# Redescription of Heterodea zeae, the Corn Cyst Nematode, with SEM Observations[1]

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*Abstract: Heterodera zeae*, the corn cyst nematode, is redescribed and illustrated with comparative details and measurements of females, cysts, and larvae from Maryland, USA; and India. Scanning electron micrographs of specimens from the United States are also presented. Revised measurements for the larval stylet and new diagnostic characters, especially in the cyst cone, for *H. zeae* are given. The relationship of *H. zeae* to close species is discussed. *Key words: Heterodera zeae*, cyst nematode, species, taxonomy, identification, distribution.

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*Heterodera zeae* was first described by Koshy, et al. (3) in 1971 from *Zea mays* L. (corn, maize) in Rajasthan State in India. In laboratory tests (29–31 C) they found that cysts of this new species hatched and produced cysts on roots of corn and barley. They also discovered *H. zeae* in corn fields in four other areas. Subsequent surveys by Koshy and Swarup (4) showed this nematode to be widely distributed in five States in India and to be of economic importance. Host range studies in India, reported in 1975 (8), showed various cultivars of corn to be good hosts for *H. zeae* but wheat, oat, and barley to be poor hosts. A final report in 1978 of a PL-480 project (B. A. Oteifa, unpublished) established the presence of *H. zeae* in Egypt and showed that all of many cultivars of corn tested were good hosts and that several cultivars of milo, wheat, barley, and one of sorghum were also good hosts. More recently *H. zeae* has been found in various areas of Pakistan (5,7).

Early in 1981 *H. zeae* was found in three corn fields in Kent County, Maryland, the first known occurrence of this cyst species in the United States (7). Subsequent limited surveys showed the nematode to be on several nearby farms; preliminary tests indicated field corn, sweet corn, and barley to be hosts. Because of the potential threat to corn production in Maryland and in the United States posed by this cyst nematode, extensive research was planned and has been initiated, including surveys, host range and pathogenicity studies, and possible control measures.

As part of this overall research effort, our report here gives an updated redescription of *H. zeae*, with SEM observations, and its taxonomic relationship to closely related species. A brief report on diagnostic features of *H. zeae* was recently given by Golden and Mulvey (2), and a key for identification of this and other cyst species in the Western Hemisphere, along with morphometric and distribution data, has been prepared by Mulvey and Golden (6).

## MATERIALS AND METHODS

Specimens used for the description and morphometric data of *H. zeae* were obtained from two primary sources. Fixed material, including paratypes, were kindly provided by Dr. Gopal Swarup, Indian Agricultural Research Institute, New Delhi, India; living material was given by Dr. Lorin Krusberg, University of Maryland, College Park, from cultures previously originating from the infested site in Kent County. In addition, specimens of *H. zeae* from Pakistan were studied; these were provided by Dr. Maqbool Ahmad, University of Karachi, Karachi. Females were removed from corn roots and cysts were sieved from soil after which larvae were hatched from cysts kept in water in Syracuse watch glasses in the laboratory. The procedures used for preparing and measuring specimens were essentially the same as those used by Golden and Birchfield (1). Photomicrographs of cysts, vulva cones, females, and larvae were

