Morphological Comparison of Second-Stage Juveniles of Six Populations of Meloidogyne hapla by SEM[1]

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Abstract: External morphology of second-stage juveniles of six populations of *Meloidogyne hapla*, belonging to two cytological races (A and B), and one population each of *M. arenaria, M. incognita*, and *M. javanica* was compared by scanning electron microscopy (SEM). Race A of *M. hapla* included three facultatively parthenogenetic populations with haploid chromosome numbers of 15, 16, and 17; race B consisted of three mitotically parthenogenetic populations with somatic chromosome numbers of 45, 45, and 48. The mitotically parthenogenetic populations of *M. arenaria, M. incognita*, and *M. javanica* had 54, 41–43, and 44 chromosomes, respectively. Observations were made on head structures, lateral field, excretory pore, anal opening, and tail. Head morphology, including shape and proportion of labial disc and lips, expression of labial and cephalic sensilla, and markings on head region, was distinctly different for each species. *M. hapla* populations of race A were distinct from each other but showed much intrapopulation variation in head morphology. Populations of race B were different from those of race A and were very stable and quite similar in head morphology. Considerable inter- and intrapopulation variation made the structure of the lateral field, excretory pore, anal opening, and tail of little value in distinguishing species or populations. The results are discussed in relation to earlier SEM observations on the genus *Heterodera*. *Key Words: M. arenaria, M. incognita, M. javanica.*

Species of the genus *Meloidogyne* Goeldi exhibit considerable variation in their morphology (10, 11, 23), physiology (12, 14, 15, 16, 17), and cytology (20, 21). In his review of the genus, Whitehead (23) showed that many morphological characters in females, males, and second-stage juveniles are of value taxonomically, but many others are variable and exhibit too much overlap among species to be useful in species differentiation. *Meloidogyne* species need to be examined more closely for distinctive morphological characters. Cytological studies of several populations of *M. hapla* Chitwood emphasized the need for a detailed morphological study, because this species appears to be made up of two distinct cytological races (21): One race includes populations that have a haploid chromosome number of 15, 16, or 17 and reproduce by facultative meiotic parthenogenesis. The other race consists of populations with a

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somatic chromosome number of 45 or 48 (personal communication, A. C. Triantaphyllou) and reproduces exclusively by mitotic parthenogenesis. Little effort has been made so far to make a detailed morphological comparision of populations of *Meloidogyne* of different chromosome number and mode of reproduction. Previous morphological studies of this genus have been limited almost exclusively to the light microscope; only recently has the electron microscope become a useful tool in clarifying the morphology of *Meloidogyne* species. A few studies on the fine structure by transmission electron microscopy have contributed greatly to our understanding of the internal morphology of these nematodes (1, 2, 3, 8, 9, 22). Scanning electron microscope (SEM) observations of the external morphology of *Meloidogyne* spp. are limited (13).

The present study was done to compare external morphological characters in second-stage juveniles of the chromosomal populations that now form the species *M. hapla* by SEM and to relate morphological findings with cytological data. In addition, one population each of *M. arenaria* (Neal) Chitwood, *M. incognita* (Kofoid and White) Chitwood, and *M. javanica* (Treub) Chitwood was observed with the SEM as a guide to the differences that may occur between species.

MATERIALS AND METHODS

Six populations of *M. hapla,* differing in mode of reproduction and chromosome number, and one population each of *M. arenaria, M. incognita,* and *M. javanica* were selected from the *Meloidogyne* collection at N.C. State University. Populations of *M. hapla* were designated by a number, an abbreviated word indicating their origin, and their chromosome number in parentheses, as follows: 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. Populations of the other three species were designated similarly, as follows: *M. arenaria* 351-Fla (54) from Florida; *M. incognita* 68-NC (41-43) from North Carolina; and *M. javanica* 78-Ga (44) from Georgia. All populations were propagated on tomato (*Lycopersicon esculentum* Mill. 'Rutgers') in a greenhouse; the *M. hapla* populations were kept at 21 C, and the other species at 28 C. Newly hatched second-stage juveniles were obtained by incubating egg masses in a moist chamber at room temperature. The specimens were transferred to 0.5 ml of filtered tap water in a BPI watch glass, and were chilled and killed by adding 3–4 drops of cold (8 C) 4% gultaraldehyde solution (buffered with 0.1*M* sodium-cacodylate at pH 7.4) every half an hour until the final concentration of the fixative reached 2%. Fixation of the material continued for an additional 72 h in the cold. The specimens were then transferred to a processing chamber modified from DeGrisse (7), and the remaining steps, except critical point drying, were taken without the top of the chamber in place. Fixation was followed by rinsing with 0.1*M* sodium-cacodylate plus 7.5% sucrose solution at pH 7.4 for 12 h in the cold. Postfixation with 2% osmium tetroxide, buffered with 0.1*M* sodium-cacodylate at pH 7.4, for 12 h was followed by rinsing with the buffer-sucrose solution for 12 h in the cold. Specimens were dehydrated with an 8-step graded series of ethanol during a 3-h period at room temperature, and Freon 13 was used as an intermediate fluid in a 5-step graded series of fifteen minutes for each step. The nematodes were then critical-point dried with Freon 113 by standard procedures (5), and dried specimens were transferred individually with a dental root-canal file onto a stub covered with double-coated tape. The specimens were propped up against a hair to ensure an unobstructed view of the entire head region; they were coated with approximately 400 Å of gold and viewed and photographed with an ETEC scanning electron microscope operated at 20 Kv. At least 100 second-stage juveniles from each population were examined.

