Morphological Comparison of *Meloidogyne* Males by Scanning Electron Microscopy[1]

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Abstract: Males of five populations of *Meloidogyne hapla* were compared by scanning electron microscopy (SEM). Three populations of race A had haploid chromosome numbers of 15, 16, and 17 and reproduced by facultative parthenogenesis. Race B consisted of two mitotically parthenogenetic populations with somatic chromosome numbers of 45 and 48. Males of one population each of *M. arenaria, M. incognita,* and *M. javanica* were also examined to delineate species differences. The populations of *M. arenaria, M. incognita,* and *M. javanica* had 54, 41–43, and 44 chromosomes, respectively, and reproduction was by mitotic parthenogenesis. Observations were made on head structures, lateral field, excretory pore, and tail. The expression of labial and cephalic sensilla, shape and proportion of labial disc and lips, and markings on the head region were distinctly different for each species. The head morphology of the two cytological races of *M. hapla* was dissimilar. Populations of race A were different from each other and showed intrapopulation variation. Populations of race B were morphologically similar and stable in head morphology. The structure of the lateral field, excretory pore, and tail was of little value in distinguishing species or populations because of inter- and intrapopulation variation. The results are discussed in relation to earlier SEM observations of second-stage juveniles of the same populations. *Key Words:* cytological races, root-knot nematodes, *Meloidogyne hapla, M. arenaria, M. incognita, M. javanica.*

Although identification of *Meloidogyne* species from morphological characters of males is very difficult by light microscopy, or in some cases impossible, male characters can often supplement an identification. Chitwood (2) pointed out many differences between males of several different root-knot nematode species, and within a single species. In the original description of *M. hapla,* he described several varieties based on morphological differences in the male. Whitehead (9) suggested that male characters were of diagnostic value. Some workers consider males of little or no value in routine identification of *Meloidogyne* species (7), and others consider only a few characters to be useful (1, 4, 5, 6). These characters include shape and annulation of the head, stylet length and shape of basal knobs, distance of the dorsal gland orifice from the base of the stylet, spicule and gubernaculum length, and the areolation and number of incisures in the lateral field. Other characters are either too variable and overlapping, or they are too small to be seen clearly enough to be of taxonomic value.

In a recent scanning electron microscope (SEM) study comparing second-stage juveniles of *M. arenaria* (Neal) Chitwood, *M. hapla* Chitwood, *M. incognita* (Kofoid and White) Chitwood, and *M. javanica* (Treub) Chitwood, we reported the cephalic morphology to be distinct for each species (3). Furthermore, within *M. hapla,* the two cytological races A and B (8) could be distinguished from each other. Race A comprises populations that reproduce by facultative parthenogenesis and have a haploid chromosome number of 15, 16, or 17. Race B includes populations that reproduce exclusively by mitotic parthenogenesis and have a somatic chromosome number of 45 or 48. The three chromosomal populations within race A could also be identified by SEM. If the morphology of second-stage juveniles is distinct between species and cytological forms of a species, the males of these same populations may possess similar discrete morphology. Although males are sometimes rare, they aid in accurate identification of species. The most widely used male characters for differentiating *Meloidogyne* spp. with the light microscope are stylet length, number of head annules, and structure of the lateral field (5). The SEM is ideal for observation of the head annules, lateral field, and other cuticular structures.

The present study compares the external morphologies of males of five chromosomal

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populations of *M. hapla* as seen with the SEM, and the morphological findings are related with cytological data. To determine differences between species, one population each of *M. arenaria, M. incognita,* and *M. javanica* was also examined with the SEM.

MATERIALS AND METHODS

Six different cytological populations of *M. hapla,* with different modes of reproduction, and one population each of *M. arenaria, M. incognita,* and *M. javanica* were selected from the *Meloidogyne* collection at North Carolina State University. Populations were designated by a number, an abbreviated word indicating their origin, and their chromosome number in parentheses, as follows: *M. hapla* populations 42-Can (15) from Canada; 6-NC (16), 86-NC (17), and 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. The *M. hapla* populations were maintained at 21 C, and the other species at 28 C. Males were obtained by incubating washed infected root systems in a moist chamber at room temperature. The specimens were then processed for SEM as described previously (3) except that they were cut in half after 24 h in the initial fixative, and fixed for an additional 36 h. At least 100 males from each population were examined with an ETEC scanning electron microscope operated at 20 Kv. The *M. hapla* population 66-Md (45) failed to produce males even though the cultures were grown under a variety of environmental conditions.

OBSERVATIONS

Head morphology: Fig. 1, based on SEM observations of *M. arenaria, M. hapla, M. incognita,* and *M. javanica,* illustrates the basic plan of the cephalic characters of males of the genus *Meloidogyne.* The opening of the prestoma is centrally located on the labial disc, slightly above the slitlike stomatal opening. The prestomatal opening may be oval-shaped in face view, when the small, pitlike openings of the six inner labial sensilla encircle it on the labial disc, or it may appear hexagonal, when the inner labial sensilla open into the prestoma. Posterior to the labial disc, the subdorsal and subventral lip pairs are fused medially, forming one dorsal and one ventral lip, respectively. These lips are termed medial lips because the symmetry of the head structures makes it impossible to distinguish dorsal from ventral. Each medial lip con-

