STEINERNEMA WEBSTERI SP. N. (RHABDITIDA: STEINERNEMATIDAE), A NEW ENTOMOPATHOGENIC NEMATODE FROM CHINA

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Summary. *Steinernema websteri* sp. n. is a new entomopathogenic nematode isolated in Beijin, China. Morphological, molecular (28S rDNA sequence and RFLP analyses), and cross-breeding tests were used for diagnostic purposes. Additionally, 28S rDNA sequence data was used to assess phylogenetic relationships of this new species with other *Steinernema* spp. Morphological diagnostic features include: maximum body width (average: 21 µm), excretory pore position (average: 36 µm), D% value (average: 31) and E% value (average: 77) of the third-stage infective juvenile; excretory pore position (average: 62 µm), cloacal body width (average: 37.5 µm), tail length (average: 29 µm), D% (average: 40) and morphology of spicules and gubernaculum of the first generation male.

Entomopathogenic nematodes (EPN) of the genus *Steinernema* Travassos, 1927 are obligate and lethal endoparasites that are symbiotically associated with *Xenorhabdus* sp. Thomas *et* Poinar, 1979 bacteria. This nematode-bacteria complex exemplifies perfectly a mutualistic association where the bacteria are vectored between insects by the nematode third-stage infective juveniles, and the bacteria, once released by the nematodes, create a favourable environment for nematode growth and development within the insect host. Steinernematids occur in soil and epigeal habitats. The successful application and commercialisation of these nematodes as biological control agents has stimulated research to improve their efficacy against insect pests and to search for new and more virulent species/isolates (Gaugler and Kaya, 1990; Gaugler, 2002).

The most recent biogeographic account indicates that steinernematids have a worldwide distribution (Hominick, 2002). Of all described *Steinernema* species, approximately fifty percent have been isolated in Asia, mainly in China and Vietnam (see Hominick, 2002).

Identification of new species and isolates is an important pursuit as it supports investigations to understand biological and ecological characteristics of these nematodes (e.g. host range, host finding behaviour, temperature tolerance, etc.), which are important factors to consider when using EPN in biological control or Integrated Pest Management (IPM) programmes.

The nematode described herein was originally identified as *Steinernema carpocapsae* Weiser, 1955, a ubiquitous species with a broad geographic distribution (see Hominick, 2002). This species has been characterized as an 'ambush' forager that 'sits and waits' for a host (Ishibashi and Kondo, 1990; Lewis *et al.*, 1993; Campbell and Lewis, 2002). However, in laboratory assays conducted by Cutler and Webster (2003) the nematode exhibited a 'cruiser-type' foraging behavior. This observation prompted a re-evaluation of its taxonomic status forming the subject of this paper. Subsequent morphological, molecular and cross-breeding studies revealed this isolate to be an undescribed *Steinernema* species. It is herein described and illustrated.

MATERIALS AND METHODS

This nematode, originally named as 'BJ isolate', was collected in Beijing, China in the early 1990s by Dr. H. Yang (Chinese Academy of Agricultural Sciences, Beijing, China) but the precise isolation site is not known. This isolate has been maintained by *in vivo* culturing, using last instar larvae of *Galleria mellonella* L., at J. M. Webster's laboratory (Simon Fraser University, Burnaby, Canada).

For identification purposes, last instar *G. mellonella* larvae were exposed to ca. 50 third-stage infective juveniles (IJs) per larva on moistened filter paper in Petri dishes and incubated at 22 °C in the dark. First- and second-generation adults and third-stage infective juveniles were randomly collected from infected cadavers following procedures described by Kaya and Stock (1997).

Twenty randomly selected specimens of each nematode stage were examined either live or heat killed and relaxed in Ringer's solution (60 °C). Killed specimens were fixed in triethanolamine formalin (TAF) at 50-60 °C (Courtney *et al.*, 1965), slowly dehydrated and processed to anhydrous glycerin (Seinhorst, 1959). Nematodes were mounted on glass slides with glass fibre used as coverglass supports to avoid flattening of the specimens. Quantitative measurements of each specimen were made using an Olympus BX60 microscope equipped with differential interference contrast optics and Scion Image software (Frederick, Maryland, USA). Illustrations were prepared from digitized camera luci-

da images.

Morphological characters measured were based on recommendations of Hominick *et al.* (1997). The following abbreviations have been used in the text or tables: ABD = anal or cloacal body diameter, D% = EP/ES x 100, E% = EP/TL x 100, EP = distance from anterior end to excretory pore, GS = GuL/SpL, GuL = gubernaculum length, MBD = maximum body diameter, ML = mucro length, NR = distance from anterior end to nerve ring, ES = distance from anterior end to base of oesophagus, SpL = spicule length (measured along the curvature in a line along the centre of the spicule), StL = stoma length, StW = stoma width, SW = SpL/ABD, TBL = total body length, TL = tail length.

