Redescription and Life History of Contortylenchus brevicomi, a Parasite of the Southern Pine Beetle Dendroctonus frontalis[1]

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Abstract: Larval and adult life stages are described for *Contortylenchus brevicomi* (Massey) Rühm parasitizing a Mississippi population of *Dendroctonus frontalis,* the southern pine beetle. Fourth-stage larvae and free-living adult females of this species are identified and described for the first time. The life cycle of *C. brevicomi* can be reconstructed from this study. The adult female nematode lays eggs in a mature beetle. Larval development progresses within the hemocoel until fourth-stage larvae exit the host. Mating occurs in beetle galleries and only females enter an immature beetle host. *Key Words:* nematode-insect interaction, Scolytidae.

Contortylenchus brevicomi (Massey) Rühm, 1960, is an endoparasitic nematode of the southern pine beetle, *Dendroctonus frontalis* Zimmerman. Laboratory-reared beetles infected with *C. brevicomi* construct shorter galleries and lay fewer eggs than do uninfected beetles (4). Although *C. brevicomi* parasitism is relatively common in southern pine beetle populations (3), little is known about the life history of this nematode.

C. brevicomi was described originally as *Aphelenchulus brevicomi* by Massey (5) and transferred to the genus *Contortylenchus* by Rühm (9). Massey's description was based on parasitic female nematodes obtained from *Dendroctonus brevicomis* LeConte. Thong and Webster (11) synonymized *C. barberus* (Massey) Rühm, another parasite described from *D. brevicomis,* with *C. brevicomi.* They redescribed the parasitic female and provided descriptions of second- and third-stage larvae. Massey (7) later amended

Received for publication 2 November 1979.

[1]Based in part on an M.S. thesis submitted to the University of Florida by the senior author. Research supported in part by SEA, CSRS Grant No. 580-15-3.

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Florida Agricultural Experiment Stations Journal Series No. 2023.

his original description of *C. brevicomi* to include characters and life-stage descriptions previously assigned to *C. barberus;* first-stage larvae and adult males were described. None of these workers identified fourth-stage larvae and free-living adult females. The objectives of this study are to identify those undescribed stages of *C. brevicomi* and to reconstruct the life cycle of this nematode.

MATERIALS AND METHODS

The specimens of *C. brevicomi* examined were obtained from *D. frontalis* collected in the Homochito National Forest, Bude, Mississippi. Adult beetles were dissected in physiological saline immediately upon emergence from naturally infested loblolly pine (*Pinus taeda*) or 1–30 days after emergence. Beetles not examined at emergence were held in gelatin capsules at 5 C. Beetles reared in pine bolts in the laboratory for 1, 2, or 3 weeks also were dissected and examined for nematodes. Bark strips containing galleries constructed by nematode-infected beetles were placed in Baermann pans to allow extraction of any nematodes present. Nematodes found in beetles or beetle galleries were placed in FAA (formalin, acetic acid, ethanol, and water) or TAF (triethanolamine, formaldehyde, and water) for a minimum of 48 hours, dehydrated by a rapid glycerin infiltration method (10), and mounted in anhydrous glycerin. Parasitic female and adult male *C. brevicomi* were compared with the type specimens of C. L. Massey now housed at Rocky Mountain Forest Research and Range Experiment station, Lincoln, Nebraska. The sex of larvae was determined by the size, position, and growth of the genital primordium as described by Hirschmann (2) and Thong and Webster (12). All specimens were observed and measured on direct light and differential interference microscopes. Measurements are presented according to the de Man formula except for T_v (distance of the vulva from the tip of the mucro).

