Mem. Inst. Oswaldo Cruz, Rio de Janeiro,
Vol. *88* (2): 335-337, apr./jun. 1993

Research Note

A COMPARISON OF LIGHT MICROSCOPIC AND SCANNING ELECTRON MICROSCOPIC STUDY ON THE CHORIONIC SCULPTURING OF SANDFLY (PHLEBOTOMUS ARGENTIPES) EGGS

K. N. GHOSH & A. BHATTACHARYA+

Immunoparasitology Research Unit, Department of Zoology, University of Calcutta, 35 Ballygunge Circular Road, Calcutta 700 019, India

Phlebotomine sandflies usually lay eggs after a blood meal except a few who are autogenous in nature i.e., follicular development is independent of blood meal (R. Killick-Kendrick, 1978, *Acta Trop., 35:* 297-313). The outer surface of the sandfly eggs show a different sculpturing pattern which has been found to be species specific. Studying sandfly eggs has been initiated by F. M. Howlett (1906, *Trans. Bombay Med. Congr., 3:* 239-242), who examined the eggs of *Phlebotomus argentipes* Annandale and Brunetti. P. P. Perfil'ev (1966, *Fauna of USSR. Diptera, Vol. III.* No. 2 *Phlebotomidae (Sandflies),* USSR Academy of Sciences, Moscow) has suggested that the difference in chorionic sculpturing of the eggs of *Sergentomyia* and *Phlebotomus* species are the supporting characters to the generic separation which has been based on other characters. Moreover, insect egg sculpturing as well as being a taxonomic character also reflects functional adaptation (R. D. Ward & P. D. Ready, 1975, *J. Ent. (A), 50:* 127-134). M. P. Barretto (1941, *Ann. Fac. Med. Univ. Sao Paulo, 17:* 357-427) used light microscope for the study on the New World sandfly eggs and found it very difficult to separate the eggs of closely related species. Later, few workers (R. D. Ward & P. D. Ready, 1975, loc. cit.; J. H. Zimmerman et al., 1977, *J. Med. Entomol., 13:* 574-579) have studied the chorionic sculpturing of different sandfly species with the help of Scanning Electron Microscope (SEM) and succeeded in identifying the various characteristic patterns. However, no attempt has been made to find out the chorionic sculpturing of the sandfly eggs with the help of light microscopic study at the follicular stage and it is also not known whether the sculpturing pattern can be seen before the eggs are laid. We have found that the light microscopic study may be helpful in identifying the different sculpturing patterns of sandfly eggs before oviposition and the SEM photograph of the same has been taken and compared.

Bloodfed females were taken from the laboratory colony of *P. argentipes* Annandale and Brunetti, maintained for the study on different aspects of their biology (K. N. Ghosh & A. Bhattacharya, 1989, *Insect Sci. Applic., 10:* 551-555). The females were kept in adult holding cage which was made after G. B. Modi & R. B. Tesh (1983, *J. Med. Entomol., 20:* 568-569) and the flies were provided with 30% glucose solution soaked in cotton. The adult holding cage with the flies was kept at 28°-29° C of temperature. After 70-80 hr of feeding, the flies were dissected in phosphate buffered saline (PBS – pH 7.2) to get the advanced stage of the ovarian follicles. The immature eggs were then placed on microscopic glass slides and allowed to dry. When the slides reached a semidry to dry condition, they were fixed in methanol, stained with Giemsa stain and washed with PBS (pH – 7.2). After washing the follicles were observed using transmission light microscopy and photographs were taken. For the SEM study, the mature eggs were taken after 24 hr of oviposition. The eggs were put into a clean glass vial with the help of a fine brush and were fixed in 3% glutaraldehyde in cold. The glutaraldehyde was removed by successive washing in PBS (pH – 7.2) and the eggs were passed through increasing grades of ethanol. Then absolute ethanol was replaced by amyl acetate and finally the specimens were dried in a critical point drying apparatus (Polaron E3000). Eggs were mounted on metal stubs with double sided adhesive tape

This work received financial assistance from the Council of Scientific and Industrial Research, India [9/28 (207) / 88-EMRI].

+Corresponding author.

Received 15 September 1992.
Accepted 15 February 1993.

Fig. 1: light microscopic observation on A: chorionic sculpturing of *Phlebotomus argentipes* eggs (Giemsa stained) 40X. B: the same 100X. r: ridges; i: interconnecting ridges; h: hexagonal areas; g: individual granules.

and coated with Au-Pd of 200-300 A° thickness in a vacuum evaporator (Edwards S150 sputter coater) and examined and photographed in a Philips SEM (PSEM 500) at varying beam of current, voltages and tilt angles.

It was found that the surface of advanced stage follicles shows characteristics pattern (Figs 1A, B) in light microscopic observation which simulates the outer chorionic sculpturing of the mature eggs as observed under SEM (Figs 2A, B). The surface had longitudinal discontinuous parallel ridges with occasional interconnecting oblique transverse short ridges that in some cases formed hexagon. Where the parallel ridges and transverse ridges meet, the ridges take the shape of 'Y', 'YY' or 'Σ' (Figs 1, 2). Each ridge consist of many small elevations which are serially arranged. These small elevations stained darkly in Giemsa at the late follicular stage. Actually, the small elevations or granules which ultimately form the ridges after arranging themselves in a particular fashion, are more clear and distinct when viewed by light microscopy.

