Postembryogenesis of Meloidodera floridensis with Emphasis on the Development of the Male[1]

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Abstract: Second-stage larvae of *Meloidodera floridensis* kept in tap water developed without feeding into small, slender males. They completed three molts, and the molted cuticles remained superimposed. All organ systems were well developed in third- and fourth-stage male larvae. Structures in the head region such as cephalic framework, stylet and esophagus were smaller and differed morphologically from those of second-stage larvae. Development of the male reproductive system was similar to that of other tylenchids, and sex was recognizable at the end of the second molt. Second-stage larvae, developing in pine roots, increased in size and molted three times to become ovoid to spherical females. Each larval stage had a stylet and fed actively. Sex could be determined at the end of the second molt. Detailed observations were made on the development of the reproductive system, cuticle, esophagus and tail region. *Key Words:* morphology, pine cystoid nematode.

Received for publication 9 October 1972.

[1] Paper No. 3886 of the Journal Series of the North Carolina State University Agricultural Experiment Station, Raleigh. This study was supported in part by Grant No. GB-29485 of the National Science Foundation. Appreciation is extended to Dr. F. G. Giesbrecht for statistical analyses of nematode measurements.

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An interesting aspect of the biology of *Meloidodera floridensis* Chitwood, Hannon and Esser is the development of second-stage larvae into males without previous feeding on a host plant (3). Larvae of this polyploid, parthenogenetic species grow, develop and differentiate as females when they infect a suitable host plant, but they develop without undergoing any growth in body size and differentiate as males under conditions of

starvation (8). These observations were responsible for our interest in the study of the morphological differentiation of starving second-stage larvae and their eventual development into adult males. For comparative purposes, development of larvae which had infected pine roots and would eventually become adult females also was studied. Some of the anatomical features of the various developmental stages and the adults, not previously described in detail, are also described and illustrated.

MATERIALS AND METHODS

Stock cultures of *Meloidodera floridensis* (population 21-NC) were maintained on loblolly pine (*Pinus taeda* L.) in the greenhouse at 25-30 C. Second-stage larvae were hatched from embryonated eggs which had been obtained by dissecting gravid females. Male development was studied by keeping second-stage larvae in tap water at 23 ± 2 C. Developing males were examined at weekly intervals (8). For morphological observations, the various larval stages and molts were killed by gentle heat and mounted in water or 1% formalin, or stained with 1% acetic orcein (2). Female development was studied by inoculating loblolly pine seedlings with second-stage larvae (8). At 5-day intervals, infected roots were stained with boiling acid fuchsin-lactophenol. Female larvae were dissected from roots, mounted in lactophenol, and their stage of development was determined. Observations on the morphology of the reproductive system of adult females were made by dissecting live specimens in a 0.9% NaCl solution and mounting their gonads in the same solution or in 1% formalin.

OBSERVATIONS

The postembryonic development of *M. floridensis* comprises four larval stages and the adult females and males. The first molt takes place within the egg. Multiplication of cells of certain organ systems, especially the female and male reproductive systems, continues throughout all stages and molts. In the case of male development, the molted cuticles of the various larval stages remain superimposed.

MORPHOLOGY OF SECOND-STAGE LARVAE: The second-stage larva has been described previously (1, 4). Additional measurements and illustrations are presented here (Table 1, Fig. 1A, B). This larval stage resembles that of the genus *Heterodera*. The coarsely annulated cuticle is laterally interrupted by the lateral fields which bear four incisures. The hypodermis is inconspicuous. The heavily sclerotized cephalic framework is characteristically shaped (Fig. 1A). The lip region is set off by a deep constriction. The robust stylet has three large knobs that are flat or slightly concave anteriorly. The vestibule extension extends over three-fourths of the conical part of the stylet. The cephalids are located on the second and eighth annule posterior to the lip constriction. The esophageal gland lobe overlaps the intestine ventro-laterally with the dorsal gland nucleus located in the dorsal sector of the body. The elongate conoid tail has a clear tail terminal and ends in a blunt tip (Fig. 1B). The large lens-like phasmids are located slightly anteriad of the middle of the tail. The genital primordium comprises two central germinal cells bordered by two somatic cells.

DEVELOPMENT OF MALES: *Second molt.* No growth occurs during the second larval stage. With the onset of molting, the larva becomes motionless. The cytoplasm in the head region, as well as in the region of the tail terminal, soon recesses, and the rounded tail of the third larval stage is formed. The hypodermis increases in thickness, and its nuclei become very distinct. Stylet shaft and knobs become faint and finally disappear. A general vacuolization appears around the esophagus, and the valve of the median bulb becomes faint. The subventral glands are difficult to identify; whereas, the dorsal gland with its large nucleus remains distinct. As molting proceeds, a general loosening of the cuticle takes place, and the new cuticle completes formation underneath the old. The conical part of the stylet, the cephalic framework and the amphidial, rectal and phasmidial linings are shed with the old cuticle of the second-stage larva. As the various structures of the third-stage larva differentiate, the conical part of the stylet and the valve of the median bulb become visible first. Numerous cells appear around the rectal area indicating the formation of the spicule primordia. The genital primordium grows continuously during this molt and consists of up to 20 cells.

