TABLE I

Media and environment for obtaining complete plants in vitro from adventive embryos of red currant cv. F. Hosszúfürtu

Medium		Effect	
1.	Mil <sup>1</sup> er <sup>1</sup>	Induction of adventive embryos	
2.	Nitsch-Nitsch <sup>3</sup> + GA 1 ppm	Proliferation of embryos	
3.	Nitsch-Nitsch-IAA	Sporadic organ formation	
4.	Murashige-Skoog <sup>2</sup> -IAA-Kinetin Temperature dilatation for 2 weeks*	Abundant organ formation the sphape of leaves is not characteristic	
5.	Nitsch-Nitsch with 1 % sucrose	First characteristic leaves	
6.	Nitsch-Nitsch with 1 % sucrose	Appearance of first complete plants	
7.	Perlite-sand-peat in pot	After hardening plants are ready for planting in open air	

<sup>\*</sup> By day: Outdoor for 9 hours, 3-8° C with occasional sunshine (characteristic of the Middle-European climate in November).

Night: Climate chamber for 15 hours, 26-27°C with 500 lux.

obtained (Table I). Procedure outlined in the table took I year approximately.

Plants obtained from adventive embryos are growing in the field for the past 2 years. They have a diploid chromosome number (16) like the mother cultivar. Their first flowers and fruits are expected in 1979. In the vegetative characters they look identical.

As far as we know this is the first case when viable plants are obtained from adventive embryos of a temperate fruit species.

Horticultural Research Station, J. M. ZATYKO. Fertod, Hungary, November 24, 1978.

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# PROLACTIN\* INFLUENCED LIPOGENESIS IN MYSTUS VITTATUS (BLOCH)

Environmental and endocrine factors which modulate the metabolic activity appear to be mediated through the hypothalamo-hypophyseal system. In fishes despite the normal fluctuations in the body lipid content due to stage of reproductive cycle the endogenous level of circulating plasma prolactin appears to influence the prevailing lipid content. Also the

specific influence of exogenous prolactin on the total body lipid reserves is contingent upon the time of the photoperiod at which the hormone is administered and also the source from which the hormone, bovine or ovaine, is obtained. While the influence of prolactin has been studied in fishes in relation to the total body lipid reserves there is paucity of information on the specific influence of prolactin on liver lipogenesis. In the present sudy the influence of bovine prolactin on liver lipogenesis has been studied both by in vivo and in vitro methods in the freshwater fish Mystus vittatus (Bloch). The hepatosomatic index in prolactin and saline injected fishes is also calculated.

Female fishes weighing between 7 and 11 gms, with gonads in maturing stage, and acclimated to a photoperiod 12L/12D were used. For in vivo studies fishes were injected with  $25 \mu$  gms of prolactin (Bovine: NIH-P-B<sub>4</sub>) in saline base six hours after the onset of the light phase for 20 consecutive days. Saline injected controls and normal fishes without treatment were maintained along with the hormone treated fishes. At the end of the desired period of experimentation fishes were sacrificed and cut pieces of liver were taken from these fishes for the estimation of lipid content. For in vitro studies liver tissues were taken from acclimated fishes and the tissues were incubated separately in teleost saline fortified with I gm/l glucose, 25 mg l lactalbumen hydrolysate, 10 mg/l streptomycin sulphate IP, 5 mg/l mycostatin, 20 mg/l fortified procaine penicilin and 25 \mu gm of bovine prolactin (de Vlaming et al.)1. The gas phase in the culture flask was maintained at 95%

oxygen and 5% carbon dixoide. The lipid content in all the liver pieces was estimated separately following the biochemical procedure of Pande et al.2. The histoarchitecture of the liver tissues in the incubated and other samp'es was studied in Bouin fixed material stained with Heidenhain's haematoxylin and counter stained with aqueous cosin. For the localization of lipid material liver pieces fixed in cobalt-calciumformalin was used and sections were stained with Sudan Black<sup>3</sup>. To understand the changes in the weight of the liver during a reproductive cycle the hepato-somatic index in fishes with ovaries in different stages of maturation was calculated. Also changes in the hepato-somatic index in prolactin and saline injected controls was calculated on the 10th, 15th and 20th days after treatment.

## Influence of prolactin and saline on liver lipids

The lipid content of the liver in fishes treated with prolactin for 20 consecutive days registered a considerable increase  $(13.55 \pm 0.5)$  over fishes maintained as untreated controls  $(8.4 \pm 0.4)$ . In saline injected controls lipid content of the liver decreased gradually, the lowest value  $(5.0 \pm 0.32)$  was noticed on the 20th day of treatment.

