# Neosteinernema longicurvicauda n. gen., n. sp. (Rhabditida: Steinernematidae), a Parasite of the Termite **Reticulitermes flavipes (Koller)**<sup>1</sup>

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Abstract: A nematode isolated from the termite Reticulitermes flavipes (Koller) was identified and described as a new genus and species, Neosteinernema longicurvicauda. Primary distinguishing characters, by contrast to members of the genus Steinernema, were females having prominent phasmids, a curved tail longer than the body width at the anus, a spiral shape in juvenile-bearing females, and juveniles becoming infective-stage juveniles before emerging from the female; males having prominent phasmids, a digitate tail tip, a characteristic shape of the spicules (foot-shaped with a hump on the dorsal side), and 13-14 pairs of genital papillae, with eight pairs preanal; and infective juveniles having prominent phasmids and a filiform curved tail as long as the esophagus. Adult nematodes are found outside the termite cadaver. Diagnosis of the family Steinernematidae was emended to accommodate the new species.

Key words: entomopathogenic nematode, morphology, nematode, Neosteinernema longicurvicauda, new genus, new species, Reticulitermes flavipes, Rhabditida, scanning electron microscopy, Steinernematidae, taxonomy, termite.

On 24 January 1992, a nematode parasite of the termite, Reticulitermes flavipes (Koller) was collected by David Price, Miles, Inc., Vero Beach, Florida. The nematode was collected from the northeast corner of Palm Beach County, Florida, just east of Lake Okeechobee and about 2 miles south of the Martin county line from the Chancey Bay citrus grove. A patent is pending for use of the nematode to control termites.

The nematode is a member of the family Steinernematidae, but it differs both morphologically and biologically from the genus Steinernema. The nematode is described herein as a new genus and new species, Neosteinernema longicurvicauda. The specific epithet is derived from Latin, based on tail shape of the infective juvenile; longi = long, curvi = curved, and cauda = tail.

## MATERIALS AND METHODS

Males, females, and infective juveniles (IJ) of N. longicurvicauda used in the description of the new species were collected by exposing termites, R. flavipes, maintained in several layers of corrugated cardboard, to IJ. After 3 to 4 days, preadult and adult nematodes emerged from termite cadavers and became immobile. Representative males and females were retained for the description. Females used for propagation were placed on filter paper (Whatman #2, 5.5 mm diam) resting on a larger piece of cloth in a petri dish  $(100 \times 15 \text{ mm})$ . After 3-4 days, I] emerged from the females and were collected. Adults and juveniles were examined live, and all measurements, except those of the holotype and allotype, were made of living specimens. Other specimens were studied after they were relaxed and killed in warm water (40 C), fixed in TAF (1), infiltrated with glycerine, and mounted in dehydrated glycerine, or were killed and fixed in hot lactophenol and mounted in dehydrated glycerine. Coverglass supports were used to avoid flattening specimens.

Scanning electron microscopy: Nematodes were fixed in 3% glutaraldehyde buffered with 0.1 M sodium cacodylate at pH 7.2 for 24 hours or longer at room temperature (25 C). They were postfixed with osmium tetroxide solution for 12 hours at 25 C, dehydrated in a graded ethanol series, transferred to a mixture of 50% amyl ace-

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tate and 50% ethyl alcohol for 10 minutes, then transferred to 100% amyl acetate. Afterwards, the nematodes were critical point dried with liquid CO<sub>2</sub>, mounted on SEM stubs, and coated with gold. Spicules and gubernacula were prepared as suggested by Nguyen and Smart (7) or were dissected from specimens in lactophenol, glycerine, or 40% lactic acid. A coverglass (12 mm diam) was placed on a glass slide; a drop of water added, and the spicules and gubernacula were arranged near the center. Excess water was removed with strips of filter paper. The coverglass was transferred to a SEM stub previously sprayed with metallic enamel. After 1 hour, spicules and gubernacula were coated with gold and examined with a Hitachi S-570 SEM at 15 kV.

