

DISTRIBUTION OF ENTOMOPATHOGENIC NEMATODES OF THE GENUS *HETERORHABDITIS* (RHABDITIDA: HETERORHABDITIDAE) IN BULGARIA

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Summary. The results from studies on entomopathogenic nematodes of the genus *Heterorhabditis* Poinar, 1976 (Rhabditida: Heterorhabditidae) in Bulgaria, conducted during 1994-2010 are summarized. Of the 1,227 soil samples collected, 3.5% were positive for the presence of *Heterorhabditis* spp. Specimens belonging to the genus were obtained from 43 soil samples collected at 27 localities in different regions of the country. Heterorhabditids were established at altitudes from 0 to 1175 m, in habitats both along the Black Sea coast and inland. The prevalent species was *H. bacteriophora* Poinar, 1976. Its identity was confirmed by detailed morphometric studies and molecular analyses of four recently obtained isolates. Inland, *H. bacteriophora* prefers alluvial soils in river valleys under herbaceous and woody vegetation. It was also found in calcareous soils with pronounced fluctuations in the temperature and water conditions. The presence of the species *H. megidis* Poinar, Jackson *et* Klein, 1987 in Bulgaria needs further confirmation.

Key words: *Heterorhabditis bacteriophora*, morphology, molecular identification, habitat preferences.

Entomopathogenic nematodes (EPNs) (Rhabditida: Steinernematidae, Heterorhabditidae) are obligate parasites of a wide range of soil insects. They are subject to intensive faunistic research worldwide as prospective agents for biological pest control (Hominick, 2002). The presence of three species of the genus *Heterorhabditis* Poinar, 1976 has been reported and confirmed in Europe: *H. bacteriophora* Poinar, 1976, *H. megidis* Poinar, Jackson *et* Klein, 1987 and *H. downesi* Stock, Griffin *et* Burnell, 2002 (Smits *et al.*, 1991; Stock *et al.*, 2002). In Bulgaria, faunistic studies on entomopathogenic nematodes started in 1994 and until now *H. bacteriophora* and seven species of *Steinernema* Travassos, 1927 have been reported (Shishiniova *et al.*, 2000; Gradinarov *et al.*, 2011). Greatest attention has been paid to species of the genus *Steinernema* and important information on the localities in which heterorhabditids are established has not yet been published.

The aim of the present study was to *i*) systematize the data on the distribution of *Heterorhabditis* in the country, *ii*) perform morphological and molecular identification of the available live cultures of *Heterorhabditis* from Bulgaria, and *iii*) analyze the inland habitat preferences of the genus *Heterorhabditis* in Bulgaria.

MATERIALS AND METHODS

The present paper summarizes the results from the

processing of 1,227 soil samples collected during the period November, 1994 to October, 2010 from different regions in Bulgaria. The samples were collected at altitudes ranging from 0 to 2690 m, in diverse habitats: from coastal to alpine, with herbaceous or woody vegetation (coniferous and deciduous forests) and agroecosystems (Table I). Most of the samples were composite, each consisting of four sub-samples from a 6 m × 6 m area that were mixed before processing. The nematodes were isolated from the soil using the 'nematode-bait' method (Bedding and Akhurst, 1975) with larvae of the greater wax moth *Galleria mellonella* L. (Lepidoptera: Pyralidae).

Both morphological and molecular identification was performed on four *Heterorhabditis* isolates from different localities in SW Bulgaria (cultures BGKB-1/2007, BGZG-24B/2009, BGSP-2B/2010 and BGSP-5/2010, listed in Table II). The identification of previously collected isolates of the genus was based only on morphological and morphometrical characters, using microscope slides from the collection of the Department of Zoology and Anthropology, Sofia University.

The morphological identification was performed on the basis of characters of third stage infective juveniles and male individuals (Poinar, 1976; Poinar and Georgis, 1990; Nguyen and Smart, 1995; Stock *et al.*, 2002). Males were obtained by dissection of parasitized *G. mellonella* larvae in 0.9% NaCl solution on the ninth day after invasion. The nematodes were fixed in 4% formaldehyde, transferred to glycerol (simple evaporation method, after Poinar, 1975), mounted within paraffin rings on microscope slides and measured at magnifications of 12×40 and 12×100 under an Olympus BX41

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microscope. The characters and ratios used for morphological identification were: body length (L), greatest body width (W), distance from anterior end to nerve ring (NR), distance from anterior end to excretory pore (EP), oesophagus length (OES), body width at oesophagus base (Woes), reflection of testis from anterior end (TR), tail length (T), body width at cloaca (Wcl), spicule length (SP), gubernaculum length (GB), body width at anus (ABW), ratios a (L/W), b (L/ES), c (L/T), d (EP/OES), e (EP/T), and GS (GB/SP). The microscope slides prepared from the four cultures are deposited in the collection of the Department of Zoology and Anthropology, Sofia University (slides BGKB-1/1-16, BGZG-24B/1-18, BGSP-2B/1-16 and BGSP-5/1-20).

