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Steinernema guangdongense sp. n. (Nematoda: Steinernematidae), a new entomopathogenic nematode from southern China with a note on *S. serratum* (*nomen nudum*)

LIHONG QIU[1], YUANYUAN FANG[1], YONG ZHOU[1], YI PANG[1] AND KHUONG B. NGUYEN[2]

[1] *The State Key Lab for Biocontrol, Zhongshan University, Guangzhou 510275, Peoples Republic of China*

[2] *Entomology and Nematology Department, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL 32611-0620; kbn@ufl.edu*

Abstract

A new species of entomopathogenic nematode, *Steinernema guangdongense* sp. n. was recovered from a soil sample collected from Jijia town in the western part of Guangdong province, the Peoples Republic of China during a survey for entomopathogenic nematodes in 2001. The nematode can be separated from other described species of *Steinernema*, by morphological, morphometrical characteristics of different stages of the nematode, by crossbreeding tests and by characterizations and phylogeny of DNA sequences of either a partial 28S or the internal transcribed spacer regions of rDNA. This nematode is closest to *S. longicaudum*. It can be distinguished from that nematode by characteristics of different stages. For infective juveniles, although the body length is almost similar (1055 μm compared to 1063 μm), body diameter of the new species is larger; values of EP (length from anterior end to excretory pore), NR (length from anterior end to nerve ring) and a body length/body width ratio are smaller, and tail with dorsal constriction. For male, the new species has longer spicule, not well curved, spicule head shorter, shaft not prominent or absent and spicule tip not suddenly tapered as shown in *S. longicaudum*. Also, the ratios SW (spicule length/anal body width) and GS (gubernaculums/spicule) are smaller. For female, the presence of a small double flapped epiptygma, a small projection on dorsal side of the tail tips and prominent post-anal swelling is typical for the new species.

Key words - 28S rDNA sequence; entomopathogenic nematode; identification; rDNA ITS sequence; *Steinernema guangdongense*; taxonomy

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Introduction

Entomopathogenic nematodes (EPN) are parasites of insects. This group of nematodes is characterized by carrying specific symbiotic bacteria of the genus *Xenorhabdus* or *Photorhabdus* in their intestine. Symbiotic bacteria play an important role in the pathogenicity of the nematode-bacteria complex to insect hosts and the subsequent reproduction of the nematode in the hosts. EPN are currently used as biopesticides for controlling several important insect pests worldwide (Shapiro-Ilan et al., 2002). Currently, there are about 44 valid species of EPN in the families of Steinernematidae and Heterorhabditidae (Nguyen, 2004). Different species or strains of EPN may possess different biological, ecological, or physiological characteristics that affect the field efficacy of the EPN-based biopesticides, such as susceptible host range, behavior, and tolerances to high or low temperature. Therefore, accumulation and correct identification of EPN species or strains are critical for the success in using them as biopesticides for controlling insect pests.

A survey of entomopathogenic nematodes has been carried out recently in Guangdong province, Peoples Republic of China, which has resulted in the recovery of more than 20 isolates of insect-parasitic nematodes. Morphological, molecular and cross-breeding studies show that an isolate of these nematodes is a new species belonging to the genus *Steinernema*. Herein, we describe the nematode as *Steinernema guangdongense* n. sp. named after the place where it was collected.

Material and Methods

NEMATODE SOURCE

The *Steinernema guangdongense* sp. n. (isolate GDc339) was isolated from a soil sample (Mracek, 1980) collected from an artificial eucalypt forest at Jijia town (latitude N20.49, longitude E109.58, precipitation = 2000 mm/year), Leizhou district in the western part of Guangdong province in November 2001 using *Galleria mellonella* (L) larvae as bait. The soil type was light yellow sandy loam. *Steinernema longicaudum* Shen & Wang, 1992 , CWL05 and CB2B strains were kindly provided by Dr. Robin Bedding of CSIRO Entomology, Canberra, Australia. CWL05 is the topotype strain of *S. longicaudum* originated from Laiyang (latitude N37.30, longitude E121.01, precipitation = 700 mm/year), Shandong province, while CB2B was isolated from Beijing (latitude N39.50, longitude E116.25, precipitation = 600 mm/year), China. Both of them were sent to CSIRO soon after they were collected and had been preserved in liquid nitrogen in the CSIRO EPN collection.

MORPHOLOGICAL CHARACTERIZATION

Light microscopy

All nematodes used in this study were produced in *Galleria mellonella* larvae. Fifteen

G. mellonella larvae were exposed to about 2000 infective juveniles (IJ) in a Petri dish (60 x15 mm) lined with two moistened filter papers at 23°C. After they died, the insect cadavers were transferred to a white trap (White, 1927) and incubated at 23°C until IJ emerged. 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. The infective juveniles used for measurements were collected 3 days after the first emergence of IJs.

All nematode samples, including IJs, the first and second generation males and females, were killed by gentle heat and then fixed in TAF (Courtney et al., 1955) and processed to anhydrous glycerol using the method describe by Seinhorst (1959). Permanent slides were made using glass slide; coverglass supports were used in all cases to avoid flattening of specimens. At least 20 each of female, male and infective juvenile were observed and measured. Measurements were conducted using a Nikon reverse microscope with 10x, 20x or 40x differential interference contrast lens.

SCANNING ELECTRON MICROSCOPY

For scanning electron microscopic (SEM) examination, adults and IJ were fixed in 3% glutaraldehyde buffered with 0.1 M sodium cacodylate at pH 7.2 for 24 hours at 8°C (Nguyen and Smart, 1995). They were post-fixed with 2% osmium tetroxide solution for 12 hours at 25°C, dehydrated in a graded ethanol series, critical point dried with liquid CO_2, mounted on SEM stubs, and coated with gold. Spicules and gubernacula were prepared as suggested by Nguyen and Smart (1997).

MOLECULAR CHARACTERIZATION

The sequences of the following two fragments of nucleic DNA were used as molecular markers to differentiate *S. guangdongense* n. sp. from other described *Steinernema* species: a fragment of 28S rDNA (460pb excluding primer sites), corresponding to nucleotide position of 4063 ~ 4557 in *Caenorhabditis elegans* (GenBank accession number X03680); and the internal transcribed spacer (ITS) regions of rDNA, including complete ITS1, ITS2 and 5.8S rDNA subunit and partial 18S and 28S rDNA subunit. The template DNA was extracted from a single first generation female using the method described previously (Joyce et al., 1994). The ITS regions of rDNA was amplified and sequenced using the method described in details by Nguyen et al. (2001). The primers for the amplification of a partial 28S rDNA (Forward: 5 -CGATAGCGAACAAGTACCGAGAG- 3; Reverse: 5 -CCTGCTCAGGCATAGTTCACCATC- 3) were selected and designed from previously published sequences (GenBank accession no. AF331888 ~ AF331909, Stock et al., 2001) using the Software Primer Select (DNAstar Inc.). The PCR products were sequenced using the same protocol as that used for sequencing rDNA ITS. The 28S and ITS rDNA sequences of CB2B, CWL05 and GDc339 obtained from this study are deposited in GenBank under accession numbers AY169553, AY170337, AY169554, AY170338, and AY169558, AY170341, respectively. Previously published 28S rDNA sequences (acces-

sion no. AF331888 ~ AF331909) and ITS region sequences of the described *Steinernema* species (Nguyen et al., 2001, Nguyen & Duncan, 2002, Nguyen & Adams, 2003) were used as reference sequences for comparison.

Multiple alignment: Sequences of studied species were aligned using the default parameters of Clustal X (Thompson et al. 1997), then optimized manually in MacClade 4.05 (Maddison & Maddison, 2002).

