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Description and SEM Observations of a New Species of Cyst Nematode *Heterodera goldeni* (Nematoda: Heteroderidae) Attacking *Panicum coloratum* in Egypt

Zafar A. Handoo[1] and I. K. A. Ibrahim[2]

Abstract: A cyst nematode, *Heterodera goldeni* n. sp., is photographed and described from Qasabagrass roots (*Panicum coloratum* L.) in Alexandria, Egypt. It is characterized in having second-stage juveniles with body length of 546 µm (450–612), stylet length of 22.6 µm (22–23.5) with anchor-shaped knobs, lateral field with 3 lines, tail 60–75 µm, hyaline tail terminus 38.4 µm (33–43); cysts are lemon-shaped, dark to light brown with an extensive sub-crystalline layer covering the entire cyst, cuticular midbody pattern zig-zag, cysts ambifenestrate, well-developed underbridge with finger-like projections, bullae present, vulva slit measuring 44–48 µm long. Males are absent, and females have heavy punctations on the cuticle. Its relationship to *H. graminophila* described from Florida and Louisiana and *H. leuceilyma* described from Florida are discussed. The present known distribution is restricted to Alexandria, Egypt. Its economic importance in rangeland grasses and cultivated crops such as rice is not known.

Key words: Alexandria, cyst nematode, Egypt, *Heterodera graminophila, H. leuceilyma*, morphology, *Panicum coloratum*, Qasabagrass roots, scanning electron microscopy, taxonomy.

In the fall of 1997, during a survey of plant-parasitic nematodes associated with various crops in Egypt, a new species of the cyst nematode *Heterodera* was found associated with Qasabagrass roots (*Panicum coloratum*) in Alexandria, Egypt. Morphological observations of the cyst perinea, white females, and second-stage juveniles (J2) isolated from soil and roots indicated that this species is different from others in the genus *Heterodera* and is described herein as *Heterodera goldeni* n. sp.

Materials and Methods

Various stages: Cysts, white females, (J2), and eggs were obtained from soil and roots associated with Qasabagrass growing in Alexandria, Egypt. Juveniles for morphological observations were separated from soil by sieving and Baermann funnel extraction or were recovered from cysts removed from fresh roots and kept in water in watch glasses. Juveniles were fixed in 3% formaldehyde and processed to glycerine by the formalin-glycerine method (Golden, 1990; Hooper, 1970). Females and some cysts were typically removed from roots after fixation for 12 hours in 3% formaldehyde solution. Photomicrographs of cyst vulval cones, females, and J2 were made with an automatic 35-mm camera attached to a compound microscope having an interference contrast system. Roots and whole cysts were photographed under a dissecting microscope, and light microscopic images of fixed nematodes were taken on a Leica WILD MPS48 Leitz DMRB compound microscope. For scanning electron microscopy (SEM), living specimens were fixed in 3% glutaraldehyde solution buffered with 0.05 M phosphate (pH 6.8), dehydrated in a graded series of ethanol, critical-point dried from liquid CO_2, and sputter-coated with a 20- to 30-nm layer of gold-palladium. Measurements were made with an ocular micrometer on a Leica WILD MPS48 Leitz DMRB compound microscope. All measurements are in micrometers unless otherwise stated.

Systematics

Heterodera goldeni n. sp.
(Figs. 1–5)

Description

Holotype (female, in glycerine): Body length with neck 813; body width 445; neck length 118, neck greatest width 65; L/W ratio 1.7; stylet 21; vulva-anus distance 62.

*Female (*n = *5, in glycerine):* Body length including neck 793–813 (803; SD 14.1); neck length 118–123 (120; SD 3.5); body width 416–475 (445; SD 41.7); neck width 60–65 (62.5; SD 3.5); L/W ratio 1.7–1.9 (1.8; SD 0.1); stylet 21–23 (22; SD 1.4); vulva-anus distance 61–65 (63; SD 2.8).

Body white to very light brown, lemon-shaped with protruding neck and vulva. Cuticle 10 µm thick with heavy punctations, generally obscuring cuticular pattern due to the heavy infestation throughout the body by *Pasteuria* sp. spores. Head slightly set off, with 2 annules. Stylet strong with slight dorsal curvature, and with well-developed stylet knobs with anterior projections. Vulval slit and anus distinct.