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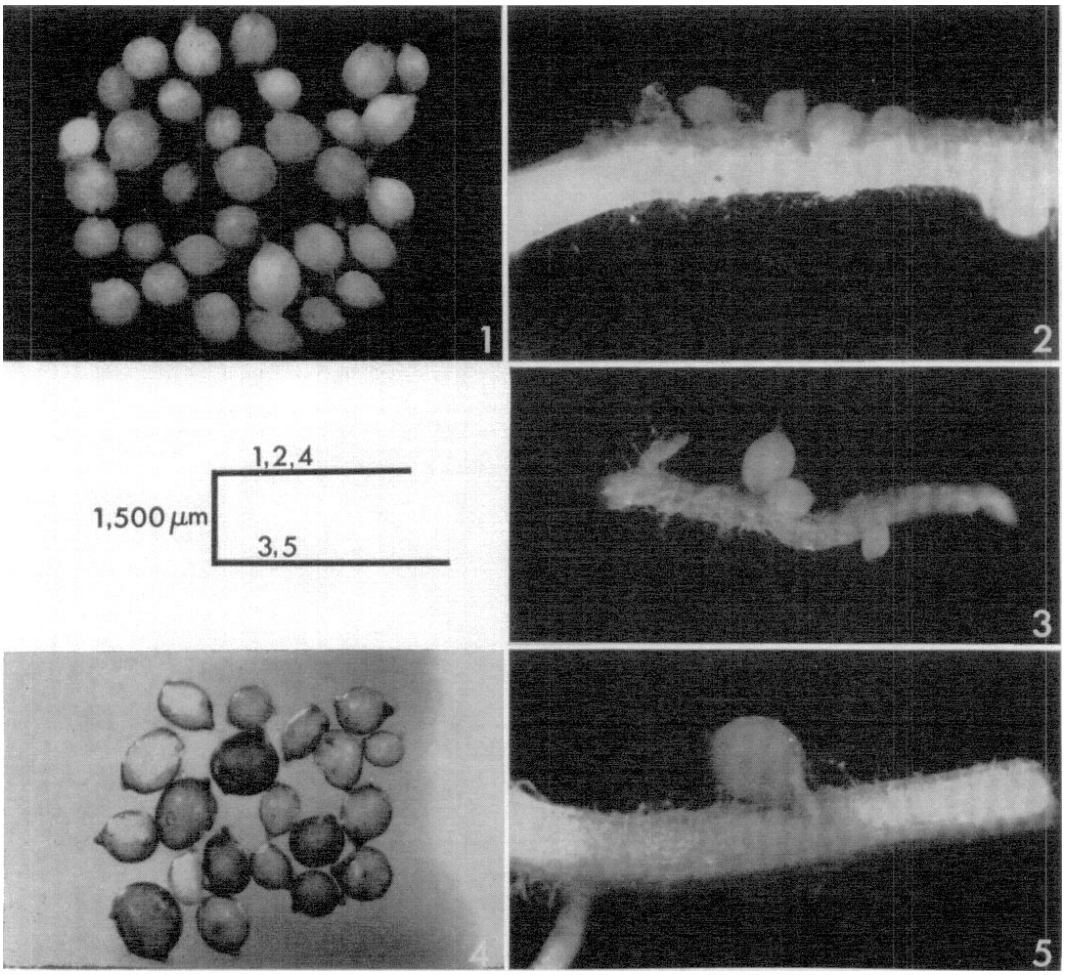
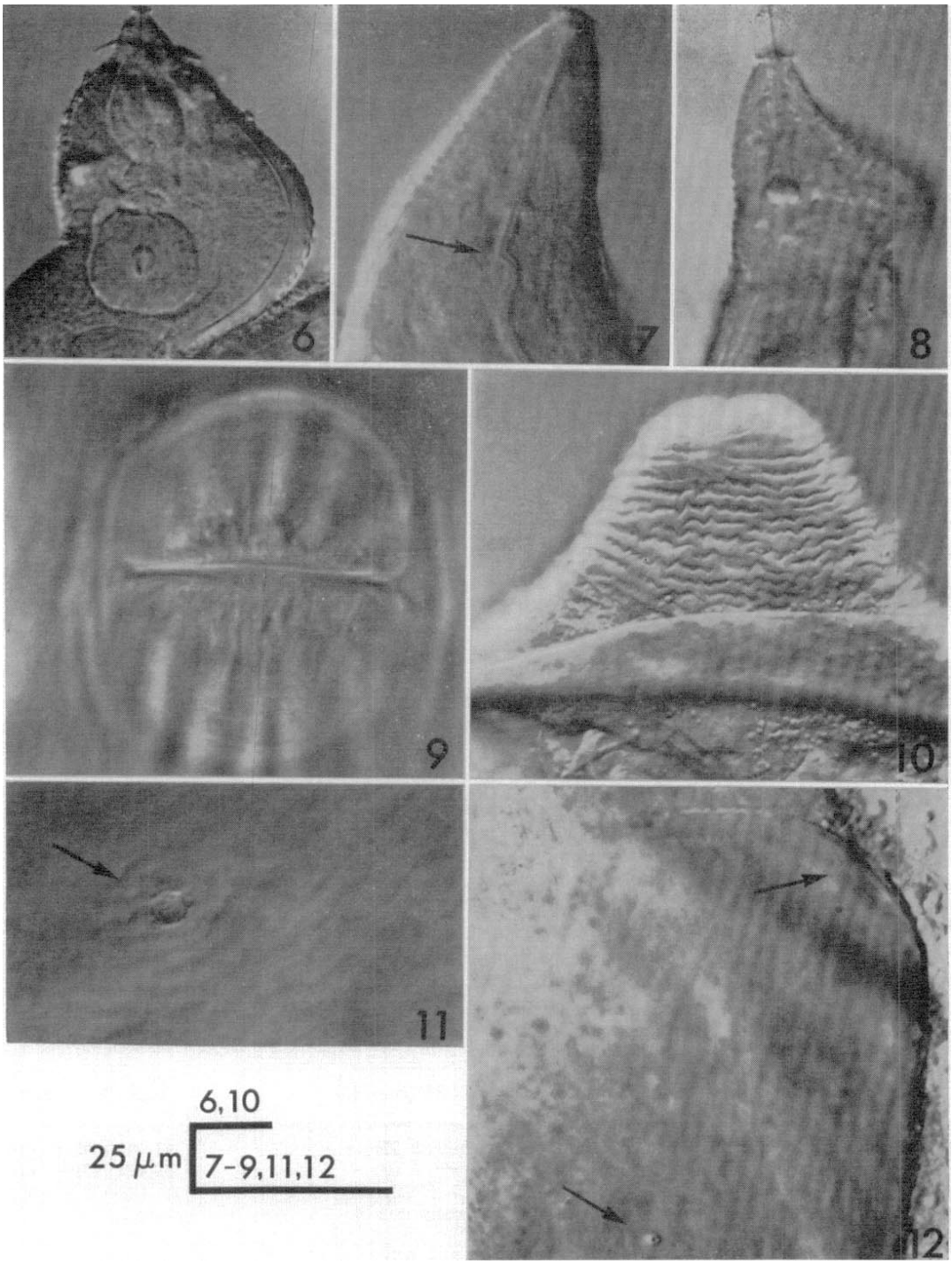



Figs. 1–5. Photomicrographs of *Heterodera zeae* specimens on, or isolated from, the roots of corn grown in Maryland, USA. 1) White females. 2, 3) Females attached to roots. 4) Whole cysts. 5) Newly formed cyst still attached to corn root.