Phylogenetic relationships: Phylogenetic trees were obtained by maximum parsimony using PAUP, 4.0b8 (Swofford, 2002). All data were assumed to be unordered, all characters have equal weight, gaps are treated as missing data and tree evaluation was made using a heuristic search (simple stepwise addition, TBR branch swapping). The species *S. intermedium* (for ITS regions) and *Panagrellus redivivus* (for D2/D3 regions) were treated as the out group taxa (Nguyen et al., 2001, Stock et al., 2001) to resolve the relationships among other species. Branch support was estimated by bootstrap analysis (100 replicates) using the same parameters as the original search.

CROSS-BREEDING

Cross-breeding tests were performed between *S. guangdongense* n. sp. GDc339 and two strains of *S. longicaudum*, CWL05 and CB2B, using the haemolymph hanging drop technique (Poinar, 1967). For the treatments of GDc339 x CWL05 and GDc339 x CB2B, one IJ of each nematode population was transferred into a drop of heamolymph of *G. mellonella* larvae on a glass slide using a hair probe under a dissecting microscope. The control treatments of GDc339, CB2B and CWL05 were also conducted in the same way except the two IJ were from the same nematode population. Fifteen replicates were made for each treatment. Slides were then incubated in Petri dishes lined with moist filter paper at 23^{o}C for 3 weeks. The development and reproduction of the nematodes was observed every day. The experiment was repeated one time. In the repeated experiment, when nematode juveniles appeared, a drop of *Galleria* haemolymph was added to the tested slide for further development of bacteria to provide more food for the nematodes.

***Steinernema guangdongense* n. sp.**

(Figs 1–8)

Description

First-generation male: Measurements are in Table 1. Body curved ventrally posteriorly, C-shaped when heat-killed. Head rounded, usually slightly swollen. Anterior end with six labial papillae, two amphids and four prominent cephalic papillae (Fig. 2D). Stoma shallow, cheilorhabdions as small and sclerotized structures at anterior end, sometimes indistinct. Excretory pore located mostly anterior to basal bulb. Esophagus with cylindrical procorpus, metacorpus slightly swollen, isthmus present, nerve ring around

isthmus, basal bulb distinct. Esophago-intestinal valve present. Gonad monorchic, reflexed. Distance from base of esophagus to anterior end of testis variable. Spicules paired, brown in color. Head (manubrium) of spicules with rounded anterior end, almost continuous with shaft (Fig. 2E). Shaft (calomus) very short or absent, blade (lamina) thick anteriorly, tapering gradually posteriorly, blade terminus blunt, velum present, sometimes very thin (Fig. 1B,C). Each spicule with two internal sclerotized ribs. Gubernaculum boat-shaped in lateral view, tapering gradually anteriorly. There are eleven pairs and one single precloacal genital papillae. Tail conoid, tail terminus rounded without a mucron.

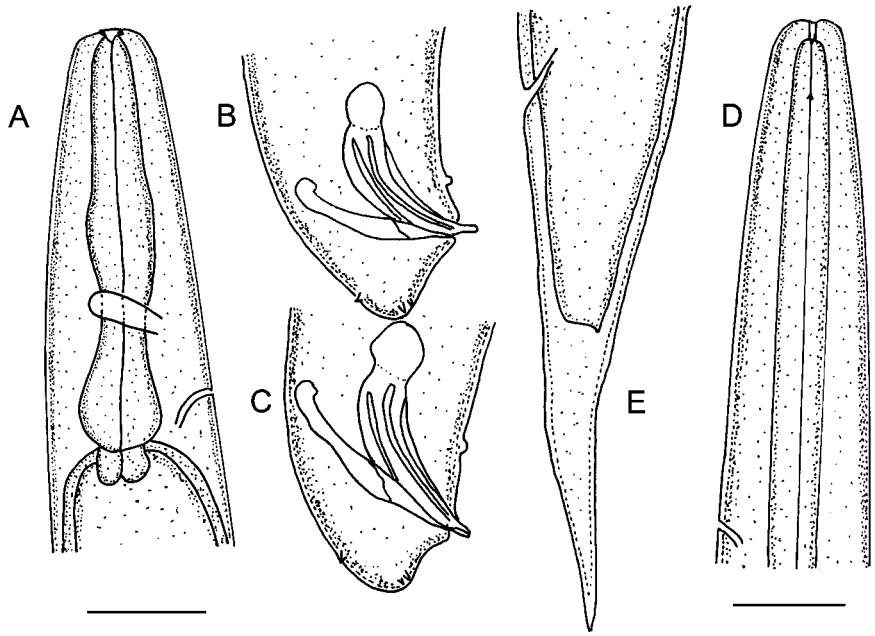

FIGURE 1. *Steinernema guangdongense* n. sp, first generation male and infective juvenile. Scale bars: A, B,C = 50 μm, D, E. = 20 μm.

Second-generation male similar to that of the first generation except body, spicule and gubernaculum shorter and thinner.

First-generation female: Measurements are in Table 1. Body cuticle smooth or with faint annules. Lateral fields and phasmids not observed. Head rounded, continuous with body; six labial and four cephalic papillae (Fig. 2A). Lips indistinct. Amphids usually inconspicuous even under SEM. Stoma shallow, subtriangular anteriorly; triradiate internally. Cheilorhabdions, well sclerotized but small (Fig. 4A). A smaller sclerotized structure posterior to cheilorhabdions (presumably the prorhabdions), observed in other species, indistinct in this species. Esophagus with procorpus cylindrical, muscular; metacorpus swollen; isthmus distinct; basal bulb valvate as in other steinernematids. Nerve ring surrounding isthmus, just anterior to basal bulb. Esophago-intestinal valve present.

Excretory pore located near mid-esophagus, anterior to nerve ring. Gonads amphidelphic, reflexed, often containing many eggs. Vulva a transverse slit situated on a protruding area, small double-flapped epiptygma present (Fig. 4D). Anterior lip larger than posterior one. Body diameter right anterior to vulva larger than that posterior to vulva. Vagina sclerotized, short. Tail shape variable, with blunt terminus, occationally, a mucron-like structure present on dorsal side (Fig. 4B,C). Ventral postanal swelling present, tail shorter than anal body width.

Second-generation female: Similar to first generation female but smaller. Vulva less protruding, epiptygma presesent but less prominent (Fig. 4E). Tail, tapering to a pointed end, longer than anal body width; ventral postanal swelling present.

