

Developmental biology of *Dorylaimus stagnalis* Dujardin, 1845 (Nematoda : Dorylaimida)

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SUMMARY

The observations are made on the embryonic and post-embryonic developments of *Dorylaimus stagnalis*. The eggs, measuring $71-95 \times 33-45$ (82×39) μm , are smooth, elongate oval, densely granulated and dark brown in colour. The first cleavage is perpendicular to the long axis of the egg and appears 15-24 h after the egg was laid. The blastula and gastrula stages are formed 40-90 and 75-90 min after the first cleavage. The tadpole stage is formed 215 min later and the juvenile hatched out of the egg 6 h after the formation of their internal organs. Maximum hatching is recorded at temperatures 30-35 °C. The first stage juveniles have two germinal and 4-6 somatic nuclei in the genital primordium; the second stage have 4-6 germinal and 8-14 somatic nuclei. The third stage have 8-12 germinal and 20-45 somatic nuclei. The fourth stage female and male have 20-60 and 18-61 germinal nuclei respectively. The first stage juveniles require 17-22 days for moulting; the second 24-28 days; the third 30-35 days. The fourth stage female and male require 46-52 and 40-45 days for their development, respectively. In the fourth and final moult, the long filiform tail of fourth stage male juveniles transforms into a short conoid tail. The total time (from hatching to adult) of males varies from 111-131 days and that of females from 117-138 days at 25-30 °C.

RÉSUMÉ

Biologie du développement de Dorylaimus stagnalis Dujardin, 1845 (Nematoda : Dorylaimida)

Des observations ont été faites sur les développements embryonnaire et postembryonnaire de *Dorylaimus stagnalis*. Les œufs, lisses, allongés-ovales, à granulation dense et de couleur brun-noir, mesurent $71-95 \times 33-45$ (82×39) μm . La première division, perpendiculaire au grand axe de l'œuf, a lieu 15-24 h après que l'œuf ait été pondu. Les stades blastula et gastrula sont réalisés 40-90 et 75-90 min, respectivement, après la première division; le stade « en têtard » est réalisé 215 min plus tard et les juvéniles éclosent 6 h après la formation de leurs organes internes. L'éclosion maximale est observée à 30-35 °C. Le primordium génital des juvéniles de 1^{er} stade comporte deux noyaux germinaux et quatre à cinq noyaux somatiques; chez les juvéniles de 2^e stade ces chiffres sont de 4-6 et 8-14, et de 8-12 et 20-45 pour les juvéniles de 3^e stade; chez les juvéniles de 4^e stade, le nombre de noyaux germinaux est de 20-60 pour les mâles et 18-61 pour les femelles. La durée du 1^{er} stade juvénile est de 17-22 jours, celle du 2^e 24-28 jours, celle du 3^e 30-35 jours, celle des 4^e stades femelle et mâle de 46-52 et 40-45 jours, respectivement. Lors de la quatrième et dernière mue, la queue des juvéniles de 4^e stade, allongée, filiforme se transforme en une queue courte, conoïde. La durée entre éclosion et apparition des adultes, à 25-30 °C, varie de 111 à 131 jours pour les mâles et de 117 à 138 jours pour les femelles.

Studies on the developmental biology of dorylaims are few, and most of the informations available relates to the phytophagous species. The life cycle of *Xiphinema index* and *X. mediterraneum* was studied by Radewald and Raski (1962), and Dalmasso and Younes (1969, 1970) respectively. Flegg (1966; 1968a; 1968b), and Cohn and Mordechai (1969) observed the yearly reproduction in the species of *Xiphinema* and *Longidorus*. Jairajpuri and Bajaj (1978), and Malik and Jairajpuri (1983) studied the embryonic development of *X. basiri*, *X. insigne* and *X. americanum*. Of the members of Dorylaimoidea, the biology of only two species have been studied in detail: *Aporcelaimellus* by Wood (1973) and *Labronema vulva-papillatum* by Grootaert and Small (1982). Pillai and

Taylor (1967) and Flegg (1969) determined the effect of different temperatures on the embryonic development and hatching of some phytophagous nematodes. The formation and development of odontostyle in some dorylaim nematodes was studied by various authors (Coomans, 1963; Coomans & De Coninck, 1963; Coomans & Van der Heiden, 1971; Carter & Wright, 1979; Grootaert & Coomans, 1980).

In the present work the embryonic and postembryonic development patterns have been studied in *Dorylaimus stagnalis* Dujardin, 1845. Besides, observations were also made on the development of odontostyle, gonad and male tail. The effect of temperatures on the embryonic development was also observed.

Material and methods

EMBRYONIC DEVELOPMENT

To obtain fresh eggs, gravid females were transferred to small cavity blocks containing 1 % water agar and allowed to lay eggs. These eggs were then placed in the cavity slide containing 1 % water agar. A cover-slip was placed gently over it to prevent desiccation of eggs.

EFFECT OF TEMPERATURE ON EMBRYONIC DEVELOPMENT

To determine the effect of different temperatures on the embryonic development, the eggs were kept in small cavity blocks containing 1 % water agar. Five eggs were placed in each cavity block and ten eggs were used at each temperature. The blocks containing eggs were then placed at different temperatures (5, 10, 15, 20, 25, 30, 35 and 40 °C) maintained in an incubator. Observations were started after 24 h, three times a day, to observe the

hatching and time required for embryonic development at each temperature.

POST-EMBRYONIC DEVELOPMENT

The moulting and inter-moulting juvenile stages were collected directly from the soil, fixed and dehydrated by slow method. Observations were made on these stages for tracing the development of odontostyle and the gonads. For the latter live starved nematodes were also stained in 1 % lacto-aceto-orcein. To observe the moulting time, gravid females were cultured in 1 % water-agar.