Third-stage larva. This larva is shorter and slightly thicker than the second-stage larva [body length: 379.8 - 540.0μ (mean 475.11μ, 95% confidence interval ± 19.42); a: 11.7 - 19.2 (15.8 ± .82); n = 20]. It has a

well-developed, finely annulated cuticle with underlying double-striated subcuticle. The cephalic framework is very faint (Fig. 1C). The stylet is much smaller than that of the second stage and very delicate with a narrow, thin conical part [stylet length: 16.5 - 20.4μ (18.53μ ± .50); n = 20]. Distinct stylet knobs are lacking, and the stylet shaft ends in a single club-like swelling. The esophageal glands are much reduced in length, and only the dorsal gland nucleus is distinct. The spicule primordia continue to increase in size (Fig. 1D). The tail is broadly rounded and terminally has two papilloid phasmids different from those of the second-stage larva. The genital primordium increases in size throughout this stage [35.5 - 59.2μ (47.71μ ± 3.62); n = 20], with a distinct narrowing near the middle in the majority of the larvae (Fig. 1F) but remaining broad in some specimens (Fig. 1E). It consists of numerous cells, most of which appear to be germinal cells, and a distinct cap cell at the posterior tip (Fig. 1E, F). No specialized ventral chord nuclei are present.

Third molt. The third molt progresses in a manner similar to the second. The cephalic framework together with the very delicate conical part of the stylet are shed with the third-molt cuticle which now appears smooth and two-layered in contrast to the annulated, single-layered second-molt cuticle. The genital primordium increases in length, and its anterior

TABLE 1. Measurements of 20 second-stage larvae of *Meloidodera floridensis* killed by gentle heat and mounted in 1% formalin.

Character	Range	Mean	95% Confidence interval
Linear measurements (μ):			
Body length	495.0 - 594.0	550.76	± 12.25
Body width	21.4 - 24.5	22.83	± .42
Head height	5.1 - 6.0	5.51	± .12
Head width at base	10.2 - 11.6	10.86	± .17
Stylet length	28.4 - 30.6	29.16	± .30
Stylet shaft and knobs	15.0 - 16.3	15.84	± .16
Stylet knobs, height	2.4 - 2.9	2.61	± .07
Stylet knobs, width	6.1 - 6.7	6.42	± .09
Dorsal gland orifice from stylet base	4.3 - 5.3	4.88	± .15
Esophagus length	123.8 - 145.9	136.96	± 2.40
Excretory pore from anterior end	109.1 - 127.1	119.79	± 2.33
Annule width	1.9 - 2.5	2.19	± .10
Tail length	52.7 - 59.6	56.66	± 1.03
Tail terminal length	20.4 - 27.9	23.97	± .99
Anal body width	14.4 - 17.8	15.85	± .38
Middle of genital primordium from anterior end	285.0 - 376.5	346.42	± 10.46
Phasmid size	3.1 - 3.6	3.33	± .08
Ratios:			
a	21.2 - 26.2	24.15	± .64
b	3.6 - 4.2	4.02	± .06
c	8.8 - 10.6	9.72	± .19
Stylet knobs width/ stylet knobs height	2.2 - 2.7	2.47	± .06
Tail terminal length/ stylet length	0.7 - 1.0	0.82	± .04
Tail length/anal body diameter	1.9 - 4.1	3.51	± .20
Percentages (in relation to body length):			
Excretory pore from anterior end	20.6 - 23.4	21.77	± .36
Middle of genital primordium from anterior end	57.6 - 64.9	62.84	± .75