Molecular identification of the four isolates was performed at the Division of Plant Protection, Institute of

Soil Science, Agrotechnologies and Plant Protection, in order to confirm the results of the morphological identification. For DNA isolation, water suspensions containing 800 live infective juveniles (IJs) per ml, of each isolates reared on *G. melonella* under laboratory conditions for years, were used. For each isolate, 1 ml of suspension was transferred to an Eppendorf tube and centrifuged at 14,000 rpm (18,300 × g) for 1 minute. The resulting precipitate was homogenized with a micropes- tle. DNA was extracted with QIAGEN DNeasy Blood and Tissue Kit following the Bench Spin-Column Protocol for Animal Tissues. A modification was made to the two DNA elution steps in the instructions of the manufacturer, where, instead of 200 µl of AE Buffer for each step, 20 µl and 10 µl were used, respectively, in order to obtain higher DNA concentrations. The resulting DNA

Table I. Distribution by altitude and by habitat types of *Heterorhabditis* in Bulgaria.

Altitude ranges / Habitats	Samples	Positive for EPNs		Positive for <i>Heterorhabditis</i>	
		Number of samples	%	Number of samples	% positive for EPNs
0-450 m altitude	170	28	16.5	11	39.3
Agricultural fields	10	1	10.0	1	100.0
Beaches	16	4	25.0	3	75.0
Coniferous woodlands	4	2	50.0	2	100.0
Deciduous woodlands	63	10	15.8	1	10.0
Meadows	67	10	14.9	4	40.0
Orchards	2	1	50.0	0	0.0
Other	8	0	0.0	-	-
500-980 m altitude	508	95	18.7	29	30.5
Agricultural fields	37	15	40.5	11	73.3
Coniferous woodlands	35	4	11.4	1	25.0
Deciduous woodlands	155	32	20.7	5	15.6
Meadows	223	33	14.8	11	33.3
Orchards	25	3	12.0	1	33.3
Other	33	8	24.2	0	0.0
1000-1450 m altitude	295	96	32.5	3	3.1
Coniferous woodlands	48	9	18.8	0	0.0
Deciduous woodlands	123	44	35.8	0	0.0
Meadows	122	43	35.3	3	7.0
Other	2	0	0.0	-	-
1500-2690 m altitude	254	115	45.3	0	0.0
Coniferous woodlands	70	28	40.0	0	0.0
Deciduous woodlands	8	3	37.5	0	0.0
Meadows	34	14	41.2	0	0.0
Subalpine meadows	70	26	37.1	0	0.0
Subalpine <i>Juniperus</i> formation	63	43	68.3	0	0.0
Alpine meadows	9	1	11.1	0	0.0
TOTAL	1227	334	27.2	43	12.9

Table II. Localities with establishment of *Heterorhabditis* in Bulgaria.

