

Identification of *Meloidogyne* Species on the Basis of Head Shape and Stylet Morphology of the Male¹

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Abstract: Head shape and stylet morphology of males of 90 populations of *M. arenaria*, *M. hapla*, *M. incognita*, and *M. javanica* from geographic regions of the world were compared by light microscopy (LM). In addition, stylets of one population each of *M. arenaria*, *M. incognita*, and *M. javanica* and three different chromosomal forms of *M. hapla* race A and two of race B were excised and examined with a scanning electron microscope (SEM). Differences among species occurred in both head and stylet morphology. Head morphology differed in size and shape of the head cap, annulation of the head region, and width of the head region relative to the first body annule. Differences in stylets occurred in size and shape of the cone, shaft, and knobs. All populations of *M. hapla*, except one, had similar head morphology, but stylet morphology was different between cytological races A and B. Populations of *M. javanica* varied with respect to the presence of head annulations. Head shape and stylet morphology of males are recommended as additional characters useful in the identification of root-knot nematodes. **Key words:** *Meloidogyne arenaria*, *M. hapla*, *M. incognita*, *M. javanica*, root-knot nematodes, cytological races, intersexes, scanning electron microscopy, taxonomy.

Identification of the four common species of root-knot nematodes—*Meloidogyne*

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arenaria (Neal) Chitwood, *M. incognita* (Kofoid and White) Chitwood, *M. javanica* (Treub) Chitwood, and *M. hapla* Chitwood—is often difficult and time consuming. Various approaches to *Meloidogyne* taxonomy include host response (12,14,17), cytology (17,18,19), biochemistry (3,4,11), and morphology (1,2,5,6,7,8,9,12,13,15,20,21,22). Host-response tests require adequate amounts of nematode inoculum, and the results may not be known for several weeks. Cytological and biochemical studies require

specific techniques and sophisticated equipment. Identification based on morphology can be rapid and practical if reliable differentiating characters can be determined. Generally, *Meloidogyne* species are identified by the perineal pattern morphology of the adult female (9,10,16). In populations of the four common species, however, perineal patterns are variable and definite identification is not always possible (5,9,10, 12,13). Additional characters that are less variable are needed to supplement perineal pattern morphology.

In a recent scanning electron microscope (SEM) study, we reported that the head morphology of males of *M. arenaria*, *M. incognita*, *M. javanica*, and *M. hapla* was distinct for each species (7). Observed differences among species, and in *M. hapla* between races, were striking. Subsequent studies revealed that some of these differences, especially in head shape, could also be detected in the light microscope (LM). When examining root-knot nematode females (8), we showed that differences occur in stylet morphology among the four common species.

The present study compares by LM the head shape of males of several populations of each of the four common species of root-knot nematodes. It also compares by LM and SEM the morphology of stylets of males of one population each of *M. arenaria*, *M. incognita*, and *M. javanica* and five different chromosomal populations of *M. hapla*.

MATERIALS AND METHODS

Ninety populations of the four common root-knot nematode species from geographic regions of the world were selected from the *Meloidogyne* collection at North Carolina State University. Thirteen populations were *M. arenaria*; 12, *M. hapla*; 44, *M. incognita*; and 31, *M. javanica*. All populations were propagated on tomato (*Lycopersicon esculentum* Mill. 'Rutgers') in a greenhouse maintained at 22–28 C. Each population had been characterized by its ability to reproduce on five host differentials (16), and many populations had been identified on a cytological basis. The populations of *M. arenaria* and *M. incognita* belonged to several host races of

