

Redescription of *Heterodera fici* (Nematoda: Heteroderidae) with SEM Observations¹

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Abstract: *Heterodera fici* is redescribed and illustrated with comparative details and revised measurements and diagnostic characters of the females, males, cysts, and juveniles from Maryland and Pakistan. This species is in the "schachtii group" (cysts lemon shaped, with bullae, and ambifenestrate) but the fenestrae in some cysts, presumably young ones, are small and widely spaced, appearing bifenestrate. It is most closely related to *H. schachtii*, *H. glycines*, and *H. cajani* but differs from these species especially in having cysts with small, scattered bullae and weakly developed underbridge; and males with four small nipples on tail tip. Scanning electron microscopy (SEM) observations of the specimens are also presented. The relationship of this species to closely related forms is discussed.

Key words: distribution, fig cyst nematode, identification, morphology, taxonomy.

The fig cyst nematode, *Heterodera fici*, was first described by Kirjanova (3) in 1954 from the roots of rubber plant (*Ficus elastica* Roxb.) from Harbin, People's Republic of China. Later Mulvey (6) and Mulvey and Golden (7) summarized the known occurrence of this cyst nematode from California, Florida, and Virginia in the United States; Brazil, Australia, Germany, Italy, Poland, South Africa, Spain, Turkey, USSR, and Yugoslavia. During a 1986 survey of a fruit orchard at Saryab, Quetta, Pakistan, *H. fici* was found heavily parasitizing the roots of fig (*Ficus carica* L.) plants, which were showing symptoms of retarded growth and yellowing of leaves (5). Since its description 33 years ago, this cyst nematode has received very little attention from taxonomists, either relative to its morphology or a revised description. Mulvey (6), while providing the basis for identification of *Heterodera* species by terminal and cone top structures, gave only measurements of the fenestra, vulval slit, and underbridge. Similarly Wouts and Weischer (9) proposed a classification, based on the larval characteristics, of 15 species of Heteroderinae commonly occurring in western Europe and included only the mea-

surements of *H. fici* larvae. We present here a redescription of *H. fici*, with SEM observations, and comparative details and measurements of females, males, cysts, and juveniles from the United States and Pakistan. The original measurements by Kirjanova (3) have also been included.

MATERIALS AND METHODS

Specimens used for the description and morphometric data of *H. fici* were obtained from *Ficus elastica* (rubber plant) in a greenhouse at Beltsville, Maryland, and living material attached to roots of *F. carica* (fig) were collected from Quetta, Pakistan. In addition, specimens of *H. fici* from California were provided by J. G. Baldwin, University of California, Riverside. Females were removed from roots, and cysts were sieved from soil; then juveniles were hatched from the cysts kept in water and fixed in 3% formaldehyde solution. Procedures used for preparing and measuring specimens were essentially the same as those used by Golden and Birchfield (1). Measurements of all stages were made with an ocular micrometer, and drawings were prepared with a drawing tube. Photomicrographs of cysts, vulval cones, males, and juveniles were made with an automatic camera attached to a compound microscope using an interference contrast system; those of infected roots, whole females, and cysts were made with a camera attached to a dissecting microscope. For SEM, female cysts, juveniles, and males were collected from a greenhouse culture

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TABLE 1. Measurements (μm) of mature females of *Heterodera fici* (range, mean and standard deviation).

	Kirjanova (3) measurements	Maryland (N = 15)	Pakistan (N = 10)
Body length (excluding neck)	320–770	360–608 (484 \pm 67.80)	335–680 (445 \pm 58.6)
Body width	320–660	232–410 (306 \pm 60.72)	220–490 (394 \pm 59.2)
Length/width ratio		1.0–2.2 (1.6 \pm 0.32)	0.9–2.3 (1.5 \pm 0.21)
Neck length		80–120 (104 \pm 19.9)	72–120 (98.5 \pm 21.6)
Stylet length	9	26–28 (27.2 \pm 0.43)	25–27.5 (27 \pm 0.56)
Stylet base to dorsal esophageal gland duct opening		4.0–5.6 (4.8 \pm 0.79)	4.0–5.6 (4.8 \pm 0.71)
Head tip to median bulb valve		56–68 (66.4 \pm 6.5)	60–69 (65 \pm 4.6)
Head tip to excretory pore		116–135 (122 \pm 3.3)	122–135 (132 \pm 1.8)
Diameter of median bulb		28–32 (30.4 \pm 2.3)	

on *F. elastica* at Beltsville, Maryland. These 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 CO₂, sputter coated with a 20–30-nm layer of gold palladium, and examined. Some females and cysts in glycerine were also examined with SEM.

SYSTEMATICS

Heterodera fici Kirjanova, 1954 (Figs. 1–68)

Females: Measurements in Table 1.

Body pearly white, basically lemon shaped (Fig. 12). Neck elongate, protruding; vulval cone prominent (Fig. 14). Cuticle with zig-zag pattern (Fig. 54). Egg sac present, easily detached from cyst. Head distinctly set off; two prominent annules, posterior one larger than anterior and generally disc shaped (Figs. 18, 52, 53). Stylet

fairly strong, conus 12 μm long; basal knobs well developed, rounded, sloping posteriorly (Fig. 18). Neck long; median bulb large, rounded, valve plates well developed (Figs. 1, 17). Esophageal glands appear as a single lobe, variable in size and shape. Excretory pore distinct, at level of esophageal glands. Ovaries paired and convoluted, fill body cavity. Vulval cone well developed; surface with wavy striae, extending to vulval slit (Figs. 57, 58). Anus prominent, located in small hyaline depression, 72 μm (60–77) from posterior end.