**Description of *Dorylaimus stagnalis*
Dujardin, 1845**

DIMENSIONS

See Table 1.

Table 1

Dimensions of adults and juvenile stages of *Dorylaimus stagnalis*
(means are between brackets, n = 50)

Characters	Adult ♀	Adult ♂	L ₁	L ₂	L ₃ ♀	L ₃ ♂	L ₄ ♀	L ₄ ♂
Length (L) mm	2.76-4.66 (3.66)	2.63-4.41 (3.30)	0.37-0.87 (0.48)	0.93-1.49 (1.24)	1.27-2.53 (2.07)	1.08-2.92 (2.33)	2.10-2.97 (2.40)	2.17-3.19 (2.56)
a	27.38-44.86 (36.68)	30.69-42.02 (35.04)	19.47-31.1 (24.37)	26.4-38.0 (32.08)	29.5-41.01 (35.07)	26.0-42.79 (33.60)	28.5-44.66 (34.49)	33.5-40.46 (36.63)
b	3.97-6.18 (5.01)	3.78-5.63 (4.73)	2.6-3.7 (2.92)	3.1-4.0 (3.60)	3.4-4.7 (4.10)	3.4-5.1 (4.29)	4.06-5.32 (4.43)	4.03-5.65 (4.68)
c	12.3-32.76 (19.41)	68.32-145.0 (86.84)	6.3-10.2 (7.82)	8.8-11.9 (10.41)	10.33-19.74 (13.53)	10.9-34.81 (15.11)	11.12-17.47 (15.46)	12.64-19.37 (14.78)
c'	2.47-6.15 (4.15)	0.5-0.84 (0.70)	3.5-6.3 (4.72)	3.8-8.0 (6.05)	2.8-6.62 (5.07)	3.0-5.81 (4.5)	2.28-6.4 (4.93)	3.25-6.54 (4.93)
V	34.88-50.24 (40.58)	—	—	—	—	—	—	—
T	—	57.95-75.59 (67.16)	—	—	—	—	—	—
G ₁	11.41-22.67 (17.45)	—	—	—	—	—	—	—
G ₂	12.8-29.77 (20.94)	—	—	—	—	—	—	—
Functional odontostyle (µm)	37.5-52.5 (42.80)	37.5-46.5 (40.61)	7.5-15.0 (9.6)	18.0-25.5 (22.70)	27-30 (28.71)	27-30 (29.35)	31.5-40.5 (34.32)	31.5-36.0 (32.25)
Replacement odontostyle (µm)	—	—	10.5-20.0 (12.45)	19.5-30 (29.19)	37.5-42 (39.04)	31.5-42 (39.94)	37.5-49.5 (42.87)	39-45 (43.43)
Odontophore (µm)	39-42 (46.86)	39-46.5 (43.67)	10.5-21 (14.22)	21-30 (26.58)	31.5-37.5 (33.67)	27-39 (34.07)	33-43.5 (37.05)	31.5-40.5 (34.75)
Oesophagus (µm)	634.6-888 (728.55)	608-814 (695.69)	125.4-250 (161.72)	292.6-364.8 (338.91)	327.4-551 (501.99)	311.6-600.4 (539)	505.4-630.8 (539.15)	513-596.6 (568.82)
Prerectum (µm)	201.4-418 (285.91)	277.4-779 (465.87)	30-57 (37.2)	60-100.5 (82.77)	76.5-186 (158.54)	115.5-235.6 (200.20)	133-201.4 (173.73)	209-273.6 (228.89)
Anal body diameter (µm)	38-57 (47.35)	49.4-68.4 (54.52)	11.4-19 (12.99)	19-22.8 (19.79)	26.6-38 (31.47)	19-45.6 (35.53)	26.6-38 (33.51)	34.2-45.6 (37.36)
Tail (µm)	102.6-304 (195.76)	30.4-41.8 (38.19)	45.6-95 (61.10)	91.2-152 (119.4)	106.4-201.4 (156.70)	64.6-197.6 (159.72)	76-212.8 (165.77)	136.8-212.8 (182.55)

DESCRIPTION

Female : Body slightly ventrally curved upon fixation, tapering gradually towards extremities but more posteriorly. Cuticle with fine transverse striations, 6-9 μm thick at mid-body and marked with 32-34 longitudinal ridges which gradually fade out towards extremities. Lateral chords 1/4-1/3 of corresponding body width wide at mid-body. Dorsal and ventral body pores 7-10 and 25-30 respectively. Lip region slightly narrower than adjoining body, marked by a slight depression, 15-23 μm wide and 6-8 μm high. *En face* view shows six amalgamated lips arranged as follows : two ventrosubdorsal, two lateral and two ventrosublateral; each lip with a papilla on the inner cirlet and a papilla on the outer cirlet; subdorsal, and subventral lips with an additional papilla; this making a total of 16 papillae.

