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Redescription of *Steinernema longicaudum* Shen & Wang (Nematoda: Steinernematidae); geographic distribution and phenotypic variation between allopatric populations

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Abstract

Steinernema longicaudum Shen & Wang is redescribed based on a comparative morphological study of specimens from the type isolate from China, and two other isolates recovered from Korea and the USA. For the first and second generation female, the location of the vulva, shape of the vulval lips, and shape and length of the tail were newly observed diagnostic characters. A more detailed description of the morphology of the male spicules and gubernaculum, and the arrangement of the genital papillae is included. A description, based on scanning electron microscopy observations, of the lateral field pattern of the third-stage infective juveniles is also provided. Additionally, restriction fragment length polymorphism profiles based on the internal transcribed spacer region, and cross-breeding tests supplement the description of this species.

Introduction

Nematodes of the family Steinernematidae are obligate parasites of insects and are common soil inhabitants in many parts of the world, having been recovered from all continents with the exception of Antarctica (Griffin *et al.*, 1991). Within the genus *Steinernema* Travassos, two species, *Steinernema carpocapsae* (Weiser) and *Steinernema feltiae* (Filipjev), appear to have a global distribution (Hominick *et al.*, 1996). However, the other *Steinernema* species seem to have a more restricted geographic distribution, having been recorded only at the continental or national level (Hominick *et al.,* 1996). Certainly, data on geographic distribution must be considered temporal, since the increase in the number of surveys conducted in many parts of the world has recently expanded our knowledge of the distribution of many entomopathogenic nematodes.

An example of this is *Steinernema longicaudum* Shen & Wang which was originally isolated from soil samples taken in an orchard in the city of Lailang, Shangdong province, China in 1985, and was formally described in 1992 (Shen & Wang, 1992). In the original description, a number of important characters, such as the shape of the tail and vulval lips of the females, the arrangement of the males' genital papillae and the lateral field pattern of the third-stage infective juveniles, were omitted or inadequately covered. Recently, the geographic distribution of this species has been expanded to include Korea (H.Y. Choo, unpublished) and North America (Stock *et al.*, 1999).

Thus, we herein redescribe *S. longicaudum*, based on

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morphological (light and scanning electron microscopy) and molecular analyses of the type isolate and two new isolates from two distant geographic locations.

Materials and methods

Origin of the isolates

The nematode material used originated from the following sources: the Chinese population (type population from Lailang, Shangdong province, China) was obtained by X. Shen in 1993. The North American isolate (B2) was recovered from sandy loam soil samples taken from lodgepole pine forests in the west side of the Sierra Nevada mountains in California (Stock *et al.*, 1999). H. Choo recovered the Korean isolate (Gongju) from sandy soil samples taken from a turfgrass area in Gongju, Chungnam province, South Korea. For this study, all isolates were propagated in last instar *Galleria mellonella* larvae according to Kaya & Stock (1997).

Molecular characterization

DNA analysis was carried out using genomic DNA extracted from both individual adult nematodes and bulked third-stage infective juveniles (IJ). In the case of individual adults, the lysis method of Joyce *et al.* (1994) was used to extract the DNA. For bulked IJ, a Phytopure DNA extraction kit from Nucleon Biosciences was used according to the manufacturer's instructions.

PCR amplification

Primers used in the polymerase chain reaction (PCR) were specific for the internal transcribed spacer (ITS) as described by Vrain *et al.* (1992). Amplifications were carried out in a volume of 100 μl, containing 50 mM KCl, 10 mM Tris (pH 9.0), 1.5 mM $MgCl_2$, 0.1% Triton X-100, 0.2 mM of each dNTP, 0.5 μM of each primer, either 5 μl of nematode lysate or 100 μg of purified DNA and 8 units of Taq polymerase (Promega Corporation). Amplifications were carried out using a Techne PHC-3 thermocycler with a heated lid. Samples were placed in the thermocycler (which was preheated to 95°C) and incubated at 94°C for 2 min followed by 40 cycles of 94°C for 30 s, 50°C for 1 min and 72°C for 1.5 min. A final step of 5 min at 72°C was included to ensure all of the final amplification products were full length.

Restriction digestion and electrophoresis of PCR products

Amplified products were immediately digested with a range of restriction endonucleases. Restriction enzymes were purchased from Amersham International or Promega and used with the buffers supplied by the manufacturers. All digestions were carried out using 4 μl of amplified product at 37°C for a minimum of 2 h. The resulting fragments were separated on 1.5% (w/v) agarose gels in TBE at 5 V/cm for 3 h. Fragments were visualized by ethidium bromide staining (Maniatis *et al.*, 1989). The restriction fragment length polymorphism (RFLP) profiles of the three *S. longicaudum* isolates were compared with each other and with three morphologically similar species: *S. arenarium*, *S. cubanum* and *S. glaseri* (for RFLP profiles see Waturu *et al.*, 1997).

Cross-breeding tests

Isolates B2 and Gongju were cross-bred with each other and with the Chinese population of *S. longicaudum* using the modified hanging drop method described by Kaya & Stock (1997). Three morphologically similar species: *S. arenarium* (unknown isolate), *S. cubanum* (type strain) and *S. glaseri* (NC isolate), were used as negative controls.

Morphological characterization

For light microscopy studies, first and second generation males (23–25 individuals of each generation) and females (22–25 individuals of each generation) of each isolate were obtained by dissecting infected *G. mellonella* larvae 3–4 and 6–7 days, respectively, after death. Infective juveniles (25–28 specimens per isolate) were obtained upon emergence from the cadavers in 10–12 days at 20–22°C. Nematodes were examined either live or heat killed and relaxed in Ringer's solution at 50–60°C, slowly dehydrated, then mounted in anhydrous glycerine on glass slides with cover supports.

Specimens were measured with a Nikon Eclipse E600 microscope using Scion Image software (1.62a version). Data were analysed using analysis of variance (ANOVA), and significance differences among means were separated by Tukey's test (SAS Institute, 1996).

For scanning electron microscopy (SEM) studies, only first generation males and females and infective juveniles were used. Specimens were placed live in a 25% aqueous solution of glutaraldehyde in 0.1 M sodium phosphate buffer. The liquid was heated for 42 s in a microwave (temperature of liquid reached about 60°C). Fixed nematodes were washed in 0.1 M sodium phosphate buffer for 10–15 min and post-fixed in 1% osmium tetraoxide in 0.1 M phosphate buffer overnight. They were rinsed in cold buffer several times, dehydrated at 10 min intervals through a graded ethanol series (10, 30, 50, 70, 80, 90 and 100%) and then critical point dried with CO_2, mounted on stubs and finally sputter coated with gold. Photographs were taken using a SES DS-130 scanning electron microscope at 10 kV and 15 kV respectively.

