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Steinernema scapterisci n. sp. (Rhabditida: Steinernematidae)[1]

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Abstract: *Steinernema scapterisci* n. sp., isolated in Uruguay from the mole cricket *Scapteriscus vicinus*, can be distinguished from other members in the genus by the presence of prominent cheilorhabdions, an elliptically shaped structure associated with the excretory duct, and a double-flapped epitygma in the first-generation female. The spicules of the male are pointed, tapering smoothly to a small terminus, and the shaft (calomus) is long, bearing a sheath. The gubernaculum has a long, upward-bent anterior part. The ratio of head to excretory pore divided by tail length of the third-stage juvenile is greater for *S. scapterisci* n. sp. than for *S. carpocapsae*. *Steinernema scapterisci* n. sp. did not hybridize with *S. carpocapsae* strain Breton. In laboratory tests, *S. scapterisci* n. sp. killed 10% or less of non-orthopteran insects, including the wax moth larva, a universal host for other species of *Steinernema*.

Key words: biological control, entomopathogenic nematode, mole cricket parasite, morphology, new species, *Neoaplectana*, *Scapteriscus*, *Steinernema scapterisci* n. sp., taxonomy, light microscopy (LM), scanning electron microscopy (SEM).

In 1985, several isolates of a steinernematid nematode collected from mole crickets in Uruguay were brought to Florida and cultured. The morphology and biology of the nematode showed that it is a new species of *Steinernema* Travassos, 1927.

The genus *Steinernema* was erected by Travassos in 1927 (6) for the species *Aplectana kraussei* which Steiner (3) had described in 1923 from a sawfly (*Cephaleia abietis*). Steiner (4) established the genus *Neoaplectana* in 1929 for *N. glaseri* which he described from the Japanese beetle (*Popillia japonica*).

Wouts et al. (7) considered *Neoaplectana* a junior synonym of *Steinernema*. We concur with these authors and describe our nematode herein as *Steinernema scapterisci* n. sp., named after its host, *Scapteriscus* (Orthoptera), a genus containing mole crickets.

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Materials and Methods

Nematodes collected in Uruguay were placed on moist filter paper in several vials, a live mole cricket (*Scapteriscus vicinus*) was placed in each vial, and the vials were placed in an insulated container with ice and hand carried to the quarantine facility in Gainesville, Florida. The populations of the nematode were increased in mole crickets (*S. vicinus* and *S. acletus*) and later in the house cricket (*Acheta domesticus*). These nematodes, or their progeny, were used for all studies.

Light microscopy (LM): First-generation and second-generation adult nematodes were obtained by dissecting infected mole crickets 2–3 and 5–7 days, respectively, after they died; J3 were obtained when they emerged from the cadavers in 7–15 days. The nematodes were killed and relaxed in warm water (40 C), then mounted in water on glass slides with coverglass supports. In addition, live specimens or those killed and stained with acid fuchsin were examined to confirm the presence and (or) detail of some structures.

Scanning electron microscopy (SEM): Specimens prepared for SEM were placed live in lactophenol at 43 C for 30 minutes, transferred to a desiccator for 2 days, removed, rinsed with water, and prepared by the method of Stone and Green (5). Spic-

ules and gubernacula were obtained from first-generation males placed in a petri dish containing water, heat killed, and stored at room temperature. After 2–3 days the bodies became softened by decay and were transferred to clean water. The posterior of each nematode was opened with two small needles, and the spicules and gubernaculum were dissected out and washed free of debris by shaking them in water. Then they were picked up with a fine needle and placed on a SEM stub close to a hair used for a marker. Specimens were examined with a Hitachi S450 SEM.

Cross hybridization: Two techniques were used. In the first, a drop of blood (hemolymph) from a mole cricket was placed in a sterile petri dish (35 × 10 mm), and one J3 of *S. scapterisci* n. sp. and one J3 of *S. carpocapsae* strain Breton were added. The dish was placed in a plastic bag containing a paper towel saturated with water, and the bag was closed, tied, and stored in the dark at 25 C. The treatment was replicated 25 times.

In the second technique, a drop of blood was placed in each of two petri dishes and 10 J3 of *S. scapterisci* n. sp. were placed in one drop and 10 J3 of *S. carpocapsae* strain Breton were placed in the other. The dishes were maintained as for the first technique. The treatment was replicated 10 times for each species. The nematodes were observed daily. When the sexes could be distinguished in the preadult stage, all males of *S. scapterisci* n. sp. were removed and placed in a new drop of blood and all males of *S. carpocapsae* strain Breton were placed in a separate drop of blood. Then the males of *S. scapterisci* n. sp. were transferred to the drop of blood containing females of *S. carpocapsae* strain Breton, and the males of *S. carpocapsae* strain Breton were transferred to the drop of blood containing females of *S. scapterisci* n. sp. The nematodes were observed for mating, continuously the first hour and then hourly for the remainder of the first day. On subsequent days they were observed hourly for mating, egg laying, or the presence of juveniles. Other specimens of each species were retained in drops of blood in two dishes as controls.

Comparative pathogenicity: Four species of insects in the order Lepidoptera—the fall army worm (*Spodoptera frugiperda*), the velvet beam caterpillar (*Anticarsia gemmatalis*), the granulate cut-worm (*Feltia subterrania*), and the greater wax-moth larva (*Galleria mellonella*)—were used to compare the rate of kill by *S. scapterisci* n. sp. to that of some other species and strains of *Steinernema.*

Two pieces of Whatman No. 2 filter paper were placed in a petri dish (100 × 15 mm), and 8,000 J3 in 2 ml water and 10 insects were added. Controls were prepared similarly, but without nematodes. Treatments were replicated four times. The dishes were maintained in the dark at 25 C. After 2 days the number of dead insects was determined.

Systematics

Steinernema scapterisci n. sp. (Figs. 1–8)

Holotype (male, first generation, in glycerine): Length 1,554 μm; width 131 μm; stoma length 3.9 μm; stoma width 6 μm; head to excretory pore 77 μm; head to nerve ring 130 μm; head to end of esophagus 189 μm; testis from reflexion to terminus 416 μm; body width at anus 37.5 μm; tail length 28 μm; spicule length 89 μm; spicule width 13.5 μm; gubernaculum length 65 μm; spicule width 13.5 μm; gubernaculum length 65 μm; gubernaculum width 7.8 μm; mucron length 4.5 μm.

