

# Morphology of the Terminal Areas of White Females and Cysts of the Genus *Heterodera* (s.g. *Globodera*)

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**Abstract:** Using a scanning electron microscope and an interference contrast microscope, distinct morphological differences were found in the terminal areas of white females and cysts of six species of the genus *Heterodera* (s.g. *Globodera*). Sufficient differences to separate species were established on the basis of length of vulval aperture in the white female; arrangement, number and size of the perineal tubercles and vulval bodies in the white female; and presence or absence of a circumfenestral area and width of the cuticular grooves in the cyst. **Key Words:** cyst nematode, morphology.

Identification of species of *Heterodera* (s.g. *Globodera* Skarbilovich, 1959) is based largely on structural differences of the cone top and terminal area of the white female and the cyst. Recently, Wilson (5) and Green (1), using a scanning electron microscope, revealed the taxonomic significance of certain features previously obscure under light microscopy. The present work using a scanning electron

microscope and an interference contrast microscope, is an extension of these studies, with particular emphasis on the mature white female.

## MATERIALS AND METHODS

White females and cysts used in the study were obtained from the following sources:

Species	Source	Host
<i>Heterodera (Globodera) millefolii</i> Kirjanova and Krall, 1965	Dr. E. Krall Estonian SSR	Unknown
<i>Heterodera (Globodera) punctata</i> Thorne, 1928	Dr. J. Willard Matador Ranch Saskatchewan, Canada	Soil and roots of native prairie grass
<i>Heterodera (Globodera) solanacearum</i> Miller and Gray, 1972	Dr. L. I. Miller Blacksburg, Virginia, U.S.A.	Roots of <i>Solanum carolinense</i> L. (Horsenettle)
<i>Heterodera (Globodera) tabacum</i> Lownsbery and Lownsbery, 1954	Dr. B. F. Lownsbery Connecticut, U.S.A.	Roots of <i>Nicotiana tabacum</i> L. (Tobacco)
<i>Heterodera (Globodera) rostochiensis</i> Wollenweber, 1923	Mr. R. F. Morris Newfoundland, Canada	Roots of <i>Solanum tuberosum</i> L. (Potato)
<i>Heterodera (Globodera) virginiae</i> Miller and Gray, 1968	Dr. L. I. Miller Blacksburg Virginia, U.S.A.	Roots of <i>Solanum carolinense</i> L. (Horsenettle)

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The material was fixed in 5% formol and stored in either 5% formol or lactophenol. For examination by interference contrast microscope, the terminal areas were prepared as described by Mulvey (3). For observations with the scanning electron microscope, the gravid white females and cysts were dehydrated to absolute alcohol, mounted on a microscope

stub (posterior end upwards) with silver paint and gold-plated in a vacuum.

From 5 to 14 white females and cysts of *H. punctata*, *H. rostochiensis*, *H. solanacearum* and *H. virginiae* were photographed and studied using the scanning electron microscope. Only two cysts of *H. tabacum* were photographed.

### MORPHOLOGY

*Perineal tubercles [Vulval papillae of Green (1)]*: Green (1), using a stereoscan microscope, was the first to reveal the presence of "vulval tubercles" in the terminal areas of *H. rostochiensis* and related species. Since these structures are knob-like modifications of the cuticular ridges, I propose the term "perineal tubercle" which is more descriptive. Because of

the minute size (approx.  $1.0\ \mu\text{m}$ ), the perineal tubercles are usually obscure due to the limits of resolution of a light microscope. Green's work established the taxonomic significance of these and other structures which are distinctly seen and easily photographed using a stereoscan microscope.

The perineal tubercles and the area surrounding the vulval aperture are best observed in gravid white females, since these structures deteriorate as the female is transformed into a cyst. The three pathotypes (races) of *H. rostochiensis* (A, B and E; British Notation), can be distinguished by the width of the tubercular crescents (vulval crescents of Green) and size and shape of the perineal tubercles. The population of *H. rostochiensis*



FIG. 1. Scanning electron micrograph of a mature white female of *Heterodera* (*G*) *virginiae*. A. Perineal tubercles. B. Striated cuticular surface. C. Vulval aperture (slit).