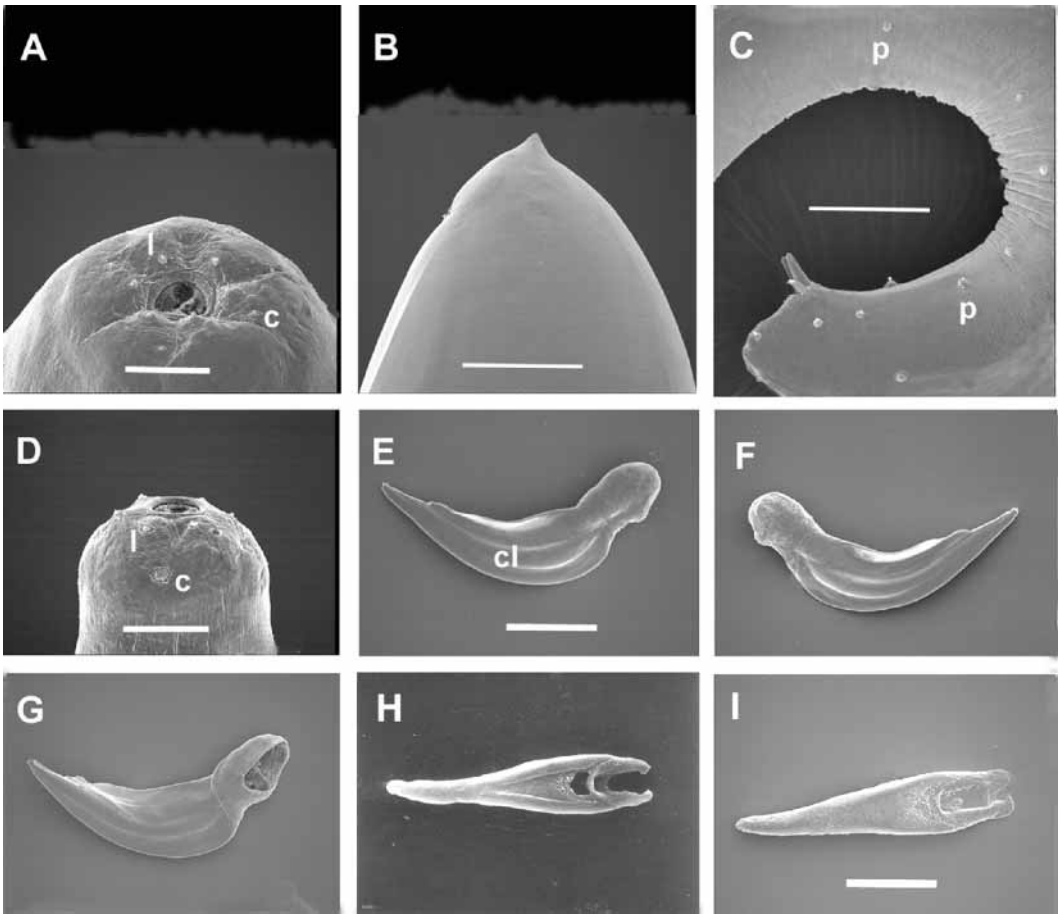

FIGURE 2. SEM photographs of the first-generation *Steinernema guangdongense* n. sp. A, head of a young female showing labial (l) and cephalic (c) papillae. B, tail of a young female. C, posterior end of a male showing genital papillae (p). E, F, spicule and gubernaculum. Scale bars: A, D = 10 μm, B = 42.9 μm, C = 42.5 μm. E, F = 20 μm.

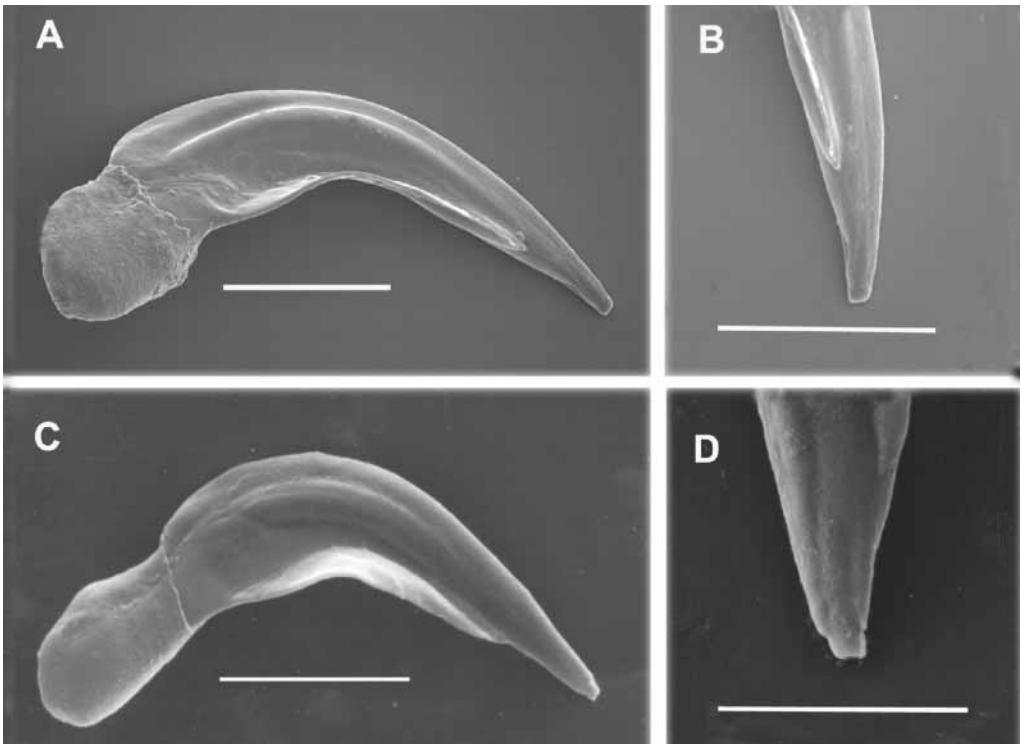

FIGURE 3. Comparative photographs of spicule shape and spicule tips of the first-generation male of *Steinernema guangdongense* n. sp. (A, B) and *S. longicaudum* (C, D). Note: Spicule tip of *S. longicaudum* suddenly narrow to form the tip but not for *S. guangdongense* n. sp. Scale bars: A, B = 20 μm, C, D = 25 μm.

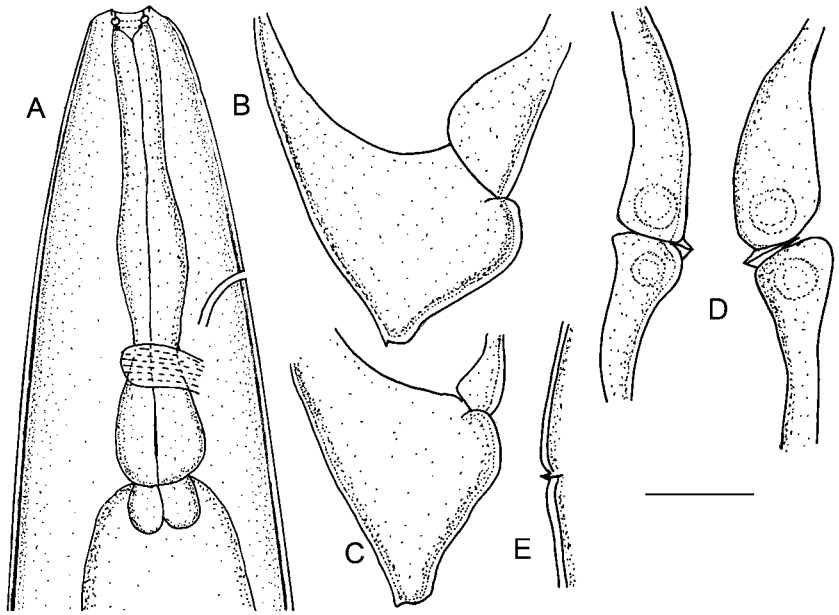

FIGURE 4. *Steinernema guangdongense* n. sp. first and second generations females. A, anterior region. B, C, variation in tail shape and mucron-like structure. D, vulva and double flapped epiptygma. E, vulva and epiptygma of the second generation female. Scale bar: A-E = 50 μm.

TABLE 1. Morphometrics (in µm) of different stages of *Steinernema guangdongense* n. sp.

Character	First generation				Second generation		Infective
	Holotype	Male	Female	Allotype	Male	Female	Juvenile
n		20	20		20	20	20
Body length	2000	1809 ± 415	5050 ± 913	6250	1441 ± 43.7	276 ± 201	1055 ± 49
		(1627–2126)	(3800–6800)		(1350–1500)	(3130–2500)	(987–1145)
Greatest body width	135	112 ± 14	218 ± 29	265	80 ± 3.5	143 ± 8	42 ± 4
		(90–135)	(175–275)		(75–88)	(130–155)	(30–48)
EP	123	120 ± 8	158 ± 7	163	119 ± 2.8	119 ± 5.6	80 ± 3.6
		(103–132)	(150–175)		(113–123)	(113–130)	(71- 85)
NR	130	127 ± 8	163 ± 15	162	136 ± 4.9	165 ± 2.5	102 ± 6
		(109–139)	(143–200)		(125–143)	(?–168)	(88–111)
ES	165	162 ± 7	199 ± 16	230	166 ± 5.9	204 ± 4.9	134 ± 5
		(150–176)	(183-238)		(160–175)	(195–215)	(123–144)
Tail length (T)	35	31 ± 3	55 ± 8	40	30 ± 1.8	80 ± 3	91 ± 8
		(24–38)	(43–65)		(28–33)	(75–85)	(82–103)
Anal body diam (ABW)	55	50 ± 5	77 ± 15	75	42 ± 3.2	54 ± 2.4	27 ± 2
		(42–58)	(63-118)		(38–48)	(50–58)	(24–32)
Spicule length (SP)	85	86 ± 3			71±3.9		
		(80–94)			(65–80)		
Gubernaculum length (GU)	70	64 ± 6			46 ± 2.9		
		(47–73)			(25-42)		
a							25 ± 3
							(22–35)
b							7.9 ± 0.3
							(7.3–8.5)
c							11.6 ± 0.7
							(10.2–12.9)
H%							57 ± 3
							(53-62)
D% =EP/ES x 100	75	70 ± 17			71 ± 3		59 ± 3
		(67–78)			(67-75)		(54–65)
E% = EP/T x 100							88 ± 7
							(74–100)
SW=SP/ABW	1.55	1.75 ± 0.20			1.83 ± 0.16		
		(1.52–2.16)			(1.58-2.00)		
GS=GU/SP	0.82	0.75 ± 0.06			0.65 ± 0.06		
		(0.59–0.82)			(0.59-0.74)		
V%			49.5 ± 0.75	50		53 ± 1.5	
			(48-50)			(51-56)	

