Embryology and Life Cycle of *Tylenchorhynchus claytoni* Steiner, 1937 (Nematoda: Tylenchoidea)[1]

L. H. WANG[2]

Abstract: Development of *Tylenchorhynchus claytoni* from unsegmented egg to hatching takes 135 hr at 22–25 C. The fourth molt lasts 5 to 6 days. During exsheathment the cast cuticle of the larva separated into two unequal parts, breaking near either the anterior or posterior end. The life cycle from egg to egg required from 31 to 38 days at 28 C on alfalfa seedlings and included four molts and four larval stages. Sexual differentiation was apparent in third-stage larvae. *Key Words:* Egg-laying, Embryogenesis, Molting, Postembryogenesis.

The tobacco stunt nematode, *Tylenchorhynchus claytoni* Steiner, is considered to be pathogenic to some agricultural and nursery crops (13, 14, 19, 21). In 1959 Krusberg (14) investigated its life cycle, reproduction, feeding habits, and host ranges. The studies reported here deal with its embryology, molting, and postembryonic development which have not previously been studied.

Materials and Methods

Nematodes were maintained on greenhouse-grown tobacco (*Nicotiana glutinosa* L.) at 22–28 C. Hand-picked gravid females were transferred to BPI dishes containing tap water for observation of egg laying. Egg development was observed in a hanging drop culture (18). Nematodes in fourth stage molt were selected and transferred to water agar (11) for observation of molting. The study of postembryonic development was conducted in a 13 × 90-mm soil-filled-container containing a 3-week-old alfalfa seedling inoculated with 100 newly-hatched second-stage larvae. The soil in containers, replicated four times, was examined at 1–3 day intervals for larval development. Measurements were made on heat-relaxed specimens mounted in 2% cold formalin. Gonad development was studied in nematodes stained with 5% acid fuchsin (22).

Results

EGG-LAYING: Eggs were laid unsegmented. Movement of the eggs in the uterus required 2–3 hr to travel one egg length. When the egg reached the vagina the nematode began to swing its tail back and forth and this, coupled with rolling of its body, continued until the vulva opened. Prior to egg deposition, movement of the female stopped, and the body became "W" shaped. Eventually, the egg was squeezed out of the vulva. The actual emergence of the egg required 5–10 seconds. After the egg was deposited the female coiled and uncoiled several times and then resumed its normal undulating movement.

EMBRYOLOGY: Eggs were 62–68 μ in length by 20–23 μ in width ($n = 40$). A vitelline membrane was first seen beneath the shell early in cleavage. The protoplasm of the egg was granular with an irregular margin which became smooth prior to the first cleavage (Fig. 1, A, B). The developmental pattern, given in detail in Table 1, was based on 75 eggs.

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[2] Department of Nematology, University of California, Riverside, California 92501. Present address: Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana 47907. The author is extremely grateful to Drs. S. A. Sher and R. Mankau, Department of Nematology, University of California, Riverside, and Dr. G. B. Bergeson, Department of Botany and Plant Pathology, Purdue University for valuable suggestions and criticisms.

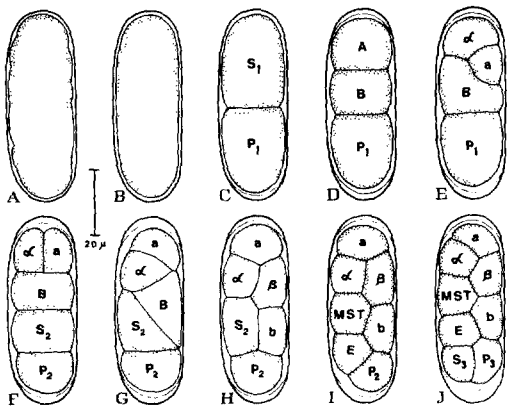

FIG. 1. Camera lucida drawings of egg cleavage in *T. claytoni*. **A.** Unsegmented egg after laying; **B.** Egg prior to the first cleavage; **C–F.** Two to five-celled stage; **G.** Shifting blastomeres at five-celled stage; **H–J.** Six to eight-celled stage.