OBSERVATIONS

Head morphology: Fig. 1 shows the basic plan of the cephalic characters for the genus *Meloidogyne,* based on SEM observations of second-stage juveniles of *M. arenaria, M. hapla, M. incognita,* and *M. javanica.* Centrally located on the labial disc is the ovoid opening of the prestoma,

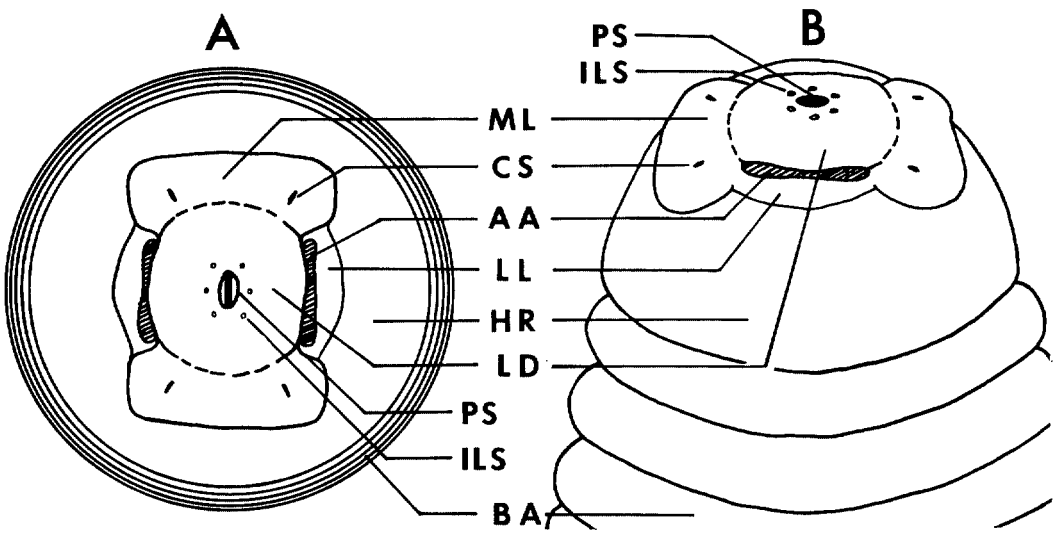


FIG. 1-A, B. Diagrams illustrating the head morphology of a second-stage juvenile of the genus *Meloidogyne*. A) Face view, B) View from the lateral side. AA, amphidial aperture; BA, body annule; CS, cephalic sensillum; HR, head region; ILS, inner labial sensillum; LD, labial disc; LL, lateral lip; ML,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

which leads to the slitlike stomatal opening medially below it. The prestoma is encircled by the small pitlike openings of the six inner labial sensilla. Posterior to the labial disc, the subdorsal and subventral lips are fused medially, forming one dorsal and one ventral lip, respectively. Because of the dorso-ventral symmetry of these head characters, however, dorsal and ventral orientations are difficult to distinguish and these lips are termed medial lips. Each medial lip contains one pair of cephalic sensilla, expressed externally in some species as small round cuticular depressions. The labial disc is often slightly raised above the medial lips. The lateral lips are smaller and lower than the medial lips, and in the same contour with the head region. The amphids appear as long slits between the labial disc and the lateral lips. Posterior to the lips, the head region may be marked by one to three incomplete annulations.

In *M. arenaria,* population 351-Fla (54) (Fig. 2, 3), the labial disc is slightly raised above the medial lips, and the lateral lips are in the same contour with the head region. The labial disc and the medial lips are dumbbell-shaped in face view, and three times as long as the width of the labial disc. The lateral lips are elongate, and in face view the junction of the medial and lateral lips is perpendicular to the lateral edge of the labial disc. Cephalic sensilla are expressed externally, and the head region is not annulated.

M. incognita, population 68-NC (41-43) (Fig. 4, 5), possesses a small round labial disc that is slightly raised above the medial lips. In face view, the labial disc and the medial lips are dumbbell-shaped, and the round shape of the labial disc is also prominent. The lateral lips are in the same contour with the head region. Cephalic sensilla are expressed externally, and in face view the junction of medial and lateral lips is perpendicular to the lateral edge of the labial disc. The head region usually bears two to three irregular incomplete annulations.

The labial disc of *M. javanica,* population 76-Ga (44) (Fig. 6, 7), is quite prominent, and the lateral lips are lower and in contour with the head region. In face view, the labial disc and the medial lips are bowtie-shaped. The lateral lips are triangular from a lateral aspect (Fig. 7). The junction of the medial and lateral lips forms an obtuse angle with the lateral edge of the labial disc. Cephalic sensilla are expressed externally, and the head region is occasionally marked by remnants of a single annulation, usually located laterally (Fig. 6).

