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Identification of Entomopathogenic Nematodes in the Steinernematidae and Heterorhabditidae (Nemata: Rhabditida)[1]

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Abstract: This paper contains taxonomic keys for the identification of species of the genera *Steinernema* and *Heterorhabditis*. Morphometrics of certain life stages are presented in data tables so that the morphometrics of species identified using the keys can be checked in the tables. Additionally, SEM photographs and diagnoses of the families and genera of Steinernematidae and Heterorhabditidae are presented.

Key words: entomopathogenic nematode, *Heterorhabditis*, Heterorhabditidae, identification, nematode, *Neosteinernema*, SEM, *Steinernema*, Steinernematidae, taxonomy.

The family Steinernematidae contains two genera, *Steinernema* Travassos, 1927 (31) and *Neosteinernema* Nguyen & Smart, 1994 (15). The family Heterorhabditidae contains one genus, *Heterorhabditis* Poinar, 1976 (18). Currently, 18 species of *Steinernema*, 1 species of *Neosteinernema*, and 7 species of *Heterorhabditis* have been described and accepted as valid. Although some authors (13,22) have constructed taxonomic keys based on both males and infective juveniles, identification to species often is attempted using infective juveniles only. Identifications based solely on infective juveniles may not be accurate because there are few differentiating morphological characteristics between species and morphometrical ranges of several species overlap. Characteristics of males and females must be used for accurate identification of most species. Recently, Nguyen and Smart (16) reported that morphometrics of *Steinernema* and *Heterorhabditis* species vary depending on the time of harvest (time after infective juveniles first appear) and whether the nematodes are reared in vitro or in vivo. These differences complicate identification in some cases. Also, structures observed with the scanning electron microscope (SEM) and the description of additional species have necessitated modification of family and generic diagnoses.

The purpose of this paper is to provide updated diagnoses of families and genera, and taxonomic keys to facilitate the identification of species. We have included SEM micrographs of females, males, and infective juveniles of *Steinernema* spp., *Neosteinernema*, and *Heterorhabditis* spp. to provide detailed illustrations of diagnostic characters. SEM micrographs of *Steinernema* spp. and *Neosteinernema longicurvicauda* are from previous publications, and the references are cited in the figure legends. SEM micrographs of *Heterorhabditis* spp. are originals; they were obtained with the method of Nguyen and Smart (17). Specimens of *Heterorhabditis bacteriophora* and *H. megidis* were obtained from H. K. Kaya and have been maintained in our laboratory on larvae of the greater wax moth, *Galleria mellonella*.

Family Steinernematidae
Figs. 1–4

Diagnosis: (After Nguyen and Smart, 1994 [15]): Alloionematoidea, Rhabditida. Obligate insect parasites. Infective juveniles carry symbiotic bacteria in the bacterial chamber of the intestine. Both males and females are necessary for reproduction.

Female (Fig. 1): Large, size variable. Cuticle smooth or annulated. Lateral fields absent. Excretory pore distinct. Head rounded or truncate, rarely offset. Six lips

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present, partly or completely fused, each lip with one labial papilla (Fig. 1A), sometimes additional papilla-like structures present near labial papillae. Four cephalic papillae. Amphids present, small. Stoma collapsed; cheilorhabdions pronounced, forming a ring resembling two large sclerotized dots in lateral view. Other parts of stoma forming an asymmetrical funnel with thick anterior end. Esophagus rhabditoid with metacorpus slightly swollen, narrow isthmus surrounded by nerve ring, and large basal bulb with reduced valve. Esophagointestinal valve usually pronounced. Reproductive system didelphic, amphidelphic, reflexed. Vulva at midbody, sometimes on a protuberance (Fig. 1B), with (Fig. 1C) or without (Fig. 1B) epiptygma. Females oviparous or ovoviviparous with juveniles developing up to the infective stage (IJ) before emerging from the body of the female. Tail longer or shorter than anal body width, with or without prominent phasmids (Figs. 1D,4B).

Male (Fig. 2): Smaller than female. Anterior end usually with six labial papillae,

