

Scanning Electron Microscope Studies of *Steinernema anomali* Kozodoi, 1984¹

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Abstract: This paper presents SEM micrographs of portions of the male, female, and infective-stage juvenile of *Steinernema anomali*. Included are micrographs of the cephalic and caudal region, spicules, and gubernaculum of the male, the cephalic and vulval region of the female, and the cephalic region of the infective-stage juvenile. Males have six labial and four prominent cephalic papillae and small amphids. There are 11-14 pairs and one single genital papillae; of these, 6-9 pairs are preanal and subventral, one pair preanal, lateral, one pair adanal, and three pairs postanal. Spicules have a short head, a long blade, and a reduced shaft. The distal end is enlarged and bears a dorsal aperture. Gubernaculum much shorter than spicules; cuneus of gubernaculum short and bifurcate anteriorly. Females have six labial and four cephalic papillae and small amphids. Vulva with a thickened posterior lip. Infective juveniles have a smooth head, prominent amphids, and four cephalic papillae. Labial papillae, if present, are not evident.

Key words: entomopathogenic nematode, morphology, nematode, scanning electron microscopy, *Steinernema anomali*.

Kozodoi (1) described *Steinernema anomali* in 1984 from soil and a dead larva of the chafer, *Anomala dubia* Scop. *Steinernema anomali* resembles *S. glaseri* Steiner, except that the spicule tip of *S. anomali* is enlarged compared to the notched or hooked spicule tip of *S. glaseri*. The two species differ, also, in that they do not interbreed, and they exhibit different protein and enzyme patterns, different symbiotic bacteria, and slightly different rates of development (3).

The purpose of this paper is to present SEM photographs of spicules, gubernacula, and genital papillae of *Steinernema anomali*. We believe those structures can be used to distinguish this nematode from the other species of *Steinernema*. We also present SEM photographs of the face view and vulva of the female, the face view of the male, and the anterior region of the infective juvenile to document structures that have not been shown previously by SEM.

MATERIALS AND METHODS

Infective-stage juveniles of *Steinernema anomali* were obtained from Biosys (Palo

Alto, CA) and reared in last instar larvae of *Galleria mellonella*. Females and males of the first generation and infective juveniles were collected and fixed in vials by one of three methods: i) specimens were placed in 1 ml of refrigerated 0.1 M sodium cacodylate buffer (pH 7.2) and 0.3 ml refrigerated 6% glutaraldehyde in 0.1 M sodium cacodylate buffer was added; 1 and 2 hours later, 0.3 and 0.4 ml, respectively, of the latter solution were added. All steps were performed under refrigeration; ii) specimens were placed in 1 ml saline solution (7.5 g NaCl, 0.35 g KCl, 0.21 g CaCl₂ and 1,000 ml water), and after 15 minutes, 1 ml of 6% glutaraldehyde in 0.1 M sodium cacodylate buffer was added. These steps were performed at room temperature (25 C); iii) specimens were placed in 2 ml of refrigerated 3% glutaraldehyde buffered in 0.1 M sodium cacodylate. After the steps indicated for each method were carried out, all specimens were refrigerated at 8 C for 24 hours. Then the nematodes were washed in five changes of 0.1 M sodium cacodylate buffer for 15 minutes in each change, post-fixed in 2% osmium tetroxide for 12 hours at 25 C, and washed in five changes of 0.1 M sodium cacodylate buffer for 10 minutes in each change. Next, they were dehydrated in a graded ethanol series, transferred to a mixture of 50% amyl acetate and 50% ethyl alcohol

Received for publication 25 November 1992.

¹ Florida Agricultural Experiment Station Journal Series No. R-02776.

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for 10 minutes, transferred to 100% amyl acetate, critical point dried with liquid CO₂, mounted on SEM stubs, and coated with gold. Spicules and gubernacula were dissected from males in lactophenol, glycerine, or 40% lactic acid and were transferred to a drop of water on a coverglass (12-mm d). 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, the specimens were coated with gold. All specimens were examined with a Hitachi S-570 SEM at 15 kV.