Male, first generation: Measurements of 10 males in Table 1. Smaller than first-generation female. Body usually rotund, curved ventrally posteriorly (Fig. 4E), spiral or C shaped when heat-killed. Gonad monorchic, reflexed. Spicules paired, uniformly curved, dark brown in color; head large somewhat angular (Figs. 3D; 4A, B; 5B); shaft and blade angle averages 110 degrees (range 100–120); shaft appears encased in a sheath (Fig. 5B); blade tapers smoothly, posterior portion thin (Figs. 4A, 7A). In SEM cross section, spicule blade has two lumina (Fig. 5C), only one aperture

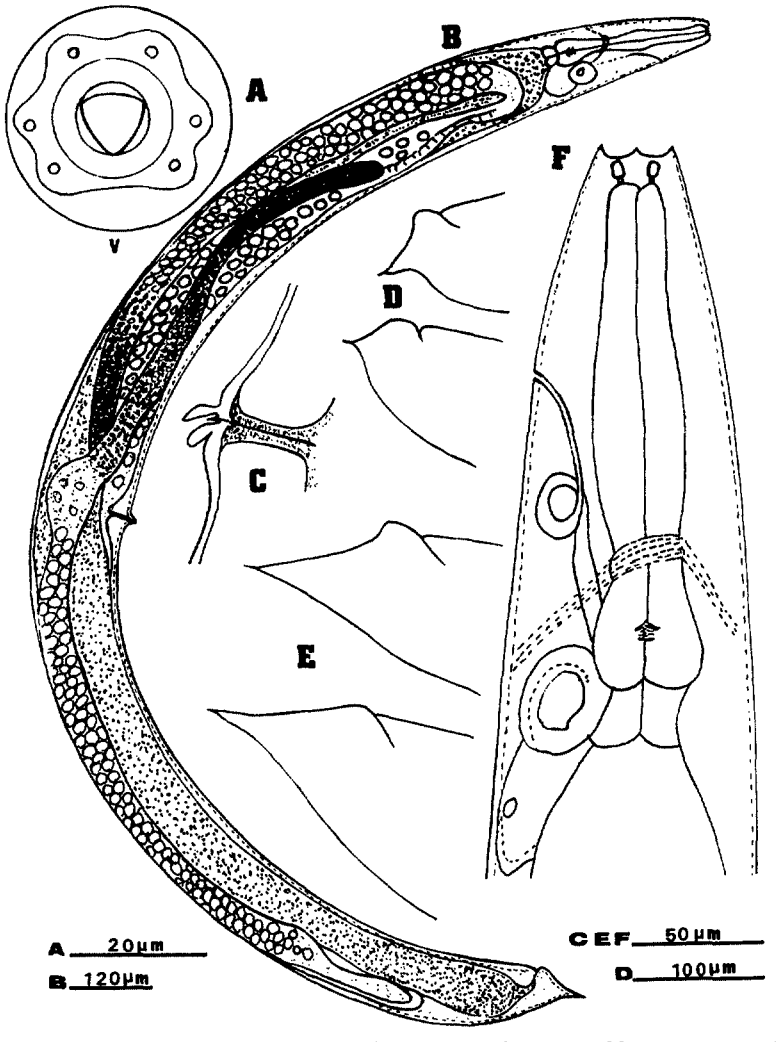


Fig. 1. Females of *Steinernema scapterisci* n. sp. A) Diagrammatic face view of first-generation female showing unevenly distributed papillae. B) Entire body of second-generation female. C) Double-flapped epiptygma on vulva of first-generation females. D) Variation in tails of first-generation females. E) Variation in tails of second-generation females. F) Anterior region of the first-generation female showing large cheilorhabdions, esophagus, nerve ring, excretory pore and duct, and elliptically shaped structure and gland cell associated with the excretory system.

observed on ventral side and close to tip (Fig. 7A). Each spicule has two internal ribs (Figs. 4A, B; 8F), with termination points variable. Gubernaculum boat-shaped, anterior part thin, ventrally curved, posterior end bifurcate (Fig. 5D). Cloacal area raised, anterior flap present. Ten pairs and one single genital papillae present (Fig. 8A). Pairs 1–5 preanal, subventral; single papilla preanal, ventral, between pairs 4 and 5; pairs 6–7 postanal, subventral; pairs 8 and 9 caudal, subventral; pair 10 caudal, subdorsal. Pairs 1 and 6 obscure. Tail conoid, mucron present (Fig. 4B).

Male, second generation: Measurements of 10 males in Table 1. Second-generation male similar to that of the first generation except that its length is shorter, 1,147 µm vs. 1,728 µm; and the width is about one-half that of the first-generation male. The

