Russian Journal of Nematology, 2004, 12 (1), 85 - 95

# *Steinernema jollieti* sp. n. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from the American Midwest

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Accepted for publication 3 March 2004

**Summary.** *Steinernema jollieti* sp. n. is described from a soil sample collected in a woodland in the Missouri valley near St. Louis, USA in 1999. The general morphology and sequences of the ITS-rDNA indicate that the new species is related to species with widespread distribution, such as *S. feltiae* and *S. kraussei*. A characteristic feature of *S. jollieti* sp. n. infective juveniles is the presence of only 6 equal and equally spaced longitudinal ridges in the lateral field at the mid-body. Infective juveniles of *S. jollieti* sp. n. are also characterized by their straight or only slightly curved body when heat-killed, by the shape of their "medium" length (average 800-900 μm), the excretory pore at the mid-pharynx position and a hyaline tail portion of about half of the total tail length. Only males of the second generation have a mucron on the tail tip. Spicules are dark-yellow, 55-70 μm or 48-63 μm long in the first and second generation, respectively. According to ITS rDNA sequences, *S. jollieti* sp. n. belongs to the *Steinernema* clade composed of species such as *S. kraussei, S. feltiae, S. weiseri,* and *S. oregonense*. The new species shows a high level of nucleotide differences in ITS rDNA sequences with all these steinernematid species.

**Key words:** entomopathogenic nematode, Midwest USA, molecular characterization, morphology, *Steinernema jollieti* sp. n.

The number of described species in the genus *Steinernema* Travassos, 1927, increased markedly in recent last years. Currently more than 30 species of steinernematids are known (Mrácek *et al.*, 2003) and it is most probable that our knowledge covers only a minor part of the steinernematid diversity. Numerous steinernematid species detected through molecular or morphological analysis remain to be described (Hominick *et al.*, 1997, Hominick, 2002).

Several separate taxonomic units at species level were detected during the phylogenetic analysis of *Steinernema* spp. ITS sequences performed at the Agricultural Research Centre, Merelbeke, Belgium. One of these *Steinernema* species was collected in 1999 in woodlands of lower Missouri valley near St. Louis. It was found after intensive *Galleria* baiting of collected soil.

## MATERIALS AND METHODS

**Isolate culturing.** The laboratory culture of this new species was established in 1999 and was initiated from juveniles extracted from soil using the *Galleria* baiting technique. For further culturing, *Galleria* larvae were inoculated in Petri-dishes lined with filter paper and the emerging progeny was collected and stored in a 5-10 mm water layer in plastic containers. *Galleria* cadavers were dissected on every day between day 4 and 10 post inoculation; adults were collected with a needle. Adults of the first generation were collected on days 4 and 5; adults of the second generation were collected on days 8 and 9. For the study of their morphology, adult nematodes were killed with 4% formaldehyde, transferred to glycerin by a slow evaporation method and

mounted in dehydrated glycerin on slides. Formaldehyde fixed juveniles were also used for SEM study.

**Molecular characterization.** About a dozen juveniles were collected in 8 µl of worm-lysis buffer (100 mM KCl, 20 mM Tris-HCl pH 8.3, 3 mM $MgCl_2$, 2 mM DDT and 0.9 % Tween 20) and homogenized (Joyce *et al.*, 1994). Primer pair of 18S (5'-TTGATTAGGTCCCTGCCCTTT-3') and 26S (5'-TTTCACTCGCCGTTACTAAGG-3'), as described by Vrain *et al.* (1992), was used for PCR amplification of the ITS rDNA. The obtained PCR products were cleaned using Qiaquick PCR or Gel Purification Kit (Qiagen Ltd, Crawley, UK). Purified PCR products were used for cloning in competent cells of *Escherichia coli* according to the Promega protocol (pGEM T Vector System, Promega Benelux b.v. Leiden, the Netherlands). Small amounts of transformant colonies were used for PCR reactions; the resulting product was cleaned and used for sequencing using the primer pairs 18S, 26S (Vrain *et al.*, 1992), and M1 (5'-ACGAGCCGAGTGATCCACCG-3') and M2 (5'-CTTATCGGTGGATCACTCGG-3'). For the sequencing reaction the mixture of 8 µl BigDye Terminator v3.0 cycle sequencing ready reaction mix (Applied Biosystems, Warrington, UK) and 0.5 µl of each primer were used with 1-3 µl of PCR product. The volume of the sequencing mix was adjusted to 20 µl with double-distilled water. Nucleotides and other DNA remnants were removed with sequencing Gel Filtration cartridges (Edge Biosystems, Inc., Gaithersburg, USA). The ITS1-5.8S-ITS2 sequence obtained for the new species was deposited in GenBank under accession number AY171265.

