Rec. zool. Surv. India, 67 : 165-189,1972

STUDIES ON THE EMBRYOLOGY AND POST-EMBRYONIC DEVELOPMENT OF *ACROBELINEMA CORNIS* KHERA (NEMATODA CEPHALOBIDAE) WITH SPECIAL REFERENCE TO DEVELOPMENT OF GONADS

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(With 2 Tables and 6 Text-figures)

INTRODUCTION

Maupas (1900), Potts (1910), Honda (1925), Nigon (1949) and others have contributed to the knowledge of reproduction of free-living nematodes. Recently, Triantaphyllou and Hirschmann (1964) have given a review of this phenomenon in plant and soil nematodes. Barring the work of Seshadri (1964) on *Criconemoides xenoplax,* which he did while in the U.S.A., the development and life cycle of plant-parasitic and free-living nematodes have not been studied in India.

Khera (1967a) reported successful culturing of *Acrobelinema cornis* Khera, a free-living nematode, in the medium containing callus tissue, on plain water-agar medium, and on nutrient medium without males, although sperm were observed in the uterus of the females (Khera, 1967). Treating it as a logical extension of the culture work, studies were taken up on the embryology and the post-embryonic development of the nematode, with special reference to gonads so as to find out as to when the sperm made their appearance in the life cycle.

The author wishes to thank Dr. A. P. Kapur, Director, Zoological Survey of India, for providing necessary facilities and Shri K. S. Pradhan for assistance.

MATERIAL AND METHODS

Stock cultures of *Acrobelinema cornis* were maintained in the laboratory on water agar and nutrient agar at room temperature (21-28°C) (Khera, 1967a).

Several methods were employed in preparing nematodes for morphological and anatomical investigations. For a detailed study of the development of the genital primordia, van Weerdt's (1960) method was employed. By this procedure the nematodes were stained pink except the genital primordia and certain nuclei of the ventral chord which were stained red. Hirschmann's (1962) method using 1% acetic orcein was also employed. Both the staining techniques gave not very constant results. The use of hot polychrome methylene blue gave better results (Roques & Jude, 1940 in : Gray, 1954) Nematodes killed by gentle heat and mounted in 4% formalin and calcium carbonate (Baker, 1945) were used for measurements and study of various systems. This method obviated granulation. The various measurements of the different larvae and adult have been represented by de Man's formula.

For embryological studies, normal uncleavaged eggs were mounted in water in Maximov tissue culture slides under cover-slip and sealed with vaseline-paraffin wax mixture. The preparation was examined from time to time till the first and the last eggs hatched.

The notation of the cells in the embryological studies is after Hyman (1951).

For the chronological development studies, fifty normal uncleavaged eggs were inoculated in each of the twenty tubes of nutrient agar after sterilization for 10-15 minutes with 0.5% hibitane diacetate. First examination of the tube was done 24 hours before the average time taken by the eggs to hatch in the embryological studies. Thereafter one tube was examined daily and the stage of development of egg/larvae recorded.

The sperm material was examined without using any stains, as well as with acidulated methyl green. Preparation of smears was of no help. Schneider's aceto-carmine as well as Delafield haematoxylin-eosin combination were used in the smears.

EXPERIMENTAL RESULTS

Egg and Embryonic Development

(Text-figures 1 & 2)

The eggs of *Acrobelinema cornis* were laid singly and at single-celled stage. In no case cleavage or further development

took place within the parent body. The eggs were oval and the two poles equally rounded. The average size of 50 eggs in different stages of embryonic development was 51 µm (range 44-57 µm) by 26 µm (range 22-31 µm) The outside cover consisted of a tough egg-shell surrounding the protoplasm which was composed of closely packed, round globules of fairly uniform size. On the shrinkage of the egg protoplasm away from the walls, presence of vitelline membrane was revealed. This shrinkage left the so-called peri-vitellar space between the vitelline membrane and the egg-shell near the poles, giving rise to extra-vitellar cavity.