Biology: Each of 35 nematode-infected termites was placed on a double layer of filter paper (1-cm<sup>2</sup>) saturated with water. These were distributed in four large petri dishes ( $100 \times 15$  mm), and several drops of water were placed in each dish to prevent drying. The termites were observed daily, and water was added as necessary. When nematodes began to emerge from the termites, each unit of filter paper containing a termite was transferred individually to a small petri dish ( $60 \times 15$  mm). Water was then added, the termite dissected, and the number and life stage of the nematodes determined. The experiment was repeated several times to collect females for further observation.

## Systematics

# Genus: Neosteinernema n. gen. Figs. 1–8

*Diagnosis:* Family Steinernematidae Chitwood and Chitwood, 1937. Only one generation occurs in the termite.

Male: Spicule human foot-shaped, with a hump on dorsal side. Gubernaculum almost as long as spicule. Genital papillae 13 or 14 pairs, eight pairs preanal (Figs. 1D,2D,7A). Phasmids prominent (Fig. 2H,I). Tail tip digitate (Figs. 1F,2D,H,I). *Female:* Body spiral (Figs. 3A,4I), usually found external to termite cadavers. Tail curved, longer than body width at anus (Fig. 3E,G–I, Table 4). Phasmids prominent, located in posterior half of tail (Fig. 4E–H). Eggs retained by the female hatch inside the body, and juveniles molt twice to become IJ before exiting the female body.

Juvenile, infective third stage (IJ): Amphid a transverse slit, situated below labial disc (Fig. 6A,B). Lateral fields with nine incisures, eight smooth ridges (Fig. 6F–H). Phasmids prominent, slit-like (Fig. 6E–G). Tail about as long as esophagus (Table 3), filiform, curved at end (Figs. 5B,C,6D,E).

Differential diagnosis: Males of Neosteinernema longicurvicauda differ from those of Steinernema by the foot-shaped appearance of the spicules (which bear a dorsal hump), by having prominent phasmids, a digitate tail terminus, a single and 13-14 pairs of genital papillae with eight pairs preanal, and by the gubernaculum being about as long as the spicules (in 32% of males studied the gubernaculum was equal to or longer than the spicules). Females of Neosteinernema differ from those of Steinernema by the prominent phasmids, a curved tail longer than the body width at the anus, a spiral body, and by retention of all eggs, which hatch and develop to IJ before emerging from the then dead female. Females are usually found outside the termite cadaver. Neosteinernema IJ differ from those of Steinernema by the thin, elongate body (a = 39), by the filiform and ventrally curved tail, which is 10 times (range 8-11) as long as the anal body width and about the same length as the esophagus, and by the prominent phasmids (Table 4).

# Type species

Neosteinernema longicurvicauda n. gen., n. sp. Figs. 1–8

Holotype (male, in glycerine): Length 1,079  $\mu$ m; width 80  $\mu$ m; stoma length 5.5  $\mu$ m; stoma width 6.3  $\mu$ m; distance from head: to excretory pore 82  $\mu$ m, to nerve ring 128  $\mu$ m, to end of esophagus 172  $\mu$ m; body

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FIG. 1. Male of *Neosteinernema longicurvicauda* n. gen., n. sp. A) Entire body. B) Anterior region. C) Enlargement of anterior end. D) Posterior region showing genital papillae, phasmid (arrow), spicules, and gubernaculum. E) At top, spicule, lateral view; bottom left, gubernaculum, ventral view; bottom right, gubernaculum sublateral view). F) Normal tail with phasmid (arrow). F–K) Variation in shape of tail tip.

width: at excretory pore 42  $\mu$ m, at anus 47  $\mu$ m; testis length from flexure to distal end 238  $\mu$ m; tail length 48  $\mu$ m; spicule 55  $\mu$ m long, 12  $\mu$ m wide; gubernaculum 50  $\mu$ m long, 9  $\mu$ m wide; digitate part of tail 9  $\mu$ m long.

