

Heterodera betulae n. sp. (Heteroderidae), a Cyst-forming Nematode from River Birch¹

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Abstract: A new species of the genus *Heterodera* A. Schmidt, 1871 parasitic on river birch, *Betula nigra* L., is described and illustrated. Females and cysts are lemon-shaped to almost spherical with slight vulval protrusion. Female cuticles have a thick subcrystalline layer. The average cyst size is 763 by 616 μ . They are circumfenestrate with small anal opening and lack a yellow phase. The cyst wall pattern is typically network-like. All eggs are retained in the cyst, although a well-developed matrix is formed. The egg shell is without markings. The second-stage larvae average 462 μ in length and have 3 incisures in the lateral field. The tail terminal is shorter than the stylet. Males are rare. They have 4 incisures in the lateral field and bifid spicules. The relationship of *H. betulae* n. sp. to other *Heterodera* species is obscure.

A cyst-forming nematode was isolated from soil samples collected along the bank of Middle Fork of White River, east of Fayetteville, Arkansas (1). Examination of the roots of other plants in the area and preliminary host tests in the greenhouse indicated that river birch, *Betula nigra* L., was the natural host. The nematode has been collected from the type locality and one other location about 5 miles away on a different stream. Males were encountered once in a temperature experiment in the greenhouse and twice from incubated roots infected with the nematode. This is the second *Heterodera* species, aside from *H. fici* Kirjanova, 1954, that parasitizes trees. It appears to be distinct from other species of this genus with respect to several characteristics and thus is described as a new species. The common name, "birch cyst nematode," is suggested.

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MATERIALS AND METHODS

Stock cultures of *Heterodera betulae* n. sp. were established from females and larvae obtained from the type locality in Arkansas. The nematodes were propagated on seedlings of river birch, *Betula nigra*, in the greenhouse at 20 to 30 C. Cultures were easily maintained by periodic subculturing.

Nematodes from greenhouse cultures were used for all morphological and morphometric studies. Permanent mounts of white females were made in the following manner: The females were fixed in FA (formalin 10 ml, glacial acetic acid 10 ml, distilled water 80 ml) for 12 to 24 hr. They were transferred from the fixative to boiling lactophenol (95 C) for 3 to 5 min, then processed through 6 mixtures of lactophenol-glycerin with increasing amounts of glycerin into pure glycerin on a hot plate at 50 C. Mounts of females were made on slides in hard glycerin jelly using small wax supports. Vulval cones were prepared from females and cysts fixed in 2% formalin. The cysts were cut around the perineal area which was mounted upright in glycerin jelly. Second-stage larvae and males were killed by gentle heat and transferred to 1% formalin for study. Type specimens of males and larvae were prepared in glycerin according to Thorne's method (2).

SPECIES DESCRIPTION

Heterodera betulae n. sp.FEMALES: *Measurements in glycerin* (20♀).

—Body length (without neck): 381–945 μ (mean 618.4 μ , 95% confidence interval \pm 56.96); body width: 342–720 μ (512.1 μ \pm 42.52); body length/body width ratio: 1.0–1.5 (1.2 \pm 0.05); stylet length: 28.8–32.6 μ (30.22 μ \pm 0.48); dorsal esophageal gland orifice: 4.1–5.3 μ (4.80 μ \pm 0.16); median bulb length: 30.6–45.0 μ (36.54 μ \pm 1.55); median bulb width: 30.2–41.0 μ (36.42 μ \pm 1.35); head end to end of median bulb: 74.1–117.0 μ (96.25 μ \pm 5.55); excretory pore from anterior end: 126.4–253.5 μ (166.87 μ \pm 16.58); vulva-anus distance (lateral view): 58.5–150.2 μ (93.02 μ \pm 10.02); thickness of cuticle: 10.2–19.5 μ (14.23 μ \pm 1.29).

Measurements in 2% formalin (50♀).—

Body length (without neck): 345–1185 μ (693.0 μ \pm 60.00); body width: 260–985 μ (585.3 μ \pm 55.42); body length/body width ratio: 1.0–1.5 (1.2 \pm 0.03).

