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THE LIFE CYCLE OF *MONONCHOIDES FORTIDENS* (NEMATODA: DIPLOGASTEROIDEA) WITH EMPHASIS ON GONAD DEVELOPMENT

BY

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Mononchoides fortidens reproduced by amphimixis. Smooth, elongate eggs measuring 60-80 × 35-45 µm were laid in single-cell stage. Embryonic and post-embryonic development lasted for 16-20 h and 3-6 days respectively. Sexes were first distinguished during second moulting by the anterior elongation of primordium in males and anterior as well as posterior elongation in females. The aggregation of cells dorsal to the rectum in males represented the site of spicule formation. In females specialized ventral chord nuclei on the mid-ventral side of genital primordium formed the vagina. A flexure developed first in the third stage male, and later in fourth stage females. The life cycle from egg to adult was completed in 4-7 days.
Keywords: nematode biology, development, reproduction, embryology.

Studies of the diplogasterids are mainly of their predatory behaviour. Aspects of reproduction and developmental biology were studied by Triantaphyllou and Hirschmann (1964), Pillai & Taylor (1968) and Hechler (1970). The life cycles of *Mesodiplogaster lheritieri* and *Eudiplogaster aphodii* were later studied by Grootaert (1976) and Poinar *et al.* (1976). Woombs and Laybourn-Parry (1984) described the growth, reproduction and longevity in *Diplogasteritus nudicapitatus*. The present study deals with embryonic and post-embryonic development of a predatory diplogasterid, *Mononchoides fortidens*.

MATERIALS AND METHODS

Soil samples containing *M. fortidens* were collected from Burdwan, West Bengal, India and processed by sieving, decantation and modified Baermann's funnel techniques. The nematodes obtained were cultured in 1.5% water agar in 5 cm diam. Petri dishes. *M. fortidens* was found to be bacteriophagous as well as predacious. The specimens studied were the progeny of a single female.

Embryonic development was studied in about 30 eggs, placed in observation chambers designed by Ahmad & Jairajpuri (1979). About 30 juveniles of each stage were stained in 1% lacto-aceto-orcein (1 g orcein + 33 cc lactic acid + 33 cc acetic acid + 34 cc distilled water) for 24 h. Nematodes were destained with 45% lactic acid, if required. All studies were conducted at 30 ± 2°C.

RESULTS

The egg laid by fertilized females were single celled, elongate and measured 60-80 × 35-45 μm (72 × 40 μm). Not more than two mature eggs were present in the uterine tract of gravid females. The cytoplasm retracted 15-20 min after egg laying indicating the commencement of cleavage.

The first cleavage furrow was transverse, dividing the egg into a larger anterior S_1 and smaller posterior P_1 blastomere (Fig. 1B). Later S_1 divided obliquely into blastomeres A and B, and after 10-15 min P_1 also divided obliquely into blastomere, S_2 and P_2 thus forming a four-cell stage (Fig. 1D). P_2 further divided into blastomeres S_3 and P_3 to form a six-cell stage 50-60 min after first cleavage. The cleavage patterns onwards could not be traced due to super-imposition of rapidly dividing blastomeres. The morula was formed 2.5-3 h after first cleavage and was followed by blastula 1.5-2 h later. Gastrulation which commenced 5.5-7 h after first cleavage marked a considerable elongation of the embryo (Fig. 1H). Invagination led to the formation of lima bean stage (Fig. 1I). Later comma (Fig. 1J) and tadpole stages were formed at 15-20 min intervals. Movements in the embryo were first observed in the tadpole stage (Fig. 1K). Plum (Fig. 1L) and loop stages were reached subsequently after 20-25 min. The loop stage embryo was two egg folds long with a median depression at the anterior end. The early pretzel stage formed 11-13 h after first cleavage brought about the appearance of refractory stomatal plates and the lining of the rectum. Twenty to 30 min after the start of the early pretzel stage, the stomal rhabdions and valvular apparatus were well formed. At the time of hatching, the forward moving juvenile retracted into the shell for 1-2 min. Total embryonation time was 16-20 h.

During post-embryonic development the multiplication of primoridal nuclei was confined to the periods of moulting until the third stage. The fourth stage showed continuous proliferation of primordial nuclei.

First stage juvenile (Fig. 2A, K)

The juveniles measured 0.26-0.40 mm (0.32 ± 0.07 mm). The genital primordium was 7-12 μm long, located at 37-50% of the body from the anterior end and was obliquely oriented. There were two germinal and two somatic nuclei. During moulting the multiplication of somatic nuclei took place while germinal nuclei remained undivided.

