundancy analysis) in the program Canoco v. 4.02. We used the presence of nematodes as dependent variable and the environmental data (type of ecosystem, habitat, and soil) as independent categorical variable. Data were scaled on inter-sample distances and centered by species. The differences in nematode species composition of various soil samples were tested by the Monte Carlo Permutation Test (MCPT). Based on the significant results we constructed ordination diagrams [program CanoDraw v. 4.0; ter Braak and Šmilauer (1998)], which expresses the similarities in species composition between soil samples. The gradient of the highest variability in the data coincides with the first ordination axis.

3. Results

3.1. Entomopathogenic nematode fauna

Out of 582 soil samples, 297 (50.6%) contained EPNs. Nine steinernematid and two heterorhabditid species were recorded from our field sampling. Our survey recovered the newly described species of *Steinernema weiseri* Mráček & Sturhan and *Steinernema silvaticum* Sturhan, Spiridonov & Mráček. *S. kraussei* and *S. feltiae* were the most frequently isolated species, representing 29 and 45% of the identified *Steinernema* isolates, respectively (Table 1). *S. affine*, *S. intermedium* (Poinar), and *S. silvaticum* occurred commonly (7–11% of identified species). Only a few samples were positive for *S. carpocapsae*, *Steinernema bicornutum* Tallosi, Peters & Ehlers, *Steinernema arenarium* (Artyukhovsky), and both heterorhabditids, *H. bacteriophora* and *Heterorhabditis megidis* Poinar, Jackson & Klein (<2% of isolates for all these species). There was only one isolate with *H. bacteriophora* (co-occurred with *S. affine* in a meadow) and *H. megidis* (co-occurred with *S. bicornutum* in a vineyard).

3.2. Distribution in ecosystems and habitats

EPNs were found in all terrestrial ecosystems, subecosystems, and in most habitats tested (Tables 1 and 2). However, they were more frequently recorded in closed and semi-open habitats, such as fruit tree hedgerows along roadsides, coniferous and deciduous forests, and perennials, than in open habitats, such as meadows either natural (flowering meadows, peat bogs, and steppe) or managed pastures (Table 1). We distinguished three groups of habitats with the EPNs. The first group, characterized by a low prevalence of entomopathogenic nematodes (10–32% samples positive), was made up of maple, beech, apple orchard (managed), walnut, cereals, and managed meadow. The second group, with an intermediate prevalence (36.4–50%), was made up of corn, acacia, willow, pine, steppe, spruce, hornbeam, ash, pear, rape, and vineyard. The third group, with high prevalence of EPNs (57–80% of samples positive), was made up of birch, plum, apple, hop-garden, flowering meadow, lime, brier-rose, poplar, alder, cherry, oak, and larch.

The differences between ecosystems with regard to the presence of different species of EPNs are shown in Fig. 1 (Test of significance of MCPT: $F=7.885$, $df=7$, $P=0.005$). Thus, *S. affine* and, to a lesser extent, *S. bicornutum* are associated mainly with open and semi-open habitats, *S. kraussei* is more closely associated with coniferous forests, whereas *S. feltiae* is more likely to occur with fruit trees and perennials. The other EPN species do not have a clear preference for certain ecosystems.

Similarly, *S. affine* prefers flowering and especially cultural meadows and *S. feltiae* prefers plum habitats and lime growths, whereas *S. kraussei* seems to occur in alder forests and steppes (Fig. 2) (Test of significance of MCPT: $F=3.295$, $df=26$, $P=0.005$), but this result is based on insufficient number of cases (only two samples). The other nematode species do not show a clear

Table 1
Occurrence of *Steinernema* species in the studied ecosystems

Ecosystem	Total number of samples	Number of positive samples	Percentage of positive samples	Number of identified samples to species	*S. kraussei*	*S. feltiae*	*S. affine*	*S. arenarium*	*S. carpocapsae*	*S. ntermedium*	*S. bicornutum*	*S. silvaticum*	*S. weiseri*
Agro													
Fields													
Perennials	25	14	56[b]	6	—	5	—	—	—	—	1	—	—
Annuals	54	14	26[a]	8	2	3	2	—	—	—	—	1	—
Apple orchards managed	16	3	19[a]	2	—	—	—	—	—	—	2	—	—
Meadows													
Natural	27	13	48[a]	6	1	2	2	—	—	—	—	1	—
Managed	41	13	32[a]	8	—	3	5	—	—	—	—	—	—
Treelforest													
Deciduous													
Fruit trees wild	146	88	60[b]	59 (8)[c]	9	39	6	—	1	2	3	1	6
Other	180	105	58[b]	58 (4)[c]	20	24	3	1	—	8	—	5	1
Coniferous	98	47	48[b]	22	17	—	—	1	—	1	—	3	—
Total	587	297		169 (12)[c]	49	76	18	2	1	11	6	11	7

[a,b] Different letters mean significant difference (G test, $p=0.05$).
[c] Number of samples with two *Steinernema* species.

