



Comparative 3D structural ornamentations on the eggs of *Aedes aegypti* (Linn.) and *Aedes albopictus* (Skuse) of Burdwan, West Bengal, India

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ABSTRACT: *Aedes aegypti* and *Aedes albopictus* are potential arboviral vectors that are responsible for spread of dengue worldwide. Studies of these vectors and their bionomics form an important part in the vector controlling strategy. In the present piece of work, efforts have been made to differentiate between the eggs of these two species morphologically through scanning electron microscope. From the scanning electron micrographs of both of the species morphological differences were very clear. The eggs of *Aedes albopictus* were found to be much smaller in structure than that of *Aedes aegypti*. Moreover the micropylar apparatus, extrachorionic structure were also significantly different. Various species can be differentiated by viewing the scanning electron micrographs of the eggs. Stereomicroscopic structures are essentially useful in determining the difference between the species. The various differences in egg structure might be due to the environmental parameters they are laid at.

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KEYWORDS: *Aedes albopictus*, *Aedes aegypti*, Micropyle, Scanning electron microscopy, Tubercle

INTRODUCTION

The global resurgence and prevalence of vector borne disease such as dengue has generated an awakening for awareness. The vectors responsible for this arbovirus are *Aedes aegypti* (Linn.) and *Aedes albopictus* (Scuse) which have attracted multiple research fields and prompted scientists and researchers to have a wider look in these mosquito species. *Ae. albopictus* is an adaptive and invasive species co-existing with or displacing *Ae. aegypti* in different regions (Paupy et al.,2009). Studies related to these species include vector competence (Boromisa et

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al., 1987; Diallo et al., 2008; Moore et al., 2007), insecticide resistance (Hidayati et al., 2005; Stasiak, 1969; Wesson, 1990) spatial, temporal and geographical analyses (Benedict et al., 2007; Castro Gomes et al., 2005; Francy et al., 1990), and ecological and evolutionary studies (Juliano et al., 2002; Pumpuni et al., 1992). Scanning electron microscopic studies are one of the most important studies related to the characterization of these species. Scanning electron microscopy (SEM) evaluation differs from transmission electron microscopy (TEM) in that the whole specimen can be viewed. In an effort to contribute to the knowledge about *Aedes* sp, it is necessary to highlight the egg morphology too. SEM reveals the 3D ultrastructural details of the egg which cannot be achieved by the traditional light microscope. Though there are a number of studies regarding the egg of *Aedes aegypti* (Sasa et al., 1971; Matsuo et al; Moriya et al., 1973) only scanty literature is available on the comparative anatomical analysis of the eggs of *Aedes aegypti* and *Aedes albopictus* prevalent in West Bengal. The present piece of work deals with the comparative 3D surface topography of the eggs of *Aedes aegypti* and *Aedes albopictus* from Burdwan, West Bengal.

MATERIALS AND METHODS

Collection of eggs: *Aedes albopictus* and *Aedes aegypti* mosquitoes has been hatched, reared, maintained and cultured for several generations in the mosquito insectary of the Parasitology and Microbiology Research laboratory, Zoology Department, The University of Burdwan. All the mosquitoes were maintained in $25\pm 2^{\circ}\text{C}$, $75\pm 5\%$ relative humidity and 12:12 h (light:dark) photoperiod in the insectary (Deng et al., 2012) where the cages measured 30 cm x 30 cm x 30 cm. 10% sucrose solution soaked in cotton pad was given prior to blood feeding. The eggs were laid on a moist filter paper and allowed to incubate in this moisture. Few eggs prior to incubation were collected for Scanning Electron Microscopy evaluation.

Scanning electron microscopy: Eggs were fixed in 2.5% glutaraldehyde (HIMEDIA) in phosphate buffer (PBS) at pH 7.4 at 4°C for 45 mins and thereafter washed in PBS giving two changes of 10 mins each, followed by post fixation in osmium tetroxide (HIMEDIA) for 1 hr at room temperature (Choochote et al., 2001). The eggs were then dehydrated by passing through an ascending series of ethanol (MERCK); 50%, 70%, 90% and 100% (10 mins each). Eggs were then immersed for 5-7 mins in 1:1 ratio of absolute alcohol and isoamyl acetate (HIMEDIA) and then in pure isoamyl acetate (HIMEDIA) for 5-7 mins again and dried by the critical point drier (HCP-2, Tokyo, Japan), mounted on stubs by just placing them directly on stubs and gold coated in an ion sputter (IB -2 Ion Coater, EICO Engineering, Japan) and viewed by the Hitachi S-150 scanning microscope and micrographs were taken.