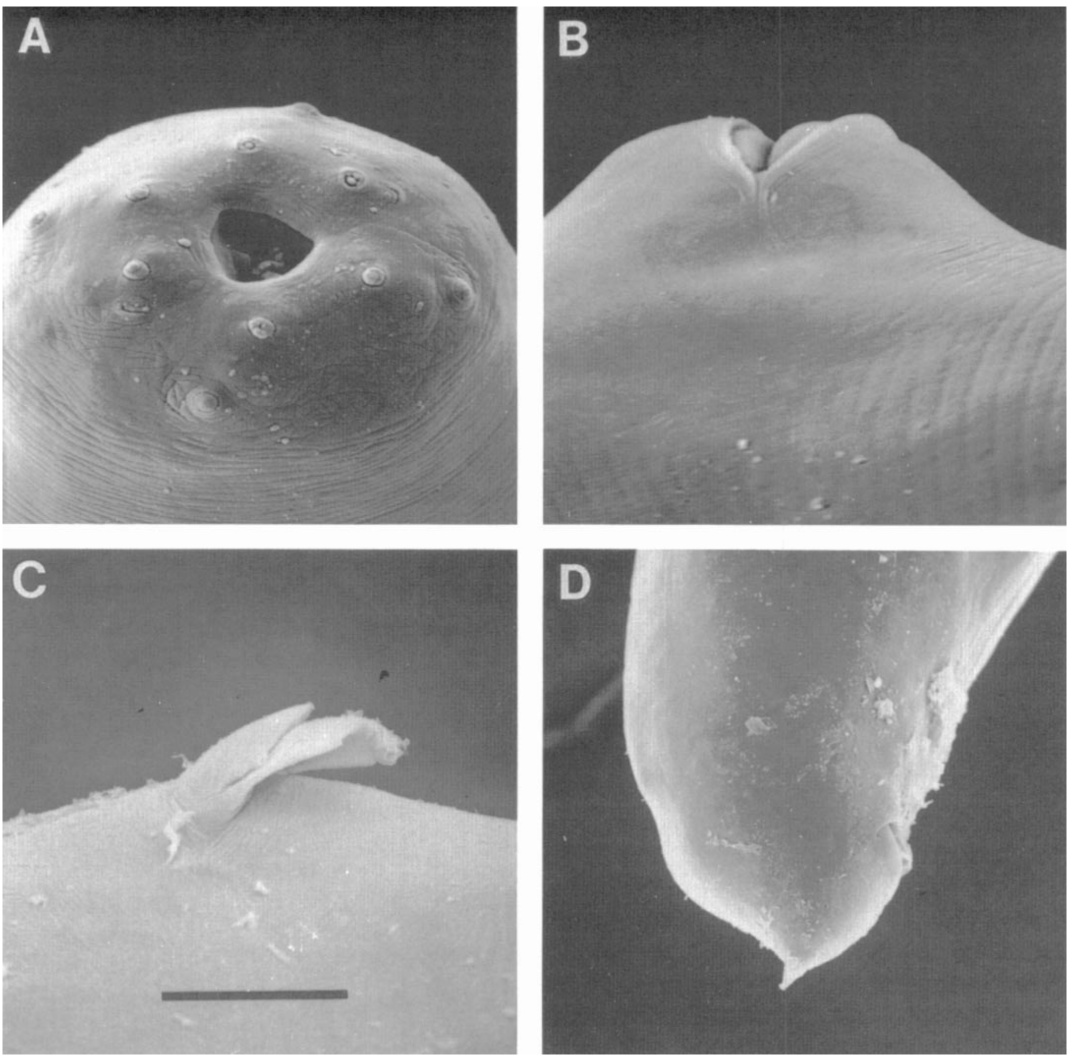

FIG. 1. *Steinernema* SEM of first-generation female. A) Face view of *Steinernema glaseri* showing cephalic papillae, labial papillae, amphids, and stoma. B) Vulva on a protuberance of *S. glaseri.* C) Vulva of *S. scapterisci* with double-flapped epiptygma. D) Tail of *S. scapterisci.* (After Nguyen and Smart [12, 17]). All magnifications based on scale bar in C: A = 13.6 μm, B = 8.6 μm, C = 15 μm, D = 38 μm.

four large cephalic papillae, and usually with perioral disc (Fig. 2A). Esophagus similar to that of female. Testis single, reflexed; spicules paired; gubernaculum long, sometimes as long as spicule (Fig. 2B,C); bursa absent. Tail tip rounded, digitate, or mucronate. One single and 10 to 14 pairs of genital papillae present with 7 to 10 pairs precloacal (Fig. 2D–F).

Infective juvenile (IJ) (= third-stage infective juvenile) (Fig. 3): Stoma collapsed. Body slender, with or without a sheath (cuticle of second-stage juvenile). Cuticle annulated. Lateral fields present with 4 to 9 incisures and 3 to 8 smooth ridges (Fig. 3B–D). Esophagus and intestine appearing reduced. Excretory pore distinct. Tail conoid or filiform (Figs. 3D,4F). Phasmids, located about mid-tail, prominent, inconspicuous, or not observed.

Type genus:	*Steinernema* Travassos, 1927
Syn.:	*Neoaplectana* Steiner, 1929
Other genus:	*Neosteinernema* Nguyen & Smart, 1994

Steinernema Travassos
Figs. 1–3

Diagnosis: Female with phasmids not observed, tail (T) shorter than anal body width (ABW) (T/ABW = 0.52 – 0.81) (Fig. 1D); oviparous but some eggs often retained in the body. Male smaller than female, posterior part usually with one single and eleven pairs of genital papillae; phasmids not observed, tail terminus rounded (Fig. 2D–F) or with mucron. Infective juvenile with phasmids small or inconspicuous, tail conoid and much shorter than esophagus (at most 65% of esophagus length), ratio c ≥ 10.

Type species: Steinernema kraussei (Steiner, 1923) Travassos, 1927. (*Steinernema kraussei* was considered the type species of the genus until 1990 when Poinar (22) designated *S. glaseri* as type species and considered *S. kraussei* as species inquirenda. In 1994 Mracek (9) redescribed *S. kraussei* from Czechoslovakia and re-established it as the type species. Recently, Reid (26), using DNA analysis of several species and isolates of *Steinernema*, constructed a phylogenetic tree that indicated that *S. kraussei* and *S. glaseri* were different species).

Neosteinernema Nguyen and Smart
Fig. 4

Diagnosis: Female with phasmids prominent, on a protuberance, located in posterior half of tail; tail longer than anal body width (T/ABW = 1.10 – 1.68) (Fig. 4B); ovoviviparous, juveniles molting and becoming infective juveniles before exiting the female body. Male smaller than female, posterior part with one ventral and 13 to 14 pairs of genital papillae, eight of the pairs preanal; phasmids prominent, tail tip digitate (Fig. 4D); spicule foot-shaped with a hump on dorsal side (Fig. 4C). Gubernaculum almost as long as spicule. Infective juveniles with head slightly swollen (Fig. 4E); phasmid large, tail elongate or filiform, as long as esophagus, usually curved at end (Fig. 4F), ratio c about 5.5.

Type and only species: Neosteinernema longicurvicauda Nguyen & Smart, 1994.

Identification of Steinernema Species

When possible, specimens used to identify species of *Steinernema* should be reared in vivo (*Galleria mellonella* or another appropriate host), and adults of the first generation should be dissected from the cadavers. Infective juveniles collected for a week after their first emergence from cadavers usually meet the criteria of the original descriptions but the body lengths of those collected after that period tend to be significantly shorter (16). All data may be obtained from either live or fixed specimens.

Species of *Steinernema* can be identified with the following key, but identity should be verified by comparing its morphomet-

