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## SOME OBSERVATIONS ON THE DEVELOPMENTAL BIOLOGY OF *CEPHALOBUS PARVUS* (NEMATODA: CEPHALOBIDAE)

by

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**Summary.** *Cephalobus parvus* reproduced by parthenogenesis and females laid elongate smooth shelled eggs, measuring 45-55x20-30  $\mu$ m. Freshly laid eggs were in single celled condition. Total embryonation period was 14-16 hr. The genital primordium of first stage juveniles showed a bulge on the dorsal wall. The division of germinal nucleus in the genital primordium started in the second moulting; prior to that only somatic nuclei divided. The primordium elongated anteriorly and formed the flexure of the ovary while elongation in the posterior direction resulted in the formation of the post-uterine sac. The multiplication of primordial nuclei became rapid in the fourth stage.

Studies on the reproductive behaviour of cephalobid nematodes have been made by Cheng and Samoiloff (1971), Jairajpuri and Azmi (1977), Duggal (1978a,b) and Ahmad and Jairajpuri (1981a,b). Developmental biology has been studied in *Acrobelus complexus* (Thomas, 1965), *Cephalobus persegnis* (Popovici, 1972), *Acrobelinema cornis* (Khera, 1973) and *Chiloplacus symmetricus* (Ahmad and Jairajpuri, 1979). Hechler (1970) studied reproduction, chromosome numbers and post-embryonic development of *Panagrellus redivivus* while post-embryonic cell lineage patterns of *Panagrellus redivivus* were compared with those of *Caenorhabditis elegans* by Horvitz and Sternberg (1982). This paper reports some observations on the developmental biology of *Cephalobus parvus* Thorne, 1937.

### Materials and methods

Soil samples containing *C. parvus* collected from the lawns of Zoology Department, A.M.U., Aligarh, were processed by using Baermann funnel technique. The nematodes obtained were cultured in 1.5% water agar (1.5 g of agar powder + 100 ml of water) in 5 cm diam Petri dishes under laboratory conditions. Five mg of milk powder (Lactogen) was spread over the surface of agar to enhance the growth of bacteria which provided food for the nematodes. The specimens used in the present study were the progeny of a single female. Observation chambers as designed by Ahmad and Jairajpuri (1979) were used to study the embryonic development. To study post-embryonic development all juvenile stages were stained in 2% Lacto-aceto-orcein (2 g orcein powder + 33 parts lactic acid +