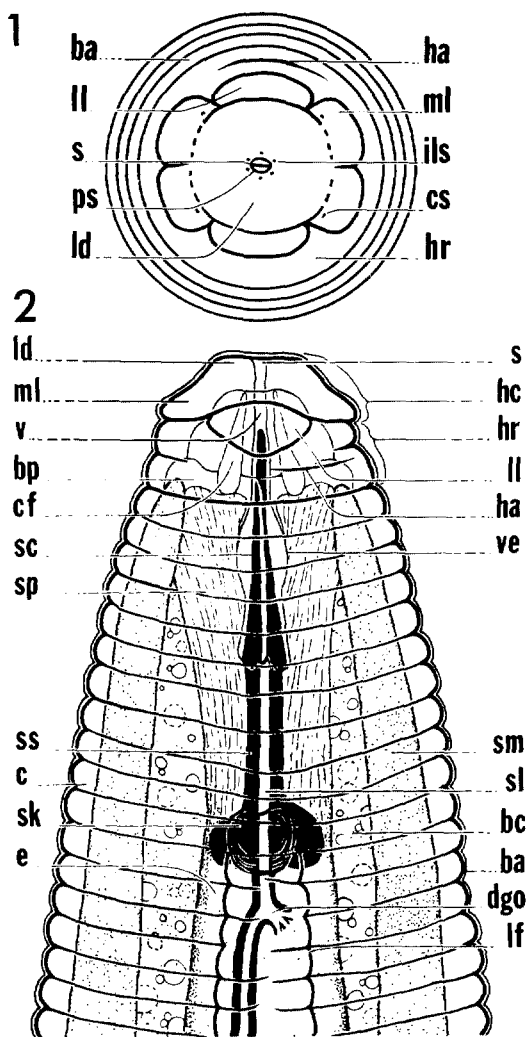
the respective species (14,16), and the populations of *M. hapla* represented the two cytological races (18).

Males were obtained by incubating washed infected root systems in a moist chamber at room temperature. The males were killed with hot TAF and mounted in the fixative for LM observations. Some specimens were killed, fixed, and mounted in glutaraldehyde (7) for additional morphological studies, and others were examined alive for comparison with fixed material. For diagnostic studies the males must be viewed in exact lateral position. Camera lucida drawings and LM photographs of the anterior body region including the stylet were made of representative populations. At least 30 specimens from each population were examined. Eight of the populations used in this study were the same populations used in previous LM and SEM observations of second-stage juveniles, males, and females (6,7,8).

Stylets of males of one population each of *M. arenaria*, *M. incognita*, and *M. javanica* and five different chromosomal populations of *M. hapla* were observed by SEM. The following populations were examined: *M. arenaria*, 351-Fla; *M. incognita*, 68-NC; *M. javanica*, 76-GA; *M. hapla* race A, 6-NC, 42-Can, and 86-NC; and *M. hapla* race B, 48-NC and 230-Chile. The technique originally developed for the removal of stylets of females (8) was adapted to males. Five males were transferred into a drop of 45% lactic acid on a glass coverslip. The specimens were cut behind the median bulb with a dental root canal file and processed for SEM observations as previously described (8). At least 30 stylets from each population were observed. The LM observations of the stylet morphology of all populations were compared with the SEM observations of the eight populations previously listed.

OBSERVATIONS

Head morphology: The basic head morphology of *Meloidogyne* males is derived from SEM and LM observations (Figs. 1, 2). Labial disc and medial lips as observed in the SEM (7) appear in the LM as a single structure, the "head cap." The annulations in the head region, so distinct in



Figs. 1-2. Diagram illustrating the generalized head morphology of a male of the genus *Meloidogyne*. 1) Face view (derived from SEM) and 2) lateral view (derived from LM and SEM); ba, body annule; bc, body cavity; bp, basal plate; cf, cephalic framework; cs, cephalic sensillum; c, cuticle; dgo, dorsal gland orifice; e, esophagus; ha, head annulation; hc, head cap; hr, head region; ils, inner labial sensillum; ld, labial disc; lf, lateral field; ll, lateral lip; ml, medial lip; ps, prestoma; sm, somatic muscles; s, stoma; sc, stylet cone; sk, stylet knobs; sl, stylet lumen; sp, stylet protractor muscles; ss, stylet shaft; v, vestibule; ve, vestibule extension.

