

Steinernema diaprepesi n. sp. (Rhabditida: Steinernematidae), a Parasite of the Citrus Root Weevil *Diaprepes abbreviatus* (L) (Coleoptera: Curculionidae)¹

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Abstract: A nematode collected from *Diaprepes abbreviatus* is identified and described as a new species, *Steinernema diaprepesi* n. sp. The new species is closely related to *S. feltiae*, *S. glaseri*, and *S. oregonense* and can be distinguished from these species by the following characteristics: *Males:* Spicule averaging 79 (71–90) μm and spicule shape; D% (distance from anterior end to excretory pore/esophagus length \times 100) about 80; the ratio SW (spicule length/anal body width) about 1.8. *Females:* Vulva with short, double-flapped epitygma; tail terminus usually with 5 papillae-like structures. *Infective juveniles:* Body averaging 1,002 (880–1,133) μm , EP (distance from anterior end to excretory pore) = 74 (66–83) μm ; tail length = 83 (65–91) μm , and E% (EP/tail length \times 100) = 89.6 (78–114). Lateral field pattern variable, the formula for the arrangement of ridges from head to tail is: 2, 6, 7, 8, 4, 2. The portion with eight ridges is the longest. This new species can be differentiated further from three closest species (*S. feltiae*, *S. glaseri*, and *S. oregonense*) by characteristic sequences of their ITS regions, including sequence lengths, ratios of similarity, composition, and differences in base characters in sequence alignment.

Key words: citrus root weevil, *Diaprepes abbreviatus*, DNA, entomopathogenic nematodes, nematodes, new species, *Steinernema diaprepesi*, taxonomy.

Entomopathogenic nematodes have been used in Florida citrus groves for more than a decade in an attempt to manage subterranean larvae of a weevil pest, *Diaprepes abbreviatus* L. Schroeder (1992) used *Steinernema carpocapsae* (Weiser, 1955) Wouts, Mracek, Gerdin and Bedding, 1982 and *Heterorhabditis bacteriophora* Poinar, 1976 to control citrus root weevil. He reported that weevil emergence was reduced by 70% compared with an untreated control. Formulated products containing *S. riobrave* Cabanillas, Poinar and Raulston, 1994, and *H. indica* Poinar, Karunakar and David, 1992 are currently used for this purpose. Field trials using commercially formulated *S. riobrave* and *H. indica* reported efficacy against *D. abbreviatus* ranging from >85% suppression to no measurable effect (Adair, 1994; Bullock et al., 1999; Duncan et al., 1996; Duncan and McCoy, 1996; McCoy et al., 2000; McCoy et al., 2001; Stansly et al., 1997). Variation in soils (Duncan et al., 2001), application rates (McCoy et al., 2000), quality of products, and application methods may contribute to unpredictability in the response to application of these nematodes. It was also demonstrated that persistence of *S. riobrave* and *H. bacteriophora* Poinar, 1976 in citrus groves is relatively low (Duncan et al., 1996; Duncan and McCoy, 1996). Efficacy is measurable only during the first week after application (McCoy et al., 2000); thus, nematodes are used periodically as inundative rather than classical biological control agents. Entomopathogenic nematodes adapted to persist at higher population density in the citrus rhizosphere might pro-

vide greater and less variable efficacy against weevil pests.

A nematode was collected from larvae of *D. abbreviatus* that were buried in cages beneath citrus trees in an experiment conducted in Polk County, Florida. During a 2-year survey, the nematode was found to be the major identifiable cause of mortality to the weevil larvae in soil (Duncan and Graham, unpubl.). In plots treated twice annually with *S. riobrave*, indigenous populations of the new nematode killed an average of 4 times more *D. abbreviatus* than did *S. riobrave*, except during treatment months. The nematode is a member of the genus *Steinernema* but is different morphologically from other species of the genus. The nematode is most similar in general morphology to *S. feltiae* (Filipjev, 1934) Wouts, Mracek, Gerdin & Bedding, 1982; *S. glaseri* (Steiner, 1929) Wouts, Mracek, Gerdin & Bedding, 1982; and *S. oregonense* Liu & Berry, 1996, but morphological characters, morphometrics, genetic characteristics, and cross hybridization suggest that this nematode is a new species. The new species is described herein as *Steinernema diaprepesi* n. sp., named after the host insect from which the nematode was collected.

MATERIALS AND METHODS

Nematodes collected from the field by baiting with caged buried larvae of *D. abbreviatus* were maintained in the laboratory on last instar *Galleria mellonella* L larvae (Dutky et al., 1964). For taxonomic studies, 10 *G. mellonella* were exposed to 5,000 infective juveniles (IJ) in a petri dish (100 \times 15 mm) lined with two moistened filter papers. First- and second-generation adult nematodes were obtained by dissecting infected insects 2 to 4 days and 5 to 7 days, respectively, after the insects died. Third-stage IJ were obtained when they emerged from the cadavers after 7 to 10 days. All observations and measurements were performed within a week after col-

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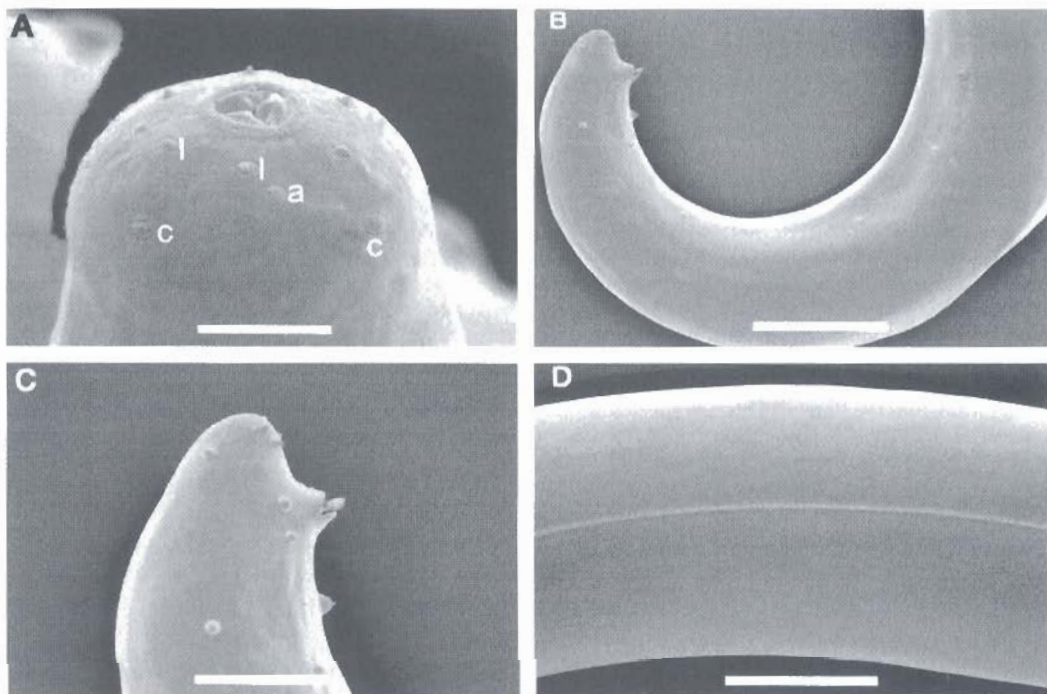


FIG. 1. *Steinernema diaprepesi* n. sp., SEM of first-generation males. A) Slightly swollen head, showing anterior end of stoma with a perianal ring, six labial papillae (l) and two of four cephalic papillae (c). B,C) Posterior region of males showing number and distribution of genital papillae (p). D) Lateral field at mid-body with a single ridge. Scale bars: A = 10 μ m, B = 66.7 μ m, C,D = 30 μ m.

lection. For light microscope observation, at least 20 males and females and 50 IJ were examined live. Additional specimens of different stages were killed in warm water (40 °C) or fixed in TAF (Courtney et al., 1955) or in lactophenol (Franklin and Goodey, 1949). These nematodes were used further when more observation was needed to confirm the morphology or variation of some structures. Nematodes fixed in TAF were processed to glycerol.

