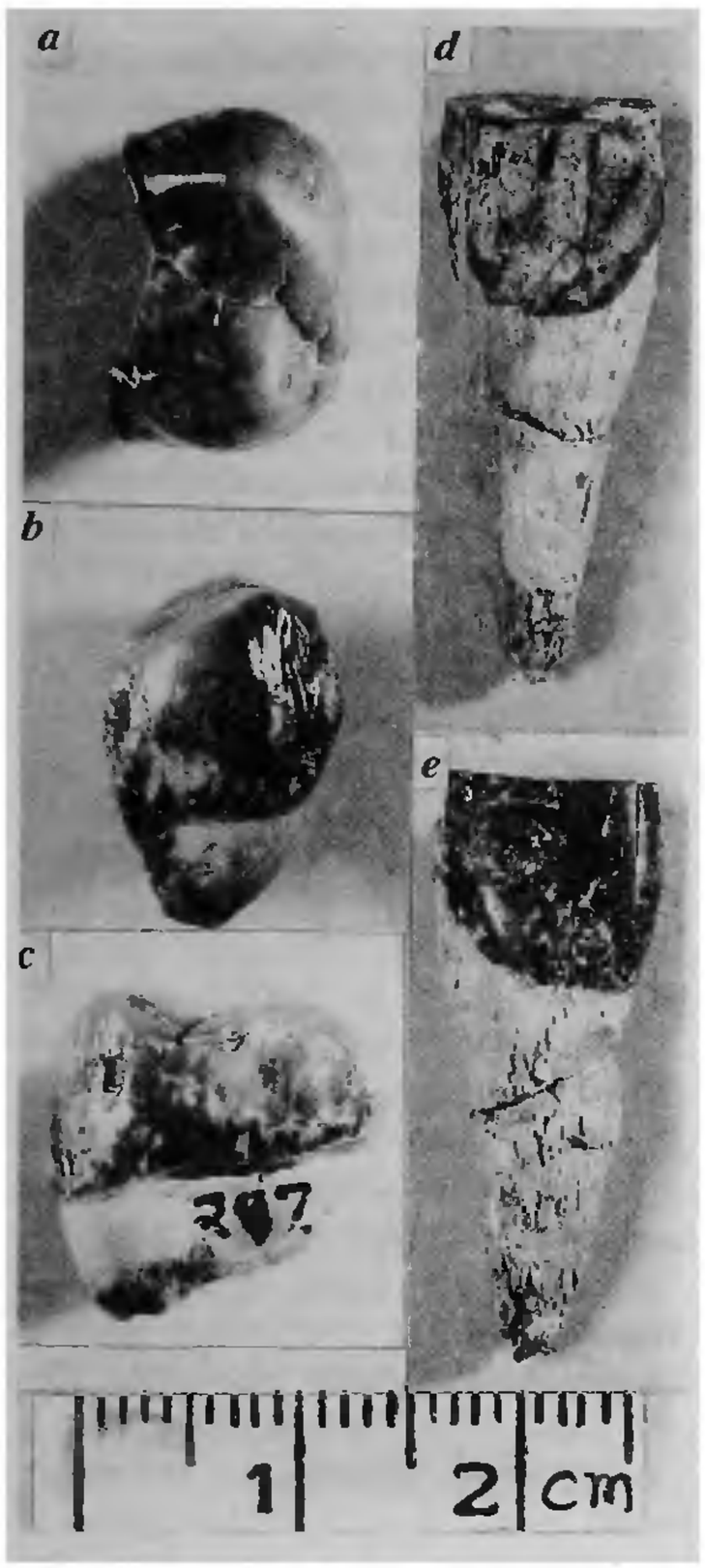


Figure 2. Hominid teeth from Ramchand *Sivapithecus* sp. (No. JVF/297), last lower left molar showing crown (*a*), lingual (*b*) and anterior (*c*) views. *Dryopithecus* sp. (No. JVF/518), upper incisor tooth showing internal (*d*) and external (*e*) views.