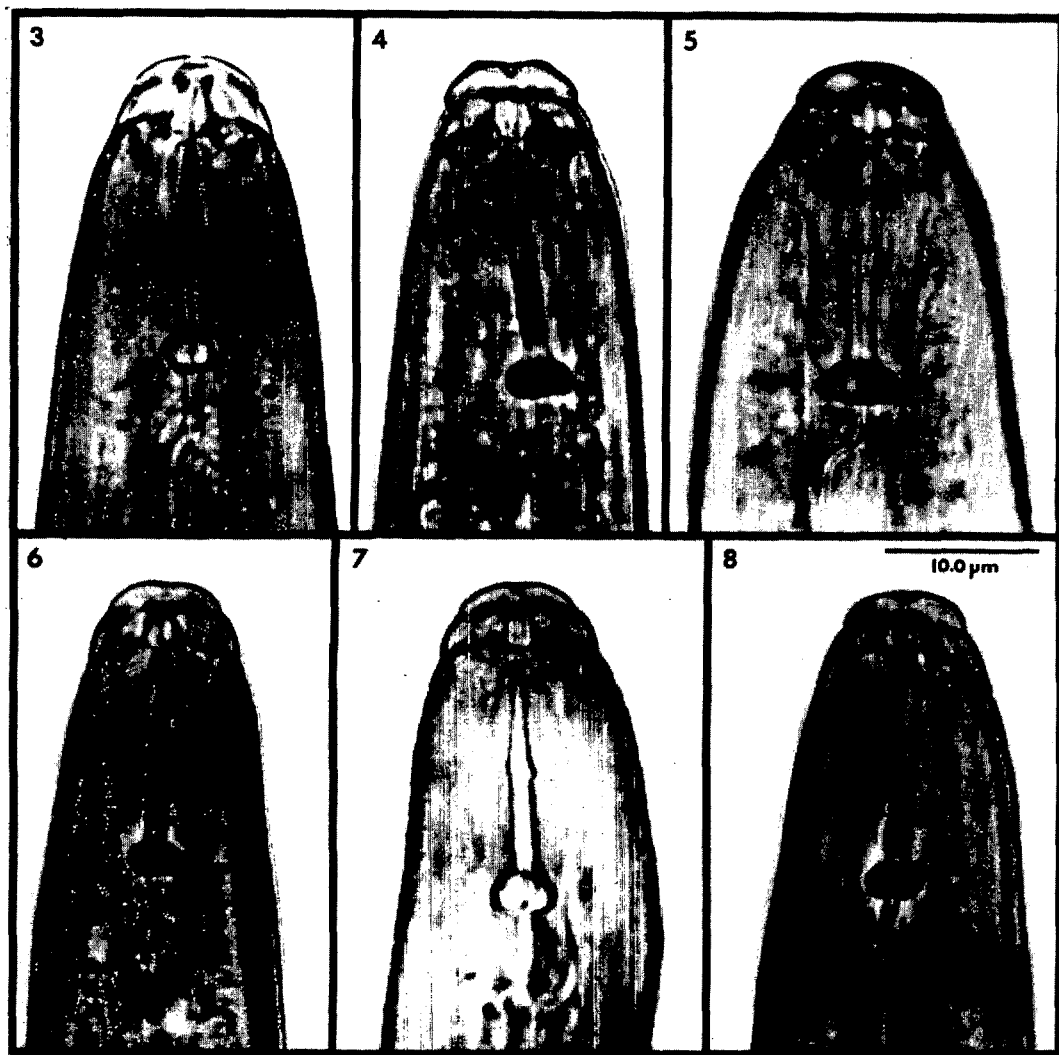
the SEM, are more difficult to observe in the LM. Generally, the head region is in the same contour with the anterior body region, but in some specimens the diameter of the head region is larger than that of the first body annule and thus the head region appears distinctly set off. The anterior body region bears annules in regular intervals,

and the lateral field begins near the level of the stylet knobs.

The four common species differ in their head morphology with respect to shape and size of the head cap, presence or absence of annulation in the head region, and the way the head region fuses with the body region. In *M. arenaria* (Figs. 3, 9) the head cap is low, slopes posteriorly, and is nearly as wide as the head region. Usually the head region is marked by 1-2 incomplete annulations and is not set off from the body region. *M. incognita* males (Figs. 4, 10) possess a high head cap formed by a large round labial disc that is raised above the medial lips and centrally concave. The head cap is as wide as the head region. The head region generally bears 2-4 incomplete annulations, although some populations may have complete annulations. The head region is not set off. In *M. javanica* (Figs. 5, 11) the head cap is high and rounded and almost as wide as the head region. The head region is not set off and is usually smooth, although 5 of the 31 populations had 2-3 incomplete annules. The head shapes of males of all populations of *M. hapla* race A (Figs. 6, 12) and race B (Figs. 7, 13) are similar except for one population. In *M. hapla* the head cap is high and much narrower than the head region which lacks annulations and is distinctly set off from the body. Generally, the distance between body annulations, as well as their diameter, decreases as they near the head region. Males of population 42-Can of *M. hapla* (Figs. 8, 14) are unique because the head cap is comparatively wider than in typical *M. hapla* populations and the head region is not set off from the body. Body annulations are indistinct.