MOLTING: Prior to the final molt, larvae were sluggish or inactive. The stylet appeared less refractive, and the esophagus became very faint. The stylet knobs disappeared first and then the shaft. The apex remained intact and was later shed with the vestibular extension and cephalic framework. A flask-shaped hyaline cavity appeared which surrounded the apex. Soon the head started to pull back from the cephalic framework. A short sclerotized tube developed behind the hyaline cavity indicating the initiation of a new apex. As the head pulled farther back, the hyaline cavity diminished and the new apex was completed. This was followed by the formation of the shaft and then the knobs. Later the knobs gradually increased in size. The lumen of the esophagus and the valve plate of the medium bulb became distinct. Development from the time the knobs disappeared to the completion of the stylet required 56–72 hr. After the stylet was completed the nematode resumed its activity. The old cuticle appeared pliable and loose around the body. An unknown

TABLE 1. Chronology of embryogenesis from egg deposition to egg hatch. The labelling of blastomere embryogenesis was according to the system of Boveri (3).

Hr since egg deposition	Developmental sequence
3–4	First division at right angles to the longitudinal axis of the egg produced cells S_1 and P_1 (Fig. 1, C).
9	S_1 blastomere (regarded the anterior cell) divided equally into an anterior "A" and a central "B" cell (Fig. 1, D).
10	"A" cell divided obliquely into an α-cell and an "a" cell (Fig. 1, E).
10.5	P_1 cell divided into S_2 and P_2 cells (Fig. 1, F).
14	Cells shifted position resulting in "B" and S_2 cells becoming triangular in outline (Fig. 1, G).
16	"B" cell divided into a β and a "b" cell (Fig. 1, H).
17.5	S_2 cell divided into an E and a MST cell (Fig. 1, I).
19–20	P_2 cell divided into a S_3 and a P_3 cell resulting in 8-celled stage egg (Fig. 1, J). Polar body between vitelline membrane and egg shell observed.
20–30	Simultaneous cleavage throughout the egg.
30–35	Formation of blastula.
35–45	Formation of gastrula. Cells began differentiating into ectoderm and endoderm.
45–68	Hyaline region formed at anterior end of egg which gave rise to the mouth, head, and esophageal region.
68	Embryo moved and rotated within egg.
70	Formation of a tadpole-shaped larva.
74	Larva became vermiform.
74–95	Larva rapidly elongated and increased its activity.
95–125	First molt occurred within the egg. A stylet was first seen during the molt.
125–135	Larva moved frequently and vigorously, alternating with several immobile periods lasting 0.5–2 hr. Egg shell appeared to become more flexible. The stylet repeatedly punctured the anterior end of the egg. Egg shell ruptured and larva emerged within seconds. The rounded polar body remained within the empty egg shell.