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.
H% = hyaline portion on tail/tail length x 100

Infective juveniles: Measurements are in Table 1. Body elongate. Sheath (second-stage cuticle) present immediately after harvesting, but many IJ will lose their sheath in storage. Labial region smooth, rounded anteriorly, continuous with body. Labial papillae not seen; four cephalic papillae prominent. Amphids slit-shaped but not prominent, sometimes covered with exudates. Cuticle marked with prominent transverse striations. Lateral field begins anteriorly with one line at annule five or six (Fig. 6A,B). Two additional lines appear at annules 10-11 to form two ridges (Fig. 6C). Near excretory pore level, the number of ridges in lateral fields increases from two to seven (Fig. 6C). Near the end of esophagus, the central ridge divides into two, making a total of eight ridges, the maximum number in the lateral field. The portion with eight ridges is the longest part (compared to portions with 2, 7, 4 ridges) of the lateral field. Near anus, the number of ridges reduced to seven. Some annules posterior to phasmid, the seven ridges in lateral field become four ridges. Three or four annules after that, the four ridges change to two ridges. With the above description, the formula of the lateral field is 2, 7, 8, 7, 4, 2.

Esophagus with thin corpus, basal bulb more or less elongate with visible valve. Tail attenuate, tapering gradually with constriction on dorsal side (Fig. 5C-F). Hyaline portion occupies about 57.5% (52-63) of tail length.

CROSS-BREEDING

No offspring were observed from any of the slides with two females indicating that none of GDc339, CB2B and CWL05 could self-fertilize and produced second-generation nematodes. Offspring were found from most of the slides in which two inoculated IJs developed into opposite sex adults for all treatments and control. Nematodes in the control developed further into adults of the second generation and many IJ were produced after that. For treatments, nematodes developed slowly, a number of them died; some of them developed to adults, but none of the females had eggs in the body. All of them died in about 10 day after becoming adults. No IJ were produced. The repeated test showed similar results. The above evidence showed that *S. guangdongense* n. sp. and *S. longicaudum* are very similar to each other; they could hybridize but would produce non-fertile second generation.

TYPE HOST, LOCALITY AND SPECIMENS

Type host and locality: The type host of this nematode in nature is unknown as it was recovered from soil using *Galleria* larvae as bait. The soil sample was collected in an artificial eucalypt forest in Jijia town (latitude N20.49, longitude E109.58), Lei Zhou, Guangdong province, China.

Type specimens: Holotype male, allotype female, 10 paratype first generation males, 5 paratype females, 15 paratype infective juveniles and other population slides deposited in the State Key Lab for Biocontrol, College of Life Sciences, Zhongshan University, Guangzhou 510275, China. Living infective juveniles are also preserved in liquid nitrogen in the nematode collection of SKLB, Zhongshan University. Slides of several males and infec-

tive juveniles were deposited in the United States Department of Agriculture Nematology, Beltsville, Maryland, USA.

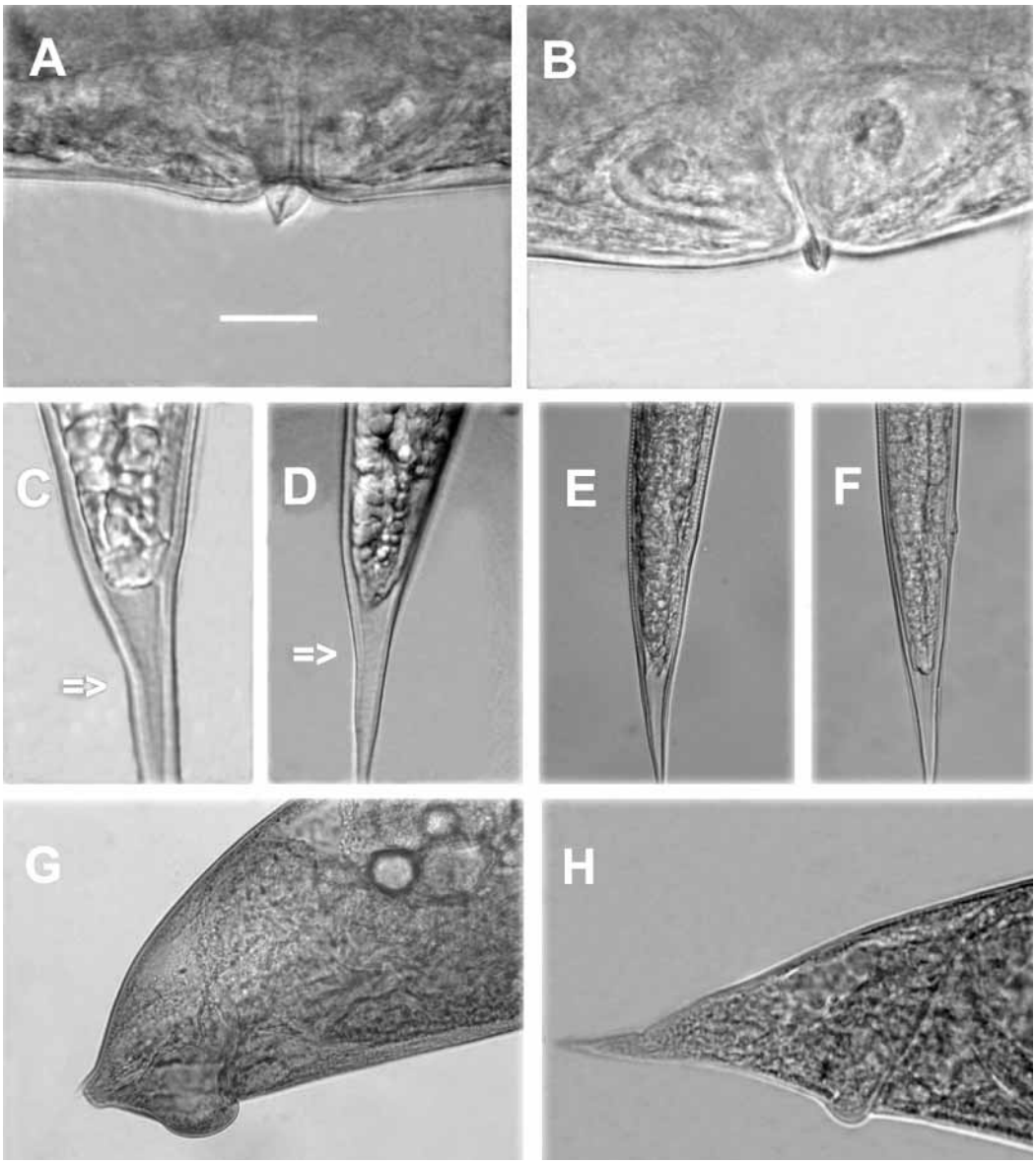

FIGURE 5. *Steinernema guangdongense* n. sp. Light-microscope photographs. A-B, epiptygma of the first generation females. C-D, tails of infective juveniles showing dorsal constriction (arrows), compared to no dorsal constriction in *S. longicaudum* in E-F. G, mature female of the first generation with prominent post-anal swelling. H, second generation female with longer tail. Scales: A, B = 18 μm, C = 24 μm, D = 22 μm, E, F = 22 μm.

