Heterodera canadensis n. sp. (Nematoda: Heteroderidae) from Spike-Rush (*Eleocharis acicularis* (L.) R. & S.) in Quebec, Canada

ROLAND H. MULVEY

Abstract: Heterodera canadensis n.sp. is described and illustrated from the roots of spike-rush, *Eleocharis acicularis* (L.) R. & S., in Deschenes, Quebec. This new abullate species is related to *Heterodera graminophila* Golden and Birchfield, 1972, but differs significantly in cyst shape, cone top structures, body length of the second-stage larva (520–600 μm, vs. 380–400 for *H. graminophila*) and tail length (110–120 μm, vs. 57–67 for *H. graminophila*). A taxonomic key based on cyst and second-stage larva characters is provided for identification of the fifteen species in the *Heterodera goettingiana* group. *Key Words:* Nematode taxonomy, morphology.

In August 1978 an undescribed species of a cyst-forming nematode (genus *Heterodera*) and a new root-knot nematode (genus *Meloidogyne*) were found attacking the roots of a spike-rush, *Eleocharis acicularis* (L.) R. & S., growing on the north shore of the Ottawa river on the outskirts of Deschenes, a small village in Quebec opposite the west end of the city of Ottawa, Ontario, Canada. This paper describes the new species of *Heterodera*. The new *Meloidogyne* species will be described at a later date.

MATERIALS AND METHODS

Specimens used herein were obtained from the roots of spike-rush collected from the type locality at Deschenes, Quebec. When the collection was made the spike-rush was growing in shallow soil on rocks about 10 feet from the water's edge whereas later, in October, this area was completely covered with water from the rising river. Spike-rush is found in all provinces of Canada and inhabits low ground and damp shores. The procedures used for fixing, preserving, measuring, and photographing specimens were the same as given by Mulvey (4). White females and second-stage larvae were prepared for micrography with the scanning electron microscope (SEM) as follows: Mature white females (mechanically cleaned in water with a very fine brush) and larvae were fixed in 3% paraformaldehyde solution for 24 h, transferred to glycerolethanol solution No. 1 for 58 h and to solution No. 2 (as described by Seinhorst [6]) for 24 h at room temperature. The females were subsequently washed in 100% alcohol (7 changes) at 10-min intervals and then placed on a stub covered with celloglue (cellulose tape dissolved in chloroform) and gold-coated in a vacuum. The second-stage larvae were washed in 100% alcohol (5–7 changes) at 5-min intervals and then transferred to epoxy resin (Ladd ultra-low-viscosity epoxy resin, which is miscible with alcohol) left for 4 h, with one change of epoxy, in a refrigerator at 4 C, and then changed again and left in the refrigerator for 16 h. The epoxy was again changed, and the larvae were left at room temperature for 2 h and placed in an oven at 58–60 C for 2 h, after which they were washed in 100% alcohol, mounted on a stub covered with celloglue, left for 16 h in an oven at 58–60 C, and then gold coated in a vacuum. A Cambridge SEM 20KV was used to photograph the specimens.

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Biosystematics Research Institute, Central Experimental Farm, Ottawa, Ontario, Canada K1A 0C6.

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Heterodera canadensis n.sp.
(Figs. 1–3; 4C, F,I)

Females (n=20). Length (L) (excluding neck) = 508 (480–520) μm; width (W) = 480 (430–510) μm; L/W ratio = 1.05 (1.01–1.17); neck length = 110–160 μm; stylet length = 24–25 μm.

Female (holotype). Length (excluding neck) = 505 μm; width = 480 μm; L/W ratio = 1.05; neck length = 150 μm.

