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Cephalobus litoralis: Biology and Tolerance to Desiccation

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Abstract: *Cephalobus litoralis* (Akhtar, 1962) Andrássy, 1984 reproduced parthenogenetically and completed its life cycle in 72–90 hours. Each female deposited 200–300 eggs. The nematodes showed synchronized movements in the rhythms of the anterior parts of the body. The nematodes were coiled when dried in culture medium or in slowly evaporating water droplets on the tops of culture plates, but in pellets they assumed irregular postures. Nematodes in pellets stored at high humidity could be reactivated after storage for 28 days.

Key words: behavior, *Cephalobus litoralis*, coiling, desiccation tolerance, life cycle, parthenogenesis, synchronized movement.

Cephalobus litoralis (Akhtar) Andrássy, 1984 was described in 1962 by Akhtar (1) as *Paracephalobus litoralis* based on only two female specimens collected from soil around the roots of sugarcane (*Saccharum officinarum* L.) in the agricultural farms of the Pakistan Council of Scientific and Industrial Research (PCSIR), Lahore, Pakistan. It had not been reported elsewhere until the authors found it in Karachi. In 1967 Andrássy (3) transferred *Eucephalobus diversipapillatus* (Altherr) Goodey to *Paracephalobus*; however, Anderson and Hooper (2) did not agree with the change. In 1984 Andrássy (4) synonymized *Paracephalobus* with *Cephalobus*.

We collected *C. litoralis* in 1984 from soil around the roots of doob grass (*Cynodon dactylon* (L.) Pers.) from a lawn at Aga Khan Medical University Hospital, Stadium Road, Karachi, and identified it as *Paracephalobus litoralis*. In the same year, however, Andrássy (4) transferred it to the genus *Cephalobus* as *C. litoralis*. This paper reports the mode of reproduction, life-cycle duration, desiccation tolerance, and behavior of *C. litoralis*.

Materials and Methods

Production of inoculum and mass rearing: Nematodes were extracted from the soil by a modified Baermann funnel method. A single gravid female was placed in a cavity slide containing water. After oviposition, a single egg was transferred to a petri dish containing finely blended pea meal paste (PMP) and water. All experiments were conducted with nematodes from single egg progeny raised in this manner.

For mass rearing, nematodes were cultured in 14-cm-d petri dishes containing pea meal paste. The dishes were placed under a bell jar lined with black paper to eliminate light. Nematodes were harvested after 10 days. The contents of petri dishes were filtered to remove the liquid and were extracted from the substrate on a Baermann funnel fitted with transparent nylon tubing. Nematodes were visible as whitish turbidity in the nylon tubing. They were collected frequently to avoid damage from oxygen shortage (anoxia). Water was added to the funnel periodically. Millions of nematodes were collected in this manner.

Mode of reproduction: A gravid female was placed in a cavity slide. After oviposition, an egg was transferred to another cavity slide and washed several times with distilled water. The slide was enclosed in a petri dish and was viewed periodically through an inverted microscope. When the egg hatched, a small amount of PMP was placed beside the newly emerged juvenile and development was observed until the juvenile became a female and laid eggs. Water was added as needed. Observations of the development of individuals were repeated 10 times at a temperature of 28 ± 5 C. The elapsed time between egg hatch-

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TABLE 1. Reactivation of, and water uptake by, dehydrated *Cephalobus litoralis*

Days stored after dehydration	Percent reactivation†	Rate of water uptake (min)
4	99	1
8	99	1
12	84	1–2
16	76	2–3
20	59	3–4
24	45	3–4
28	42	4–5

† Mean of 10 replicates.

ing and oviposition by the emerged individual was considered the length of life cycle. Increase in number of nematodes was considered proof of parthenogenesis.

Desiccation: Nematodes were placed in watch glasses, and excess water was aspirated with a fine-tipped dropper. Remaining moisture was removed by passing hot air from a hair dryer over the watch glasses while simultaneously moving the nematode mass with a small spatula to form aggregates called pellets (5). The pellets were dried further by rolling them on absorbent paper till they ceased to leave a moist spot. To slow down the rate of water loss, the dried pellets were coated with paraffin oil by rolling them in a petri dish containing a thin layer of oil. Excess oil was removed by rolling the pellets again on absorbent papers. The pellets, each weighing 60–100 mg, were placed in a humidity chamber containing a glycerine–water solution (R.H. = 94–98%) for 4–28 days. Every 4 days a pellet was removed from the chamber and rehydrated. The pellet was observed in a petri dish with a stereoscopic microscope while drops of water were poured over it. The time required for the worms to regain their natural posture, turgidity, and movement was recorded.

Behavior: Culture plates containing nematodes were observed with an inverted microscope. The plates were stored under a bell jar, and the nematodes were observed periodically for a period of 4 weeks at 28 ± 5 C.

RESULTS AND DISCUSSION

Mode of reproduction: Cephalobus litoralis reproduced parthenogenetically and completed its life cycle in 3–4 days (72–90 hours) at 28 ± 5 C. Each female deposited 200–300 eggs which soon began embryogenic development.

Desiccation: Dehydrated nematodes in pellets were initially irregularly shrunken, twisted, and apparently lifeless. Upon rehydration, the nematodes separated from each other and became turgid in 1–5 minutes. Most of the reactivated nematodes were either juveniles or young females, but some were gravid females. As many as 11 juveniles were observed moving about inside the body of a female ("endotokia"). Nematodes were noted to survive in pellets for 28 days (Table 1). Within this period, the nematodes stored longer resumed activity more slowly upon rehydration.

Behavior: C. litoralis showed synchronized movement in the rhythms of the anterior part of the body in the medium (pea meal paste). When the culture medium became dry, the nematodes migrated to water droplets on the top of the petri dish. As these droplets evaporated, the nematodes aggregated and about 90% of them became coiled while the others were twisted irregularly. Coiled nematodes were also observed in the slowly dried culture medium. When placed in slowly evaporating water in petri dishes, the nematodes aggregated in spindle-shaped masses as the water evaporated.

Cephalobus litoralis survived water stress and withstood unfavorable environmental conditions. Coiling under water stress has been observed in several anhydrobiotic nematodes including both parasitic and free-living forms (6). Saeed and Roessner (8) studied the behavior of some plant-associated nematodes in paraffin oil and noted a marked reduction in body surface area. Coiling and transverse and longitudinal folding of the body cuticle is generally an indication of anhydrobiotic capability in nematodes. *C. litoralis* seems to have these characteristics, but further studies will be

needed to ascertain whether this nematode is merely desiccation tolerant or has also the capability of surviving anhydrobiotically.

Since *Cephalobus litoralis* matures rapidly and has a short life cycle, it appears to be ideal for the study of nematode morphogenesis. It is also inexpensive to maintain as it feeds on bacteria which can be easily grown on a medium as simple as pea meal, and it seems to be quite malleable to a variety of laboratory purposes. Therefore, although it has not been widely reported geographically, it meets all the criteria (7) for a suitable eucaryote model for aging research and genetic manipulation.

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