For control, the crosses between IJ of the same species were conducted. All treatments were replicated 30 times. All petri dishes were kept in closed plastic bags containing a piece of moistened facial tissue to keep



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the hemolymph from drying. Cross hybridization between *S. diaprepesi* n. sp. and *S. feltiae* and *S. oregonense* were conducted in the same way.

Extraction of DNA: DNA of *S. feltiae*, *S. oregonense*, *S. glaseri*, and *S. diaprepesi* n. sp. was extracted from a single female using the modified method reported by Joyce et al. (1994).

PCR amplification: The ITS region of the ribosomal DNA was amplified by the polymerase chain reaction (PCR) in a 100- μ l reaction with a DNA polymerase kit (AMRESCO, INC., Solon, OH). All tubes were kept on ice, and the following PCR mix was added to each tube: 10 μ l of 10 \times PCR buffer (100 mM Tris-HCl (pH 8.8), 15 mM MgCl₂, 500 mM KCl, 1% Triton X-100), 2 μ l of

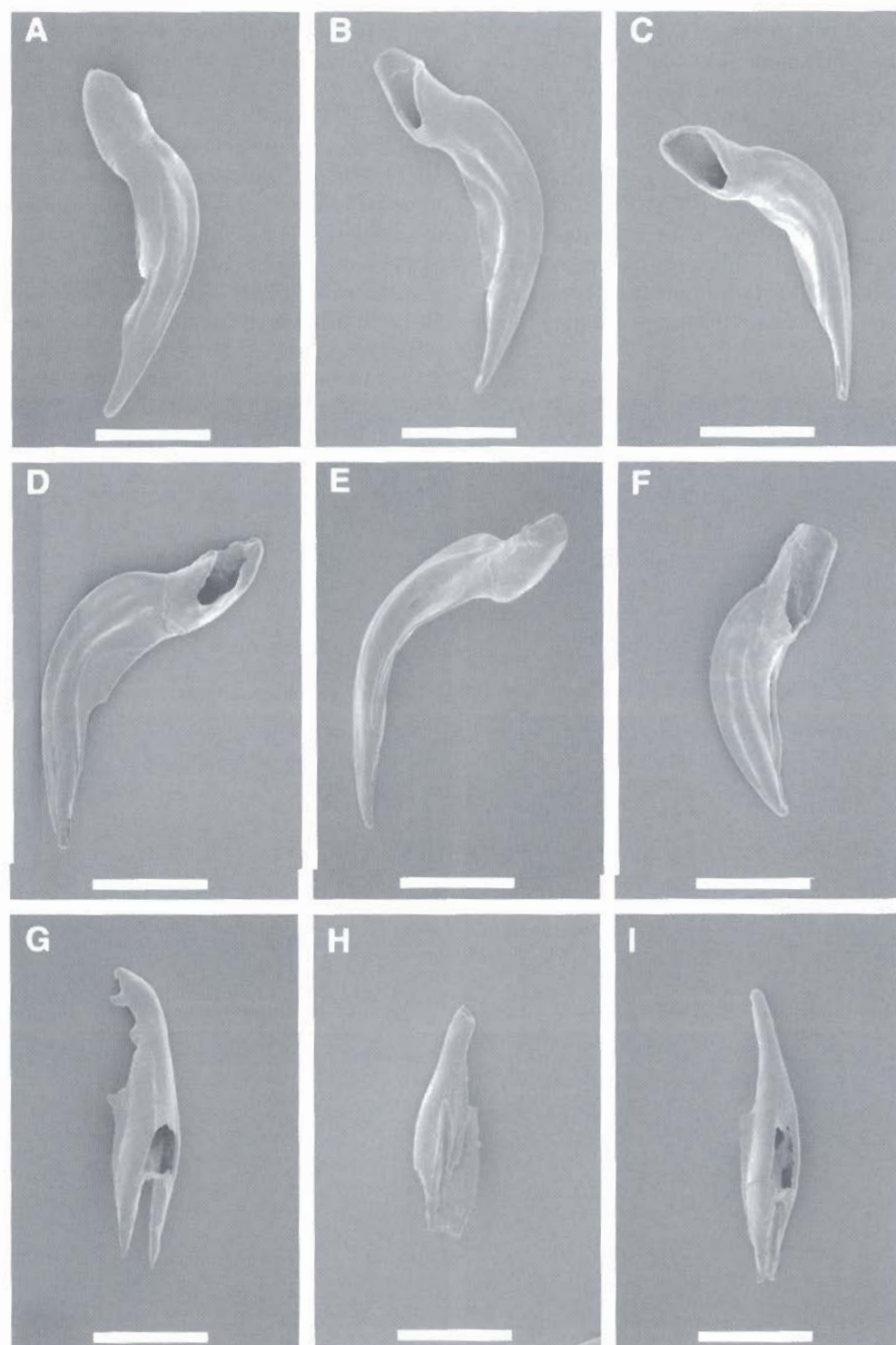


FIG. 3. *Steinernema diaprepesi* n. sp., scipules and gubernacula of first-generation males. A-D) Common types of scipules. E,F) Uncommon types of scipule. G-I) Gubernacula in different views. Scale bars: all scale bars = 20 μ m.

dNTP mixture (10 mM each), 2 μ l of 5 pM forward primer, 2 μ l of 5 pM reverse primer, 0.5 μ l of Thermo-lase Tbr (AMRESCO, INC., Solon, OH) (2 U/ μ l), 74 μ l of distilled water, and 10 μ l of DNA. Mineral oil (100 μ l) was placed on top of the solution in the tube to minimize evaporation. The primers used in this study were reported by Hominick et al. (1997): AB28: 5'-ATATGCTTAAGTTCAGCGGGT-3' (forward) and TW81: 5'-GTTTCCGTAGGTGAACCTGC-3' (reverse). All PCR reactions were run in a PTC-100 Thermocycler (MJ Research, Inc., Waltham, MA) with the cycling profile: 1 cycle of 94 °C for 2 minutes followed by 40 cycles of 94 °C for 30 seconds, 45 °C for 60 seconds, 72 °C for 90 seconds. The last step was 72 °C for 15 minutes.

Sequencing: PCR products were purified with a QIAquick PCR purification kit (QIAGEN Inc., Santa Clarita, CA). Purified DNA was sequenced directly using an ABI PRISM™ Dye Terminator Cycling Sequencing Ready Reaction kit (PerkinElmer, Inc., Foster City, CA). For each reaction the following reagents were

added to a 0.5-ml tube: 4 μ l Terminator Ready Reaction Mix, 1.5 μ l primer (3.2 pM), 2.5 μ l DNA from the PCR reaction, 2 μ l water, and 10 μ l of mineral oil to avoid evaporation. The primers used in this step were the AB28 and TW81 described earlier and two internal primers suggested by Nguyen et al. (2001): KN58 = 5'-GTATGTTTGGTTGAAGGTC-3' and KNRV = 5'-CAGGCTCATACAACCTGCTC-3'. The sequences flanked by the AB28 and TW81 primers were assembled using Auto-Assembler Version 2 (PerkinElmer, Inc., Foster City, CA).