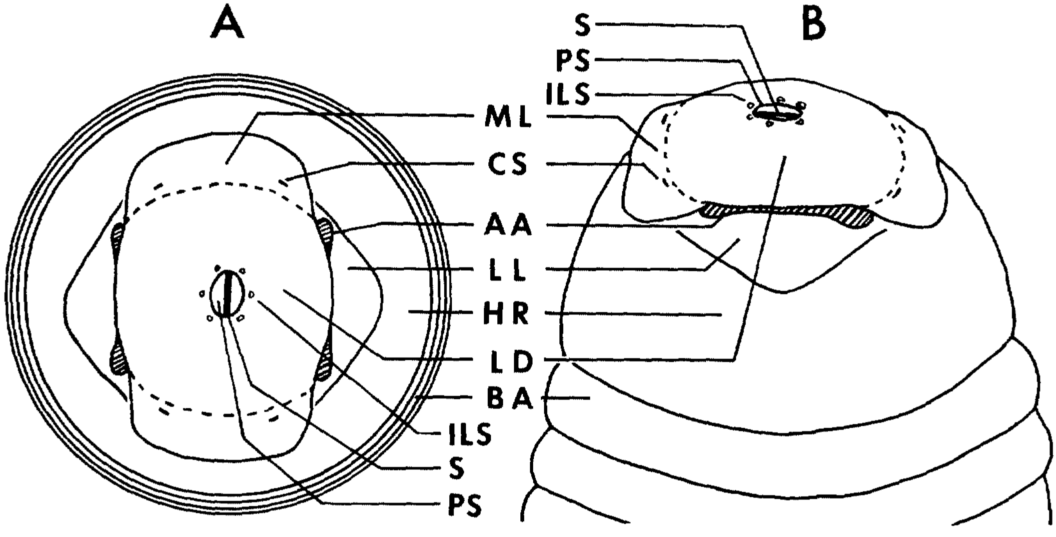

FIG. 1-A, B. Diagram illustrating the generalized head morphology of a male of the genus *Meloidogyne.* A) Face view. B) View from the lateral side. AA, amphidial aperture; BA, body annule; CS, cephalic sensillum; HR, head region; ILS, inner labial sensillum; LD, labial disc; LL, lateral lip; ML, medial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

tains one pair of cephalic sensilla that in some species are expressed externally as small cuticular depressions. The labial disc, often slightly raised above the medial lips, may form a continuous structure with the medial lips. Lateral lips, when present, constitute an area on the head region that is marked off by grooves beginning near the lateral edges of the medial lips and extending into the head region posteriorly. Amphidial openings are long slits between the labial disc and the lateral lips. Posterior to the lips, the head region may be smooth as illustrated in Fig. 1, or marked by one to three incomplete annulations.

The slightly rounded labial disc of *M. arenaria,* population 351-Fla (54) (Figs. 2, 3), is raised above the medial lips, which are crescent-shaped in face view and extend for some distance onto the head region. Remnants of lateral lips are visible on the head region as short grooves originating near the middle of the lateral edges of the medial lips. Except for the lateral sensilla, the inner labial sensilla open into the prestoma and are thus partly obscured. Small cephalic sensilla are expressed externally, and the head region has either one or two incomplete annulations.

M. incognita, population 68-NC (41–43) (Figs. 4, 5), possesses a round labial disc that is distinctly raised above the medial lips and depressed for some distance around the prestomatal opening. The medial lips are