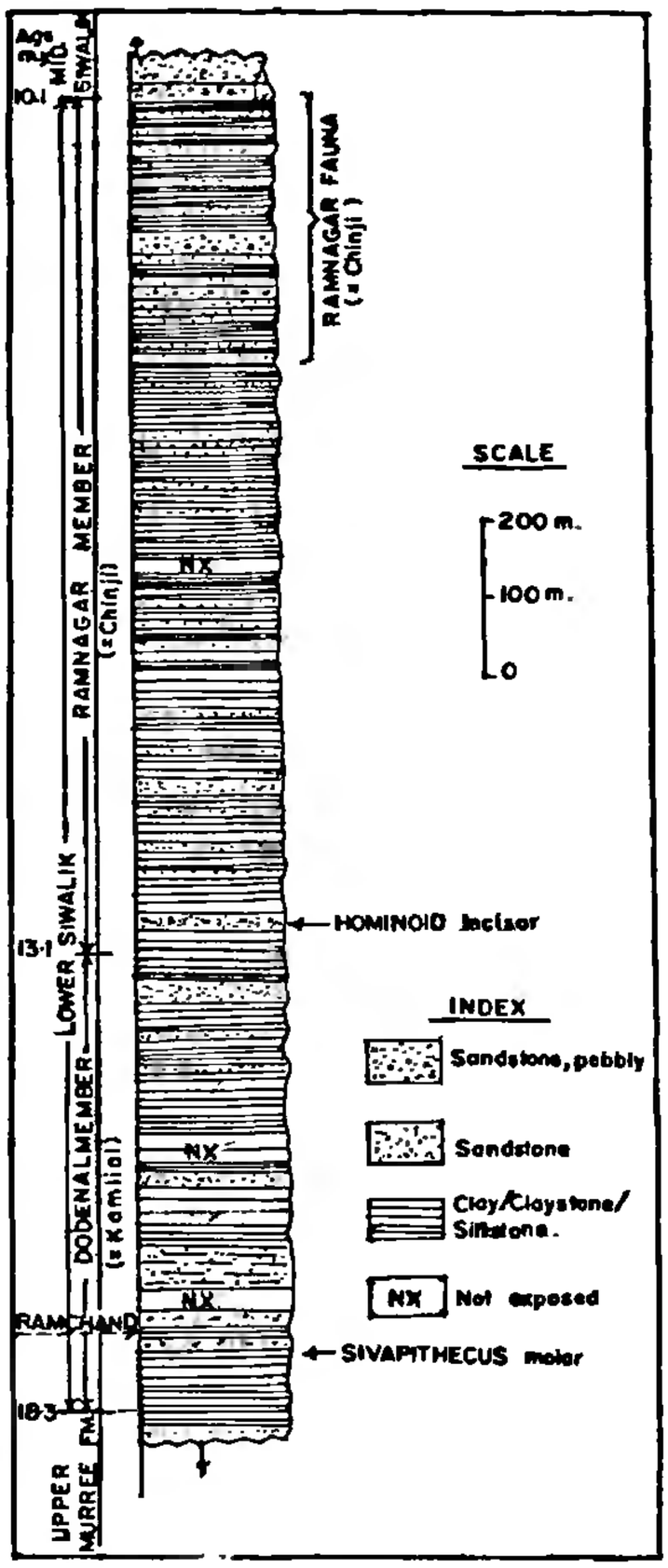


Figure 3. Lithocolumn along Ramchand–Ramnagar section showing stratigraphic position of the hominid teeth.

cates a Burdigalian affinity as well. In fact the approximate 18 m.y. datum for hominid existence in the Siwalik region is within expectations[9] and supports the earlier view of Andrews and Tobein[10] that the initial hominid diversification took place 'outside Africa and probably in central Asia'. The evolved character of the present *Sivapithecus* molar also indicates the pre-existence of its ancestral stock in Murree times as well and further suggests the Siwalik region to be the probable centre of hominid diversification and radiation. Further work, currently in progress in the area, is likely to substantiate the present find.