Two previously published ITS sequences from GenBank - *S. monticolum* – AF331914 and of *S. oregonense* - AF122019 - were used for the analysis of the taxonomic position of the new species. Several unpublished sequences obtained during wider taxonomic study of *Steinernema* phylogeny (Spiridonov *et al.*, unpublished) were also used (*S. feltiae* from Armenia: A 171256; *S. feltiae*: from St. Bernard; Switzerland: AY171247; *S. feltiae* from Tomsk, Siberia: AY171272; *S. feltiae* from Izhevsk, Russia (topotype culture): AY171246; *S. kraussei* from Moscow region: AY171264; *S. kraussei* from Altai mountains in Siberia: AY171270; *S. kraussei* from Belgium: AY171250. The sequence obtained from the German population of *Steinernema weiseri*: AY171268, and that for an undescribed *Steinernema* sp. "B": AY171255 were also used in the analyses. Alignments of these sequences were generated using Clustal X program under default values (Thompson *et al.*, 1994). Maximum parsimony analyses were conducted with PAUP* 4.0b8 (Swofford, 1998). The heuristic search procedure was used with 100 replicates of random taxon addition. Gaps were treated as missing data. Bootstrap (BS) analysis with 100 replicates was conducted to assess the degree of support for each branch on the tree (Felsenstein, 1985) using simple addition sequences with TBR swapping. Trees were displayed with TreeView 1.6.1 (Page, 1996).

## DESCRIPTIONS

### *Steinernema jollieti* sp. n. (Figs. 1–5, Tables 1-4)

**Morphometrics.** Measurements of infective-stage juveniles and of adults of the first and second generation are presented in Tables 1-3.

**Infective third-stage juveniles**. Bodies of heat-killed juveniles straight or slightly curved on ventral side, tapering toward anterior and posterior ends. Cuticular annulation (about 1.4-1.9 µm wide at mid-body) visible throughout body, except at lip region and tail terminus. Cephalic end rounded, continuous with body profile. Lateral field with six well developed ridges in the middle of body. Equal spaces between ridges in anterior body half and near body middle (Fig. 3C, D). Wider gaps between marginal and submarginal ridges in tail region. Deirids at basal bulb level, with prominent hemizonid as a 4 µm long cuticular depression closely anterior to deirids. Outer body cuticle over hemizonid slightly swollen or impressed. Labial papillae indistinct, four cephalic sensilla and amphidial pouches occasionally visible. Pharynx corpus slender, spindle-shaped with only minor widening in its middle. Excretory pore at level of mid-pharynx, encircled with refractive ring contacting body cuticle. Strongly cuticularised 2-3 µm long tube linking excretory duct with excretory channel situated usually at bulbus-isthmus junction. Cardia 8-9 µm long, with rounded posterior end. Bacterial vesicle with well developed 1 µm thick walls. Intestine with lumen visible only around vesicle. Rectum about 1 anal body diameters long. Genital primordium 130-180 µm long. Phasmidial opening near mid-tail. Tail conical, straight or slightly bent to ventral side. Tail tip pointed, usually with spike-like mucron. Refractive inclusion discernible inside tail tip in majority of specimens. Tail hypoderm ending with

rounded protuberance rarely bearing one or two pointed projections.

**First generation males.** Body quite slender, usually "J"-shaped in heat-killed specimens or with coiled posterior end. Cuticle annulation undistinguishable throughout the body. Anterior end rounded. Six labial papillae present, which appear as small (less than 0.5 µm high) pointed spikes around the oral opening. Circle of four more protruding cephalic papillae (around 1 µm high) behind labial ones. Cheilostom cuticle connected to a 3 µm thick and 3.5 µm long strongly cuticularised ring, forming the middle part of stoma walls (Fig. 1A). Pharynx lumen tightly closed up to 7 µm from stoma bottom. Pharynx corpus widening from 12-13 µm diameter near bottom of stoma up to 19-20 µm before isthmus. Isthmus 12-14 µm wide. Basal bulb subpyriform; valves as thin folded platelets. Cardia protruding up to 15 µm into intestine proventriculus, often asymmetrical. Deirids behind level of pharynx-intestine junction. No cuticle elevation around excretory pore. Anterior part of intestine with widened lumen and pronounced microvillar layer. Testis flexure 250-400 µm behind basal bulb. Intestinal lumen collapsed at body middle but discernible near cloaca. Rectal glandular cells present around spicular muscles. Typical steinernematid set of genital papillae with a row of usually nine pairs of subventral papillae extending from tail terminus to body middle, one unpaired large precloacal papilla in midventral position, one pair of papillae in lateral position at manubrium level when spicules retracted, and one pair of papillae in dorso-lateral position in postcloacal area. This latter pair usually in mid-part of tail, being closer to tail terminus only in a few of the males examined. Posteriormost pair of subventral papillae close to tail tip, with neighboring pair situated 7-8 µm anteriorly. Next pair situated at cloaca level. In two cases ten pairs of subventral papillae were observed with eight in precloacal position. Spicules of slightly yellowish colouration, moderately to strongly curved, tapering toward distal end, with rounded tip. Manubrium more or less elongated. Velum poorly visible, usually expanding over only one quarter to one third of spicular length. Boat-shaped gubernaculum with narrowed and flattened distal end and wide inner space in the middle.

**Second generation males.** Body quite slender, always "J"-shaped in heat-killed specimens. Cheilostom cuticle connected to a 6-7 µm wide cuticularised ring, which forms buccal cavity. In some specimens buccal cavity widely opened (Fig. 1 C). Pharynx lumen collapsed up to 5-6 µm from stoma bottom. Pharynx corpus is widening from 10-12 µm diameter near stoma bottom up to 16-17 µm before isthmus. Isthmus 11-12 µm wide. Cuticle slightly elevated around excretory pore. Testis flexure 165-240 µm behind basal bulb. Intestinal lumen collapsed around body middle. Genital papillae as in first generation males. Prominent 5-8 µm long conical mucron. Posteriormost pair of subventral papillae behind mucron, with next subventral pair situated in 5 µm anteriorly. Spicules with velum longer and manubrium more elongated than in first generation males.