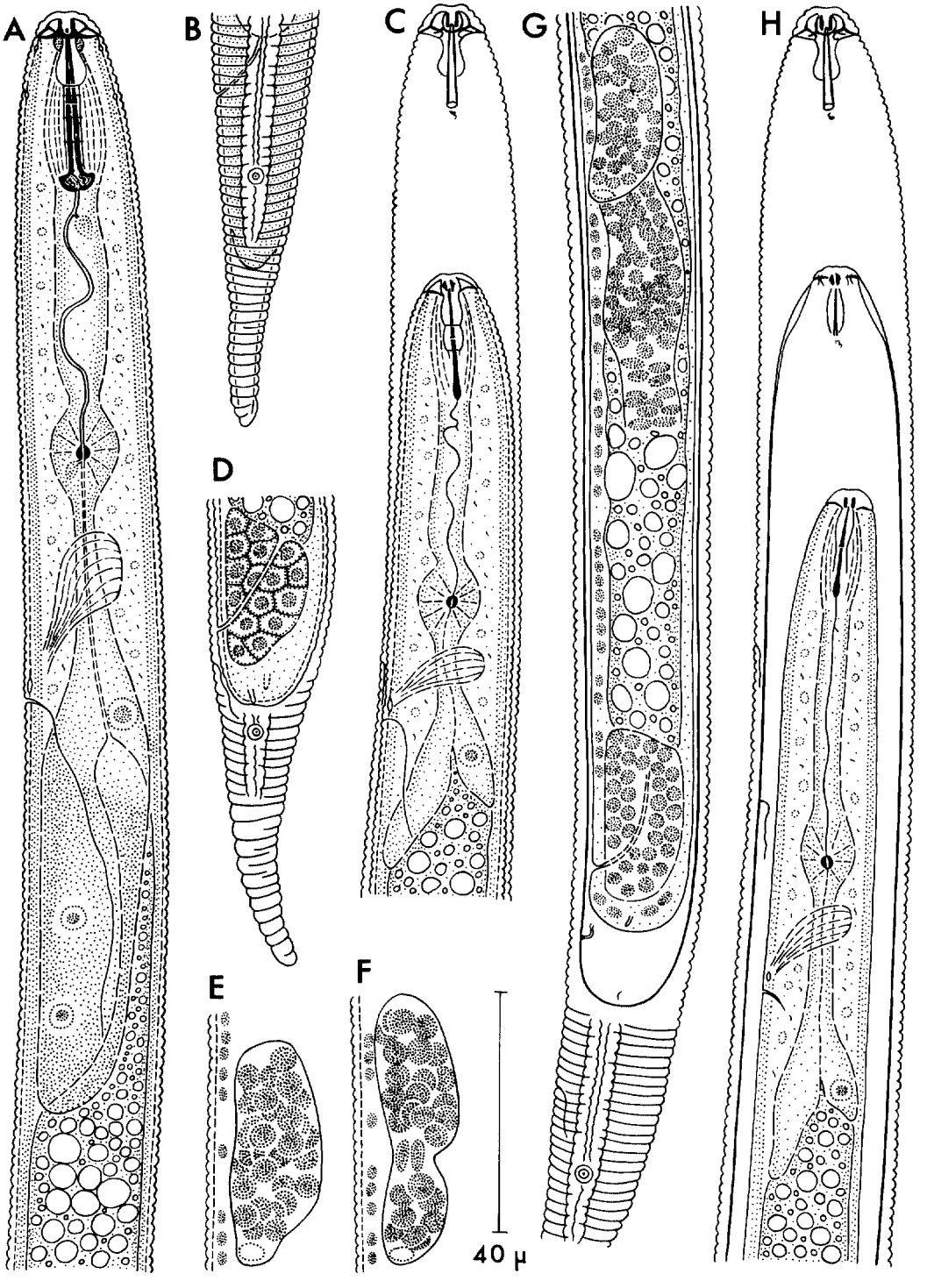

FIG. 1. *Meloidodera floridensis*, male development - second to fourth stage. **A.** Anterior portion of second-stage larva; **B.** Tail of second-stage larva; **C.** Third-stage male larva within second-molt cuticle; **D.** Tail of third-stage male larva within second-molt cuticle; **E, F.** Gonads of third-stage male larvae; **G.** Posterior part of third-molt male larva within superimposed cuticles; **H.** Fourth-stage male larva, anterior part.

end turns and elongates posteriorly forming the male gonoduct (Fig. 1G). Thus, during this early stage, the developing gonad becomes U-shaped but straightens out later on, as the anterior zone which contains the cap cell becomes outstretched. Most of the anterior part of the gonad is filled with spermatogonia, whereas the posterior gonoduct consists of a double row of epithelial nuclei. The spicule primordia increase in size.

Fourth-stage larva. This stage seems to be of very short duration, since it was rarely encountered. Fourth-stage male larvae possess well-developed organ systems and a very thin cuticle. The cephalic framework is extremely faint (Fig. 1H). The stylet is even smaller and more delicate than that of the third-stage larva but has the same general shape, with club-like swelling posteriorly instead of stylet knobs [stylet length: 13.1 - 15.0μ (14.3μ); n = 5]. The esophageal glands appear further reduced in size. The gonad continues to increase in length. The posteriorly developing gonoduct makes contact with the region of the spicule primordia. The tail is broadly rounded and has terminally two minute phasmids.

Fourth molt. During the fourth molt, the adult male is formed within the superimposed cuticles of the previous larval stages (Fig. 2A, B). The conical part of the stylet of the fourth stage and the very faint framework are shed with the very thin, finely annulated cuticle. The hypodermis is thick with distinct nuclei. The conical part of the stylet first appears as a small point located centrally at the bottom of the stomatal cavity (Fig. 2A). The gonad structure of late fourth-stage and early fourth-molt individuals is similar (Fig. 2B). Most of the gonad is filled with spermatocytes and spermatozoa. Spicule and gubernaculum pouches begin to form posteriorly. The short tail is broadly rounded.

Male. The only description of *M. floridensis* males existing in the literature is that by

TABLE 2. Measurements of 25 males of *Meloidodera floridensis* killed by gentle heat and mounted in 1% formalin.

