SCANNING ELECTRON MICROSCOPY OF CEPHALIC REGIONS OF HETERODERA GLYCINES AND THREE RELATED CYST NEMATODE SPECIES

Barbara A. Stanger¹ and Gregory R. Noel⁷

Department of Crop Sciences¹ and Crop Protection Research Unit, U.S. Department of Agriculture, Agricultural Research Service,² University of Illinois at Urbana-Champaign, IL 61801, U.S.A.

ABSTRACT


Cephalic regions of second-stage juveniles and white females of the four closely related species, Heterodera glycines races 1-5, H. lespedezae, H. schachtii, and H. trifolii, were examined by scanning electron microscopy. Interspecific and intraspecific variation occurred among the populations, but differences among female cephalic regions could not be used in species identification of the populations studied. Differences in morphology of dorsal and ventral lips and partial head annulation in second-stage juveniles of H. lespedezae, H. schachtii, and H. trifolii may aid in species identification but cannot be used as the sole criteria to identify the species evaluated in this study.

Key words: Heterodera glycines, H. lespedezae, H. schachtii, H. trifolii, morphology, nematode races, scanning electron microscopy.

RESUMEN


Las regiones cefálicas de segundos estadios juveniles y hembras blancas de cuatro especies estrechamente relacionadas (Heterodera glycines razas 1-5, H. lespedezae, H. schachtii, y H. trifolii) fueron examinadas bajo microscopio electrónico de barrido. Se observaron variaciones inter e intraespecíficas entre las poblaciones, pero las diferencias entre las regiones cefálicas de las hembras no podrían ser usadas en la identificación de especies de las poblaciones estudiadas. Las diferencias en la morfología de los labios dorsales y ventrales y en el anillamiento parcial de la cabeza en los segundos estadios juveniles de H. lespedezae, H. schachtii, y H. trifolii pueden ayudar en la identificación de especies pero no deben ser usadas como un único criterio para indentificar las especies evaluadas en este estudio.

Palabras clave: Heterodera glycines, H. lespedezae, H. schachtii, H. trifolii, microscopio electrónico de barrido, morfología, razas de nematodos.

INTRODUCTION

Scanning electron microscopy (SEM) has become increasingly useful in studies of nematode morphology. The cephalic region, including lips, labial disc, sensory organs, and the oral opening, has been studied most frequently. Differences in cephalic regions of males, females, and second-stage juveniles (J2) have been found in several species of Heterodera and Globodera (Momota & Oshima, 1976; Oth-

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man et al., 1988; Stone, 1972). Species of the schachtii group of cyst nematodes often are differentiated by using J2 stylet and tail length, but measurements of these characters often overlap among species (Hirschmann & Triantaphyllou, 1979; Mulvey & Golden, 1983). Finding stable characters that can clearly distinguish the species would greatly benefit taxonomists. Scanning electron microscopy of the lip region of various life stages of cyst nematodes has great potential in species identification. The species, H. glycines Ichinohoe, H. lespedezae Golden & G. Cobb, H. schachtii Schmidt, and H. trifolii Goffart, are closely related members of the schachtii group of cyst nematodes (Triantaphyllou, 1970). Matings between H. schachtii and H. glycines have resulted in viable progeny (Potter & Fox, 1965). The cephalic region of J2 of H. glycines races 1-5 were studied previously, but differences in cephalic morphology which would provide differentiation of the races were not found (Noel & Stanger, 1985). The objective of this study was to determine whether females of H. glycines races 1-5, H. lespedezae, H. schachtii, and H. trifolii, and J2 of the four species could be separated on the basis of differences in cephalic regions observable with SEM.

MATERIALS AND METHODS

Populations studied: Female cephalic regions of H. glycines races 1-5, as determined by host range tests (Golden et al., 1970; Riggs & Schmitt, 1988), H. lespedezae, H. schachtii, and H. trifolii were examined by SEM. Photomicrographs of H. glycines race 2 (HgR2) from North Carolina, U.S.A. were used for comparison with J2 of the other species. Heterodera glycines race 1 (HgR1) also was obtained from North Carolina, U.S.A. Races 3 (HgR3), 4 (HgR4), and 5 (HgR5) were obtained from Illinois, U.S.A., Tennessee, U.S.A., and Hokkaido, Japan, respectively. All H. glycines populations were cultured on soybean (Glycine max (L.) Merr. cv. Williams 82). Heterodera lespedezae was obtained from North Carolina and cultured on striate lespedeza (L. striata (Thunb.) Hook. & Arn. cv. Kobe), and H. schachtii and H. trifolii from California were cultured on sugar beet (Beta vulgaris L.) and white clover, (Trifolium repens L. cv. Dutch White), respectively. All cultures were maintained at 22-28°C in a greenhouse.