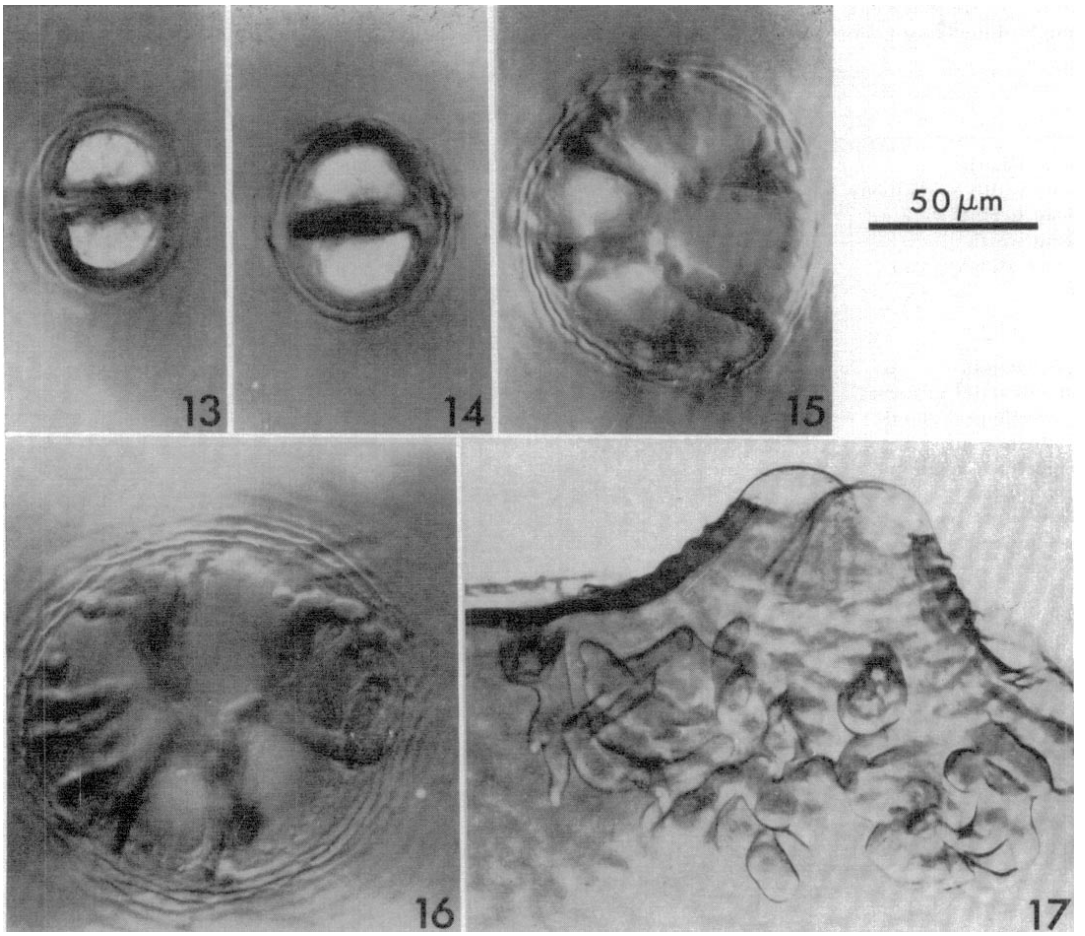
Table 1. Measurements (μm) of mature females of *Heterodera zeae* from corn (range, mean, and standard deviation).

| | Maryland, USA (N = 40) | India (N = 26)* |
|---|---|---|
| Body length (including neck) | 459–796 (617 ± 77) | 427–716 (592 ± 15) |
| Body width | 275–515 (366 ± 69) | 325–684 (491 ± 18) |
| Length/width ratio | 1.3–2.3 (1.7 ± 0.2) | 1.0–1.5 (1.2 ± 0.02) |
| Stylet length | 22.4–25.8 (24.7 ± 1.0) | 24–25 |
| Stylet base to dorsal esophageal gland duct opening | 3.4–6.2 (4.8 ± 0.7) | ... |
| Head tip to median bulb valve | 53.8–92.4 (73.2 ± 10.1) | ... |
| Head tip to excretory pore | 110–199 (159 ± 18) | Usually 90 |
| Diameter of median bulb | 31.7–35.6 (33.6 ± 1.6) | ... |
| Length of vulva slit | 31.8–34.3 (32.9 ± 0.8) | ... |
| Anus to vulva slit distance | 54.6–61.0 (59.5 ± 2.9) | ... |
| Cuticle width at midbody | 6.4–8.9 (8.0 ± 0.9) | 7–9 |

*After Koshy et al. (3).




Figs. 6–12. Photomicrographs of *Heterodera zeae* females from Maryland, USA. 6) Esophageal region showing head, stylet, and large valvated median bulb. 7) Dorsal esophageal gland duct opening (arrow) at about 5 μm from base of stylet. 8) Head area showing two annules and protruded stylet. 9) Vulva slit. 10) Ridge pattern on vulva cone. 11) Excretory pore (arrow), located approximately 150 μm posterior to head tip. 12) Anus (lower arrow) about 60 μm anterior to vulva cone tip (arrow).




Figs. 13–17. Photomicrographs of *Heterodera zeae* vulva cones of newly formed cyst from Maryland, USA. 13) Fenestra and vulva slit. 14) Underbridge. 15) Four finger-like bullae immediately below underbridge. 16) Bullae immediately below finger-like bullae. 17) Lateral view of vulva cone showing bullae.