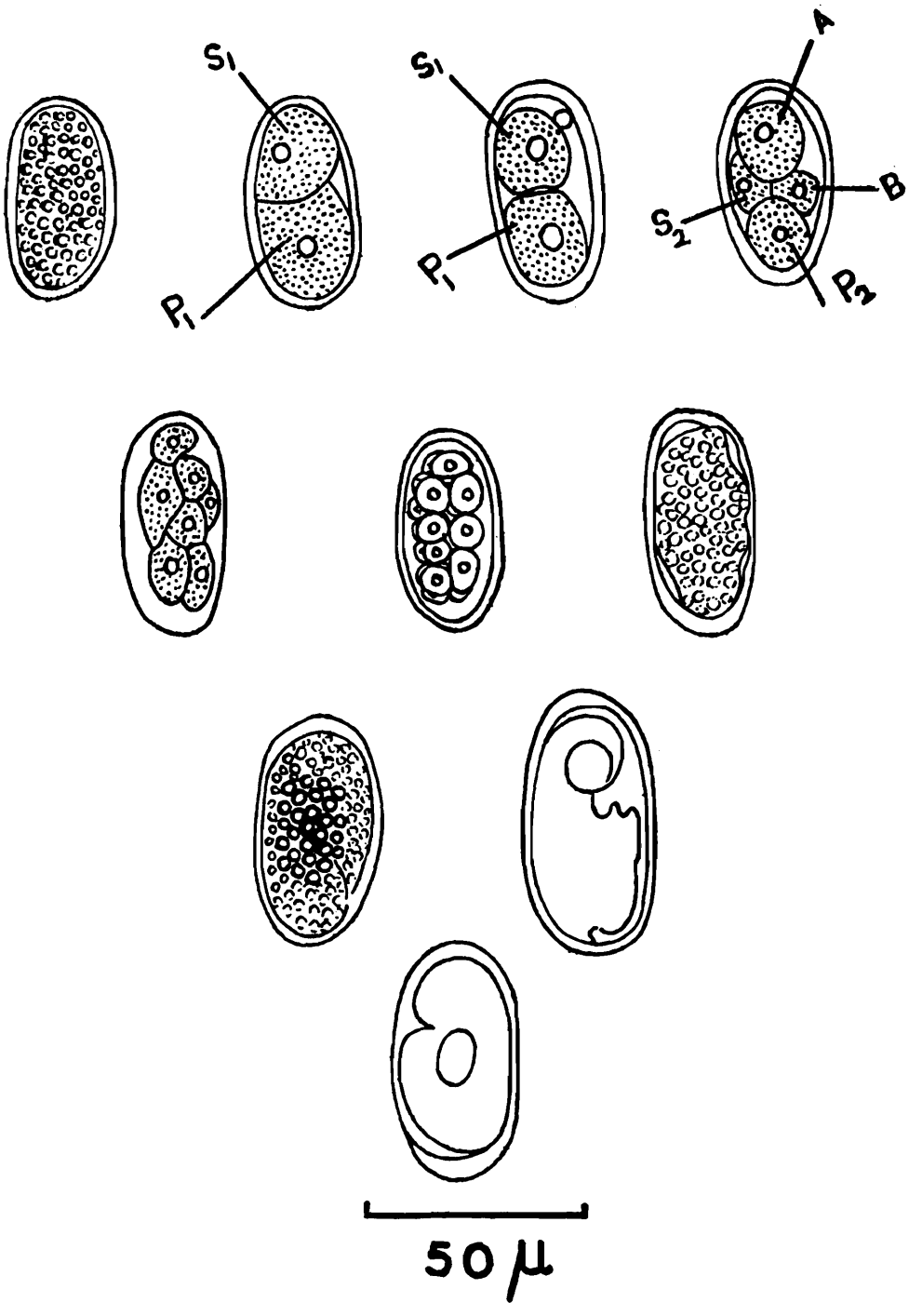
When eggs were placed in an aqueous solution of 5% crystal violet, the vitelline membrane was not penetrated by the stain and the blastomeres remained unstained. After heating (up to *ca* 60°C) or after treating with ordinary fat solvents like choloroform, acetone or benzene, the crystal violet coloured the entire egg dark violet indicating a possible lipoidal mechanism in the vitelline membrane.

As much as 15% of all the eggs recovered did not develop any further, most of the undeveloped eggs being of abnormal proportions (length/width either 2.25 or more, or 1.75 or less)

The usual time between the appearance of eggs in the uterus and egg laying was about eight hours.

After cleavage had started, the presence of a single polar body in some eggs became apparent. Since the polar body occurred inside the vitelline membrane, it follows that it was formed after the vitelline membrane had been laid down.

The protoplasm of the unicellular egg exhibited continuous streaming movement and its rearrangement marked the beginning of the first cleavage of the unicellular egg. The cleavage was holoblastic, and as would be seen later, spiral. The first division took place at right angles to the longitudinal axis of the egg, resulting in a large posterior blastomere (P_1) and a slightly smaller anterior cell (S_1). The polar body was located near the anterior pole, although not exactly at the pole. In the two-celled stage, the two nuclei showed up as large rounded clear spaces, one approximately in the centre of each cell. The protoplasm became finely granular and streamed continually in each blastomere, especially immediately before cell division. The second cleavage was longitudinal, both the anterior and posterior cell dividing : S_1 into A and B, and P_1 into S_2 and P_2. Simul-



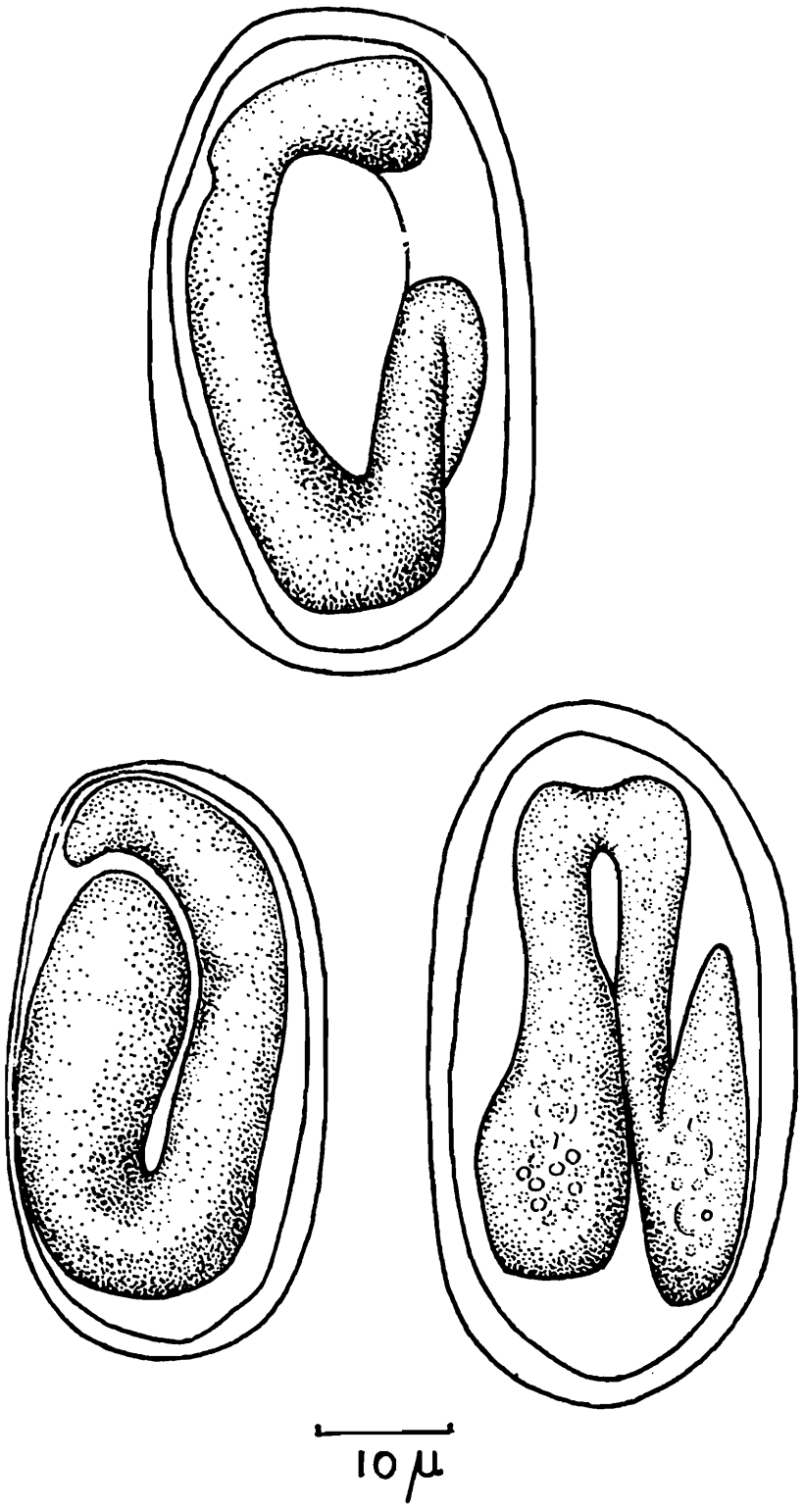
Text-fig. 1. *Acrobelinema cornis*. Eggs in various stages of development.