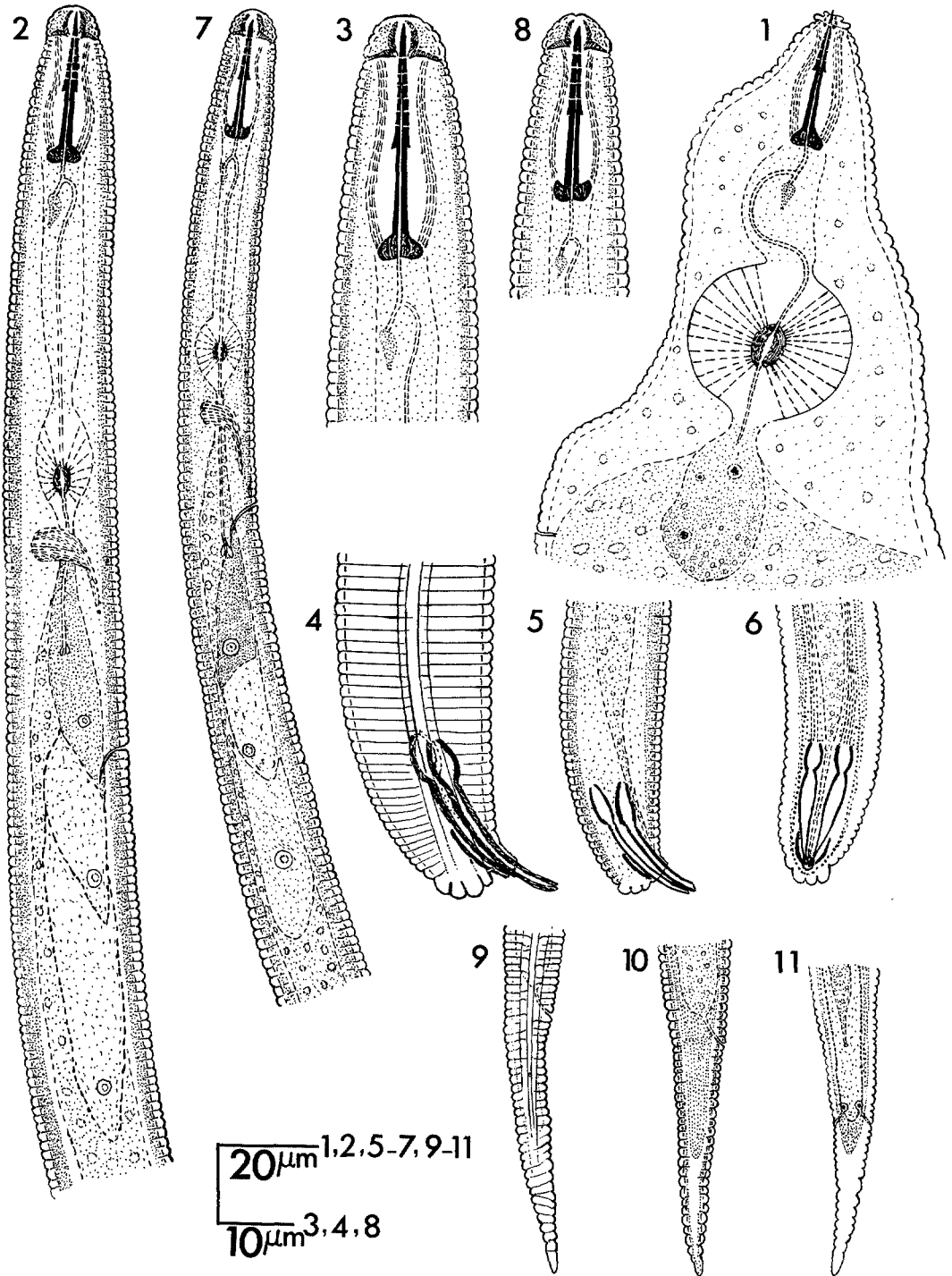
Cysts: Measurements in Table 2.

Body light to dark brown, basically lemon shaped, neck and vulval cone distinct. Neck protruding, curved posteriorly. Cuticle thin, without subcrystalline layer. External wall pattern at mid-body with interlocking ridges, forming a zig-zag pattern (Figs. 61, 62). Terminus of vulval cone with strongly developed zig-zag ridges surrounding vulval slit and fenestra (Figs. 63–