*Male:* Measurements of 25 males in Table 1. No second-generation males found. Male smaller than female. Body curved ventrally or C-shaped when heat-killed. Lateral field present with one incisure, sometimes indistinct. Stoma shallow, cheilorhabdions prominent; other parts of stoma appear funnel-shaped. By light microscopy the walls of the stoma, especially the dorsal side, appear thickened anteriorly and raised to form a subtriangular structure, which in lateral view resembles a dorsal and ventral tooth (Fig. 1C). Esophagus steinernematoid but somewhat thinner and more elongate than in *Steinernema*. Esophagointestinal valve pronounced. Excretory pore at or anterior to middle of esophagus; excretory duct sclerotized an-



FIG. 2. SEM photographs of male *Neosteinernema longicurvicauda* n. gen., n. sp. A) Spicule and gubernaculum. B) Enlargement of distal end of spicules and gubernaculum; tip of left spicule extended beyond gubernaculum (arrow); tip of right spicule about at level of end of right side of gubernaculum. C) Left and right spicules; note dorsal hump (arrow). D) Posterior region showing genital papillae (note that the second papilla, anteriorly to posteriorly, is subdorsal), cloacal tube (tubus), protruding spicules, and digitate tail terminus. E) Enlargement of the most anterior genital papilla. F) Enlargement of a part of male tail showing single ventral gapilla (s), and one each of pairs 8 and 9. G) Genital papillae, one each of pairs 10, 11, and 12. H) Genital papillae, pair 13, and one of pair 14, phasmid (p), and digitate tail terminus. I) Genital papillae, one of pair 13, phasmid (p), and digitate tail terminus. Scale (in A): A = 23  $\mu$ m, B = 2.5  $\mu$ m, C = 20  $\mu$ m, D = 43  $\mu$ m, E = 2.3  $\mu$ m, F = 12  $\mu$ m, G = 10  $\mu$ m, H = 6  $\mu$ m, I = 5  $\mu$ m.



FIG. 3. Female of *Neosteinernema longicurvicauda* n. gen., n. sp. A) Entire body. B) Anterior end. C) Protruding vulva of a female with juveniles in body. D) Nonprotruding vulva of a female without juveniles in body. E, G–I) Variation in tail shape; E is the more normal shape. F) Anterior region.

teriorly. Monorchic, gonad reflexed. Spicules paired, foot-like in shape, a prominent hump present on dorsal side of spicules; spicule head enlarged anteriorly; spicule tip pointed, slightly curved ventrally; two prominent ribs present in each spicule (Figs. 1E–K,8B,D). Gubernaculum long (gubernaculum/spicule ratio 0.97, Table 4), eight of 25 males with gubernaculum equal to or longer than spicule (Fig. 8C,D), usually the distal end of gubernaculum is difficult to see in situ (Fig. 1F–K), but after they are dissected out they can be seen and measured accurately (Figs. 2A,C,8C,D). Cuticle of cloacal area forming a tubus (2) around spicules (Fig. 2D,F). Genital papillae, 13 or 14 pairs and one single ventral precloacal papilla (Fig. 2D– I); eight pairs precloacal, mostly subventral, more or less variable in position (Figs. 2D,7A), one pair in cloacal area (Fig. 2F), two or three pairs caudal, subventral and two pairs caudal, subdorsal (Figs. 1F,2G,H,I). Phasmids prominent (Fig.



FIG. 4. SEM photographs of female *Neosteinernema longicurvicauda* n. gen., n. sp. A–C) Face views; note several papilla-like structures between the labial papillae (1) and the cephalic papillae (c), and the raised triradiate opening of the stoma in A and B. D) Anterior region of a young female showing offset swollen lip region, labial papillae (1), cephalic papillae (c), and amphid (a). Note the papilla-like structures near the labial papillae. E–G) Variation in tail showing prominent phasmids (p) on a protuberance. H) Enlargement of a phasmid showing a raised, round area with an apparent opening at the center. I) Entire body. Scale (in A): A = 12 µm, B, C = 7.5 µm, D = 10 µm, E = 13.6 µm, F = 30 µm, G = 17.6 µm, H = 7.5 µm, I = 475 µm.

2H,I). Tail conoid, tail tip digitate (Fig. 2I) but variable, especially on young males.

Allotype (female, in glycerine): Length 3,909 µm; width 213 µm; stoma length 8.6

 $\mu$ m; stoma width 11.7  $\mu$ m; distance from head to: excretory pore 115  $\mu$ m, nerve ring 153  $\mu$ m, end of esophagus 231  $\mu$ m; width at anus 81  $\mu$ m; tail length 113  $\mu$ m;



FIG. 5. Third-stage infective juvenile of *Neosteinernema longicurvicauda* n. gen., n. sp. A) Anterior region showing long, sinuous excretory duct terminating in an excretory cell. B) Tail, ventral view showing the two prominent phasmids. C) Posterior region with long, curved tail. D) Enlargement of a portion of the esophageal region showing a sclerotized structure where the excretory duct and cell join.

tail length divided by width at anus 1.38; V 57.

