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Scanning Electron Microscopy of Second-Stage Juvenile Cephalic Morphology in *Heterodera glycines* Races[1]

GREGORY R. NOEL AND BARBARA A. STANGER[2]

Abstract: Scanning electron microscopy (SEM) was used to compare juvenile cephalic morphology of the five described races of *Heterodera glycines*. Races 1, 2, 3, and 4 were obtained in the United States and race 5 was obtained from Japan. Differences in the gross morphology of labial discs; ventral, dorsal and lateral lips; amphidial apertures; and fissures on the labial disc of some specimens were observed. There was considerable interracial and intraracial variation which precluded separation of juveniles of *H. glycines* races by SEM.

Key words: *Heterodera glycines*, soybean cyst nematode, morphology, races, SEM.

Populations of the soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, are highly variable in their ability to reproduce on various soybean (*Glycine max* (L.) Merr.) cultivars (7). When SCN populations are challenged by resistant cultivars, the resistance may be overcome within several nematode generations. There may be several reasons for the instability of resistance in soybeans to SCN, including the vertical or race specific nature of the resistance that may allow SCN populations in which a large percentage of the nematodes have genes for pathogenicity to defeat the resistant cultivar. Populations of SCN vary widely in gene frequencies for pathogenicity (5). Currently, there are five described races differing in their ability to reproduce on soybean differentials (3,4). The race test using soybean differentials has been helpful to researchers but has limitations. Second-stage juvenile (J2) tail morphology has been used to aid in race identification, but tail measurements are not always race specific (3). It is desirable to find characters that either supplant the race test or supplement information obtained from race tests. Our objective was to determine if scanning electron microscopy (SEM) of the cephalic region of *H. glycines* J2 could aid race determinations.

MATERIALS AND METHODS

Populations of races 1 and 2 from North Carolina, race 3 from Illinois, race 4 from Tennessee, and race 5 from Hokkaido, Japan, were increased on 'Williams' soybean in a greenhouse. Before experimentation SCN race identifications were verified using soybean differentials (3,4). Freshly hatched, J2 obtained by mist chamber incubation of cysts were placed in BPI watch glasses containing 0.5 ml of 0.1 M phosphate buffer (PB) pH 7.2. Juveniles then were chilled to 4 C, and cold 4% glutaraldehyde buffered with PB was added dropwise twice daily until a 2% solution was obtained. Specimens were rinsed in 7% sucrose in PB, postfixed in 2% osmium tetroxide in PB, and dehydrated in a 9-step ethanol series at 4 C. Juveniles were dried in a critical point dryer using carbon dioxide. SEM stubs were prepared by affixing double adhesive tape to each surface, ap-

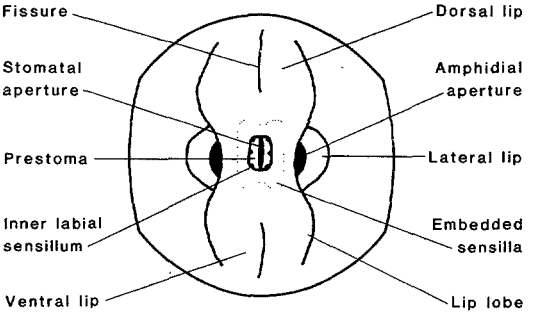

FIG. 1. Diagrammatic face view of second-stage juvenile of *Heterodera glycines* observed with scanning electron microscopy.

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[2] Research Plant Pathologist, USDA ARS, and Assistant Plant Pathologist, Department of Plant Pathology, University of Illinois, Urbana, IL 61801.