#### Liver incubation studies

Samples of liver incubated with bovine prolactin alone showed a greater quantity of lipid  $(9.6 \pm 0.15)$  compared to controls incubated in saline medium without the hormone  $(7.3 \pm 0.19)$ . During the short period of incubation (4 hours) prolactin has promoted lipogenesis implicating the liver as the target organ in the physiological process of lipid synthesis.

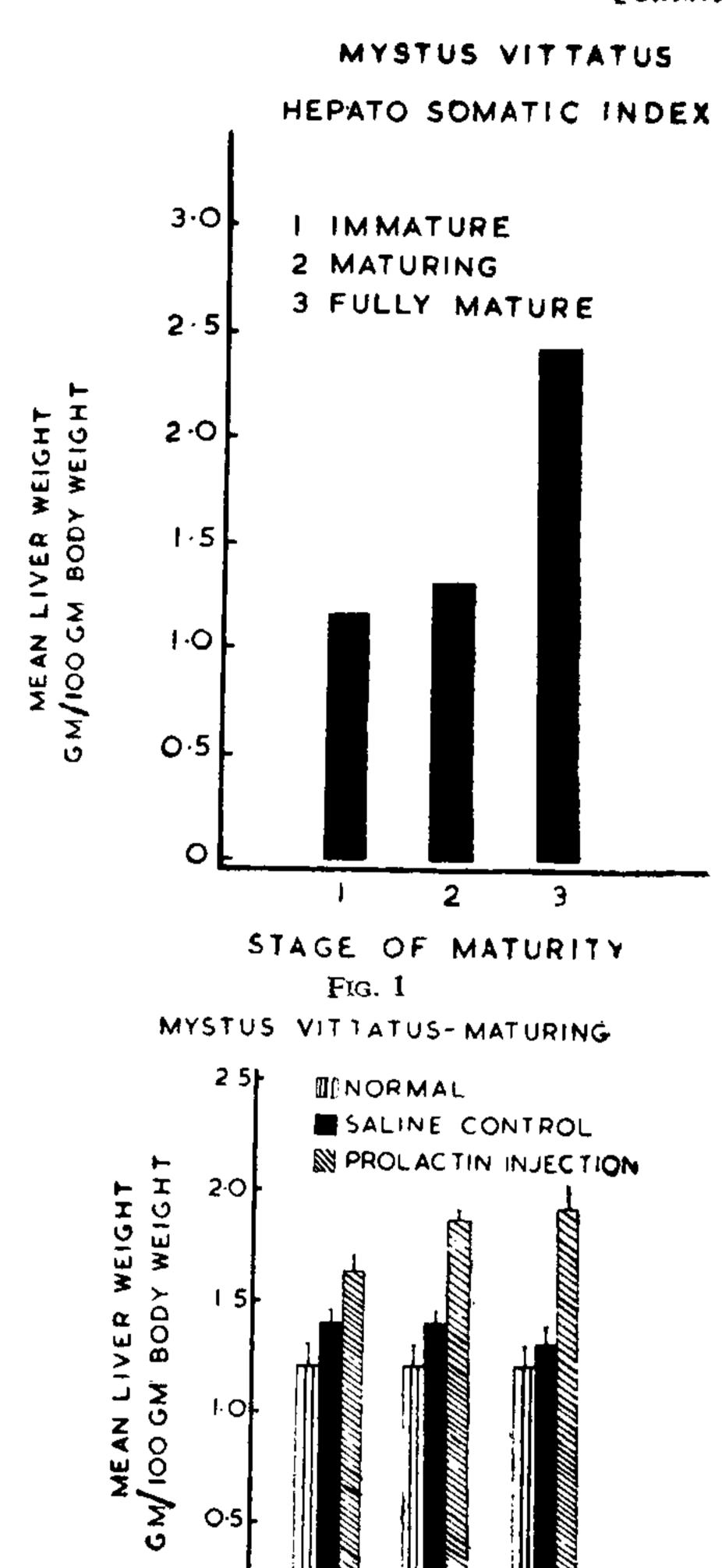
### Hepato-somatic index

A gradual but steady increase in the weight of the liver was noticed along with the growth and sexual maturation of the fish during a normal reproductive cycle (Fig. 1). In maturing fishes administration of bovine prolactin has elicited a gradual increase in the weight of the liver during the 20 days of hormone treatment; the increase in liver weight was significantly greater than normal and saline injected controls in fishes examined on the 20th day following prolactin treatment (Fig. 2).