1. Johnson, N. M., Opdyke, N. D., Johnson, G. D., Lyndsay, E. H. and Tahirkheli, R. A. K., *Palaeogeogr. Palaeoclimatol. Palaeoecol.*, 1982, **37**, 17–42.
2. Colbert, E. H., *Trans. Am. Philos. Soc. Philadelphia*, 1935, **26**, 1–401.
3. Vasishat, R. N., Gaur, R. and Chopra, S. R. K., *Nature*, 1978, **275**, 736–737.
4. Verma, S. N. and Verma, B. C., Geol. Surv. India, unpublished report, Field Season 1968–69, 1–13.
5. Gupta, S. S. and Shali, A. K., *Geol. Surv. India Rec.*, 1989, **122**(8), 28–28b.
6. Lewis, G. E., *Am. J. Sci.*, 1937, **34**, 139–147.
7. Simons, E. L., *Primate Evolution*, The Macmillan Series in Physical Anthropology, New York, 1972, pp. 1–322.
8. Johnson, N. M., Stix, J., Tauxe, L., Cerveny, P. F. and Tahirkheli, R. A. K., *J. Geol.*, 1985, **93**, 27–40.
9. Delson, E., *Nature*, 1985, **318**, 107–108.
10. Andrews, P. and Tobein, H., *Nature*, 1977, **268**, 699–701.

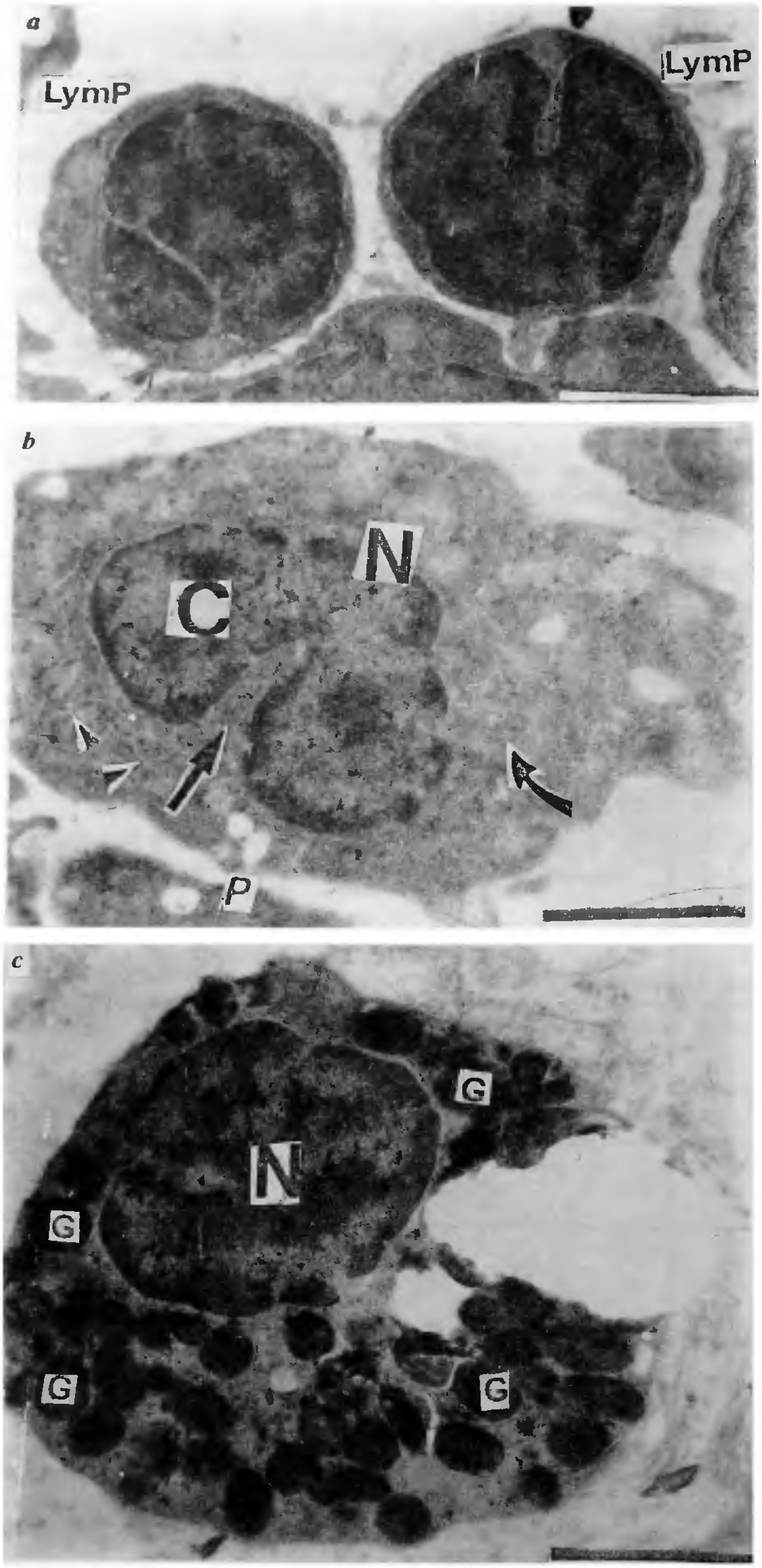
ACKNOWLEDGEMENTS. We thank Ravi Shanker, Dy. Director General, GSI, NR, Lucknow for his keen interest in the study and providing logistics and P. K. Basu, Director, GSI, Bhopal for critical discussions.