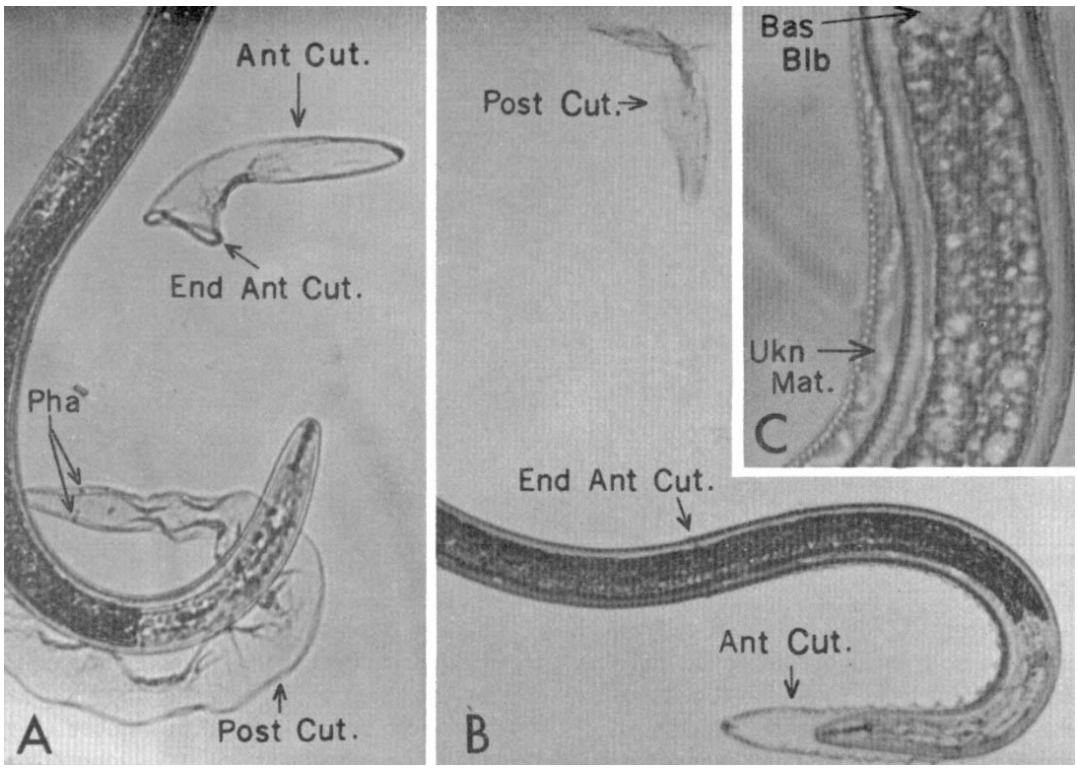

FIG. 2. Exsheathment in *T. claytoni*. **A.** Anterior part of cuticle (Ant Cut.) split; **B.** Posterior part of cuticle (Post Cut.) split; **C.** Prior to exsheathment unknown material (Ukn Mat.) accumulated on the old cuticle. (Bas Blb = Basal Bulb; End Ant Cut. = End of anterior part of cuticle).

material gradually accumulated between the old and the new cuticle near the end of either the esophagus (Fig. 2, C) or the anal area. Later the old cuticle split at one of these two areas (Fig. 2, A, B), and at exsheathment the shorter part of the old cuticle, either the head or the tail portion, was exsheathed first followed by the rest of the cuticle. The break between cuticle segments was usually a smooth circular split, but occasionally it was jagged. The fourth molt lasted 5–6 days.

DEVELOPMENTAL STAGES: Three larval stages occur between hatching and the adult stage. Characteristics important for distinguishing the various larval stages were: structure and length of gonad, body length, tail length, and tail width. The measurements of the different larval stages were presented in Table 2.

Second-stage larva.—Genital primordium measured 8–10 μ long and was located at the right of the ventral chord at 58–62% of body length. It consisted of four cells, two large germinal cells between two small cap cells (Fig. 3, A). During the molt the genital primordium enlarged and measured 12–14 μ in length.

Third-stage female larva.—The female gonad differed markedly from the male gonad at this stage. Two germinal cells of the genital primordium were separated by two to four somatic cells, one germ cell entering each end of gonad (Fig. 3, B). The gonad measured 20–26 μ long. During the molt the gonad extended in length and

TABLE 2. Dimensions of Postembryonic Stages of *T. claytoni*.

Stage		Length	Width	Stylet	Tail length	Tail width
Second-stage		212–214† (228.0)	12–15 (13.5)	15–17 (16.2)	23–29 (26.0)	10–12 (11.2)
Third-stage	♀	382–432 (400.6)	16–20 (18.0)	18–21 (19.0)	30–37 (32.3)	12–15 (13.9)
Third-stage	♂	330–402 (358.4)	15–19 (16.9)	17–19 (18.2)	23–33 (29.5)	10–14 (12.5)
Fourth-stage	♀	504–598 (553.3)	22–24 (23.0)	19–21 (20.1)	32–40 (36.2)	14–16 (15.3)
Fourth-stage	♂	435–523 (501.0)	20–23 (22.2)	18–20 (19.0)	33–38 (37.3)	16–20 (18.4)
Female		638–720 (689.5)	24–28 (26.6)	21–23 (22.3)	35–40 (37.7)	15–19 (17.6)
Male		595–695 (648.1)	21–25 (23.4)	20–22 (21.4)	42–51 (44.1)	15–17 (16.5)

† Measurements in μ (n = 20).

ranged from 62–86 μ. A cap cell was located at both ends of the gonad and each was adjacent to a big germinal cell and several somatic cells. At the middle of the gonad a group of somatic cells multiplied to form a widening region. Four specialized vaginal primordium cells were differentiated in the ventral chord opposite the widening region of the gonad (Fig. 3, C).

Third-stage male larva.—The number of cells of the gonad remained the same as in the second-stage larva, but the posterior cap cell increased in size. The gonad measured 17–24 μ in length. Early in the molt the gonad was six or seven cells (Fig. 3, I). This was followed by the multiplication of cells at the posterior end of the gonad resulting in a spindle-shaped arrangement (Fig. 3, J). The gonad increased to 50–55 μ long. By this time the cloacal primordium became visible and was oval in shape (Fig. 3, J). It consisted of a group of cells which later gave rise to the spicule and gubernaculum.