Stylet morphology: The excised stylet and attached cuticular lining of the esophagus of the male as seen in the SEM (Fig. 15) is similar to that of the female (8) except for a few structural differences. In the male the opening of the stylet lumen is located one-fourth the length of the cone from the stylet tip, the esophageal lumen is smaller in diameter, and the triradiate lining of the smaller metacarpus pump is of the same thickness throughout.

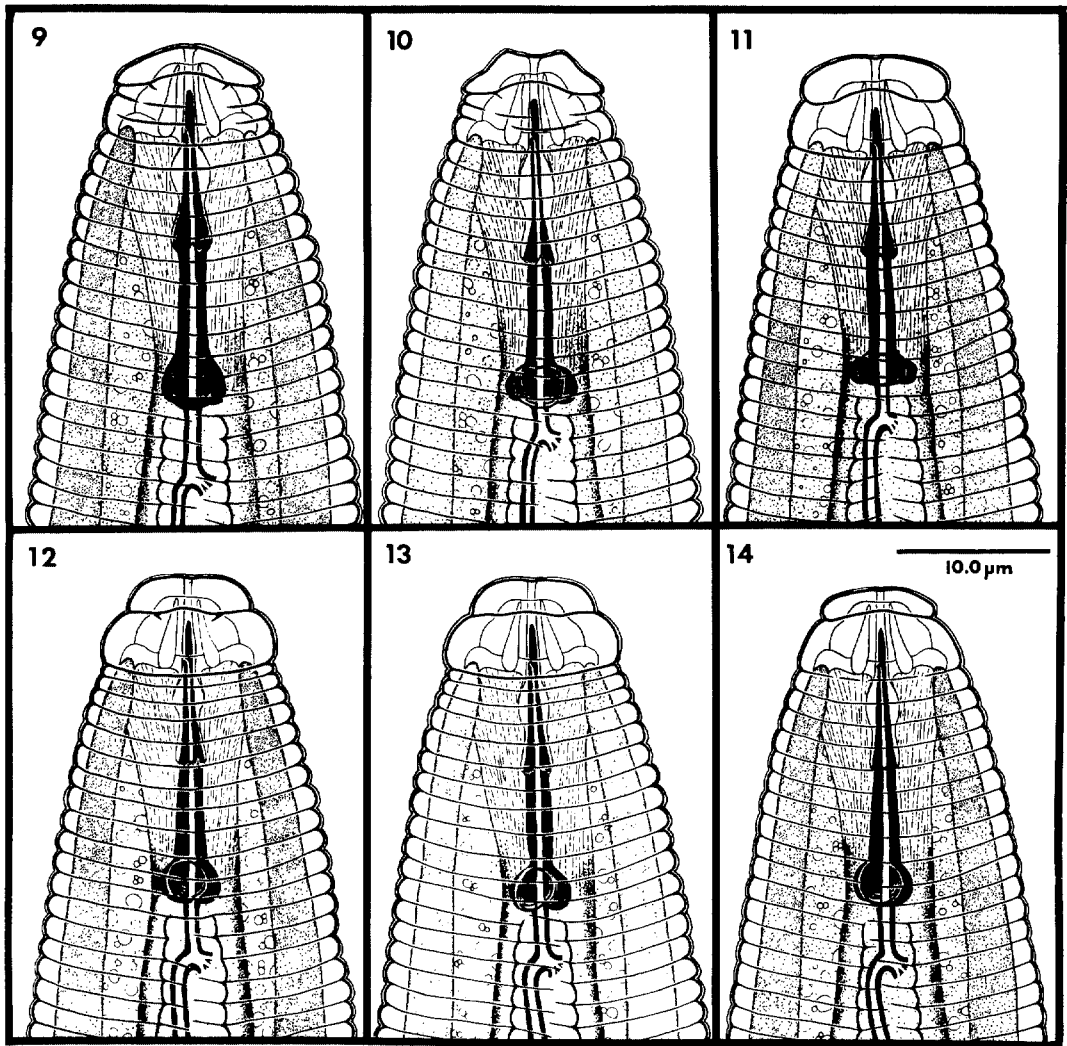
Differences in stylet morphology of the male as seen in LM and SEM occur in the shape of the cone, shaft, and knobs. In *M.*



Figs. 3-8. LM photographs of lateral view of males. 3) *Meloidogyne arenaria*. 4) *M. incognita*. 5) *M. javanica*. 6) *M. hapla* race A. 7) *M. hapla* race B. 8) *M. hapla* race A population 42-Can. All figures are same scale as Fig. 8.