Female: Measurements of 25 females in Table 2. No second-generation females found. Females much larger than males. Body of mature female spiral (Figs. 3A,4I), usually found near infected dead termites. Cuticle annulated. Lateral field not observed. Head rounded, truncated (Fig. 3F), apex sometimes enlarged, especially in young females (Fig. 4D). Labial papillae six, prominent; many additional papillalike structures often present together with labial papillae (Fig. 4A,B,D). Stoma as in male but structures larger and more prominent; raised ring prominent in stoma (Fig. 4A,B) appearing tooth-like in lateral view by LM (Fig. 3B,F). Esophagus typical of steinernematids (Fig. 3F), procorpus cylindrical, muscular; metacorpus slightly swollen, nonvalvate; isthmus present, not prominent; basal bulb muscular, enlarged, valvate. Nerve ring surrounding isthmus. Esophagointestinal valve prominent (Fig. 3F). Excretory pore at or anterior to midesophagus. Excretory duct large, anterior part well sclerotized. Didelphic, amphidelphic, gonads reflexed. Vulva a transverse slit, not protruding in females containing eggs, slightly protruding in females containing juveniles. Epiptygma absent. Vagina sclerotized. Tail conoid, curved, longer than body width at anus (Figs. 3G– I,4E–H); tail length divided by body width at anus 1.40 (1.10–1.70). Phasmids prominent, on a protuberance, in posterior half of tail (Fig. 4E–H).

Juvenile, infective third stage (IJ): Measurements in Table 3. Juveniles become IJ before emerging from female. Body thin, elongate (a = 39), annulated (Fig. 6A,C), annule width 0.8 (0.6-1.0) µm. Sheath (second-stage cuticle) present, sometimes lost. Head region smooth, mostly slightly enlarged (Figs. 5A,6A). Oral aperture closed, triradiate (Fig. 6C), six lips (Fig. 6A-C). Labial papillae not observed; four cephalic papillae prominent, situated well behind anterior end (Fig. 6A-C). Amphids large, slit like, postlabial but anterior to cephalic papillae (Fig. 6A-C). Esophagus reduced, basal bulb elongate with valve; cardia present, sometimes obscure. Excretory pore prominent; duct long, sinuous, seen clearly in living specimens. A sclerotized structure associated with excretory duct just anterior to excretory cell, appearing as two black dots in lateral view (Fig. 5A,D). Lateral fields with nine incisures and eight smooth ridges, prominent in anterior and posterior regions (Fig. 6F-H); at midbody, lateral field greater than onethird body width, usually only border ridges seen clearly (Fig. 6I). Tail elongate, 10 times (8-11) as long as body width at anus, posterior end always curved ventrally. Tail length about as long as esophagus length. Phasmids prominent, slit like (Fig. 6F,G), not at same level (Figs. 5B,8A), usually 25 to 50 µm posterior to anus, occupying three or four lateral bands.

## Biology

The nematode developed only in the head of the termite. The adults emerged when the tissues of the termite began to decay. The number of adults in each termite varied from 1 to 4 (26% with 1; 40% with 2; 20% with 3; 14% with 4). Twenty of 35 termites harbored both males and fe-



FIG. 6. SEM photographs of infective juveniles of *Neosteinernema longicurvicauda* n. gen., n. sp. A) Anterior region showing smooth, swollen head region, amphid (a), and two of the four cephalic papillae (c). B) Enlargements of anterior end showing six lips, oral aperture, prominent amphid, and two of the four cephalic papillae. C) Anterior region with sheath showing amphid, triradiate oral aperture (m), and cephalic papillae (c). D) Posterior region showing filiform, ventrally curved tail. E) Tail showing large phasmid and hook-shaped terminus. F) Lateral fields with eight lateral bands (9 incisures) and large phasmid. G) Lateral field and large phasmid. H) Lateral field with eight bands. I) Lateral field at midbody with two border ridges and vague inside ridges. Scale (in A): A = 3  $\mu$ m, B = 1.8  $\mu$ m, C = 2.5  $\mu$ m, D = 43  $\mu$ m, E = 17  $\mu$ m, F = 4.3  $\mu$ m, G = 1.8  $\mu$ m, H = 5  $\mu$ m, I = 10  $\mu$ m.

males, and 15 harbored either males or females. Where only one sex was present, no reproduction occurred. When both males and females were present, the females moved a short distance away from the termite cadavers, assumed a spiral shape (Fig.

