THE LIFE CYCLE OF THE FREE-LIVING MARINE NEMATODE *INNOCUONEMA TENTABUNDA* DE MAN, 1890

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Summary. The free-living marine nematode *Innocuonema tentabunda* was collected from the mangrove sediment of a mud-flat at Chorao Island, Goa, India, and reared in the laboratory to investigate its life cycle at 27 ± 2 °C. The nematodes were fed with cultured bacteria and diatoms. Copulation was observed one day after females and males were put together in cavity blocks. Eggs were clearly seen inside females 2 days after they were fertilized, when egg laying started, and a single female could lay 10-20 eggs. The egg diameter varied from 40 µm (newly laid) to 57 µm (before hatching). Eggs hatched 3 days after being laid. The first stage juvenile, after hatching, took about 20 days to reach the adult stage. The length of juveniles averaged 150 µm when they first emerged from the egg and 505 µm when they were 9 days old. The length of fully grown specimens varied between 700 and 900 µm.

Keywords: Biology, laboratory rearing, development, India.

Because of overlapping generations and of difficulties in making repeated observations in the matrix sediment where marine nematodes live in nature, laboratory studies are necessary to understand their ecology, growth rate, generation time, embryonic development, feeding behaviour, and energy flow (Vranken *et al.*, 1981). This requires that marine nematodes are cultured under laboratory conditions. The large majority of marine nematode species that have so far been cultured belong to the Monhysteridae and Rhabditidae (Moens and Vincx, 1998). There are a few exceptions in the Chromadoridae, such as in the genus *Innocuonema,* but to date no report is available on how to culture *I. tentabunda* De Man under controlled laboratory conditions. This study is on a population of *I. tentabunda*, reported for the first time from a tropical mangrove mud-flat located along the west coast of India. Samples were collected with an elongated meiocorer at Chorao Island Goa, India (latitude 15° 00'-15° 52'N and longitude. 73° 30'-74° 44'E). The cores were strained through a 45 µm sieve and the sieve contents transferred to a Petri dish (14 cm diameter), which was filled with sediment from the same locality with the addition of 10 g agar per litre. With this procedure, we were able to maintain the nematodes for more than 1 month at room temperature. From this stock we established monospecific sub-cultures of *I. tentabunda* in small cavity blocks (3.7 cm diameter and 2.3 cm height).

To study the reproductive behaviour and life cycle of *I. tentabunda*, 25 males and 25 females were selected for observation and pairs of males and females were put separately into the above-mentioned cavity blocks (at 27 ± 2 °C; 12 h light, 12 h dark). Feed was provided in the form of mixed cultures of colony forming bacteria, standardized to 85 x 10^7 CFU (Colony Forming Units)/litre. The medium was nutrient agar containing the following constituents per litre of seawater: peptic digest of animal tissue 5 g, sodium chloride 5 g, beef extract 1.5 g, yeast extract 1.5 g, and Agar (Himedia®) 20 g.

The average length of adult *I. tentabunda* was 0.7-0.9 mm (Fig. 1A) and they thrived well in the media provided (Fig. 1B). Our observations showed that the nematodes fed actively on the feed provided; however, their activity slowed down as the amount of food decreased. Copulation was observed after one day in the cavity block. The male was usually orientated at an angle to the female and often coiled around her. The eggs were clearly seen in fertilized females 2 days after copulation, when egg deposition began (Fig. 1C). The eggs were oval in shape (Fig. 1D). Newly laid eggs could be clearly observed at the bottom of the cavity block in a mass of mucilage secreted by the female. Males survived only for a short time after mating and females were in general more active than males. Females deposited eggs either singly or in pairs and the total number of eggs deposited by a single female ranged from 10 to 20. The female usually laid eggs in a mass of secreted mucilage, which also favoured the growth of unidentified micro-algae and ciliates, as these were also observed in the mucus. Measurement of egg diameter and the length of each developmental stage was done daily. To monitor the growth of the nematode, the lengths of five juvenile specimens, which were the progeny of different females, were measured daily for nine consecutive days. The newly laid eggs had an ovoid shape with an average diameter of 40 µm (Table I). Two hours after egg deposition, the first cleavage occurred and two equal blastomeres appeared (Fig. 1D). The nucleus of the largest blastomere usually lay closer to the eggshell. After this stage, it was difficult to observe other stages of cell division. Further cell division produced an irregular blastu-