arenaria (Figs. 3, 9, 16) the stylet tip is pointed and there may be a slight projection on the ventral side of the cone, posterior to the orifice. The anterior two-thirds of the cone gradually increase in width and the posterior one-third greatly increases in width. The shaft can be wider in the middle, but generally remains cylindrical. The rounded, robust knobs gradually merge with the shaft. The stylet tip of *M. incognita* (Figs. 4, 10, 17) is blunt and the anterior portion of the cone is blade-like. Often there is a pronounced projection posterior to the stylet opening. The cylindrical shaft may narrow slightly at the junc-

tion with the knobs which are broadly elongate to round, sometimes anteriorly indented, and set off from the shaft. In *M. javanica* (Figs. 5, 11, 18) the anterior two-thirds of the cone gradually increase in width. Stylet lumen openings were found in only 7 of 30 specimens. The shaft is cylindrical and the stylet knobs are low and wide, often anteriorly indented, and set off from the shaft. The stylet morphology of the *M. hapla* populations is unique for the species but the two races have slightly different stylets. In populations of both races the stylet cone gradually increases in width, and the base of the cone is



Figs. 9-14. Line drawings of lateral view of males. 9) *Meloidogyne arenaria*. 10) *M. incognita*. 11) *M. javanica*. 12) *M. hapla* race A. 13) *M. hapla* race B. 14) *M. hapla* race A population 42-Can. All figures are same scale as Fig. 14.

only slightly wider than the anterior portion of the shaft. In populations of race A (Figs. 6, 8, 12, 14, 19) the shaft is wider near its junction with the knobs than at the junction with the cone, whereas in race B populations (Figs. 7, 13, 20) the shaft remains cylindrical except for a slight narrowing near its junction with the knobs. The rounded, set-off stylet knobs are larger in race B populations. The stylets of *M. hapla* are smaller than those of the other three common root-knot nematode species.

DISCUSSION

The present study of *Meloidogyne* males

of many populations from various sources around the world shows that head shape and stylet morphology are good, reasonably reliable taxonomic characters for the identification of the four common root-knot nematode species by light microscopy. We also illustrated the value of the SEM in recognizing morphological details in the LM that might otherwise be missed.

The populations of *M. arenaria* belonged to cytological race A and represented host races 1 and 2 (16). Although minor morphological differences were noticed among populations, certain basic characteristics are common for this species.

