Reproduction, Chromosome Number, and Postembryonic Development of *Panagrellus redivivus* (Nematoda: Cephalobidae)

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Abstract: *Panagrellus redivivus* (L.) T. Goodey reproduced amphimictically; the sexual primordia of males had nine chromosomes, those of females had ten. Eggs contained five chromosomes, sperm four or five. There were four molts, all after hatching. The sexes could be separated at the second molt by development of a lobe of somatic cells in the gonad, anteriorly in males, posteriorly in females. The lobe in males reflexed posteriorly at the third molt and joined the rectum at the fourth molt. Third molt females had a thickened vaginal primordium and at the fourth molt the spermathecal and uterine primordia were evident. The uterus elongated enormously in the adult. The 15 ventral chord nuclei between esophagus and rectum in the first stage increased to approximately 63 during the first molt; specialized nuclei, not evident until the third molt, participate in vaginal lining formation in fourth molt females. Sperm were first produced at the late fourth molt. Eggs, not produced until after copulation, hatched within the uterus. *Key Words*: *Panagrellus redivivus*, Chromosome number, Reproduction, Development, Morphogenesis.

Panagrellus redivivus (L.) T. Goodey and related forms have often been used in research on the nutrition, biochemistry and growth of nematodes (3, 4, 6). However, except for allometric studies (5) and a description of the fine structure of the cuticle of developmental stages of *P. silusiae* (de Man) (8), no attempt has been made to describe the various stages in the life cycle of a member of this genus. The following is a report on gametogenesis, chromosome number, mode of reproduction, and postembryonic development of *P. redivivus*.

MATERIALS AND METHODS

P. redivivus, progeny of an isolate from Florida soil, were reared in petri dish cultures each containing about 50 ml Gerber's oatmeal and water and a few grains of baker's yeast. A few nematodes were added which multiplied to several thousand in seven to ten days. The nematodes were stained with acetic orcein as described by Hechler (7). Camera-lucida drawings were prepared from these specimens as well as living specimens and specimens fixed in formalin and mounted in formalin or glycerine. Single larval cultures, established to verify amphimictic reproduction, were prepared by placing single pre-adult nematodes in 8-mm diameter microtiter wells with a medium of 1% yeast extract, 1% casein, 4% soy peptone, and 4% heated liver extract.

REPRODUCTION

Spermatogonia multiplied in the distal part of the testis, with a rachis beginning about ten cell layers from the terminus. Mitotic divisions were seen throughout about ⅓ of its length, although individual chromosomes were too crowded to count. The spermatogonia enlarged until they were about five times the diameter of newly formed cells. Prophase was not studied since nuclei at that stage were poorly stained, with only the nucleolus and a few faintly defined chromosomes visible. Meiosis was limited to a short section of the testis consisting of about five layers of cells. At Metaphase I four bivalents and one univalent were present (Fig. 1, K_1). At the first division usually one daughter nucleus received five univalents, the other four (Fig. 1, K_4). Rarely the first division was equational for the heterochromosome, so