Results

Molecular characterization

The RFLP analysis of the ITS region of the rDNA repeat produced identical restriction fragment bands for

Fig. 1. Polymerase chain reaction amplified products from the internal transcribed spacer digested with 17 different enzymes. Fragments were separated with ethidium bromide stained 1.5% (w/v) agarose gel. Lane 1 is a digest of *Steinernema feltiae* (UK, site 76) with Alu I. Lanes 2–18 are individual digests of *S. longicaudum* with the following restriction enzymes: 2, *Alu* I; 3, *Bst*0 I; 4, *Dde* I; 5, *Eco*R I; 6, *Hae* III; 7, *Hha* I; 8, *Hind* III; 9, *Hinf* I; 10, *Hpa* I; 11, *Kpm* I; 12, *Pst*I; 13, *Pvu* I; 14, *Rsa* I; 15, *Sal* I; 16, *Sau* A I; 17, *Sau* 96; 18, *Xba* I. Lane 19, molecular weight marker. The band sizes are shown in base pairs. A. B2 isolate (US); B. Chinese isolate; C. Gongju isolate (Korea).

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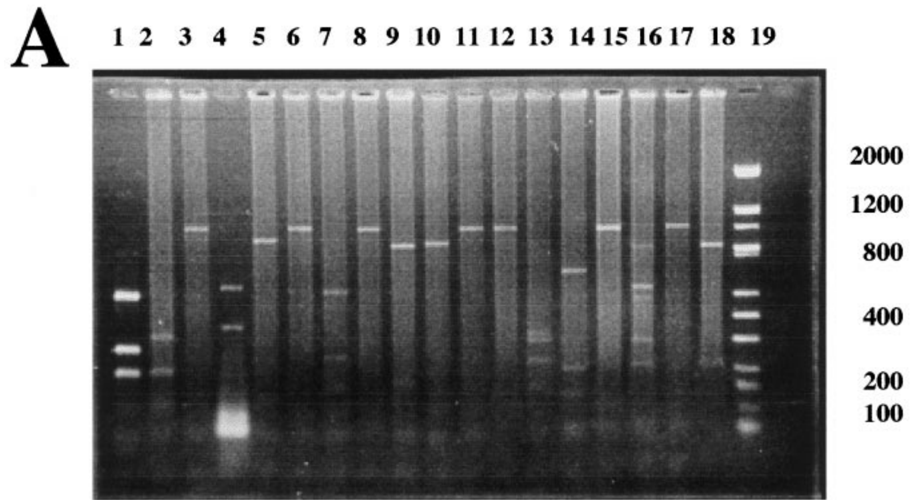

