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Heterorhabditis floridensis n. sp. (Rhabditida: Heterorhabditidae) from Florida

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Abstract

In a survey of entomopathogenic nematodes associated with plants and trees in areas adjacent to production citrus groves in Florida, a new species of nematode in the genus *Heterorhabditis* was found based of morphological and molecular studies. The new nematode is described as *Heterorhabditis floridensis* n. sp. *H. floridensis* n. sp. is characterized by males, females, and infective juveniles. For males, the number of papillae in the terminal group of bursa is variable, either with 2 pairs of papillae (40%), with 3 papillae on one side and 2 papillae on the other side (30%), with one pair of papillae (20%), or with three pairs of papillae (10%). SW and GS values are 179 and 50, respectively. Females have a typical vulva pattern, which is different from that of closely related nematode species *H. bacteriophora*, *H. mexicana*, and *H. indica*. For infective juveniles, EP=109 (101–122) μm, ES=135 (123–142) μm, tail length=103 (91–113) μm, and a=27.6 (25–32) are different from those of the above-mentioned three related nematodes. Phylogenetic analysis based on ITS regions show that the new species forms a clade with *H. mexicana*, *H. baujardi* and *H. indica* and differs from these species by several nucleotide autapomorphies.

Key words: entomopathogenic nematodes, ITS rDNA, morphology, nematode, phylogeny, SEM, systematics, taxonomy

Introduction

Entomopathogenic nematodes (EPN) are important biological control agents of a variety of economically important pests (Shapiro-Ilan *et al.*, 2002; Klein, 1990). At present, over 40 species of *Steinernema* and nine species of *Heterorhabditis* (Nguyen, 2005) have been

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reported, and the number of nominal species is increasing rapidly. In the years 2000–2005, fifteen species (twelve species of *Steinernema* and three species of *Heterorhabditis*) of EPN have been described. The rapid increase in the rate of EPN species descriptions is due to the potential new species have for improving biological control applications. Several biocontrol successes have been based on the discovery of new nematode species, (for example, the effectiveness of *Steinernema scapterisci* Nguyen & Smart, 1990, and *S. riobrave* Cabanillas *et al.*, 1994 against target insects).

In searching for indigenous nematodes with potential to control the citrus root weevil (*Diaprepes abbreviatus*), a survey of nematodes associated with plants and trees in areas adjacent to production citrus groves in Florida was carried out. Several nematode isolates were found. Three hundred-eight samples were taken, of which 26 contained EPN (8.4%). Four samples (1.3%) contained *S. glaseri* (Steiner, 1929) Wouts, Mracek, Gerdin & Bedding, 1982, and 22 samples (7.1%) contained *Heterorhabditis* spp. Morphological and molecular studies showed that most *Heterorhabditis* isolates were *H. indica* Poinar, Karunaka & David, 1992, while one isolate from the survey represents a new species. The new species is described herein as *Heterorhabditis floridensis* n. sp.; the species is named after the state where the nematode was collected.

Materials and methods

Light microscopy

Nematodes were collected from the soil by the insect-baiting technique (Bedding & Akhurst, 1975) and maintained in the laboratory on last instar *Galleria mellonella* (L.) larvae (Dutky *et al.*, 1964). For morphological studies, ten larval *G. mellonella* were exposed to about 300 infective juveniles in a petri dish (100 x 15 mm) lined with two moistened filter papers. The first generation hermaphrodites and second-generation males and females were obtained by dissecting infected insects 4–5 days and 7–8 days respectively after the insects died. Third-stage infective juveniles (IJ) were obtained during the first 2 days after emerging from insect cadavers as suggested by Nguyen and Smart (1995a). All observations and measurements were performed within a week after collection. For light microscope observation, 20 males and females and 25 infective juveniles were examined live. Additional specimens of different stages were killed in warm water (40°C), and fixed in either TAF (Courtney *et al.*, 1955) or lactophenol (Franklin & Goodey, 1949). These nematodes were used when more observations were needed to confirm the morphology or variation of some structures. Nematodes fixed in TAF were processed to glycerin using the Seinhorst method (Seinhorst, 1959). Type specimens were mounted in glycerin. Coverglass supports were used in all cases to avoid flattening of specimens.

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Morphology of bursa

The nematodes were reared and collected as stated above. Living males were transferred into a small disc of lactophenol (with 0.002% acid Fuchsin) on a hot plate at 65–70ºC. After 30 minutes, a male was transferred to a drop of lactophenol on a glass slide. The anterior three fourths of the body was severed and removed. A coverglass was placed on top of the drop of lactophenol containing the posterior part of the nematode. The coverglass was moved slowly by a needle to rotate the nematode posterior part to a ventral view. Ten nematode males were observed.

Scanning electron microscopy

Adults and IJ were fixed in 3% glutaraldehyde buffered with 0.1 M sodium cacodylate at pH 7.2 for 24 hours at 8ºC (Nguyen & Smart, 1995b). They were post-fixed with 2% osmium tetroxide solution for 12 hours at 25ºC, 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 (1995b).

Molecular characterization

Heterorhabditis species used in this study were: *Heterorhabditis bacteriophora* Poinar, 1975, strain HP88 (NCBI Genbank, Accession #AY321477), *H. baujardi* Phan, Subbotin, Nguyen & Moens, 2003, strain Vietnam (AF548768), *H. downesi* Stock, Griffin & Burnell, 2002, strain K122 (AY321482), *H. floridensis* n. sp. (DQ372922), *H. indica* Poinar, Karunakar & David, 1992, strain India (AY321483), *H. marelatus* Liu and Berry, 1996, strain OH10 (AY321479), *H. megidis* Poinar, Jackson & Klein 1987, strain AGC (AY321480), *H. mexicana* Nguyen, Shapiro-Ilan, Stuart, McCoy, James & Adams, 2004, strain MX4 (AY321478), and *H. zealandica* Poinar, 1990, strain Florida (AY321480), DNA was extracted from individual nematode and the entire internally transcribed spacer regions (ITS) were PCR amplified as described previously (Nguyen & Duncan, 2002; Nguyen *et al.*, 2001). PCR products were sequenced bidirectionally in their entirety. Internal primers used for sequencing were: 58P = 5'-ACGAATTGCAGACGCTTAG-3' (forward) and H58R = 5'-GTGCGTTCAAAACTTCACC-3' (reverse). The sequencing primers were designed with the Prime program of the Genetic Computer Group (GCG) Package, Madison, Wisconsin. Sequences of the complete ITS array were aligned to previously published sequences of the ITS1 region (Adams *et al.*, 1998) using the profile alignment option of Clustal X (Thompson *et al.*, 1997), then optimized manually in MacClade 4.05 (Maddison & Maddison, 2002). Following alignment optimization, the partial sequences of the original alignment (Adams *et al.*, 1998) were removed from the matrix.