**First generation females.** Body "C"-shaped to semicircular in heat-killed specimens. Cuticle annulation not discernible. Stoma 8 µm wide and 6 µm deep, with cylinder-like inner space and strongly cuticularised walls. Excretory opening with slightly protruding pore margins. Vulva opens on body swelling with vulvar lips being lower than maximal protrusions of these swellings (Fig. 1E). Genital tract amphidelphic with reflexed ovaries. Short chains of 3 to eight spermatozoa, of 6-8 µm diameter each, in distal part of uterus lumen. Anus without postanal swelling. Tail conical, with spike-like terminal mucron.

**Second generation females.** Buccal cavity funnel-shaped, widening from 3-4 µm at bottom up to 10-11 µm anteriorly. Vulvar lips prominently protruding.

**Molecular characterization.** Different methods of the phylogenetic analyses were applied to the alignments obtained for *Steinernema jollieti* sp. n. *Steinernema monticolum* was choosen as the outgroup, as the maximum parsimony phylogenetic analysis of a wider set of steinernematid species consistently placed this species as a sister taxon to the clade composed of *S. feltiae, S. kraussei, S. oregonense, S. weiseri, Steinernema jollieti* sp. n., *Steinernema* sp. "B". The latter species is reported throughout Europe and designated as species "B" (Reid *at al.* 1997) or "B3" (Sturhan, 1997, 1999). Maximum parsimony analysis based on Clustal X generated alignments of *S. jollieti* sp. n. and related species placed this new species into a clade with *S. feltiae* and *S. weiseri* (Fig. 4). Maximum parsimony analysis of distances between the studied sequences supported

**Table 1**. Comparative morphometrics for infective-stage juveniles of *Steinernema jollieti* sp. n. and two closely related species (mean, standard deviation, range; all measurements in μm).

| Character | *Steinernema jollieti sp. n.* (n=25) | *Steinernema weiseri* (after Mracek et al., 2003 – paratypes) | *Steinernema feltiae* (n=25) topotype isolate) |
|---|---|---|---|
| Body length | 711±59 (625-820) | 740±68 (586-828) | 901±42(830-997) |
| Greatest width | 23±1.9 (20-28) | 25±1.4 (24-29) | 31±2.4 (27-38) |
| Pharynx length | 123±5.8 (115-135) | 113±6.2 (95-119) | 135±7.4 (126-163) |
| Anterior end to excretory pore | 60±3.1 (53-65) | 57±5.3 (43-65) | 64±3( 61-72) |
| Tail length | 68±3.7 (60-73) | 60±5.2 (49-68) | 77±4.2(71-86) |
| Anal body width | 15±1.2 (13-18) | 17±1.3 (14-19) | 18±1.4 (16-20) |
| Hyaline tail length (H) | 37±3.5 (28-43) | 22±1.8 (18-24) | 36±4.2 (29-42) |
| a | 30.5±2.1 (25-34.1) | 29±2.0 (25-33) | 29.6±2.0 (25.4-34.6) |
| b | 5.7±0.33 (4.9-6.4) | 6.6±0.5 (5.7-7.2) | 6.7±0.3 (6.0-7.3) |
| c | 10.5±0.67 (9.0-11.7) | 12±0.9 (10-14) | 11.6±0.7 (10.3-12.9) |
| D % | 48±1.5 (46-50) | 51±3.0 (44-55) | 47±2 (44-53) |
| H % | 55±3.3 (46-60) | 36±1.3 (34-39) | 49±3.4 (38-59) |

**Table 2**. *Steinernema jollieti sp. n.*, morphometrics of holotype and paratype males (mean, standard deviation, range; all measurements in μm).

| Character | Holotype – first generation male | First generation (4-5 days post inoculation) (n=12) | Second generation (8-9 days post inoculation) (n=12) |
|---|---|---|---|
| Body length | 1653 | 1662± 203(1296 – 1952) | 1000±74.8 (891 – 1134) |
| Greatest width | 108 | 115±13 (98 – 135) | 57±3.7 (50 – 63) |
| Pharynx length | 151 | 156±16 (110 – 168) | 126±6.5 (113 – 135) |
| Anterior end to excretory pore | 106 | 98±8.0 (83 – 110) | 79±6.8 (70 – 93) |
| Tail length | 35 | 33±4.2 (24 – 38) | 24.6±2.4 (20 – 30) |
| Anal body width | 44 | 44±2.9 (40 – 50) | 31±4.9 (24 – 38) |
| Spicule length | 68 | 64±5.4 (55 – 70) | 58±5.6 (48 – 63) |
| Gubernaculum length | 53 | 54±4.8 (45 – 60) | 42±3.4 (35 – 45) |
| a | 15.3 | 14.5±1.9 (12.1 – 18.9) | 17.7±1.7 (15.3 – 21.4) |
| b | 11.0 | 10.7±1.5 (8.1 – 14.1) | 7.9±0.6 (7.1 – 8.9) |
| c | 47.2 | 51.1±9.0 (53 – 86) | 40.1±3.7 (35.4 – 46.1) |
| D % | 70 | 64±8.9 (53 – 83) | 63±5.2 (53 – 71) |