Nematodes were processed for electron microscopy observation following procedures described by Stock and Koppenhöfer (2003). A SES DS-130 scanning electron microscope equipped with a digital image camera and Imagecap™ software were used for this study. An accelerating voltage of 15 kV was used for all observations.

Reproductive compatibility of the new species was tested using the following *Steinernema* spp.: *S. carpocapsae* Weiser, 1955 (ALL strain), *S. riobrave* Cabanillas, Poinar *et* Raulston, 1994 (TX strain) and *S. abbasi* Elawad, Ahmad *et* Reid, 1997 (type strain). The modified hanging-blood assay described by Kaya and Stock (1997) was used for this study.

Molecular characterization of the new species was conducted by analyzing the large-subunit (28S) of ribosomal DNA (LSU rDNA) sequences. Total genomic DNA isolation, PCR amplification (reaction, cycling conditions and primers) and sequence analysis followed protocols described by Stock *et al.* (2001). LSU rDNA sequences and phylogenetic relationships with other *Steinernema* species were compared using an existing library of more that 20 *Steinernema* spp. (Stock *et al.*, 2001). Additionally, restriction fragment length polymorphism (RFLP) analysis was done according to procedures described by Reid *et al.* (1997).

Phylogenetic analyses (maximum parsimony analysis) of LSU sequence data were made using PAUP* v 4.0b (Swofford, 2001) following criteria described by Stock *et al.* (2001).

DESCRIPTION

STEINERNEMA WEBSTERI SP. N.
(Figs. 1-3, Table I)

Male: body slender, ventrally curved posteriorly, "J"-shaped when heat killed (Fig. 1A). First generation male larger (average 1712 µm) than second generation male (average 1122 µm). Cuticle smooth under light microscopy, but fine transverse striae are visible under SEM. Lateral field and phasmids inconspicuous. Head truncated to slightly round, continuous with the body. Six lips amalgamated but tips distinct, and with one labial papilla each. Four cephalic papillae. Amphids small, located posterior to lateral labial papillae. Stoma reduced (cheilo- gymno- and stegostom vestigial), short and wide, with inconspicuous sclerotised walls. Oesophagus muscular; procorpus cylindrical; metacorpus slightly swollen and non-valvated; indistinct isthmus followed by pyriform basal bulb containing reduced valve. Oesophagus set off from intestine. Nerve-ring usually surrounding isthmus or anterior part of basal bulb. Excretory pore opening circular, located above the nerve ring at anterior 1/3 of metacorpus (Figs. 1A; 2B). Single reflexed testis, consisting of germinal growth zone leading to seminal vesicle. Vas deferens with inconspicuous walls. Spicules paired, symmetrical, curved, with ochre-brown coloration (Figs. 1H, 2E, 2F). Manubrium continuous dorsally with lamina and rounded (Figs. 1H, 2E, 2F). Calomus (shaft) inconspicuous. Lamina with rostrum or retinaculum and 2 internal ribs. Velum present, extending to the terminus (Figs. 1H, 2E). Gubernaculum arcuate, about 2/3 length of spicules. First generation male with conoid and mucronated tail (Fig. 1G). Second generation male tail with or without mucro. There are 23 genital papillae (11 pairs and one single) arranged as follows: six precloacal subventral pairs, one single ventral precloacal papilla (located between precloacal pairs 5 and 6); one pair subventral adcloacal (or precloacal in some specimens); one pair subventral postcloacal, one pair subdorsal postcloacal, 2 pairs terminal postcloacal.

Female: cuticle, lip region, stoma and oesophageal region as in male. Body "C"-shaped when heat-killed (Fig. 1B). First generation females larger (average 5542 µm) than second generation females (average: 2223 µm). Excretory pore located about the middle level of procorpus (Figs. 1C, 2A). Ovaries opposed, reflexed in dorsal position; oviduct well developed; glandular spermatheca and uterus in ventral position. Vagina short, with muscular walls. Vulva located near middle of body with non protruding lips (Figs. 1E, 2C). Second generation female with vulval lips slightly protruding. First generation female tail blunt, conoid, with a mucro (Figs. 1F, 2D). Second generation female tail conoid. First generation female without postanal swelling. Second generation female with or without postanal swelling.

Third-stage infective juvenile: body of heat-relaxed specimens almost straight, slender, gradually tapering posteriorly. Cuticle with transverse striae. Lateral field distinct with six longitudinal ridges in mid-body region (Fig. 2G). Head region continuous with body, slightly truncated (Fig. 1J). Labial and cephalic papillae distinct. Amphids visible. Lip region smooth and continuous; stoma closed. Oesophagus long, narrow, with slightly expanded procorpus, narrower isthmus and pyriform basal bulb with valve. Cardia present. Nerve-ring located at level of isthmus. Excretory pore located about the middle of corpus (Fig. 1J). Anterior portion of intestine with dorsally displaced pouch containing

