Nematol. medit. (1985), *13*: 73-80.

Istituto di Nematologia Agraria, C.N.R.
70126 Bari, Italy

OBSERVATIONS ON THE MORPHOLOGY AND HISTOPATHOLOGY OF *HOPLOLAIMUS PARAROBUSTUS* ATTACKING COFFEE IN SAO TOMÉ

by
N. VOVLAS and F. LAMBERTI [1]

A bisexual species belonging to *Hoplolaimus* Daday, was extracted from soil and root samples of *Coffea arabica* L. collected in September 1984 during a nematode survey in Sao Tomé. Several specimens from a number of localities have been examined and determined as *Hoplolaimus pararobustus* (Schuurmans Stekhoven *et* Teunissen 1938), Sher in Coomans 1963. In this article, supplementary descriptive data, supported by scanning electron microscope (SEM) observations, are given and some histopathological effects caused by the nematode feeding on coffee roots are discussed and illustrated.

Materials and Methods

Specimens from the soil and root samples were killed and fixed in 4% hot aqueous formaldehyde and then processed to glycerin by Seinhorst's (1959) method. Several specimens (females and males) were prepared for observations of the external morphology with the scanning electron microscope (SEM) following the method of De Grisse (1973), but ethanol was used for dehydration instead of acetone. Glycerin mounted specimens were also used for SEM observations. For histopathological observations living nematode-infected plant

[1] The technical assistance of Mrs. A. Agostinelli is gratefully acknowledged.

material was fixed in FAA (formalin-acetic acid-ethyl alcohol) dehydrated through a tertiary-butyl alcohol series, and embedded in paraffin. Serial sections (10 μm) were stained with safranin-fast green and photographed using a light microscope (Johansen, 1940).

Description of a Sao Tomé population of *Hoplolaimus pararobustus* from coffee.

Female: (n = 25). L = 1.306 mm (1.067-1.629); a = 29 (27-39); b = 7.8 (8.0-10.3); b' = 6.4 (5.1-8.5); c = 62 (53-81); V = 55 (51-58)%; anterior ovary = 22% (15-28); posterior ovary = 23% (18-28); anterior phasmid = 27% (22-33); posterior phasmid = 83% (80-86); stylet length = 41 (40-43) μm; DGO = 6-7 μm behind the stylet base.

Body curved ventrally to varying degrees: width 40 μm (38-43). Transverse body annules 2.12 (2.0-2.15) μm wide interrupted by a single incisure more evident in the posterior body portion and often inconspicuous for most of the body. Head region hemispherical (7-8 μm high) set off from the body by a distinct constriction with a terminal disc and 4-5 post labial annules (Figs. 2B, C). Head region divided into two halves by a deep dorsal and ventral groove; each half subdivided by shallower grooves that delimit the lateral sectors (Fig. 2B). The basal head annule is divided in 16-20 segments of variable size by numerous longitudinal striations (Fig. 2C). Head width about 14-18 μm.

Excretory pore, about half an annule width in diameter, 90 (83-133) μm posterior to head end and always at level of, or anterior to, median bulb (Figs. 1A, B, C). Hemizonid 143 (124-163) μm from anterior end 30-48 μm posterior to excretory pore. Oesophagus 202 μm (183-245) long, 147 (130-165) μm to oesophago intestinal valve. Basal oesophageal glands extending 60 (52-70) μm posterior to oesophago intestinal valve with three round nuclei 6-8 μm in diameter.

Prominent scutellum-like phasmids, 4.5-6.0 μm in diameter and highly refractive (Fig. 2D). Anal opening (arrowed in Fig. 2E) present on 10-16th annule from mid tail terminus, appearing in ventral view as a circular pore half an annule wide (Fg. 2E). Tail 20 (19-25) μm long, with terminus broadly rounded and with distinct annulations; cuticle markedly thickened in two distinct layers (Fig. 1D).

Male: (n = 20). L = 1.323 mm (1.162-1.460); a = 33 (30-36); b = 8.4 (7.2-10.4); b' = 6.5 (5.3-7.5); c = 58 (52-66); stylet length = 51 (40-42) μm; spicule length = 45 (38-40) μm; gubernaculum = 21 (20-22) μm; T = 40 (35-48)%; anterior end to excretory pore distance = 96

