Russian Journal of Nematology, 1995, 3 (2), 71 - 80

Steinernema bicornutum sp. n. (Rhabditida: Steinernematidae) from Vojvodina, Yugoslavia

Bela Tallosi[*], Arne Peters[**] and Ralf-Udo Ehlers[1***]

[*]Ady Endre 16 (VIII/9), 5600 Solnok, Hungary.
[**]ECOGEN EUROPE S.r.l., 3A Parco Technologico Agro-Alimentare Dell'Umbria, Frazione Pantalla 06050, Todi (PG), Italy.
[***]Institute for Phytopathology, Christian-Albrechts-University Kiel, Dep. Biotechnology and Biological Control, Klausdorfer Strasse 28-36, 24223 Raisdorf, Germany.
[1] Corresponding author.

Accepted for publication 2 March 1995

Summary. *Steinernema bicornutum* sp. n., isolated from soil samples taken in Strazilovo, Vojvodina, Yugoslavia by baiting with last instar *Galleria mellonella*, is distinguished from other members of the genus *Steinernema* by two protruding horn-like papillae on the head of the dauer juvenile. The average length of the dauer stage is 770 µm, the lateral field has 8 ridges. Only second generation females and males bear a small tail mucron. Two different spicule types occur in first generation males. The species did not hybridize with *S. carpocapsae* and *S. feltiae*, the latter also isolated from the type locality. Its restriction fragment length pattern of the internal transcribed spacer region of the ribosomal DNA repeat unit differ from other *Steinernema* species. Dauer juveniles with similar horn-like structures have been recorded from Spain and Germany. Axenic and monoxenic cultures of the new species were established on artificial media. An improved time-saving method to dehydrate nematodes for permanent preparations is described.

Key words: entomopathogenic nematode, *Steinernema bicornutum* sp. n., Vojvodina, Yugoslavia, description.

The search for new species and strains of entomopathogenic nematodes during the last two decades has been intensified due to the possible use of these nematodes for the biological control of soil-borne insect pests (Ehlers & Peters, 1995). The objectives of a survey carried out in Vojvodina, Yugoslavia were to isolate and identify entomopathogenic nematodes from this region and to test their potential as biological control agents against the sugar beet weevil, *Bothynoderes punctiventris* (Tallosi et al., 1993). Besides *Steinernema feltiae* Filipjev, 1934 a new species was isolated which is described herein as *Steinernema bicornutum* sp. n. The species is named «*bicornutum*» for its most typical character; a double horn-like structure on the lip region of the dauer juvenile.

MATERIAL AND METHODS

Soil samples of 1 kg were collected at Strazilovo, Vojvodina, Yugoslavia and four last instar *Galleria mellonella* were added. The insects were caged in a 5 x 1 cm tube of copper net (200 µm mesh size). After five days dead insects were removed and each was transferred to a moist chamber for emergence of nematodes from the cadaver. Two insects contained entomopathogenic nematodes. Non-entomopathogenic rhabditid and diplogasterid nematodes were separated from the two steinernematid populations, which were subsequently cultivated on larvae of *G. mellonella*. Permanent microscopical slides were prepared of dauer juveniles and adults. The latter were isolated from final instar *G. mellonella* which were infested with approximately 50 dauer juveniles/larva. Four days later, first generation males and females were removed. After a further four days second generation adults were sampled from another batch of insects. The nematodes were killed in 80° C Ringer's solution and fixed in 4% formaldehyde. Dehydration was done in three steps on a heating plate at 80° C in the well of a microscopic slide. Nematodes were transferred with a needle into 15% glycerol adjusted

to pH 12 with 5M NaOH. After 3-4 min the nematodes were transferred into 30% glycerol for another 3-4 min. To stain the nematodes 0.03% methylene blue (w/w) was added to the second solution. In a third step the nematodes were kept in 40% glycerol for approximately 15 min until all water had evaporated. Dehydrated nematodes were finally mounted in a drop of glycerol on a flat microscopic slide. Glass supports were used to avoid flattening of the adult nematodes. The cover slip was fixed to the slide by melting a mixture of 3 parts paraffin with 1 part bee wax.

Morphometric studies of dauer juveniles (DJs) and first and second generation adults were done using living specimens which were video recorded under the microscope and measurements were taken from the video screen. Dauer juveniles were fixed in 2% glutaraldehyde in 0,05% phosphate buffer at 6° C for 4 h prior to scanning electron microscopy. After 3 rinses with buffer they were transferred in increments of 30 min to 15, 30, 50, 70, 90, 95 and 100% ethanol. The ethanol was then replaced in 3 steps by acetone. The DJs were then dried in a CO_2-critical point drier (CPD 020, Balzers, Liechtenstein) and sputtered at 19 mA for 2,5 min with gold. Specimens were examined with a Leitz SEM - AMR 1000.