Table 2
Presence of *Steinernema* species in different habitat types

Habitat	Total number of samples	Number of positive samples	Percentage of positive samples	Number of identified samples to species	*S. kraussei*	*S. feltiae*	*S. affine*	*S. arenarium*	*S. carpocapsae*	*S. intermedium*	*S. bicornutum*	*S. silvaticum*	*S. weiseri*
Pine	11	5	45	3	1	—	—	1	—	—	—	1	—
Spruce	82	38	46	17	15	—	—	—	—	1	—	1	—
Larch	5	4	80	2	1	—	—	—	—	—	—	1	—
Oak	55	42	76	27 (3)[a]	9	12	—	1	—	5	—	2	1
Lime	15	10	66	5	1	4	—	—	—	—	—	—	—
Poplar	27	19	70	6	1	4	1	—	—	—	—	—	—
Brier-rose	6	4	66	1	—	1	—	—	—	—	—	—	—
Horn-beam	4	2	50	0	—	—	—	—	—	—	—	—	—
Alder	14	10	71	6	2	2	—	—	—	1	—	1	—
Birch	14	8	57	6 (1)[a]	4	—	1	—	—	—	—	2	—
Ash	6	3	50	1	—	—	1	—	—	—	—	—	—
Acacia	5	2	40	2	1	1	—	—	—	—	—	—	—
Willow	5	2	40	1	1	—	—	—	—	—	—	—	—
Beech	12	2	16	2	—	—	—	—	—	2	—	—	—
Rowan	3	0	0	0	—	—	—	—	—	—	—	—	—
Chestnut	4	0	0	0	—	—	—	—	—	—	—	—	—
Maple	10	1	10	1	1	—	—	—	—	—	—	—	—
Cherry	22	16	72	8	1	6	1	—	—	—	—	—	—
Pear	14	7	50	6	—	5	—	—	—	—	—	—	1
Apple wild	81	49	60	34 (7)[a]	6	19	5	—	1	2	3	1	4
Plum	25	15	60	11 (1)[a]	2	9	—	—	—	—	—	—	1
Walnut	4	1	25	0	—	—	—	—	—	—	—	—	—
Corn	11	4	36	2	—	2	—	—	—	—	—	—	—
Rape	2	1	50	1	—	—	1	—	—	—	—	—	—
Cereals	36	9	25	5	2	1	1	—	—	—	—	1	—
Horse bean and peas	1	0	0	0	—	—	—	—	—	—	—	—	—
Lucerne	2	0	0	0	—	—	—	—	—	—	—	—	—
Potatoes	2	0	0	0									
Vineyard	6	3	50	3	—	2	—	—	—	—	1	—	—
Currant	1	0	0	0									
Hop-garden	18	11	61	3	—	3	—	—	—	—	—	—	—
Apple orchard	16	3	18	2	—	—	—	—	—	—	2	—	—
Flowering meadow	13	8	61	4	—	1	2	—	—	—	—	1	—
Steppe	11	5	45	2	1	1	—	—	—	—	—	—	—
Peat-bog	3	0	0	0									
Managed meadow	41	13	31	8	—	3	5	—	—	—	—	—	—
All habitats	587	297	50.6	169 (12)[a]	49	75	18	2	1	11	6	11	7

[a] Number of samples with two *Steinernema* species.

preference for certain ecosystems. Clusters of points representing individual types of habitats roughly correspond to the groups already indicated in Table 2.

3.3. Effect of soil type

Out of four basic soil types, nematodes were significantly most abundant in the light brown soil. Generally, nematodes occurred mainly in the light soils, such as sand/silt soil (61% of samples), rather than in the heavy soils either with low or high organic content, 49 and 32% nematode positive samples, respectively (Table 3). However, soil texture is probably more important for infective juvenile survival and movement than for the nematode prevalence. Fig. 3 clearly indicates that all species strongly prefer light, sand/silt soils, with the exception of *S. kraussei*, which tolerates both silt/clay and silt/clay organic soils (Test of significance of MCPT: $F=4.202$, $df=3$, $P=0.005$).

3.4. Effect of altitude

Nematodes were recovered from lowlands (below 300 m), hilly countryside (300–500 m), and highlands (500–800 m) up to the mountains (above 800 m) (Table 4). *S. kraussei* was most frequently found in the hilly countryside and highlands, *S. feltiae* was most frequently found in lowlands and hilly countryside, and *S. affine* was most frequently found in hilly countryside and highlands (Table 4). There was no difference among these sub-ecosystems. The highest altitude at which *S. kraussei* occurred was 1100 m in Šumava Mts., the locality where the web-spinning sawfly outbreak lasted for several years.