taneously with the cleavage, cells B and S_2 underwent spiral movement with the result that rhomboid shape, characteristic of the four-celled stage of nematodes, was obtained.

About twelve hours after the single-celled egg was laid, it had developed to a seven-celled form ; in the subsequent cleavages the picture became more and more obscure and ultimately a multicellular stage was reached after another twelve hours.

Details of gastrulation were difficult to follow but some stages could be discerned. A band of small, lighter ectodermal

cells was formed around large, darker endoderm cells in the centre ; cell differentiation thus becoming obvious. Protoplasmic streaming was considerable, the ectoderm cells divided actively, especially at the anterior end, forming a clear area of small cells, the hyaline region. This stage is known as the early "tadpole stage" [Anderson and Darling (1964) and Clark (1967)]. This took another eight hours after the formation of the multicellular stage.



Text-fig. 2. *Acrobelinema cornis.* Embryonated eggs.

Twenty four hours after the formation of the multicellular stage, the embryo had lengthened considerably, resulting in a single flexure. From this stage onward the embryo could be observed to move about within the egg-shell. Lengthening of the "tadpole" continued until the end of the third day after the egg was first laid. The embryo now acquired another flexure. The embryo at this stage has often been termed as "pre-larva" Four days after the egg formation in the uterus, the larva was fully developed. Hatching occurred within twelve hours; its details were not observed except that the larva, to begin with, moved vigorously within the egg-shell, then became relatively immobile and finally uncoiling itself just before hatching.

It thus took about 100 hours for the egg to hatch after being laid.

Careful examination did not reveal the presence of moulted cuticle either in the egg-shell with the larva or within the empty egg-shell. From this and from the facts ensuing, it seems that all the four moults are undergone outside the egg-shell and it is the first larval stage that hatches.

Process of Moulting

Larvae approaching the moulting stage became more sluggish in their movement. Later they became quiescent with occasional contractions, and finally straight and immobile. The first indication of moulting was a retraction of the protoplasm in the cephalic region away from the cuticle (hereafter called the moulted cuticle). The entire stoma, as far as could be distinguished, remained in the moulted cuticle. The cuticular lining of the terminal excretory duct was also shed. The protoplasm then reorganized to form a new cephalic region with a faintly visible stoma. Simultaneously with these processes in the anterior region, the cuticular lining of the rectum moulted. The old cuticle apparently stretched, since its annulations became more and more indistinct. This caused the cuticle to loosen around the body. In the final stage of transition period (from one moult to next stage) the new organs became clearly defined : the stoma became distinct ; the lining of the excretory pore became sclerotized ; and the internal lining of the rectum became reorganized. In one case observed the cast cuticle broke somewhere near the nerve ring and the excretory pore.

With the completion of stoma, the new cuticle was formed,

although the next stage was surrounded by the moulted cuticle for some time.

Larval stages

As has been shown by Conte (1900), Clapham (1930), Bingefors (1957), and van Weerdt (1958, 60), the measurements of actual size of nematode larvae alone are not a reliable indication of the stage of development because size is influenced by environmental factors. The various stages of *Acrobelinema cornis* were, therefore, studied in terms of the anatomical differentiation and development of gonads. The development of gonads only was found useful in distinguishing different stages.

Post-embryonic Development

First Larval Stage[1]

(Text-figure 3)

Length = 169-190 μm; a = 18-21; b = 2.4-2.6; c = 13-15.

The first stage larvae at hatching were fully developed and resembled the adults and other larval stages in the general shape. The larvae possessed very small probolae whose arrangement and structure were difficult to interpret. Stoma also was not clearly visible. The oesophagus measured 68-73 μm in length and possessed a poorly delineated isthmus and only a slightly swollen oesophageal bulb, with doubtfully discernible valvular apparatus. Nerve ring and excretory pore could not be seen. Tail measured 13-14 μm in length. The minute rounded genital primordium, formed of a single cell, was located at 70% of the body-length from the anterior end. The larva went into first moult about sixteen hours after hatching.