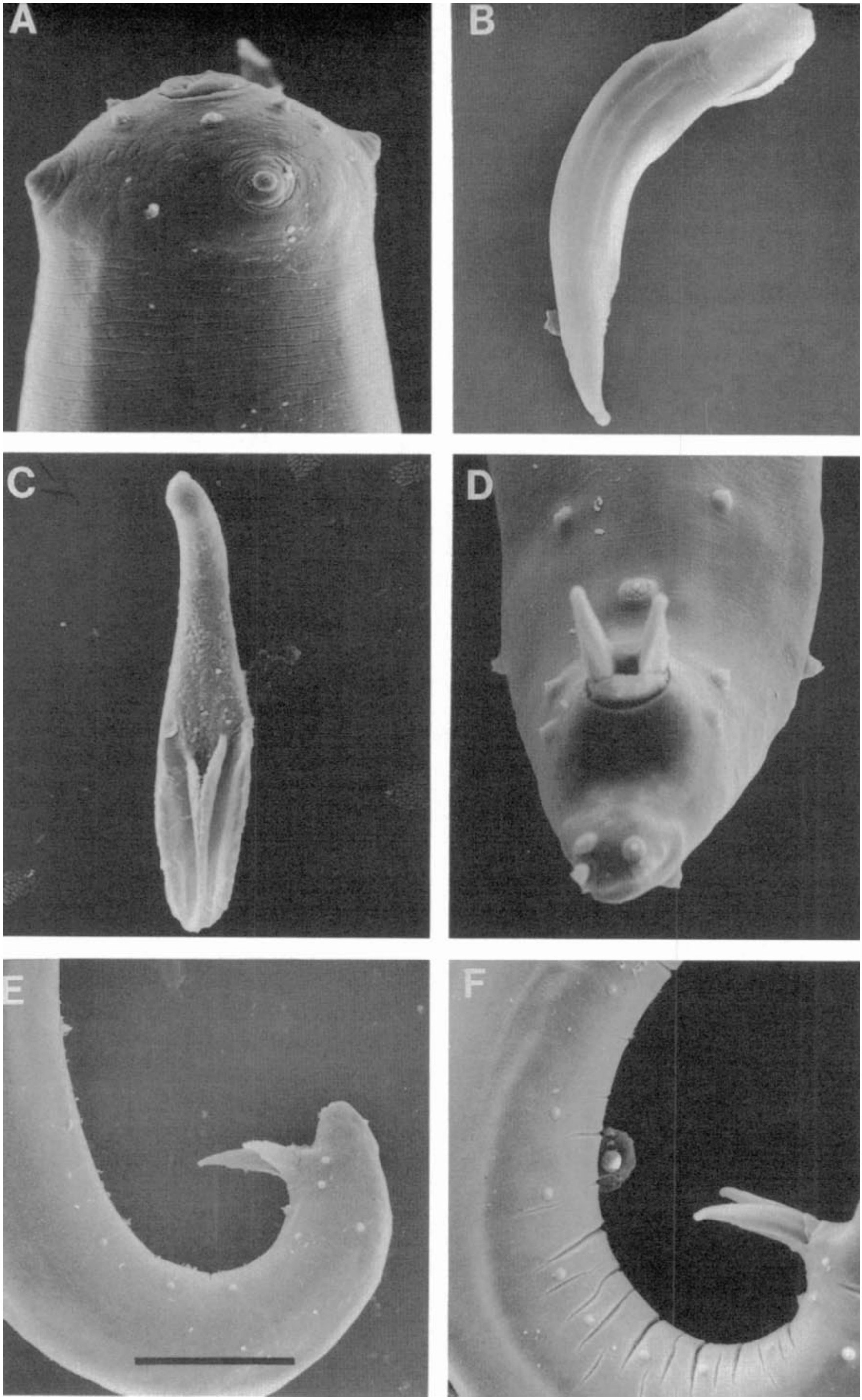

FIG. 2. *Steinernema* SEM of first-generation male. A) Anterior region of *S. glaseri* with large cephalic papillae and smaller labial papillae. B) Spicule of *S. anomali,* lateral view. C) Gubernaculum of *S. glaseri,* ventral view. D) Caudal region of *S. anomali* showing spicule and gubernaculum tips and posterior genital papillae. E,F) Posterior region of *S. anomali* showing 7 (E) to 10 (F) of the paired preanal genital papillae, the single ventral preanal papilla, and extended spicules. (After Nguyen and Smart [14, 16]). All magnifications based on scale bar in E: A = 10 μm, B = 23.1 μm, C = 17.6 μm, D = 20 μm, E = 60 μm, F = 50 μm.

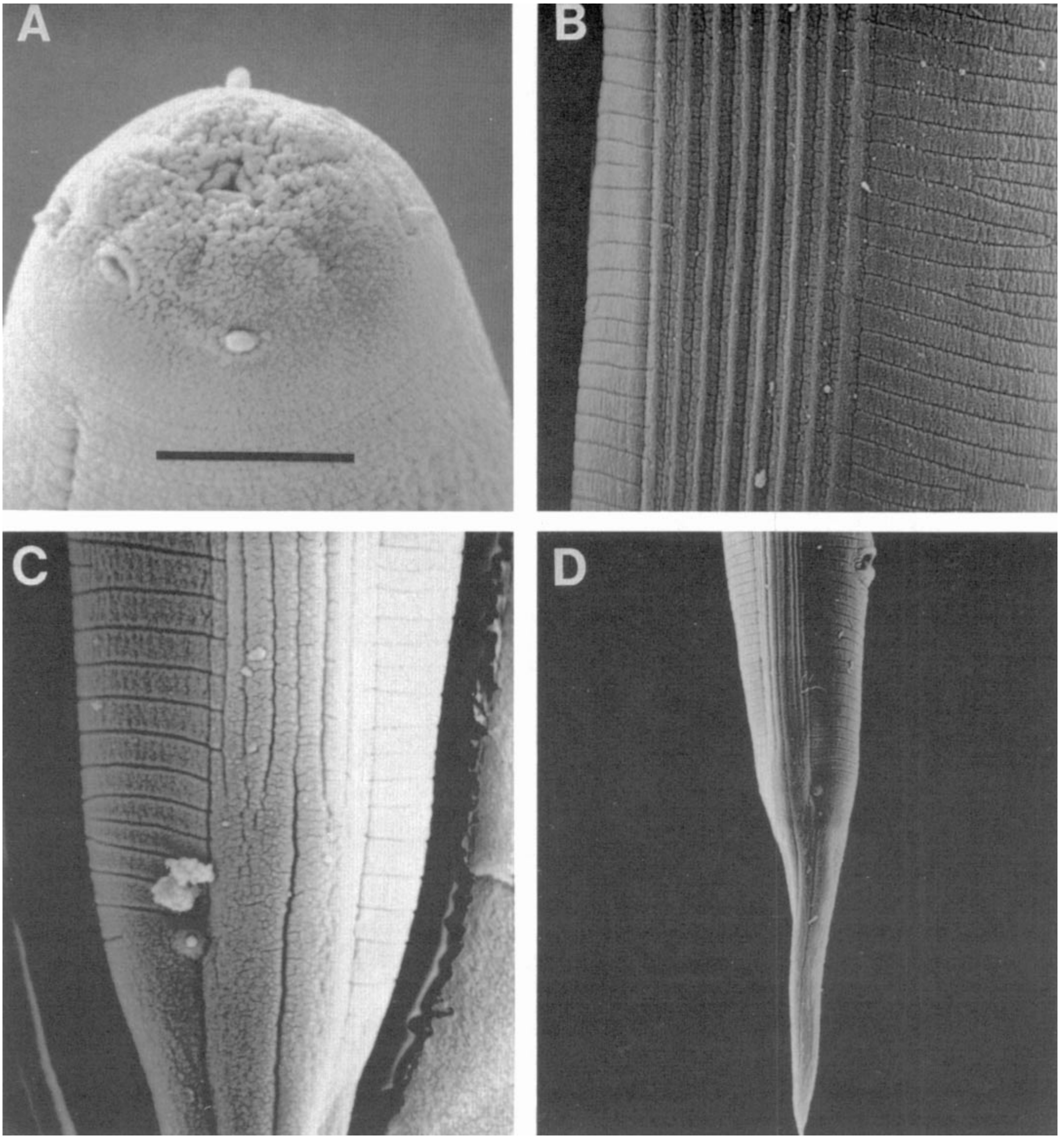

FIG. 3. *Steinernema glaseri* SEM of infective juvenile. A) Anterior region with four cephalic papillae, one of two amphids, stoma, and the beginning of the lateral field. B) Lateral field with eight ridges. C) Lateral field at phasmid level showing eight ridges becoming two large, smooth bands. D) Tail showing anus, and phasmid near midtail. (After Nguyen and Smart [17]). All magnifications based on scale bar in A: A = 6 μm, B = 12 μm, C = 7.5 μm, D = 25 μm.

rics with the data from original descriptions listed in Tables 1 and 2, and from original descriptions, if possible.