Table 1.	Measurements	(in	μm)	of	25	males	of
Neosteinernema	longicurvicauda	n.	gen.,	n.	sp.		

P-00					
Character†	Mean	SD	Range		
Body length	1,236	228	854-1,713		
Greatest width	97	18	67 - 140		
Stoma length	5.5	1	5-8		
Stoma width	7.2	1.4	5-11		
EP	82	12	59 - 103		
EPW	44	5	33-52		
NR	126	15	103-156		
ES	187	22	147 - 227		
Testis reflexion	277	55	178-394		
Anal body width	60	10	41 - 78		
Tail length	48	6	36-63		
Spicule length	61	4	52 - 67		
Spicule width	13	1	11 - 15		
Gubernaculum					
length	59	4	52 - 66		
Gubernaculum					
width	9	1	8-13		
Digitate part					
of tail	8	3	3-14		
D = EP:ES(%)	44	6	30 - 54		
EW	1.88	0.17	1.49-2.19		
SW	1.03	0.18	0.8 - 1.5		
GS	0.97	0.06	0.84 - 1.08		

† Distance from anterior end to: EP = excretory pore, NR = nerve ring; ES = base of esophagus; EPW = body width at excretory pore; EW = EP divided by body width at excretory pore; SW = spicule length divided by anal body width; GS = gubernaculum length divided by spicule length. Testis reflexion = distance from flexure to distal end.

7B), and became immobile. The females retained all eggs that hatched, and the juveniles underwent two molts to become IJ. When most of the juveniles were IJ, they moved vigorously, broke through, and

TABLE 2. Measurements (in µm) of 25 females of Neosteinernema longicurvicauda n. gen., n. sp.

Character†	Mean	SD	Range		
Body length	3,444	784	2,250-4,766		
Greatest width	177	37	122-250		
Stoma length	8	1.3	6-11		
Stoma width	14	1.6	11-17		
EP	112	16	84-147		
NR	177	21	150 - 225		
ES	262	28	219-334		
Tail length	105	12	88-134		
ABW	76	10	63-103		
T/ABW	1.4	0.1	1.1 - 1.7		
Vulva %	55	3	49-60		
$\mathbf{D} = \mathbf{EP:}\mathbf{ES} \ (\%)$	43	7	31-64		

 $\dagger$  Distance from anterior end to: EP = excretory pore, NR = nerve ring, ES = base of esophagus; T = tail; ABW = anal body width.

TABLE 3. Measurements (in  $\mu$ m) of 50 thirdstage infective juveniles of *Neosteinernema longicur*vicauda n. gen., n. sp.

Character†	Mean	SD	Range
Body length	926	60	789–1,084
Greatest width	24	2	20-31
EP	68	4	61-76
EPW	18	2	10-20
NR	107	7	92 - 125
ES	164	10	144-188
Tail length	167	11	141-190
ABW	17	1	14-20
T/ABW	10	1	8-11
а	39	3	30-46
b	5.6	0.5	5-7
с	5.5	0.3	4.7 - 6.5
D = EP:ES(%)	41	2	38-46
E = EP:tail			
length (%)	41	3	37-48

 $\dagger$  Distance from anterior end to: EP = excretory pore, NR = nerve ring, ES = base of esophagus; EPW = body width at excretory pore; T = tail; ABW = anal body width; a = body length divided by greatest width; b = body length divided by ES; c = body length divided by T.

emerged from the then female cadaver in the region of the tail or stoma. No firstand second-stage juveniles were observed in or surrounding termite cadavers. During development of juveniles, large numbers of bacterial cells were observed in the female bodies. No second generation was observed.

#### Type host

Head of the termite *Reticulitermes flavipes* (Koller).