Phylogenetic analysis was performed using PAUP* (Swofford, 2002). For the parsimony analysis, shortest trees were obtained using the branch and bound algorithm. Gaps in the matrix were treated as either missing data or a 5th base. *Pellioditis typica* (AF036946) and *Caenorhabditis elegans* (X03680) were used as outgroup taxa and to root

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the tree. Models of sequence evolution were evaluated using ModelTest 3.06 (Posada & Crandall, 1998) and favored the Hasegawa-Kishino-Yano model (Hasegawa *et al.*, 1985). The HKY+Γ model was used to correct for genetic distances in the construction of Neighbor Joining trees, and to search for the maximum likelihood solution (heuristic search, starting tree gained stepwise, stepwise addition sequence as-is, and TBR branch swapping).

***Heterorhabditis floridensis* sp. n.**
(Figs. 1–7)

Description

Male: Measurements are in Table 1. Body curved ventrally when heat killed. Head truncate, sometimes slightly swollen. Labial papillae 6, cephalic papillae not observed. Amphid prominent. Stoma with sclerotized rod- or barrel-shaped cheilorhabdions; other rhabdions not distinguishable forming a funnel-shaped structure below cheilorhabdions. Pharynx with cylindrical corpus, metacorpus sometimes slightly enlarged. Nerve ring surrounding isthmus just anterior to basal bulb. Basal bulb with reduced valve. Cardia present, protruding into intestine. Excretory pore usually posterior to basal bulb. Testis monorchic, reflexed; distance from end of basal bulb to testis flexure variable. Vas deferens well developed. Spicules paired, separate, rostrum absent, ventral side usually flattened. Gubernaculum slightly curved ventrally. Bursa peloderan. The number of genital papillae from one to six (from anterior end) are unchanged and typical for *Heterorhabditis* spp. (Nguyen et al. 2004). The distribution of these papillae is variable. From anterior to posterior, pair one is well anterior to the cloaca, its tips reach beyond the bursal rim; pairs two and three usually (90%) occur in a group (Fig. 2F), sometimes (10%) separated (Fig. 2E). The two papillae standing immediately anterior to cloaca also reach beyond the bursal rim. Pairs four, five and six usually (40%) form a group separately, or papillae four and five form a group, with pair six standing separately (20%). These papillae are situated just posterior to the cloaca, with pair 4 curved outward (laterally). Frequently (40%), papillae four, five, six, and seven form a group (Fig. 3D). The number and distribution of papillae in the terminal group are also highly variable. The observation of papillae in the terminal group of ten bursa yield the following results: 4/10 (40%) with 2 pairs of papillae (Fig. 3B); 3/10 (30%) with 3 papillae on one side and 2 papillae on the other side (Fig. 3D); 2/10 (20%) with one pair of papillae Fig. 3A), and 1/10 (10%) with three pairs of papillae (Fig. 3C). In general, the first papilla in the terminal group is prominent; the last two papillae, if present, are weaker, shorter, or appear as small sclerotized dots. Under SEM observation, only pairs seven was seen in the terminal group; in one male eight pair were observed (Fig. 2D). In addition to bursal papillae, a pair of smaller papillae was observed on the posterior edge of the cloacal opening (Fig. 2F). The tail is conoid, slightly curved ventrally.

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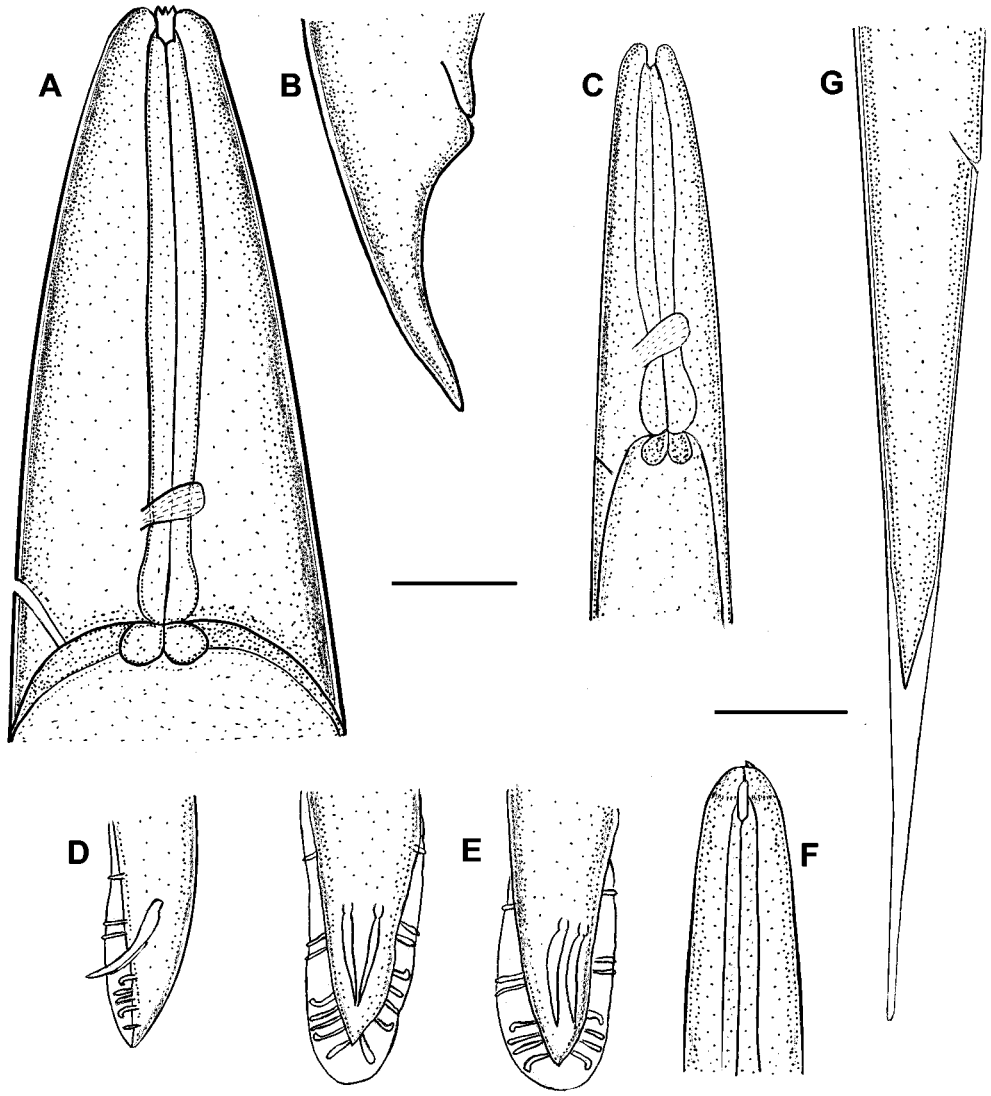

FIGURE 1. *Heterorhabditis floridensis* n. sp. A, B: Anterior and posterior regions of a hermaphrodite; C–E: Head and tail of a male; F–G: Infective juvenile; E: Anterior region showing a dorsal tooth; F: Tail of an exsheathed infective juvenile; G: Tail of an ensheathed infective juvenile showing terminus of second stage cuticle and third stage juvenile. Scale bar: A–E = 50 μm, F, G = 15 μm.

