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Scanning Electron Microscopic Observations of Eggs of *Anopheles Fluviatilis* (T) Mosquito (Diptera: Culicidae).

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ABSTRACT

In the present investigations, Scanning Electron Microscopy of eggs of *An. fluviatilis* is carried out to differentiate the *An. fluviatilis* significantly from other mosquito species in egg size, float size, structure of lobed tubercles, pattern of deck tubercles, pattern of chorionic cells and structure of tubercles on under float area. The shape and size of tubercle is different in *An. fluviatilis* as compared to *An. culicifacies*, *An. nyssorhynchus*, *An. nuneztovary* and *An. apicimacula*. The eggs of *An. fluviatilis* are similar to *An. culicifacies* in micropylar rays but different from *An. darlingi*, *An. rangeli* and *An. dunhami*. These characters along with different shapes present on different surface and/or ends of egg were used to differentiate various mosquito species.

INTRODUCTION

Anopheles species which transmit malaria in India have distinct biological characters and specific distribution pattern. Out of the 60 *Anopheles* species reported from India 9 species are considered to be transmitting malaria- *Anopheles culicifacies*, *Anopheles dirus*, *Anopheles fluviatilis*, *Anopheles minimums*, *Anopheles stephensi* and *Anopheles syndics* are major vectors and *Anopheles annularis*, *Anopheles philippinensis* and *Anopheles vicuna* are of local importance and play a secondary role.

Anopheles fluviatilis is one of the primary vector of malaria in India and contributes around 15% of the total malaria cases in the country. Rest of the malaria is caused by *Anopheles culicifacies* and *An. stephensi*.

Studies of egg morphology of several Anophelines species with scanning electron microscope (SEM) have been documented (Damrongphol *et al.*, 1989; Linley *et al.*, 1996; Rodriguez *et al.*, 1992,1996 &1999; Forattion *et al.*, 1997 & 1998; Lounibos *et al.*, 1997; Junkum *et al.*, 2004; Chaudhry & Gupta, 2003 & 2004; Gupta & Chaudhary, 2005), because they provide better description of fine structures.

To overcome the inherent limitations of above techniques in identifying *Anopheles fluviatilis* from other mosquito species SEM of eggs could be an important alternative. In addition, the present study could help to understand the structure of chorion in *Anopheles fluviatilis* to prepare eggs for microinjection after chorion removal for development of transgenesis technology for this mosquito. We present herein a detailed description of the eggs of this species by SEM.

MATERIAL AND METHODS

Adults of *Anopheles fluviatilis* were obtained from Nation Institute for Malaria Research, New Delhi. The gravid females laid eggs in the small plastic containers lined with filter paper. For forced egg laying, the complete dark condition was provided by wrapping the container with black cloth/chart paper. The gravid females oviposited their egg on the wet filter paper. The egg were collected with the help of fine

point brush and fixed in Carnoy's fixative for 36 hr. After that, fixed eggs transferred to phosphate buffer for further use.

Eggs were initially examined under a dissecting microscope for measurements of length, shape, width etc. For SEM, the eggs were air dried and mounted on aluminum stubs with double stick tape. The specimens then coated with gold in a sputter-coating apparatus and examined in a Hitachi S-510 Scanning Electron Microscope.

RESULTS

The scanning electron microscopic observations of the *An. fluviatilis* are shown in fig. 1-9. The diagnostic differences have been summarized in Table-1. However, the general and common characters of the species are described below-

Overall appearance

In general, the appearance of eggs of *An. fluviatilis* are black in color and broadly boat shaped in lateral and vertical view, the contour is straight ventrally, dorsal surface curved, more acutely near ends, float centered near midline in lateral view (fig 1), floats are closer to ventral than dorsal surface and extending approximately 2/3 total length of egg.

Egg Ornamentation

Ventral (upper surface)

Anterior part of deck is slightly longer than the posterior part. Both the ends are surrounded by frill along the periphery (fig 1). Frill moderate in height across the length of egg (fig 1). The outer chorion is sculptured with various size tubercles. The outlines of chorionic cells are not visible on deck, (fig 1&3). Lobed tubercles are present at both the ends of eggs. Generally Lobed tubercles are oval shaped and 3 lobed tubercles (fig 2 & 4) are present at each posterior end and anterior end.

Dorsal (lower) and lateral surfaces

Chorionic cell boundary is not visible on dorsal surface (fig 3). Plastron pores of different sizes are present on dorsal surface. Chorionic cell boundary is more apparent on lateral side, polygonal cells are present at both ends of lateral sides (fig 1 & 3). Float is divided into ribs (fig 8). Under float area on lateral side is also covered by variously shaped tubercles (fig 5).