*Cysts (*n = *20):* Body length including neck 510–1150 (756; SD 204); neck length 50–124 (93; SD 21.3); body width 257–995 (466; SD 196); neck width 25–65 (45.7; SD 11.9); L/W ratio 1.3–2.1 (1.6; SD 0.20); vulval slit measuring 42 µm (35–50).

Cysts light brown to slightly darker brown, basically lemon-shaped, with protruding neck and vulva. Extensive thick sub-crystalline layer present, covering cysts

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[1] Microbiologist, USDA, ARS, Nematology Laboratory, Plant Sciences Institute, Beltsville Agricultural Research Center, Beltsville, MD 20705-2350, and
[2] Department of Plant Pathology, Faculty of Agriculture, Alexandria University, El-Shatby, Alexandria, Egypt.
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E-mail: *handooz@ba.ars.usda.gov*
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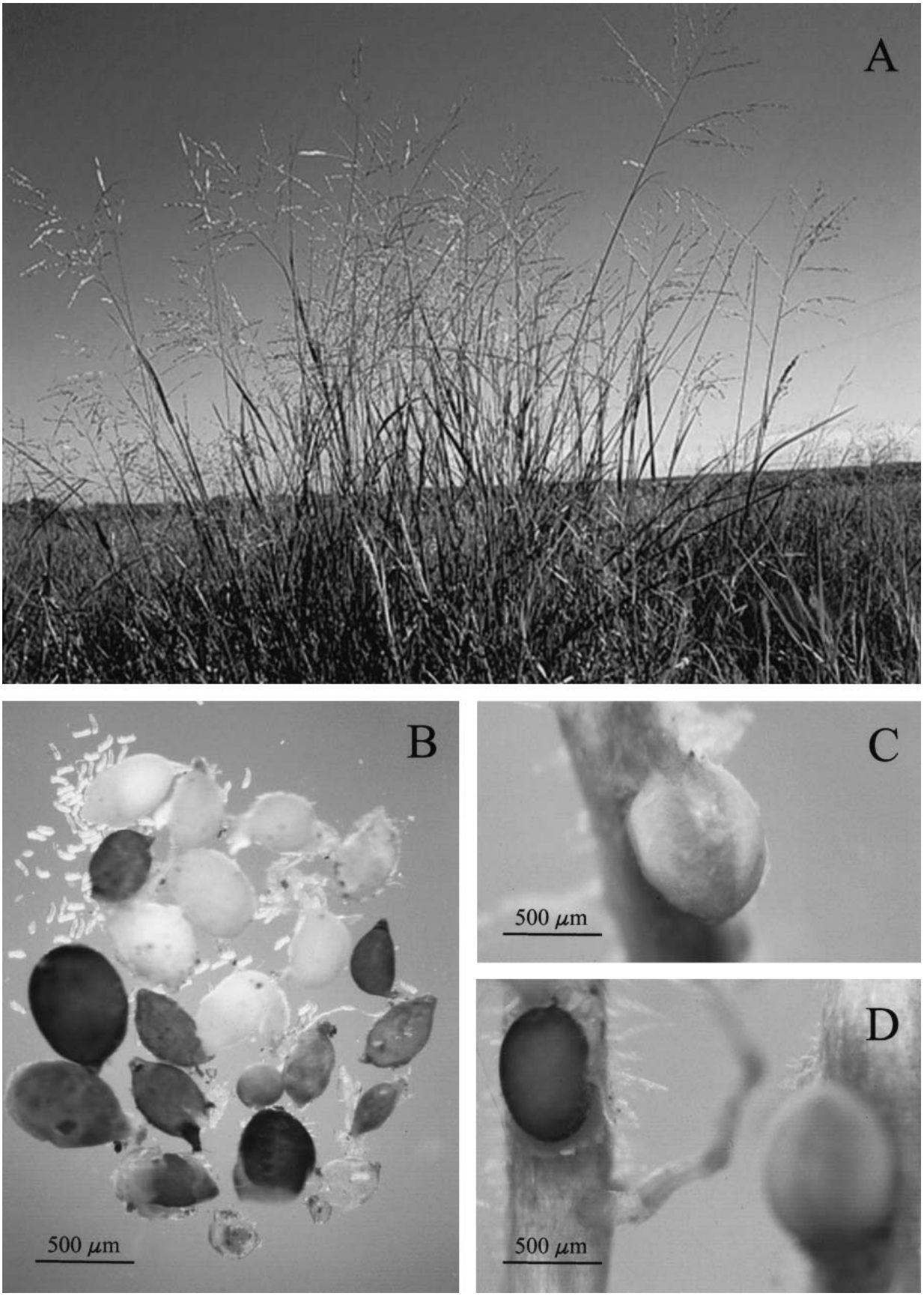

FIG. 1. A) *Panicum coloratum* plants, B–D Photomicrographs of *Heterodera goldeni* n. sp., on or isolated from *Panicum coloratum* roots. B) Cysts and white females and some eggs cracked out of cysts. C,D) Cysts on or isolated from roots (note the heavy sub-crystalline layer covering the cyst).