Female: Hermaphroditic female C-shaped in fixative, body robust, always with many eggs in young females and many eggs and juveniles in mature females. Cuticle smooth under light microscope but finely annulated with SEM. Head region tapering anteriorly. Labial region with six prominent lips appearing protruding into stoma (Fig. 4A,B), each lip with a labial papilla on top. Cephalic papillae not observed with SEM. In face view, mouth hexagonal in shape (Fig. 4A). Amphid present, pore-like. Stoma with refractile

cheilorhabdions. Posterior part of stoma funnel-shaped, enclosed by anterior part of pharynx. Pharynx with cylindrical corpus. Isthmus very short or undistinguishable. Nerve ring near anterior end of basal bulb. Basal bulb prominent with inconspicuous valve, but lumen of pharynx in basal bulb well sclerotized. In young female, anterior end of intestine with thick wall. Intestinal cells prominent, especially between basal bulb and flexure of anterior ovary, and from rectum to flexure of posterior ovary. Intestinal lumen well defined in these two regions. Cardia present. Gonads didelphic, amphidelphic. Vulva with a transverse slit, located on a slightly protruding area, anterior to mid-body (V=44–49%). In ventral view, vulva elliptical encircled by arch-shaped annules anteriorly and posteriorly (Fig. 4D). Vagina short. Tail longer than anal body diameter, conoid with pointed terminus (Fig. 4C,E,F). Sometimes, near the end, tail widens then narrows down to a pointed tip. Post-anal swelling present. Phasmid inconspicuous. Amphimictic female similar to hermaphroditic female but smaller. Vulva rarely protruding, usually (67%) covered with exudates or copulation plug after mating (Fig. 5F). Post-anal swelling much smaller.

TABLE 1. Morphometrics (μm) of *Heterorhabditis floridensis* n. sp.

Character	Holotype	Male	Herma	Female	IJ
n	1	20	20	20	25
Body length (L)	837	862 ± 44	4885 ± 69	2302 ± 48	562±24
		(785–924	(3731–5865)	(2054–2548)	(554–609)
Greatest body diam (D)	42	47.6 ± 2.2	271 ± 16	139 ± 12	21.2±4.6
		(43–50)	(217–331)	(120–156)	(19–23)
Stoma length			11.9 ± 3	10.9 ± 3.3	
			(9–15)	(10.6–12.1)	
Stoma width			11.8 ± 3	9.8 ± 3.1	
			(9–15)	(9.1–10.6)	
EP	119	117 ± 6	246 ± 15	136 ± 12	**109±10.4**
		(104–128)	(211–301)	(110–168)	(101–122)
NR	79.7	80 ± 5	209 ± 14	107 ± 10.3	86±9.2
		(73–90)	(169–271)	(86–122)	(68–107)
ES	105.8	105 ± 4	308 ± 17	149 ± 12.2	**135±11.6**
		(97–111)	(271–391)	(126–178)	(123–142)
Testis reflexion	78	93 ± 11			
		(78–116)			
Tail length with sheath (T)	34.8	34 ± 5.8	109 ± 10	77 ± 8.8	**103±10.1**
		(29–40)	(84–126)	(69–87)	(91–113)

.....continued on the next page

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TABLE 1 (continued)

Character	Holotype	Male	Herma	Female	IJ
Tail length without sheath					63 ± 7.9
					(48–68)
Anal body diam (ABD)	24.6	26.3 ± 3	57 ± 7	35.4 ± 5.9	14 ± 3.7
		(20–31)	(42–78)	(32–42)	(12–16)
Spicule length (SP)	40.6	**42 ± 3.5**			
		(36–46)			
Spicule width	4.3	4.8 ± 0.7			
		(4.3–5.8)			
Gubernaculum length (GU)	21.7	**23 ± 3.7**			
		(17–30)			
V			46 ± 7	48.1 ± 6.9	
			(44–49)	(44–50)	
a					27.6 ± 5.2
					(25–32)
b					4.3 ± 2.1
					(3.9–4.9)
c					5.6 ± 2.4
					(5.3–6.6)
D% = EP/ES x 100	112	112 ± 4			81 ± 8.9
		(105–119)			(71–90)
E% = EP/T x 100					105 ± 10.2
					(95–134)
SW% = SP/ABD x 100	165	157 ± 25			
		(133–209)			
GS% = GU/SP x 100	53.4	53.8 ± 6			
		(47–65)			

n = number of specimens measured.
EP = distance from anterior end to excretory pore.
NR = distance from anterior end to nerve ring.
ES = distance from anterior end to end of pharynx.
V = distance from anterior end to vulva/body length X 100.

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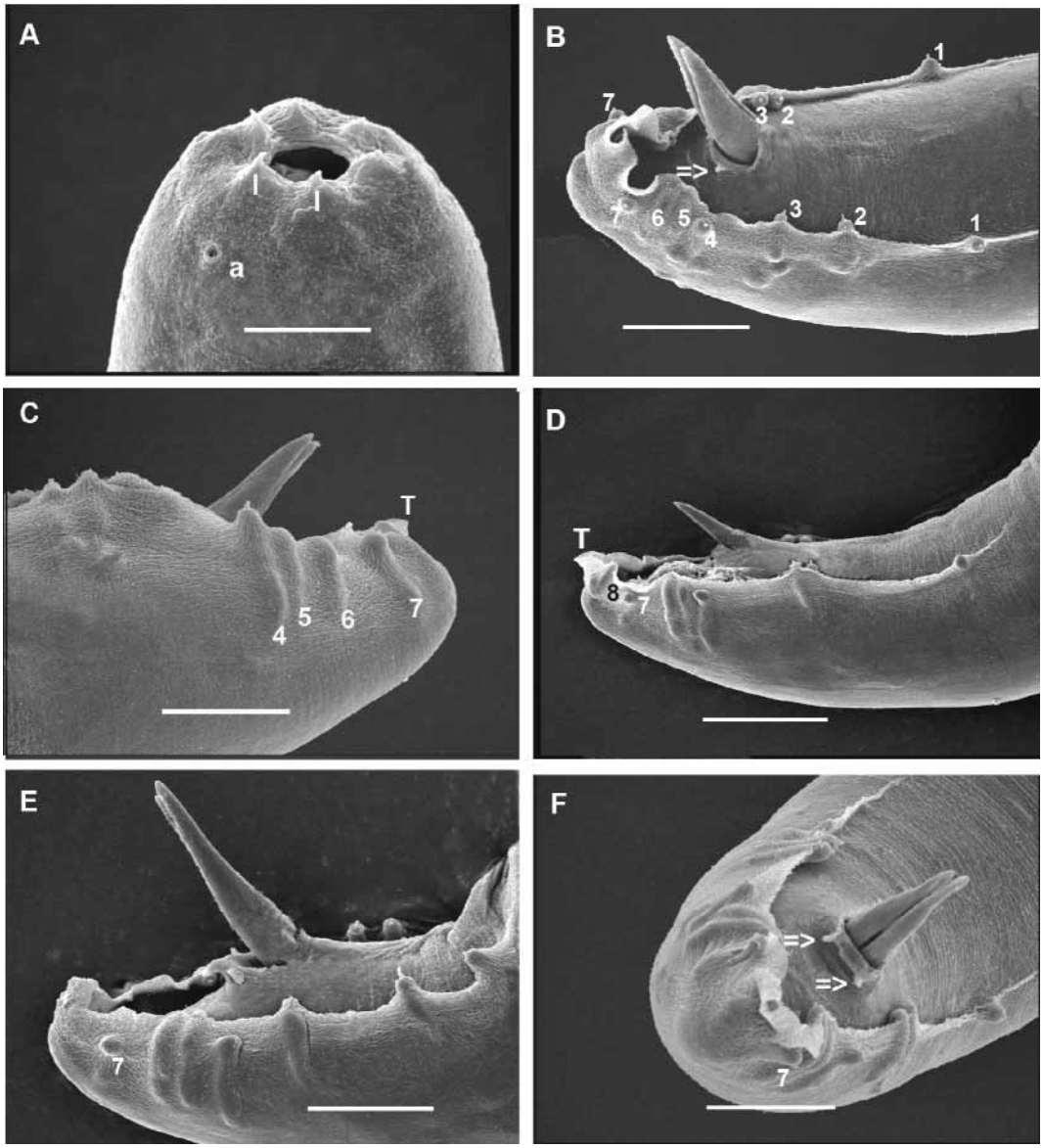

