Nematology, 2007, Vol. 9(3), 379-396

Steinernema texanum n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from Texas, USA

Khuong B. NGUYEN [1,*], Robin J. STUART [2], Vanessa ANDALO [3], Ugur GOZEL [4] and Michael E. ROGERS [2]

[1] *Entomology and Nematology Department, Institute of Food and Agricultural Science, University of Florida, Gainesville, FL 32611-0620, USA*

[2] *Citrus Research and Education Center, Institute of Food and Agricultural Science, University of Florida, 700 Experiment Station Road, Lake Alfred, FL 33850, USA*

[3] *Entomology Department, University of Lavras, Lavras, MG, 37200-000, Brazil*

[4] *Canakkale Onsekiz Mart University, Faculty of Agriculture, Department of Plant Protection, 17100 Canakkale, Turkey*

Received: 8 December 2006; revised: 28 February 2007
Accepted for publication: 1 March 2007

Summary – *Steinernema texanum* n. sp. is characterised by morphometrics of the infective juvenile with body length = 756 μm, distance from anterior end to the excretory pore = 59 μm, tail = 73 μm, ratio a = 25, H% = 59 and E% = 81. The lateral field pattern of the new species is 2, 7, 2, and is typical for the species. The male of the first generation can be recognised by the spicule and the gubernaculum lengths and shapes, position of the excretory pore, D% = 67 and GS% = 75. The female can be recognised by the vulva with very low epiptygma and two wart-like structures anterior to the tail tip that are always present on the ventral side. *Steinernema texanum* n. sp. is characterised genetically by the sequence of the ITS region (sequence length = 956 bp, the length of ITS1 = 263 bp, ITS2 = 286 bp, composition of its sequence and by 18 autapomorphies) and by sequence of D2D3 regions (sequence length = 860 bp, its composition and 15 autapomorphies). *Steinernema texanum* n. sp. is closely related to species in the *feltiae*-group, which include *S. akhursti*, *S. feltiae*, *S. hebeiense*, *S. jollieti*, *S. kraussei*, *S. kushidai*, *S. litorale*, *S. monticolum*, *S. oregonense*, *S. sangi*, *S. silvaticum* and *S. weiseri.* Isolates of the new species were obtained using the *Galleria*-baiting technique from soil samples taken near Kingsville, Texas, USA.

Keywords – D2D3, description, entomopathogenic nematodes, molecular, morphology, morphometrics, phylogeny, SEM, taxonomy.

Entomopathogenic nematodes have been known since 1923 but interest in the use of nematodes as biological control agents waned until the 1970s and 1980s. During that period, increasing environmental concerns about the use of chemical pesticides and their reduced availability reawakened interest in entomopathogenic nematodes for insect control. In 2001, about 100 different laboratories were exploring these nematodes and their bacterial symbionts in more than 60 countries from every inhabited continent (Gaugler, 2002). Many surveys have been conducted all over the world in search of species to control economically important insect pests (Hominick, 2002). Currently, 56 species of entomopathogenic nematodes in the family Steinernematidae and 12 species in Heterorhabditidae have been reported (Nguyen, 2006).

Laboratory experiments, and field releases of entomopathogenic nematodes in the past, show that they have been used successfully to control insect pests (Klein, 1990; Shapiro-Ilan *et al.*, 2002). The isolation of *S. scapterisci* Nguyen & Smart, 1990 from Uruguay and *S. riobrave* Cabanillas, Poinar & Raulston, 1994 from Texas are good examples of successful searches for, and subsequent use of, entomopathogenic nematodes for biological control (Parkman *et al.*, 1993, 1994; Parkman & Smart, 1996; Shapiro-Ilan *et al.*, 2002). These two nematodes are commercialised and sold for controlling mole crickets and citrus root weevils, respectively, in the USA.

Steinernema riobrave has proved to be one of the most effective entomopathogenic nematodes yet tested for inundative biological control of root weevils such as *Diaprepes abbreviatus* (L.) in Florida citrus groves (Shapiro & McCoy, 2000a, b; Shapiro-Ilan *et al*., 2002). However, until recently, only a single strain of this species was known. Therefore, in an effort to find new strains

* Corresponding author, e-mail: kbn@ufl.edu

Also available online - www.brill.nl/nemy

of *S. riobrave* for potential use in biological control we conducted two soil-sampling surveys in the vicinity of the type locality near Weslaco, Texas (Cabanillas *et al.*, 1994). In the first survey (17-19 April 2001), we found ten new isolates of *S. riobrave* (Stuart *et al.*, 2004) and a previously undescribed heterorhabditid species, *Heterorhabditis mexicana* Nguyen, Shapiro-Ilan, Stuart, McCoy, James & Adams, 2004. Here we report the results of our second survey and the discovery of a new steinernematid species.

This isolate differs from all other species of *Steinernema* in morphological, morphometrical and molecular characteristics, and as indicated in cross-hybridisation tests with closely related nematodes. This new nematode is described and illustrated here as *Steinernema texanum* n. sp.

Materials and methods

Survey

Soil samples were collected at ten sites in southern Texas during the period 19-22 December 2003. The collection sites were roadside areas located between Kingsville, Brownsville, and Laredo, mostly along state roads 77 and 83, in areas with mixed weedy vegetation. Soil sampling followed the procedures of Stuart and Gaugler (1994) and involved taking a series of 20 soil cores (2.5 cm diam. × 21 cm depth) per site (total n = 200 samples). Soil cores were generally taken at *ca* 4 m intervals along linear transects at each site. Soil samples were kept on ice in coolers for transport to the laboratory.

In the laboratory, each sample was baited with a single wax moth larva, *Galleria mellonella* (L.) (Webster's Waxie Ranch, Webster, WI, USA). Soil samples were checked weekly for dead larvae which were removed and replaced with live larvae for additional rounds of baiting. Dead larvae that exhibited signs of infection with entomopathogenic nematodes were placed in modified White traps (Hara *et al.*, 1991). Nematodes that emerged from any individual bait larva were considered a separate isolate.

Nematode source

Steinernema texanum n. sp. was isolated in the laboratory from soil samples by means of trapping with *G. mellonella* as stated above. The isolates were maintained in the laboratory by recycling through *G. mellonella* (Dutky *et al*., 1964; Nguyen, 1988) every 3-4 months and were stored in culture flasks at 16°C.

Morphological observations

For taxonomic studies, 100 × 15 mm Petri dishes lined with moistened filter paper were inoculated with 200 infective juveniles (IJ) per *G. mellonella* and kept in the dark at 25°C in a growth chamber. *Galleria* larvae died 2 days after inoculation. First generation males and females were obtained 3 days after the *Galleria* died and second generation males and females after 5-7 days, by dissecting the cadavers in 1% NaCl solution. Third-stage infective juveniles were obtained after 10 days and harvested during the first week of emergence. Third-stage infective juveniles were obtained during the first 2 days after emergence from insect cadavers as suggested by Nguyen and Smart (1995a). For light microscope observations, 20 males and females and infective juveniles (IJ) were examined alive. In order to see morphological structures better, additional specimens from different stages were killed in warm water (40°C), or fixed in triethanolamine formalin (TAF) as suggested by Courtney *et al*. (1955), or in lactophenol as reported by Franklin and Goodey (1949). These nematodes were used when further observation was needed to confirm the morphology or variation of particular structures. Nematodes fixed in TAF were processed to glycerin by the Seinhorst (1959) method. Type specimens were mounted in glycerin. Cover glass supports were used in all cases to avoid flattening of specimens. Measurements and drawings were made by using a compound microscope with a drawing tube.

Scanning electron microscopy (SEM)

Adults of the first generation and IJ were fixed in 4% formalin buffered with 0.1 M sodium cacodylate at pH 7.2 for 24 h at 8°C. They were post-fixed with 2% osmium tetroxide solution for 12 h at 25°C, dehydrated in a graded ethanol series, critical point dried with liquid CO_2, mounted on SEM stubs and coated with gold (Nguyen & Smart, 1995b). Spicules and gubernacula were prepared as suggested by Nguyen and Smart (1990, 1997).

Cross-hybridisation

Infective juveniles of *S. feltiae* (Filipjev, 1934) Wouts, Mráček, Gerdin & Bedding, 1982 (CN strain) and *S. oregonense* Liu & Berry, 1996 (Oregon strain) were tested for reproductive compatibility with *S. texanum* n. sp. The method used was suggested by Nguyen and Duncan (2002) using *G. mellonella* haemolymph. Other related species were not available for the tests.

Molecular characterisation

Extraction of DNA

DNA was extracted from a single female using a DNeasy tissue kit (Qiagen, Valencia, CA, USA). Methods in the instruction book were followed in this study.

PCR amplification

The methods reported by Nguyen *et al.* (2006) were used for this study.

Sequencing

PCR products were purified with a QIAquick PCR purification kit (Qiagen). Purified DNA was sequenced in both directions on automated sequenchers as reported previously (Nguyen *et al*., 2001). Sequencher™, version 4.2 (Gene Code, Ann Arbor, MI, USA) was used for sequence editing and verifying base-calls.

Multiple alignments

Sequences of studied species were aligned using the default parameters of Clustal X (Thompson *et al*., 1997), then optimised manually in MacClade 4.0 (Maddison & Maddison, 2002).

Phylogenetic relationships

Sequences of the partial 28S ribosomal DNA (D2D3 regions) and the internal transcribed spacer regions (ITS) of *Steinernema* species have been used by different authors (Nguyen *et al*., 2001, 2006; Stock *et al*., 2001; Nguyen & Duncan, 2002; Nguyen & Adams, 2003; Spiridonov *et al.*, 2004) in taxonomic and phylogenetic studies. In this paper, sequences of these two regions were used. For ITS regions and D2D3, phylogenetic relationships were done as reported by Nguyen *et al.* (2004, 2006) except that the sequence of D2D3 for *S. monticolum* Stock, Choo & Kaya, 1997 was new. Distance analysis was by neighbour joining. Accession numbers of sequences of nematode species are cited on the phylogenetic trees. Branch support was estimated by bootstrap analysis (1000 replicates) using the same parameters as the original search. For further species delimitation, we traced the phylogenetic relationships among *Steinernema* species in the *feltiae*-group and maps of ITS and D2D3 character states that could be polarised unambiguously (MacClade 4.05). The results of this process show the numbers of transition/transversion sites and autapomorphies (unique, derived characters) that, when fixed within and among lineages, indicate lineage independence.

Steinernema texanum* **n. sp.**

(Figs 1-5)

Measurements

See Table 1.