DIAGNOSIS AND RELATIONSHIP

Steinernema guangdongense n. sp. can be recognized by large IJ body diam. 42 (30-48) μm, distance from anterior end to nerve ring 102 (88-111) μm; pharynx length 134

(123-144) μm and a = 25 (22-35). Lateral field pattern variable, the formula for the arrangement of ridges from head to tail is 2, 7, 8, 7, 4, 2 (Fig. 6). The new species can be recognized further by males with spicules length averaging 86 (80-94) μm, spicule shape, spicule tip (Fig. 3) and the ratios SW and GS (Table 2). The female of the new species is characterized by the presence of an epiptygma (Fig. 4, 5) and prominent post-anal swelling.

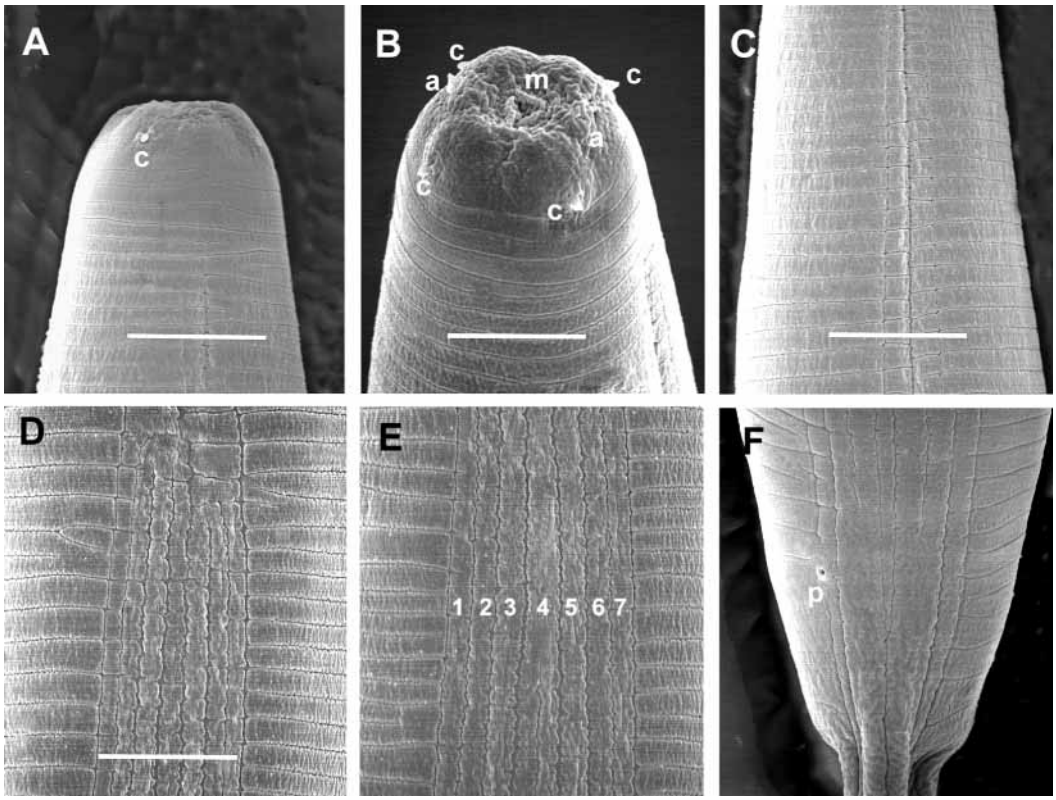

FIGURE 6. SEM photographs of *Steinernema guangdongense* n. sp. infective juvenile. A-B, anterior region showing one lateral line, closed mouth (m), amphids (a) and cephalic (c) papillae. C, lateral field with 2 ridges (3 incisures). D, lateral field showing the change of lateral field pattern from 2 to 7 ridges. E, lateral field showing 7 ridges and the middle one (number 4) is divided into 2 making 8 ridges in lateral field. F, lateral field showing phasmid (p) and 7 ridges changing to 4 then 2. Scales: A = 6.67 μm, B = 5 μm, C = 8.60 μm, D - F = 6.67 μm.

S*teinernema guangdongense* n. sp. can be distinguished from the closest species S. *longicaudum* by characteristics of infective juveniles (IJ), males and females. For IJ, although the body length, EP, E% is almost similar (1055 μm compared to 1063 μm, 80 μm to 82 μm and 88% to 87% respectively), body diameter is larger; value of NR and the ratio body length/body width are smaller (Table 2) and tail with dorsal constriction (Fig. 5C,D). For male, the new species has longer spicule, not well curved, spicule head shorter, shaft not prominent or absent and spicule tip not suddenly tapered as shown in *S. longicau-*

dum (Fig. 3). Also, the ratios SW and GS are smaller. For female, the presence of a small double flapped epiptygma (Fig. 5 A,B), a small projection on dorsal side of the tail tip and prominent post-anal swelling (Fig. 5G) differentiate this nematode from *S. longicaudum*. *Steinernema guangdongense* n. sp. can be distinguished from other related species by morphometrical characteristics listed in the Table 2.