### Histology and histochemistry (Figs. 3-6)

Histological preparations of incubated liver as well as hormone treated fishes showed no signs of distortions of the constituent cells. In such hormone treated liver tissues a considerable amount of sudanophilic granules were noticed when stained with Sudan black. In saline injected controls and samples incubated in saline medium no such sudanophilic material was noticed.

Treatment with bovine prolactin during early photoperiod has interfered with the prevailing lipid



FIGS. 1-2. Fig. 1. Changes in the hepato-somatic index in female Mystus vittatus during normal reproductive cycle. Fig. 2. Effects of bovine prolactin treatment on the hepatosomatic index in maturing female Mystus vittatus acclimated to 12L/12D photoperiod. Each fish received  $25 \mu$  gm of prolactin for 20 consecutive days.

Fig. 2

10

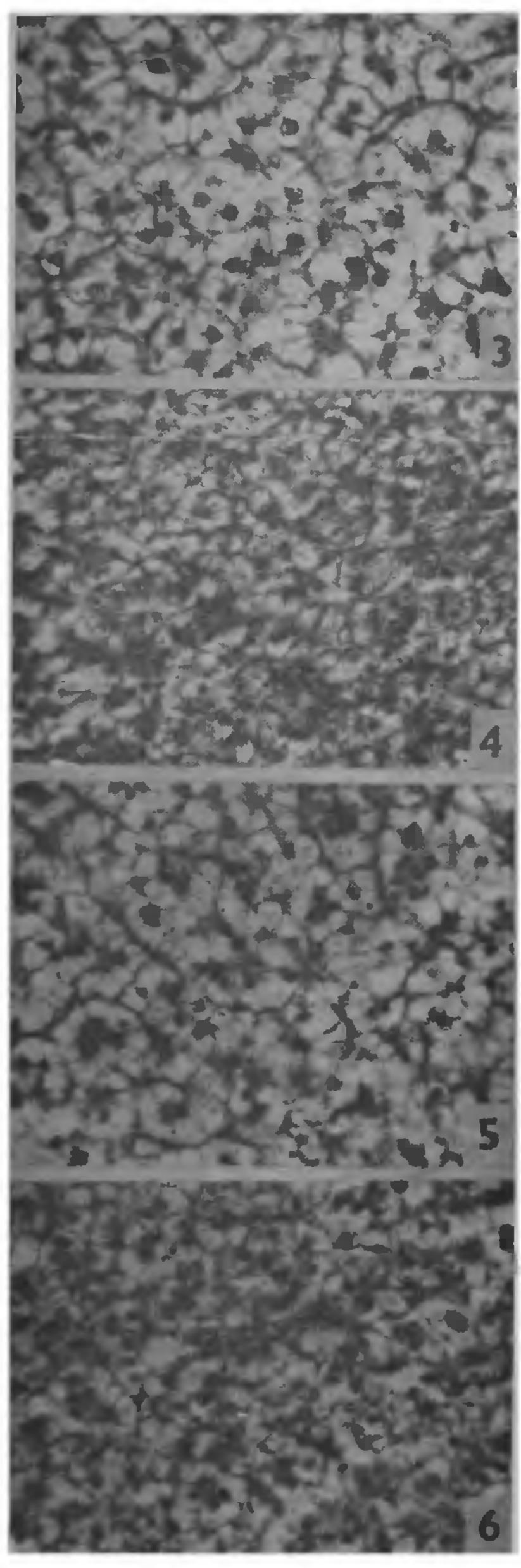
15

DAYS OF BOVINE

PROLACTIN INJECTION

50

0



Figs. 3-6. Fig. 3. Section of the liver of normal Mystus vittatus. Note the appearance of the cells and the distribution of sudanophilic material, x ca.

metabolism in Mystus vittatus. The increase in the lipid content of the liver is due to an enhanced lipogenesis brought about by the influence of the administered hormone since saline and normal untreated controls registered no such increase in the lipid content. The results obtained with in vitro studies are consistent with in vivo studies, for in both instances bovine prolactin has promoted lipogenesis in fish liver. de Vlaming et al1 have reported that while liver pieces of Ictalurus natalis incubated with bovine prolactin induced lipid accumulation in the tissue, liver pieces of Notemigonus crysolucas incubated with ovaine prolactin showed depletion of lipid from the tissues, The differential response noticed due to treatment with bovine and ovaine prolactin in different fishes subjected to the same photoperiod (16L/8D) is interesting.