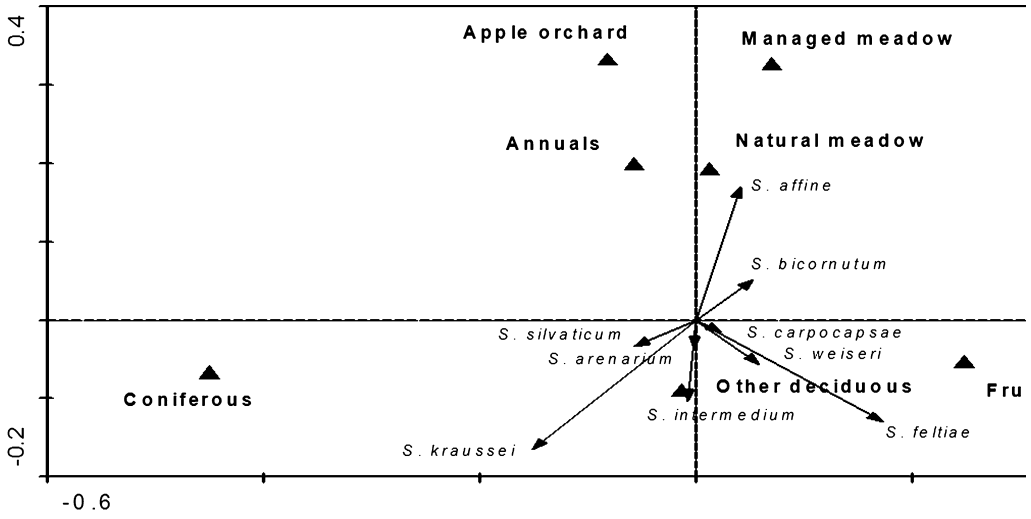

Fig. 1. Differences between ecosystems with regard to the presence of different species of entomopathogenic nematodes. Clusters of points represent ecosystems with similar nematode fauna. Arrows of similar direction mean the species exist in similar ecosystems. The prevalence of nematode species increases in the direction of arrow. The I and II ordination axes explain 5.9 and 8.0% of variability in data composition, respectively.

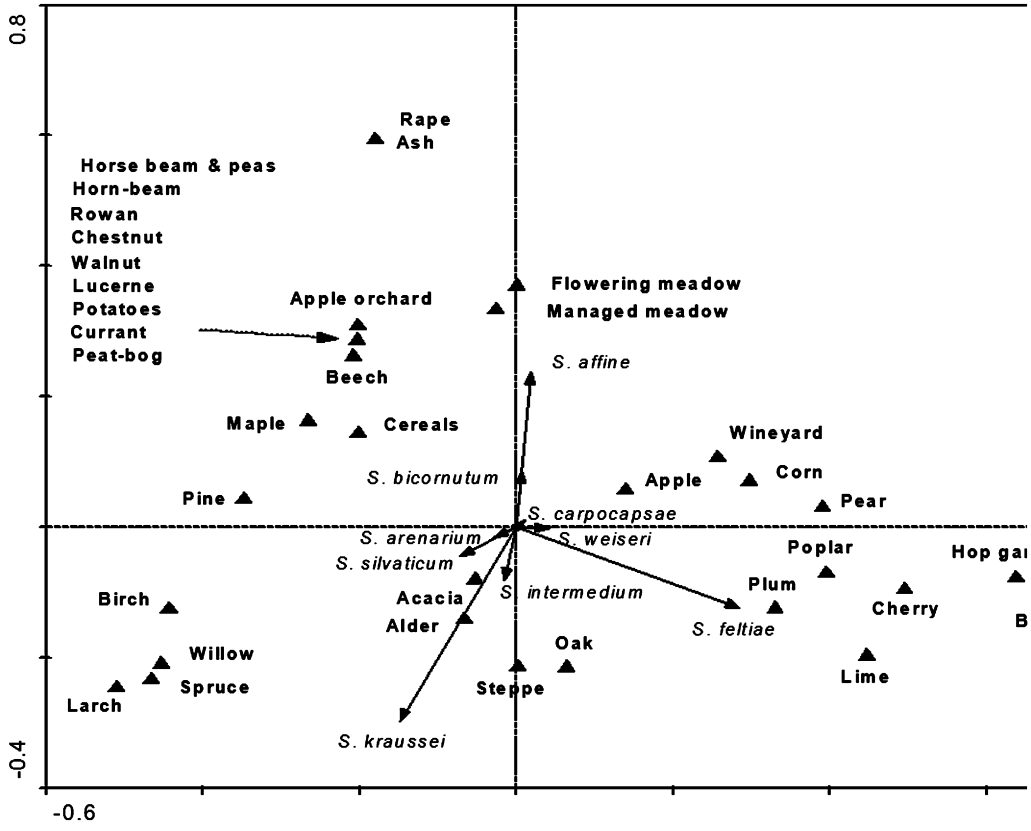

Fig. 2. Differences between habitats with regard to the presence of different species of entomopathogenic nematodes. Clusters of points contain habitats with similar nematode fauna. Arrows of similar direction mean the species exist in similar habitats. The prevalence of nematode species increases in the direction of arrow. The I and II ordination axes explain 10.2 and 14.5% of variability in data composition, respectively.

3.5. Effect of season

Nematodes were isolated from soil samples in all seasons (Table 5). The majority of soil samples were taken from April to September. However, in early spring in March and early winter in November and December (of eight soil samples four were with EPNs), nematodes were also present. There was no significant effect of season on nematode prevalence. No sampling was possible during the winter months (January–February) due to cold or frozen conditions.