Description of female. Body white, basically spherical (Fig. 1F) with long pro-

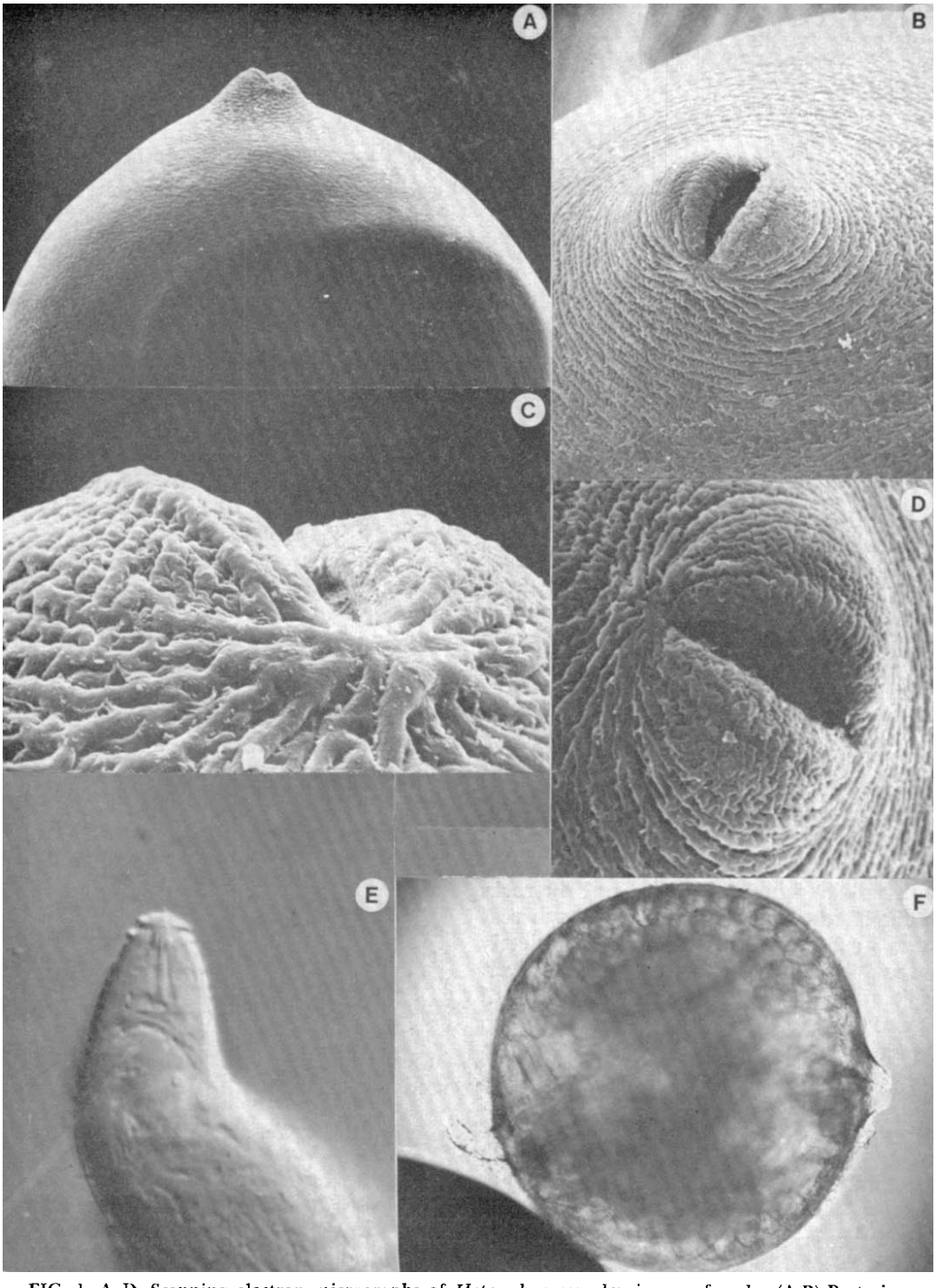


FIG. 1. A–D. Scanning electron micrographs of *Heterodera canadensis* n.sp., female. (A,B) Posterior end of female showing short vulva cone. (C,D) Enlarged micrograph of vulva cone showing heavy striae at vulval slit and wavy striae extending from outer edge of fenestra to vulva slit. E–F. Photomicrographs of *Heterodera canadensis* n.sp. female. (E) Head end showing head annule and apex of stylet. (F) Spherical-shaped female with long neck and short posterior protuberance (vulval cone).