Measurements of vulval cone tops in glycerin jelly (30♀).—

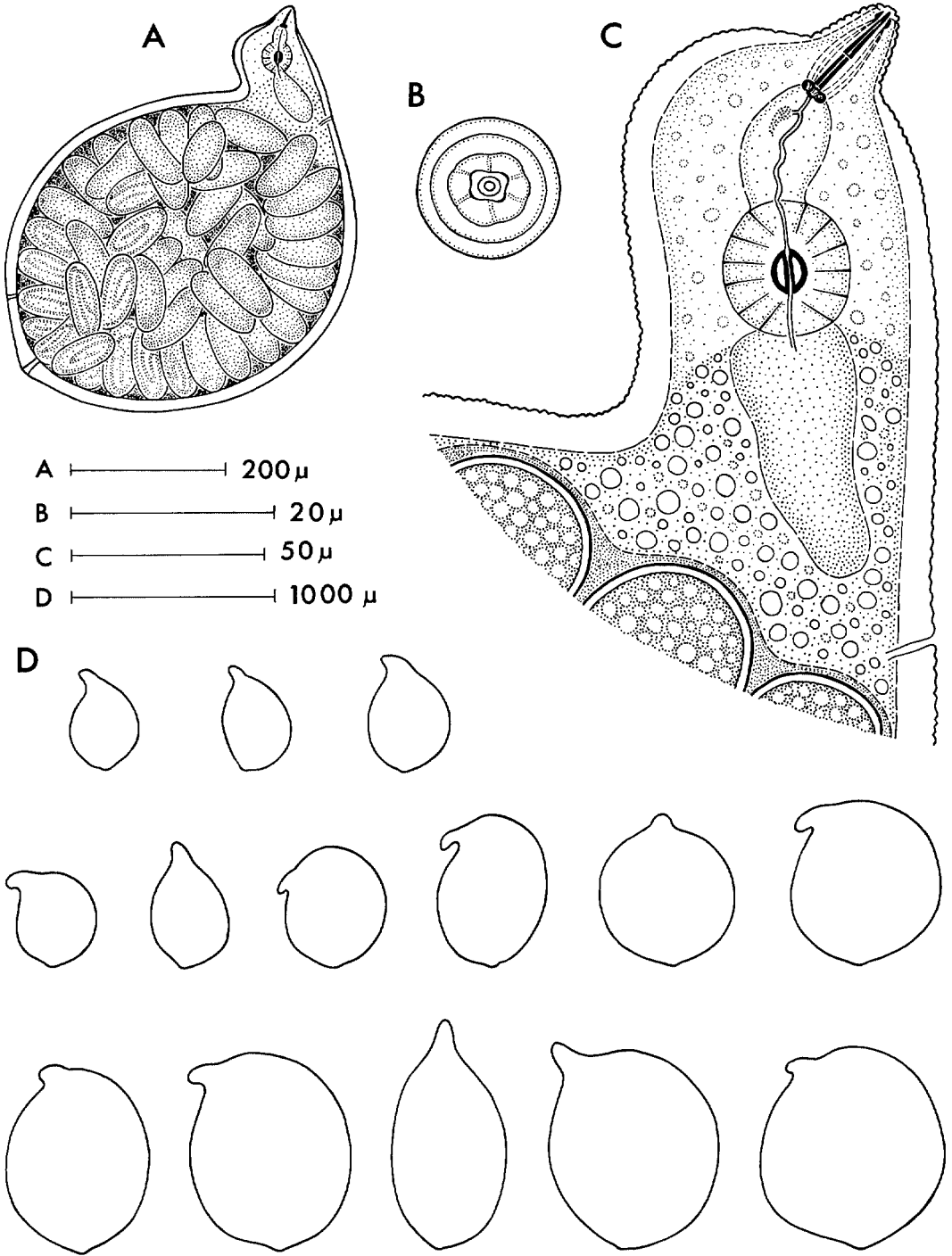
Length of vulval slit: 5.1–6.6 μ (5.84 μ \pm 0.16); width of vulval slit: 1.0–5.0 μ (2.20 μ \pm 0.33); distance from anus to nearest edge of vulva: 42.6–153.0 μ (80.87 μ \pm 7.95).

Description (Figs. 1, 2).—Body pearly-white, varying from slightly lemon-shaped in small individuals to pear-shaped and spheroidal in large specimens. Protrusion of vulval cone very slight (Fig. 1A), in some specimens hardly noticeable. Neck frequently slightly subterminal and characteristically curved posteriad. Abrupt bend between neck and body dorsally, in vicinity

of root; ventral side away from root bearing excretory pore and vulva (Fig. 1A). Cuticle thickest where neck and body join. Anterior region at level of stylet annulated (Fig. 1C); neck area with pronounced pimples; neck-to-body transition zone with broken longitudinal ridges; cuticle pattern over rest of body slightly zig-zag, wavy or network-like depending on size of individual. Subcrystalline layer thick, chalky-looking, crazed deposit, covering body, neck and head completely, with definite pattern in surface view and striated in longitudinal optical section (Fig. 2B). Labial disc tetroradial, distinctly sclerotized at the four corners (Fig. 1B). First lip annule hexaradial indicating 6 lips. Labial disc and first annule distinctly set off from rest of neck annules by constriction (Fig. 1C). Cephalic hexaradial framework indistinct (Fig. 1B). Stylet strong with well-developed backward-sloping knobs measuring about 3.5 μ in height. Brownish secretion sometimes adhering to head around oral opening. Dorsal esophageal gland orifice slightly behind stylet knobs; median bulb large, rounded, with big valve plates; esophageal glands contained in single lobe, overlapping ventrally. Excretory pore distinct, located approximately at end of gland lobe or slightly behind (Fig. 1C). Gonads two, characteristic of genus; germinal zone short, growth zone elongated, oviduct short and narrow, spermatheca well differentiated, hemispherical, uterus long and broad. Both gonads forming pronounced angle at spermatheca-uterus junction. Sperm absent in females examined. Vulva located on very small protrusion; vulval slit short but distinct, transparent halo around it indicating future opening (Fig. 2F). Gelatinous matrix pres-

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FIG. 1. Drawings of females and cysts of *H. betulae* n. sp. A—Female filled with eggs (lateral); B—Face view of female; C—Anterior portion of female (lateral); D—Shape and size ranges of cysts.



ent, no eggs deposited in it. Anus small but distinct (Fig. 2E).