Amphids stirrup-shaped with slit-like apertures, 8-11 μm wide, occupying about 1/2 of corresponding body width, 8-9 μm from anterior end. Odontostyle cylindroid, 5-6 lip region widths long, forked at its junction with odontophore, aperture 13-15 μm wide or 29-35 % of odontostyle length. Odontophore simple, rod-like, slightly longer than odontostyle, embedded in the oesophageal tissues. Guiding ring "double"; fixed ring at 17-32 μm from anterior end. Length of guiding sheath variable, depending upon the position of odontostyle. Oesophagus dorylaimoid. Nerve ring encircling anterior slender part of oesophagus at 165-247 μm from anterior end. Expanded part of oesophagus 327-460 μm long or 51-52 % of oesophageal length. Location of oesophageal gland nuclei and their orifices are as follows :

DO = 50-54 % (52 %),	DN = 53-56 % (55 %)
S ₁ N ₁ = 68-83 % (76 %),	S ₁ O ₁ = 66-78 % (72 %)
S ₁ N ₂ = 71-86 % (79 %),	S ₁ O ₂ = 70-83 % (77 %)
S ₂ N ₁ = 84-92 % (88 %),	S ₂ O ₁ = 83-90 % (87 %)
S ₂ N ₂ = 84-93 % (89 %),	S ₂ O ₂ = 85-89 % (87 %)

Cardia well developed, 23-57 μm long, elongate-cylindroid with a disc and surrounded by intestinal tissues. Vulva transverse, slit-like, pre-equatorial or equatorial. Reproductive system amphidelphic, each sexual branch consisting of a reflexed ovary, oviduct and uterus. Prerectum 201-418 μm long, 5-7 anal body widths. Rectum 45-68 μm long or 1.1-1.2 anal body widths. Tail elongate, tapering gradually, 2.7-5.3 anal body widths; three caudal pores on each side.

Male : Similar to females in general body shape and morphology except for the ventrally curved posterior extremity. Reproductive system diorchic. Spicules dorylaimoid, ventrally curved, 78-112 μm long, lateral guiding pieces 9-17 μm long. Supplements consisting of an adanal pair and a contiguous series of 41-47 ventromedian. Prerectum 6-11 anal body widths long, extending beyond range of supplements. Tail short, conoid with bluntly rounded terminus, caudal pores varying from 3-5 on each side.

Results

EMBRYONIC DEVELOPMENT (Fig. 1)

Each sexual branch of the gravid female possessed 10-19 oocytes at one time. Generally not more than four mature eggs were present in the uterus. The egg shell is made up of three distinct layers (Fig. 1, A) and measures 71-95 \times 33-45 (82 \times 39) μm (n = 20). The eggs are smooth, elongate, densely granulated and dark brown in colour. The single-celled egg divided perpendicularly to the long axis into two equal blastomeres 15-24 h after

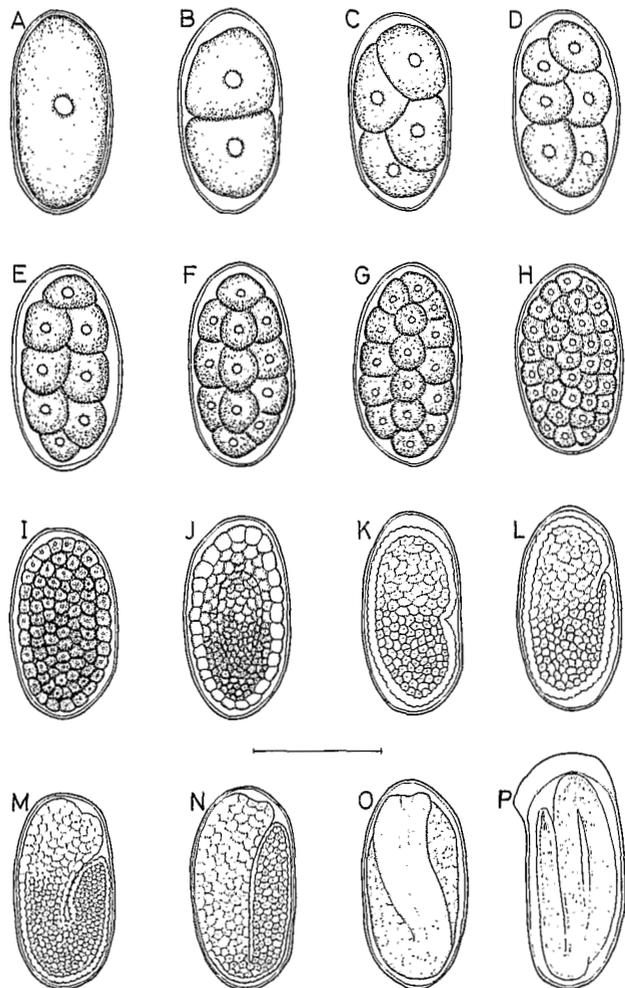


Fig. 1. Embryonic development. A : Single-celled stage; B : Two-celled stage; C : Four-celled stage; D : Six-celled stage; E : Eight-celled stage; F : Twelve-celled stage; G : Sixteen-celled stage; H : Thirty six-celled stage; I : Blastula stage; J : Gastrula stage; K : "Lima bean" stage; L : "Comma" stage; M : "Tadpole" stage; N : One fold embryo; O : Two fold embryo; P : First stage juvenile ready to hatch (Bar = 50 μm).