Table 3
Occurrence of *Steinernema* species in different soil types

Soil type	Number of samples	Number of positive samples	Percentage of positive samples	Number of identified samples to species	*S. kraussei*	*S. feltiae*	*S. affine*	*S. arenarium*	*S. carpocapsae*	*S. intermedium*	*S. bicornutum*	*S. silvaticum*	*S. weiseri*
Sand/silt	288	176	61[b]	110 (11)[c]	31	51	11	2	1	7	4	9	5
Silt/clay	98	48	49[a]	22	11	7	1	—	—	1	—	2	—
Sand/silt organic	170	63	37[a]	30 (1)[c]	3	16	5	—	—	3	2	—	2
Silt/clay organic	31	10	32[a]	7	4	2	1	—	—	—	—	—	—
Total	587	297	50.6	169 (12)[c]	49	76	18	2	1	11	6	11	7

[a,b] Different letters mean significant difference (G test, $p = 0.05$).
[c] Number of samples with two *Steinernema* species.

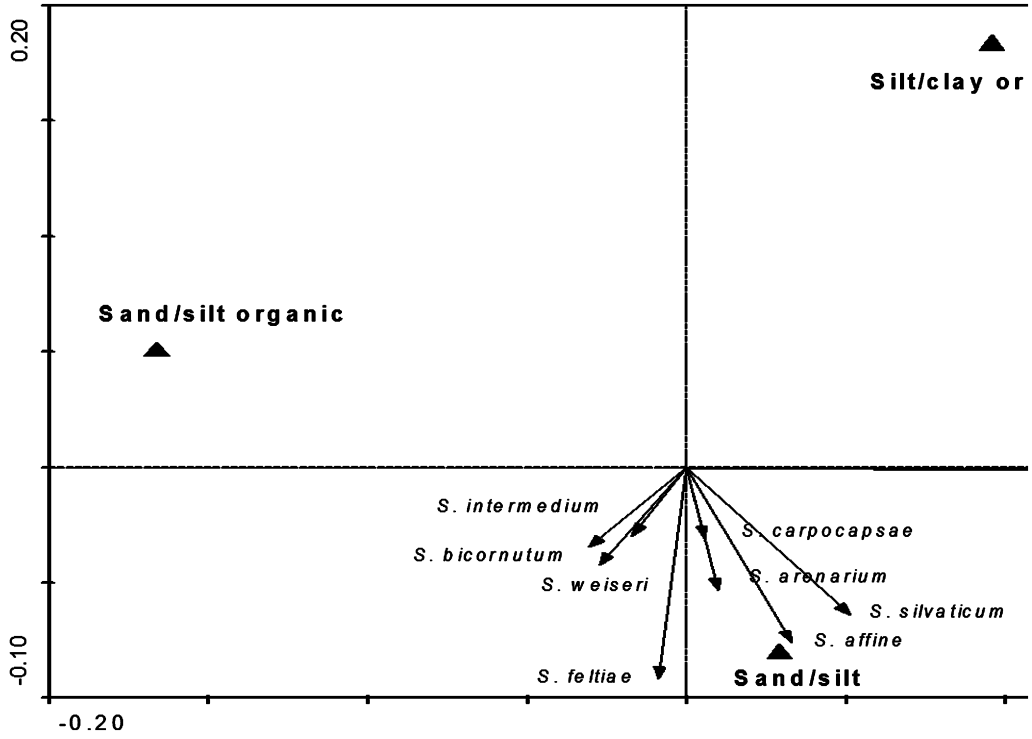

Fig. 3. Differences between soil types with regard to the presence of different species of entomopathogenic nematodes. Clusters of points represent soil types with similar nematode fauna. Arrows of similar direction mean the species exist in similar soil types. The prevalence of nematode species increases in the direction of arrow. The I and II ordination axes explain 1.4 and 2.1% of variability in data composition, respectively.

3.6. Effect of insect host

Table 6 shows the effect of insect host on the occurrence of nematodes. The percentages of samples positive were: 100% of samples where the fly families Bibionidae and Sciaridae were present (deciduous tree habitats), 69% of samples with *Cephaleia abietis* (L.) (Pamphilidae) outbreaks (spruce monocultures), 76% with lepidopteran hosts (deciduous tree habitats), and 58% of samples where *Otiorhynchus sulcatus* (Fabr.) (Curculionidae) was present (coniferous forests). *S. kraussei* appeared mainly in samples with *C. abietis*, whereas *S. feltiae* mainly where lepidopteran hosts and *O. sulcatus* were present (Table 6).