Cross-breeding experiments were conducted with *S. carpocapsae* (strain pieridarum) and *S. feltiae* (strain YuS-WO3). The latter was isolated in the vicinity of the *locus typicus* of *S. bicornutum* sp. n. and identified by morphological characters and by successful hybridization with *S. feltiae* (strain NLS-OBSIII) from the Netherlands. Individual DJs of either *S. carpocapsae* or *S. feltiae* were injected into *G. mellonella* larvae together with one DJ of the new species. Controls were injected with two dauer juveniles of the same isolate.

Cross-breeding was also done in *vitro*: *G. mellonella* were treated with dauer juveniles and 3 days later the cadavers were dissected. The resulting haemolymph was diluted with 1 ml yeast-salt solution (Dye, 1968), centrifuged at 1000 rpm and 25 µl of the supernatant was transferred into cell wells. Three pre-adult (J_3/J_4) males of one isolate were combined with one pre-adult female of the new species in the cell well. Experiments were also done with preadult males of the new species and pre-adult females of the other species. *In vitro* cross-breeding experiments were carried out in suspensions of the symbiotic bacterium from one nematode isolate and repeated with bacteria of the other isolate. Additionally, nematodes were also grown on symbiotic bacteria of the third nematode isolate. Nematodes were also grown in mono- and axenic *in vitro* cultures according to Lunau et al. (1993).

Dauer juveniles of the new species were sent to Alex Reid, CABI, International Institute of Parasitology, St. Albans, UK, for RFLP analysis of the internal transcribed spacer region of the ribosomal DNA repeat unit (Reid, 1994).

RESULTS

The method described here to prepare permanent mounts is straightforward, reducing the time required to approximately 30 min. Since their preparation, in summer 1992, the nematodes have preserved their morphological characters. The rapid dehydration process nicely preserves all morphological characters and no shrinkage occurred.

Interbreeding experiments between the new steinernematid and the previously described species *S. carpocapsae* and *S. feltiae* were negative, whilst controls using specimens of the same species were positive in both, *in vivo* and *in vitro* cross-breeding tests. Dauer juvenile offspring of male and female *S. bicornutum* developed as two distinct male morphological types (description below). When pre-adults of the same species were combined, development to adult stages and reproduction was observed with all three *Xenorhabdus* isolates tested. The new species was also propagated mono- and axenically.

The interbreeding experiments separate *S. bicornutum* sp. n. from the morphologically similar species *S. carpocapsae* and *S. feltiae* and together with the morphological data and the results of the molecular analysis (Reid, 1994) indicate that this steinernematid is a new species and it is described below.

DESCRIPTION

Steinernema bicornutum sp. n. (Figs. 1-3, Tables 1-3)

Dauer juvenile. Measurements are given in Table 1. Head hemispherical, labial region bearing a horn-like structure composed of two protuberances, which

possibly are laterally protuding labial papillae, located on an oval ring around the oral opening (Fig 3A and B). The horn-like structure is exclusively found in the dauer juvenile and, in lateral view, the two horns might be misidentified as the similar but single tooth observed on heterorhabditid dauer juveniles (Fig. 3 C). The third stage dauer juvenile is generally ensheathed by the pre-dauer second stage cuticle. Observation of the horn-like structure is occasionally prevented by the presence of the pre-dauer cuticle.

Amphidial openings lateral, a little further back from the lip region, almost covered by the two horns. Four distinct cephalic papillae arranged medially on the head at a 45° angle to the horn-like structure (Fig. 3A). Excretory pore situated half-way between head and bulbus (Fig. 1). Pharynx and intestine lumen collapsed. In the anterior portion of the intestine a pouch is present containing cells of the symbiotic bacterium, probably *Xenorhabdus* sp. Body slender with transverse annulations. Lateral field with 8 ridges of which the submarginal ridges are less distinct than the others (Fig. 3 D). Tail constricted with a pointed end (Fig. 1).

Adults. Cuticle smooth, head rounded, not offset from rest of the body. Each of the 6 lips bearing a single labial papilla. Outer circle of 4 cephalic papillae further back on the head. Stoma short and wide, pharynx muscular and almost cylindrical, but widening towards the basal end. Metacorpus nonvalvated; isthmus much thinner than metacorpus; nerve ring surrounding pharynx between isthmus and basal bulb. Valve ridges of basal bulb indistinct; basal bulb slightly broader or as wide as metacorpus. Excretory pore situated slightly anterior to isthmus, almost at the middle of the pharynx. Sexes are separate and reproduction is by amphimixis.

Female. Measurements of first and second generation females are given in Tab. 2. First generation females often produce 'giant' forms >1 cm. *Tractus genitalis* amphidelphic with opposed reflexed ovaries. Vulva a transverse slit protruding only in second generation, and never in first generation females. Protruding of vulva lips less pronounced than in other steinernematids. Tail of first generation short, blunt, with a short wedge-shaped projection and always with mucron (Fig. 2A). Tail of second generation longer and always with mucron.

