Morphological Comparison of *Meloidogyne* Female Head Structures, Perineal Patterns, and Stylets[1]

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Abstract: The external morphology of female heads of three populations of each of two cytological races of *Meloidogyne hapla* (race A—meiotic, race B—mitotic) and single populations of *M. arenaria, M. incognita,* and *M. javanica* was compared by light (LM) and scanning electron microscopy (SEM). Perineal patterns of all nine populations were observed with a LM and then examined with a SEM. In addition, female stylets of each population were excised, viewed with a SEM, and compared with observations made with a LM. Head morphology of the females, including shape of medial and lateral lips, expression of sensilla, and head annulation, was distinct for each species, each race of *M. hapla,* and each population of *M. hapla* race A. The morphology of a given perineal pattern appeared similar with the SEM and the LM. The SEM emphasized surface details, whereas the LM revealed subcuticular structure as well. Stylet morphology was unique for each species but similar in all populations of *M. hapla.* There were differences between species in the shape of the cone, shaft, and knobs and in the distance of the dorsal esophageal gland orifice from the stylet knob base. Several of the morphological characters first detected in the SEM were seen subsequently with the LM and are helpful in species identification. *Key Words:* cytological races, root-knot nematodes, *Meloidogyne hapla, M. arenaria, M. incognita, M. javanica,* scanning electron microscopy.

The morphology of the adult female is usually the most important character in the identification of species of *Meloidogyne* (root-knot nematodes) (2,9,11,17,23,24,31). Since Chitwood (2) re-established the genus and described five new species, the structure of the perineal pattern has become the major character used in species identification. Other characters, such as shape of the stylet knobs and various measurements, were listed as useful; but because the measurements were variable and overlapped between species, they were not generally accepted by later investigators.

Researchers soon realized that the perineal patterns were quite variable, and many aberrant and intermediate forms were found (1,2,6,17,18,30). Whitehead (31) recognized that with the number of described species increasing rapidly, it was becoming progressively more difficult to identify species from perineal patterns alone. He reemphasized the morphology and morphometrics of second-stage juveniles and males and provided pertinent information about many species, pointing out differences among them. Still the perineal pattern remained the most important character, and the morphology of second-stage juveniles and males was useful only in a supplemental way. Some investigators find these supplemental characters of juveniles and males useful (9,11,23), but others do not (17). Host range (19,20,21,23), biochemical data (3,4,5,10,14), and cytological data (13,25,26, 27,28,29) were found to be helpful in species identification, especially of populations with aberrant or intermediate perineal patterns.

The morphology of the female head and stylet has received very little attention. Even though Chitwood (2) pointed out differences in female stylet morphology, its taxonomic value has not been documented. The stylet length and the distance of the dorsal gland orifice from the stylet knob base are the only characters used by taxonomists.

Use of the scanning electron microscope (SEM) for morphological studies of *Meloidogyne* females has been limited to perineal patterns (15,16,22,32) until recently when SEM observations of the female head were included in the descriptions of two new species (12).

In recent SEM studies we have shown that the external morphology of second-stage juveniles (7) and males (8) of *Meloidogyne arenaria* (Neal) Chitwood, *M. hapla* Chitwood, *M. incognita* (Kofoid and White) Chitwood, and *M. javanica* (Treub) Chitwood is different for each species.

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Furthermore, within *M. hapla* the two cytological races, A and B (27), are morphologically different; and within race A the three cytological forms can be distinguished morphologically from each other. Race A includes populations which have a haploid chromosome number of 14, 15, 16, or 17 and reproduce by facultative meiotic parthenogenesis. Race B consists of populations which have a somatic chromosome number of 43 to 48 and reproduce exclusively by mitotic parthenogenesis. Since second-stage juveniles and males of different species and cytological forms of a species show distinct morphological differences, we wanted to determine whether females of the same populations also differed.

The present study compares the external morphology of female heads of six cytologically different populations of *M. hapla* and single populations of *M. arenaria, M. incognita,* and *M. javanica.* Perineal patterns of each population were examined with a SEM and light microscope (LM) to compare the two methods. In addition, the stylets of females of all populations were excised and studied with the SEM and LM.

MATERIALS AND METHODS

Populations studied: Three different chromosomal forms of race A and two chromosomal forms of race B of *Meloidogyne hapla* were selected from the root-knot nematode collection at North Carolina State University. In addition, single populations of *M. arenaria, M. incognita,* and *M. javanica* were examined. Each population was designated by the collection number, an abbreviated word indicating its origin, and the chromosome number in parentheses. The following populations were studied: *M. hapla* race A–42-Can (15) from Canada, 6-NC (16), and 86-NC (17) from North Carolina; *M. hapla* race B–48-NC (45) from North Carolina, 66-Md (45) from Maryland, and 230-Chile (48) from Chile; *M. arenaria,* 351-Fla (54) from Florida; *M. incognita,* 68-NC (41–43) from North Carolina; and *M. javanica,* 76-Ga (44) from Georgia. All populations were propagated on tomato (*Lycopersicon esculentum* Mill. 'Rutgers') in a greenhouse maintained at 22–28 C. Each population was tested for its ability to reproduce on five host differentials, as suggested by Taylor and Sasser (23). The results (not included in this paper) confirmed that each population had a host range typical of the species to which it was identified on morphological and cytogenetic bases. Furthermore, the populations of *M. arenaria* and *M. incognita* belonged to race 1 of the respective species (21). The nine populations used in this study were the same populations used in previous SEM observa-