Locality	Collection date	Altitude (m)	Soil type	Habitat	Positive samples	Source
Lozen (42°36'N; 23°29'E)	Nov 1994	700	Alluvial	Mesophilic meadow	1	Shishinova <i>et al.</i> , 1997
Lozen (42°36'N; 23°29'E)	Dec 1994	700	Alluvial	Riverbank forest	1	Shishinova <i>et al.</i> , 1997
Svishtov (43°37'N; 25°26'E)	Aug 1995	80	Loess	Agricultural field	1	Shishinova <i>et al.</i> , 1997
Jeleznitsa (42°32'N; 23°21'E)	Sep 1997	1175	Brown forest	Mesophilic meadow	3	Shishinova <i>et al.</i> , 2000
Varna (43°14'N; 28°00'E)	Jul 1997	60	Grey forest	Coniferous forest	2	Shishinova <i>et al.</i> , 2000
Razhdavitsa (42°23'N; 22°42'E)	May 1998	550	Cinnamonic	Pine forest	1	Shishinova <i>et al.</i> , 2000
Zemen (42°28'N; 22°43'E)	Sep 1998	580	Alluvial	Riverside meadow	1	Shishinova <i>et al.</i> , 2000
Gorubljane (42°36'N; 23°24'E)	Oct 1998	600	Alluvial	Walnut orchard	1	New data
Zemen (42°28'N; 22°43'E)	Apr 1999	620	Calcareous	Xerophilic meadow	1	New data
Aheloi (42°38'N; 27°39'E)	Mar 1999	0-1	Sand	Beach	2	New data
Ravda (42°38'N; 27°40'E)	Mar 1999	0-1	Sand	Beach	1	New data
Ichtiman (42°28'N; 23°47'E)	Mar 2000	650	Alluvial	Meadow to an irrigation canal	1	New data
Zemen (42°28'N; 22°43'E)	Apr 2000	580	Alluvial	Meadow	1	New data
Razhdavitsa (42°23'N; 22°42'E)	Mar 2001	500	Alluvial	Riverbank forest	2	New data
Stara Kresna (41°47'N; 23°11'E)	May 2001	640	Alluvial	Meadow to an irrigation canal	1	New data
Kresna Gorge (41°45'N; 23°09'E)	May 2002	200	Alluvial	Meadow	1	Gradinarov <i>et al.</i> , 2011
Novo Selo (42°11'N; 22°40'E)	Jun 2002	850	Alluvial	Riverside meadow	1	New data
Kokaljane (42°35'N; 23°25'E)	Aug 2002	600	Alluvial	Mixed deciduous forest	1	New data
Kostinbrod (42°48'N; 23°10'E)	Aug 2002	550	Alluvial	Strawberry, raspberry	11	Gradinarov, 2003
Melnik (41°31'N; 23°24'E)	Nov 2002	450	Cinnamonic	Meadow	1	New data
Blatska (41°32'N; 23°52'E)	Apr 2003	590	Alluvial	Meadow	1	New data
Hadzhidimovo (41°30'N; 23°53'E)	Apr 2003	500	Alluvial	Riverside meadow	1	New data
Kostinbrod (42°48'N; 23°11'E), BGKB-1/2007	Jun 2007	550	Alluvial	Riverbank forest	1	New data
Zemen (42°28'N; 22°44'E), BGZG-24B/2009	Sep 2009	580	Alluvial	Riverside meadow	2	New data
Kojucha (41°27'N; 23°15'E)	Oct 2009	175	Calcareous	Xerophilic meadow	1	New data
Katuntci (41°26'N; 23°25'E), BGSP-2B/2010	Oct 2010	150	Alluvial	Riverbank forest	1	New data
Rupite (41°27'N; 23°15'E), BGSP-5/2010	Oct 2010	95	Alluvial	Meadow to an irrigation canal	1	New data

Table III. Morphometrics of infective juveniles (n = 30) of four *H. bacteriophora* isolates. All measurements are in μm .

Character	BGKB-1/2007		BGZG-24B/2009		BGSP-2B/2010		BGSP-5/2010	
	Mean \pm STD	Range						
L	578 \pm 16.79	540-619	599 \pm 23.02	520-651	570 \pm 23.63	517-614	585 \pm 20.82	542-619
W	24 \pm 0.88	22-25	22 \pm 0.84	21-25	23 \pm 1.23	21-25	23 \pm 1.15	21-25
EP	105 \pm 1.70	101-109	108 \pm 2.90	97-111	103 \pm 2.79	99-108	105 \pm 2.95	99-110
NR	82 \pm 1.66	79-85	83 \pm 2.17	77-87	81 \pm 2.31	77-87	82 \pm 2.51	78-89
OES	124 \pm 2.29	121-129	128 \pm 3.03	120-134	122 \pm 3.85	109-126	127 \pm 2.85	121-131
T	90 \pm 4.04	82-97	94 \pm 4.66	79-104	86 \pm 4.39	77-93	89 \pm 4.54	79-95
ABW	16 \pm 0.83	15-17	15 \pm 1.13	12-17	14 \pm 0.93	12-17	15 \pm 0.79	14-16
ratio <i>a</i>	24.18 \pm 0.80	22.50-25.56	26.69 \pm 0.90	24.50-28.35	24.71 \pm 1.04	22.80-26.94	25.38 \pm 0.67	24.21-26.71
ratio <i>b</i>	4.65 \pm 0.12	4.45-4.90	4.68 \pm 0.16	4.33-5.06	4.67 \pm 0.19	4.35-5.24	4.61 \pm 0.13	4.38-4.90
ratio <i>c</i>	6.42 \pm 0.23	5.95-6.90	6.37 \pm 0.20	6.03-6.74	6.67 \pm 0.18	6.27-7.10	6.61 \pm 0.22	6.34-7.25
ratio <i>d</i>	0.84 \pm 0.02	0.81-0.87	0.84 \pm 0.02	0.80-0.91	0.84 \pm 0.03	0.81-0.92	0.83 \pm 0.02	0.80-0.87
ratio <i>e</i>	1.16 \pm 0.05	1.09-1.26	1.15 \pm 0.04	1.07-1.22	1.20 \pm 0.05	1.12-1.31	1.19 \pm 0.06	1.12-1.36