RESULTS AND DISCUSSION

All three methods used for fixing nematodes produced acceptable specimens. The first method provided the best specimens of males and infective-stage juveniles, and the second and third methods provided satisfactory specimens of males, females, and infective-stage juveniles. The photomicrographs in this paper were taken from specimens prepared by the first (Figs. 1,2,5), second (Fig. 4A) and third (Figs. 4B,C,D) methods.

The number and arrangement of labial and cephalic papillae for the female is as reported for the family Steinernematidae (2).

Male: Head truncate, with six labial and four large cephalic papillae (Fig. 1), small amphids (Fig. 1A), and perioral disc (Fig. 1B). Body curved ventrally after death, tail without mucron (Fig. 2B). Number of caudal papillae ranges from 11 to 14 pairs with one single ventral, preanal papilla (Fig. 2A,B; 30 specimens observed). Kozodoi (1) reported 11 pairs and one single, ventral preanal papilla. When 11 pairs of papillae are present, the arrangement (Fig. 2B) is as described by Kozodoi (1), except that he called the posteriormost lateral papilla adanal, whereas it is preanal. In general, the arrangement of papillae is five to eight pairs preanal and subventral, one pair preanal and lateral, two pairs adanal (Fig. 2C,D; sometimes one pair is preanal, Fig. 2A), two pairs postanal and subventral, and one pair postanal and subdorsal (Fig. 2C,D). Spicules unique for the species (see Fig. 6). Spicule head (manubrium) short, about 22% of spicule length, slightly variable in shape (Fig. 3A–D); shaft (calomus) reduced; blade (lamina) thick, more

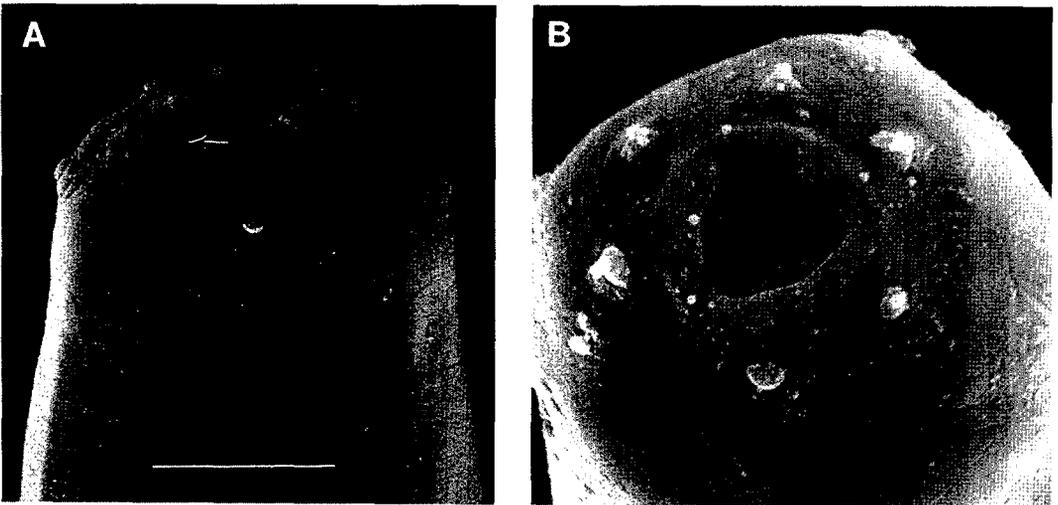


FIG. 1. *Steinernema anomali* male head. A) Sublateral view showing six labial papillae, three of the four cephalic papillae, and one of the two amphids (arrow). B) Enface view showing perioral disc and triradiate mouth. Scale (in A): A = 8.8 μ m, B = 6 μ m.