RESULTS

C. brevicomi was the only internal nematode parasite found in *D. frontalis.* Beetles dissected immediately upon emergence, as well as beetles examined 1–30 days after emergence, contained mature females, eggs, and second- and third-stage larval nematodes. Beetles retrieved from galleries after 1, 2, or 3 weeks contained gravid females, eggs, and second-, third-, and fourth-stage larvae. Fourth-stage larvae were recovered also from beetle galleries. Free-living adult male and female nematodes were found only in beetle galleries. Nongravid adult females were found in beetle larvae with head capsules measuring .203–.482 mm (probably first and second instars). Male and female nematodes were recovered from beetle egg niches, though no nematode parasitism of eggs was observed. Descriptions of *C. brevicomi* life stages follow:

Adult parasitic female (n = 25): L = 2.2 mm (1.6–3.3 mm); a = 30.5 (20.6–45.6); c = 62.8 (40.5–104.6); V = 94.6% (93.4–95.9%); T_v = 117.2 μm (86.0–160.0 μm). Females brownish-yellow in color, sausage-shaped, narrowing at anterior end. Body bent dorsally, lying in a slightly curved to spiral position when relaxed. Cuticle thick, moderately to coarsely striated. Head region annulated in some specimens. Stylet slender, 9–11 μm long with small basal knobs. Lip region conical, protuberant. Esophagus straight, lacking metacorpus; obscured by gonad. No nerve ring or excretory pore observed. Gonad monodelphic, prodelphic; ovary outstretched or reflexed for short distance. Spermatheca present, filled with spermatozoa. Uterus packed with eggs at various stages of development. Post-uterine sac not observed. Body constricted at vulva. Terminus rounded, ending in mucro. Rectum and anus not visable in all specimens. Tail shape and distance from vulva to terminus variable.

Adult free-living female (n = 12): L = 576.5 μm (486.0–636.0 μm); a = 43.8 (38.5–64.2); c = 25.7 (22.6–34.1); T_v = 46.5 μm (40.8–55.2 μm). Free-living female about same length as fourth-stage female but only half as wide. Stylet 11.2–12.0 μm long with prominent basal knobs. Nerve ring *ca.* 80 μm (73.0–102.0 μm) from anterior end. Excretory pore slightly anterior to nerve ring. Esophagus less clearly defined than in fourth-stage larvae. Esophageal glands long, extending dorsally 40% of total body length over intestine. Prodelphic gonad occupies posterior half of body. Ovary few-celled;