RESULTS AND DISCUSSION

Comparisons of the two species' eggs depict evident distinctions between them. The terminology followed here is of Harbach and Knight (1980). Out of the various attributes like egg dimensions, micropylar apparatus, tubercle type, chorionic structure etc studied, these species' eggs were found to be only 48.48% different from each other (Suman et al., 2011). Eggs of *Aedes albopictus* were found to be much smaller in structure than that of *Aedes*



Figure 1: Scanning electron micrograph of *Aedes aegypti* (a) Entire egg length

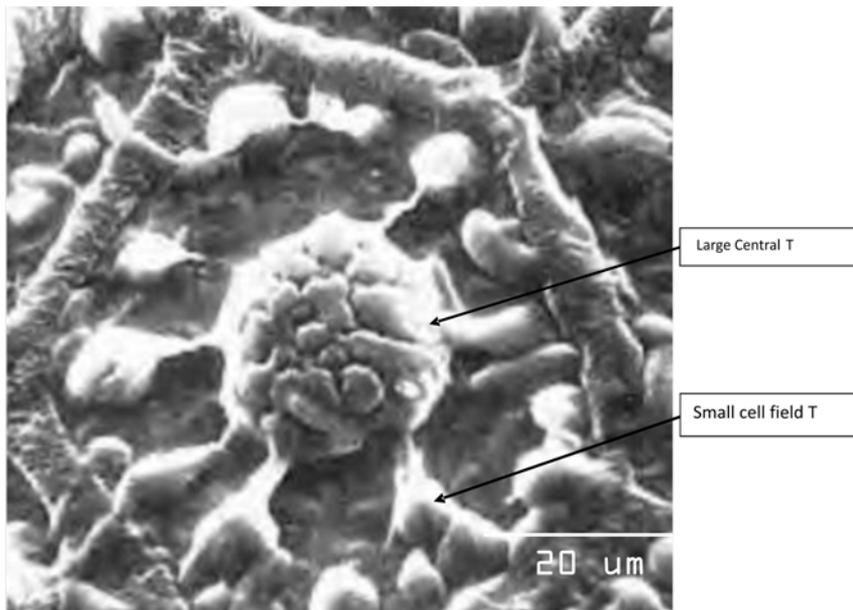


Figure 1: Scanning electron micrograph of *Aedes aegypti* (b) a single reticulum showing the central tubercle. T= Tubercle.

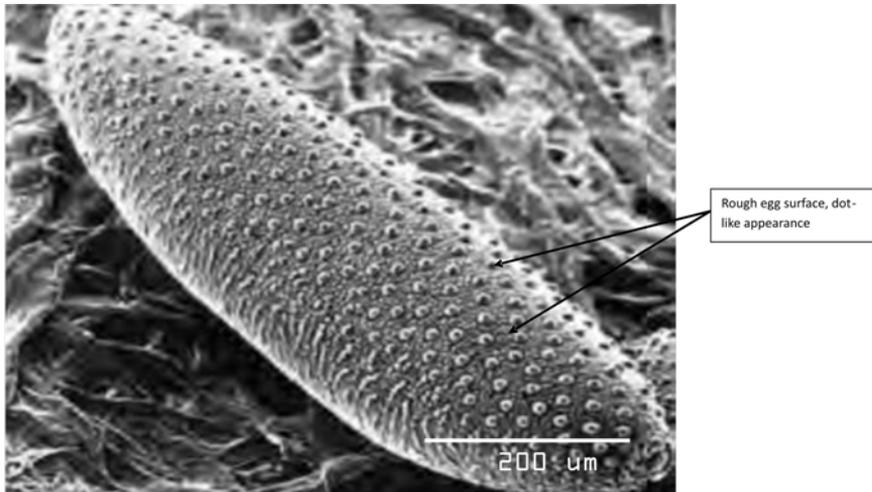


Figure2: Scanning electron micrograph of *Aedes albopictus* (a) An entire egg length.