Table 2. Measurements (µm) of cysts of *Heterodera zeae* from corn (range, mean, and standard deviation).

| | Maryland, USA (N = 40) | India (N = 38) | India (N = 80)* |
|---|---|---|---|
| Body length (including neck) | 454–785 (588 ± 88) | 428–785 (565 ± 92) | 342–684 (501 ± 10) |
| Body width | 255–551 (347 ± 63) | 245–525 (380 ± 74) | 260–537 (396 ± 9) |
| Length/width ratio | 1.4–2.2 (1.7 ± 0.2) | 1.2–1.8 (1.4 ± 0.3) | 1.0–1.5 (1.3 ± 0.01) |
| Fenestral length | 35–45 (40.4 ± 3.3) | 39–57 (46.0 ± 4.5) | 37–53 (44.0 ± 0.8) |
| Fenestral width | 16–34 (26.3 ± 3.9) | 19–38 (27.4 ± 5.3) | 20–32 (27.8 ± 0.8) |
| Vulva slit length | 29–42 (36.5 ± 3.7) | 38–45 (40.4 ± 3.5) | 36–58 (44.4 ± 1.0) |
| Underbridge length | 30–41 (36.8 ± 3.0) | 34–51 (38.7 ± 3.5) | ... |
| Underbridge width | 7.6–12.0 (10.3 ± 1.4) | 7.6–10.2 (9.1 ± 1.9) | ... |
| Underbridge depth | 29–38 (34.6 ± 4.7) | ... | ... |

*After Koshy et al. (3)

Table 3. Measurements (μm) of second-stage larvae of *Heterodera zeae* from corn (range, mean, and standard deviation).

| | Maryland, USA (N = 50) | India (N = 30) | India (N = 25)* |
|---|---|---|---|
| Body length | 399–460 (431 ± 14) | 392–451 (423 ± 15) | 360–440 (410 ± 40) |
| Body width at midbody | 18.5–20.7 (19.6 ± 0.7) | 19.6–20.2 (19.7 ± 0.1) | ... |
| Head height | 3.4–4.5 (3.9 ± 0.1) | 3.9–4.5 (4.3 ± 0.3) | ... |
| Head width | 8.4–9.5 (9.1 ± 0.2) | 9.0–9.5 (9.1 ± 0.2) | ... |
| Width/height ratio | 2.3–2.7 (2.3 ± 0.1) | 2.0–2.4 (2.1 ± 0.1) | ... |
| a | 20.4–23.4 (22.1 ± 0.9) | 20.0–23.3 (21.6 ± 1.2) | 19.0–25.4 (22.3 ± 0.3) |
| b | 2.5–3.0 (2.8 ± 0.9) | 2.4–3.0 (2.7 ± 0.2) | 2.9–4.2 (3.4 ± 0.1) |
| c | 9.3–10.5 (9.9 ± 0.3) | 8.8–9.8 (9.2 ± 0.3) | 8.0–13.0 (8.8 ± 0.3) |
| Stylet length | 19.0–20.7 (19.9 ± 0.4) | 19.6–20.2 (19.9 ± 0.3) | 20–25 (23.3 ± 0.3) |
| Base of stylet to dorsal esophageal gland duct opening | 3.4–5.0 (4.3 ± 0.4) | 3.9–5.6 (4.3 ± 0.4) | 4–5 |
| Head tip to median bulb valve | 61–67 (65.3 ± 2.7) | 63–77 (69.5 ± 3.1) | ... |
| Head tip to base of esophageal gland lobe | 137–168 (154 ± 8) | 143–182 (159 ± 20) | ... |
| Tail length | 40–49 (44.2 ± 2.4) | 40–51 (45.9 ± 2.2) | 52–50 (41.0 ± 1.1) |
| Hyaline tail terminal | 16.8–25.2 (21.9 ± 1.7) | 17.9–26.3 (22.7 ± 2.3) | 16–30 (24.0 ± 0.7) |
| Caudal ratio A | 2.1–3.7 (3.0 ± 0.3) | 2.5–3.8 (3.1 ± 0.3) | ... |
| Caudal ratio B | 9.2–20.9 (12.9 ± 2.9) | 8.0–20.0 (12.5 ± 2.7) | ... |
| Lateral lines | 4 | 4 | 4 |

*After Koshy et al. (3).

made with an automatic 35 mm camera attached to a compound microscope having an interference contrast system; those of infected roots, whole females, and intact cysts were made with a 8.3 × 10.8 cm sheet-film camera attached to a dissecting microscope. In making the scanning electron micrographs, living specimens were fixed in 3% glutaraldehyde solution with 0.05 M phosphate buffer (pH 6.8), dehydrated in a graded series of ethanol, critical-point dried from liquid $CO_2$, sputter-coated with a 20–30-μm layer of gold-palladium, and examined with a scanning electron microscope (SEM).