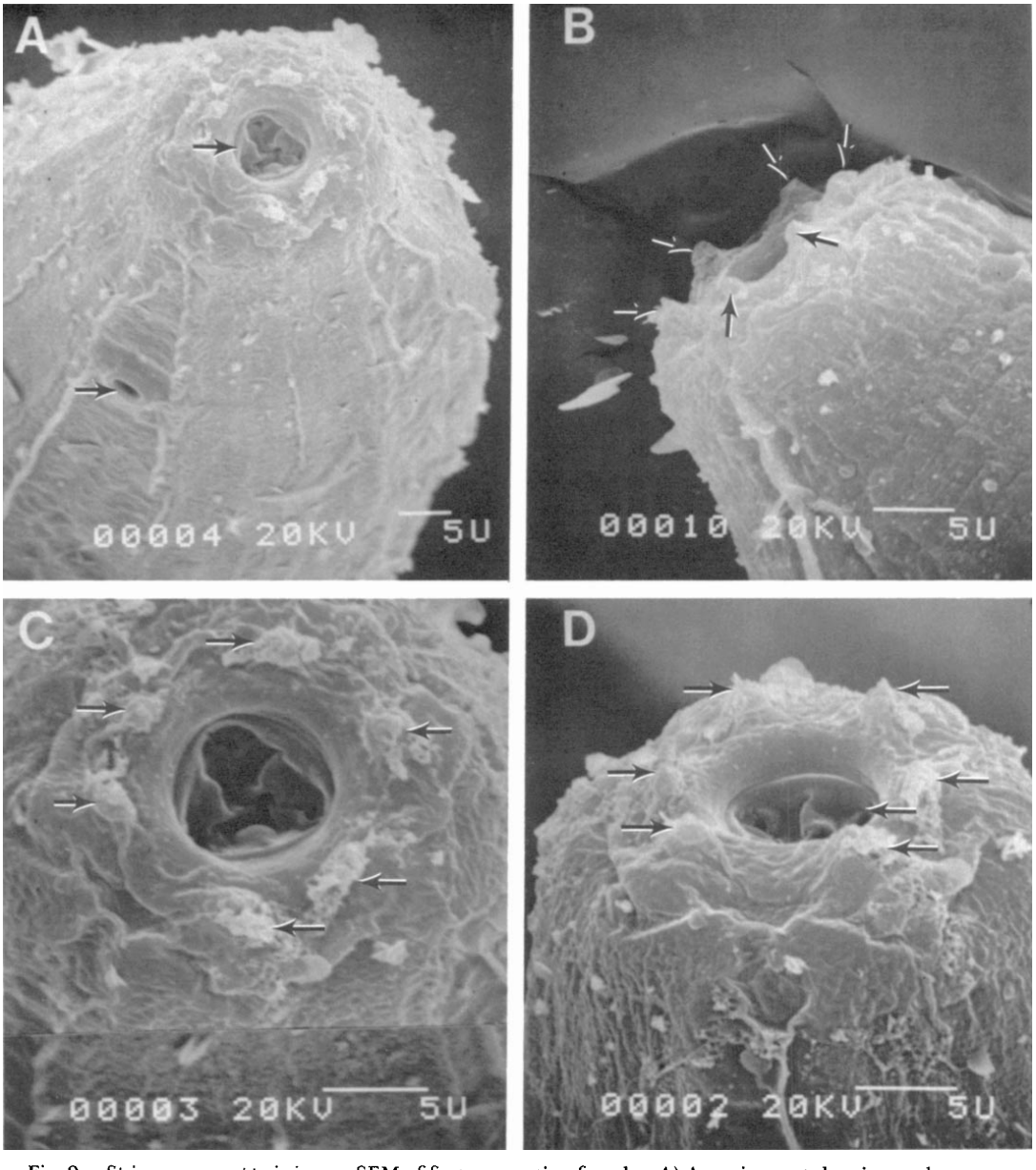


Fig. 2. *Steinernema scapterisci* n. sp. SEM of first-generation females. A) Anterior part showing oral aperture (arrow), lips, cephalic sensory papillae and excretory pore (arrow). B) Head showing six labial papillae (arrows) and faint transverse striae (annuli). C) Enlargement of Figure 2A showing unevenly distributed labial papillae (arrows) and the circular oral aperture which becomes subtriangular more posteriorly. Note the white (electron lucent) material covering the papillae. D) Face view showing the circular oral aperture (arrow) and labial papillae (arrows).

spicules have an elongate head (Fig. 4C, D) compared to an angular head in the first-generation male (Fig. 4A, B).

Allotype (female, first generation, in glycerine): Length 4,875 μm; width 200 μm; stoma length 7.8 μm; stoma width 11 μm; head to excretory pore 78 μm; head to nerve ring 181 μm; head to end of esophagus 265 μm; body width at anus 73 μm; V 50%.

Females, first generation: Measurements of 10 females in Table 2. Body cuticle smooth by LM faint annules by SEM; lateral fields and phasmids not observed. Head

TABLE 1. Measurements (in μm) of first-generation and second-generation males of *Steinernema scapterisci* n. sp. (n = 10).

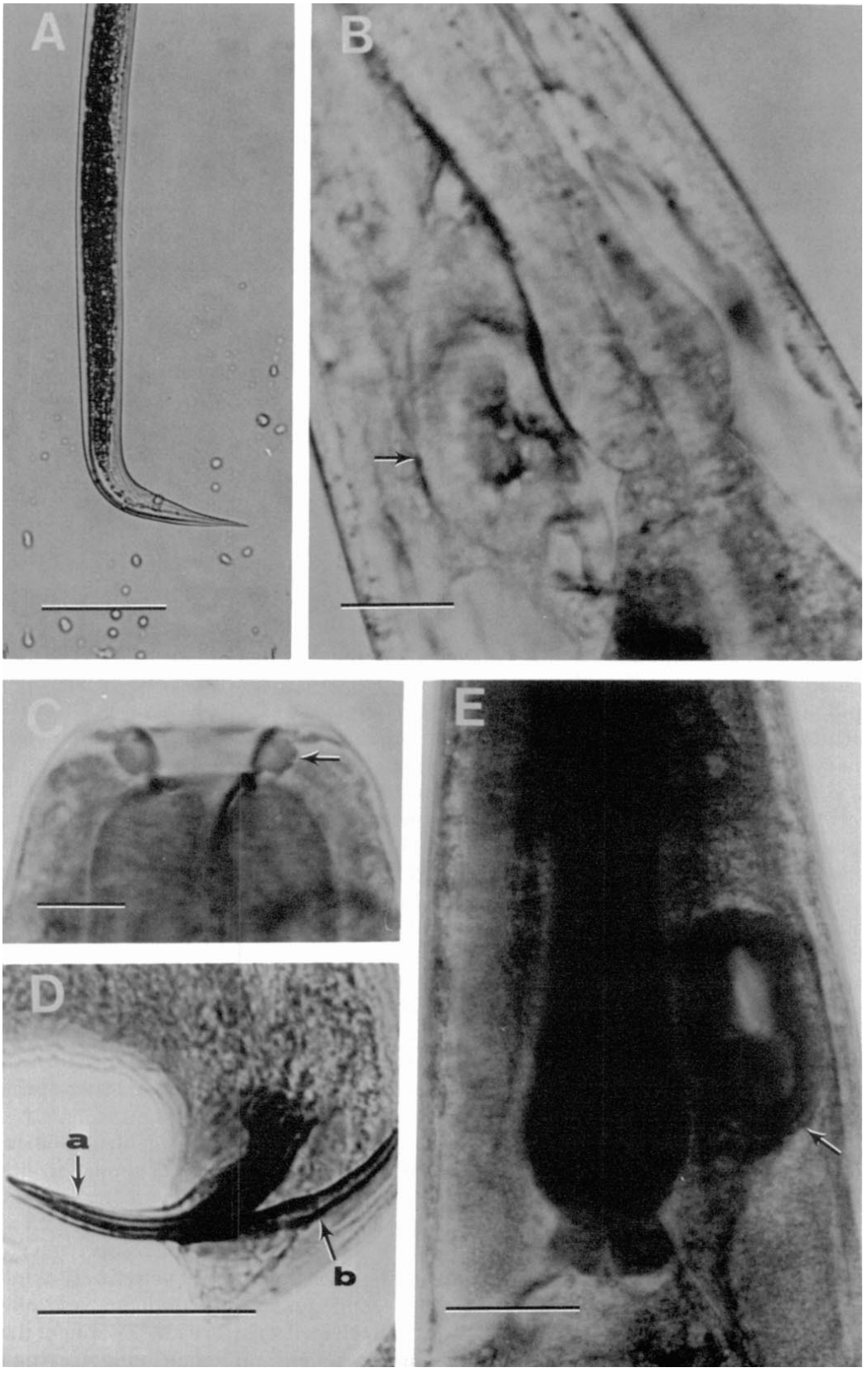
Character	First generation			Second generation		
	Mean	(SD)	Range	Mean	(SD)	Range
Body length	1,728	(358)	1,319–2,271	1,147	(95)	1,031–1,342
Greatest width	156	(49)	97–231	73	(8)	62–84
Stoma length	4.4	(1)	3–5	4.3	(1)	3–6
Stoma width	6.1	(1)	5–8	6.0	(1.2)	5–8
EP†	71	(11)	63–98	68	(7)	50–75
NR‡	136	(11)	120–152	121	(10)	103–131
ES§	187	(21)	164–216	168	(13)	138–181
Testis reflexion	374	(52)	306–447	205	(19)	176–234
Anal body width	33	(5)	31–45	33	(4)	28–41
Tail length	25	(3)	21–30	25	(3)	22–30
Spicule length	83	(5)	72–92	78	(3)	75–83
Spicule width	13	(4)	13–14	12	(1)	11–14
Gubernac. length	65	(5)	59–75	54	(3)	47–59
Gubernac. width	8	(0.5)	8–9	6	(0.7)	5–8
EP:ES	0.36	(0.02)	0.32–0.39	0.40	(0.06)	0.29–0.52
Mucron length	4.3	(0.6)	3.1–4.7	3.9	(0.6)	3.1–4.6