Measurements of holotype in glycerin.—Body length (without neck): 450 μ ; body width: 403 μ ; body length/body width ratio: 1.1; stylet length: 30.6 μ ; dorsal esophageal gland orifice: 4.5 μ ; median bulb length: 33.2 μ ; median bulb width: 36.1 μ ; head end to end of median bulb: 87.8 μ ; excretory pore from anterior end: 140.4 μ ; vulva-anus distance (lateral view): 78.0 μ ; thickness of cuticle: 12.7 μ . Female as in general description. Body almost spheroidal, neck not curved posteriad, cuticle pattern network-like. Subcrystalline layer not retained completely.

CYSTS: Measurements of 50 cysts in 2% formalin.—Length (without neck): 435–1160 μ (mean 762.6 μ ; 95% confidence interval \pm 55.67); width: 340–920 μ (616.4 μ \pm 42.83); length/width ratio: 1.0–1.8 (1.2 \pm 0.04).

Measurements of 50 cyst cone tops in glycerin jelly.—Long axis of vulval fenestra (A): 11.9–29.8 μ (21.96 μ \pm 1.16); short axis of vulval fenestra: 10.2–27.7 μ (18.76 μ \pm 1.12); distance from anus to nearest edge of vulval fenestra (B): 44.9–153.0 μ (80.96 μ \pm 5.50); Granek's ratio B/A: 1.8–7.5 (3.8 \pm 0.34).

Description (Figs. 1, 2).—Size and shape of cysts similar to white females (Fig. 1D). As in females, neck in many cysts curved posteriad and not terminal in long axis of cyst. Cyst color ranging from light brown to dark brown to blackish brown, some cysts appearing almost black throughout. Yellow phase lacking as females age; white females turning first light brown, then darker in color. Thick subcrystalline layer present also in young brown cysts, covering whole cyst including neck; adhering for some time. Underlying cyst wall rather transparent, larvae in eggs are easily visible through it

with magnification in incident light. Cyst wall in neck area very thick and dark colored. Cyst wall pattern varying with age and size of cysts; in large cysts appearing as continuous irregular network-like pattern in the middle of the body with intermittent short broken lines (Fig. 2A); around vulva and anus exhibiting long, broken zig-zag lines (Fig. 2E); where cyst body merges into neck, regular pattern changing to broken longitudinal ridges which, further anterior in the neck region, become arranged in transverse rows of pronounced wart-like, pimples (Fig. 2C). Pimples, longitudinal ridges in transitional area, and general reticulate pattern over the body represent raised areas on the cyst surface which give a rugose surface contour in optical section (Fig. 2D). Cyst surface dull-matte when viewed under low magnification in incident light. No punctations observed in inner, deeper layers of cyst wall. Vulval cone very insignificant and obtuse (Fig. 1D). Vulval protrusion in most cysts darker colored than rest of cyst. Cysts circumfenestrate. As females age and turn into brown cysts, thin-walled area surrounding vulval slit (Fig. 2F) breaks down forming a single fenestra; remains of vulval slit lost. Vulval fenestra circular to oval-shaped (Fig. 2E, G). Occasionally, even in older brown cysts, vulval fenestra not broken through, still covered by a thin membrane. Vaginal remnant and underbridge not observed. In several cysts, deeper focus at fenestra and anal openings revealed brown, bullae-like structures clustered in the cone (Fig. 2H). In some cysts 2 V-shaped lines running anterior from anus caused an interruption in the anal area. Anus located in small hyaline depression (Fig. 2E); lining of rectum thick and well visible. In one cyst the thin transparent area around anus was broken through leaving a circular opening.