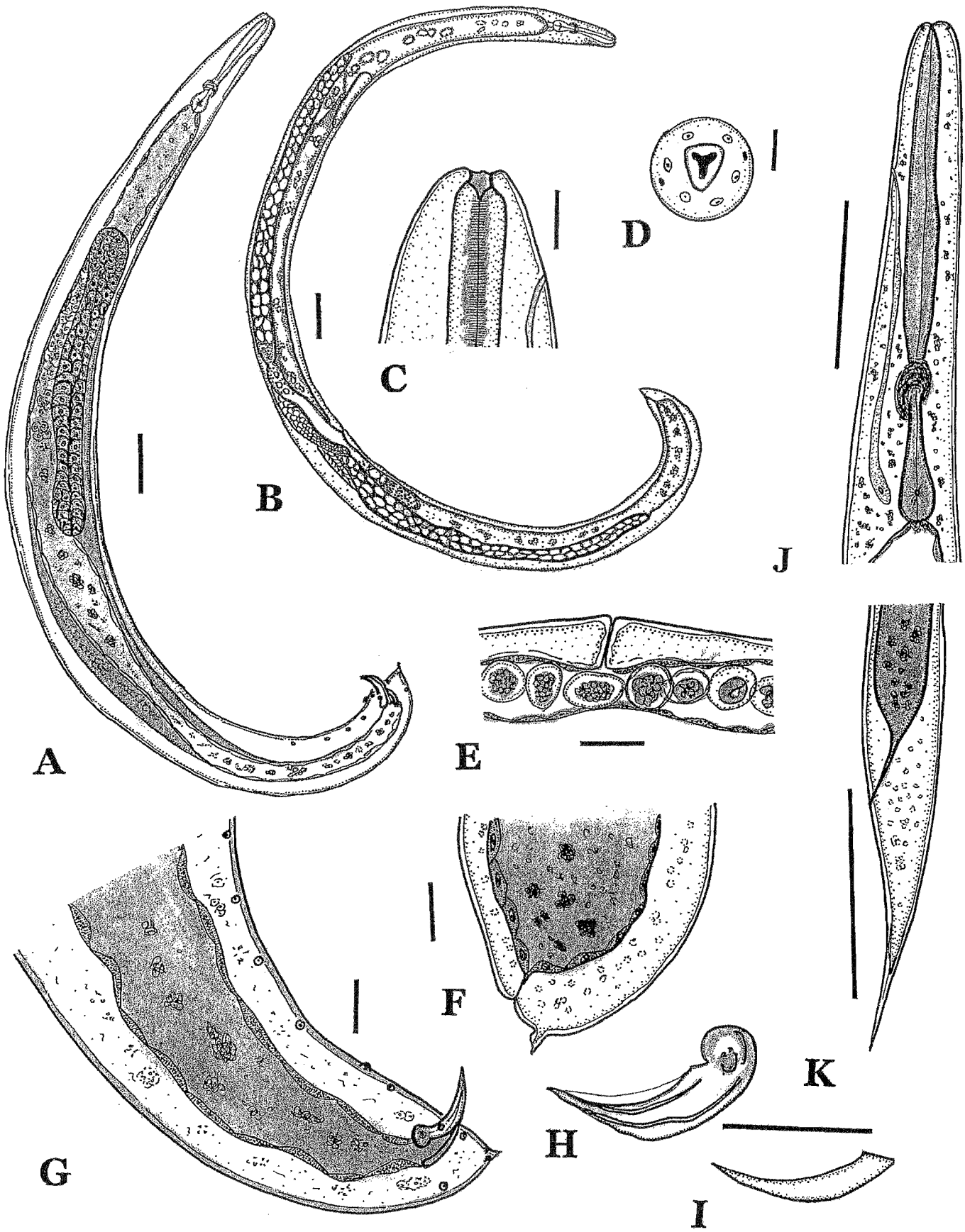

Fig. 1. *Steinernema websteri* sp. n. A, first generation male, entire body; B, first generation female, entire body; C-D, female, anterior end in lateral view (C) and en face view (D); E, vulva, first generation female in lateral view; F, tail, first generation female in lateral view; G, tail, first generation male in lateral view; H, spicule in lateral view; I, gubernaculum in lateral view; J-K, third-stage infective juvenile: J, anterior end in lateral view, K, tail in lateral view. Scale bars: A, B, J = 100 μm; C, E = 50 μm; D = 25 μm; F = 35 μm; G = 30 μm; H, I, K = 50 μm.

Table I. Morphometrics of *Steinernema websteri* sp. n. All measurements are in µm. Ranges, means and standard deviation (in parenthesis) are provided.