Description

First generation male

Body C-shaped to strongly curved posteriorly when heat-relaxed. Cuticle smooth under light microscope but with striation under SEM. Lateral field not observed. Head rounded, gently tapering anteriorly, slightly swollen and flattened anteriorly in some specimens. Face with six labial papillae, two amphidial apertures and four cephalic papillae. Labial papillae longer than cephalic papillae. Stoma shallow, narrow, usually with pronounced cheilorhabdions, posterior part funnel-shaped, well cuticularised. Excretory pore anterior to nerve ring, anterior part of excretory duct well cuticularised, excretory cells not observed. Pharynx with cylindrical procorpus, metacorpus slightly swollen, isthmus present. Nerve ring surrounding isthmus, basal bulb distinct. Pharyngo-intestinal valve present. Gonad monorchic, reflexed. Testis with ventral reflexion, germinal zone, growth zone and *vas deferens*. Distance from base of pharynx to anterior end of testis variable, longer than pharynx length. Spicules paired, dark brown in colour. Head (manubrium) of spicules, somewhat elongate (spicule head length/ width = 1.20-1.40); shaft (calomus) very short or absent; rostrum present; blade (lamina) thick, tapering slightly posteriorly; blade terminus blunt; velum prominent. Each spicule with two internal ribs. Under SEM, spicule with three lobes: dorsal lobe prominent, well curved anteriorly; lateral lobe well defined on dorsal edge and divided into two posteriorly; ventral lobe short. Gubernaculum boat-shaped in lateral view, cuneus needle-shaped, anterior end curved ventrally. Usually a single precloacal papilla and 11 pairs of genital papillae arranged in normal position for *Steinernema* (*i.e*., six or seven pairs precloacal subventral, one pair adcloacal, one pair lateral, two pairs subterminal and one pair subdorsal). Number of caudal papillae is constant (three pairs), but number of precloacal subventral pairs variable. Tail conoid, without phasmid; tail terminus without mucron.

* The specific epithet is derived from Texas.

Table 1. *Morphometrics of* Steinernema texanum *n. sp. Measurements are in μm and in the form: mean ± sd (range).*

Character	First generation			Second generation		Infective juvenile
	Holotype (Male)	Male	Female	Male	Female	
n	–	20	20	20	20	20
L	1384	1296 ± 66 (1197-1406)	3058 ± 268 (2720-3623)	828 ± 34 (773-890)	1717 ± 227 (1250-2105)	756 ± 13.4 (732-796)
a						25 ± 1.5 (22-27)
b						6.5 ± 0.2 (6.2-7.0
c						10.4 ± 0.6 (9.6-12.5)
c′						4.1 ± 0.3 (3.3-4.6)
V			52 ± 1,4 (50-55)		55 ± 1.8 (52-59)	
Body diam.	128	99 ± 10 (81-116)	163 ± 15 (130-202)	50 ± 3 (45-58)	95 ± 11 (75-114)	30 ± 2.2 (29-34)
EP	80	90 ± 4.7 (79-100)	88 ±7.5 (78-107)	75 ± 5.4 (64-83)	94 ± 8 (68-104)	59 ± 2.6 (52-62)
NR	123	104 ± 4.8 (94-114)	122 ± 7.8 (111-135)	97 ± 8.7 (81-117)	127 ± 11 (113–159)	92 ± 4.8 (84-102)
ES	155	135 ± 6.8 (123-147)	172 ± 9 (160-189)	129 ± 10.2 (109-151)	178 ± 17 (159-236)	115 ± 2.7 (111-120)
Testis reflexion	267	269 ± 15.8 (273-325)		130 ± 24.6 (90-165)		
Tail length (T)	28	23 ± 3.7 (19-30)	40 ± 5.7 (30-52)	28 ± 1.7 (25-30)	53 ± 6 (43-61)	73 ± 4.5 (60-79)
Hyaline (H)						43 ± 2.9 (37-47)
Anal body diam. (ABD)	38	38 ± 4.1 (31-45)	60 ± 5.6 (50-71)	31 ± 1.7 (27-33)	43 ± 4 (38-52)	18 ± 0.9 (17-20)
Spicule length (SL)	58	60 ± 2.9 (55-66)		42 ± 1.9 (39-46)		
Spicule width	10	12.6 ± 1.4 (10-14)		7.4 ± 1.0 (5.8-8.7)		
Gubernaculum length (GL)	49	45 ± 3.9 (39-53)		24 ± 2.1		
Gubernaculum width	7.2	6.7 ± 1.1 (5.8-10.0)				
D% = EP/ES × 100	52	67 ± 4.7 (58-73)				51 ± 2.2 (46-53)
E% = EP/T × 100						81 ± 3.2 (76-88)
SW% = SL/ABD × 100	152	157 ± 19.2 (127-203)				
GS% = GL/SL × 100	84	75 ± 5.5 (62-84)				
H% = H/T × 100						59 ± 2.7 (53-64)

EP = distance from anterior end to excretory pore; NR = distance from anterior end to nerve ring; ES = distance from anterior end to end of pharynx.

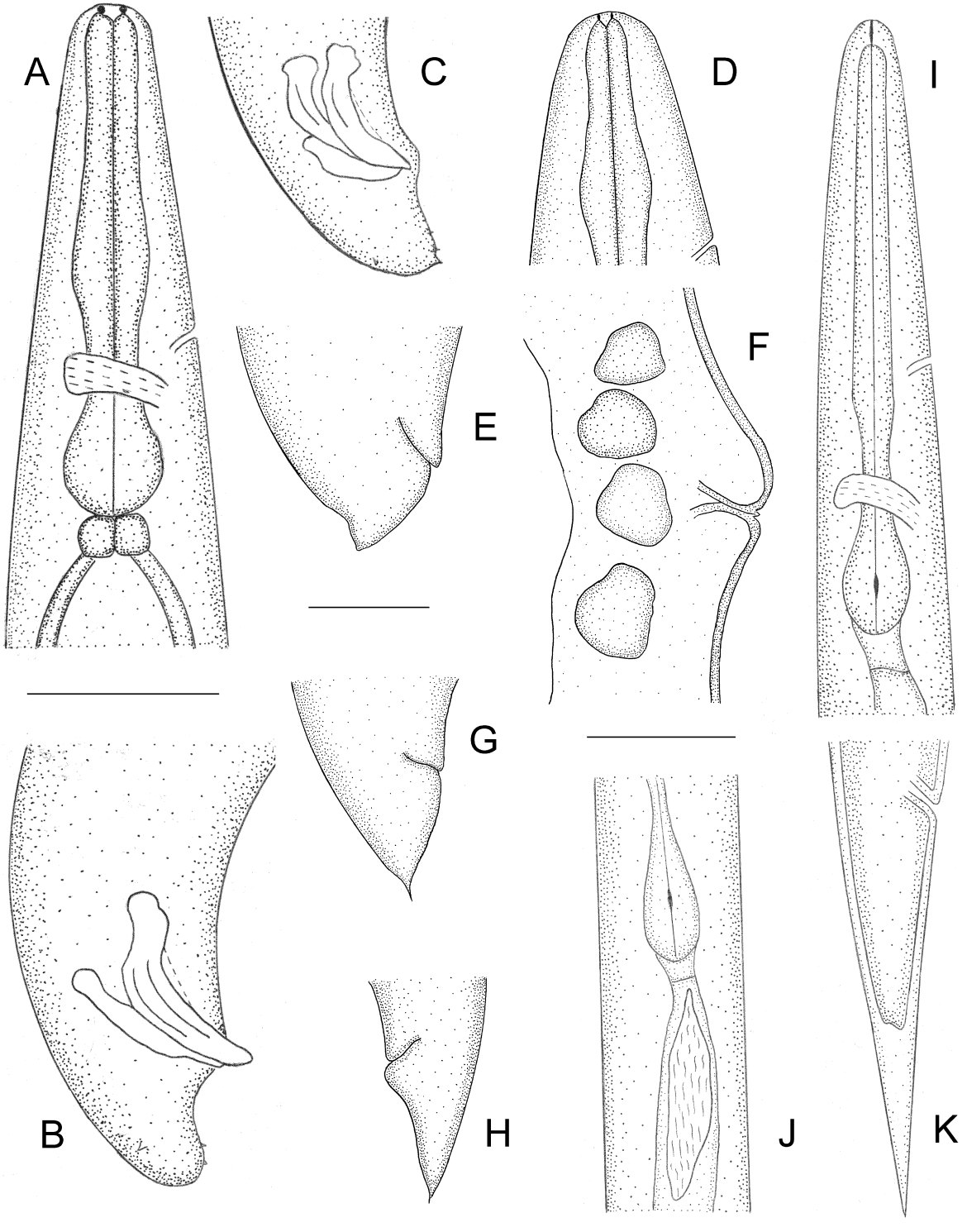

Fig. 1. Steinernema texanum *n. sp. A, B: Anterior region and tail of first generation male; C: Second generation male tail. D-F: First generation female head, tail and vulva. G, H: Second generation female tail shapes. I-K: Infective juvenile: I: Anterior region; J: Posterior part of pharynx showing basal bulb and bacterial chamber, K: Tail. (Scale bars: Left and central bars for males and females = 50 μm, right bar for infective juvenile = 25 μm.)*