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la (Fig. 1D). Gastrulation started after ten hours, after which time rotation of the embryo was clearly observed (Fig. 1E). Eight hours after gastrulation, a vermiform stage appeared and the embryo moved continuously within the shell. Accordingly, the position of different embryos was observed up to hatching. The eggs

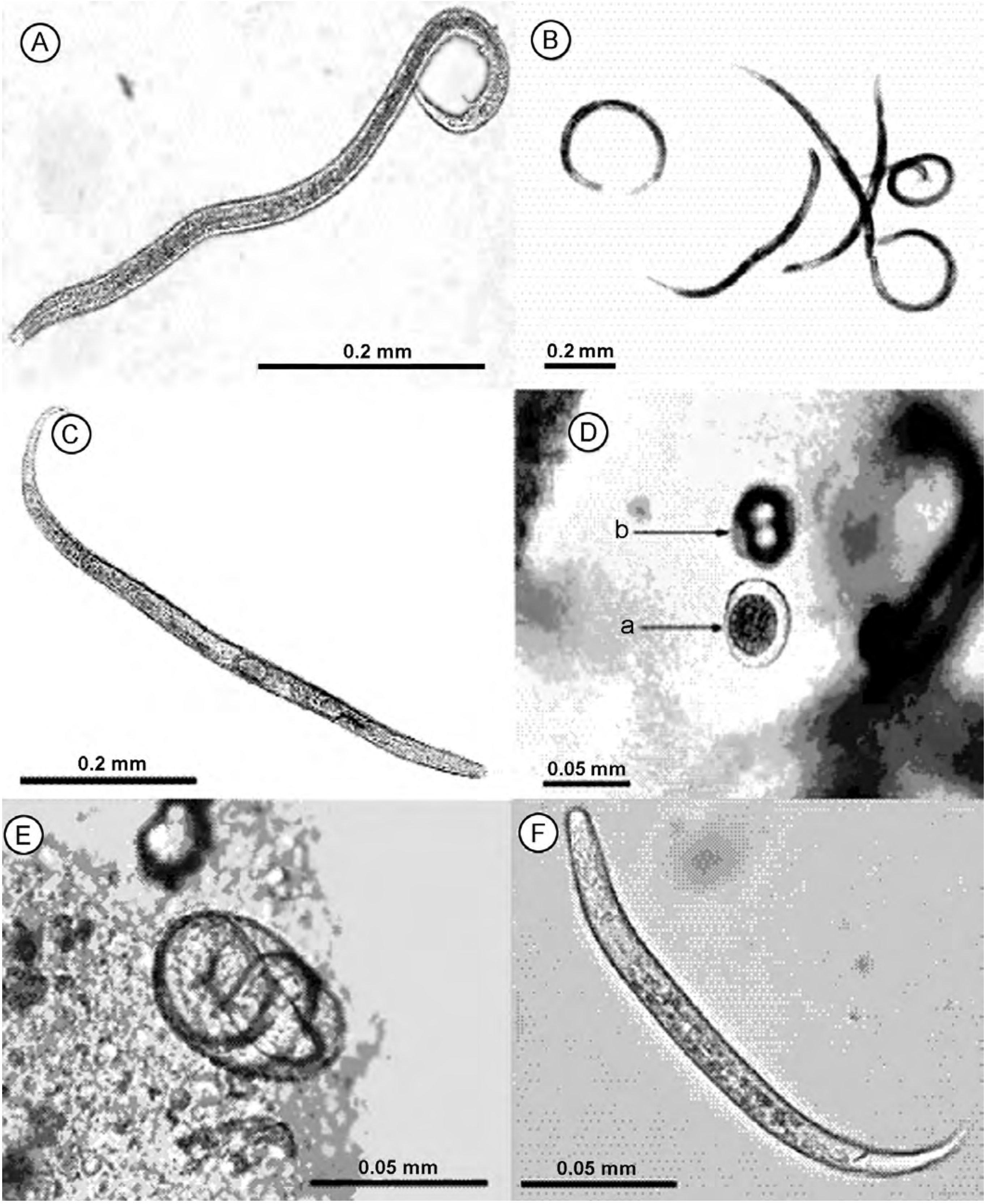

Fig. 1. *Innocuonema tentabunda*. A: adult male (length = 700 µm); B: adult male and female in the culture plate; C: gravid female; D: eggs in the culture plate (a: single-celled stage; b: egg at first cleavage stage); E: developing embryo; F: juvenile (length = 50 µm).