M. hapla race A, population 42-Can (15) (Fig. 8, 9), is the most variable popu-

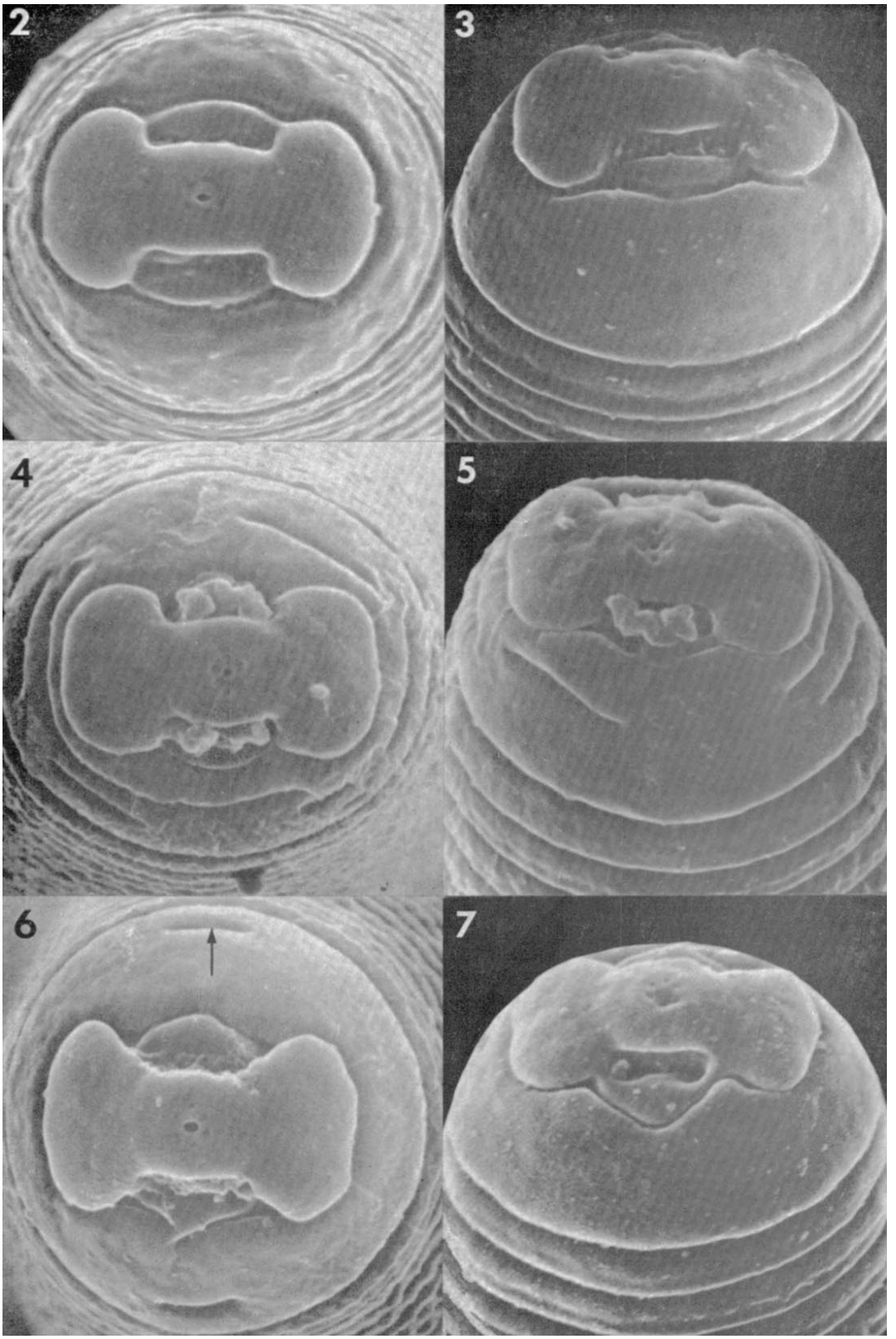


FIG. 2–7. 2, 3) Face and approximate lateral views, respectively, of *M. arenaria* population 351-Fla (54). 4, 5) Face and lateral views, respectively, of *M. incognita* population 68-NC (41-43). 6, 7) Face and lateral views, respectively, of *M. javanica* population 76-Ga (44) (Arrow, Fig. 6, incomplete head annulation). All approximately 20,000×.