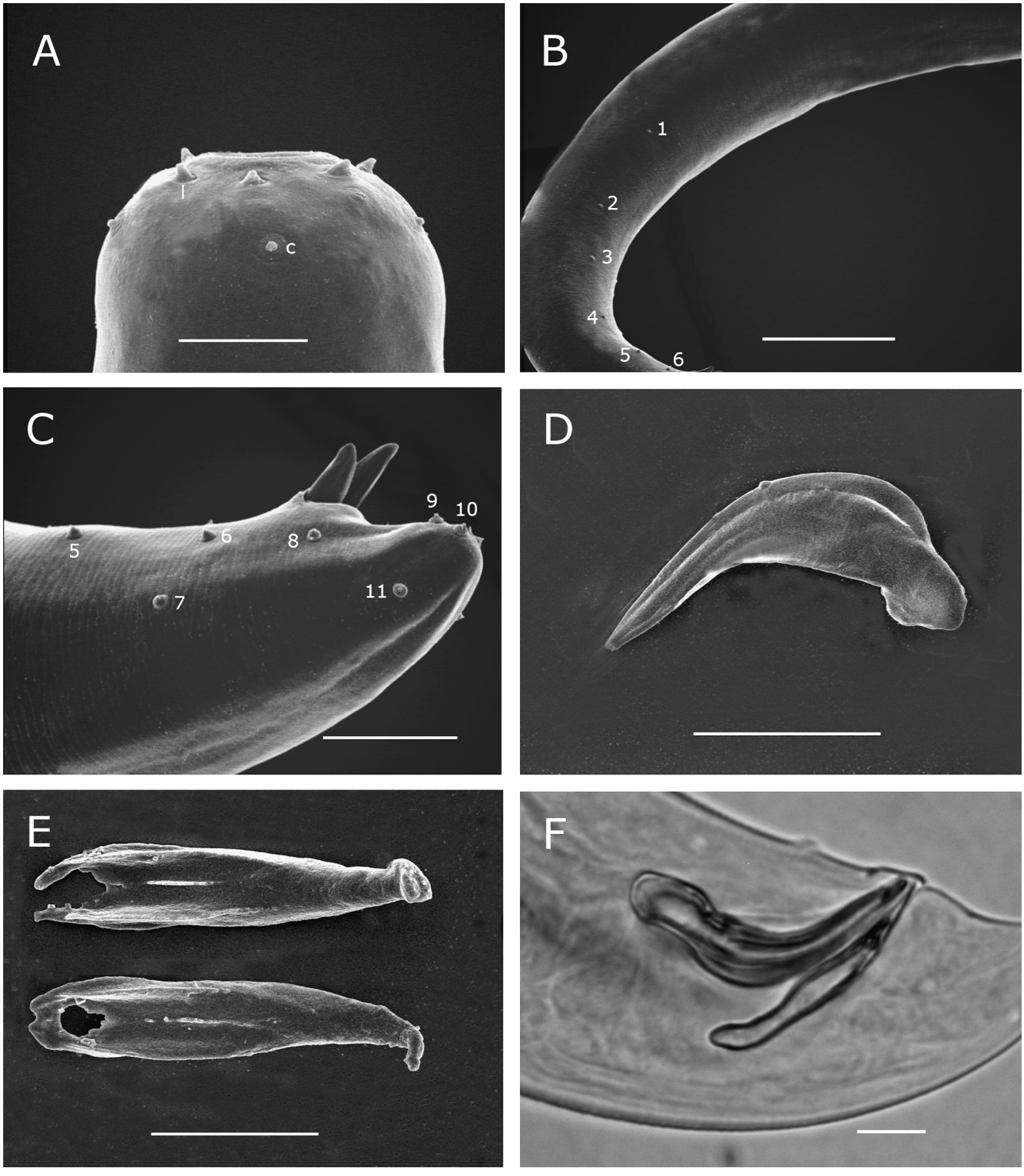

Fig. 2. *SEM of* Steinernema texanum *n. sp. first generation males. A: Male head showing labial papillae (l), and cephalic papillae (c); B: Posterior region showing genital papillae from pair 1 to pair 6; C: Papillae pairs 5-11; D: Spicule; E: Gubernacula ventral view showing needle-shaped cuneus; F: LM photograph of spicules and gubernaculum. (Scale bars: A = 12.0 μm, B = 66.7 μm, C = 20 μm, D = 27.3 μm, E = 16.7 μm, F = 12.4 μm.)*

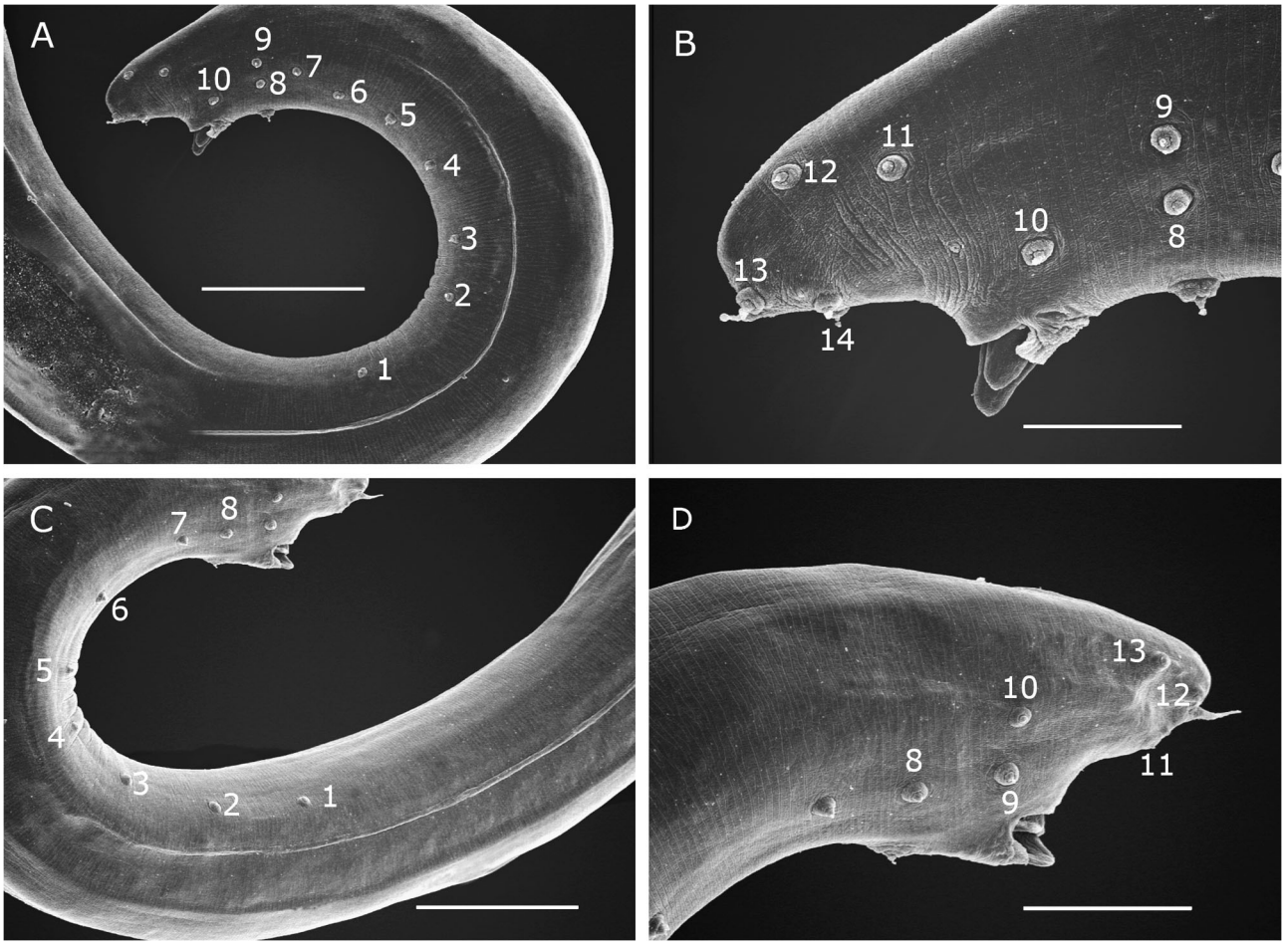

Fig. 3. *SEM of* Steinernema texanum *n. sp. A-D: Two second generation males showing 13 and 14 pairs of genital papillae, single-ridged lateral field and variation of mucron position. (Scale bars: A = 30 μm, B = 10 μm, C = 30 μm, D = 15 μm.)*

Second generation male

Similar to first generation male except body shorter, body diam. less and the following characteristics: Head usually somewhat swollen. Excretory pore more anterior, in posterior part of corpus. Posterior part of body with a single precloacal papilla and 13-14 pairs of genital papillae of which four pairs caudal compared to three in the first generation. Lateral field prominent with one ridge. Tail terminus with a mucron, usually subterminal subventral in position.

First generation female

Body C-shaped or strongly spiralled when heat-relaxed and fixed with 4% formalin. Head rounded, continuous with body. Cuticle smooth or with faint annules. Lateral fields with one line present on all females observed. Phasmids inconspicuous. Cheilorhabdions prominent, well sclerotised. Another smaller sclerotised structure present posterior to cheilorhabdions (presumably the prorhabdions), posterior part funnel-shaped. Pharynx with procorpus cylindrical, muscular; metacorpus swollen; isthmus distinct; basal bulb enlarged, valvate. Nerve ring surrounding isthmus, just anterior to basal bulb. Pharyngo-intestinal valve prominent. Excretory pore position variable, near mid-pharynx. Pharyngo-intestinal valve present. Vulva a transverse slit on a protruding area, small epiptygma present. Tail with bluntly pointed tip, one or two projections present. Postanal swelling present in most females, more pronounced in fully mature females. Tail shorter than anal body diam., tapering to a blunt terminus. Two projections usually present at tail end. Two