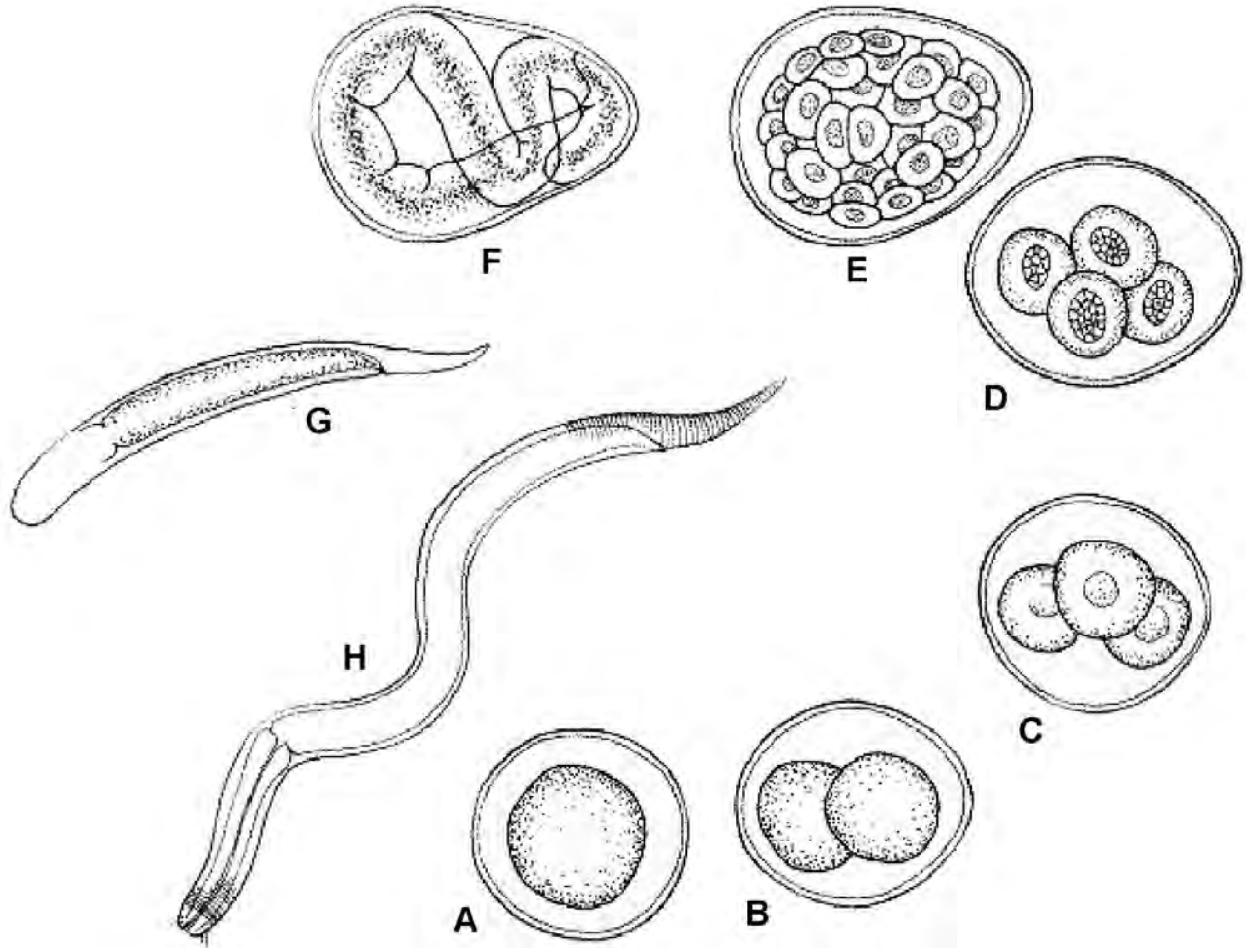

Fig. 2. Schematic life history of *I. tentabunda*. A: newly laid egg; B: egg at first cleavage stage; C: three-celled stage; D: four-celled stage; E: egg with mass of cells after repeated cell division; F: developing embryo; G: newly emerged juvenile; H: adult male.

hatched about 3 days after they had been deposited. Just before hatching, the average diameter of the egg was 150 µm (Table I). Juvenile *I. tentabunda* emerged as J1. At this stage, most of the body structures were similar to those of older stages, except that the reproductive system was lacking and the body had not yet reached full size. During development to adults, juvenile *I. tentabunda* underwent some progressive changes, especially of the posterior end and the buccal capsule (Fig. 1F). Development from J1 to J2 took 2 days and approximately the same time was taken for development of the following two stages (J3 and J4) on the way to the adult stage (Table II). The digestive system (gut) was observed in the J2 stage (Figs 2 A-H). In the J3 stage, the oesophageal bulb and the oesophagus were evident. Juveniles started feeding on the bacteria growing in the agar medium at stage J3. The sex was differentiated at the J3 stage as male spicules were visible, but the mouthparts, the