# Type locality

The northeast corner of Palm Beach County, Florida, just east of Lake Okeechobee and about two miles south of the Martin county line in the Chancey Bay citrus grove.

#### Type specimens

Holotype (male): Isolated from the head of the termite Reticulitermes flavipes, deposited in the United States Department of Agriculture Nematode Collection (USDANC), Beltsville, Maryland. Allotype (female): Same data as holotype, deposited in the USDANC, Beltsville, Maryland. Paratype (males, females, third-stage infective juveniles):

Criteria† AFF T 66 (64–74) a 23 (21–28) c 11	ANO 75 (64-84) 26 (17-34) 14 (9-17) 119 3) (106-130) 15	CAR 53 (46-61) 21 (19-24) 10 (9-11) 60 (54-66)	FEL 81 (70–92) 31 (29–33) 10 (9–13) 78	GLA 78 (62–87) 29 (26–35) 15 (14–16)	INT Juvenile 66 (53–74) 23 (20–26) 10	KUS 50 (44-59) 23 (19-25) 12	NEO 80 (64–97) 26 (22–29)	RAR 51 (44–56) 23 (20–26)	RIT 49 (4454) 24 (1931)	54 (48-60) 24 (20-31)	N. LON 167 (141–190) 39 (30–46)
T 66 (64–74) a 23 (21–28) c 11	$\begin{array}{c} 75\\ (64-84)\\ 26\\ (17-34)\\ 14\\ (9-17)\\ 119\\ 3) (106-130)\\ 15\end{array}$	$53 \\ (46-61) \\ 21 \\ (19-24) \\ 10 \\ (9-11) \\ 60 \\ (54-66)$	81 (7092) 31 (2933) 10 (913) 78	78 (62–87) 29 (26–35) 15 (14–16)	Juvenile 66 (53–74) 23 (20–26) 10	50 (44–59) 23 (19–25) 12	80 (64–97) 26 (22–29)	51 (44–56) 23 (20–26)	49 (44–54) 24 (19–31)	54 (48–60) 24 (20–31)	167 (141–190) 39 (80,46)
T 66 (64–74) a 23 (21–28) c 11	$\begin{array}{c} 75\\ (64-84)\\ 26\\ (17-34)\\ 14\\ (9-17)\\ 119\\ 3) (106-130)\\ 15\end{array}$	$53 \\ (46-61) \\ 21 \\ (19-24) \\ 10 \\ (9-11) \\ 60 \\ (54-66)$	81 (70-92) 31 (29-33) 10 (9-13) 78	78 (62-87) 29 (26-35) 15 (14-16)	66 (53–74) 23 (20–26) 10	50 (4459) 23 (1925) 12	80 (64-97) 26 (22-29)	51 (44–56) 23 (20–26)	49 (44–54) 24 (19–31)	54 (48–60) 24 (20–31)	167 (141–190) 39 (30,46)
a (64-74) a 23 (21-28) c 11	$\begin{array}{c} (64-84) \\ 26 \\ (17-34) \\ 14 \\ (9-17) \\ 119 \\ 3) (106-130) \\ 15 \end{array}$	(46-61) 21 (19-24) 10 (9-11) 60 (54-66)	(7092) 31 (2933) 10 (913) 78	(62-87) 29 (26-35) 15 (14-16)	(53–74) 23 (20–26) 10	(4459) 23 (1925) 12	(64–97) 26 (22–29)	(44–56) 23 (20–26)	(44–54) 24 (19–31)	(48-60) 24 (20-31)	(141–190) 39 (30, 46)