Fourth-stage female larva.—Each germ cell divided into two or three cells of equal size, the somatic cells and the vaginal primordium cells increased in number (Fig. 3, D). The gonad was 96–105 μ long. During the molt the gonad was distinctly more developed than in the previous stage. The anterior part of the gonad ranged from 115–162 μ in length, the posterior one measured from 126–173 μ (Fig. 3, E).

Fourth-stage male larva.—The gonad measured 71–97 μ in length. The cloacal primordium increased in size (Fig. 3, K). During the molt the gonad joined with the cloacal primordium (Fig. 3, L). By this time spermatocytes and sperms were observed. Development of the spicule, gubernaculum, and the caudal alae was completed at the same time as the stylet. The gonad increased to 290–330 μ.

Adult.—Measurements of adults in the present study generally confirmed those given by Steiner (2) and Allen (1) in their

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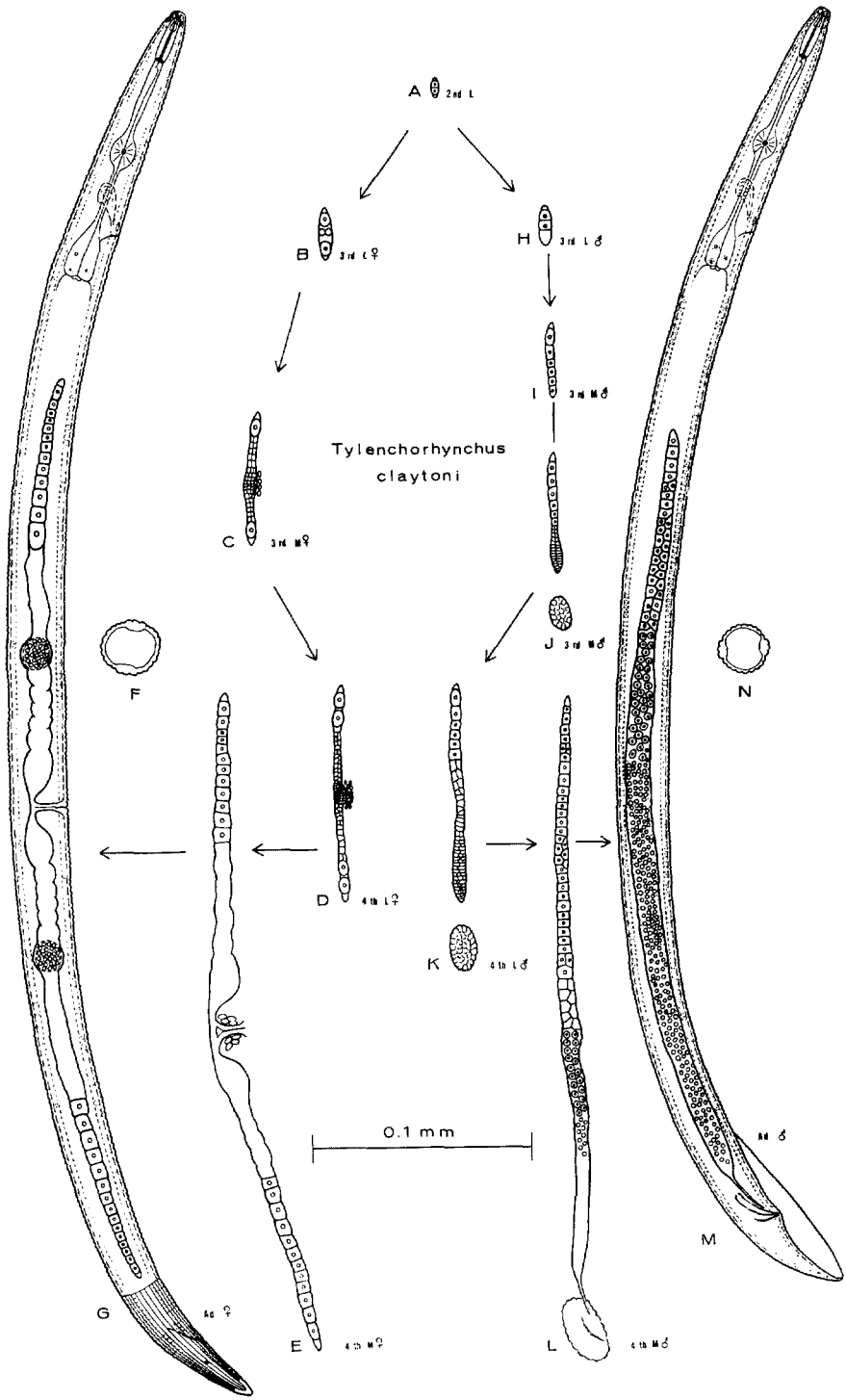
FIG. 3. Stages in the development of male and female gonad (L = Larva; M = Molting; Ad = Adult).


