

Feeding, Egg-Laying, and Embryology of the Columbia Lance Nematode, *Hoplolaimous columbus*

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Abstract: Feeding and egg-laying of *Hoplolaimous columbus* were observed on excised alfalfa (cultivar 'DuPuits') root cultures in 1.0% nutrient agar. Feeding was ectoparasitic on cortical cells in the maturation zone of the root. Oocytes were first observed in the anterior ovary of the feeding female 8-9 days after feeding began. Globular secretions emanating from the vagina and vulva preceded migration of the posterior-most oocyte of the anterior gonad into the columella. The egg shell was formed within 8 h, and the egg was laid within 12-24 h. The eggs differed from those of other plant-parasitic nematodes in having a stalk on the distal end. The average time required from egg-laying to hatching was 12 days in water and in alfalfa root cultures. **Key Words:** root culture, alfalfa, reproductive system.

The Columbia lance nematode, *Hoplolaimous columbus* Sher, 1963, is a polyphagous species with a wide host range (7). It is often associated with damage to soybean [*Glycine max* (L.) Merr.] and cotton [*Gossypium hirsutum* L.] in the sandy soils of South Carolina and Georgia (8). The present work is part of a series of studies on the biology of the nematode. This paper presents in vitro studies on feeding, egg-laying, and embryology of *H. columbus*.

MATERIALS AND METHODS

Feeding and egg-laying: Several tylenchid species have been cultured monoxenically on excised alfalfa roots and callus cultures (13). Preliminary laboratory trials indicated that *H. columbus* readily penetrated and fed on alfalfa roots. Adult feeding and egg-laying were observed under sterile conditions on 'DuPuits' alfalfa root cultures growing on 1.0% nutrient agar in plastic petri dishes.

Seeds were scarified with concentrated sulfuric acid for 20 min, rinsed three times

with sterile distilled water, and germinated on 1% water agar in petri dishes at 27 C. Roots, 1-2 cm long, were excised and transferred to petri dishes containing a modified Linsmeier and Skoog (RM-1964) nutrient agar medium supplemented with 20 g/liter sucrose and 0.400 mg/liter thiamine:HCl(11). The pH was adjusted to 5.5.

H. columbus was extracted from infested soil collected from a fallow soybean field near Holly Hill, South Carolina, by the modified Baermann funnel technique. Nematodes were surface sterilized in 0.5% solution of hibitane diacetate for 15 min, rinsed three times with sterile tap water, and transferred with a micropipette to BPI (Bureau of Plant Industry) dishes. About 20 nonvacuolated, motile adult nematodes were hand-picked and transferred to 3-week-old root cultures.

Several nematodes were observed during feeding and egg-laying. The total number of eggs laid by each female could not be determined because of their migratory behavior.

Embryology: For embryological studies, gravid females were picked from a nematode suspension and kept in water in BPI dishes. Newly-laid eggs were washed in 0.05% NaOCl for 5 min, and rinsed three times with sterile distilled water. This treatment was enough to

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keep the eggs relatively free of microorganisms during the observation period. For photomicrographs and visual observations of developing embryos at higher magnification, hanging drop preparations of the eggs were prepared (3). Photomicrographs were taken at periodic intervals during embryogenesis. In addition, the location of newly-laid eggs in the root cultures was marked on the dishes and the duration of development to the second-stage larva was recorded.

OBSERVATIONS

Feeding: Nematodes feeding on roots appressed to the bottom of the petri dish were observed under an inverted light microscope. Feeding occurred only in the maturation zone of both young and older roots. Although the behavior of *H. columbus* leading to feeding varied somewhat, a general pattern existed. The nematode migrated along the length of the root for 20-90 min, and pressed its lips intermittently against the epidermis. When a suitable site was found, the nematode focused its attention on a three- or four-cell area, and its stylet probed the epidermis. The stylet penetrated the epidermal cell readily and proceeded to penetrate the cortical cell, where it met with some resistance. The stylet was thrust against the cell wall at a rate of 28-40 times per min. With each thrust, there was a corresponding backthrust of the nematode's body as contact was made with the cell wall. Probing lasted 17-41 min, and ceased after the stylet penetrated the cortical cell.