Another aspect worth mentioning relates to the enhanced weight of the liver of maturing Mystus vittatus subjected to bovine prolactin treatment, which incidently has raised the hepato-somatic index over the untreated controls. The contributory factor for the increment in weight appears mainly due to an increase in the production and accumulation of lipid by the liver under the influence of the hormone administered. In most of the fishes investigated lipogenesis appears to be a seasonal phenomenon associated with the reproductive cycle of the fish and in particular, the percentage of fat found in the liver of female fishes is higher than in the males. An increase in the size of the liver is also noticed in Coregonus lavaretus, as evidenced by an increase in the value of the hepatosomatic index<sup>6</sup>. In the chicklid fish, Aequidens pulcher prolactin seems to influence ovarian steroids by accentuating the activity of  $3-\beta$ -hydroxysteroid dehydrogenase and promotes lipogenesis in the liver. The present study leaves little room to doubt the influence of bovine prolactin in promoting lipogenesis in the liver of Mystus vittatus, but whether such a system as reported for A. pulcher is operative in M. vittatus also merits consideration.

320. Fig. 4. Section of liver of Mystus vittatus subjected to 25  $\mu$  gm of prolactin for 20 consecutive days. Note the heavy accumulation of sudanophilic material,  $\times$  ca. 320. Fig. 5. Section of liver of Mystus vittatus incubated for 3 hours in teleost saline without prolactin in the medium. Note sparce distribution of sudanophilia,  $\times$  ca. 320. Fig. 6. Section of liver of Mystus vittatus incubated for three hours in teleost saline with 25  $\mu$  gm of prolactin. Note the Intense accumulation of sudanophilic material,  $\times$  ca. 320.

(Note: Tissues were fixed in Cobalt-calcium formal fixative and sections were stained with Sudan black B.)

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Department of Zoology, M. R. Parvathi.
Annamalai University, Vijayam Sriramulu.
Annamalainagar 608 101.
September 12 i 1978.

- \* Prolactin NIH-P-B4 Bovine supplied by National Health Institute, Bethesda, USA, has been used in the present study.
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## IOTONCHUS PRABHOOI SP. N. (MONONCHIDA-NEMATODA) A NEW PREDATORY NEMATODE FROM THE SOILS OF KERALA

In a survey of the predatory nematodes in the soils of Kerala State a form of *Iotonchus* which does not fit into any known species of the genus has been collected and is described here as a new species. A few other predatory nematodes collected in the survey has been reported earlier<sup>1,2</sup>.

Iotonchus prabhooi n.sp.

Paratypes, 14  $\stackrel{\frown}{}$   $\stackrel{}$ 

Holotype Q: L = 1.69 mm; a = 28; b = 3.8; c = 7.3; c' = 6.1; tail length = 0.23 mm, 13.6% of body length; V = 60; buccal cabity = 24 × 48  $\mu$ m. Ventrally arcuate on fixation. Maximum thickness of cuticle 4, 3 and 5  $\mu$ m at neck, midbody and tail region respectively. Lip region slightly expanded, 14  $\mu$ m high and 38  $\mu$ m wide. Amphid opening very close to lip region, 4  $\mu$ m wide and 6  $\mu$ m from anterior end (7-10  $\mu$ m in paratypes). Buccalcavity about twice as long as wide, wall about 2.5  $\mu$ m thick. Dorsal tooth small, basal, 10-12  $\mu$ m from base of stoma. Anterior surface of dorsal tooth concave

and the posterior surface convex. Oblique subventral wall of the stoma with two small teeth. Nerve ring 144  $\mu$ m from anterior end. Gonad didelphic; ovary reflexed; proximal portion of oviduct enlarged. Vagina short, less than half of vulval body-width. Cuticular pieces present at vulva. Tail arcuate, and conoid. Caudal glands three, its opening terminal.

Male: Not found.

Type habitat and locality: Soil around roots of rubber, Kottayam, collected on 31-12-1973.

Holotype and two paratype females deposited with National Collection of Nematodes, Zoological Survey of India, Calcutta and three paratype females deposited with National Nematodes Collection, Division of Nematology, I.A.R.I., New Delhi-12.