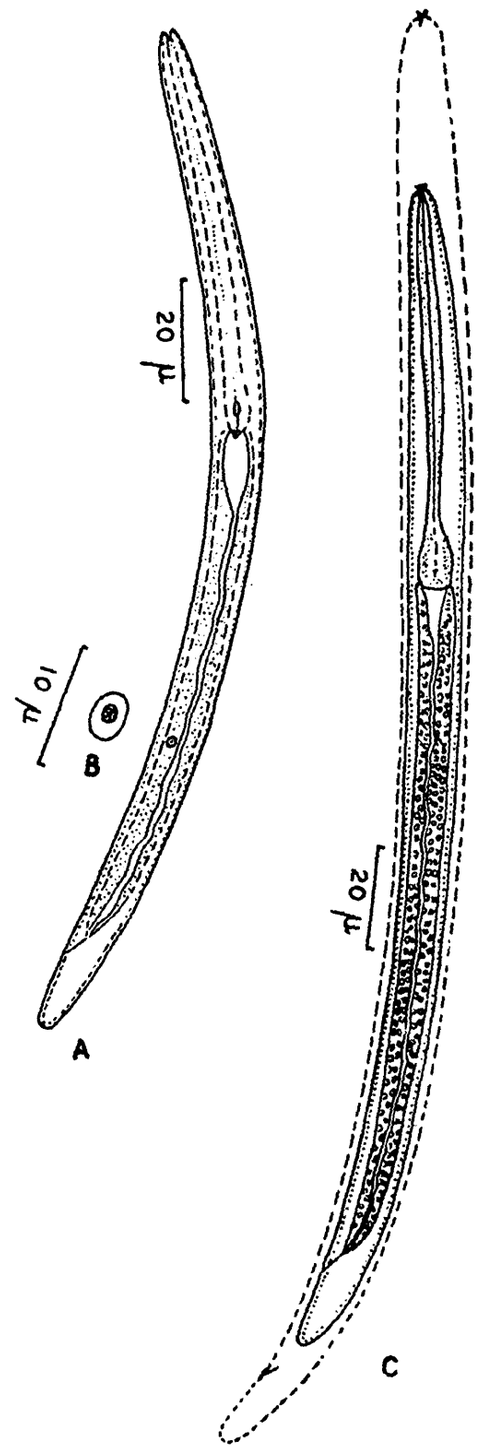
First Moult[2]

(Text-figure 3)

Length = 195-235 μm; a = 20-22; b = 2.5-2.9; c = 13-16.

The stage being small in size and with additional cuticle, the details could not be worked out. Oesophagus measured

[1] Description based on 25 freshly hatched first stage larvae.
[2] Description based on 14 specimens.



Text-fig. 3. *Acrobelinema cornis*. A. First larval stage.
B. Genital primordium of first larval stage.
C. First moult stage.

78-85 µm in length. Isthmus started getting delineated and the characteristic valvular apparatus of the end bulb made its appearance. Tail measured 13-17 µm long. The first moult lasted for eight hours on an average.

Second Larval Stage[3]

(Text-figure 4)

Length = 228-260 μm; a = 20-23; b = 2.8-3; c = 14-17

Probolae were slightly larger in size than in the first stage; their details still could not be observed. The small stoma, 3 μm in length, could now be distinguished; its components still could not be determined. The oesophagus increased in length to 80-87 μm and took on a normal shape, the isthmus, posterior bulb and its valvular apparatus assuming the shape as found in the adult. Nerve ring was situated at 48-52 μm from anterior end. Tail measured 14-18 μm in length. A spindle-shaped genital primordium was located at approximately 64-67% of the body-length. The genital primordium consisted of three nuclei—one large central germinal nucleus, bordered anteriorly and posteriorly by two smaller somatic nuclei. The germinal portion of the primordium was thus set apart from the somatic portion at this stage only. Two of the nuclei in the region of ventral chord nuclei became more prominent and stained moderately heavy. Both were situated posterior to the genital primordium. This stage lasted for sixteen hours on an average after which it went into second moult.

Second Moult[4]

(Text-figure 4)

Length = 240-320 μm; a = 20-24; b = 3.0-3.3; c = 15-19.

Details of stoma could not be worked out. Oesophagus and tail respectively measured 80-100 μm and 17-21 μm in length. The two somatic nuclei of the genital primordium underwent a division. The anterior somatic nucleus divided once, resulting in the formation of two cells that would, after further divisions, form the female gonoduct. The division of the posterior nucleus resulted in two nuclei. One of these remained as cap cell nucleus at the posterior tip of the gonad throughout the life of the nematode. The other nucleus, the epithelial nucleus, migrated anterior to the germinal nucleus.

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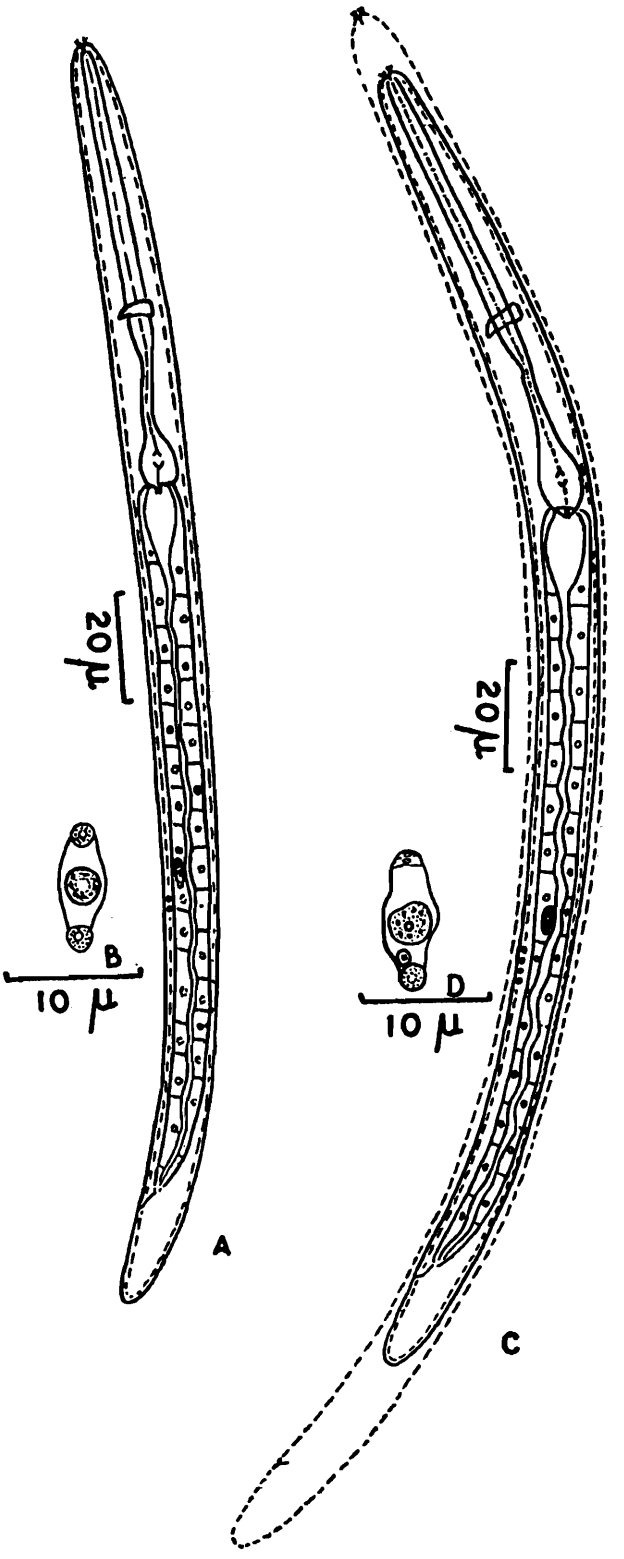
[3]Description based on 20 specimens.