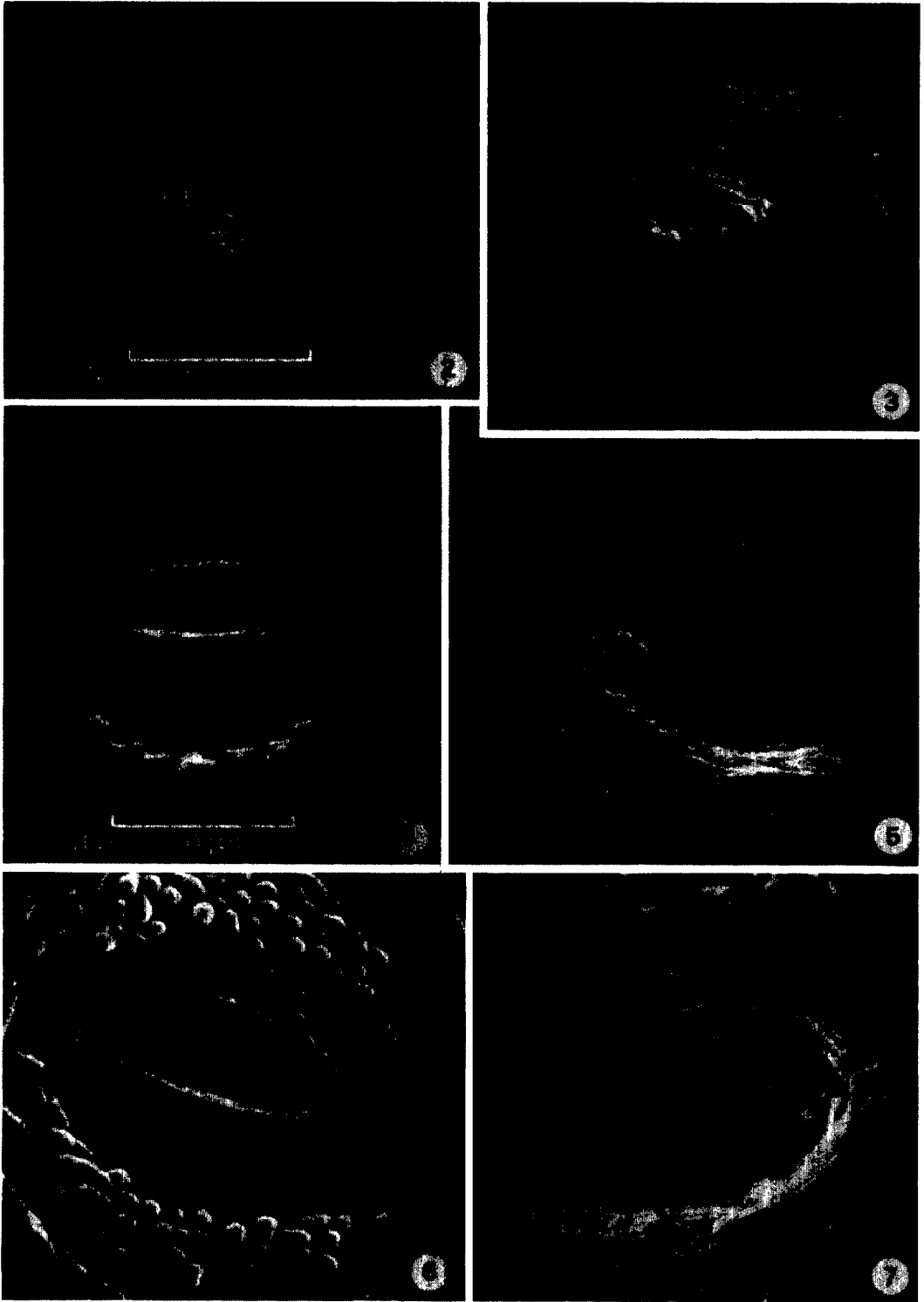


FIG. 2-7. Scanning electron micrographs of the terminal areas of mature white females and cysts of *Heterodera* (G) spp. 2. *H. punctata*, anal area of white female. 3. *H. punctata*, vulval area of white female. 4. *H. rostochiensis*, vulval aperture and perineal tubercles of white female. 5. *H. solanacearum*, vulval aperture and tubercles of white female. 6. *H. virginiae*, vulval aperture and tubercles of white female. 7. *H. tabacum*, perineal tubercles and deteriorated vulval aperture of cyst.