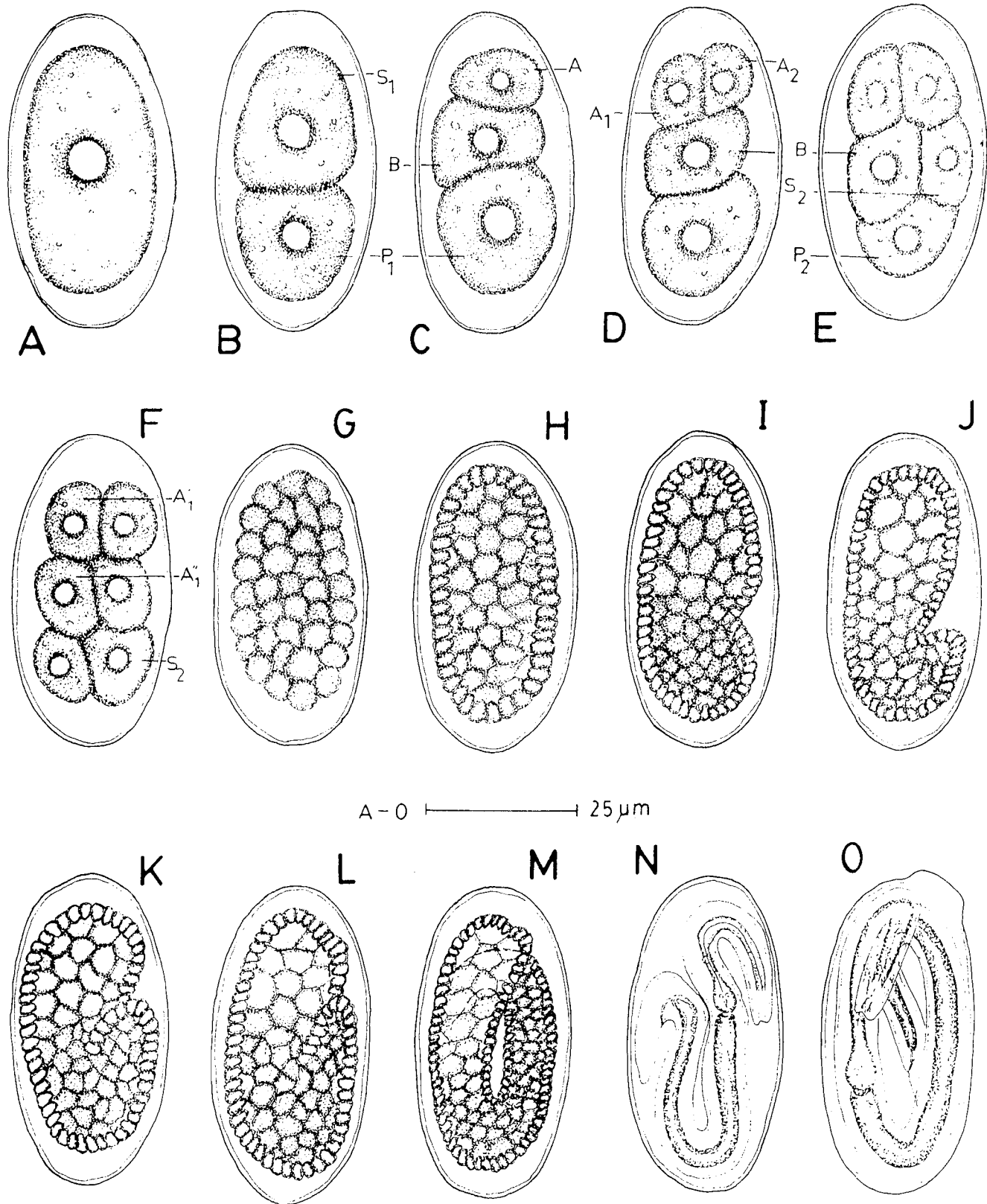
33 parts acetic acid + 34 parts distilled water) for 2 h. Overstained nematodes were transferred to 45% lactic acid for 1-2 min. The observations were made at  $28 \pm 2^\circ\text{C}$  temperature.

### Results

*C. parvus* was found to be amictic. Gravid females at the maximum possessed two mature eggs in their uterine tract.

Intra-uterine egg development was not observed and freshly laid eggs were in single celled condition. They were elongate, smooth shelled measuring 45—55x20—30  $\mu$ m (Fig. 1A).

The first cleavage occurred after the retraction of cytoplasm 15-30 min after egg laying. The cleavage was transverse dividing the egg into unequal  $S_1$  and  $P_1$  blastomeres. The anterior  $S_1$  was slightly larger than the posterior  $P_1$  (Fig. 1B). The  $S_1$  divided horizontally 5-10 min later to form A and B blastomeres (Fig. 1C). After a further 10-15 min the anterior A cell divided longitudinally into  $A_1$  and  $A_2$  (Fig. 1D). The two reoriented themselves so that they were placed obliquely to one another. The posterior  $P_1$  blastomere divided longitudinally into  $S_2$  and  $P_2$  producing the five-celled stage (Fig. 1E) and subsequently after 10-15 min  $A_1$  divided into  $A'_1$  and  $A''_1$  forming a total of six cells (Fig. 1F). Beyond this stage it was difficult to trace the divisions because of the super-imposition of cells over one another. The sixteen-celled condition was formed 30-40 min after the six-celled stage and a morula formed 40-50 min later (Fig. 1G).



