
IDENTIFICATION TO ENTOMOPATHOGENIC NEMATODE SPECIES OF THE GENUS *STEINERNEMA*^{1/}

Khuong B. Nguyen^{2/}

INTRODUCTION: The genus *Steinernema* contains entomopathogenic nematode species which are important in controlling several noxious insects, especially those having cryptic habits and inhabiting soil. Steinernematid nematodes have acquired great economic importance in agriculture because they have been used as the active component of several biotic insecticides. The Florida Department of Agriculture and Consumer Services is involved in the evaluation of the purity, viability, and identification of biological agents in biotic insecticides marketed in the State. The purpose of this circular is to assist nematologists in the identification of species in the genus *Steinernema*.

TAXONOMY STATUS: The genus *Steinernema*, family Steinernematidae, order Rhabditida, was erected by Travassos in 1927 (4) for the species *Aplectana kraussei* which Steiner had described in 1923 (2) from a sawfly, *Cephalaea abietis*. Another genus in the family, *Neoaplectana*, described by Steiner in 1929 (3), was considered a junior synonym of *Steinernema* by Wouts *et al.* in 1982 (5). Currently, the genus *Steinernema* contains 11 species.

MORPHOLOGY AND LIFE CYCLE: *Steinernema* adults are found in association with a dead insect. Female (Fig. 1A, Fig. 2A-C,G) large with collapsed stoma (Fig. 2A). Male (Fig. 1B-F, Fig. 2D) much smaller than female; with collapsed stoma; and unique spicules (Fig. 1C,D, Fig. 2D) and gubernaculum (Fig. 1E,F, Fig. 2D) for the genus. Third-stage infective juvenile (Fig. 2E,F,H) with a sheath (sometimes lost); stoma absent; esophagus degenerate. The nematode life cycle is always associated with a symbiotic bacterium (*Xenorhabdus* sp.). The third-stage infective juveniles (IJ) enter the hemocoel of an insect, and release a pellet of bacteria into the hemolymph. The bacteria multiply rapidly and kill the insect by septicemia in about 24 hours. The IJ change into the feeding form and feed on bacteria. Fourth stage juveniles and first generation adults appear after 3-5 days. The adults mate and eggs are laid. First-stage juveniles (J1) emerge from the eggs. J1 molt to the J2 and then to the third-stages IJ which retain the J2's cuticle. The IJ leave the cadaver and seek a new host to repeat the life cycle.

IMPORTANT IDENTIFICATION CRITERIA: The following morphological parameters must be calculated before using the key for identification.

Infective juveniles (IJ): 1) Body length (the sheath must be disregarded). 2) Distance from anterior end to excretory pore (EP). 3) Tail length. 4) $E\% = EP \text{ divided by tail length} \times 100$.

First generation males: 1) Esophagus length. $D\% = EP \text{ divided by esophagus length} \times 100$. 3) Spicule and gubernaculum length (proper care should be used in measuring the gubernaculum because the distal end of gubernaculum is thin, closely associated with spicules and difficult to see). 3) The following ratios:

EW = EP/Width of body at excretory pore

SW = Spicule length/Width of body at cloaca

GS = Gubernaculum length/Spicule length

First-generation males can be obtained by exposing 2-10 wax moth (*Galleria* sp.) larvae (or any caterpillar) to about 20,000 IJ in a petri dish lined with two pieces of filter paper saturated with water. Nematode males and IJ can be found in the larval cadavers 2-3 and 7 days after the insects' death, respectively.

¹ Contribution No. 447, Bureau of Entomology, Nematology, and Plant Pathology - Nematology Section., P.O. Box 147100, Gainesville, FL 32614-7100

² Nematologist, Dept. of Entomology & Nematology, IFAS, University of Florida, Gainesville, FL 32611