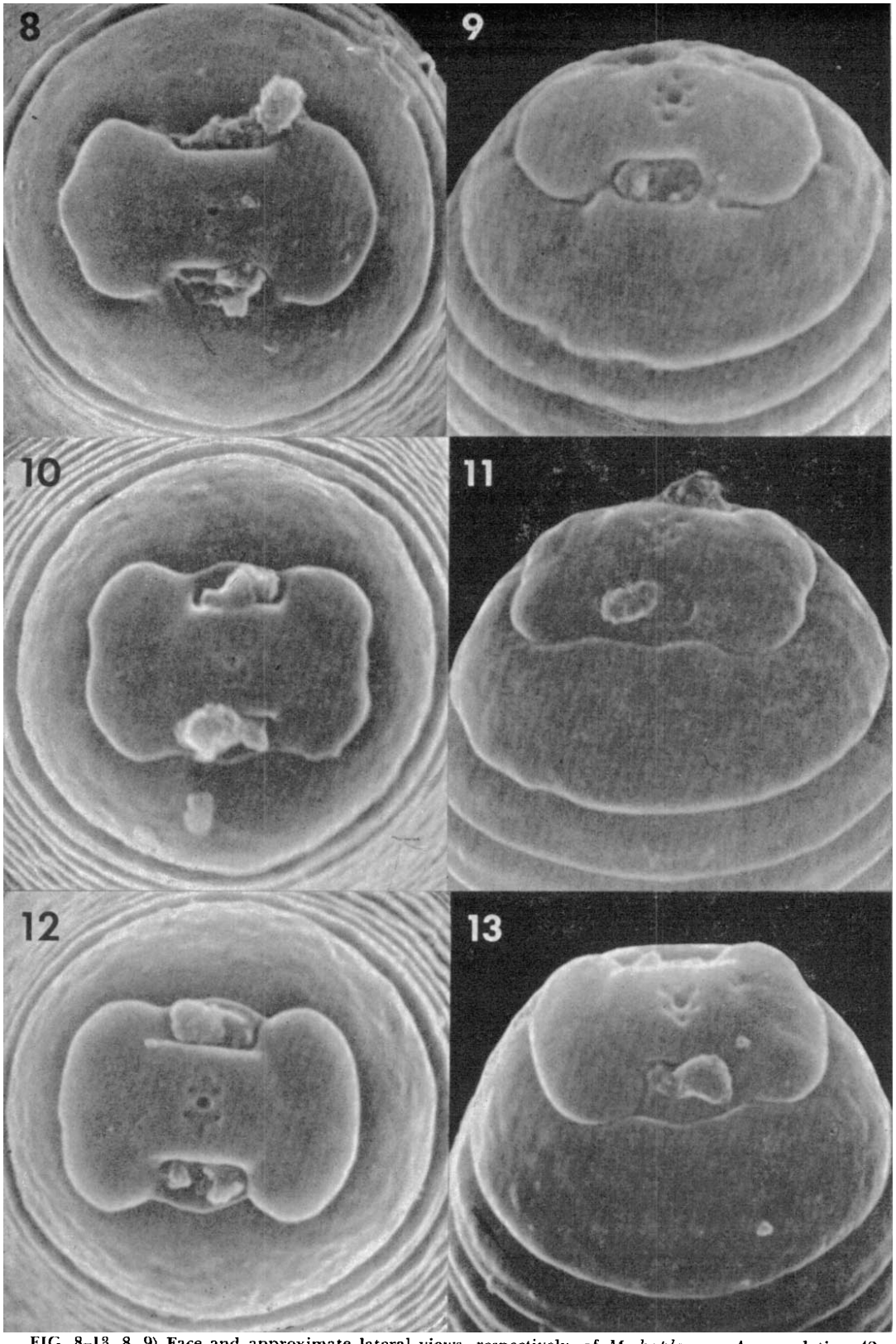


FIG. 8–13. 8, 9) Face and approximate lateral views, respectively, of *M. hapla* race A, population 42-Can (15). 10, 11) Face and lateral views, respectively, of *M. hapla* race A, population 6-NC (16). 12, 13) Face and lateral views, respectively, of *M. hapla* race A, population 86-NC (17). All approximately 20,000×.

lation studied. Its cephalic characters are distinctly different from those of the other populations of *M. hapla*. Medial lips and labial disc are in the same contour and form a smooth continuous structure. The medial lips are usually pointed (Fig. 8, 21, 22) but one lip may occasionally be rounded or straight. Often the lateral edges of the labial disc are not parallel to each other (Fig. 21). The six inner labial sensilla openings are arranged symmetrically around the prestoma, although in an occasional specimen one sensillum may be displaced a short distance (Fig. 21). The cephalic sensilla are not visible externally and, only rarely, one cephalic sensillum may be expressed (Fig. 20, 21). The most striking characteristic of this population is the absence of lateral lips. As a consequence, the medial lips fuse laterally with the head region instead of the lateral lips. In some specimens, remnants of lateral lips are discernible (Fig. 20). The head region is not annulated.

In specimens of *M. hapla* race A, population 6-NC (16), the labial disc and medial and lateral lips are in the same contour (Fig. 10, 11) and form a rectangular-shaped structure in face view, 2½ times as long as the width of the labial disc. Cephalic sensilla are not visible, and the head region is not annulated.

Although the labial disc and the medial lips of specimens from *M. hapla* race A, population 86-NC (17) (Fig. 12, 13), are fused and in the same contour, the lateral lips are lower and in contour with the head region. In face view, the medial lips and the labial disc are dumbbell-shaped and measure 2½ times the width of the labial disc. The medial lips are rounded off, and the cephalic sensilla are not expressed externally. The head region is not annulated in this population.