Anterior end and Micropyle

Anterior end is smaller in size and surrounded with frill smaller in size (fig 1). Three lobed tubercles are present on ventral surface at anterior end (Fig 3 & 5). The micropyle is surrounded by a polygonal smooth collar with an irregular outer margin, separated from frill margin by plastron pore (fig 7). Micropylar disc surface is smooth with six thorns like micropylar rays extending from the inner margin towards the central micropylar orifice.

Posterior end

Posterior end is rounded as anterior end (fig 4), frill well developed, slightly smaller in size. Three lobed tubercles are present (fig 3 & 5).

Table 1: Characteristic features of *Anopheles fluviatilis*

Sr. No.	Character	<i>Anopheles fluviatilis</i>
1.	Size (a) Egg Length (b) Egg width (c) Length/width	371.5um 141.0um 2.634um
2.	Egg color	Black
3.	Egg Shape	Boat shaped
4.	Float (a) Relative Size (b) No. of ridges	Moderate, 2/3 of total egg length 16-17 ridges
5.	Lobed Tubercles	Oval in shape, 3 in number at both ends, eight lobed
6.	Under float area	Less branched polygonal tubercles
7.	Micropylar apparatus	Irregularly rounded, incomplete hexagonal rays
8.	Plastron pomes	Comparatively smaller in number and fewer
9.	Frill	Moderate in height
10.	Deck	Restricted to anterior and posterior ends. More elongate at the anterior end
11.	Chorionic cells	On anterior and posterior-lateral with distinct boundaries, moderate in number

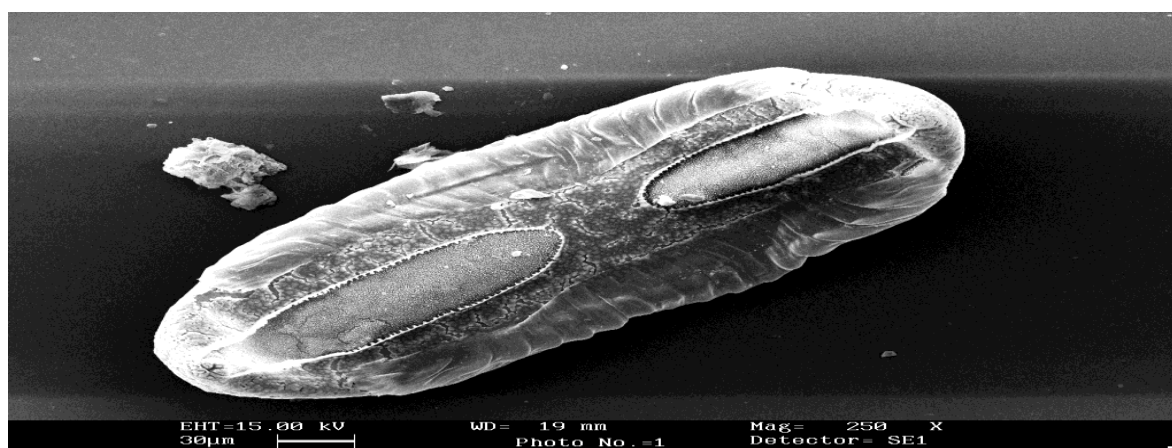


Fig.1. Anterior end showing micropylar apparatus (micropylar collar, micropylar orifice, micropylar rays)



Fig.2 Anterior end showing lobed tubercles at the anterior deck, frill, micropylar apparatus