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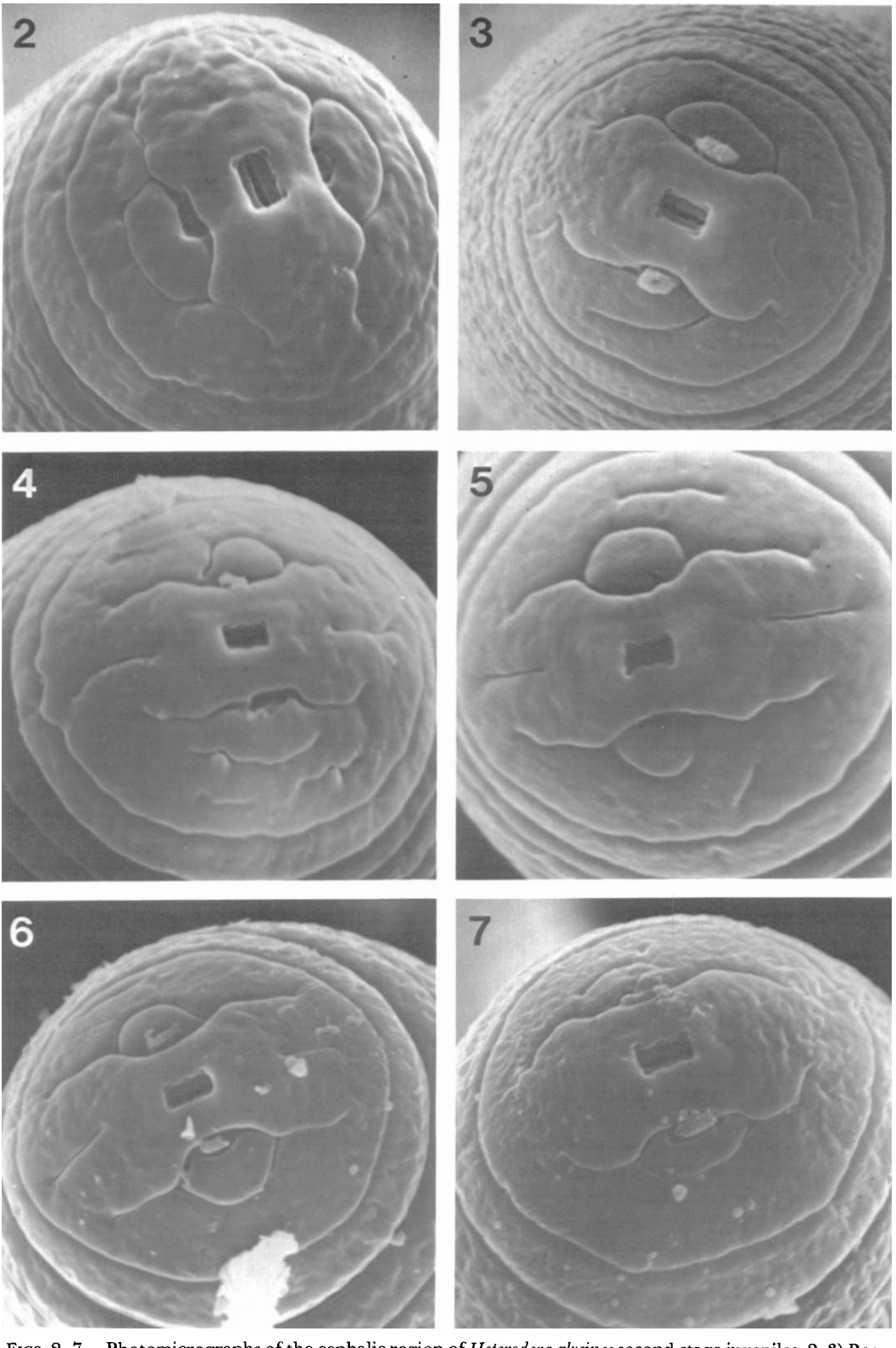
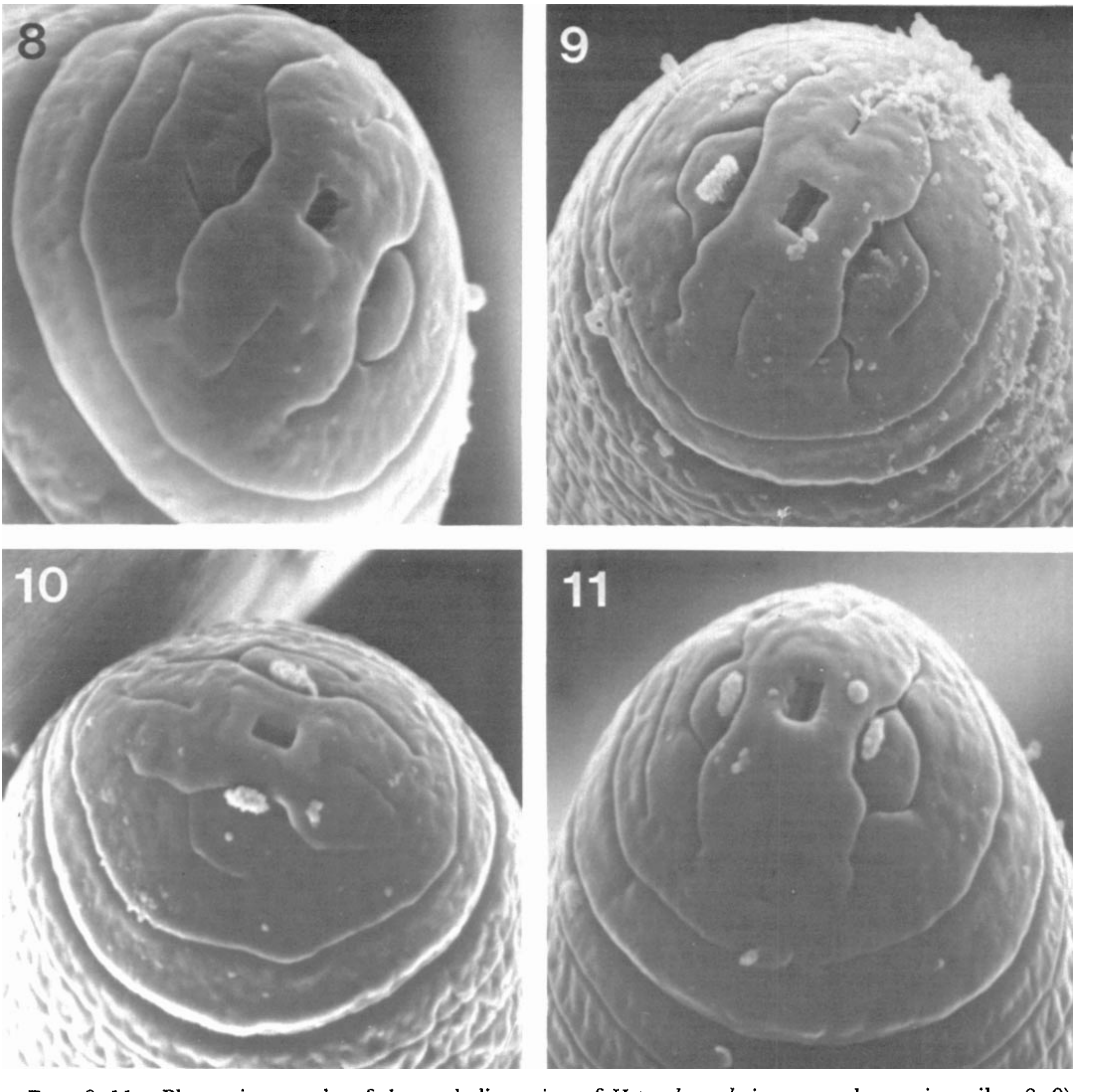