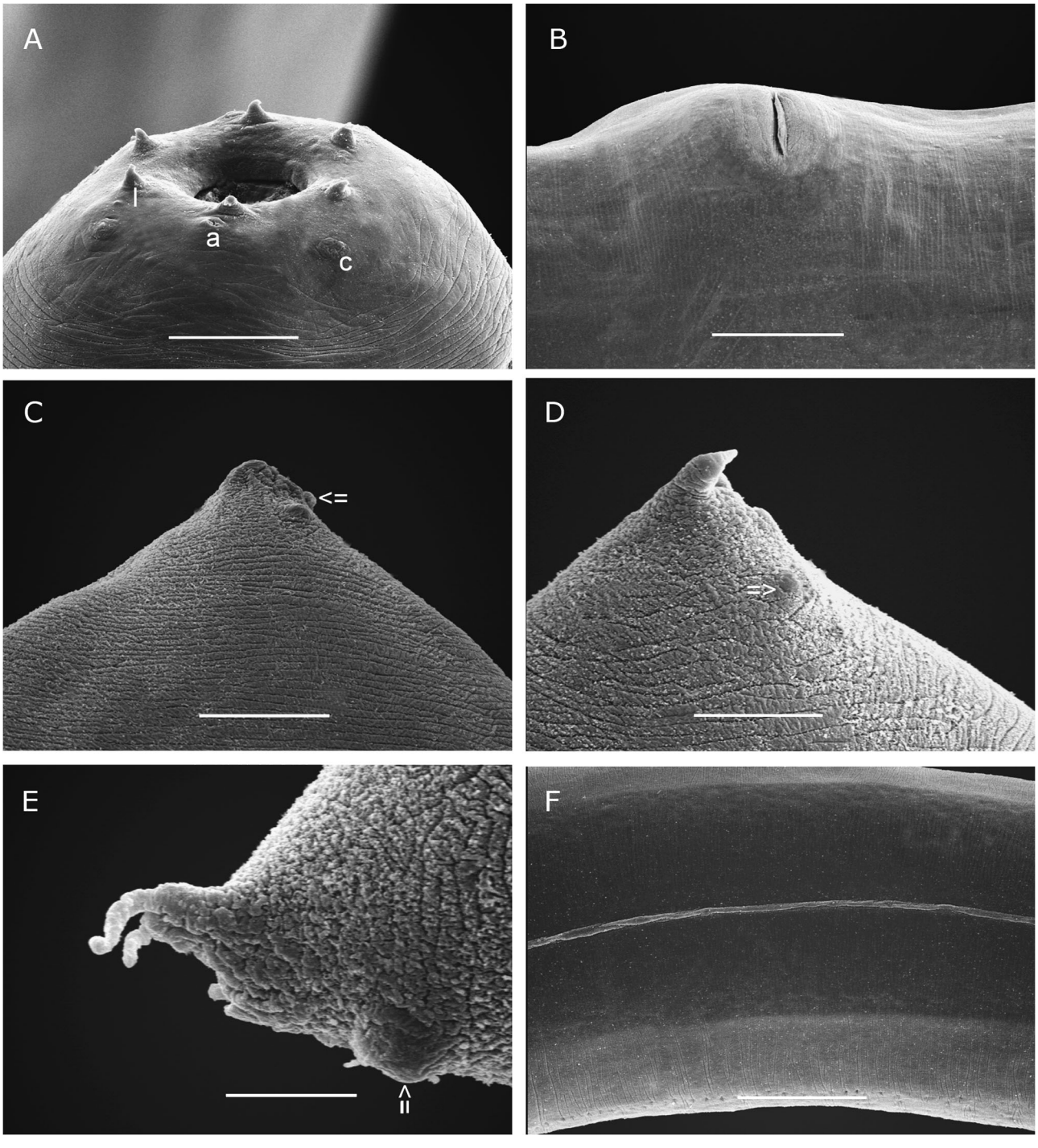

Fig. 4. *SEM of* Steinernema texanum *n. sp. females. A: First generation female face view showing mouth, amphidial aperture (a), six labial papillae (l) and four cephalic papillae (c); B: First generation female vulva; C-E: Tail tip with posterior projections and wart-like structures (arrow) on tail; F: First generation female body showing lateral field with single line. (Scale bars: A = 8.57 μm, B = 37.5 μm, C = 6.0 μm, D = 3.7 μm, E = 2.0 μm, F = 15.0 μm.)*

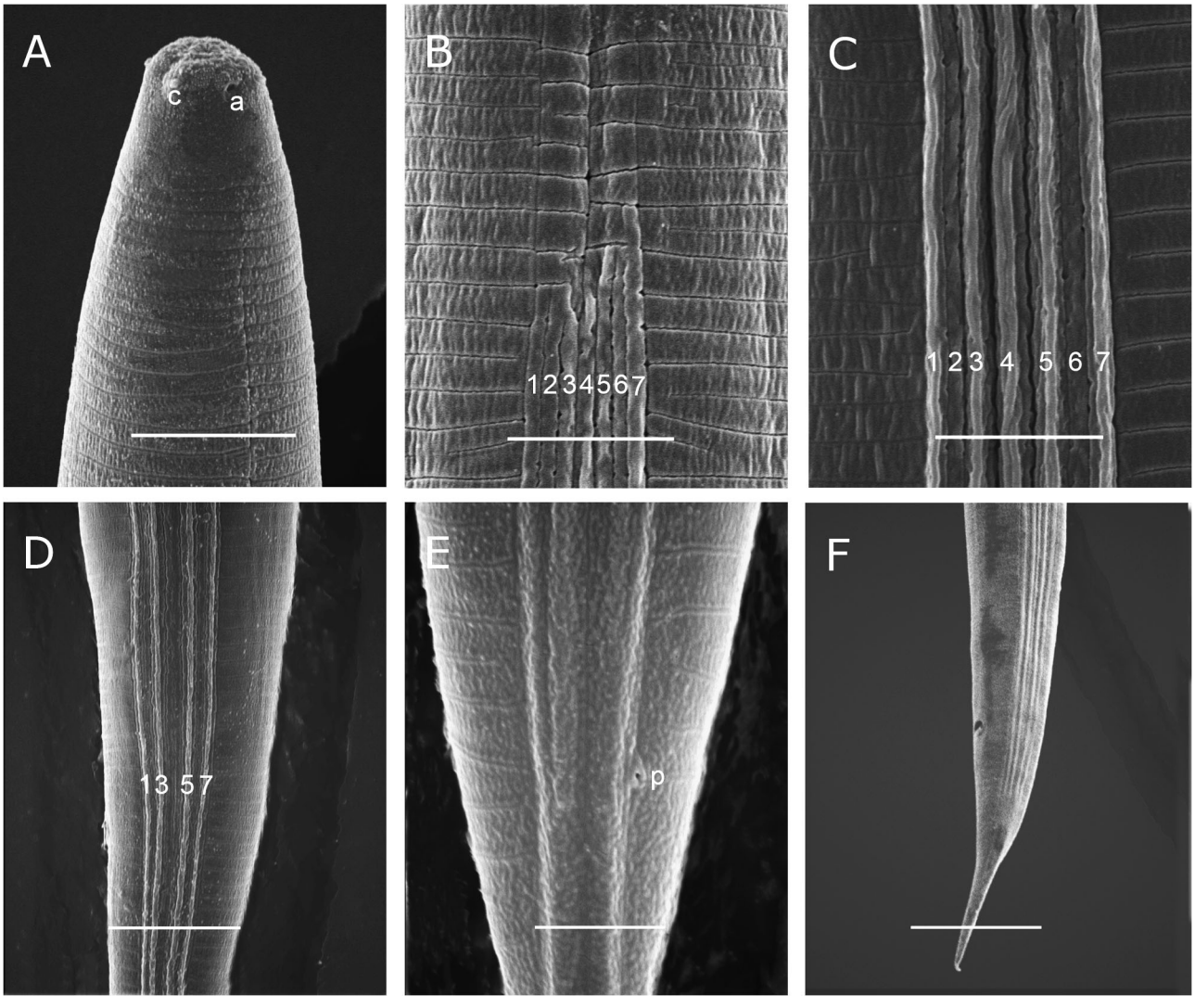

Fig. 5. *SEM of* Steinernema texanum *n. sp. infective juvenile. A: Anterior region showing one of two amphidial apertures (a), three of four cephalic papillae (c) and single line in lateral field; B: Lateral field showing the change from two to seven ridges; C: Lateral field showing seven ridges; D: Lateral field showing lateral field narrowing posteriorly; E: Lateral field in posterior region showing change to two ridges and phasmid (p); F: Posterior region showing lateral field and anus. (Scale bars: A = 6.6 μm; B, C = 5.0 μm; D = 10.0 μm; E = 3.3 μm; F = 20.0 μm.)*

wart-like structures anterior to tail tip always present on ventral region.

Second generation female

Body an open C when heat-relaxed and fixed with 4% formalin. Similar to first generation female but smaller. Body diam. greater anterior to vulva than posterior. Vulva on asymmetrical protuberance and situated at midbody; epiptygma not observed. Postanal swelling present. Tail mostly longer than anal body diam., tapering gently to a sharp point. For some large females (about 5%), tail shorter than anal body diam.

Infective juvenile

Body elongate, almost straight or slightly curved. Sheath (second-stage cuticle) present immediately after harvesting, but many infective juveniles losing sheath in storage. Exsheathed juvenile with four cephalic papil-

lae. Labial region smooth, continuous with body. Amphidial apertures prominent. Cuticle marked with prominent transverse striations. Excretory pore anterior to nerve ring. Lateral field beginning anteriorly with one line at fourth or fifth annule. A short distance posteriorly, two additional lines appearing to form two ridges. Near excretory pore level, number of ridges in lateral fields increasing from two to seven. Seven ridges remaining unchanged from excretory pore to anus except that two submarginal ridges not as raised as others in middle part of body. Near anus all ridges become smaller, only ridges 1, 3, 5, 7 raised and seen clearly, others gradually disappearing. Near phasmid, only two ridges (poorly separated) observed in lateral field. With above arrangement, formula of lateral field is 2, 7, 2. Pharynx with narrow corpus, metacorpus slightly swollen, isthmus present, nerve ring usually at middle of isthmus; basal bulb elongate with visible valve. Cardia present. Bacterial pouch located just posterior to cardia, 7-8.5 μm in diam., 26-31 μm in length, containing bacterial cells, variable in shape, mostly fusiliform or oval. Hemizonion and hemizonid not observed. Tail four times as long as anal body diam. and attenuate. Phasmid present near mid-tail, just ventral to lateral field. Hyaline portion occupying 58 (54-61)% of tail length.

Type host and locality

Natural host unknown as *S. texanum* n. sp. was isolated using the *Galleria*-baiting technique from soil samples taken at a site along state road 77 south of Kingsville between the towns of Sarita and Armstrong in Kennedy County, TX, USA, in an area with sandy soil, weedy vegetation, and shaded by mature live oak trees (*Quercus* sp.).

Type material

Holotype (male, first generation): isolated from haemocoel of *G. mellonella* deposited in the United States Department of Agriculture Nematode Collection (USDANC), Beltsville, MD, USA. Allotype (female, first generation): same data as holotype, deposited in the USDANC, Beltsville, MD, USA. Paratypes: same data as holotype. Many males and females of the first generation and several third-stage infective juveniles in 4% formalin deposited in USDANC, Beltsville, MD, USA. Several males, females and infective juveniles in formalin deposited in the Department of Entomology and Nematology, University of Florida, Gainesville, FL, USA.

Diagnosis and relationships

Steinernema texanum n. sp. is characterised by morphometrics of the infective juvenile with body length 756 μm, distance from anterior end to the excretory pore = 59 μm, tail length = 73 μm, ratio a = 25, H% = 59 and E% = 81 (Table 2). Lateral field pattern of the new species is 2, 7, 2, and is very typical for the species. Male of the first generation can be recognised by the spicule and the gubernaculum lengths and shapes, position of the excretory pore, D% = 67 and GS% = 75. Female can be recognised by the vulva with very low epiptygma, and two wart-like structures anterior to tail tip always present on ventral region (Fig. 4C-E).

