Heterodera graminophila n. sp. (Nematoda:Heteroderidae) from Grass with a Key to Closely Related Species[1]

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Abstract: Heterodera graminophila n. sp., a member of the *H. goettingiana* group, is described and illustrated from roots of barnyard grass, *Echinochloa colonum* (L.) Link, in Baton Rouge, La. This new abullate species, having second-stage larvae with only three lines in the lateral field, is most closely related to *H. cyperi* Golden, Rau & Cobb, 1962, and *H. graminis* Stynes, 1971, but differs particularly in having a small, inconspicuous anus without a circum-anal pattern and located about 20% of the cyst length from the vulval cone terminus, and a longer vulval slit averaging 45 μ in length. A key, based on cyst and larval characters, is presented for identification of the 10 *Heterodera* species in the *H. goettingiana* group. *Key words:* taxonomy, morphology, new *Heterodera* species, *Echinochloa colonum*, barnyard grass.

In 1965, a cyst nematode was found in large numbers parasitizing the roots of barnyard grass, *Echinochloa colonum* (L.) Link, about five kilometers west of St. Joseph, La., on U.S. Highway 65. Subsequent greenhouse and laboratory studies on its biology, host range, and pathogenesis (1, 2) showed that this nematode also attacked rice, *Oryza sativa* L., and Johnson grass, *Sorghum halepense* (L.) Pers., but did not develop on several other grass and dicotyledonous plants. Giant cells were not observed in the host tissue, although phloem enlargement was noted near the head of the nematode. This nematode is described herein as a new species of *Heterodera*, and a key is provided for identification of the 10 species in the *H. goettingiana* group.

MATERIALS AND METHODS

Specimens used in this description were collected from barnyard grass grown in a greenhouse at Baton Rouge, La., and were fixed in 3% formaldehyde solution. Measurements on all stages were made with an ocular micrometer, and drawings prepared with a camera lucida. Gross measurements and photomicrographs of intact females and cysts, and all measurements and drawings of males and larvae, were made on specimens in the formalin fixative. These and other specimens then were processed to glycerine by Golden's method, a summary of which is given by Hooper (6).

The drawing of the female anterior portion was made on a glycerine-mounted specimen, as were the detailed photomicrographs of other females and cysts (except the cone tops which were mounted in Euparal). Specimens of related species examined in preparation of the key were obtained from the USDA Nematode Collection, Beltsville, Md.

Heterodera graminophila, n. sp.

Measurements: 50 females—length (including neck), 460 μ (290-650); width, 310 μ (160-560); l/w ratio, 1.5 (1.1-2.1); stylet, 22.6 μ (21.3-23.8); outlet of dorsal esophageal gland from base of stylet, 5.0 μ (4.0-6.7).

Holotype (female)—Length, 530 μ; width, 310 μ; l/w ratio, 1.8; stylet, 22.0 μ; dorsal esophageal gland, 5.0 μ.

Description of females: Body pearly white, basically lemon-shaped, with protruding neck and vulva (Fig. 2). Thick cuticle with zig-zag pattern, subcuticular punctation (Fig. 7), and commonly a granular surface obscuring details of pattern. Head variable in shape, set off from neck, bearing two annules with second one often larger than first (Fig. 1-C). Cephalic sclerotization distinct but weak. Stylet strong and curving dorsally, with well-developed knobs sloping posteriorly. Esophageal region appearing about as illustrated, with excretory pore commonly at base of neck. Anterior portion of neck on some specimens seen with peculiar protrusions (Fig. 8, 9). Vulva prominent, and surface with wavy striae extending to vulva slit which averages about 38 μ (35-43) in length (Fig. 4, 5, 6). Anus small, inconspicuous, and located approximately 20% of body length from vulva.