TABLE I. Comparison of terminal areas of *Heterodera (Globodera)* spp.

Species	Vulval aperture length ( $\mu\text{m}$ ) white female (No. of specimens)	Fenestra meas. ( $\mu\text{m}$ ) (No. of specimens)	Circumfenestral area, yellow-mature cyst	Tubercular crescents	Vulva bodies
<i>H. (G.) solanacearum</i>	5-6 (10)	26-30 $\times$ 18-20 (15)	Distinct in most specimens 35-45 $\times$ 28-30	Wide, tubercles discrete	Regularly arranged, 7-9 glands
<i>H. (G.) rostochiensis</i>	8-9 (20)	15-30 $\times$ 15-25 (50)	Distinct in few specimens	Narrow, tubercles large	Irregularly arranged, many
<i>H. (G.) tabacum</i>	8-9 (6)	28-30 $\times$ 14-20 (20)	Mostly obscure	Wide, tubercles clumped	Regularly arranged, 7-9 glands
<i>H. (G.) virginiae</i>	8-9 (6)	18-23 $\times$ 16-22 (20)	Mostly obscure	Wide, tubercles discrete	Regularly arranged, 7-9 glands

(Fig. 4) from Newfoundland resembles pathotype A since the tubercular crescents are narrow and the tubercles are large and irregularly shaped. The perineal tubercles of *H. solanacearum* (Fig. 5), *H. tabacum* (Fig. 7) and *H. virginiae* (Fig. 1, 6) are more uniform in size than *H. rostochiensis* and appear as single, distinct elements, or in groups of 2 to 3. The tubercular crescents are much wider than those of *H. rostochiensis*.

Surrounding the vulval aperture is an area which Green (1) describes as smooth cuticle. The finely, uniformly striated surface (Fig. 1) suggests that the subcuticular structure may be muscular, possibly a sphincter muscle controlling the size of the vulval aperture.

A few cysts of *H. millefolii* were available for study, and, although these were in poor condition, the perineal tubercles were present. The tubercular crescents were similar in width and tubercular size and shape to those of *H. solanacearum*. No perineal tubercles were found in *H. punctata* females and cysts (Fig. 3).

**Fenestral and vulval area of white female:** The vulval and anal fenestra of *H. punctata* are very similar in size (Figs. 13 and 15) but can be differentiated by the presence of a distinct circumfenestral area (Fig. 13) around the vulval fenestra and which is absent for the anal fenestra (Fig. 15). The very minute anus, which is located in the outer area of the fenestra (Fig. 15), is obscured in older cysts. The circumfenestral areas of *H. rostochiensis* (Fig. 29), *H. tabacum* and *H. virginiae* were mostly

obscure (Table 1) but that of *H. solanacearum* (Fig. 25) was distinct in most specimens. This difference is of some taxonomic value in separating these species.

There are also distinct differences in the length of the vulval aperture (Table 1). *H. solanacearum* (Fig. 14) has a very short vulval aperture (5-6  $\mu\text{m}$ ); whereas that of *H. rostochiensis* (Fig. 18), *H. tabacum* (Fig. 17) and *H. virginiae* (Fig. 16) is much longer (9-10  $\mu\text{m}$ ).

**Cuticular ridges:** The cuticular ridges of *H. punctata* (Figs. 2, 3, 12) in the anal-vulval area are different from those of the other species in this sub-genus. *H. solanacearum* cuticular ridges (Figs. 9, 24) are wider and more distinct than those of *H. rostochiensis* (Figs. 8, 28), *H. tabacum* (Fig. 11) and *H. virginiae* (Figs. 10, 26, 27). These differences in cuticular ridge structure complement the other diagnostic characters used to separate the cysts of the four closely related species mentioned.