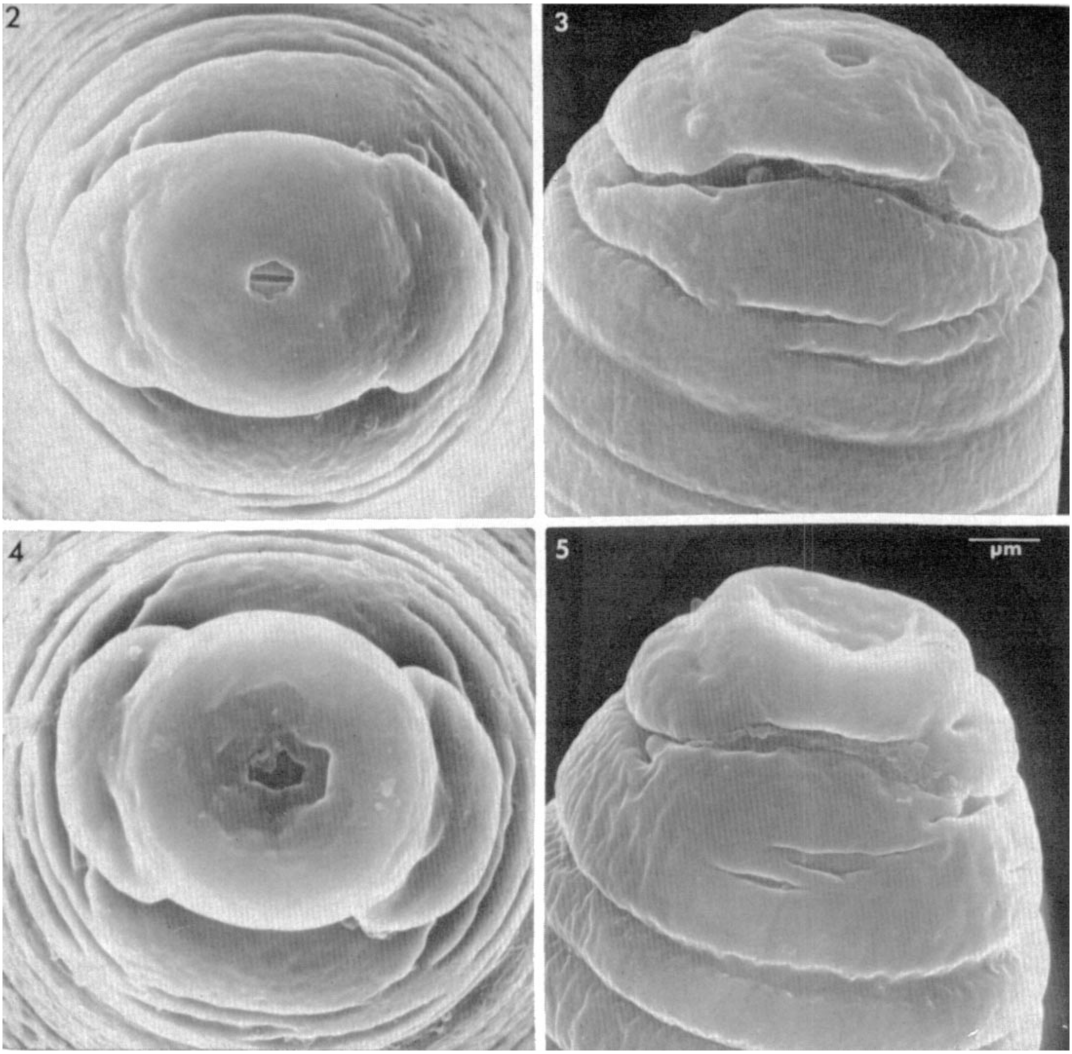

FIG. 2–5. 2,3) Face and approximately lateral views of *M. arenaria.* 4,5) Face and lateral views of *M. incognita.* All figures are same scale as Fig. 5.

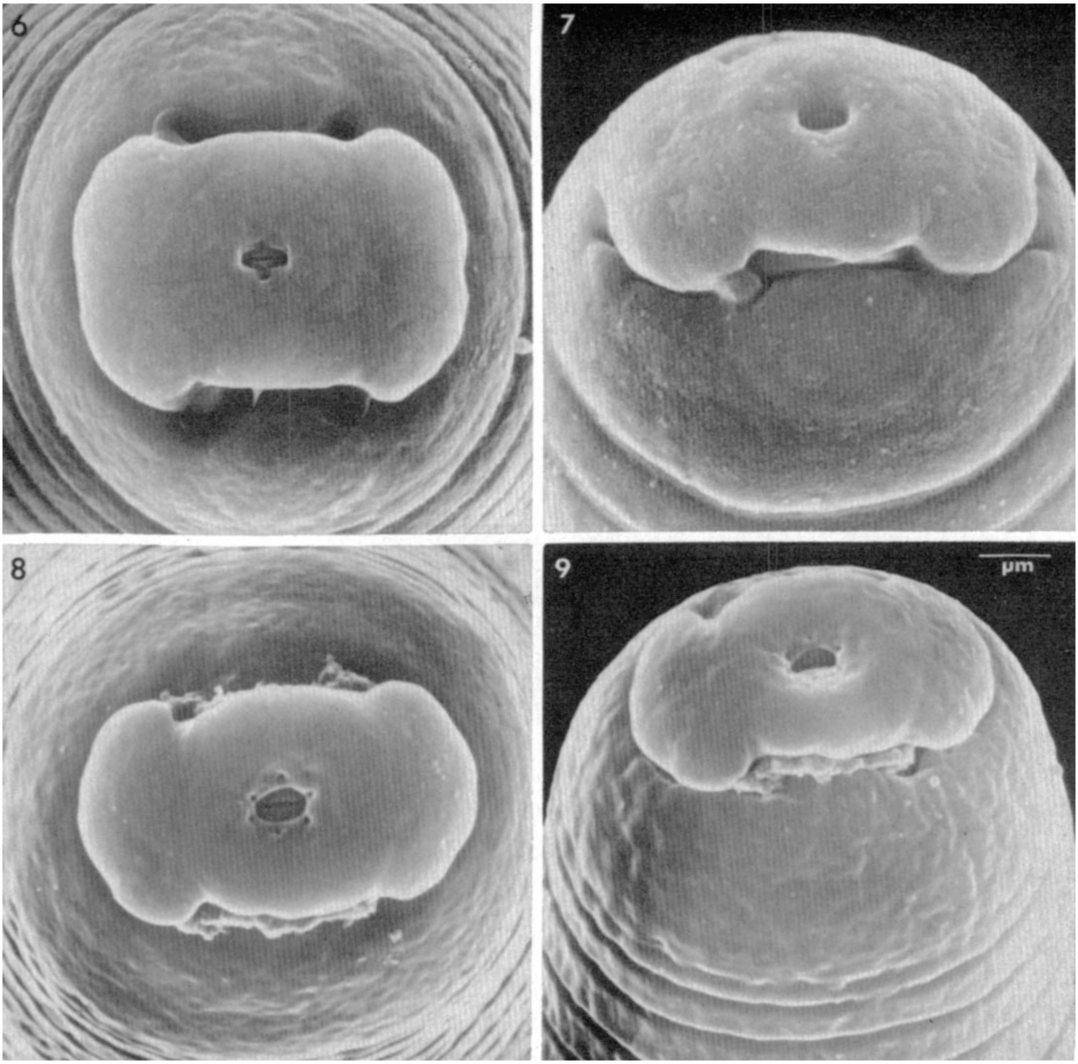

FIG. 6–9. 6,7) Face and approximately lateral views of *M. javanica.* 8,9) Face and lateral views of *M. hapla* race A, population with 15 chromosomes. All figures are same scale as Fig. 9.

crescent-shaped in face view and extend for some distance onto the head region. The lateral lips are generally not visible, although remnants may be present on the head region, appearing as grooves near the middle of the lateral edge of the medial lips. Large cephalic sensilla are present and the head region usually bears either two or three irregular incomplete annulations.

In *M. javanica,* population 76-Ga (44) (Figs. 6, 7), labial disc and medial lips are in the same contour and fuse to form a continuous elongate structure. The lateral edges of the labial disc are almost straight in face view, so that the large amphid openings are exposed. The medial lips are wider than the labial disc, and lateral lips are absent. The six inner labial sensilla open into the prestoma, although the lateral sensilla are usually more prominent. Cephalic sensilla are not expressed externally, and the head region is not annulated.