3.7. Importance of two baiting temperatures

Two baiting temperatures gave different results in nematode isolation from the same soil sample. In 50.3% cases, nematodes were isolated simultaneously at both experimental temperatures. In 30.8% of the soil samples, nematodes were obtained only when reared at 15 °C, and in 18.9% of the soil samples, nematodes were obtained only when reared at 22 °C. Thus our results confirm the importance of using several different temperatures in laboratory evaluation using *Galleria* baits. If only one temperature was used, 30.8 or 18.9% of nematode isolates would have been determined as containing no

Table 4
Presence of *Steinernema* spp. at different altitudes

Altitude	Below 300 m	300–500 m	500–800 m	Above 800 m
Total number of samples	150	267	120	50
Number of positive samples	64	151	61	21
% of positive samples	43	57	51	42
Number of identified samples to species	40 (1)[a]	82 (7)[a]	35 (4)[a]	12
S. kraussei	6	19	19	5
S. feltiae	23	41	10	2
S. affine	1	10	6	1
S. arenarium	1	1	—	—
S. carpocapsae	1	—	—	—
S. intermedium	2	5	1	3
S. bicornutum	3	3	—	—
S. silvaticum	2	6	2	1
S. weiseri	2	4	1	—

[a] Number of samples with two *Steinernema* species.

Table 5
Occurrence of *Steinernema* species at different times of the year

Season (months)	March–April	May–June	July–August	September–October	November–December
Total number of samples	77	153	157	145	55
Number of positive samples	39	70	82	73	33
% of positive samples	51	46	52	50	60
Number of identified samples to species	23 (1)[a]	45 (8)[a]	41 (3)[a]	41	19
S. kraussei	4	16	9	16	4
S. feltiae	13	17	26	9	11
S. affine	1	11	3	3	—
S. arenarium	—	—	1	1	—
S. carpocapsae	—	—	—	—	1
S. intermedium	3	1	2	3	2
S. bicornutum	2	—	—	4	—
S. silvaticum	—	4	2	5	—
S. weiseri	1	4	1	—	1

[a] Number of samples with two *Steinernema* species.

Table 6
Occurrence of *Steinernema* species in samples taken from sites at which different insects were abundant

Insect host	Bibionidae and Sciaridae	*C. abietis*	Lepidoptera	*O. sulcatus*
Total number of samples	7	26	84	19
Number of positive samples	7	18	64	11
% of positive samples	100	69	76	58
Number of identified samples to species	3	9	46 (6)[a]	3
S. kraussei	1	8	11	—
S. feltiae	—	—	27	3
S. affine	—	—	5	—
S. intermedium	1	1	4	—
S. bicornutum	—	—	2	—
S. weiseri	1	—	2	—

[a] Number of samples with two *Steinernema* species.

nematodes (Table 7). *S. kraussei* was more frequently isolated at temperature of 15 °C and *S. feltiae* at 22 °C. There were insufficient data for other EPN species.

3.8. Abundance of infective juveniles in habitat

Baiting was most frequently replicated only once. The largest observed number of baiting replicates was five for habitats with the prevalence of suitable insect host and three for the habitats without observation of the suitable hosts. Its average was higher for the former habitats (Table 8). The average number of dead *Galleria* larvae per one trap and the average number of recovered nematodes per one infected *Galleria* larva were almost double in habitats with the prevalence of suitable insects than in habitats without observation of the suitable

Table 7
Impact of two baiting temperatures on success of isolation of *Steinernema* species from 355 soil samples in the laboratory

Isolation	Only 15 °C	Only 22 °C	15 °C and 22 °C	Total
Number of positive samples	44	30	75	149
% of positive samples	12	8	21	42
Number of identified samples to species	12 (1)[a]	16	50 (2)[a]	78 (3)[a]
S. kraussei	6	2	26	34
S. feltiae	5	7	16	28
S. affine	1	3	3	7
S. arenarium	—	—	1	1
S. intermedium	—	2	4	6
S. silvaticum	1	1	1	3
S. weiseri	—	1	1	2

Note. Tested samples did not contain *S. carpocapsae* and *S. bicornutum*.
[a] Number of samples with two *Steinernema* species.

Table 8
Abundance of entomopathogenic nematodes in 27 randomly selected soil samples

	Soil samples from habitats with abundance of suitable insect hosts	Soil samples from habitats without observation of suitable insect hosts
Number of soil samples	11	16
Average number of baiting replicates in one soil sample	2.9	1.9
Average number of infected *Galleria* larvae in one soil sample	9.5	5.3
Total number of infected *Galleria* larvae	105	85
Percentage of infected *Galleria* larvae	65.6	54.8
Total number of recovered nematodes	662	320
Average number of recovered nematodes per one infected *Galleria* larva	6.3	3.8

insects. The absolute numbers of dead *Galleria* larvae per one trap varied from 1 to 17 in habitats with the prevalence of suitable insects and from 1 to 12 in habitats without the prevalence of suitable insect. The absolute numbers of recovered nematodes per one infected *Galleria* larva varied from 1 to 149 in habitats with the prevalence of suitable insects and from 1 to 116 in habitats without the prevalence of suitable insects.