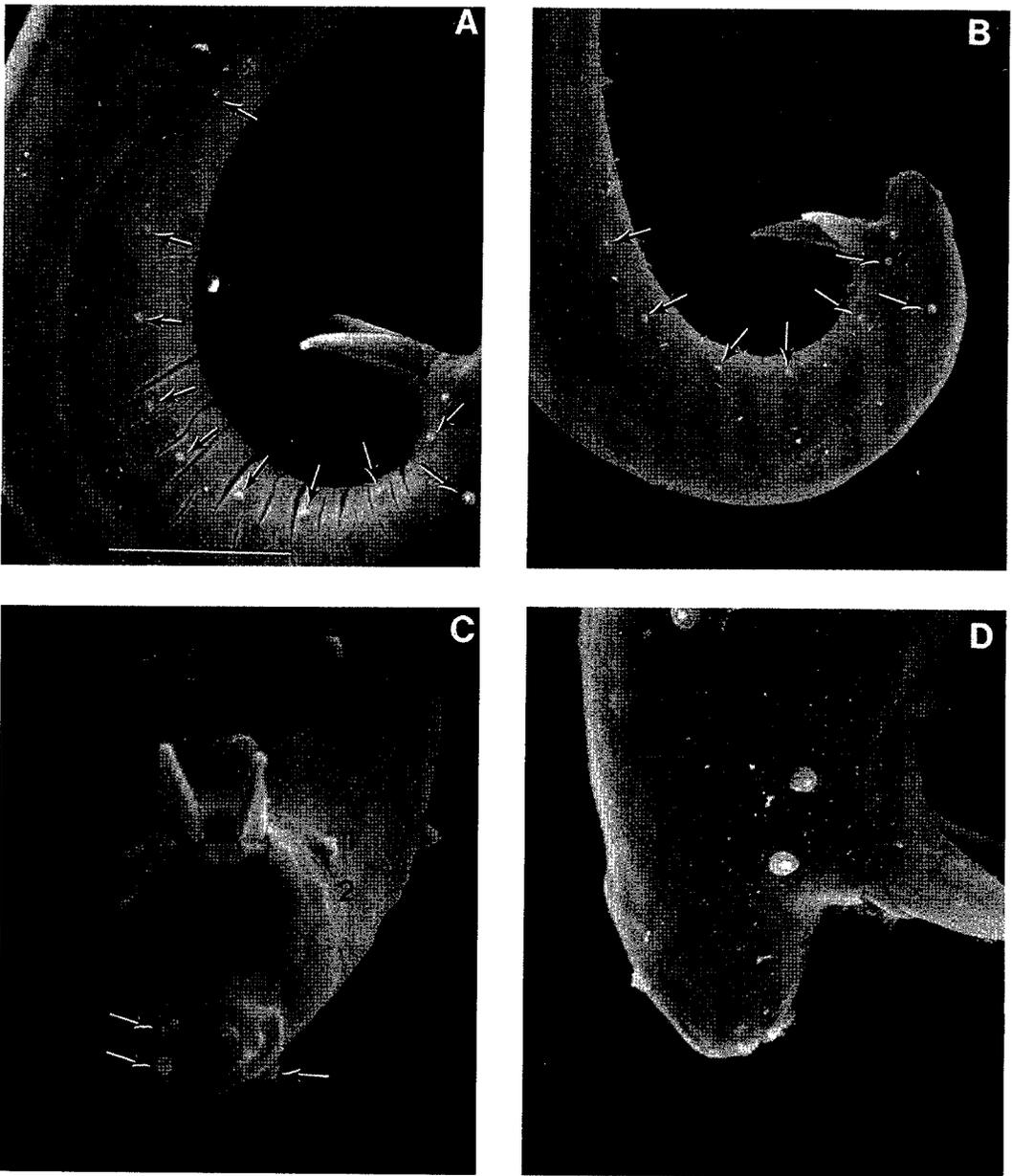


FIG. 2. *Steinernema anomali* male, posterior region. A) Lateral view showing 10 of the paired preanal genital papillae (arrows), nine subventral and one lateral, the single ventral preanal papilla, and extended spicules. B) Lateral view, showing seven of the paired preanal genital papillae (arrows, six subventral and one lateral), the single ventral preanal papilla, tail without mucron, and protruding spicules. C) Ventral view, showing seven pairs and one single preanal papillae, the two spicule tips, and the distal end of the gubernaculum. Subventral genital papillae pairs number 1 and 2 always present with pair 1 subventral, preanal and pair 2 subventral adanal. D) Enlargement of end portion of Fig. 2B. Scale (in A): A = 50 μm , B = 60 μm , C,D = 20 μm .

than 3.5 times as long as head, its curvature variable (Fig. 3A–F), velum absent. Spicule tip enlarged with a prominent dor-

sal aperture (Fig. 3H), making the spicule tip appear constricted. *Steinernema glaseri* males also have an aperture at the spicule