FIGS. 2–7. Photomicrographs of the cephalic region of *Heterodera glycines* second-stage juveniles. 2, 3) Race 1 from North Carolina. 4, 5) Race 2 from North Carolina. 6, 7) Race 3 from Illinois.



FIGS. 8–11. Photomicrographs of the cephalic region of *Heterodera glycines* second-stage juveniles. 8, 9) Race 4 from Tennessee. 10, 11) Race 5 from Hokkaido, Japan.

adhesive side up on top of the sticky surface, and bordering the edges of the tape with colloidal graphite. Charging was ameliorated by this preparation. Individual J2 were propped against a hair on the sticky tape (1) and sputter coated with gold-palladium for 3 minutes. Either a JEOL JSM U3 or an ISI DS-130 SEM operated at 15 kV was used to observe and photograph the J2.

RESULTS

The basic cephalic morphology of *H. glycines* J2 observed with SEM is illustrated in Figure 1. The labial disc boundary was incomplete, and the subdorsal and subventral lips were fused to form one dorsal and one ventral lip. There were differences in the shapes of labial discs, lateral lips, ventral and dorsal lips, and amphidial apertures (Figs. 2–11). There were varying amounts of indentation at the interface of the disc and lateral lips. Lobes of the dorsal and ventral lips varied from arcuate (Fig. 5) to subacute (Fig. 2). The lateral lips delimited the amphidial aperture and frequently had an incomplete outer margin (Figs. 3, 7–9); they varied in shape from crescentic (Fig. 8) to angular (Fig. 6). The prestoma appeared as a rectangular-shaped structure oriented similarly to the labial disc (Figs. 1–11). The stomatal aperture

was evident in most micrographs. Inner labial sensilla in the prestoma were visible on most specimens, depending on specimen preparation and angle of view during SEM. The prestoma was surrounded by a slightly elevated rectangular-shaped structure containing an outer labial and a cephalic sensillum embedded in the cuticle of each of the four lobes (Figs. 1–11). One (Fig. 3) or both lip pairs (Figs. 4–6, 8–11) were often fused to form one dorsal or one ventral lip, but sometimes they were divided by a fissure (Figs. 2, 7). Incomplete annulation on the cephalic region was observed on some specimens of all five races (Figs. 4–6, 8, 10).

Discussion

The morphology of the *H. glycines* J2 lip region was typical of *Heterodera* species and similar to the type 5 pattern of Stone (10). During a previous ultrastructure study, embedded outer labial and cephalic sensilla were observed in the *H. glycines* cuticle adjacent to the prestoma (2). The locations of the sensilla observed in that study correspond to the four lobes of the raised area surrounding the prestoma that we observed. These sensilla have no external openings.

The significant intraracial variation among *H. glycines* J2 prevented identification of races on the basis of their cephalic morphology. After observing and photographing about six specimens from each race during initial phases of this study, the utility of SEM for race identification seemed promising. However, after observations were made on several hundred J2 and photographs were taken of about 250 individuals, the intraracial variability became quite apparent.

Electrophoretic differences among females of *H. glycines* races (6) were not reflected in juvenile morphological differences. Stone and Williams (9) using SEM and electrophoresis were unable to observe differences between *H. avenae* Woll. races 1 and 2. SEM and electrophoretic studies revealed differences in potato cyst nematode pathotypes A and E leading to their separation into two species, *Globodera rostochiensis* (Woll.) Behrens and *G. pallida* (Stone) Behrens (8,12).

Eisenback and Hirschmann (1) differentiated races A and B of *Meloidogyne hapla* Chitwood using SEM. The populations they studied differed in chromosome number as well as method of reproduction. Presumably, all races of *H. glycines* and *H. avenae* have a haploid chromosome number of 9 and are amphimitic. Of interest for future study are *H. glycines* polyploids (11). Perhaps their genetic differences may be expressed morphologically.

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