[4]Description based on 12 specimens.



Text-fig. 4. *Acrobelinema cornis*. A. Second larval stage.
B. Genital primordium of second larval stage.
C. Second moult stage. D. Genital primordium of second moult stage.

Each of the moderately heavy staining ventral chord nuclei divided once during the second moult, thus resulting in group of four nuclei all situated *in tandem* posterior to the primordium.

The derivatives of the various nuclei could be traced separately throughout the development of the gonad, since they appeared different and stained differentially in cases where such preparations were successful. The large germinal nucleus with coarse granules stained heavily. The cap cell nucleus and the epithelial nuclei of the gonad stained faintly. The nuclei of the cells of gonoduct were small and stained rather dark.

The second moult lasted for eight hours on an average.

Third Larval Stage[5]

(Text-figure 5)

Length = 380-480 μm; a = 21-24; b = 3.2-3.3; c = 21-24.

In the early third larval stage there occurred a sudden increase in size of the nematode as well as in the activity of the genital primordium.

Three labial probolae, each with two bifurcations, could be seen at this stage. Stoma measured 5 μm in length and could be distinguished as segmented. Oesophagus measured 120-145 μm and tail 18-22 μm in length. Nerve ring was situated at 80-88 μm from anterior end. Excretory pore, situated just behind nerve ring at 83-92 μm from anterior end, could be detected for the first time in the life-cycle.

The anterior two cells meant to produce gonoduct divided rapidly and instead of proceeding anteriad were reflexed, growing posteriad along the ventral side. In the rest of the primordium, which was pushed dorsally, the germinal nucleus divided once, the two resultant nuclei being arranged side by side. The epithelial nucleus in the meantime divided twice to form four epithelial nuclei. Cap cell was present as usual. Four ventral chord nuclei were still there.

The third larval stage lasted for about 36 hours after which period it went through the third moult.

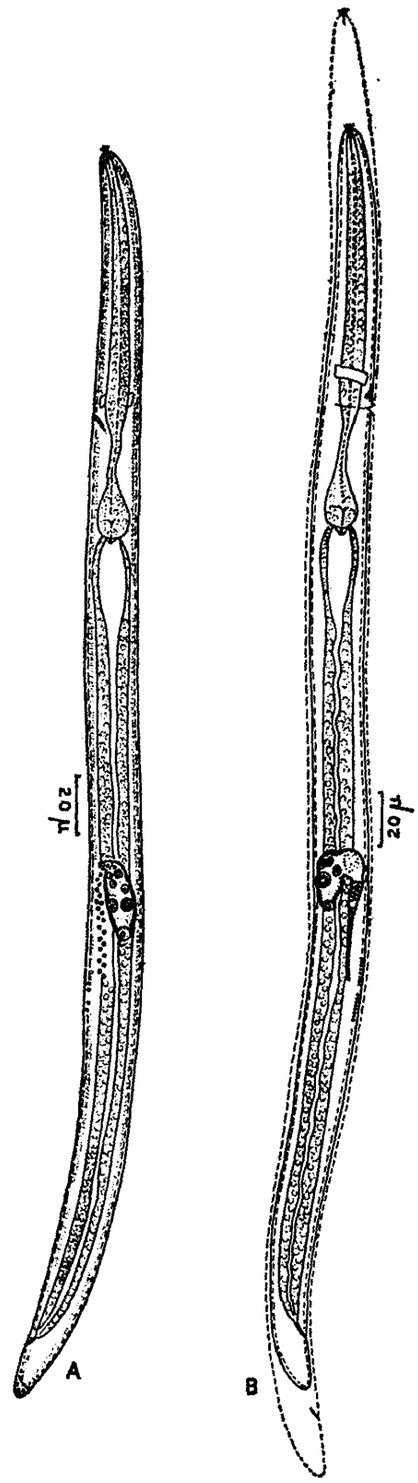
Third Moult[6]

(Text-figure 5)

Length = 450-520 μm; a = 21-24; b = 3.25-3.5; c = 21-24.

Oesophagus measured 140-160 μm long. Nerve ring was

[5] Description based on 22 specimens.
[6] Description based on 14 specimens.



Text-fig. 5. *Acrobelinema cornis.* A. Third larval stage. B. Third moult stage.

situated at 86-92 µm and excretory pore at 96-104 µm from anterior end. Tail measured 19-24 µm in length.

Slight differentiation in the gonoduct had set in. One of the germinal nuclei and one epithelial nucleus disappeared. Connected to the gonad antero-ventrally, a chamber (? recepta-

culum seminis) containing a large number of rounded refringent sperm could be seen. This chamber was marked off from both the gonad and the gonoduct by constrictions at either end. Probably the germinal nucleus and the epithelial nucleus which disappeared, had something to do with the formation of the sperm. The chamber might have been formed either by the cells that formed the gonoduct or by the epithelial cell whose nucleus had disappeared. This being a very transitory phase, the details could not be worked out. Cap cell was present as usual.