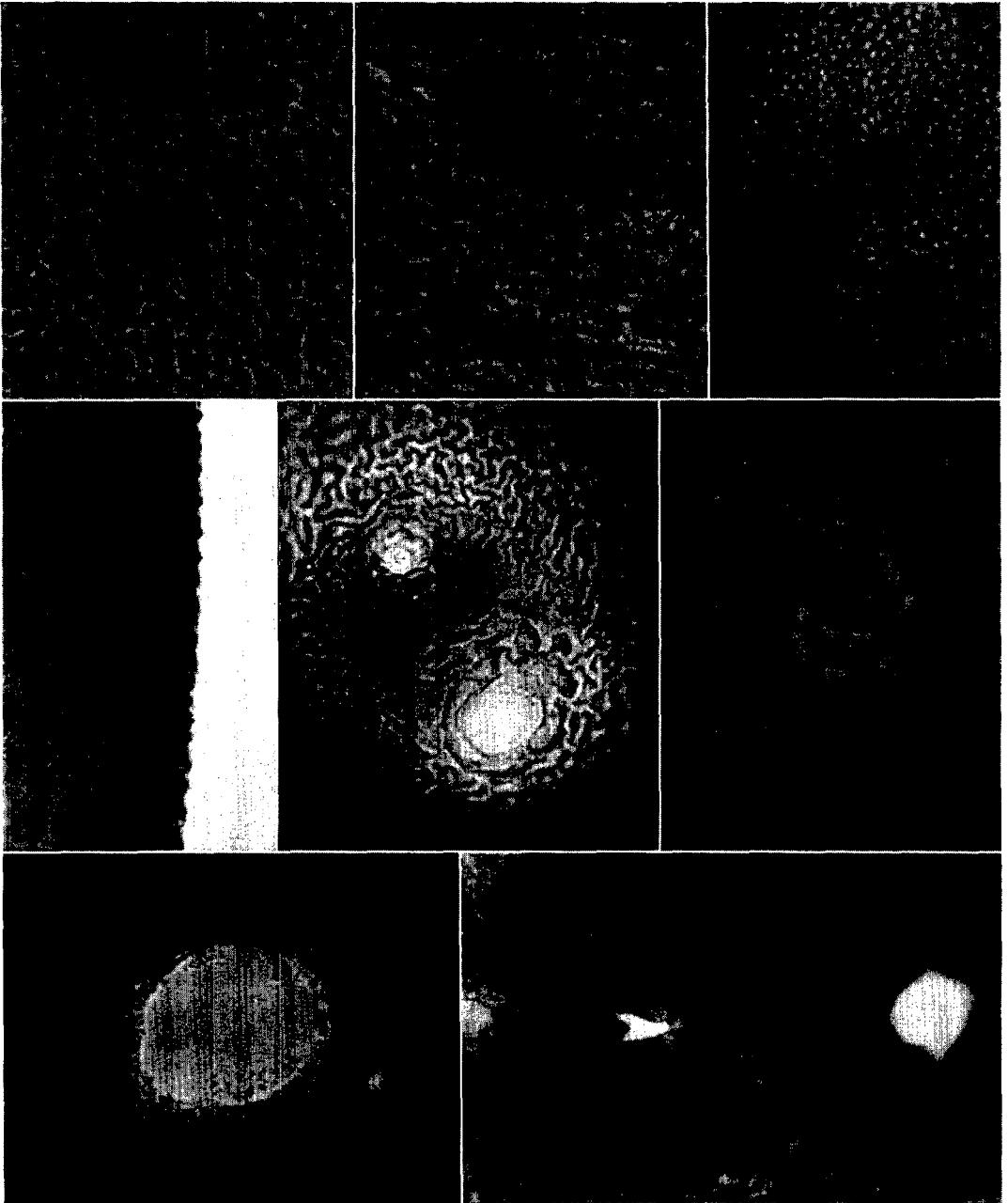


FIG. 2. Photomicrographs of female and cyst characters of *H. betulae* n. sp. A-D, F, G $\times 900$; E, H $\times 300$. A—Typical network-like cyst wall pattern from middle portion of large cyst; B—Pattern of subcrystalline layer of young white female; C—Pronounced pimples in neck region of cyst; D—Cyst wall in longitudinal section showing rugose surface contour; E—Surface view of cyst cone top with single fenestra (large opening) and anus (small opening); F—Vulval area of white female during early stage of fenestration; G—Fenestra of brown cyst broken through completely; H—Bullae-like structures clustered around fenestra and anus on inside of cyst cone at lower focusing level.

TABLE 1. Measurements of second-stage larvae of *Heterodera betulae* n. sp.