a 23 (21-28) c 11	$\begin{array}{c} 26\\ (17-34)\\ 14\\ (9-17)\\ 119\\ 3) (106-130)\\ 15 \end{array}$	$ \begin{array}{r} 21\\ (19-24)\\ 10\\ (9-11)\\ 60\\ (54-66) \end{array} $	31 (29–33) 10 (9–13) 78	29 (26–35) 15 (14–16)	23 (20–26) 10	23 (19–25) 12	26 (22–29)	23 (20–26)	24 (19–31)	24 (20-31)	39
с (21–28) с 11	$\begin{array}{c} (17-34) \\ 14 \\ (9-17) \\ 119 \\ (106-130) \\ 15 \end{array}$	(19-24) 10 (9-11) 60 (54-66)	(29–33) 10 (9–13) 78	(26–35) 15 (14–16)	(20–26) 10	(19–25) 12	(2229)	(20–26)	(19-31)	(20 - 31)	(20 46)
c 11	$ \begin{array}{c}     14 \\     (9-17) \\     119 \\     3) (106-130) \\     15 \\ \end{array} $	10 (9–11) 60 (54–66)	10 (9–13) 78	15 (14–16)	10	12	11	()	(		1.00-401
	$\begin{array}{c} (9-17) \\ 119 \\ 3) (106-130) \\ 15 \end{array}$	(9-11) 60 (54-66)	(9–13) 78	(14-16)	(0, 1, 1)		11	10	11	11	5.5
(10-12)	119 3) (106–130) 15	60 (54–66)	<b>`7</b> 8 ´	· /	(9-11)	(10 - 13)	(9-14)	(9-11)	(9-13)	(9-12)	(4.7-6.5)
E% 94	3) (106–130) 15	(54-66)		131	<b>9</b> 6	<u>92</u>	23	72	88	73	41
(74-10)	15	(01-00)	(69-86)	(122 - 138)	(89-108)	(84-95)	(18 - 30)	(63-80)	(79 - 97)	(60-80)	(37-48)
N 15	1.0	25	25	25	25	<b>50</b>	50	25	20	20	50
Ref (13)	(15)	(14)	(14)	(14)	(14)	(6)	(8)	(14)	(4)	(7)	PS
· · · ·		()		( -)	Male	(-)	(~)	. ()	(-)	(•)	
SP 70	84	66	70	77	91	63	58	47	69	83	61
(67-86)	(81 - 91)	(58-77)	(65-77)	(64-90)	(84-100)	(48 - 72)	(52-64)	(42-52)	(58-75)	(72 - 92)	(52-67)
GU 46	<b>55</b>	47	<u>`41</u> ´	55	64	44	52	34	44	65	59
(37-56)	(49-60)	(39–55)	(34-47)	(44-59)	(56 - 75)	(39-60)	(44-59)	(23 - 38)	(33-50)	(59-75)	(52-66)
GS% 66	65	71	59	71	69	70	89	71	64	78	97
(55-65)	(60-66)	(59-88)	(52 - 61)	(64-85)	(62 - 77)		(82-93)	(55 - 73)	(57-67)	(69 - 84)	(84-108)
N 10	10	60	10	10	10	20	10	20	30	10	25
Ref (13)	(5)	PS	(11)	PS	PS	(6)	(8)	(3)	(4)	(7)	PS
( )	~ /		( )		Female	(-)	(-)	(0)	(-)	(•)	
T 53	56	36	44	45	71	38	61	44	27	46	105
(47-63)	(49-67)	(28 - 47)	(37-49)	(43 - 53)	(60-89)	(30-45)	(47-78)	(37-50)	(19-36)	(34-59)	(88-134)
ABW 73	83	69	70	75	101	64	65	84	66	58	76
(54-95)	(61 - 104)	(50-87)	(6185)	(56 - 93)	(73 - 127)	(54-84)	(50-100)	(65-110)	(45 - 95)	(41 - 72)	(63-103)
c'% 73	68	52	63	60	73	59	81	52	41	79	140
· ···	(60-80)	_	(51 - 73)		_	_	_			(73-95)	(110-170)
N 10	10	25	10	25	10	20	20	20	93	10	95
Ref (13)	PS	(9)	PS	(10)	(12)	(6)	(8)	(3)	(4)	(7)	PS