Steinernema texanum n. sp. is closely related to species of the *feltiae*-group, which includes *S. akhursti* Qiu, Hu, Zhou, Mei, Nguyen & Pang, 2005, *S. feltiae*, *S. hebeiense* Chen, Li, Yan, Spiridonov & Moens, 2006, *S. jollieti* Spiridonov, Krasomil-Osterfeld & Moens, 2004, *S. kraussei* (Steiner, 1923) Travassos, 1927, *S. kushidai* Mamiya, 1988, *S. litorale* Yoshida, 2004, *S. monticolum*, *S. oregonense*, *S. sangi* Phan, Nguyen & Moens, 2001, *S. silvaticum* Sturhan, Spiridonov & Mráček, 2005 and *S. weiseri* Mráček, Sturhan & Reid, 2003.

The infective juvenile of *S. texanum* n. sp. differs from the closely related species, *S. feltiae*, *S. kraussei* and *S. oregonense* by its short body length, short pharynx length and lower a ratio (Table 2). The E% of the new species is similar to that of *S. kraussei* but smaller than that of *S. feltiae* and *S. oregonense*. The new species has a similar body length to *S. jollieti* and *S. weiseri* but body diam. is different in both means and ranges. Consequently, ratio a value is different, being 25 compared to 30.5 and 29, respectively. Additionally, the E% of the three species are different (Table 2). The new species is unique in the *feltiae*-group in having seven ridges in the lateral fields compared to six in *S. akhursti* and *S. jollieti* and eight in all others. *Steinernema texanum* n. sp. can be distinguished from all other members of the *feltiae*-group by data in Table 2.

The first generation male of the new species differs from all related species by lengths and shapes of spicules and gubernaculum (Fig. 2D-F). D% and GS% are also different (Table 3). The second generation male differes from other species by the presence of 13-14 pairs of genital papillae.

The first generation female of *S. texanum* n. sp. differs from all other species by the presence of two wart-like structures on the ventral region of the tail (Fig. 4C, D).

Table 2. *Comparative morphometrics of third-stage infective juveniles of* Steinernema texanum *n. sp. and related* Steinernema *spp. (in descending order of body length). Measurements are in µm and in the form: mean (range).*

Species[b]	Morphometric character[a]											
	L	Body diam.	EP	NR	ES	T	a	b	c	D%	E%	n
S. oregonense	980	34	66	–	132	70	30	7.6	14.0	50	100	20
	(820-1110)	(28-38)	(60-72)		(116-148)	(64-78)	(24-37)	(6-8)	(12-16)	(40-60)	(90-110)	
S. litorale	909	31	61	96	125	83	29.5	7.3	11	49	73	25
	(834-988)	(28-33)	(54-69)	(89-104)	(114-133)	(72-91)	(27.2-30.9)	(6.7-7.9)	(9.7-11.9)	(44-56)	(68-84)	
S. silvaticum	860	30	62	96	121	75	28.6	7.1	11.4	50	–	21
	(670-975)	(26-35)	(51-73)	(75-109)	(100-141)	(63-86)	(22.5-32.5)	(6.3-7.7)	(9.9-13.1)	(46-56)	–	
S. kraussei	951	33	63	105	134	79	29	7.1	12.1	47	80	?
	(797-1102)	30-36	50-66	(99-111)	(119-145)	(63-86)	–	–	–	–	–	
S. feltiae	849	26	62	99	136	81	31	6	10.4	45	119	25
	(736-950)	(22-29)	(53-67)	(88-112)	(115-150)	(70-92)	(29-33)	(5.3-6.4)	(9.2-12.6)	(42-51)	(69-86)	
S. akhursti	812	33	59	90	119	73	24	6.8	11	47	77	20
	(770-835)	(33-35)	(55-60)	(83-95)	(115-123)	(68-75)	(23-26)	(6.6-7.2)	(10-12)	(45-50)	(73-86)	
S. texanum n. sp.	756	30	59	92	115	73	25	6.5	10.4	51	81	20
	(732-796)	(29-34)	(52-62)	(84-102)	(111-120)	(60-79)	(22-27)	(6.2-7.0)	(9.6-12.5)	(46-53)	(76-88)	
S. sangi	753	35	51	91	127	81	22	5.9	9.3	40	62	50
	(704-784)	(30-40)	(46-54)	(78-97)	(120-138)	(76-89)	(19-25)	(5.6-6.3)	(8.7-10.2)	(36-44)	(56-70)	
S. weiseri	740	25	57	84	113	60	29	6.6	12	51	95	20
	(586-828)	(24-29)	(43-65)	(72-92)	(95-119)	(49-68)	(25-33)	(5.7-7.2)	(10-14)	(44-55)	–	
S. jollieti	711	23	60	–	123	68	30.5	5.7	10.5	48	88	25
	(625-820)	(20-28)	(53-65)	–	(115-135)	(60-73)	(25-34-1)	(4.9-6.4)	(9.0-11.7)	(46-50)	–	
S. monticolum	706	37	58	88	124	77	19	5.7	9.3	47	76	?
	(612-821)	(32-46)	(54-62)	(81-93)	(120-131)	(71-95)	(14-22)	(5.0-6.4)	(7.6-11.1)	(44-50)	(63-86)	
S. hebeiense	658	26	48	78	107	66	26	6.2	10	45	72	20
	(610-710)	(23-28)	(43-51)	(73-83)	(100-111)	(63-71)	(24-28)	(5.7-6.7)	(9.4-11)	(40-50)	(65-80)	
S. kushidai	589	26	46	76	111	50	22.5	5.3	11.7	41	92	50
	(424-662)	(22-31)	(42-50)	(70-84)	(106-120)	(44-59)	(19.3-25.2)	(4.9-5.9)	(10-13)	(38-44)	–	

[a] Abbreviations as in Table 1.
[b] After the original authors except *S. feltiae* after Poinar (1990) *S. kraussei* after Mráček (1994).
– Measurements not available.

Cross-hybridisation tests

Cross-breeding between males and females of *S. texanum* n. sp. with *S. feltiae* and *S. oregonense* produced no progeny. In the control, when all species were self crossed, males and females produced offspring normally.

Molecular characterisation

Steinernema texanum n. sp. is characterised genetically by the ITS and D2D3 regions. The sequence of ITS regions of *S. texanum* n. sp., flanked by primers 18S and 26S is characterised by its length 956 base pairs (bp), ITS1 = 263 bp, ITS2 = 286 bp and its composition (Table 4). The sequence length of ITS1 of the new species is longer than that of *S. sangi* but shorter than all other species in the *feltiae*-group (Table 4). ITS2 is longer than that of *S. monticolum* but shorter than all others in Table 4. For more interspecific relationships, pairwise distances (Table 5) show that the new species differs from *S. jollieti*, its closest taxon, by 67 bp and from the most divergent species, *S. monticolum*, by 141 bp. Distances from the new species and others are presented in Table 5. Among these species, the less divergent species are *S. litorale* and *S. weiseri* with 27 bp difference; the most divergent species are *S. monticolum* and *S. kushidai* with 150 bp difference.

For D2D3 regions, the sequence of *S. texanum* n. sp. is 855 bp, composition, A = 0.24912, C = 0.19532, G = 0.30292, T = 0.25263. Pairwise distances (Table 6) show that the new species differs from its closest taxon, *S.*

Table 3. *Comparative morphometrics of first generation males of* Steinernema texanum *n. sp. and related* Steinernema *spp. (in descending order of spicule length). Measurements are in μm and in the form: mean (range).*

Species[b]	Morphometric characters[a] (Range)							
	Spicule	Gubern.	Body diam.	D%	SW%	GS%	MUC	n
S. akhursti	90 (85-100)	64 (58-68)	131 (115-150)	56 (52-61)	180 (140-200)	71 (65-77)	P	20
S. litorale	75 (67-89)	53 (44-64)	96 (82-111)	40 (34-56)	174 (154-200)	71 (62-81)	P	25
S. oregonense	71 (65-73)	56 (52-59)	138 (105-161)	73 (64-75)	151 –	79 –	A	20
S. feltiae	70 (65-77)	41 (34-47)	75 (60-90)	60 (51-64)	113 (99-130)	59 (52-61)	P	25
S. monticolum	70 (61-80)	45 (35-54)	160 (117-206)	55 (49-61)	140 (120-150)	60 (50-70)	P	20
S. weiseri	68 (62-72)	53 (46-57)	112 (84-138)	49 (39-60)	180 (150-140)	80 (70-85)	A	20
S. jollieti	64 (55-70)	54 (45-60)	115 (98-135)	64 (53-83)	145 –	84 –	A	12
S. kushidai	63 (48-72)	44 (39-60)	97 (75-156)	51 (42-59)	150 –	70 –	A	20
S. sangi	63 (58-80)	40 (34-46)	159 (120-225)	49 (42-63)	150 (120-160)	60 (50-70)	P	20
S. hebeiense	57 (51-63)	46 (38-50)	86 (74-98)	51 (48-59)	140 (120-170)	80 (60-90)	A	20
S. texanum n. sp.	60 (55-66)	45 (39-53)	99 (81-116)	67 (58-73)	157 (127-203)	75 (62-84)	A	20
S. silvaticum	51 (42-64)	37 (30-43)	65 (52-78)	60 (45-63)	–	–	P	26
S. kraussei	49 (42-53)	33 (29-37)	128 (110-144)	53 –	110 –	67 –	P	?

[a] Abbreviations as in Table 1.
[b] After the original authors except *S. feltiae* after Poinar (1990), *S. kraussei* after Mráček (1994).
– Measurements not available.

feltiae, by 28 bp and from the most divergent species, *S. monticolum*, by 51 bp. *Steinernema texanum* n. sp. differs from other closely related species by 28-37 bp. These data indicate that the new nematode is a good new species when comparing these distances with those of other species, the distances between some valid species actually being as low as zero bp (Nguyen *et al*., 2006; Table 7). There are some other described species that have low pairwise distances; for example, pairwise distances between *S. hermaphroditum* Stock, Griffin & Chaerani, 2004 and *S. scarabaei* Stock & Koppenhöfer, 2003 and *S. cubanum* Mráček, Hernandez & Boemare, 1994 and *S. longicaudum* Shen & Wang, 1992 are 1 bp, and between *S. cubanum* and *S. glaseri* and *S. anatoliense* Hazir, Stock & Keskin, 2003 and *S. websteri* Cutler & Stock, 2003 are 3 bp (Stock *et al*., 2004, Table 3).