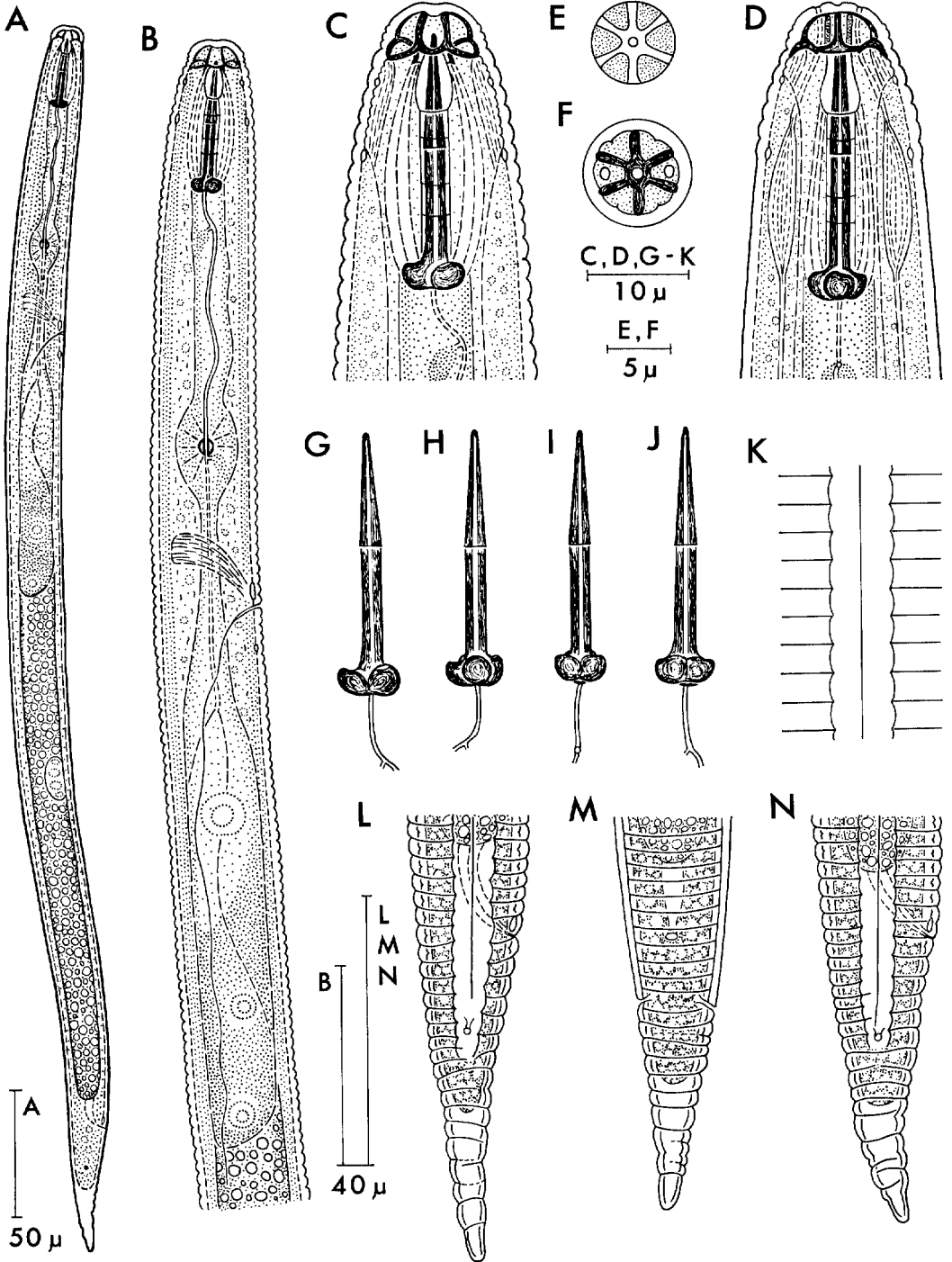
Character	Range	Mean	Confidence Interval		Standard Deviation	Coefficient of Variability (%)
			95%	99%		
<i>Linear</i> (μ)						
Body length	407.7–508.5	461.67 \pm 3.59 \pm 4.75			18.8172	4.1
Body width	20.4– 24.6	21.81 \pm .19 \pm .25			.9931	4.6
Stylet length	24.6– 27.6	25.95 \pm .11 \pm .15			.5941	2.3
Stylet knobs (height)	2.4– 3.7	2.93 \pm .05 \pm .06			.2519	8.6
Stylet knobs (width)	5.3– 6.6	5.93 \pm .05 \pm .06			.2527	4.3
Stylet shaft and knobs	13.2– 14.9	13.98 \pm .08 \pm .10			.3940	2.8
Dors. esoph. gl. orifice	5.0– 7.8	6.00 \pm .10 \pm .13			.5000	8.3
Esophagus length	112.7–139.6	126.55 \pm .90 \pm 1.19			4.7154	3.7
Excretory pore	95.5–117.2	107.09 \pm .75 \pm 1.00			3.9472	3.7
Annule width	1.9– 2.4	2.16 \pm .02 \pm .03			.0979	4.5
Tail length	39.0– 51.0	43.84 \pm .46 \pm .61			2.4044	5.5
Tail terminal length	14.7– 25.5	20.79 \pm .31 \pm .41			1.6057	7.7
Width of term. at beginning	7.0– 8.2	7.51 \pm .04 \pm .05			.2017	2.7
Width of term. 5μ from end	2.6– 3.7	3.11 \pm .05 \pm .06			.2507	8.1
Phasmids (from tail end)	25.0– 37.4	31.56 \pm .47 \pm .62			2.4564	7.8
Genital primordium (from tail end)	144.4–191.4	173.16 \pm 1.89 \pm 2.51			9.9203	5.7
<i>Ratios</i>						
a	18.5– 23.3	21.20 \pm .19 \pm .26			1.0191	4.8
b	3.3– 4.2	3.65 \pm .03 \pm .04			1.462	4.0
c	9.0– 11.9	10.55 \pm .09 \pm .12			4.731	4.5
Caudal ratio A	2.0– 3.5	2.77 \pm .04 \pm .06			.2209	8.0
Caudal ratio B	4.1– 8.4	6.73 \pm .14 \pm .18			7.105	10.6
Tail terminal/stylet length	.6– 1.0	.80 \pm .01 \pm .02			.0608	7.6
<i>Percentages</i>						
Excretory pore	21.9– 25.2	23.21 \pm .11 \pm .14			.5618	2.4
Genital primordium	33.8– 42.2	37.51 \pm .31 \pm .42			1.6465	4.4

EGGS: *Measurements of 100 eggs in 1% formalin.*—Length: 97–120 μ (mean 109.0 μ , 95% confidence interval \pm 0.88); width: 45–53 μ (48.4 μ \pm 0.39); length/width ratio: 1.9–2.6 (2.3 \pm 0.03).