	Males			Females			Third-stage infective juveniles
	1st. Generation		2nd. Generation	1st. Generation		2nd. Generation	
	Holotype	Paratypes	Paratypes	Alloty	Paratypes	Paratypes	Paratypes
n		19	20	pe	19	20	20
TBL	1542	1523-1865 (1712 ± 92)	900-122 (1122 ± 109)	5187	3497-10450 (5542 ± 1559)	1895-3119 (2523 ± 116)	553-631 (584 ± 13)
MBD	145	119-175 (147 ± 17)	53-75 (91 ± 8.5)	177	116-223 (177 ± 28.4)	99-132 (115 ± 22)	17-25 (21 ± 1.7)
StL	5	3-8 (5.5 ± 1)	2-3 (2.5 ± 0.3)	9	7-41 (12.5 ± 10)	4-5 (4.5 ± 0.2)	-
StW	8	3-11 (7.5 ± 2)	6-9 (12 ± 0.5)	6	5.5-13.6 (8 ± 2.7)	10-12 (11 ± 0.5)	-
ES	166	135-180 (163 ± 12)	47-53 (49 ± 1.5)	174.5	19-237 (181 ± 58)	154-200 (178 ± 8)	107-122 (115 ± 4.4)
EP	55	54-73 (62 ± 6)	100-165 (131 ± 9.5)	59	57-6805 (739 ± 2075)	55-68 (60 ± 7)	29-40 (36 ± 3.1)
NR	125	100-139 (119 ± 10)	86-124 (118 ± 2.3)	120	119-174 (144 ± 14)	91-133 (118 ± 11)	83-95 (88 ± 3.6)
TL	25	25-33 (29 ± 2)	18-25 (22 ± 1.5)	34	3.6-45.5 (32 ± 11.7)	25-39 (33 ± 4)	37-56 (47 ± 4.5)
M	3	2.-5 (3.5 ± 0.5)	1.5-2.5 (2 ± 0.5)	10	8.5-13 (10 ± 1)	-	-
ABD	36	34-41 (37.5 ± 2)	25-35 (28 ± 2.4)	51	51-87 (69 ± 13)	31-42 (38 ± 3.5)	10-14 (12 ± 1.2)
SpL	67	64-72 (68 ± 2)	54-64 (58 ± 4.2)	50.5	-	-	-
GuL	56	42-56 (49 ± 3.2)	37-42 (40 ± 3.1)	-	-	-	-
SW	1.9	1.6-2.1 (1.8 ± 0.1)	1.5-1.7 (1.6 ± 0.1)	-	-	-	-
GS	0.8	0.6-0.8 (0.7 ± 0.1)	0.6-0.8 (0.7 ± 0.1)	-	-	-	-
V	-	-	-	-	49-59 (53 ± 2.8)	51-54 (52 ± 1.4)	-
a	-	-	-	-	-	-	24-35 (28 ± 2.7)
b	-	-	-	-	-	-	4.8-5.6 (5.1 ± 0.2)
c	-	-	-	-	-	-	11-15.5 (12.6 ± 1.3)
D%	33	30-50 (40 ± 10)	47-53 (49 ± 3.5)	-	-	-	24-34 (31 ± 2.7)
E%	217	180-250 (210 ± 20)		-	-	-	62-102 (77 ± 11)
H	-	-	-	-	-	-	10-14 (11 ± 0.5)
H%	-	-	-	-	-	-	30-34 (33 ± 0.6)

symbiotic bacterium. Intestine filled with numerous fat globules, lumen of intestine narrow. Rectum long and straight; anus distinct. Genital primordium evident. Tail conoid with pointed terminus. Hyaline portion occupying about 1/3 of the tail length (Fig. 1K).

Type-host: no type-host known in nature. This isolate was recovered by baiting soil with *G. mellonella* larvae.

Type-locality: precise location unknown, Beijing, China.

Type-specimens: holotype male first generation, allotype female first generation, five paratype males first generation, five paratype females first generation, five paratype third stage infective juveniles deposited in the University of California Nematode Collection, Davis, California, USA

Etymology: this species is dedicated to John M. Webster (Simon Fraser University, Burnaby, BC, Canada), a leading scientist in nematology.

Attempts to cross-hybridize *S. websteri* sp. n. with *S. riobrave* and *S. abbasi* yielded no progeny. Control crosses using individuals of the same species produced viable offspring. When crossing the new species with *S. carpocapsae*, progeny production was observed in about 30% of the replicates. However, when pre-adults of the

