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PRELIMINARY OBSERVATIONS ON MORPHOLOGY AND EMBRYOGENESIS OF *RHABDITIS KARACHIENSIS* N. SP. FROM PAKISTAN

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Cultures of *Rhabditis karachiensis* n. sp. were prepared on potato dextrose agar from wild population of this nematode taken from marine algae (*Sargassum* spp.) in the laboratory. After successful culturing, single male and female were inoculated in sterilized (PDA) plates and left at room temperature (28 ± 5°). After three weeks population of these nematodes was increased. Embryological studies were made under compound microscope.

Rhabditis karachiensis n. sp. completed its embryonic development in about 19.66 hr. and deposited 8-10 eggs in 1 hr. Usually the early cleavage division occured within uterus. Development of the embryo was fast and to some aspects similar to *Pelodera teres*. Lima bean stage was reached within 6.33 - 9.50 hr., from P stage. Comma stage followed by Tadpole stage within 8.33 - 12.16 hr. Plum stage transformed into first larval stage within 9.66 - 13.66 hr. from P stage.

Key words: *Rhabditis karachiensis* n. sp., Morphology, Egg, Female gonad, Embryogenesis.

Introduction

Goette, (1982) studied embryology of *Rhabditis nigrovenosa* [46], made observation on early embryology of genus *Rhabditis*. Neuhaus [47], traced the development of the fat of three germ layers. However, knowledge of the early embryology of *Rhabditis* is incomplete. Maupas [48] studied moulting and post-embryonic growth of the larvae *Rhabditis (chorio-rhabditis) caussandeli* and *Rhabditis (C.) pellio*. Chuang [34] studied embryonic and postembryonic development of *Pelodera terricola* larvae of marsh- cranely which is a major pest of grass-lawns and pastures. Poinar [51] studied *Heterorhabditis*. During study of their life cycle it was found that infective larvae of this genus possessed a symbiotic bacterium at the anterior end of the intestine, which is lethal for their host. They shared this character with the species of the genus *Neoaplectena* from which they differed most conspicuously by the character of the male and heterogonic life cycle. Male and female of *Rhabditis karachiensis* n. sp. are described in detail with their embryogenesis and possible use as biological control [33]. These studies were made from single egg progeny reared on potato dextrose agar.

Materials and Methods

Cultures of *Rhabditis karachiensis* n. sp. were taken from petri plates and sieved according to Cobb gravity sieving method [49] and later by Baermann funnel method [50]. Water containing nematodes were drawn off into a syracause watch glass. Nematodes were fixed in 5% hot formalin and left for 24 hr. Later, they were transferred into 1.5% glycerine and left in desiccator for slow evaporation. After several weeks some quantity of anhydrous-glycerine was added and again left for a week. Permanent mounts were made in anhydrous glycerine.

Measurements were made with an ocular disc micrometer, calibrated with the aid of stage micrometer. For very curved specimens, projections were made with camera lucida and the line measured with the aid of plastic ruler, which was calibrated with the aid of stage micrometer. Drawings were made with a camera lucida attachement or with a first aid surface mirror and prism according to the setup recommended by Thorne [10].

The population of *R. karachiensis* n. sp. was isolated from marine algae, procured from Karachi coast (Cape Monze). Stock cultures of *R. karachiensis* n. sp. were maintained on potato dextrose agar at room temperature (28 ± 5°). Eggs were obtained by dissecting the gravide female of *R. karachiensis* n. sp. Eggs were washed in 0.5% sodium hypochlorite solution (commercial bleach) for 5 min., followed by rinsing with sterile distilled water to prevent the contamination of other microorganisms during observation.

Normal eggs were randomly selected and mounted in a drop of distilled water placed in a shallow cavity slide, covered with a glass coverslip and sealed with wax. Embryogenesis was observed under high power compound microscope.

Review of literature. The genus *Rhabditis* was established by Dujardin but diagnosed rather scantily, especially by modern standards. Dujardin listed four new species. Bastian [11] added four new species. Butschli [30] was the first to analyse the genus *Rhabditis* in detail. Schneider [3] rejected the name *Rhabditis*, and divided Dujardin's genus into two genera *viz. Leptodera* and *Pelodera* [18, 19] described some more new species and added 37 species in the genus *Leptodera and Pelodera*. Orley [24] was the first to try to accomodate the genus *Rhabditis* into the system Nematoda and proposed a family Rhabditidae for genera *Anguillula, Cephalobus,*