4. Discussion

Even though a lot of national surveys gave valuable data on the world EPN distribution, the species habitat preferences remain inadequately known. However, the enhanced knowledge of relationships among EPNs, their habitats, and insect species should give the higher potential for the efficient biological control of pests. A main shortcoming of the majority of surveys is poor identification of species which is mostly confined to the well-known species *S. kraussei*, *S. feltiae*, and *H. bacteriophora*. Other isolates are referred to as *Steinernema* sp. and *Heterorhabditis* sp. or by letters (e.g., Akhurst and Bedding, 1986; Liu and Berry, 1995), which do not contribute much to the knowledge of EPN distribution. Some of these isolates were recently described as new species and their designation by letters was justified (Mráček et al., 2003; Sturhan and Mráček, 2000). In a minority of surveys, the EPN fauna is completely identified. For example, Stock et al. (1999) identified five steinernematids and two heterorhabditids from a Californian survey. The recovery of *Steinernema longicaudum* (Shen & Wang) was the first report from North America. Many surveys published before 2000 had poor identification to species and so surveys such as ours with comprehensive identifications will contribute to the improvement of EPNs zoogeography.

In the past, the EPN fauna of the Czech Republic contained six described species (Mráček et al., 1999; Mráček and Bečvář, 2000). Recently, the nematode of the "*glaseri*" group was identified as *S. arenarium*, two unknown species were described as *S. weiseri* (Mráček et al., 2003) and *S. silvaticum* (Sturhan et al., in press), and *H. bacteriophora* was found at low prevalence. The number of species found is comparable with Germany, where 11 *Steinernema* and two *Heterorhabditis* species were reported by Sturhan (1995), and in the UK where eight *Steinernema* and two *Heterorhabditis* species were reported by Hominick et al. (1995). The Czech EPN fauna is richer than the three *Steinernema* and one *Heterorhabditis* species found in West-Flanders, Belgium (Miduturi et al., 1996) and the six and one species, respectively, reported by Steiner (1996) from Switzerland.

The choice of sampling sites probably contributed to the relatively high percentage of nematode positive soil samples. Many of the soil samples were taken at random, but some sampling sites were focused on sites, where a higher presence of suitable hosts was expected. It is likely that such focusing resulted in our survey having a higher

number of positive samples (50.6%) than that reported in the majority of recent surveys, e.g., 2.2% for Scotland (Boag et al., 1992), 5% for Italian soils (Ehlers et al., 1991), 10% for Japan (Yoshida et al., 1998), 10.5% for the Republic of Ireland (Griffin et al., 1991), 12.3% in West-Flanders, Belgium (Miduturi et al., 1996), 26.3% for California natural habitats (Stock et al., 1999), and 27% in Swiss Alps (Steiner, 1996). Only a few national surveys have reported prevalences close to 50% including Great Britain with 48.6% (Hominick and Briscoe, 1990a), and Czech Republic with 53.8% (Mráček et al., 1999). The higher percentage of the nematode positive soil samples in our studies was because we used two baiting temperatures, instead of only one, as in other studies.

The Czech Republic seems to be the northwestern border for the distribution of the Pannonian and Mediterranean species *S. arenarium*, which was described from Ukraine (Artyukhovski, 1967) and recently was recorded at low prevalence in Slovakia (Sturhan and Lišková, 1999), Bulgaria (Shishiniova et al., 1997), Switzerland (Kramer et al., 2001), and Spain (Garcia del Pino and Palomo, 1996). This species seems to be the European vicariant of *S. glaseri* described from and distributed widely in the USA. Two species (*S. feltiae* and *S. carpocapsae*) are considered to have a global distribution (Hominick et al., 1996; Hominick, 2002); the former is frequently found in a variety of habitats, whereas the latter is very rare or the method of *Galleria* baiting underestimates its field frequency due to its ambush behavior. These findings correspond with our results in which *S. feltiae* occurred commonly, whereas *S. carpocapsae* was found only in two soil samples.

Several species show a distinct habitat preference. Open and semi-open habitats, such as arable, meadows, verges, and roadsides, are suitable for *S. affine* and *S. feltiae* that was found in our survey. Hominick et al. (1995), Sturhan (1995), and Sturhan and Lišková (1999) reported similar frequency. However, *S. feltiae* occurred in deciduous tree habitats and was never recovered from coniferous tree habitats. *S. intermedium* and *S. kraussei* dominate in woodlands with the exception of alpine altitudes (2000–2500 m) where *S. kraussei* occurred frequently in meadows (Steiner, 1996). *S. silvaticum* (former *Steinernema* sp. B) prevailed in forest habitats in Germany (Sturhan, 1995) and the Czech Republic, as reported here, it is a new location for this species with no data from other countries. The recently described species *S. weiseri* may prefer semi-open habitats (Sturhan, 1995) that is supported by our findings from fruit tree habitats (roadside verges, hedgerows).