Alignment of ITS regions

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                          1        .         .         .         .        50
S. guangdongense          GTACACACTGCCCGTCGCTGCCCGGGACTGAGTTGTTTCGAGAAAAGCGG
S. longicaudum CB2B       GTACACACCGCCCGTCGCTGCCCGGGACTGAGTTGTTTCGAGAAAAGCGG
S. diaprepesi             GTACACACCGCCCGTCGCTGCCCGGGACTGAGTTGTTTCGAGAAAAGCGG
S. glaseri                GTACACACCGCCCGTCGCTGCCCGGGACTGAGTTGTTTCGAGAAAAGCGG
S. longicaudum CWLO5      GTACACACCGCCCGTCGCTGCCCGGGACTGAGTTGTTTCGAGAAAAGCGG
                          ******** ****************************************
                          51       .         .         .         .       100
S. guangdongense          AGACTGCTTCTCTGAGCGTTTTCGGACGTGAATTGAGGCGAGAACCGCGT
S. longicaudum CB2B       AGACTGCTTCTCTGAGCGCTTTCGGGCGTGAATTGAGGCGAGAACCGCGT
S. diaprepesi             AGACTGCTTCTCTGAGCGCTTTCGGGCGTGAATTGAGGCGAGAACCGCGT
S. glaseri                AGACTGCTTCTCTGAGCGCTTTCGGGCGCGAATTGAGGCGAGAACCGCGT
S. longicaudum CWLO5      AGACTGCTTCTCTGAGCGCTTTCGGGCGTGAATTGAGGCGAGAACCGCGT
                          ****************** ****** ** ********************
                          201      .         .         .         .       250
S. guangdongense          GATGATGATTGTTCGGAACG--GCACTGC-TTCGTTTCTAGGTGTCGATT
S. longicaudum CB2B       GATTAGAA--GTTCGGAACG--GGACTG--TGCGCATCTAAGTGTCGATT
S. diaprepesi             GTTACGTATCGTTCGGAACG--ACACTGTCCACGTTTCTAAGTGTCGATT
S. glaseri                TATGATCACTGTTCGGAACGCGGCACTGT---CGTTTCTAGGTGTCGCGA
S. longicaudum CWLO5      GATTAGAA--GTTCGGAACG--GCACTGT--GCGCATCTAGGTGTCGATT
                            *    *  **********    ****    **  **** ******
                          301      .         .         .         .       350
S. guangdongense          ATGAGCGTGGCTGTGGTGAAGGACATTTGACATCCTATGCCAGACGTCTT
S. longicaudum CB2B       ATG-GCGTGGCTGTGGTGAAGGACATTTTACATCC--------------
S. diaprepesi             ATGAGCGTGGCTGTGATGAAGGACATTTAACATCCTATGCCAGACGTCTA
S. glaseri                ATGAGCGTGGCTGTGGTGAAGGACATTTGACATCGCGT------------
S. longicaudum CWLO5      ATGAGCGTGGCTGTGGTGAAGGACATTTTACATCGC-------------
                          *** *********** ************ *****
                          351      .         .         .         .       400
S. guangdongense          G-TGTTTCTAGCGTTTGGTGATGT-AGAATTAAAGAGGTCAGGTCGGAGG
S. longicaudum CB2B       ------------ATTTGCTGATGT-AGAATTAAAGAAGTCAG-TCGGAGA
S. diaprepesi             GCTGTCTCTTGCGTTTGGTGATG--AGAATTAAAGAGGTCAG-TCGGAGA
S. glaseri                -------------CTCGACGCGGTGAGAATTGAAGAGGTCAG-TCGGAGA
S. longicaudum CWLO5      -------------TTTGCTGATGT-AGAATTAAAGAGGTCAGTCCGGAGA
                                      * *  *  *  ****** **** *****  *****
                          401      .         .         .         .       450
S. guangdongense          CCCG--CCGTTCACAAACCCTACT-ATTAACATTT----ACTTGATGCTG
S. longicaudum CB2B       CCCCGCCCGTTCACAAACCCTACT-ATTAACATTTT---ACTTGATGATG
S. diaprepesi             CCCG--CCGTTCAAAAACC-TACC-ATTAACATTTT---CCATACTAA-G
S. glaseri                CCCG--CCGTTCACAAACCCTACC-ATTAACAATTTTACACACGATGACA
S. longicaudum CWLO5      CCCG--CCGTTCCCAAACCCTACTTATTACCATTTT---ACTTGATGATG
                          ***   ******  ***** ***  **** ** **     *    *
```

FIGURE 7. Selective blocks of sequence alignments of ITS regions and partial 28S showing diagnostic characters (red letters) of *Steinernema guangdongense* n. sp. (continued on the next page).


```
                          651       .         .         .         700
S. guangdongense          TCGTGACTTGCAGTCAGCTGAGACTGTTTTTTCGATTAGCTACTCTTCTT
S. longicaudum CB2B       TCGTGACTTGCAGTCAGCTGAGACTGTTTTTTCGATGAGCTACTTTTTT-
S. diaprepesi             TCGTTACTTGCAGTCAGCTTCGACTGTTTATTCGATAAGCTACTTTCGAG
S. glaseri                ACGTTACTTGCAGTCAGC---GACTGTTTTTTCGACGAGCTATGTACGTT
S. longicaudum CWLO5      TCGTGACTTGCAGTCAGCTGAGACTGTTTTTTCGATGAGCTACTTTTTT-
                           *** *************   ******** *****  *****
                          701       .         .         .         750
S. guangdongense          TT-CGGAGGGACCTT-TTCGGTATGGTCGC---AATTGAAAAATGCGAT-
S. longicaudum CB2B       -----GAAGTACCTT-TTCGGTATGGTCGC---AAT-GAAAAGCGCGAT-
S. diaprepesi             CTGCGAAAGTACCTT-TTCGGTGTGAACGCTTCAATGCGATAGGCTAATG
S. glaseri                ---CGTATGTACCTCGTTCGGTGTGAACGTTCCCCCGGCACTGGGGGCGA
S. longicaudum CWLO5      -----GAAGTACCTT-TTCGGTATGGTCGC---AAT-GAAAAACGCGAT-
                                * * ****  ****** **  **          *
                          851       .         .         .         900
S. guangdongense          G--GACAGC---GT-TCGTGCGTA-GTTTCTAGAAGTCGGTAGCCACGTG
S. longicaudum CB2B       G--GACAGCTTCGT-TCGTGCGTAAGTTTCTAGAAGTCGGTAGCCATTTT
S. diaprepesi             GCAGACGTAACTGTCTCGTATGTAAGCTTCTTGAAGTCGGCTGCCACAT-
S. glaseri                G----TAATTTTTT-GCGTATGTAAGCTTCTTGAAGTCAGT-GTTGCCAG
S. longicaudum CWLO5      G--GACAGCTTCGT-TCGTGCGTAAGTTTCTAGAAGTCGGTAGCCATTTT
                          *            *  ***  *** * **** ****** *  *
                          901       .         .         .         950
S. guangdongense          G---TGACTCAGCTTGTTTCCGTTGGTCAACGGACGCACTTGGAACTA--
S. longicaudum CB2B       AGTTTGACTCAACTTGTTTCCGTTGGTCAACGGACGTACGTG-AACTT--
S. diaprepesi             ------GTTCGACC----TTTGCGGGTTGACGAACGCAACTGGAACTTGC
S. glaseri                CAAGCGTTTGAGCCTGT-ACGGTTCGGCGCGCGACGTAGCTGGGACTT--
S. longicaudum CWLO5      AGTTTGACTCAACTTGTTTCCGTTGGTCAACGGACGTACGTG-AACTT--
                                 *   *        *   *       *** *  **  ***
```

Alignment of partial 28S sequences

```
                          201       .         .         .         250
S. guangdongense GDC339   TCGGCGTGCGATGCGTGGTATGGCTAAGGTTT---CGCCGGTCTTGAA-G
S. longicaudum CB2B       TCGGCGTGCGATGCGTGGTATGGCTAGGGTG----CGCCGGTCTTGAA-G
S. longicaudum CWL05      TCGGCGTGCGATGCGTGGTATGGCTAGGGTGC----GCCGGTCTTGAA-G
S. cubanum                TCGGCGTACGATGCGTGGTATGGCTAAGGTTCTGTCGCCGGTCTTGAAAG
S. longicaudum USA        TCGGCGTGCGATGCGTGGTATGGCTAGGGTGC----GCCGGTCTTGAA-G
S. glaseri                TCGGCGTACGATGCGTGGTATGGCTAAGGTTCTGTCGCCGGTCTTGAA-G
S. longicaudum CH         TCGGCGTACGATGCGTGGTATGGCTAAGGTTCTGTCGCCGGTCTTGAA-G
                          ******* ****************** ***      ************ *