† EP = distance from anterior end to excretory pore.
‡ EP = distance from anterior end to nerve ring.
§ ES = distance from anterior end to end of esophagus.

rounded, continuous with body. Ten sensory papillae: six labial, four cephalic. Six lips, distinct, each with one papilla. Labial papillae not evenly distributed, two lateral papillae located ventrolaterally making ventral and lateral papillae closer together than lateral and dorsal papillae (Figs. 1A; 2A, C, D). Each papilla often covered with electron lucent material (Fig. 2A, C, D). Cephalic papillae sometimes obscure. Amphids not observed. Stoma shallow (Figs. 1F, 3C); *en face,* stoma circular anteriorly, subtriangular posteriorly (Fig. 2A, C).

Cheilorhabdions prominent, heavily thickened (Figs. 1F, 3C), appearing circular or hexagonal *en face* (Fig. 2C). Posterior to cheilorhabdions, another sclerotization, presumably the prorhabdions, distinct. Esophagus typical of family, procorpus cylindrical, muscular; metacorpus slightly swollen, nonvalvate; isthmus distinct; basal bulb muscular, with small, distinct valve. Nerve ring surrounds isthmus. Esophagointestinal junction large, prominent (Figs. 1F; 3B, E). Excretory pore anterior to midmetacorpus. Excretory duct prominent forming small loop between excretory pore and base of esophagus, then extending to right of esophagus, sometimes posteriorly to anterior of intestine then anteriorly on ventral side of intestine to esophago-intestinal junction; here it forms an elliptically shaped structure with center appearing as a cavity (Figs. 1F; 3B, E). (Note: This structure does not occur in males.) Uninucleate glandular cell occurring posterior to st' .cture. Gonads didelphic, amphidelphi' reflexed as in second generation fema! . (Fig. 1B). Vulva a transverse slit, prominent double-flapped epiptygma present (Figs. 1C, 6C). Vagina sclerotized, about one-third body width at vulva. Body width anterior to vulva greater than posterior to vulva. Tail conoid, shape variable, usually with ventral postanal swelling, mucron usually present (Fig. 1D). Tail length less than body width at anus. Pigmy form referred to by Bovien (1) for other species not observed.

Female, second generation: Measurements of 10 females in Table 2, similar to first-generation female but smaller. Length about 2,209 μm and width about 123 μm vs. 4,162 μm and 179 μm, respectively, for first-generation female; valve in basal bulb of esophagus more prominent, and elliptically shaped structure less prominent than in first-generation female; tail, tapering to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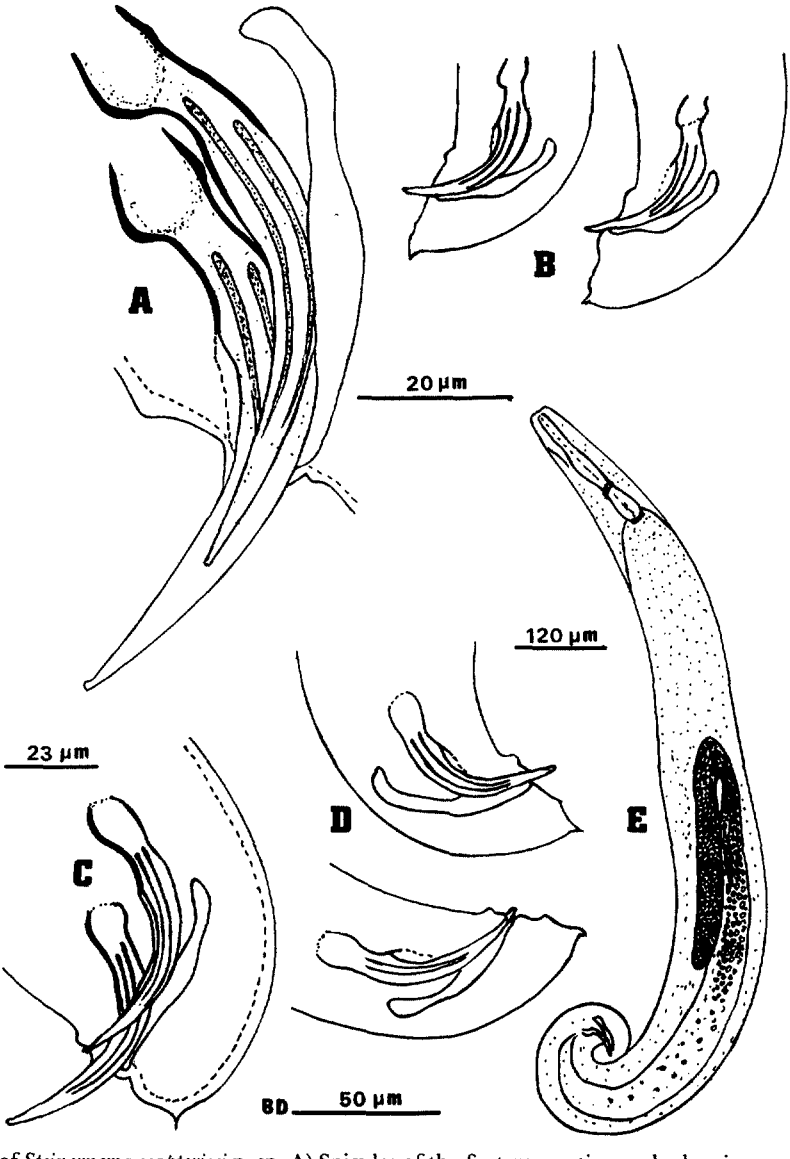