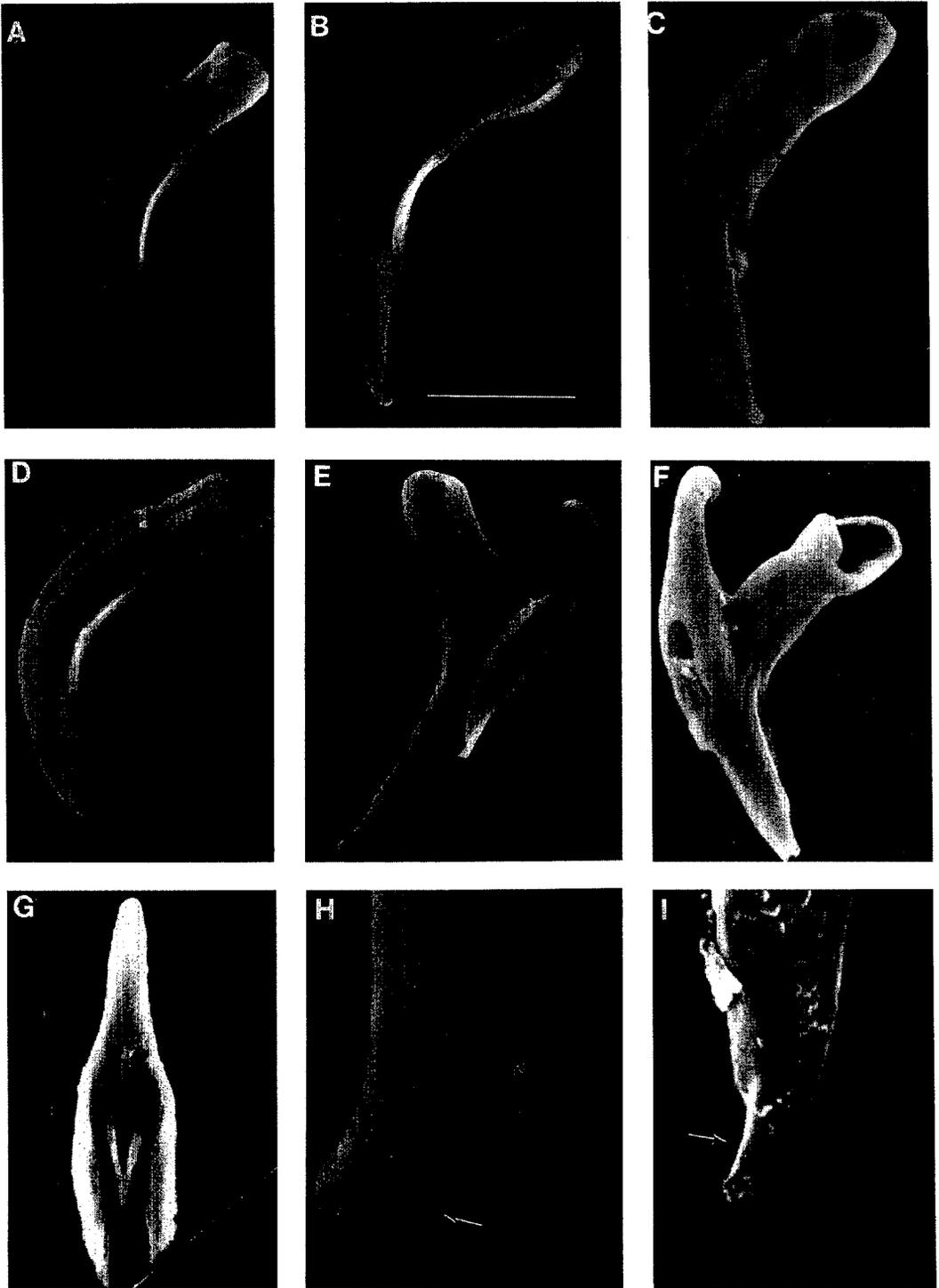


FIG. 3. *Steinernema anomali*. A–D) Spicules with spicule head short, shaft reduced, and tips enlarged. E, F) Spicules and gubernacula, with gubernaculum in lateral and subdorsal views, respectively. Note the forked posterior end of the gubernaculum in F. G) Gubernaculum, ventral view. Note the short and anteriorly bifurcate cuneus. H) Spicule tip (enlarged) with a dorsal aperture. I) Spicule tip of *S. glaseri* (not enlarged) with a ventral aperture, which is longer than that of *S. anomali*. Scale (in B): A = 30 μm , B, D = 23 μm , C, F = 25 μm , E = 27 μm , G = 20 μm , H = 2.5 μm , I = 7 μm .