DNA analysis

Multiple alignment: Sequences of studied species were aligned using the default parameters of Clustal W (Thompson et al., 1994). The alignment would be used to compare the similarity and difference of nucleotides of the four sequences.

Ratios of similarity: This ratio was obtained using default parameters of the Olddistances program (Wiscon-

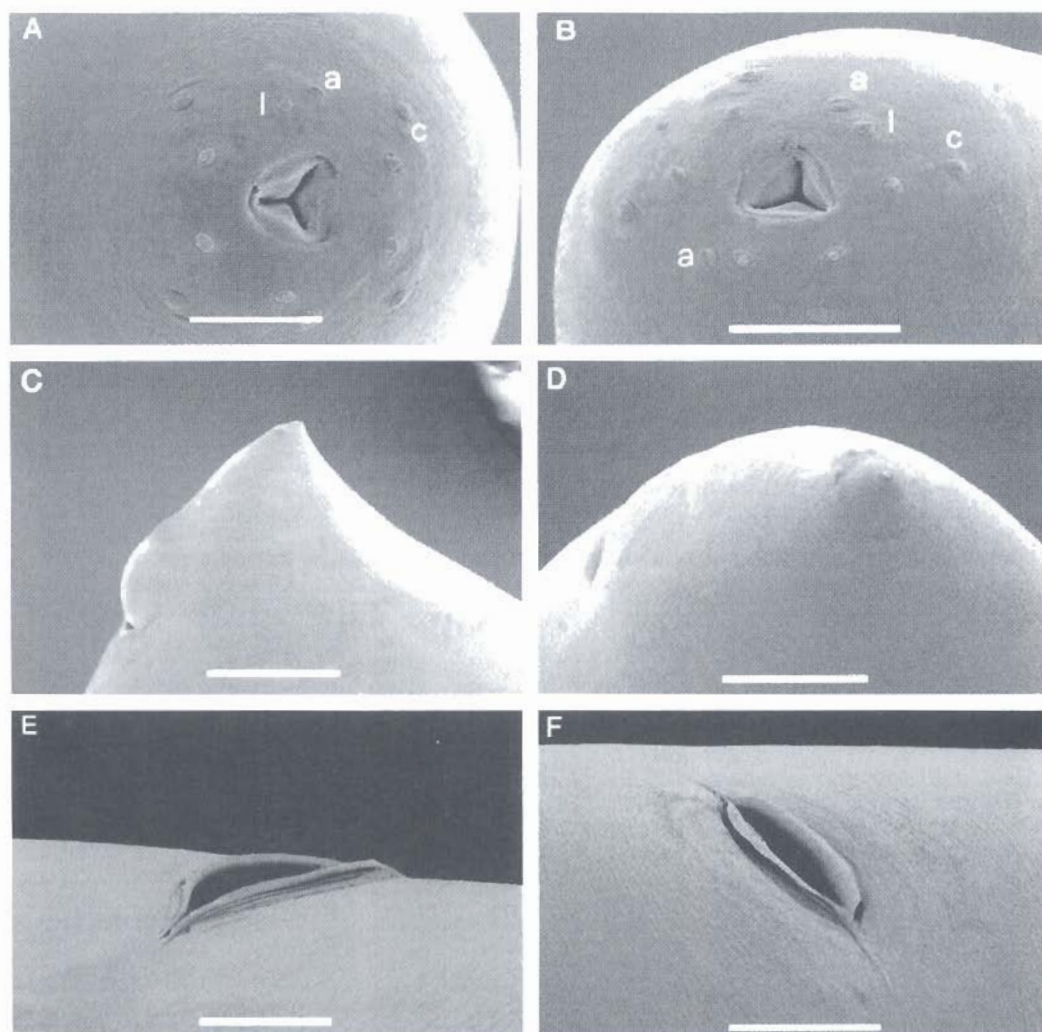


FIG. 4. *Steinerema diaprepesi* n. sp., first-generation females. A,B) Anterior end showing stoma, 6 labial papillae (l), 2 amphids (a), and 4 cephalic papillae (c). C,D) Tails showing anus, ventral postanal swelling (C), and tail terminus with papillae-like structures (D, arrow). E,F) Low double-flapped epiptygma. Scale bars: A,B = 10 μ m, C = 20 μ m, D = 15 μ m, E = 10 μ m, F = 15 μ m.

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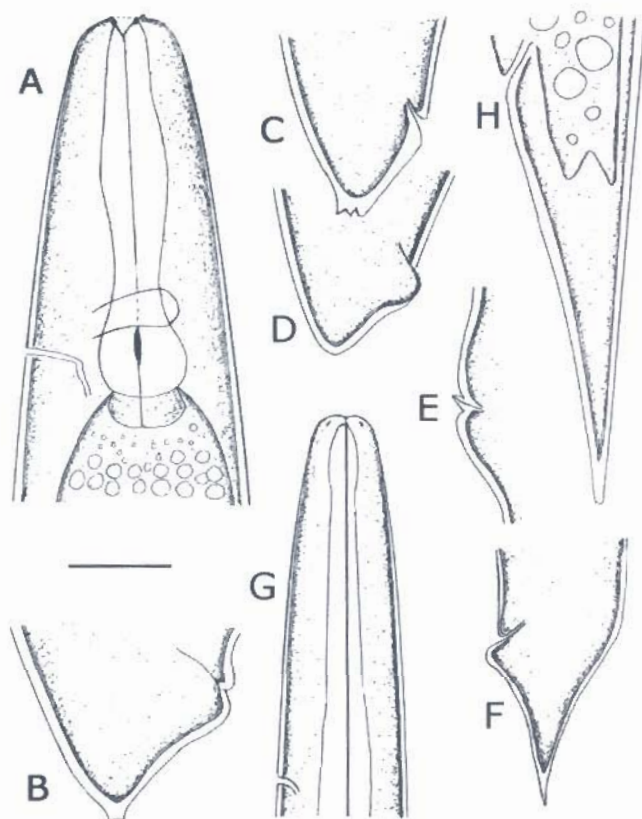


FIG. 5. *Steinernema diaprepesi* n. sp., first- and second-generation females and infective juvenile. A) Anterior region of a first-generation female. B-D) Variation in tail shapes of first-generation females. E) Epitygma of a second-generation female. F) Posterior region of a second-generation female. G, H) Anterior and posterior regions of an infective juvenile, note that the hyaline portion is longer than half the tail length. Scale bar: A-F = 33 μ m, G, H = 20 μ m.

SYSTEMATICS

Steinernema diaprepesi n. sp. (Fig. 1-6)

Holotype (male, first generation): Length 1,669 μ m; width 127 μ m; stoma width 15.6 μ m; stoma length 7.6 μ m; distances from anterior end to excretory pore (EP) 106 μ m; to nerve ring 97 μ m; to end of esophagus (ES) 132 μ m; testis reflexion 533 μ m; tail length 21 μ m; spicule length 77 μ m; spicule width 16.6 μ m; gubernaculum length 53 μ m; gubernaculum width 10.6 μ m; SW (spicule length/anal body width) 2.02; GS (gubernaculum length/spicule length) 0.7; D = EP/ES \times 100 = 80.