M. hapla race A, population 42-Can (15) (Figs. 8, 9), was the most variable population studied. Its head structures are generally smaller than those of other species. The rounded labial disc is slightly raised above the pointed medial lips, and both structures indent markedly at the lateral junction. These indentations are usually more pronounced at one of the crescent-shaped medial lips, making the lip structures asymmetric. Also, in a few specimens, the labial disc is set off from the medial lips by a groove, and, occasionally, one of the medial lips is partially reduced. Lateral lips are always lacking. The six inner labial sensilla open out on the labial disc close to the oval-shaped prestomatal opening, though they sometimes open into the prestoma. Rarely, one of the inner labial sensilla is not expressed externally, or an additional one is present. Cephalic sensilla are not visible externally. The head region is not annulated, but a remnant of a short annulation is occasionally present. The head region of this population of *M. hapla* merges smoothly into the gradually enlarging body contour (Fig. 18). In all other populations of this species, the first body annule is smaller in diameter than the head region, and thus the head region is distinctly set off from the rest of the body (Fig. 19).

In specimens of *M. hapla* race A, population 6-NC (16) (Figs. 10, 11), the labial disc is slightly raised above the medial lips. The lip structures form a rectangle in face view, but the medial lips are wider laterally than the labial disc. Lateral lips are present. The inner labial sensilla generally open out at the surface of the labial disc, very close to the prestoma, although a few occasionally open into the prestoma. Cephalic sensilla are not expressed externally and the head region is not annulated. The head region is set off from the body annules, because the

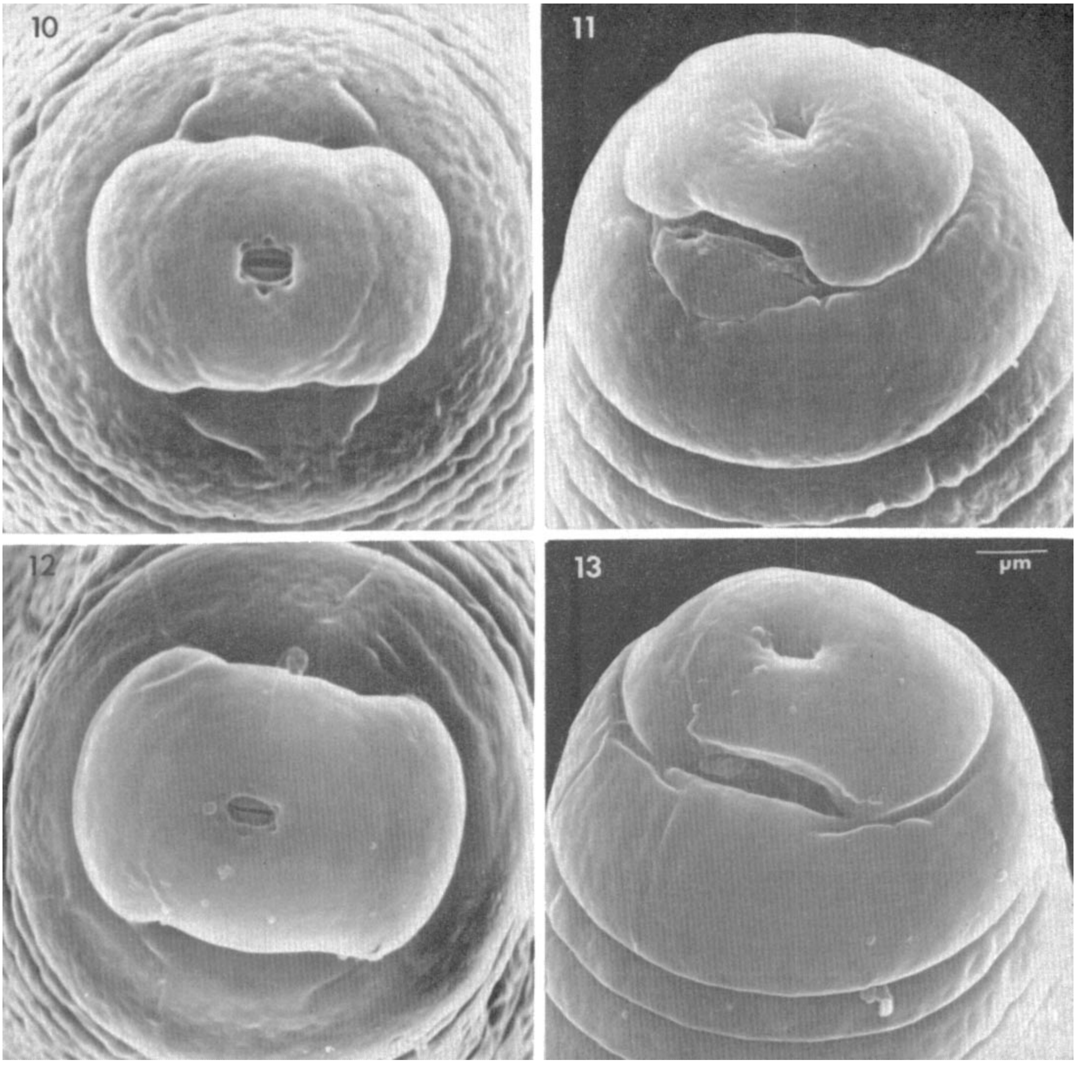

FIG. 10–13. 10,11) Face and approximately lateral views of *M. hapla* race A, population with 16 chromosomes. 12,13) Face and lateral views of *M. hapla* race A, population with 17 chromosomes. All figures are same scale as Fig. 13.

first annule is smaller in diameter than the head region.