egg laying (Fig. 1B). The anterior cell divided after 16 min forming a three celled stage whereas the posterior one divided 10 min later resulting in four equal blastomeres with conspicuous nuclei (Fig. 1C). The plan of division was oblique to the long axis in both cases. After another 25 min the two anterior cells divided perpendicularly forming a six-celled egg with four small anterior and two large posterior cells (Fig. 1D). 40 min later the two posterior cells divided leading to an eight-celled stage with equal blastomeres (Fig. 1E). The 12-celled stage appeared after 45 min (Fig. 1F) and the 16-celled stage within about 60 min (Fig. 1G). In the next 2 h the 16 cells divided to form the 36-celled stage (Fig. 1H). Afterwards the cells multiplied rather rapidly and it became difficult to follow the sequence and plan of cell divisions. 40-90 min later the blastula was formed (Fig. 1I). The gastrula stage was formed 75-90 min after the formation of blastula stage. The gastrula forming cells were differentiated into two types: the outer layer of cuboidal cells forming the hyaline portions and the anterior and posterior cells of the inner mass forming the granular portions (Fig. 1J). The anterior region was broad having large cells formed the future stomodeum (feeding apparatus, oesophagus) while the posterior region with smaller cells formed the intestine and tail. The gastrula transformed into the "lima-bean" stage after 105 min by a slight invagination in the middle of the developing embryo (Fig. 1K) and to "comma" stage after another 75 min (Fig. 1L). The "tadpole" stage appeared 35 min later as a result of the formation of deep invagination (Fig. 1M). The mouth depression was formed at the broader (anterior) end of the developing embryo in the late "tadpole" stage. 160 min later gradual and intermittent movements began in the anterior region of the embryo. The movements were very slow during the first 15 min but became vigorous 45 min later and spread over the entire body. The developing embryo increased in length, became two-fold within 25 min (Fig. 1O) and three-fold in another 160 min (Fig. 1P). The movements within the egg shell continued during entire process of elongation of embryo. The different regions like the oesophagus and intestine made a faint appearance after the three-fold stage was reached. About 150 min later the tip of the functional odontostyle appeared. The odontostyle secreting cell was not visible at this stage due to heavy granulation and continuous movement. First the tip of the functional odontostyle was formed and then it grew further posteriorly. Near the middle of the odontostyle two refractive dots appeared on the two sides of odontostyle and formed the future guiding ring. The complete guiding ring along with the intestine appeared about 4 h after the odontostyle tip was formed. The formation of a functional odontostyle took 5 h, followed by the formation of the replacement odontostyle and the walls of the odontophore. The movement of the embryo became very slow after the formation of the digestive system. This de-

crease in the movement was an indication that the juvenile is ready to hatch. Some secretions on the tip of odontostyle of the new fully formed juvenile were seen.

HATCHING (Fig. 1P)

The juveniles hatched out of the eggs approximately 6 h after the formation of their internal organs. The egg shell became thin, flexible, increased in width, and form a blister-like structure at the anterior region. After the formation of the blister, continuous activity of juvenile ruptured the egg membrane. The total duration of embryonic development i.e., from single cell stage to hatching varied from 30 to 59 h.

EFFECT OF TEMPERATURE ON THE EMBRYONIC DEVELOPMENT (Fig. 2)

Maximum hatching (70-80%) was recorded at 25, 30 and 35 °C. However, the duration of embryonic development was more at 25 °C (86 h) than at 30 °C (54 h) or at 35 °C (44 h). Least number of eggs hatched at 15 °C (40%) whereas all the eggs remained single celled at 5 and 10 °C and no hatching was recorded up to a period of 10 days. Maximum time required for hatching was 144 h at 15 °C and minimum 24 h at 40 °C.

POST-EMBRYONIC DEVELOPMENT

The post-embryonic development of *D. stagnalis* was studied with particular emphasis on the development of odontostyle, oesophagus, cardia, gonads and tail.

Development of odontostyle (Fig. 3)

The odontostyle develops from the cell present in the submedian wall of the anterior slender part of oesophagus. The position of the cell varies in different juvenile

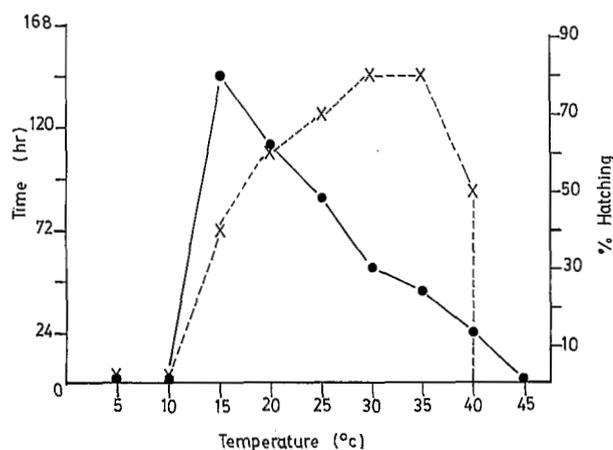


Fig. 2. Effect of temperature on embryogenesis (●) and % hatching (x) of *Dorylaimus stagnalis*.