Oxyuris, Rhabditis and *Teratocephalus*. He placed this family in the higher "Rhabditiforme formae" which formed a "Connecting link" between free living and animal parasitic nematodes. Micoletzky [15] described seven new species, but his system was artificial. Baylis and Daubney [40] divided the family Rhabditidae into three sub-families *viz*. Rhabditinae, Cylindrolaiminae, Bunonematinae [16, 17] containing 64 genera. Reiter [26] worked on Rhabditids in a classical manner and gave detailed description and good illustrations about 16 nematodes. Schneider [4] placed Rhabditids in the sub- family Rhabditinae and distinguished 4 genera i.e. *Cheilobus, Diploscapter, Rhabditis, Poikilolaimus*. T. Goody [35] distinguished three sub-families *viz*. Rhabditinae, Diploscapterinae, Bunonematinae and nine genera within the family Rhabditidae. Earlier other workers [9,14,22,38] contributed a great knowledge to Rhabditidae and added 60 new species with some new data on ecology and biology. Osche [9] also provided a check list of 163 valid species and 7 species *inquirenda*. Dougherty [41] developed the system of Rhabditidae. Meyl [43] delt with 18 genera of the family Rhabditidae and enumerated 121 species from Central Europe. In the same year Thorne [10] divided the Rhabditidae into five sub-families Rhabditinae, Protorhabditinae, Poikilolaminae, Diploscapterinae, Bunonematinae and 18 genera in the sub-family Rhabditinae. Paramonov [1] placed 2 free living families (Bunonematidae, Rhabditidae) and 5 zooparasitic ones (Rhabdiasidae, Neoaplactanidae, Carabonematidae, Angiostomatidae, Strongyloididae) in the superfamily Rhabditoidae. Sudhaus [39] made the greatest contribution to the knowledge of morphology, taxonomy and ecological studies. Later, Andrassy [18] published a taxonomic review of the suborder Rhabditina and research the generic diagnosia of the genus *Rhabditis*.

Rhabditis karachiensis n. sp. (Fig. 1)

Holotype♀: L= 0.9 mm; a= 7.9; b=4; c= 13; c^1= 4; V=59.

Paratype ♀♀ (n= 16): L= 0.86 - 1.35 mm; (1.1±0.34); a=7.2 - 9 (8.1±1.2), b= 0.49 - 7.0 (3.7±4.6); c= 9.61 - 14.2 (11.9±3.24); c= 2.02 - 14.2 (3.21±1.55); V= 52 - 64 (58±8.4); Ova= 34.2 -35µm, x 21.6 -24 (34.6±0.56) x (22.8±1.6) µm.

Paratype ♂♂ (n= 16): L= 0.45 - 1.12 mm; (0.94±0.25); a= 10.2- 31.8 (25.87±13.8); b= 1.79 - 4.8 (3.3±2.12); c= 6.43 - 13.78 (11.4±5.7); c' = 1 -3.7 (3.3±0.53); Spicules=33.8-55 (44.4±14.9); Gubernaculum= 20.8 -33 (26.9±8.6) µm.

Description. Body of the heat relaxed specimens slightly curved, narrow towards extremities. Cuticle thin 1.5 µm in thickness; lateral field 1/5th wide, as body marked by single bright line; lip low rounded; lip region 10.8 µm in breadth; metarhabdiation contained 4 or 5 rounded and minute verrucae; stoma tubular measuring 18.7 - 19.4 µm in length and 4.9–14.5 µm in breadth. Rhabditions of stoma symmetrical; Pharyngeal collar 26% stomal length. Pharynx containing an anterior cylindrical and muscular corpus, 35µm in length and 18.9 µm in breadth; a swollen median bulb, 41–42.4 µm long and 21.6–22 µm in breadth with centrally located concentric valve plates; isthmus 39 - 40 µm long, encircled by nerve ring located at 80.33 - 110 µm from anterior region. Female; Vulva median, vagina transverse to body axis extending into two branches of the gonads being reflexed at overy and developed unequally. Intestine prominent and ending into a long rectum. Rectal glands present; anal portion markedly annulated. Female tail convex into a spicated terminus. Ova measured 42.4 x 23.3 µm; near about 16 eggs were reported in females in different stages of development.

Male. Similar to female in general shape of the body. Male is smaller in length than female, spicules paired, 33.8 µm, cephalated; gubernaculum, 20. 8 µm with obtused distal end. Tail with a short spicated terminus extending past the leptoderm bursa; bursal rays 8–9 in number, rather uniformly spaced, but often in 1, 1-2, 3-3 series.