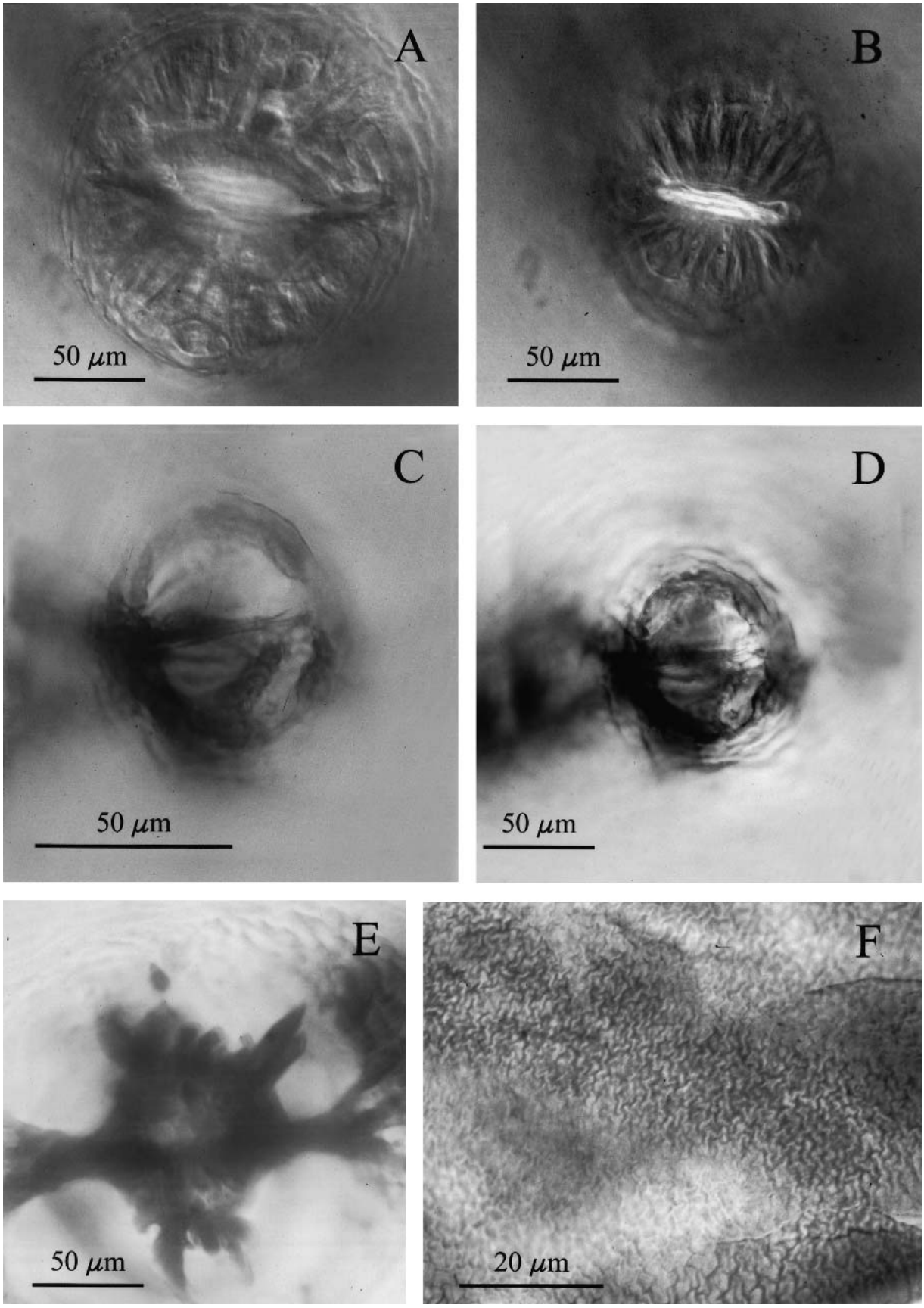

FIG. 2. Photomicrographs of *Heterodera goldeni* n. sp., Cyst cones. A) Surface. B) Beneath surface. C,D) Ambifenestrae and vulval slit. E) Underbridge with finger-like projections. F) Cyst wall pattern (zig-zag).