M. hapla race A, population 86-NC (17) (Figs. 12, 13), possesses a labial disc that is fused with the medial lips to form a smooth, continuous, rectangular lip structure in face view. The medial lips are rounded off medially and are generally the same width as the labial disc. Remnants of lateral lips are marked off by grooves in the head region near the middle of the lateral edges of the medial lips. The inner labial sensilla open out on the labial disc around the oval-shaped prestoma, and no cephalic sensilla are visible. The head region is not annulated, but is distinctly set off from the rest of the body.

The head morphology of the two populations of *M. hapla* race B, populations 48-NC (45) and 230-Chile (48) (Figs. 14–17), is very similar and stable among all individuals. The labial disc is not raised above the medial lips, and the lip structures are the same width and form a rectangle in face view. Lateral lips are not present in these populations. The labial sensilla open at the edge of or into the prestoma, and cephalic sensilla are sometimes faintly visible on the medial lips. The head region is not annulated, except that a remnant of a short

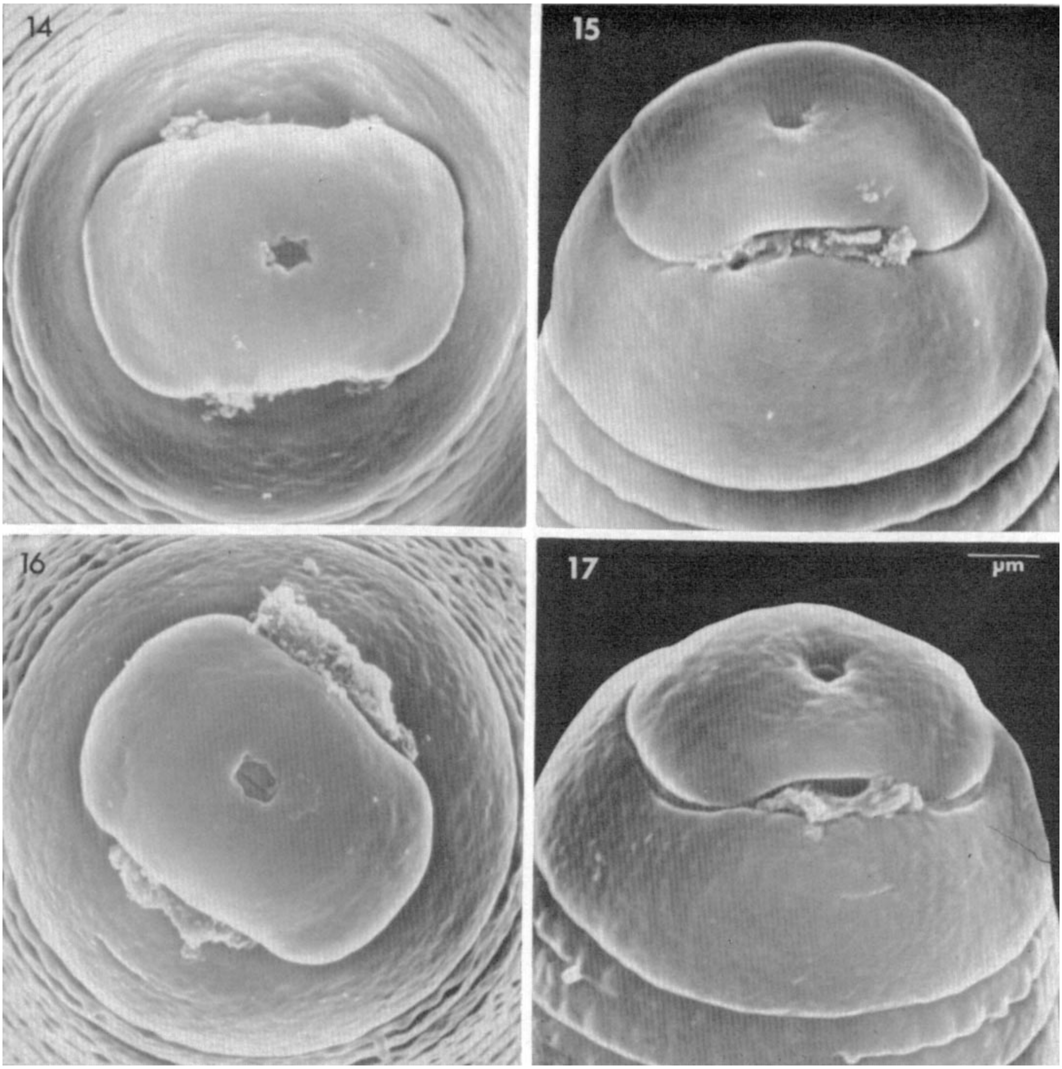

FIG. 14–17. 14,15) Face and approximately lateral views of *M. hapla* race B, population with 45 chromosomes. 16,17) Face and lateral views of *M. hapla* race B, population with 48 chromosomes. All figures are same scale as Fig. 17.

annulation is occasionally present laterally on one or both sides. In most specimens, the head region is set off from the body.