Fig. 4. Males of *Steinernema scapterisci* n. sp. A) Spicules of the first-generation male showing angular head, ribs, and gubernaculum with anterior end bent upward. B) Variation in tail shape of first-generation males. C) Tail of the second-generation male showing elongate spicule head. D) Variation in tail shape of second-generation males. E) Entire body of the first-generation male.

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Fig. 3. Light micrographs of *Steinernema scapterisci* n. sp. A) Posterior region of a living but inactive J3 showing the typical ventral curvature of the tail region (bar = 60 µm). B) Live first-generation female showing elliptically shaped structure (arrow) associated with the excretory duct (bar = 25 µm). C) Head of the first-generation female showing the thickened cheilorhabdions (bar = 10 µm). D) Spicules (a) and gubernaculum (b) of the first-generation male (bar = 50 µm). E) Fixed first-generation female showing elliptically shaped structure associated with the excretory duct (bar = 40 µm).

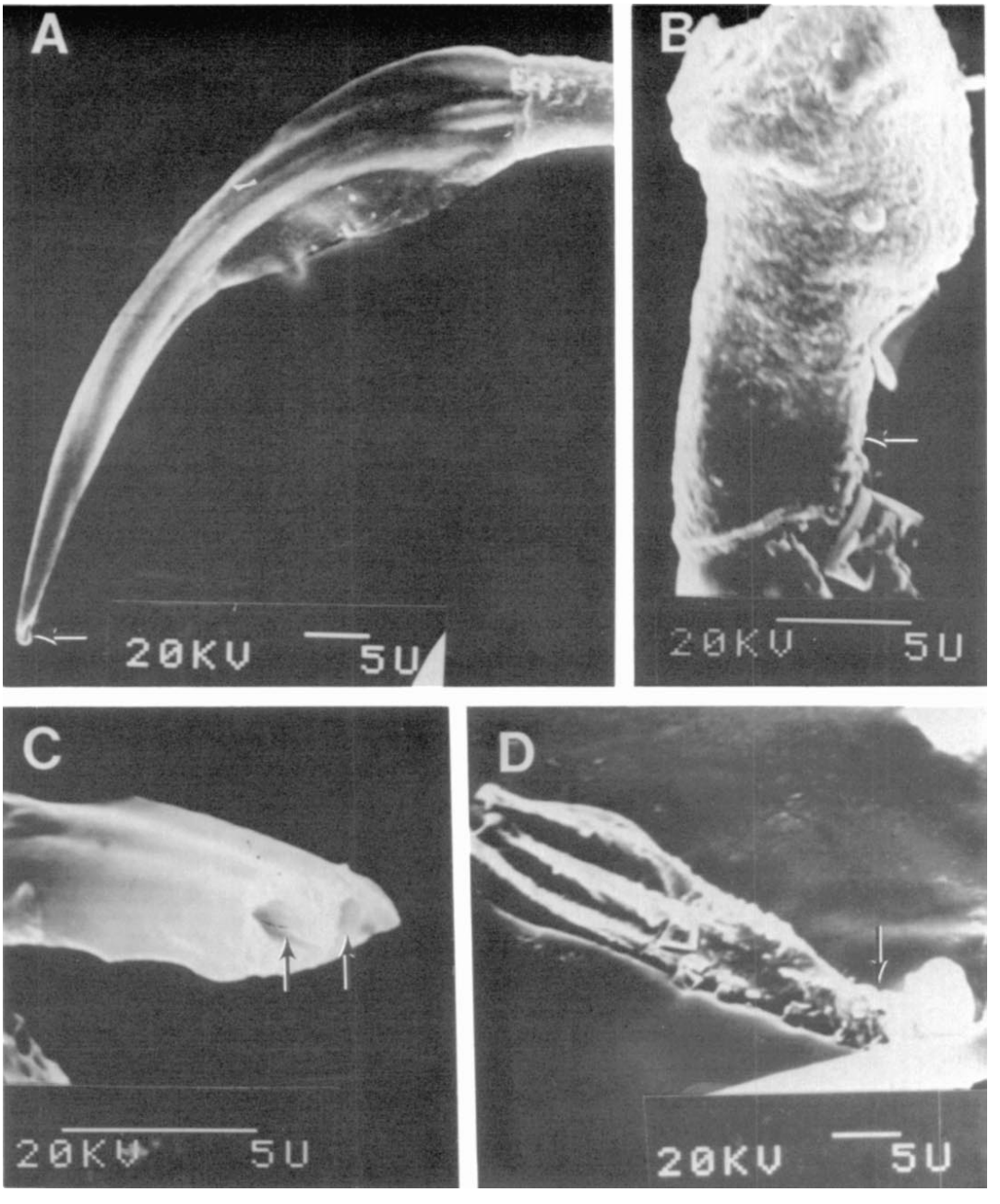

Fig. 5. *Steinernema scapterisci* n. sp. SEM of spicule and gubernaculum of the first-generation male. A) Spicule blade showing the thin posterior part with a small aperture at the tip (arrow). B) Spicule showing the angular head and a sheath (arrow) around the shaft. C) Cross section of the spicule showing two lumina (arrows) in the spicule blade. D) Gubernaculum showing the long anterior part which bends upward (arrow).

a point bearing a mucron, longer than body width at anus (Fig. 1B, E).