## SYSTEMATICS

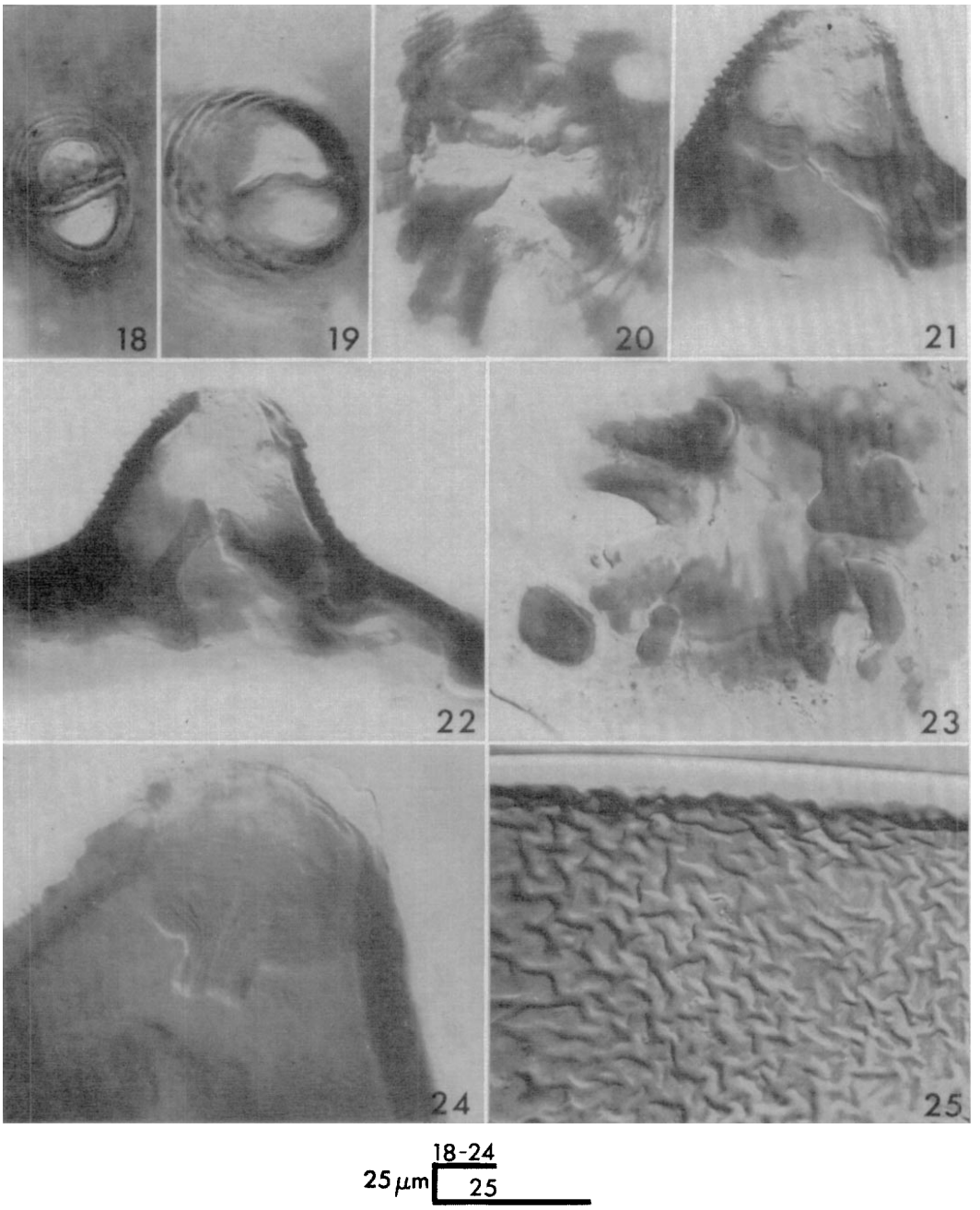
### *Heterodera zeae* Koshy, Swarup & Sethi, 1971 (Figs. 1–52)

*FEMALES. Measurements:* See Table 1.

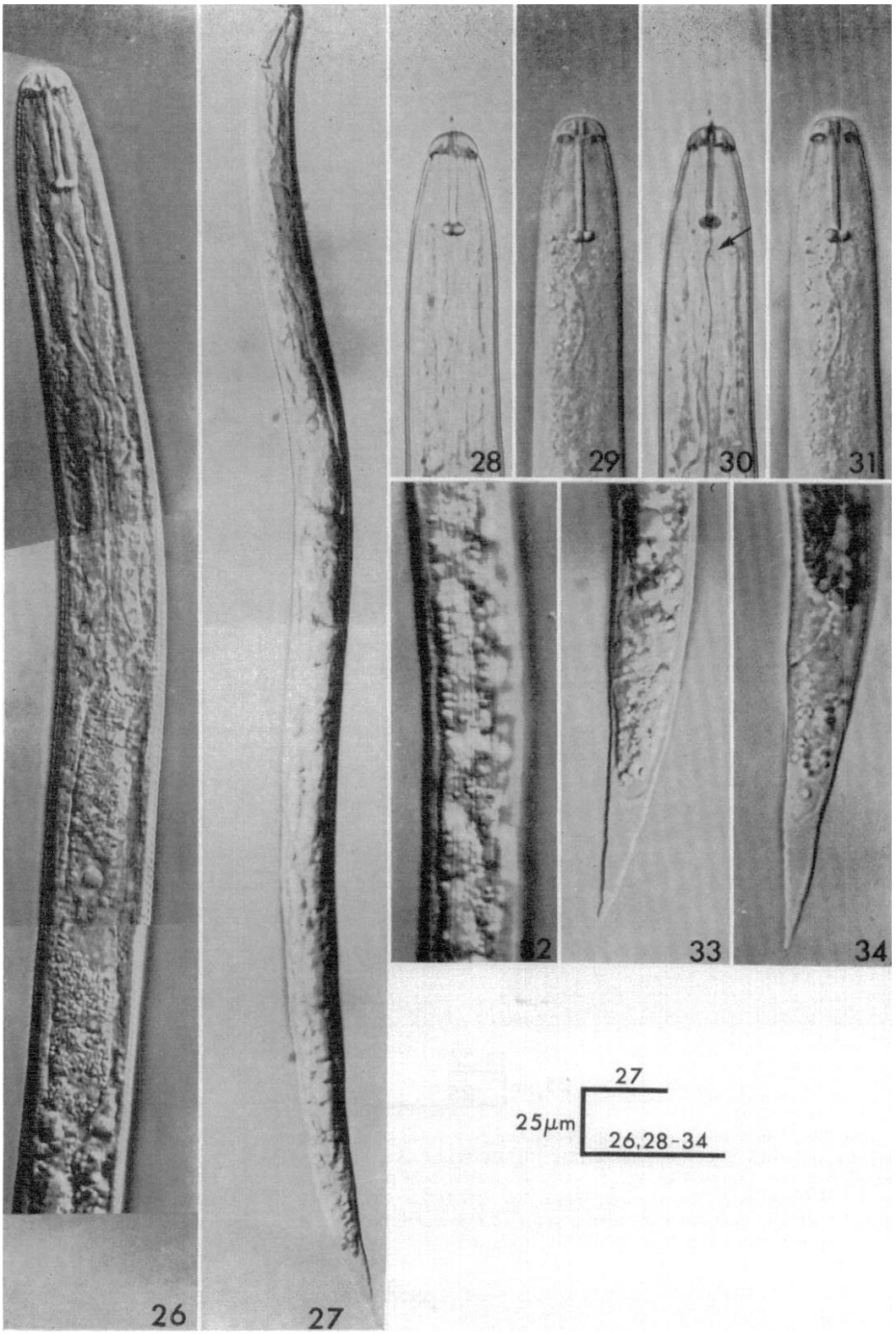
*Description:* Body pearly white, basically lemon-shaped (Fig. 1), cuticle thin, external wall pattern zig-zag (Fig. 37) without subcrystalline layer. Head with two annules (Figs. 8, 35) second larger than first. Stylet slender (Fig. 8) with fairly wide (4 μm), anteriorly flattened knobs, dorsal esophageal gland duct opening (Fig. 7) about 6 μm from stylet base. Neck long, median bulb (Fig. 6) large, rounded with well-developed valve plates. Esophageal gland a single lobe, variable in size and shape. Excretory pore (Fig. 11) well posterior to head tip, generally at level of base of esophageal gland lobe. Ovaries two, mature female filled with eggs in various stages of development. Vulva cone well developed (Fig. 10), vulva slit fairly long (Fig. 9; Table 1). Egg sac (Fig. 2) generally present, small to large. Anus very small (Fig. 12), difficult to detect, and fairly close to vulva slit.