Body morphology: The structure of the lateral field, excretory pore, and tail were of little value in distinguishing species or populations, since considerable variation existed among individuals of a given population. The lateral field of specimens of all species and populations examined begins as a ridge that originates about 8–12 body annules from the head region (Fig. 20). Slight irregularities in body annulation may be present near the beginning of the lateral field. At a short distance posteriorly, two incisures develop separately or from a single incisure and subdivide the ridge longitudinally. In some specimens, the incisures start near the anterior end of the ridge, and in others they develop further posteriorly. The lateral field extends the entire length of the nematode and broadens slightly before it merges into the tail tip (Figs. 21, 22). The two inner incisures may continue close to the tail tip or may disappear in the tail region. The lateral field may fuse with the

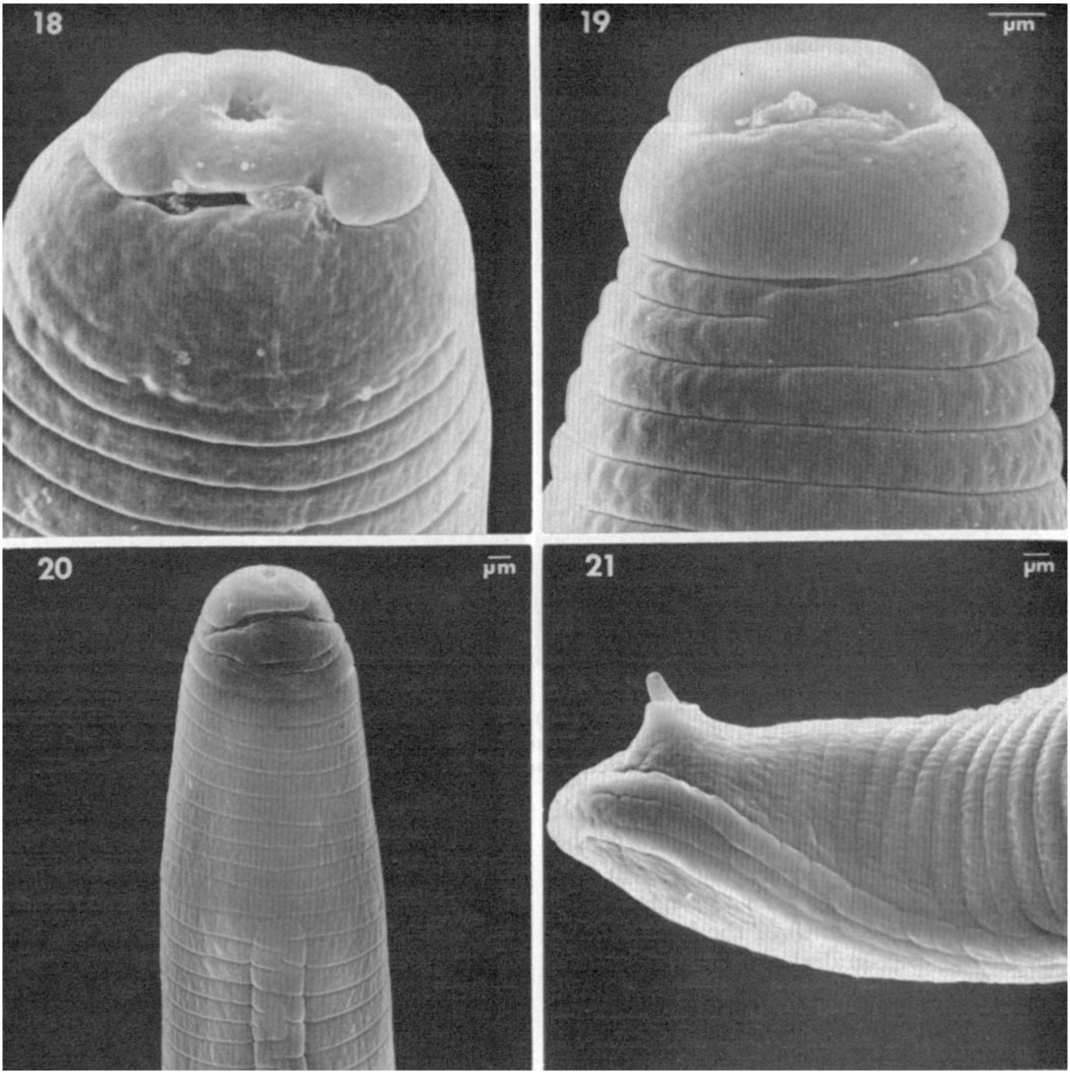

FIG. 18–21. 18) lateral view of *M. hapla* race A, population 42-Can (15), showing a head region that is not set off from the body. 19) Lateral view of *M. hapla* race A, showing the set-off head region that is typical for all populations of *M. hapla* except 42-Can (15). Figure 18 is same scale as Fig. 19. 20) Side view of the lateral field and the anterior end of a *Meloidogyne* male typical of *M. arenaria, M. hapla, M. incognita,* and *M. javanica.* 21) Side view of the posterior end of the lateral field and tail typical of *M. arenaria, M. hapla, M. incognita,* and *M. javanica.*