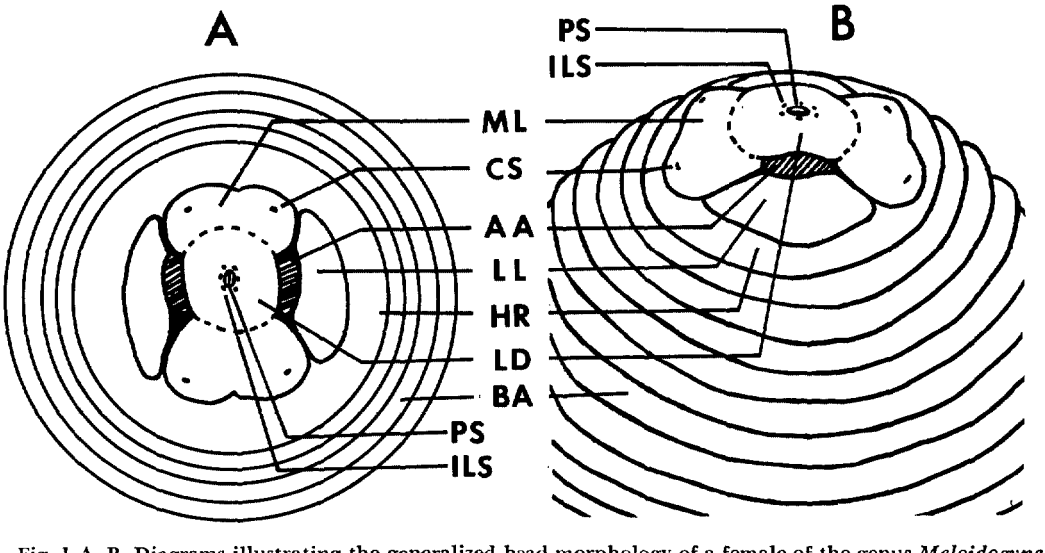
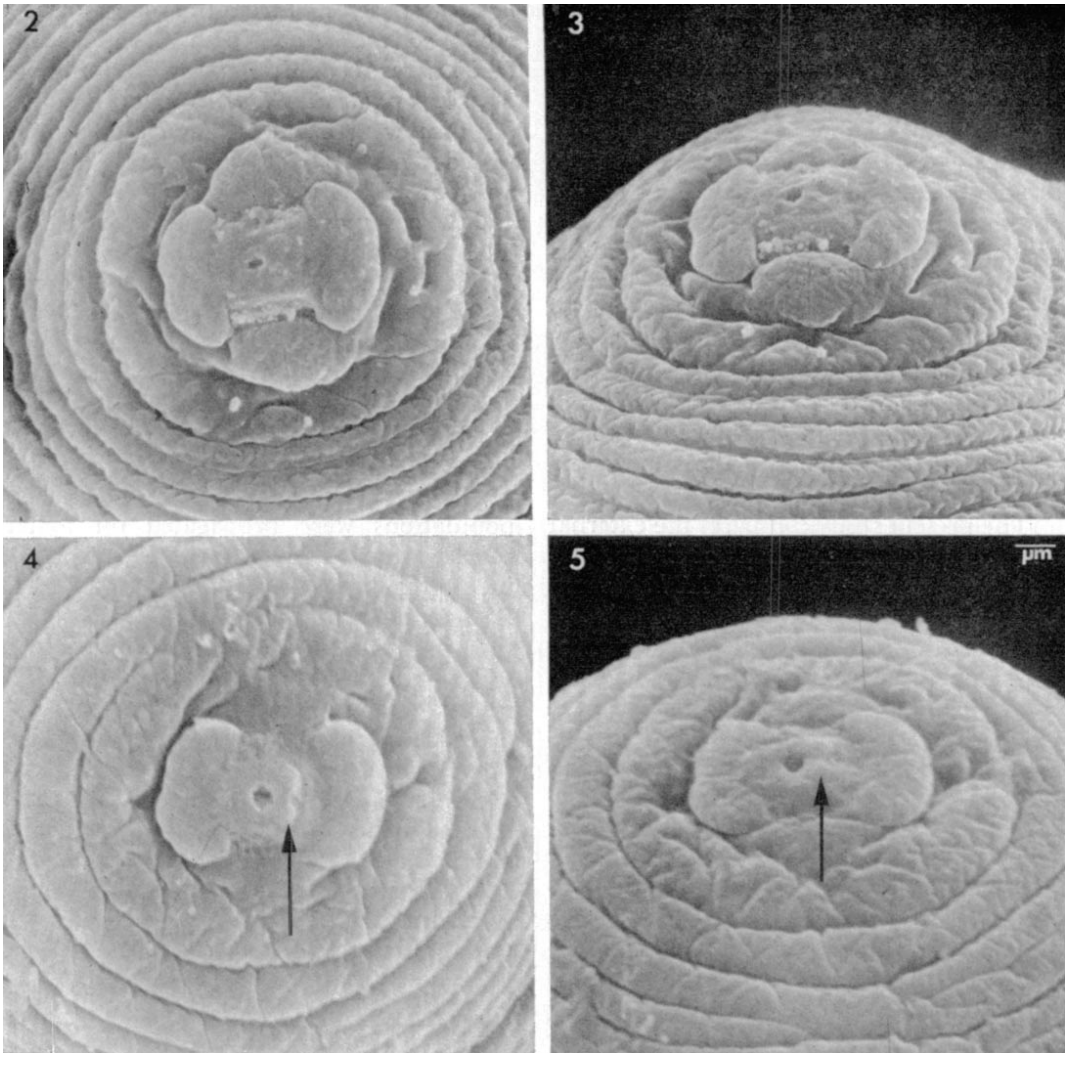


Fig. 1-A, B. Diagrams illustrating the generalized head morphology of a female of the genus *Meloidogyne.* A) Face view. B) View from the lateral side. AA, amphidial aperture; BA, body annule; CS, cephalic sensillum; HR, head region; ILS, inner labial sensillum; LD, labial disc; LL, lateral lip; ML, medial lip; PS, prestoma.



Figs. 2-5. SEM photographs. 2, 3) Face and lateral views of *M. arenaria*. 4, 5) Face and lateral views of *M. incognita*. Bumps on labial disc are marked by a single arrow. All figures are same scale as Fig. 5.