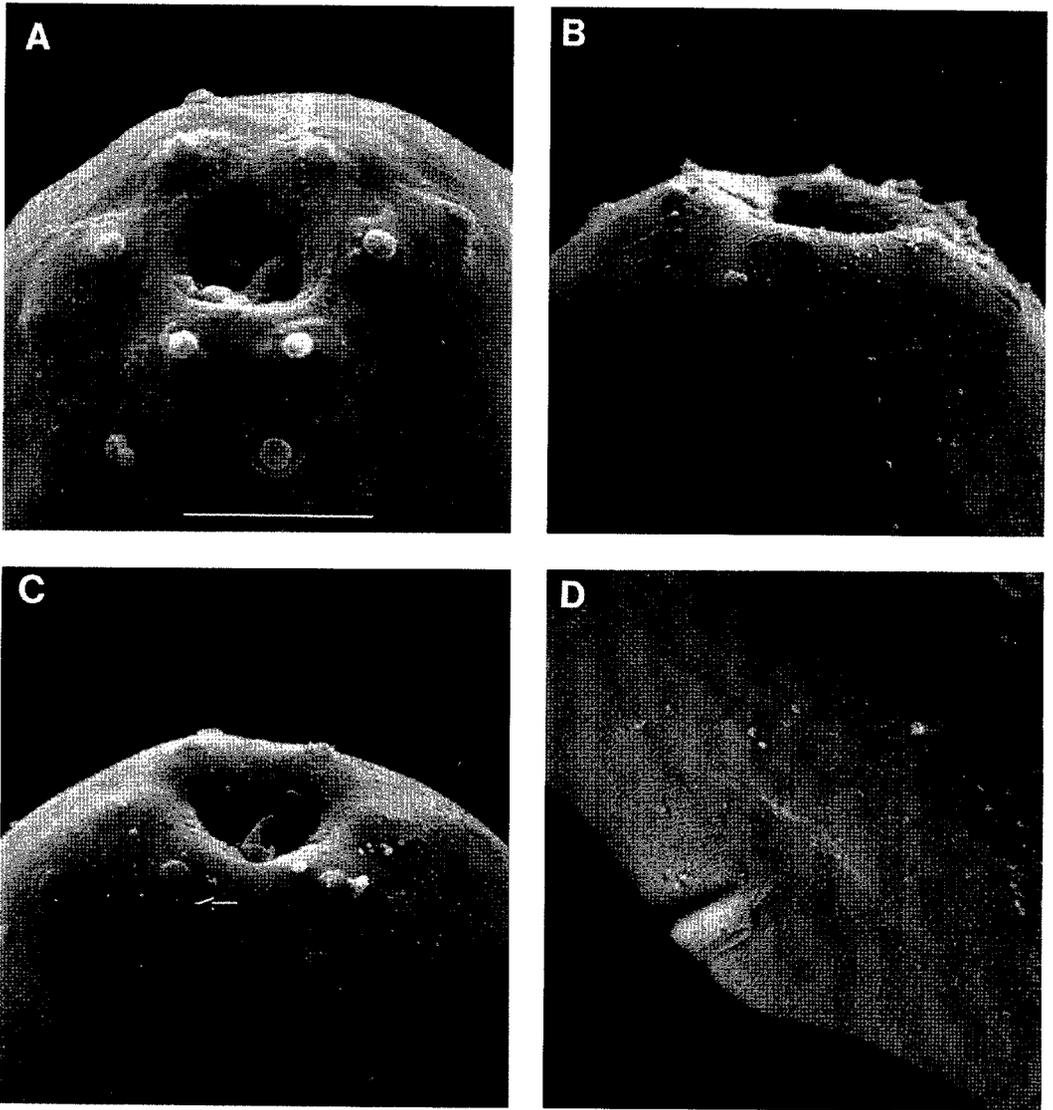


FIG. 4. *Steinernema anomali*, female. A) Face view showing six labial and four cephalic papillae. B) Anterior end, sublateral view. C) Anterior end, sublateral view showing one of the two small amphids (arrow), six labial papillae, and three of the four cephalic papillae. D) Vulval region showing a thickened posterior lip. Scale (in A): A = 10 μm , B,D = 12 μm , C = 13.6 μm .

tip, but the aperture is longer and located ventrally (Fig. 3I). Gubernaculum much shorter than spicule, its anterior part curved ventrally (Fig. 3E,F), and its posterior part forked (Fig. 3F); corpus wide with its two "wings" curved ventrally then inward when the spicules are removed (Fig. 3G,6C); cuneus short, bifurcate anteriorly (Fig. 3G). Length of gubernaculum

divided by its width (ventral view) about 3.8.

Female: Head with six prominent labial papillae and four cephalic papillae (Fig. 4A–C). Amphids present, small (Fig. 4C). Vulva with a thickened posterior lip (Fig. 4D).