Differential diagnosis. *Rhabditis karachiensis* n. sp. comes close to *Rhabditis terricola* (Dujardin, 1845) but varies in body length, 'a' ratio, shape and location of vulva. In *R. terricola* vulva median where as post median in *Rhabditis karachiensis* n. sp. *Rhabditis karachiensis* is also similar to *R. gracilicauda* (DeMan, 1876) in general shape of the body but differs in 'a' ratio, length of the stoma, vulva percentage and length of the tail. *Rhabditis karachiensis* n. sp. is similar to *R. seychellensis* Polts (1910) but differs in body length, shape, length of the collar, arrangement of papilla and bursal rays.

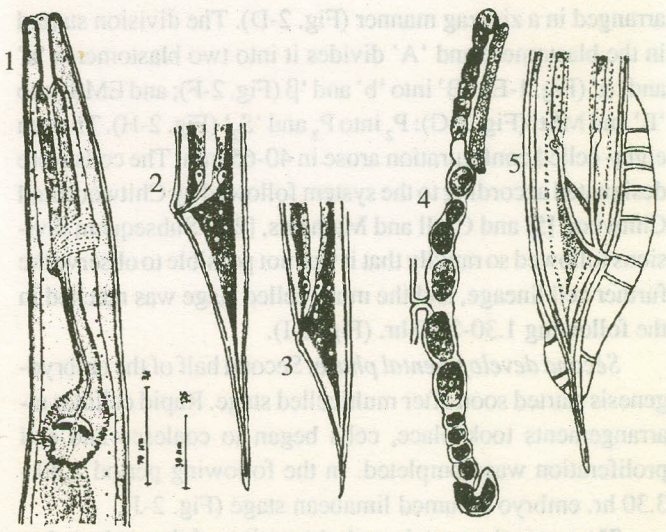

Fig. 1. *Rhabditis karachiensis* n. sp. (1) Female anterior end, (2) Female posterior end, (3) Female posterior end, (4) Female reproductive organ, (5)Male posterior end.

Egg. Eggs of *Rhabditis karachiensis* n. sp. were hyaline and oblong in shape 59.8–72.8 µm (66.30±9.19) in length and 23.4–33.8 µm (28.6±7.3) in breadth and the average egg size was 64.56 x 31.32 µm. It appeared to be enveloped in two layers, an inner thin layer and an outer thick layer. The egg membrane was transparent and stretchable, so it could change the shape from a relatively short and broad.

Female gonad. In a well fed female the ova in each ovary developed in batches. Approximately 25–30 ova were observed in one batch. It was also observed that in 1 hr. 8-10 eggs were laid and cleavage divisions occurred in the uterus and mostly eggs were laid in larval stages.

EMBROYOGENESIS

First development phase. In the mature one-celled egg (P) the first cleavage division occurred in about 40-60 min. (Fig.2-A), the division being transverse giving rise to two blastomeres of an unequal size, an anterior blastomere (S_1) and posterior blastomere (P_1). The P_1 was slightly larger than the S_1 (Fig. 2- B). The nuclei appeared as large, less dense and rounded bodies which could be easily distinguished from the rest of the cytoplasm by their lighter colour and one in the centre of each cell.

The second cleavage division was perpendicular to the long axis, which appeared in the larger blastomere (P_1) dividing it into two sister blastomeres, P_2 and EMSt, resulting into three celled stage (Fig. 2-C). This division occurred within the period of 5-15 mins.

Then the division started in the anterior blastomere 'S_1' dividing it into two sister blastomeres 'A' and 'B'. The division was completed in about 5-10 min. The four cells were arranged in a zig-zag manner (Fig. 2-D). The division started in the blastomere and 'A' divides it into two blastomeres 'a' and 'α' (Fig. 1-E), 'B' into 'b' and 'β (Fig. 2-F); and EMSt into 'E' and MSt; (Fig. 2-G): P_2 into P_3 and 'S_2' (Fig. 2-H). Thus an eight- celled configuration arose in 40-65 min. The cells were designated according to the system followed by Chitwood and Chitwood [5] and Croll and Mathews, [29]. Subsequent divisions followed so rapidly that it was not possible to observe the further cell lineage, and the multi celled stage was reached in the following 1.30-2.30 hr. (Fig. 2-I).

Second developmental phase. Second half of the embryogenesis started soon after multicelled stage. Rapid cellular rearrangements took place, cells began to coalesce and cell proliferation was completed. In the following period 2.30 - 3.30 hr. embryo assumed limabean stage (Fig. 2-J).