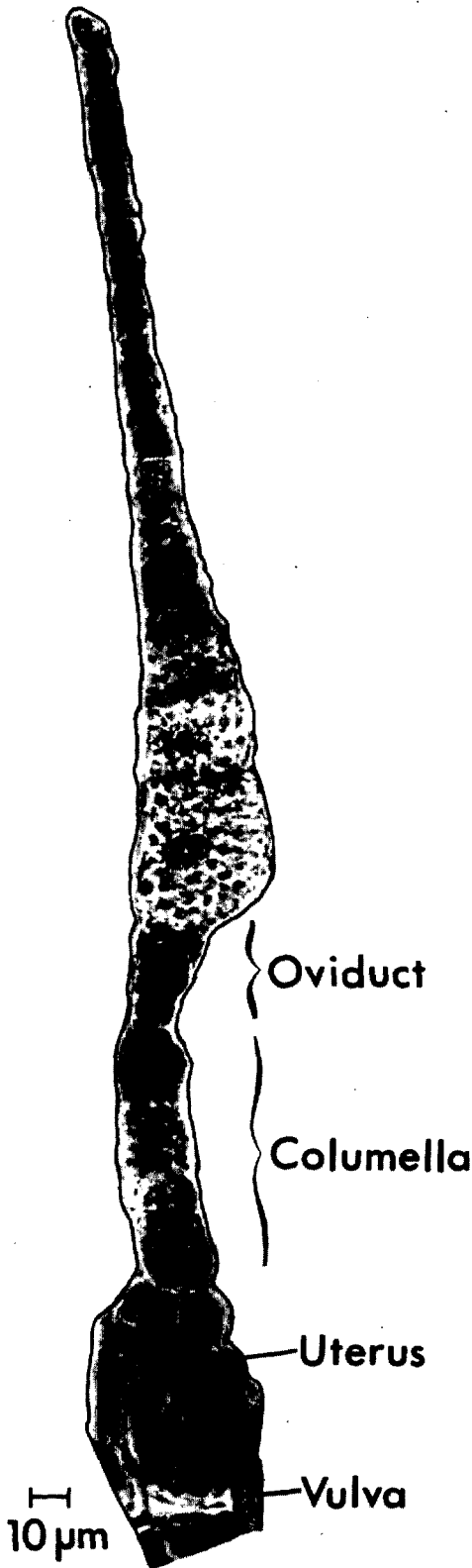
I could not determine whether penetration was due to only the physical force exerted by the stylet against the cell wall or whether the cell wall was partially digested through enzymatic secretions that emanated from the stylet. The depth to which the nematode penetrated appeared to depend on the age of the root. Nematodes on young white roots usually were shallow feeders and penetrated no deeper than one or two cells. In older roots the nematodes penetrated several cells deep, and became embedded to the level of the valve of the esophageal metacarpus.

During ingestion, the nematodes were quiescent and the stylet protruded about 10 μ m beyond the lip region, exposing the subterminal aperture of the stylet. Although the median bulb pulsated at 200 beats per min,

no material was observed to pass through the lumen of the esophagus. The nematodes fed actively for periods of 1-45 min, rested for 3-28 min, and then resumed feeding. Occasionally, during rest periods, the head was withdrawn from the feeding site for 15-20 sec and then reinserted to resume feeding as evidenced by the pulsation of the median bulb. At other times, after the head was reinserted into the feeding site, the stylet probed adjacent cells within the cortex before the nematode resumed feeding. The time spent by the nematode at one feeding site was variable, and ranged from 1-9 days with obvious lesions developing on the root within 2-3 days after feeding began. In young white roots, cells around the head of the nematode turned yellow and then brown; in the older yellow roots, the cell turned brown. After leaving the original feeding site, the nematode often migrated to a new root or remained on the same root and selected a new feeding site some distance away.