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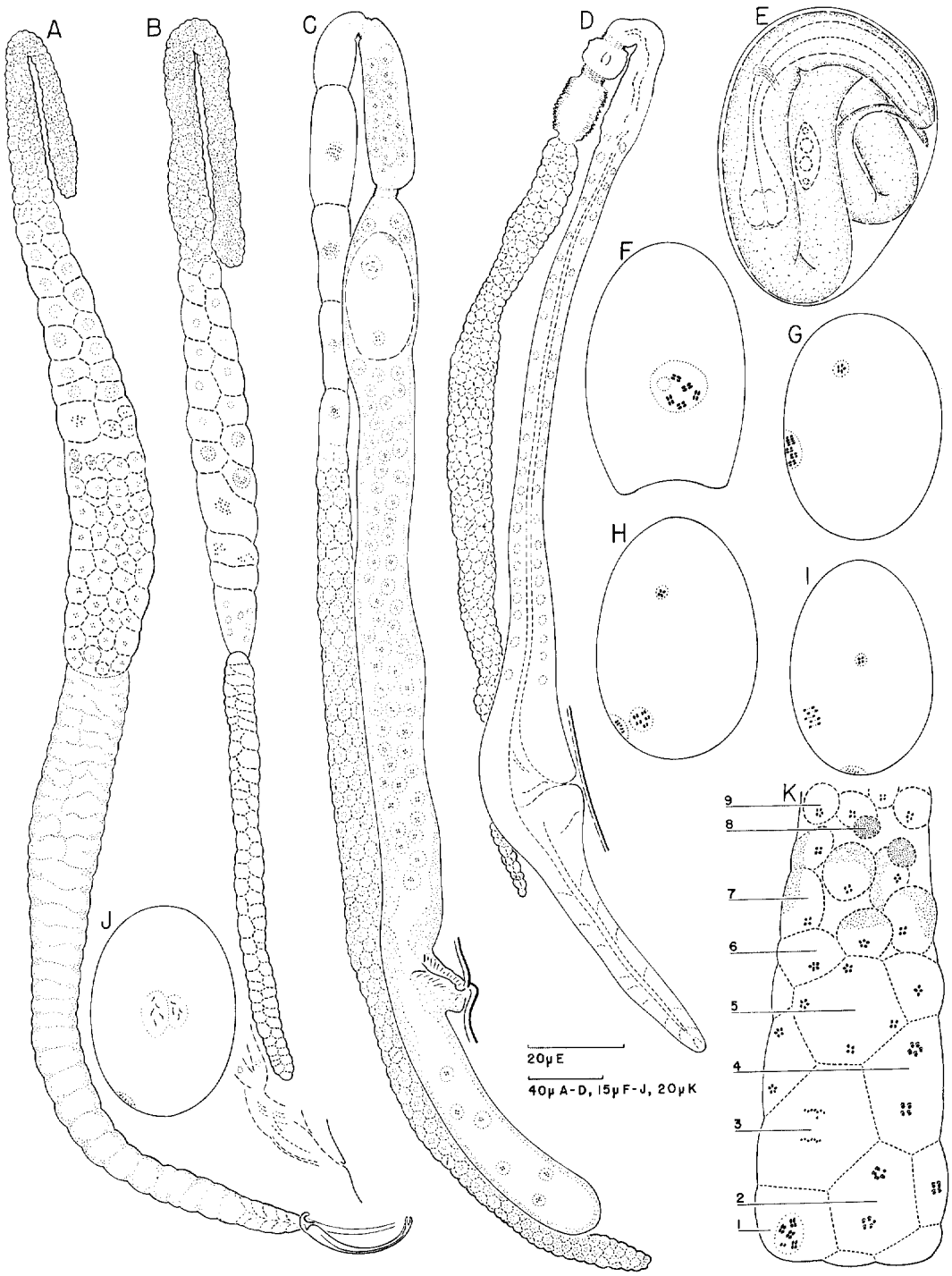
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that the secondary spermatocyte had two nuclei, each with four univalents and a single chromosome (Fig. 1, K_2). Very rarely a lagging chromosome was seen (Fig. 1, K_3). At the second division half the nuclei received four chromosomes, half five (Fig. 1, K_5). Cytokinesis was not evident until after both divisions had occurred.

Cytoplasm in spermatids just proximal to cells undergoing meiosis stained uniformly light pink with orcein (Fig. 1, K_6). In older spermatids a portion of the cytoplasm stained more deeply, whereas the remainder, containing the nucleus, was hyaline (Fig. 1, K_7). Proximally to these cells a few smaller rounded bodies were seen stained deeply throughout (Fig. 1, K_8). Possibly the latter deeply stained cytoplasm represents an anucleate extruded body, as suggested by Cobb (1, 2). Occasionally the smaller bodies appeared blackened and were filled with many small vacuoles, but an accumulation of structureless material in the gonad, which could be interpreted as the disintegrated extruded material, was not found. Mature sperm were nearly as large as the spermatids and did not appear to have lost the material contained in the round bodies.

Mature sperm were hyaline with only their chromosomes, which remained discrete and easy to count, taking the stain (Fig. 1, K_9). Sperm were rarely found in the posterior third of the male gonad except in older specimens; most sperm were crowded into a short section proximal to the meiotic zone (Fig. 1, A).