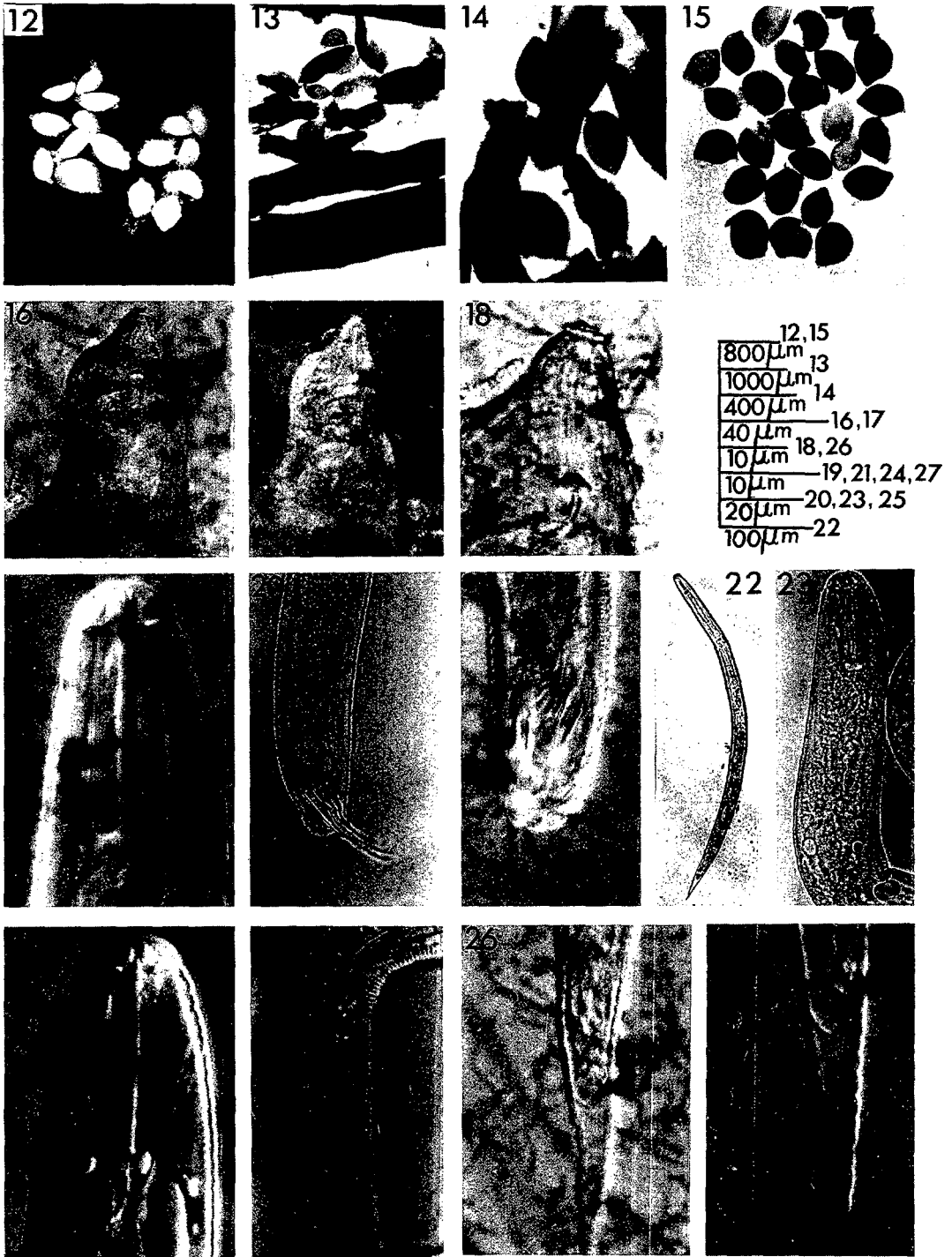
TABLE 2. Measurements (μm) of cysts of *Heterodera fici* (range, mean, and standard deviation).†

	Mulvey (6) (N = 50)	Maryland (N = 30)	Pakistan (N = 30)
Body length (excluding neck)	432–688	(550 \pm 88.58)	400–640 (510 \pm 80.7)
Body width	280–560	(384 \pm 100.29)	272–500 (370 \pm 85.5)
Length/width ratio		1.1–2.0 (1.46 \pm 0.25)	1.0–1.6 (1.36 \pm 0.21)
Fenestral length	45–68	58–64 (62.0 \pm 5.0)	48–62 (58.8 \pm 4.9)
Fenestral width	22–40	30–41 (35 \pm 4.9)	32–40 (36.8 \pm 3.28)
Vulva slit length	35–60	40–56 (47.6 \pm 5.4)	35–48 (43 \pm 3.17)
Basin width		8–12 (10.5 \pm 2.54)	7–12 (10 \pm 1.49)
Underbridge length	53–75	65–81 (75 \pm 5.98)	62–80 (70 \pm 8.59)
Underbridge width	15–20	13–18 (14.2 \pm 1.84)	14–16 (15 \pm 1.79)