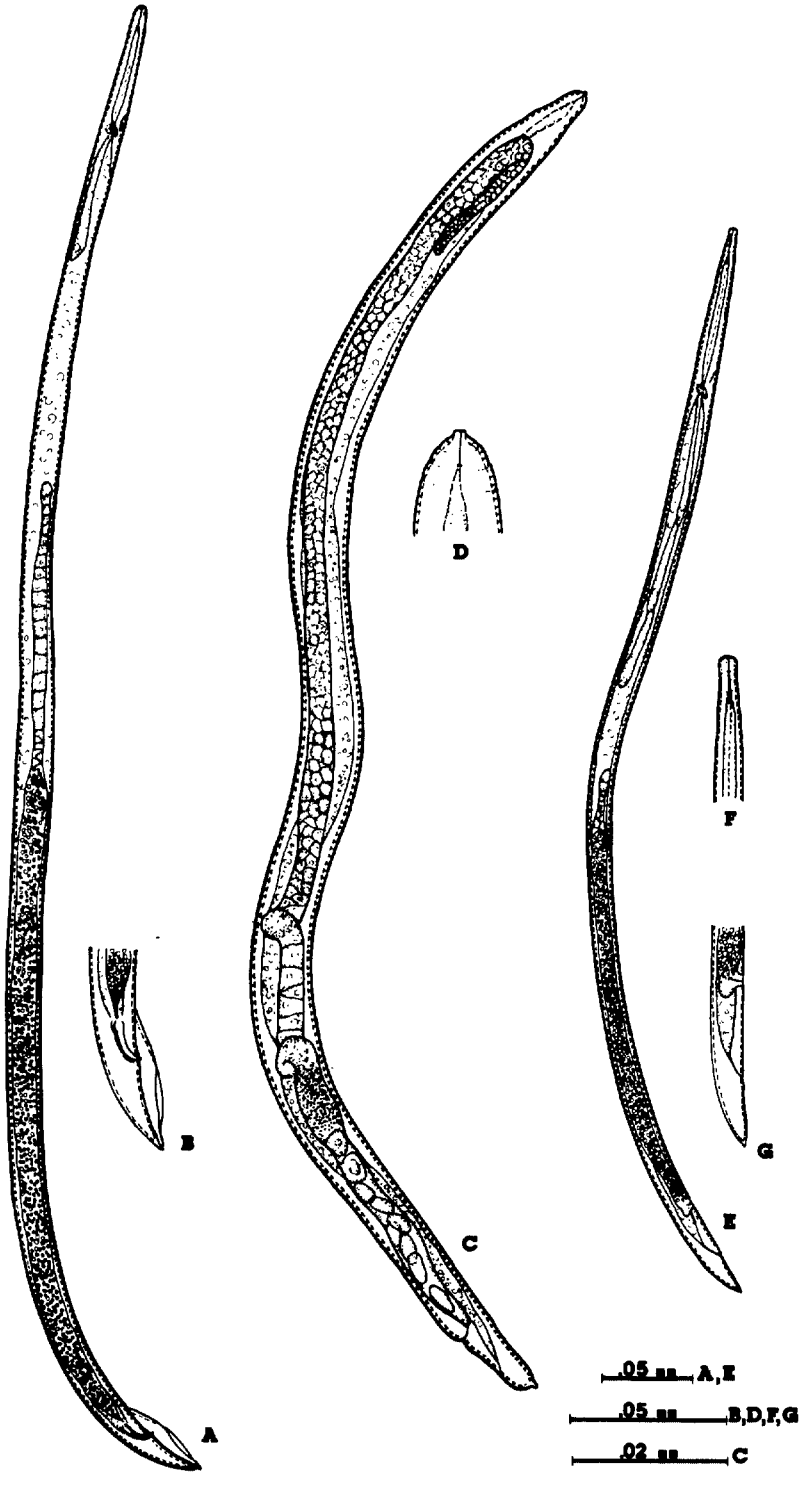

FIG. 1. Male and female adult stages of *Contortylenchus brevicomi*. A) Male. B) Tail region of male. C) Adult parasitic female. D) Head region of parasitic female. E-G) Adult infective female. F) Head region. G) Tail region.