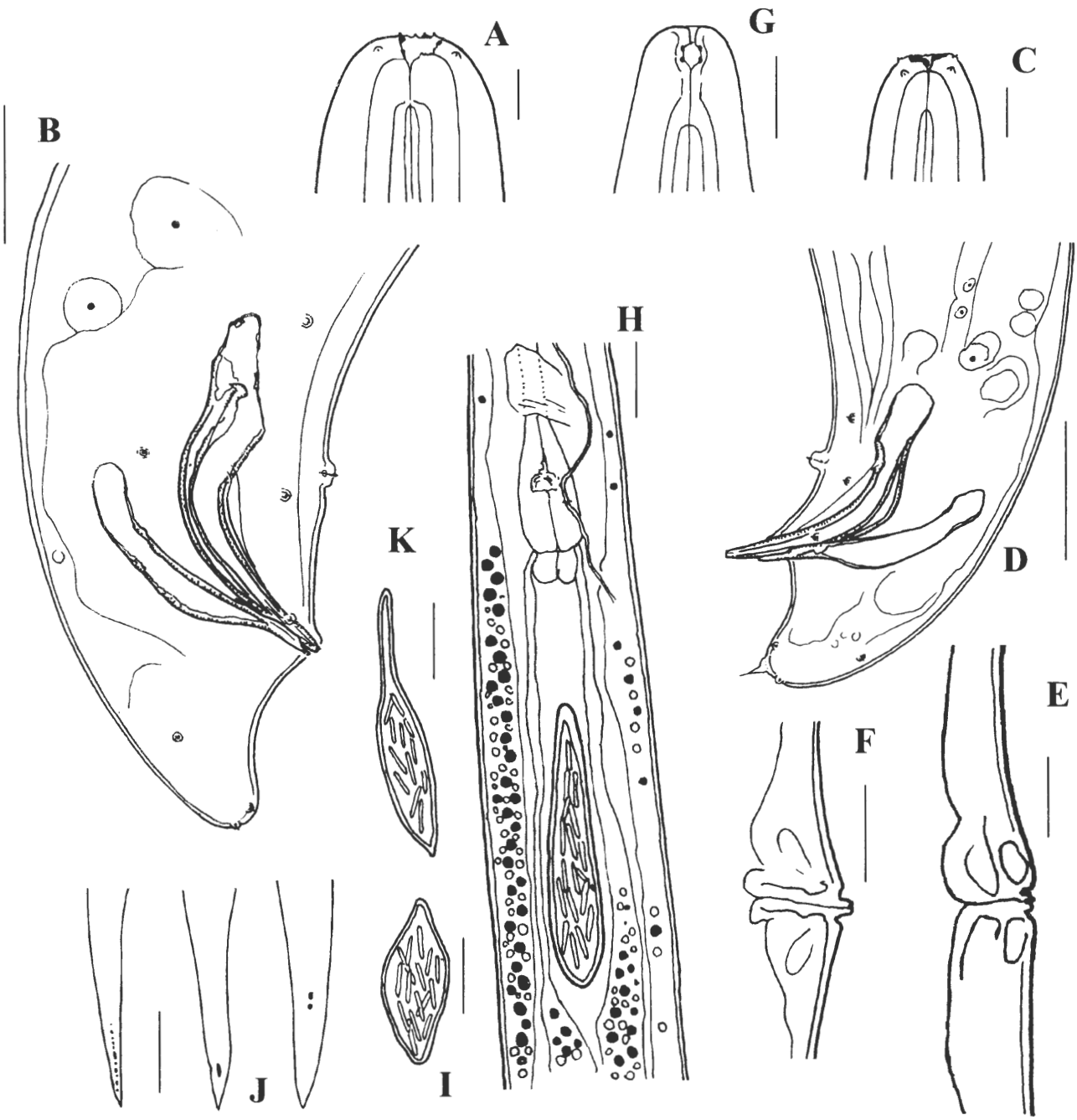


**Fig. 1.** Morphology of *Steinernema jollieti sp. n.* A: anterior end of first generation male; B: tail region of first generation male; C: anterior end of second generation male; D: tail region of second generation male; E: vulva lips of first generation female; F: vulva lips of second generation female; G: anterior end of infective juveniles; H: Pharynx-intestine junction of infective juvenile; K,I: shapes of bacterial vesicle in infective juveniles; J: variation in tail mucron tips of infective juveniles. (All drawings are in lateral aspect, Scale bars: A, C, G, H, K, I: 10 μm, B, D: 25 μm; E,F: 25 μm.)