The present study shows that it is possible to see the chorionic sculpturing pattern of sandfly eggs with the help of light microscopic study and it will help to separate the eggs of closely related species. This is in contrary to earlier observation of M. P. Barretto (1941, *loc. cit.*) who was not able to differentiate the eggs of closely related species with the help of light microscope. Probably the earlier workers who observed the chorionic sculpturing pattern of sandfly eggs with the help of light microscopy, used mature eggs as the specimens. It appears from the present study that the use of light microscope may be an alternative method to the SEM for examining the surface structures of sandfly eggs. This new method would help to study the chorionic sculpturing of more species which in turn would

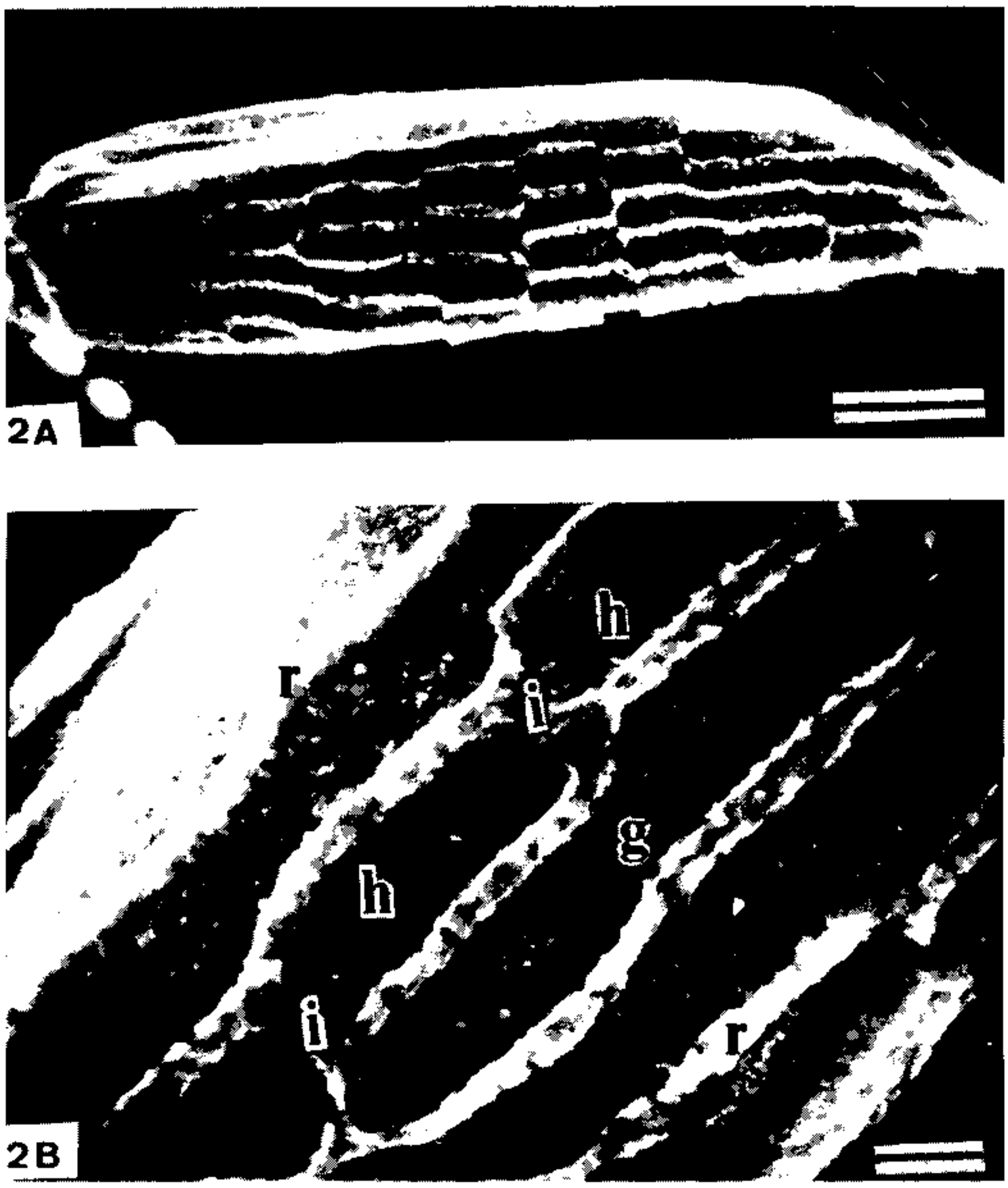

Fig. 2: scanning electron microscopic observation on A: chorionic sculpturing of *Phlebotomus argentipes* eggs (Bar = 50 μ). B: the same at higher magnification (Bar = 10 μ). r = ridges; i: interconnecting ridges; h: hexagonal areas; g: individual granules.

help to assess further the value of this character for identification. The findings that surface structure are present on immature eggs could help in identifying a sandfly when a gravid fly dies before oviposition, if the particular sculpturing pattern of the sandfly species in question is known. Light microscopy is readily available, economic, simple and the process is less time consuming than SEM. The present study shows that the sculpturing pattern on the outer surfaces of the eggs starts appearing at the late follicular stage i.e., before mature eggs are laid. It will be interesting to see the chemical nature of the granules along the ridges using different stains or dyes.

Acknowledgements: to the staff of the Scanning Electron Microscope Unit, Regional Sophisticated Instrumentation Centre (RSIC), Bose Institute, Calcuta. To the Distributed Information Centre (DIC0, Bose Institute, Calcuta for providing computer facility in typing the manuscript. To the technical assistance of J. Mukherjee in SEM studies.