Ratios and abbreviations used in the following key are: D% = EP/ES × 100 (EP = distance from anterior end to excretory pore; ES = esophagus length); E% = EP/T × 100 (EP = distance from anterior end to excretory pore; T = tail length); IJ = infective juvenile; SW = spicule length divided by anal body width.

KEY TO SPECIES OF THE GENUS STEINERNEMA

1. Infective juvenile (IJ) with a double horn-like structure on labial region ---------- *S. bicornutum* Tallosi et al. 1995 (30)

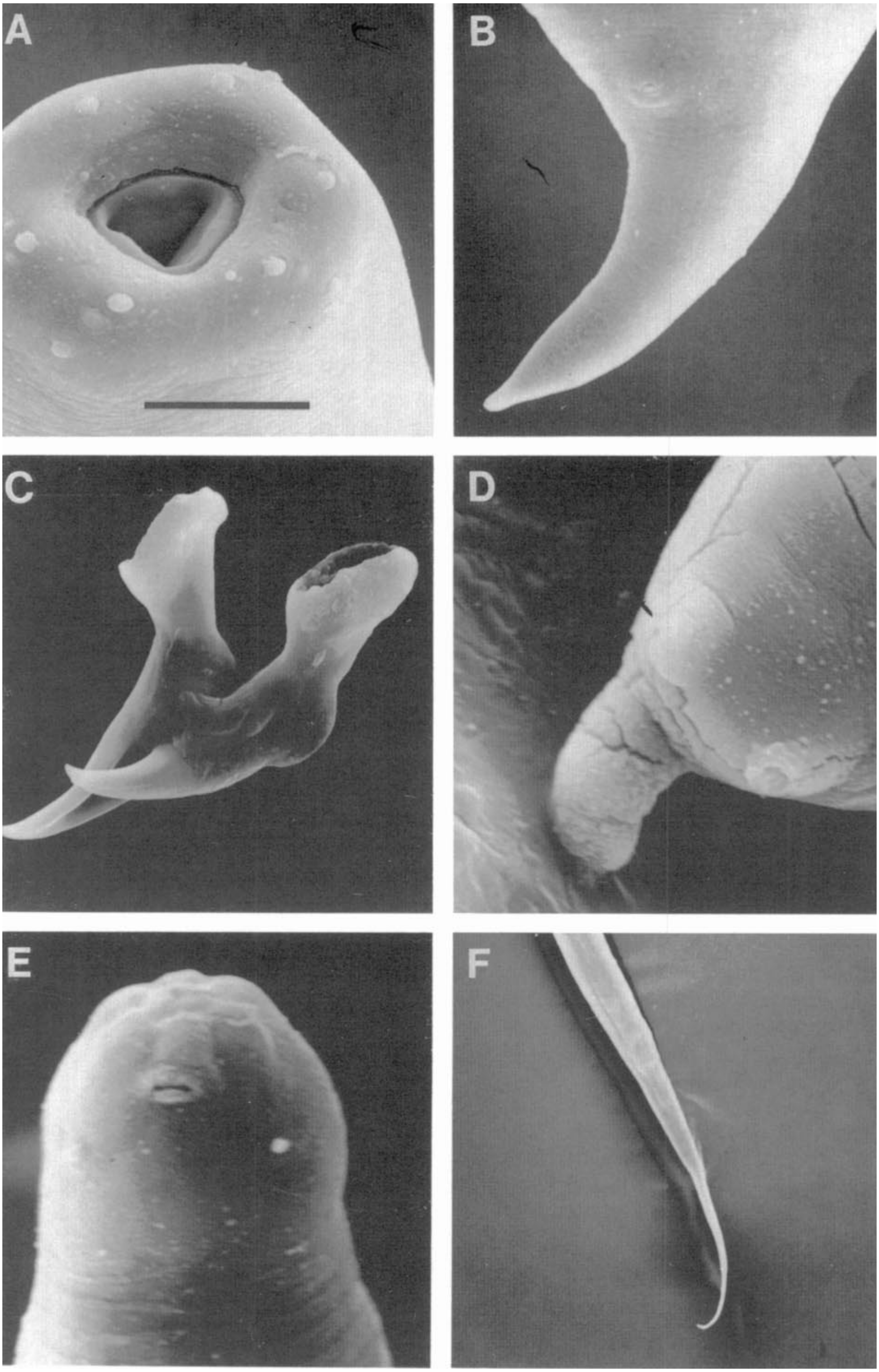

FIG. 4. *Neosteinernema longicurvicauda* SEM. A) Female face view. B) Female tail with prominent phasmid. C) Spicules with a hump on dorsal side. D) Male tail with digitate terminus and prominent phasmid. E) Third-stage infective juvenile head. F) Infective juvenile tail. (After Nguyen and Smart [15]). All magnifications based on scale bar in A: A = 12 μm, B = 17.6 μm, C = 20.1 μm, D = 5.1 μm, E = 3 μm, F = 43 μm.

TABLE 1. Morphometrics (in µm) of third-stage infective juveniles of *Steinernema* and *Neosteinernema* species (in descending order of body length).