cuticle and setae were not completely developed. The J4 stage finally moulted into the adult stage, when all body organs were well developed. The measurements of adults were: average body length 0.7-0.9 mm (Fig. 1A), oesophagus length 100 µm, tail length 75 µm in males and 100 µm in females with a tip bent to one side and curved dorsally, spicule length 20-22 µm, cephalic setae 6-7 µm long. Figure 3 shows the reproductive system of a female (Fig. 3A), an adult male (Fig. 3B) and a more mature female (Fig. 3C). All of the five

Table I. Mean diameter (± Standard Deviation) of five eggs of *Innocuonema tentabunda* at different ages.

Time of observation	Mean diameter (mm)	Shape/stage
Newly laid eggs	0.040±0.0158	Round
After 12 hrs	0.050±0.0122	Oval
After 24 hrs	0.050±0.01	Oval (dividing mass of cells)
After 48 hrs	0.055±0.0122	Oval with embryo
After 72 hrs	0.057±0.0217	Before hatching

Table II. Measurements (mm) (± Standard Deviation) of juvenile *I. tentabunda* of different ages. (Each figure is the mean of five specimens).

Age (days)	Mean length (mm)	Stages after hatching
1	0.15±0.018	J1
2	0.21±0.022	J1
3	0.279±0.0495	J2
4	0.303±0.0599	J2
5	0.335±0.0548	J3
6	0.375±0.0637	J3
7	0.415±0.0548	J4
8	0.455±0.0274	J4
9	0.505±0.0570	Adult

specimens studied had almost the same growth pattern.

