## Observations on the Inner Labial Sensilla of Second-stage Larvae of Heterodera glycines'

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Scanning electron microscopic (SEM) studies of the cephalic region of members of the family Heteroderidae have failed to report inner labial sensilla of second-stage larvae, although the labial sensilla of males have been observed (1,4,6,7,9,11,12,13). The existence and location of the labial sensilla in the families Heteroderidae and Meloidogynidae have been discussed among nematologists conducting SEM studies of head morphology (1,2,6,13). Endo (3), in a recent transmission electron microscopic study of Heterodera glycines Ichinohe larvae, illustrated inner labial receptor canals which opened into the prestoma. While conducting SEM research with second-stage larvae of H. glycines, six inner labial sensilla were observed in the prestoma. This paper reports the location and appearance of these sensilla.

Freshly hatched second-stage larvae were placed in a BPI watch glass containing 0.5 ml of 0.2 M phosphate buffer, pH 7.2 (PB). Larvae were then chilled to 6 C and one drop of a 4% solution of 6 C glutaraldehyde buffered with PB was added daily for approximately I week until the final glutaraldehyde concentration approximated 2%. Larvae then were rinsed at room temperature in PB, fixed in 1% osmium tetroxide (buffered with PB), dehydrated in an 8-step ethanol series during a 3-day period, and critical point dried with CO<sub>2</sub> using the apparatus described by McClure and Stowell (5). A SEM stub was prepared by affixing double sticky tape to the surface, applying a  $7 \times 7$ -mm piece of silver tape on top of the sticky surface, and bordering the edges of the tape with colloidal graphite. Stubs

prepared in this manner ameliorated charging. Individual larvae were propped against a hair on the sticky tape (2) and sputter coated with gold for 3 min. A JEOI. JSM U3 scanning electron microscope operated at 15 kv was used to observe and photograph the head morphology of the secondstage larvae.

The configuration of the larval lip region was typical of the Heterodera type illustrated by Stone (10) and also similar to the type 5 pattern (13) in which the labial disc boundary is incomplete and the submedian lips are fused to form one dorsal and one ventral lip (Fig. 1). In nematodes with the type 5 pattern, the dorsal and ventral portions of the labial disc may or may not be marked by a fissure (Fig. 1). The labial morphology of the H. glycines second-stage larva presented by Momota and Ohshima (6) closely resembles the labial pattern shown in Fig. 1B. Although the labial sensilla were not reported during that study, the subdorsal and subventral sensilla are discernable. The specimen was distorted and the sensilla would not be readily apparent without knowledge of their exact location. The prestoma of the specimen photographed by Momota and Ohshima (6) is similar to the prestoma shown in Fig. 1C. de Grisse (1) hypothesized that the inner labial sensilla would be located in the corners of the prestoma. The inner labial sensilla of H. glycines appear as small pore-like apertures in the rectangular-shaped prestoma (Fig. 1A,B). Two sensilla are positioned subdorsally, two subventrally, and two laterally (Fig. 1).

Most heteroderid specimens illustrated in previous SEM papers appear distorted due to shrinkage. The larval specimen of H. fici Kirjanova presented by de Grisse (1) was not distorted, yet labial sensilla were not observed. In that study the specimen was infiltrated with Spurr's low viscosity epoxy resin (8). This medium may have occluded the sensilla and prevented their detection, or perhaps the sensilla are more readily visible in H. glycines. The four

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1. SEM micrographs of en face views of *Heterodera glycines* second-stage larvae. A, B) Labial sensilla visible as pore-like apertures in prestoma. Note that the dorsal and ventral portions of the labial disc are not marked by a fissure in A. C) Position of labial sensilla in prestoma apparent but pore-like nature not discernable.

outer labial sensilla and four cephalic sensilla which were visible with the transmission electron microscope (3) were not observed in this study nor in previous SEM studies of other researchers. Apparently, these sensilla have "blind endings" (1) in the cuticle and are only visible with the transmission electron microscope.

## LITERATURE CITED

I. de Grisse, A. 1979. SEM observations on the sensory organs in the head region of tylenchid nematodes. Scanning Electron Microsc. 3:487-496.

2. 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.

3. Endo, B. Y. 1980. Ultrastructure of the anterior neurosensory organs of the larvae of the soybean cyst nematode, Heterodera glycines. J. Ultrastruc, Res. 72:349-366.

4. Green, C. D., A. R. Stone, R. H. Turner, and S. A. Clark. 1975. Preparation of nematodes for scanning electron microscopy. J. of Microsc. 103:89-99. 5. McClure, M. A., and L. J. Stowell. 1978. A simple method of processing nematodes for electron microscopy. J. Nematol. 10:376-377.

6. Momota, Y., and Y. Ohshima. 1976. Scanning electron microscopy of some cyst nematodes. JPN J. Nematol. 6:14-23.

7. Rivoal, R. 1974. Observations de caractères morphologiques de espèces d'Heterodera au microscope electronique a balayage. Sci. Agron. Rennes. pp. 43-49.

8. Spurr, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26:31-43.

9. Stone, A. R., and C. D. Green. 1971. A simple method of preparing nematodes for scanning electron microscopy. Nematologica 17:490-491.

10. Stone, A. R. 1972. The round-cyst species of Heterodera as a group. Ann. Appl. Biol. 71:280-283.

11. Stone, A. R. 1972. Heterodera pallida n. sp. (Nematoda: Heteroderidae), a second species of potato cyst nematode. Nematologica 18:591-606.

12. Stone, A. R., and T. D. Williams. 1974. The morphology and soluble protein electrophoresis of Heterodera avenae pathotypes. Ann. Appl. Biol. 76:231-236.

13. Stone, A. R. 1975. Head morphology of second-stage juveniles of some Heteroderidae (Nematoda: Tylenchoidea). Nematologica 21:81-88.