Male. Measurements of first and second generation males are given in Tab. 3. Males have a single reflexed testis. Sperms have a diameter of 4-5 μm. Anterior to the cloacal opening a row of 6 pairs of genital papillae in ventro-lateral position and a single ventral precloacal papilla. Three pairs posterior to the gubernaculum and another 3 pairs surrounding the tail tip (Fig. 2C). Spicules paired and light brown in colour. In first generation males two different types of spicules occur of which type II was present only in 10-20% of the population. Type I spicules are curved; capitulum distinct and well separated from lamina, proximally broad and flattened; calamus with a small pointed protrusion. From this point a distinct velum extends almost to the proximal end of the lamina. Spicule tip with a transverse incision, similar to the spicule of *S. carpocapsae* (Fig. 2 C). Type II spicules are much less curved; lamina extending to capitulum without distinct demarcation; velum absent or indistinct; spicule tip without incision; colour usually a little darker; proximal hook of gubernaculum usually more pronounced than in type I males. Spicule morphology similar to *S. feltiae* (Fig. 2 D). Tails of first generation males of both types are short and round and without a mucron. Second generation males (Fig. 2 E) have a longer tail usually with a small mucron. The gubernaculum is spindle-like. Only one type of spicule is present similar to type II of the first generation.

Type locality. Strazilovo, Vojvodina, south of Novi Sad, Yugoslavia, in the Furska Gora Mountains. No type host can be named as the species was isolated using wax moth larvae as bait.

Type material. Holotype and paratypes deposited in the German Nematode Collection at Biologische Bundesanstalt, Institute for Nematology and Vertebrate Research, Münster, Germany. The strain designation of the type population is YuS-Wo 6.

Differential diagnosis. *Steinernema bicornutum* sp. n. can be readily distinguished from all other described *Steinernema* spp. by the presence of a double