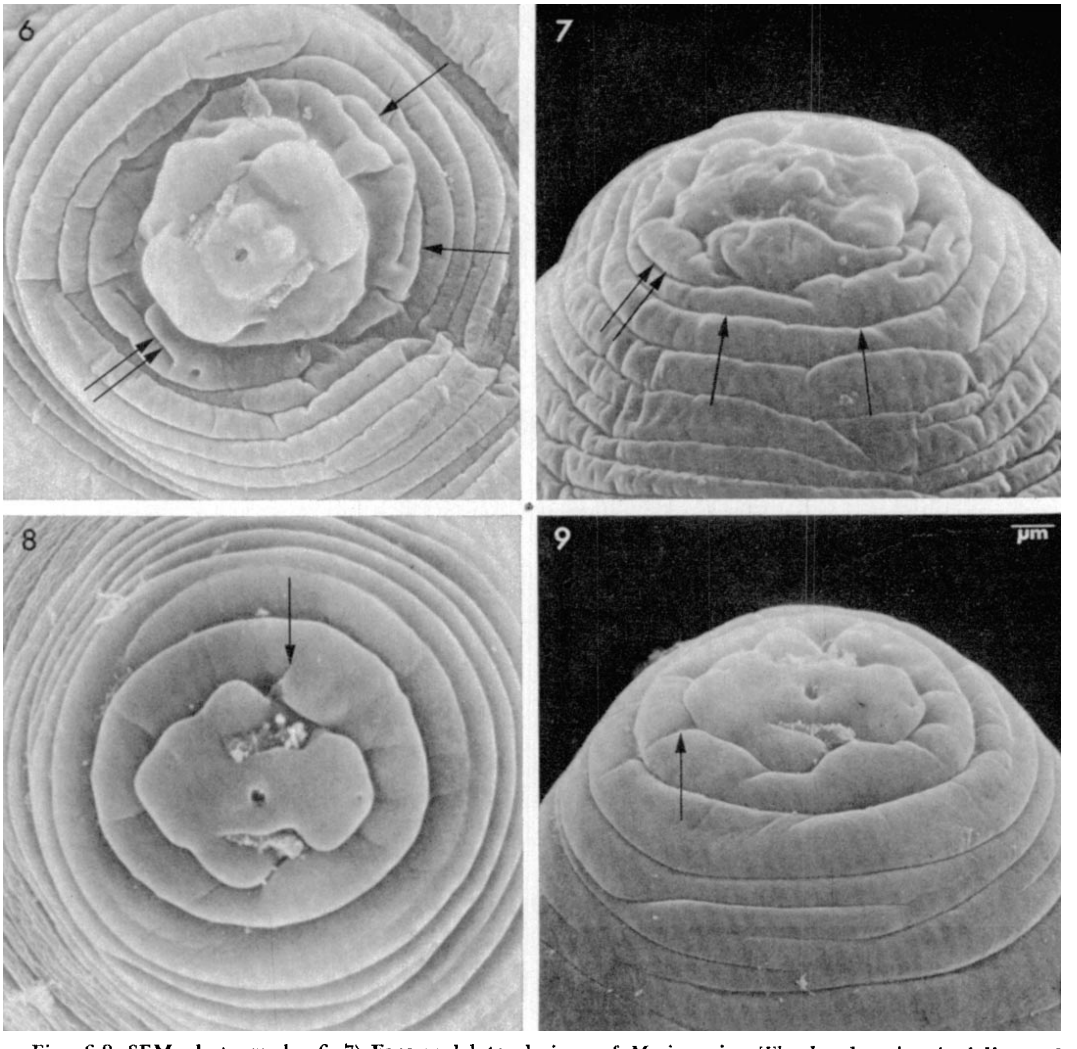
tions of second-stage juveniles and males (7,8).

SEM of female heads: Small pieces of galled root tissues containing egg-laying females were placed in 0.5 ml of cold (8 C) 4% glutaraldehyde solution buffered with 0.1 *M* sodium-cacodylate at pH 7.2. After fixation for at least 4 d, whole females were removed from the root tissues. The specimens were washed with several changes of the buffer, post-fixed for 12 h in 2% osmium tetroxide, dehydrated, and critical point dried (7). Dried specimens were transferred with a dental root canal file onto a stub covered with double-sided adhesive tape. They were mounted with their necks perpendicular to the surface of the stub, sputter coated with 400 Å of gold, and viewed and photographed with an ETEC scanning electron microscope operated at 20 KV accelerating voltage. At least 50 females from each population were examined.

LM and SEM of perineal patterns: Galled roots with females were fixed in 5% formalin for several days. Females were removed from the tissue and perineal patterns were cut and cleaned in lactic acid. The perineal patterns were mounted in glycerin on glass slides, viewed, and photographed with a bright field light microscope equipped with an apochromatic objective. For SEM comparisons the perineal patterns

were removed from the slides and placed on lint-free filter paper in an oven at 50 C for 1 h to drain most of the glycerin. They were then placed in a desiccator over anhydrous $CaSO_4$ for 1 wk. The desiccated patterns were mounted on double-sided adhesive tape on SEM stubs, coated with gold, viewed, and photographed. LM and SEM photographs of the same pattern were compared.

SEM of female stylets: Adult females were dissected from galled roots and placed in 45% lactic acid in a plastic petri dish. After 5–15 min in the acid, the female neck was cut behind the median bulb with a sharp eye-knife. Using a stereoscope at 80x magnification, the stylet and attached lumen lining of the esophagus were pushed out posteriorly with a dental root canal file. The muscles of the median bulb, which usually remained attached to the lining, were cleaned off in the lactic acid. The stylet, with the attached lining, was transferred into a new drop of lactic acid on a glass coverslip. After 5–7 stylets had been placed on one coverslip, a drop of 2% formalin was pipetted onto the lactic acid every 2–3 min until the coverslip was flooded with formalin. The formalin was drained off the coverslip 5–10 min later. The stylets were air dried, coated with gold, viewed, and photographed. At least 35 stylets from each popu-



Figs. 6-9. SEM photographs. 6, 7) Face and lateral views of *M. javanica*. The head region is delineated by a single arrow and head annulations are marked by double arrows. 8, 9) Face and lateral views of *M. hapla* race A, population with 15 chromosomes. Longitudinal lines on the head region are marked by a single arrow. All figures are same scale as Fig. 9.

Table 1. Head characteristics for females of different *Meloidogyne* species.

Species	Prestoma shape	Bumps on labial disc	Symmetry of labial disc and medial lips	Medial lip shape	Lateral lip size	Lateral lip shape	Head region annulation
M. arenaria	Hexagonal	No	Symmetric	Angular	Large	Set-off	Yes
M. incognita	Hexagonal	Yes	Symmetric	Rounded	Large	Fused with head region	Yes
M. javanica	Oval	Yes	Symmetric	Indented	Large	Set-off	Yes
*M. hapla**	Hexagonal	No	Asymmetric	Squared	Small	Fused with medial lip	No
Population 42-Can†	Hexagonal	No	Asymmetric	Triangular	Small	Fused with medial lip	No