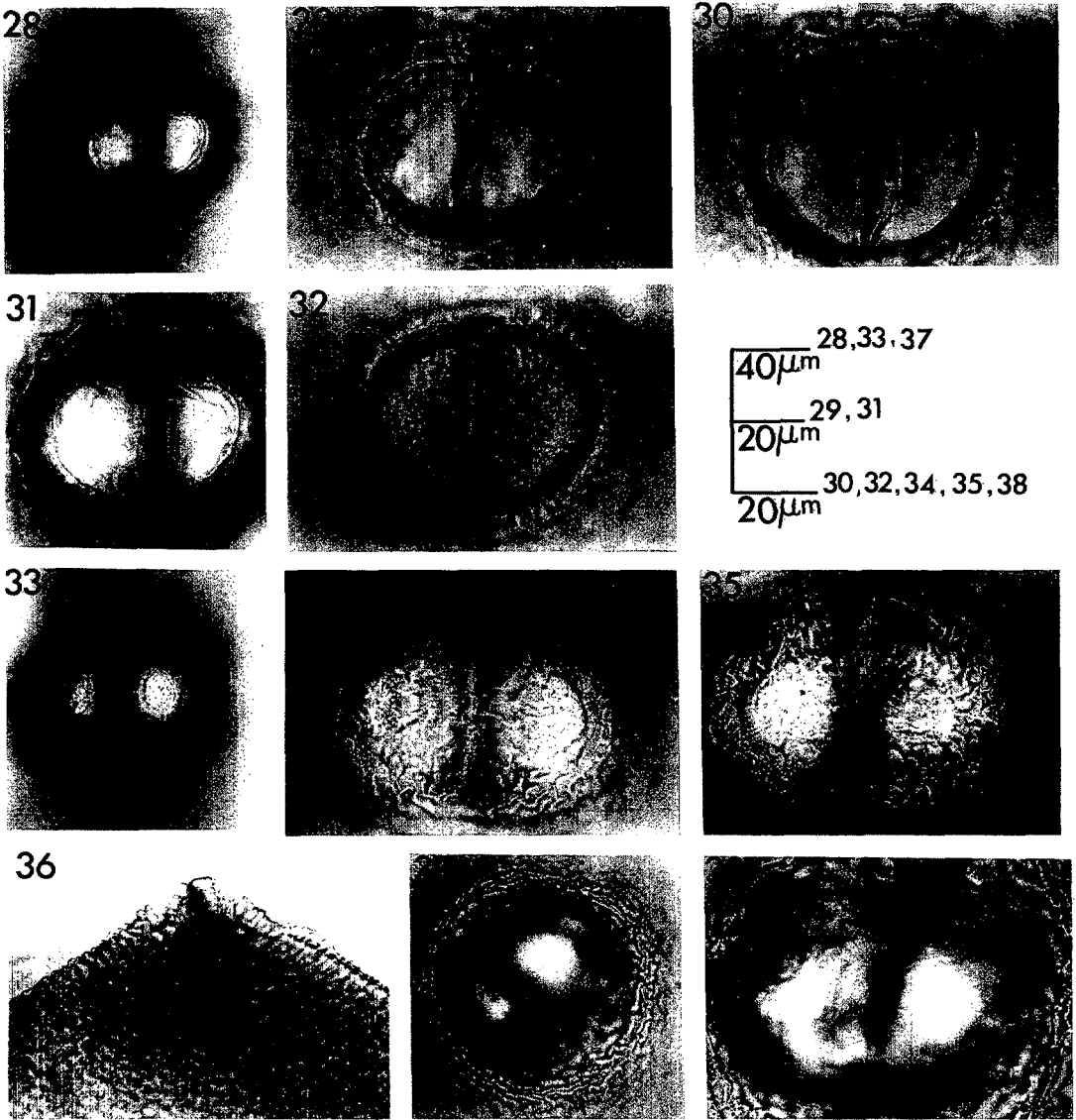
† Measurements not given by Kirjanova (3).



FIGS. 1-11. Drawings of *Heterodera fici* from Pakistan. 1) Female, anterior region. 2-6) Male. 2) Esophageal region. 3) Head. 4-6) Tail. 7-11) Second-stage juveniles. 7) Esophageal region. 8) Head. 9-11) Variations in tail morphology.



FIGS. 12-27. Photomicrographs of *Heterodera fici* from Pakistan. 12) White females. 13, 14) Females attached to roots. 15) Whole cysts. 16-18) Females. 16) Esophageal region showing head, stylet, dorsal esophageal gland duct opening. 17) Valvated median bulb. 18) Enlarged head and stylet. 19-21) Male, anterior and posterior regions. 22-27) Second-stage juveniles. 22) Whole juvenile. 23) Anterior region showing head, stylet, and median bulb. 24) Anterior region showing head, stylet, and dorsal esophageal gland duct opening. 25) Four lateral lines. 26, 27) Tails, anus (26) hyaline portion (27).



FIGS. 28–38. Photomicrographs of *Heterodera fici* cyst vulval cones from Pakistan. 28–31) Fenestrae. 32) Fenestrae, distinct wavy lines extending from the outer edge to vulval slit. 33–35) End-on view with focus at outer cuticular surface, appearing bifenestrate. 36) Lateral view. 37, 38) Bullae small, scattered about level of underbridge.

68). Fenestra ambifenestrate, sometimes top of the cone appears bifenestrate (Figs. 33–35, 64). Semifenestra symmetrical, separated by vulval bridge, surrounded by well-developed basin (Figs. 28–32). Semifenestra with distinct wavy lines, extends from the outer edge to vulval slit; lines commonly persist in older cysts (Fig. 32). Bullae dome shaped, small, scattered around underbridge plane (Figs. 37, 38). Under-

bridge weakly developed, with furcate ends. Vulval slit about same length as bridge. Anus distinct, without surrounding pattern (Figs. 59, 60), located about $72\ \mu\text{m}$ (65–80) from posterior end.

Males: Measurements in Table 3.

Body slender, vermiform, slight ventral curvature. Cuticular annulation prominent, annules $1.5\ \mu\text{m}$ (1.0–2.0) wide at mid-body. Lateral field areolated, four inci-

TABLE 3. Measurements (μm) of males of *Heterodera fici* (range, mean, and standard deviation).