Measurements: 25 older cysts (washed from soil)—length (including neck), 650 μ (520-780); width, 450 μ (350-550); l/w ratio = 1.5 (1.2-1.8). Twenty-five young cysts (removed from roots)—length (including neck), 660 μ (520-780); width, 470 μ (350-560); l/w ratio = 1.4 (1.1-1.6). Cysts light to dark brown in color, basically lemon-shaped, with protruding neck and vulva (Fig. 3); abullate,

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ambifenestrate (Fig. 16), with prominent underbridge having furcate ends (Fig. 10, 11, 12, 13). Vulval slit averaging about 45 μ (40-52) in length. External cyst pattern zig-zag (Fig. 17, 18), often appearing granular, and with conspicuous, irregularly arranged subcuticular punctation (Fig. 19). On the semifenestrae, which commonly persist even in older cysts, distinct wavy lines extend from the outer edge to vulval slit (Fig. 14, 15). Anus small, inconspicuous, without surrounding pattern, and located about 20% of cyst length from vulval cone terminus.

Measurements: 25 males–length, 990 μ (850-1110); a = 32 (28-37); b = 6.0 (5.3-6.5); c = –; stylet, 24.7 μ (22.4-25.8); outlet of dorsal esophageal gland from base of stylet, 5.3 μ (4.0-6.2); spicules, 34 μ (31-36); gubernaculum, 13 μ (11-14).

Allotype (male)–Length, 1040 μ; a = 35; b = 5.8; c = –; stylet, 24.4 μ; outlet of dorsal esophageal gland, 5.0 μ; spicules, 33 μ; gubernaculum, 12.4 μ.

Description of males: Body slender, vermiform, tapering slightly at both extremities. Cuticle with prominent annulation; subcuticular annulation less distinct and appearing twice as often as on cuticle. Lateral field without areolation, measuring about 6 μ in width at midbody, generally with only three equally spaced lines except two or one in anterior portion. At middle, body averages 31 μ (27-35) in width. Head high, broadly hemispherical, slightly set off, with five annules. Cephalic framework heavily sclerotized. Stylet, knobs, and cephalids commonly appearing as illustrated (Fig. 1-B). Median bulb ellipsoidal with its center 83 μ (74-90) from anterior end. Excretory pore about three annules posterior to the distinct hemizonid. Spicules slightly arcuate, with bidentate tips. Tail generally shaped as illustrated (Fig. 1-A), extremely short and with cloaca often extending beyond tail terminus.

The stylet guide in both males and larvae appears as the usual lyre-shaped structure, plus a sleeve-like extension reaching to approximately halfway of the basal shaft of the stylet and ending in a ring encircling the shaft at that point (1-B, D). This is the same arrangement as described by Golden & Ellington (4) for *H. rostochiensis* Wollenweber, 1923.

Measurements: 25 second-stage larvae–length, 430 μ (380-460); a = 20 (18-22); b = 2.4 (2.1-2.8); c = 6.9 (6.4-7.4); stylet, 22.7 μ (21.8-23.5); outlet of dorsal esophageal gland from base of stylet, 5.6 μ; tail, 62 μ (57-67); hyaline tail terminal, 32 μ (25-38); caudal ratio, A = 4.8 (3.8-5.7); caudal ratio, B = 18.6 (13.3-34.0).

Description of second-stage larvae: Body vermiform, tapering at both extremities, but much more so posteriorly. Cuticle clearly annulated; subcuticular annulation less distinct but twice as numerous. Lateral field without areolation, and composed of only three lines (Fig. 1-E). At widest part, body measures 22 μ (20-23). Head slightly set off, possessing five annules, and being wider at its base than in height. Cephalic framework heavily sclerotized. Stylet strong, with prominent knobs. Anterior and posterior cephalids present (Fig. 1-D). Prominent median bulb ellipsoidal, its valvated center located 64 μ (59-71) from anterior end. Excretory pore posterior and almost adjacent to hemizonid. Tail long, tapering to a fine, rounded terminus. Phasmids exceptionally prominent for a *Heterodera* species and located on lateral field in anterior fifth portion of tail (Fig. 1-E). Within eggs, larvae generally in four complete folds plus a partial fifth one for the tail or portion of it.