A 2nd L
B 3rd L♀
H 3rd L♂
I 3rd M♂
Tylenchorhynchus
claytoni
C 3rd M♀
J 3rd M♂
F
N
D 4th L♀
K 4th L♂
0.1 mm
G Ad ♀
E 4th M♀
L 4th M♂
M Ad ♂

description of the species. Steiner reported 29 longitudinal striations on the middle region of body regardless of the sex, whereas I consistently found females with 26 longitudinal striations, and males with 24 or 26 (Fig. 6, F, N).

LIFE CYCLE: Males were required for reproduction (14). The life cycle from egg to egg required from 31–38 days at 28 C on alfalfa seedlings, with four molts and four larval stages. The egg stage lasted 3–4 days, the second-stage 7–8 days, the third-stage 6–7 days, the fourth-stage 7–9 days and the preoviposition period of the adult was from 8–10 days.

DISCUSSION

The first cleavage of the egg of *T. claytoni* results in two blastomeres. At the two-celled stage the cleavage of the eggs is delayed in P_1 cell, but the S_1 cell continued to divide. The P_1 cell does not undergo cleavage until the four-celled stage. Delayed cleavage has been also reported in other nematodes: one blastomere at the two-celled stage does not divide until the six-celled stage in *Ditylenchus dipsaci* (25), the five-celled stage in *D. destructor* (2), and the four-celled stage in *Aphelenchoides dactylocercus* (17). By contrast, the two-celled eggs of *Radopholus similis* (22), *Hemicriconemoides chitwoodi* (8), *Criconemoides xenoplax* (18), *Helicotylenchus vulgaris* (24), *Nacobbus serendipiticus* (5), *Rotylenchulus parvus* (6), *Xiphinema diversicaudatum* (10), *Pratylenchus scribneri* (16) and *Hoplolaimus indicus* (7) divide consecutively resulting in 4 cells in tandem.

In an animal-parasitic nematode, *Ascaris megalocephala* (4), the third cleavage gives the T-shaped four-celled stage which produces a bilaterally symmetrical embryo. In *T. claytoni* a bilaterally symmetrical embryo is observed at the five-celled stage. This stage also exists in *H. indicus* (7).

Cleavage in *T. claytoni* begins shortly after egg-laying. It takes about 14% of the total development time to reach the 8-celled stage and about 54% to reach the vermiform larval stage. In some *Xiphinema* spp. and *Longidorus* spp. (10), 15% of the total embryonic development time is required for the formation of the eight-celled stage and 45% for the appearance of a vermiform larva.

During the molt the formation of the new stylet of *T. claytoni* follows the same pattern as described for *R. similis* (22), *D. destructor* (3) and *P. scribneri* (16). The stylet apex is formed followed by the shaft and knobs, but no sclerotized rings in the region of the shaft is observed in *T. claytoni* which is similar to *R. similis* (22). During the exsheathment in *Paratylenchus nanus* (9), *Seinura* spp. (11) and *Longidorus africanus* (15) the entire cuticle is shed in one piece. In contrast to this, the cast cuticle of *T. claytoni* separates in two pieces as reported for *Haemonchus contortus* (20) and *Caenorhabditis briggsae* (12). In the latter two species the splitting of the cast cuticle is only from the anterior end. The splitting of the cast cuticle of *H. contortus* (20) is initiated by the formation of a refractive ring in the cuticle near the anterior end. In *T. claytoni* no refractive ring is found. The sites of cuticle split are associated with an accumulation of unknown material observed during molting. It is possible that the unknown material produced by nematodes plays a role in splitting the cuticle.

The life cycle of *T. claytoni* is similar to that of other parasitic nematodes with four molts and four larval stages. Sex can be differentiated in the third-stage larvae as also reported in *D. destructor* (2) and *N. serendipiticus* (4).

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