Redescriptions of *Neodiplogaster tropica* Cobb and *N. pinicola* Steiner (Nematoda: Diplogasteridae)

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Abstract: Neodiplogaster tropica is redescribed from syntypes, and *N. pinicola* from syntypes and newly collected material. Information on the previously undescribed female tail and reproductive system is given for *N. tropica*, and details of the stoma, female gonad, spicules, gubernaculum, and excretory system are emphasized for *N. pinicola*. Lectotypes are designated for both species. The predaceous feeding of *N. pinicola* is described. *Key Words:* Taxonomy, Stoma, Excretory system, Predaceous feeding, White pine beetle.

Syntypes of *Neodiplogaster tropica* Cobb, 1924 and *N. pinicola* Steiner, 1930 were found in the U.S.D.A. Nematode Collection. These specimens, as well as newly collected specimens of *N. pinicola*, were used to redescribe these species. In addition, a brief description of the predaceous feeding of *N. pinicola* is presented below.

MATERIALS AND METHODS

Syntypes of *N. tropica* and *N. pinicola* mounted in glycerine were from the U.S.D.A. Nematode Collection, Beltsville, Maryland. The *N. pinicola* specimens were in good condition, but unfortunately the *N. tropica* specimens were so poorly preserved that little could be added to the original description of Cobb (1) except information on the female tail shape and reproductive system. Lectotypes were chosen from the syntypes of both species.

Living specimens of *N. pinicola* were extracted from frass of the white pine beetle taken from under the bark of white pine terminal shoots. Beetle-infested white pine material was collected at Orange, Connecticut and Branford, Connecticut in late June of 1969, and at Branford in June, 1970. Specimens from all collections were fixed in formalin and transferred to glycerine by the slow method. Heads and male tails were severed and mounted in glycerine jelly.

Nematodes from the 1970 collection were placed together with *Panagrellus redivivus* on water agar in petri dishes, where they fed and reproduced. They were studied alive or relaxed in water, or relaxed and mounted in formalin or formalin and methylene blue, or formalin-fixed specimens were processed to glycerine. The excretory system was studied according to the method of Sanwal (3).

Measurements of all populations were made with an ocular micrometer on specimens mounted in glycerine. Spicule measurements were taken along the curve from *camera lucida* sketches. The terminology of J. B. Goodey (2) is used for parts of the stoma.

Neodiplogaster tropica Cobb, 1924

Measurements (♂ Lectotype): L = 0.680 mm; a = 41; b = 5.4; c = 23; spicules = 0.043 mm; gubernaculum = 0.017 mm.

(♀ Allolectotype): L = 0.810 mm; a = 34; b = 6.2; c = 7.7; V = 50%.

(2 ♂♂ Paralectotypes): L = 0.499, 0.487 mm; a = 37, 32; b = 4.2, 4.2; c = 17, 25; spicules = 0.040, 0.038 mm; gubernaculum = 0.014, 0.014 mm.