Measurements: 25 eggs–length, 109 μ (101-121); width, 41 μ (37-45); l/w ratio, 2.6 (2.3-3.1). Egg shell hyaline, without visible markings.

HOLOTYPE (female): Collected from greenhouse culture by Wray Birchfield at Baton Rouge, Louisiana on April 1, 1971. Slide T-206t, United States Department of Agriculture Nematode Collection, Beltsville, Md.

ALLOTYPE (male): Slide T-207t, same data and collection as holotype.

PARATYPES: Males, females, cysts, larvae and eggs; United States Department of Agriculture Nematode Collection, Beltsville, Maryland; and California Nematode Survey Collection, Davis.

TYPE HOST AND LOCALITY: Roots of barnyard grass, *Echinochloa colonum*, about five kilometers west of St. Joseph, Louisiana.

DIAGNOSIS: Heterodera graminophila n. sp. differs from the closely related *H. cyperi* (5) and *H. graminis* (8) in that second-stage larvae possess only three lines in the lateral field, and cysts have a small, inconspicuous anus without circum-anal pattern and located about 20% of cyst length from vulval cone terminus; in *H. cyperi* and *H. graminis*, the conspicuous anus has a definite circum-anal pattern and is located about 10% of cyst length from the vulval cone terminus. This new species differs further from *H. cyperi* by having: (i) a massive, furcate underbridge in cysts; (ii) vulval slit about 45 μ in length; and (iii) larval stylet and hyaline tail terminal averaging 22.7 and 32 μ in length, respectively; in *H. cyperi*, (i) the underbridge is weak and inconspicuous; (ii) vulval slit about 30 μ in length; and (iii) larval stylet and hyaline tail terminal averaging 20 and about 25 μ, respectively. From *H. graminis*, this new species can be further differentiated by (i) moderately slender cysts (l/w ratio = 1.6); (ii) vulval slit about 45 μ; and (iii) males with only three lines in lateral field; in the former species, (i) the cysts are stout (l/w ratio = 1.3); (ii) vulval slit averages 38 μ in length; and (iii) there are males with four lines in lateral field.

Two points about this interesting new species deserve further comment. At first glance in the vulval cone, bullae appear to be present. However, these structures, though seemingly bullae, are in fact furcations of the ends of the massive underbridge. They appear to have the same texture as the underbridge, are commonly attached to it, and further unlike bullae, occur only at either end of the underbridge. The other feature of this species is the persistence of each semifenestra. Even in many old cysts examined, the semifenestrae could still be seen, and the disintegration and disappearance of the semifenestrae as generally occurs in related species was not observed.

The known distribution of *H. graminophila* is limited to several locations in Louisiana.

KEY TO THE *HETERODERA* SPECIES OF THE *H. GOETTINGIANA* GROUP

(Cysts lemon-shaped, zig-zag pattern, abullate, though inconspicuous bullae may sometimes be seen in certain species, mainly *fici, humuli, latipons,* and *urticae.* Specimens of all species except *urticae* were examined in preparation of the key.)[3]