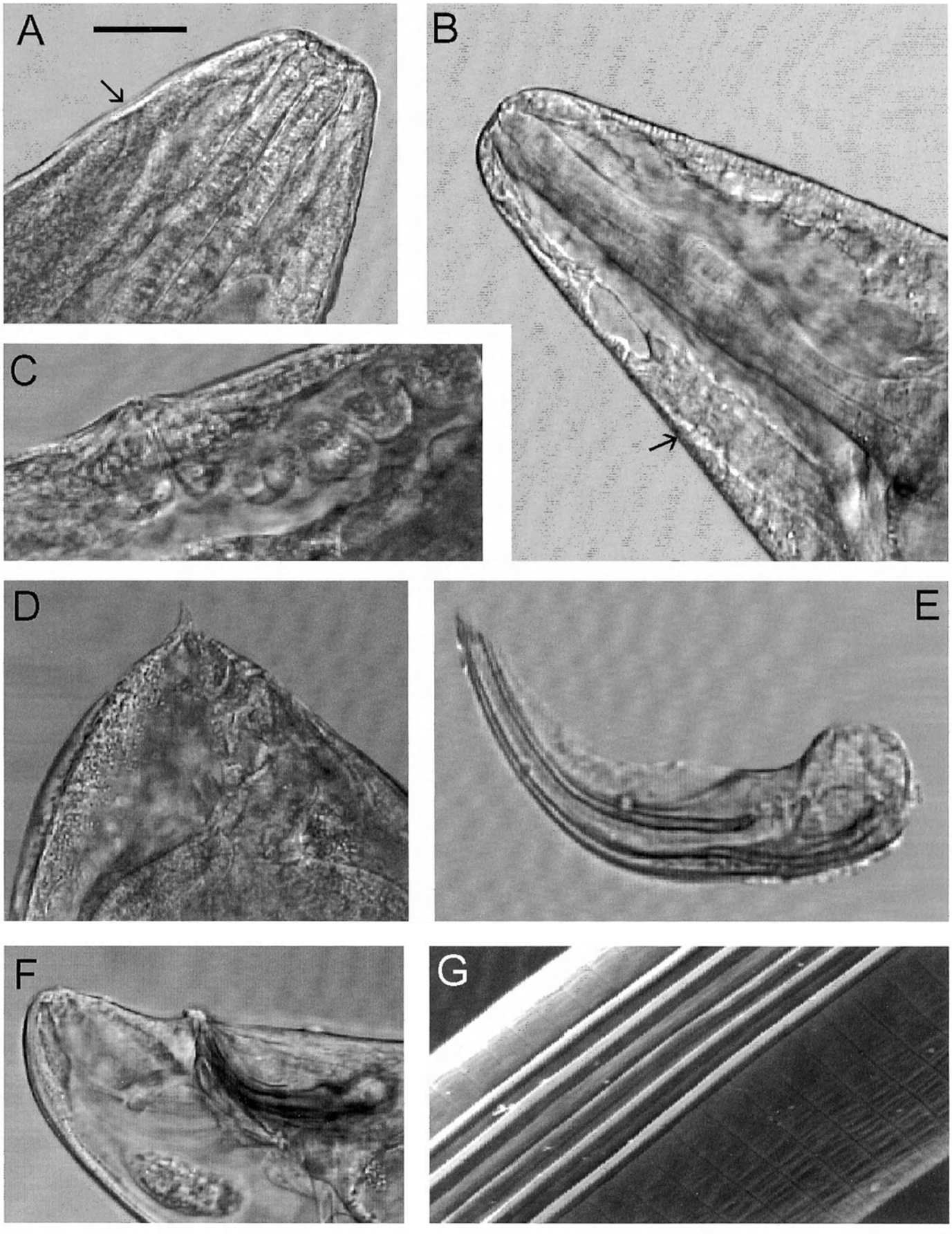

Fig. 2. Photomicrographs of *Steinernema websteri* sp. n. (lateral views): A, first generation female, anterior end showing excretory pore position (arrow); B, first generation male, anterior end showing excretory pore position (arrow); C, first generation female, vulva; D, first generation female tail; E, spicule; F, first generation male tail ; G, third-stage infective juvenile lateral field pattern. All scale bars are based on the scale bar in A. A-C, E = 45 µm; D = 55 µm; F = 30 µm; G = 10 µm.