SP[b]	Morphometric character[a] (Range)										
	L	W	EP	NR	ES	T	a	b	c	D%	E%
					Steinerema						
CUB[c]	1,283	37	106	116	148	67	35	8.6	19.2	70	160
	(1,149–1,508)	(33–46)	(101–114)	(106–130)	(135–159)	(61–77)	—	—	—	—	—
PUE[c]	1,171	51	95	117	143	94	23	8.2	12.4	66	101
	(1,057–1,238)	(47–54)	(90–102)	(111–121)	(138–147)	(88–107)	(20–24)	(7.4–8.6)	(11.6–13.6)	(662–74)	(88–108)
GLA[d]	1,130	43	102	120	162	78	29	7.3	14.7	65	131
	(864–1,448)	(31–50)	(87–110)	(112–126)	(158–168)	(62–87)	(26–35)	(6.3–7.8)	(13.6–15.7)	(58–71)	(122–138)
LON[c]	1,063	40	81	107	145	95	27	7.3	11.2	56	85
	—	—	—	—	—	—	—	—	—	—	—
ANO[d]	1,034	46	83	109	138	75	26	7.6	13.8	55	119
	(724–1,408)	(28–77)	(76–86)	(100–120)	(123–160)	(64–84)	(17–34)	(5.9–10.8)	(9.4–16.9)	(52–59)	(106–130)
KRA[c]	951	33	63	105	134	79	29	7.1	12.1	47	80
	(797–1,102)	(30–36)	(56–66)	(99–111)	(119–145)	(63–86)	—	—	—	—	—
NEO[c]	885	34	18	107	144	80	26	6.1	11.2	12	23
	(741–988)	(28–42)	(14–22)	(100–119)	(130–159)	(64–97)	(22–29)	(5.4–6.7)	(9.1–14.2)	(10–15)	(18–30)
FEL[d]	849	26	62	99	136	81	31	6.0	10.4	45	78
	(736–950)	(22–29)	(53–67)	(88–112)	(115–150)	(70–92)	(29–33)	(5.3–6.4)	(9.2–12.6)	(42–51)	(69–86)
BIC[c]	769	29	61	92	124	72	27	6.2	10.7	50	84
	(648–873)	(25–33)	(53–65)	(88–100)	(113–135)	(63–78)	(23–29)	(5.6–6.9)	(9.7–12)	(40–60)	(80–100)
AFF[c]	693	30	62	95	126	66	23	5.5	10.5	49	94
	(608–880)	(28–34)	(51–69)	(88–104)	(115–134)	(64–74)	(21–28)	(5.1–6.0)	(9.5–11.5)	(43–53)	(74–108)
INT[d]	671	29	65	93	123	66	23	5.3	10.0	51	96
	(608–800)	(25–32)	(59–69)	(85–99)	(110–133)	(53–74)	(20–26)	(5.0–6.0)	(9.3–10.8)	(48–58)	(89–108)
RIO[c]	622	28	56	87	114	54	23	5.4	11.6	49	105
	(561–701)	(26–30)	(51–64)	(84–89)	(109–116)	(46–59)	(20–24)	(4.9–6.0)	(10.1–12.4)	(45–55)	(93–111)
KUS[c]	589	26	46	76	111	50	22.5	5.3	11.7	41	92
	(524–662)	(22–31)	(42–50)	(70–84)	(106–120)	(44–59)	(19–25)	(4.9–5.9)	(9.9–12.9)	(38–44)	(84–95)
SCA[c]	572	24	39	97	127	54	24	4.5	10.7	31	73
	(517–609)	(18–30)	(36–48)	(83–106)	(113–134)	(48–60)	(20–31)	(4.0–4.6)	(9.2–11.7)	(27–40)	(60–80)
CAR[d]	558	25	38	85	120	53	21	4.4	10.0	26	60
	(438–650)	(20–30)	(30–60)	(76–99)	(103–190)	(46–61)	(19–24)	(4.0–4.8)	(9.1–11.2)	(23–28)	(54–66)
RAR[d]	511	23	38	70	102	51	23	4.7	9.8	35	72
	(443–573)	(18–26)	(32–40)	(60–88)	(89–120)	(44–56)	(20–26)	(4.1–5.6)	(8.7–11.0)	(30–39)	(63–80)
RIT[c]	510	21.5	43	73	91.5	49	24.1	5.5	10.6	46	88
	(470–590)	(19–24)	(40–46)	(68–85)	(85–95)	(44–54)	(19–31)	(4.9–6.3)	(9.2–13.1)	(44–50)	(79–97)
					Neosteinernema						
LONC[c]	926	24	68	107	164	167	39	5.6	5.5	41	41
	(789–1,084)	(20–31)	(61–76)	(92–125)	(144–188)	(141–190)	(30–46)	(5.0–7.0)	(4.7–6.5)	(38–46)	(37–48)

[a] L = length; W = greatest width; EP = distance from anterior end to excretory pore; NR = distance from anterior end to nerve ring; ES = esophagus length; T = tail length; a = L/W; b = L/ES; c = L/T; D% = EP/ES × 100; E% = EP/T × 100.
[b] SP = species: AFF = *affinis;* ANO = *anomali;* BIC = *bicornutum;* CAR = *carpocapsae;* CUB = *cubana;* FEL = *feltiae;* GLA = *glaseri;* INT = *intermedia;* KRA = *kraussei;* *KUS* = *kushidai;* LONC = *longicurvicauda;* LON = *longicaudum;* NEO = *neocurtillis;* PUE = *puertoricensis;* RAR = *rara;* RIO = *riobravis;* RIT = *ritteri;* SCA = *scapterisci.*
[c] After original author cited in table 2.
[d] After Poinar (22).
— No data available.

TABLE 2. Morphometrics (in μm) of first-generation males used for the identification of *Steinernema* and *Neosteinernema* species (in descending order of spicule length).

Species	Criteria[a] (Range)							N	Reference
	Spicule	Gubern	W	D%	SW	GS	MUC		
				Steinernema					
intermedia	91 (84–100)	64 (56–75)	168 (113–207)	67 (58–76)	1.24 (1.03–1.39)	0.69 (0.62–0.77)	a	10	(13)
anomali	84 (81–91)	55 (49–60)	188 (184–219)	93 (88–102)	2.10 —	0.65 (0.60–0.66)	a	10	(5)
scapterisci	83 (72–92)	65 (59–75)	156 (97–213)	38 (32–44)	2.52 (2.04–2.80)	0.78 (0.69–0.84)	p	10	(11)
puertoricensis	78 (71–88)	40 (36–45)	101 (67–148)	77 —	1.52 —	0.51 —	a	10	(27)
glaseri	77 (64–90)	55 (44–59)	72 (54–92)	70 (60–78)	2.05 (1.64–2.43)	0.71 (0.64–0.85)	a	10	(13)
longicaudum	77 —	48	155 —	62 —	1.60 —	0.62	a	10	(28)
affinis	70 (67–86)	46 (37–56)	118 (95–164)	61 (60–66)	1.17 —	0.66 —	p	10	(21)
feltiae	70 (65–77)	41 (34–47)	75 (60–90)	60 (51–64)	1.13 (0.99–1.30)	0.59 (0.52–0.61)	p	25	(19)
ritteri	69 (58–75)	44 (33–50)	130 (110–176)	47 (44–50)	1.56 (1.44–1.57)	0.64 (0.57–0.67)	a	30	(3)
riobravis	67 (62.5–75)	51 (47.5–56.2)	133 (116–159)	71 (60–80)	1.14 —	0.76 —	a	10	(1)
carpocapsae[b]	66 (58–77)	47 (39–55)	101 (77–130)	41 (27–55)	1.72 (1.40–2.00)	0.71 (0.59–0.88)	p	60	(12)
bicornutum	65 (53–70)	48 (38–50)	109 (80–127)	52 (50–60)	2.22 (2.18–2.26)	0.72 —	a	20	(30)
kushidai	63 (48–72)	44 (39–60)	97 (75–156)	51 (42–59)	1.50 —	0.70 —	a	20	(8)
cubana	58 (50–67)	39 (37–42)	97 (77–117)	70 —	1.41 —	0.67 —	a	20	(10)
neocurtillis	58 (52–64)	52 (44–59)	111 (77–144)	19 (13–26)	1.43 (1.18–1.64)	0.89 (0.82–0.93)	p	10	(13)
kraussei	49 (42–53)	33 (29–37)	128 (110–144)	53 —	1.10 —	0.67 —	p	?	(9)
rara	47 (42–52)	34 (23–38)	123 (100–142)	50 (44–51)	0.94 (0.91–1.05)	0.71 (0.55–0.73)	p	20	(2)
				Neosteinernema					
longicurvicauda	61 (52–67)	59 (52–66)	97 (67–140)	44 (30–54)	103 (0.80–1.50)	0.97 (0.84–1.08)	a	25	(15)