**Vulval bodies:** Wilson (4) first described and illustrated endocuticular bodies closely associated with the vulval area of white females of *H. rostochiensis*. She named them "vulval bodies". I have examined these bodies in the white females of *H. rostochiensis* (Figs. 20, 21), *H. solanacearum* (Fig. 19), *H. tabacum* (Fig. 23) and *H. virginiae* (Fig. 22) and found their arrangement and number is sufficiently different to separate *H. rostochiensis* from the other three species. These bodies, which are located 7-10  $\mu\text{m}$  below the level of the vulval

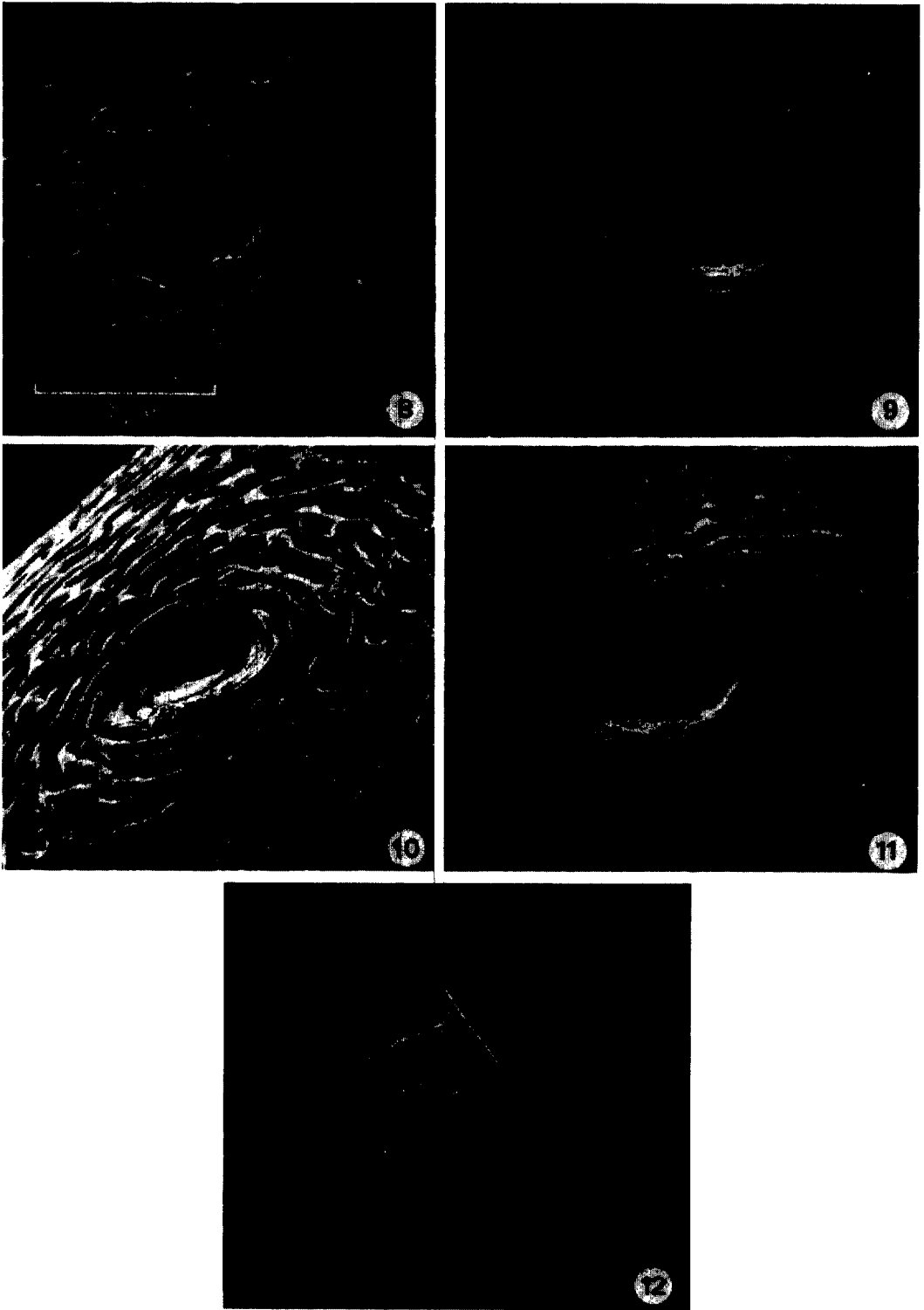


FIG. 8-12. Scanning electron micrographs of cuticular ridges around the vulval aperture of *Heterodera* (G.) spp. 8. *H. rostochiensis*, cyst. 9. *H. solanacearum*, mature female. 10. *H. virginiae*, cyst. 11. *H. tabacum*, cyst. 12. *H. punctata*, cyst. (All figures of same magnification).