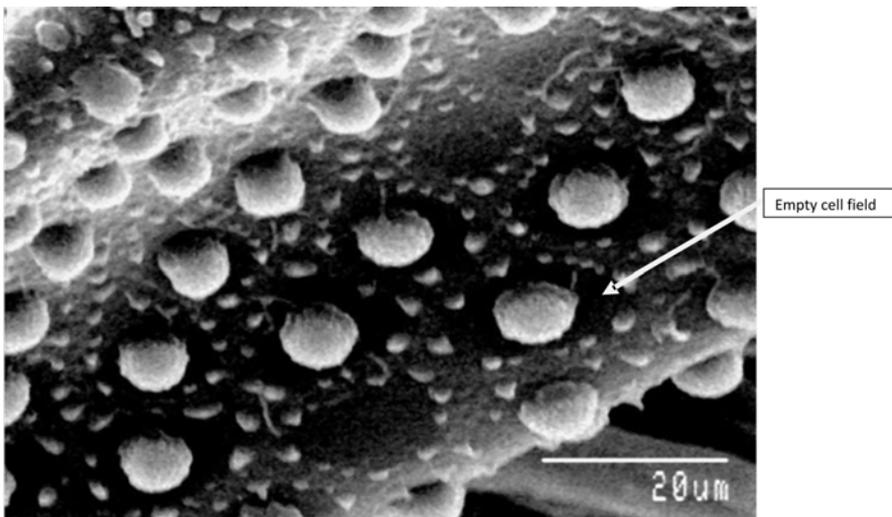


Figure2: Scanning electron micrograph of *Aedes albopictus* (b)dorsal view showing empty cell field.

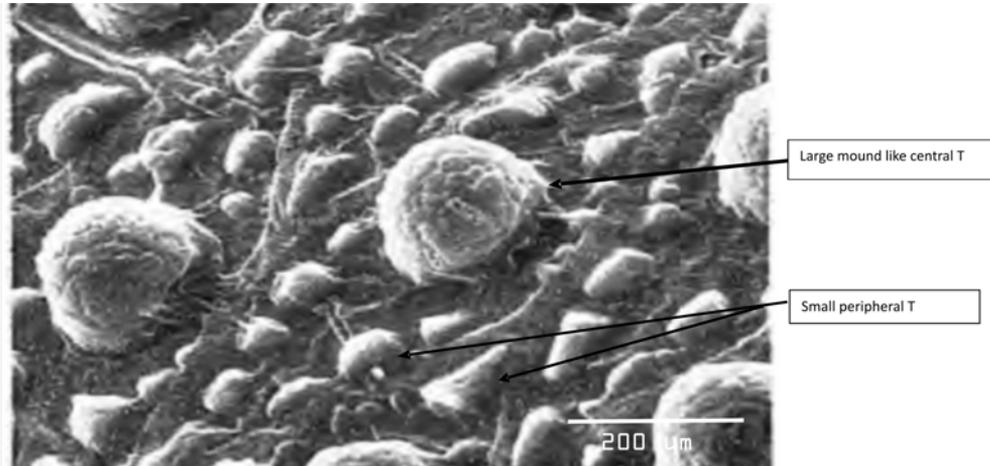


Figure2: Scanning electron micrograph of *Aedes albopictus* (c) large swollen tubercle.T= Tubercle.

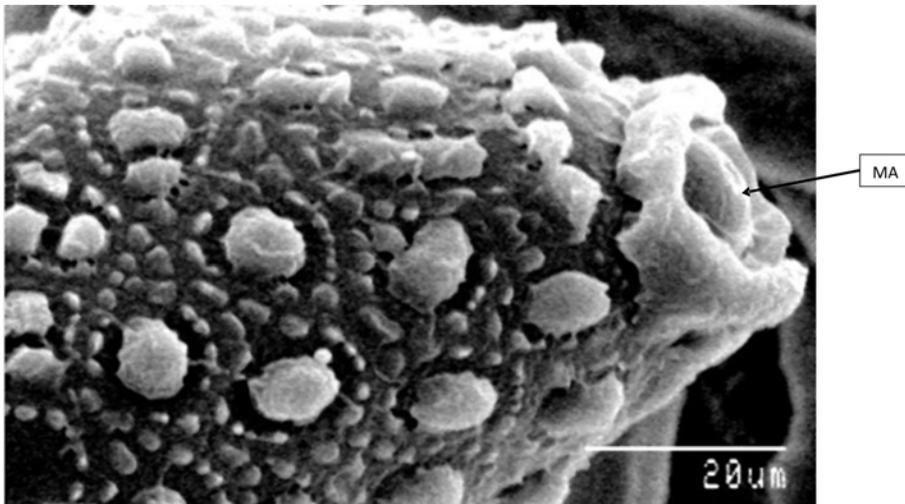


Figure2: Scanning electron micrograph of *Aedes albopictus* (d) micropylar apparatus (MA)