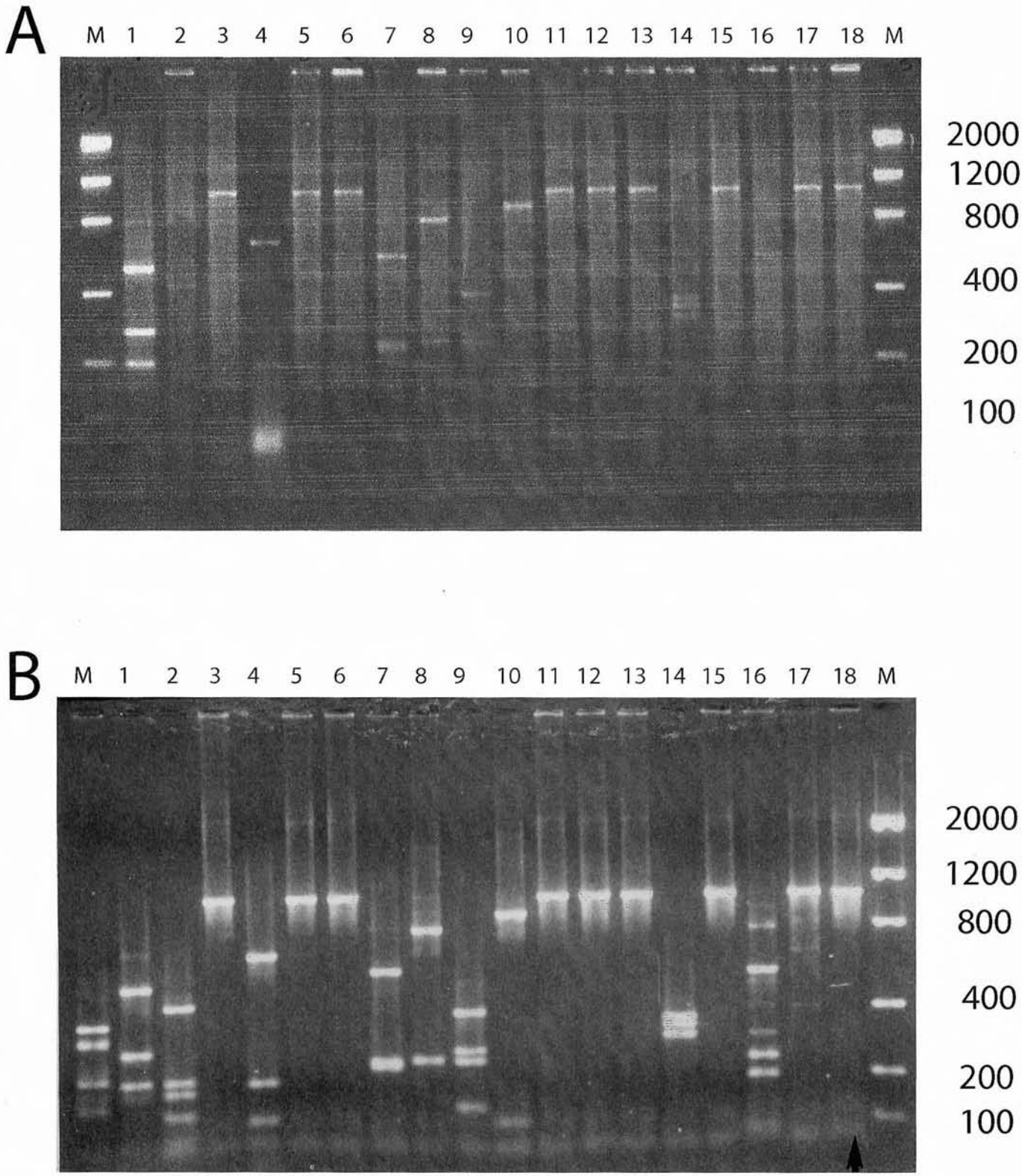

Fig. 3. PCR amplified products from the internal transcribed spacer (ITS) digested with 17 restriction enzymes. A, *Steinernema websteri* sp. n.; B, *S. carpocapsae*. Lane 1 is the digest of *S. feltiae* (UK, site 76) with *Alu* I. Lanes 2-18 are individual digests of the respective species for that gel with the following restriction enzymes: 2. *Alu I*; 3. *BstO* I; 4. *Dde* I; 5. *EcoR* I; 6. *Hae* III; 7. *Hha* I; 8. *Hind* III; 9. *Hinf* I; 10. *Hpa* II; 11. *Kpn* I; 12. *Pst* I; 13. *Pvu* II; 14. *Rsa* I; 15. *Sal* I; 16. *Sau* 3 A I; 17. *Sau* 96; 18. *Xba* I; Lane M is the molecular weight marker. Band sizes are shown in base pairs.

Table II. Comparison of morphometrics (range and mean) of infective juveniles of *Steinernema websteri* sp. n. and other morphologically similar *Steinernema* spp. All measurements in µm.

Species	TBL	MBD	EP	TL	D%	E%
S. websteri sp. n.	553-631	17-25	29-40	37-56	24-34	62-102
	(584)	(21)	(36)	(47)	(31)	(77)
S. carpocapsae[1]	438-650	20-30	29-38	46-61	23-28	54-66
	(558)	(25)	(35)	(53)	(26)	(60)
S. abbasi[2]	496-579	27-30	46-51	52-61	51-58	79-94
	(541)	(29)	(48)	(56)	(53)	(86)
S. riobrave[3]	561-701	26-30	51-64	46-59	45-55	93-111
	(622)	(28)	(56)	(54)	(49)	(105)

[1]After Poinar, 1990; [2]after Elawad *et al.*, 1997; [3]after Cabanillas *et al.*, 1994.

Fig. 4. Evidence of large subunit (LSU) ribosomal DNA lineage independence for *S. websteri* sp. n. based on maximum parsimony analysis. Numbers in bold refer to bootstrap values.

resulting hybrids were crossed with each other no offspring was produced.

Figure 3 shows the RFLP pattern yielded for S. *websteri.* sp. n. Comparison of RFLP profiles of the new species with those for S. *carpocapsae* shows these two species have very similar restriction digest profiles. Differences are mainly observed in the digest pattern of *Alu* I and *Hinf* I and *Sau3* AI.