Infective juvenile: Head smooth, four prominent cephalic papillae, amphids pro-

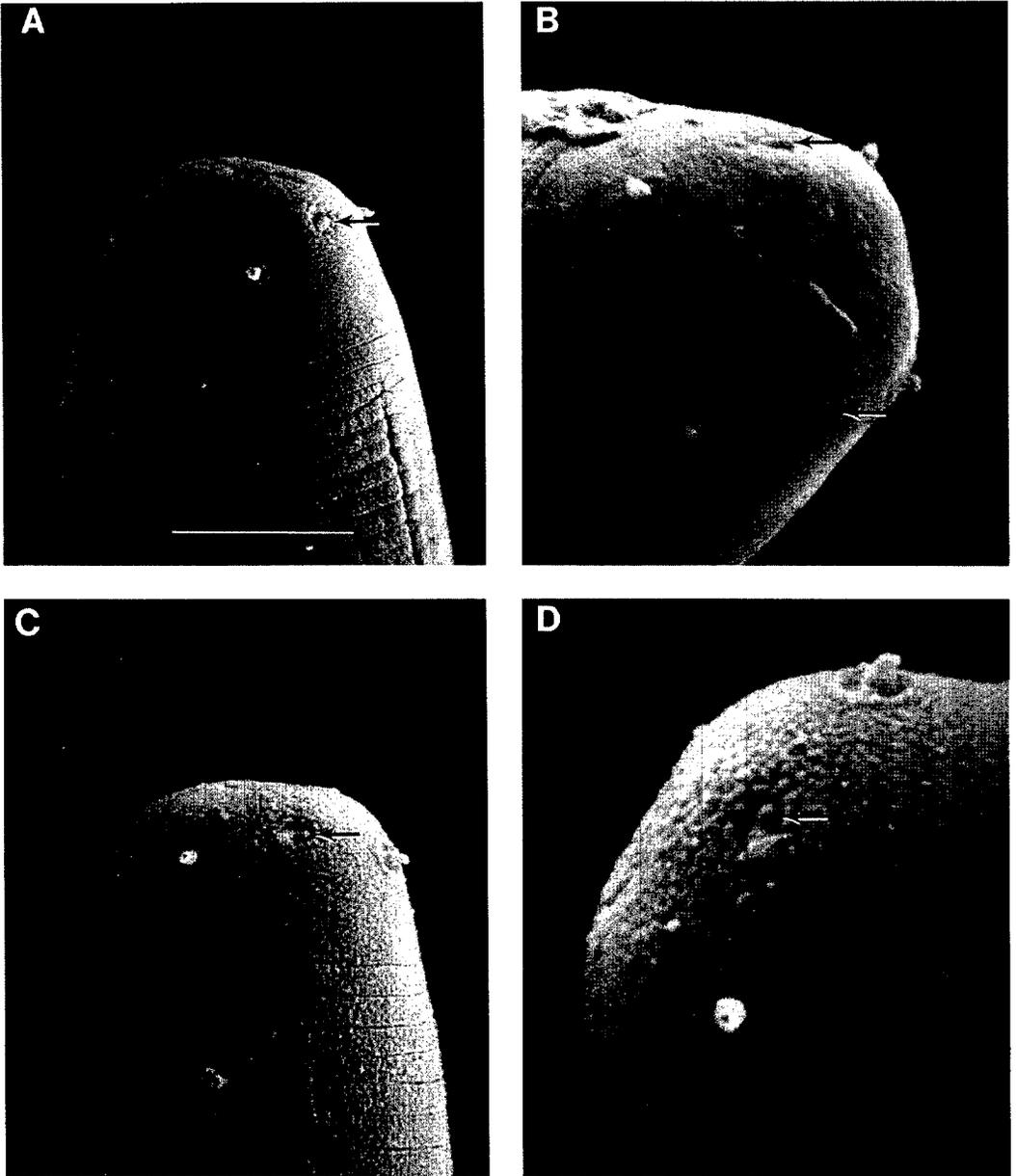


FIG. 5. *Steinernema anomali* third-stage infective juvenile (IJ). A) Anterior end showing smooth head, one of the two amphids (arrow), three of the four cephalic papillae and the origin (one incisure) of the lateral field. B) Anterior end showing triradiate mouth, four cephalic papillae and the two amphids (arrows). C) Anterior end showing one of the two amphids, three of the four cephalic papillae, and the origin (one incisure) of the lateral field. D) Enlargement of C showing one of the two amphids (arrow) and three of the four cephalic papillae. Scale (in A): A = 6 μm , B = 3.8 μm , C = 5 μm , D = 2.5 μm .