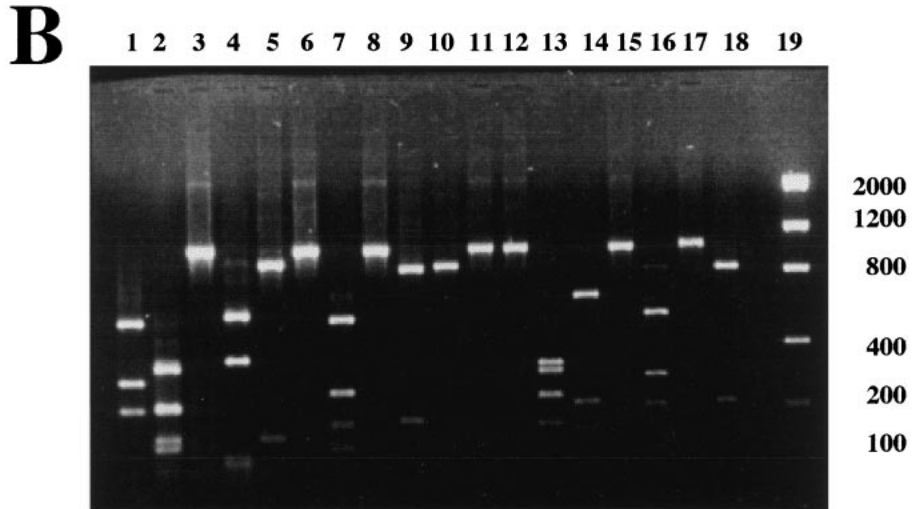

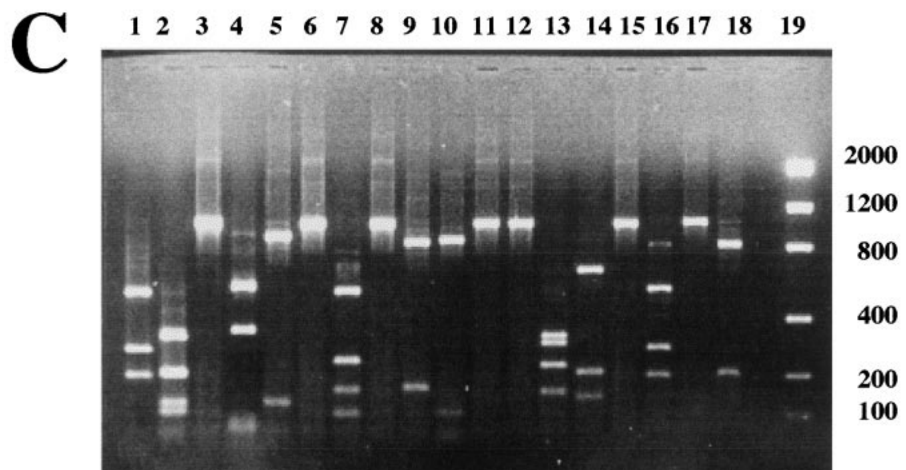

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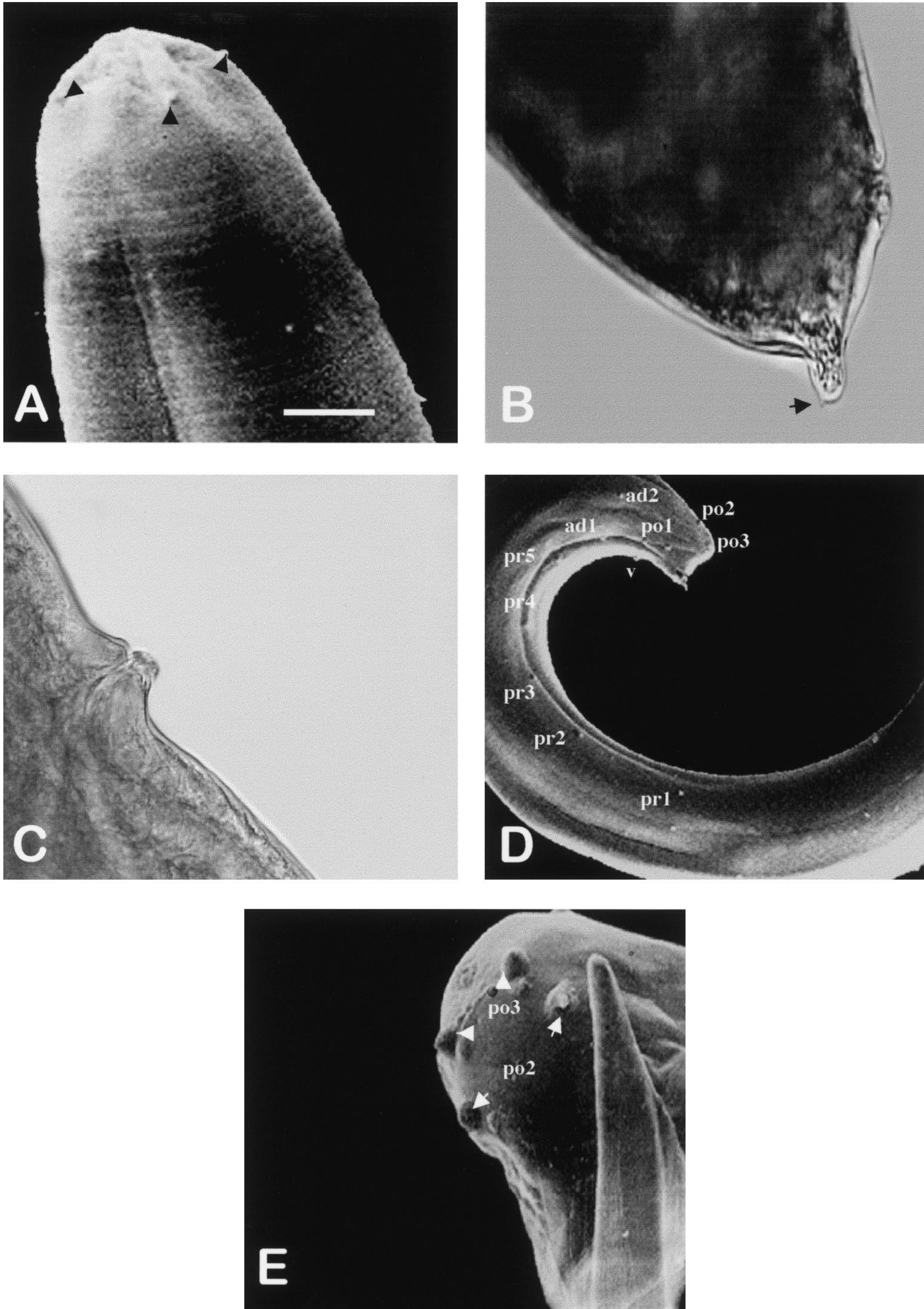

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the three *S. longicaudum* isolates (fig. 1). The RFLP profiles of *S. longicaudum* were unique to this species and distinguishable from the profiles of three morphologically similar species: *S. arenarium*, *S. cubanum* and *S. glaseri* (see Waturu *et al.*, 1997 for RFLP profiles). From the 17 restriction enzymes tested, *S. longicaudum* differed in seven enzyme profiles with *S. arenarium*. Similarly, ten out of 17 restriction enzyme profiles were different between *S. longicaudum* and both *S. cubanum* and *S. glaseri*.

Cross-breeding tests

Cross-breeding tests revealed that B2, Gongju and the Chinese isolates could interbreed with each other and produced progeny. Crosses of these three isolates with *S. arenarium*, *S. cubanum* and *S. glaseri* resulted in no progeny.

Morphological characterization

Our morphological analysis indicated that the three isolates possessed characteristics of *S. longicaudum* as described by Shen & Wang (1992). However, some differences, at the intraspecific level, were observed and are discussed below.

Chinese specimens

When compared with the original description, first and second generation males of this population were significantly shorter (average: 1788 vs. 2048 μm) ($P \leq 0.05$) than the specimens previously studied by Shen & Wang (1992). The excretory pore of the first and second generation males is more anteriorly located (average: 127 vs. 139 μm, 114 vs. 128 μm, respectively) when compared with the original description. First generation males had typically 23 papillae although there may be as few as 21, the right hand series losing one or two papillae in some specimens.

When compared with the original description, the females (both first and second generation) herein studied differed in the location of the excretory pore and nerve ring, and the tail length. The excretory pore was more anteriorly located in our specimens (average: 129 vs. 162 μm and 90 vs. 76 μm, for first and second generation, respectively). First and second generation females had the nerve ring more posteriorly located than the specimens studied in the original description (average: 176 vs. 159.6 μm; 136 vs. 127 μm, for first and second generation, respectively). The tail length in both first and second generation females was significantly shorter ($P \leq 0.05$) than in the description given by Shen & Wang (1992) (average: 57 vs. 76.4 μm; 50 vs. 67.5 μm, for first and second generation, respectively).

Infective juveniles of the studied isolate had the excretory pore (average: 82 vs. 80.9 μm) and the nerve ring (average: 111 vs. 96.7 μm) more posteriorly located than those of the original description.

Korean specimens

No morphometric or morphological differences were observed between adults of the Gongju and the Chinese isolate. The IJ of the Gongju isolate had more prominent cephalic papillae, clearly distinct with light microscopy and SEM (fig. 2A).

Californian specimens

First and second generation males of isolate B2 had the excretory pore more posteriorly located than the males of the Chinese isolate (table 1). The nerve ring of the first generation males was more anteriorly located than in the Chinese isolate (table 1).

The vulva in the Californian females (first generation) was more posteriorly located than that of the Chinese specimens (table 2). The tail of the first generation females was significantly longer than that of the Chinese isolate ($P \leq 0.05$) (table 2), and had one minute mucronate process (fig. 2B).

The IJ of isolate B2 had the excretory pore more anteriorly located than those of the Chinese isolate (table 3).

Based on these observations, we present below a redescription of this species.