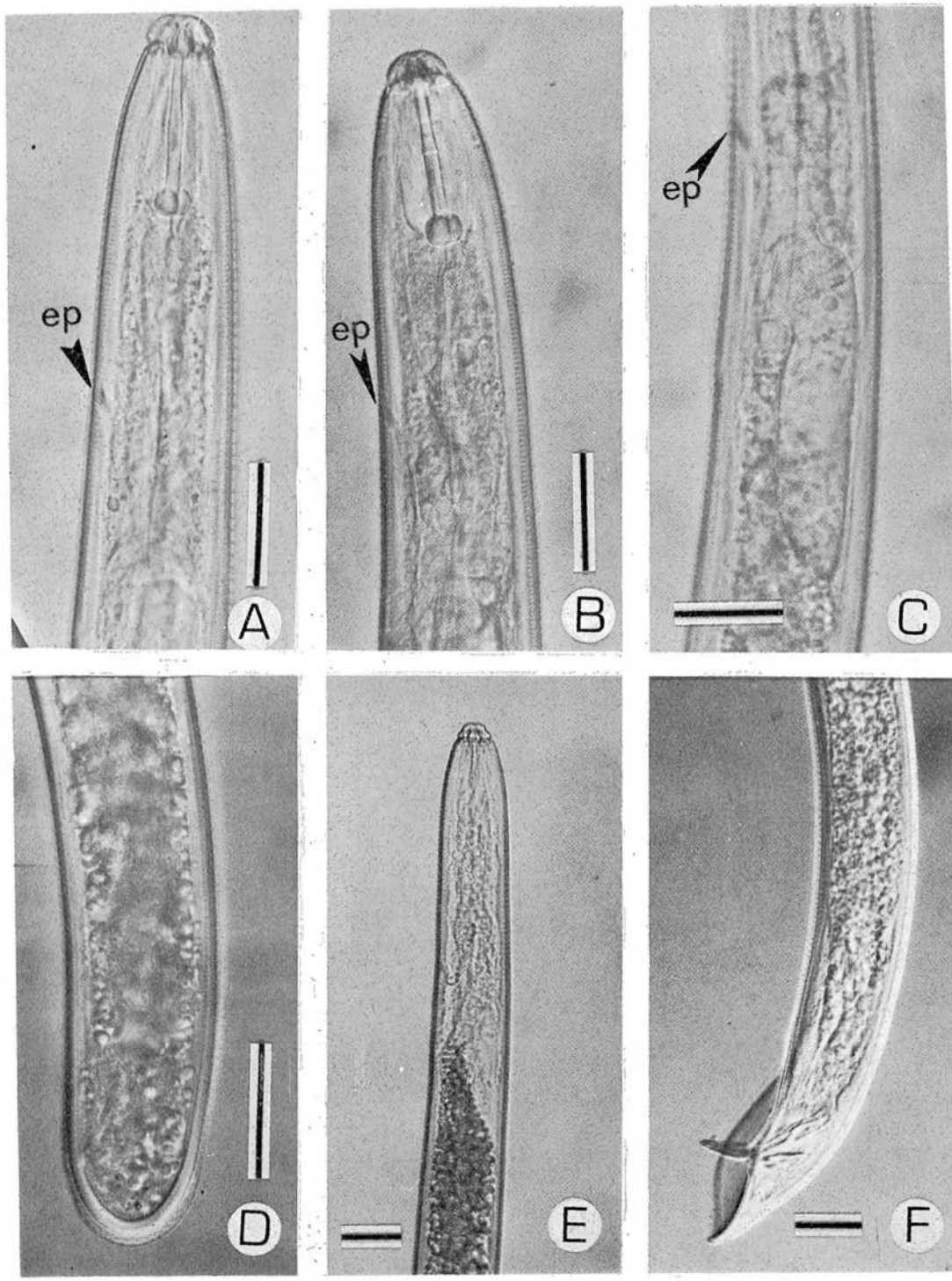

Fig. 1 A-F - *Hoplolaimus pararobustus* A, B, C) Female oesophageal region (ep = excretory pore); D) Female tail end; E) Male anterior body portion; F) Male tail. Scale bar = 25 μm.