The three populations of *M. hapla* race B, populations 48-NC (45), 66-Md (45), and 230-Chile (48) are very similar in head morphology (Fig. 14–19). The labial disc is slightly raised above the medial lips, and the lateral lips are lower than the medial lips and in the same contour with the head region. Occasionally, the lateral lips fuse with the head region for a short distance laterally (Fig. 16). The junction of the medial and lateral lips is perpendicular to the lateral edge of the labial disc. The medial lips are slightly indented, suggesting a subdivision of the lip into a pair of submedial lips (Fig. 23). Cephalic sensilla are not expressed externally in any of these populations. Rarely, one short, incomplete annulation is present on one side or both lateral sides of the head region (Fig. 17). All of the specimens of these three populations are very stable in their head morphology.

Body morphology: The structure of the tail, lateral field, excretory pore, and anal opening was of little value in distinguishing species or populations since considerable variation existed among individuals of a given population. The shape of the tail and its terminus (Fig. 25) was quite variable among individuals and differences between populations could not be determined. The lateral field (Fig. 24) of specimens of all populations examined begins as a ridge about 10–15 body annules from the head region. At a certain distance, one longitudinal incisure develops that splits further posteriad into two incisures which subdivide the ridge longitudinally. In some specimens, this incisure appears near the beginning ridge; in others it is further posteriad. The lateral field extends the entire length of the nematode and narrows in the tail region (Fig. 25). Near the middle of the tail, the two inner incisures fuse, resulting in one incisure. This incisure gradually disappears also, and the two outer edges of the ridge join, terminating the lateral field near the tail tip. The distance over which this change takes place varies among individuals. All species exhibit areolation over the entire length of the lateral field. The areolation generally corresponds with the body annulation, although the distance between two lines of areolation may vary by one to four body annulations. Areolation does not necessarily involve the entire width of the lateral field. Phasmidial apertures are not expressed externally but may be hidden by areolation or incisures occurring in the lateral field.

A simple oval-shaped opening, the excretory pore (Fig. 26, 27), is located in a depression in the cuticle. The regular body annules are interrupted or deviate from their course around the excretory pore. Except for population 48-NC (48), which has a much larger excretory pore, all popu-