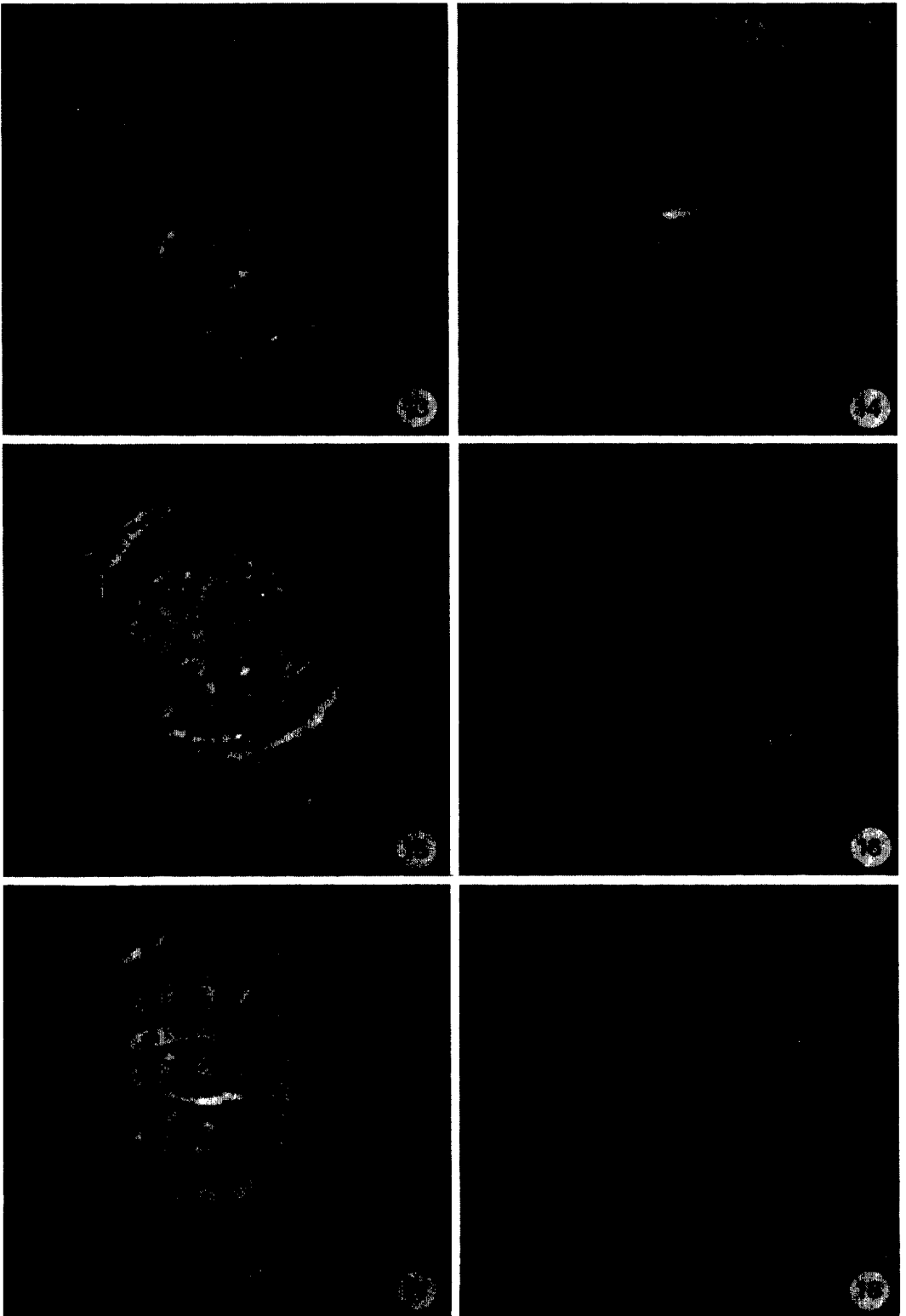


FIG. 13-18. Photomicrographs of the terminal areas of white females. 13. *H. punctata*, vulval fenestra and circumfenestral area. 14. *H. solanacearum*, vulval fenestra. 15. *H. punctata*, anal fenestra showing minute anal aperture at bottom right. 16. *H. virginiae*, vulval fenestra showing minute perineal tubercles. 17. *H. tabacum*, vulval fenestra with well-defined fenestration. 18. *H. rostochiensis*, vulval fenestra. (All figures of same magnification).

aperture, are regularly arranged in *H. solanacearum*, *H. tabacum* and *H. virginiae*. Dorsally there are 2-3 bodies, ventrally 3-4 and one to either side of the vulval aperture. The vulval bodies of *H. rostochiensis* are asymmetrically grouped and viewed laterally,

