

to Prof. K. M. Alexander for facilities in the department.

Department of Zoology,
University of Kerala,
Kariavattom 695 581
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C. MOHANDAS.*

* Present address: Scientist S-1 (Nematology),
Central Rice Research Institute, Cuttack 753 006
(Orissa).

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ADAPTIVE CHANGES IN THE BLOOD BICARBONATE LEVELS OF THREE AIR-BREATHING FISHES FOLLOWING AERIAL EXPOSURE

Introduction

FISHES, which depend upon water-breathing only, have very low carbon dioxide and bicarbonate concentrations in the blood. In water, the high solubility of carbon dioxide helps its release to a very great extent. But in the case of air-breathing fishes, when exposed to aerial conditions during torrid seasons, the elimination of carbon dioxide becomes a problem particularly in those fishes which have thick covering of scales. A rise in carbon dioxide tension in the blood then, would, result in a fall in blood pH, unless there is an adaptive increase in buffering capacity of the blood. Reduction in oxygen uptake and accumulation of lactic acid in blood of fishes following aerial exposure, further necessitates the operation of an efficient buffering mechanism to minimize the change in blood pH and delay the onset of physiological disturbances.

An adaptive increase in buffering capacity with increased importance of air-breathing in fishes is reported and bicarbonates acting as buffering agents in blood are demonstrated for a number of air-breathing fishes¹⁻⁵.

In the present investigation, an attempt is made to evaluate the operation of the buffering system by estimating the blood bicarbonate levels of three species of air-breathing fishes, *Anabas scandens* (Cuvier), *Ophiocephalus gachua* (Hamilton-Buchanan) and *Mystus vittatus* (Bloch) under normal and exposed conditions.

Materials and Method

The Climbing perch, *Anabas scandens* (Order : Acanthopterygii; Family : Anabantidae) inhabits

tropical freshwater ponds (dissolved oxygen content, 1 to 8.5 ppm; pH, 7.6 to 8.0) with thick growth of vegetation, where the oxygen content will be greatly depleted, particularly during the nights in summer. *A. scandens* is reported to habitually leave the waters and migrate overland at times of danger from drought^{6,7}. When out of water, the fish utilizes atmospheric oxygen, making use of the accessory respiratory organs consisting of suprabranchial chambers and labyrinthine organs⁸.

The snake-headed fish, *Ophiocephalus gachua* (Order : Acanthopterygii; Family : Ophiocephalidae) is also a tropical fish living under habitat conditions similar to those of *A. scandens*. *O. gachua* is known to survive for prolonged periods out of water (about 18 hrs) and obtain oxygen from air using a pair of air chambers, dorsal to the branchial area and partly enclosed within the cavity of the skull⁹.

The catfish, *Mystus vittatus* (Order : Physostomi; Family : Bagridae) is a fast swimmer, occurring in rivers and nearby ponds fed by those rivers and is not known to leave waters. It has no well-defined accessory air-breathing organs, though it is known to survive out of water for short periods (5-8 hrs) by breathing atmospheric air¹⁰.

Samples of *A. scandens* (size range : 16-30 g), *O. gachua* (size range : 16-35 g) and *M. vittatus* (size range : 9-16 g), collected from local ponds, were maintained in separate cement cisterns at $28 \pm 1^\circ\text{C}$ and fed with boiled eggs and earthworms alternatively, every two days. Feeding was stopped one day before the fishes were used in the experiments.

Fishes were removed from the cement cisterns and the blood samples for estimation of bicarbonate levels were collected from ductus Cuvier as described by Hawk *et al.*¹¹. Microtitration method¹¹ was employed for the estimation of bicarbonate levels in the blood.

Bicarbonate levels of blood were estimated in *A. scandens* and *O. gachua* before and after exposure to aerial conditions for 5 and 10 hrs and in *M. vittatus* before and after exposure for only 5 hrs (since the fish survived out of water for only 5 to 8 hrs). Exposure of fishes was carried out by keeping individual fishes separately in empty glass jars (at $28 \pm 1^\circ\text{C}$ and 80% R.H.) covered by wire gauze for aeration.

Bicarbonate levels of blood were estimated and expressed in mM per litre of plasma (mM/l) and the changes observed in the blood of fishes under different experimental conditions were statistically tested for their significance, using Student's 't' test at 5% level. Mean values of bicarbonate levels, under each experimental condition, were obtained from 8 estimations for each species.

Results and Discussion

The results obtained for the bicarbonate levels in blood of the three species under different experimental conditions are shown in Fig. 1.

