

RESEARCH NOTES

Journal of Nematology 19(3):384-386. 1987.
© The Society of Nematologists 1987.

Evaluation of the Utility of a Stylet Extraction Technique for Understanding Morphological Diversity of Several Genera of Plant-Parasitic Nematodes¹

J. D. EISENBACK² AND A. RAMMAH³

Key words: identification technique, morphology, scanning electron microscopy, stylet, taxonomy.

Dimensions and morphology of stylets of plant-parasitic nematodes include useful taxonomic characters. Light microscopy of stylets in whole mounts of nematodes is sometimes difficult to interpret because of specimen orientation, interference caused by surrounding tissues, and limited resolving power of the microscope. Scanning electron microscopy (SEM) of excised stylets, however, eliminates or reduces these problems and reveals the stability and usefulness of stylet morphology for species identification (3-6). Removal of the stylet from the nematode is necessary for SEM because the image is only of the surface of the specimen. After details of stylet morphology are clarified by SEM, many of them become readily apparent by light microscopy (3-6).

This study evaluates the utility of the technique developed to dissect stylets from root-knot nematodes (*Meloidogyne* spp.) for understanding detailed morphological diversity of various other genera of plant-parasitic nematodes. Stylets are usually excised in 45% lactic acid, washed in 2% formalin, air dried, and sputter coated with

200 Å of gold. Details of the extraction procedure have been published previously (2).

Generally the stylets were dissected in 45% lactic acid; however, other solutions or concentrations may be better for certain genera (1,2,8). One exotic fluid, an extract from the digestive tract of a toad (*Bufo bufo* L.), has been used successfully (8). In this study, lactic acid was used to excise the stylets of all genera except *Heterodera*. Stylets of *Heterodera glycines* Ichinohe second-stage juveniles were extracted with 0.5% sodium hypochlorite. The solution of choice minimized formation of artifacts, particularly dissolution of the shaft and knobs (2), and (or) separation of the cone from the remainder of the stylet (1). Long exposures or high concentrations of some solutions caused swelling of the knobs and shaft or caused their lumen to collapse. Comparisons between the images seen in the light and SEM insured that the extraction procedure did not produce unacceptable artifacts.

Stylets were extracted from various genera, species, and life stages of plant-parasitic nematodes and from a *Dorylaimus* sp. (Fig. 1). Plant parasites examined included *Belonolaimus longicaudatus* Rau, *Hemicycliophora* sp., *Criconemella ornata* (Raski) Luc and Raski, *Xiphinema americanum* Cobb, *Hoplolaimus galeatus* (Cobb) Thorne, *H. colymbus* Sher, *Scutellonema* sp., *Heterodera glycines*, *Meloidogyne javanica* (Treub) Chitwood, *M. incognita* (Kofoid and White) Chitwood, *M. hapla* Chitwood, *Pratylenchus brachyurus* (Godfrey) Filipjev and Schuurmans Stekhoven, and *Sphaeronema sasseri*

Received for publication 13 October 1986.

¹ Partial support provided by U.S. Agency for International Development Contract No. ta-C-1234 to Dr. J. N. Sasser.

² Assistant Professor, Department of Plant Pathology, Physiology and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

³ Assistant Professor, Department of Phytopathologie, Institut Agronomique et Veterinaire Hassan II, B.P. 6202, Rabat-Instituts, Rabat, Morocco.