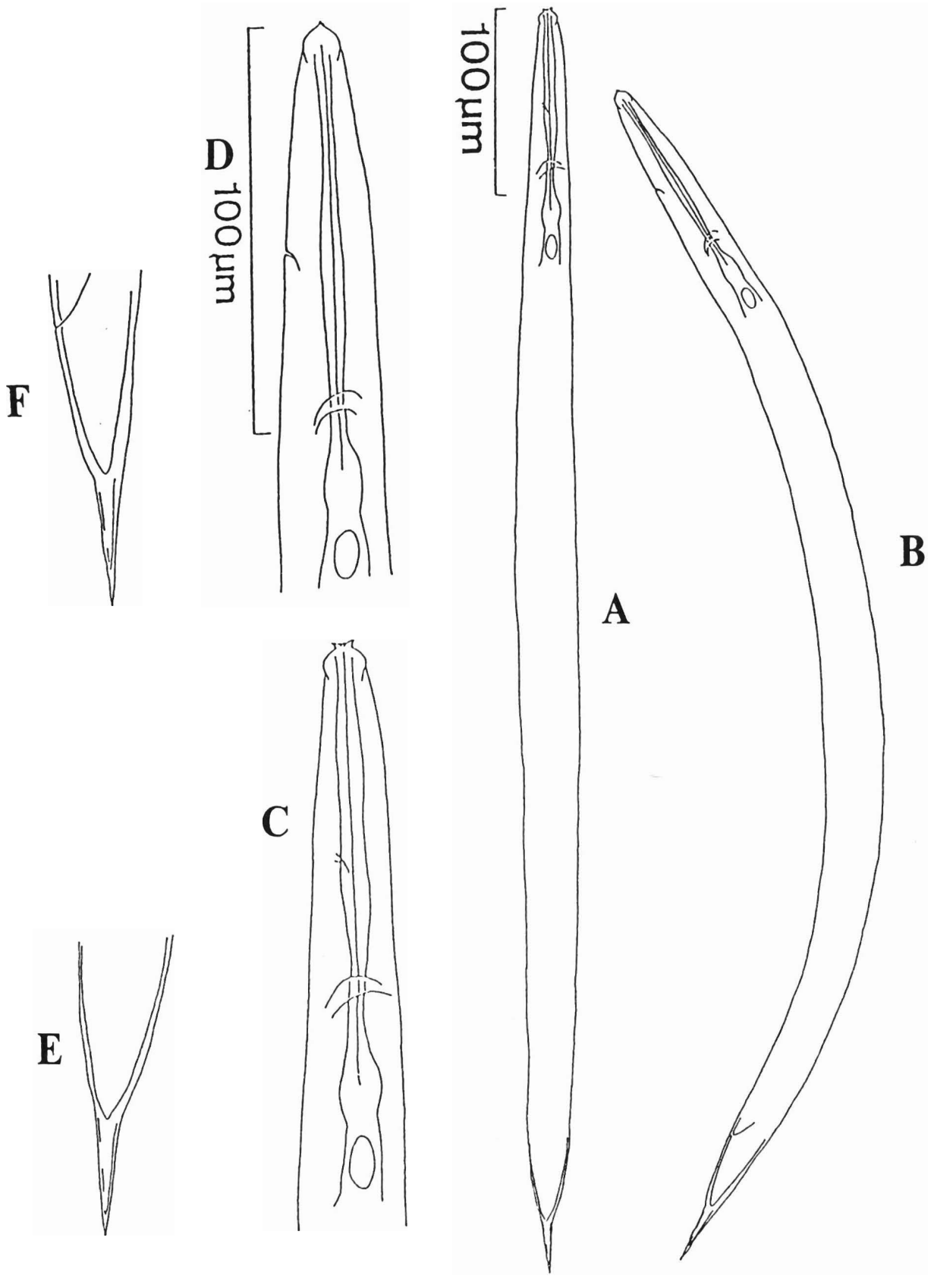

Fig. 1. A dauer juvenile of *Steinernema bicornutum* sp. n. A: Dorso-ventral view; B: Lateral view; C: Dorso-ventral view of anterior portion; D: Lateral view of anterior portion; E: Dorso-ventral view of tail; F: Lateral view of tail.

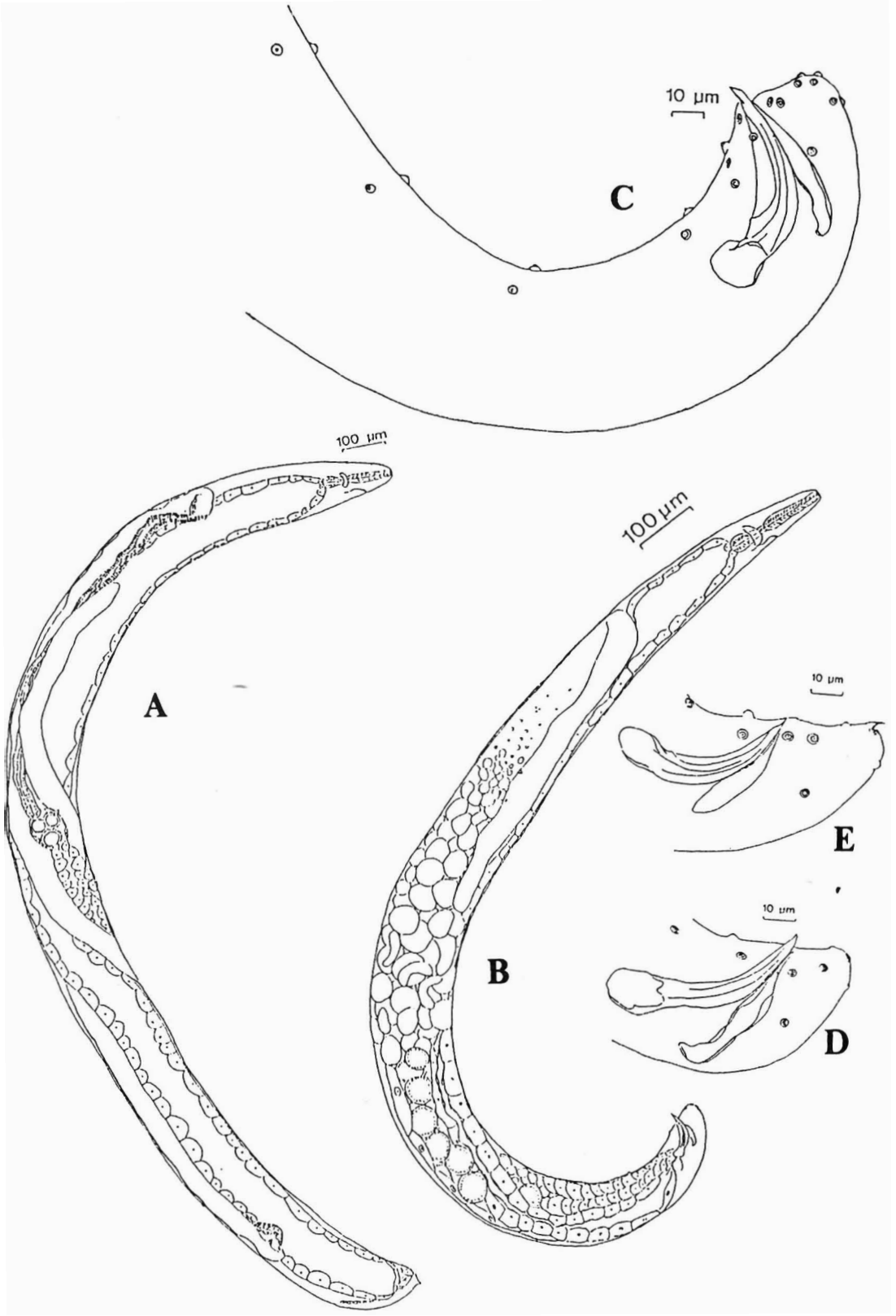

Fig. 2. Adults of *Steinernema bicornutum* sp. n. A: First generation female; B: First generation male; C: Posterior view of first generation male with type I spicule; D: Posterior view of first generation male with type II spicule; E: Posterior view of second generation male.