As in the male gonad, mitotic divisions were seen in the distal part of the ovary and a rachis was present. As the developing oogonia moved proximally they grew to many times the size of the young cells; most of their growth occurred after separation of the cell from the mass in the ovary. In older females they were rarely contiguous, but each one moved toward the spermatheca separately. Staining was unsatisfactory in the growth zone; occasionally nucleoli were visible but the chromosomes were not stained. As an oocyte approached the flexure the nucleolus disappeared and faintly stained chromosomes were visible. At Metaphase I, which occurred just before the oocyte entered the spermatheca, staining was quite satisfactory and five bivalents were clearly visible (Fig. 1, F). As the egg entered the spermatheca a sperm cell penetrated it, and immediately thereafter the first polar nucleus was formed, usually at the distal end of the egg but occasionally at the middle (Fig. 1, G). Five univalents were left in the egg (Fig. 1, H). The second polar nucleus was formed after the egg entered the uterus (Fig. 1, I), leaving the egg with five chromosomes. A single polar body could be seen within the shell of living eggs but it was not possible to determine which polar nucleus it contained. After the second division the chromosomes of egg and sperm pronuclei elongated and the nuclei fused (Fig. 1, J). At the first cleavage, grouping according to origin of chromosomes from sperm or egg was not seen. The chromosomes could not be counted accurately at this division, but about ten were present.

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FIG. 1. *Panagrellus redivivus*, gonads and chromosome configurations. **A.** Adult male gonad; **B.** Fourth molt male gonad; **C.** Gonad, very young adult female; **D.** Fourth molt female gonad; **E.** Larva just before hatching; **F.** Egg from distal end of oviduct, late Prophase I; **G.** Egg in spermatheca, sperm has penetrated, Metaphase I; **H.** Egg in uterus, with sperm and first polar nucleus, Telophase I; **I.** Egg, Anaphase II; **J.** Egg, fusion of pronuclei; **K.** Portion of male gonad showing phases of spermatogenesis: K_1. Metaphase I; K_2. Metaphase II, equational division of heterochromosome at first division (rare); K_3. Anaphase I, lagging chromosome (rare); K_4. Secondary spermatocyte, 5 dyads in one nucleus, 4 in the other; K_5. Telophase II; K_6. Spermatid; K_7. Spermatid, portion of cytoplasm deeply stained; K_8. Rounded deeply stained cytoplasm; K_9. Mature sperm cell.

Embryogenesis and hatching occurred within the uterus. Older females contained eggs in all stages of development, with the more advanced ones near the vulva. In even older females, hatched larvae filled the entire uterus.

As shown previously by Cryan *et al.* (3), female larvae isolated from males before their final molt failed to produce progeny, showing that males are necessary for reproduction.

Postembryonic Development

First Stage: Embryonated eggs, both within live females and released from the uterus artificially, were observed for several hours using a 95× oil immersion objective. When the developing nematode first began to move it had no recognizable esophagus, although the stomatodeum could be identified. Just before hatching a fully developed esophagus with valvate basal bulb was present, and the stoma was completely cuticularized (Fig. 1, E). No evidence of a molt was seen between these two stages.

At hatching the gonad primordium contained two large centrally-located germinal nuclei and a smaller somatic nucleus at each end. Between the base of the esophagus and the rectum 15 ventral chord nuclei were present in a single row, spaced as shown in Fig. 2, A. Dimensions of the smallest first stage larva measured, released artificially from the female after it had hatched, were: L = 0.285 mm; a = 19; b = 3.1; c = 5.0; genital primordium = 0.012 mm, located 52.6% of body length from the head. Dimensions of the largest first stage larva measured were: L = 0.408 mm; a = 21; b = 3.6; c = 4.8; genital primordium = 0.017 mm, located 51.5% of body length from the head.