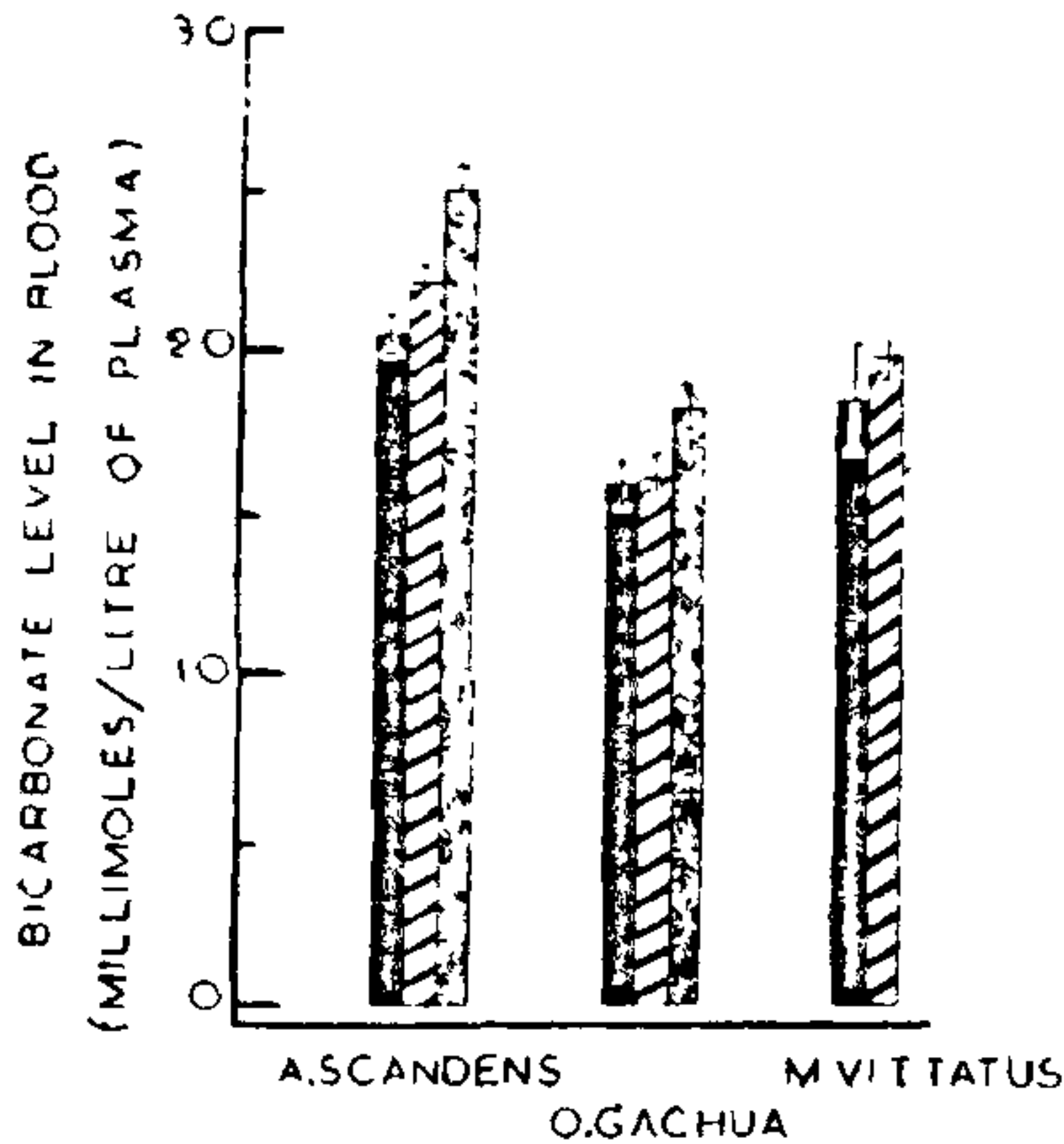


FIG. 1. Bicarbonate levels in the blood of normal fishes (closed bar) and fishes exposed for 5 hours (striped bar) and 10 hours (variegated bar).

The normal bicarbonate level in the blood of *A. scandens* was 20.64 ± 0.627 mM/l. It revealed no significant change in its blood bicarbonate level upon exposure for a period of 5 hrs, the estimated value being 22.23 ± 0.552 mM/l ($P > 0.05$). On the contrary, fishes exposed for 10 hrs revealed a significant rise from normal level to 25.13 ± 0.841 mM/l ($P < 0.01$).

O. gachua, with a normal blood bicarbonate level of 16.06 ± 0.686 mM/l, also showed no significant change in its blood bicarbonate level upon exposure for a period of 5 hrs (16.23 ± 0.616 mM/l; $P > 0.5$). Exposure of this species for 10 hrs, however, resulted in a significant rise in its blood bicarbonate level, the value reaching 18.40 ± 0.593 mM/l ($P = 0.02$).