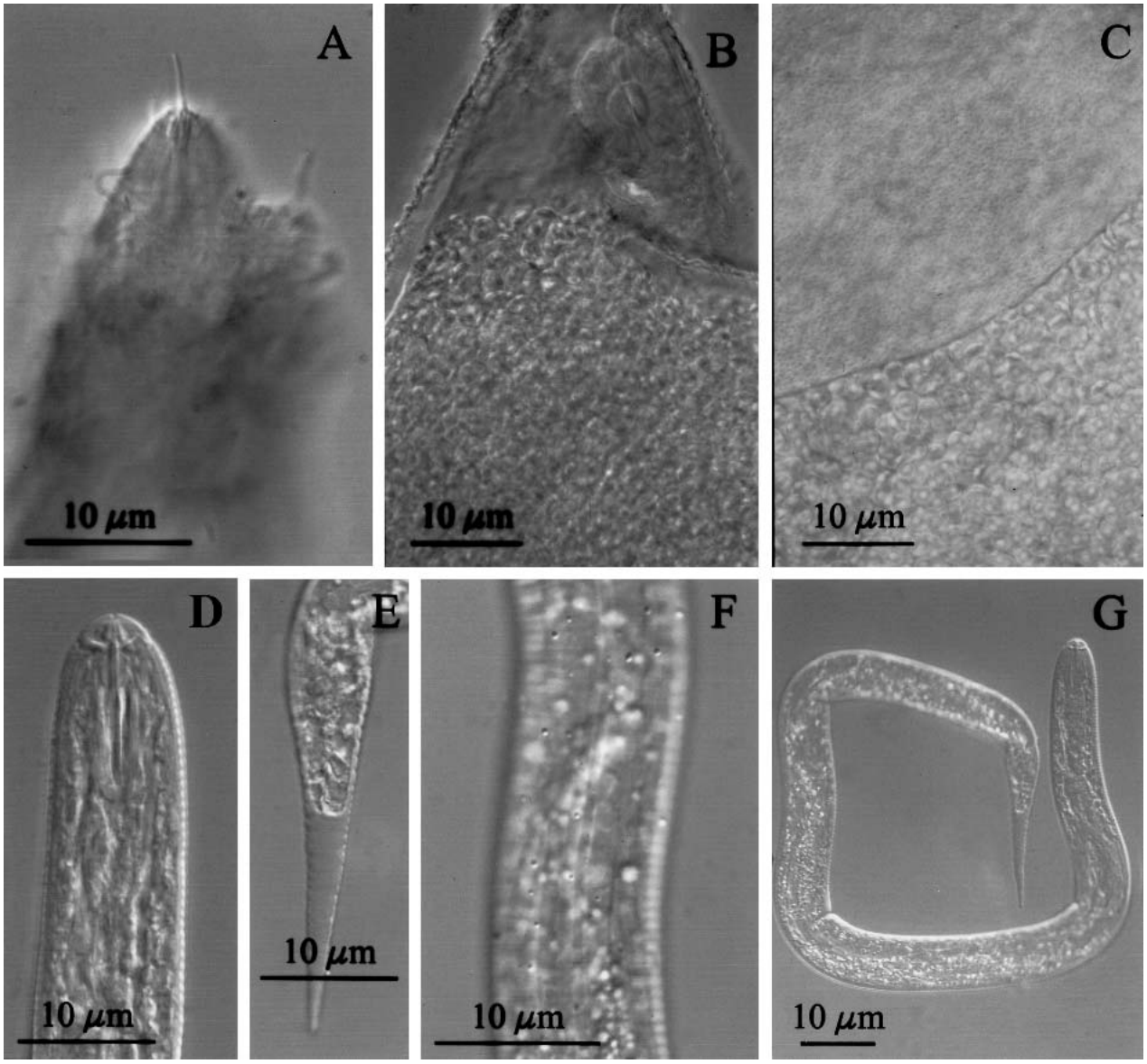

FIG. 3. Photomicrographs of *Heterodera goldeni* n. sp., White females. A) Anterior B) Anterior end heavily attacked by *Pasteuria* sp. spores. C) Cuticular punctations and *Pasteuria* sp. spores. D–G) Second-stage juveniles. D) Anterior. E) Posterior. F) Lateral field. G) Whole juvenile.

completely. Cyst wall pattern zig-zag with heavy punctations. Ambifenestrate. Heavy underbridge with characteristic finger-like projections present, measuring 102–150 (117: SD 28.3). Bullae present. Fenestra diam. average 52 (33–65).