Description.—Eggs thin-shelled, unsculptured. Fully developed larvae in eggs folded 4 times. Number of eggs in cysts correlated with size of cyst, however, cyst and egg size not correlated. Number of eggs in small cyst of 435 by 340 μ was 86; in average size cyst of 790 by 640 μ was 725; and in large cyst of 1160 by 875 μ , was 1969 eggs. No giant eggs were observed.

SECOND-STAGE LARVAE: *Description* (Fig. 3).—Measurements of 108 larvae (3 larvae from each of 36 cysts) in 1% formalin are shown in Table 1. Body cylindrical, tapering posteriorly more than anteriorly (Fig. 3A). When killed by gentle heat, larvae slightly twisted, true lateral position infrequently encountered. Lip region slightly set off from body, lip annules indistinct, only 3–4 very fine annules anterior to constriction of lip region observed in lateral or median view (Fig. 3 C, D). Cuticular annulation over rest of body distinct (Fig. 3B). Lateral field with 3 incisures, 4.59 μ wide (range: 4.1–

FIG. 3. Drawings of second-stage larvae of *H. betulae* n. sp. A—Full-length outline (lateral); B—Esophagus region (lateral); C—Cephalic region (lateral); D—Cephalic region (dorsal); E—Face view at level of lips; F—Face view at level of basal plate; G—Stylet (lateral); H—Stylet (dorsal); I—Stylet (ventral); J—Stylet (dorsolateral); K—Lateral field; L, N—Tails (lateral); M—Tail (ventral).



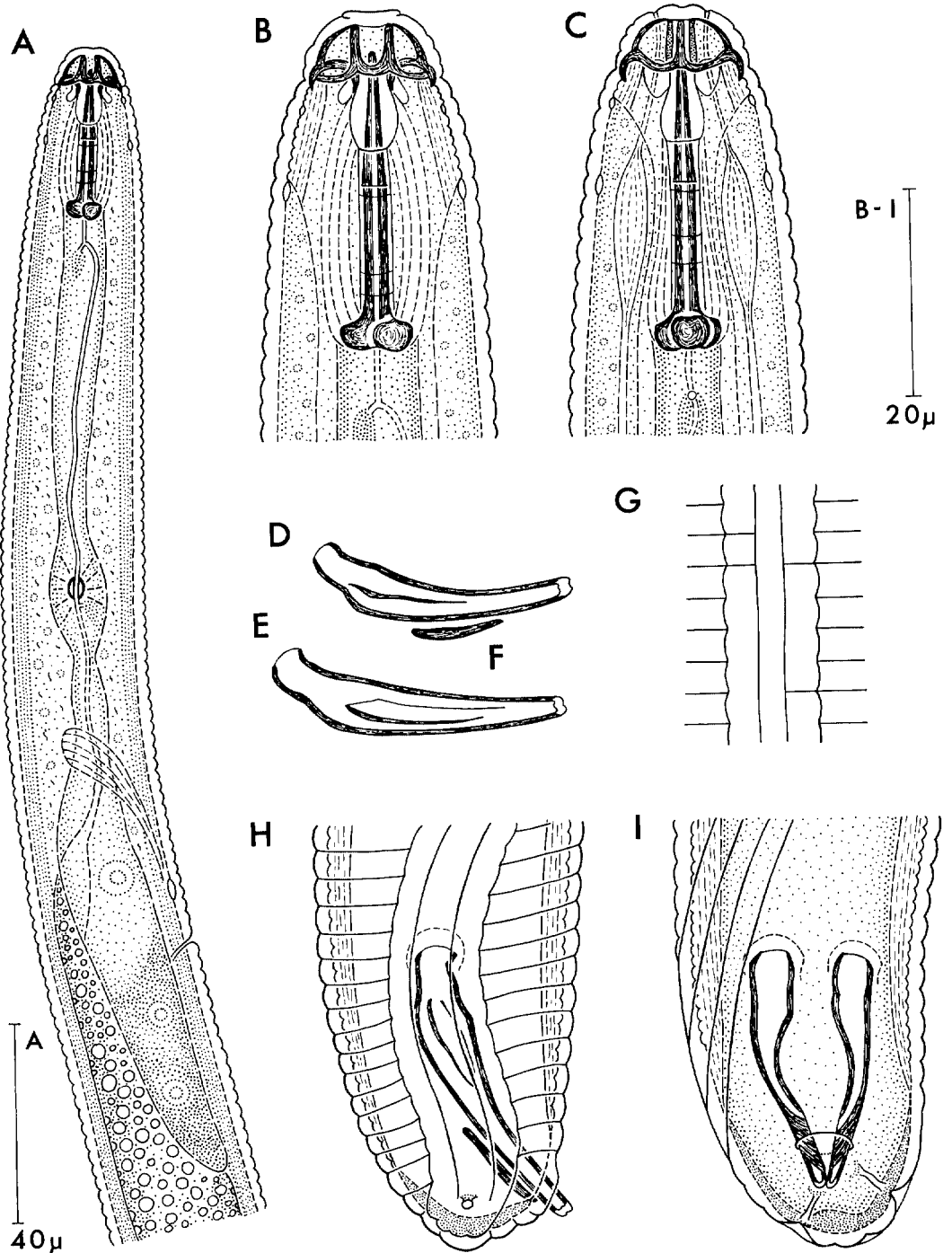
5.1 μ ; n = 28) in region of genital primordium, beginning at level of stylet shaft as 2 incisures, increasing to 3 incisures at dorsal gland orifice; outer incisures usually faintly crenate (Fig. 3K); middle incisure ending shortly in front of phasmids, outer incisures continuing a short distance posteriad (Fig. 3 L, N); very slight areolation in anterior and posterior end of lateral field. Cephalic framework heavily sclerotized, radial blades extending short distance over lip constriction into body (Fig. 3 C, D). Anterior cephalids located 2 annules, posterior cephalids 6 annules behind lip constriction. Stylet strong with well-developed knobs, slightly concave on anterior surface; shape of knobs appeared variable depending upon position of larva (Fig. 3 C, D, G–J). Dorsal gland orifice difficult to observe. Amphidial pores, ducts and pouches distinct in median view; pouches with sensillae extending approximately the length of stylet shaft (Fig. 3D). Esophagus with valvate, oval median bulb and ventrolateral gland overlap (Fig. 3B); intestine in area of well-defined junction located dorso-laterad. Difference in texture between 3 esophageal gland bodies pronounced: dorsal gland lobe clear and light, 2 subventral gland bodies granular and dark. Hemizonid distinct, 1½ annules long, located anterior and adjacent to excretory pore; terminal excretory duct visible up to end of esophageal gland lobe. Hemizonion ¾ annule long, 3 to 5 annules posterior to excretory pore. Genital primordium distinct, consisting of 2 small epithelial cells and 2 large central germinal cells (Fig. 3A). Anus distinct; caudalid, one annule long, located anterior and adjacent to anus. Shape of tail and hyaline tail terminal as illustrated in Fig.