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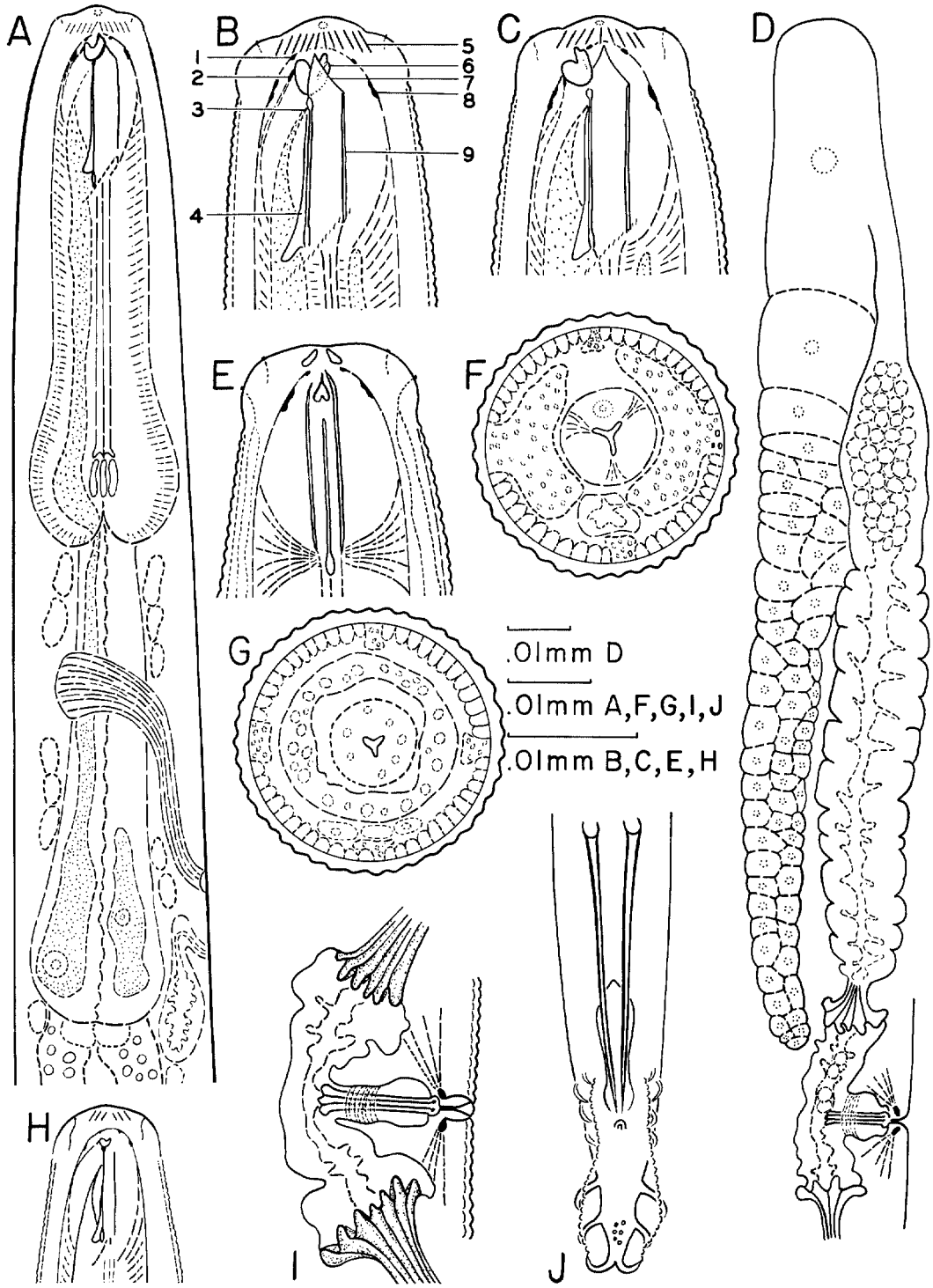
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(6 ♀♀ Paralectotypes): L = 0.587 (0.465–0.802) mm; a = 32 (31–35); b = 5.3 (4.1–6.5); c = 7.5 (6.6–8.2); V = 49 (44–53)%.

Females.—Cuticle with fine transverse striae and coarse longitudinal striae. Head continuous with body. Cheilorhabdions surrounding oral aperture, number not determined. Dorsal metarhabdion a claw-shaped tooth at anterior end of narrow cylindrical telostom. Two basal wing-like muscles attached to base of telostom. Remaining details of stoma not verifiable because the specimens were poorly preserved. Esophagus diplogasteroid. Nerve ring just behind median bulb. Excretory pore ventral to basal bulb, leading to excretory sinus. Remainder of excretory system not visible.

Female reproductive system similar to that of *N. pinicola.* Vulva pore-like, vagina a narrow cylinder. Ovaries opposed, reflexed. Oviduct at flexure leading to convoluted uterus. Uterine sphincter present 2½ to 3 body widths from vulva.

Tail elongate conoid, tapering to filiform terminus (Fig. 2J). Phasmids not visible.

Males.—Anterior body similar to that of female. Tail conical, with bursa supported by seven pairs of long papillae (Fig. 1J, 3C). Posterior-most papillae form bifurcate tail terminus. Four to six small mid-ventral papillae present just in front of terminus. Spicules cephalated, slightly curved. Gubernaculum trough-like, triangular in lateral view (Fig. 3F).