SEM of nematodes: Second-stage juveniles were obtained by placing cysts in a mist chamber and collecting nematodes after 48 to 72 hrs. Freshly emerged J2 were selected individually, removed from the water with a nematode pick and placed in distilled water. The nematodes were rinsed 1-4 times in water, depending upon the amount of surface contamination. The following protocol was used for J2 illustrated in the photomicrographs. Nematodes were placed in 0.5 ml distilled water in a Bureau of Plant Industry dish and chilled at 4°C for 15 min. One drop of 4% glutaraldehyde in 0.1M phosphate buffer pH 7.2 (later referred to as buffer) chilled to 4°C was added twice each day for 10 days. Fixation continued for an additional 4 days at 4°C.

Roots with white females attached were rinsed gently to remove soil and cut into 1-cm-long segments and were placed in 2 ml of distilled water and refrigerated. Chilled glutaraldehyde in buffer was added 12 times over a 3-day period until a 2% glutaraldehyde solution was obtained. Roots with attached females were fixed for 2 weeks at 4°C, then rinsed with buffer. Females were dissected from the roots, sonicated for 40 sec, and rinsed in buffer for 12 hrs at 4°C. After fixation and rinsing, all nematodes were maintained at 4°C. The methods for post-fixation and dehydration were described previously.
(Noel & Stanger, 1985). Briefly, all nematodes were rinsed in buffer for 12 hrs, postfixed in 2% buffered osmium tetroxide for 12 hrs, and rinsed in buffer for 12 hrs. Nematodes then were dehydrated in an eight-step alcohol dehydration series during a 3-day period and dried in a critical point dryer using carbon dioxide.

In order to ameliorate charging, SEM stubs were prepared as described previously (Noel & Stanger, 1983). The J2 were cut in half to further reduce charging, mounted on the sticky side of silver tape, and leaned against a hair with the anterior end up. Females were mounted with the anterior end up. At least 40 nematodes per population were viewed on either a JEOL JSM U3 or ISI DS 130 scanning electron microscope operated at 10-15 kV. Photomicrographs of at least 20 specimens en face, and one side view per population were taken.

RESULTS

Interspecific and intraspecific variability was observed among J2 of the four species. In all species, the incomplete labial disc was surrounded by two lateral lips, a dorsal lip, and a ventral lip (Fig. 1). The amalgamated dorsal and ventral lips often were divided by a fissure which delimited the subdorsal and subventral lips. In H. schachtii, the fissure was either V-shaped (Fig. 2) or a narrow line oriented subdorally or subventrally (Figs. 3,4), or in the dorsoventral plane. About 25% of the H. schachtii J2 had V-shaped fissures. Very few H. glycines (Fig. 5) and H. lespedezae (Fig. 6), and none of the H. trifolii (Fig. 7) had V-shaped fissures. All H. schachtii J2 (Figs. 2-4) and most of the H. lespedezae J2 (Fig. 6) had two fissures. Approximately one-half of the H. trifolii J2 had two fissures, and the remaining specimens had one (Fig. 7). The number of fissures on H. glycines J2 varied from none to two (Fig. 5). Fissures were usually shorter in H. trifolii than in the other three species. The dorsal and ventral lips of some H. schachtii J2 were distinctly set off from the lip area and had poorly developed fissures (Fig. 4). A partially amalgamated labial disc was characteristic of H. glycines and H. lespedezae whereas complete dorsal and ventral lips were common in H. schachtii and H. trifolii. The lateral lip lobes of H. glycines were usually more arcuate than the other species and varied considerably in the degree of amalgamation of the lateral lips.

The J2 of all species had one or two complete annules anterior to the annule which separates the cephalic region from the body. Both H. lespedezae (Fig. 6) and H. trifolii (Fig. 7) usually had an additional, incomplete cephalic annule which was seldom present in H. schachtii (Figs. 2-4). About one-half of the HgR2 had an incomplete cephalic annule (Fig. 5).