Egg-laying: Although the reproductive system is fully developed after the last molt, the female must feed before eggs are laid (12). *H. columbus* is didelphic, and the reproductive system with outstretched ovaries occupies about 61% of the body length. Both gonads appear equally developed, but the anterior one is often slightly longer than the posterior one. Each gonad contains 20-35 oogonia and oocytes. The germinal zone contains 14-20 oogonia, which are not attached to a rachis. Proximally, the germinal zone unites with the growth zone, which contains as many as 10 oocytes in various stages of maturity (Fig. 1).

Oocytes were first observed in the proximal part of each gonad 8-9 days after feeding began. When six oocytes were apparent in the anterior gonad, globular secretions emanated from the vulva. Shortly thereafter, the nematode stopped feeding, withdrew from its feeding site, and became very active. The proximal end of the posterior-most oocyte in the anterior gonad assumed a V-shape and slowly squeezed through the constriction of the oviduct leading to the columella (Fig. 2-A to E); a process which took approximately 5 min. The egg assumed its typical shape in the columella (Fig. 2-E). The nematode became quiescent, and the shell formed around the egg within 8 h. Within 12-24 h, the egg passed into the uterus, and the nematode became very active. The moment of oviposition almost



could be predicted by the action of the female. The nematode undulated intensely, and pseudocoelomic fluid circulated rapidly around the uteri and vagina. Within 5-10 min, the egg moved directly adjacent to the vagina. After 20 sec, the vulva and vagina dilated to about $7 \mu\text{m}$ (measured from photomicrographs). At first the egg shell appeared as a bud (5) through the vulva opening. The ovum then flowed into the shell as it proceeded to emerge, until the egg was laid (Fig. 2-F). In one observation, the egg was ejected with some force when the egg was half way out. It took about 10 sec for the egg to be laid. The nematode became quiescent again for 1-12 h. The second egg laid was from the posterior gonad, and the third from the anterior gonad. After 4-6 eggs were deposited, the nematode temporarily returned to the root and resumed feeding before laying more eggs. A total of 15 eggs were counted that could be attributed definitely as being laid by a single female.

Embryology: Eggs of *H. columbus* were deposited in the one-celled stage. They were oblong and slightly kidney-shaped, measuring $112.5 \times 39.5 \mu\text{m}$ ($109.5-120.5 \times 32.9-43.8 \mu\text{m}$; 15 measured). They differed from eggs of other plant-parasitic nematodes in having a stalk on the end of the egg that emerged last. The stalk is cuticular and varied in length from a barely discernible nub to $15\text{-}\mu\text{m}$ long (Fig. 2-G, 3). The first cleavage, within 4-12 h, was equatorial and unequally holoblastic; the anterior-most cell (S) was slightly smaller than the posterior cell (P_1) (Fig. 3-B). The second cleavage, about 12 h later, divided the anterior blastomere transversely, forming cells A and B (Fig. 3-C). One or two polar bodies were usually evident between cell B and P_1 . About 6 h later, cell P_1 divided transversely to form the P_2 and EMST blastomeres; the latter cell to give rise to future endoderm, mesoderm, and stomodeum tissues. The polarity of the embryo had no relationship to the end of the egg possessing the stalk.

The linear arrangement of the four blastomeres was often set askew, due to a shifting of the EMST blastomere around and over the B blastomere. Blastomere A divided into a,a and B into b,b. Beyond this point, it

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 FIG. 1. Montage of anterior gonad of female *Hoplolaimus columbus* in ventral view, showing various gonad regions.

was difficult to follow the sequence of cleavages. The gastrula stage was reached 5-6 days after oviposition (Fig. 3-H), and within 24 h the cells had differentiated into two zones of differing densities; the light zone to become the esophagus and the dark zone the intestine of the larva (Fig. 3-I). By the 8th day after oviposition, the first-stage larva differentiated (Fig. 3-J). Progenitors of the esophagous and stylet were barely discernible in the first stage larva (Fig. 3-K). During the first molt, the conical part of the stylet appeared, and by about 24 h later, the shaft and knobs had differentiated. The cuticle of the first-stage larva was smooth, but that of the second-stage larva was annulated. The second-stage larva hatched within 2-3 days after the first molt. The average time required from egg-laying to hatching was 12 days, but it varied from 9-15 days in water and in alfalfa root cultures.