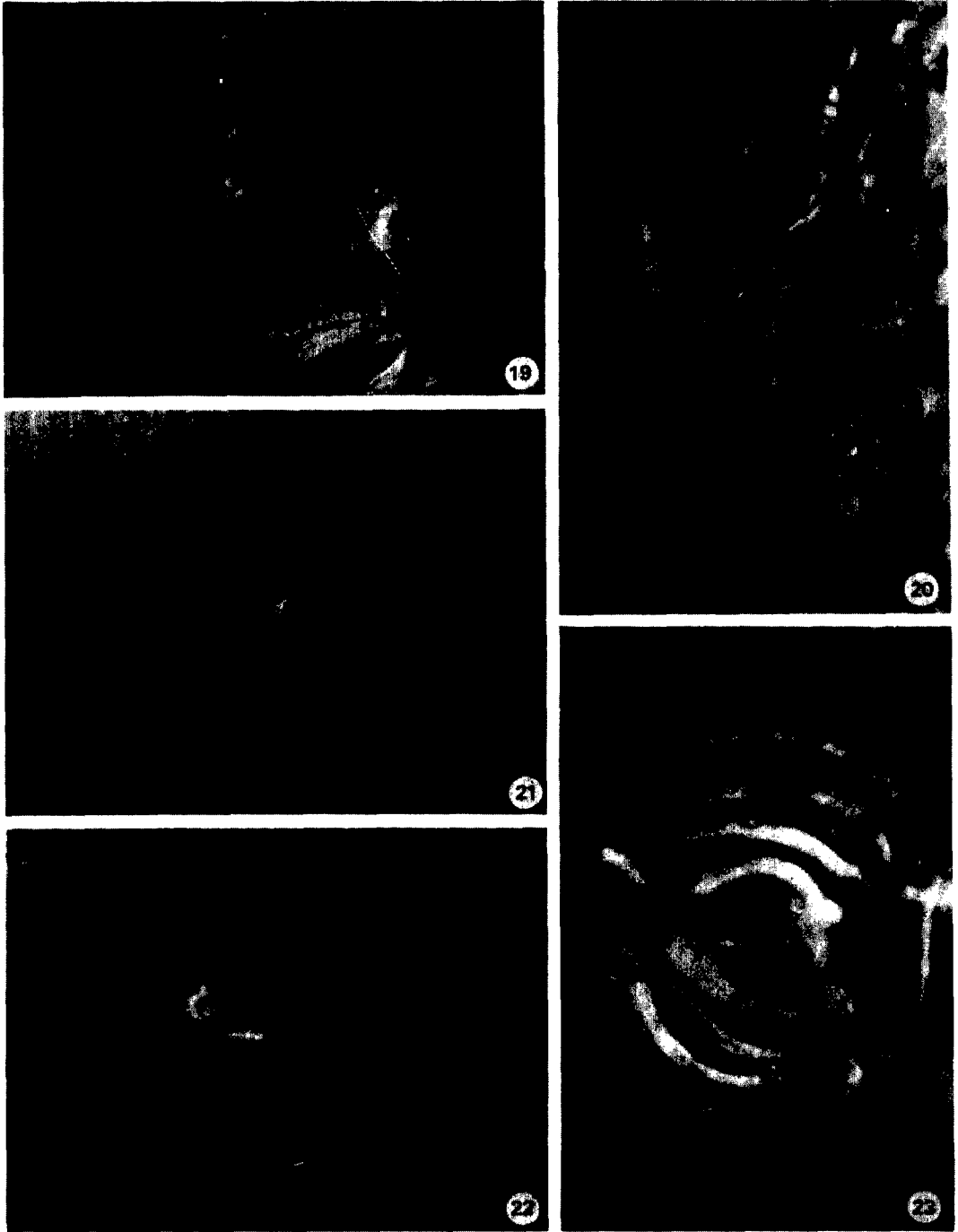


FIG. 19-23. Photomicrographs of the vulval bodies of white females. 19. *H. solanacearum*, showing regular arrangement of bodies. 20. *H. rostochiensis*, side view of bodies which appear to lead into the vagina. 21. *H. rostochiensis*, showing irregular arrangement of bodies. 22. *H. virginiae*. 23. *H. tabacum*. (All figures of same magnification).