FIGURE 2. *Heterorhabditis floridensis* n. sp. SEM photographs of males. *A*, head of a male showing six labial papillae (l), and one of the two amphids (a). B–F, posterior region in different views showing bursal papillae (1,2,3,4,5,6,7,8) with variation in shape and position, small papillae on posterior edge of cloaca (arrows), tail tip (T), and spicules. *Scale bars:* A = 3.75 μm, B = 12 μm, C = 8.75 μm , D = 15 μm, E = 12 μm, F = 10 μm.

Infective juvenile: Body elongate. Sheath (second-stage cuticle) present immediately after harvesting, but many infective juvenile (IJ) losing sheath in storage. Ensheathed infective juvenile with body length close to that of *H. bacteriophora* and *H. indica*. Labial region with six annules without longitudinal incisures (Fig. 6B). Anterior part of body

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(about 3–5 body diameters from anterior end) with tessellate pattern. Posterior part with longitudinal ridges. Tail long, pointed. Exsheathed IJ body annulated, without longitudinal ridges. Labial region with a dorsal tooth, labial and cephalic papillae absent. Amphid prominent, pore-like (Fig. 6B). Excretory pore posterior to nerve ring but just anterior to base of pharynx (Fig. 6A). Excretory duct pronounced, well cuticularized except in anterior part, 3–5 µm from excretory pore. Hemizonid when observed, 3–4 annules anterior to excretory pore (Fig. 6A). Pharynx typical for *Heterorhabditis*. Lateral field at the center of the body with 2 ridges. Phasmid not observed under light microscope. Tail elongate conoid with pointed terminus. Tail length without sheath is about 61% of tail with sheath.

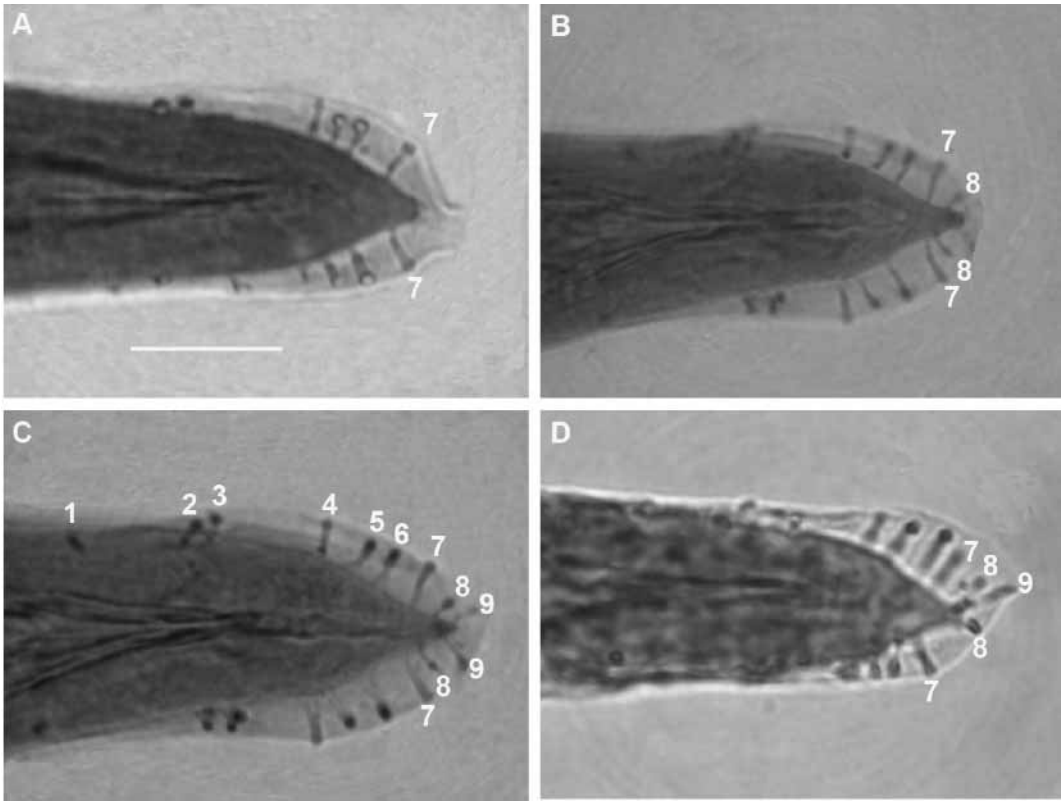

FIGURE 3. *Heterorhabditis floridensis* n. sp. Male tails with bursal papillae. A, bursa with one pair of bursal papillae in terminal group. B, bursa with two pairs of bursal papillae in terminal group. C, bursal with three pairs of bursa papillae in terminal group. D, bursa with three papillae in one side and two papillae on the other side in terminal group. Scale bar (in A) = 20 µm.

Type host and locality

Natural host unknown. The nematode was collected by baited soil sample in Desoto county, from a wooded area next to the highway (SR 70), GPS coordinates: N 27.20855 and W 81.75035.