3L–N, tail with bluntly rounded terminus. Phasmids small but distinct, location as in Table 1. No giant larvae observed, although a few dwarfed specimens were encountered.

MALES: Measurements of 10 males in 1% formalin.—Body length: 831–1280 μ (mean 1063.2 μ , 95% confidence interval \pm 121.42); body width: 26.5–36.4 μ (30.9 μ \pm 2.32); stylet length: 28.5–29.6 μ (29.1 μ \pm 0.27); stylet knobs (height): 2.9–3.5 μ (3.2 μ \pm 0.12); stylet knobs (width): 5.8–6.8 μ (6.3 μ \pm 0.24); stylet shaft and knobs: 15.0–16.3 μ (15.9 μ \pm 0.31); dorsal esophageal gland orifice: 3.6–5.8 μ (4.5 μ \pm 0.49); esophagus length: 136.2–163.8 μ (148.5 μ \pm 5.78); excretory pore: 130.1–185.4 μ (153.9 μ \pm 12.24); tail length: 5.1–10.2 μ (6.2 μ \pm 1.03); testis length: 335–710 μ (538.8 μ \pm 99.79); spicule length: 23.3–30.6 μ (27.8 μ \pm 1.45); gubernaculum length: 8.2–10.9 μ (9.2 \pm 0.57); size of sperm: 3.6–4.6 μ (4.0 μ \pm 0.31); a: 27.2–38.9 (34.3 \pm 2.38); b: 5.6–8.7 (7.2 \pm 0.81); c: 122.5–251.0 (176.2 \pm 26.00); T % : 39.0–59.1 (50.2 \pm 5.44); excretory pore % : 12.3–16.8 (14.6 \pm 1.08).

Description (Fig. 4).—Body cylindrical, slender, assuming the shape of an open C when killed by gentle heat; tapering more anteriorly than posteriorly. Posterior part of some males twisted through 90° after killing with gentle heat. Lip region of normal height, hemispherical, slightly offset from body (Fig. 4B, C). Annules in lip region large, irregular in number varying within and between specimens from 3 to 5 (not including lip cap). This may indicate that some of the transverse lip striations do not complete a full circle or that one striation

FIG. 4. Drawings of males of *H. betulae* n. sp. A—Esophagus region (lateral); B—Cephalic region (lateral); C—Cephalic region (dorsal); D, E—Spicules of different males (lateral); F—Gubernaculum (lateral); G—Lateral field; H—Tail (lateral); I—Tail (ventral).