Second stage juvenile (Fig. 2B, G)

The second stage juveniles varied in length from 0.43-0.50 mm (0.46 ± 0.03 mm). The primordium measured 9-15 μm in length and was located 30-36% from the anterior end of body. The number of germinal and somatic nuclei was two and six respectively. The differentiation of sexes became evident in the

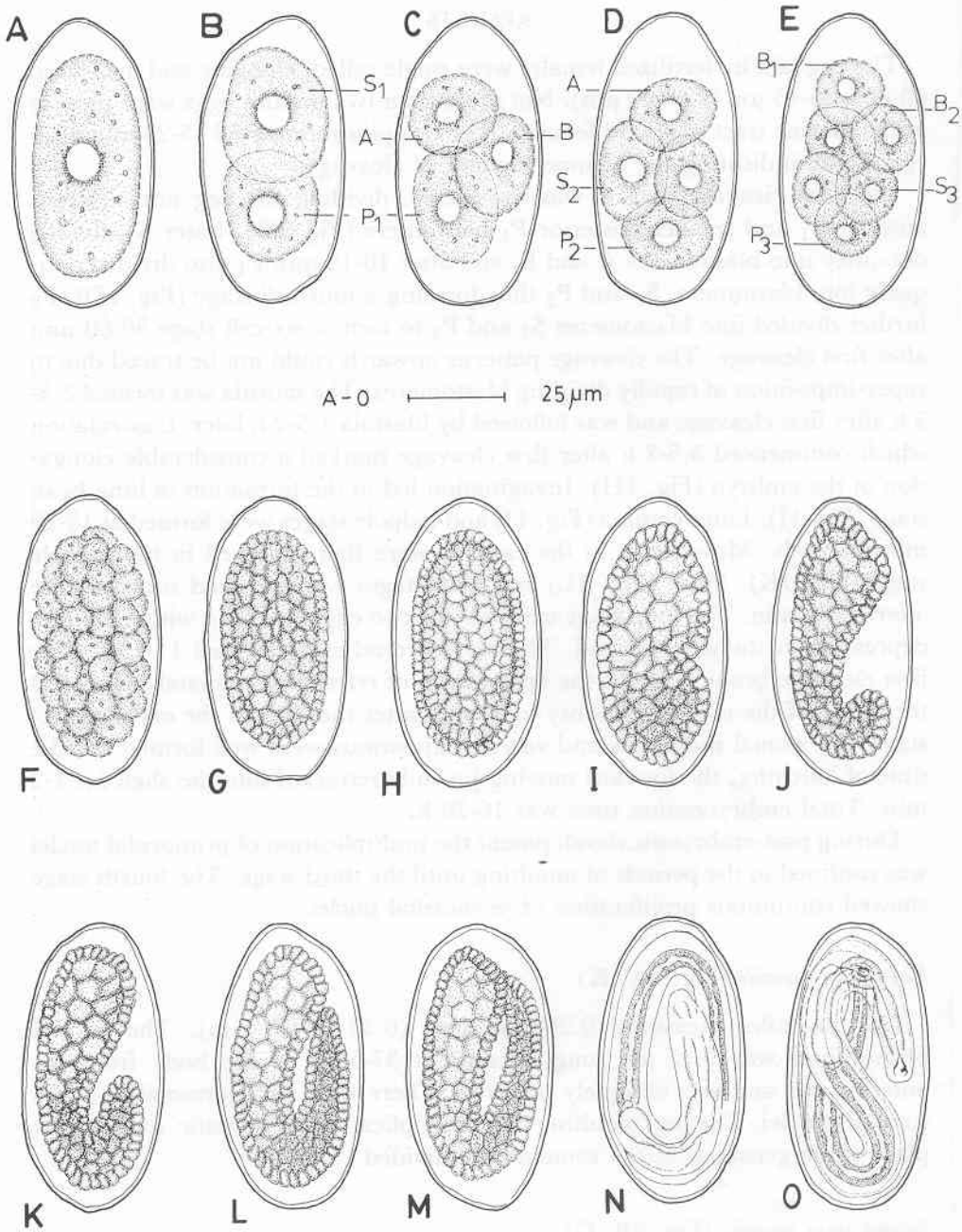

Fig. 1. *Mononchoides fortidens* - Embryonic development. A. Single call stage; B. Two cell stage; C. Three cell stage; D. Four cell stage; E. Six cell stage; F. Morula stage; G. Blastula stage; H. Gastrula stage; I. Lima bean stage; J. Comma stage; K. Tadpole stage; L. Plum stage; M. Loop stage; N. Early pretzel stage; O. Late pretzel stage.