Character	Range	Mean	95% Confidence interval
Linear measurements (μ):			
Body length	414.9 - 594.0	513.30	± 21.65
Body width	15.5 - 22.4	19.62	± .67
Stylet length	20.4 - 23.7	22.39	± .32
Stylet shaft and knobs	10.2 - 11.9	11.16	± .21
Stylet knobs, height	1.2 - 1.8	1.56	± .08
Stylet knobs, width	3.1 - 3.9	3.53	± .09
Dorsal gland orifice from stylet base	2.6 - 3.6	2.93	± .11
Esophagus length	84.7 - 113.0	94.93	± 3.03
Excretory pore from anterior end	88.6 - 106.6	98.57	± 2.21
Annule width	1.2 - 1.6	1.42	± .05
Tail length	5.3 - 7.7	6.58	± .25
Anal body width	12.2 - 16.8	14.87	± .47
Testis length	155.7 - 251.1	205.80	± 8.31
Spicule length	21.8 - 24.8	23.28	± .37
Gubernaculum length	4.6 - 5.9	5.29	± .14
Ratios:			
a	22.0 - 37.6	26.28	± 1.33
b	4.4 - 7.0	5.42	± .25
c	62.6 - 97.1	78.42	± 3.74
Stylet knobs width/ stylet knobs height	1.8 - 2.9	2.29	± .11
Percentages (in relation to body length):			
Excretory pore from anterior end	14.9 - 25.2	19.39	± .93
Testis length (T)	33.2 - 50.5	40.34	± 1.89

Hopper (4) which was based on five specimens. Wouts and Sher (10) gave an emended diagnosis of the genus and illustrated portions of the male of another species, *M. charis*. Additional observations are reported here on the male of *M. floridensis* including measurements and

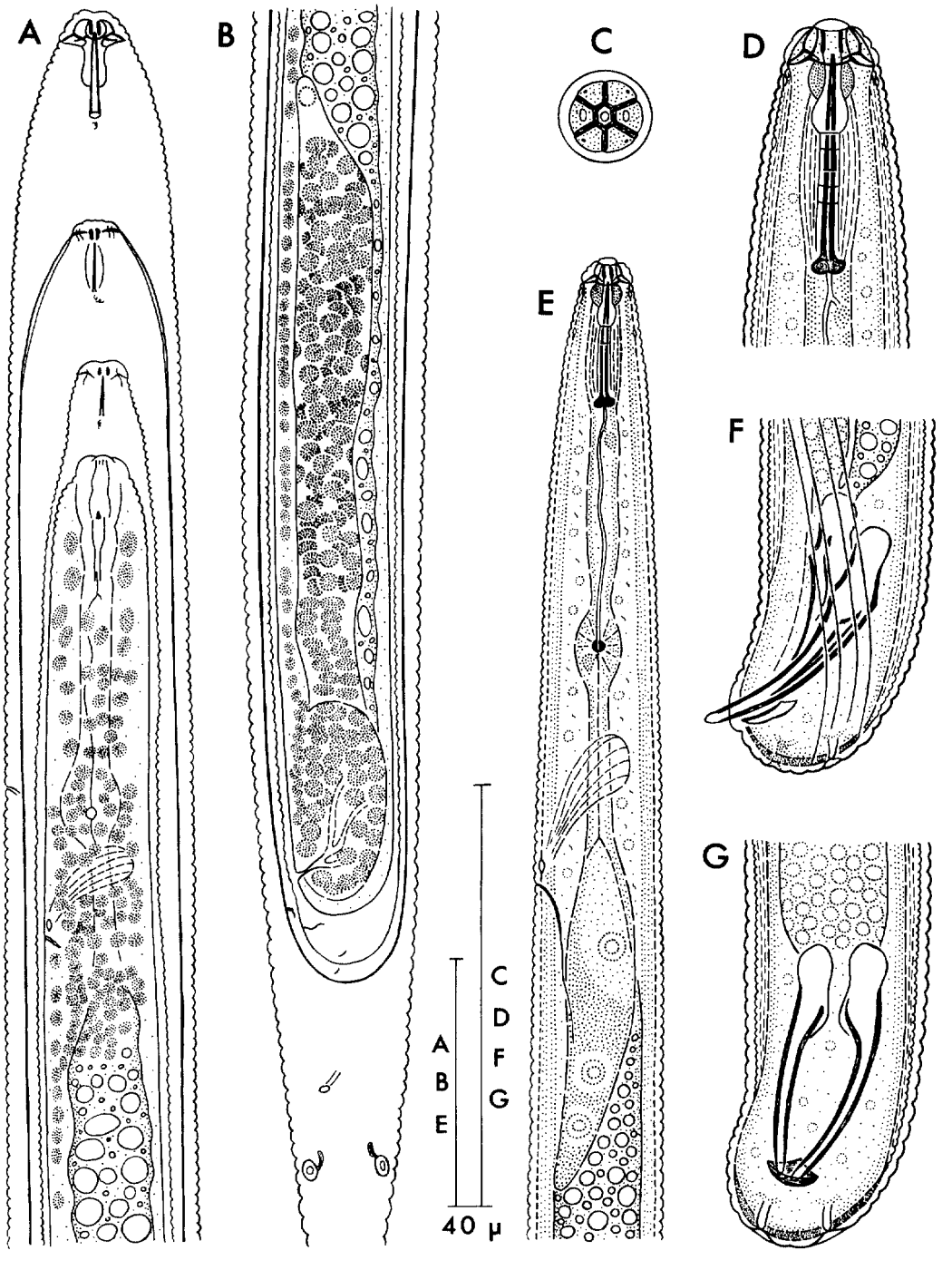

FIG. 2. *Meloidodera floridensis*, male development - fourth molt to adult. **A.** Fourth molt, anterior portion; **B.** Fourth molt, posterior portion; **C.** Face view at level of basal lip annule; **D.** Head of male; **E.** Anterior portion of male, esophagus region; **F.** Male tail, lateral; **G.** Male tail, ventral.