Then morphogenesis and elongation of the embryo began. At coma stage (Fig. 2-K) invagination appeared within the mouth portion of the embryo at an interval of 80-100 min. Now the embryo acquired a bluntly rounded shape and converted into the Tadpole stage within the period of 40-60 min. (Fig. 2-L). After an interval of another 30-50 min. The embryo reached plum stage; the length of the embryo became twofold the egg length and moved slowly (Fig. 2-M). By the end of 50-90 min. the embryo attained the larval stage and had fourfold egg length. The juvenile moved in rotatory and longitudinal fashion. It gradually became thin and long (Fig. 2-N-O). Pressure on egg shell applied by flexure and rotation of the body. Finally the egg shell ruptured and hatching was achieved in about 7-9 hr.

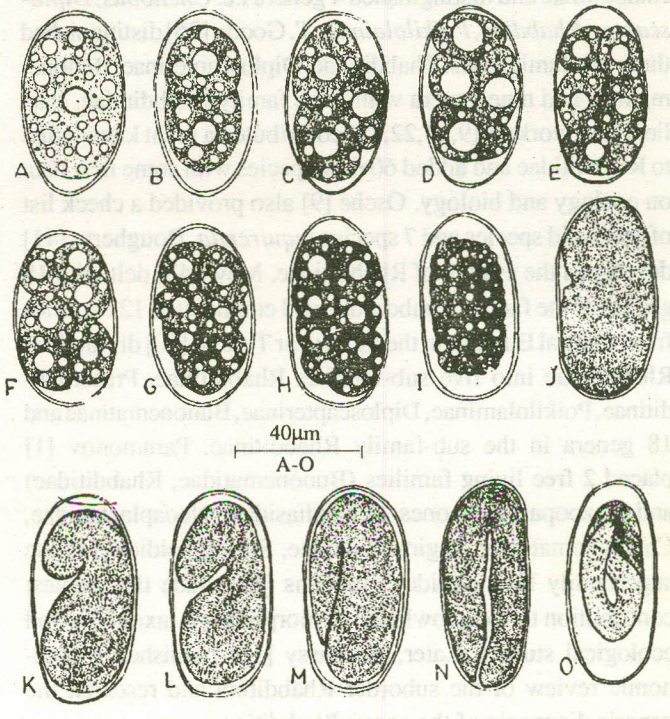

Fig. 2. *Rhabditis karachiensis* n. sp. Embryonic development. (A) One-celled stage; (B) Two-celled stage; (C) Three-celled stage; arranged in a zig-zag manner; (D) Four-celled stage; (E) Five-celled stage; (F) Six-celled stagge; (G) Seven-celled stage; (H) Eight-cell stage; (I) Multi-celled stage; (J) Lima bean stage; (K) Comma stage; (L) Tadpole stage; (M) Plum stage; (N) Early larval stage; (O) Late larval stage.

Discussion

It was observed that when the nematodes were left in tap water in petri plates they started egg laying and approximately 8- 10 eggs were laid in one hr. The embryogenesis in *Rhabditis karachiensis* n. sp. is similar in certain stages to *Pelodera teres* [34]. The second cleavage occurred in the larger 1/2 blastomere (P_1). It was further noted that its cleavage plane was perpendicular to the plane of the first cleavage [44] while the plane of the smaller 1/2 blastomere (S_1) was parallel to give rise a sort of 'T' form arrangement of the four blastomeres like in *P.teres* and *Caenorhabditis elegans* [21]. Further development of the embryo in *R. karachiensis* n. sp. is similar untill hatching to *P. teres* [34], *C. elegans* [20], *Cephalobus litroralis*

[26], *Acrobeles complexus* [30], *Panagrolaimus nigophilus* [24] and *Panagrolaimus tipulae* [2].

The life cycle of *R. karachiensis* n. sp. was completed in about 3.29 days at room temperature (28±5°). Embryonic development lasted for about 19.66 hr. In contrast, the life cycle, duration of *P. teres* was 3.25 days, whereas, embryonic development was completed in about 20 hr. and postembryonic development in about 58 hr. [34]. In *C. elegans* it was 3.5 days (6,32, 8, 37, 28 and 45) and embryogenesis was completed in about 11.5 hr. according to Sulston and Horvitz [21]. In the case of *C. litoralis* [27], the duration of the life cycle was 3-4 days and embryogenesis was completed in about 18.5 hr. Thomas [31], studied the life cycle of *A. complexus* which was completed within 32 days and embryonic development in about 6 days. The life span of *Panagrellus redivivus* (L.) T. Goodey, was completed within 5-6 days [35].

It is clear from the present study that developmental characteristics of *R. karachiensis* n. sp. are similar to *P. teres* [34]. Some phases of development of *R. karachiensis* are like that of *C. elegans* [21]; *C. litoralis* [27]; *A. complexus* [31].

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