Type Locality and Habitat.—Notation found among Cobb's records: "Cocoa rinds from Nicaragua, brought in by Dr. Irwin F. Smith. Published as from cocoa pods, Guatemala, in Helminthological Society of Washington, D.C. 12/17/24."

Distribution of Specimens.—Lectotype ♂ and allolectotype ♀ on slide T-193t, paralectotypes 2 ♂♂, 6 ♀♀ on slides T-929p to T-932p and slide T-193t, U.S. Dept. of Agric. Nematode Collection, Beltsville, Maryland.

Neodiplogaster pinicola Steiner, 1930.

Measurements (♂ Lectotype): L = 0.730 mm; a = flattened; b = 5.9; c = 22; spicules = 0.049 mm; gubernaculum = 0.019 mm.

(♀ Allolectotype): L = 0.900 mm; a = flattened; b = 7.0; c = 13; V = 52%.

(7 ♂♂ Paralectotypes): L = 0.624 (0.558–0.727) mm; a = flattened; b = 6.0 (5.2–7.2); c = 28 (25–32); spicules = 0.044 (0.039–0.049) mm; gubernaculum = 0.019 (0.018–0.020) mm.

(3 ♀♀ Paralectotypes): L = 0.768 (0.640–0.915); a = 28 (one specimen only, remainder flattened); b = 6.6 (5.6–7.6); c = 13 (12–14); V = 53 (50–55)%.

(93 ♂♂, Connecticut): L = 0.683 (0.525–0.960) mm; a = 25 (18–44); b = 5.7 (4.6–6.8); c = 23 (18–30); spicules = 0.044 (0.037–0.049) mm; gubernaculum = 0.020 (0.017–0.022) mm.

(53 ♀♀, Connecticut): L = 0.768 (0.558–1.245) mm; a = 27 (20–43); b = 6.3 (4.6–9.7); c = 12 (10–13); V = 53 (50–68)%.