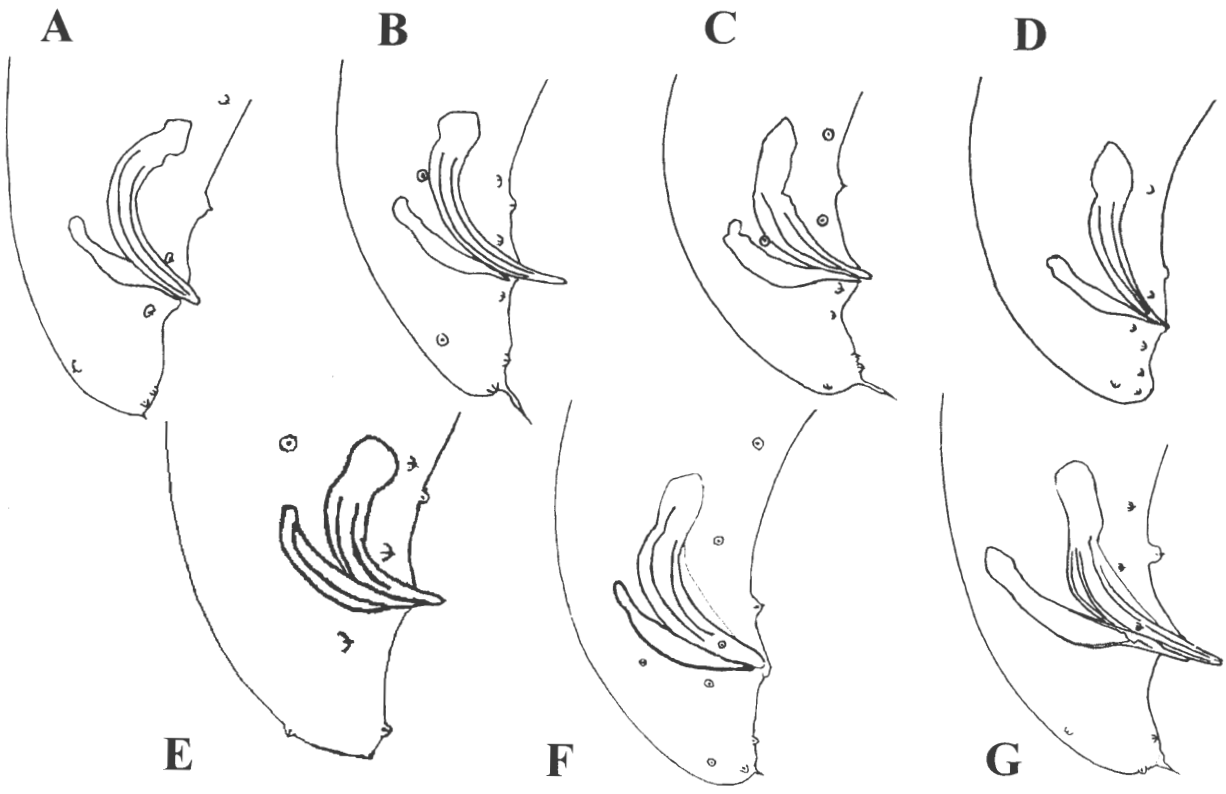


**Fig. 2.** Disposition of papillae in steinernematid males. A: *Steinernema kraussei*; B: *S.* sp. "B", C: *S. feltiae*; D: *S. oregonense*; E: *S. sangi*; F: *S. weiseri*; G: *S. jollieti* sp. n. (No scale; A - C, G – original drawings from second generation males; D - F – redrawn from original decsriptions).

**Table 3**. *Steinernema jollieti sp. n.*, morphometrics of paratype females (mean, standard deviation, range; all measurements in µm).

| Character | First generation (4-5 days post inoculation) (n=12) | Second generation (8-9 days post inoculation) (n=12) |
|---|---|---|
| Body length | 5148±662 (3746-6030) | 1509±126 (1332-1692) |
| Greatest width | 259±24 (212-298) | 103±17 (85-130) |
| Pharynx length | 214±33 (184-310) | 155±7 (145-165) |
| Anterior end to excretory pore | 111±13 (96-136) | 64±3.3 (60-68) |
| Tail length | 43±8.0 (31-55) | 49±7.3 (38-61) |
| a | 20±2.7 (15-24) | 15±2.2 (12.5-19.1) |
| b | 25.6±3.8 (19-31) | 9.7±0.8 (8.3-10.7) |
| c | 128±38 (72-185) | 31±5.2 (26-43) |
| V | 51±3.8 (44-56) | 54±2.8 (52-59) |