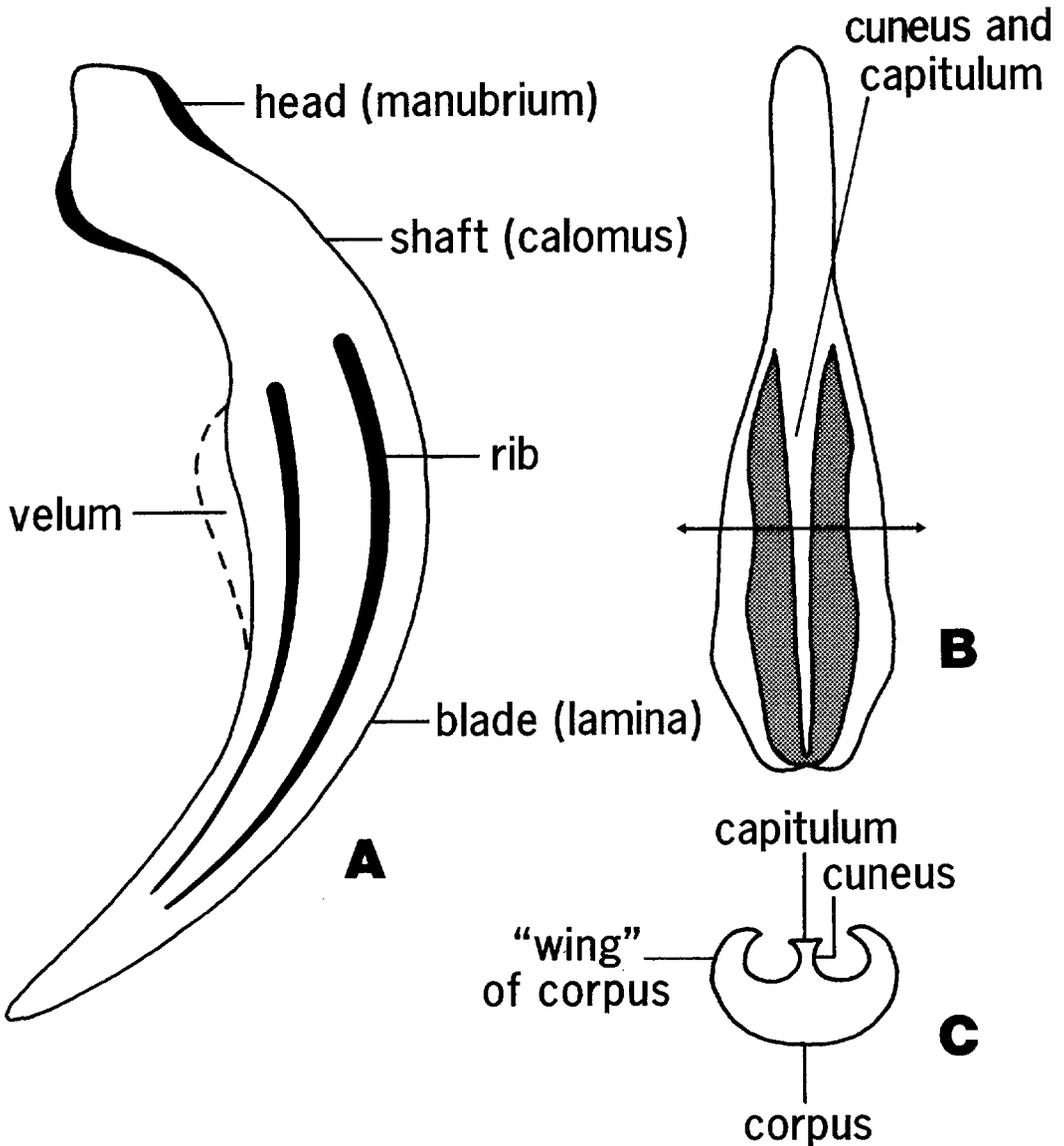


FIG. 6. Diagrammatic illustrations of the morphology of the spicule and gubernaculum of steinernematid nematodes. A) Spicule. B) Gubernaculum. C) Cross section of gubernaculum at the arrow on B. Note that the two "wings" of the corpus curve upward, then inward when the spicules are retracted. When the spicules are protracted the two "wings" flare out.

nounced (Fig. 5). Labial papillae not observed.

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