(7 ♂♂ from culture): L = 0.721 (0.600–0.783); a = 24 (23–26); b = 5.2 (4.8–

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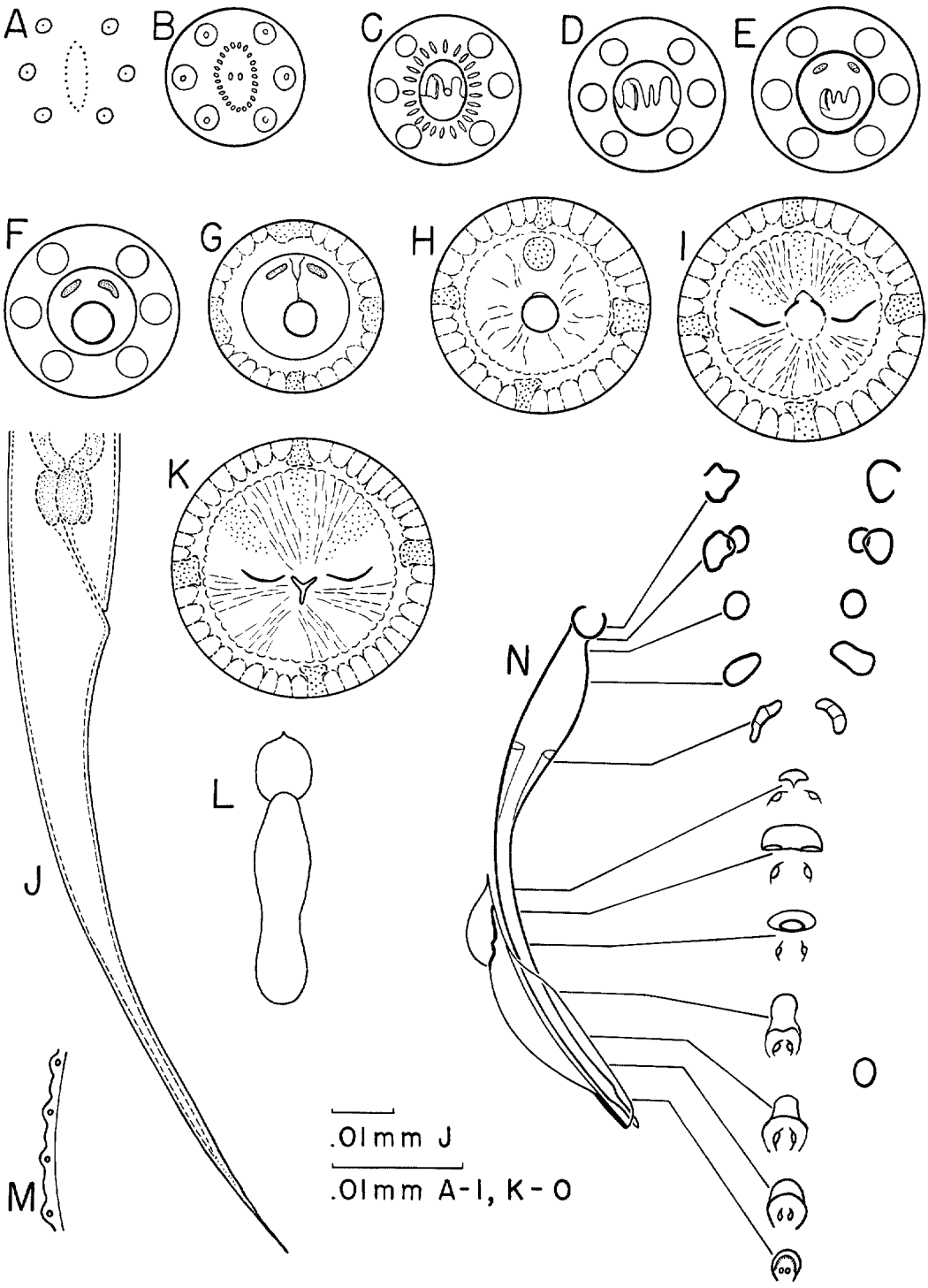
FIG. 1. **A–I.** *Neodiplogaster pinicola*: **A.** anterior end, large-toothed female; **B.** head, large-toothed female, lateral view; 1. prorhabdion, 2. muscle attachment of dorsal metarhabdion, 3. dorsal gland orifice, 4. tube dorsal to telostom, 5. cheilorhabdion, 6. dorsal metarhabdion, 7. right subventral metarhabdion, 8. Mesorhabdion, 9. ventral telorhabdion; **C.** head, large-toothed female, lateral view, tooth in feeding position; **D.** anterior half female reproductive system; **E.** head, large-toothed female, dorsal view; **F.** cross section through basal bulb and excretory sinus; **G.** cross section through cardia; **H.** head, small-toothed female, lateral view; **I.** vulva, vagina, and uterine sphincters; **J.** *N. tropica,* male tail, ventral view.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5.5); c = 23 (22–25); spicules = 0.044 (0.040–0.046) mm; gubernaculum = 0.020 (0.020–0.020) mm.

(10 ♀♀ from culture): L = 0.877 (0.787–0.990) mm; a = 24; (22–27); b = 5.9 (5.1–6.6); c = 12 (11–13); V = 51 (49–52)%.

Females.—Cuticle with fine transverse striae and longitudinal ridges (Fig. 1 F, G). At mid-body, cuticle between ridges slightly raised (Fig. 2M). Ridges marked with punctations in pattern described by Steiner (4). Cuticle not modified to lateral field over lateral chords.

Head smooth, nearly continuous with body, comprised of six amalgamated lips, each with a minute papilla (Fig. 2A). Amphid apertures on lateral lips, small, porelike.