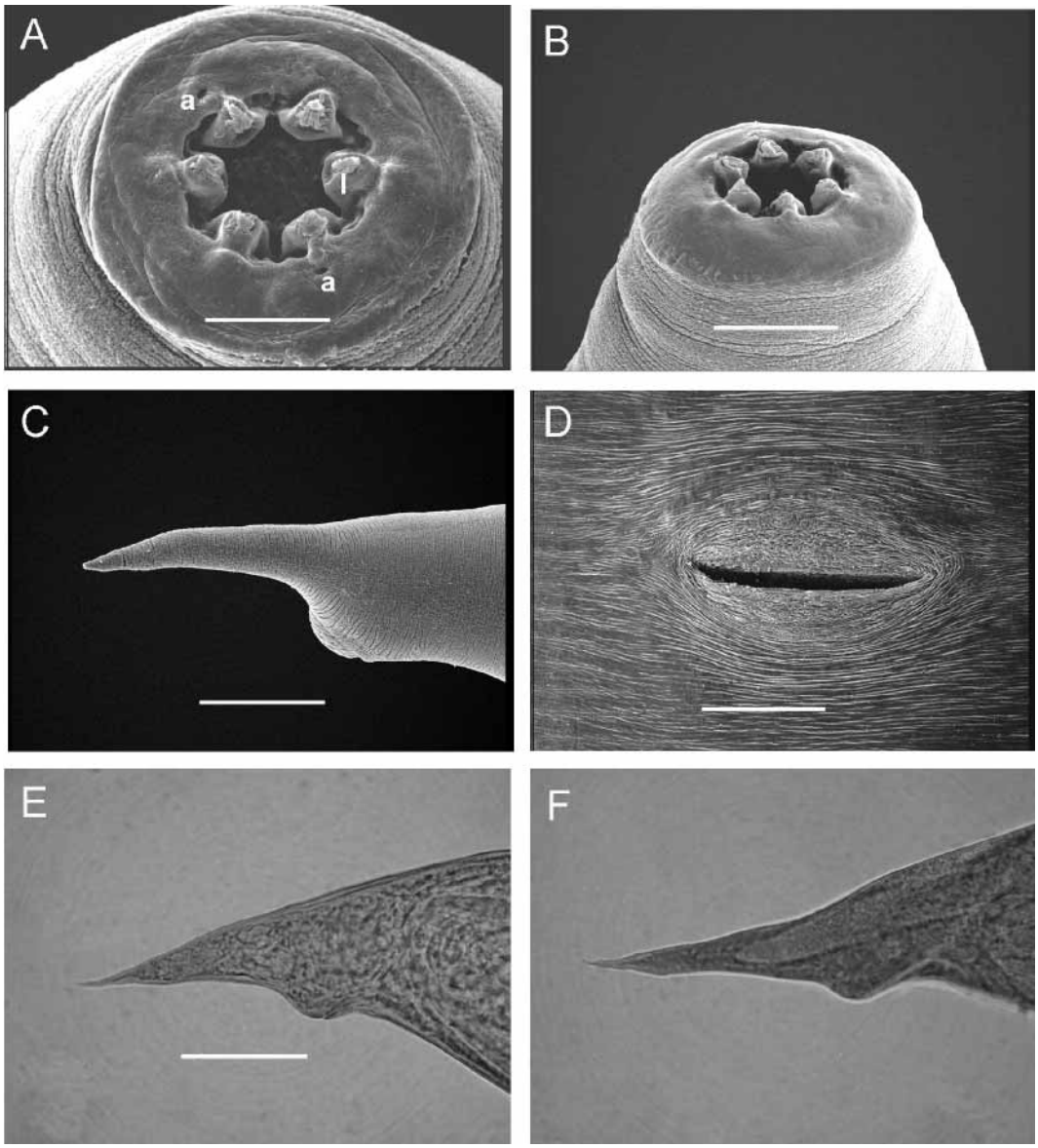

FIGURE. 4. *Heterorhabditis floridensis* n. sp. A – D, SEM photographs of hermaphroditic and amphimictic females. A, SEM of a face view of a hermaphroditic female showing 6 lips protruding into stoma with labial papillae on top, 2 pore-like amphids. B, face view of an amphimictic female showing 6 prominent lips, 6 labial papillae on top, amphids not seen. C, SEM of a hermaphroditic female showing post-anal swelling. D, vulva pattern. E, F, light microscope photographs of 2 different tail shapes of hermaphroditic female. Scale bars: A = 5.99 μm, B = 10 μm, C = 20 μm, D = 10 μm, E, F (in E) = 60 μm.

Type material

Holotype (male), and paratype (hermaphrodites, females, males, and infective juve-

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niles in 3% formalin) isolated from haemocoel of *G. mellonella* deposited in the United States Department of Agriculture Nematode Collection (USDANC), Beltsville, Maryland.

Slides of one male and one female of the second generation, and several infective juveniles deposited in the California Collection of Nematodes, University of California Davis Nematode Collection, Davis, California.