Males, first generation: Measurements of 20 males are given in Table 1. Body curved ventrally posteriorly (Fig. 1B), C-shaped when heat-killed. Lateral field present in mid-body with one narrow ridge (Fig. 1D). Head rounded, usually slightly swollen (Fig. 1A). Anterior end with a perioral disc around stoma; six labial papillae, two amphids (usually covered with exudate), and four cephalic papillae (Fig. 1A). Stoma shallow, cheilorhabdions as small and sclerotized structures at anterior end (Fig. 2A), sometimes indistinct. Excretory pore near nerve ring (Fig. 2A), located mostly anterior to

basal bulb. Esophagus with cylindrical procorpus, metacarpus absent or slightly swollen, isthmus present, nerve ring around isthmus, basal bulb distinct. Esophago-intestinal valve present, usually weak. Gonad monorchic, reflexed. Distance from base of esophagus to anterior end of testis variable. Spicules (Fig. 3) paired, brown in color. Head (manubrium) of spicules elongate, the ratio length/width from 1.4 to 2.0 (averaging 1.7 ± 0.2) in some specimens (4/20), twice as long as wide; shaft (calomus) very short or absent; blade (lamina) thick, tapering gradually posteriorly, blade terminus blunt with a longitudinal depression slit in ventral side (Fig. 3C,D); velum present. Each spicule with two internal ribs. Gubernaculum boat-shaped in lateral view, anterior part usually with one or two ventral projections (Fig. 2C,G). Eleven pairs [occasionally 12 (Fig. 1B)] and one single precloacal genital papillae distributed as in Fig. 1B. Tail conoid; tail terminus without mucron (Figs. 1B,2B).

General morphological characteristics of males collected from *D. abbreviatus* appear similar to those collected from *G. mellonella*, but they differ morphometrically (Table 1). Most measurements of first-generation males from *D. abbreviatus* are shorter than those collected

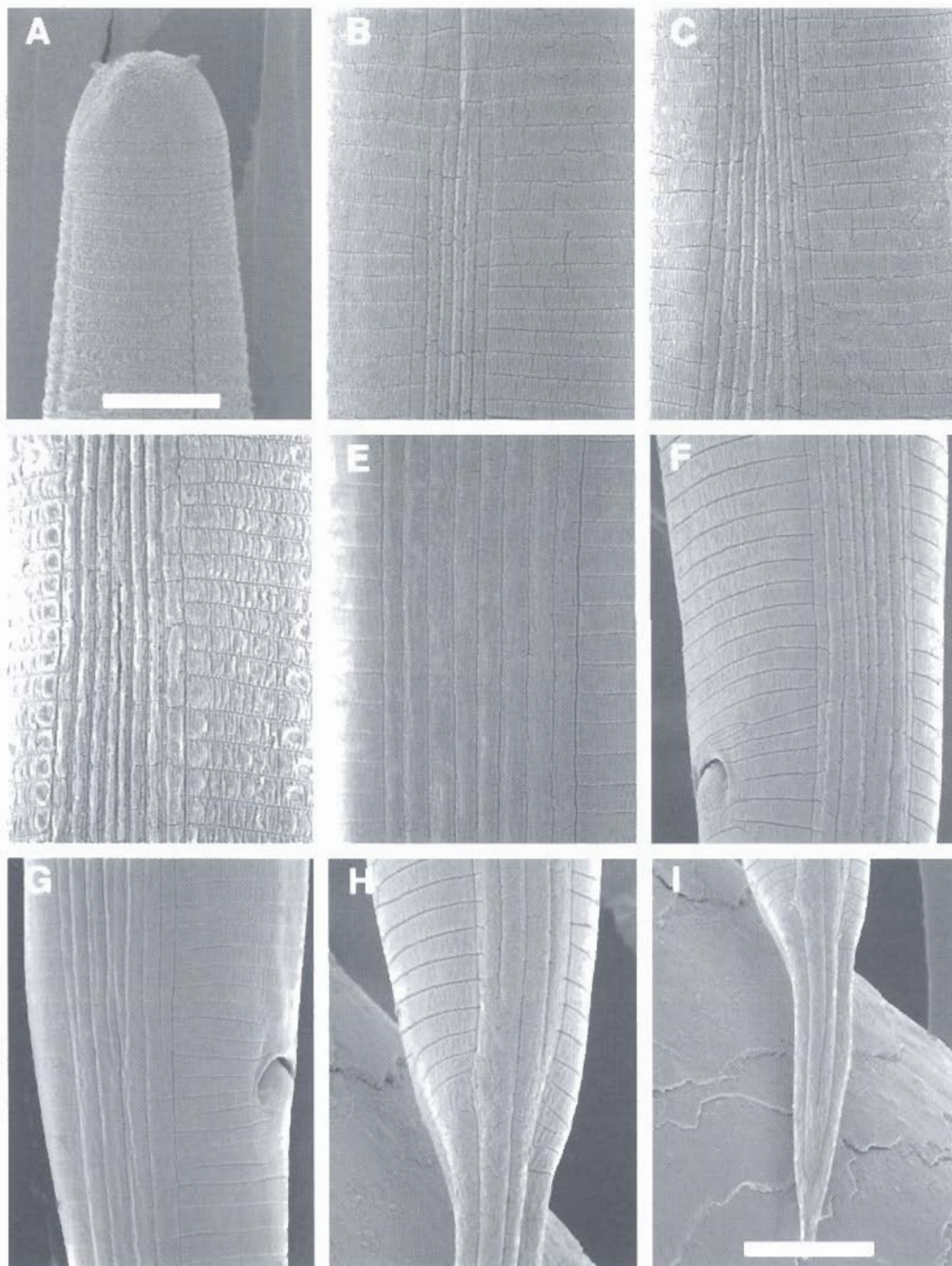


FIG. 6. *Steinernema diaphanes* n. sp., lateral field of infective juvenile. A) Anterior region showing prominent cephalic papillae. B) Lateral field with two ridges anteriorly, six ridges posteriorly. C) Lateral field showing the seventh ridge arising in the middle of lateral field. D) Lateral field showing the middle ridge dividing into two making a total of eight. E) Eight ridges in lateral field. F,G) Left and right lateral fields showing the disappearance of two marginal and two central ridges making the lateral fields become four. H) Number of ridges in lateral field reduce to two. I) Tail showing the end of lateral field. Scale bars: A-H = 7.5 μ m, I = 12 μ m.

from *G. mellonella*. This result concurs with the previous suggestion by Nguyen and Smart (1995b) that, when possible, *Steinernema* species should be reared in *G. mellonella* for nematode description and (or) identification.

Males, second generation: Measurements of 20 males reared in *Galleria* are given in Table 1. Second-generation male similar to that of the first generation except body, spicule, and gubernaculum shorter and thinner, excretory pore much more anterior, and isthmus

more distinct (Table 1). Gubernaculum short. Mucron on tail terminus sometimes present (Fig. 2D).

Females, first generation

Allotype (Female, first generation): Length 5,242 μ m; width 211 μ m; stoma width 9.1 μ m; stoma length 7.6 μ m; distance from anterior end to excretory pore 109 μ m, to nerve ring 132 μ m, to end of esophagus 182 μ m; anal body width 61 μ m; tail length 32 μ m; V% = 52.