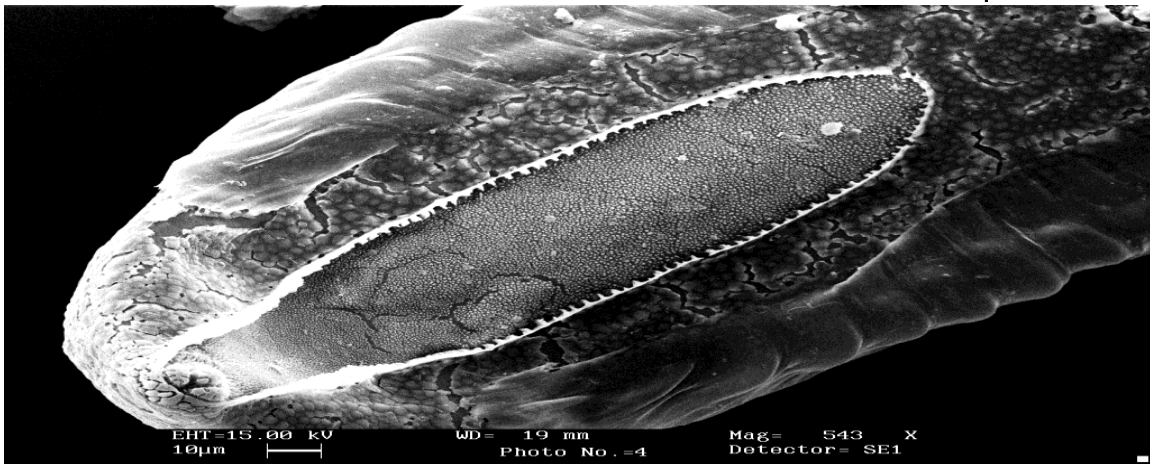


Fig.3. Anterior end showing anterior deck, chorionic cells, floats, lobed tubercles, tubercles

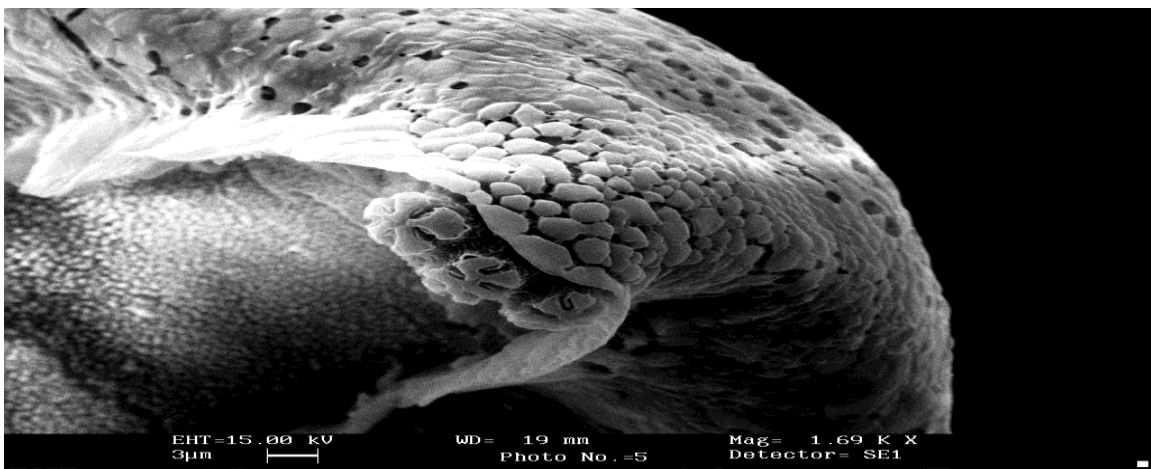


Fig.4. Posterior end showing lobed tubercles on deck surface and frill

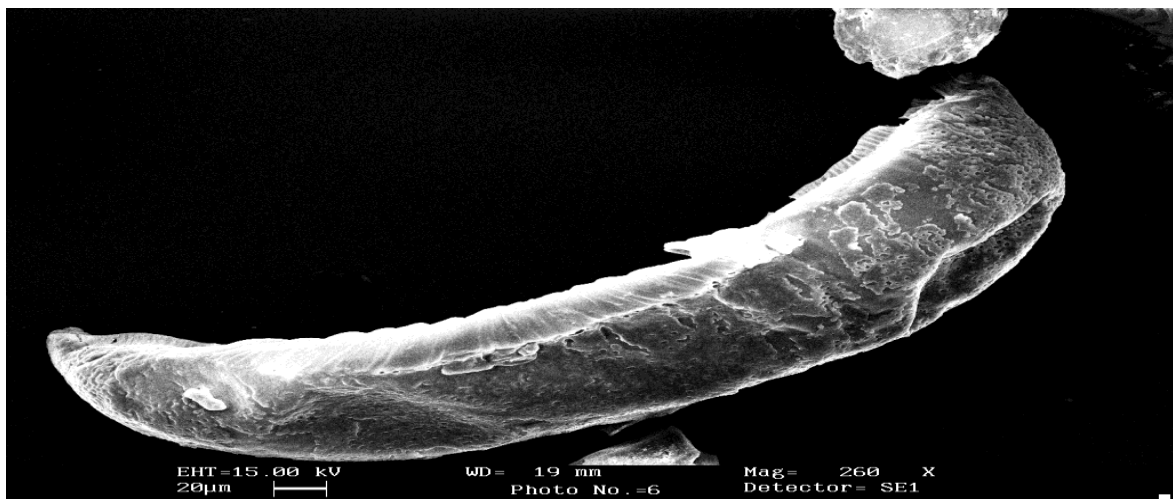


Fig.5. Lateral view showing frill, float extending 2/3rd of the egg, anterior and posterior end showing boat shape of the egg