***Steinernema longicaudum* Shen and Wang, 1992**

Adults. Body usually C- or J-shaped when heat relaxed and anteriorly truncated to rounded (fig. 3A,F). Cuticle finely striated anteriorly, but otherwise appearing smooth under the light microscope, although under the SEM fine transverse striae are visible on the body. Lateral field and phasmids inconspicuous. Head truncate to slightly rounded, more or less continuous with body contour; mouth opening circular to slightly triangular (fig. 3B). Ten sensory papillae: six labial, four cephalic; six distinct lips present, each bearing a papilla. Labial papillae evenly distributed. Stoma partially collapsed, oesophageal collar absent, oesophagus extending near to mouth opening. Cheilorhabdions located beneath the lips and composed of a thick, cuticularized ring. Oesophagus typical of family, procorpus cylindrical and muscular; metacorpus slightly swollen, non-valvate, isthmus distinct; basal bulb muscular with small cuticularized valve plates (fig. 3B). Nerve ring surrounding the isthmus and anterior portion of basal bulb. Oesophagus-intestinal valve almost bilaterally symmetrical and projecting into the intestine. Excretory pore opening located at level of procorpus/isthmus junction or slightly anterior somewhat variable in location.

Female. Body usually spiral or C-shaped when heat

Fig. 2. *Steinernema longicaudum*. A. Scanning electron microscopy of third-stage infective juvenile showing (arrow) prominent cephalic papillae. B. Tail of first generation female showing the mucronated process (arrow). C. Vulva of first generation female. D. Tail of first generation male showing arrangement of genital papillae (pr1–pr5, pre-cloacal paired papillae; ad1–2, ad-cloacal paired papillae; po1–po3, postcloacal paired papillae; v, ventral single papillae). E. Tail of first generation male showing two pairs of postcloacal papillae (po2, po3). All magnifications based on scale bar in A. A = 8 μm; B = 40 μm; C, D = 45 μm; E = 15 μm; F = 35 μm.

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Table 1. Morphometrics of first and second generation males of *Steinernema longicaudum*.

Character	Chinese isolate (n = 23) First generation	Chinese isolate (n = 23) Second generation	Gongju isolate (Korea) (n = 25) First generation	Gongju isolate (Korea) (n = 25) Second generation	B2 isolate (USA) (n = 25) First generation	B2 isolate (USA) (n = 25) Second generation
Body length	1788 ± 309 (1412–2733)	826 ± 36 (774–911)	1600 ± 256 (1398–1999)	816 ± 29 (757–905)	1946.5 ± 378 (1678–2856)	805 ± 31 (725–909)
Greatest body width	136 ± 27.3 (86–194)	82 ± 3.6 (77–90)	120.5 ± 18.6 (81–178)	79 ± 3.6 (75–88)	137.5 ± 25.5 (98–191)	84 ± 3.0 (79–93)
Stoma length	4.9 ± 1.1 (3.4–6.8)	5.5 ± 1.2 (3.4–7.9)	5.0 ± 1.0 (3.5–6.5)	5.5 ± 1.1 (3.5–7.5)	4.0 + 0.3 (3.5–6.5)	4.5 ± 0.9 (4.0–7.5)
Stoma width	9.5 ± 1.3 (7.9–12.4)	8.5 ± 1.4 (4.5–11.3)	9.5 ± 1.1 (7.5–11.9)	8.5 ± 1.2 (4.5–10)	7.8 + 1.1 (7.5–12)	8.0 ± 1.3 (6.5–9.5)
Anterior end to excretory pore	127 ± 20.3 (79–162)	114 ± 6.2 (101–124)	125.0 ± 15.4 (81–157)	108 ± 6.0 (98–120)	129.0 + 19.7 (85–175)	121 ± 4.5 (109–125)
Body width at excretory pore	70 ± 11.1 (45–92)	46.8 ± 1.8 (45–50)	63.0 ± 9.5 (55–89)	45 ± 1.2 (43–49)	68.5 + 8.7 (51–81)	47 ± 1.5 (42–54)
Anterior end to nerve ring	151 ± 16.2 (120–176)	144 ± 6.3 (131–156)	148.5 ± 11.3 (123–175)	143 ± 6.0 (130–153)	143.0 + 15.3 (118–165)	146 ± 3.5 (135–155)
Anterior end to oesophagus	165 ± 22.5 (79–192)	164 ± 4.3 (153–171)	163.0 ± 15.5 (89–191)	153 ± 4.0 (151–158)	173.0 + 16.5 (95–197)	163 ± 8.0 (153–175)
Anterior end to testis reflexion	340 ± 84 (167–479)	195 ± 27.9 (147–241)	335 ± 57 (189–463)	195 ± 25 (145–245)	357.0 + 33 (250–410)	200 ± 5.5 (171–227)
Body width at cloaca	58 ± 9.9 (43–74)	48 ± 4.0 (43–59)	55.0 ± 6.7 (42–71)	49 ± 3.2 (41–58)	58.0 + 5.3 (45–69)	45 ± 3.5 (42–57)
Tail length	30 ± 5.8 (20–43)	33 ± 3.5 (25–41)	30.0 ± 4.2 (21–42)	38 ± 3.5 (29–45)	46.0 + 5.5 (28–48)	34 ± 3.1 (27–45)
Spicule length	91 ± 3.8 (72–108)	75 ± 3.4 (56–86)	88.5 ± 3.1 (75–102)	71 ± 4.0 (50–83)	84.0 + 3.8 (75–97)	74 ± 3.3 (56–83)
Gubernaculum length	60 ± 3.4 (54–65)	37 ± 3.9 (30–45)	57.5 ± 2.5 (55–60)	35 ± 3 (30–42)	63.0 + 3.7 (55–68)	34 ± 2.5 (31–45)
D%*	75.4 ± 2.2 (56–92)	70 ± 2.0 (63–75)	74 ± 2.5 (63–89)	64 ± 2.0 (61–69)	75.0 + 2.2 (58–92)	70.5 ± 2.0 (64–74)
EW†	1.85 ± 0.05 (1.36–2.3)	2.44 ± 0.05 (2.25–2.70)	1.81 ± 0.05 (1.46–1.98)	2.33 ± 0.01 (2.27–2.40)	1.81 ± 0.05 (1.41–2.1)	2.37 ± 0.01 (2.28–2.67)
SW‡	1.61 ± 0.27 (1.16–2.25)	1.58 ± 0.19 (1.14–2.00)	1.60 ± 0.15 (1.43–1.78)	1.45 ± 0.25 (1.19–2.23)	1.56 ± 0.21 (1.21–1.95)	1.56 ± 0.2 (1.19–2.05)
GS§	0.66 ± 0.08 (0.56–0.88)	0.5 ± 0.07 (0.39–0.68)	0.65 ± 0.05 (0.55–0.73)	0.60 ± 0.02 (0.55–0.66)	0.67 ± 0.05 (0.55–0.87)	0.51 ± 0.05 (0.41–0.65)