TABLE 1. Me

Character	Measurement
Body length	5,242 μ m
Greatest width	211 μ m
EP	109 μ m
NR	132 μ m
ES	182 μ m
Testis reflexion	182 μ m
T	32 μ m
ABW	61 μ m
Spicule length	10 μ m
Spicule width	1.5 μ m
Gub. length	10 μ m
Gub. width	1.5 μ m
D = EP/ES (%)	59.9
SW	1.5 μ m
GS	10 μ m

EP = distance
NR = distance
ES = distance
T = tail length
ABW = anal b
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TABLE 2.

Character	Measurement
Body length	5,242 μ m
Stoma length	7.6 μ m
Stoma width	9.1 μ m
Greatest width	211 μ m
EP	109 μ m
NR	132 μ m
ES	182 μ m
T	32 μ m
ABW	61 μ m
V%	52
D = EP/ES	59.9
EP = dis	
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TABLE 1. Measurements (in μm) of males of *Steinernema diaprepesi* n. sp. from *Galleria mellonella* and *Diaprepes abbreviatus* ($n = 20$)

Character	Reared using <i>Galleria</i> (First generation)			Reared using <i>Diaprepes</i> (First generation)			Reared using <i>Galleria</i> (Second generation)		
	Means	SD	Range	Means	SD	Range	Means	SD	Range
Body length	1,735	156	1,506-2,078	1,403	185	1,060-1,693	1,176	117	1,036-1,343
Greatest width	113	15	90-145	78	11	61-100	64	7	54-78
EP	115	9	100-130	100	13	74-117	83	8	71-108
NR	119	6	109-129	121	11	100-139	110	8	100-136
ES	150	7	136-162	148	11	127-168	138	12	129-168
Testis reflexion	391	68	241-542	289	51	211-380	264	16	187-428
T	25	3	20-32	25	3.2	21-32	24	5	18-30
ABW	42	11	36-50	40	4	30-45	34	6	29-42
Spicule length	79	5	71-90	66	5	57-76	69	8	61-76
Spicule width	16	2	14-20	12.3	1.7	9-15	12.5	3	11-15
Gub. length	54	5	45-61	44	3.8	38-53	40	6	30-53
Gub. width	8.7	1.4	6-12	6.7	1.3	4.6-9.1	6.7	1.4	5-9
D = EP/ES (%)	80	6	68-86	68	8	52-80	60	8	54-67
SW	1.8	1.3	1.5-2.0	1.7	0.3	1.2-2.1	2	0.2	1.66-2.33
GS	0.69	0.1	59-79	0.66	0.04	0.62-0.74	0.59	0.07	0.46-0.73

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.
 T = tail length.
 ABW = anal body width.
 Gub. = gubernaculum.
 SW = spicule length/ABW.
 GS = gubernaculum length/spicule length.
 SD = standard deviation.

Measurements of 20 females are presented in Table 2. Body cuticle smooth or with faint annules. Lateral fields and phasmids absent. Head rounded, continuous with body; six labial papillae, four cephalic papillae (Fig. 4A,B). Lips indistinct. Amphids present. Stoma shallow, subtriangular anteriorly; triradiate internally (Fig. 4A,B). Cheilorhabdions (Fig. 5A) well sclerotized but small. A smaller sclerotized structure posterior to cheilorhabdions (presumably the prorhabdions), observed in other species, indistinct in this species. Esophagus with procorpus cylindrical, muscular; metacarpus swollen; isthmus distinct; basal bulb valvate (Fig. 5A) as in

other Steinernematids. Nerve ring surrounds isthmus, just anterior to basal bulb. Esophago-intestinal valve present. Excretory pore located just anterior to, or at the middle of, basal bulb (Fig. 5A). Gonads amphidelphic, reflexed, often containing many eggs. Vulva, a transverse slit; protruding or not; low double-flapped epiptygma present (Fig. 4E,F). Vagina sclerotized, short. Body width greater anterior to vulva than posterior to vulva. Tail shape variable (Fig. 5B,C), ventral postanal swelling present, tail shorter than anal body width. Most females with five papillae-like structures on tail tip (Fig 4C,D). Size of these structures is variable

TABLE 2. Measurements (in μm) of females of *Steinernema diaprepesi* n. sp. collected from *Galleria mellonella* ($n = 20$).

Character	First generation			Second generation		
	Means	SD	Range	Means	SD	Range
Body length	6,508	1,512	4,061-9,878	2,433	260	2,030-3,030
Stoma length	10.3	2.2	7.6-13.6	10.6	1.1	9.1-12.1
Stoma width	12.1	2.8	9-18.2	10.4	1.5	9.1-13.6
Greatest width	272	44	190-343	158	12	137-180
EP	156	39	98-214	116	15	94-139
NR	150	21	105-179	144	12	120-153
ES	200	25	119-232	182	11	161-211
T	52	10	38-68	78	6	67-91
ABW	94	22	59-138	50	7	39-59
V%	50	2.8	44-57	54	3	49-60
D = EP/ES (%)	80	19	46-105	64	8.2	47-74

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.
 T = tail length.
 ABW = anal body width.
 SD = standard deviation.

depending on age of females, usually longer in young females (Fig. 5C), becoming smaller and short as the female bodies enlarge, disappearing in fully mature females (Fig. 5D).

Females, second generation: Measurements of 20 females reared in *G. mellonella* are given in Table 2. Similar to first-generation female but smaller (length = 2,385 μm , width = 149 μm compared to 6,512 μm and 264 μm , respectively, for first-generation female). Vulva less protruding, epitygma (Fig. 5D) usually more prominent than that of first-generation females. Tail, tapering to a pointy end, longer than anal body width; ventral post-anal swelling present (Fig. 5E).

Infective juveniles: Measurements of 50 juveniles are given in Table 3. Body elongate. Sheath (second-stage cuticle) present immediately after harvesting, but many IJ will lose their sheath in storage (in our study, all IJ observed have sheath). Labial region smooth, continuous, rounded anteriorly (Fig. 6A). Labial papillae not seen; four cephalic papillae prominent (Fig. 6A). Amphids slit-shaped but not prominent. Cuticle marked with prominent transverse striations. Lateral field begins anteriorly with one line (Fig. 6A). Two additional lines appear at annules 14 to 16 to form two ridges (Fig. 6B). Near excretory pore level, the number of ridges in lateral fields increases from two to six (Fig. 6B). A new central ridge appears more posteriorly, about a body width posterior to excretory pore, making a total of seven ridges in the lateral field (Fig. 6C). Near the end of the esophagus, the central ridge divides into two, making a total of eight ridges—the maximum number in the lateral field (Fig. 6D,E). The portion with eight ridges is the longest part (compared to portions with 2, 6, 7, 4 ridges) of the lateral field. At the level of the anus

the two marginal and two central ridges disappear (Fig. 6F,G), only four ridges remain in the lateral field. At about mid-tail the four ridges in the lateral field become two ridges (Fig. 6H). Near the tail terminus, the two marginal lines in the lateral field converge (Fig. 6I) and the central line disappears before reaching the end of the lateral field.

Esophagus with thin corpus (Fig. 5F), basal bulb more or less elongate with visible valve. Tail attenuate, tapering gradually (Fig. 5G). Hyaline portion occupies about 57% (50–63) of tail length, sometimes up to 70% (Fig. 5G).