*Second-stage juveniles (*n = *38):* Length 450–612 (546; SD 41.6); a = 28–36 (31; SD 2.1); b = 2.3–3.4 (2.5; SD 0.2); c = 7.7–9.3 (8.3: SD 0.4); stylet 22–23.5 (22.6; SD 0.4); dorsal esophageal gland orifice 2.5–6.0 (4.0; SD 0.9) from base of stylet; tail 60–75 (65.4; SD 3.8); hyaline tail terminal 33–43 (38.4; SD 2.9); caudal ratio A = 4.2–5.7 (4.7; SD 0.5); caudal ratio B = 10.5–21.5 (15.1; SD 0.3).

Body vermiform, tapering at both extremities, more so posteriorly. Cuticular annulations distinct. Excretory pore near the beginning of the basal esophageal bulb. Lateral field with occasional aerolation, consisting of three incisures. Widest part of body measures 16–20 (18.6; SD 2.1). Head slightly set off with cephalic sclerotization, bearing three annules. Stylet with prominent anchor-shaped knobs. Tail long with a conical-shaped terminus.

Males: The absence of males suggests that the species is to be considered a monosexual species or simply not found during the present survey.

Type host and locality

Associated with Qasabagrass roots (*Panicum coloratum* L.) growing in Montazah Palace Garden, Alexandria, Egypt.

Type specimens

Holotype (female): Isolated from roots from the type host and locality. Slide T-545t, deposited in the U.S. Department of Agriculture Nematode Collection, Beltsville, Maryland.