In some of the females collected from frass the stoma was about half the size of the stoma in other females of about the same body length from the same collection (Fig. 1B, H). After several generations of culture all females had a large stoma. Cheilorhabdions narrow wedges arranged around dorso-ventrally elongate oral aperture, 12 present in specimens with small stoma, 18, 20, 22, or 24 in those with large stoma (Fig. 1B, C, H; 2A–C). Because of elongate oral aperture, only two cheilorhabdions visible in one plane in dorso-ventral view (Fig. 1E). Prorhabdions round in cross section, forming a ring around anterior metastom (Fig. 1B, 2C). Mesorhabdions fused into a short cylinder surrounding base of metarhabdions, sclerotization irregularly thickened in cross section (Fig. 1B). Dorsal metarhabdion a claw-shaped tooth (Fig. 1B, E; 2B–E), with two conspicuous muscles attached to dorsal surface (Fig. 1B; 2E–G), about 2 μ long in specimens with small stoma, about 4 μ long in those with large stoma. Right subventral metarhabdion a delicate triangular tooth (Fig. 1B, C, E; 2B–E), usually obscured by the more robust dorsal tooth unless dorsal tooth pulled back in feeding position (Fig. 1C). No tooth seen in left subventral sector of metastom. Telostom cylindrical, telorhabdions thickening slightly from anterior to posterior, dorsal telorhabdion also with anterior thickening. A thin-walled tube, triangular in cross section present dorsal to telostom (Fig. 1B, C, E, H; 2G–I), widening and extending dorsally at base of stoma. Two lateral wing-like groups of muscles attached to base of telostom, positioned obliquely, with anterior margin slightly ventral to telostom, posterior margin dorsal, slightly curved in cross section (Fig. 1B, C, E, H; 2I, K).

Procorpus muscular, muscles extending to base of mesorhabdions. Median bulb valved. Isthmus and basal bulb glandular, diameter of basal bulb less than that of median bulb. Lumen of esophagus and cardia triquetrous (Fig. 1F, G; 2K). Dorsal esophageal gland extending from basal bulb to telostom (Fig. 1A), orifice emptying into telostom just behind dorsal tooth (Fig. 1B, C; 2G).

Nerve ring surrounding isthmus just be-

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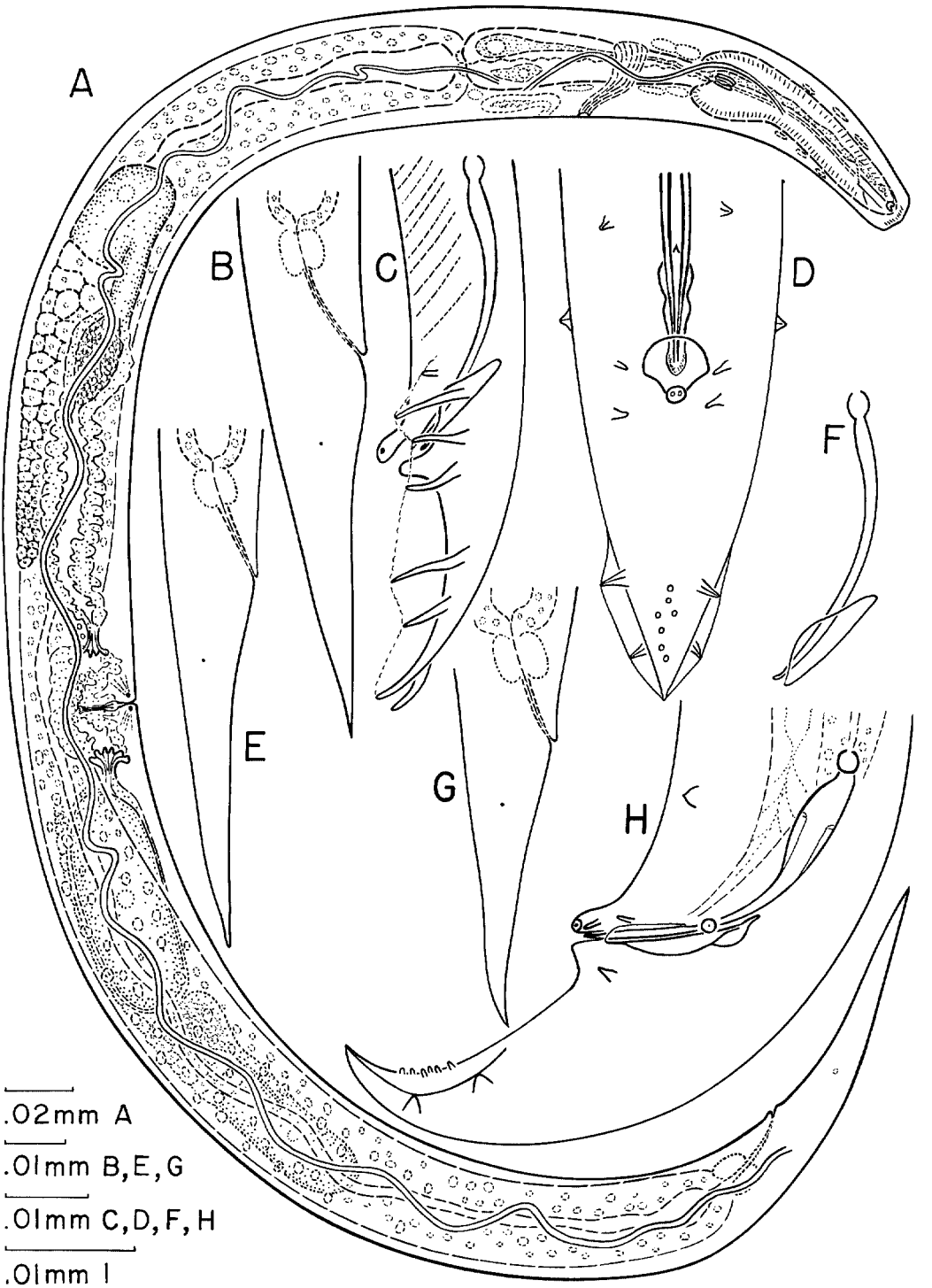
FIG. 2. **A–I.** *Neodiplogaster pinicola*: **A.** extreme anterior end, showing papillae and cheilorhabdions; **B.** cross section through cheilorhabdions, showing anterior ends of metarhabdions; **C.** cross section, anterior metastom; **D.** cross section, middle of metastom; **E.** cross section, base of metastom, showing muscles of dorsal metarhabdion; **F.** cross section, anterior telostom; **G.** cross section, level of dorsal esophageal gland orifice; **H.** cross section, base of telostom; **I.** cross section, basal stomatal musculature; **J.** *N. tropica*, female tail; **K–O.** *N. pinicola*: **K.** cross section, anterior esophagus; **L.** gubernaculum, dorsal view; **M.** cross section cuticle at mid-body; **N.** spicule and gubernaculum, lateral view; **O.** series of cross sections through spicule and gubernaculum (corresponding levels on spicule shown in **N.** indicated by pointers).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hind median bulb. Hemizonid just anterior to basal bulb, about 6 annules anterior to excretory pore. Deirids slightly behind pore.