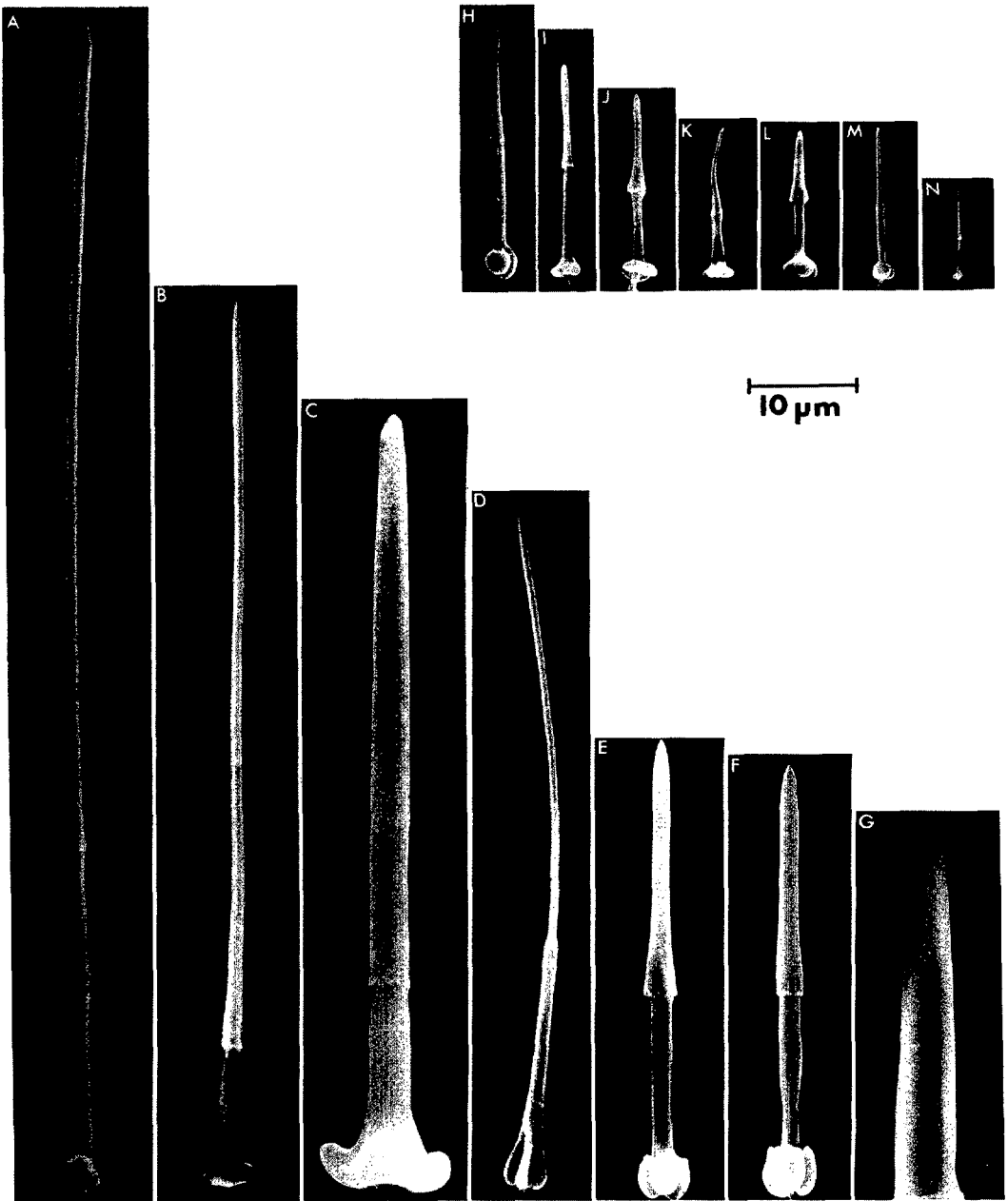


FIG. 1. Scanning electron micrographs of excised stylets of various genera, species, and life stages of nematodes. A) *Belonolaimus longicaudatus*. B) *Hemicycliophora* sp. C) *Criconemella ornata*. D) *Xiphinema americanum*. E) *Hoplolaimus galeatus*. F) *Hoplolaimus columbus*. G) *Dorylaimus* sp. H) *Scutellonema* sp. I) *Heterodera glycines* second-stage juveniles. J) *Meloidogyne javanica* male. K) *M. incognita* male. L) *Pratylenchus brachyurus*. M) *Sphaeronema sasseri* second-stage juveniles. N) *M. hapla* second-stage juvenile. All stylets are illustrated at the same magnification. Unless specified, all specimens were adult females.

Eisenback and Hartman. The technique or its modifications (2) gave good results for the species examined, and SEM of stylet morphology may be useful for species iden-

tifications for several genera in addition to *Meloidogyne* (3-6).

Recently, other studies have revealed the usefulness of stylet extraction for the com-

parison of stylet morphology among taxa in the Longidoridae (9). Stylets of several species and genera were examined by SEM, and details not previously seen by light microscopy were elucidated. Application of the extraction technique also may contribute to a better understanding of the interrelationships within Longidoridae.

Spicules and gubernacula also have been removed successfully by an adaptation of the stylet extraction technique (2,7). Comparisons of spicule morphology of several genera and species of plant-parasitic and free-living nematodes demonstrated the value of the technique for many diverse groups of nematodes, including those without stylets (7).

Extraction of hard, cuticularized structures from nematodes by adaptation of the stylet extraction technique developed for *Meloidogyne* spp. is possible for many genera. These techniques may identify new morphological characters useful in the routine identification of species, and may also clarify relationships among the higher groups of nematodes.

LITERATURE CITED

1. Chen, T. A., and G. Y. Wen. 1980. Electron microscopy of the stomatostylet and esophagus of *Cri-conemoides curvatum*. *Journal of Nematology* 12:72-83.
2. Eisenback, J. D. 1985. Techniques for preparing nematodes for scanning electron microscopy. Pp. 79-105 in K. R. Barker, C. C. Carter, and J. N. Sasser, eds. An advanced treatise on *Meloidogyne*, vol. 2. Methodology. A Cooperative Publication of the Department of Plant Pathology, North Carolina State University and United States Agency for International Development, Raleigh, North Carolina.
3. Eisenback, J. D. 1982. Morphological comparison of head shape and stylet morphology of second-stage juveniles of *Meloidogyne* species. *Journal of Nematology* 14:339-343.
4. Eisenback, J. D., and H. Hirschmann. 1981. Identification of *Meloidogyne* species on the basis of head shape and stylet morphology of the male. *Journal of Nematology* 13:513-521.
5. Eisenback, J. D., H. Hirschmann, and A. C. Triantaphyllou. 1980. Morphological comparisons of *Meloidogyne* female head structures, perineal patterns, and stylets. *Journal of Nematology* 12:300-313.
6. Jepson, S. B. 1983. Identification of *Meloidogyne* species; a comparison of stylets of females. *Nematologica* 29:132-143.
7. Rammah, A., and H. Hirschmann. 1987. Morphological comparison and taxonomic utility of copulatory structures of selected nematode species. *Journal of Nematology* 19:314-323.
8. Shen, B. K., and J. J. Lui. 1985. [Method for preparing the specimen of the root-knot nematode stylet.] *Journal of Nanjing Institute of Forestry* 1: 142-150.
9. Swart, A., and J. Heyns. 1987. Comparative morphology of the stylet of some species of Longidoridae by means of the scanning electron microscope. *Revue de Nematologie* 9:311 (Abstr.).