1. Second-stage larvae with 4 lines in lateral field .. 4
   Second-stage larvae with only 3 lines in lateral field 2
2. Cyst surface often granular, with conspicuous, irregularly arranged punctation; anus small, without circum-anal pattern, and located about 20% of body length from vulval cone terminus; vulval slit on cyst averaging about 45 μ in length ..... *H. graminophila* n. sp.
   Cyst surface without granulation or conspicuous punctation; anus prominent, with definite circum-anal pattern, and located about 10% of body length from vulval cone terminus; vulval slit averaging less than 40 μ in length 3
3. Stylet and tail terminal of larvae averaging 20 and 24 μ, respectively; cysts slender, 1/w ratio = about 2.0; vulval slit averaging 30 μ in length ..... *H. cyperi* Golden, Rau & Cobb, 1962
   Stylet and tail terminal of larvae averaging 22 and 29 μ, respectively; cysts stout, 1/w ratio = 1.3; vulval slit averaging 38 μ in length ..... *H. graminis* Stynes, 1971
4. Cysts ambifenestrate (semifenestrae longer on axis with vulval slit than on angle to it, and close together, being separated by very narrow vulval bridge) 7
   Cysts bifenestrate (semifestrae circular or partially so, and well separated by wide vulval bridge) 5
5. Semifenestrae always circular, with distance between the two greater than the diameter of a single semifenestra; vulval slit very short, averaging 7 μ in length ..... *H. latipons* Franklin, 1969
   Semifenestrae may be only partially circular, with distance between the two always less than the diameter of a single semifenestra; vulval slit over 25 μ in length 6
6. Cyst wall pattern extremely coarse, appearing maze-like to rugose; vulval slit averaging about 42 μ in length ..... *H. fici* Kirjanova, 1954
   Cyst wall pattern smoother, clearly zigzag, without rugose effect; vulval slit averaging about 35 μ in length .. *H. humuli* Filipjev, 1934
7. Second-stage larvae with stylet averaging 27 μ in length, and outlet of dorsal esophageal gland 8 μ from base of stylet; outer two lines of lateral field crenate, outer two bands areolated ..... *H. urticae* Cooper, 1955
   Second-stage larvae with stylet measuring around 22-24 μ in length, and outlet of dorsal esophageal gland about 5 μ or less from base of stylet; outer two lines of lateral field smooth, outer two bands not areolated 8
8. Second-stage larvae with tail averaging less than 50 μ (generally 45-47 μ); hyaline tail terminal measuring 22-25 μ in length ..... *H. cruciferae* Franklin, 1945
   Second-stage larval tail averaging more than 50 μ in length; hyaline tail terminal 28 μ or more in length 9
9. Cysts relatively small, averaging 450 μ in length and 340 μ in width; vulval slit measuring around 45 μ in length; average larval tail and tail terminal lengths about 54 and 30 μ, respectively ..... *H. carotae* Jones, 1950
   Cysts relatively large averaging 630 μ in length and 460 μ in width; vulval slit measuring 50 μ or more in length; average larval tail and tail terminal lengths about 60 and 35 μ, respectively... *H. goettingiana* Liebscher, 1892

[3] **Note: The inclusion of Heterodera urticae in the key is based on the recent complete description by Mathews (7). A case for an opinion on the priority and authorship of this and the other provisional names proposed by Cooper (3) is pending before the International Commission on Zoological Nomenclature.**