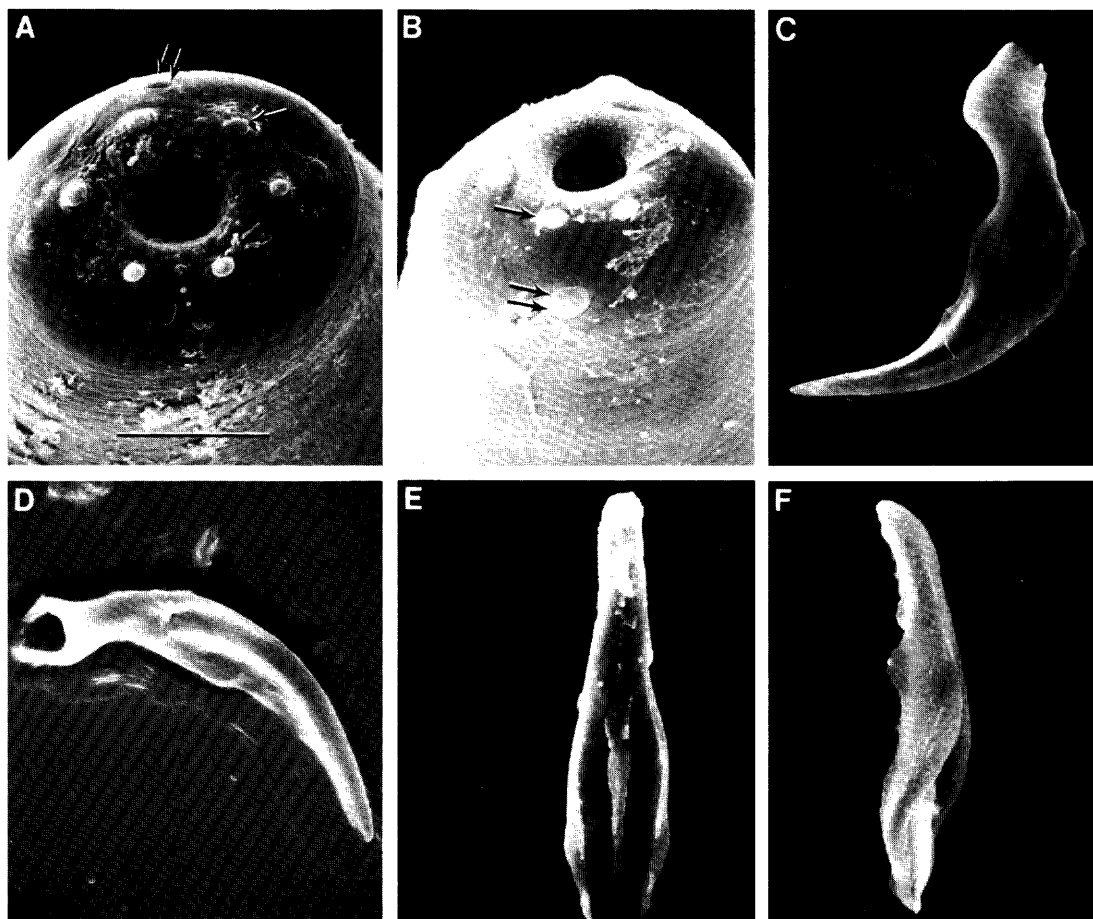


Fig. 1. SEM photographs of some structures of *Steinernema scapterisci* female and male. A) Female head showing four cephalic papillae (double arrows) and six labial papillae (single arrow). B) Male head showing four cephalic papillae (double arrows) and six labial papillae. C, D) Spicules, variation in shape. E, F) Gubernacula, ventral and lateral view respectively.