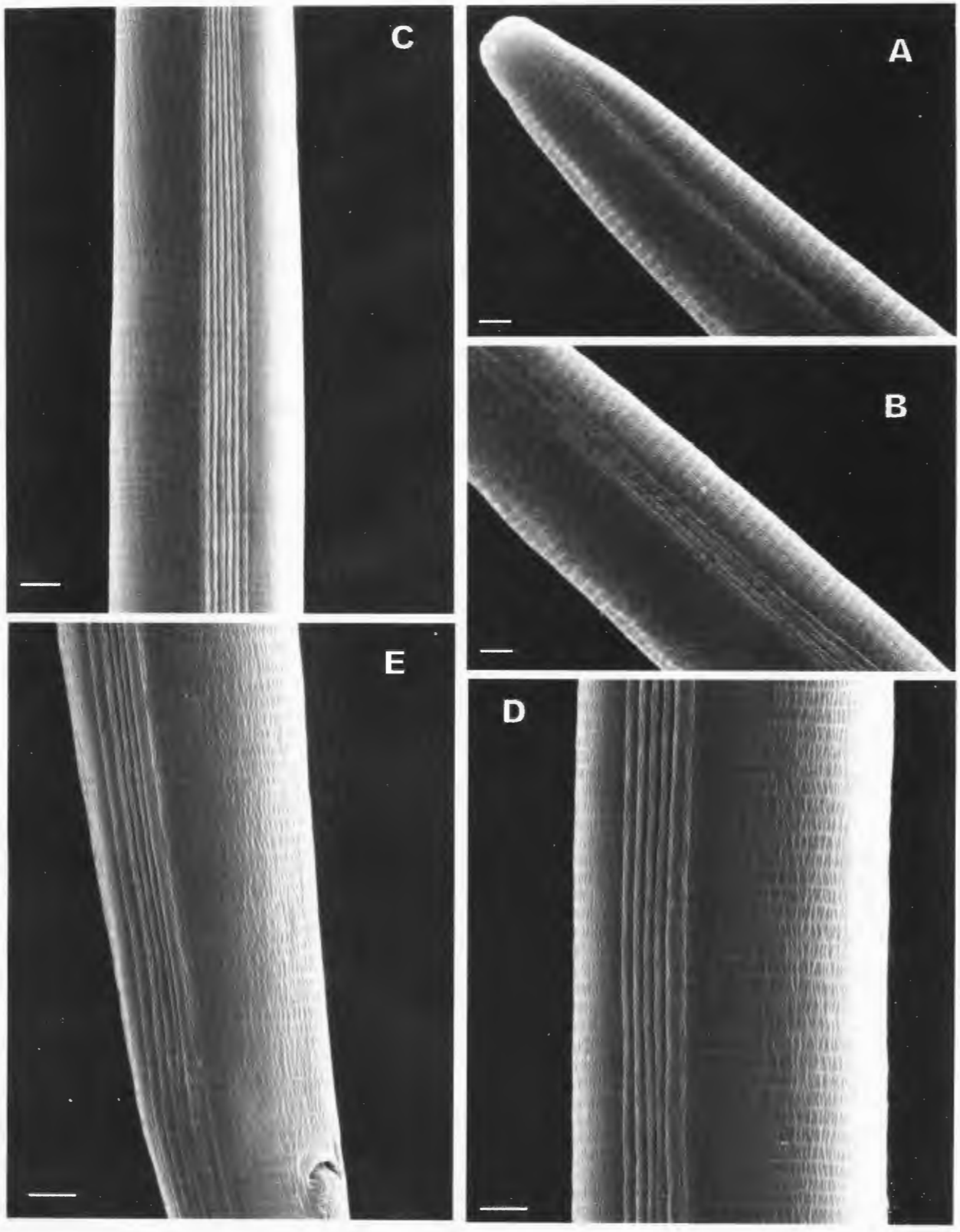


**Fig. 3**. *Steinernema jollieti sp. n.,* SEM morphology of infective juveniles. A: - anterior end; B: beginning of prominent ridges in lateral field; C: lateral field appearance in anterior third of body length ("neck" region); D: lateral field at body middle; E: lateral field near the tail. Scale – 5 μm.

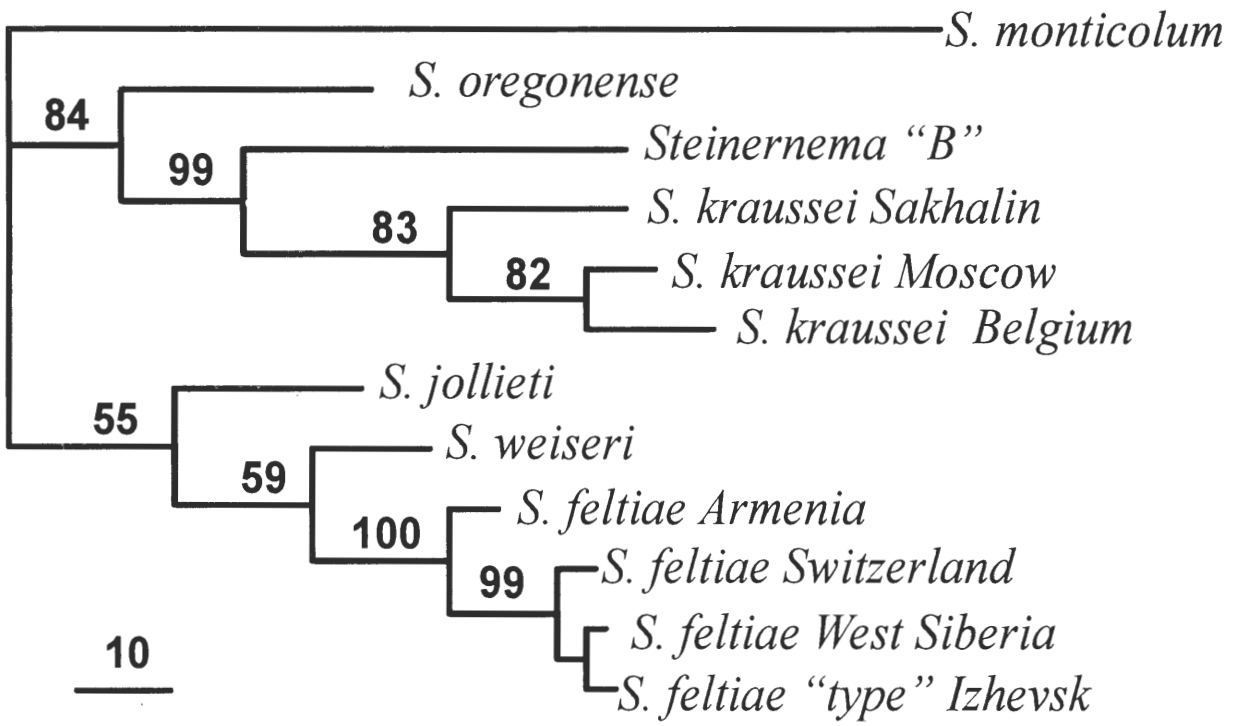


**Fig. 4.** Maximum parsimony phylogram of relationships between *Steinernema jollieti* sp. n. and related species based on ITS sequences of rDNA. (of 803 total characters - 604 characters are constant, 109 variable characters are parsimony-uninformative and 90 are parsimony-informative; tree length = 304; consistency index = 0.8388; homoplasy index = 0.1612; retention index = 0.8008.