DISCUSSION

Although *H. columbus* is frequently encountered as an ecto-endoparasite on soybean and cotton (12), it behaves as an ectoparasite on excised alfalfa root cultures. Its feeding habits are similar to the basic pattern outlined for *H. indicus* (10) and other tylenchids and involves exploration, penetration, ingestion, and rest periods (4). Judging from the necrotic reactions on the roots around the head of the nematode and at abandoned feeding sites, the older roots appeared to be damaged by the feeding nematodes more severely than were the younger white roots. In the older roots, the nematodes fed for a longer period, and inserted their heads deeper.

Nematode migration in the root cultures appeared to be random on transfer to the dishes, and no evidence was observed of an attractive stimulus to the roots. However, after the nematode stopped feeding and withdrew from the root, it appeared to orient itself toward the root, and had no difficulty in finding the original feeding site or a new one. Possibly the stimulus came from the necrotic tissues. Wound tissues have been shown to attract *Aphelenchus avenae*, and to stimulate feeding (9).

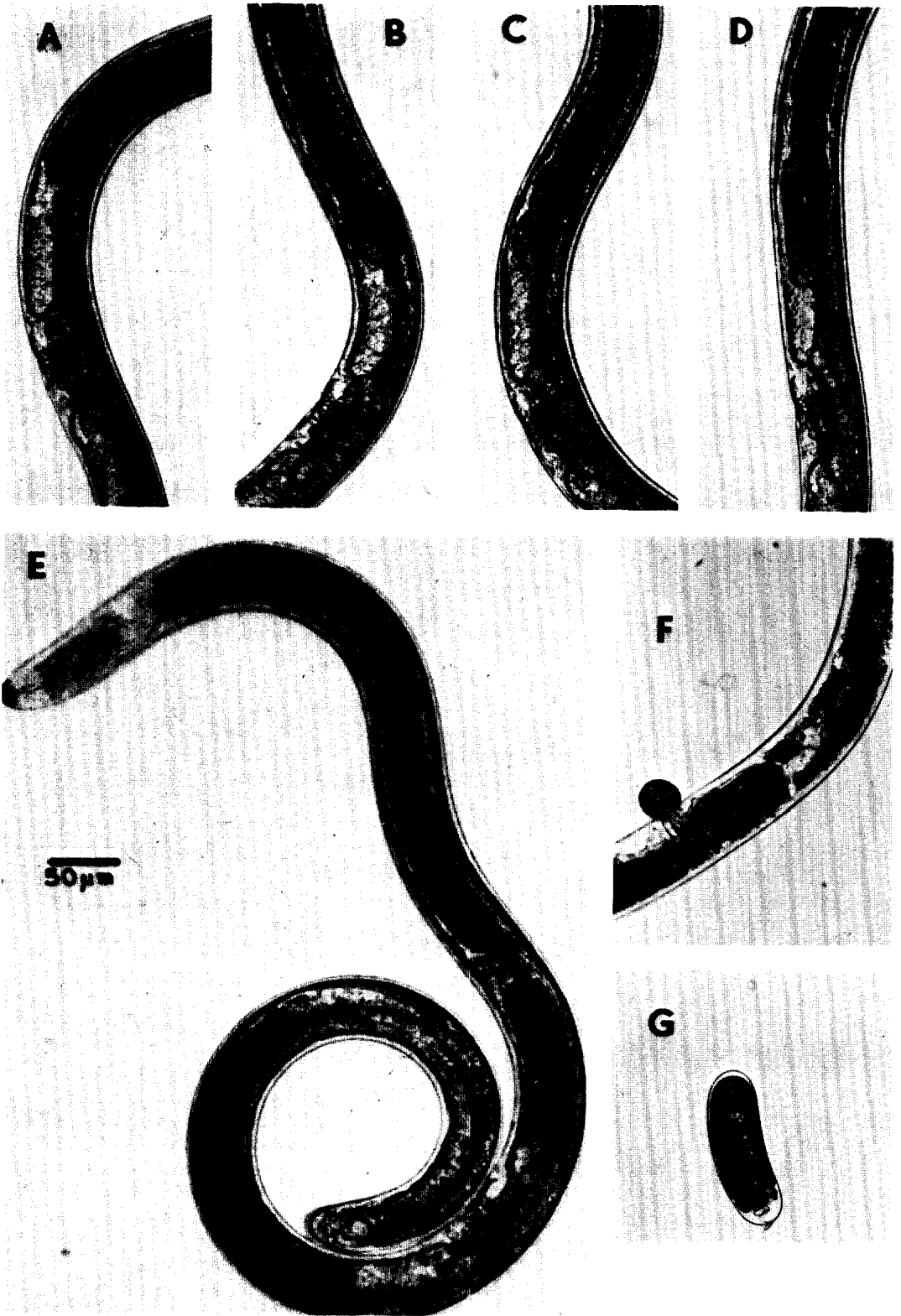
Oocyte development in adult females was initiated only after the nematode began feeding. It could not be induced by our bathing the females in root exudates