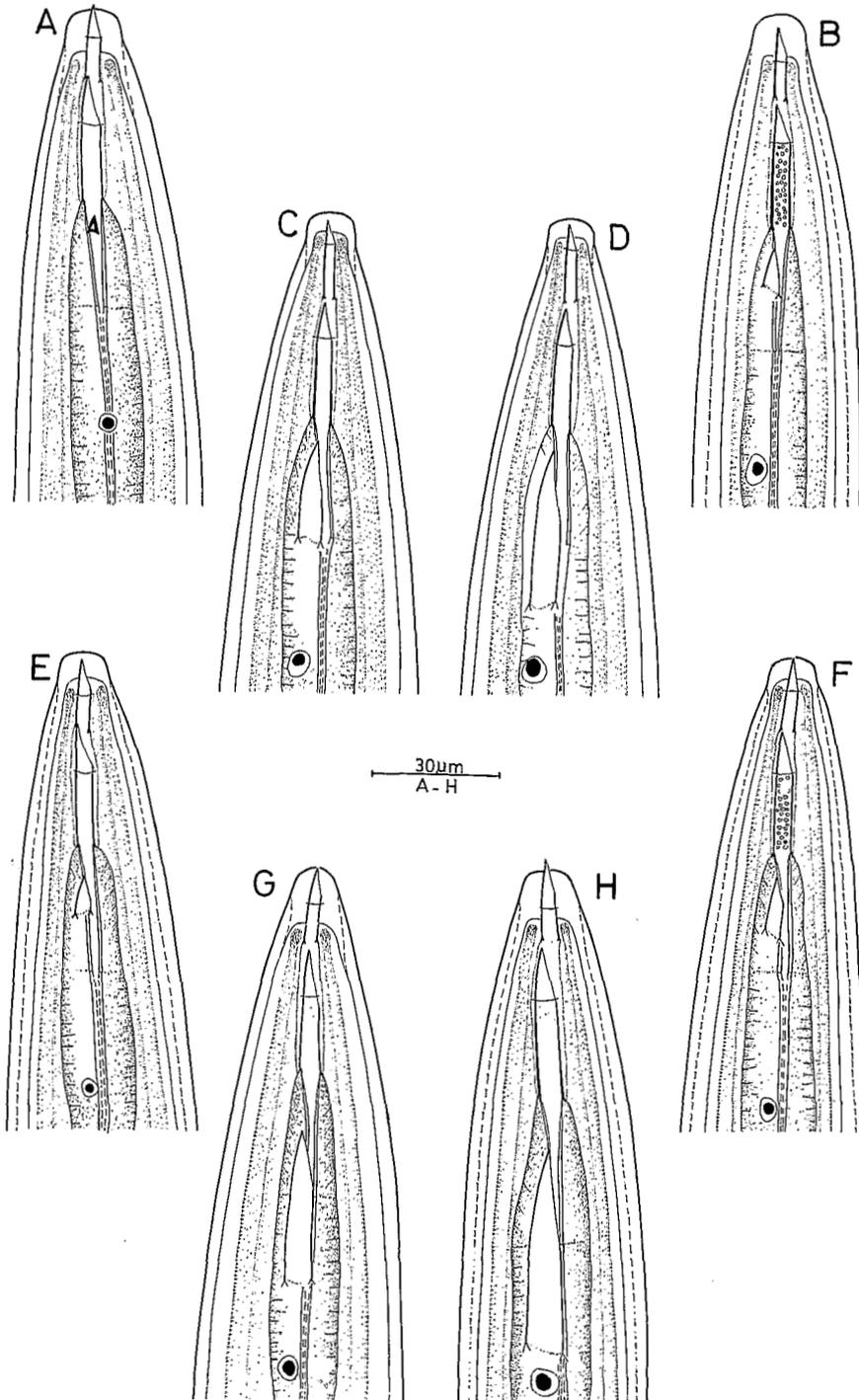


Fig. 3. Development of odontostyle in second and third stage juveniles. A-D : Second stage juveniles; E-H : Third stage juveniles.

stages. The tip of the replacement odontostyle appears first close to the posterior region of odontophore. After tip formation, the basal forked part of the odontostyle is formed. Generally two odontostyles (functional and replacement) are present in first, second and third juveniles during intermoult stages. As the moulting process is initiated, the functional odontostyle migrates anteriorly and the replacement odontostyle turns on its axis shifting to occupy the position adjacent to the functional odontostyle. The ecdysis starts with the separation of old cuticle from the new one which is secreted by the hypodermal cells. All the cuticular structures like lining of the labial papillae, amphids, guiding ring, odontostyle, lumen of odontophore and rectum, etc., remain attached to the old cuticle. The fourth and final moult differs from all the other moulting stages due to absence of a new replacement odontostyle.

Development of oesophagus

Along with the shedding of odontostyle during moulting, the lumen of odontophore is also cast off. The shed odontophore lining can be seen along with that of the lining of stoma (odontostyle) in the moulted cuticle. At the time of moulting the structure of oesophagus is partly obscured due to accumulation of granules.

Development of cardia

The oesophago-intestinal junction or cardia is an elongated cone-like structure. The cone shaped structure is made up of tissues in adults but of cardiac cells in juveniles. At each moulting stages, with the formation of odontostyle and other body parts, a new cardia is formed by the multiplication of cardiac cells. These cells appear dark upon staining. The number of cardiac cells varies in different juveniles stages. The first and second stage juveniles have 5-8 and 9-12 cardiac cells respectively, while the third and fourth stages have 11-14 and 13-16 cells respectively.

Development of gonads

First stage juveniles; Table 1 (Fig. 4A) : The primordium ($9-21 \times 6-12 \mu\text{m}$) consists of two large coarse, granular germinal nuclei and four to six small somatic nuclei. The ventral chord nuclei varies from 14-35 from the base of oesophagus to the middle of the primordium. The time taken from hatching to the completion of the first moulting varies from 17-22 days.

Second stage juveniles; Table 1 (Fig. 4B) : The germinal primordium is $15-30 \mu\text{m}$ long, consisting of four to six germinal and 8-14 somatic nuclei. There are 40-75 ventral chord nuclei between base of oesophagus and middle of germinal primordium. The second stage juveniles transform into third stage in 24-28 days.

Third stage juveniles; Table 1 (Fig. 4C-E et 5A-D) : The sexes can be differentiated at this stage. The length of male and female primordia measured $21-143 \mu\text{m}$ and $42-135 \mu\text{m}$ respectively and each possess 8-12 germinal and 20-45 somatic nuclei. The number of ventral chord nuclei varies from 170-190.

The juveniles that develop into females have two-four dark oval shaped specialized ventral chord nuclei (Fig. 4C). In the late third stage the middle part of the primordium bulges out slightly to form a "cone" like structure (Fig. 4E) representing the future vagina and vulva.

The juveniles destined to become males have a small compact mass of dark stained nuclei of spicular primordium in the anal region below the rectum (Fig. 5D). In the third stage male during moulting a backwardly directed tube (gonoduct) appears in the middle of the genital primordium which forms the future *vas deferens* and ejaculatory duct in the adults (Fig. 5B). The time interval between the completion of the second and third moulting varies from 30-35 days.