In the Czech Republic, deciduous trees and shrubs have many defoliators from the orders Lepidoptera, Hymenoptera, and Coleoptera, which pupate in the soil. Similarly, many fly larvae (Bibionidae, Sciaridae, and Tipulidae) feed on organic matter and roots in the soil. All these insects create an ideal environment for EPNs by their presence (Mráček and Sturhan, 2000). The nematodes occurred frequently (from 57 to 80%) in the oak, poplar, birch, lime, and alder habitats, which are especially rich in insects, whereas in maple, beech, and walnut habitats, which have a low abundance of insects, the occurrence of EPNs was <25%. Thus, the EPNs occurred in both forested and open habitats, but they were more frequent in habitats rich in insects, such as tree (especially deciduous tree) habitats. The habitats from which the highest numbers of EPNs were recovered were apple and cherry verges along roadsides, lime and poplar hedgerows, and oak forests. The nematodes were recorded in 67% of habitats with high or moderate insect abundance and in only 15% of habitats with few insect numbers (Mráček et al., 1999). Accordingly, the abundance of insect hosts seems to be crucial for the EPN occurrence and distribution. This is true, however, only for certain insect groups which can be considered as suitable hosts. There were some links among insect hosts, habitats, and EPNs. Thus, older spruce forests with the web-spinning sawfly had *S. kraussei* and *S. feltiae* which were prevalent in different habitats with the high numbers of lepidopteran species. Outbreaks and aggregations of other "unsuitable" insect hosts, such as Psocoptera, Elateridae, Staphylinidae, and Formicidae, did not positively influence nematode occurrence (Půža and Mráček, in press).

Baiting temperature may influence the efficacy of the *Galleria* bait method. Recently published EPN national surveys were mostly based on only one baiting, usually laboratory, temperature (for example, Yoshida et al., 1998). Mráček and Bečvář, 2000) exposed the *Galleria* baits in soil samples at two temperatures of 15 and 22 °C. They inferred that if only 15 or 22 °C had been used, 7 and 23%, respectively, of EPN isolates would have not been found. Bioassays conducted by Hominick and Briscoe (1990b) did not show a not statistically significant difference between baiting temperatures of 15 and 20 °C. However, 24 nematode positive samples (108 total) of 945 bioassays were higher at 15 °C when compared with bioassys conducted at 20 °C. In our laboratory baiting that confirm our recent results (Mráček and Bečvář, 2000), the difference between one and two baiting temperatures was statistically significant. This may be explained by the origin of isolated nematodes, which came either from cooler or warmer locations of temperate zone of the Czech Republic. EPN species differ in their thermal optima (Grewal et al., 1994; Mráček et al., 1997). Therefore assays at least at two baiting temperatures are recommended.

Abundance of EPNs in a soil sample can be assessed by the number of killed *G. mellonella* larvae and by the number of adult nematodes recovered from *Galleria* cadavers (Mráček, 1982; Bednarek, 1986). In our study, soil samples with highest average number of baiting replicates, average number of infected *Galleria* larvae, and

number of recovered nematodes per infected *Galleria* larva originated from sites with a high numbers of suitable insects. Such trait of EPN/insect relationship mentioned in Mráček and Bečvář (2000) and supported by our results has not been studied satisfactorily.

The altitude did not influence EPN occurrence, and we found *S. kraussei* in more spruce forests above 1000 m altitude. This confirms that EPNs, especially *S. kraussei*, occur at high altitudes as shown previously: *S. kraussei* was located at 2530 m in the Swiss Alps and the majority of isolates were recovered at altitudes between 1500 and 2100 m (Steiner, 1996). In Bulgaria *S. kraussei* was recovered from subalpine meadows at 1290 m in Vitosha Mts and from mountain forests at altitudes ranging from 700 to 1300 m (Shishiniova et al., 1997).

Our survey of EPNs in the Czech Republic contributes and extends information concerning these nematodes in relation to some abiotic and biotic factors. However, it is still a future goal to have reliable knowledge on habitat preference and host specifcity of EPNs for biological control programs.

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References

Artyukhovski, A.K., 1967. *Neoaplectana arenaria* sp.n. (Steinernematidae)—vozbuditel nematodoza majskogo zhuka v Voronezhskoi oblasti. Trudy Voronezh. Gos. Zap. 15, 94–100.

Akhurst, R.J., Bedding, R.A., 1986. Natural occurrence of insect pathogenic nematodes (Steinernematidae and Heterorhabditidae) in soil in Australia. J. Austr. Entomol. Soc. 25, 241–244.

Bedding, R.A., Akhurst, R.J., 1975. A simple technique for the detection of insect parasitic rhabditid nematodes in soil. Nematologica 21, 109–110.

Bednarek, A., 1986. Estimation of the occurrence in soil of infective juveniles of entomogenous nematodes (Rhabditida: Steinernematidae, Heterorhabditidae). Rev. Nematol. 9, 414–416.

Boag, B., Neilson, R., Gordon, S.C., 1992. Distribution and prevalence of the entomopathogenic nematode *Steinernema feltiae* in Scotland. Ann. Appl. Biol. 121, 355–360.

Ehlers, R.-U., Deseö, K.V., Stackebrandt, E., 1991. Identification of *Steinernema* spp. (Nematoda) and their symbiotic bacteria *Xenorhabdus* spp. from Italian and German soils. Nematologica 37, 360–364.

Fisher, P., Führer, E., 1990. Effect of soil acidity on the entomophilic nematode *Steinernema kraussei* Steiner. Biol. Fertil. Soils 9, 174–177.

Garcia del Pino, F., Palomo, A., 1996. Natural occurrence of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Spanish soil. J. Invertebr. Pathol. 68, 84–90.