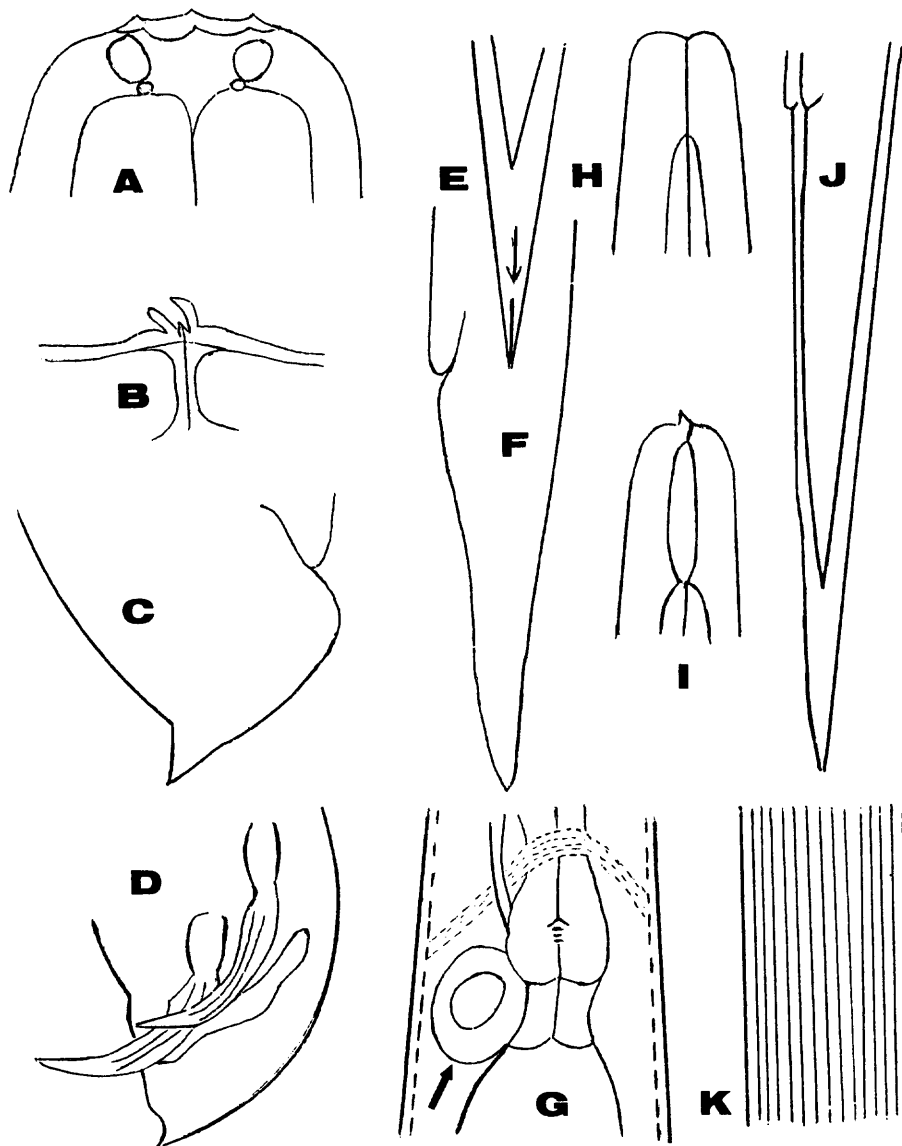


Fig. 2. A-H) Generalized anatomy of *Steinernema*. A) Female head with stomal structure. B) Double-flapped epiptygma. C) First-generation female tail. D) Male tail. E) Infective juvenile (IJ) tail of the species *S. affinis*; note the spine-like structure in the sheath (arrow). F) Normal shape of IJ. G) Elliptically-shaped structure (arrow) close to base of esophagus of *Steinernema scapterisci* female. I-K) *Heterorhabditis* IJ with some structures used to distinguish *Steinernema* from *Heterorhabditis*. I) IJ head with anterior tooth. J) IJ tail, long and thin. K) Ridges on IJ body. Note IJ of *Steinernema* do not have those characteristics.

Key to Species of the Genus *Steinernema*¹ Based on IJ and First-generation Male Characters

- 1 Average length of IJ > 800 μ m 2
 Average length of IJ < 800 μ m 5
- 2 Average length of IJ greater than 950 μ m (1034-1130); E% > 100. Male without mucron, ratio SW greater than 1.60 3
 Average length of IJ less than 950 μ m (849-885), E% < 100. Male with or without mucron, ratio SW less than 1.60 4
- 3 In IJ, distance from anterior end to excretory pore 76-86 μ m. In male, D% about 93 (88-102); spicule tip swollen *S. anomali* (Kozodoi) Poinar
 In IJ, distance from anterior end to excretory pore 87-100 μ m. In male, D% about 70 (60-78); spicule tip with large aperture, under light microscope, resembling a notch *S. glaseri* (Steiner) Wouts *et al.*
- 4 In IJ, distance from anterior end to excretory pore extremely short, 18 μ m (14-22 μ m). In male, D% averages 19 (13-26); ratio GS greater than three fourth (0.82-0.93) ... *S. neocurtillis* Nguyen & Smart
 In IJ, distance from anterior end to excretory pore 62 μ m (53-67 μ m). In male, D% averages 60 (51-64); ratio GS less than three-fourth (0.52-0.61) *S. feltiae* (Filipjev) Poinar
- 5 Average length of IJ 682 \pm 11 μ m (608-880 μ m) 6
 Average length of IJ 550 \pm 40 μ m (443-662 μ m) 7
- 6 IJ sheath with spine-like structure inside the tail tip (Fig. 2E). Male spicules 70 μ m (67-86 μ m) long and tail with a mucron *S. affinis* (Bovien) Poinar
 IJ sheath without spine-like structure inside the tail tip. Male spicules 91 μ m (84-100 μ m) long and tail without a mucron *S. intermedia* (Poinar) Poinar
- 7 Average body length of IJ about 510 μ m 8
 Average body length of IJ > 540 μ m 9
- 8 In IJ, E% averages 88 (79-97). Male tail without mucron; EW = 1.35; SW = 1.56 ... *S. ritteri* Doucet
 In IJ, E% averages 72 ((63-80). Male tail with mucron; EW = 1.88; SW = 0.94 *S. rara* (Doucet) Poinar
- 9 In IJ, E% averages 92(84-95). Male without mucron; EW about 1.90 *S. kushidai* Mamiya
 In IJ, E% less than 92 (54-80). Male with mucron; EW about 1.45 10
- 10 In IJ, E% averaging 73 (60-80). In male, SW averaging 2.52 (2.04-2.80); spicule shaft long, spicule length 83 (72-92) μ m *S. scapterisci* Nguyen & Smart
 In IJ, E% averaging 60 (54-66). In male, SW averaging 1.72 (1.4-2.00), spicule shaft short, spicule length 66 (58-77) μ m *S. carpocapsae* (Weiser) Poinar

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¹*S. kraussei* was considered as a *species inquirenda* (1).