Maximum parsimony (MP) analysis of the molecular data set yielded 312 parsimony informative characters and produced eight equally parsimonious trees with a tree length of 771steps (CI = 0.61) (Fig. 4). *Steinernema websteri* sp. n. was found to be most closely related to a clade that comprises four *Steinernema* species which are characterized by the presence of horn-like cephalic papillae: S. *abbasi* Elawad, Ahmad *et* Reid, 1997., *S. riobrave* Cabanillas, Poinar *et* Raulston, 1994, *S. ceratophorum* Jian, Reid *et* Hunt, 1997 and *S. bicornutum* Tallosi, Peters *et* Ehlers, 1995 (Fig. 4). Within this clade, *S. bicornutum* and *S. ceratophorum* have a high bootstrap support (99%), but the position of *S. abbasi* and *S. riobrave* in this clade could not be resolved. Bootstrap support for the association of *S. websteri* n. sp to this clade is considered low (<55%). Adjusted distance matrix showed that the BJ isolate and *S. carpocapsae* differ in 156 characters (base pairs) (Table III).

Diagnosis and relationships

Steinernema websteri sp. n. is characterized by the infective juvenile maximum body width of 21 (17-25) µm, excretory pore position 36 (29-40 µm), D% value 31 (24-34), E% value 77 (62-102), and the presence of six longitudinal ridges in the IJ lateral field. First genera-

Table III. Adjusted character distance matrix based on 28S rDNA sequence comparison between *Steinernema* spp.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1. *S. abbasi*	-																						
2. *S. affine*	83	-																					
3. *S. arenarium*	74	83	-																				
4. *S. bicornutum*	62	95	60	-																			
5. *S. carpocapsae*	160	139	138	158	-																		
6. *S. ceratophorum*	63	100	65	25	157	-																	
7. *S. cubanum*	82	93	26	72	152	73	-																
8. *S. feltiae*	64	83	32	52	142	59	42	-															
9. *S. glaseri*	84	95	22	72	148	75	6	40	-														
10. *S. intermedium*	83	10	85	97	137	102	93	85	97	-													
11. *S. karii*	74	91	28	66	144	71	42	40	40	93	-												
12. *S. kraussei*	62	83	34	54	142	61	44	6	44	85	42	-											
13. *S. kushidai*	61	80	33	53	143	56	43	15	43	84	43	15	-										
14. *S. longicaudum*	82	93	26	72	152	73	3	42	6	93	42	44	43	-									
15. *Steinernema*	69	86	21	63	151	66	39	35	37	92	31	39	40	39	-								
16. *S. onticolum*	160	139	138	158	2	157	152	142	148	137	144	142	143	152	151	-							
17. *S. oregonense*	60	83	32	52	144	59	42	4	42	85	40	4	15	42	35	144	-						
18. *S. puertoricense*	69	70	15	57	137	62	29	27	29	74	31	29	28	29	24	137	29	-					
19. *S. rarum*	75	80	55	69	149	78	63	49	63	84	65	47	44	63	56	149	45	46	-				
20. *S. riobrave*	61	92	59	53	161	58	67	57	67	96	65	57	50	67	60	161	55	58	78	-			
21. *S. scapterisci*	159	138	137	157	1	156	151	141	147	136	143	141	142	151	150	2	143	136	148	160	-		
22. *S. siamkayai*	163	142	141	161	5	160	155	145	151	140	147	145	146	155	154	5	147	140	152	162	4	-	
23. *S. websteri sp. n.*	70	85	60	60	156	59	68	60	70	89	66	58	53	68	59	156	56	55	69	65	155	159	-

tion males are distinguished by the position of the excretory pore (average: 62 µm), cloacal body width (average: 37.5 µm), tail length (average: 29 µm), D% (average: 40) and morphology of spicules and gubernaculum as well as the number and arrangement of genital papillae. First generation females of the new species are characterized by having a mucronate tail and non-protruding to slightly protruding vulval lips.

Phylogenetic analysis of LSU sequence data placed *S. websteri* sp. n. close to a clade that includes four species, *S. abbasi*, *S. riobrave*, *S. ceratophorum* and *S. bicornutum*, characterized by the presence of horn-like cephalic papillae. However, the new species can be differentiated from these taxa by the absence of such features and by several morphometric differences of the IJs (i.e. MBW, EP, TL) and first generation males (i.e. EP, ABD, D%, E%).

Steinernema websteri sp. n. is most similar to *S. carpocapsae, S. abbasi* and *S. riobrave* in the general morphology of the infective juveniles and 1st generation males, but can be separated from these species using a combination of morphological and molecular traits.

IJs and first generation males of *S. websteri* sp. n. most resemble *S. carpocapsae* in many morphological/morphometric traits. However, these two species can be differentiated by the tail length of the IJ, which is shorter in the new species (average: 47 *vs* 53 µm), and the E% value (average 77 *vs* 60). Males of the new species can be separated from *S. carpocapsae* by the morphology of the spicules, which have a more rounded manubrium and an inconspicuous calomus in the former species. The arrangement of the genital papillae in these two species is slightly different. *Steinernema websteri* sp. n. has one subdorsal pair in a postcloacal position, whereas *S. carpocapsae* has two postcloacal pairs located subdorsally.