**Table 4**. Pairwise ITS rDNA nucleotide differences between *Steinernema jollieti* sp. n. and related species. (Below diagonal: Total character differences – number of different nucleotides in the sequences under comparison, which were considered by maximum parsimony analysis as "parsimony-informative". Above diagonal: Mean character differences (adjusted for missing data) expressed as the percentage of differences in nucleotide sequences; the range of distances is given when several populations of the same species were tested.

| | *S. kraussei* | *Steinernema* sp. B | *S. oregonense* | *S. feltiae* | *S. weiseri* | *S. jollieti* | *S.monticolum* |
|---|---|---|---|---|---|---|---|
| *S. kraussei* | | 3.5-5.1 | 5.3-6.9 | 9.3-10.5 | 8.3-9.3 | 8.5-9.4 | 16.7-17.8 |
| *Steinernema* sp.*B* | 26-37 | | 6.7 | 9.8-10.3 | 9.1 | 9.1 | 18 |
| *S. oregonense* | 39-51 | 49 | | 7.6-8.6 | 6.3 | 7.5 | 16.8 |
| *S. feltiae* | 66-76 | 72-76 | 55-63 | | 4.2-5.3 | 6.5-7.2 | 16.8-18.3 |
| *S. weiseri* | 61-68 | 66 | 46 | 37 | | 5.7 | 16.3 |
| *S. jollieti* | 62-68 | 66 | 54 | 50 | 41 | | 15.5 |
| *S. monticolum* | 111-117 | 118 | 110 | 110-121 | 107 | 102 | |

close relationships between *S. jollieti* sp. n. and *Steinernema weiseri* (Table 4). Observed level of nucleotide differences between these two species was equal or greater than that between morphologically distinct species such as *S. feltiae* and *S. weiseri* or *S. kraussei* and *S. oregonense*.

**Life cycle.** Development of *S. jollieti* sp. n. development was observed in *Galleria mellonella* caterpillars inoculated with 20-50 IJs in Petri dishes lined with wet filter paper and kept at 20-24°C. First generation males and females were abundant on the 4-5th day after inoculation, and second generation individuals were found inside insects on 9th-10th days. The beginning of migration of infective juveniles was recorded on 13-17 day after inoculation.

**Bacterial symbiont.** The bacterium associated with *Steinernema jollieti* sp. n., *Xenorhabdus bovienii,* is currently sequenced under a grant from the USDA. Data for the sequence of the bacterium is at www.xenorhabdus.org

**Type locality**. *Steinernema jollieti* sp. n. originated from a soil sample collected close to stream waterbed in the Bush-Augusta State Park region near St. Louis. The vegetation consisted of several North American species of oaks with shrubs as undergrowth. The sample was collected from heaps of soil blocks displaced by the stream and alluvial sediments. The new steinernematids were accompanied in the sample by a *Heterorhabditis* sp. Hosts for both entomopathogenic nematode species are unknown.

**Type material.** Holotype males and paratype males, females and juveniles are deposited in the Institute of Parasitology, Russian Academy of Sciences, Moscow. Additional paratypes are deposited in the British Museum of Natural History, Musee National d'Histoire Naturelle in Paris and Museum voor Dierkunde, University Gent, Belgium. The type isolate is presently maintained on *G. mellonella* in the Institute of Parasitology, Russian Academy of Sciences, Moscow.

**Differential diagnosis.** Steinernema jollieti *sp. n. is distinguished from described species of the* genus by the following characters: body of the infective-stage juveniles slightly C-shaped or straight with medium length (625-820 μm), lateral field with equally spaced and developed six ridges at body middle, cephalic region rather flat and broad, excretory pore at level of middle of pharynx and average hyaline tail portion around half of total tail length. Males have yellowish spicules (55-70 μm long in the first generation, 48-63 μm in the second generation), with elongated manubria and a boat-shaped gubernaculum (45-60 μm long in the first generation, 35-45 μm in the second generation). Only two pairs of subventral and one pair of subdorsal papillae are situated in the postcloacal area of male tail and only second generation males have a mucronate tail.

**Taxonomic remarks.** The phylogenetic analysis of a broad set of steinernematid species and populations placed *Steinernema jollieti* sp. n. in a clade composed of S. *feltiae, S. kraussei, S. oregonense, S. weiseri* and the undescribed *Steinernema* sp. "B" (Spiridonov *et al.*, unpublished). Spermatozoa of *S. jollieti* sp. n. are amoeboid cells of 6-8 μm diameter, forming short chains of 5-8 cells. Such a structure is also found in *S. feltiae* and *S. kraussei* (Spiridonov *et al.*, 1999). The length of both spicules and gubernaculums of *S. jollieti* sp. n. is very similar to those of S. *feltiae and S. weiseri.* (Mrácek *et al.*, 2003).