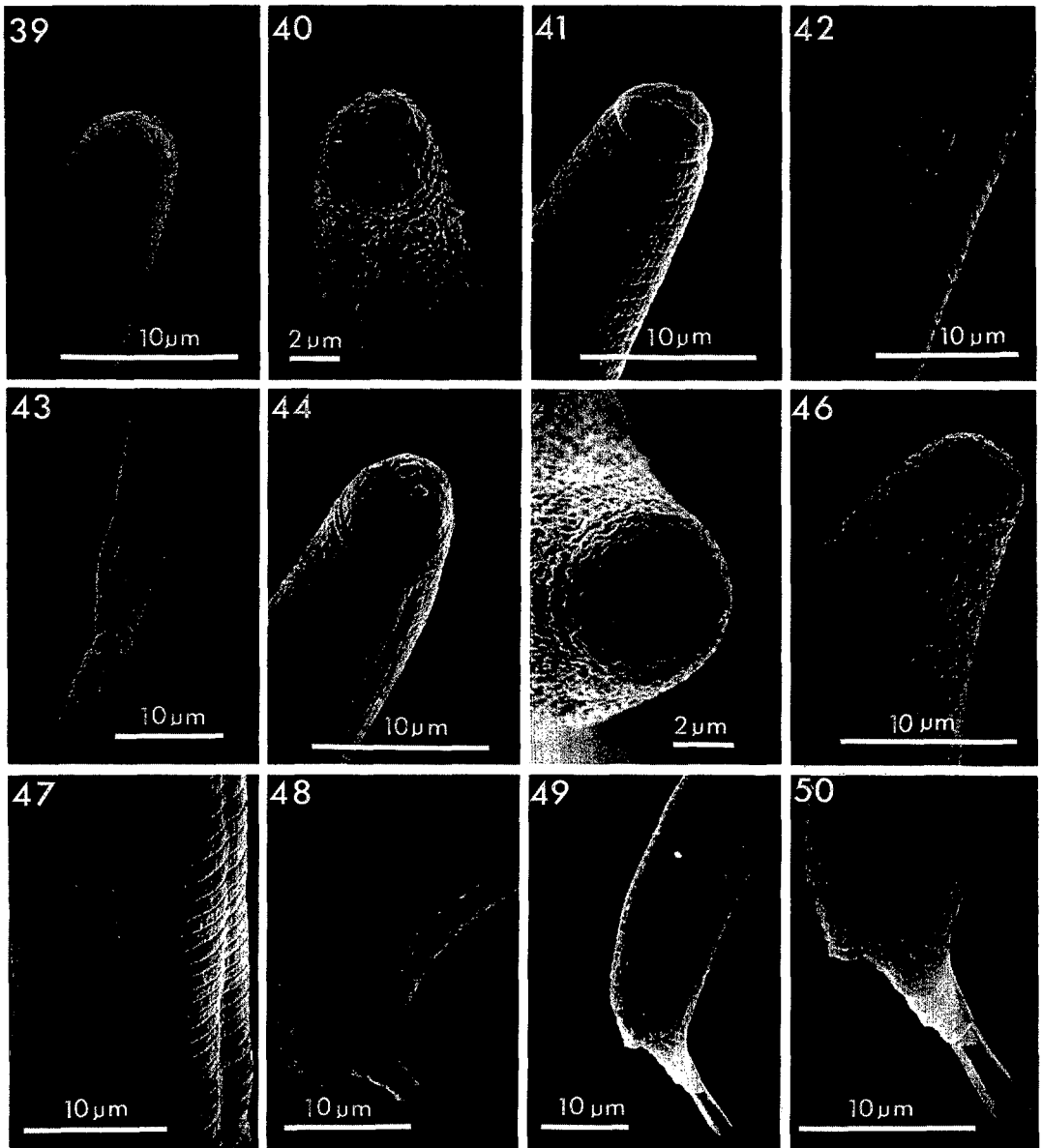
	Kirjanova (3) measurements	Maryland (N = 20)	Pakistan (N = 10)
Body length	865–900	781–1002 (943 \pm 0.08)	760–900 (828 \pm 0.07)
Body width at mid-body	25–32	18–27 (24.1 \pm 1.72)	24–25.6 (24.5 \pm 0.92)
Head height	7–8	4.5–6.5 (5.2 \pm 0.56)	5.6–6.9 (5.8 \pm 0.46)
Head width		7.5–11.5 (8.7 \pm 0.41)	10.4–11.2 (10.9 \pm 0.46)
Head width/height ratio		1.8–2.6 (2.05 \pm 0.28)	1.7–2.0 (1.8 \pm 0.14)
a	29–36	33–42 (38.7 \pm 3.4)	31–40 (35.7 \pm 4.78)
b	4.5	3.9–5.1 (4.19 \pm 0.34)	2.7–4.8 (3.6 \pm 1.06)
c	144–150	112–151 (118.9 \pm 12.42)	105–136 (108 \pm 16.0)
Stylet length	30	27.2–32.0 (28.7 \pm 2.68)	26.4–30.0 (27.6 \pm 1.03)
Base of stylet to dorsal esophageal gland duct opening		4.0–6.4 (5.0 \pm 0.78)	4.0–5.8 (4.5 \pm 0.46)
Head tip to median bulb valve		79–97.6 (89.9 \pm 6.25)	83–108 (97.2 \pm 13.1)
Head tip to base of esophageal gland lobe		183–256 (224 \pm 25.8)	204–272 (242 \pm 34.4)
Tail length	6	7.2–8.0 (7.8 \pm 0.33)	6.0–8.0 (7.6 \pm 0.46)
Spicule length	30–35	27.2–32.0 (28.8 \pm 1.72)	27–31 (28.5 \pm 2.34)
Gubernaculum		6.4–8.8 (7.6 \pm 0.86)	8.0–8.8 (8.5 \pm 0.46)

tures, about $\frac{1}{5}$ body width (Fig. 47). Head slightly set off, hemispherical, with three or four annules (Figs. 2, 3). Cephalic framework heavily sclerotized. Stylet very strong, basal knobs rounded. Median bulb oval, 21 μm (20–22) \times 11 μm (10–12), valvular apparatus moderately developed. Excretory

pore 148 μm (132–150) from anterior end. Hemizonid prominent, three or four annules long, three annules anterior to excretory pore. Testis single, sometimes reflexed, occupying 40–55% body length. Tail short, obtusely rounded, four prominent nipples on tail tip (Fig. 4). Spicules

TABLE 4. Measurements (μm) of second-stage juveniles of *Heterodera fici* (range, mean, and standard deviation).