                          351       .         .         .         400
S. guangdongense GDC339   TGTAGC-TCGATCTACTGAATTGGGATGCGTTGTCTCTT-GTGGACGGCG
S. longicaudum CB2B       TGTAGC-TCGATCTACTGACTTGGGATGCGTTGTCTCCT-GTGGACAGCG
S. longicaudum CWL05      TGTAGC-TCGATCTACTGACTTGGGATGCGTTGTCTCCT-GTGGACAGCG
S. cubanum                GGTGAC-GTAAGTTGCTGACTTGGGATGCGCTGTCTTCTTGTGGACGGCG
S. longicaudum USA        TGTAGC-TCGATCTACTGACTTGGGATGCGTTGTCTCCT-GTGGACAGCG
S. glaseri                GGTGACGTCAAGTTGCTGACTTGGGATGCGCTGTCTCCT-GTGGACGGCG
S. longicaudum CH         GGTGACGTAAGTTTGCTGACTTGGGATGCGCTGTCTTCT-GTGGACGGCG
                           **  *       * **** ********** *****  * ****** ***
```

* No differences found among nematode species.
\- Gaps.

TABLE 2. Comparative morphometrics (in µm) of males and infective juveniles of *Steinernema guangdongense* sp. n and related Steinernema spp.

Character	Male				Infective juvenile			
	S. guangdongense	*S. longicodum*	*S. arenarium*	*S. glaseri*	*S. guangdongense*	*S. longicodum*	*S. arenarium*	*S. glaseri*
	Present study	Stock *et a*l. 2001	Kozodoi 1984	Poinar 1978	Psesent study	Stock *et al.* 2001	Poinar 1990	Poinar 1990
n	20	23	-	25	20	28	25	25
Body length	1809 (1627–2126)	1788 (1412–2733)	2282 (2091–2550)	1700 (1500–1900)	1055 (987–1145)	1043 (929–1170)	1034 (724–1408)	1130 (864–1448)
Greatest body width	112 (90–135)	136 (86–194)	188 (184–219)	72 (54–92)	42 (30–48)	37 (34–40)	46 (28–77)	43 (31–50)
EP	120 (103–132)	127 (79–162)	164 (153–187)	145 (121–178)	80 (71–85)	82 (74–92)	83 (76–86)	102 (87–110)
NR	127 (109–139)	151 (120–176)	-	132 (99–183)	102 (88–111)	111 (98–129)	109 (100–120)	120 (112–126)
ES	162 (150–176)	165 (79–192)	176 (173–184)	160 (155–187)	134 (123–144)	142 (134–150)	138 (123–160)	162 (158–168)
Tail length (T)	31 (24–38)	30 (20–43)	49 (41–57)	30 (28–44)	91 (82–103)	94 (79–105)	75 (64–84)	78 (62–87)
Spicule length (SP)	86 (80–94)	91 (72–108)	84 (81–91	77 (62–90)				
Gubernaculum length (GU)	64 (47–73)	60 (54–65)	55 (49–60)	46 (40–50)				
a					25 (22–35)	28.2 (25.9–30.7)	26 (17–34)	29 (26–35)
D% =EP/ES x 100	70 (67–78)	75.4 (56–92)	93 (88–102)	90	59 (54–65)	57.4 (52.4–62.5)	55 (52–59)	65 (58–71)
E% = EP/T x 100					88 (74–100)	86.9 (75.5–104.1)	119 (106–130)	131 (122–138)
SW=SP/ ABW	1.75 (1.52–2.16)	1.61 (1.16–2.25)	2.1	2.56				
GS=GU/SP	0.75 (0.59–0.82)	0.66 (0.56–0.88)	0.65 (0.60–0.66)	0.59				

- not available
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.
ABW = anal body width.

MOLECULAR CHARACTERIZATION

The lengths of ITS and partial 28S rDNA (flanked by the above-mentioned primers) of *S. guangdongense* n. sp. GDc339 and *S. longicaudum* CB2B and CWL05 were 989, 463; 902, 462; 903, 462bp, respectively. The phylogenetic tree of ITS regions (Fig. 8) shows that the 5 nematodes (*S. guangdongense* n. sp. *S. longicaudum* CB2B and CWL05, *S. diaprepesi* and *S. glaseri*) form a monophyletic group. Additionally, the sequence alignment (Fig. 7) of this group shows that *S. guangdongense* n. sp. has 24 diagnostic character states and differs from its sister taxon, *S. longicaudum* at 35 (CB2B) and 38 (CWL05) total characters of the ITS sequence (Tables 3, 4).

TABLE 3: Sequence length of ITS regions, composition, and diagnostic characters of 5 isolates of *Steinernema* spp.

Species (Seq length)	ITS1 (bp)	ITS2 (bp)	A (%)	C (%)	G (%)	T (%)	Diagnostic character*
S. guangdongense (986)	285	293	24.1	19.6	26.7	29.6	24
S. longicaudum CB2B (955)	257	292	25.1	19.6	25.8	29.5	5
S. diaprepesi (1022)	301	313	24.9	21.2	24.9	29	91
S. glaseri (988)	279	302	23.6	21.8	27.1	27.5	117
S. longicaudum CWLO05 (956)	257	292	24.7	19.9	25.9	29.5	7

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

TABLE 4. Pairwise distances of ITS regions betwwen taxa. Below the diagonal:Total character differences; above diagonal: Mean character differences (adjusted for missing data).

Species	GUA	CB2B	DIA	GLA	CWL05
S. guangdongense	-	0.03704	0.11191	0.14752	0.04013
S. longicaudum CB2B	35	-	0.10650	0.14952	0.01368
S. diaprepesi	109	100	-	0.16046	0.10957
S. glaseri	140	141	155	-	0.14799
S. longicaudum CWLO05	38	13	103	140	-

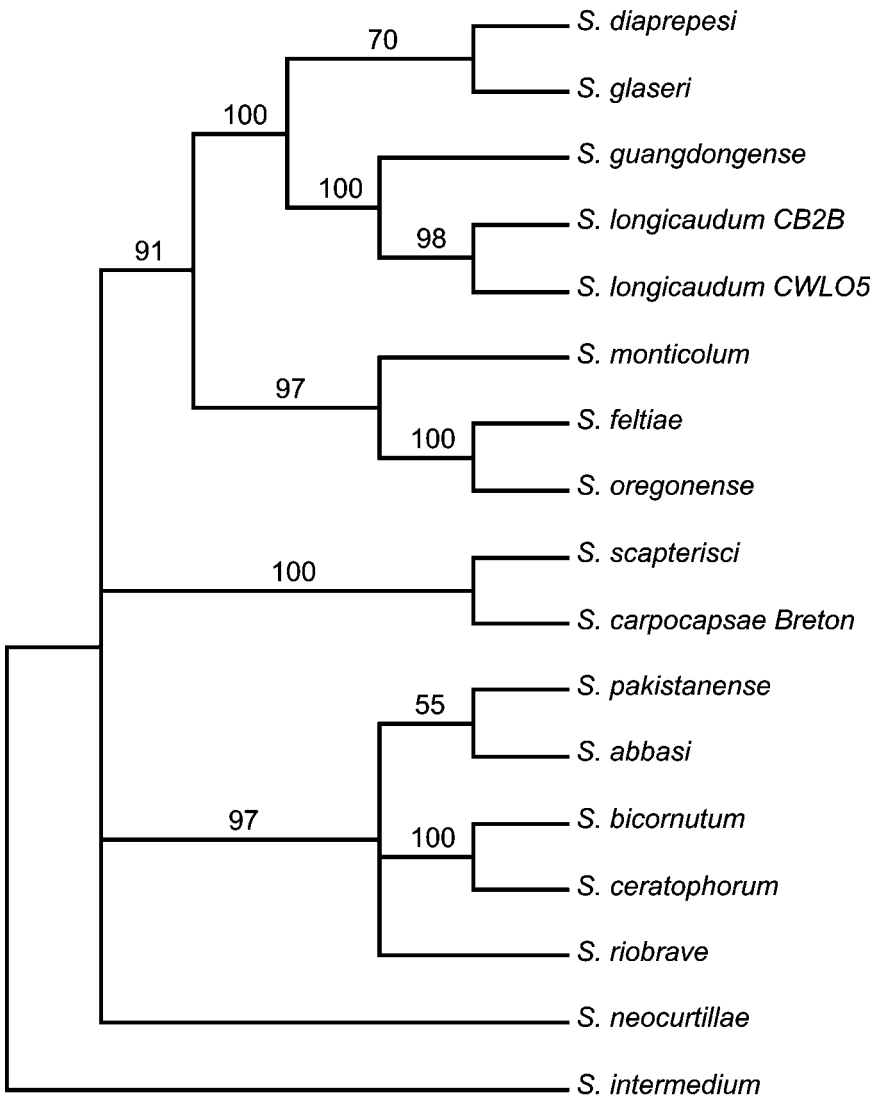

FIGURE 8. Phylogenetic relationships between 17 species of *Steinernema* with bootstrap analysis of ITS regions. The five species *S. diaprepesi*, *S, glaseri*, *S. guangdongense*, *S. longicaudum* (strains CB2B and CWL05) form a monophyletic group. Numbers at the nodes represent bootstrap proportion.