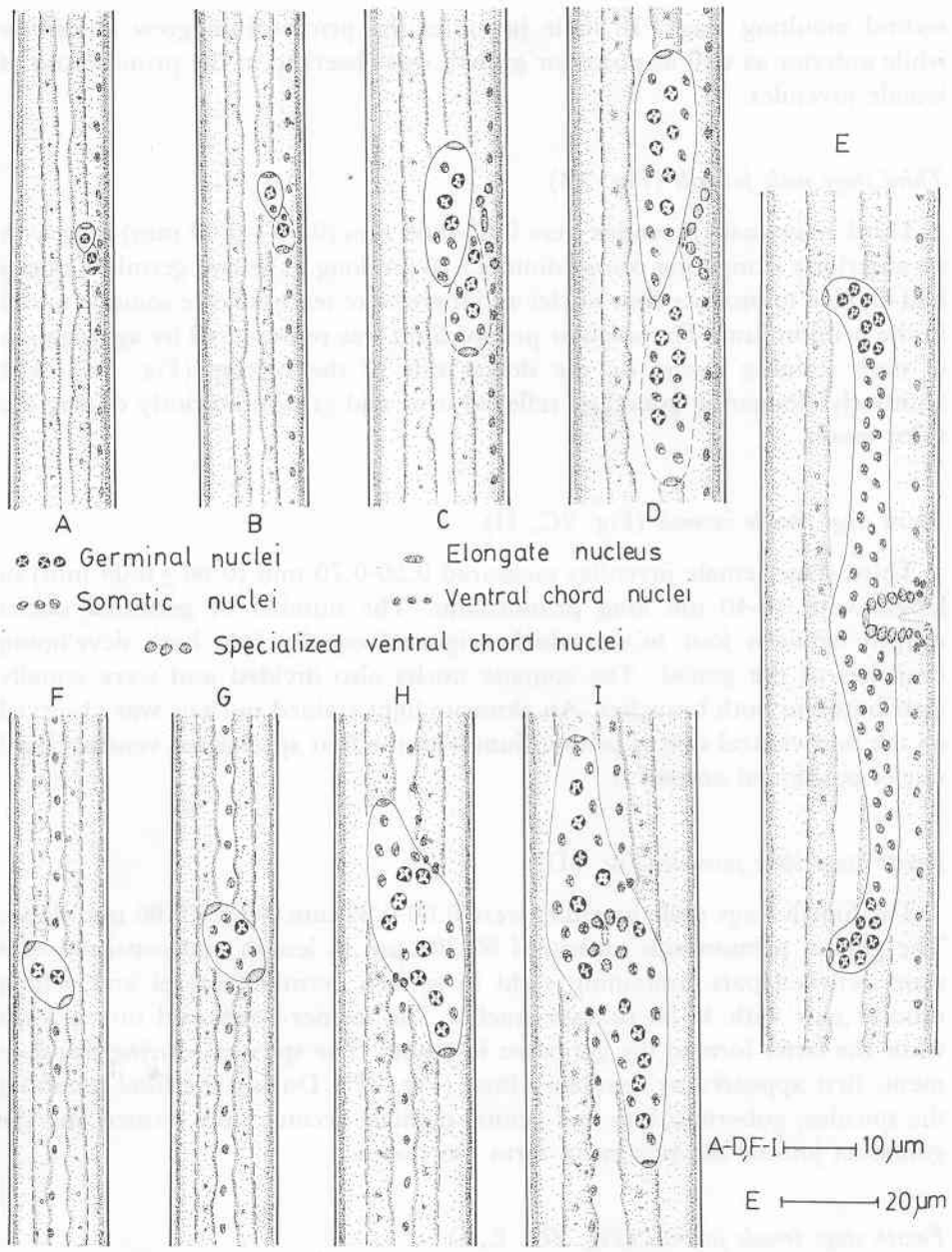

Fig. 2. *Mononchoides fortidens* female - Post-embryonic development. A-E. Developing genital primordia (lateral view); F-I. Developing genital primordia (ventral view); A, F. First stage juvenile; B, G. Second stage juvenile; C, H. Third stage juvenile; D, I. Early fourth stage juvenile; E. Late fourth stage juvenile.

second moulting stage. In male juveniles the primordium grew anteriorly while anterior as well as posterior growth was observed in the primordium of female juveniles.