	Kirjanova (3) measurements	Wouts & Weischer (9) (N = 10)	Maryland (N = 50)	Pakistan (N = 50)
Body length	320–460	(402 \pm 5.5)	386–421 (402 \pm 0.47)	372–405 (389 \pm 11.14)
Body width at mid-body	16	(15.8 \pm 0.28)	14–20 (18 \pm 1.26)	17–22 (20.6 \pm 1.02)
Head height	6	(4.13 \pm 0.11)	3–5.6 (4.4 \pm 0.55)	4–4.8 (4.5 \pm 0.41)
Head width		(8.75 \pm 0.08)	6–9 (7.5 \pm 0.38)	7.2–9.0 (8.5 \pm 1.002)
Head width/height ratio			1.7–2.4 (2.1 \pm 0.28)	2.0–2.4 (2.1 \pm 0.19)
a	22		20.1–24.2 (22.4 \pm 1.26)	17–21 (19.1 \pm 1.23)
b			2.1–3.1 (2.5 \pm 0.33)	2.5–3.1 (2.7 \pm 0.21)
c			8–10 (8.5 \pm 0.61)	7–9 (8.0 \pm 0.68)
Stylet length	25–26	(23.2 \pm 0.2)	22.4–23.4 (22.6 \pm 0.38)	21–23 (22 \pm 0.59)
Base of stylet to dorsal esophageal gland duct opening			4–5.6 (4.5 \pm 0.65)	4–5.6 (4.6 \pm 0.66)
Head tip to median bulb valve		(68.9 \pm 1.16)	60–82 (75 \pm 7.47)	57–75 (62.3 \pm 3.0)
Head tip to base of esophageal gland lobe			116–198 (152.7 \pm 38.08)	126–152 (138.5 \pm 9.97)
Tail length		(51 \pm 1.36)	40–52 (47.2 \pm 2.30)	40.8–53.6 (48.3 \pm 4.33)
Hyaline tail terminal		(26.5 \pm 0.87)	20–28 (22.2 \pm 2.55)	17.6–25.6 (22.1 \pm 3.22)
Caudal ratio A			2.5–3.8 (2.9 \pm 0.36)	2.4–3.2 (2.8 \pm 0.29)
Caudal ratio B			8.3–15.0 (11.2 \pm 2.33)	10.0–16 (12.5 \pm 2.33)
Lateral lines		4	4	4



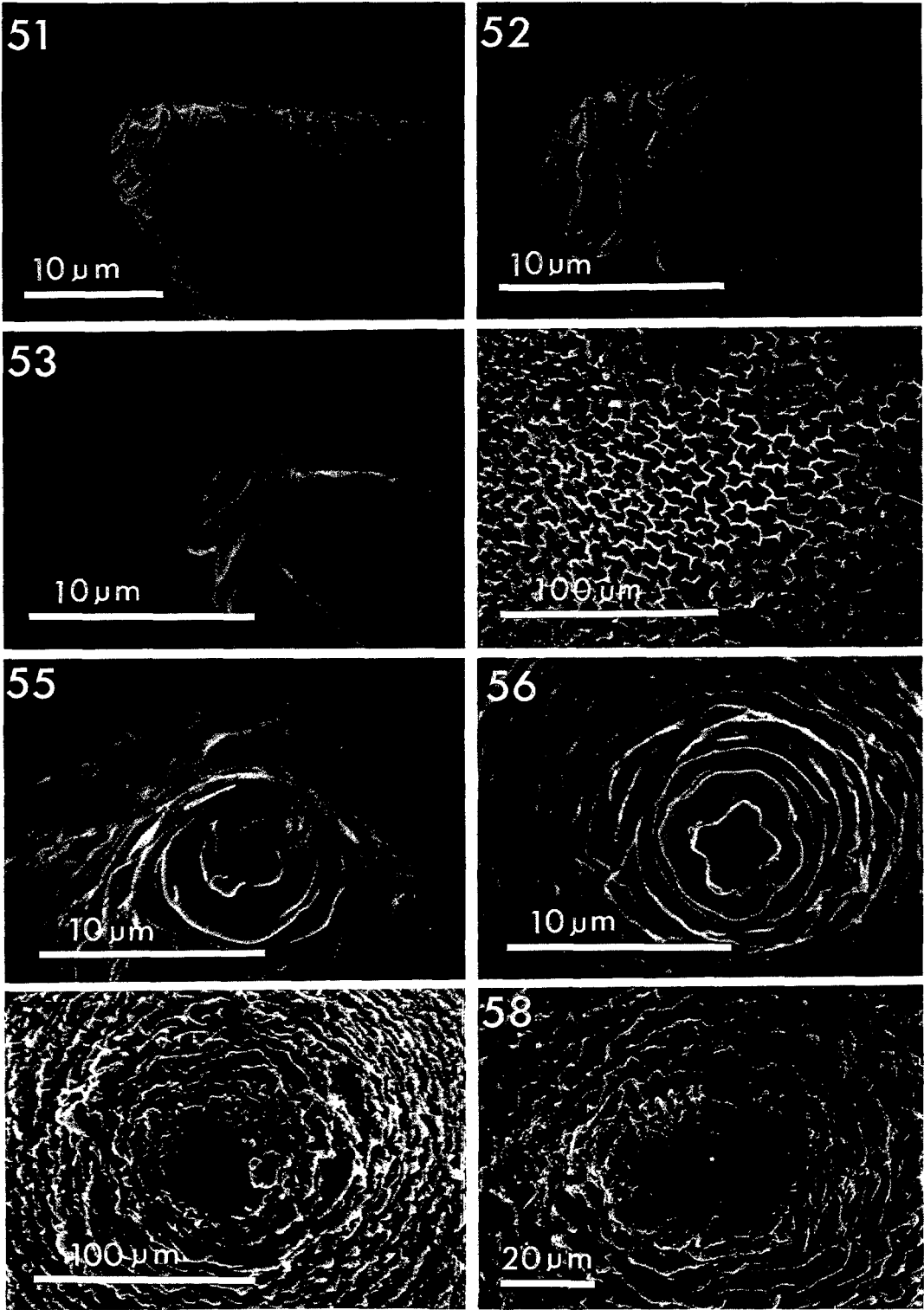
FIGS. 39–50. Scanning electron micrographs of second-stage juveniles (J2) and males of *Heterodera fici* from Maryland. 39–43) J2. 39) Lateral view of head. 40) En face. 41) Medio-lateral. 42) Lateral field at mid-body, areolated, narrow inner band, wide outer bands. 43) Tail region. 44–50) Male. 44) Lateral. 45) En face. 46) Slight oblique view, anterior end of lateral field. 47) Lateral field at mid-body, areolated outer bands. 48) Tail, lateral view. 49, 50) Tail region showing protruded spicules.