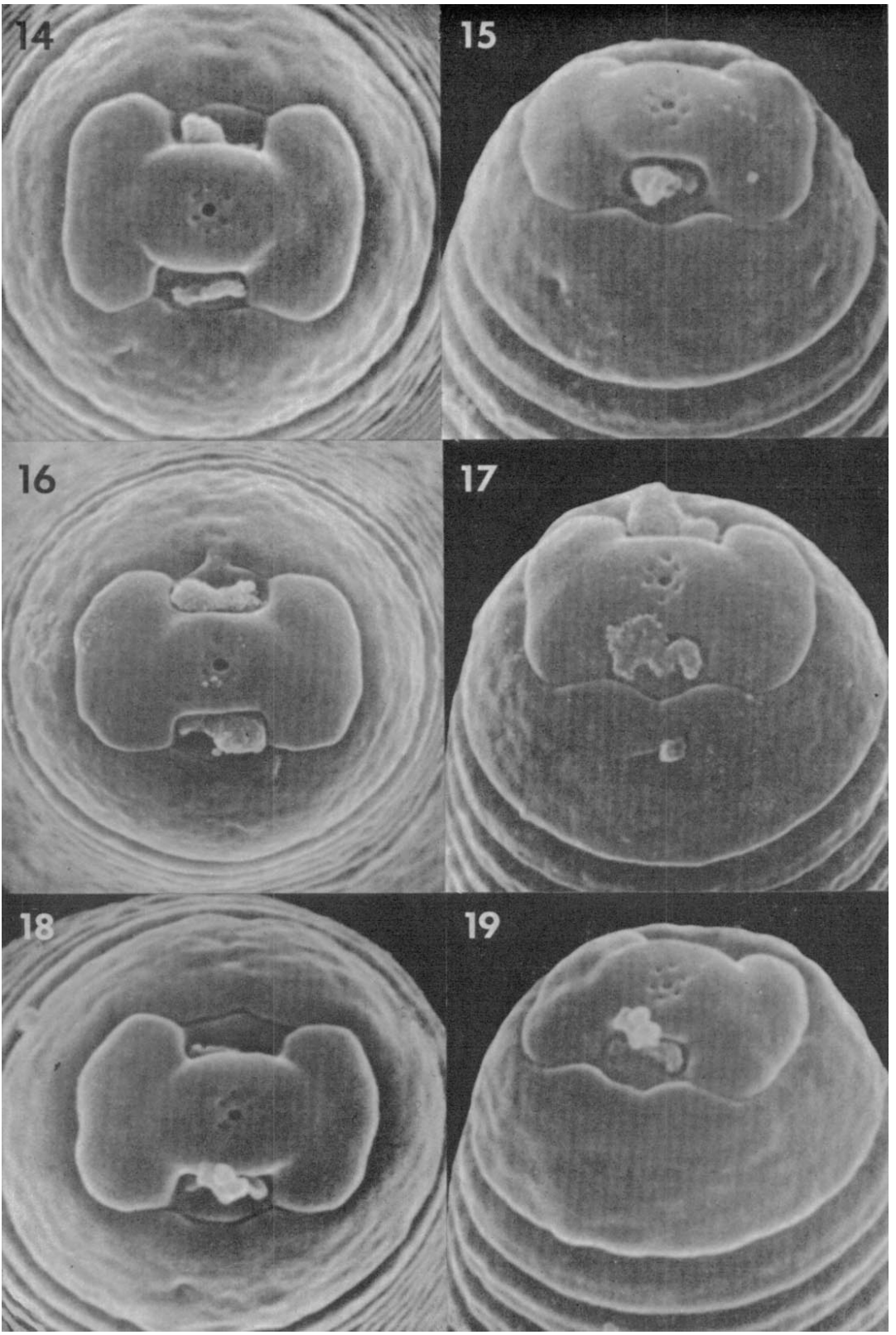


FIG. 14–19. 14, 15) Face and approximate lateral views, respectively, of *M. hapla* race B, population 48-NC (45). 16, 17) Face and lateral views, respectively, of *M. hapla* race B, population 66-Md (45). 18, 19) Face and lateral views, respectively, of *M. hapla* race B, population 230-Chile (48). All approximately 20,000×

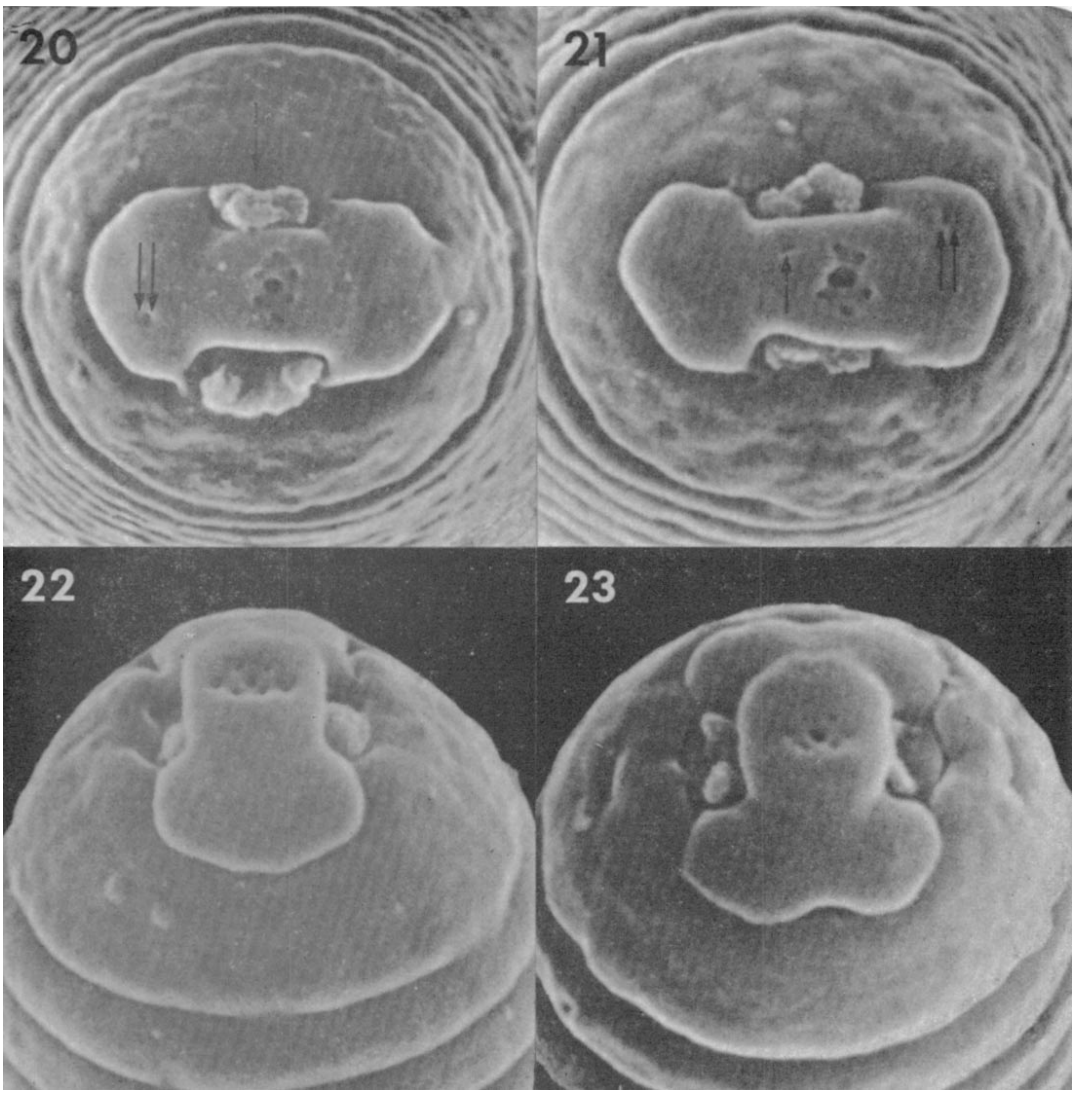


FIG. 20–23. 20) Face view of *M. hapla* race A, population 42-Can (15), illustrating one cephalic sensillum (double arrow) and remnants of a lateral lip (single arrow). 21) Face view of *M. hapla* race A, population 42-Can (15), showing the displacement of an inner labial sensillum (single arrow), one cephalic sensillum (double arrow), and the nonparallel lateral edges of the labial disc. 22) Approximate medial view of *M. hapla* population 42-Can (15), illustrating a pointed medial lip. 23) Medial view of *M. hapla* race B, population 48-NC (45), showing a medial lip that suggests a subdivision of the lip into a pair of submedial lips. All approximately 20,000×.

lations possess a typical excretory pore (Fig. 26). The anal opening is a simple oval-shaped opening through the cuticle.