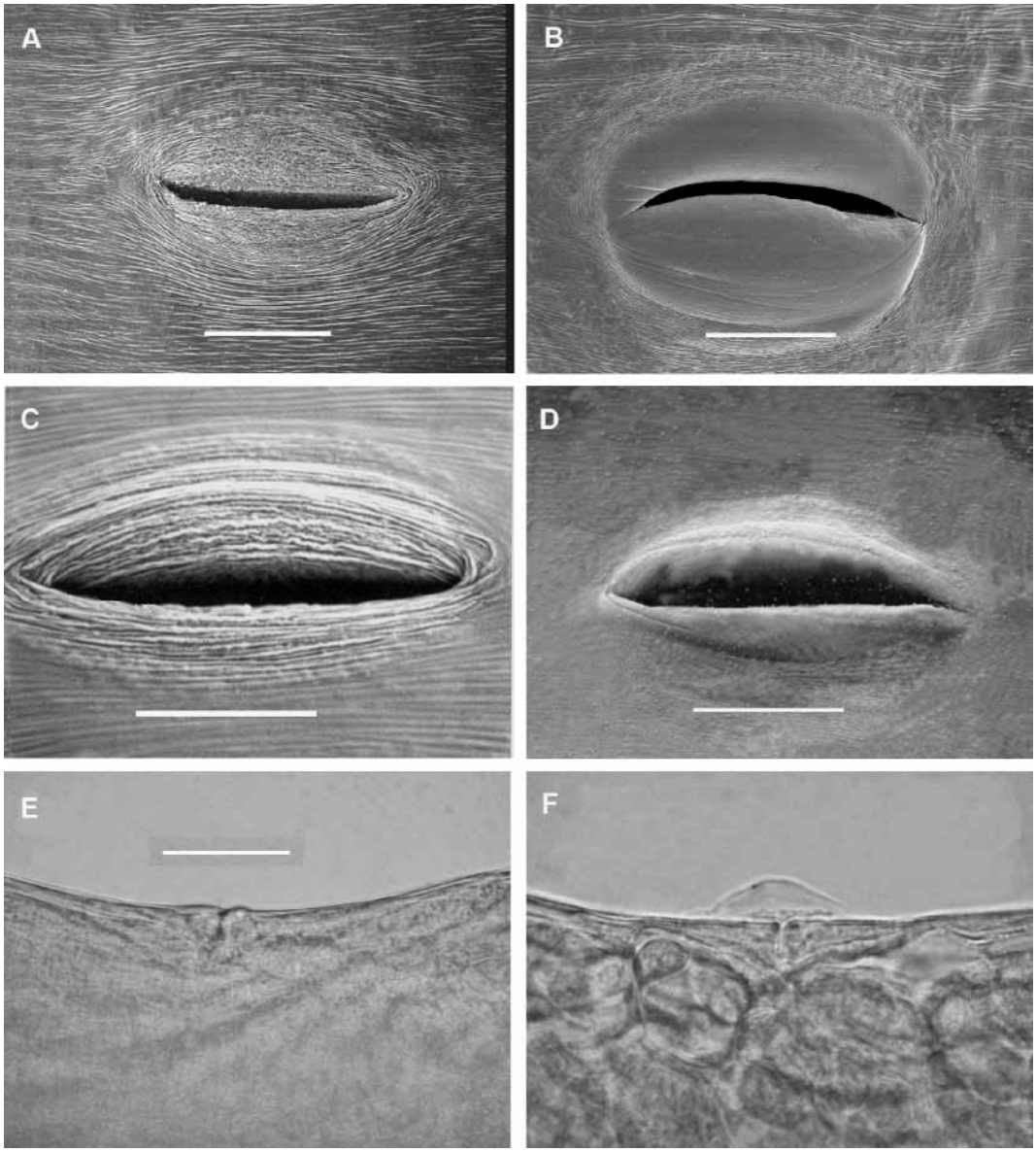

FIGURE 5. Comparison of vulva patterns of *Heterorhabditis floridensis* n. sp. and 3 closely related species. A, *Heterorhabditis floridensis* n. sp. B, *H. mexicana*. C, *H. bacteriophora*. D, *H. indica*. E, F, lateral views of vulva of hermaphroditic and amphimictic females, note that vulva of hermaphroditic female slightly protruding and vulva of amphimictic female covered with exudates or copulation plug after mating. *Scale bars:* A = 10 μm, B-D = 8.6 μm, E F (in E) = 60 μm.

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Several slides of hermaphrodites, males, females and all stages in 4% formalin are maintained in the Department of Entomology and Nematology, University of Florida, Gainesville, Florida, USA.

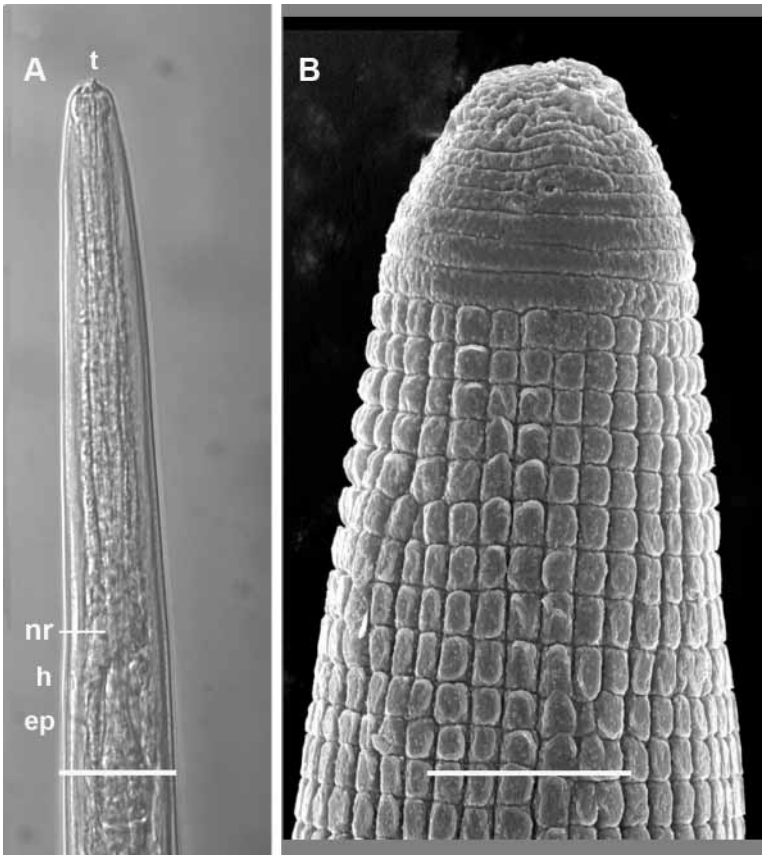

FIGURE 6. *Heterorhabditis floridensis* n. sp. infective juvenile. A, anterior region showing dorsal tooth (t), nerve ring (nr), hemizonid (h), and excretory pore and excretory duct (ep). B, SEM of anterior region showing labial region with 6 annules, and one of 2 pore-like amphids, and anterior part of body with tessellate pattern. Scale bars: A = 22 μm, B = 3.75 μm .

Diagnosis

Heterorhabditis floridensis n. sp. is characterized by unique male, female, and infective juvenile characters. For males, the number of papillae in the terminal group of bursa varies, either with 2 pairs of papillae (40%), 3 papillae on one side and 2 papillae on the other side (30%), one pair of papillae (20%), or with three pairs of papillae(10%). SW and GS values are 157 and 54, respectively. Females possess a typical vulva pattern (Fig. 4D). For infective juveniles, EP=109 (101–122) μm, ES=135 (123–142) μm, tail length=103 (91–113) μm, and a=27.6 (25–32). This new species can be further characterized by molecular characteristics of ITS regions of ribosomal DNA.