The four specialized ventral chord nuclei underwent further division and at the third moult were arranged in two groups of eight nuclei each in the vicinity of the posterior region of the developing gonad. These nuclei later took part in the formation of vagina. Their arrangement in two groups had already indicated the future position of vagina which would be located ventrally between them.

The third moult lasted for about twelve hours on an average.

Fourth Larval Stage[7]

(Text-figure 6)

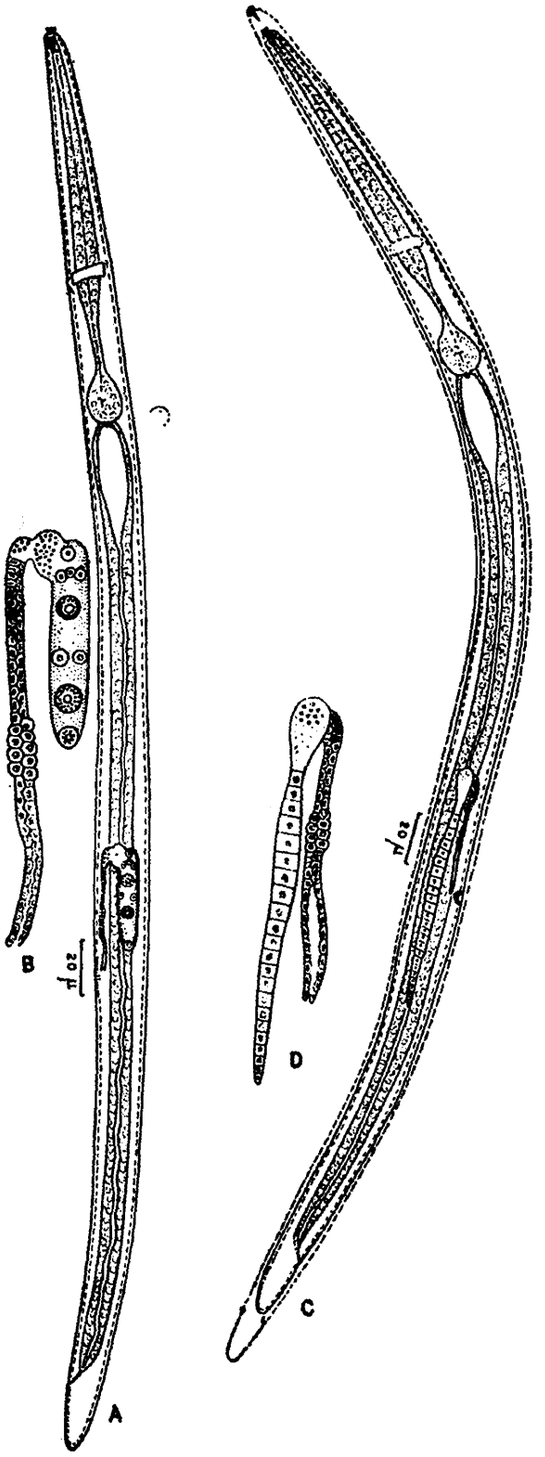
Length = 500-550 μm ; a = 21-26 ; b = 3.45-3.6 ; c = 22-24 ; V = 64-65.

Stoma measured 7 μm, oesophagus 140-165 μm and tail 21-25 μm in length. Nerve ring and excretory pore were situated respectively at 87-95 μm and 92-100 μm from anterior end.

During the fourth stage the germinal portion of the primordium started increasing in size and the gonoduct became more or less differentiated. The remaining one germinal nucleus divided once ; the two resultant oögonial nuclei were arranged this time *in tandem*. The epithelial nuclei also increased in number but their ultimate fate could not be determined. Probably they formed the epithelium of the ovary. The anterior narrow part of the gonoduct, the oviduct, consisted of two rows of five columnar epithelial cells with transversely elongated nuclei. The oviduct was followed by a region of 5-6 larger cells in two rows with oval nuclei which formed one or two temporary spermathecae in the adult. The spermatheca(e) constituted the modified anterior end of uterus and was followed by a

[7]Description based on 15 specimens.

slightly widened part consisting of sixteen large, coarsely granulated cells with spherical nuclei, the quadricolumella. Behind the quadricolumella, the uterus narrowed to from another short, constricted region with two rows of three narrow epithelial



Text-fig. 6. *Acrobelinema cornis*. A. Fourth larval stage.
B. Developing gonad of the fourth larval stage.
C. Fourth moult. D. Developing gonad of the fourth moult stage.

cells each. This region was followed in sequence by a number of other flattened epithelial cells, the uterus proper. Some of the specialized ventral chord nuclei started migrating so as to form, later on, the vagina and the vulva.

Fourth larval stage lasted for about 30 hours on an average.

Fourth Moult[8]

(Text-figure 6)

Length = 525-610 μm ; a = 21-27 ; b = 3.5-3.85 ; c = 22-25 ; V = 66-70.

Oesophagus measured 145-170 μm in length. Nerve ring and excretory pore were situated respectively at 90-100 μm and 98-110 μm from anterior end. Tail measured 22-28 μm long.

The ovary became very much elongated owing to increase in the number of oögonial nuclei, and the cell membranes became indistinct. The epithelial nuclei could not be traced. The vagina and vulva completed their development. The two groups of eight specialized ventral chord nuclei migrated inside, creating the lumen of vagina between them. Four nuclei came to lie posteriad, four anteriad in the median plane, and four took a dorsolateral position on each side of the vagina. After the last moult the cuticular lining of the vagina was formed and the uterus connected with the vagina.

The fourth moult lasted for about 18 hours.

After the hatching it took about six days for the larva to undergo all the moults and become a young female, or about ten days from a single celled egg to a young female.

The female started laying eggs within forty hours.

The average number of eggs laid by a single female was 70 (range 50-85).