Juvenile, third stage: Measurements of 20 J3 in Table 3. Body thin, not enlarged. Cuticle of J2, as a sheath, present or absent (may be lost). Lip region continuous (Fig. 8C). Oral aperture not observed. Esophagus degenerate, obscure; basal bulb elongate, valve present. Lateral field with six incisures, five striae (Fig. 6D). Tail attenuate; tapering gradually dorsally but abruptly ventrally (Fig. 8D). When re-

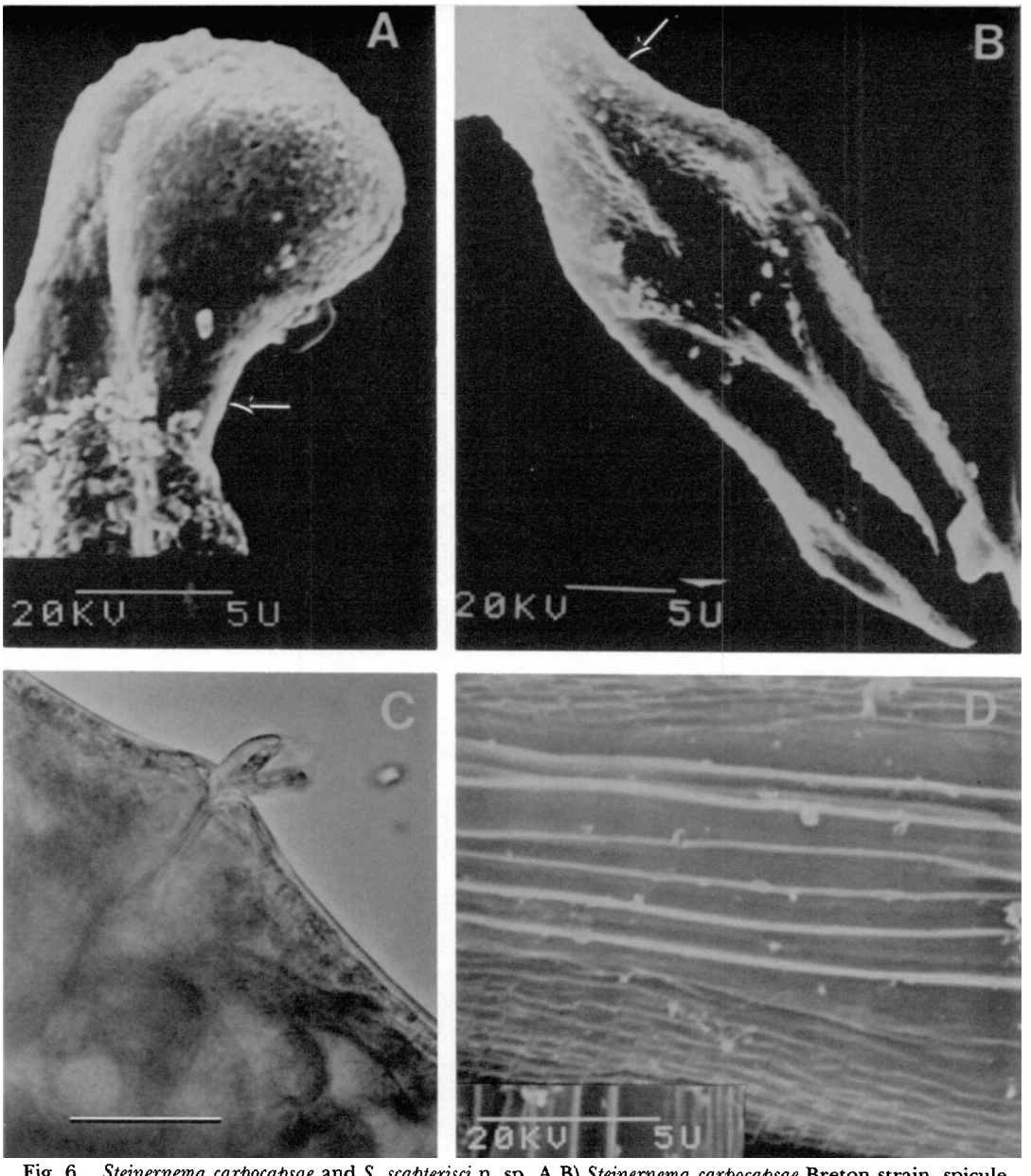

Fig. 6. *Steinernema carpocapsae* and *S. scapterisci* n. sp. A,B) *Steinernema carpocapsae* Breton strain, spicule and gubernaculum of the first-generation male. A) Spicule showing the short shaft without a sheath and with a rounded head. B) Gubernaculum showing short anterior part. C,D) *Steinernema scapterisci* n. sp. C) Double-flapped epiptygma of the first-generation female (bar = 32 μm). D) Mid-body of the J3 showing transverse striae (annuli) and lateral field with six incisures.

laxed, tail usually curved ventrally forming an angle ca. 110 degrees with body (Fig. 3A).

Type host and locality

Hemocoel of the mole cricket, *Scapteriscus vicinus* collected near Rivera, Uruguay.

Type specimens

Holotype (male, first generation): Isolated from the hemocoel of the mole cricket (*Scapteriscus vicinus*) derived from the original population from Uruguay. Slide no. T-432t, deposited in the United States Department of Agriculture Nematode Collection (USDANC), Beltsville, Maryland.