The new species also can be differentiated from other closely related nematodes by characteristics of its 28S sequence. Phylogenetic tree of 28S partial sequence (Fig. 9) shows that *S. guangdongense* n. sp. and *S. longicaudum* comprise a monophyletic group. Pairwise distances from Table 5 can be used to separate the new species from other closely related nematodes. The sequence alignment (Fig. 7) of this group shows that *S. guangdongense* n. sp. has three diagnostic character states and differs from its sister taxon, *S. longi-*

caudum at nine base-pair characters. It is interesting that the pairwise distances show that there is no difference between a strain from USA and two Chinese strains (CB2B and CWL05) of *S. longicaudum* (Table 5). Also, the distances between *S. longicaudum* strain CH, and CB2B, CWL0, and USA are 33 base pairs. It is possible that the strain CH was misidentified as a strain of *S. longicaudum*. The identification of this isolate needs to be re-evaluated.

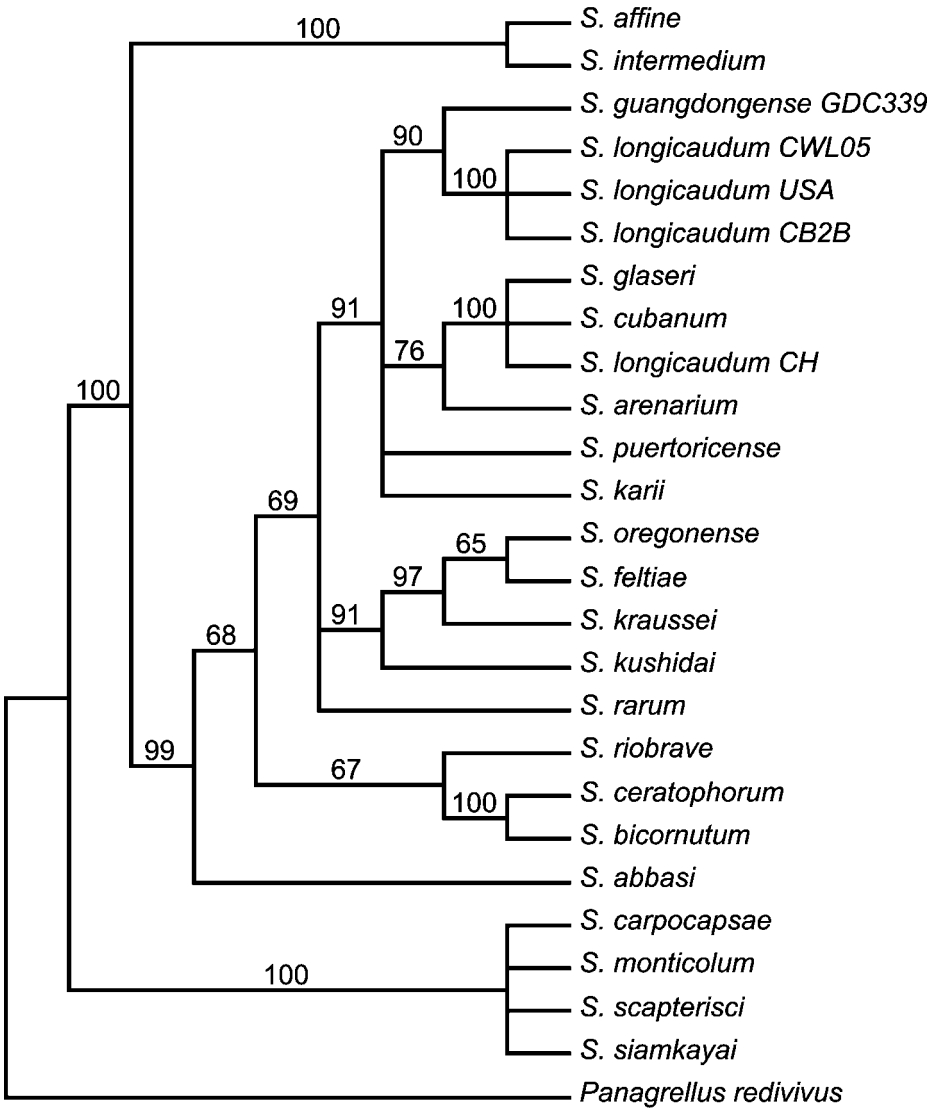

FIGURE 9. Phylogenetic relationships between 25 species of *Steinernema* with bootstrap analysis of partial 28S sequences. The eight species *S. guangdongense* n. sp., *S. longicaudum* (strains CWL05, USA and CB2B) *S. glaseri*, *S. cubanum*, *S. longicaudum* CH, *S. arenarium*, *S. puertoricense* and *S. karii* form a monophyletic group. Numbers at the nodes represent bootstrap proportion.

TABLE 5. Pairwise distances of the partial 28S between taxa. Below diagonal: Total character differences. Above diagonal: Mean character differences (adjusted for missing data).

Species	GUA*	GLA*	CWL05	CUB*	ARE*	USA	CB2B	CH
S. guangdongense	-	0.06263	0.01952	0.06061	0.05832	0.01952	0.01952	0.06479
S. glaseri	29	-	0.07143	0.00644	0.03656	0.07143	0.07143	0.01071
S. longicaudum CWL05	9	33	-	0.07359	0.05411	0.00000	0.00000	0.07143
S. cubanum	28	3	34	-	0.04095	0.07359	0.07359	0.01073
S. arenarium	27	17	25	19	-	0.05195	0.05195	0.04516
S. longicaudum USA	9	33	0	34	24	-	0.00000	0.07143
S. longicaudum CB2B	9	33	0	34	24	0	-	0.07143
S. longicaudum CH	30	5	33	5	21	33	33	-

* GUA = guangdongense, GLA = glaseri, CUB = cubanum, ARE = arenarium.

Both morphological and molecular studies showed that the new nematode species belongs to *S. glaseri* group, and is closely related to *S. longicaudum* (with long body of IJ, morphometrical similarity, small pairwise distances, and monophyletic group). Geographic distribution shows that *S. guandongense* n. sp. was found in a very humid region (latitude N20.49, precipitation = 2000mm/year) while *S. longicaudum* was found in a colder and drier region (latitude N39.50, precipitation = 700 mm/year). The two nematodes may be derived from the same ancestors; the differences of the two nematodes may be due to their adaptations for their survival in different geographic conditions.

A NOTE ON STEINERNEMA SERRATUM

While working with *S. guangdongense* n. sp. we contacted Professor Wang who is one of the authors of *S. longicaudum*, Shen & Wang, 1992. In a telephone conversation, we asked him about *S. serratum*, his answer was, *S. serratum* is only a strain of *S. longicaudum*. Our conversation could be summarized in the following letter from Professor Wang:

> To whom it may concern
>
> This is to certify that: 1. The entomopathogenic nematode C8506 strain was isolated from a soil sample collected in an orchard of Laiyang Agricultural University, Shangdong Province, China in 1985 by Dr Shen and myself and it was described as S. longicaudum in 1992; 2. The WL05 strain which was also known as CWL05 in some publications was isolated from the same orchard in 1986 by Dr Liu Jie, this strain was described by Dr Liu as S. serratum in his PhD dissertation; 3. I had successfully crossed C8506 with CWl05.
>
> Yours faithfully
>
> Prof. Guohang Wang
> School of Plant Protection
> Southern Agriculture University of China

Professor Wang can be reached at telephone number 86-20-85281910.

According to articles No 8, and 9 (11) of the International Code of Zoological Nomenclature, the third edition, a dissertation is not considered as a publication; hence the species name *S. serratum* in Lius dissertation is not valid. As stated by Prof. Wang in the above letter, he had successfully crossed isolate CWL05 (*S. serratum*) and isolate C8506 (*S. longicaudum)*; we confirm that the name *S. serratum* is not valid and the nematode used to described *S. serratum* was a strain of *S. longicaudum.*

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