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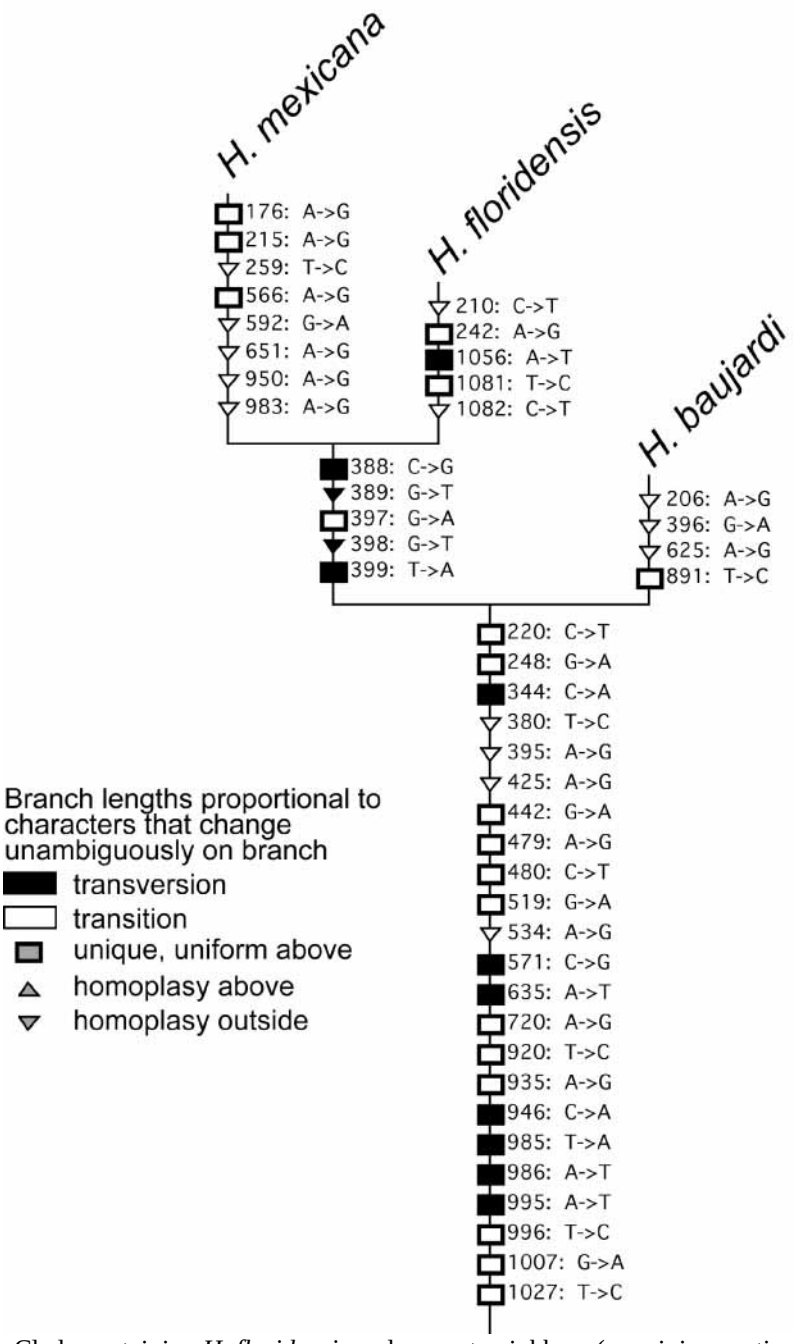

FIGURE 7. Clade containing *H. floridensis* and nearest neighbors (remaining portion of tree containing all other nominal taxa and characters not shown). Phylogeny reconstructed from ITS rDNA sequences (maximum likelihood, distance, and parsimony solutions were congruent). Mapped character states (unambiguous changes only; MacClade 4.06) identify character support and autapomorphies (positions 242, 1056 and 1081 of the transformation series). Character polarity was established by the outgroup taxa *Caenorhabditis elegans* and *Pellioditis typica* and compared with the other nominal species of the genus included in the analysis (see text for discussion of included taxa and GenBank accession numbers).

Relationships

Heterorhabditis floridensis n. sp. can be distinguished from other species of *Heterorhabditis* by morphological characters of males, females, and infective juveniles. As shown in the phylogenetic tree (Fig. 7) and morphological comparisons, the new species is most similar to *H. mexicana, H. baujardi,* and *H. indica.* Measurements of infective juveniles of the new species are different from these three species by bold-faced numbers in Table 3. For males of *H. floridensis* n. sp., the number of papillae in the terminal group is variable as stated in the diagnosis, and measurements of some characteristics differ from other taxa as presented in Table 2. For females, the vulval pattern of the new species (Fig. 5A) is quite different from that of the other morphologically closely related species, *H. mexicana* (Fig. 5B), *H. bacteriophora* (Fig. 5C), *H. indica* (Fig. 5D). Unfortunately, the vulval pattern of *H. baujardi* could not be obtained for comparison. The new species can be distinguished from *H. megidis, H. zealandica,* and *H. marelatus* by the presence of three pairs of bursal papillae in the terminal group (Fig. 6, Nguyen et al. 2004), and the body length (more than 600 µm) of the infective juvenile of these three species.

DNA characterization

The ITS rDNA regions, flanked by primers 18S and 26S, of *Heterorhabditis floridensis* n. sp. are characterized by length (1012 base pairs [bp], ITS1= 393 bp, ITS2=214 bp), and nucleotide usage composition (Table 4). Compared to sequences of eight other species in the genus *Heterorhabditis*, the sequence length of the new species is longer than that of seven other species, and shorter than that of only one species, *H. bacteriophora* (1021 bp). The ITS1 sequence length of the new species is longer than most of the other species (4–17 bp longer) but is one bp shorter than *H. mexicana* (ITS1=394 bp), The sequence length of the ITS2 region differs (1–3 bp) from six species (*H. baujardi, H. downesi*, *H. indica*, *H. marelatus, H. mexicana*, *H. zealandica*), but is six bp shorter than *H. megidis* and 14 bp shorter than *H. bacteriophora*. The nucleotide composition of all nine species of *Heterorhabditis* is presented in Table 4. The reconstructed nucleotide character transformations (Figure 7) show that the new species differs from its closest taxon *H. mexicana* at 16 aligned positions three of which are unambiguous, polarized autapomorphies. It is most divergent from *Heterorhabditis zealandica* (193 aligned positions). Genetic distances between the new species and others are presented in Table 5.

Phylogenetic Analysis

Parsimony, likelihood and distance based tree building approaches produced a single, concordant phylogenetic hypothesis for the genus *Heterorhabditis*. The topology of this tree is identical with that produced for *H. mexicana* (Nguyen et al., 2004) except the branch for *H. mexicana* in the former is replaced by the new clade (Fig. 7). The new species and its sister taxon *H. mexicana* form a clade with *H. baujardi*.

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TABLE 2. Comparative morphometrics (µm) of male of *Heterorhabditis floridensi*s n. sp. other related species.