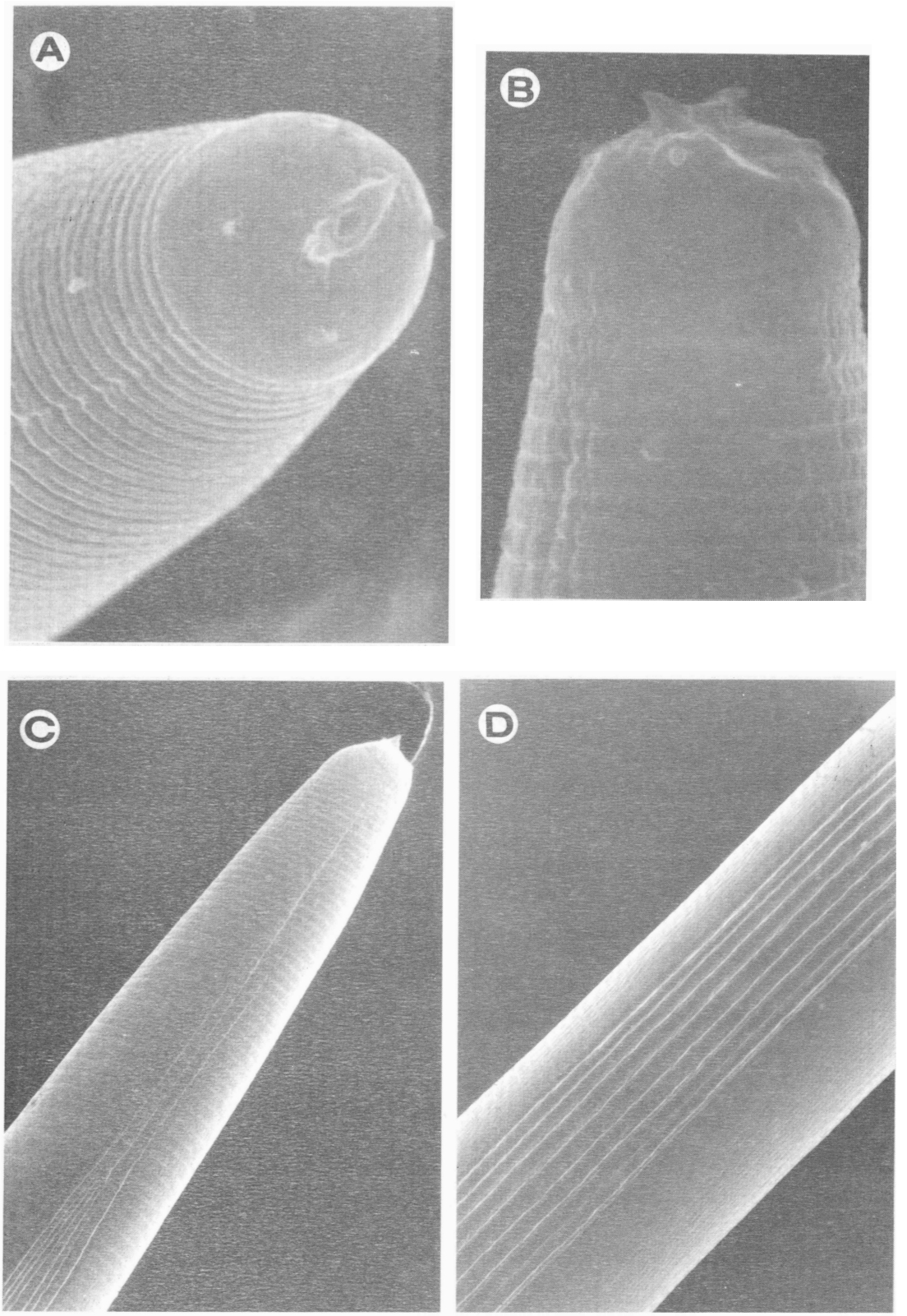

Fig. 3. Exsheathed dauer juveniles of *Steinernema bicornutum* sp. n. A: Face view showing oral opening surrounded by «horn-like» structure, 4 cephalic papillae and amphid almost covered by protruding horn-like structure; B: Anterior region showing the elevated position of the «horn-like» structure; C: Lateral view of anterior part showing similarity with the «tooth-like» structure of heterorhabditid dauer juveniles when viewed from lateral side; D: Lateral field with 8 lines.