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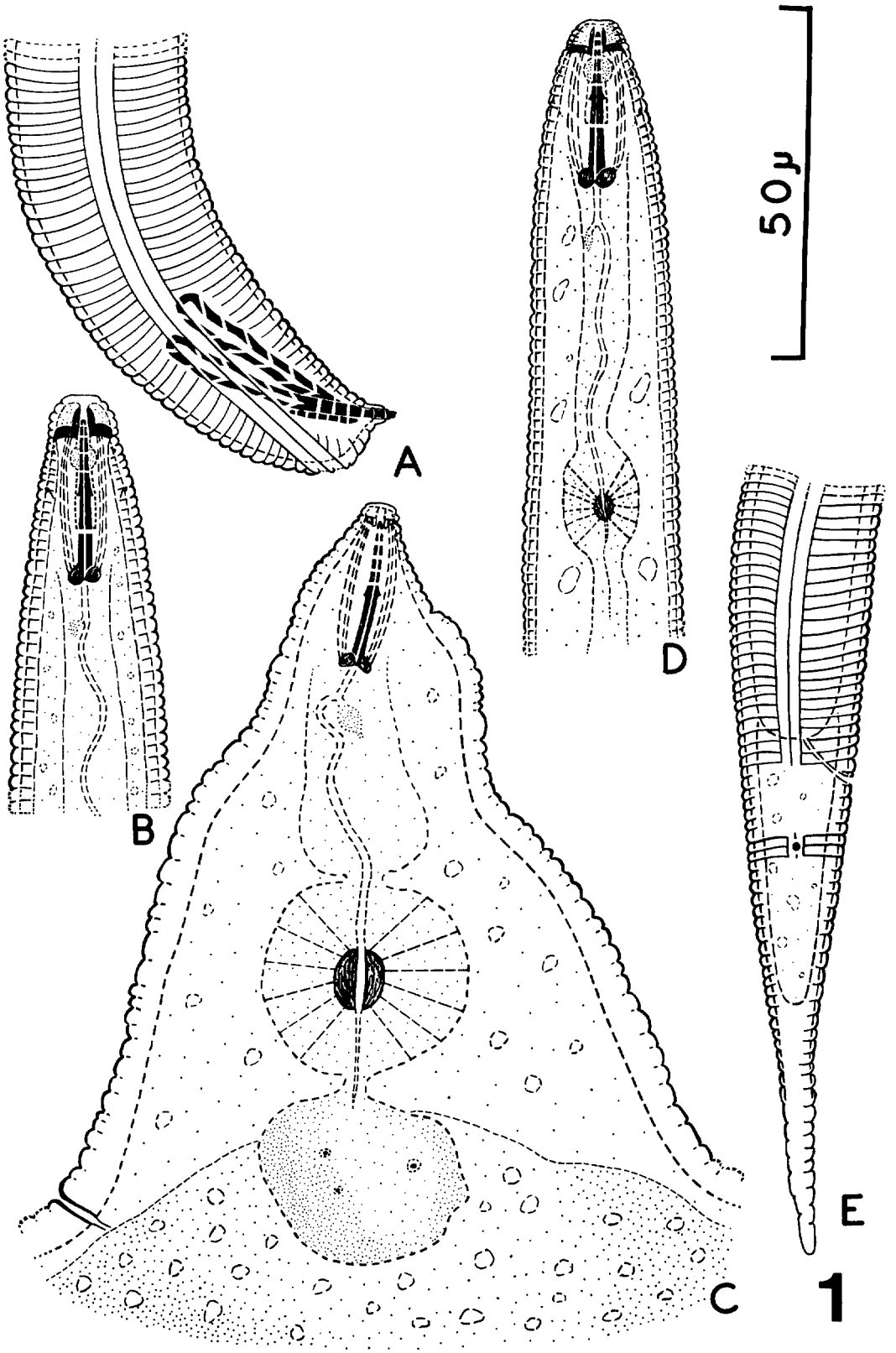
FIGURE LEGENDS

FIG. 1. Drawings of *Heterodera graminophila*, n. sp. Male: A = Posterior; B = Anterior. Female: C = Anterior. Second-stage larva: D = Anterior; E = Posterior.

FIG. 2-3. Photomicrographs of whole specimens of *Heterodera graminophila*, n. sp. **2.** White females. **3.** Cysts of varying ages. 30 X.

FIG. 4-9. Photomicrographs of portions of females of *H. graminophila*, n. sp. **4.** End-on view of vulval area at outer surface. **5.** Same view as 4 but at deeper focus. **6.** Lateral view of vulval area at outer surface. **7.** Punctation near midbody. **8, 9.** Peculiar protrusions on necks of two females. 1200 X.

FIG. 10-19. Photomicrographs of portions of cysts of *H. graminophila*, n. sp. **10, 11.** Deep-focus views of two different vulval cones showing vaginal sheath and massive underbridge with variable furcations at ends. **12.** Deep-focus, end-on view of a vulval cone. **13.** An underbridge with furcate ends as seen from inside a vulval cone top. **14.** End-on view of vulval cone with focus at outer cuticular surface. **15.** End-on view of vulval cone with focus at inner cuticular surface. **16.** End-on, deep-focus view of vulval cone of young cyst (note the two distinct semifenestrae, incompletely fenestrated). **17, 18.** Cyst walls of two different cysts at midbody, showing variation in zig-zag pattern. **19.** Punctations in cyst wall near midbody. 10-13 at 350 X; 14-19 at 760 X.