Fourth stage juveniles (Females); Table 1 (Fig. 4F) : The number of germinal nuclei in the primordium varies from 20-60. The somatic nuclei can not be counted because of their large numbers and overlapping arrangement. Few somatic nuclei are also arranged on both the sides of the anterior and posterior arms along the wall of the developing gonad which form the epithelial covering of the gonad. The number of specialized ventral chord nuclei increases to 14-20 and arrange themselves around the "cone". With the completion of moulting, the cuticular linings of the vagina and vulva are formed (Fig. 4H-J). The final moulting took 46-52 days after the third moulting.

Fourth stage juveniles (Males); Table 1 (Fig. 5C, F, G) : The development of gonads in the fourth stage male juveniles is faster than females. The number of germinal nuclei varies from 18-61. The backwardly directed tube (gonoduct) increases in size as the anterior and posterior arms of the developing system elongate. The somatic nuclei are arranged alternately along the walls of the gonoduct. During early stages of moulting, the spicular primordium becomes more compact and the spicules appear as faint hyaline refractory lines which get shortened and become hard at later stage assuming their normal shape and size (Fig. 6). Many rows of darkly stained nuclei are arranged on the ventral side of the body between the cloaca and intestine-prerectum junction (Fig. 5E) and give rise to copulatory muscles and the ventromedian supplements. The male fourth stage juveniles moult finally into the adults 40-45 days after the third moulting.

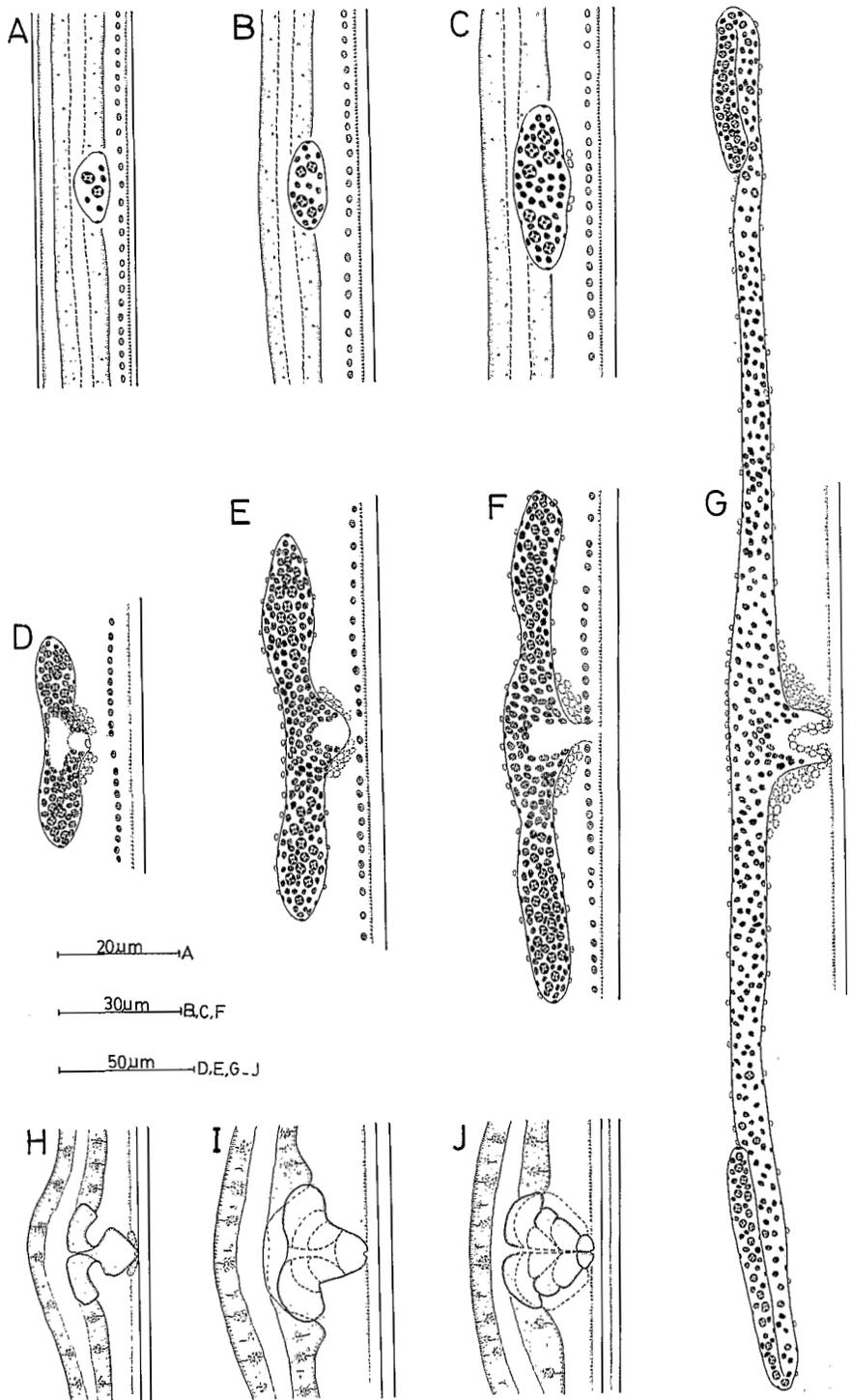


Fig. 4. Development of female genital system. A : Primordium of the first stage juvenile; B : Primordium of the second stage juvenile; C : Primordium of the third stage juvenile; D : Abnormal primordia of third stage juvenile; E : Primordium of the moulting third stage juvenile; F : Primordium of fourth stage juvenile; G : Primordium of the moulting fourth stage juvenile (formation of vulva); H : Early fourth stage juvenile; I : Mid-fourth stage juvenile; J : Late fourth stage juvenile.