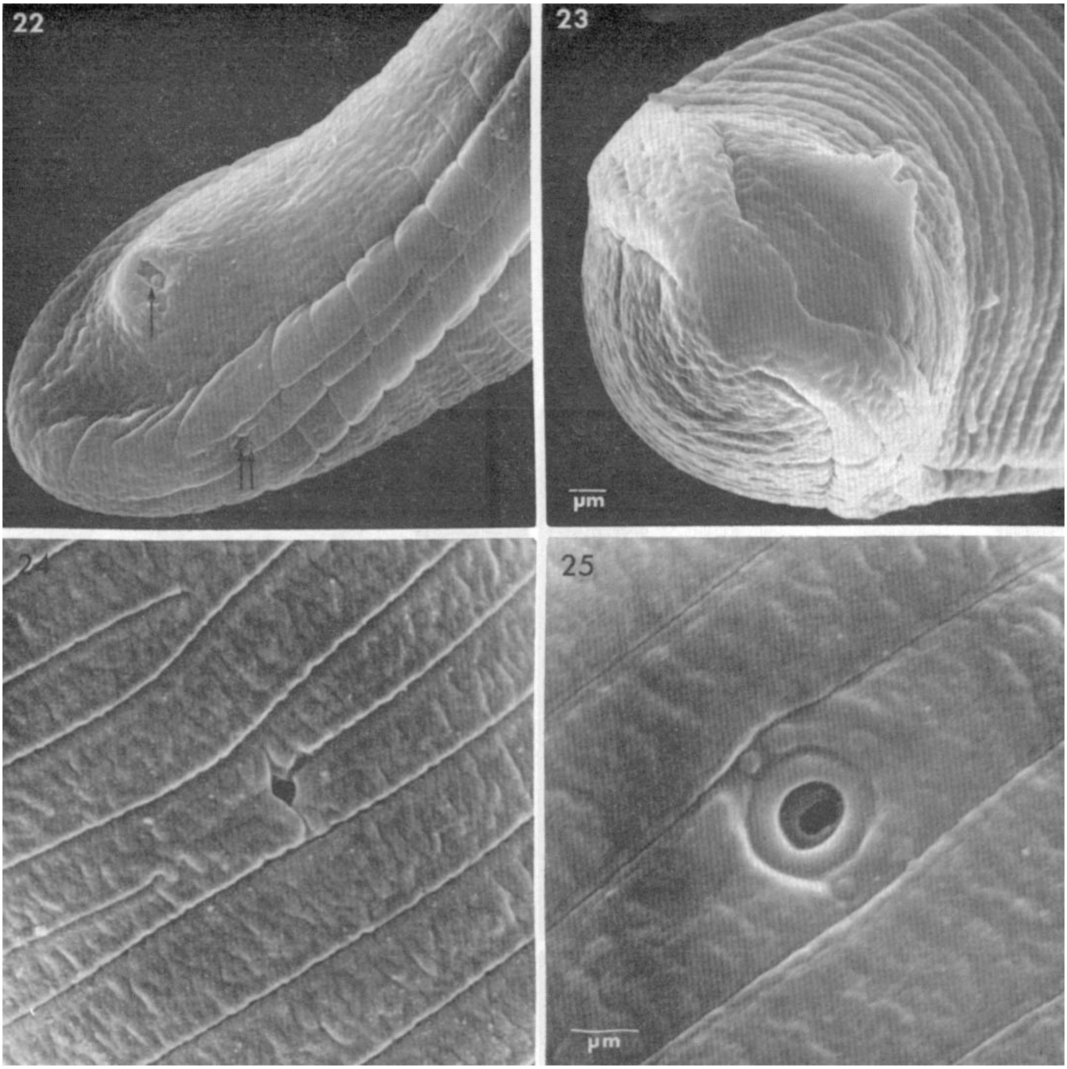

FIG. 22–25. 22) Approximately lateral view of a tail, showing cloacal opening with retracted spicules (arrow) and phasmidial aperture (double arrow). 23) End view of tail illustrating a lateral field that extends completely around the tail tip. Fig. 22 is same scale as Fig. 23. 24) Excretory pore typical of *M. arenaria, M. hapla,* and *M. incognita.* 25) Excretory pore of *M. javanica.* Fig. 24 is same scale as Fig. 25.

tail tip or may extend completely around the tail tip (Fig. 23). Areolation of the lateral field usually corresponds with the body annulations but does not necessarily involve the entire width of the lateral field. The slitlike phasmidial aperture is located between the two inner incisures of the lateral field (Fig. 22). The cloacal opening is a simple transversely elongate opening (Fig. 22). The spicules, which have two sensilla openings near their tips, are usually retracted, and not enough specimens were examined to establish a relationship between spicule morphology and species. In most populations, the excretory pore is a simple oval-shaped opening (Fig. 24), but in *M. javanica* it is located in a depression in the cuticle (Fig. 25). The excretory pore is usually contained within one body annule, but two or three body annules in its vicinity sometimes deviate from the regular pattern.

DISCUSSION

These studies of males of *Meloidogyne arenaria, M. incognita, M. javanica,* and several chromosomal populations of *M. hapla* revealed that the cephalic morphology is distinct for each species. Furthermore, the two cytological races of *M. hapla* and the three chromosomal populations of race A have different head morphologies.

M. arenaria males possess a raised labial disc and crescent-shaped medial lips. The head region usually has 2–3 annules. *M. incognita* is similar to *M. arenaria* but is characterized by a very prominent labial disc that is centrally concave. The medial lips are crescent-shaped in face view, and the head region has 2–3 incomplete annulations. *M. javanica* can be differentiated from *M. arenaria* and *M. incognita* because the labial disc and medial lips are elongate in face view and the head region is not annulated. *M. hapla* can be distinguished from the other species by the small rectangular labial disc and medial lips. In all populations of *M. hapla* race A except 42-Can (15), the head region is set off from the body annules because the first body annule is smaller in diameter than the head region. The populations of *M. hapla* race B also have a set-off head region. As in *M. javanica,* the head region is not annulated in the populations of *M. hapla.*

Each population of *M. hapla* race A has distinct cephalic morphology. Population 42-Can (15) can be identified by the deep indentations at the junction of the labial disc and the medial lips. The medial lips are pointed and the head region is not set off from the body contour. Population 6-NC (16) has squared-off medial lips and an elevated labial disc. The almost completely formed lateral lips that are set off from the head region are unique to this population. Population 86-NC (17) is very similar to population 6-NC (16), but the labial disc is not raised above the medial lips, and the lateral lips are not as complete. Among the populations of *M. hapla* race A studied, the head morphology of population 42-Can (15) was the most variable. The shape of the medial lips, demarcation of the labial disc, and placement of the inner labial sensilla were variable among individuals of this population. Individuals of populations 6-NC (16) and 86-NC (17) were also variable, though less than the population with 15 chromosomes.