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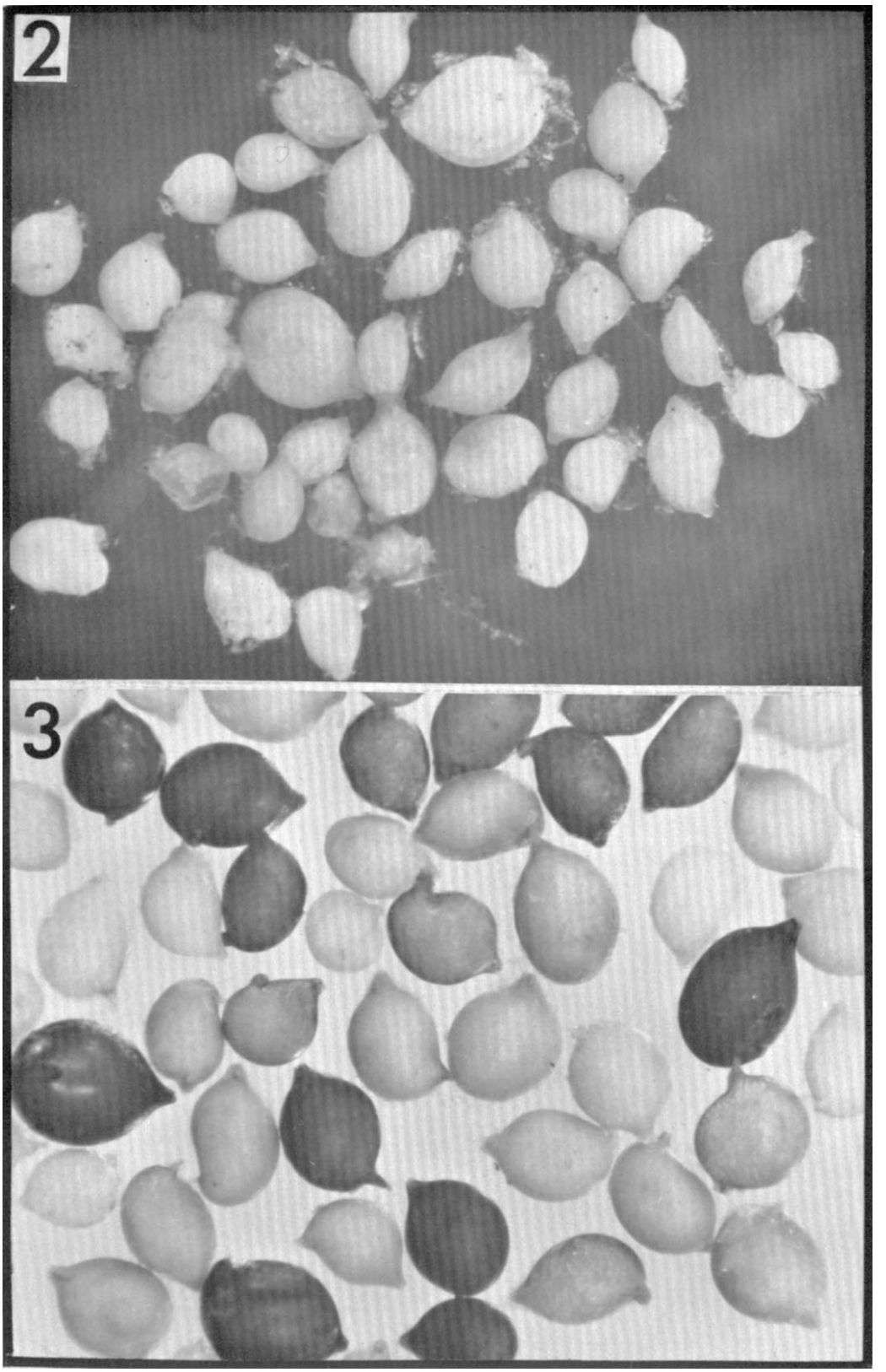