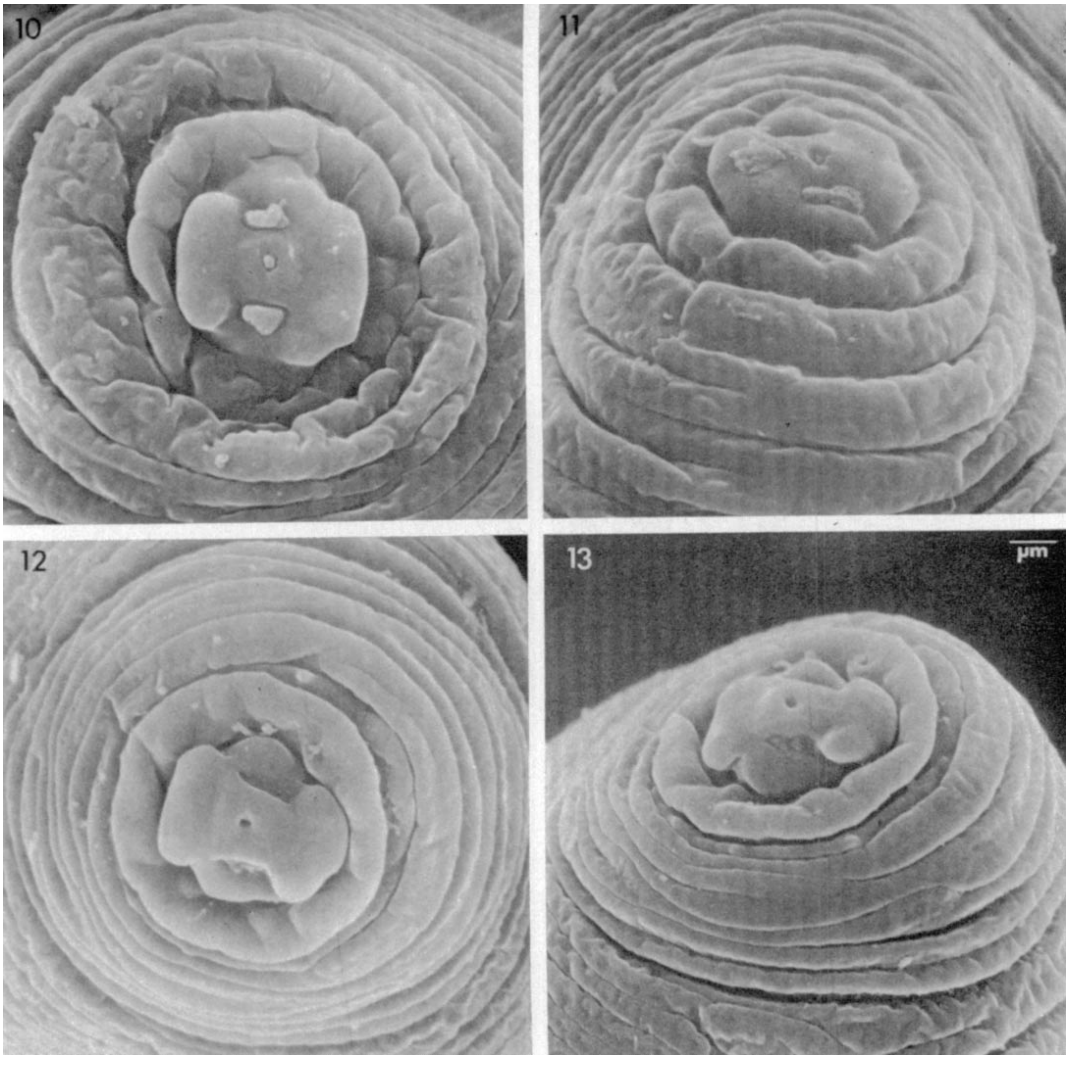
*Typical characters for most of the populations of *M. hapla*.
†*M. hapla* population that was atypical in medial lip shape.

lation were observed. They were photographed with the surface of the stub perpendicular to the electron beam to avoid foreshortening of the image.

LM of female heads: Egg-laying females were removed from galled roots and placed in 2% glutaraldehyde buffered with 0.1 *M* sodium-cacodylate at pH 7.2. Following a 24–72 h fixation at 8 C, the heads of the females were cut off and mounted in glutaraldehyde on glass slides. Camera lucida drawings and photographs were made of each population. At least 40 specimens of each population were examined.

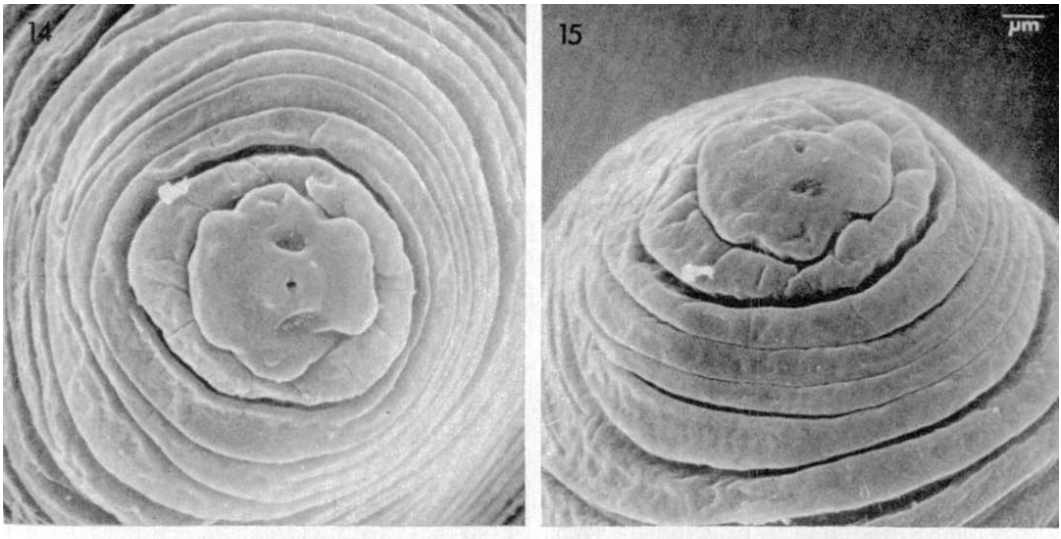
OBSERVATIONS

SEM of female heads: Fig. 1 illustrates the basic morphology of the head of a *Meloidogyne* female based on SEM observations of *M. arenaria, M. hapla, M. incognita,* and *M. javanica.* The slitlike stoma is located centrally on the labial disc within the oval prestoma. The porelike openings of the six inner labial sensilla surround the prestoma. In some species they open into the prestoma, making the prestomatal opening appear hexagonal. Often the four medial inner labial sensilla open into the