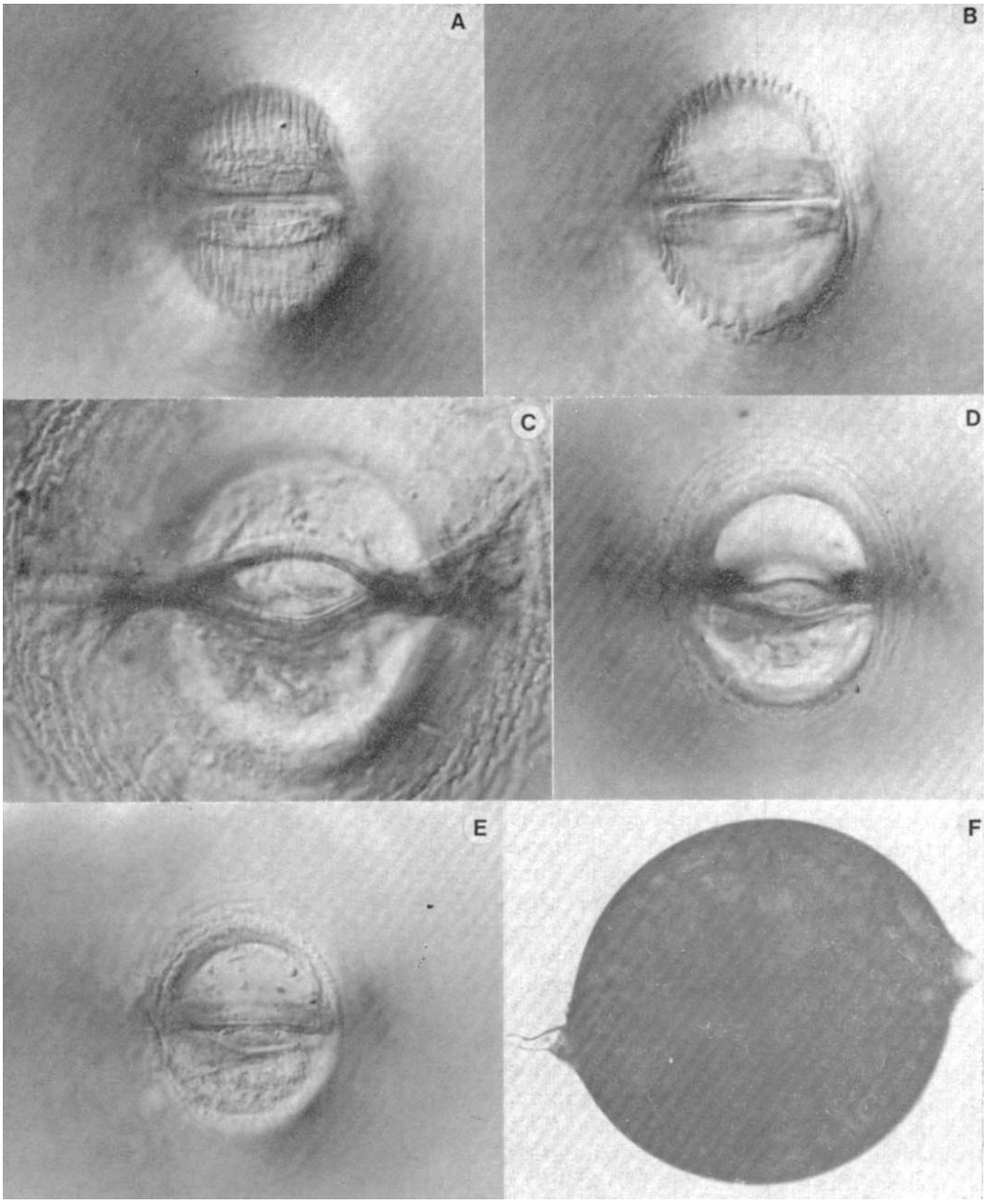


FIG. 2. Photomicrographs of *Heterodera canadensis* n.sp., of female and cyst. (A) Female, surface area above level of fenestra showing wavy striae extending from edge of fenestra to vulva slit. (B) Female, fenestral area slightly below that of figure A. (C) Cyst, underbridge with furcations at each end. (D) Cyst, fenestra and wide vulva bridge. (E) Cyst, surface area showing vulva slit and fenestra, the wavy striae have deteriorated. (F) Cyst, spherical, with long neck and short posterior protuberance.

truding neck and short posterior protuberance (Figs. 1A, F) bearing the vulva. Cuticle with zigzag pattern (Fig. 1C) strongly developed at both ends of the vulva. Head set off from neck and bearing two annules (Fig. 1E), the anterior one much larger. Stylet with fairly large rounded knobs. Medium bulb with large, well developed valve. Reproductive system typical of *Heterodera*. Vulva prominent (Figs. 1D, 2B), surface area with wavy striae (Figs. 1C, D; 2A) extending to vulva slit which averages 47 (40–50) μm. Anus small, inconspicuous, 50–60 μm from vulva.