Within the clade *S. jollieti* sp. n. can be distinguished from the other species by the structure of the lateral field in infective juveniles (Fig. 3). The presence of only 6 equally developed ridges separated by gaps of similar width near the middle of the body has not been reported for any other species. *Steinernema kraussei* is characterized by the presence of three ridges in the central part of the lateral field, two well developed marginal ridges, and two less developed submarginal ridges (total number of ridges: 7). Both *S. weiseri* and *Steinernema* sp. "B" have a lateral field composed of 8 equal ridges. *Steinernema feltiae* is characterized by the presence of four ridges in the centre of the lateral field, two well developed marginal ones and two less developed submarginal ridges (total number of ridges: 8). In some aspects, the gaps between ridges of lateral field of *S. jollieti* sp. n. do not look equal (e.g. near the tail, Fig. 3 E), but with submarginal ridges being absent, the general pattern is still very different from that of *S. feltiae* (Kozodoi & Spiridonov, 1988). A lateral field pattern similar to that of *S. feltiae* was reported for *S. sangi* Phan, Nguyen and Moens, 2001 described from Vietnam (Phan *et al.*, 2001). This latter species can also be distinguished from *S. jollieti* sp. n. by the presence of submarginal ridges. In the original description of *S. oregonense,* the presence of six or eight ridges of the lateral

field is mentioned (Liu & Berry, 1996). Our examination of some populations identified as *S. oregonense* via sequencing of the ITS revealed a lateral field pattern similar to that of *S. kraussei, i.e.* five well developed ridges and two weak submarginal ones. Additional studies are needed to supplement the original description of *S. oregonense* using the specimens from type culture, but lateral fields of *S. jollieti* sp. n. do not demonstrate the presence of eight ridges at any part of juvenile body.

The phylogenetic analysis of ITS sequences clusters *S. jollieti* sp. n. closely to *S. weiseri* and *S. feltiae*. Infective juveniles of these three species are quite similar in their general morphometrics (Table 1). *Steinernema feltiae* infective juveniles, however, are usually longer than those of *S. jollieti* sp. n. and have longer tails. The hyaline part of tail is shorter in *S. weiseri* and correspondingly the H% value is lower (Table 1).

The distribution of the genital papillae of *S. jollieti* sp. n. is also very specific (Fig. 2). Only two terminal pairs of subventral papillae and one pair of subdorsal papillae are present in the postcloacal region of the tail in this species. All other steinernematids of this group are characterized by the presence of at least one pair of subventral papillae behind the cloaca level.

The maximum parsimony phylogenetic tree obtained for a clade of species related to *S. jollieti* sp. n. were in good concordance (Fig. 5). Bootstrap support for nodes linking *S. jollieti* sp. n. with the closely related species (*S. feltiae, S. weiseri*) was always weak. Pairwise nucleotide differences between these steinernematids are presented in Table 4. *S. jollieti* sp. n. differs from the closest steinernematid – *S. weiseri* in 5.7% of nucleotides. The reported level of differences between the two closest species (*S. feltiae* and *S. weiseri*) was lower: 4.2-5.3%.

**Etymology.** The species is named after Louis Jolliet, the first European explorer of this region of American Midwest during his voyage down the Mississippi River together with Jacques Marquette in 1673.

## ACKNOWLEDGEMENTS

Support was provided for the Russian author by Monsanto Company and Agricultural Research Center in Merelbeke, Belgium. Steinernematid researches inside Russia were supported by RFBR grant 02-04-48389.

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**Спиридонов С.Э., Красомил-Остерфельд К., Мунс М.** *Steinernema jollieti* sp. n. (Rhabditida: Steinernematidae), - новый вид энтомопатогенных нематод со Среднего Запада США.

**Резюме.** В почвенных пробах, собранных в лесах нижней долины Миссури близ Сент-Луиса, США, в 1999 обнаружен новый вид штейнернематид - *Steinernema jollieti* sp. n. По морфологии и нуклеотидным последовательностям новый вид близок к широко распространенным видам *S. feltiae* и *S. kraussei*. Характерная особенность инвазионных личинок *S. jollieti* sp. n. – наличие только на уровне середины тела шести кутикулярных ребер латерального поля, разделенных равными промежутками. Кроме того, инвазионные личинки *S. jollieti* sp. n. отличаются по их прямому или лишь слегка изогнутому телу у особей, зафиксированных с нагреванием, длиной около 800-900 мкм, положением экскреторной поры на уровне середины пищевода, гиалиновой частью, составляющей около половины хвостового конца. Лишь у самцов второго поколения этого вида отмечено наличие мукро. Спикулы *S. jollieti* sp. n. темно-желтые, длиной 55-70 мкм и 48-63 мкм у самцов первого и второго поколений, соответственно. По последовательностям ITS rDNA, *S. jollieti* sp. n. принадлежит к группе видов *Steinernema,* включающей *S. kraussei, S. feltiae, S. weiseri* и *S. oregonense*. Новый вид демонстрирует высокий уровень нуклеотидных различий ITS – участка рибосомальной ДНК со всеми этими видами.