[a] Gubern = gubernaculum; W = greatest body width; D% = distance from anterior end to excretory pore divided by esophagus length × 100; SW = spicule length divided by anal body width; GS = gubernaculum length divided by spicule length; MUC = mucron: a = absent, p = present; N = number of specimens measured.
[b] Measurements from 10 males each of six satrains: Agriotos, All, Breton, DD-136, Italian, and Mexican.
— Data not available.

IJ without a double horn-like structure on labial region ---------- 2
2. Body length of infective juvenile (IJ) >800 μm ---------- 3
Body length of IJ < 800 μm ---------- 10
3. Average length of IJ greater than 1,000 μm (1,034–1,171); male tail without mucron ---------- 4
Average length of IJ less than 1,000 μg (849–951); male tail with or without mucron ---------- 8
4. Tail of IJ less than 85 μm (67–78); E% > 106 (119–160); female without epiptygma ---------- 5
Tail of IJ averaging more than 90 μm (95–95); E% < 105 (85–101); female with or without double-flapped epiptygma ---------- 7
5. SW ratio about 1.4, spicule about 58 μm (50–67), E% about 160 ---------- *S. cubana* Mracek et al., 1994 (10)
SW ratio about 2.0, spicule length >72 μm (77–84); E% less than 140 ---------- 6
6. In IJ, distance from anterior end to excretory pore 76–86 μm; in male, D% about 93 (88–102), spicule 84 μm long with tip swollen ---------- *S. anomali* (Kozodoi, 1984) Poinar and Kozodoi, 1988 (5, 25)
In IJ, distance from anterior end to excretory pore 87–110 μm; in male, D% about 70 (60–78), spicule 77 μm long, spicule tip with large aperture resembling a notch ---------- *S. glaseri* (Steiner, 1929) Wouts et al. 1982 (33)
7. E% averaging 101 (88–108); in male D% about 77 ---------- *S. puertoricensis* Roman & Figueroa, 1994 (27)
E% averaging 85 (range not known); in male D% about 62 ---------- *S. longicaudum* Shen & Wang, 1991 (28)
8. In IJ, distance from anterior end to excretory pore extremely short, 18 μm (14–22), E% = 23; in male, D% averaging 19 (13–25) ---------- *S. neocurtillis* Nguyen & Smart, 1992 (13)
In IJ, distance from anterior end to excretory pore 53–67 μm, E% about 80; in male, D% > 50 ---------- 9
9. IJ body length averaging 951 μm (797–1,102); spicule length about 49 μm (42–53) ---------- *S. kraussei* (Steiner, 1923) Travassos, 1927 (9, 31)
IJ body length averaging 849 μm (736–950); spicule length about 70 μm (65–77) ---------- *S. feltiae* (= *bibionis*) (Filipjev, 1934) Poinar, 1990 (22)
10. Average length of IJ > 600 μm (622–693) ---------- 11
Average length of IJ < 600 μm (510–589) ---------- 13
11. Spine-like structure inside IJ tail tip; mucron present ---------- *S. affinis* (Bovien, 1937) Poinar, 1990 (21)
No spine-like structure inside IJ tail tip; mucron absent ---------- 12
12. Spicule length about 93 μm (80–106); E% about 94 (74–108) ---------- *S. intermedia* (Poinar, 1985) Poinar, 1990 (20, 22)
Spicule length about 67 μm (63–75); E% about 105 (93–111) ---------- *S. riobravis* Cabanillas et al., 1994 (1)
13. Average body length of IJ about 510 μm ---------- 14
Average body length of IJ > 540 μm ---------- 15
14. First-generation male without mucron; spicule length 69 μm (58–75), SW = 1.56 (1.44–1.57); in IJ, E% averaging 88 ---------- *S. ritteri* Doucet & Doucet, 1990 (3)
First-generation male with mucron; spicule length 47 μm (42–52), SW = 0.94 (0.91–1.05); in IJ, E% averages 72 ---------- *S. rara* (Doucet, 1986) Poinar, 1990 (2, 22)
15. First-generation male without mucron; in IJ, E% averaging 92 ---------- *S. kushidai* Mamiya, 1988 (8)
First-generation male with mucron; in IJ, E% less than 92 ---------- 16
16. In IJ, E% averaging 73; in male, SW ratio averaging 2.52 (2.04–2.80), spicule length 83 μm (72–92); female with large, double-flapped epiptygma ---------- *S. scapterisci* Nguyen & Smart, 1990 (11)
In IJ, E% averaging 60; in male, SW ratio averaging 1.72 (1.40–

2.00), spicule length 66 μm (58–77); double-flapped epiptygma rarely present ---------*S. carpocapsae* (Weiser, 1955) Poinar, 1990 (22)

Note: Steinernema caudatum was described by Xu and Wang, 1991 in Chinese. We have been unable to obtain a translation of the publication and cannot include the species in this key.

Based on data in Tables 1 and 2, *Steinernema puertoricensis* and *S. longicaudum* are closely related. Cross-hybridization studies need to be performed to clarify the situation.

Family Heterorhabditidae
Fig. 5

Diagnosis (emended): Rhabditoidea, Rhabditida. Obligate insect parasites. Infective juveniles carrying symbiotic bacteria. Both hermaphroditic and amphimictic females present.

Hermaphroditic female (Fig. 5A,B): After entry into an insect host, infective juveniles developing into hermaphroditic females. Head truncate to slightly rounded, six conical lips well developed (Fig. 5A), separate, each with a terminal papilla; one or two small raised structures sometimes visible at the base of each lip; amphidial opening small. Stoma wide but shallow; cheilorhabdions present, forming a ring, in lateral view resembling two refractile dots. Other parts of the stoma fused to form a collapsed posterior portion. Posterior part of stoma covered by esophagus. Esophagus without metacorpus; isthmus slender; basal bulb swollen; valve in basal bulb reduced. Nerve ring at middle of isthmus. Excretory pore usually posterior to end of esophagus. Vulva median, slit-like, surrounded by elliptical rings (Fig. 5B); ovotestis amphidelphic, reflexed. Oviparous, later becoming ovoviviparous. Tail pointed, longer than anal body width, postanal swelling usually present.