Phylogenetic analysis

For ITS regions, maximum parsimony analysis shows that the alignment resulted in 1247 characters of which 152 in ambiguous regions are excluded, 276 are constant, 261 variable characters are parsimony-uninformative and 558 characters (included) are parsimony-informative. Parsimony and distance based treebuilding approaches produce almost identical trees. The phylogenetic relationships between 33 species of *Steinernema* are presented in Figure 6 (for MP, tree length = 3622, CI = 0.4268, RI = 0.4616, RC = 0.1970, HI = 0.4616). In this con-

Table 4. *Sequence lengths and composition of ITS and D2D3 regions of species of* Steinernema *closely related to* S. texanum *n. sp.*

Species	ITS1 (bp)	ITS2 (bp)	A (%)	C (%)	G (%)	T (%)	Sequence length (bp)
ITS regions							
S. akhursti	271	295	0.25268	0.19165	0.24304	0.31263	934
S. feltiae	275	298	0.25714	0.18367	0.23061	0.32857	980
S. hebeiense	260	292	0.25648	0.1513	0.22046	0.37176	694
S. jollieti	266	289	0.25552	0.16575	0.21685	0.36188	724
S. kraussei	264	314	0.25899	0.19361	0.23844	0.30896	973
S. kushidai	279	304	0.24867	0.19235	0.24867	0.31031	941
S. litorale	264	290	0.26383	0.18191	0.23085	0.3234	940
S. monticolum	264	245	0.27402	0.17358	0.23908	0.31332	916
S. oregonense	267	298	0.25385	0.19322	0.23535	0.31757	973
S. sangi	255	308	0.23469	0.18513	0.23324	0.34694	686
S. texanum n. sp.	**263**	**286**	**0.25628**	**0.18515**	**0.23117**	**0.32741**	**956**
S. weiseri	265	297	0.25171	0.16553	0.22025	0.36252	731
D2D3 regions							
S. feltiae			0.24683	0.19377	0.30219	0.25721	860
S. kushidai			0.24683	0.18454	0.30334	0.26528	867
S. kraussei			0.25029	0.19235	0.30127	0.25608	863
S. monticolum			0.24501	0.19041	0.29827	0.26631	751
S. texanum n. sp.			0.24912	0.19532	0.30292	0.25263	860
Panagrellus redivivus			0.23541	0.19683	0.31553	0.25223	1011

Table 5. *Pairwise distances of ITS regions between taxa in the* feltiae*-group.*

Species	*S. tex*	*S. jol*	*S. sil*	*S. san*	*S. fel*	*S. ore*	*S. wei*	*S. kra*	*S. lit*	*S. heb*	*S. akh*	*S. kus*	*S. mon*	*C. ele*
S. texanum n. sp.	–													
S. jollieti	67	–												
S. silvaticum	85	57	–											
S. sangi	87	78	88	–										
S. feltiae	88	49	70	95	–									
S. oregonense	90	69	69	93	60	–								
S. weiseri	91	54	76	102	51	79	–							
S. kraussei	95	77	62	104	66	70	93	–						
S. litorale	102	59	88	104	62	88	27	102	–					
S. hebeiense	107	83	101	118	81	101	84	115	83	–				
S. akhursti	109	83	98	83	115	108	102	122	105	120	–			
S. kushidai	127	108	116	110	127	116	122	128	123	138	59	–		
S. monticolum	141	111	130	123	122	134	119	136	128	135	135	150	–	
C. elegans	474	372	475	383	469	476	387	478	469	380	469	471	447	–

sensus tree, 13 species of the *feltiae*-group form a monophyletic assemblage in which the new species, *S. feltiae*, *S. hebeiense*, *S. jollieti*, *S. kraussei*, *S. litorale*, *S. oregonense*, *S. sangi*, *S. silvaticum* and *S. weiseri* form a monophyletic group. The new species and *S. sangi* do not group with any others. Bootstrap support in this clade is from low (50) to high (99). The reconstructed nucleotide character transformations (Fig. 7) show that the new species differs from other species of the *feltiae*-group by 18 unambiguous, polarised autapomorphies. For D2D3 regions, maximum parsimony analysis shows that the alignment resulted in 1032 characters of which 554 are constant, 128 variable characters are parsimony-uninformative and 350 characters are parsimony-informative. The phylogenetic

Table 6. *Pairwise distances of D2D3 regions between taxa in the* feltiae*-group.*

	S. tex	*S. fel*	*S. ore*	*S. kra*	*S. kus*	*S. mon*	*P. red*
S. texanum n. sp.	–						
S. feltiae	28	–					
S. oregonense	31	227	–				
S. kraussei	36	230	15	–			
S. kushidai	37	235	27	28	–		
S. monticolum	51	49	48	52	42	–	
P. redivivus	237	239	239	238	238	231	–

relationships between 26 species of *Steinernema* are presented in Figure 8 (tree length = 1125, CI = 0.6009, RI = 0.6999, RC = 0.4205, HI = 0.3991). The six species, *S. texanum* n. sp., *S. feltiae*, *S. kraussei*, *S. kushidai*, *S. monticolum* and *S. oregonense* form a monophyletic group that is well supported by bootstrap proportion (99). The fact that *S. monticolum* (using the new sequence EF439651) clustered with the *feltiae*-group is different from previous studies (Stock *et al*., 2001; Nguyen *et al*., 2006) in which *S. monticolum* (using sequence AF331895) clustered with the *carpocapsae*-group. The anomaly between the D2D3 sequence reported here and that of Stock *et al*. (2001) is clearly significant and needs to be resolved. Our D2D3 sequence was derived from material of *S. monticolum* supplied in 2005 by H. Kaya, one of the original authors of the species, and is in agreement with a sequence obtained from the same source by Spiridonov (pers. comm., 2005). The fact that our sequence puts *S. monticolum* within the *feltiae*-group rather than the *carpocapsae*-group also makes sense as this placement is supported by morphological characters (see Spiridonov *et al*., 2004b). In conclusion, it seems plausible that the D2D3 sequence reported by Stock *et al*. (2001) may actually refer to a different species to that attributed.

In this clade, the new species, *S. feltiae*, *S. kraussei* and *S. oregonense*, formed a monophyletic group in which the new species is the sister taxon to the group formed by others. Bootstrap support in this clade are from medium to high (85-99). The two species *S. monticolum* and *S. kushidai* stand separately. Additionally, the reconstructed nucleotide character transformations (Fig. 9) show that *S. texanum* n. sp. differs from species of the *feltiae*-group by 15 unambiguous, polarised autapomorphies.

Survey

A total of 15 of the 200 soil samples (7.5%) from three of the ten sampling sites (30%) proved positive for entomopathogenic nematodes. At these sites, one (5%), five (25%) and nine (45%) of the 20 soil cores taken were positive. One site, located on state road 186 near the junction with state road 281, Hidalgo County, produced a single *H. mexicana* isolate. A second site, located on state road 83 between the towns of Zapata and Falcon, Zapata County, produced five *H. mexicana* isolates. The third site, located on state road 77 south of Kingsville, between the towns of Sarita and Armstrong, Kennedy County, produced one *H. mexicana* isolate, seven *S. glaseri* (Steiner, 1929) Wouts, Mráček, Gerdin & Bedding, 1982 isolates (from six cores) and seven isolates of the new species (from two cores).

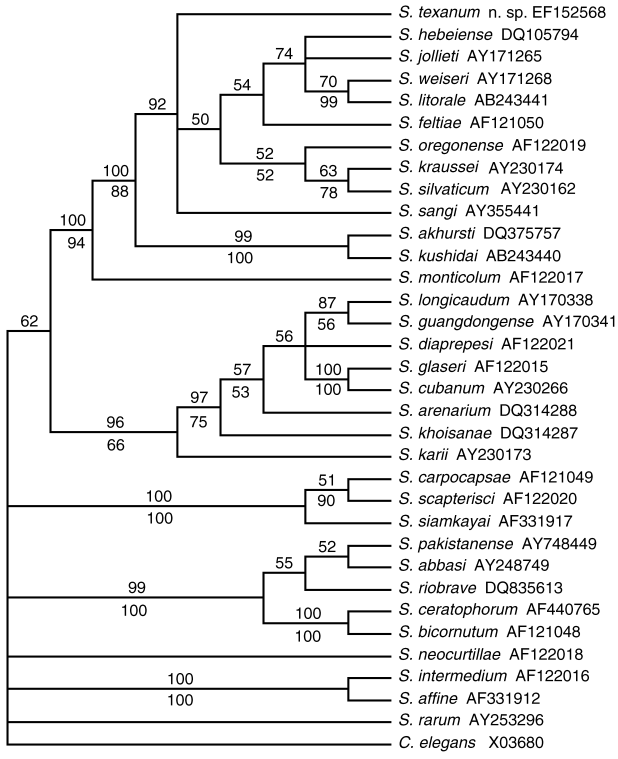

Fig. 6. *Phylogenetic relationships of 33 species of* Steinernema *based on analysis of ITS rDNA regions. The species in the* feltiae*-group (*S. akhursti, S. feltiae, S. hebeiense, S. jollieti, S. kraussei, S. kushidai, S. litorale, S. monticolum, S. oregonense, S. sangi, S. silvaticum, S. weiseri *and* S. texanum *n. sp.) form a monophyletic group.* S. texanum *n. sp.,* S. oregonense, S. monticolum *and* S. sangi *do not group with any other species. Note:* Steinernema neocurtillae *with an IJ body length similar to that of nematodes in* feltiae*-group but not clustering with that group. Similarly,* S. rarum *does not cluster with the* carpocapsae*-group. Numbers at the nodes represent bootstrap proportion for MP (below) and neighbour joining (above) (50% or more).*