The two populations of *M. hapla* race B studied, one with 45 and one with 48 chromosomes, were similar in head morphology. Their cephalic morphology was different from the populations of race A and, unlike populations of race A, the head morphology of the populations of race B was very stable. Populations of race B can be distinguished from populations of race A by the absence of lateral lips. As in race A, the head region is set off from the body annules in both populations of race B.

Results of recent studies (3) of second-stage juveniles of *M. hapla, M. arenaria, M. incognita,* and *M. javanica* are comparable to those of the present study of males. As in the juveniles, morphological differences were observed in males of different species, in races of *M. hapla,* as well as in populations of *M. hapla* race A. Juveniles and males had similar head morphology with respect to the basic cephalic characters, though the expression of these characters was considerably different between the two life stages. The prestoma is small and oval-shaped in the second-stage juvenile but large and either oval or hexagonal in the male. The shape of the prestoma in the male is determined by the position of the inner labial sensilla. If they encircle the prestoma, as in the second-stage juvenile, the prestoma is oval; if the inner labial sensilla open into the prestoma, the prestoma becomes hexagonal. In the male, the labial disc is larger than both medial lips combined, but in the juvenile the labial disc is smaller or equal in size to one medial lip. The large labial disc in all of the males except *M. javanica* overlaps the head region laterally and, in face view, covers the amphidial openings. In the second-stage juvenile, the labial disc is much smaller and the amphidial apertures are generally visible in face view. The lateral lips of juveniles are joined with the medial lips, but in the few populations of males that had lateral lips, they are areas on the head region marked off by grooves. The number of annules on the head region of the male do not necessarily correspond to the annulation on the head region of the second-stage juvenile. The head region of *M. arenaria* juveniles is not annulated, whereas the males have 2–3 incomplete annulations. In *M. incognita* both the second-stage juvenile and the male have 2–3 incomplete annulations in the head region.

The annulations on the head region could not be seen clearly and counted accurately with the light microscope, because they are irregular and incomplete. This difficulty has caused this character to be rejected by some workers (4). Although Whitehead (9) thought there was considerable intraspecific variation for this character within *M. incognita,* he considered it stable for several other species. The present studies show that the presence or absence of annulations in the head region is a useful differentiating character. Annulations are present in *M. arenaria* and *M. incognita* but absent in *M. hapla* and *M. javanica.* The apparent intraspecific variation, as seen by Whitehead, may be caused by the irregularities of the annulations. Since they are often incomplete and annulations may overlap for a short distance (Figs. 3, 5), it is nearly impossible to count the exact number. Annulations are usually more numerous on the medial sectors of the head region, and thus the position of the specimen in the light microscope would affect the number of annules seen.

The lateral fields of the four species of *Meloidogyne* males studied were similar in structure and of little value taxonomically. As in the second-stage juvenile, the lateral field of the male is a ridge divided by two inner incisures. The two edges of the ridge and the two incisures make up the four incisures seen in the light microscope. All species examined had four incisures that were areolated over the entire lateral field. The presence or absence of areolation was previously considered (4) to be a useful character in the taxonomy of *M. arenaria, M. hapla, M. incognita,* and *M. javanica.* Areolation and number of incisures in the lateral field may be useful characters for some species, but they should first be confirmed by SEM observations. Once they are seen with the SEM, they can probably be recognized with the light microscope.

The remaining body characters, except for the excretory pore, were not helpful in differentiating the species. The spicules may be different structurally between the species, but the observations made in this study were too few for a critical evaluation.

LITERATURE CITED

1. BALDWIN, J. G. 1976. Morphology of Meloidogyne spp.—summary and prospects for further investigation. Proc. Research Planning Conf. on Root-knot Nematodes, Meloidogyne spp. N.C.S.U., Raleigh, pp. 25-28.

2. CHITWOOD, B. G. 1949. "Root-knot nematodes." I. A revision of the genus Meloidogyne Goeldi, 1887. Proc. Helminthol. Soc. Wash. 16:90-104.

3. EISENBACK, J. D., and H. HIRSCHMANN. 1979. Morphological comparison of second-stage juveniles of six populations of Meloidogyne hapla by SEM. J. Nematol. 11:5-16.

4. ESSER, R. P., V. G. PERRY, and A. L. TAYLOR. 1976. A diagnostic compendium of the genus Meloidogyne (Nematoda: Heteroderidae). Proc. Helminthol. Soc. Wash. 43:138-150.

5. FRANKLIN, M. T. 1971. Taxonomy of Heteroderidae. Pages 139-162 *in* B. M. Zuckerman, W. F. Mai and R. A. Rhode, eds. Plant Parasitic Nematodes, Vol. I. Academic Press, N. Y.

6. GOLDEN, A. M. 1976. Meloidogyne taxonomy —current status, some problems, and needs. Proc. Research Planning Conf. on Root-knot Nematodes, Meloidogyne spp. N.C.S.U., Raleigh, pp. 13-21.

7. TAYLOR, A. L., and J. N. SASSER. 1978. Biology, identification, and control of root-knot nematodes (Meloidogyne species). A cooperative publication of the Department of Plant Pathology, North Carolina State University, and the United States Agency for International Development. North Carolina State Graphics. 111 pp.

8. TRIANTAPHYLLOU, A. C. 1966. Polyploidy and reproductive patterns in the root-knot nematode Meloidogyne hapla. J. Morphol. 118:403-413.

9. WHITEHEAD, A. G. 1968. Taxonomy of Meloidogyne (Nematodea: Heteroderidae) with descriptions of four new species. Trans. Zool. Soc. Lond. 31:263-401.