may split into two. Coarse cuticular annulation over rest of body (Fig. 4A). Lateral field with 4 equally spaced incisures, (inner incisures sometimes slightly closer); outer lines only very slightly crenate, in most places straight, a few areolations present (Fig. 4G); ending as broad band in tail region (Fig. 4H, I). Cephalic framework heavily sclerotized (Fig. 4B, C). Anterior cephalids located 2 annules, posterior cephalids 6 annules, behind lip constriction. Stylet strong with well-developed knobs that are anteriorly straight or slightly hollowed out (Fig. 4B, C). Dorsal gland orifice short distance behind stylet knobs. Amphidial pores, ducts and pouches distinct in median view; pouches with sensillae extending over middle part of stylet (Fig. 4C). Cylindrical procorpus of esophagus followed by oval elongate median bulb with well-developed valve plates (Fig. 4A); gland lobe varying in length, glands retaining distinct entity, of similar difference in texture as in larvae; junction with intestine distinct. Hemizonid $1\frac{1}{2}$ to 2 annules long, $\frac{1}{2}$ to 5 annules in front of excretory pore. Hemizonion not observed. No spermatogonia observed in testis of any of the specimens studied. Gonad packed with spermatozoa behind cap cell. Lower part of vas deferens glandular, not filled with sperm. Spicule characteristically shaped (Fig. 4D, E) rather straight with slightly bifid end; gubernaculum simple, $\frac{1}{3}$ length of spicule (Fig. 4F). Tail very short, rounded, coarse annulations extending around it (Fig. 4H, I). Phasmids present, subterminal, indistinct.

Measurements of allotype in glycerin.—

Body length: 1110 μ ; body width: 29.1 μ ; stylet length: 29.1 μ ; stylet knobs (height): 3.3 μ ; stylet knobs (width): 6.5 μ ; stylet shaft and knobs: 16.3 μ ; dorsal esophageal gland orifice: 4.8 μ ; esophagus length: 136.2 μ ;

excretory pore: 151.5 μ ; tail length: 9.0 μ ; testis length: 473.8 μ ; spicule length: 23.3 μ (ventral); gubernaculum length not measured; size of sperm: 3.6 μ ; a: 38.1; b: 8.2; c: 123.3; T % = 42.7; excretory pore % = 13.6. Male as in general description. Stylet knobs anteriorly slightly hollowed out. Amphidial pouches not visible. Hemizonid $1\frac{1}{2}$ annules long, located immediately in front of excretory pore. Posterior body twisted short distance in front of spicules. Spicules were measured in ventral view. Gubernaculum could not be measured.

DIAGNOSIS: *H. betulae* n. sp. is distinct among known *Heterodera* species by its morphology and that it parasitizes *Betula nigra*. Since it has circumfenestrate cysts with slight vulval protrusion, a comparison with *H. cacti* Filipjev & Schuurmans Stekhoven, 1941 and *H. weissii* Steiner, 1949, which have similar cysts, may be most useful.

H. betulae n. sp. can be distinguished from *H. cacti* on the basis of the following characteristics: Cyst size (averaging 763 by 616 μ in *H. betulae*, 497 by 447 μ in *H. cacti*); cyst shape (lemon-shaped, pear-shaped or spheroidal with very slight vulval protrusion in *H. betulae*, lemon-shaped or spheroidal with prominent vulval cone in *H. cacti*); cyst wall pattern in middle part of cyst (irregular network-like in *H. betulae*, basic pattern in *H. cacti* consisting of parallel lines running transversely around cyst, interrupted by short vertical or oblique lines); yellow cyst phase (lacking in *H. betulae*, present in *H. cacti*); subcrystalline layer (heavily developed in *H. betulae*, slightly developed in *H. cacti*); egg shell (unsculptured in *H. betulae*, exhibiting punctations in *H. cacti*); number of incisures in lateral field of second-stage larva (3 incisures in *H.*

betulae, 4 incisures in *H. cacti*); occurrence of males (rare in *H. betulae*, appear to be common in *H. cacti*); spicule length (averaging 27.8 μ in *H. betulae*, 33.5 μ in *H. cacti*).

H. betulae n. sp. differs from *H. weissi* in the following respects: Cyst size (averaging 763 by 616 μ in *H. betulae*, 541 by 348 μ in *H. weissi*); cyst shape (lemon-shaped, pear-shaped or spheroidal with very slight vulval protrusion in *H. betulae*, oval lemon-shaped with small obtuse vulval cone in *H. weissi*); cyst wall pattern in middle of cyst (irregular network-like in *H. betulae*, basic pattern in *H. weissi* consisting of parallel lines running transversely around cyst, interrupted by short vertical or oblique lines); stylet length of second-stage larvae (averaging 26.0 μ in *H. betulae*, 21.4 μ in *H. weissi*); tail terminal/stylet length ratio of second-stage larvae (0.8 in *H. betulae*, 1.0 in *H. weissi*); occurrence of males (rare in *H. betulae*, common in *H. weissi*); stylet length of male (averaging 29.1 μ in *H. betulae*, 24.7 μ in *H. weissi*); spicule length of male (averaging 27.8 μ in *H. betulae*, 33.5 μ in *H. weissi*).

Holotype.—Female. Isolated from greenhouse culture propagated on river birch, derived from original population collected by R. D. Riggs from type locality in Arkansas, U.S.A. Slide No. T-129t, United States Department of Agriculture Nematode Collection, Beltsville, Maryland.

Allotype.—Male. Same data as holotype. Slide No. T-130t, same collection.

Paratypes.—Females, cysts, larvae, eggs and males. Same data as holotype; United States Department of Agriculture Nematode Collection, Beltsville, Maryland and University of California Survey Collection.

Type Host and Habitat.—River birch, *Betula nigra* L., roots.

Type Locality.—South bank of Middle Fork of White River 1.75 miles upstream from Highway 16 bridge in Washington County, Arkansas.

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