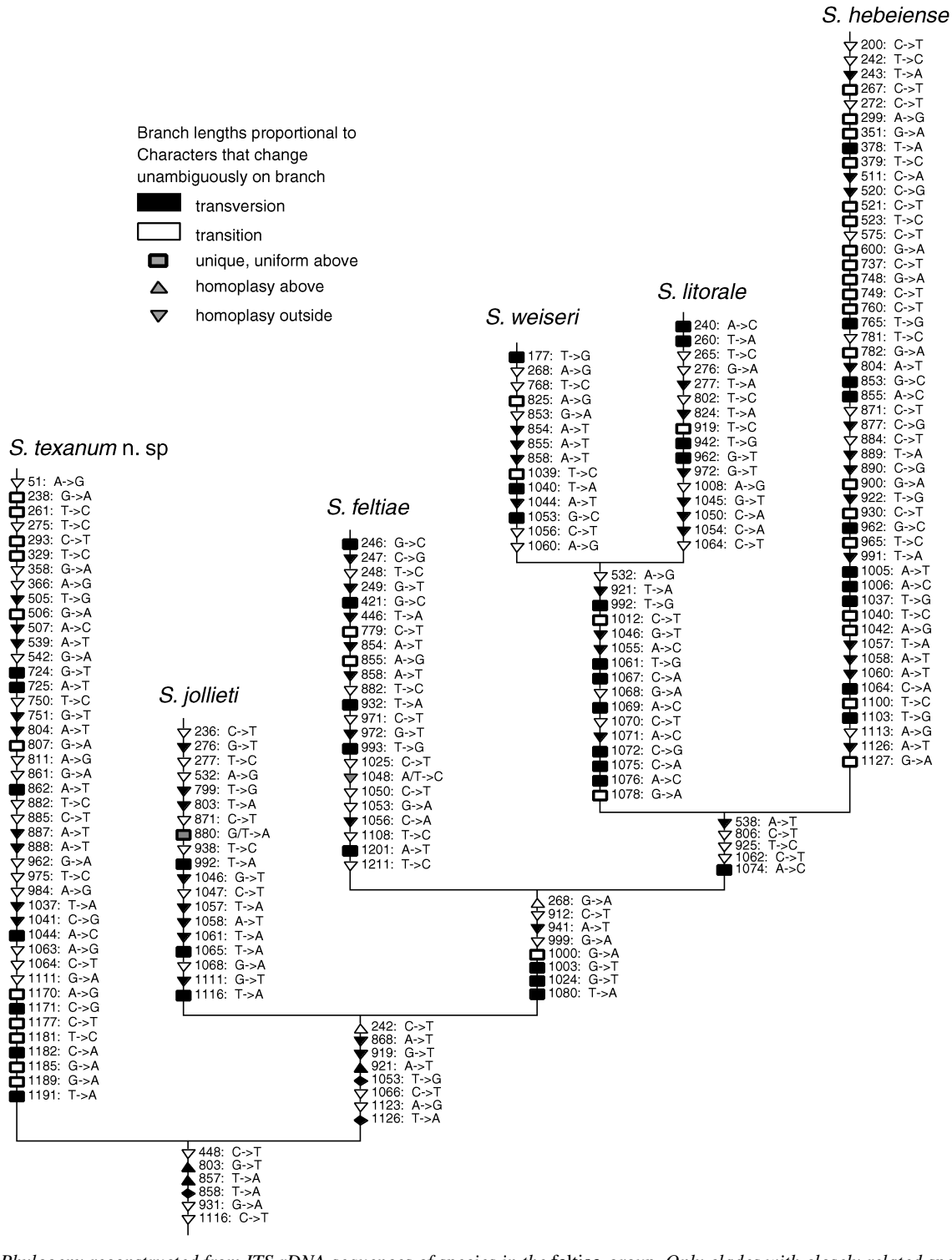

Fig. 7. *Phylogeny reconstructed from ITS rDNA sequences of species in the* feltiae*-group. Only clades with closely related species are shown.* Steinernema texanum *n. sp. has 18 autapomorphies (= unique, derived characters, rectangular boxes at the positions 238, 261, 293, 329, 506, 724, 725, 807, 862, 1044, 1170, 1171, 1177, 1181, 1182, 1185, 1189 and 1191); seven of them are transversion and 11 are transition.*

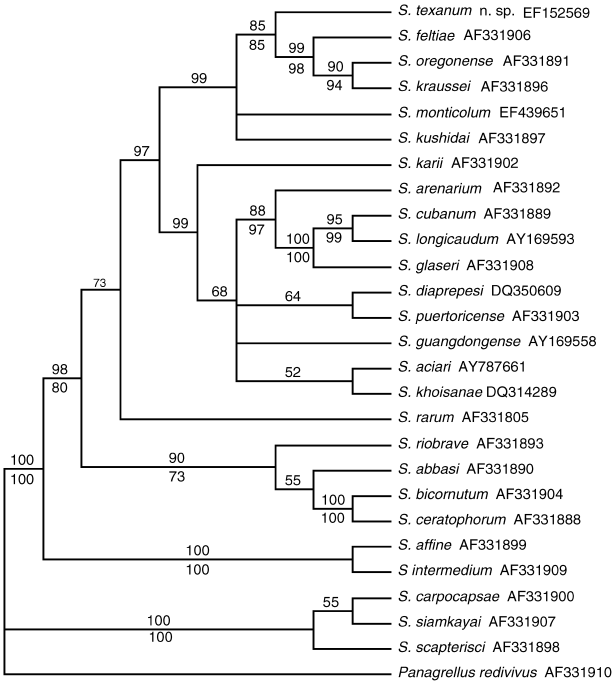

Fig. 8. *Phylogenetic relationships of 26 species of* Steinernema *based on analysis of D2D3 rDNA regions using* Panagrellus redivivus *as an outgroup. The six species of the* feltiae*-group in this tree (*S. feltiae*,* S. kraussei*,* S. kushidai*,* S. monticolum*,* S. oregonense *and* S. texanum *n. sp.) form a monophyletic group. Numbers at the nodes represent bootstrap proportion (50% or more).*

Acknowledgements

The authors thank the South African Deciduous Fruit Producers Trust and a USDA Special Project Grant for funding of the project and the EM Core, University of Florida, for use of the scanning electron microscope to produce SEM photographs for this paper.

References

CABANILLAS, H.E., POINAR JR, G.O. & RAULSTON, J.R. (1994). *Steinernema riobravis* n. sp. (Rhabditida: Steinernematidae) from Texas. *Fundamental and Applied Nematology* 17, 123-131.

CHEN, S.L., LI, X., YAN, A., SPIRIDONOV, S.E. & MOENS, M. (2006). A new entomopathogenic nematode, *Steinernema hebeiense* sp. n. (Rhabditida: Steinernematidae), from North China. *Nematology* 8, 563-574.

COURTNEY, W.D., POLLEY, D. & MILLER, V.L. (1955). TAF, an improved fixative in nematode technique. *Plant Disease Reporter* 39, 570-571.

DUTKY, S.R., THOMPSON, J.V. & CANTWELL, G.E. (1964). A technique for the mass propagation of the DD-136 nematode. *Journal of Insect Pathology* 6, 417-422.

FILIPJEV, I.N. (1934). Miscellania nematologica 1. Eine neue Art der Gattung *Neoaplectana* Steiner nebst Bemerkungen über die systematische Stellung der letzteren. *Parazitologicheskiy* 4, 229-240.

FRANKLIN, M.T. & GOODEY, J.B. (1949). A cotton blue-lactophenol technique for mounting plant-parasitic nematodes. *Journal of Helminthology* 23, 175-178.

GAUGLER, R. (2002). Preface. In: Gaugler, R. (Ed.). *Entomopathogenic nematology*. Wallingford, UK, CABI Publishing, pp. ix-x.

HARA, A.H., GAUGLER, R., KAYA, H.K. & LEBECK, L.M. (1991). Natural populations of entomopathogenic nematodes (Rhabditida: Heterorhabditidae, Steinernematidae) from the Hawaiian Islands. *Environmental Entomology* 20, 211-216.

HOMINICK, W.M. (2002). Biogeography. In: Gaugler, R. (Ed.). *Entomopathogenic nematology*. Wallingford, UK, CABI Publishing, pp. 115-143.

KLEIN, M.G. (1990). Efficacy against soil-inhabiting insect pests. In: Gaugler, R. & Kaya, H.K. (Eds). *Entomopathogenic nematodes in biological control*. Boca Raton, FL, USA, CRC Press, pp. 195-214.

LIU, J. & BERRY, R.E. (1996). *Steinernema oregonensis* n. sp. (Rhabditida: Steinernematidae) from Oregon, USA. *Fundamental and Applied Nematology* 19, 375-380.

MADDISON, W.P. & MADDISON, D.R. (2002). *MacClade version 4.0*. Sunderland, MA, USA, Sinauer Associates.

MAMIYA, Y. (1988). *Steinernema kushidai* n. sp. (Nematoda: Steinernematidae) associated with scarabaeid beetle larvae from Shizuoca, Japan. *Applied Entomology and Zoology* 23, 313-320.

MRÁČEK, Z. (1994). *Steinernema kraussei* (Steiner, 1923) (Nematoda: Rhabditida: Steinernematidae): redescription of its topotype from Westphalia. *Folia Parasitologica* 41, 59-64.

MRÁČEK, Z., STURHAN, D. & REID, A. (2003). *Steinernema weiseri* sp. n. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from Europe. *Systematic Parasitology* 56, 37-47.

NGUYEN, K.B. (1988). *A new nematode parasite of mole crickets: its taxonomy, biology and potential for biological control*. Ph.D. dissertation, University of Florida, Gainesville, FL, USA, 154 pp.

NGUYEN, K.B. (2006). Morphology and taxonomy of entomopathogenic nematodes. Available from http://kbn.ifas.ufl.edu/kbnstein.htm (accessed October 1, 2006).

NGUYEN, K.B. & ADAMS, B.J. (2003). SEM and systematic studies of *Steinernema abbasi* Elawad *et al.*, 1997 and *S. riobrave* Cabanillas *et al.*, 1994 (Rhabditida: Steinernematidae). *Zootaxa* 179, 1-10.