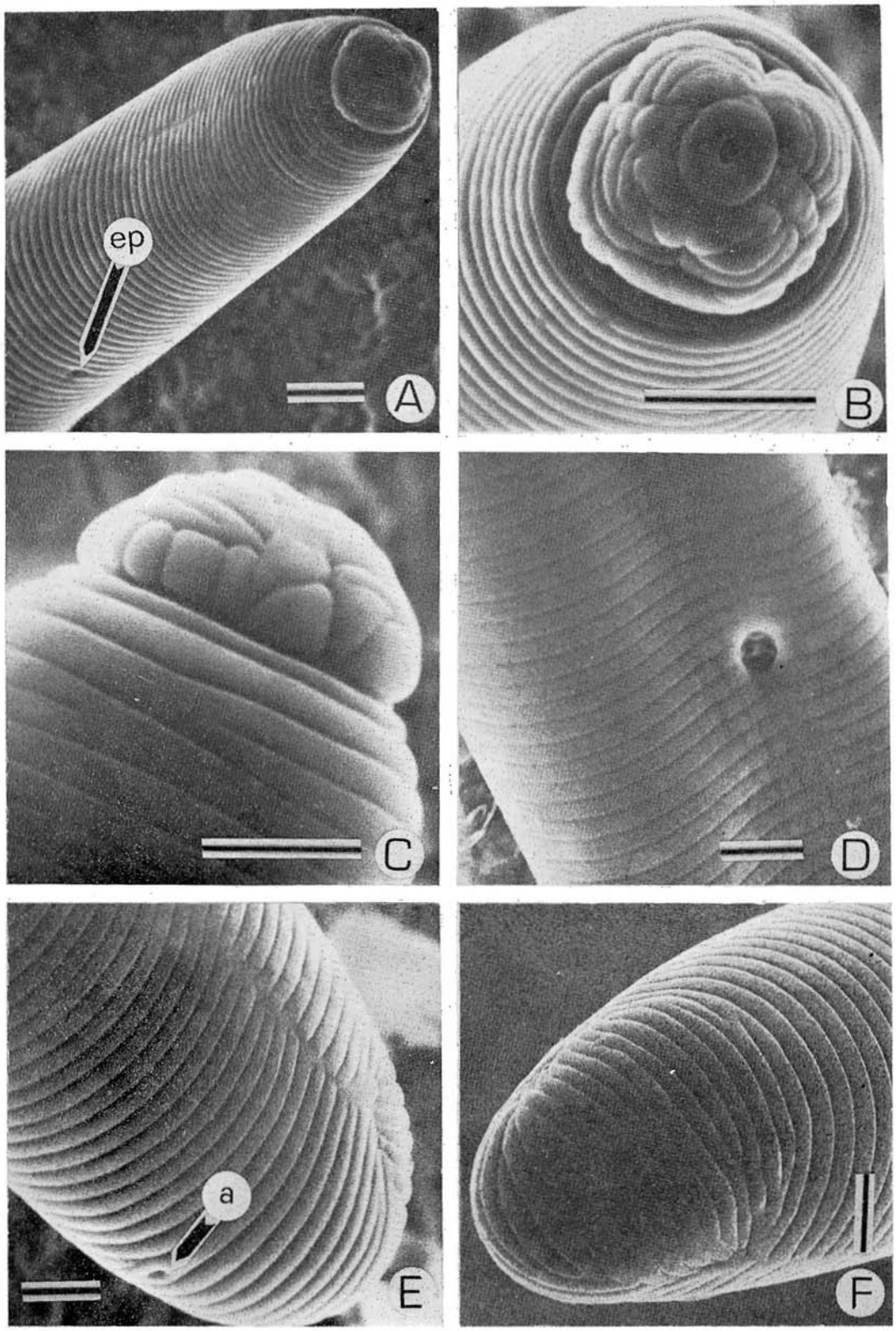

Fig. 2 A-F - SEM micrographs of *H. pararobustus* A) Ventro-lateral view of anterior region (excretory pore arrowed); B, C) En face view and profile of head region; D) Posterior scutellum-like phasmid; E, F) Female tail in ventro-lateral and dorsal view (anus arrowed). Scale bar = 10 μm.