The blastula was formed 50-60 min after the morula stage (Fig. 1H). Gastrulation occurred 2-2<sup>1</sup>/<sub>2</sub> h after blastulation and marked the differentiation of hyaline and granular zones in the developing embryo. The invagination developed at the centre or in some cases slightly towards the granular region (Fig. 1I) and the 'lima bean' stage was formed 10-15 min after the initiation of invagination. The 'comma' stage (Fig. 1J) of the embryo appeared 10-15 min later. After 20-30 min the 'tadpole' stage (Fig. 1K) was formed with a broad shallow depression at the anterior end. Movement in embryo started in the late phase. Subsequently the 'plum' stage (Fig. 1L) was formed with a much elongated narrow posterior part. This lasted for 30-35 min and during this stage the embryo moved its head to and fro and also showed a rolling and sliding movement of the body. The embryo attained two egg-fold length with an anterior invagination representing the stomal cavity in the 'loop' stage (Fig. 1M). The embryo of the early 'pretzel' stage was 2-2<sup>1</sup>/<sub>2</sub> egg folds long without differentiation of any organ. Later the oesophageal outline and the oesophago-intestinal junction were formed and the rectum appeared in the form of a broad hyaline band (Fig. 1N). With the passage of time the labial probolae also became prominent. After 60-80 min early pretzel stage, the late 'pretzel' stage was reached which showed refractory lining of stoma along with well formed oesophagus but no valvular apparatus. Later the lumen of the rectum appeared and the junction of intestine and rectum became prominent. The valvular apparatus, chilorhadions and stoma became visible when the juvenile attained a three egg-fold length (Fig. 1O). The lateral lines appeared very faint and were prominent only in the oesophageal region. The juvenile moved continuously prior to hatching, the shell became stretched as a result and a blister (Fig. 1O) formed which served as the point of exit for the moving juvenile. The hatching varied from 14-16 h.

During post-embryonic development the reproductive organs were the only structures which showed proliferation and multiplication of cells. In other organs such as those of the alimentary tract, nerve tissue and muscles etc. cell multiplication did not occur and as development proceeded the nematodes increased in size primarily because of the enlargement of body cells.

The first stage juvenile (Fig. 2A,F) measured 0.18-0.21 mm. The primordium was 4-8 $\mu$ m long and located at 56-62% from anterior end of the body. The genital primordium was slightly asymmetric because of a slight bulge on the dorsal wall. There was one germinal and two somatic nuclei, the former very large in size with a light staining pattern. Moulting was initiated after 5-6 hr and the somatic nuclei multiplied increasing their number to three

including two cap nuclei at the poles. The germinal nucleus did not divide.