During the first molt the ventral chord nuclei began to divide and approximately 63 were present between esophagus and rectum by the end of the molt (Fig. 2, B). Dimensions of larvae measured during the first molt were: (n = 15) L = 0.407 (range 0.353–0.495) mm; a = 21.8 (18.7–22.9); b = 3.7 (3.2–4.4); c = 5.1 (4.8–5.7); genital primordium = 0.014 (0.012–0.015) mm; located 50.9 (47–54)% of body length from head.

Second Stage: In the second stage, which is differentiated from the first stage by the presence of 63 ventral chord nuclei, the genital primordium was similar to that of the first, with four nuclei (Fig. 2, B). Early in the second molt mitotic divisions of the somatic nuclei of the genital primordium were seen and a small lobe set off from the germ cells appeared anteriorly in males (Fig. 2, E) and posteriorly in females (Fig. 2, D); thus, sexual differentiation could be detected as early as the second molt. The primordial spicule pouch was occasionally evident in males (Fig. 2, E). No specialized ventral chord nuclei opposite the genital primordium could be recognized in females. Dimensions of second molt females were: (n = 15) L = 0.609 (0.518–0.690) mm; a = 25 (18–29); b = 4.0 (3.1–4.6); c = 5.1 (3.8–5.7); genital primordium = 0.036 (0.023–0.045) mm, located 52 (47–65)% of body length from head. Second molt males measured: (n = 15) L = 0.596 (0.545–0.645) mm; a = 24 (22–26); b = 4.1 (3.8–4.5); c = 5.4 (5.1–5.9); genital primordium = 0.032 (0.027–0.068) mm, located 48.7 (46–51)% of body length from head.

Third Stage: During the third stage the genital primordium grew considerably in both sexes. In females (Fig. 2, F) the anterior end of the ovary remained near the middle of the