Table 1. Measurements (in μm) and ratios of dauer juveniles (n=20) of *Steinernema bicornutum* sp.n.

Character	Min.	Max.	Mean	±SD
Length	648	873.0	769.5	52.38
Greatest width	25	32.5	29.5	1.67
Head-excretory pore (EP)	52.5	65.0	60.6	3.33
Head-nerve ring (NR)	87.5	100.0	92.0	3.49
Head-base oesophagus (ES)	112.5	135.0	123.9	6.04
Tail	62.5	77.5	72.0	4.97
Hyaline part of tail	27.5	37.5	32.9	3.74
Length/greatest width	23.3	29.4	26.5	1.51
Length/EP	10.2	13.5	12.7	0.76
Length/ES	5.6	6.9	6.2	0.30
Length/tail	9.7	12.0	10.7	0.66
EP/ES	0.4	0.6	0.5	0.03
EP/tail	0.8	1.0	0.8	0.06
Greatest width/tail	0.3	0.5	0.4	0.03
Tail/hyaline tail	1.8	2.7	2.2	0.21

Table 2. Measurements (in μm) and ratios of first and second generation females (n=20) of *Steinernema bicornutum* sp.n.

Character	First generation				Second generation			
	Min.	Max.	Mean	±SD	Min.	Max.	Mean	±SD
Length	2727.0	10071.0	6328.8	2068.34	1269.0	1809.0	1615.9	145.87
Greatest width	170.0	305.0	243.1	34.96	80.0	122.5	101.5	11.37
Width vulva region	170.0	312.5	250.1	34.23	87.5	127.5	108.9	10.43
Head-excretory pore (EP)	57.5	95.0	78.0	11.22	60.0	72.5	66.5	4.32
Head-nerve ring (NR)	100.0	157.5	126.1	14.11	95.0	110.0	103.6	4.55
Head-base oesophagus (ES)	162.5	210.0	188.4	13.18	135.0	162.5	146.1	8.13
Tail	20.0	40.0	29.5	5.0	57.5	75.0	62.1	5.34
Head-Vulva	1362.0	4860.0	3168.9	1017.65	675.0	999.0	870.3	93.5
Length/greatest width	15.3	33.5	25.5	5.77	14.1	20.1	16.0	1.62
Length/EP	37.6	140.4	80.7	23.35	20.4	29.7	24.3	2.11
Length/ES	15.4	50.4	33.2	9.59	9.2	12.9	11.1	0.99
Width/tail	4.2	12.2	8.5	2.04	1.2	2.0	1.6	0.25
EP/ES	0.3	0.5	0.4	0.06	0.4	0.5	0.5	0.04
EP/tail	1.7	3.8	2.7	0.65	0.9	1.3	1.1	0.10

Table 3. Measurements (in μm) and ratios of first and second generation males (n=20) of *Steinernema bicornutum* sp.n.

Character	First generation				Second generation			
	Min.	Max.	Mean	±SD	Min.	Max.	Mean	± SD
Length	945.0	1539.0	1352.7	149.66	864.0	1134.0	1007.1	67.30
Greatest width	80.0	127.5	108.8	11.23	62.5	67.5	64.1	1.68
Head-excretory pore (EP)	67.5	97.5	82.0	7.80	60.0	80.0	71.0	4.96
Head-nerve ring (NR)	107.5	137.0	123.3	8.04	95.0	110.0	100.5	4.41
Head-base oesophagus (ES)	137.5	167.5	156.2	7.28	125.0	140.0	130.8	3.99
Tail	25.0	35.0	31.5	2.49	25.0	35.0	29.7	2.55
Length spiculum	52.5	70.0	65.0	4.29	47.5	52.5	50.5	1.54
Length gubernaculum	37.5	50.0	47.9	3.47	25.0	37.5	33.1	2.91
Length/greatest width	10.8	13.7	12.4	0.82	13.8	17.4	15.7	1.04
Length/EP	14.0	18.4	15.5	1.14	12.8	15.7	14.2	0.74
Length/ES	6.6	9.6	8.6	0.76	6.8	8.5	7.7	0.42
Length/tail	31.5	49.7	43.1	4.85	27.8	40.2	34.1	3.74
EP/ES	0.5	0.6	0.5	0.03	0.5	0.6	0.5	0.03
EP/tail	2.2	3.1	2.6	0.24	1.8	2.9	2.4	0.30
Width/tail	2.7	4.0	3.5	0.37	1.8	2.6	2.2	0.20

horn-like papillae on the lip region of the dauer juvenile, a character unique within the genus *Steinernema*. Consequently, the dauer juvenile is the preferred stage to separate *S. bicornutum* sp. n. from other species of the genus.