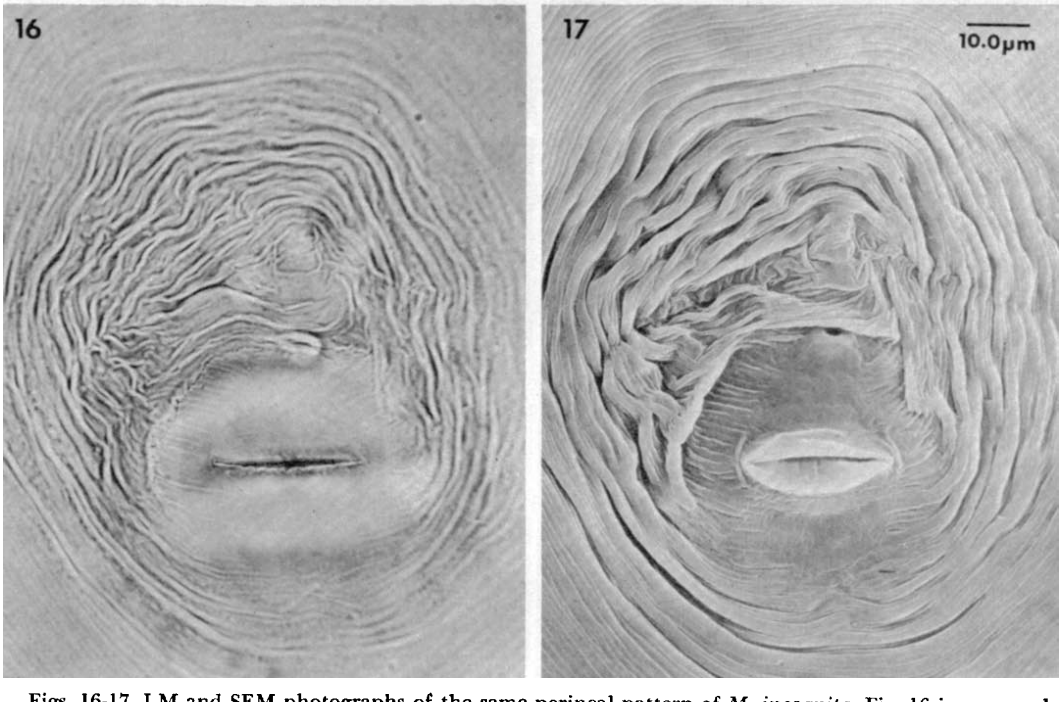


Figs. 10-13. SEM photographs. 10, 11) Face and lateral views of *M. hapla* race A, population with 16 chromosomes. 12, 13) Face and lateral views of *M. hapla* race A, population with 17 chromosomes. All figures same scale as Fig. 13.

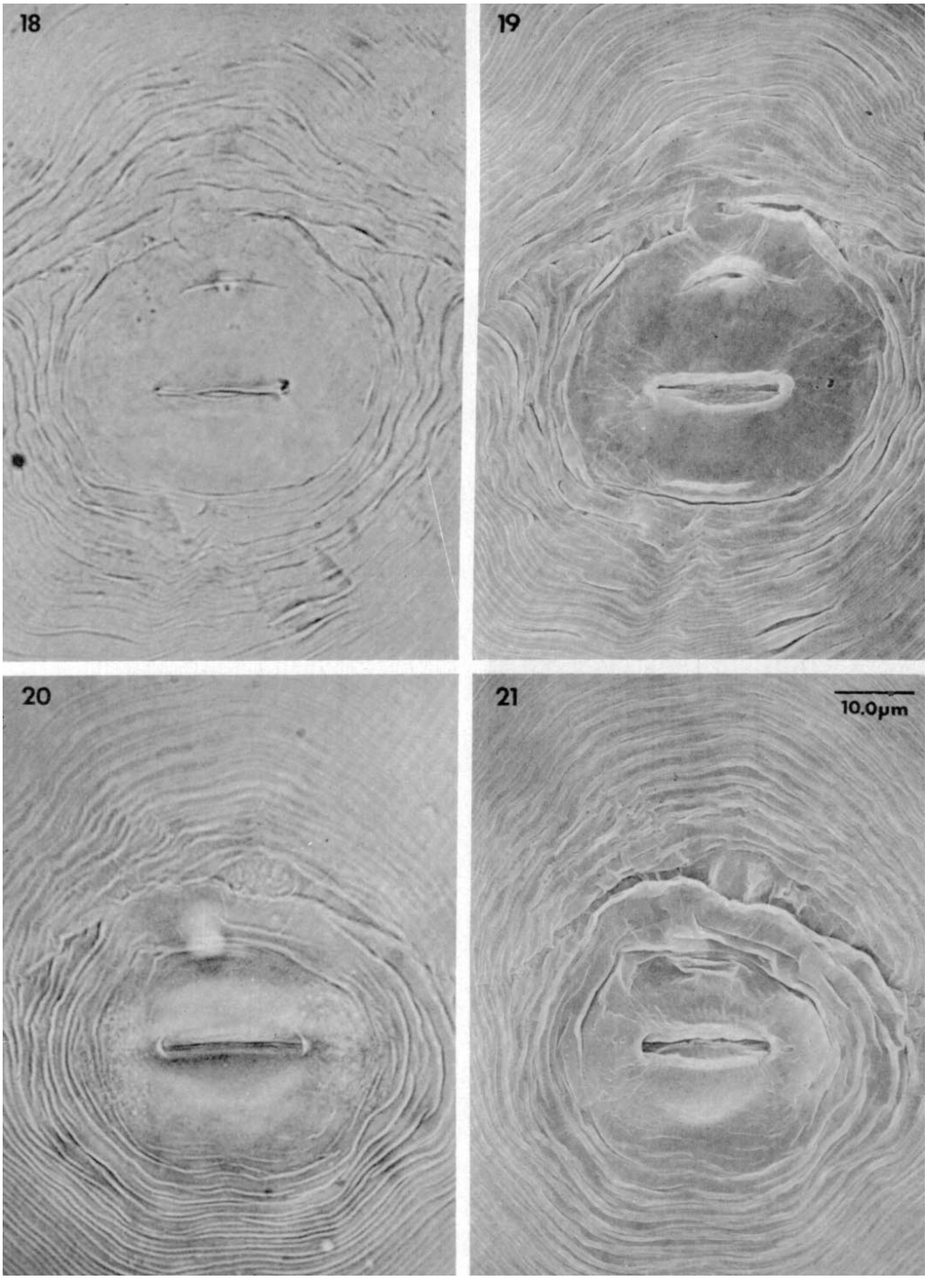
prestoma, but the lateral ones open onto the labial disc. Six lips surround the labial disc posteriorly. The subdorsal and subventral lips are fused medially, forming one dorsal and one ventral lip. These lips are termed medial lips because it is often difficult to distinguish the dorsal and ventral sectors from each other. Each medial lip contains a pair of cephalic sensilla which are expressed in some speices as small round depressions in the cuticle. The labial disc is raised above the medial lips in most species but is in the same contour and fused with the medial lips in others. The lateral lips are large and may be fused with the medial lips or the head region. The amphidial openings appear oval shaped and are between the labial disc and lateral lips. The



Figs. 14-15. SEM photographs. Face and lateral views of *M. hapla* race B. Fig. 14 is same scale as Fig. 1



Figs. 16-17. LM and SEM photographs of the same perineal pattern of *M. incognita*. Fig. 16 is same scale as Fig. 17.



Figs. 18-21. 18, 19) LM and SEM photographs of the same perineal pattern of *M. hapla* showing the square type of pattern. 20, 21) LM and SEM photographs of the same perineal pattern of *M. hapla* showing a round type of pattern. All figures are same scale as Fig. 21.