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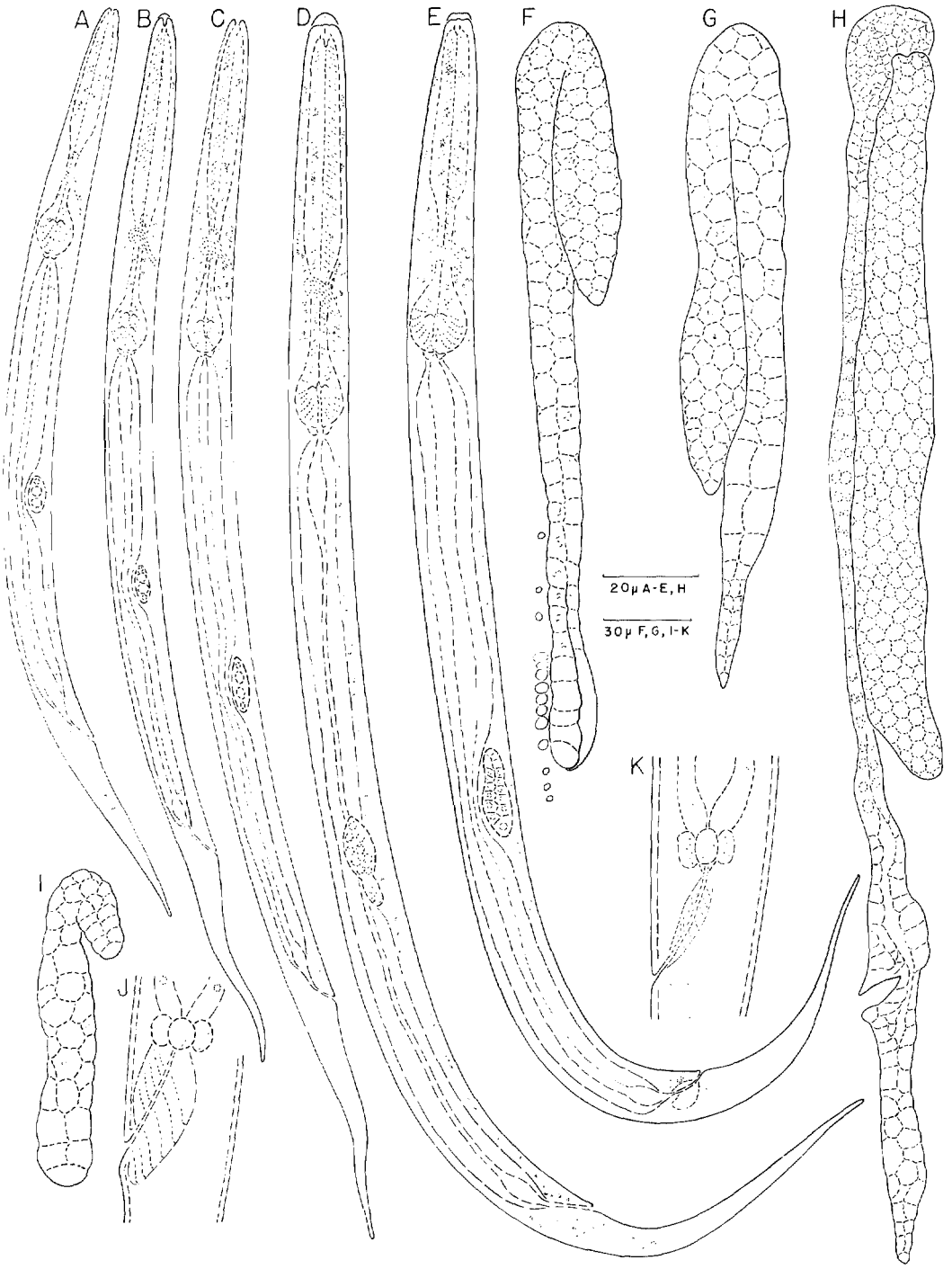
Fig. 2. *Panagrellus redivivus*, larval stages and gonad development. **A.** First stage; **B.** First molt; **C.** Second stage; **D.** Second molt, female; **E.** Second molt, male; **F.** Third molt female gonad; **G.** Fourth stage male gonad; **H.** Fourth stage female gonad; **I.** Third molt male gonad; **J.** Third molt male rectum; **K.** Third molt female rectum.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body [48 (46–52)% of body length from the head]; although the germ cells multiplied, most of them were contained in a reflexed lobe. The greatest elongation was in the somatic part of the gonad which lengthened posteriad until, at the third molt, it had reached about 65% of body length from the head. The vaginal primordium appeared as a slight swelling at the posterior terminus, with three longitudinal rows of cells; opposite it several ventral chord nuclei had increased in size. Between the vaginal primordium and the germ nuclei were four rows of smaller cells comprising the primordial uterus. Dimensions of third molt females were: (n = 10) L = 0.878 (0.762–0.960) mm; a = 27 (25–29); b = 4.7 (4.0–5.3); c = 6.4 (6.0–6.9); genital primordium = 0.158 (0.105–0.188) mm, with the vaginal primordium located 65 (61–68)% of body length from the head.

In third stage males the genital primordium (Fig. 2, I) extended slightly further forward than in females, reaching 46 (44–47)% of body length from the head. The germ cells were in the longer posterior lobe; most of the somatic nuclei were contained in the shorter anterior lobe, which reflexed on the dorsal side and began to grow posteriad during the third stage. Thus the flexure in adults was ventral in males, dorsal in females. The primordial spicule pouch was uniformly evident as a thickening surrounding the rectum (Fig. 2, J) and much more conspicuous than the corresponding tissue in females of the same age (Fig. 2, K). Dimensions of third molt males were: (n = 10) L = 0.875 (0.750–0.980) mm; a = 27 (25–28); b = 4.8 (3.6–5.3); c = 6.4 (5.8–6.9); genital primordium = 0.112 (0.078–0.171) mm.