extracts were used in PCR reactions with two sets of primers to amplify both the rDNA ITS-regions and the D2D3 expansion segment of the 28S rRNA gene: TW81 = 5'-GTTTCCGTAGGTGAACCTGC-3' and AB28 = 5'-ATATGCTTAAGTTCAGCGGGT-3' as described by Joyce *et al.* (1994); D2A = 5'-ACAAGTACCGT-GAGGGAAAGTTG-3' and D3B = 5'-TCGGAAG-GAACCAGCTACTA-3' as described by De Ley *et al.* (1999). The PCR mix for one reaction contained 5 μl of PCR Taq buffer 10x, 4 μl of 25 μM MgCl₂, 1 μl of 100 μM dNTPs, 0.55 μl of each primer, 0.4 μl 2U Taq Polymerase, 5 μl DNA extract and ddH₂O to a final volume of 50 μl . The PCR products were purified using QIAGEN QIAquick PCR Purification Kit in accordance with the instructions of the manufacturer and sent for sequencing to Macrogen's Laboratory for Genomics and Bioinformatics. The obtained sequences were processed and aligned manually using MEGA5 software (Tamura *et al.*, 2011).

RESULTS

During the period of the investigation, entomopathogenic nematodes were isolated from a total of 334 soil samples (27.2% of all collected samples). Nematodes of the genus *Heterorhabditis* were established in 43 of them (12.9% of the positive samples or 3.5% of the total number of samples) (Table I), collected from 27 different localities in Southwest and North Bulgaria, and the Black Sea coast (Table II). The genus is reported for the first time in Bulgaria for 18 of these localities (labelled as *new data*).

Based on the morphological and morphometrical characters, all isolates, except for one, were identified as *H. bacteriophora*. The following characters were decisive for the identification of the species: body length and ratios *b*, *c*, *d* and *e* of infective juveniles; ratios *d*, *e*, GS and morphology of the tail region of males (Fig. 1A - D). The morphometrical characters of males and IJs from cultures BGKB-1/2007, BGZG-24B/2009, BGSP-2B/2010 and BGSP-5/2010 are presented in Tables III and IV.

The mean values of the morphometrics and ratios of the infective juveniles of one isolate from Gorubljane (2002) deviate significantly from the specific characteristics of *H. bacteriophora*: L (775.1 \pm 15.12); W (27.8 \pm 0.55); EP (125.9 \pm 2.28); OES (150.0 \pm 2.86); T (122.1 \pm 5.31); ratio *a* (27.9 \pm 0.75); ratio *b* (5.2 \pm 0.12); ratio *c* (6.4 \pm 0.23) (n = 10). These values correspond with the description of *H. megidis* (after Stock *et al.*, 2002). However, molecular identification of the isolate was not performed due to the lack of suitable material and the identity of the species cannot yet be confirmed.

The morphological identification of the four investigated isolates of *H. bacteriophora* was confirmed by molecular analysis. The sequences obtained with each of the two sets of primers used were identical for all four

Table IV. Morphometrics of male individuals (n = 15) of four *H. bacteriophora* isolates. All measurements are in μm .