The morphometrics of IJ reared from *G. mellonella* and from *D. abbreviatus* appear different (Table 3).

Cross hybridization tests: Males and females of *S. diaprepesi* n. sp. did not interbreed with *S. feltiae*, *S. glaseri*, and *S. oregonense*. In the control, males and females of the each species mated and produced offspring normally.

Molecular diagnosis: Because PCR-RFLP analysis should be used with caution in determining affinities among species (Dowling et al., 1996), a sequencing approach was undertaken. The alignment of the sequences of the ITS regions of four species (*S. feltiae*, *S. oregonense*, *S. glaseri*, and *S. diaprepesi* n. sp.) is presented in Figure 7. The partial 18S (nucleotides 1–12), 5.8S gene sequence (319–466), and 28S portion (792–814) show little variation among the four species. The ITS1 (13–318) and ITS2 (467–791) regions are much more variable and provide most of the base differences (Table 4) that are important for species diagnosis (Adams et al., 1998). The lengths of the amplified sequences and sequence similarity (Table 5) also are different among the four species (Table 4) (Nguyen et al., 2001). The phylogenetic relationships among the 11 studied *Steinernema* species are presented in Figure 8. *Steinernema diaprepesi* n. sp. appears as the sister to *S. glaseri*, and the clade *S. glaseri* + *S. diaprepesi* n. sp. is well supported (100%) by bootstrap analysis.

Habitat and biology: *Steinernema diaprepesi* n. sp. was isolated from the larvae of *D. abbreviatus* that were buried in cages beneath citrus trees as part of an experiment to evaluate the insecticidal efficacy of commercially available entomopathogenic nematodes. During 2 years of the experiment, indigenous populations of *S. diaprepesi* n. sp. infected and killed 13% to 50% of the buried insects within 7 days, with higher rates of parasitism during the summer months compared to spring and autumn months. The nematode was isolated with much lower frequency from the rhizosphere of native plants growing between citrus tree rows than from the rhizosphere of citrus trees.

The toptype locality is an irrigated, commercial citrus orchard c. 6 km east of Bartow, Florida, and immediately southeast of the intersection of Cowpen Road and 80-Foot Road. Mean monthly temperatures range from 16.1 to 27.9 °C, and cumulative annual precipitation averages 1,364 mm with most rainfall occurring

TABLE 3. Measurements (in μm) of infective juveniles of *Steinernema diaprepesi* n. sp. collected from *Galleria mellonella* and *Diaprepesi abbreviatus* (n = 50).

Character	Reared using <i>Galleria</i>			Reared using <i>Diaprepesi</i>		
	Means	SD	Range	Means	SD	Range
Body length	1,002	53	880–1,133	957	31	807–1,030
Greatest width	34	3.6	30–42	36	6	29–42
EP	74	6.4	66–83	69	8	55–77
NR	102	6	74–109	105	5	98–114
ES	138	7.4	111–152	144	5	136–156
T	83	5	65–91	81	9	76–88
ABW	23	2.3	21–27	22	1.8	18–24
a	30	2.6	23–35	26.5	1.6	23–29
b	7.3	0.5	6.5–8.3	6.6	0.4	6.1–7.6
c	12.1	0.7	10.4–13.2	11.7	0.7	10–13
D = EP/ES (%)	54	5	30–70	48	2.2	47–56
E = EP/T (%)	90	6.5	78–114	85	9	71–96

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.
T = tail length.
ABW = anal body width.
a = body length/greatest width; b = body length/ES; c = body length/T.
SD = standard deviation.

FIG. 7.
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<i>Steinernema feltiae</i>	GGAAGGATCA	TTATTGAGCT	TATCCATTTA	CTTG---GAT	TCAAATGAAT
<i>S. oregonense</i>	GGAAGGATCA	TTATTGAGCT	TATCCATTTA	CTTG---GAT	TCAAATGAAT
<i>S. glaseri</i>	GGAAGGATCA	TTATTGAGCC	TACTCTCATA	CATGTGAGTA	TTATGATCAC
<i>S. diaprepesi</i>	GGAAGGATCA	TTATTGAGCT	TA-ACACTTC	GTTGTTTATA	CGTTACGTAT
	*****	*****	** * *	**	*
	51				100
<i>Steinernema feltiae</i>	CGAGCTGAAT	-TTTCGCTGT	TCGTTTCAAA	GCGTTGTATT	CTCTCAACTA
<i>S. oregonense</i>	CGAGCTGAAT	CGTTTGCCTGT	TTGTCTCGAG	GCAATGTATT	CTCTCATCTA
<i>S. glaseri</i>	TGTTCCGGAAC	GCGGCACT-G	TCGTTTCTAG	GTGTCGCGAC	CGTTTCG-ACA
<i>S. diaprepesi</i>	CGTTCCGGAAC	GACACTGTCC	ACGTTTCTAA	GTGTCGATTG	CGTTCA-CTA
	* * **	*	** ** *	* *	* ** *
	101				150
<i>Steinernema feltiae</i>	ACGGCTATGA	ATGGTTTCTA	TAGGTGTCTG	GAGCAGTTGT	ATGAGCGTGA
<i>S. oregonense</i>	ACGGCTATGA	ATGGTTTCTA	TAGGTGTCTG	GAGCAGTTGT	ATGAGCGTGA
<i>S. glaseri</i>	ACGGCTTTGA	ATGGTTTCTA	TAGGTGTCTG	GAGCAGCTGT	ATGAGCGTGG
<i>S. diaprepesi</i>	ACGGCTTCGA	ATGGTTTCTA	TAGGTGTCTG	GAGCAGCTGT	ATGAGCGTGG
	*****	**	*****	*****	**
	151				200
<i>Steinernema feltiae</i>	CTGTGGTGAT	GGACATTTTG	-----	-----	-GTGGCTCCT
<i>S. oregonense</i>	CTGTGGTGAT	GGACATTTGA	-----	-----	-GT-----
<i>S. glaseri</i>	CTGTGGTGAA	GGACATTTGA	CATCGCGT--	-----	-----
<i>S. diaprepesi</i>	CTGTGGTGAA	GGACATTTGA	CATCCATATG	CAGACGTTTA	GCTGTCTCTT
	*****	**	*****	*****	*****
	201				250
<i>Steinernema feltiae</i>	TAGTCGGGTC	ACTAGAATTA	AAGAAGTCTG	TTATGACTCG	CCGTTCTT-A
<i>S. oregonense</i>	---TCTTGTC	ACTAGAATTA	AAGAAGTCTG	ATACGACTCG	CCGTTCTTAA
<i>S. glaseri</i>	-CTCGACGCG	GTGAGAATTG	AAGAGGTCAG	-TCGGAGACC	CGCCGTTTCA
<i>S. diaprepesi</i>	GCGTTTGGTG	ATGAGAATTA	AAGAGGTCAG	-TCGGAGACC	CGCCGTTTCA-
	*	*****	*****	** *	* *
	251				300
<i>Steinernema feltiae</i>	AAAAACTTCA	ATTAACGTTT	GATCAATTTG	ACTGCACCAG	CCGT----AG
<i>S. oregonense</i>	AAAAACTTCA	ATTAACGTTT	GATCAATTTG	ACTGCACCAG	CCGT----AG
<i>S. glaseri</i>	AAACCCTACC	ATTAACAATT	TTACACACGA	TGACAAGCAT	CGTTGATGCT
<i>S. diaprepesi</i>	AAACCCTACC	ATTAACATTT	TCCATACTAA	GCTCCATATG	TATTTATGGT
	** * *	*****	**	*	*
	301				350
<i>Steinernema feltiae</i>	GTGTACTTAA	AGATTTATCA	AGTCTTGT-C	GGTGGATCAC	TCGGTTCGTA
<i>S. oregonense</i>	GTGTACTTAA	AGATTTATCA	AGTCTTGT-C	GGTGGATCAC	TCGGTTCGTA
<i>S. glaseri</i>	GTGTTATACA	ACTGTTACCA	AGTCTTAT-C	GGTGGATCAC	TCGGTTCGTA
<i>S. diaprepesi</i>	GGCGAAAACA	ATGTTATCCA	AGTCTTATCC	GGTGGATCAC	TCGGTTCGTA
	*	* * *	**	*****	*****
	351				400
<i>Steinernema feltiae</i>	GTTTCGATGAA	AAACGGGGCA	AAAACCGTTA	TTTGGCGTGA	ATTGCAGACA
<i>S. oregonense</i>	GTTTCGATGAA	AAACGGGGCA	AAAACCGTTA	TTTGGCGTGA	ATTGCAGACA
<i>S. glaseri</i>	GTTTCGATGAA	AAACGGGGCA	AAAACCGTTA	TTTGGCGTGA	ATTGCAGACA
<i>S. diaprepesi</i>	GTTTCGATGAA	AAACGGGGCA	AAAACCGTTA	TTTGGCGTGA	ATTGCAGACA
	*****	*****	*****	*****	*****