FOURTH STAGE: In fourth stage females (Fig. 2, H) the vaginal primordium developed as a hollow tube surrounded by wedge-shaped cells. Just before the fourth molt it became lined with the specialized ventral chord nuclei. The primordial uterus lengthened considerably, consisting of four rows of epithelial cells; occasionally the lumen of the gonoduct was visible. Two thickenings were seen at the flexure, and in the reflexed lobe the germ cells continued to multiply. The greatest growth was in the uterus, so that by the final molt the flexure was located only 24 (14–28)% of body length from the head. By the final molt three definite constrictions were present near the flexure, setting off a rounded and an elongated expansion, both with thick walls, the elongate one quite plicated (Fig. 1, D). The terminus of the ovary was posterior to the vulva at the final molt. A thickened posterior uterine sac was present, flattened dorsoventrally. The vagina became lined with cuticle and the external vulval opening appeared. At the final molt dimensions of females were: (n = 12) L = 1.312 (1.220–1.425) mm; a = 26.3 (22.6–30.0); b = 6.1 (5.7–6.4); c = 8.0 (7.2–8.7); gonad = 0.654 (0.585–0.810) mm, with the vulva located 65.6 (63.8–67.1)% of body length from the head.

In fourth stage males (Fig. 2, G) the ventral lobe of the gonad lengthened only slightly with multiplication of the spermatogonia. The dorsal lobe, consisting mostly of somatic cells, grew posteriorly toward the rectum, with four rows of cells throughout most of its length but only two rows near the terminus. Later the gonad grew in both directions, the multiplication of germ cells pushing the flexure forward to 31 (25–37)% of body length from the head, the somatic cells continuing to grow toward the rectum. At the final molt (Fig. 1, B) the gonad became linked to the rectum in a cloaca. The membranes of cells forming the gonoduct were thickened and conspicuous during the late fourth stage. At the final molt they became thinner in the center of the gonad, whereas the outer membranes remained thick and each cell seemed to contain a single large vacuole. Later, a

few membranes disintegrated near the center, presumably forming the lumen through which the sperm would pass at copulation (Fig. 1, A). A few cells just anterior to the cloaca in the adult remained similar to those of the preadult except for large vacuoles near the center (Fig. 1, A). During the molt the spicules and gubernaculum were formed. Dimensions of males at the final molt were: (n = 10) L = 1.177 (1.130–1.237) mm; a = 28 (25–32); b = 5.9 (5.3–6.3); c = 8.0 (7.5–8.3); gonad = 0.650 (0.555–0.735) mm.

By the beginning of the final molt, meiosis in the testis had begun and several mature sperm were present in the gonad when the nematode emerged from the preadult cuticle.

ADULT: In females (Fig. 1, C) the vulva was a transverse slit and the vagina was anteriorly directed, with extremely muscular walls and a sinuous lumen. The epithelium of the uterus was thickened near the vagina, becoming gradually thinner anteriorly. Faint striae marking the epithelial cell boundaries were sometimes visible in surface view throughout the length of the uterus. Immediately after copulation the uterus was packed with sperm between the flexure and the vulva, and smaller numbers were found in the post-vulvar sac. The ovary extended posteriorly behind the post-vulvar sac and sometimes behind the anus. Rarely the terminus of an extremely long ovary was directed forward. Two sphincters at the flexure separate the spermatheca from the uterus and oviduct. The conspicuous elongated plicated structure distal to the spermatheca in preadults and interpreted to be an oviduct, had expanded considerably longitudinally, causing the plication to disappear and leaving the wall smooth. In older specimens it extended from the flexure to a point behind the vulva. Its walls were very elastic so that it was quite narrow except where it contained an egg.

The adult testis had a short reflexed portion at the anterior end. Spermatogonia, increasing in size posteriorly, were located in the anterior third to half of the gonad, with mature sperm stored in a section usually a little behind the middle. Sperm were rarely found in the posterior portion (vas deferens) except in older specimens (Fig. 1, A).

Adult measurements: (30 ♀ ♀) L = 1.679 (1.470–2.090) mm; a = 25.7 (20–31); b = 7.6 (6.4–9.5); c = 9.1 (7.0–11.0); V = 66.4 (59.7–69.6)%; gonad flexure 19 (13–25)% of body length from anterior end. (20 ♂ ♂) L = 1.509 (1.250–1.800) mm; a = 28.7 (24–35); b = 6.9 (6.0–7.8); c = 8.6 (7.6–11.5); gonad = 0.930 (0.760–1.070) mm, flexure 26 (14–46)% of body length from head, spicules, measured in a straight line between the ends, = 0.058 (0.054–0.060) mm; gubernaculum = 0.030 (0.029–0.031) mm.

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