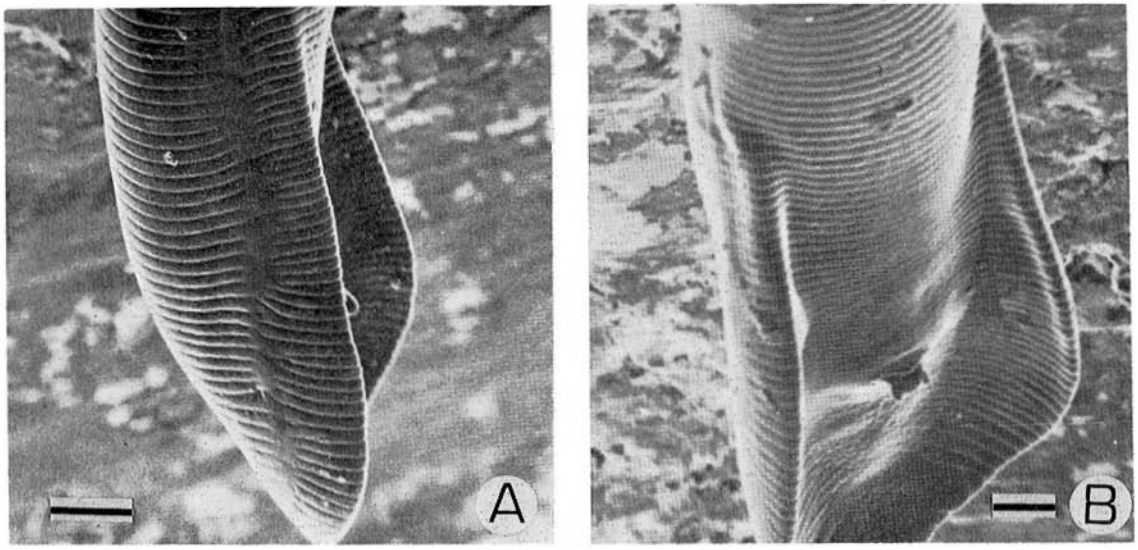

Fig. 3 A-B - Male tail of *H. pararobustus* in lateral and ventral view. Scale bar = 10 μm.

(83-110) μm; oesophagus = 201 (190-220) μm long; 150 (130-160) μm to oesophago intestinal valve.

Male morphological characters are generally similar to those described for the female but with slightly smaller dimensions.

Well developed caudal alae (with distinct annulation and protruding ventrally half a body width, at the cloacal level) originating near the level of retracted spicule head and enclosing the tail which is conoid (Figs 1F, 3A, B).

Histopathology

Hoplolaimus pararobustus has generally been observed feeding semi-endoparasitically in coffee roots (Fig. 4A). The surface of infested roots had numerous, irregular brown necrotic lesions. The anterior end of the nematode was either parallel with, or perpendicularly positioned to the vascular system (Fig. 4B, C, D).

Examination of sectioned and stained material showed that nematode penetration through the cortex resulted in cavities and ruptured cells with brown and necrotic cells near the nematode body