*CYSTS. Measurements:* See Table 2.

*Description:* Cyst light brown in color, basically lemon-shaped (Fig. 4), cuticle thin walled, without subcrystalline layer. Cuticular midbody pattern (Figs. 25, 50) zig-zag. Vulva cone prominent (Figs. 24, 44), end-on view (Figs. 47, 49) shows concentric lines of cuticular ridges around vulva slit and fenestra. Fenestra ambifenestrate, semifenestra separated by fairly wide vulva bridge (Figs. 13, 18), fenestral length and width variable (Table 2), basin (Fig. 13) wide but generally poorly defined. Bullae prominent, characteristic arrangement for




Figs. 18–25. Photomicrographs of *Heterodera zeae* vulva cones of *mature* cysts from Maryland, USA. 18) Fenestra and vulva slit.. 19) Underbridge slightly below level of fenestra. 20) Four finger-like bullae immediately below underbridge. 21, 22) Dorso-ventral view of two of the four finger-like bullae at same level. 23) Bullae immediately below finger-like bullae. 24) Dorso-ventral view of underbridge showing striated vaginal wall. 25) External culticular pattern of cyst at midbody.


26
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26,28-34

this species; immediately below (anteriad) the underbridge are four finger-like bullae (Figs. 20–22) in a distinct formation which was observed in all but a few of the several hundred cysts examined. Immediately below the finger-like bullae are a number of randomized bullae (Fig. 23). Underbridge (Figs. 14, 19, 24) simple, short, and thin, and found in all but a few of the cysts examined. Vulva slit fairly long (Figs. 13, 48; Table 2), anus indistinct.

*SECOND-STAGE LARVAE. Measurements:* See Table 3.

*Description:* Body typically vermiform (Fig. 27), tapering at both ends. Head slightly set off, rounded, with low profile, (Figs. 29, 38). Lip pattern (Fig. 40) type "4" of Stone's (9) designation, 4–5 head annules, and with moderately developed cephalic framework. Stylet (Figs. 28, 29) slender, with knobs shallowly concave anteriorly. Dorsal esophageal gland duct opening (Figs. 30, 31) close to base of stylet. Anterior cephalids 1–2 annules behind base of cephalic framework, posterior cephalids 7–8 annules behind base of framework. Excretory pore distinct, hemizonid immediately anterior to level of excretory pore. Median bulb nearly twice as long as wide, with well-developed valve plates (Fig. 26). Basal part of glandular lobe (Fig. 26) generally distinct, with three large nuclei, and overlapping the anterior part of the intestine. Esophagus about one-third total body length. Lateral field with four distinct lines (Figs. 32, 41), generally visible in most specimens. Phasmids very small but conspicious, about midway between anus and anterior end of hyaline tail terminal. Tail (Figs. 33, 34) short, conically tapering, with acutely rounded terminus, hyaline terminal about one-half tail length.

*EGGS. Measurements:* Length = 97–105 μm (102.6 ± 2.1); width = 37.8–47.2 μm (43.3 ± 2.6); L/W ratio = 2.1–2.4 (2.3 ± 0.1)

*Description:* Shell hyaline (Figs. 42, 43), without any visible markings under light (optical) and SEM microscopy.