DISCUSSION

Observing two different types of spicules, we initially concluded that the population consisted of two different species. This possibility was later excluded as progeny from the controls in the cross-breeding experiments also produced both types of spicule morphology in the first generation. Further evidence for spicule polymorphism within first generation of one species, *S. bicornutum* sp. n., is that a DNA analysis would have yielded unreadable RFLP patterns, but, these were not detected (A. Reid, personal communication). Also, the morphologically most similar species *S. carpocapsae* and *S. feltiae*, the latter widely distributed in Europe (e.g. Hominick & Briscoe, 1990; Ehlers et al., 1991), were excluded by cross-breeding experiments. Currently, diagnosis of *Steinernema* species is ideally done using morphometric characters of the DJs and the male spicule shape (Poinar, 1990). However, after comparing measurements of the new species with data of previously described species, very few morphometric characters can be used to reliably distinguish *S. bicornutum* sp. n. from species like *S. affinis, S. intermedium, S. kraussei, S. ritteri, S. feltiae* or *S. carpocapsae*. The number of ridges in the lateral field of the dauer juvenile may be used as a distinguishing feature from *S. affinis, S. intermedium* and *S. kraussei* which have less than 8 (Mracek & Bednarek, 1991), and from *S. ritteri* with 6 ridges (De Doucet & Doucet, 1990). However, this character does not differentiate *S. bicornutum* sp. n. from *S. feltiae* and *S. carpocapsae*. The mean length of the dauer juvenile is not a reliable character to distinguish species within the genus *Steinernema*, as too many new species have recently been described with considerable overlap in their measurements. However, *S. bicornutum* sp. n. can be separated by this character from the two species *S. glaseri* and *S. anomali*, which form DJs with a total length > 800 μm. Measurements of *S. bicornutum* sp. n. dauer juveniles (Tab. 1) when compared with previously described species (Nguyen & Smart, 1992), reveal that few morphometric characters can be used for identification, especially if only a small number of individuals are measured. Substantial intra-population variation occurs with many of these measurements and such morphometric variation is even larger between isolates of one species recorded from different geographical origins.

Morphometric variation also occurs in characters of adult stages and therefore measurements are rarely used for diagnostic purposes. Previously, qualitative morphological characters of males *viz.* shape of spicules, proved useful for identification of *Steinernema* species. However, with three different types of spicule morphology having been recorded for *S. kraussei* (Mracek, 1977) and dimorphism identified within the first generation of *Steinernema bicornutum* sp. n. the reliability of this character for species identification is much reduced. A generation polymorphism and within-generation variability is now evident and, to our knowledge, for the first time the mucron was recorded only in second generation adults, as well as two distinct spicule types within the first generation. Also, selection of the different types of males for identification is largely dependent on the sample size and on the percentage of the different forms in a population, which could be influenced also by environmental conditions.

Therefore, with highly variable morphology and overlapping of characters within the genus *Steinernema*, cross-breeding experiments appear the most reliable method for species identification. Also, molecular techniques such as those described for reliably distinguishing species of the genera *Steinernema* and *Heterorhabditis* by Curran & Driver, (1994), Joyce et al., 1994 and of Reid, (1994) will be used more frequently in taxonomic investigations. A comparison of the restiction enzyme maps for the ribosomal DNA repeat units of *Steinernema* spp. confirmed the genetic distance of *S. bicornutum* sp. n. from other species of the genus and clustered *S. bicornutum* sp. n. within the *S. feltiae* and *S. kraussei* group (Reid, 1994).

Two horn-like structures, similar to those described for *S. bicornutum* sp. n., have been reported from dauer juveniles isolated at 5 different localities in

Germany by D. Sturhan (personal communication) and from the Canary Islands, Spain by F. Garcia del Pino (personal communication). However, it was not possible to confirm whether these isolates also belong to *S. bicornutum* sp. n. as living material of these isolates was not available.

ACKNOWLEDGEMENTS

We thank Dr. Alex Reid, CABI, St.Albans, UK, for providing results from his molecular studies; to Brigitte Friedrich, University Kiel, for preparing the SEMs and Dr. Dieter Sturhan for critical comments on the manuscript. Financial support for cooperation with Prof. Dr. Radosav Sekulic, University Novi Sad, Vojvodina, Yugoslavia, by the BMFT (Bundesministerium für Forschung und Technologie) is greatfully acknowledged. We also thank DAAD (Deutscher Akademischer Austauschdienst) for providing a scholarship to the first author to support his visit to Raisdorf, and the European Union COST Action 819 for supporting the international scientific cooperation.

REFERENCES

Curran, J. & Driver, F. 1994. Molecular taxonomy of *Heterorhabditis*. In: *COST 812 Biotechnology: Genetics of entomopathogenic nematode-bacterium complexes*. Proceedings of a Symposium & Workshop, St. Patrick's College, Maynooth, Co. Kildare, Ireland. (A. M. Burnell, R.-U. Ehlers & J. P. Masson. Eds.) pp. 41-48. Luxembourg, European Commision, DG XII.