The normal bicarbonate value of 18.64 ± 0.580 mM/l in the blood of *M. vittatus* also, did not show a statistically significant change upon exposure for 5 hrs, though a slightly higher value of 19.92 ± 1.550 mM/l was obtained following exposure ($P > 0.5$).

Hughes¹² suggested that during the evolution of the land forms, and particularly because of the increasing use of the lungs, it was necessary for the buffering mechanism of the blood to become adapted to an increased P_{CO_2} . Cameron and Randall¹³ have stated that a rise in P_{aCO_2} will result in a fall in blood pH unless there is a concomitant rise in the blood bicarbonate level and they suggested the possibility of bicarbo-

nate-chloride mechanism operating in maintaining the blood pH upon CO_2 accumulation in the blood.

In the present study, both *A. scandens* and *O. gachua*, when exposed to air for 5 hrs, revealed no significant change in their blood bicarbonate levels (Fig. 1). This is, perhaps, correlated with the higher rate of oxygen uptake and a decline in the lactic acid level in the blood of these fishes following exposure for 5 hrs¹⁴. The lack of significant rise in blood bicarbonate levels of *M. vittatus*, following 5 hrs of exposure, in spite of a drop in oxygen uptaks and an increase in the blood lactic acid level in 5 hrs exposed fishes¹⁴, is suggestive of a poor buffering potential of blood of this species. The possibility of diffusive elimination of gaseous carbon dioxide through the naked skin of the catfish, as a factor responsible for lack of an adaptive increase in the blood bicarbonate levels, may not be totally ruled out.

The increase in the bicarbonate levels of blood of *A. scandens* and *O. gachua*, exposed for a period of 10 hrs, may, however, be considered to adaptively increase the buffering potential of blood, thereby preventing or at least minimizing a sudden drop in blood pH due to accumulation of metabolites like lactic acid in higher quantities reported in the blood of these species exposed to air for 10 hrs¹⁴. Such an adaptive mechanism in blood could be of tremendous help to these air-breathing fishes for their prolonged aerial existence in the laboratory conditions and in their natural habitat conditions, particularly during torrid seasons.

In conclusion, it may be stated that the problem of carbon dioxide elimination and the consequent need for buffering mechanism upon exposure to aerial conditions is met by adaptive changes in the bicarbonate levels of blood in *A. scandens* and *O. gachua* enabling them to survive out of water for prolonged periods (18 hrs.) and also during their overland migration (particularly in the former species) in torrid seasons. However, a similar adaptive change is totally lacking in *M. vittatus* which is known to survive out of water only for short periods (5 to 8 hrs.) and also never known to habitually leave water.

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Department of Zoology, M. RAMASWAMY,
Govt. Arts College,
Coimbatore 641 018, India,

and
Department of Zoology, T. GOPALAKRISHNA REDDY,
Nagarjuna University,
Nagarjunanagar 522 510, India,
October 9, 1978.

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ON THE OCCURRENCE OF *SPINOSTRONGYLUS INDICUS* LOVEKAR, 1970
(NEMATODA: TRICHOSTRONGYLIDAE) IN TWO MICRO-BATS (NEW HOSTS) FROM NAGPUR AND A NOTE ON COPULATION

THIRTEEN male and fifteen female worms were recovered from the intestine of two micro-bats, viz., *Taphozous melanopogon* and *Rhinolophous luctus* at Nagpur. One specimen was obtained in the act of copulation.

Measurements

Male ($n = 5$): Length 5.5–7.0 mm and breadth 0.22–0.24 mm; head diameter 0.10–0.12 mm; oesophagus 0.42–0.48 mm long; nerve ring and excretory pore 0.22–0.24 mm and 0.22–0.27 mm from head end, respectively. Spicules equal, 0.37–0.39 mm long; gubernaculum pyriform, 0.10–0.13 mm in length. Bursa 0.15–0.18 mm long and 0.24–0.37 mm broad.

Female ($n = 7$): Length 7–13 mm and breadth 0.24–0.27 mm; head diameter 0.12–0.15 mm; oesophagus 0.34–0.48 mm long; nerve ring and excretory pore 0.24–0.25 mm and 0.24–0.28 mm from head end, respectively. Vulva 2.02–4.00 mm

from posterior end. Eggs 0.06–0.10 × 0.05–0.06 mm in size. Tail 0.12–0.15 mm long.