(Fassuliotis, *unpublished*). The stalk on one end of the egg of *H. columbus* is of special interest, since it can possibly be used as a diagnostic character for species identification. I am not aware of its occurrence on eggs of other species of *Hoplolaimus*. Polar filaments and other modifications of the protein coat are common on the eggs of animal-parasitic nematodes and appear to be associated with the life cycle to ensure survival (1). The stalk in *H. columbus* appears to serve no such purpose, because eggs are usually laid within the root cortex or in the soil close to the root. Egg shell formation in plant-parasitic nematodes is apparently an endogenous process (1). I have not determined with certainty how the stalk is formed, and could not see it in in toto preparations, either under light- or phase-microscopy (Fig. 2-E, F). However, dissection of females with oocytes that had recently migrated to the columella, showed that the stalk is an integral part of the egg morphology before the shell is fully formed.

The embryology of *H. columbus* is essentially similar to that reported for other plant-parasitic nematodes. Dasgupta, et al. (2) described the embryology of *H. indicus*, and found that it took 8-10 days for the egg to hatch after oviposition. Eggs of *H. columbus* hatched 9-15 days after oviposition in water and in alfalfa root cultures.

The fecundity of *H. columbus* is yet to be determined. It was difficult to count with certainty the number of eggs deposited by each female, because the nematode migrated in the alfalfa root cultures during egg-laying.

Although the nematode can survive under adverse conditions of high osmotic pressures, desiccation, and high soil temperatures (6), it is difficult to rear in large quantities in the greenhouse. Only a few plant-parasitic nematodes have been raised monoxenically on excised roots or callus cultures. In the current studies, we had no difficulty in culturing the nematode on alfalfa root cultures. Corn roots also are readily penetrated and fed upon (Fassuliotis, *unpublished*). Monoxenic culture methods to rear *H. columbus* will increase the capabilities for expanded research on this nematode, and facilitate studies on its biology and physiology. Other plant species are being tested to find more suitable substrates for culturing the nematode, and to verify greenhouse and field observations on the host