## DISCUSSION

*Heterodera zeae* differs from the closely related species—*H. glycines, H. schachtii,* and *H. trifolii*—by arrangement of bullae in the vulva cone; there are four finger-like bullae immediately below a short, thin underbridge and many randomly located bullae below these versus no finger-like bullae and many large, randomized bullae below a strongly developed underbridge in the other three species. The second-stage larva of *H. zeae* also differs from those of *H. glycines, H. schachtii* and *H. trifolii* in head shape, having a low, rounded head for *H. zeae* versus a high, less rounded head for the other three species; and a shorter stylet (average length 19.9 μm) for *H. zeae* versus a longer stylet (average 23.0, 25.6, and 27.5 μm) for *H. glycines, H. schachtii,* and *H. trifolii,* respectively. The tail of *H. zeae* second-stage larva is shorter (average length 41.0 μm) than that of *H. schachtii* (av. 48.5 μm), *H. trifolii* (av. 59.9 μm), and races one, three, and four of *H. glycines.* The tail of *H. zeae* is also thinner and more sharply pointed than the other three species.

In the original description, the stylet length of second-stage larvae of *H. zeae* was given as 20–25 μm (23.0 ± 0.26). In our measurements of larval stylets from India and Maryland, we continually found it to average about 20.0 μm (Table 3). Though data is not included, our measurements of specimens from Pakistan were also the same as those of specimens from Maryland and India.

Scanning electron microscope examination of the females, eggs, cysts, and second-stage larvae confirmed observations made with optical microscopy and showed greater detail, especially en face views of the lip pattern of the larvae.