DISCUSSION

When Chitwood (4) revised the genus *Meloidogyne* and designated five species, he pointed out the existence of varieties within these species and suggested that certain variations in morphological characters depended on genetic factors. In the original description of *M. hapla*, Chitwood described several varieties based on morphological differences in the male, such as shape of the labial annule, length of the stylet, and the distance of the dorsal esophageal gland orifice to the stylet base. Several investigators have detected physiological differences in populations of *M. hapla* (12, 14, 15, 16). Triantaphyllou (21) studied several populations cytologically and subdivided the species into race A, those popu-

lations reproducing by meiotic parthenogenesis or amphimixis and having chromosome numbers of n = 15, 16, and 17; and race B, those populations reproducing by mitotic parthenogenesis and having somatic chromosome numbers of 45.

Of the populations of *M. hapla* race A studied in this paper, population 42-Can (15) showed the most extensive variation in head morphology. The shape of the medial lips, demarcation of the lateral lips, placement of inner labial sensilla, and insertion of cephalic sensillum were variable between individuals of this population. Populations 6-NC (16) and 86-NC (17) also showed variation among individuals within the populations, although less than in the population with 15 chromosomes. Each population of race A is distinct in its cephalic morphology. Population 42-Can (15) can be identified by the absence of lateral lips and a tendency toward pointed medial lips. Population 6-NC (16) has squared-off medial lips and elevated lateral lips that are in contour with the labial disc and the medial lips. Population 86-NC (17) is very similar to population 6-NC (16), but the medial lips are rounded-off, and the lateral lips are lower than the medial lips and in contour with the head region.

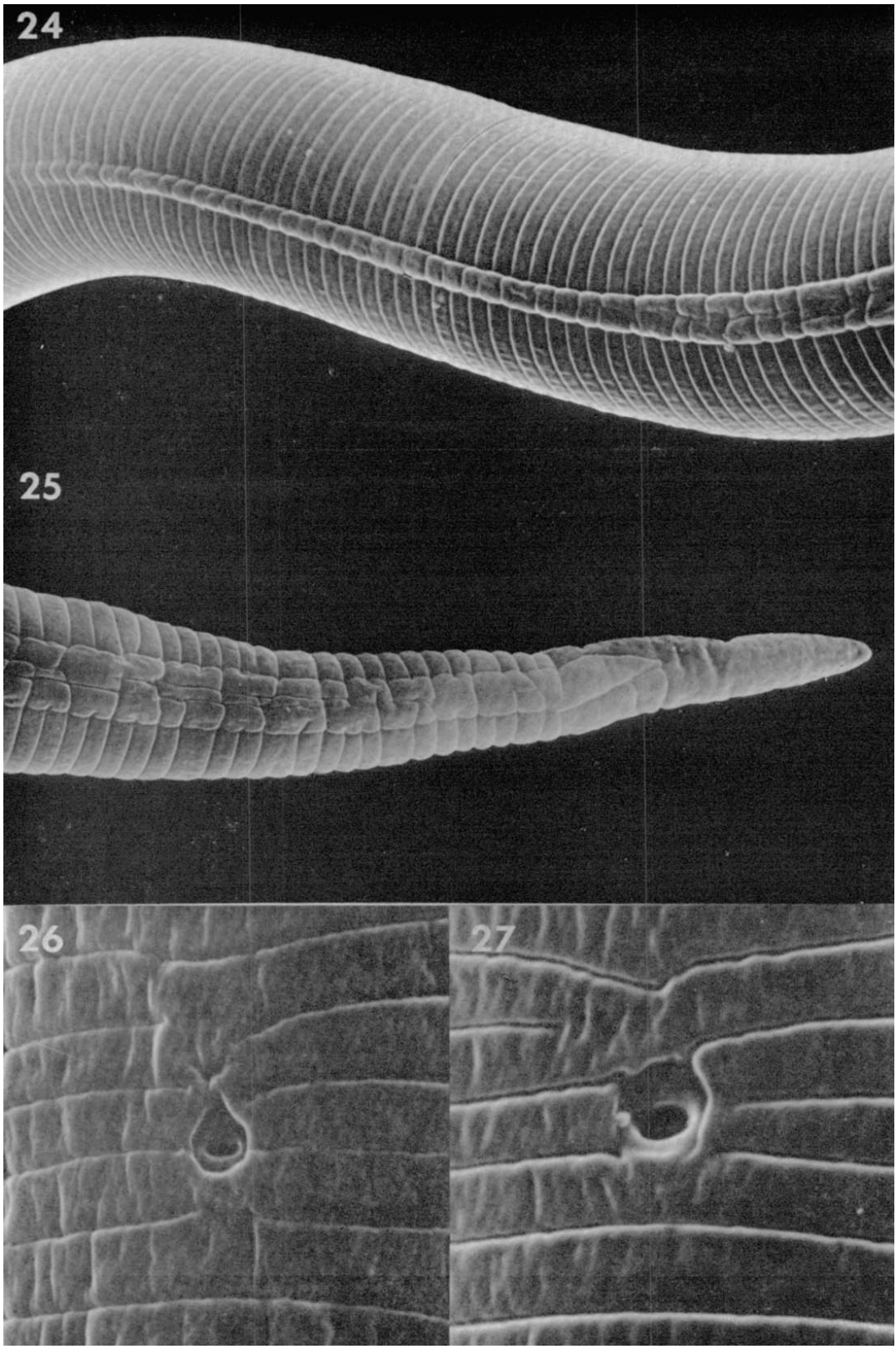
Populations belonging to race B of *M. hapla* were very stable in morphology and different from race A. All populations studied, including two with a somatic chromosome number of 45 and one with 48 chromosomes, were similar in head morphology. However, one of the populations, 48-NC (45), possessed an excretory pore that was different from the other populations of *M. hapla*. Each population of race B can be distinguished from the populations of race A by an elevated labial disc. The lateral lips are lower than the medial lips and in contour with the head region and often become fused with the head region for a short distance laterally.