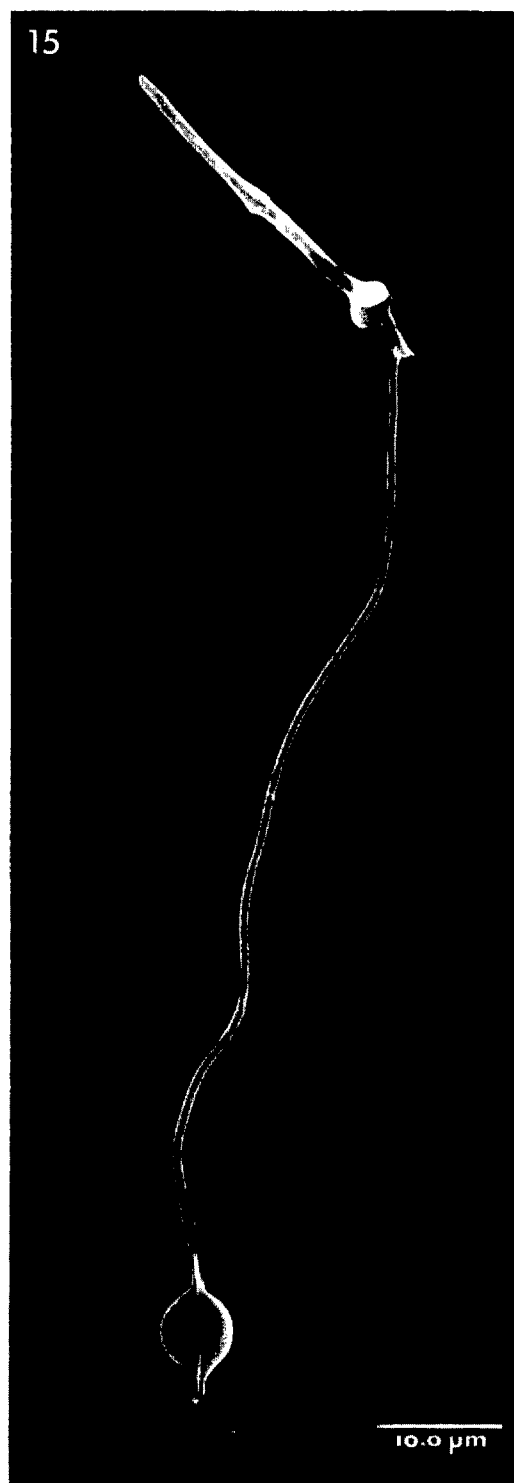


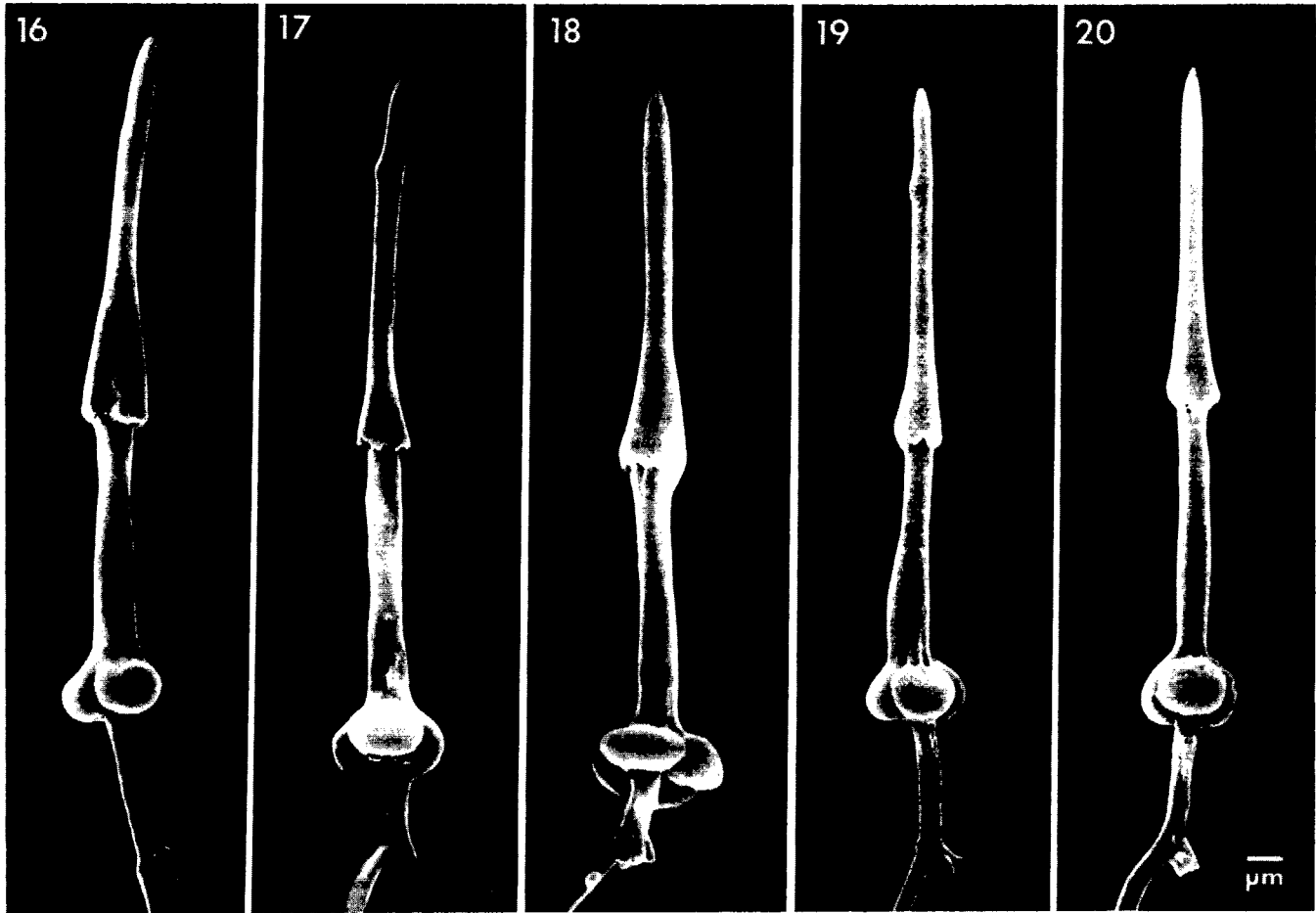
Fig. 15. SEM photograph of an excised stylet and attached cuticular lumen lining of the esophagus of a *Meloidogyne* male.