The cephalic morphology of females was simpler than that of the J2. The lip region consisted of a prominent, square shaped labial disc usually lobed at each corner (Figs. 8-16). The prestone and stoma were present, but the inner labial sensilla observed in the prestone of males and J2 were not visible. The lips were fused

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Fig. 1. Diagram of the cephalic region of a second-stage juvenile of the schachtii group.
Figs. 2-7. Scanning electron micrographs of cephalic regions of second-stage juveniles. 2) *Heterodera schachtii*, 6000x. 3) *H. schachtii*, 6200x. 4) *H. schachtii*, 6100x. 5) *H. glycines* race 2, 8000x. 6) *H. lespedezae*, 6200x. 7) *H. trifoli*., 6300x.
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into a circular structure below the labial disc. Lateral lips were not observed on any specimen of the four species studied nor were amphidial apertures apparent. Cephalic morphology of females was similar among specimens of all four species (Figs. 9-16). The variation in lip regions that is evident in Figures 9-16 is representative of the variation observed within each population.

**DISCUSSION**

In an earlier study, *G. pallida* and *G. rostochiensis*, which formerly had been considered two races of the same species (Pozdol & Noel, 1984), could be differentiated using SEM of J2 cephalic areas. However, SEM also was used in an attempt to differentiate *H. avenae* pathotypes (Stone & Williams, 1974) but with no success. In the present study, morphology of the J2 cephalic region including shape of labial discs and lips varied among *H. glycines*, *H. lespedeza*, *H. schachtii*, and *H. trifolii* and within species.

The observations reported in this paper support the hypothesis that the ventral and dorsal lip pairs of J2 have become fused with the labial disc rather than having been lost during the elongation of the labial disc (Stone, 1975). Thus, the fissure would represent an incomplete division between either the ventral or dorsal lip pairs. Similarities in cephalic morphology of *H. glycines*, *H. lespedeza*, *H. schachtii*, and *H. trifolii* J2 found in this study are important in demonstrating the close taxonomic relationship among these species. *Heterodera schachtii* specimens with complete dorsal and ventral lip margins appeared as intermediate forms between Stone’s types 3 and 4 heteroderid lip patterns (Stone, 1975), whereas specimens with incomplete lip lobes and V-shaped or straight fissures were typical of type 5 into which Stone classified these four species. *Heterodera trifolii* specimens that had varying completeness of dorsal and ventral lip margins, exhibited forms intermediate between types 4 and 5. *Heterodera glycines* and *H. lespedeza* were typical of Stone’s type 5 pattern, with *H. glycines* having a large number of J2 with incomplete lateral lip margins. The greater degree of variability in *H. schachtii* supports the hypothesis that this species is the ancestral type for the *schachtii* group (Triantaphyllou, 1970), since the ancestor would have more traits of the less closely related species in the genus than would the descendents of *H. schachtii*.

Some differences in the morphology of female labial discs of *H. glycines*, *H. lespedeza*, *H. schachtii*, and *H. trifolii* were found, but as reported previously (Momota & Oshima, 1976) for *H. avenae, H. elachista* and *H. glycines*, there was much variability among the species, and accurate species identification using morphology of the female cephalic region was not possible. Demarcation of lateral lips of females in the Heteroderidae may be characteristic of certain species. Lateral lips were not found on the four species investigated in this study nor on *H. avenae* and *H. elachista* (Momota & Oshima, 1976). Similarly, lateral lips were not observed on females of *G. rostochiensis*, *Cactodera eremica*, and *Punctodera punctata* (Othman et al., 1988). On females of
G. solanacearum, G. tabacum, G. virginiae, and P. chalcoensis, lateral lips were observed (Othman et al., 1988).

Electrophoresis of females of the four species used in this study also demonstrated a close taxonomic relationship, especially between H. lespedezae and H. trifolii (Pozdol & Noel, 1984). The inability to use cephalic morphology for species identification is in contrast to our earlier work in which we found differences in vulval cones that could be used to identify the same populations of H. glycines, H. lespedeza, H. schachtii, and H. trifolii evaluated in the present study (Stanger & Noel, 1988).

The cephalic regions of females of H. glycines, H. lespedezae, H. schachtii, and H. trifolii are quite similar morphologically. Use of SEM of the cephalic region as the sole criterion to identify species in a mixed population is not possible. In contrast to females, substantial interspecific and
Figs. 9-12. Scanning electron micrographs of the cephalic regions of females of *Heterodera glycines* races. 9) Race 1, 1000×. 10) Race 2, 5300×. 11) Race 3, 8700×. 12) Race 4, 4400×.
intraspecific differences exist in the morphology of the cephalic region of J2, but these differences would preclude identification of species to a high degree of certainty.

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