arcuate, tapering distally. Gubernaculum slightly curved ventrally.

Second-stage juveniles: Measurements in Table 4.

Body vermiform; tapering at both extremities, more so posteriorly. Cuticular annulation prominent, $1.5\ \mu\text{m}$ (1.0–1.6)

wide at mid-body. Lateral field with four incisures, areolated (Figs. 25, 42). Head slightly set off, rounded, three or four annules (Fig. 8). Cephalic framework moderate. Stylet well developed, basal knobs rounded, directed slightly anteriorly (Figs. 23, 24). Median bulb ovoid, $13.6\ \mu\text{m}$ (13–



FIGS. 51-58. Scanning electron micrographs of females of *Heterodera fici* from Maryland. 51, 52) Head region lateral view. 53) Anterior region, anterior smaller head annule, posterior annule much larger, everted stylet tip. 54) External cyst wall pattern at mid-body. 55, 56) En face views. 57, 58) White female vulval cone, in early stage of fenestration.

TABLE 5. Measurements (μm) of eggs of *Heterodera fici* (range, mean, and standard deviation).

	Kirjanova (3) measurements	Maryland (N = 30)	Pakistan (N = 30)
Length	93–104	86–106 (94 \pm 5.25)	78–92 (84 \pm 5.47)
Width	38–58	38–44 (42 \pm 1.83)	38–42 (40 \pm 1.0)
L/W ratio	2	2.04–2.6 (2.34 \pm 0.12)	1.95–2.31 (2.1 \pm 0.88)

14) \times 9.6 μm (9–10), valvular apparatus well developed. Esophageal lobe usually distinct, three large nuclei, overlaps anterior part of intestine. Excretory pore 92 μm (88–102) from anterior end. Hemizonid two annules long, just anterior to excretory pore. Genital primordium posterior to mid-body, 228 μm (220–230) from anterior end; usually two celled. Tail long, tapering, terminus rounded (Figs. 9–11, 27). Anus distinct (Figs. 10, 26). Phasmid small but distinct, 13 μm (11–15) posterior to anus and anterior to middle of tail. Hyaline terminal about $\frac{1}{2}$ tail length.

Eggs: Measurements in Table 5.