Means ± standard deviation and range (between brackets) are given in μm.
* Distance from anterior end to excretory pore divided by oesophagus length × 100.
† Distance from anterior end to excretory pore divided by body width at excretory pore.
‡ Spicule length divided by body width at cloaca.
§ Gubernaculum length divided by spicule length.

relaxed. First generation females larger (average length: 7660 μm) than those of the second generation (average length: 2530 μm). Vulva a transverse slit; vulval lips rounded, protruding (fig. 2C) and apparently lacking epiptygma (in several specimens a small volume of secretion extruded from the vagina could possibly be mistaken for an epiptygma-like structure). Vagina short, initially angled slightly anteriad and leading into paired, amphidelphic uteri (fig. 3A). Eggs initially deposited externally but later hatching inside the female body (especially true of the first generation). First generation female tail shorter than anal body width, usually dorsally convex-conoid to subconoid in shape with an irregular, serratulate terminus bearing several (at least two) minute projections (figs 2B and 3D). In contrast, tail of the second generation female is relatively longer and usually more evenly conoid to a pointed terminus which may be markedly offset. (fig. 3E).

Male. Cuticle, lip region, stoma and oesophageal region as in female. Body curved posteriorly, C- or J-shaped when heat-killed (fig. 3F). First generation male (average length: 1778 μm) larger than second generation male (average length: 815 μm). Gonad monorchic, reflexed. In the first generation male there are typically 23 papillae (fig. 3G) although there may be as few as 21, the right hand series losing one or two papillae in some specimens. Papillae arranged thus: a well spaced precloacal series of five subventral pairs, all of which are anterior to the midventral papilla (fig. 2E); one pair of subventral adcloacal papillae located between the cloaca and the midventral papilla and somewhat variable in position; another pair of subventral adcloacal papillae located at about cloacal level (fig. 2E); three pairs of postcloacal papillae near the tail terminus, one pair subventral, one pair almost terminal and the other subdorsal; a subdorsal pair located at about the level of the cloaca or slightly more anterior and a single midventral papilla situated some distance anterior to

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Table 2. Morphometrics of first and second generation females of *Steinernema longicaudum*.

	Chinese isolate (n = 22)		Gongju isolate (Korea) (n = 25)		B2 isolate (USA) (n = 25)	
Character	First generation	Second generation	First generation	Second generation	First generation	Second generation
Body length	6935 ± 1977 (3188–10999)	2385 ± 293 (1847–2986)	7500 ± 878 (5500–9600)	2442 ± 250 (1987–3011)	8500 + 625 (7500–8956)	2756 ± 598 (2456–3652)
Greatest body width	347 ± 65.0 (227–518)	144 ± 19 (113–214)	343 ± 44 (250–489)	148 ± 15 (115–218)	315.5 ± 33.8 (281–454)	376 ± 31.2 (276–525)
Stoma length	5.5 ± 1.3 (4.5–9.0)	4.4 ± 0.8 (2.3–6.8)	6.5 ± 1.5 (5.5–7.5)	4.5 + 0.7 (2.5–5.5)	5.5 ± 0.9 (4.9–8.5)	5.5 ± 0.9 (3.7–7.5)
Stoma width	11.4 ± 1.3 (9.0–13.5)	9.1 ± 0.7 (6.7–11.3)	9.9 ± 1.5 (9.0–12.3)	9.1 ± 0.5 (6.5–11.5)	11.0 ± 1.2 (9.3–13.5)	9.5 ± 0.5 (6.5–11.5)
Anterior end to excretory pore	129 ± 23.3 (83–169)	90 ± 12.2 (63–124)	135 ± 25.6 (100–171)	92 ± 10.5 (70–128)	132.5 ± 11.5 (89–143)	91 ± 9.5 (75–119)
Body width at excretory pore	127 ± 29.1 (68–210)	69 ± 9.2 (54–97)	125.5 ± 20.0 (89–188)	70 ± 8.7 (58–89)	125.5 ± 20.5 (93–222)	74 ± 6.5 (59–105)
Anterior end to nerve ring	176 ± 23.1 (140–221)	136 ± 5.7 (126–149)	175.5 ± 22.5 (143–199)	135 ± 5.5 (125–150)	165.5 ± 7.7 (151–188)	144 ± 3.5 (135–149)
Anterior end to oesophagus	224 ± 19.1 (176–268)	177 ± 4.6 (169–183)	220 ± 18.3 (175–273)	172 ± 3.9 (167–190)	235.5 ± 11.8 (191–262)	182 ± 5.1 (175–197)
Body width at anus	97 ± 39.9 (54–216)	40 ± 4.6 (27–50)	102 ± 26.4 (68–158)	43 ± 4.4 (29–51)	83.5 ± 11.4 (81–121)	45 ± 3.1 (35–55)
Tail length	57 ± 24.3 (26–130)	50 ± 8.7 (18–65)	66 ± 19.5 (25–109)	53 ± 6.7 (22–68)	86.5 ± 25.7 (49–148)	57.5 ± 9.1 (31–87)
Anterior end to vulva	3492 ± 950 (1708–5579)	1241 ± 128 (998–1540)	3458 ± 897 (1814–5875)	1256 ± 115 (1110–1580)	3789.5 ± 756 (1954–5876)	1289 ± 121 (1175–5635)
Body width at vulva	377 ± 62.1 (255–509)	151.0 ± 19 (110–223)	367.5 ± 55.5 (245–499)	155 ± 17.5 (111–225)	353.5 ± 51.3 (285–526)	171 ± 18 (115–231)
V%*	50.6 ± 2.8 (44.4–57.0)	52.2 ± 2.2 (47.8–56.4)	54 ± 1.8 (48–58)	53 ± 1.5 (49–55)	55.0 ± 3.1 (52–60)	53 ± 1.8 (51–54)
D%†	57.4 ± 5.3 (40–72.8)	51 ± 3.4 (36.4–68.8)	61.5 ± 3.5 (57–64)	53 ± 4.3 (40–67)	54 ± 2.1 (49–62)	52 ± 2.5 (39.5–65.5)

Means ± standard deviation and range (between brackets) are given in μm.
* Distance from anterior end to vulva divided by body length × 100.
† Distance from anterior end to excretory pore divided by oesophagus length × 100.

the cloaca (fig. 2D,E). Spicules paired, symmetrical, dark yellowish brown in colour and ventrally arcuate with a well developed, somewhat angular capitulum about 1.5 times as long as wide. There is a short ventral velum. Spicule lamina with two prominent cuticular ribs running along its length. Distal region of lamina narrowed and tips finely rounded (fig. 3H). Gubernaculum boat-shaped, distally bifurcate in ventral or dorsal view; capitulum of variable form being rounded to irregularly hamate in shape (fig. 3I,J). The second generation male also has a papillal complement of 23 arranged more or less as in the first generation male. The precloacal series is somewhat closer spaced, probably due to the shorter body length of the second generation male and there are two, distinctly subventral pairs of postcloacal papillae plus one pair subdorsal. The spicules are similar to the first generation male, but the capitulum is slightly different in shape and somewhat more elongate. The gubernaculum is more consistent in shape with a less variable capitulum. Bursa absent. Tail short conoid, dorsally convex with a rounded terminus and no mucron.