illustrations (Table 2, Fig. 2 C-G). The posterior part of the body is slightly twisted when specimens are killed, and the cuticle is distinctly annulated. The hemispherical lip region has one distinctly set off, anterior labial annule (labial disc) (Fig. 2D). The remainder of the lip region is slightly asymmetric and bears two to three irregularly arranged annules. No distinct longitudinal striae were observed on the basal lip annule (Fig. 2C). The well-sclerotized hexaradiate framework is symmetrical, the lateral sectors being only slightly larger than the subventral and subdorsal ones. One cephalic papilla is present in each of the subventral and subdorsal sectors, and the oval-shaped amphidial ducts are located laterally. The stylet is delicate with small, rounded to slightly backward-sloping knobs. Faint cephalids are present at the second and seventh annules behind the lip constriction. The hemizonid is distinct and located 1-2 annules in front of the excretory pore and extends over 1 1/2 annules (Fig. 2E). The length of the esophageal gland lobe varies among different specimens. The single, outstretched male gonad terminates anteriorly in a cap cell. The entire testis and the vas deferens are filled with spermatozoa. The ejaculatory duct is highly glandular. The phasmids are very distinct and located terminally on the short tail (Fig. 2F, G). The spicules are characteristically tylenchoid. The gubernaculum is short and triangular (Fig. 2G).

Abnormalities in male development. In some cases only a low percentage of the larvae developed to adult males. Other larvae underwent the second and third molts but died before reaching adulthood. In these cases, various developmental abnormalities were observed which involved mainly incomplete development of structures in the anterior and posterior body regions. The stylet was not fully developed, i.e., only the conical part was differentiated, or the stylet knobs were only partially developed. The cephalic framework was hardly sclerotized, or completely absent. The distal part of the spicules formed projections, or one of the spicules was half the normal length. The tail was not properly formed in some instances.

DEVELOPMENT OF FEMALES: *Second molt.* The second-stage larva grows slightly in width before the onset of the second molt which proceeds exactly as described for the male. The dorsal esophageal gland, however, enlarges, whereas the subventral glands diminish. The genital primordium is multicellular and slightly kidney-shaped and no spicule primordia are recognizable.

Third-stage larva. Measurements of 30 specimens in lactophenol.—Body length: 405.0 - 513.0μ (mean 463.23μ, 95% confidence interval ± 10.13); body width: 34.2 - 95.4μ (67.87μ ± 5.48); stylet length: 25.8 - 28.5μ (27.13μ ± .30); dorsal gland orifice from stylet base: 4.2 - 5.6μ (4.83μ ± .20); median bulb length: 16.9 - 28.0μ (23.29μ ± .76); median bulb width: 14.8 - 24.3μ (19.76μ ± .61); excretory pore from anterior end: 52.0 - 112.2μ (85.33μ ± 10.23); gonad size: 35.2 - 105.3μ (62.09μ ± 6.50); middle of gonad from anterior end: 253.8 - 350.1μ (306.0μ ± 8.99); tail length: 10.2 - 18.1μ (13.76μ ± .56); a: 4.9 - 13.2 (7.14 ± .63); excretory pore from anterior end (expressed as % of body length); 11.3 - 26.7 (18.66 ± .24); middle of gonad from anterior end (expressed as % of body length); 59.4 - 70.6 (66.0 ± .89). The third-stage larva is shorter and wider than the second stage (Fig. 3A). The thick cuticle is marked by very fine transverse striations that are resolvable into rows of minute punctations. The hypodermis is thick. The lip region is distinctly set off and bears anteriorly a lip cap followed by two to three labial annules. The cephalic framework is slightly sclerotized. The stylet is less robust and smaller than that of the second-stage larva and has three well-developed, backward-sloping knobs (Fig. 3B). The median bulb increases in size and becomes very muscular with large valve plates. The isthmus is short, and the gland lobe is also shortened. The dorsal gland is enlarged, whereas the subventral glands remain small (Fig. 3A). Excretory pore and hemizonid are distinct. The tail is short and bluntly rounded with large papilloid phasmids (Fig. 3C, D). Three large gland-like bodies (2 subventral, 1 dorsal), similar to rectal glands, appear to open into the rectum (Fig. 3C, D). During the third stage, the gonad elongates to form an anterior and a posterior branch (Fig. 3E, F). The middle part of the gonad begins to make contact with specialized ventral chord tissue in larvae approaching the third molt (Fig. 3F).

Third molt. During the third molt, the hypodermis continues to enlarge. A new cuticle is formed underneath the old which is shed together with the conical part of the stylet, the cephalic framework and the amphidial, rectal and phasmidial linings. The gonads continue to elongate anteriorly and posteriorly each

showing an epithelial tubular region, a region with the germinal cells and a distinct cap cell at the apex. The specialized ventral chord area is well developed.