Copulation

In copulation the male is oriented at an angle to the body of female in the region where the vulva is located. The lateral lobes of the bursa fold over the body of female. The lateral bursal rays hold the female worm laterally while the dorsal and ventral rays rest ventrally. The forked tip of the dorsal ray assists in holding the female body. The spicules and genital cone are inserted in the vulva to widen its opening. The gubernaculum directs the spicules towards the anus and prevents their piercing the cloacal wall.

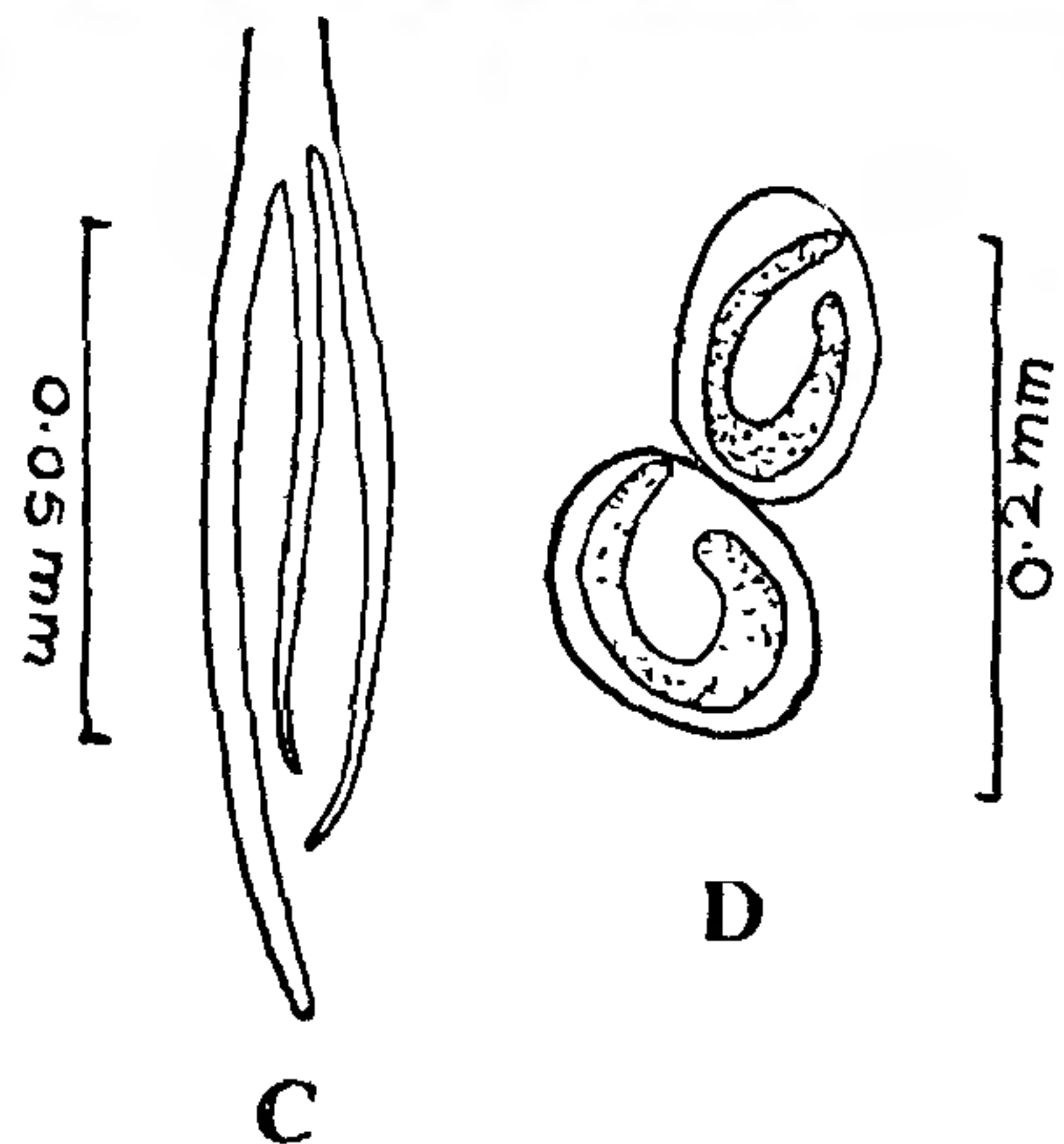


FIG. 1. *Spinostrongylus indicus* Lovekar 1970. A. Male: Microphotograph of anterior end of *Spinostrongylus indicus* Lovekar, 1970: Dorsal view × 100. B. Micro photograph of *Spinostrongylus indicus* Lovekar, 1970, in the act of copulation, × 100. C. Trifurcated tip of spicule. D. Eggs with larvae.

Remarks

Lovekar¹ described *Spinostrongylus indicus* from a micro-bat *Taphozous kucchensis* at Aurangabad, M.S.,