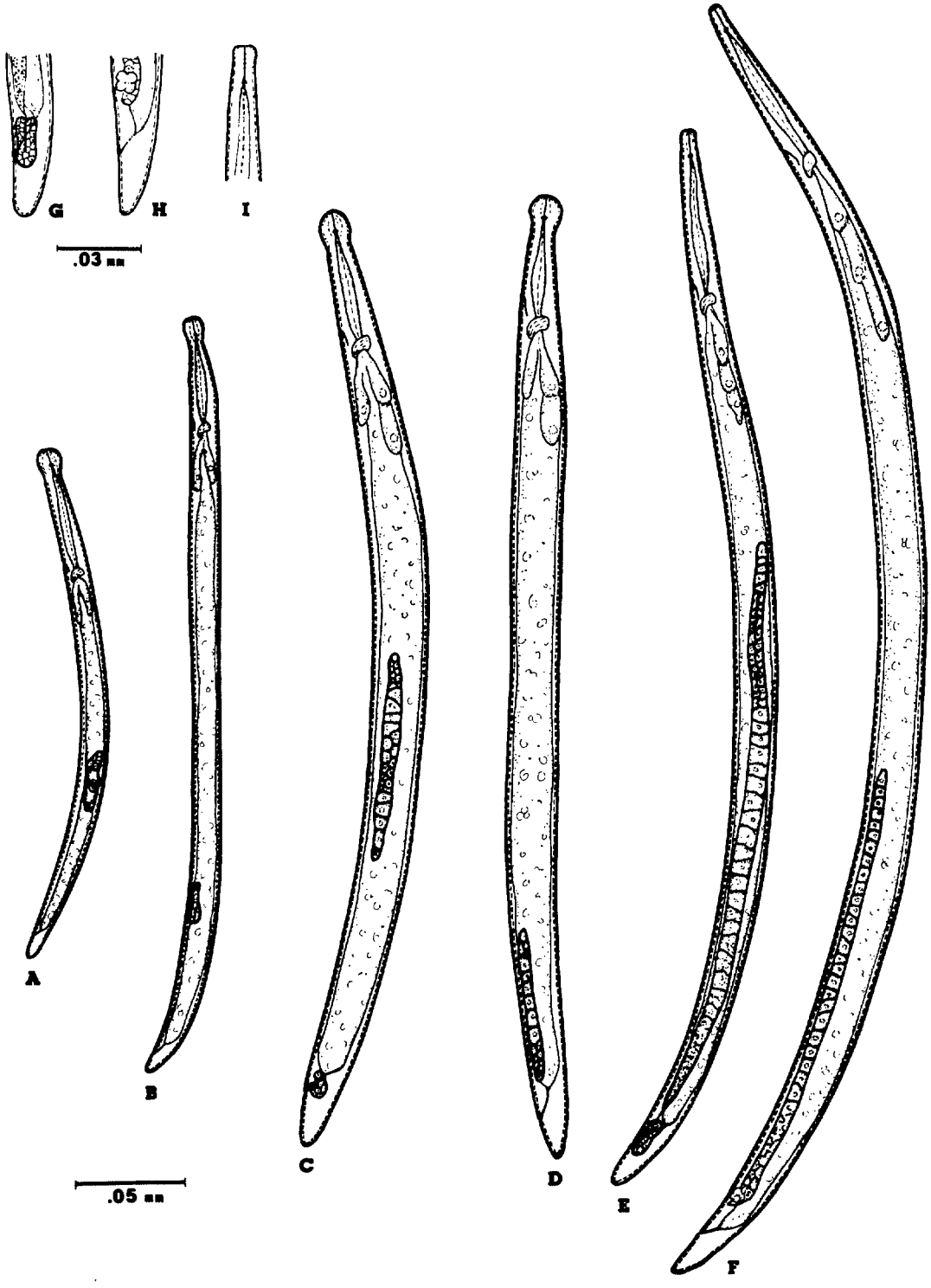

FIG. 2. Male and female larval stages of *Contortylenchus brevicomi*. A-B) Second-larval stage. A) Male. B) Female. C-D) Third-larval stage. C) Male. D) Female. E-I) Fourth-larval stage. E) Male. F) Female. G) Male tail region. H) Female tail region. I) Female head region.

uterus long and densely packed with spermatozoa. No eggs produced by this stage. Unlike gravid females, body not constricted at vulva. Rectum and anus not always distinct. Anal opening 22.6 μm (17.6–25.6 μm) from terminus. Developing mucro present in some specimens. This stage observed in beetle galleries.

Adult male (n = 10): L = 767.6 μm (674.9–880.0 μm); a = 50.7 (44.0–62.3); tail length 28.7 μm (27.2–32.8 μm). Body longer but about same width as free-living infective female. Cuticle finely striated. Stylet slender, 11 μm (11.0–12.0 μm) long with distinct basal knobs. Esophagus straight, narrowing at nerve ring. Nerve ring distinct, *ca.* 78 μm (72.0–88.0 μm) from anterior end. Esophageal glands shorter than in female, extending 140.0–183.0 μm from anterior end. Excretory pore obscure, less than one body width anterior to nerve ring. Testis outstretched, gonad 432.0–636.0 μm long. Spicules curved, *ca.* 17 μm long with slight enlargement at anterior end. Gubernaculum curved, troughlike, 5.2–6.0 μm long. Bursa enveloping tail. Male found only in beetle galleries.

Second-stage larvae, female (n = 13): L = 275.6 μm (201.6–352.8 μm); a = 22.1 (14.0–36.5); c = 23.6 (18.7–36.5). *Male* (n = 9): length 251.3 μm (170.4–324.0 μm); a = 20.8 (15.1–28.8); c = 18.1 (16.7–20.7). Male and female about same size. Cuticle very finely striated. Head region expanded, rounded. Stylet slender, 8.0 μm (7.9–9.6 μm) long with small delicate knobs. Nerve ring distinct, *ca.* 48.0 μm (36.0–62.0 μm) from anterior end. Excretory pore slightly anterior to nerve ring. Esophagus cylindrical, narrowing as it passes through nerve ring. Only two esophageal glands observed, one ventral and one dorsal to intestine. Genital primordium conspicuous, germinal nucleus visible. Cell division in male primordium in anterior somatic cell. Cell division in female promordium confined to posterior somatic cell. Rectum evident but anus obscure in most specimens. Smallest larval stage found free in host hemocoel.