Similar SEM studies (19) with six populations of *Heterodera avenae* Wollenweber did not reveal any consistent differences between the two pathotypes of that species. SEM studies (18) of second-stage juveniles of several species of *Heterodera* A. Schmidt showed that head morphology agrees with recognized species groups of the genus. Identification to species level, however, was not possible using external head morphology.

In *Meloidogyne*, the present SEM studies of second-stage juveniles of several chromosomal populations of *M. hapla, M. arenaria, M. incognita,* and *M. javanica* revealed that the cephalic morphology was distinct for each species. *M. hapla* second-stage juveniles are characterized by the absence of surface manifestations of cephalic sensilla and smaller labial disc, medial, and lateral lips. *M. arenaria* juveniles possess very long lateral lips, and labial disc and medial lips are also elongate. *M. incognita* is similar to *M. arenaria* but has a small round labial disc, reduced lateral lips, and, in most specimens, two to three incomplete head annulations. *M. javanica* can be differentiated from the other species by its bow-tie-shaped labial disc and medial lips, and triangular-shaped lateral lips in side view.

The structure of the lateral field of second-stage juveniles of the four *Meloidogyne* species studied was very similar and of no value in identification. Number of incisures and type of areolation may, however, differ in other species of the genus. The outer incisures, as seen in the light microscope, are not true incisures but are the outer edges of the ridge that constitutes the lateral field in SEM micrographs. The remaining body characters, including shape of the tail and tail terminus and structure of the excretory pore and anal opening, were of little taxonomic value, since they were quite variable among individuals.

A comparison of the genera *Heterodera* and *Meloidogyne* shows that the prestoma is much larger in *Heterodera* second-stage juveniles than in *Meloidogyne* second-stage juveniles. The inner labial sensilla of *Heterodera* second-stage juveniles open into the prestoma and are thus obscured in face view. In *Meloidogyne* second-stage juveniles, on the other hand, the inner labial sensilla are always visible. Also, in most species of *Meloidogyne* the cephalic sensilla are expressed externally, whereas no *Heterodera* species examined (18) have shown any external indication of cephalic sensilla. The expression of cephalic sensilla, however, could be obscured by shrinkage of the specimen. Although the latter sen-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silla are on the lips, we term them "cephalic sensilla" since they correspond to the cephalic sensilla in DeConinck's scheme of the head structures of a generalized nematode (6). Also, transmission electron microscope studies have shown that second-stage juveniles of *M. incognita* have six inner labial and four cephalic sensilla but lack the six outer labial sensilla (9).

Compared with the lip patterns of *Heterodera* groups illustrated by Stone (18), the lip patterns of *Meloidogyne* species are similar to patterns 3 and 4. In our opinion, it is more likely that the large labial disc in patterns 3, 4, and 5 results from a fusion of the labial disc with the medial lips rather than an elongation of the labial disc and subsequent loss of the medial lips, as suggested by Stone (18). The medial division of patterns 3 and 5 is indicative of the presence of medial lips. Posterior to these lips, the head region of many species of *Heterodera* had 1–3 annulations which were regular and complete. In the few species of *Meloidogyne* with annulations, they were irregular and incomplete. Also, the annulation separating the head from the body was pronounced in *Heterodera* but in *Meloidogyne* was similar to the other body annulations.

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FIG. 24–27. 24) Side view of the lateral field and its anterior origin, typical of *Meloidogyne hapla, M. arenaria, M. incognita,* and *M. javanica.* 25) Side view of tail with posterior end of lateral field, typical of *Meloidogyne hapla, M. arenaria, M. incognita,* and *M. javanica.* (24 and 25, approximately 5,000X.) 26) Excretory pore typical of *Meloidogyne hapla, M. arenaria, M. incognita,* and *M. javanica.* 27) Atypical excretory pore of *M. hapla* race B, population 48-NC (45). (26 and 27, approximately 15,000×. The top of Fig. 26 and 27 is anteriad.)

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