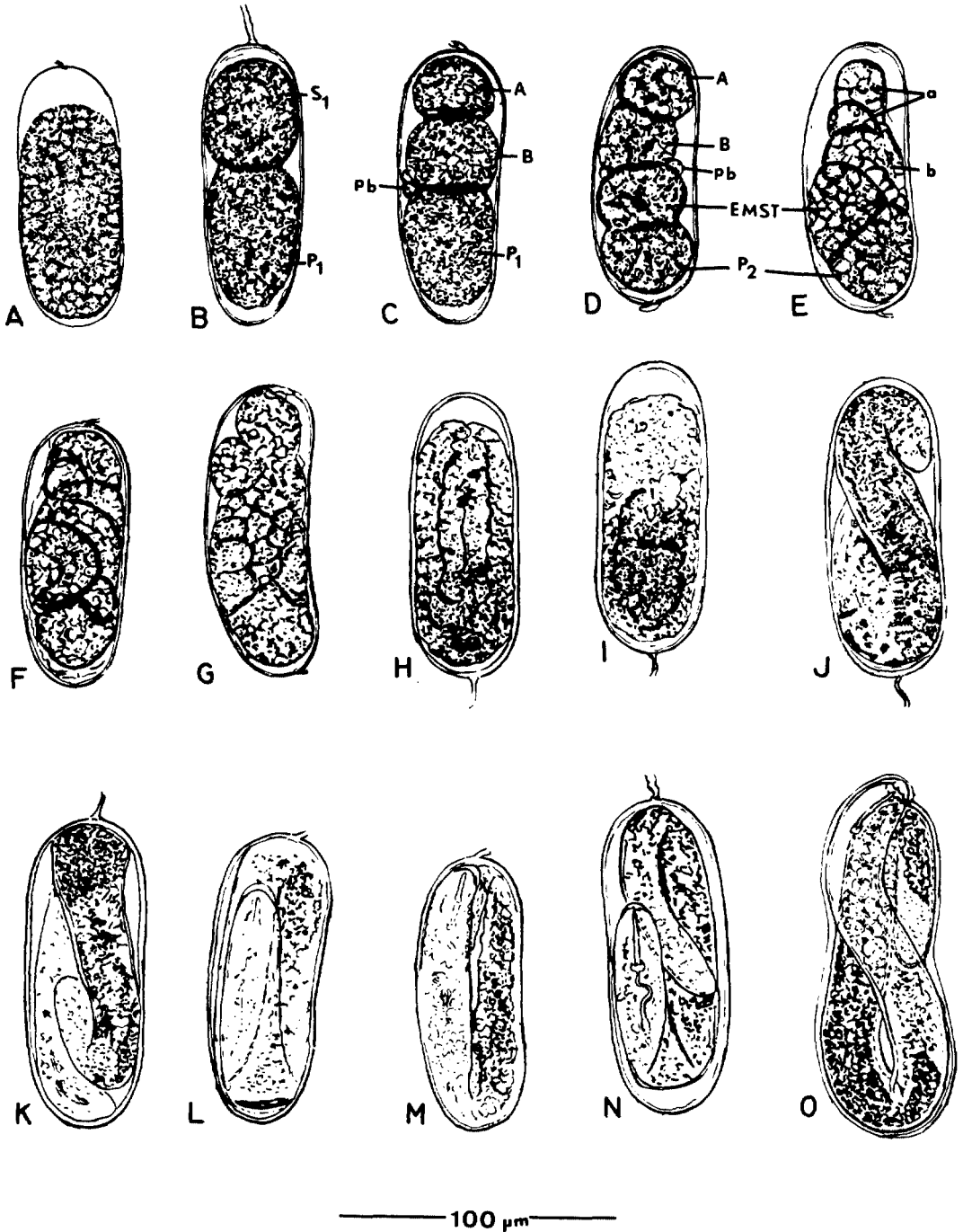


FIG. 3-(A to O). Eggs of *Hoplolaimus columbus* in various stages of development. A) One-celled stage; B) two-celled stage; C) three-celled stage showing two polar bodies between cells B and P₁; D) four-celled stage with a polar body on either side between B and EMST; E) seven-celled stage; F-G) Multiple-celled stages; H) Gastrula; I) Early 'tadpole' stage; J-K) First-stage larva; L-N) Molting first-stage larva; O) Second-stage larva ready to hatch.

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FIG. 2-(A to G). Female reproductive system of *Hoplolaimus columbus*. A-E) Oocyte migration from oviduct to columella. Note that the stalk is not visible on the egg while in the columella or as the egg is being laid; F) Egg being deposited; G) Egg immediately after being deposited. Note stalk at distal end of egg.

range of *H. columbus* (7). If behavior patterns in root cultures are comparable to those of intact plants, it may be possible to develop methods to screen plants for resistance to *H. columbus* in this manner.

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