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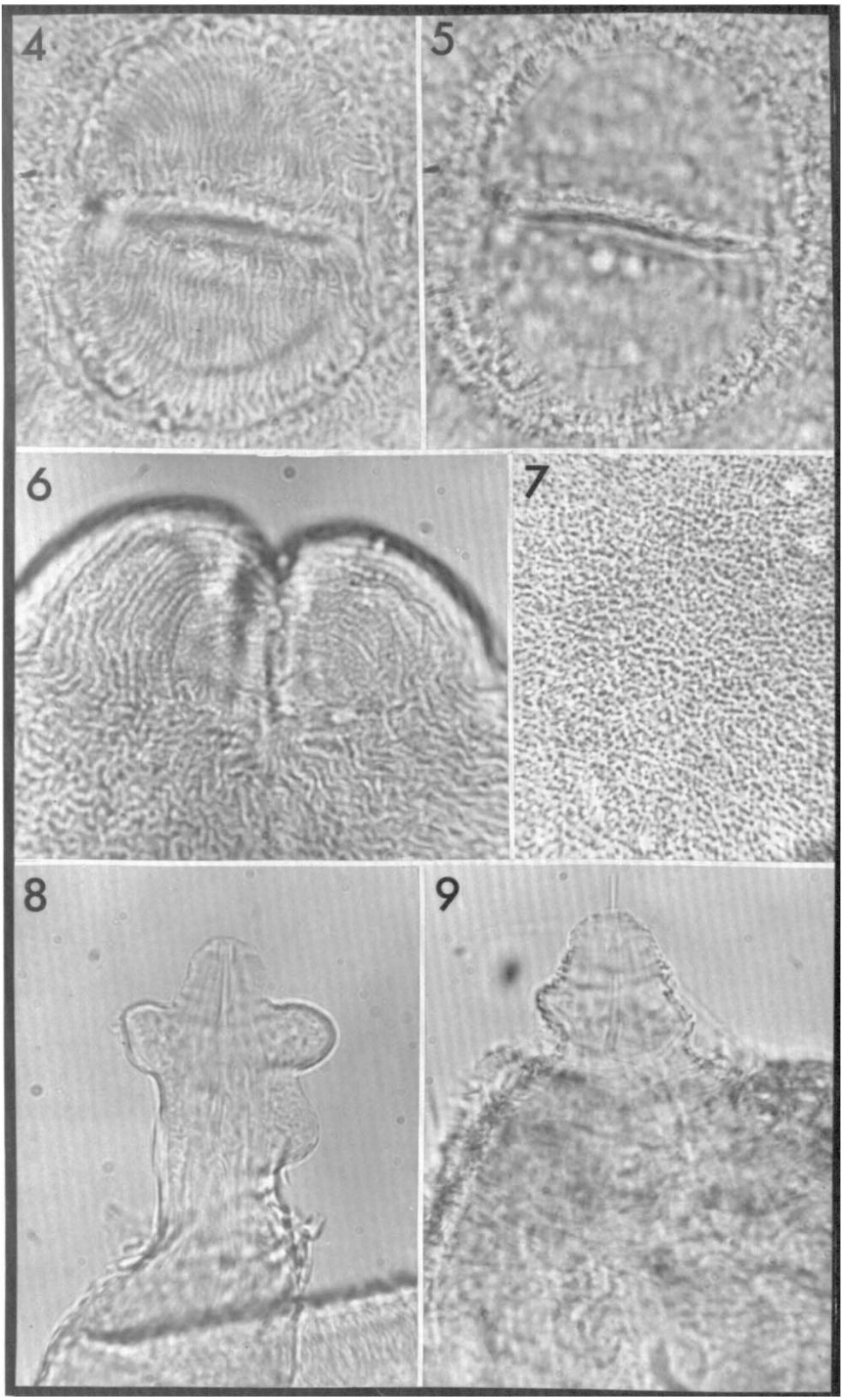
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