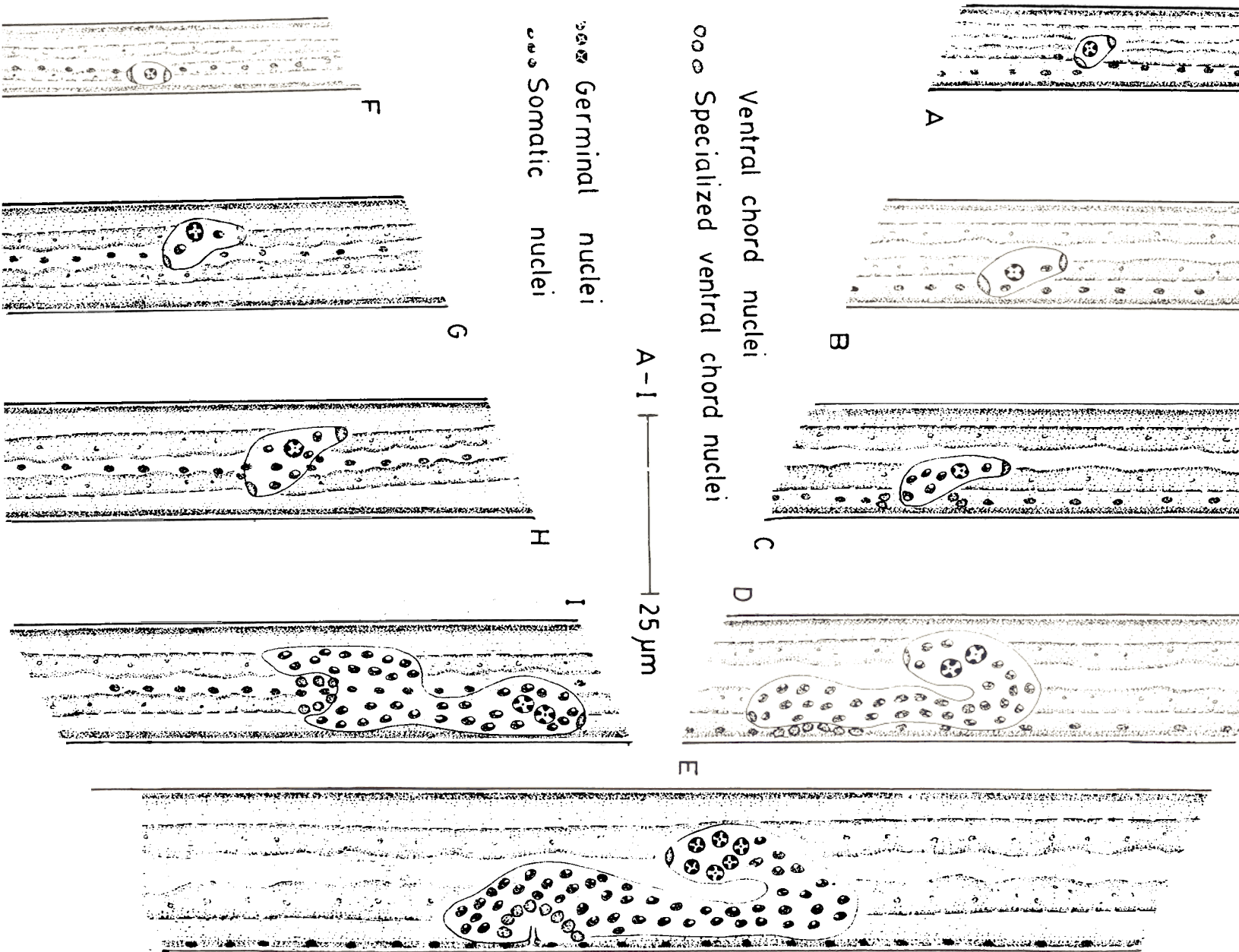
The second stage juvenile (Fig. 2B,G) measured 0.22-0.27 mm. The primordium had increased in length to 7-11  $\mu$ m and was located 50-62% from anterior end. There was one germinal and four somatic nuclei, two of which formed the cap nuclei at the ends. When moulting, this stage had 1-2 germinal nuclei and 5-7 somatic nuclei including two cap nuclei at the tips. Moulting took 3-5 hr.

The third stage juveniles (Fig. 2C,H) measured 0.24-0.29 mm. The primordia in all juveniles showed a similar trend of development as the population contained females only. The primordium was located 55-61% from anterior end and was 14-30  $\mu$ m long. As a result of anterior and posterior proliferation of somatic nuclei, the primordium increased in size. The anterior part of the primordium was narrow containing germinal nuclei compared with the broader posterior part which contained somatic nuclei. The primordium contained 2 germinal and 8-10 somatic nuclei inclusive of the cap ones. Four specialized ventral chord nuclei derived from ventral chord nuclei, appeared at the posterior side of primordium. During moulting due to the multiplication of the primordial nuclei, the anterior part containing 2 germinal nuclei reflexed over. The posterior broader part which formed the uterus in the late stage showed posterior elongation beyond the level of the specialized ventral chord nuclei. Germinal and somatic nuclei were in the ratio of 2: 11-17 at the time of moulting.