Cyst (N=20). Length (excluding neck) = 580 (490–615) μm; width = 543 (450–610) μm; L/W ratio = 1.06 (1.00–1.19). Cysts light to medium brown, basically spherical (Fig. 2F) with relatively long neck, mostly terminal but sometimes sharply offset. Cyst wall with zigzag pattern. Vulva cone (Fig. 2F) distinct and protruding posteriorly, ambifenestrate averaging 44 (41–48) μm in fenestral length, vulva bridge usually strongly developed. Semifenestra (Fig. 2D), which commonly persist even in older cysts, have distinct wavy striae extending from the outer edge to the vulva slit. Fenestral length 51 (47–55) μm, width 45 (42–50) μm. Underbridge length 88 (80–95) μm (Fig. 2C), width 18–20 μm. Underbridge ends furcated. Bulla absent. Anus small, inconspicuous.

Male. Unknown

Second-stage larvae (n=20, in lactophenol). Length = 558 (520–600) μm; width = 19–21 μm; a = 27.5 (26–30); b = 2.3 (2.2–2.4); c = 4.9 (4.7–5.2); tail length = 115 μm (110–120) hyaline tail terminal = 60 (55–65) μm; stylet length = 24 (23–25) μm; stylet knob width = 4–5 μm; outlet of dorsal esophageal gland from base of stylet = 8 (7–9) μm.

Cuticle annulated, lateral field without areolation and composed of three lateral lines (Fig. 3C). Head slightly set off from body, dome shaped (Fig. 3B), possessing 2–3 annules (Figs. 3A, G), cephalic framework heavily sclerotized, amphids small (Fig. 3E), oral disc not clearly demarcated. Stylet robust with well developed rounded knobs (Fig. 1A). Anterior cephalids 3 annules behind head constriction, posterior cephalids at about the eighth annule. Median bulb with prominent valve (Fig. 3D) located 80 (77–82) μm from anterior end of body. Esophagus (Fig. 3D) 250–275 μm long, nearly half body length. Excretory pore posterior to median bulb and hemizonid slightly anterior to pore. Tail very long (Fig. 3F), tapering to acutely pointed terminus. Phasmids small, located posterior to anus about one-third tail length.

Eggs (N=25). Length = 118 μm (110–125); width = 45 μm (42–50); egg shell hyaline, without any visible markings as seen by optical microscope.