Steinernema websteri sp. n. also resembles *S. abbasi* in the total body length of the IJs. However, IJs of the new species can be distinguished by having a narrower body width (average: 21 *vs* 29 µm), and a more anteriorly located excretory pore (average: 36 *vs* 48 µm). These morphometric differences are also reflected in the values of D% and E% (Table II). Males of the new species can be separated from those of *S. abbasi* by the absence of a tail mucro, the overall morphology of the spicules, the morphology and size of the gubernaculum (average: 49 *vs* 45 µm), and the arrangement of the genital papillae. Females of the new species can be distinguished from *S. abbasi* by the morphology of the vulval lips (non – to slightly protruding *vs* protruding).

IJs of the new species can be separated from *S. riobrave* by their body length and width, which are shorter and narrower, respectively, in the new species (Table II). The excretory pore of *S. websteri* sp. n. is more anteriorly located (Table II). First generation males of *S. websteri* sp. n. can be separated from those of *S. riobrave* by having a narrower cloacal body diameter (average: 37.5 µm *vs* 50 µm) and by the position of the excretory pore, which is more anteriorly located in the new species (average: 62 µm *vs* 103 µm). The morphology and size of the spicules and gubernaculum, and the arrangement of the genital papillae, also separate these two species.

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LITERATURE CITED

Cabanillas H.E., Poinar G.O. and Raulston J.R., 1994. *Steinernema riobravis* n. sp. (Rhabditida: Steinernematidae) from Texas. *Fundamental and Applied Nematology, 17*: 123-131

Campbell J.F. and Lewis E.E., 2002. Entomopathogenic nematode host-search strategies. Pp. 13-38. *In*: The behavioural ecology of parasites. Lewis E.E., Campbell J.F. and Sukhdeao M.V.K. (eds). CABI Publishing, Wallingford, UK.

Courtney W.D., Polley D. and Miller V.L., 1965. TAF, an improved fixative in nematode techniques. *Plant Disease Reporter, 39*: 570-571.

Cutler G.C. and Webster J.M., 2003. Host-finding ability of three entomopathogenic nematode isolates in the presence of plant roots. *Nematology* (in press).

Elawad S., Ahmad W. and Reid A.P.,1997. *Steinernema abbasi* sp. n. (Rhabditida: Steinernematidae) from the Sultanate of Oman. *Fundamental and applied Nematology, 20*: 433-442.

Gaugler R., 2002. *Entomopathogenic nematology*. CAB International, New York, New York, U.S.A. 400 pp.

Gaugler R. and Kaya H.K., 1990. *Entomopathogenic nematodes in biological control.* CRC Press, Boca Raton, Florida, U.S.A., 365 pp.

Hominick W.M., 2002. Biogeography. Pp.115-143. *In*: Entomopathogenic nematology. R. Gaugler (ed.). CABI, Wallingford, UK.

Hominick W.M., Briscoe B.R., del Pino F.G., Heng J., Hunt D.J., Kozodoy E., Mracek Z., Nguyen K.B., Reid A.P., Spiridonov S., Stock S.P., Sturhan D., Waturu C. and Yoshida M., 1997. Biosystematics of entomopathogenic nematodes: current status, protocols and definitions. *Journal of Helminthology, 71*: 271-298.

Ishibashi N. and Kondo E., 1990. Behavior of infective juveniles. Pp. 139-152. *In*: Entomopathogenic nematodes in biological control. Gaugler R. and Kaya H. K. (eds). CRC Press, Boca Raton, Florida, U.S.A.

Kaya H.K. and Stock S.P., 1997. Techniques in insect nematology. Pp. 281-324. *In*: Manual of techniques in insect pathology. Lacey L. (ed.). Academic Press Ltd., San Diego, California, U.S.A.

Lewis E., Gaugler R. and Harrison R., 1993. Response of cruiser and ambusher entomopathogenic nematodes to host volatile cues. *Canadian Journal of Zoology, 71*: 765-769.

Poinar G.O. Jr., 1990. Taxonomy and biology of Steinernematidae and Heterorhabditidae. Pp: 23-60. *In*: Entomopathogenic nematodes in biological control. Gaugler R. and Kaya H.K. (eds). CRC Press, Boca Raton, Florida, U.S.A..

Reid A.P., Hominick W.M. and Briscoe B.R., 1997. Molecular taxonomy and phylogeny of entomopathogenic nematode species (Rhabditida: Steinernematidae) by RFLP analysis of the ITS region of the ribosomal DNA repeat unit. *Systematic Parasitology, 37*: 187-193.

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

Stock S.P., Campbell J.F. and 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. and Koppenhöfer A.M., 2003. *Steinernema scarabaei* sp. n. (Rhabditida: Steinernematidae), a natural pathogen of scarab beetle larvae (Coleoptera: Scarabaeidae) from New Jersey, USA. *Nematology, 5*: 191-204.

Swofford D.L., 2001 - *PAUP*. Phylogenetic analysis using parsimony (and other methods), version 4.* Sinauer Associates, Sunderland, Massachusetts, U.S.A., 257 pp.

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