FIG. 7. Alignment rDNA sequences from four species of *Steinernema*: *S. diaprepesi* n. sp., *S. feltiae*, *S. glaseri*, and *S. oregonense*. Asterisk (*), no differences found among nematode species. Hyphen (-), gap.

May through September and frequent periods of drought from mid-autumn to late-spring. Soil texture is astatula sand (97:1:2, sand:silt:clay). *Steinernema diaprepesi* n. sp. has been isolated from other orchards located on the central ridge of Florida, which is characterized by deep sandy soils. Attempts to isolate the nematode in heavier-textured soils off of the central ridge have been unsuccessful; however, no comprehensive survey has yet been conducted.

Type host and locality

The nematode was collected from an infested citrus root weevil, *Diaprepes abbreviatus* (L), in a commercial citrus orchard in Polk County, Florida.

Type specimens

Holotype (male, first generation): Isolated from hemo-coel of *Galleria mellonella* deposited in the U.S. Depart-

	401				450
<i>Steinernema feltiae</i>	TATTGAACGC	TAAAATTTTG	AACGCAAATG	GCACTATCAG	GTTTATATCT
<i>S. oregonense</i>	TATTGAACGC	TAAAATTTTG	AACGCAAATG	GCACTATCAG	GTTTATATCT
<i>S. glaseri</i>	TATTGAACGC	TAAAATTTTG	AACGCAAATG	GCACTATCAG	GTTTATATCT
<i>S. diaprepesi</i>	TATTGAACGC	TAAAATTTTG	AACGCAAATG	GCACTATCAG	GTTAATATCT
	*****	*****	*****	*****	*** *****
	451				500
<i>Steinernema feltiae</i>	GTTAGTATGT	TTGGTTGAGG	GTCGATTAAT	TCGTAACCTG	CAGTCTGCTG
<i>S. oregonense</i>	GTTAGTATGT	TTGGTTGAGG	GTCGATTAAT	TCGTAACCTG	CAGTCTGCTG
<i>S. glaseri</i>	GATAGTATGT	TTGGTTGAGG	GTCGACTAAC	ACGTTACTTG	CAGTCAG---
<i>S. diaprepesi</i>	GATAGTATGT	TTGGTTGAGG	GTCGATTAAC	TCGTTACTTG	CAGTCAGCTT
	*	*****	*****	*** ** *	***** *
	501				550
<i>Steinernema feltiae</i>	TGACTGTTTT	TTCGATTAGT	TA----TTTG	GTTTTTTTAT	CGAGTACCTT
<i>S. oregonense</i>	TGACTGTTTT	TCCGATTAGT	TACTCGATTG	GCTCGCTGAT	CGAGTACCTT
<i>S. glaseri</i>	CGACTGTTTT	TTCGACGAGC	TATGTAC--G	TTCGTATGTA	CCTCGTTCGG
<i>S. diaprepesi</i>	CGACTGTTTA	TTCGATAAGC	TACTTTCGAG	CTGCGAAAGT	ACCTTTTCGG
	*****	* ** *	**	*	*
	551				600
<i>Steinernema feltiae</i>	TTTGGAATGT	GAA--TTGAT	TGTCTAATTC	GTTTCCTAAT	CGAA---ACG
<i>S. oregonense</i>	CTAGGTATGT	GAATTTTGAT	AGTCTAATTC	GTTTCCTAAT	CGAA---ACG
<i>S. glaseri</i>	TGTGAACGTT	CCCCGGCCAC	TGGGGCCGAT	AGTGCATGG	ACAAGGCTTT
<i>S. diaprepesi</i>	TGTGAACGCT	TCAATGCCGAT	AGGCTAATGG	AGGTCGTTAG	GCGAGTGTCT
	*	*	*	*	*
	601				650
<i>Steinernema feltiae</i>	AGCTATTTTT	TATTTCT-GT	GCAATGTATT	TTTGGTGTTT	CGGCGTTTTT
<i>S. oregonense</i>	AGCTATCTTT	GAATTCTGGT	GCGTTGTATC	TTTGGTGTTT	CGGCGCGTTT
<i>S. glaseri</i>	-----TGT	CGTGTCCGCT	ATCACATCGG	TTCCGTGCGT	T-GATGGCTT
<i>S. diaprepesi</i>	CTTTCGCTAA	CGCTTCTGCT	ATCATATCGG	TTCTGTGCGT	TACGTGGCTA
	*	** *	*	** ** *	*
	651				700
<i>Steinernema feltiae</i>	CTTGCCGACT	GATTGGTACA	AACTTAACAG	TTCGTATATT	TTTCAGAATT
<i>S. oregonense</i>	CTTGCCGACT	GATCTGTACG	TA-----	ACCGTATATG	CTTCA-ATTT
<i>S. glaseri</i>	TGGCGTGTCT	CTTGCCAGCT	GACTTGTACG	TAATTTTTTG	CGTATGTAAG
<i>S. diaprepesi</i>	TGGCGTGTCT	CTTGCCAGTT	GACTTGTACG	CAGACGTAAC	TGTCCTCGTAT
	*	** *	*	*	*
	701				750
<i>Steinernema feltiae</i>	TTTCAGAGGC	CCTTACAATA	CATCACTTGA	CACAACACGT	ATCGTTTGTC
<i>S. oregonense</i>	GATCAGATGC	CCTTAGCTTA	CTTCACTCGA	CACAACACGT	TTCGTTTGTT
<i>S. glaseri</i>	CTTCTTGAAG	TCAGTGTTCG	CAGCAAGCGT	TTGAGCCTGT	ACGGTTCGGC
<i>S. diaprepesi</i>	GTAAGCTTCT	TGA-AGTCGG	CTGCCACATG	TTCGACCTTT	GCGGGTTGAC
			* *	* *	* ** *
	751				800
<i>Steinernema feltiae</i>	GAGGAATTGC	GCAAGAA---	-----AGAAA	CTTTTCGTTT	TACGACCTCA
<i>S. oregonense</i>	GAGTAATCGC	GCAAAAA---	-TTGTAAACT	TTTTTCGTTT	TACGACCTCA
<i>S. glaseri</i>	GCGCGACGTA	GCTGGGACTT	CGTGTTCGAT	GTTTTCGAAT	GACGACCTCA
<i>S. diaprepesi</i>	GAACGCAACT	GGAACCTTGT	CG-ATTCGAT	GTTTTCGAAT	TACGACCTCA
	*	*		*** *	*****
	801	814			
<i>Steinernema feltiae</i>	ACTCAAGCAA	GATT			
<i>S. oregonense</i>	ACTCAAGCAA	GACT			
<i>S. glaseri</i>	ACTCAAGCAA	GACT			
<i>S. diaprepesi</i>	ACTCAAGCAA	GACT			
	*****	** *			

* no differences found among nematode species.
- gaps.