Fourth-stage larva. Measurements of 20 specimens in lactophenol.–Body length: 407.7 - 520.2μ (mean 464.81μ, 95% confidence interval ± 13.33); body width: 90.0 - 184.5μ (135.23μ ± 12.53); stylet length: 30.6 - 34.6μ (32.17μ ± .52); dorsal gland orifice from stylet base: 5.1 - 6.3μ (5.72μ ± .17); median bulb length: 25.5 - 35.7μ (29.85μ ± 1.09); median bulb width: 20.4 - 28.6μ (23.35μ ± .80); excretory pore from anterior end: 95.9 - 150.6μ (119.79μ ± 7.00); middle of gonad from anterior end: 283.5 - 379.8μ

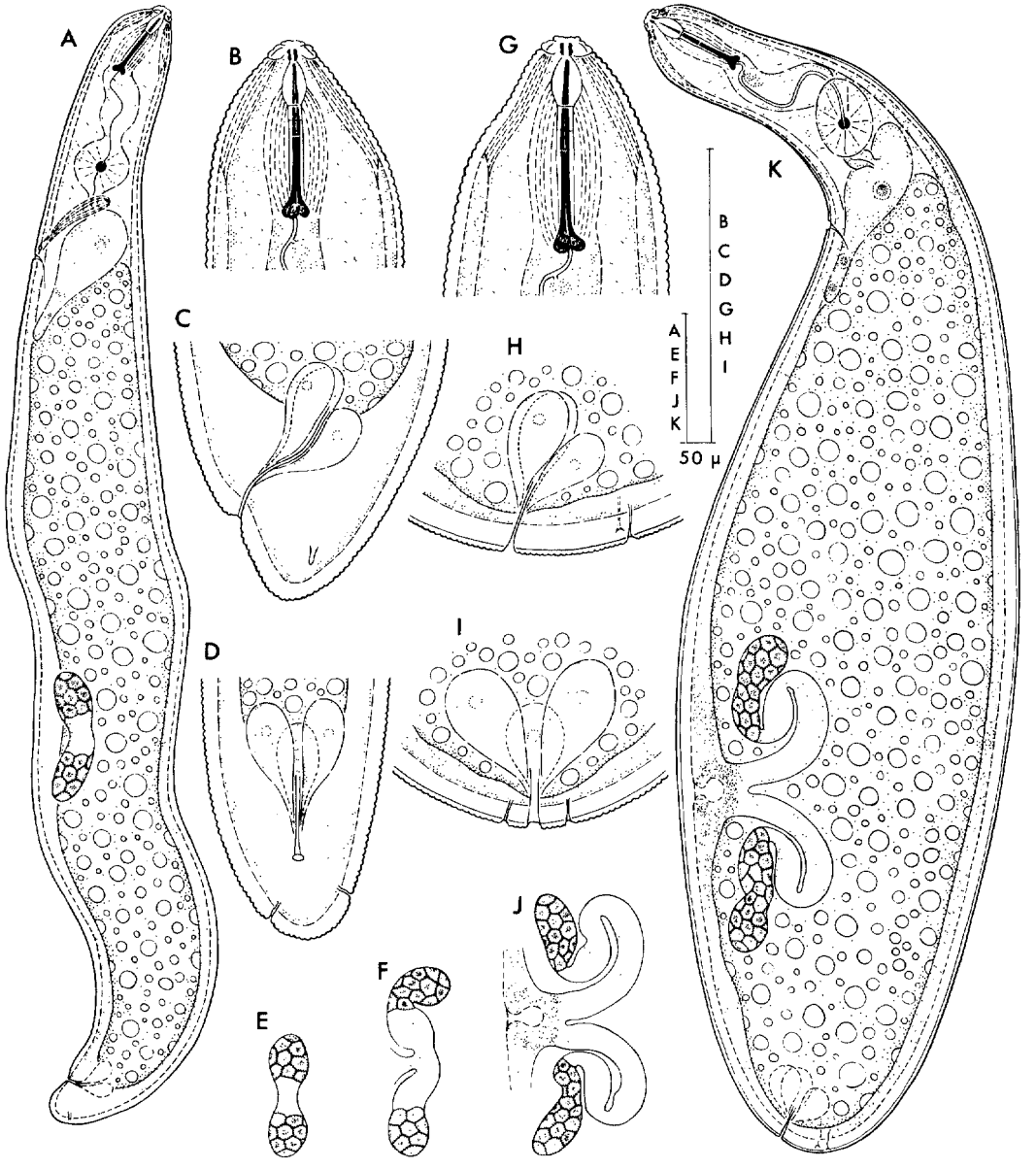

FIG. 3. *Meloidodera floridensis*, female development. **A.** Third-stage larva; **B.** Head of third-stage larva, lateral; **C.** Tail of third-stage larva, lateral; **D.** Tail of third-stage larva, ventral; **E, F.** Gonads of third-stage larvae; **G.** Head of fourth-stage larva, lateral; **H.** Posterior part of fourth-stage larva, lateral; **I.** Posterior part of fourth-stage larva, ventral; **J.** Gonads of fourth-stage larva; **K.** Fourth-stage larva.