Infective stage juvenile. Emerging third stage juveniles often enclosed in the second stage cuticle. Heat relaxed form straight to slightly arcuate ventrally. Body slender, gradually tapering from base of oesophagus to anterior end and from anus to terminus. Cephalic region rounded and not offset from body. Cuticle finely annulated (fig. 4A), the striae stopping at the point where the hyaline region commences. Lateral field distinct with eight, equally spaced longitudinal ridges (i.e. nine lines or incisures) visible in the midbody region with SEM observation (fig. 4B). Mouth and anus closed. Oesophagus non-functional and of usual form for the genus. Excretory pore located in postmedian region of oesophagus (average D% = 55) with a long, cuticularized excretory duct extending posteriorly (fig. 3K). Prominent, lenticular hemizonid, together with smaller hemizonion located near anterior margin of the weakly developed basal bulb. Anterior portion of intestine with a pouch containing cells of the symbiotic bacterium. Tail straight or slightly ventrally directed, initially sub-conoid but narrowing slightly posterior to the beginning of the hyaline portion before tapering evenly to a finely pointed terminus; terminal spine absent (fig. 4C,D). Hyaline region prominent, comprising about half the tail length; cytoplasmic contents terminating somewhat irregularly and extending slightly further posteriorly, particularly dorsally, around the periphery. Phasmids located in anterior half of tail at about 30–40% of tail length.

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Table 3. Morphometrics of third-stage infective juveniles of *Steinernema longicaudum*.

Character	Chinese isolate (n = 28)	Gongju isolate (Korea) (n = 25)	B2 isolate (USA) (n = 25)
Body length	1043 ± 73 (929–1170)	960 ± 54 (900–1075)	1040 ± 65 (931–1194)
Greatest body width	37 ± 1.4 (34–40)	33 ± 1.5 (31–38)	44 ± 1.4 (37–47)
Anterior end to excretory pore	82 ± 4.0 (74–92)	81 ± 3.0 (75–94)	76 ± 3.1 (71–85)
Body width at excretory pore	27 ± 1.3 (24–30)	25 ± 0.9 (22–29)	26 ± 1.7 (24–32)
Anterior end to nerve ring	111 ± 6.9 (98–129)	108 ± 5.5 (95–125)	109 ± 5.5 (99–132)
Anterior end to oesophagus	142 ± 4.8 (134–150)	140 ± 3.5 (130–145)	145 ± 3.5 (133–159)
Body width at anus	23 ± 1.3 (21–27)	21 ± 1.1 (19–25)	28 + 1.7 (24–40)
Tail length	94 ± 6.9 (79–105)	95 ± 4.5 (78–109)	95 ± 3.4 (81–110)
Ratio a	28.2 ± 1.4 (25.9–30.7)	26.5 ± 1.2 (25.7–29.8)	27.8 ± 1.4 (25.2–28.3)
Ratio b	7.4 ± 0.5 (6.3–8.1)	7.3 ± 0.3 (6.7–7.9)	7.3 ± 0.5 (6.4–7.9)
Ratio c	11.1 ± 0.4 (10.5–12.1)	11.3 ± 0.2 (10.5–11.8)	10.9 ± 0.7 (10.4–12.3)
D%*	57.4 ± 2.4 (52.4–62.5)	55 ± 1.9 (53–57)	56.5 ± 1.5 (53.6–59.4)
E%†	86.9 ± 7.1 (75.5–104.1)	87.5 ± 5.5 (79–97)	85.5 ± 4.3 (76.5–98.3)

Means ± standard deviation and range (between brackets) are given in μm.
* Distance from anterior end to excretory pore divided by oesophagus length × 100.
† Distance from anterior end to excretory pore divided by tail length × 100.

Type-host and locality. No type host known as this species (type isolate) was recovered from soil using *G. mellonella* larvae as bait. The soil samples were collected by Shen in an apple orchard in Laiyang City (North 37°, East 121°28′), Shangdong province in June, 1985.

Type specimens. Holotype first generation male, allotype first generation female, paratype first generation males and paratype females, paratype infective juveniles (accession numbers/codes not known) deposited by X. Shen in the Insect Collection of the Department of Plant Protection, Laiyang Agricultural College, Laiyang 265200, Shandong province, China (details of number of specimens unknown). Additional paratype first generation males, paratype first generation females and paratype infective juveniles in the Department of Plant Protection, South China Agricultural University, Guangzhou, China (details of number of specimens unknown). Topotype material of both sexes (provided by Shen) deposited in the nematode collection, Institute of Biological Control, CAAS, Beijing 10081, China. Living material from China also deposited in the nematode collection, CABI Bioscience (formerly the International Institute of Parasitology), Bakeham Lane, Egham, Surrey, UK.

Voucher specimens. Five males of isolate B2 (UCDNC 3692); five males of isolate Gongju (UCDNC 3693); five juveniles of isolate B2 (UCDNC 3694); five juveniles of isolate Gongju (UCDNC 3695) deposited at the University of California Davis Nematode Collection.

Diagnosis and relationships. *Steinernema longicaudum* is characterized by the following combination of characters: infective stage juvenile with a body length of about 1014 (900–1194) μm, a relatively long tail (mean length of about 95 μm) and lateral field with eight longitudinal ridges; tail tip of both first and second generation males lacking a cuticular mucron; spicules curved with a distinct dark yellow coloration and about 87 μm and 73 μm long in first and second generation respectively; 11 pairs of copulatory papillae plus a single midventral papilla located some distance anterior to the cloaca; first generation female tail subconoid with serratulate terminus; second generation female tail usually relatively longer and more conoid with a pointed terminus. *Steinernema longicaudum* is morphologically similar to *S. arenarium*, *S. cubanum*, and *S. glaseri*, but can be separated from these species by a combination of morphological characteristics and DNA analysis. Only *S. longicaudum* is recorded as having a serratulate projection on the tail of the first generation female and this feature can be used to separate it from the other species listed above. The IJ and first generation male of *S. longicaudum* can be distinguished from all the above species by a combination of characters (see tables 4, 5).