NGUYEN, K.B. & DUNCAN, L.W. (2002). *Steinernema diaprepesi* n. sp. (Rhabditida: Steinernematidae), a parasite of

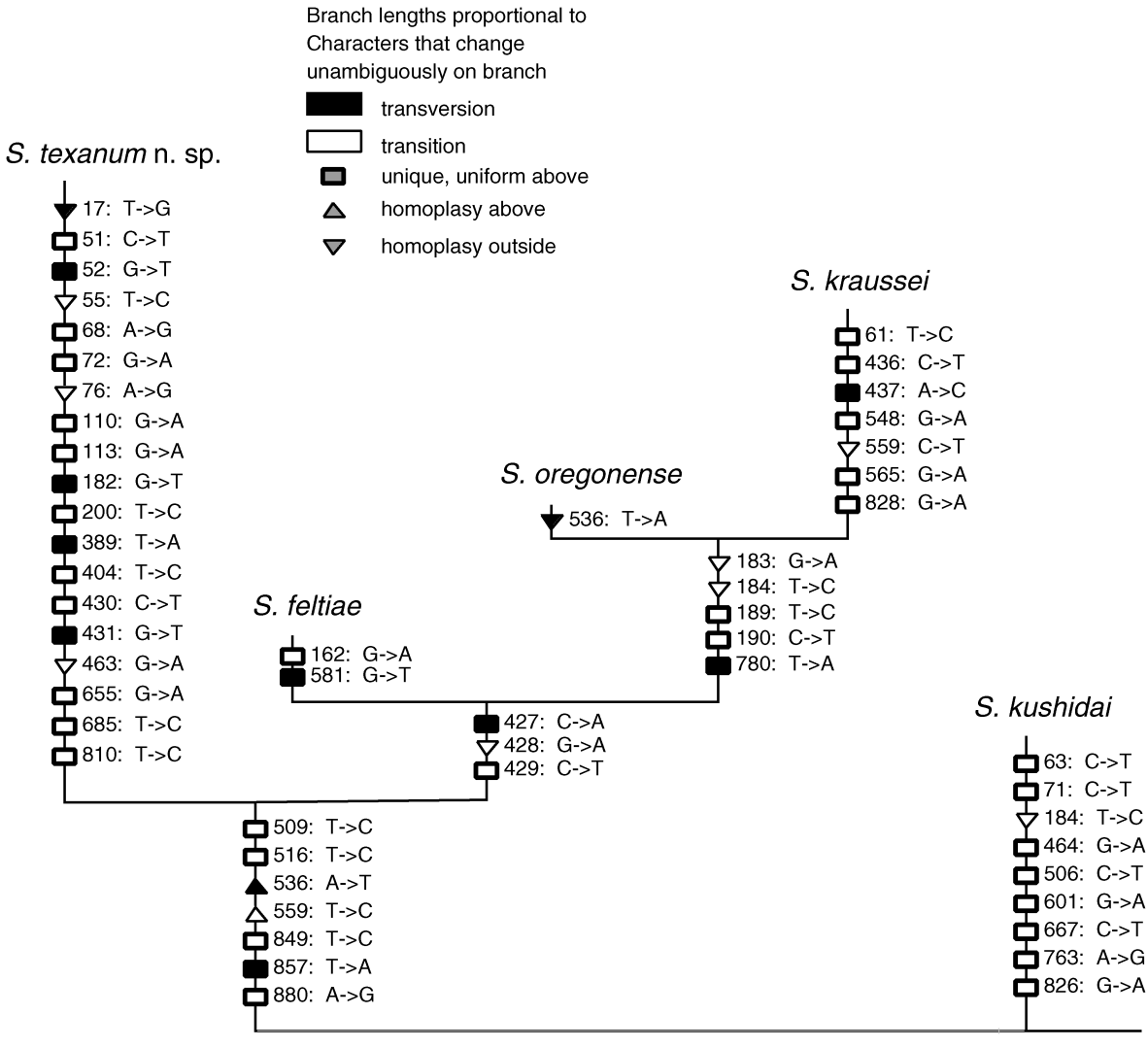

Fig. 9. *Phylogeny reconstructed from D2D3 rDNA sequences of species in the* feltiae*-group.* Steinernema texanum *n. sp. has 15 autapomorphies (= unique, derived characters, rectangular boxes at the positions 51, 52, 68, 72, 110, 113, 182, 200, 389, 404, 430, 431, 655, 685 and 810); four of them are transversion and 11 are transition.*

the citrus root weevil *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae). *Journal of Nematology* 34, 159-170.

NGUYEN, K.B. & SMART JR, G.C. (1990). *Steinernema scapterisci* n. sp. (Rhabditida: Steinernematidae). *Journal of Nematology* 22, 187-199.

NGUYEN, K.B. & SMART JR, G.C. (1992). *Steinernema neocurtillis* n. sp. (Rhabditida: Steinernematidae) and a key to species of the genus *Steinernema*. *Journal of Nematology* 24, 463-477.

NGUYEN, K.B. & SMART JR, G.C. (1995a). Morphometrics of infective juveniles of *Steinernema* spp. and *Heterorhabditis bacteriophora* (Nemata: Rhabditida). *Journal of Nematology* 27, 206-212.

NGUYEN, K.B. & SMART JR, G.C. (1995b). Scanning electron microscope studies of *Steinernema glaseri* (Nematoda: Steinernematidae). *Nematologica* 41, 183-190.

NGUYEN, K.B. & SMART JR, G.C. (1997). Scanning electron microscopic studies of spicules and gubernacula for *Steinernema* spp. (Nemata: Steinernematidae). *Nematologica* 43, 465-480.

NGUYEN, K.B., MARUNIAK, J. & ADAMS, B.J. (2001). The diagnostic and phylogenetic utility of the rDNA internal transcribed spacer sequences of *Steinernema*. *Journal of Nematology* 33, 73-82.

NGUYEN, K.B., SHAPIRO-ILAN, D.I., STUART, R.J., JAMES, R.R. & ADAMS, B.J. (2004). *Heterorhabditis mexicana* n.

sp. (Heterorhabditidae: Rhabditida) from Tamaulipas, Mexico, and morphological studies of the bursa of *Heterorhabditis* spp. *Nematology* 6, 231-244.

NGUYEN, K.B., MALAN, A.P. & GOZEL, U. (2006). *Steinernema khoisanae* n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from South Africa. *Nematology* 8, 157-175.

PARKMAN, J.P. & SMART JR, G.C. (1996). Entomopathogenic nematodes, a case study: introduction of *Steinernema scapterisci* in Florida. *Biocontrol Science and Technology* 6, 413-419.

PARKMAN, J.P., HUDSON, W.G., FRANK, J.H., NGUYEN, K.B. & SMART JR, G.C. (1993). Establishment and persistence of *Steinernema scapterisci* (Rhabditida: Steinernematidae) in field population of *Scapteriscus* spp., mole crickets (Orthoptera: Gryllotalpidae). *Journal of Entomological Science* 28, 182-190.

PARKMAN, J.P., FRANK, J.H., NGUYEN, K.B. & SMART JR, G.C. (1994). Inoculative release of *Steinernema scapterisci* (Rhabditida: Steinernematidae) to suppress pest mole crickets (Orthoptera: Gryllotalpidae) on golf courses. *Environmental Entomology* 23, 1331-1337.

PHAN, K.L., NGUYEN, N.C. & MOENS, M. (2001). *Steinernema sangi* sp. n. (Rhabditida: Steinernematidae) from Vietnam. *Russian Journal of Nematology* 9, 1-7.

POINAR JR, G.O. (1990). Taxonomy and biology of Steinernematidae and Heterorhabditidae. In: Gaugler, R. & Kaya, H.K. (Eds). *Entomopathogenic nematodes in biological control*. Boca Raton, FL, USA, CRC Press, pp. 23-60.

QIU, L., HU, X., ZHOU, Y., MEI, S., NGUYEN, K.B. & PANG, Y. (2005). *Steinernema akhursti* sp. n. (Nematoda: Steinernematidae) from Yunan, China. *Journal of Invertebrate Pathology* 90, 151-160.

SEINHORST, J.W. (1959). A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica* 4, 67-69.

SHAPIRO, D.I. & MCCOY, C.W. (2000a). Virulence of entomopathogenic nematodes to *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in the laboratory. *Journal of Economic Entomology* 93, 1090-1095.

SHAPIRO, D.I. & MCCOY, C.W. (2000b). Susceptibility of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) larvae to different rates of entomopathogenic nematodes in the greenhouse. *Florida Entomology* 83, 1-9.

SHAPIRO-ILAN, D.I., GOUGE, D.H. & KOPPENHÖFER, A.M. (2002). Factors affecting commercial success: case studies in cotton, turf and citrus. In: Gaugler, R. (Ed.). *Entomopathogenic nematology*. Wallingford, UK, CABI Publishing, pp. 333-356.

SPIRIDONOV, S.E., KRASOMIL-OSTERFELD, K. & MOENS, M. (2004a). *Steinernema jollieti* sp. n. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from the American Midwest. *Russian Journal of Nematology* 12, 85-95.

SPIRIDONOV, S.E., REID, A.P., PRODRUCKA, K., SUBBOTIN, S.A. & MOENS, M. (2004b). Phylogenetic relationships within the genus *Steinernema* (Nematoda: Rhabditida) as inferred from analyses of the sequences of the ITS1-5.8S-ITS2 region of rDNA and morphological features. *Nematology* 6, 547-566.

STEINER, G. (1923). *Aplectana kraussei* n. sp., eine in der Blattwespe *Lyda* sp. parasitierende Nematodenform, nebst Bemerkungen über das Seitenorgan der parasitischen Nematoden. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. Abteilung I* 59, 14-18.

STOCK, S.P., CHOO, H.Y. & KAYA, H.K. (1997). An entomopathogenic nematode, *Steinernema monticolum* sp. n. (Rhabditida: Steinernematidae) from Korea with a key to other species. *Nematologica* 43, 15-29.

STOCK, S.P., CAMPBELL, J.F. & NADLER, S.A. (2001). Phylogeny of *Steinernema* Travassos, 1927 (Cephalobina: Steinernematidae) inferred from ribosomal DNA sequences and morphological characters. *Journal of Parasitology* 87, 877-889.

STOCK, S.P., GRIFFIN, C.T. & CHAERANI, R. (2004). Morphological and molecular characterisation of *Steinernema hermaphroditum* n. sp. (Nematoda: Steinernematidae), an entomopathogenic nematode from Indonesia, and its phylogenetic relationships with other members of the genus. *Nematology* 6, 401-412.

STUART, R.J. & GAUGLER, R. (1994). Patchiness in populations of entomopathogenic nematodes. *Journal of Invertebrate Pathology* 64, 39-45.

STUART, R.J., SHAPIRO-ILAN, D.I., JAMES, R.R., NGUYEN, K.B. & MCCOY, C.W. (2004). Virulence of new and mixed strains of the entomopathogenic nematode *Steinernema riobrave* to larvae of the citrus root weevil *Diaprepes abbreviatus*. *Biological Control* 30, 439-445.

STURHAN, D., SPIRIDONOV, S.E. & MRÁČEK, Z. (2005). *Steinernema silvaticum* sp. n. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from Europe. *Nematology* 7, 227-241.

THOMPSON, J.D., GIBSON, T.J., PLEWNIAK, F., JEANMOUGH, F. & HIGGINS, D.G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acid Research* 25, 4876-4882.

TRAVASSOS, L. (1927). Sobre o genera *Oxystomatium*. *Boletim Biologico* 5, 20-21.

WOUTS, W., MRÁČEK, M.Z., GERDIN, S. & BEDDING, R.A. (1982). *Neoaplectana* Steiner, 1929, a junior synonym of *Steinernema* Travassos, 1927 (Nematoda: Rhabditida). *Systematic Parasitology* 4, 147-154.

YOSHIDA, M. (2004). *Steinernema litorale* n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from Japan. *Nematology* 6, 819-838.