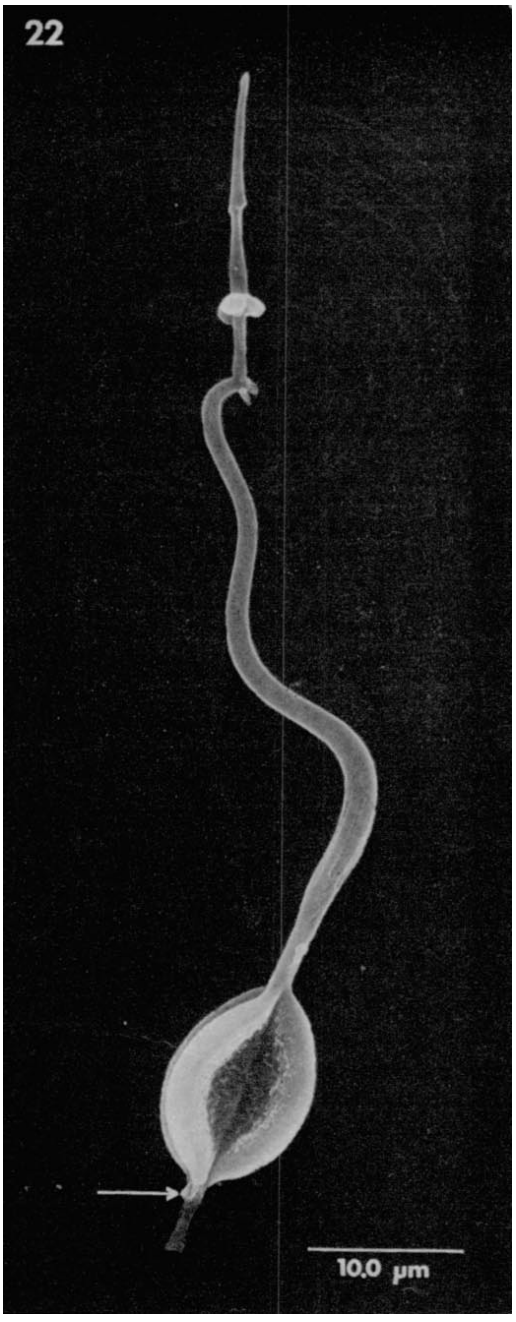


Fig. 22. SEM photograph of an excised stylet and attached cuticular lumen lining of the esophagus typical of *Meloidogyne* spp. females. Arrow indicates one of the subventral esophageal gland orifices.

head region may appear as one large smooth annule, posterior to the lips as illustrated in Fig. 1, or it may be marked by one broken annulation (Fig. 6). Usually the head region is marked by longitudinal lines (Fig. 8).

The head structures of females of *M. arenaria* population 351-Fla (54) (Figs. 2, 3), *M. incognita* 68-NC (41–43) (Figs. 4, 5), and *M. javanica* 76-Ga (44) (Figs. 6, 7) are similar in many respects (Table 1). In all three species the labial disc and medial lips are dumbbell shaped in face view and the lateral lips are large and extend the entire length of the labial disc and medial lips. The lateral lips are separated from the medial lips, and the head region is usually marked by one broken annulation. There are some distinguishing characteristics between the species. In *M. arenaria* and *M. incognita* the prestoma is hexagonal, but in *M. javanica* it is oval. *M. incognita* and *M. javanica* have two bumps on the ventral side of the labial disc, but *M. arenaria* has none. The shape of the lateral sides of the medial lips is different for each species; in *M. arenaria* they are angular, in *M. incognita* they are rounded, and in *M. javanica* they are usually indented medially, often dividing the lip into a pair of medial lips. The lateral lips of *M. incognita* are unique because in general they fuse laterally with the head region for a short distance.

The populations of *M. hapla* (Figs. 8–15) were quite different in head morphology from *M. arenaria, M. incognita,* and *M. javanica* (Table 1). In all populations of *M. hapla* the labial disc and medial lips are asymmetric; the lateral lips are small and triangular and fused with the ventral lip; the head region is not annulated. The prestoma is hexagonal, and bumps are not present on the labial disc. The medial lips are either square, angular, or triangular; and the shape of the ventral lip is different from that of the dorsal lip.

Populations of *M. hapla* race A are similar to each other in many respects, but some general differences do occur. Population 42-Can (15) (Figs. 8, 9) is distinct from the other *M. hapla* populations because the medial lips are triangular in face view. In population 6-NC (16) (Figs. 10, 11) the medial lips and labial disc are square in face view, whereas, in population 86-NC (17) (Figs. 12, 13) they are rectangular. Populations of race B—48-NC (45), 66-Md (45), and 230-Chile (48)—are similar to each other (Figs. 14, 15). They are different from race A populations because the ventral lip is even more enlarged and rounded in face

view and the lateral lips are fused almost totally with the ventral lip and partially with the dorsal lip.

LM and SEM of perineal patterns: Examination of the same perineal pattern by light and scanning electron microscopy revealed essentially the same basic features. The SEM three-dimensional image shows more details of surface morphology in the anal and vulval areas. Any existing vulval depression or protuberance is more obvious, and the cuticular annules or folds of the pattern proper appear very pronounced. The same perineal pattern viewed with the LM has less depth of field, but subcuticular details as well as surface structure are visible (Figs. 16, 17).

When *M. hapla* (Figs. 19, 21) is examined by SEM, some characteristics of the pattern, such as the covered anal opening and fine striations in the perineum, are emphasized; whereas, other features, such as the typical subcuticular punctations which are visible by LM (Figs. 18, 20), are obscured. The perineal pattern of *M. hapla* consists of two distinct types, one with a squared-off dorsal arch (Figs. 18,19) and another with a rounded-off arch (Figs. 20, 21). In the rounded type the whole pattern is more circular. Patterns of both types were found in all six populations of *M. hapla*.

SEM and LM of female stylets: Fig. 22 illustrates the excised stylet and attached cuticular lumen lining of the esophagus typical of *Meloidogyne* spp. The conical part of the stylet is curved dorsally, and the stylet lumen opens out ventrally near the stylet tip. The irregular margin of the cone overlaps the cylindrical shaft posteriorly, and the three basal knobs of the stylet may gradually fuse with the shaft or they may be distinctly set off. Directly connected to the knobs, the lumen lining of the esophagus extends posteriorly; at a short distance the three branched channels of the dorsal esophageal gland duct enter into the lumen. At this junction the lining of the lumen curves sharply ventrally and then continues for some distance as a smooth round tube. In the metacorpus (median bulb) the esophageal lumen lining greatly enlarges and becomes the triradiate lining of the metacorpus pump. The triradiate lumen lining of the median bulb appears thick along the outside edges but is thin centrally. Immediately posterior to the lining of the median bulb, two ducts from the subventral esophageal gland enter the lumen. The lumen lining of the esophagus is extremely thin posterior to the subventral gland ducts.