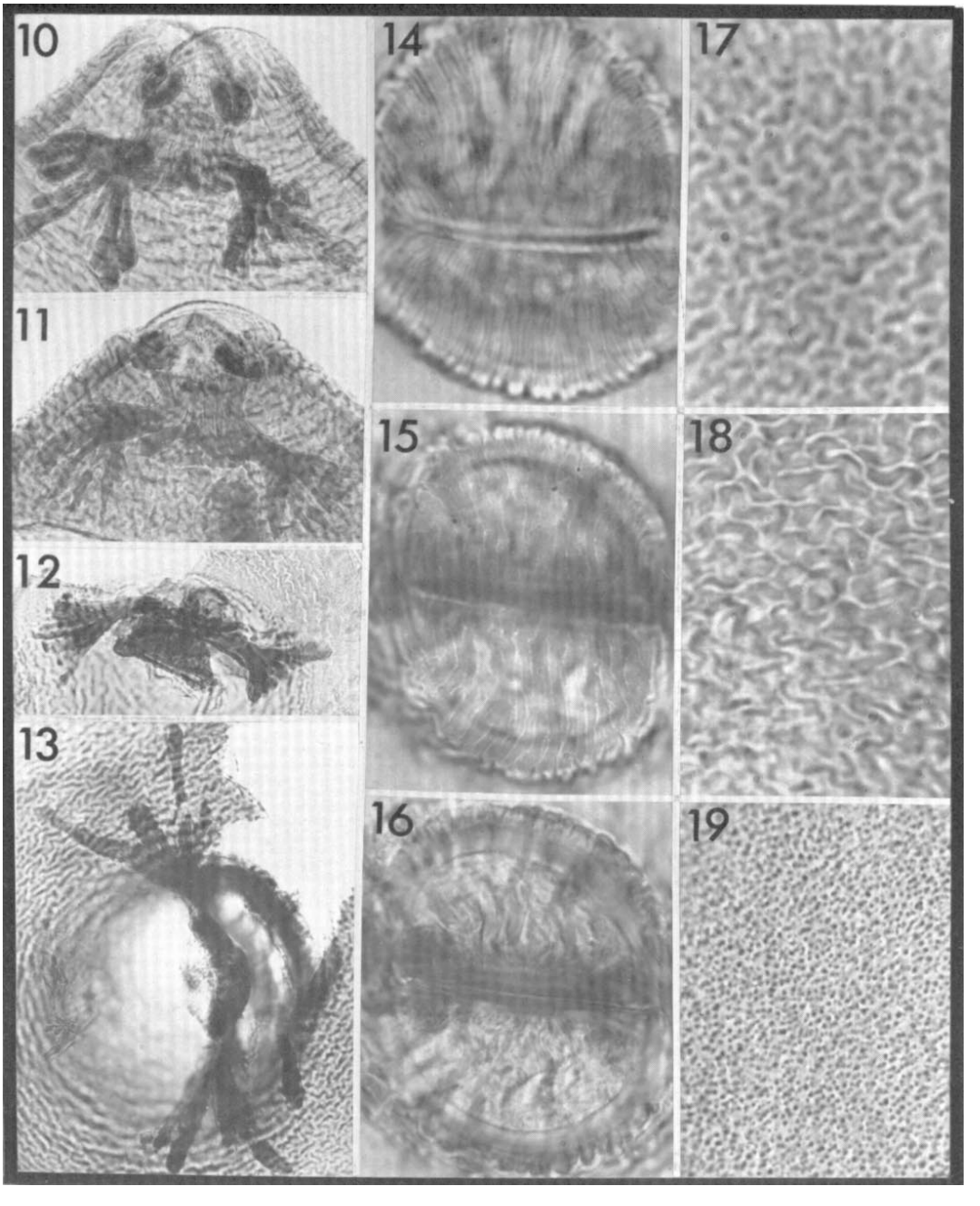
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