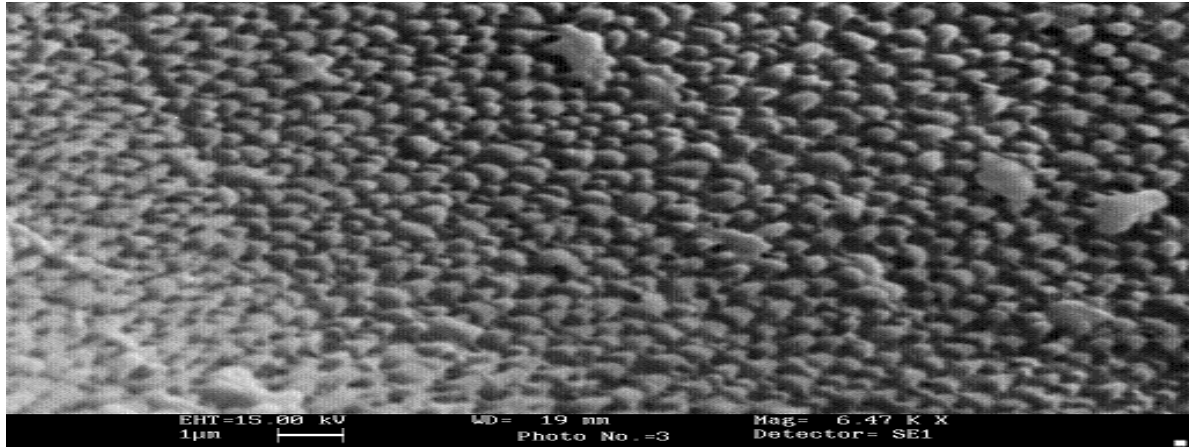


Fig.6. Ventral surface of *An.fluviatilis* egg showing tubercles

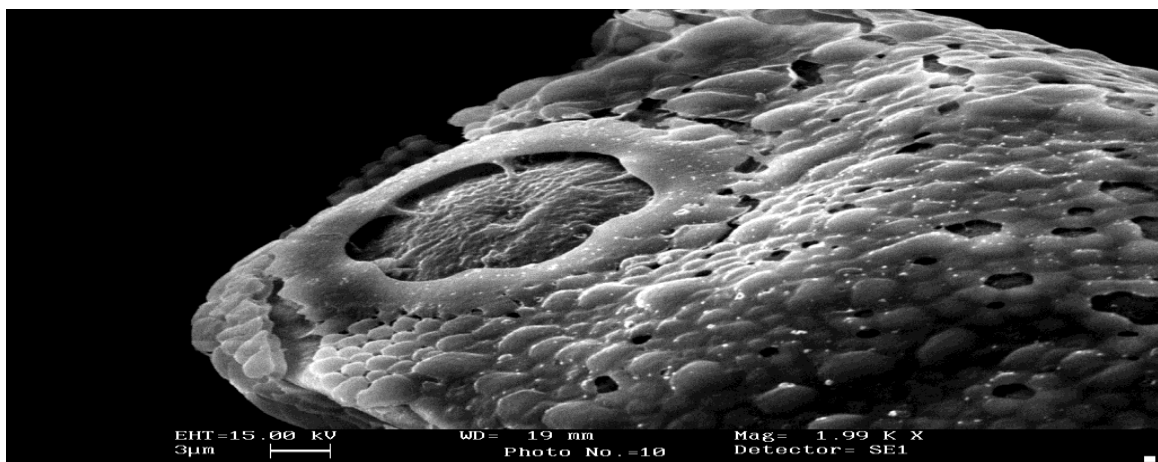


Fig.7. Anterior end showing micropylar apparatus (micropylar collar, micropylar orifice, micropylar rays)



Fig.8. Floats of *Anopheles fluviatilis* egg ridges

DISCUSSION

The general characters of eggs of *An. fluviatilis* appear rather similar under the light microscope. However, the ornamentation of the exochorion is an excellent parameter for making comparisons and has been found useful in differentiating species from other mosquito species.