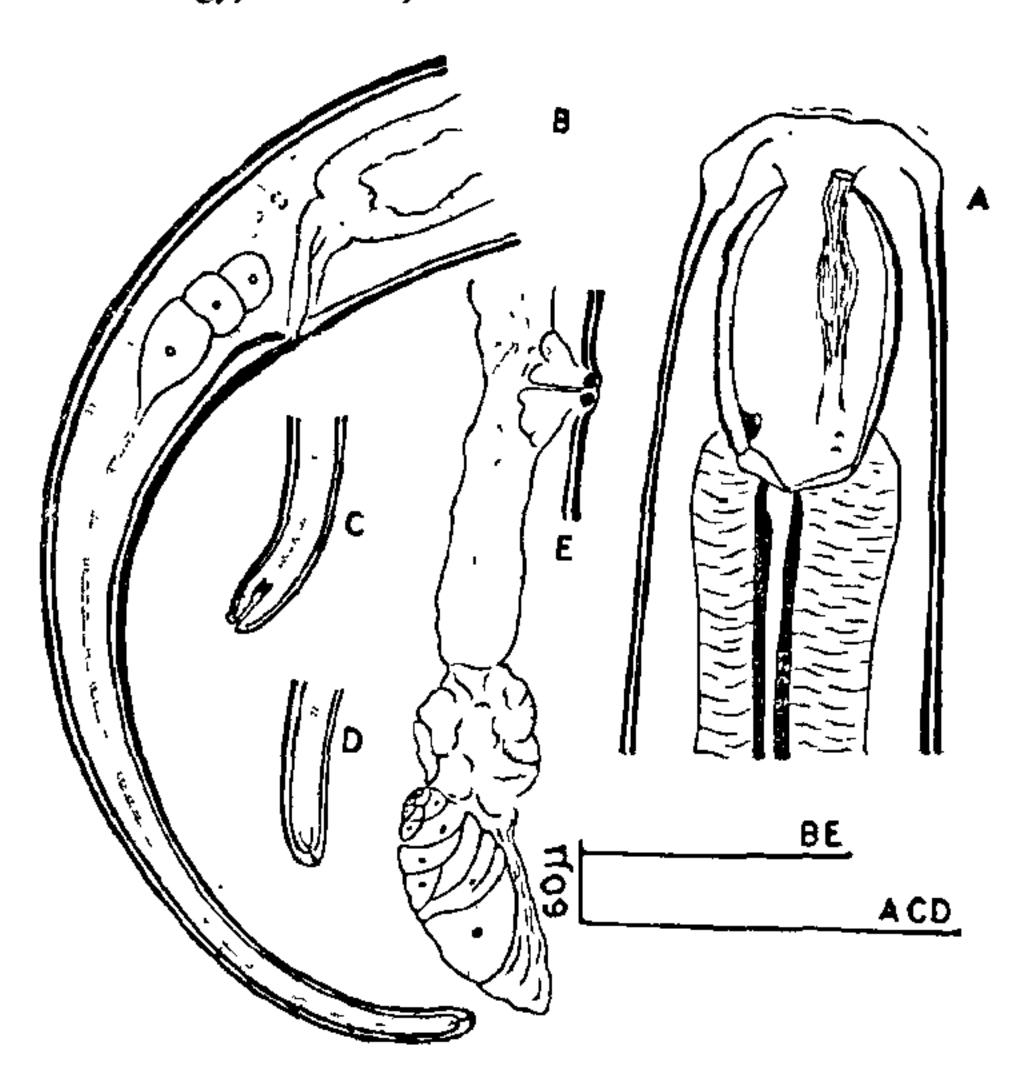


Fig. 1. Iotonchus prabhooi sp. n. female. A—anterior end; B—posterior end; C, D—tail tips; E—posterior gonad.

Differential diagnosis: This species resembles I. basidontus Clark<sup>3</sup>, but differs in the absence of males, smaller body and smaller tail. (L = 1·8-2·4 mm and tail length = 0·26-0·36 mm in I. basidontus). This species also differs in the presence of caudal glands and spinneret which are obscure in I. basidontus. It also resembles I. risoceiae (Carvalho<sup>4</sup>). Andrássy, 1958 but differs in the shape and size of buccal cavity, smaller body and absence of males.

The species is named after Dr. N. R. Prabhoo, my teacher, in appreciation of his guidance and encouragement. Thanks are due to late Prof. K. K. Nayar and