Third stage male juvenile (Fig. 3B)

Third stage male juveniles were 0.48-0.68 mm (0.59 ± 0.09 mm) long with an anteriorly elongating primordium 25-70 µm long. The two germinal nuclei had divided to produce four nuclei and there were ten to twelve somatic nuclei in the primordium. The spicular primordium was represented by aggregation of dark staining nuclei on the dorsal side of the rectum (Fig. 3E). The anteriorly elongating gonoduct reflexed over and grew posteriorly during the third moult.

Third stage female juvenile (Fig. 2C, H)

Third stage female juveniles measured 0.50-0.70 mm (0.60 ± 0.09 mm) in length with 15-40 µm long primordium. The number of germinal nuclei ranged between four to six which migrated equally into both developing branches of the gonad. The somatic nuclei also divided and were equally distributed in both branches. An elongate light stained nucleus was observed on the mid-ventral side of primordium with the four specialized ventral chord nuclei positioned around it.

Fourth stage male juvenile (Fig. 3D)

The fourth stage male juveniles were 0.60-0.82 mm (0.67 ± 0.06 mm) long. The genital primordium measured 60-300 µm in length and consisted of a short reflexed part containing eight to sixteen germinal nuclei and a long tubular part with 40-80 somatic nuclei. The former developed into a testis while the latter formed the gonoduct in adults. The spicules, during development, first appeared as refractory lines (Fig. 3F). During the final moulting the spicules, gubernaculum and genital papillae became fully formed and the gonoduct jointed the rectum to form the cloaca.

Fourth stage female juvenile (Fig. 2D, E, I)

Fourth stage female juveniles varied in length from 0.60-0.87 mm (0.72 ± 0.1 mm) with a primordial length of 60-196 µm. There were 12-30 germinal nuclei and 22-58 somatic nuclei in the early fourth stage. The specialized ventral chord nuclei, 6-12 in number now aggregated at the site of the future vagina with the elongate nucleus lying in the centre. These nuclei marked the boundary of the vagina and formed the lumen by migrating inwards. During