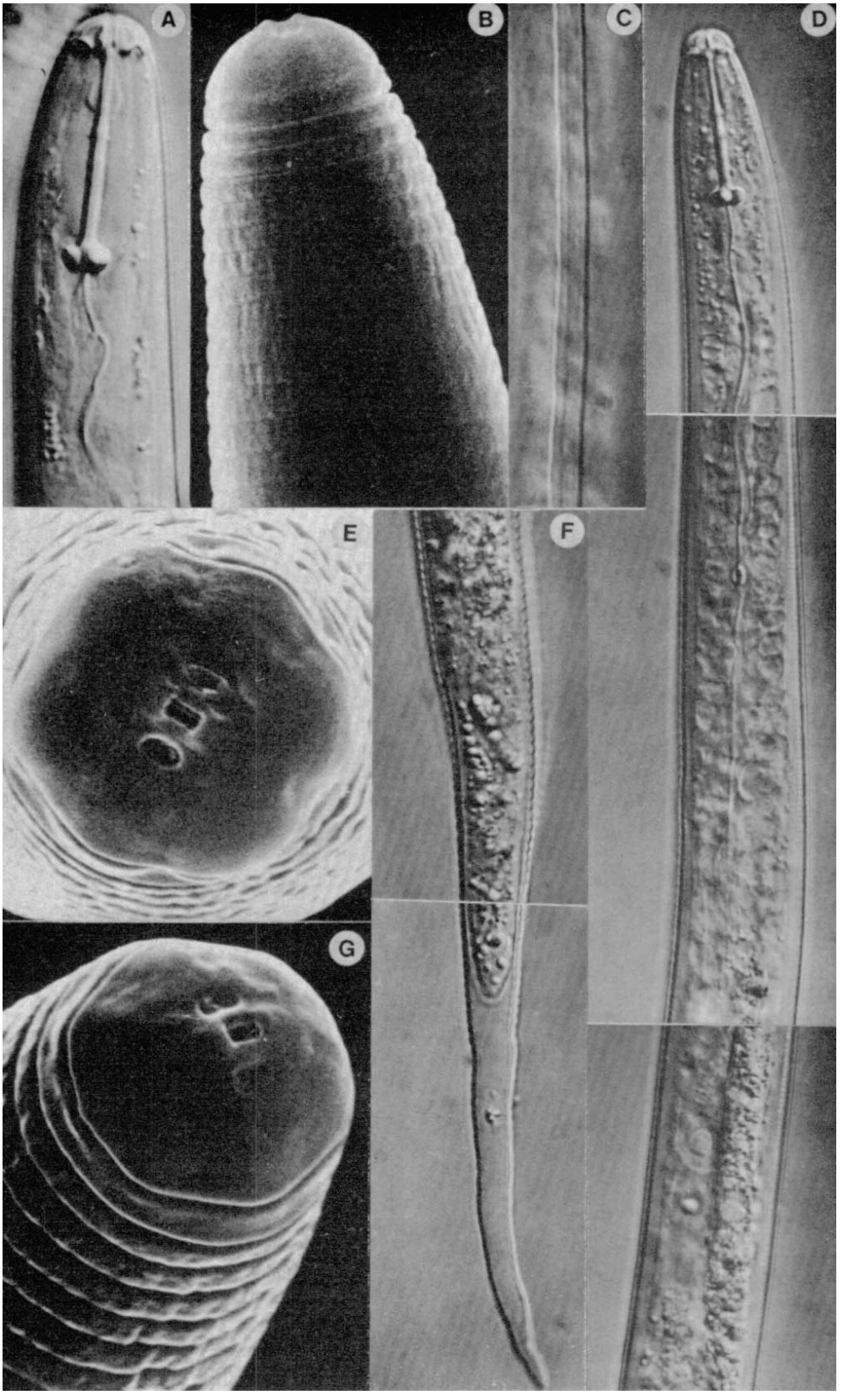
Holotype (female). Collected 21 August 1978 by Mr. R. Sewell, Biosystematics Research Institute, Ottawa. CNC of Nematodes Collection No. 7328, Type slide No. 246.

Paratypes. Females, cysts, second-stage larvae, and cone tops deposited in CNC of Nematodes, Ottawa, Canada; United States Department of Agriculture Nematode Collection, Beltsville, Maryland; Nematology Department, Rothamsted Experimental Station, England.

Type host and locality. Roots of spikerush (*Eleocharis acicularis* (L.) R. & S.) growing on the north bank of the Ottawa river on the outskirts of the town of Deschenes, Province of Quebec.

Diagnosis. Heterodera canadensis n.sp. fits the *goettingiana* group and is related most closely to *H. graminophila* Golden & Birchfield, 1972, and *H. graminis* Stynes, 1971 (see Table 1). It differs from both those species in having females and cysts basically spherical versus basically lemon-shaped for *H. graminophila* and *H. graminis* and in larval length (see Table 1). *H. canadensis* differs from *H. graminophila* in having the wavy striae extending to the vulva slit (Fig. 4C) less strongly developed than that of *H. graminophila* (Fig. 4B, also Mulvey (5), Figs. 1 & 2). *H. graminis* (Fig. 4A) has less strongly developed wavy striae than *H. canadensis* (Fig. 4C). The second-stage larvae of *H. canadensis* are much longer in body length than those of *H. graminophila* (520–600 vs. 380–460 μm) and in tail length (110–120 vs. 57–67 μm for *H. graminophila*). Scanning *en face* views of *H. canadensis* second-stage larva head (Figs. 3E, G) show an oral disc poorly demarcated compared with that illustrated by Stone (7;