Amphimictic female (Fig. 5C): Similar to, but usually smaller than, hemaphroditic female; labial papillae prominent. Reproductive system amphidelphic, vulva not functional for egg deposition, but functional for mating.

Male (Fig. 5D–F): Testis one, reflexed. Spicules paired, separate, slightly curved ventrally (Fig. 5E). Spicule head short, offset from lamina by a constriction. Gubernaculum (Fig. 5F) usually about half as long as spicule length. Bursa peloderan (Fig. 5D) with nine pairs of genital papillae.

Infective juvenile (Fig. 5G–I): Third-stage infective juvenile (IJ) usually with sheath (cuticle of second-stage juvenile). Sheath with anterior tessellate pattern (Fig. 5G) and longitudinal ridges (Fig. 5H); IJ cuticle striated with one smooth band marginated by two ridges in lateral fields. Head with prominent dorsal tooth (Fig. 5I). Mouth and anus closed. Stoma appearing as a closed chamber with parallel walls. Esophagus and intestine reduced. Excretory pore posterior to nerve ring. Symbiotic bacterial cells found in intestine. Tail pointed.

Type and only genus: *Heterorhabditis* Poinar, 1976 *Heterorhabditis* Poinar

Type species: *Heterorhabditis bacteriophora* Poinar, 1976

Diagnosis: As for family.

Identification of Heterorhabditis Species

Currently the genus contains seven species. Species are identified based mainly on morphometrics of the IJ, but the following key uses a combination of characteristics of both the IJ and male. After a species is identified using the key, the identity should be verified by comparing its morphometrics with the data from original descriptions listed in Tables 3 and 4, or from original descriptions, if possible.

Ratios and abbreviations used in the following key are: E% = EP/T × 100 (EP = distance from anterior end to excretory

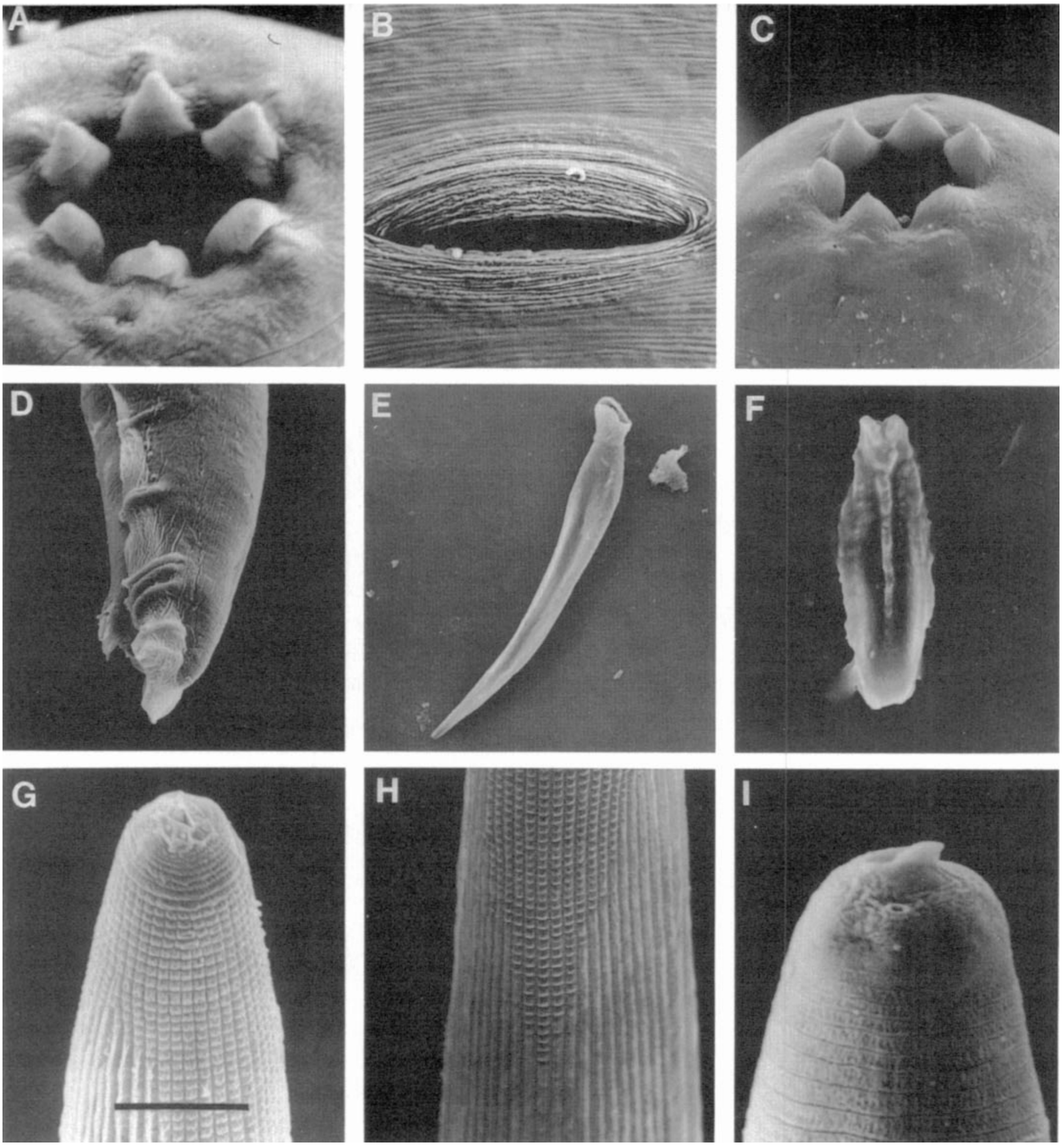

FIG. 5. *Heterorhabditis* spp. SEM: A,B,D,E,I) *H. bacteriophora.* A) Face view of hermaphroditic female. B) Vulval region of hermaphroditic female showing elliptical rings around vulva. D) Male posterior region showing bursa and genital papillae. E) Spicule, lateral view. I) IJ (without sheath) anterior region with prominent dorsal tooth and amphid. C,F,G,H) *H. megidis.* C) Face view of amphimictic female. F) Gubernaculum, ventral view. G) Anterior region of a third-stage infective juvenile (IJ) in cuticle of second-stage juvenile, showing anterior tessellate structure. H) Body of IJ (with sheath), showing longitudinal ridges and termination of tessellate pattern. All magnifications based on scale bar in G: A = 5 μm, B = 8.6 μm, C = 6 μm, D = 15 μm, E = 15 μm, F = 8.6 μm, G = 8.6 μm, H = 10 μm, I = 3.8 μm.

pore; T = tail length); GS% = gubernaculum length divided by spicule length × 100; IJ = infective juvenile.