Discussion

According to Shen & Wang (1992), *S. longicaudum* can be distinguished from other species of the genus *Steinernema* by the size of the IJ (about 1074 μm long),

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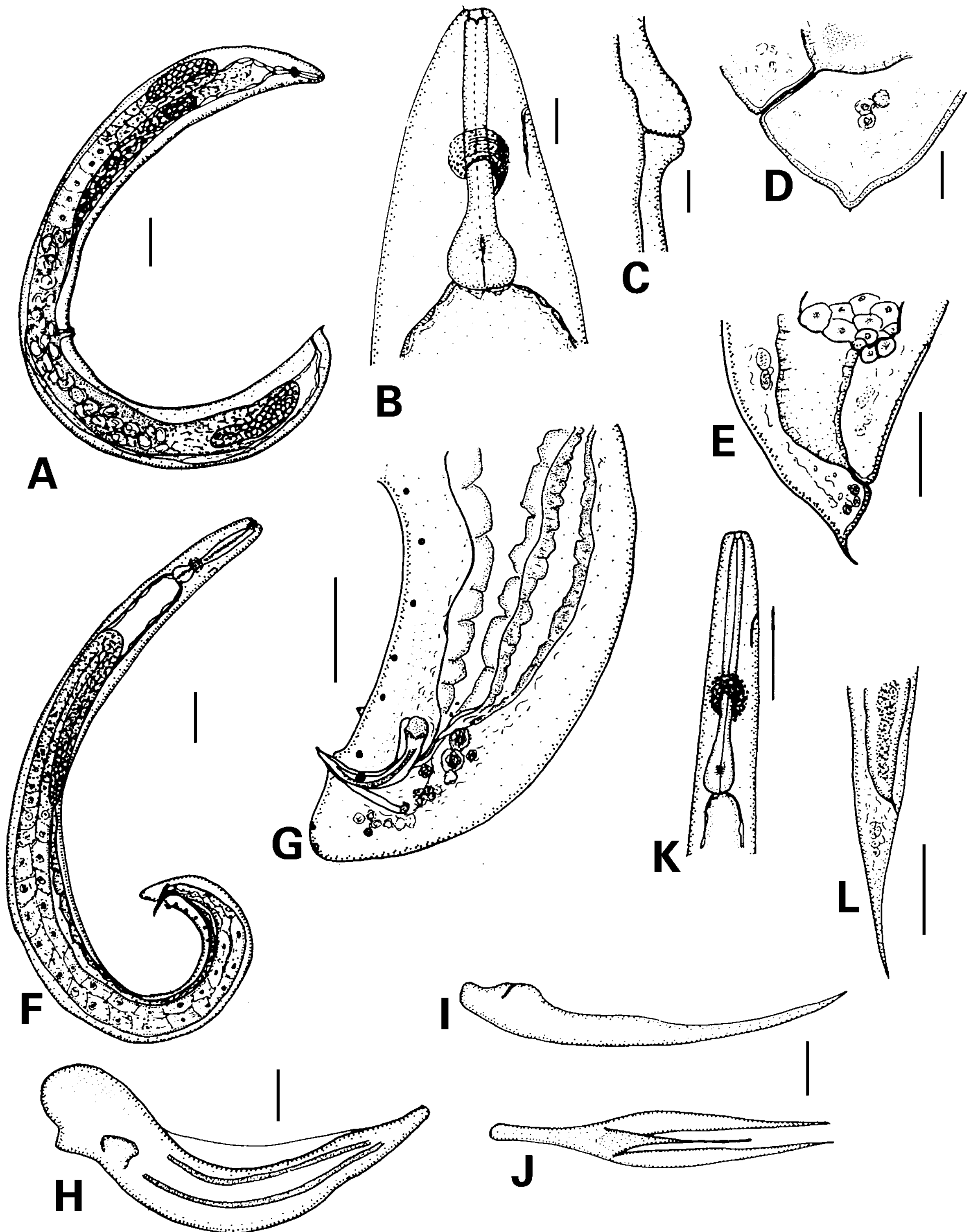

Fig. 3. *Steinernema longicaudum*. A. First generation female, in toto. B. Anterior end of first generation female. C. Vulva of first generation female. D. Tail of first generation female indicating variation in shape. E. Tail of second generation female. F. First generation male, in toto. G. Tail of first generation male. H. Spicule of first generation male. I. Gubernaculum in lateral view. J. Gubernaculum in dorsal view. K. Anterior end of third-stage infective juvenile. L. Tail of third-stage infective juvenile. Bars = A: five 300 μm; B, C, E, G, K, L: 50 μm; D, F: 100 μm; H, J: 10 μm.