As illustrated in Fig. 2, the life cycle (from egg-hatching to adult) of *I. tentabunda* is completed in about 20 days. Mean day-to-day growth measurements of five specimens are presented in Table II. In comparison, Hopper and Meyers (1966) and Thun (1968) observed life cycles of approximately one month for several chromadorid species maintained in a laboratory culture. Mutsumi *et al.* (1998) observed a 40 days life cycle for two chromadorids, *Prochromadorella* sp. and *Spiliphera* sp.

Compared to the 10-20 eggs laid by a female of *I. tentabunda*, specimens of *Chromadora macrolaimoides* Steiner are known to lay fewer eggs (10 to 12 eggs; Tietjen and Lee, 1973), while 14 to 19 eggs were reported for *Prochromadorella* sp. and *Spiliphera* sp. (Mutsumi *et al.*, 1998). Egg deposition in *I. tentabunda* began 48 hours after copulation and eggs hatched 3 days after they had been laid. Mutsumi *et al.* (1998) reported that eggs of *Prochromadorella* sp. hatched in 3.5 to 4 days after spawning and those of *Spiliphera* sp. in 5 to 6.5 days. The embryonic stage of *I. tentabunda* could be clearly observed in the eggs (Fig. 1D) and was similar to that reported for *Monhystera parelegantula* De Coninck (Vranken *et al.*, 1981). As *I. tentabunda* has a very short life cycle (about 20 days) it can develop several generations per year under optimal environmental conditions. Finally, our results indicate that cultures of *I. tentabunda* can be successfully reared in the laboratory and that they could be used for experimental studies of, for example, physiology, reproductive biology and ecotoxicology.

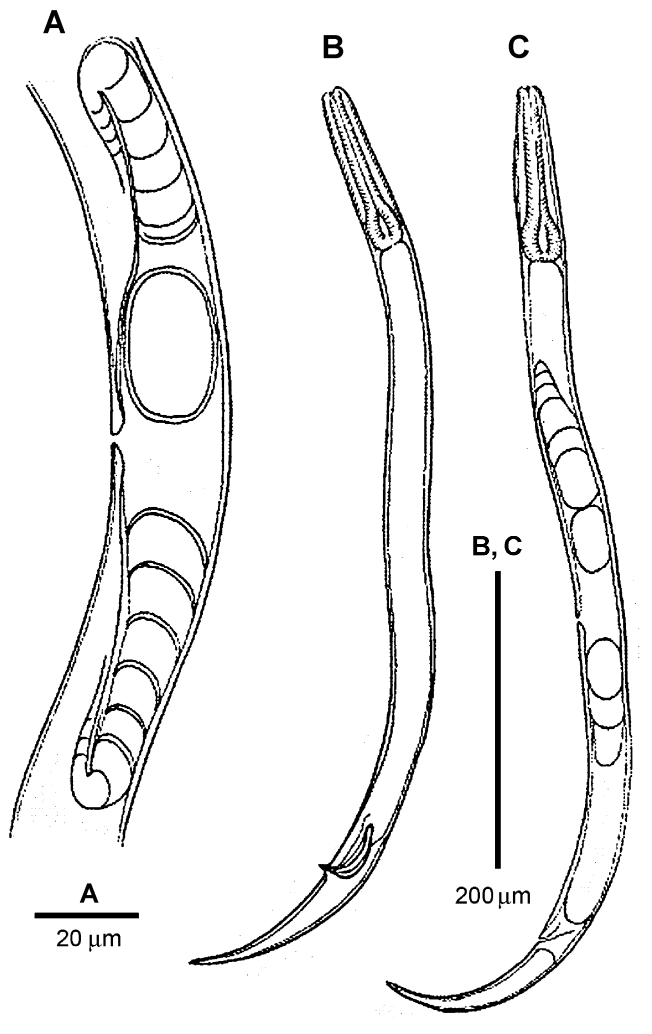

Fig. 3. *I. tentabunda.* A: female reproductive system; B: adult male; C: mature female.

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