DISCUSSION

A single polar body has been found in *Acrobelinema cornis.* Hirschmann (1962) and Clark (1967) have described a single polar body in *Ditylenchus triformis* and *Nacobbus serendipiticus,* respectively, whereas van Weerdt (1960) has described up to two polar bodies in *Radopholus similis,* of which at least one is extra-vitellar. The polar body is extra-vitellar in *D. triformis*

[8] Description based on 8 specimens

also. In *A. cornis* and *N. serendipiticus* it is enclosed within the vitelline membrane.

Many workers (Pai, 1928 ; Stewart, 1921 ; Gadd & Loos, 1941 ; Raski, 1950 ; Skotland, 1957 ; van Gundy, 1958 ; Triantaphyllou & Hirschmann, 1960 ; van Weerdt, 1960 ; Yuksel, 1960 ; French & Barraclough, 1961 ; Rhoades & Linford, 1961 ; Hirschmann, 1962 ; Loos, 1962 ; Seshadri, 1964 ; and Clark, 1967), while working on different species of nematodes, have reported that first moult takes place inside the egg-shell and the egg hatches into second stage larva. Linford & Oliveira (1940), however, failed to detect the pre-hatch moult in *Rotylenchus uniformis*. The pre-hatch moult, according to Maggenti (1961), may also be absent in *Plectus parietinus*. Thomas (1965) has also described the four larval stages outside the egg-shell in *Acrobeles complexus*. In *A. cornis* also first stage larva is hatched. This is further supported by Lee's (1965) observation that in free living nematodes the eggs usually release a first stage larva.

Mechanism of the egg-hatching seems to be as under : The vigorous movement of larva inside the egg-shell, coupled probably with the enzymatic activity of the larva was possibly responsible for breaking down the vitelline membrane of the egg-shell, thus making the latter permeable. The larva could then take up water, which resulted in an increase in hydrostatic pressure in pseudocoelom. As a result, the larva became relatively immobile, enlarged and exerted pressure on the shell, eventually rupturing it. Uncoiling of the larva at this stage could also be partially responsible for rupturing the egg-shell. This mechanism, except the uncoiling part, has already been proposed by Wilson (1958) in *Trichostrongylus*.

In plant nematodes, on the contrary, the larva generally exhibits vigorous movements inside the egg-shell just before hatching (Dropkin *et al.*, 1958 ; di Edwardo, 1960 ; Rhoades & Linford, 1961 ; Shepherd, 1961 and Clark, 1967) although Ellenby (1957) was unable to detect any movement in the larvae of *Heterodera rostochiensis* within the egg. Dropkin *et al.* (1958), Shepherd (1961) and Clark (1967) showed that the larvae made a series of rapid thrusts with the mouth spear against the egg-shell until the latter was pierced. A hatching factor in *Heterodera* spp. is fairly, and in *H. rostochiensis* absolutely, essential (Shepherd, 1962 ; Wallace, 1963). Onions (1955) reported no hatch of *H. rostochiensis* in water. Vigilier-

chio & Lownsberry (1960) showed increased hatching in three species of *Meloidogyne* with root diffusates. In some zooparasitic nematodes the egg-shell is pierced with the pointed tail (Silvermann & Campbell, 1959)

Process of moulting has been studied by various workers (Linford & Oliveira, 1940 ; van Gundy, 1958 ; Rhoades & Linford, 1961 ; and Hirschmann, 1962) Their results indicate that the nematode stops feeding and becomes sluggish or inactive just before moulting. In *Acrobelinema cornis* also the larvae approaching moulting stage first become sluggish, and later quiescent and immobile.

van Gundy (1958) and Wallace (1963) stated that old cuticle might be discarded by simple abrasion against soil particles. Since the present studies were carried out in the soft medium (agar), the question of abrasion does not arise. In *A. cornis* the breaking of cuticle took place just after the formation of the new cuticle, stoma, and excretory anl rectal linings when the nematodes again started moving vigorously. The breaking of the cuticle in the nervous cum excretory region is similar to that found in *Trichostrongylus, Haemonchus* and *Ostertagia* —all animal nematodes (Lapage, 1935), although its mechanism was not studied.

In water, the development from the single cell stage to larva took five days for *Paratylenchus projectus* (Rhoades & Linford, 1961), about five days for *Ditylenchus dipsaci* (Yuksel, 1960), 3-5 days for *Hemicycliophora arenaria* (van Gundy, 1959), 4-11 days for *Radopholus similis* (van Weerdt, 1960) 11 days for *Plectus parietinus* (Maggenti, 1961), 11-13 days for *Criconemoides xenoplax* (Seshadri, 1964), 12-17 days for *Nacobbus serendipiticus* (Clark, 1967). In *A. cornis* it took about four days for the larva to appear from the single cell stage.

The general development of the gonad of *A. cornis* resembles that of *Ditylenchus triformis* (Hirschmann, 1962). There is only one germinal nucleus in the genital primordium in both these species but the difference lies in the stage at which the germinal nucleus starts dividing. The development is also similar to that of *Turbatrix aceti* (Pai, 1928) except in the number of germinal nuclei in the genital primordium. Hirschmann's surmise (1962) that a similar pattern may be obtained in various monodelphic nematodes seems to be correct.

The presence of a single germinal nucleus in the germinal primordium disproves the contention of Chitwood & Chitwood

(1950) and van Weerdt (1960) that all nematodes have two germinal nuclei regardless of the number of gonads in the adult. Genital primordia with one germinal nucleus have been described by Strassen (1892) and Wülker (1923) in animal parasitic nematodes, and by Hirschmann (1962) in a plant nematode.

Pai (1928) first distinguished clearly between germinal and somatic nuclei in the genital primordium of *Turbatrix aceti*. He also traced the derivative of each nucleus throughout the development of gonad. van Weerdt (1960) was able to distinguish between germinal and somatic cells up to third moult in *Radopholus similis*. Hirschmann (1962) traced the derivatives of germinal nucleus and two epithelial nuclei of the primordia of *Ditylenchus triformis* up to the adult stage. In the present case, the derivatives of the germinal and somatic nuclei of the genital primordium have been traced separately and the differentiation into various parts of the gonad of the adult followed, as far as possible.