FIG. 3. Photomicrographs and scanning electron micrographs of *Heterodera canadensis* n.sp., second-stage larva. (A) Head area, robust stylet, large basal knobs and dorsal esophageal gland duct opening about 6 μm posterior to base of stylet knobs. (B) Scanning electron micrograph of second-stage larva head area showing helmet-like shape. (C) Three lateral lines in lateral field. (D) Esophageal region. (E) Scanning electron micrograph of *en face* view of head, showing mouth, amphids on either side of mouth. and characteristic head pattern. (F) Tail, anal area, and hyaline terminal area. (G) Scanning electron micrograph, side view of head area, showing annulation.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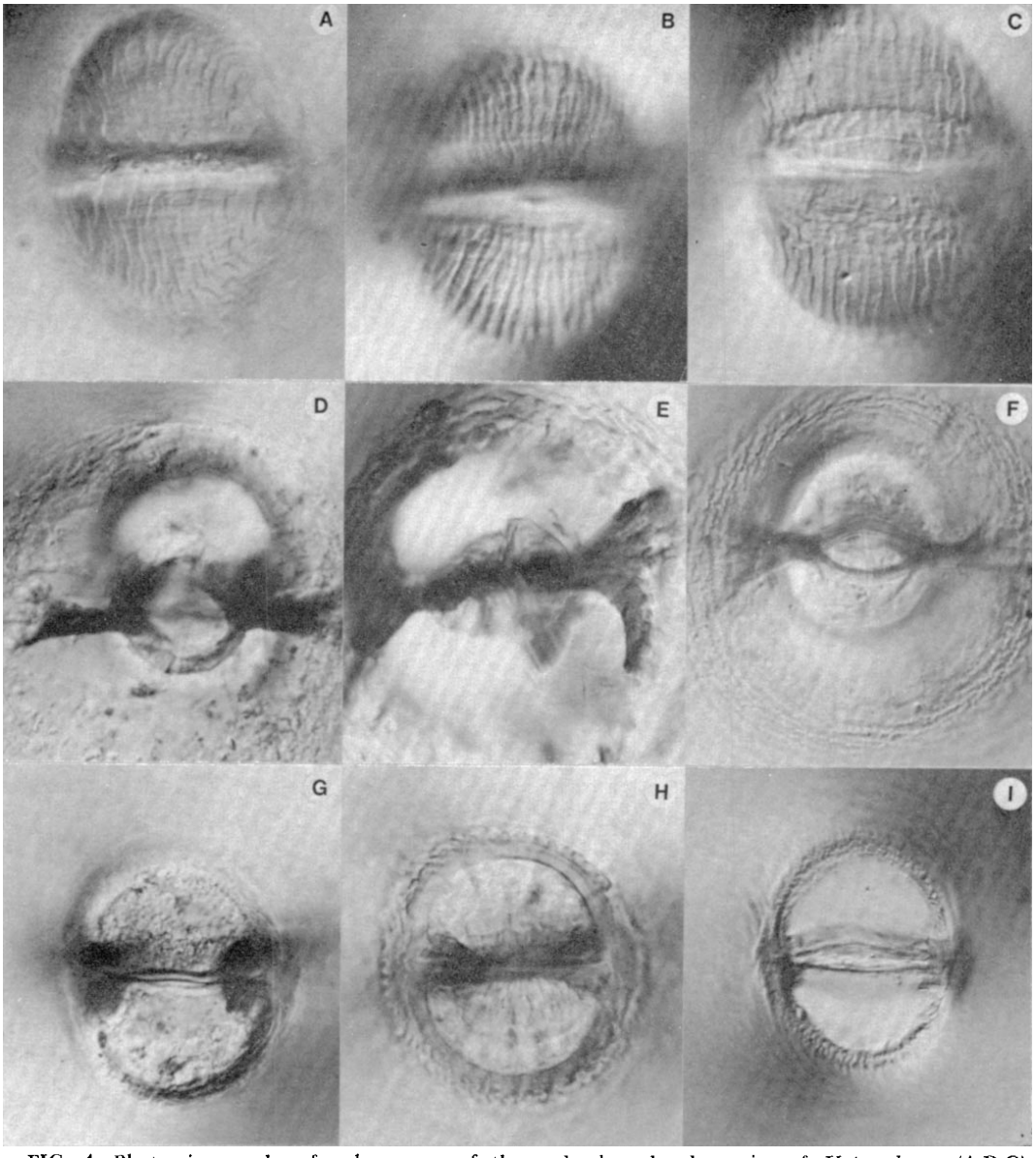