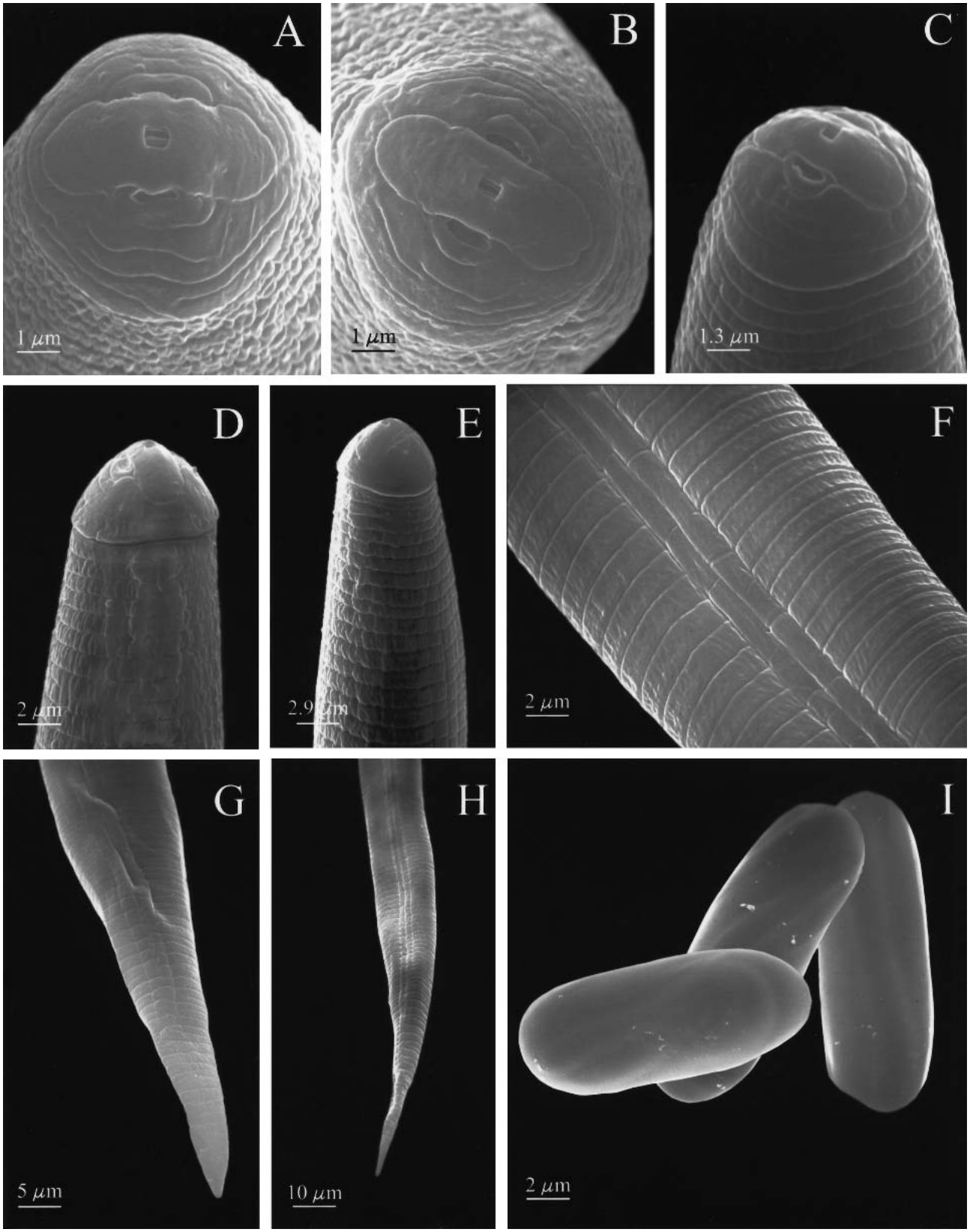

FIG. 4. SEM micrographs of second-stage juveniles of *Heterodera goldeni* n. sp., A–E) Head region and *enface* view showing slit-like amphidial apertures. F) Lateral field showing three incisures. G,H) Posterior region. I) Eggs from cysts.

Paratypes (cysts, J2, and females): Same data and repository as holotype. Slides T-4956p–T-5031p. Additional paratypes deposited in University of California–Riverside Nematode Collection (UCRNC), Riverside, California; The Nematode Collection of the Nematology Department, Rothamsted Experimental Station,