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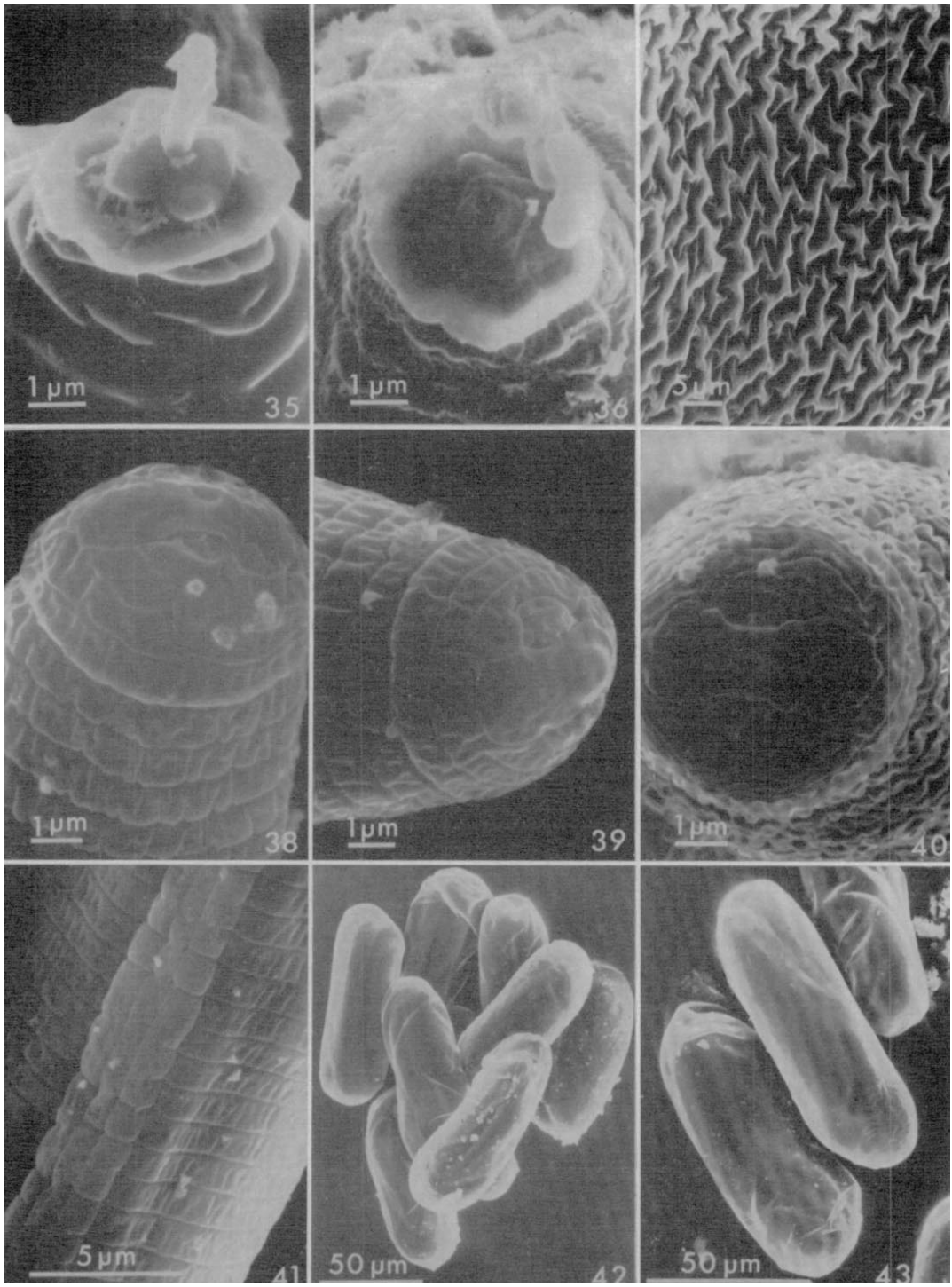
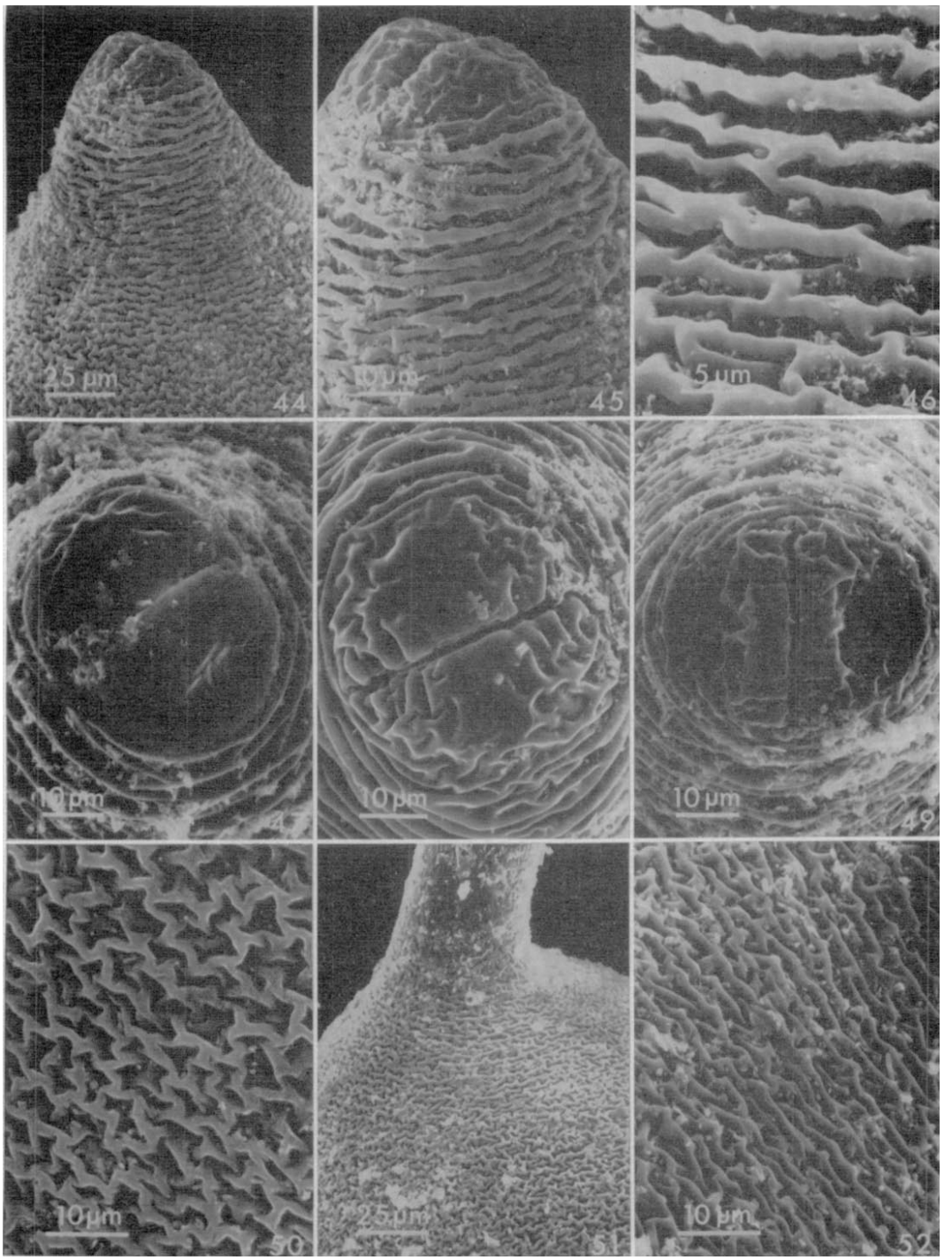
Figs. 26–34. Photomicrographs of *Heterodera zeae* second-stage larvae from Maryland, USA. 26) Esophageal region showing head area, stylet, median bulb, and esophageal gland lobe. 27) Whole larva. 28) Head area showing everted stylet; fixed mount. 29) Head area showing stylet; water mount. 30) Stylet and dorsal esophageal gland duct opening (arrow); fixed mount. 31) Stylet showing dorsal esophageal gland duct opening; water mount. 32) Four lateral lines. 33) Tail; fixed mount. 34) Tail; water mount.




Figs. 35-43. Scanning electron micrographs of female, second-stage larva, and eggs of *Heterodera zeae* from Maryland, USA. 35) Anterior region of female showing small first head annule, second much larger annule, and everted stylet tip. 36) En face view female showing four inner labial sensillae surrounding mouth aperature. 37) External cyst wall pattern at midbody of female. 38) Lateral view of second-stage larva head region showing head annulation, lip pattern, oral, and amphid apertures. 39) Dorso-ventral view of second-stage larva head region. 40) En face view of second-stage larva lip pattern showing oral aperature surrounded by an elongated oral disc, amphid apertures, and lateral lips, 41) Lateral field at midbody of second-stage larva showing narrow inner band and areolated, wide outer bands. 42, 43) Eggs, shells without visible external markings.




Figs. 44–52. Scanning electron micrographs of *Heterodera zeae* cysts from Maryland, USA. 44) Lateral view of vulva cone region showing differences in cuticular pattern on cone and adjacent area. 45, 46) External cuticular ridges around vulva cone showing interlocking branching. 47-49) Vulva slit, fenestra, and concentric rings of cuticular ridges surrounding fenestra. 50) External cuticular pattern at midbody, interlocking ridges to form a zig-zag pattern. 51, 52) External cuticular pattern posterior to level of neck.

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