The female stylet of *M. arenaria* population 351-Fl (54) (Figs. 23, 28, 33) is unique and very characteristic. The cone and shaft are thick. The shaft broadens posteriorly and gradually merges with the rounded backward sloping stylet knobs. In *M. incognita* population 68-NC (41–43) (Figs. 24, 29, 34) the anterior half of the stylet cone is cylindrical and distinctly curved dorsally, whereas the posterior half is conical. The shaft is only slightly wider posteriorly. The stylet knobs of *M. incognita* are set off from the shaft. They are wide and low, and the tip of each knob is indented. This is so pronounced in some specimens that each knob appears as if it were two. Female stylets of *M. javanica* population 76-Ga (44) (Figs. 25, 30, 35) are similar to those of *M. incognita,* but the stylet cone is only slightly curved dorsally and the shaft does not widen posteriorly.

Populations of *M. hapla* race A—42-Can (15), 6-NC (16), and 86-NC (17)—(Figs. 26, 31, 36) and race B—48-NC (45), 66-Md (45), and 230-Chile (48)—(Figs. 27, 32, 37) have similar stylet morphology. *M. hapla* female stylets in both races have cones that curve only slightly; the shaft becomes broader posteriorly, and the large rounded knobs are distinctly set off from the shaft. The differences observed between races were slight except for stylet length. The populations of race B have longer stylets, and the shaft generally broadens more in populations of race A.

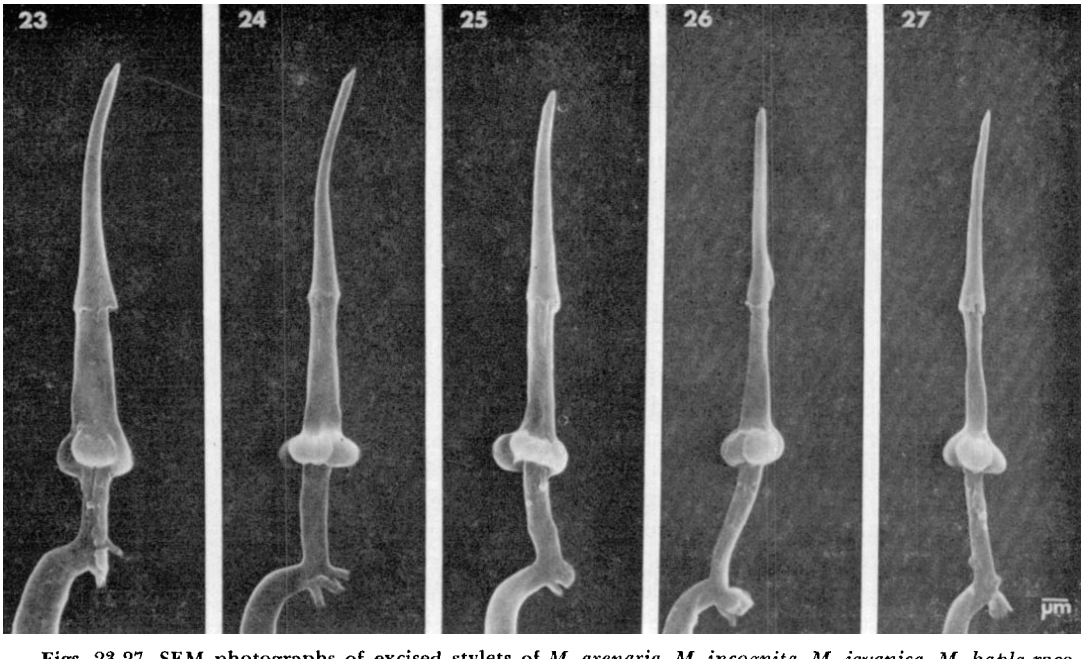
DISCUSSION

The present study of female head morphology is comparable to previous SEM observations of second-stage juveniles and males of the same populations (7,8). As with the juveniles and males, differences in head morphology of the females were found between the four species, the two races of *M. hapla,* and the three chromosomal forms of *M. hapla* race A. Juveniles, males, and females possess the same basic cephalic characters, but their expression is different. The

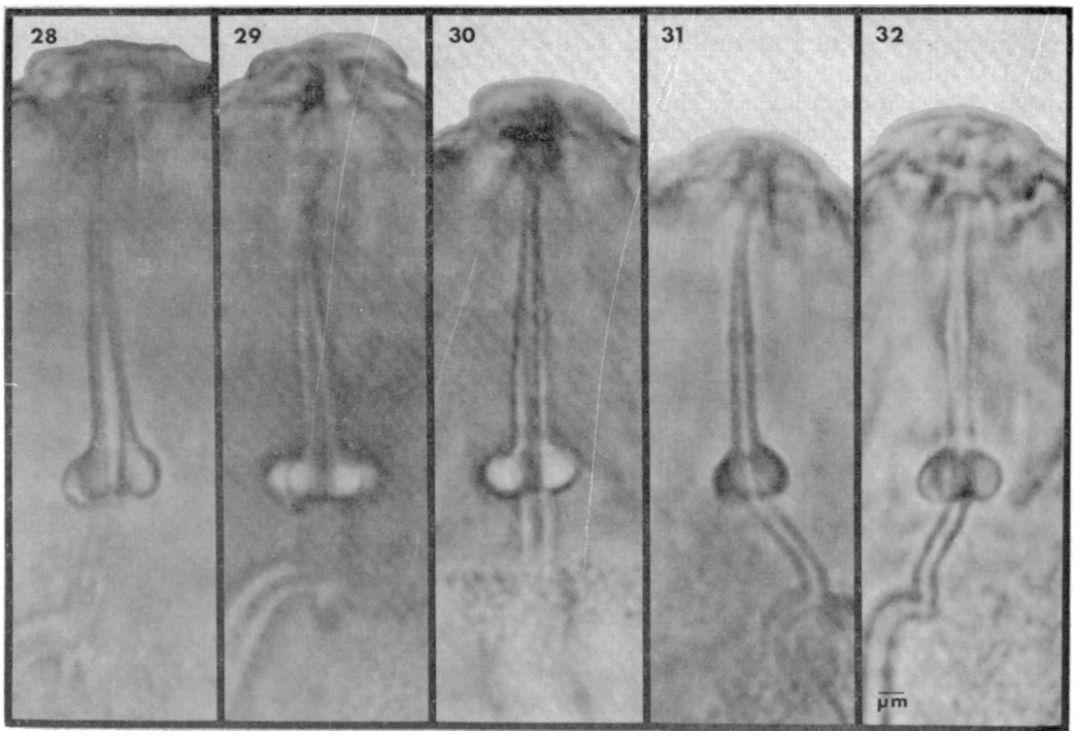
stoma is slitlike in juveniles, males, and females, and the prestoma is oval shaped in the juvenile and either oval shaped or hexagonal in the adult stages, depending on the location of the openings of the inner labial sensilla. In the juvenile the sensilla encircle the prestoma, which remains oval shaped, but in the male and female the sensilla often open into the prestoma, which appears hexagonal. The shape of labial disc and medial lips of the female resembles that of second-stage juveniles more than that of males. The amphidial openings of the females and juveniles are visible in face view; they are covered by the labial disc in the male. Lateral lips of the females are similar to those of juveniles; the males usually do not have lateral lips. Head annulation occurs in *M. arenaria, M. incognita,* and *M. javanica* females but not in *M. hapla.* Juveniles and males of *M. incognita* have head annules, as do males of *M. arenaria.* Juveniles of *M. arenaria* do not have annulations in the head region, nor do males or juveniles of *M. javanica* and *M. hapla.* In general the head morphology of the female resembles the second-stage juvenile more than the male, although certain distinguishing characters may be expressed in all three life stages; e.g., juveniles, males, and females of *M. hapla* population 42-Can (15) have pointed medial lips.