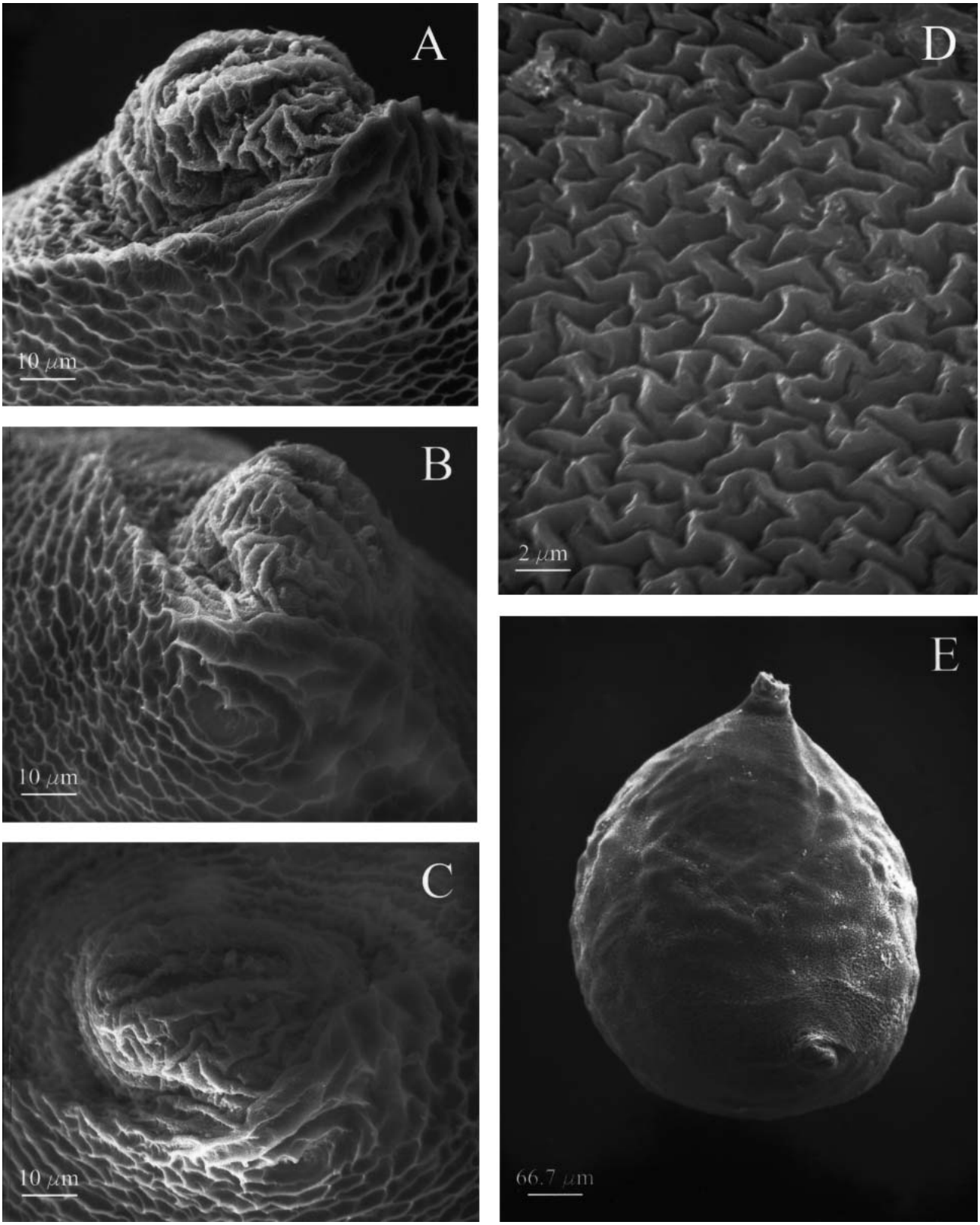

FIG. 5. SEM micrographs of posterior end and surface patterns of *Heterodera goldeni* n. sp., Cysts. A–C) Posterior ends showing vulval slit and anal area. D) Zig-zag cuticular pattern. E) Whole cyst.

Harpenden, Herts., England; Canadian National Collection of Nematodes, Ottawa, Canada; Collection Nationale de Nématodes, Laboratorie des Vers, Muséum National d'Histoire Naturelle, Paris, France; Nematode Collection of the Landbouwhogeschool, Wageningen, The Netherlands; Commonwealth Institute of Parasitology Collection, St. Albans, Herts., England.

Diagnosis

Heterodera goldeni n. sp. is characterized in having J2 with body length of 546 µm (450–612), stylet length of 22.6 µm (22–23.5) with anchor-shaped knobs, lateral field with 3 incisures, tail 65.4 µm (60–75) long, hyaline tail terminus 38.4 µm (33–43); cysts are lemon-shaped, light to dark brown, with an extensive sub-crystalline layer covering the entire cyst, cysts ambifenestrate, well-developed underbridge with finger-like projections, bullae present. The absence of males suggests that the species is to be considered a monosexual species or simply not found during the present survey. Females have heavy punctations on the cuticle.

Relationships

Heterodera goldeni n. sp. is closely related to *H. graminophila* Golden & Birchfield, 1972 and *H. leuceilyma* Di Edwardo & Perry, 1964. It differs from *H. leuceilyma* in having J2 with a shorter stylet (length 22.6 (22–23.5) vs. 26 (23–28 µm)) and in the shape of J2 stylet knobs, anchor-shaped vs. prominently rounded. The new species differs from *H. graminophila* in that it has J2 with longer bodies and with longer, more hyaline tail termini than those described from Louisiana (Golden and Birchfield, 1972) and Florida (Inserra et al., 1989) populations: body length 546 (450–612) vs. 430 (380–460 µm) in the Louisiana population on barnyard grass (*Echinochloa colonum*), and 391 (374–412 µm) in the Florida population on roots of *Panicum rigidulum*, hyaline tail terminus 38.4 (33–43) vs. 32 (25–38 µm) in the Louisiana population and 27 (23–31 µm) in the Florida population. *H. goldeni* n. sp. also has bullae in the cysts, which are absent in *H. graminophila*. Also, the J2 stylet length in the Florida population of *H. graminophila* is shorter than that of *H. goldeni* n. sp. from Egypt 18.2 (17.6–18.6) vs. 22.6 (22–23.5 µm). Male specimens reported in *H. graminophila* and *H. leuceilyma* were absent in *H. goldeni* n. sp.

Etymology

The species name is given in honor of Dr. A. Morgan Golden for his outstanding contributions to our knowledge of cyst nematodes.

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