FIG. 7. CONTINUED.

TABLE 4.
Diagnostic char

Species (Seq length)
<i>S. diaprepesi</i> (808 bp)
<i>S. feltiae</i> (766 bp)
<i>S. glaseri</i> (774 bp)
<i>S. oregonense</i> (759 bp)

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S. feltiae
S. oregonense
S. glaseri
S. diaprepesi

TABLE 4. Sequence length of ITS regions, composition, and diagnostic characters of four species of *Steinernema*.

Species (Seq length)	ITS1 (bp)	ITS2 (bp)	A (%)	C (%)	G (%)	T (%)	Diagnostic character	
<i>S. diaprepesi</i> (808 bp)	301	158	313	24.1	20.6	24.1	31.2	144
<i>S. feltiae</i> (766 bp)	275	157	298	25.5	16.7	21.5	36.3	45
<i>S. glaseri</i> (774 bp)	279	157	302	22.5	21.2	26.7	29.6	136
<i>S. oregonense</i> (759 bp)	267	157	297	24.9	18.1	22.1	34.9	41

Diagnostic characters = numbers of characters (in the same column of the alignment) present in one sequence but not in others.

ment of Agriculture Nematode Collection (USDANC), Beltsville, Maryland.

Allotype (female, first generation): Same data as holotype, deposited in the USDANC, Beltsville, Maryland.

Paratypes (first-generation males and females, and third-stage IJ): Same data as holotype. Many males and females of the first generation and several third-stage IJ in TAF deposited in USDANC, Beltsville, Maryland; several males, and females of the first generation, and several third-stage IJ deposited in the Florida Collection of Nematodes, Florida Department of Agriculture and Consumer Services, Gainesville, Florida; one male and one female of the first generation, and several IJ deposited in the California Collection of Nematodes, University of California Davis Nematode Collection, Davis, California.

Diagnosis. Males: Spicule averaging 79 (71–90) μm ; D% about 80; the ratio SW about 1.8. Lateral field with one narrow ridge. *Females*: Vulva with short, low double-flapped epiptygma; tail terminus usually with 5 papillae-like structures. *Infective juveniles*: Body length averaging 1,002 (880–1,133) μm , EP = 74 (66–83) μm ; tail length = 83 (65–91) μm , and E% = 89.6 (78–114). Lateral field pattern variable; the formula for the arrangement of ridges from head to tail is: 2, 6, 7, 8, 4, 2 (Fig. 6). The portion with eight ridges is the longest. The new species is characterized genetically by sequence lengths of ITS region (808 bp), ITS1 (301 bp), ITS2 (313 bp), and composition of its sequence.

Relationships: *Steinernema diaprepesi* n. sp. body length of IJ (1,002 μm) is intermediate between that of the *S. glaseri* group (*S. arenarium*, *S. cubanum*, *S. glaseri*, *S. longicaudum*, *S. puertoricense*, body length > 1,000 μm) and

TABLE 5. Pairwise similarity among four species of *Steinernema* expressed as the number of matches between each sequence pair divided by sequence length.

	<i>S. fel</i>	<i>S. ore</i>	<i>S. gla</i>	<i>S. dia</i>
<i>S. feltiae</i>	1.0000	0.8972	0.6097	0.6332
<i>S. oregonense</i>		1.0000	0.6008	0.6271
<i>S. glaseri</i>			1.0000	0.7429
<i>S. diaprepesi</i>				1.0000

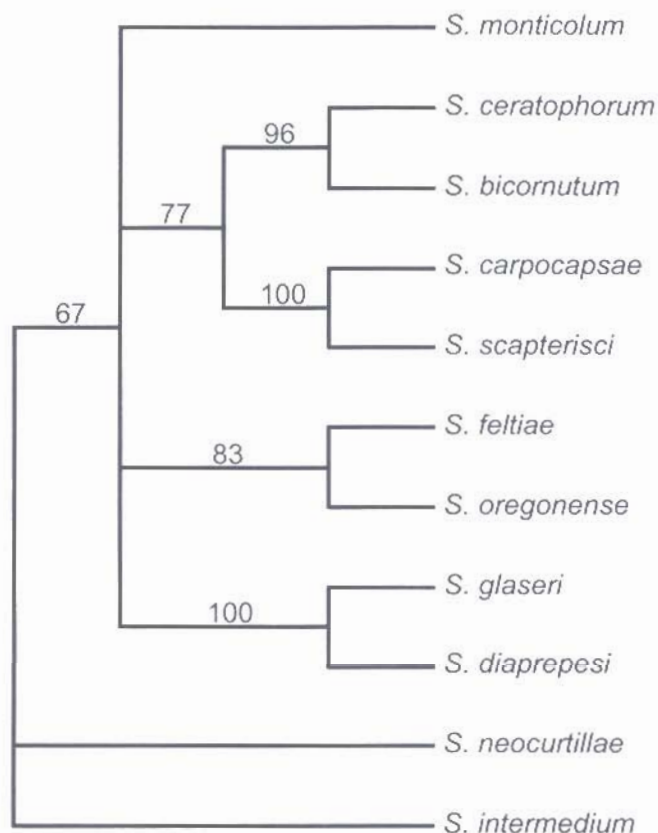


FIG. 8. Phylogenetic relationship between 11 species of *Steinernema*. Numbers over branches indicate bootstrap proportion. The clade *S. glaseri* + *S. diaprepesi* n. sp. is well supported (100%) by bootstrap analysis.

S. kraussei group (*S. feltiae*, *S. kraussei*, *S. neocurtillae*, *S. oregonense*, 800 μm < body length < 1,000 μm). The new species can be distinguished from these *Steinernema* species by several morphological characters of males, females, and IJ. Males can be differentiated from other Steinernematids by shape and length of spicules and gubernaculum (Fig. 3; Nguyen and Smart, 1997), D% = 80, and SW = 1.8. Females can be distinguished from other species by the presence of five papillae-like structures on the tail tip (Figs. 4,5) and vulva with low double-flapped epiptygma (Figs. 4,5). Infective juveniles of the new species can be recognized by body length, distance from anterior end to excretory pore, tail length, and E%. Finally, *Steinernema diaprepesi* n. sp. can be differentiated from the three closest species (*S. feltiae*, *S. oregonense*, *S. glaseri*) by sequence analysis and by reproductive incompatibility, as previously stated.

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