Character	BGKB-1/2007		BGZG-24B/2009		BGSP-2B/2010		BGSP-5/2010	
	Mean \pm STD	Range						
L	897 \pm 65.35	755-1000	854 \pm 56.99	785-965	963 \pm 70.89	817-1082	929 \pm 56.94	772-1047
W	51 \pm 2.71	47-57	45 \pm 3.13	40-51	49 \pm 3.27	45-54	51 \pm 2.80	47-57
NR	73 \pm 4.15	67-82	73 \pm 4.34	67-82	78 \pm 2.55	74-82	78 \pm 4.71	69-85
EP	144 \pm 13.91	104-163	138 \pm 10.39	124-161	149 \pm 10.83	121-166	140 \pm 5.88	124-149
OES	107 \pm 4.77	99-116	112 \pm 4.02	104-119	112 \pm 2.72	106-116	115 \pm 3.97	106-121
Woes	36 \pm 1.22	35-38	32 \pm 2.48	27-35	33 \pm 1.47	30-35	35 \pm 2.46	31-40
TR	102 \pm 22.87	67-134	105 \pm 7.06	94-114	102 \pm 6.01	89-111	100 \pm 7.12	87-109
SP	44 \pm 3.20	37-47	42 \pm 1.61	40-45	45 \pm 3.42	38-50	46 \pm 2.20	42-50
GB	21 \pm 3.11	15-27	21 \pm 2.22	15-24	23 \pm 1.51	20-26	22 \pm 1.68	20-25
T	29 \pm 2.28	25-33	29 \pm 1.46	26-32	30 \pm 2.50	25-33	30 \pm 2.01	27-35
Wcl	22 \pm 1.63	20-25	20 \pm 1.09	19-22	20 \pm 1.72	17-22	19 \pm 1.66	15-22
ratio <i>a</i>	17.64 \pm 0.75	16.05-18.78	18.93 \pm 1.09	16.68-21.08	19.54 \pm 0.98	17.80-21.89	18.11 \pm 1.08	15.60-19.53
ratio <i>b</i>	8.35 \pm 0.52	7.09-9.18	7.66 \pm 0.45	7.12-8.45	8.61 \pm 0.61	7.33-9.50	8.09 \pm 0.41	7.26-8.91
ratio <i>c</i>	30.80 \pm 2.45	25.42-35.00	29.54 \pm 2.05	26.42-32.96	32.25 \pm 3.26	27.50-39.40	31.26 \pm 1.65	28.08-33.73
ratio <i>d</i>	1.34 \pm 0.11	0.98-1.42	1.23 \pm 0.07	1.13-1.40	1.33 \pm 0.09	1.09-1.46	1.22 \pm 0.04	1.14-1.26
ratio <i>e</i>	4.95 \pm 0.54	3.50-6.00	4.76 \pm 0.37	4.23-5.42	5.00 \pm 0.50	4.08-6.20	4.71 \pm 0.33	4.14-5.36
GS	0.48 \pm 0.05	0.40-0.58	0.51 \pm 0.06	0.35-0.59	0.53 \pm 0.05	0.45-0.65	0.47 \pm 0.05	0.40-0.56

isolates (GenBank accession numbers JX993984 and JX993985). Full sequences were obtained for ITS1, the 5.8S rRNA gene and ITS2 and a partial sequence was obtained for the 28S rRNA gene. Nucleotide queries were submitted to the BLAST database at GenBank. The comparison of the obtained products to the database sequences yielded average top similarity values of 100% for *H. bacteriophora* for both the rDNA ITS-region and the D2D3 expansion segment of the 28S rRNA gene. The sequence of the ITS-region (ITS1-5.8SrRNA-ITS2) of the investigated cultures corresponds to "strain 2" of *H. bacteriophora* from Southern France (Emelianoff *et al.*, 2008).

In Bulgaria, the habitats where *Heterorhabditis* was established are at altitudes from 0 to 1175 m, with sandy and alluvial soils and less frequently calcareous, brown and cinnamonic soils of meadows, riverbank forests, agro-ecosystems and less commonly in open forests (Table II). Heterorhabditids have not been found in dense forests. Inland, they were almost always found in proximity to waterways (rivers or irrigation canals). In one of the agrocenoses, a nursery for transplanting strawberries and raspberries, *H. bacteriophora* was isolated from eleven out of a total of sixteen randomly collected samples (Table II).