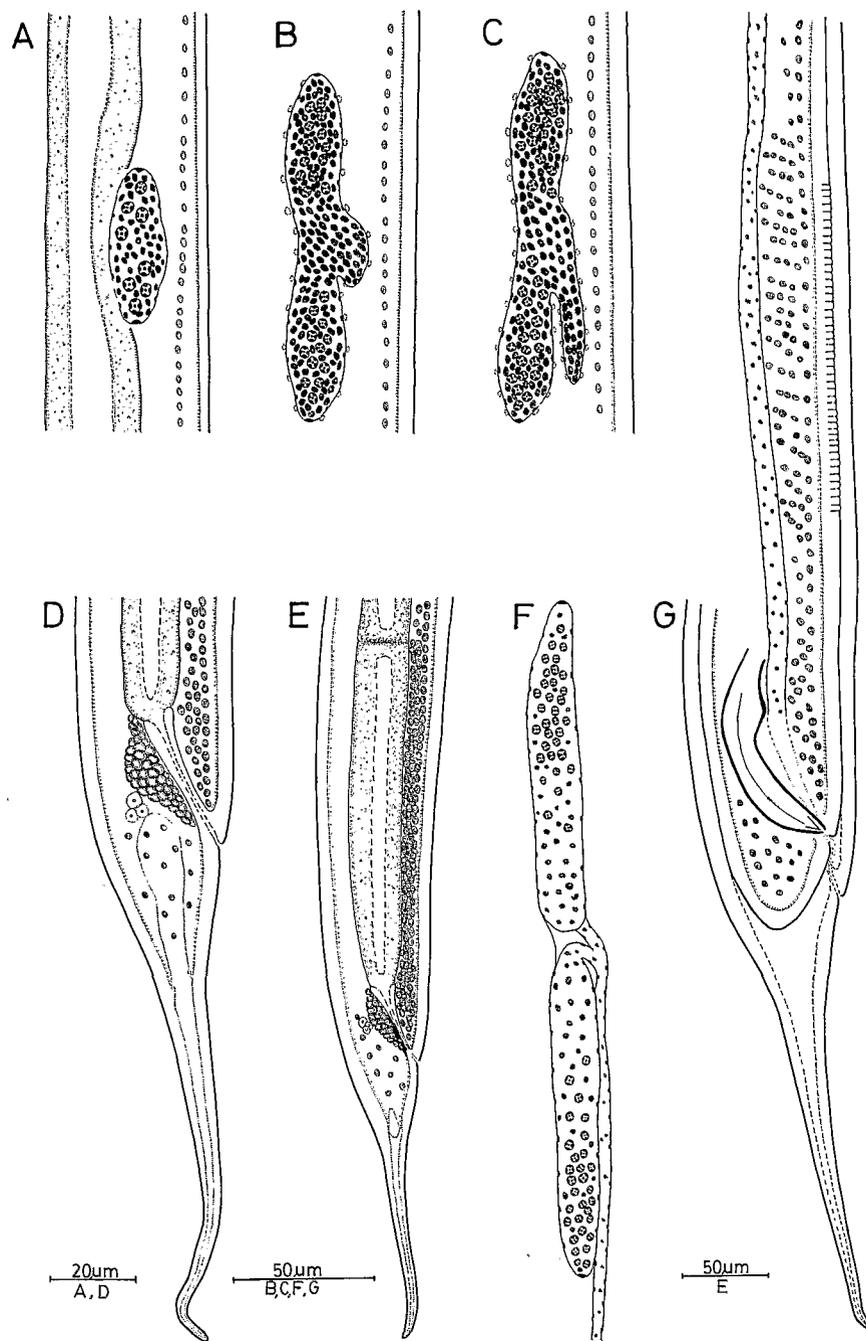


Fig. 5. Development of male genital system. A : Primordium of the third stage juvenile; B : Primordium of the moulting third stage juvenile; C : Primordium of the fourth stage juvenile; D : Posterior region of the third stage juvenile; E : Posterior region of the fourth stage juvenile; F : Fully developed testes of the fourth stage moulting juvenile; G : Moulting fourth stage juvenile.