De Doucet, M. M. A. & Doucet, M. E. 1990. *Steinernema ritteri* n. sp. (Nematoda: Steinernematidae) with a key to the species of the genus. *Nematologica* 36: 257-265.

Dye, D. W. 1968. A taxonomic study of the genus *Erwinia*: I. The «*amylovora*» group. *New Zealand Journal of Science* 11: 590-607.

Ehlers, R.-U., Deseo, K. V. & Stackebrandt, E. 1991. Identification of *Steinernema* spp. (Nematoda) and their symbiotic bacteria *Xenorhabdus* spp. from Italian and German soils. *Nematologica* 37: 360-366.

Ehlers, R.-U. & Peters, A. 1995. Entomopathogenic nematodes in biological control. Feasibility, perspectives and possible risks. In: *Biological Control - Benefits and Risks*. (H. Hokkanen & J. Lynch. Eds.) in press. Cambridge University Press.

Hominick, W. M. & Briscoe, B. R. 1990. Occurrence of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in British soils. *Parasitology* 100: 295-302.

Joyce, S. A., Reid, A., Driver, F. & Curran, J. 1994. Application of polymerase chain reaction (PCR) methods to the identification of entomopathogenic nematodes. In: *COST 812 Biotechnology: Genetics of entomopathogenic nematode bacterium complexes*. Proceedings of a Symposium & Workshop, St. Patrick's College, Maynooth, Co. Kildare, Ireland. (A. M. Burnell, R.-U. Ehlers & J. P. Masson. Eds.) pp. 178-187. Luxembourg, European Commision, DG XII.

Lunau, S., Stoessel, S., Schmidt-Peisker, A. J. & Ehlers, R.-U. 1993. Establishment of monoxenic inocula for scaling up in vitro cultures of the entomopathogenic nematodes *Steinernema* spp. and *Heterorhabditis* spp. *Nematologica* 39: 385-399.

Mracek, Z. 1977. *Steinernema kraussei*, a parasite of the body cavity of the sawfly, *Cephaleia abietes*, in Czechoslovakia. *Journal of Invertebrate Pathology* 30: 87-94.

Mracek, Z. & Bednarek, A. 1991. The morphology of lateral field of infective juveniles of entomogenous nematodes of the family Steinernematidae (Rhabditida). *Nematologica* 37: 63-71.

Nguyen, K. B. & Smart, G. C. 1992. *Steinernema neocurtillis* n. sp. (Rhabditida: Steinernemaditae) and key to species of the genus *Steinernema*. *Journal of Nematology* 24: 463-477.

Poinar, G. O. Jr. 1990. Biology and taxonomy of Steinernematidae and Heterorhabditidae. In: *Entomopathogenic Nematodes in Biological Control* (R. Gaugler & H. K. Kaya. Eds.) pp. 23-61. Boca Raton, CRC Press.

Reid, A. P. 1994. Molecular taxonomy of *Steinernema*. In: *COST 812 Biotechnology: Genetics of entomopathogenic nematode-bacterium complexes*. Proceedings of a Symposium & Workshop, St. Patrick's College, Maynooth, Co. Kildare, Ireland. (A. M. Burnell, R.-U. Ehlers & J. P. Masson. Eds.) pp. 49-58. Luxembourg, European Commision, DG XII.

Tallosi, B., Sekulic, R. & Keresi, T. 1993. Investigations on entomopathogenic nematodes in Vojvodina and possibility of their use for some agricultural pest control. *Zastita Bilja* 44: 213-219.

Tallosi B., Peters A., Ehlers R.-U. *Steinernema bicornutum* sp. n. (Rhabditida: Steinernematidae) из Воеводина, Югославия.

Резюме. Представители нового вида *Steinernema bicornutum* sp. n. были выделены из почвенных проб, собранных в Стразилово (Воеводина, Югославия) с использованием в качестве приманки гусениц последнего возраста большой вощинной моли *Galleria mellonella*. *S. bicornutum* sp. n. отличается от других видов рода наличием двух выступающих рожковидных папилл на головном конце инвазионной личинки. Средняя длина инвазионных личинок 770 мкм, в латеральном поле 8 кутикулярных ребер. Только самцы и самки второй генерации имеют небольшой мукро. Описаны два типа спикул у самцов первой генерации. Особи нового вида не скрещиваются с *S. carpocapsae* и *S. feltiae*, в том числе с особями последнего вида, выделенными из места обнаружения *S. bicornutum* sp. n. По длине фрагментов, получаемых при рестрикции одной из повторяющихся спейсерных последовательностей рибосомальной ДНК, новый вид отличается от всех известных видов штейнернем. Инвазионные личинки с аналогичными рожковидными придатками были обнаружены также в Германии и Испании. Аксенные и моноксенные культуры нового вида поддерживаются на искусственных средах. Предлагается модификация быстрого метода обезвоживания нематод при изготовлении постоянных препаратов.