In some specimens of *M. arenaria* the head morphology may be similar to that of *M. javanica* males, particularly if the head cap is high or does not slope much posteriorly. However, the two species can easily be separated by stylet morphology. In *M. javanica* the stylet knobs are low, wide, and set off from the shaft, whereas in *M. arenaria* the knobs are rounded and gradually merge with the shaft.

The populations of *M. incognita* belonged to host races 1, 3, and 4 (16) and included only cytological race A. All populations had similar head shape and stylet morphology. The stylet morphology of *M. incognita* resembles that of *M. javanica* in the LM because the uniquely shaped stylet cone of *M. incognita* cannot always be seen clearly; also, in some specimens the stylet knobs appear as wide as in *M. javanica*. These two species, however, can easily be distinguished on the basis of head morphology.

Presently, only one cytological and one host race of *M. javanica* are recognized. The head shape of all populations in this study was similar. Most populations did not have head annulations, although 4 of the 26 populations examined had 2–3 head annulations. Chitwood, in his original description of *M. javanica*, described males with three head annulations (2). Future investigations should attempt to correlate head annulation with other taxonomic characters. Fourteen populations of *M. javanica* had male intersexes in large proportions, whereas the remaining populations did not produce intersexes, even though many males were present. Chitwood (2) stated that true males were rare, and Triantaphyllou (17) has reported on intersex formation in this species. Further research should elucidate if all populations of *M. javanica* have the ability to produce male intersexes.

Populations of *M. hapla* included four each from cytological race A and B (18). The populations of the two cytological races were similar in head shape, except for population 42 from Canada with 15 chromosomes. Stylet morphology of the races, however, is quite different, and individual populations can be identified according to stylet length and shape of stylet shaft. In his original description of *M. hapla*, Chit-



Figs. 16–20. SEM photographs of excised stylets. 16) *Meloidogyne arenaria*. 17) *M. incognita*. 18) *M. javanica*. 19) *M. hapla* race A. 20) *M. hapla* race B. All figures are same scale as Fig. 20.

wood (2) pointed out two varieties based on differences in male stylet length. In one group the males had short stylets (17–18 μm), in the other long stylets (20 μm). According to our findings, populations with short stylets belong to race A, populations with long stylets to race B.

A comparison of the stylet and attached cuticular lumen lining of the female (8) with that of the male reveals some structural differences. In the female the esophageal lumen lining of the procorpus is thick and the triradiate lining of the metacarpus pump is large and thick. The thick, rigid edges of the lining support the metacarpus pump, and the thin, flexible inner portions are pulled apart by the pump muscles, thus producing the suction required for feeding. In the male the triradiate lining is thin throughout and the pump probably is not functional. Stylet orifices were not seen in 70% of the *M. javanica* males which further indicates that males do not feed. The opening of the stylet lumen in the male is located more posteriad on the cone than in the female.

When stylets of the two sexes are compared within the same species (8) similar morphological trends are apparent. In *M. arenaria* the stylet is robust and the knobs gradually merge with the stylet shaft in the male and female. In *M. incognita* the stylet cone is uniquely shaped in both sexes and the overall shape of the knobs is similar, even though the female stylet knobs are wider. In *M. javanica* the stylet cone is straight and the knobs are short and wide and anteriorly indented in both sexes. Males and females of both races of *M. hapla* have stylets with narrow, pointed cones and rounded, set off knobs. Some differences were noted in the shape of the stylet shaft between the sexes of race B. In race B males the stylet shaft remains cylindrical throughout and often narrows near the knobs, whereas in the females the shaft is wider near the knobs.

Previously, the value of males in root-knot nematode taxonomy has not been emphasized because of their apparent morphological variability and scarcity. Males, however, were numerous in most of our greenhouse cultures, and head shape and stylet morphology of males appear to be

useful supplemental taxonomic characters. Our observations of male head shape and stylet morphology of these four species further emphasize the importance of male head shapes and stylet morphology in the identification of the four common species of root-knot nematodes. Our findings confirm some of Chitwood's (2) earlier findings as illustrated in his figures 1A (*M. arenaria*), 5A,K (*M. incognita*), 2E (*M. javanica*), and 3C,V,X,Z (*M. hapla*).

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