The egg of *An. fluviatilis* are 371.5µm in length and 141.0 µm in width. In the range (398 µm - 649 µm) are *An. laneanus* (Forattini et al., 1997), *An. nuneztovari* (Linley et al., 1996), *An.culicifacies* (Chaudhary et al., 2003), *An.albimanus* (Rodriguez et al., 1992), *An. apimacula* (Rodriguez et a., 1996), *An.gambiae* complex (Lounibos et al.,1997), *An. dirus* complex (Damrongphol et a., 1989), *An. vestitipennis* (Rodriguez et al.,1999). In *Aedes triseriatus* (680.8 µm, Linley et al., 1989). The second range from *An. strode* (Linley et al., 1996), *An. benarrochi* (Linley et al., 1996) and *An. randoni* (Forattini et al., 1998) are larger than *An. fluviatilis*. The third range (345.9 µm - 397.8 µm) *An. dunhami* (Linley et al., 1996) and *An. apicimacula* (Lounibos et al., 1997) are lying in the range of *An.fluviatilis*.

The floats have also been used in differentiating the eggs of various species. The float of *An. fluviatilis* egg extends approximately 2/3 of total length and has 16-17 ridges. The float can be divided into two categories, depending upon the pattern of float. Firstly in the species viz., *An. nuneztovari* (Linley et al., 1996), *An. gambiae* complex (Lounibos et al.,1999), *An. dirus* complex (Damrongphol et al.,1989), *An. culicifacies* (Chaudhari et al., 2003) and *An. antunesi* (Forattini et al.,1997). In these species the pattern of float is similar to that of the *An. fluviatilis* as observed in the present study. Secondly, the float of eggs in the case of *An. albimanus* (Lounibos et al., 1997), *An. vestitipennis* (Rodriguez et al.,1999), and *An. apicimacula* (Rodriguez et al., 1996) showed highly variable pattern.

No significant difference in the form of frill was observed between three strains of *An. fluviatilis*. All other previously examined *Anopheles* species complexes viz., *An. apicimacula* (Rodriguez et al.,1996), *An. laneanus* (Forattini et al.,1997), *An. gambiae* complex (Lounibos et al.,1999), and *An dirus* (Damrongphol et al.,1989) also do not show any difference in the frill of their species complex. However, in *An. albimanus* complex, the frill pattern is significant in differentiating six species (Lounibos et al., 1997).

The presence of discontinuous deck the eggs of *An. fluviatilis* is observed during present study is different to that present in *An. laneanus*, *An. antunesi*, *An. vestitipennis*, and in *An. apicimacula*. However, in *An. albimanus* (Rodriguez et al.,1992; Lounibos et al,1997), the float covers most part of the deck and while in *An. gambiae* complex the deck surface is slightly narrower in the middle part and normal at two ends (Lounibos et al.,1999).

There is no difference in the tubercles distribution. Previously examined species, *An. albimanus*, *An. nuneztovari* and *An. apicimacula* also showed prominent polygonal tubercles without any interconnection (Lounibos et al.,1997; Linley et al.,1996; Rodriguez et al.,1996). However, in *An. dirus* complex, some interconnections in between tubercles at deck are reported (Damrongphol et al., 1989). The similarity in this character indicates the proximity *An. fluviatilis* with *An. dirus* complex.

There is significant difference in the structure of multilobed large tubercles present at both ends of egg in *An. fluviatilis* and *An.culicifacies*. In *An. fluviatilis* at both end three lobed tubercles are present whereas in *An. culicifacies* numbers of lobed tubercles are not same at both ends. All other previously studied species complex had oval shaped lobed tubercles. No such type of species-specific difference had been reported on the basis of shape of lobed tubercles.

There is no species - specific differences on basis of chorionic cells in *An. fluviatilis* species. Similarly *An. gambiae* complex and *An. vestitipennis* also could not be distinguished on the basis of chorionic cell (Lounibos et al., 1999 & Rodriguez et al., 1999). On the contrary, *An. dirus* species complex could be distinguished on the basis of pattern of chorionic cells between the frill and float (Damrongphol et al., 1989).

The micropylar collar is irregularly rounded with incomplete hexagonal rays in *An. fluviatilis* similar to *An. culicifacies*. No such type of differences has been observed in other *Anopheles* species complex (Damrongphol et al., 1989; Linley et al., 1996; Lounibos et al., 1999; Rodriguez et al., 1999).

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