Differences in female heads observed among species are too obscure to be detected with the light microscope. Furthermore, the morphology of the head is greatly influenced by the age of the female and possibly by environmental factors. Difficulty in specimen preparation is an additional factor limiting the usefulness of female head morphology in the taxonomy of the genus. The specimens must be fixed in the root tissues because removal of unfixed specimens from the root causes the neck to be stretched unnaturally. Specimens must also be fixed and prepared for SEM with extreme care because the large size of the body makes the preservation of the nematode difficult and unnatural wrinkling is likely to occur. Although there are many difficulties in preparing females for SEM, new species descriptions would be more complete if face views and approximate lateral views of the female heads were included.

Perineal patterns probably remain the single most important character of females used in species identifications. The present study shows that the SEM and the LM reveal similar information and that the light microscope is quite adequate. Although the

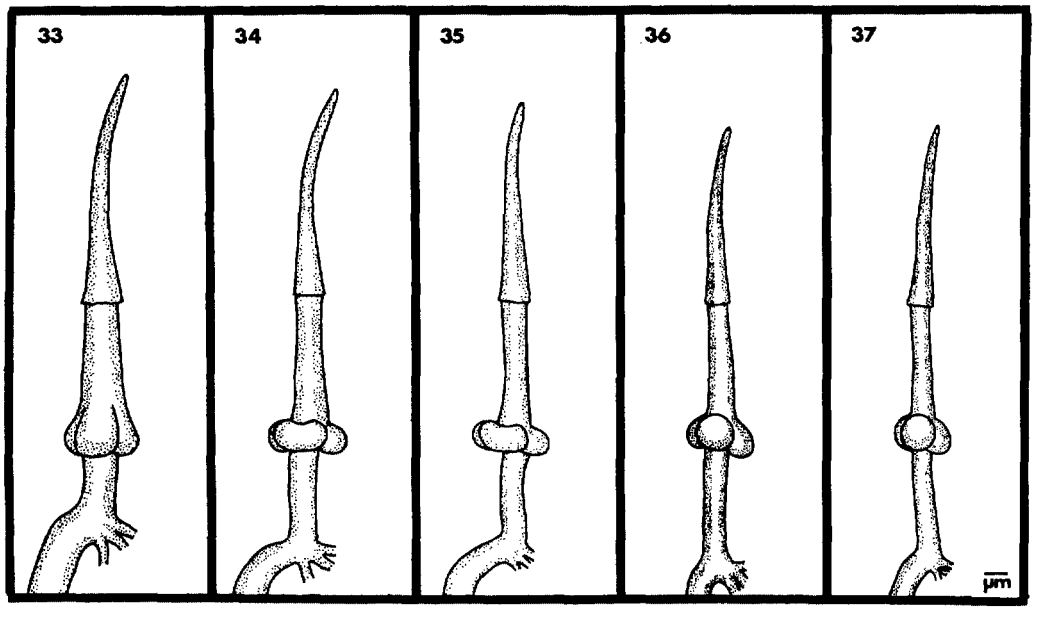


Figs. 23-27. SEM photographs of excised stylets of *M. arenaria, M. incognita, M. javanica, M. hapla* race A, and *M. hapla* race B. All figures are same scale as Fig. 27.



Figs. 28-32. LM photographs of stylets of *M. arenaria, M. incognita, M. javanica, M. hapla* race A, and *M. hapla* race B. All figures are same scale as Fig. 32.

SEM gives a three-dimensional image and provides a higher degree of resolution, it is limited to surface morphology. It is useful in clarifying finer surface details of the pattern, but the overall shape of the pattern is similar to the image provided by LM. Even though LM and SEM provide similar information, it is difficult to identify species



Figs. 33-37. Line drawings of stylets of *M. arenaria, M. incognita, M. javanica, M. hapla* race A, and *M. hapla* race B. All figures are same scale as Fig. 37.

from SEM photographs because the criteria used in species identifications established from LM photographs are sometimes obscure in SEM photographs; e.g., the punctations of *M. hapla* patterns are not seen in the SEM. In future studies with the SEM it may be possible to describe new criteria that would enable identification of species from SEM observations alone. SEM studies may also help determine the extent of intraspecific variation in perineal patterns. Future investigators should be aware of the difficulty of preparing specimens for SEM observation. Certain preparation procedures can cause artifacts, such as small wrinkles within the lines of a perineal pattern that could mistakenly be described as new characters. In our opinion the light microscope is sufficient for resolving details of the patterns useful in species identifications and it is more practical in routine morphological studies. For this reason we did not include, in this paper, SEM photographs of the perineal patterns of all species studied. Such photographs are available in the literature for a number of species (12,16,22,32).

The morphology of the female stylets is distinct for each species, and all populations of *M. hapla* had similar stylet morphology. Close examination of the female stylets illustrated by Chitwood (2) shows many of the differences in stylet morphology revealed in this study. Chitwood's Fig. 1 (E, N) of *M. arenaria* female stylets is similar to our figures of a typical *M. arenaria* stylet. Our description of *M. incognita* stylets is comparable to Chitwood's Fig. 5 (D), and our description of *M. javanica* stylets is like his Fig. 2 (A, L, P, Q). Likewise, the illustrations by Chitwood of *M. hapla* female stylets, Fig. 3 (F, G, W, Y), resemble our figures of the same species.

Observation of excised stylets with the SEM has made it possible to recognize with the light microscope differences in stylet morphology of the different species. We suggest that female stylet morphology be used as a supplemental character in the identification of *Meloidogyne* species.

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