(323.82μ ± 14.34); tail length: 3.1 - 13.0μ (6.25μ ± 1.21); a: 2.4 - 5.3 (3.57 ± .35); excretory pore from anterior end (expressed as % of body length): 20.2 - 32.1 (25.86 ± 1.65); middle of gonad from anterior end (expressed as % of body length): 63.2 - 75.1 (69.65 ± 1.79). This stage further swells to attain an ovoid shape without increasing in length (Fig. 3K). The cuticle is thicker than that of the third stage and is marked by fine transverse rows of small punctations which become very coarse in the anal area. Lip region and esophagus are similar to that of the third-stage larva. The stylet is longer and the knobs are larger (Fig. 3G). The anus is located subterminally, the phasmids subterminally to terminally (Fig. 3H, I, K). The three glands in the vicinity of the rectum do not increase in size. The reproductive system extends in length. The two gonads are characteristically twisted and begin to differentiate into the various parts (Fig. 3J, K, 5). Specialized ventral chord tissue is well defined, and the beginning of vagina formation is indicated.

Fourth molt. The fourth molt proceeds in a similar manner as the two previous molts. The hypodermis increases in thickness especially in the vulva and tail regions. In the vulva region, invagination of specialized ventral chord tissue is completed and the vagina gains access to the exterior by formation of the subequatorially located vulva. The gonads increase in length and the various parts become fully differentiated. The transversely annulated cuticle of the female is much folded beneath the fourth-stage punctated cuticle.

Female. The female which was described earlier (1) attains an ovoid to spherical shape (Fig. 4). Cuticle and hypodermis are thicker than in the previous stages. The cuticle is distinctly annulated with underlying punctations arranged in regular rows. Lip region and esophagus are similar to that of the fourth-stage larva and the stylet is slightly longer. The female is didelphic with the vulva located subequatorially. Each gonad has typically four flexures, and the various gonad regions are well defined. The germinal zone of the ovary is followed by a short growth zone which ends in one large oocyte, the size of an egg. This oocyte passes through a short oviduct with high columnar cells into a small spermatotheca and then into an enlarged region with large flat cells that appear to be glandular. Formation of the egg membranes seems to be completed in this crustaformeria-like structure which is set off from the uterus proper by a constricted region comprising six cells. The uterus in young females is short and wide, but later, as more eggs accumulate, it appears to extend in depth throughout the body cavity, and the eggs become arranged around the female perpendicular to the long axis of the body. The anus is located subterminally with the phasmids nearly terminal. The rectal gland-like cells are distinct, but do not increase in size.

DISCUSSION

There are only a few cases in the literature where plant-parasitic nematodes develop to adults without feeding. In *Tylenchulus semipenetrans* Cobb, male second-stage larvae are morphologically distinguishable from female larvae and develop to adult males without feeding when kept in tap water or in soil (9). Female larvae, however, do not develop beyond the second stage, unless they feed on a suitable host plant. It is evident that the pattern of postembryogenesis of *T. semipenetrans* is similar to that of *M. floridensis*. The major difference is that second-stage larvae of *T. semipenetrans* are sexually differentiated and their further development proceeds according to their genetic sex. Second-stage larvae of *M. floridensis*, on the other hand, are sexually undifferentiated and their differentiation as females or males depends on whether they start feeding or remain under conditions of starvation, respectively.

Similarly, second-stage larvae of *Rotylenchulus reniformis* Linford and Oliveira held in water or in soil develop without feeding into small vermiform adult males and females (5). The males do not feed and remain small and vermiform throughout their life. The young females start feeding in the roots of a host plant and grow to attain the kidney shape, characteristic of mature females. The pattern of postembryogenesis again resembles that of *M. floridensis*, although development of males and females of *R. reniformis* proceeds normally and according to the genetic sex of the larvae. Only three molts have been observed in *R. reniformis* (5), but we re-examined this nematode and found that an additional molt (the first molt) occurs in larvae within the egg. This suggests that the molting pattern of *R. reniformis* is similar to that of *M. floridensis* and of most other plant-parasitic nematodes.