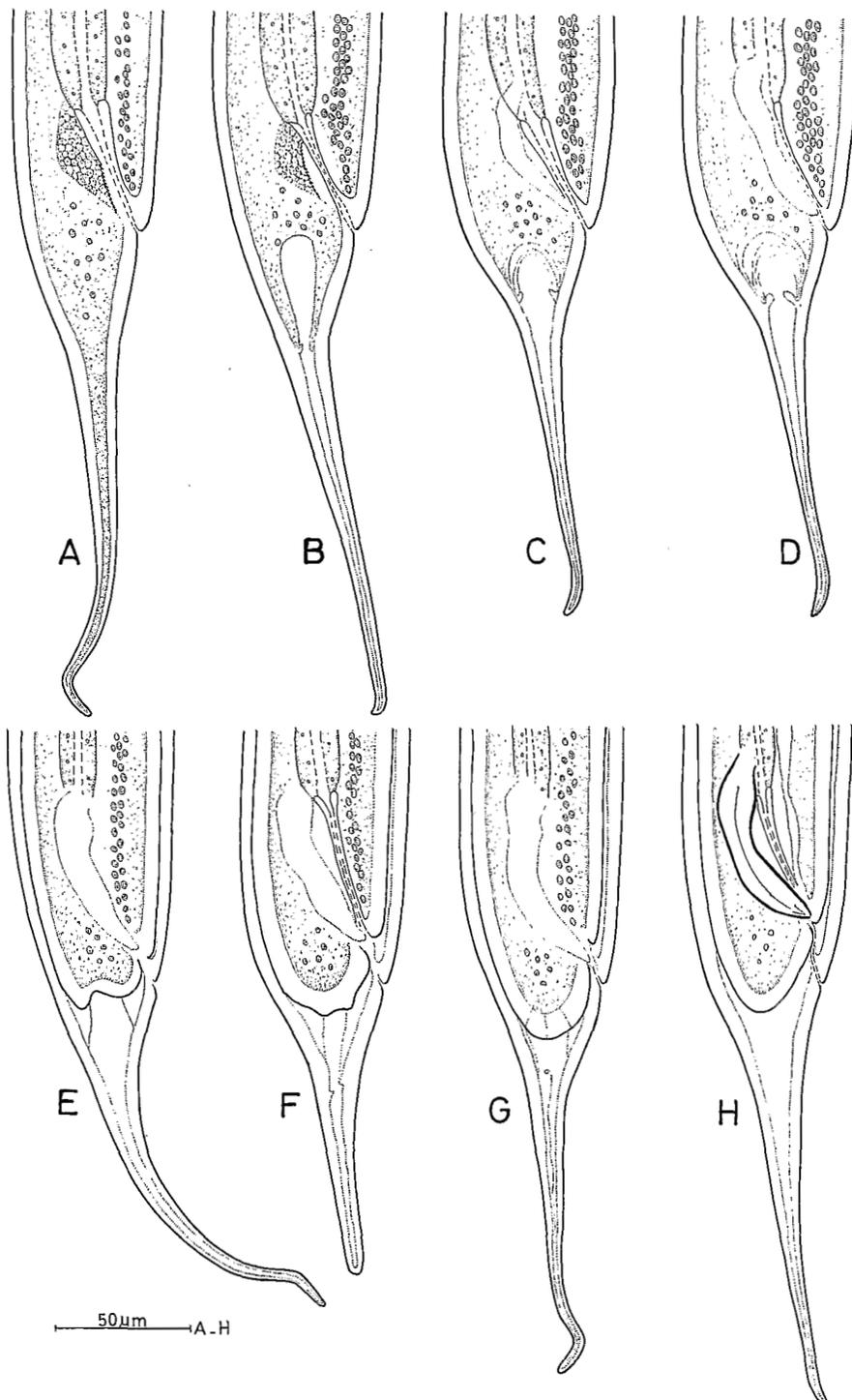


Fig. 6. Development of spicule and tail. A : Posterior region of late third stage juvenile; B : Early fourth stage juvenile; C, D : Mid fourth stage juvenile; E : Late fourth stage juvenile; F, G : Moulting fourth stage juvenile; H : Fourth stage juvenile just before final moult.

Development of tail in males (Fig. 6)

The adult males have a short conoid tail as compared to females which possess a long filiform tail. The male juveniles of the third and fourth stages also have filiform tails much like that of adult females. The development of male tail starts rather late during the third stage of male juvenile. At the time of fourth or the last moulting, when the old cuticle of tail is getting separated, a new short and conoid tail is formed. The tail of the old elongated juvenile is detached from the newly formed conoid tail of adult male.

Discussion

The embryonic development of *Dorylaimus stagnalis* is on the same pattern as those of *Xiphinema* spp. (Flegg, 1968a; 1968b); *Aporcelaimellus* sp. (Wood, 1973); *Xiphinema basiri*, *X. insigne* (Jairajpuri & Bajaj, 1978); *X. americanum* (Malik & Jairajpuri, 1983) except for the differences in the arrangement of blastomeres after the second cleavage. *Aporcelaimellus* sp., took 230-250 h (Wood, 1973); *Xiphinema* sp. 19-30 days (Flegg, 1968a; 1968b); *X. basiri*, seven days (Jairajpuri & Bajaj, 1978) and *X. americanum* 100-120 h (Malik & Jairajpuri, 1983) to hatch. However, *D. stagnalis* took only 30 to 59 h to hatch out of the egg. Pillai and Taylor (1967) and Flegg (1969) observed the effect of temperatures on the mycophagous nematodes and *X. diversicaudatum* and found decrease in the time of hatching with the increase in temperature to a certain limit. During present observations the development and hatching of *D. stagnalis* is also affected by various temperatures in a similar fashion.

The position and length of odontostyle, replacement odontostyle, odontophore, size of primordia as well as the number of primordial cells were used for differentiating juvenile stages. The development of odontostyle which originate from an elongated flask-shaped cell is similar to that of other dorylaim nematodes (Coomans & De Coninck, 1963; Grootaert & Coomans, 1980; Coomans & Van Der Heiden, 1971). The cardia cells were observed in juveniles only. The primordium of the first stage juvenile is oval with two germinal and four to six somatic nuclei. With the increase in body and primordial length, the number of germinal, somatic nuclei and ventral chord nuclei also increases. The sexes could be differentiated at the third stage due to the presence of specialized ventral chord nuclei in females and spicular primordia in males (Hirschmann, 1962). The third and fourth juvenile stages could not be differentiated on the basis of primordial lengths as these overlap.

The fourth stage juveniles mature sexually earlier than the females as was also observed by Coomans and Lima (1965) in *Anatonchus amiciae*, and Ahmad and Jairajpuri (1982) in *Parahadronchus shakili*. *Labronema*

ferox and *L. thornei* took 3-4 months and 5-6 months respectively to complete their life cycles (Ferris, 1968) whereas *L. vulvapapillatum* required 27-126 days for its development (Grootaert & Small, 1982). *Aporcelaimellus* sp. completed its cycle in 75-114 days as was observed by Wood (1973). During present observations, *D. stagnalis* took 17-22 days for the completion of first moulting, 24-28 days for the second and 30-36 days for the third moulting. The fourth stage male juveniles required 40-45 days whereas female juveniles took 46-52 days to complete the final moult. The total time in *D. stagnalis* from the first stage (hatching) to the adult varied from 111-131 days in males and 117-138 days in case of females.

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