Excretory pore ventral to basal bulb, excretory duct short, wide, leading to thick-walled sinus with rayed lumen (Fig. 1A, F). Lateral canals (Fig. 3A) present on both sides of body, sinuous, often looped, leading ventrally toward excretory sinus, but connections with sinus not seen. Canals visible anteriorly as far as half the distance between median bulb and head, and posteriorly to behind anus, but their termini were not seen.

Vulva porelike. Vagina cylindrical, surrounded by a sphincter (Fig. 1 I), with muscle attachment thickenings present just inside vulva. Gonads opposed, reflexed (Fig. 3A). Each uterus convoluted, with very conspicuous sphincter at proximal end, less than one body width from vulva (Fig. 1 D, I). Sperm stored at distal end of uterus; a few sperm just within vagina seen in some specimens. Oviduct narrow, joining ovary at flexure. Oogonia arranged in several rows.

Tail conical, tail length and shape of terminus variable (Fig. 3B, E, G). Phasmids at about 30% of tail length from anus. Anus a transverse slit, slightly curved with center more anterior.

Males.—Anterior portion of male body similar to that of females. Stoma of all males taken from frass small (dorsal tooth less than 2 μ). After several generations in culture some males with larger stomata, and after about ten weeks in culture, all males with large stomata (dorsal tooth 3 to 4 μ).

Testis single, reflexed. Spicules (Fig. 2N, O) separate, manubrium round, shoulder slightly wider than manubrium. Spicule tapering gradually to very slender terminus. Each spicule a single tube in cross section proximally, divided behind shoulder by two thin septa into a triple tube, distal half a single tube with dorsal and ventral flanges. Spicules flexible, so that the distance between distal and proximal ends could be less on a longer spicule than on a shorter one. Gubernaculum (Fig. 2L, N, O) constricted at about ⅓ its length, proximal terminus pointed, thickenings present at constriction, distal portion surrounding spicules dorsally and laterally.