Georgis, R., Poinar Jr., G.O., 1983. Effect of soil texture on the distribution of *N. carpocapsae* (Nematoda: Steinerenematidae). J. Nematol. 15, 308–311.

Grewal, P.S., Selvan, S., Gaugler, R., 1994. Thermal adaptation nematodes: niche breadth for infection, establishment, and reproduction. J. Therm. Biol. 19, 245–253.

Griffin, C.T., Moore, J.F., Downes, M.J., 1991. Occurrence of insect-parasitic nematodes (Steinernematidae, Heterorhabditidae) in the Republic of Ireland. Nematologica 37, 92–100.

Hominick, W.M., 2002. Biogeography. In: Gaugler, R. (Ed.), Entomopathogenic Nematology. CABI Publishing, Wallingford, UK, pp. 115–143.

Hominick, W.M., Briscoe, B.R., 1990a. Occurrence of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in British soil. Parasitology 100, 295–302.

Hominick, W.M., Briscoe, B.R., 1990b. Survey of 15 sites over 28 months for entomopathogenic nematodes (Rhabditida: Steinernematidae). Parasitology 100, 289–294.

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

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. Technol. 6, 317–331.

Kramer, I., Hirschy, O., Grunder, J.M., 2001. Survey of baited insect parasitic nematodes from the Swiss lowland. In: Griffin, C.T., Burnell, A.M., Downes, M.J., Mulder, R. (Eds.), COST 819: Developments in Entomopathogenic Nematode/bacterial Research. European Commission, Luxembourg, pp. 178–182.

Kung, S.P., Gaugler, R., Kaya, H.K., 1990. Soil type and entomopathogenic nematodes persistence. J. Invertebr. Pathol. 55, 401–406.

Liu, J., Berry, R.E., 1995. Natural distribution of entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae) in Oregon soils. Environ. Entomol. 24, 159–163.

Midduturi, J.S., Moens, M., Hominick, W.M., Briscoe, B.R., Reid, A.P., 1996. Naturally occurring entomopathogenic nematodes in the province of West-Flanders, Belgium. J. Helminthol. 70, 319–327.

Mráček, Z., 1980. The use of *Galleria* traps for obtaining nematodes parasite of insects in Czechoslovakia (Lepidoptera:Nematoda, Steinernematidae). Acta Entomol. Bohemoslov. 77, 378–382.

Mráček, Z., 1982. Estimate of the number of infective larvae of *Neoaplectana carpocapsae* (Nematoda: Steinernematidae) in soil samples. Nematologica 28, 303–306.

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

Mráček, Z., Sturhan, D., 2000. Epizootic of the entomopathogenic nematode, *Steinernema intermedium* (Poinar) in a nest of the bibionid fly, *Bibio marci* (L). J. Invertebr. Pathol. 76, 149–150.

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

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

Mráček, Z., Bečvář, S., Iezáč, P., Kindlmann, P., Webster, J.M., 1997. Canadian steinernematid (Nematoda isolates and their cold activity to greater wax moth (*Galleria mellonella*) larvae). Biol. Control 8, 160–164.

Půža, V., Mráček, Z., in press. 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.

Shishiniova, M., Budurova, I., Gradinarov, D., 1997. Contribution to fauna of entomopathogenic nematodes (Rhabditida: Steinernemat-

idae, Heterorhabditidae) from Bulgaria. Biotechnol. Biotechnol. Eq. 11, 45–52.
Steiner, W.A., 1996. Distribution of entomopathogenic nematodes in the Swiss Alps. Rev. Suisse Zool. 103, 439–452.
Stock, S.P., Pryor, B.M., Kaya, H.K., 1999. Distribution of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) in natural habitats in California, USA. Biodivers. Conserv. 8, 535–549.
Sturhan, D., 1995. Prevalence and habitat specificity of entomopathogenic nematodes in Germany. Proc. Workshop held at Todi, Perugia, Italy, 16 to 29 May, 1995. European Commission, COST 819, Luxembourg, pp. 123–132.
Sturhan, D., Lišková, M., 1999. Occurrence and distribution of entomopathogenic nematodes in the Slovak Republic. Nematology 1, 273–277.
Sturhan, D., Mráček, Z., 2000. Comparison of the *Galleria* baiting technique and a direct extraction method for recovering Steinernema infective-stage juveniles from soil. Folia Parasitol. 47, 315–318.
Sturhan, D., Spiridonov, S., Mráček, Z., in press. *Steinernema silvaticum* sp.n. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from Europe. Nematology.
ter Braak C.J.F., Šmilauer, P., 1998. CANOCO reference manual and users guide to Canoco for Windows: software for canonical community ordination (version 4), Microcomputer Power, Ithaca, New York.
Yoshida, M., Reid, A., Briscoe, B.R., Hominick, W.M., 1998. Survey of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Japan. Fundam. Appl. Nematol. 21, 185–198.
Zar, J.H., 1984. Biostatistical Analysis. Prentice Hall Press, Englewood Cliffs, NJ.