B. C. VERMA
S. S. GUPTA*

Geological Survey of India, Lucknow 226 020, India
**Geological Survey of India, Jammu 180 004, India*

Ultrastructural evaluation, an useful method for fish leucocyte identification

Clarias batrachus is an air-breathing fish inhabiting unhygienic hypoxic waters of the swamp. Because of its air-breathing habit and survival in hypoxic water it is used as one of the model species for aquaculture in swampy water. Routine differential blood count reflects the pathophysiological status of the fish in culture. Sometimes difficulty was encountered in differentiating various leucocyte types by light microscopy[1–3] and scanning electron microscopy[4,5]. Identification of leucocytes at ultrastructural level is more authentic[6] and therefore, forms the basis of the present report.



Live specimens of *C. batrachus* (62 g) were cold anaesthetized[7] and blood samples were obtained in heparinized micro-haematocrit tube from the caudal vein and centrifuged at 1000 *g* for 30 min to sediment the leucocytes. The plasma of the haematocrit tube was carefully removed and replaced by chilled (4°C) 1.5% phosphate buffered (pH 7.4) glutaraldehyde for preliminary fixation of the packed mass of the leucocytes. After 1 h, the fixative (1.5% phosphate-buffered glutaraldehyde) was removed and replaced by 1% phosphate-buffered (pH 7.4) osmium tetraoxide at 4°C for 2 h. After 2 h the buffy coat of the packed leucocyte mass was sufficiently hardened to be removed from the micro-haematocrit tube, dehydrated in ascending concentrations of ethanol and acetone, cleared in toluene and embedded in araldite. Ultrathin (60–90 nm) sections obtained through Reichert Jung Supernova Ultramicrotome were transferred to copper grids and stained in uranyl acetate and lead citrate and viewed under Philips EM-10 transmission electron microscope at SIC-EM facility, AIIMS, New Delhi.

A merz grid of known dimension was projected over the transmission electron micrographs to estimate the surface volume ratios of the various leucocytes.

Lymphocytes, thrombocytes and granulocytes were the three distinct types of leucocytes identified in the section. The lymphocytes are the smallest (cross sectional area – 4.79 μm^2) and the most abundant leucocytes discernible in the section (Figure 1 *a*). The large clefted nucleus (3.55 μm^2) occupies nearly 74% of the entire interior of the cell and is surrounded only by a thin (1.24 μm^2) rim of cytoplasm. The nucleus–cell (N/C) ratio was very high (0.74). The surface/volume ratios of each leucocyte and its nucleus were 0.865 and 1.2 respectively. The nuclear chromatin was abundantly distributed on the periphery and the central part of the nucleoplasm. These ultrastructural

Figure 1 *a–c*. ***a***, Transmission electron micrographs of the packed leucocyte showing lymphocytes (Lymp), bar = 1.33 µm. ***b***, TEM of the fusiform thrombocyte showing chromatic (C), nuclear pore (arrow) of the nucleus (N). Pinocytic vesicles (P), electron dense material-filled vacuoles (curved arrow) and microtubules (arrowheads) are also seen, bar = 1.33 µm. ***c***, TEM of granulocyte showing nucleus (N) and osmiophilic granules (G), bar = 1.33 µm.