KEY TO SPECIES OF THE GENUS HETERORHABDITIS

1. Average body length of IJ > 700 μm (736–800)......*megidis* Poinar et al., 1987 (23)
   Average body length of IJ < 700 μm (528–685)........................ 2
2. IJ tail short, averaging 76 μm (68–80), E% about 147 ....*brevicaudis* Liu, 1994 (6)

TABLE 3. Morphometrics (μm) of third-stage infective juveniles of *Heterorhabditis* species (in descending order of infective juvenile length)

	Morphometric character[a] (Range)										
SP[b]	L	W	EP	NR	ES	T	a	b	c	D%	E%
MEG[c]	768	29	131	109	155	119	26	5.0	6.5	85	110
	(736–800)	(27–32)	(123–142)	(104–115)	(147–160)	(112–128)	(23–28)	(4.6–5.9)	(6.1–6.9)	(81–91)	(103–120)
ZAE[c]	685	27	112	100	140	102	25	4.9	6.6	80	108
	(570–740)	(22–30)	(94–123)	(90–107)	(135–147)	(87–119)	(24–26)	(4.2–5.0)	(6.2–6.7)	(70–84)	(103–109)
ARG[d]	657	31	107	95	132	84	21	5	7.8	81	127
	(610–710)	(24–38)	(68–122)	(82–116)	(101–150)	(70–105)	—	—	—	—	—
MAR[d]	654	28	102	99	133	107	24	4.9	6.1	77	96
	(588–700)	(24–32)	(81–113)	(83–113)	(121–139)	(99–117)	(21–29)	(4.7–5.4)	(5.5–6.6)	(60–86)	(89–110)
BAC[c]	588	23	103	85	125	98	25	4.5	6.2	84	112
	(512–671)	(18–31)	(87–110)	(72–93)	(100–139)	(83–112)	(17–30)	(4.0–5.1)	(5.5–7.0)	(76–92)	(103–130)
HAW[d]	575	25	114	92	133	90	23	4.3	6.4	86	127
	(506–631)	(21–28)	(95–132)	(79–103)	(115–181)	(82–108)	—	—	—	—	—
BRE[d]	572	22	111	101	124	76	26	4.6	7.6	90	147
	(528–632)	(20–24)	(104–116)	(96–104)	(120–136)	(68–80)	—	—	(6.6–8.6)	—	—
IND[d]	528	20	98	82	117	101	26	4.5	5.3	84	94
	(479–573)	(19–22)	(88–107)	(72–85)	(109–123)	(93–109)	(25–27)	(4.3–4.8)	(4.5–5.6)	(79–90)	(83–103)

[a] L = length; W = greatest width; EP = distance from anterior end to excretory pore; NR = distance from anterior end to nerve ring; ES = esophagus length; T = tail length; a = L/W; b = L/ES; c = L/T; D% = EP/ES × 100; E% = EP/T × 100.

[b] Sp = species: ARG = *argentinensis;* BAC = *bacteriophora;* BRE = *brevicaudis;* HAW = *hawaiiensis;* IND = *indicus;* MAR = marelatus; MEG = *megidis;* ZEA = *zealandica.*

[c] After Poinar (22).

[d] After the original author.

— No data available.

TABLE 4. Morphometrics (μm) of males used for the identification of *Heterorhabditis* species.

	Criteria[a] (Range)						
Species	Spicule	Gubern	W	D%	GS%	N	Reference
argentinensis	46 (42–49)	23 (20–26)	56 (42–70)	138	50	25	(29)
bacteriophora	40 (36–44)	20 (18–25)	43 (38–46)	117	50	15	(18)
brevicaudis	47 (44–48)	22 (20–24)	43 (40–48)	88	47	20	(6)
hawaiiensis	47 (40–51)	22 (18–26)	63 (49–84)	110	47	20	(4)
indicus	43 (35–48)	21 (18–23)	42 (35–46)	122	49	12	(24)
megidis	49 (46–54)	21 (17–24)	47 (44–50)	122	43	15	(23)
marelatus	45 (42–50)	19 (18–22)	51 (48–56)	113	41	?	(7)
zealandica	51 (48–55)	22 (19–25)	41 (36–45)	118	43	15	(32)

[a] Gubern = gubernaculum; W = greatest body width; D% = distance from anterior end to excretory pore divided by esophagus length × 100; GS% = gubernaculum length divided by spicule length multiplied by 100 (calculated from original author's measurements); N = number of specimens measured.

IJ tail longer, averaging > 80 μm (84–119), E% = 127 or less 3

3. IJ body length averaging > 600 μm 4

   IJ body length averaging < 600 μm 6

4. In IJ, E% > 120, c > 7; lamina of spicule with ventral expansion ... *argentinensis* Stock, 1993 (29)

   In IJ, E% < 120, c < 7; lamina of spicule without ventral expansion 5

5. IJ body length averaging 654 μm, E% about 96, c about 6.1; male body width averaging 51 μm, spicule length averaging 45 μm ..... *marelatus* Liu & Berry, 1996 (7)

   IJ body length averaging 685 μm, E% about 108, c about 6.6; male body width averaging 41 μm, spicule length averaging 51 μm ..... *zealandica* (Wouts, 1979) Poinar, 1990 (22, 32)

6. IJ body length averaging 528 μm, E% about 94 .... *indicus* Poinar et al., 1992 (24)

   IJ body length averaging 570 μm, E% > 100 7

7. E% of IJ about 127; spicule averaging 47 μm, lamina with ventral expansion ...... *hawaiiensis* Gardner et al. 1994 (4)

   E% of IJ about 112, spicule averaging 40 μm, lamina without ventral expansion .... *bacteriophora* Poinar, 1976 (18)

To identify species of *Steinernema* and *Heterorhabditis* the following should be considered:

(i) IJ morphometrics usually are insufficient for species identification, and male and female characteristics must be considered.

(ii) IJ produced on artificial media (laboratory reared or commercial products) are shorter (rarely longer) than those produced in vivo (16), and usually do not meet the criteria of the original description. Males and females collected 4 or 5 days after the host dies, and IJ collected for one week after they first appear from cadavers, usually meet original species descriptions.

(iii) Measurements of at least 10 individuals should be obtained before trying to identify the species.

(iv) Morphological and morphometric characteristics of different stages of the identified nematode should be verified with the original description (morphometrics are listed in Tables 1–4) to confirm the identification.

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