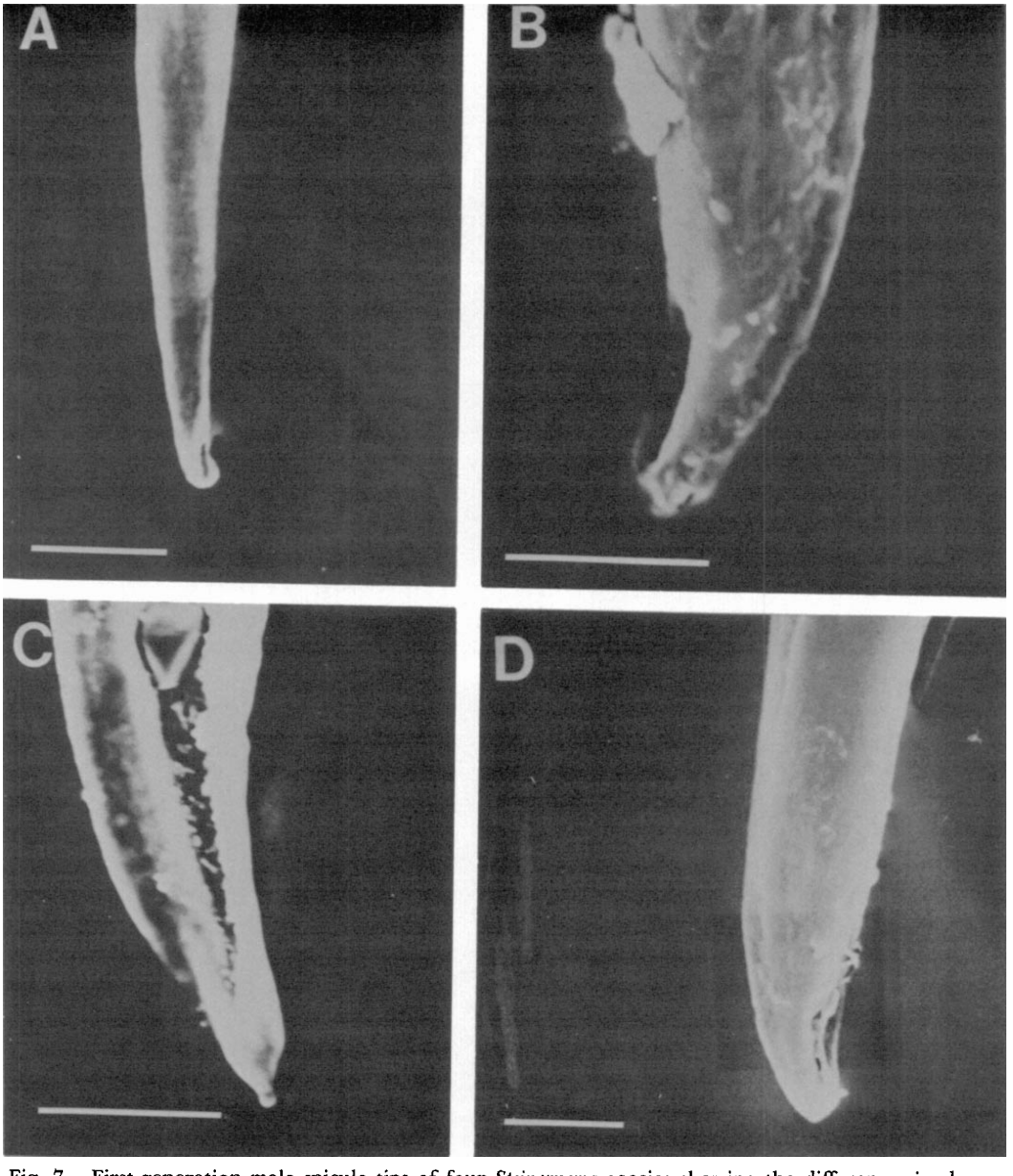

Fig. 7. First-generation male spicule tips of four *Steinernema* species showing the differences in shape, thickness, and size of the aperture. A) *Steinernema scapterisci* n. sp. B) *S. glaseri.* C) *S. carpocapsae.* D) *S. bibionis.* Each bar = 5 μm.

Allotype (female, first generation): Same data as holotype. Slide no. T-433t, deposited in the USDANC, Beltsville, Maryland.

Paratypes (first-generation males, first-generation females and third-stage juveniles): Same data as holotype. Ten males and ten females of the first generation and several third-stage juveniles in lactophenol, vial no. T-318p USDANC, Beltsville, Maryland; one male and one female of the first generation, slide no. UCNC 2406; and 11 third-stage juveniles, slide no. UCNC 2407 deposited in the California Collection of Nematodes, University of California Davis Nematode Collection (UCDNC), Davis, California; one male and one female of the first generation, slide no. T 99 N89-694, and 10 third-stage juveniles, slide no. T

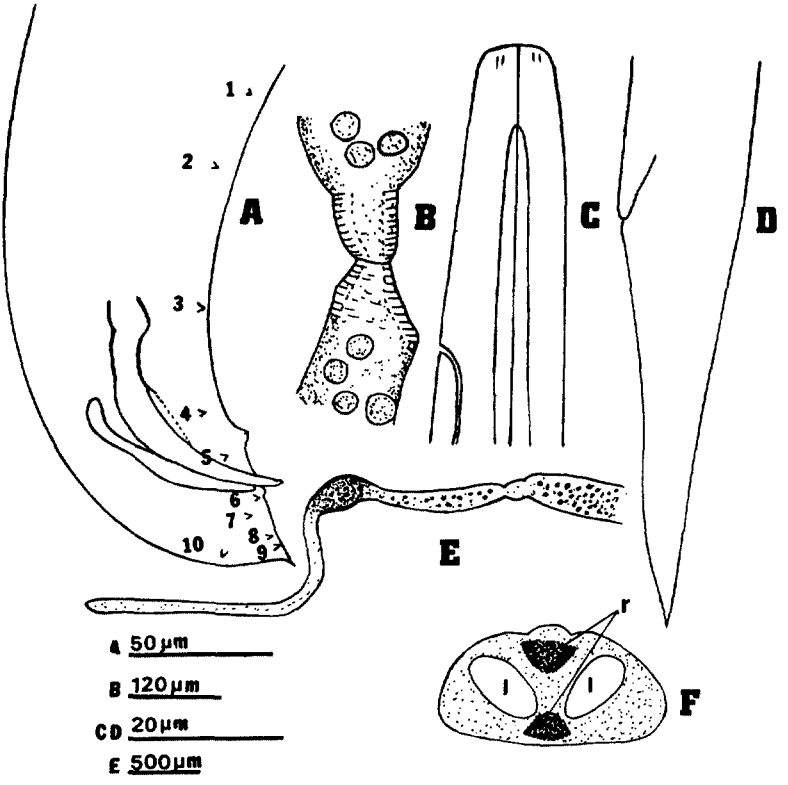

Fig. 8. *Steinernema scapterisci* n. sp. A) First-generation male tail showing 10 pairs of papillae and one single, preanal ventral genital papilla. B) Structure of a gonad of the first-generation female where the ovary reflexes (gonad, when dissected from the female, lies straight, not reflexed). C) Head of J3. D) Tail of J3. E) Diagrammatic ovary of the first-generation female showing spermatheca. (In the body the gonad is reflexed and no flexure is present.) F) Diagrammatic cross section of the spicule blade of the first-generation male showing two lumina (l), and two internal ribs (r).

100 N89-694, deposited in the Florida Collection of Nematodes, Florida Department of Agriculture and Consumer Services, Gainesville, Florida.