The cap nucleus in *A. cornis* is derived from the first division of the posterior somatic nucleus. It does not divide later, but remains part of the epithelium of the ovary. This, while supporting the viewpoint of Pai (1928), Chitwood and Chitwood (1950), Hirschmann (1962) and others, is contrary to Musso's (1930) opinion, that the cap cell, in general, can be considered as a germinal stem cell that gives rise to germinal nuclei as well as epithelial nuclei. In *Ditylenchus triformis* it is the anterior somatic nucleus which forms the cap cell but this is due to outstretched condition of ovary in *D. triformis* as compared to the reflexed ovary of *A. cornis*.

The various parts of the female gonoduct are similar to those described for *Ditylenchus* spp. by Wu (1958) and Hirschmann (1962). Even the quadricolumella is present and it is composed of sixteen cells. Only the post-vulvar sac is absent. van Weerdt (1960) reported several ventral chord nuclei with a different staining capacity, appearing for the first time during the third larval stage of the female in *Radopholus similis*. Hirschmann (1962) could see two such nuclei in second stage, four in the second moult and the third larval stage, eight in the third moult stage, sixteen (two groups of eight cells) in the fourth larval stage and fourth moult stage. In the latter, these cells were responsible for the formation of vagina. Observations similar to Hirschmann's (1962), are also recorded in case of *A. cornis*.

The most significant finding is the syngonic protandrous condition of the nematode, the most interesting feature being that the sperm appear in third moult stage and are stored in a sac-like chamber. The exact source of sperm and the formation of the chamber is not evident, but probably one of the germinal nuclei is responsible for sperm formation and the epithelial cell for the formation of the chamber. It is of interest to note that hermaphroditism (syngonic or digonic) has been described only in the adults so far (Triantaphyllou & Hirschmann, 1964). Formation of sperm in a larval stage is being recorded for the first time.

Overgaard Nielson (1949) determined the time required for development from egg to egg in a number of fresh-water nematodes as follows : *Alaimus primitivus, Prismatolaimus dolichurus,* and *Plectus cirratus,* 20-30 days ; *Achromadora dubia, Wilsonema auriculatum,* and *Plectus parvus,* 20 days ; *Anaplectus granulosus,* 25 days ; *Tripyla setifera,* 30-40 days. *Acrobeles complexus,* on asparagine-mannitol agar with micro-organisms takes 32 days from egg to egg : six days for embryonic and 25 days for post-embryonic development (Thomas, 1965). The length of life cycle (egg to adult) varies in plant parasitic nematodes from 10-14 days in *Aphelenchoides ritzemabosi* (French & Barraclough, 1961 ; Wallace, 1960) to 42-56 days in *Tylenchulus semipenetrans* (van Gundy, 1958). *Acrobelinema cornis,* a seemingly saprozoic form, completes its life cycle in about ten days (four days for embryonic development and six days for post-embryonic development), and it takes twelve days from egg to egg. A high rate of reproduction in *A. cornis* can be attributed to the short duration of life cycle as well as high rate of egg production. The latter confirms Chitwood and Allen's (1965) view that saprozoic rhabditids would present a higher figure of egg production.

Hirschmann (personal communication) commenting on the author's work (1967a) has expressed her opinion that it was unlikely that nematodes would thrive and multiply on water agar without any N-source. The present author agrees with Hirschmann, but is of the view that it was quite likely that the nematodes utilize nitrogenous material from the excretory matter produced by the katabolic activities or from other nematodes which died in the culture. In that case there seems to be no hope of ever attaining an ideal of a holidic medium

or even of a meridic medium as enunciated by Dougherty (1960).

SUMMARY

In *Acrobelinema cornis* all the four moults occur outside the egg shell. It takes about twelve days from egg to egg stage.

A single germinal nucleus has been found in the genital primordium.

A. cornis is syngonic and protandrous; the sperm appear in the third moult stage and are stored in a sac-like chamber. Thereafter, it develops, more or less, like any other female nematode. Formation of sperm in a larval nematode is being recorded for the first time.

The time taken for development and percentage recovery, and the measurements and de Manian values of different stages of larvae and adult are given in tables no. 1 and 2, respectively.

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*Not seen in original.

Table I.—Development of *Acrobelinema cornis* in Nutrient Agar at 21-28°C

Number of eggs inoculated in each tube = 50
Recovery in %

Age (hours after inoculation)	I L	I M	II L	II M	III L	III M	IV L	IV M	A
76									
88									
100	94								
112	80	12							
124		20	70						
136		2	80	4					
148			8	34	40				
160				4	72				
172				2	70				
184					20	54			
196						8	60		
208							62		
220							54	6	
232*									
244								12	46

*Not examined at 232 hours.

A = Adult; L = Larval Stage; M = Moult Stage.

Table II.—Measurements & de Manian values of various stages and adults of *Acrobelinema cornis*

	I L	I M	II L	II M	III L	III M	IV L	IV M	A
Lth	169-190 (177)	195-235 (210)	228-260 (245)	240-320 (290)	380-480 (425)	450-520 (485)	500-550 (530)	525-610 (570)	620-750 (670)
Oes	68-73	78-85	80-87	80-100	120-145	140-160	140-165	145-170	165-190
T	13-14	13-17	14-18	17-21	18-22	19-24	21-25	22-28	25-31
a	18-21	20-22	20-23	20-24	21-24	21-24	21-26	21-27	22-28
b	2.4-2.6	2.5-2.9	2.8-3	2.9-3.3	3.2-3.3	3.25-3.5	3.45-3.6	3.5-3.85	3.8-4
c	13-15	13-16	14-17	15-19	21-24	21-24	22-24	22-25	22-26
Gp	70	?	64-67						
V							64-66	66-70	70-73

A = Adult; Gp = Position of genital primordium in percentage from anterior end; L = Larval Stage; Lth = Total length in μm; M = Moult Stage; Oes = Oesophageal length in μm; T = Tail length in μm. Other values as in de Manian formula.