FIG. 4. Photomicrographs of vulva cones of three closely related species of *Heterodera*. (A,D,G) *Heterodera graminis,* respectively surface area above level of female fenestra, underbridge of cyst and fenestra of cyst. (B,E,H) *Heterodera graminophila,* respectively surface area above level of female fenestra, underbridge of cyst, and fenestra of cyst. (C,F,I) *Heterodera canadensis* n.sp., respectively surface area above level of female fenestra, underbridge of cyst and fenestral area of cyst (Figs. A–C same magnification; Figs. D–I same magnification).

Figs. 7A,B) for *H. graminophila* and *H. graminis,* which are well developed.

DISCUSSION

H. canadensis females and cysts were found mostly near the crown of the host plant, although some were observed farther down on the roots. None of the females examined had an egg sac. Golden et al. (1) discussed the nature of the furcations of the underbridge in *H. graminophila* and their similarity to bulla. These furcations, which are present in the underbridge of *H. canadensis* (Fig. 4F) and *H. graminis* (Fig. 4D), are much less strongly developed than those of *H. graminophila* (Fig. 4E).

Krall (3) reviewed the status of cyst-forming nematodes described from the

TABLE 1. Measurements of *Heterodera* spp. of the *H. goettingiana* group.

Species	Cyst				Larva			
	Shape	Fenestra (vulva slit) (μm)	Underbridge (L/W) (μm)	Bulla	Length (μm) (average)	Stylet length (μm)	Tail length (μm)	Lateral lines
amygdali	Lemon	37-54 x 32-48 (41-64)	none	yes	405-540 (500)	24-27	52-64	4
cardiolata	Lemon	51-60 x 50-39 (40)	short, thin	none	320-370 (340)	18-20	20-21	4
canadensis n. sp.	Spherical	47-55 x 42-50 (41-48)	80-95 x 18-20	none	520-600 (558)	23-25	110-120	3
carotae	Spherical	34-40 x 38-40 (45-50)	none	none	420-454	23-24	48-53	4
cruciferae	Spherical	20-38 x 30-50 (45-55)	90-110 x 12-33	none	415	22-24	45-48	4
cyperi	Lemon	27-35 x 20-28 (30-35)	40-60 x 10-12	none	414-565	19-21	56-63	3
goettingiana	Lemon	40-50 x 30-45 (43-50)	90-100 x 10-18	few	450-580	24-25	56-60	4
graminis	Lemon-spherical	45-60 x 25-45 (40-48)	70-100 x 10-35	none	343-444	19-24	44-65	3
graminophila	Lemon	52-58 x 43-50 (40-52)	115-140 x 15-20	none	380-460	22-24	57-67	3
humuli	Lemon	40-65 x 18-35 (32-45)	40-70 x 6-15	none	340-450	21-25	51-58	4
longicolla	Lemon	48 x 40 (45)	none	none	312-378	17-19	45-54	3
mentha	Lemon	45-63 x 22-45 (38-46)	70-108 x 6-7	none	280-320	20-24	34-42	4
mothi	Lemon	33-40 x 30-38 (38-46)	55x10 (rare)	yes	380-430	16-17	60-68	3 ?
oryzae	Lemon	26-30 x 30-42 (37-45)	52-70 x 5-10	few	370-500 (440)	20-22	60-75	3
urticae	Spherical	32-35 x 38-40 (44-45)	rare	few	467-615 (540)	25-31	44-67	4

TABLE 2. Key to *Heterodera* species of the *H. goettingiana* group. (Cysts basically lemon-shaped or spherical, bullae when present small and scattered, underbridge present in most species and strongly to weakly developed, cone top either ambifenestrate or bifenestrate.)