TABLE 4. Selective differentiating data between species of Steinernema and Neosteinernema longicurvicauda.

 $\dagger$  T = tail length; a = body length/greatest width; c = body length/tail length; E% = see Table 3; N = number of specimens measured; SP = spicule; GU = gubernaculum; GS% = GU/SP × 100; ABW = anal body width; c'% = T/ABW × 100; Ref = reference number in Literature Cited.

 $\pm$  Species of *Steinernema*: AFF = affinis; ANO = amomali; CAR = carpocapsae; FEL = feltiae; GLA = glaseri; INT = intermedia; KUS = kushidai; NEO = neocurtillis; RAR = rara; RIT = ritteri; SCA = scapterisci; N. LON = *Neosteinernema longicurvicauda*.

- Data not available; PS = present study.



FIG. 7. Neosteinernema longicurvicauda n. gen., n. sp. A) Posterior region of a male showing eight preanal genital papillae. B) Spiral-shaped females, which emerged from termite cadavers. Scale (in A):  $A = 43 \mu m$ ; B = 855  $\mu m$ .

Same data as holotype; in lactophenol in a vial, deposited in USDANC, Beltsville, Maryland. One male, one female, and several IJ deposited in the Florida Collection of Nematodes, Florida Department of Agriculture and Consumer Services, Gainesville, Florida; one male, one female, and several IJ deposited in the California Collection of Nematodes, University of California Davis Nematode Collection, Davis, California.

## DISCUSSION

Neosteinernema longicurvicauda has some morphological and biological features that are new for the family Steinernematidae. In Steinernema generally two generations are produced in the insect cadaver, and adults emerge only when overcrowded. In N. longicurvicauda only one generation is produced, and the adults—and sometimes preadults—emerge from the insect cadaver. In Steinernema the female lays most of her eggs, but in N. longicurvicauda the female retains the eggs, which hatch and develop to the IJ.

In the male, the shape of the spicules

(foot-shaped with a hump on the dorsal surface), the digitate tail terminus, and the 13–14 pairs of genital papillae with eight pairs preanal are new for steinernematids. In the female, the tail being longer than the anal body width and the presence of prominent phasmids in first-generation females has not been reported in *Steinernema* (Table 4). In infective juveniles, the filiform, long, curved tail, and prominent phasmids are distinctive characteristics for the new genus. To accommodate these new characteristics for the family Steinernematidae, its diagnosis is emended.

# FAMILY STEINERNEMATIDAE Chitwood & Chitwood 1937

Diagnosis (emended): Rhabditoidea, Rhabditida. Members of this family of obligate insect parasites carry a symbiotic bacterium in the digestive system of the infective juvenile. Both males and females are necessary for reproduction.

*Female:* Large nematode, size variable. Cuticle smooth or annulated. Lateral fields absent. Excretory pore distinct. Head rounded or truncate, rarely offset. Six lips



FIG. 8. Light microscope photographs of *Neosteinernema longicurvicauda* n. gen., n. sp. A) Tail of an infective juvenile (ventral view) showing the two prominent phasmids. B) Spicule, lateral view, showing its foot-shaped form with dorsal hump. C) Gubernaculum (subventral view). D) Spicule (lateral view); note that the spicule is shorter than gubernaculum in C. C and D were from the same male. Scale (in A):  $A = 17 \mu m$ ;  $B = 30 \mu m$ ; C, D = 31  $\mu m$ .

present partly or completely fused, each with one labial papilla; sometimes additional papilla-like structures present near labial papillae. Four cephalic papillae. Amphids present, small. Stoma collapsed; cheilorhabdions pronounced, forming a ring which in lateral view resembles two large sclerotized dots. Other parts of stoma forming an asymmetrical funnel with thick anterior end. Esophagus rhabditoid with metacorpus slightly swollen, somewhat narrow isthmus surrounded by a nerve ring, and large basal bulb with reduced valve. Esophagointestinal valve usually pronounced. Didelphic, amphidelphic, gonads reflexed. Vulva at mid-body, with or without epiptygma. Females oviparous or

ovoviviparous with juveniles developing up to the infective juvenile stage before emerging from the body of the female. Tail longer or shorter than anal body width, with or without prominent phasmids.

*Male:* Smaller than female but similar to it except for the reproductive structures. Testis single, reflexed; spicules paired; gubernaculum long, sometimes as long as spicule; burså absent. Tail tip rounded, digitate or with mucron. Twenty-one to 29 genital papillae present with 5 to 10 pairs precloacal (10 pairs occasionally precloacal in *S. anomali*).

Infective juvenile (= third-stage infective juvenile): Also called dauer larva. Body slender with or without a sheath (cuticle of second-stage juvenile). Cuticle annulated. Lateral fields present with 4–9 incisures and 3–8 smooth ridges. Digestive system reduced. Excretory pore distinct. Tail conoid or filiform. Phasmids, prominent, inconspicuous, or absent.

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