aegypti, and were more tapered cylindrically at the posterior end, whereas the eggs of *Aedes aegypti* showed much wider posterior side. Both species' eggs were shiny, pitch black in outlook and looked rice-like when laid. The egg surface was found to be rough in case of both the species', but the tubercles looked evenly placed in the micrographs in case of *Aedes albopictus* (Fig 2a) and irregularly placed with distinct gaps between each tubercle in case of *Aedes aegypti* (Fig 1a).

The outer chorionic cell field is the space between the hexagonal or polygonal boundary. It is the space where the tubercle lies centrally. The boundary guarding the cell field is known as "outer chorionic reticulum". In this work the ventral chorionic structure has been highlighted. In case of *Aedes albopictus*, the outer chorionic reticulum was mostly hexagonal (Fig 2b), with very few pentagonal structures. Within these polygons tubercles were present, which again differ from species to species and act as prominent species identification marker. *Aedes albopictus* eggs showed to have a large central tubercle (Fig 2c), swollen mound-like and a bit protruding with a slight dent in the middle; whereas eggs of *Aedes aegypti* also showed the same but often two tubercles were seen to be present in the same reticulum in the same cell field (Fig 1b). The cell field was seen to be completely empty in case of *Aedes albopictus* with smaller peripheral tubercles arranged in the outer chorionic reticulum (Fig 2 b-d), but cell field failed to be empty in case of *Aedes aegypti*. Smaller tubercles were often found to be in connection with the large central tubercle (Fig 1b). The collar of the micropylar apparatus of the *Aedes albopictus* was seen to be circular without any sectors and the micropyle was seen to be inserted into a shallow groove-like structure (Fig 2d); however the collar of the micropylar apparatus of *Aedes aegypti* had sectors.

Scanning electron microscopy provides a greater depth into the fine ornamentations of the eggs which enable to distinguish between various species. Though SEM structures of *Aedes albopictus* and *Aedes aegypti* are hard to differentiate, there are still certain features that bring out the difference between the species. Very little work has been done on the scanning electron microscopy of *Aedes* sp eggs. The shiny black colour of the *Aedes* eggs is thought to be mainly due to the darkening of the endochorion after the eggs are laid (Hinton and Service, 1969). Though the function of the exochorion or the outer layer of the *Aedes* eggs is not properly understood, Hinton and Service (1969) reported that in other species like *Culex* it holds a thin film of air. The outer egg shell of the aedine eggs is roughly polygonal but often hexagonal. The shapes of polygons differ from species to species and that is a remarkable distinguishing feature of identification (Hinton and Service, 1969). In *Aedes lineatopennis*, the micropylar collar were found to be fragmented and the exochorion reticulum was irregular (Choochote et al., 2001), which was a distinguishing feature specific to this species only and differed from the other *Aedes* species. The findings of Linley (1989) agreed with our study with respect to the length of the eggs, which stated that eggs of *Aedes aegypti* are longer than eggs of *Aedes albopictus*. Similar studies by the same author showed eggs of *Aedes bahamensis* to be significantly longer and larger than the two species studied in our work. The micropylar collar of *Aedes bahamensis* was not seen to be prominent but discontinuous, while those of *Aedes aegypti* were prominent. *Aedes albopictus* showed the same feature as

Aedes bahamensis (Linley, 1989). According to Suman et al. (2011) the strong solid wall like exochorions of *Aedes albopictus* might be responsible for their protection from desiccation when laid in containers, whereas exochorions of *Aedes aegypti* were found to be reticulated and interwoven. Nevertheless, the present work correlated with the findings done in the past.

From the present study, minute differences in the egg ornamentations were easily distinguished through SEM and hence can be used as a relevant tool to identify the differences in species. Stereomicroscopic structures are essentially useful in determining the difference between the species. The differences in the architecture of the egg structure of the species may be adapted to the environment and their habitat.

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