Character	FL332	MXA	BAU	IND	BAC
n	20	20	14	12	15
L	862 ± 44	686 ± 38	889 ± 45	721 ± 64	820
	(785–924	(614–801)	(818–970)	(573–788)	(780–960)
Greatest body diam (D)	47.6 ± 2.2	42 ± 3	49 ± 2	42 ± 7	43
	(43–50)	(38–47)	(43–53)	(35–46)	(38–46)
EP	**117 ± 6**	124 ± 10	81 ± 7	123 ± 7	121
	(104–128)	(108–145)	(71–93)	109–138)	(114–130)
NR	80 ± 5	71 ± 6	65 ± 7	75 ±4	72
	(73–90)	(61–83)	(54–77)	(72–85)	(65–81)
ES	105 ± 4	96 ± 5	116 ± 10	101 ± 4	103
	(97–111)	(89–108)	(105–132)	(93–109)	(99–105)
Tail length(T)	34 ± 5.8	27 ± 4	33 ± 3	28 ±2	28
	(29–40)	(21–36)	(28–38)	(24–32)	(22–36)
Anal body diam (ABD)	26.3 ± 3	24 ± 1.3	22 ± 1	23 ± 8	23
	(20–31)	(23–27)	(20–24)	(19–24)	(22–25)
Spicule length (SP)	**42 ± 3.5**	41 ± 3.8	40 ± 3	43 ± 3	40
	(36–46)	(30 – 47)	(33–45)	(35–48)	(36–44)
Gubernaculum length (GU)	**23 ± 3.7**	23 ± 3	20 ±1.5	21 ± 3	20
	(17–30)	(18–32)	(18–22)	(18–23)	(18–25)
D% = EP/ES x 100	**112 ± 4**	129 ± 9	70	122	117
	(105–119)	(114–149)	-	-	-
SW% = SP/ABD x 100	**157 ± 25**	167 ± 2	182 ±18	187	174
	(133–209)	(130–196)	(138–208)	-	-
GS% = GU/SP x 100	53.8	56 ± 7	50 ± 5	50 ± 10	50
	(47–65)	(43–70)	(44–61)	(40–60)	-

n = number of specimens measured.
EP = distance from anterior end to excretory pore.
NR = distance from anterior end to nerve ring.
ES = distance from anterior end to end of pharynx.
V = distance from anterior end to vulva/body length X 100.

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TABLE 3. Comparative morphometrics (µm) of infective juveniles of *Heterorhabditis floridensis* n. sp. and other related species.

Character	F332 IJ present study	MXA IJ Nguyen *et al.* 2004	BAU IJ Phan *et al.* 2003	IND IJ Poinar 1992	BAC IJ Poinar 1990
n	25	25	25	25	25
Length (L)	562 ± 24	578 ± 23	551 ± 27	528 ± 26	558
	(554–609)	(530–620)	(497–595)	(479–573)	(512–671)
Greatest body diam (D)	21.2±4.6	23 ± 0.8	20 ± 2	20 ± 6	23
	(19–23)	(20–24)	(18–22)	(19–22)	(18–31)
EP	**109 ± 10.4**	102 ± 5.2	97 ± 3	98 ± 7	103
	(101–122)	(83–109)	(91–103)	(88–107)	(87–110)
NR	86 ± 9.2	81 ± 4.2	81 ± 3	82 ± 4	85
	(68–107)	(74–88)	(75–86)	(72–85)	(72–93)
ES	**135 ± 11.6**	122 ± 27	115 ± 3	117 ± 3	125
	(123–142)	(104–142)	(107–120)	(109–123)	(10–139
Tail length with sheath (T)	**103 ± 10.1**	99 ± 4.2	90 ± 4	101 ± 6	98
	(91–113)	(91–106)	(83–97)	(93–109)	(83–112)
Tail length without sheath	63 ± 7.9	66 ± 3	-	-	-
	(48–68)	(59–73)	-	-	-
Anal body diam (ABD)	14 ± 3.7	15 ± 1.2	13 ± 0.7	-	-
	(12–16)	(12–17)	(11–14)	-	-
a =L/D	**27.6 ± 5.2**	25.8	28 ± 1	26 ± 4	25
	(25–32)	(23.6–28.4)	(26–30)	(25–27)	(17–30)
b = L/ES	4.3 ± 2.1	4.6	4.8 ± 0.2	4.5 ± 0.34	4.5
	(3.9–4.9)	(4.2–5.1)	(4.5–5.1)	(4.3–4.8)	(4–5.1
c = L/T	5.6 ± 2.4	5.9	6 ± 0.3	5.3 ± 0.5	6.2
	(5.3–6.6)	(5.5–6.3)	(6–6.7)	(4.5–5.6)	(5.7–7)
D% = EP/ES x 100	81 ± 8.9	81 ± 3	84 ± 3	84 ± 5	84
	(71–90)	(72–86)	(78–88)	(79–90)	(76–92)
E% = EP/T x 100	105 ± 10.2	104 ± 5.2	108 ± 4	94 ± 7	112
	(95–134)	(87–111)	(98–114)	(83–103)	(103–130)

n = number of specimens measured; EP = distance from anterior end to excretory pore;
NR = distance from anterior end to nerve ring; ES = distance from anterior end to end of pharynx;
V = distance from anterior end to vulva/body length X 100.
MXA = *Heterorhabditis mexicana,* BAU = *H. baujardi*, IND = *H. indica,* BAC = *H. bacteriophora.*

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TABLE 4. Sequence length (base pairs =bp) and composition of ITS regions of nine species of *Heterorhabditis*.

Species (Seq length)	ITS1 (bp)	5.8S (bp)	ITS2 (bp)	A	C	G	T
H. zealandica (1003 bp)	387	154	212	0.22233	0.21834	0.27318	0.28614
H. downesi (990 bp)	374	154	212	0.23838	0.21717	0.26162	0.28283
H. megidis (1005 bp)	384	154	220	0.23284	0.2199	0.27164	0.27562
H. marelatus (995 bp)	379	154	211	0.23417	0.21608	0.26432	0.28543
H. bacteriophora (1021 bp)	389	154	228	0.26347	0.1998	0.25661	0.28012
H. indica (988 bp)	370	154	215	0.26316	0.20547	0.25506	0.27632
H. mexicana (1010 bp)	394	154	213	0.25545	0.20198	0.2604	0.28218
H. floridensis (1012 bp)	393	154	214	0.25988	0.20059	0.25494	0.28458
H. baujardi (795 bp)[1)]	?	154	211	0.25732	0.19677	0.25089	0.29502

1) This sequence is not as complete as in other species.
? Not available.

TABLE 5. Pairwise distances between taxa. Below diagonal: Total character differences; above diagonal: Mean character differences (adjusted for missing data).

		1	2	3	4	5	6	7	8	9	10
1	*H. zealandica*	-	0.07614	0.09959	0.08595	0.15923	0.18352	0.19652	0.19734	0.23553	0.42261
2	*H. downesi*	75	-	0.03959	0.03741	0.12590	0.15497	0.16770	0.16960	0.20561	0.41340
3	*H. megidis*	98	39	-	0.05894	0.14051	0.17140	0.17890	0.18060	0.22222	0.42437
4	*H. marelatus*	85	37	58	-	0.12513	0.15641	0.16872	0.17061	0.20530	0.41598
5	*H. bacteriophora*	157	123	137	123	-	0.15328	0.17725	0.17912	0.21476	0.43216
6	*H. indica*	176	148	163	150	147	-	0.07857	0.07959	0.09449	0.42990
7	*H. mexicana*	192	162	173	164	173	77	-	0.01586	0.03034	0.43996
8	*H. floridensis*	**193**	**164**	**175**	**166**	**175**	**78**	**16**	-	0.02399	0.44254
9	*H. baujardi*	179	154	168	155	163	72	24	**19**	-	0.49035
10	*C. elegans*	415	401	418	406	430	417	439	**439**	381	-

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