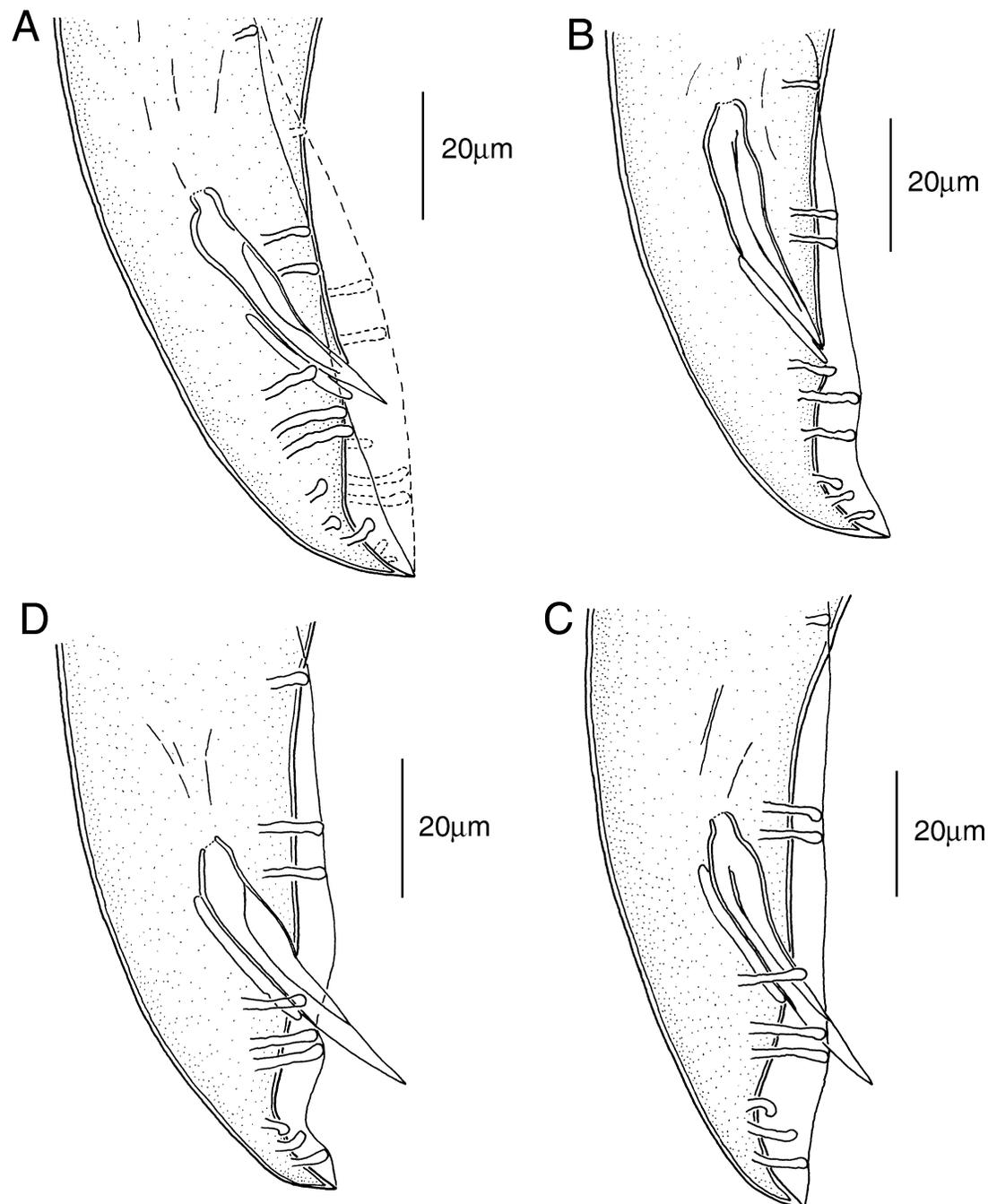


Fig. 1. Drawings of the lateral view of the tail of male individuals from the investigated isolates: A, Kostinbrod isolate (BGKB-1/2007); B, Zemen isolate (BGZG-24B/2009); C, Katunci isolate (BGSP-2B/2010); D, Rupite isolate (BGSP-5/2010).