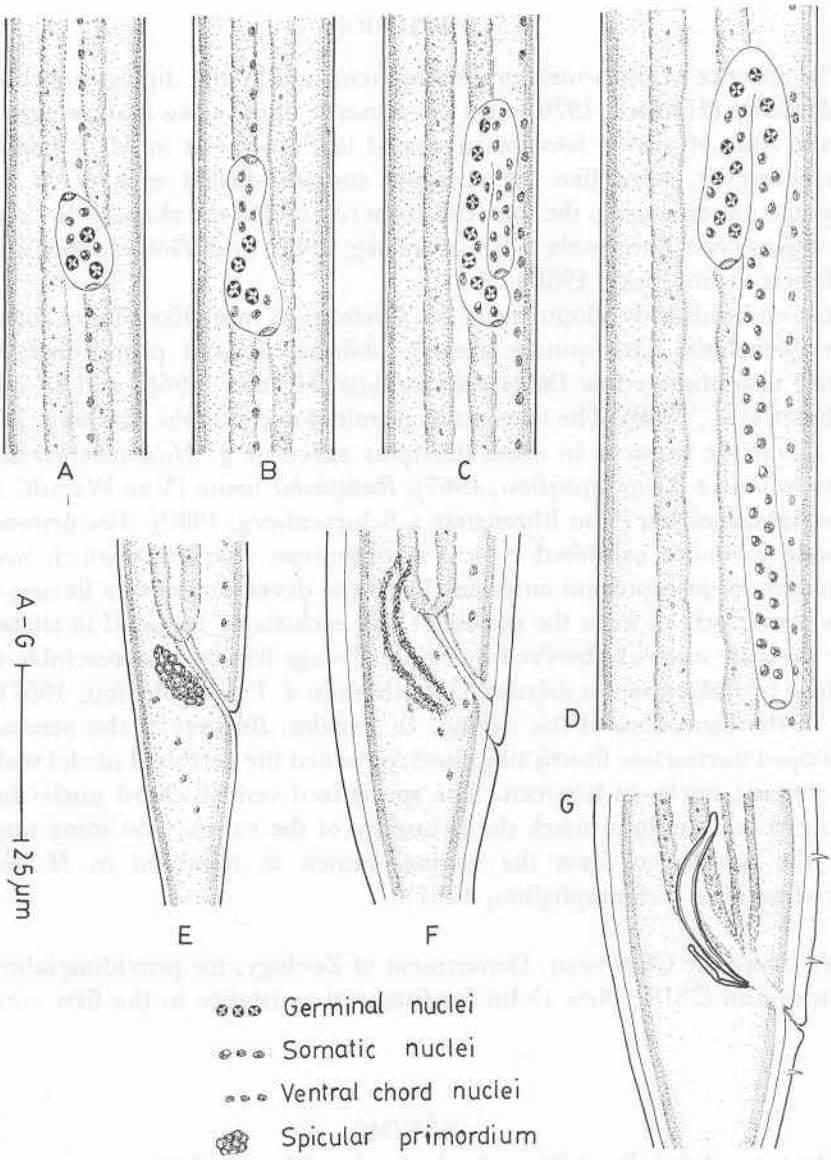

Fig. 3. *Mononchoides fortidens* male - Post-embryonic development. A-D. Developing genital primordia (lateral view); E-G. Developing spicular primordia (ventral view); A, C. Second/third moulting stage juvenile; B, E. Third stage juvenile; D, F, G. Fourth stage juvenile.

the final moult the primordium increased in length and developed flexures at the tips. The germinal nuclei were confined to the flexure which formed the ovary. On completion of the moult the entire reproductive tract was completely formed. The total time taken for post-embryonic development was 3-6 days.

DISCUSSION

The absence of intra-uterine development, unlike the diplogasterid *Mononchoides changi* (Hechler, 1970), and the presence of only two mature eggs in the uterine tract of gravid females suggested late oogenesis in *M. fortidens*. The eggs, however, were like the elongate smooth shelled eggs of *M. changi*. Cleavage patterns up to the four-cell stage resembled the rhabditids *Caenorhabditis elegans* (von Ehrenstein & Schierenberg, 1980) and *Teratorhabditis andrassyi* (Tahseen & Jairajpuri, 1988).

Post-embryonic development of *M. fortidens* was more like that of rhabditids than tylenchids. The spindle shaped, obliquely placed primordium was a feature also observed in *Diploscapter coronata* (Hechler, 1968) and *D. orientalis* (Tahseen *et al.*, 1990). The number of germinal nuclei in the first stage juvenile was about the same as in other didelphic species e.g. *Helicotylenchus dihystera* (Hirschmann & Triantaphyllou, 1967), *Radopholus similis* (Van Weerdt, 1960), *Caenorhabditis elegans* (Von Ehrenstein & Schierenberg, 1980). The primordium of male juveniles exhibited typical secernentean characters which involved elongation of primordium anteriad. Later the development of a flexure which grew posteriorly to form the gonoduct was exclusively reported in rhabditids. The elongate nucleus observed in the third stage female may resemble the 'I' nucleus of *Helicotylenchus dihystera* (Hirschmann & Triantaphyllou, 1967) in its role in the formation of the vagina. In females, flexures of the primordium developed during late fourth stage and contained the germinal nuclei with very few somatic nuclei in between. The specialized ventral chord nuclei became arranged in a circle to mark the boundary of the vagina and some migrated into the middle to form the vaginal lumen as observed in *H. dihystera* (Hirschmann & Triantaphyllou, 1967).

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RÉSUMÉ

Etude du cycle biologique de Mononchoides fortidens *(Nematoda: Diplogasteroidea) et plus particulièrement du développement de la gonade*

Mononchoides fortidens se reproduit par amphimixie. Les oeufs, lisses et allongés — 60-80 × 35-45 µm — sont pondus au stage unicellulaire. Les développements embryonnaire et post-embryonnaire durent 16-20 h et 3-6 jours, respectivement. Le sexe peut être distingué au moment de la deuxième mue par l'allongement vers l'avant du primordium génital chez le mâle, tandis que cet allongement a lieu à la fois vers l'avant et vers l'arrière chez la femelle. L'accumulation de cellules observée sur la face dorsale du rectum correspond, chez le mâle, au site de formation des spicules. Chez la femelle, des noyaux différenciés de la corde ventrale sur la face médio-ventrale du primordium génital donnent naissance au vagin. Le repli se forme chez le mâle au troisième stade juvénile et chez la femelle au quatrième. Le cycle d'oeuf à adulte est accompli en 4 à 7 jours.

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