The fourth stage female juveniles (Fig. 2D,E,I) measured 0.27-0.35 mm. They possessed a primordium, 30-125  $\mu$ m long, located at 50-58% from the anterior end. The reflexed part of the primordium grew posteriorly with proliferation of germinal and somatic nuclei. The 2-6 germinal nuclei were at the tip of the reflexed part while the somatic nuclei numbering 18-34, were placed behind. The number of primordial nuclei increased and further elongation of the primordium beyond the level of the specialized ventral chord nuclei resulted in the formation of a post-uterine sac. The vagina was formed by specialized ventral chord nuclei, arranged in a circular area.

The final moulting stage of *C. parvus* showed a reflexed germinal part running beyond vulva and sometimes reaching to the rectum. A hyaline area was formed at the site of the future vagina and the specialized ventral chord nuclei became arranged in a circle and formed the vagina. The number of germinal nuclei varied from 7-15 while the somatic ones ranged from 50-70. The somatic nuclei of the oviduct and uterus were flattened. At the completion of moulting all the reproductive organs including ovary, oviduct, uterus, vagina, vulva and a short post-uterine sac were well formed.

Fig. 1 (Front page) - Stages of development in *Cephalobus parvus*: A, single celled; B, two celled; C, three celled; D, four celled; E, five celled; F, six celled; G, morula; H, blastula; I, 'lima bean'; J, 'comma'; K, 'tadpole'; L, 'plum'; M, 'loop'; N, early 'pretzel'; O, late 'pretzel'.



The elongate smooth shelled eggs of *C. parvus* resembled those of cephalobids, *Acrobelinema cornis* (Khera, 1973) and *Chiloplacus symmetricus* (Ahmad and Jairajpuri, 1979). In *C. parvus* intra-uterine development was not observed and eggs laid were always single-celled unlike in *C. symmetricus* where up to three division could occur inside the body. The early cleavage patterns showed a more or less similar trend as observed in *C. symmetricus* and *Acrobelus complexus* (Thomas, 1965). Blastulation was not well marked. The first sign of movement in embryo was observed in the tadpole stage. Formation of a blister in the egg shell of *C. parvus* was a unique phenomenon, not observed in any cephalobid. The embryonation period was 14-16 h at  $28 \pm 2^\circ\text{C}$  similar to *Teratorhabditis andrassyi* (Tahseen and Jairajpuri, 1988) which completed its embryonic development in 12-14 h. Hatching time was much less than that of *C. symmetricus* (72 h).

The presence of one germinal nucleus in the primordium of first stage juvenile conforms with monodelphic *C. symmetricus* but the shape of the primordium differed from the latter. The direction of elongation of the primordium was anterior and posterior unlike other monoprodelfic species where the elongation has been reported in posterior direction. Another contrasting feature was the formation of flexure in the third moult in *C. parvus* as compared to *Diploscapter coronata* (Hechler, 1968) and *T. andrassyi* (Tahseen and Jairajpuri, 1988) where it was observed in the fourth moult. The flexure in *C. symmetricus* was seen in the fourth stage (Ahmad and Jairajpuri, 1979) but as growth of gonad in the species was restricted to moulting periods, it may be assumed that it was formed in the late third moulting. The fourth stage primordium of *C. parvus* contained few germinal nuclei, a character that was observed by Hirschmann (1962) and Van Gundy (1958) in *Ditylenchus triformis* and *Tylenchulus semipenetrans*. Total period of transformation of newly hatched juvenile into a young adult female was 3-6 days which is much closer to post-embryonation period of *T. andrassyi* i.e. 3-5 days.

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Fig. 2 (Front page) - Development of female gonad in *C. parvus*: A-E, developing primordia (lateral view); F-I, developing primordia (ventral view); A and F, first stage juvenile; B and G, second stage juvenile; C and H, third stage juvenile; D and I, early fourth stage; E, Late fourth stage.