1. Semifenestra separated by wide vulval bridge (bifenestrate), fenestra generally more than twice as long as wide ..... *humuli* Filipjev, 1934
   Semifenestra separated by narrow to medium-wide vulval bridge fenestra mostly much longer than wide 2
2. Underbridge rarely present (*mothi* an exception) 3
   Underbridge weakly to strongly developed 7
3. Bullae absent in cone top of cyst ..... *carotae* Jones, 1950
   Bullae present in cone top of cyst 4
4. Cysts basically spherical in shape ..... *urticae* Cooper, 1955
   Cysts basically lemon-shaped 5
5. Vulval denticles present in cone top, fenestral length 32–40 µm ..... *mothi* Khan & Husain, 1965
   Vulval denticles absent in cone top, fenestral length 37–54 µm 6
6. Larvae with three lines in lateral field, stylet length 17–19 µm ..... *longicolla* Golden & Dickerson, 1973
   Larvae with four lines in lateral field, stylet length 24–27 µm ..... *amygdali* Kirjanova & Ivanova, 1975
7. Underbridge strongly to very strongly developed, with furcations at ends, semifenestra with wavy lines extending from outer edge to vulval slit 8
   Underbridge weakly developed, without furcations at ends, semifenestra without wavy lines 10
8. Underbridge very strongly developed, 115–140 µm in length, semifenestra of older cysts with distinct wavy lines extending from outer edge to vulval slit ..... *graminophila* Golden & Birchfield, 1972
   Underbridge less strongly developed, 70–100 µm in length, semifenestra of older cysts without wavy lines 9
9. Cysts basically spherical, larval length 520–600 µm, tail length 110–120 µm ..... *canadensis* n.sp.
   Cysts basically lemon-shaped, larval length 340–450 µm, tail length 44–65 µm ..... *graminis* Stynes, 1971
10. Cysts basically spherical, fenestral arch very low, L/W ratio more than 2 ..... *cruciferae* Franklin, 1945
    Cysts basically lemon-shaped, fenestral arch higher, L/W ratio Less than 1.5 11
11. Circumfenestral area (basin) with brick-like pattern ..... *goettingiana*, Liebscher, 1892
    Circumfenestral area without brick-like pattern 12
12. Underbridge long, 70–108 µm, larval length 280–320 µm ..... *mentha* Kirjanova & Narbaev, 1977
    Underbridge short, 40–70 µm, larval length 320–500 µm 13
13. Cysts mostly heart-shaped, fenestral length 51–60 µm, larvae with four lines in lateral field ..... *cardiolata* Kirjanova & Ivanova, 1969
    Cysts basically lemon-shaped, fenestral length 27–35 µm, larvae with three lines in lateral field 14
14. Cone top with vulval denticles and few scattered bullae, cysts stout, L/W ratio less than 1.5 ..... *oryzae* Luc & Brizuela, 1961
    Cone top without denticles or bullae, cysts slender, L/W ratio about 2 ..... *cyperi* Golden, Rau & Cobb, 1962

USSR, and two of those species, *Heterodera amygdali* and *H. cardiolata,* belong in the *goettingiana* group. *Heterodera graduni* Kirjanova *in* Kirjanova & Krall, 1971, which was described on the basis of ten cysts, may possibly belong also to the *goettingiana* group. The cysts of *H. graduni* are lemon-shaped, ambifenestrate, fenestral length 28–40 µm and width 32–48 µm. The semifenestra are rectangular and bullae are present although not illustrated. No male or larva was found. Krall considers it a possible valid species but suggests reexamination of type material and new samplings for the male and larva. Therefore, this species is not included in the present key.

Golden and Raski (2) proposed the name "vulval denticles" for small tooth-like structures found below the fenestral surface and within the vulval cone of some species belonging to the *Heterodera cacti* group. Examination of *H. oryzae, H. urticae,* and *H. mothi* revealed these structures present in the vulval cone of these species which belong to the *H. goettingiana* group. Since these structures are of taxonomic significance they are used in the key in Table 2 to separate these three species from closely related species.

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