details will be useful in identifying lymphocytes in the leucocyte sections of fish. The lymphocytes are important leucocytes with immunoglobulin (I_g) molecules on their surface membrane[8]. To the contrary of the high percentage of lymphocytes only a few thrombocytes were discernible in the leucocyte section of the air-breathing catfish. Each elongated fusiform thrombocyte was characterized by clefted nucleus (Figure 1 *b*) and its large size (18.07 μm^2). The nucleus (5.21 μm^2) occupies about 29% of the total cellular cross section with comparatively small (N/C) ratio (0.29). The surface/volume ratios of the cell and the nucleus were 0.50 and 0.93 respectively. Nuclear pores were clearly discernible in the nuclear membrane. The denser chromatin granules were mostly distributed on the periphery of the nucleus. However, centrally placed chromatin patches in the nucleoplasm were also seen. Presence of microtubules, clear vacuoles and vesicles full of osmiophilic granules were the other features of thrombocytes of the catfish in question. Each vesicle contains electron dense material similar to that of cytoplasm. Such vesicles loaded with secretory granules indicate their secretory nature. The granulocytes are larger (19 μm^2) with eccentrically placed nucleus (4.78 μm^2) and large number of osmiophilic granules of varied dimensions (0.46 ± 0.12 μm^2). The N/C ratio was comparatively smaller (0.25). Denser nuclear chromatin was more patchy in distribution. The cytoplasm was packed with numerous large, round to elongated osmiophilic granules (Figure 1 *c*). The surface/volume ratios of the cell and the nucleus were 0.42 and 0.88 respectively.

1. Srivastava, A., *Anat. Anz.*, 1968, **123**, 520–533.
2. Mishra, N., Ojha, J. and Munshi, J. S. D., *Arch. Hydrobiol.*, 1984, **100**, 267–272.
3. Mishra, N., Towheed, M. A., Das, A. K., Singh, B. N. and Ojha, J., *Proc. Indian Natn. Sci. Acad.*, 1992, **B58**, 341–346.
4. Ahmad, M., Ghosh, T. K., Moitra, A. and Munshi, J. S. D., *Cytologia*, 1990, 15–19.
5. Ojha, J., Singh, Bishankha and Mishra, N., *Indian J. Exp. Biol.*, 1991, **29**, 492–493.
6. Savage, A. G., *J. Morphol.*, 1983, **178**, 187–206.
7. Mittal, A. K. and Whitear, M., *J. Fish. Biol.*, 1992, **13**, 519–520.
8. Ellis, A. E., *J. Fish. Biol.*, 1977, **11**, 453–491.

ACKNOWLEDGEMENTS. We thank University Grants Commission, New Delhi for financial aśsistance and SIC-EM Facility, AIIMS, New Delhi, for TEM facilities.

JAGDISH OJHA
NEERA PANDEY

P. G. Department of Zoology,
T. M. Bhagalpur University,
Bhagalpur 812 007, India

FORM IV

Particulars of *Current Science*—as per Form IV under the Rule 8 of the Registration of Newspapers (Central) 1956.

1. Place of Publication: Bangalore
2. Periodicity of Publication: Fortnightly
3. Printers' Name and Address:
   P. Balaram and S. Ramaseshan
   Current Science Association, Bangalore 560 080
4. Publishers' Name, Nationality and Address:
   P. Balaram and S. Ramaseshan,
   Indian,
   Current Science Association, Bangalore 560 080
5. Editors' Name, Nationality and Address:
   P. Balaram and S. Ramaseshan,
   Indian,
   Current Science Association, Bangalore 560 080
6. Name and Address of the owner:
   Current Science Association,
   Bangalore 560 080

We, P. Balaram and S. Ramaseshan, hereby declare that the particulars given above are true to the best of our knowledge.

Bangalore
1 March 1997

(Sd/-)
P. Balaram and S. Ramaseshan
Publishers, *Current Science*