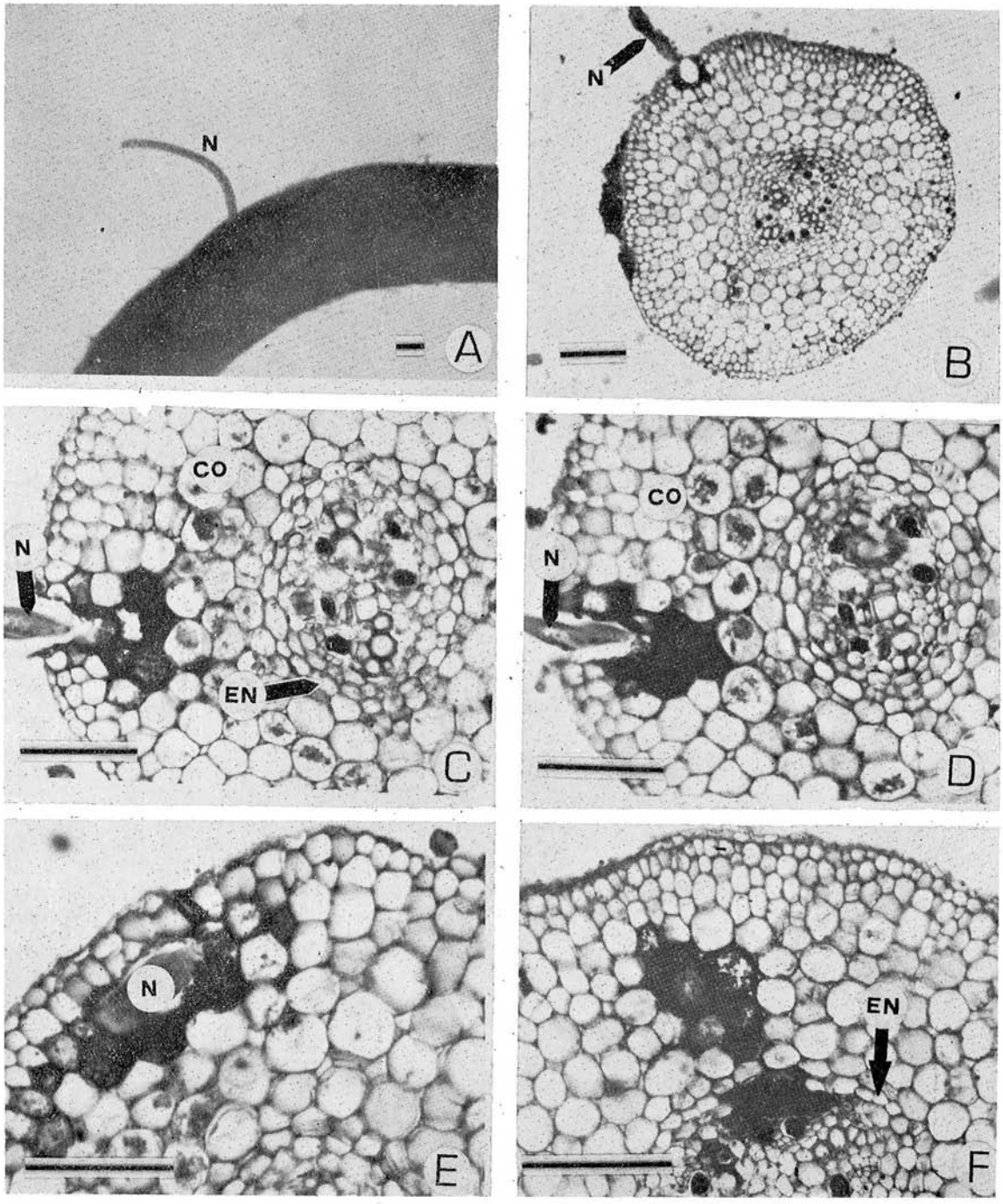

Fig. 4 A-F - Anatomical changes induced by *H. pararobustus* on coffee roots: A) Penetration of mature female in a feeder root; B) Cross section of a primary root showing the damage to the epidermal cells; C, D, E) Cross section showing cavities and necrotic cells around the nematode body in the epidermal-cortical region of the root; F) Cross section showing cortical and vascular damage in a primary root. N = nematode; CO = cortex; EN = endodermis. Scale bar = 100 μm.

(Fig. 4E). The cortical damage observed in coffee roots was similar to that caused by *H. columbus* on alfalfa roots (Fassuliotis, 1975) and cotton (Lewis *et al.*, 1976) and by *H. galeatus* on cotton (Krusberg and Sasser, 1956). Feeding damage was most often observed in the epidermal-cortical region but severe damage also occurred in the vascular region (Fig. 4F).

In Sao Tomé *H. pararobustus* occurred in great numbers (40-120/g of fresh root) indicating that coffee is a good host. *H. pararobustus* is reported from several countries in Africa, usually from the rhizosphere of banana (Coomans, 1963). Also it has been found in association with the roots of coffee, tea and sugarcane (Siddiqi, 1974).

Field observations (made during the nematode survey) show that this nematode is widely distributed in the coffee plantations of Sao Tomé; it was present in about 46% of the samples examined. Although *H. pararobustus* damages coffee roots, its effect on crop yields has not been assessed. Other plant parasitic nematodes were also observed in the root systems infested with *H. pararobustus*.

SUMMARY

Hoplolaimus pararobustus was found on the roots of coffee (*Coffea arabica* L.) in Sao Tomé. Supplementary descriptive data, supported by scanning electron microscope observations, are given which extend the known range of variability in this species. Nematode infestations caused extensive damage to the cortical parenchyma, and occasionally to the endodermal-vascular region, of the roots.

LITERATURE CITED

COOMANS A., 1983 - Observations on the variability of morphological structures in *Hoplolaimus pararobustus*. *Nematologica, 9*: 241-254.

DE GRISSE A., 1973 - A method for preparing nematodes and other soft tissues for SEM. *Meded. Fac. Landbovet. Rijksuniv. Gent, 38*: 1685-1703.

FASSULIOTIS G., 1975 - Feeding, egg-laying and embryology of the Columbia lance nematode *Hoplolaimus columbus*. *J. Nematol.*, 7: 152-158.

JOHANSEN D. A., 1940 - Plant Microtechnique. N. York, McGraw Hill, 523 pp.

KRUSBERG L. R. and SASSER J. N., 1956 - Host-parasite relationships of the lance nematode in cotton roots. *Phytopathology, 46*: 505-510.

LEWIS A. S., SMITH F. H .and POWELL W. M., 1976 - Host-parasite relationships of *Hoplolaimus columbus* on cotton and soybean. *J. Nematol., 8*: 141-145.

SEINHORST J. W., 1959 - A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica, 4*: 67-69.

SHER A. S., 1963 - Revision of the Hoplolaiminae (Nematoda) II *Hoplolaimus* Daday, 1905 and *Aorolaimus* n. gen. *Nematologica, 9*: 267-295.

SIDDIQI M. R., 1974 - C.I.H. Descriptions of Plant Parasitic Nematodes. Set. 3, n. 33, 3 pp. St. Albans, Herts, England 1974.

Accepted for publication on 20 January 1985.