Third-stage larvae, female (n = 16): L = 324.1 μm (276.0–446.4 μm); a = 21.0 (17.2–26.8); c = 20.3 (18.5–23.6). *Male* (n = 17): L = 317.8 μm (256.0–447.2 μm); a = 21.3 (16.1–26.7); c = 20.9 (19.0–23.7). Male and female about same size. Cuticle finely striated. Head region rounded, expanded as in second-stage larvae. Stylet slender, 8.0 μm (7.2–9.6 μm) long with small basal knobs. Esophagus cylindrical, narrowing at prominent nerve ring. Nerve ring *ca.* 52 μm (43.6–60.8 μm) from anterior end. Excretory pore less than one body width anterior to nerve ring. Anteriad growth of genital primordium in male larvae. Small spicular primordium present in rectal region. Posteriad growth of genital primordium in female larvae. Developing female gonad smaller than male gonad. Terminus of both sexes rounded. Rectum and anus evident. Anal opening *ca.* 16.0 μm from terminus. Third-stage larvae found only in host hemocoel.

Fourth-stage larvae, female (n = 10): L = 626.4 μm (446.0–758.0 μm); a = 28.2 (22.6–37.5); c = 29.9 (25.4–36.0). *Male* (n = 10): L = 593.4 μm (552.0–682.0 μm); a = 28.1 (21.7–34.2); c = 25.6 (20.5–28.5). Fourth-stage females slightly longer but about same width as fourth-stage males. Other measurements comparable. Stylet slender, 11.2–12.0 μm long. Excretory pore less than one body width anterior to nerve ring. Nerve ring prominent, *ca.* 72.0 μm (68.0–79.0 μm) from anterior end. Three long overlapping esophageal glands extend *ca.* 65 μm behind nerve ring. Genital primordium more strongly developed in males (320.0–420.0 μm long) than in females (90.0–340.0 μm long). Differentiation of male gonad evident; vas deferens not connected to rectum. Spicular primordium present surrounding rectum. Cell mass, which will develop into vulva, visible in females *ca.* 46 μm from terminus. Tail lengths of males slightly longer than females. Male terminus more rounded than female terminus. Rectum and anus visible in most specimens. Fourth-stage larvae found in host hemocoel, hindgut, and beetle galleries.

DISCUSSION

The life cycle of *C. brevicomi* parasitizing *D. frontalis* can be reconstructed from this study. Our observations support findings on the life history of *Contortylenchus elongatus* (Massey) Rühm by Massey (6) and Nickle (8). Fourth-stage larvae exit the host beetle through the digestive or repro-

ductive tract. After mating, males die in the beetle galleries and impregnated adult females enter immature beetles. The mode of nematode entry into *D. frontalis* was not observed. Maturation of nematode females and host occur simultaneously. Mature nematodes lay eggs in adult beetles, which hatch as second-stage larvae. Second-stage larvae molt to the third stage in emerging adult beetles but do not develop into fourth-stage larvae until the beetle host attacks a tree. Synchrony of the life cycles of nematodes and their insect hosts was suggested by Massey (6,7) and others (1). Our investigations indicate that they were correct and explains why fourth-stage larvae were not identified by Massey or by Thong and Webster; their descriptions were based on nematodes found only in teneral adult beetles.

Massey (5,7) and Thong and Webster (11) disagree on several characters of the parasitic female. In agreement with Massey, we observed a spermatheca filled with spermatozoa and did not observe a postuterine sac in mature females. In agreement with Thong and Webster, the gonad in the majority of our specimens was reflexed.

Our descriptions of fourth-stage larvae and free-living adult females of *C. brevicomi* are similar to descriptions of other *Contortylenchus* spp. Contrary to illustrations of all stages of *Contortylenchus* spp. by other workers (7,8), however, the majority of our larval and preparasitic specimens did not lie in a dorsally bent position.

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