Diagnosis: Spicules of males of both generations are pointed and taper smoothly to the end; distal end of the blade narrow with a small aperture on ventral side; shaft long, bearing a sheath; gubernaculum with long, upward-bent anterior part. First-generation female with large cheilorhabdions (about 4.8 µm thick by 5.8 µm long in lateral view of a normal-sized female), with an elliptically shaped structure in the excretory system, and bearing a prominent double-flapped epiptygma. The ratio of head to excretory pore divided by tail length of the J3 is 0.73 (0.60–0.80), and the ratio of head to excretory pore divided by head to end of esophagus is 0.31 (0.27–0.40).

Relationships: Steinernema scapterisci n. sp. can be distinguished from other species of *Steinernema* as follows: from *S. glaseri* by the presence of a mucron on the male tail, and by the shorter J3 of *S. scapterisci* n. sp. (517–609 µm vs. 860–1,500 µm for *S. glaseri*); from *S. bibionis* and *S. intermedia* by the shorter J3 (700–1,000 µm for *S. bibionis* and 608–800 µm for *S. intermedia*); also from *S. bibionis* by yellow-orange spicules of *S. bibionis* vs. brown of *S. scapterisci* n. sp.; from *S. carpocapsae* by the ratio of head to excretory pore divided by tail length of the J3, (0.73 [0.60–0.80] in *S. scapterisci* n. sp. and 0.60 [0.54–0.66] *in S. carpocapsae* [2]); also by the grey-yellow spicules of *S. carpocapsae* vs. brown of *S. scapterisci* n. sp.,

TABLE 2. Measurements (in μm) of first-generation and second-generation females of *Steinernema scapterisci* n. sp. (n = 10).

Character	First generation		Second generation	
	Mean (SD)	Range	Mean (SD)	Range
Body length	4,162 (540)	3,531–5,156	2,209 (223)	1,841–2,530
Greatest width	179 (13)	159–203	123 (14)	94–141
Stoma length	7.5 (1)	6–9	6.7 (1.4)	5–9
Stoma width	10 (3)	9–12	8.9 (0.9)	8–11
EP†	89 (5)	78–94	78 (6.8)	66–88
NR‡	174 (13)	153–194	169 (12)	147–184
ES§	242 (17)	219–269	241 (15)	222–266
Tail length	46 (8)	34–59	58 (4)	48–64
Anal body width	58 (9)	41–72	47 (2.8)	43–52
Vulva%	53 (2)	50–54	52 (2)	52–60
EP:ES	0.37 (0.03)	0.32–0.41	0.32 (0.3)	0.28–0.36

† EP = distance from anterior end to excretory pore.
‡ NR = distance from anterior end to nerve ring.
§ ES = distance from anterior end to end of esophagus.

and by the longer anterior part of the gubernaculum of *S. scapterisci* n. sp. The J3 can be distinguished by the ratio of head to excretory pore divided by head to end of esophagus which is 0.31 for *S. scapterisci* n. sp. vs. 0.26 for *S. carpocapsae*, 0.45 for *S. bibionis*, 0.51 for *S. intermedia* and 0.65 for *S. glaseri* (2).

Cross hybridization: In cross hybridization experiments, males and females were never observed to mate and no eggs were laid within the 10-day observation period. In the controls, males and females mated and eggs were laid which hatched and developed to J3 in 10 days. No further observations were made.

Comparative pathogenicity: Except for *S. scapterisci*, all species of *Steinernema* tested, including all strains of *S. carpocapsae*, killed from 20–100% of the test insects; *S. scapterisci* n. sp. killed no more than 10% (Table 4).

Biological diagnosis: S. scapterisci n. sp. could not be cultured on the greater wax moth larva (*Galleria mellonella*), but sometimes a few larvae were killed by the nematode. When this occurred, the bodies of the larvae turned black, whereas those killed by other species of *Steinernema* turned whitish or yellowish but never black. Other species of *Steinernema* developed well in wax moth larvae. Finally, this nematode can be distinguished from other species by bioassay on three insects: fall army worm, velvet

TABLE 3. Measurements (in μm) of the third-stage juvenile of *Steinernema scapterisci* n. sp. (n = 20).

Character	Mean	SD	Range
Body length	572	27	517–609
Greatest width	24	4	18–30
EP†	39	4	36–48
NR‡	97	1.1	83–106
ES§	127	6	113–134
Tail length	54	3	48–60
EP:ES	0.31	0.03	0.27–0.40
EP:Tail length	0.73	0.06	0.60–0.80

† EP = distance from anterior end to excretory pore.
‡ NR = distance from anterior end to nerve ring.
§ ES = distance from anterior end to end of esophagus.

TABLE 4. Four species of lepidopterous insects† killed (%) within 48 hours by *Steinernema* spp.

Nematode	FAW	VBC	GCW	WML
S. glaseri	100	90	50	100
S. bibionis	100	90	55	100
S. carpocapsae				
Breton	100	100		100
Italian	100	100		100
Mexican	100	100	80	100
Agriotos	100	100	20	100
All	100	100		100
S. scapterisci	8	3	10	9
Control	0	0	0	0

Average of four trials.
† FAW = fall army worm; VBC = velvet bean caterpillar; GCW = granulate cut worm; WML = wax moth larva.

bean caterpillar, and wax moth larva. In 2 days, other species of *Steinernema* killed 90-100% of the test insects, but *S. scapterisci* n. sp. killed no more than ca. 10% (Table 4).

Discussion

In the genus *Steinernema,* females usually are not used to identify species. For *S. scapterisci* n. sp., however, the elliptically shaped structure in the excretory system and the epiptygma are so prominent in every first-generation female that the two structures can be used as diagnostic characters. Thus, the first-generation female as well as the first-generation male and J3 can be used for species identification.

SEM photographs of the spicules of *S. scapterisci* show two lumina, which, when considered in conjunction with the two distinctive ribs shown by LM, indicates that the ribs are strengthening structures of the upper and lower walls between the two lumina (Fig. 8F).

Up to the present, larvae of *Galleria mellonella* have been used as universal hosts for all species and strains of *Steinernema* spp. Since this has been the case, the concept of differential hosts has not been used for this genus. However, *G. mellonella* is neither a good host for *S. scapterisci* n. sp. nor does it reproduce in it. This difference in infectivity and reproduction indicates that *G. mellonella* can be used as a test insect to differentiate between *S. scapterisci* n. sp. and all other known species and strains of *Steinernema.* We have shown also that three other insects can be used to distinguish *S. scapterisci* n. sp. from others, and perhaps in time other insects will be found which will permit the establishment of a series of differential insect hosts that could be used to help distinguish between species or even strains of the genus.

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