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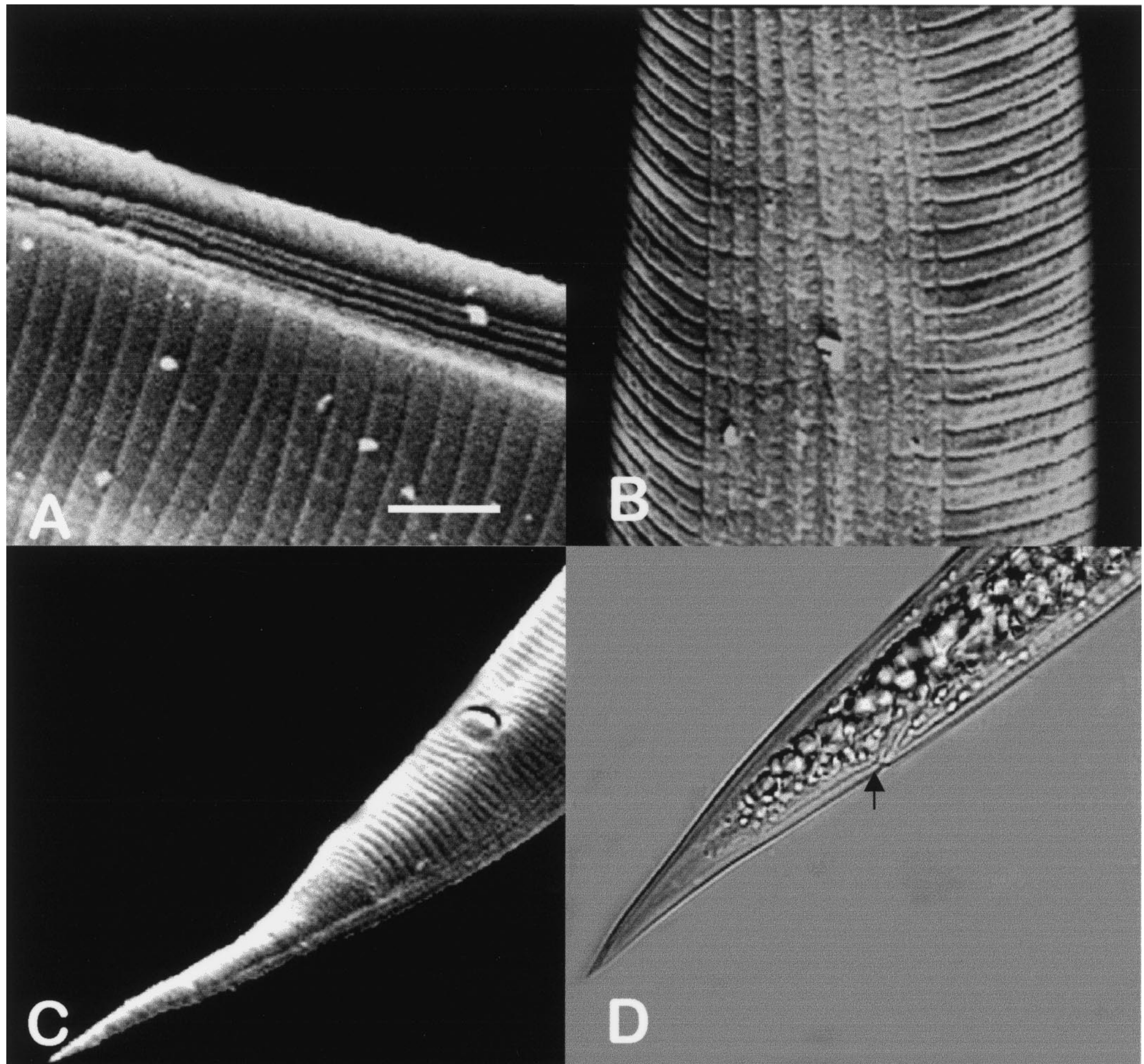

Fig. 4. *Steinernema longicaudum*: third-stage infective juvenile. A. Scanning electron micrograph of the lateral field at the anterior region of the body. B. Scanning electron micrograph of the pattern of lateral field pattern at the midbody with eight ridges. C. Scanning electron micrograph of the tail region showing position of the anus. D. Light microscopy image of the tail region showing position of the anus (arrow). All magnifications based on scale bar in A. A, B = 10 μm; C = 15 μm; D = 40 μm.

Table 4. Comparison of some important characters of infective juveniles of similar species of *Steinernema*.

Species	Body length (μm)	Body width (μm)	EP* (μm)	ES† (μm)	Tail length (μm)	a	b	c	D%‡	E%§	Source
S. arenarium	1034	46	83	138	75	26	7.6	13.8	55	119	Nguyen & Smart, 1996
S. cubanum	1283	37	106	148	67	35	8.6	19.2	70	160	Nguyen & Smart, 1996
S. glaseri	1130	43	102	162	78	29	7.3	14.7	65	131	Nguyen & Smart, 1996
S. longicaudum	1014	39	79	144	96	27	7.3	10.8	55	85.5	Present study

* Distance from head to excretory pore.
† Oesophagus length.
‡ EP/ES × 100.
§ EP/tail length × 100.

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Table 5. Comparison of some important characters of first generation males of similar species of *Steinernema*.

Species	Spicule length (μm)	Gubernaculum length (μm)	D%*	SW†	GS‡	Source
S. arenarium	84	55	93	2.1	0.65	Nguyen & Smart, 1996
S. cubanum	58	39	70	1.41	0.67	Nguyen & Smart, 1996
S. glaseri	77	55	70	2.05	0.71	Nguyen & Smart, 1996
S. longicaudum	87	58	74	1.55	0.66	Present study

* Distance from anterior end to excretory pore divided by oesophagus length × 100.
† Spicule length divided by body width at cloaca.
‡ Gubernaculum length divided by spicule length.

and by having the greatest average tail length (more than 90 μm long); the shape of the tail of both first and second generation males, lacking a cuticular mucron; and the shape of the spicules, with a markedly curved lamina and a distinct dark yellow coloration.

The present study provides a more detailed characterization of this species including new morphological and molecular diagnostic features. For instance, for the first and second generation female, the location of the vulva, shape of the vulval lips and shape and length of the tail are newly observed diagnostic characters. Moreover, the presence of a minute serrulate terminus bearing minute projections is also observed in the first generation females. Although Shen & Wang (1992) did not acknowledge this character, the presence of a minute mucro is indicated in the original illustration of the tail of the first generation female. With respect to males, a more detailed characterization of the morphology of the spicules, gubernaculum and location of the genital papillae is provided. Our SEM observations of the IJ indicate that the lateral field pattern is characterized by the presence of eight equally spaced longitudinal ridges, and not six as originally described by Shen & Wang (1992).

The use of DNA analysis, supplemented by cross-breeding data provides a more objective means of assessing species validity in this study. By comparing the restriction enzyme profiles for the ITS region of the ribosomal DNA repeat unit, the identity of all three isolates of *S. longicaudum* was confirmed. Additionally, a comparison of the RFLP profiles of this species with other morphologically similar species (*S. arenarium*, *S. cubanum* and *S. glaseri*), demonstrates its distinctiveness.

Intra-specific morphological variation has been observed among steinernematids. Poinar (1992) reported morphological and morphometric differences between a Californian population and the SN strain of *S. feltiae*, suggesting the observed variation could be attributed not only to their geographic origin but also to different environmental conditions and host interactions. Similarly, Stock *et al.* (1997) described intra-specific variation between populations of *S. glaseri* from Korea, and highlighted the value of male and IJ features for identification and characterization of these different populations of steinernematids. Despite these observations, it is unknown to what extent morphological differences that are observed among populations reflect underlying genetic differences as distinct from environmental influences and the role of natural selection.

In the past, descriptions of new entomopathogenic nematode species have only been based on the characterization of a single population. In the present study, we considered not only topotype specimens but also included specimens from two geographically distant populations of *S. longicaudum*. We recognize these studies are necessary not only to understand the existing intraspecific variations among steinernematid species but will provide a firm base for the establishment of species among this group of nematodes.

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