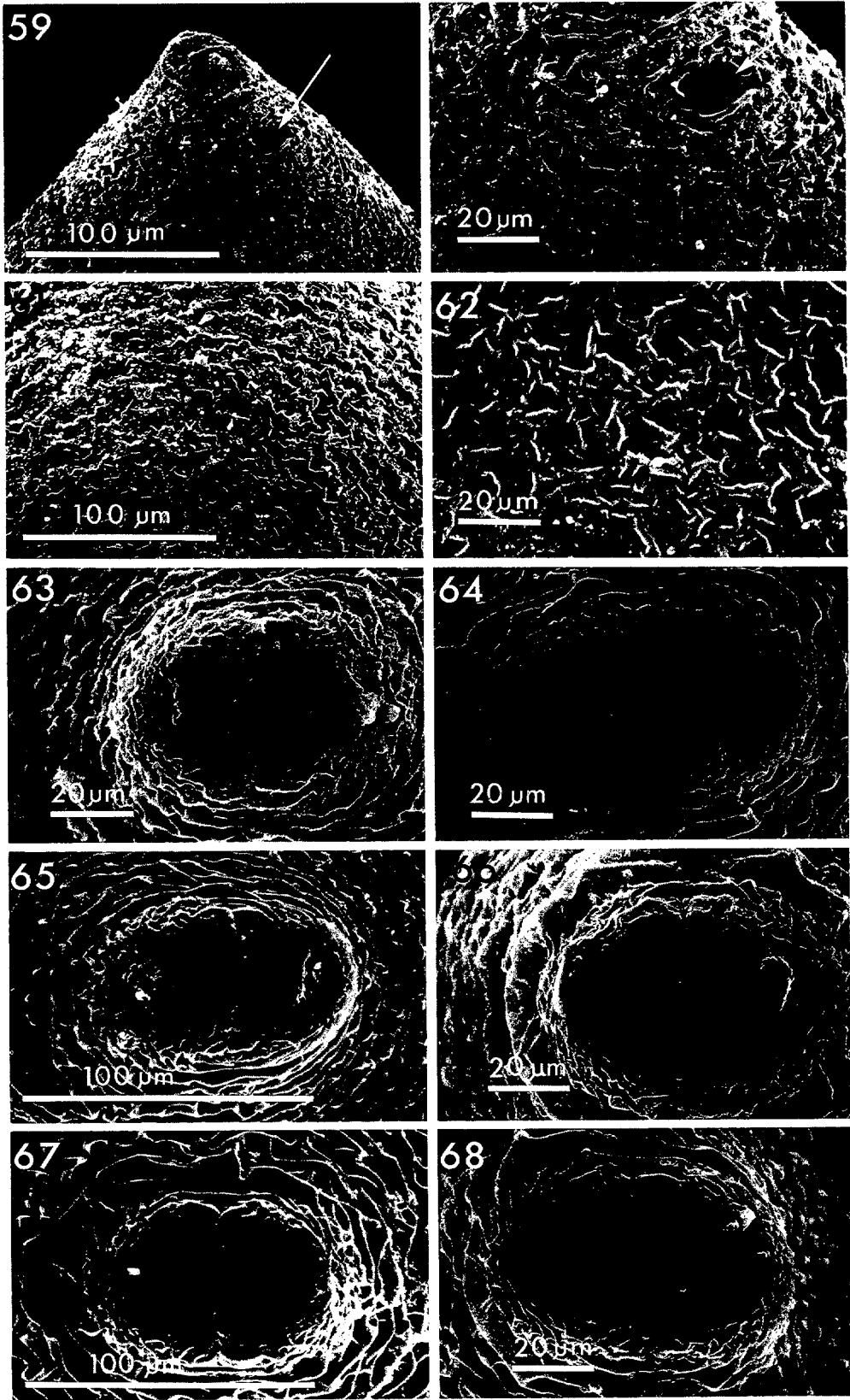
Shells hyaline, without surface markings. Juveniles form five folds within egg.

Diagnosis: *Heterodera fici* is most closely related to *H. schachtii* Schmidt, 1871, *H. glycines* Ichinohe, 1952, and *H. cajani*, 1967 on the basis of bullae randomly located at one level; second-stage juveniles with four distinct lateral incisures; and mean stylet length ranges of 23–26 μm (2,4,8). It differs from these species by the presence of a weakly developed underbridge and small, scattered bullae (vs. well-developed underbridge and prominent bullae). It can be further separated from *H. schachtii* by the absence of the typically molar shape bullae and larger fenestra length. Males of *H. fici* differ from these three species by having an obtusely rounded tail with four small nipples at the tip, from *H. schachtii* and *H. glycines* by a shorter body length, and from *H. cajani* by having posteriorly sloping stylet knobs. In *H. schachtii*, *H. glycines*, and *H. cajani* the male tail tip is without nipples; body length in *H. schachtii* = 1,120–1,440 μm and *H. glycines* = 1,200–1,400 μm ; and in *H. cajani* the stylet knobs are anteriorly directed.

DISCUSSION

In 1972 Golden and Birchfield (1) placed *Heterodera fici* under “goettingiana group.” In the same year Mulvey (6) categorized this species under his group 4 of *Heterodera*. (Cysts with posterior protuberance, lemon shaped to spherical, vulval slit long [$>$ 35 μm], underbridge and bullae generally strongly developed, ambifenestrate.) Later Mulvey and Golden (7) in their classification transferred this species to the “schachtii group” (females and cysts with posterior protuberance, basically lemon shaped, vulval slit long [$>$ 30 μm], bullae present, underbridge generally strongly developed, ambifenestrate; male present or absent). We agree that *H. fici* properly belongs in the “schachtii group” of species.

In the original description of *H. fici* by Kirjanova (3), stylet length of the female was 9 μm , whereas the female stylets from Pakistan and Maryland ranged from 25 to 28 μm (Table 1). Stylet length of the second-stage juvenile is 25–26 μm , compared with 21–23 μm from Pakistan, Maryland, and England (Table 4). In Kirjanova’s original description egg width was 38–58 μm , but specimens from Pakistan and Maryland ranged from about 38 to 44 μm (Table 5). It is noteworthy that Kirjanova (3) illustrated and named the four nipples (direct translation from Russian) on the tail tip of *H. fici* males. These structures appear to be associated with termination of the lateral field on the tail tip, but the exact nature and origin of these nipples are not known at this time. They are of value, however, in distinguishing males of this species. In the present study, the fenestrae in the vulval cone of some cysts were small and widely spaced, giving the appearance of



being bifenestrate. Presumably these were young cysts. This bifenestrate appearance could be misleading unless many vulval cones are examined to reveal the true ambifenestrate vulval cone of this species.

Scanning electron microscope examination of the females, cysts, second-stage juveniles, and males confirmed the observations made with light microscopy but showed greater detail of various structures.

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FIGS. 59-68. Scanning electron micrographs of *Heterodera fici* cysts from Maryland. 59) Lateral view of vulval cone region, cuticular pattern on cone and anal area. 60) Anal area of cyst cone, cuticular pattern. 61, 62) External cuticular pattern at mid-body. 63-65) Vulval slit, fenestra, and cuticular pattern, fenestrae appearing bifenestrate. 66-68) Vulval slit, fenestrae, and cuticular zig-zag ridges around fenestra.