DISCUSSION

The morphometric features of the four examined cultures of *H. bacteriophora* are within the ranges reported for the species (Poinar, 1976; Poinar and Georgis, 1990; Nguyen and Smart, 1995) and differentiate them from the other two European species, *H. megidis* and *H. downesi* (Stock *et al.*, 2002). There are notable differences in the morphometric features of the four isolates, in particular regarding the male individuals. For example, the body length and the length of spicules and gubernaculum of the southern isolates BGSP-2B/2010 and BGSP-24B/2010 show greater mean values (Table IV). There also are notable differences in the spicule morphology of the investigated isolates (Fig. 1A - D). The spicules of males from isolate BGKB-1/2007 (Fig. 1A) exhibit a dorsal expansion on the blade, which is present to a different degree in all studied individuals. Such an expansion is not present in the rest of the isolates, even though it is mentioned in the original description of *H. bacteriophora* (Poinar, 1976). It appears that the morphology of the proximal spicule end of the species can show significant variations.

Given the results from the molecular identification, the established morphological differences between the investigated isolates appear to be intraspecific and complement the available data on morphological variations within *H. bacteriophora*. The four investigated populations were isolated from inland habitats, similarly to the French isolates of *H. bacteriophora*, belonging to "strain 2" (Emelianoff *et al.*, 2008). It seems that the investigated cultures belong to the same population of the species. The established morphological differences are due either to environmental influences or to random factors.

The presence of *H. megidis* in Bulgaria needs further confirmation. The species was reported from neighbouring Greece (Menti *et al.*, 1997) and Turkey (Yilmaz *et al.*, 2009) and, therefore, is quite possibly present in Bulgaria. In any case, the present investigations reveal that the prevalent heterorhabditid species in Bulgaria is *H. bacteriophora*.

Heterorhabditis bacteriophora has been established on all continents except Antarctica (Hominick, 2002). In Europe it has been reported from Spain, Italy, Moldova, Hungary, Southern France (Smits *et al.*, 1991), The Azores, Germany, Switzerland (Hominick, 2002), South Russia (Ivanova *et al.*, 2000), the European part of Turkey (Hazir *et al.*, 2003) and Slovenia (Laznik *et al.*, 2009). *Heterorhabditis bacteriophora* seems to be more widespread in the southern and central parts of the continent while to the north and the northeast the dominant species of the genus are *H. megidis* and *H. downesi* (Smits *et al.*, 1991; Griffin *et al.*, 1999; Stock *et al.*, 2002).

Numerous faunal studies carried out in Europe show that EPNs from the genus *Steinernema* are much more common than those from the genus *Heterorhabditis*. During investigations in Great Britain (Hominick and Briscoe, 1990), Ireland (Griffin *et al.*, 1991), Spain

(García del Pino and Palomo, 1996), Czech Republic (Mráček *et al.*, 1999) and Slovakia (Sturhan and Lišková, 1999), heterorhabditids were either established in a few localities or were not established at all (Campos-Herrera *et al.*, 2007). In Bulgaria, the percentage of samples positive for heterorhabditids is relatively high (12.9% of all positive samples). The rate of samples positive for *Heterorhabditis* reaches 39.3% in the low altitude range from 0 to 450 m and 30.5% in the range 500 - 980 m (Table I). According to Griffin *et al.* (1994), the rare establishment of the genus *Heterorhabditis* in various regional investigations in Europe is due to their marked preference for sandy soils, in particular along sea coasts. During selective investigations in suitable habitats, heterorhabditids have been found at relatively high frequencies in Estonia, Denmark and Hungary (Griffin *et al.*, 1999). In Bulgaria, given the high percentage of positive samples collected inland, limiting factors for the distribution of the genus, apart from the soil type, are the elevation and its inherent temperature regime. In Turkey, where the climate is warmer, nematodes of the genus *Heterorhabditis* are relatively common, accounting for 31.8% of all samples positive for EPNs (Hazir *et al.*, 2003). In both Bulgaria and Turkey, the high incidence of heterorhabditids is mainly accounted for by the presence of *H. bacteriophora*, which prefers warmer temperatures and is dominant in inland areas of the continent (Hominick, 2002).

In Bulgaria, *H. bacteriophora* exhibits preference for alluvial soils under herbaceous (often pastures) and woody vegetation on river terraces and along irrigation canals (Table I). It has also been found in calcareous soils with xerophilic herbaceous vegetation. In both cases, the soil has poor water retention ability and its water and temperature regimes exhibit significant seasonal fluctuations. It is possible that the tolerance of *H. bacteriophora* to high temperatures (Grewal *et al.*, 1994) and low soil humidity (Glazer *et al.*, 1993) contributes to its ability to compete in such habitats, even if it feeds on the same hosts as other species of EPNs.

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