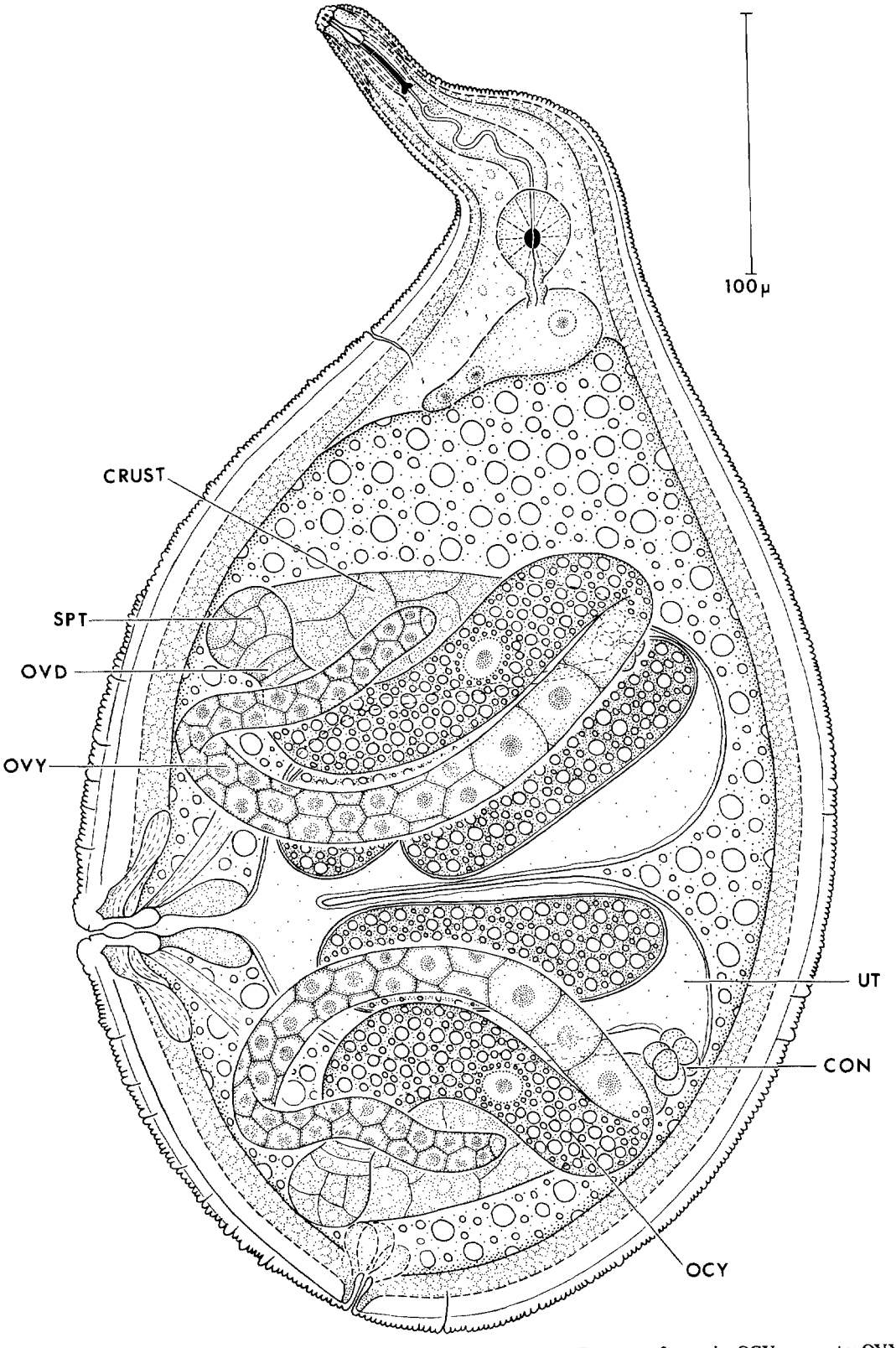

FIG. 4. *Meloidodera floridensis*. Female. CON = constriction; CRUST = crustaformeria; OCY = oocyte; OVY = ovary; OVD = oviduct; SPT = spermatotheca; UT = uterus.

Third- and fourth-stage male larvae of *M. floridensis, T. semipenetrans* and *R. reniformis* also share the common characteristics of having degenerate stylets and esophagi. These structures remain degenerate in adult males of *T. semipenetrans*, but become well defined in adult males of *M. floridensis* and *R. reniformis*.

No obvious metamorphosis occurs during the development of males of *M. floridensis*. Fourth-stage male larvae of the related genera *Heterodera* and *Meloidogyne* are saccate and undergo metamorphosis to become elongate and vermiform adult males (6, 7). This apparent lack of metamorphosis in *M. floridensis*, however, may not constitute a real developmental difference between these genera. Since *M. floridensis* males develop without feeding, developing larvae do not grow in size and therefore, cannot become truly saccate. Our observations show that third- and fourth-stage male larvae are indeed shorter and wider than the resulting adult males. Therefore, some change in shape occurs in the fourth larval stage of *Meloidodera*, but it is not striking enough to be characterized as metamorphosis.

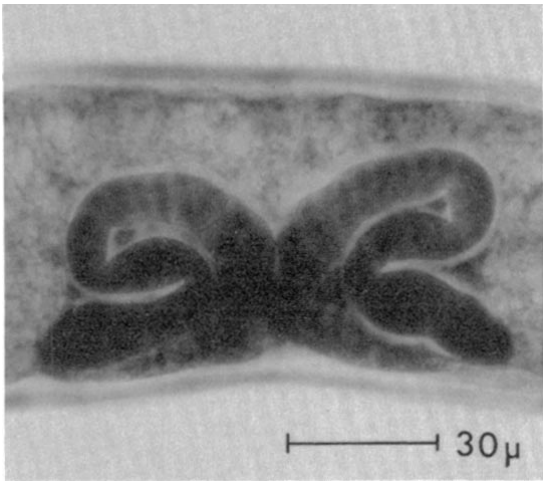

FIG. 5. Photomicrograph of reproductive system of fourth-stage female larva of *Meloidodera floridensis*.

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