Tail conical, curved ventrally (Fig. 3D, H). Six pairs of caudal papillae present: i) subventral preanal, ii) subventral adanal, iii) lateral adanal, iv) subventral postanal, v) subventral at ⅔ tail length, vi) subdorsal near terminus. In addition, six to eight small papillae occur in two uneven rows ventrally near terminus. Small bursa present posteriorly. Anterior anal lip protruding. Phasmids obscure, not observed. However, ducts leading from phasmids in preadult cuticle of molting specimens could be traced to two of the small ventral papillae, usually near the middle of the group of papillae.

Type Locality and Habitat.—Mines produced by the white pine beetle on terminal shoots of white pine, Boston, Massachusetts.

Distribution of Specimens.—Lectotype ♂ on slide T-191t; allolectotype ♀ on slide T-192t; paralectotypes 7 ♂♂, 3 ♀♀, 2 y, broken specimens, on slides T-915p to T-928p; Branford, G-2868, G-2869; Orange, G-2870, G-2871; *Panagrellus* culture, G-2872, G-2873. U.S. Dept. Agric. Nematode Collection, Beltsville, Maryland.

Bionomics.—*N. pinicola* specimens extracted from beetle frass and placed with

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FIG. 3. **A.** *Neodiplogaster pinicola*, female; **B.** *N. pinicola*, variation of female tail; **C.** *N. tropica*, male tail, lateral view; **D.** *N. pinicola*, male tail, ventral view; **E.** *N. pinicola*, variation of female tail; **F.** *N. tropica*, spicules and gubernaculum; **G.** *N. pinicola*, variation of female tail; **H.** *N. pinicola*, male tail, lateral view.

Panagrellus redivivus on water agar fed predaceously on the *Panagrellus* and reproduced. When the cultures were first established only the females with large dorsal metarhabdial teeth were seen to kill *Panagrellus* specimens. Males and larvae fed on dead prey, but were never seen to kill nematodes. Males attempted to penetrate the cuticle of living prey, but the prey always escaped before penetration was achieved. After about three weeks all females taken from the cultures had large teeth, and a few large-toothed males were also seen. After about ten weeks of subculturing only adults with large teeth were seen, and both males and females killed prey. It is probable that only large-toothed specimens were capable of penetrating the cuticle of prey rapidly enough to prevent its escape, and that, in the early stages of the cultures, the large-toothed females provided food for the small-toothed males and juveniles.

Penetration was accomplished by simultaneously pressing the lips against the prey, pulling back the dorsal tooth to expose its point, and moving telorhabdions and metarhabdial teeth forward so that the metarhabdions extended out of the oral aperture and punctured the prey. Often the body contents of the prey gushed out of the wound and was immediately ingested by the predator by action of its anterior esophagus. Usually the dorsal tooth was held back during this process (Fig. 1C), probably to allow easier passage of the food. If eggs developing within the prey came out with the body contents, the predator punctured them one by one as they emerged and ingested their contents. Once the ejected body contents were consumed, the remaining contents of the prey were removed through fresh wounds made in the cuticle, or the prey was abandoned.

When all *Panagrellus* in a dish were killed, the predators fed cannibalistically on adults, larvae, and eggs of their own species.

Discussion

The occurrence of two stoma sizes among adults of *N. pinicola* is interesting, but the cause is unknown. It is possible that there are two different genotypes in the population, or that the two stoma sizes may be the result of varying nutrition, with larger stomata possessed by better-fed specimens. The disappearance of specimens with small stomata after several generations in culture sheds little light on the problem, since the change could result either from an uninterrupted supply of suitable food, or from selective pressures leading to elimination of specimens with the small stoma.

N. pinicola has been shown here to feed predaceously on other nematodes. Attempts to establish it in culture with only bacteria or protozoans as food failed. It is rarely found in association with other nematode species and its food source in nature remains unknown. The species is found in association with the white pine beetle, but it has never been shown to kill or feed on any stage of the insect. This study shows that the larvae and males and many of the females collected from nature, with small dorsal teeth, cannot kill prey as large and active as an adult *Panagrellus redivivus*. Thus if the species feeds on any stage of the insect, it may be the small stages such as the eggs or newly hatched larvae.

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