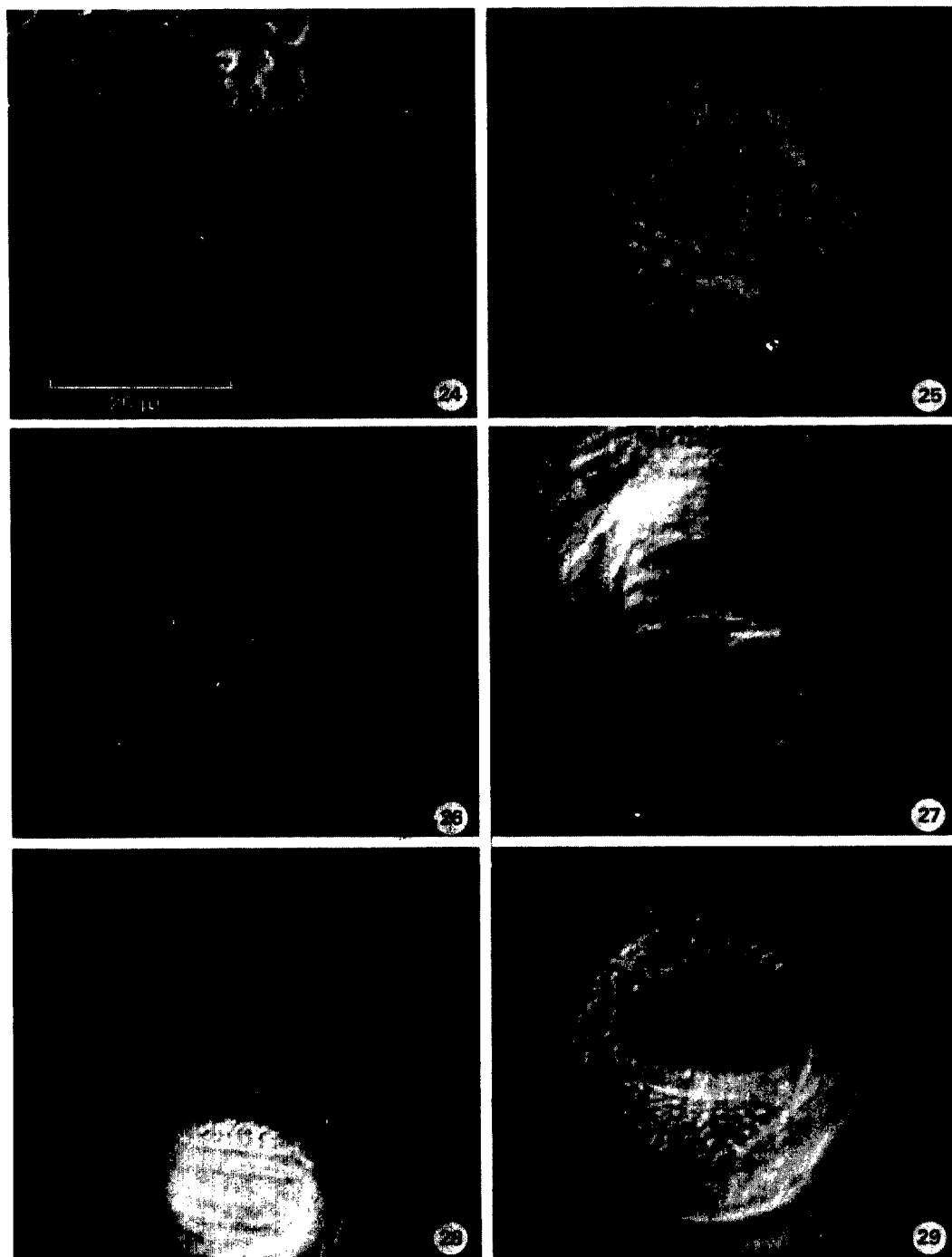


FIG. 24-29. Photomicrographs of cuticular ridges and variations in terminal areas of cysts. 24. *H. solanacearum*, showing wide, distinct grooves. 25. *H. solanacearum*, showing large circumfenestral area. 26. *H. virginiae*. Shallow narrow grooves. 27. *H. virginiae*, shingle effect associated with fenestration. 28. *H. rostochiensis*, shallow grooves. 29. *H. rostochiensis*, large circumfenestral area. (All figures of same magnification).



they appear to lead into the vagina (Fig. 20). Possibly they have a secretory function and may atrophy as this function ceases. Their association with the vagina and their disappearance as the female matures supports this contention. Maggenti and Allen (2) described and illustrated matrix-producing glands for the genus *Meloidogyne* Goeldi, 1887 and this further substantiates that these bodies found in *Heterodera* have a secretory function although they may not serve the same function as those of *Meloidogyne* since a gelatinous matrix is seldom found for species of *Heterodera* (Globodera). Wilson reported finding "vulval bodies" in *Heterodera* (*Heterodera*) *schachtii* and *H. (H.) cacti*; in the former, the white female produces a gelatinous matrix.

Key to species of *Heterodera* (*Globodera*)

- 1. Vulval and anal fenestra of nearly equal size, perineal tubercles absent . . . . .*punctata*  
     Vulval fenestra much larger than anal area, perineal tubercles present . . . . . 2
- 2. Anal area encircled by cuticular rings. . . . .  
     . . . . .*millefolii*  
     Anal area not encircled by cuticular rings . . 3
- 3. Vulval bodies many, irregularly arranged; perineal tubercles large, clumped. . . . .  
     . . . . .*rostochiensis*  
         (pathotype A)

- Vulval bodies 7-9, regularly arranged; perineal tubercles small, discrete . . . . . 4
- 4. Vulval aperture length 5-6  $\mu\text{m}$ , circumfenestral area distinct in most cysts . . . . .*solanacearum*  
     Vulval aperture length 8-9  $\mu\text{m}$ , circumfenestral area seldom distinct in cysts . . . . . 5
- 5. Fenestral length 18-23  $\mu\text{m}$ , pattern of lines between anus and vulval maze-like . . . . .  
     . . . . .*virginiae*  
     Fenestral length 28-30  $\mu\text{m}$ , pattern of lines between anus and vulval not maze-like . . . . .  
     . . . . .*tobacum*

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