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Research Article

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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 HitachiS-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 pomes 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 pome (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*

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

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 2/3 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. nunezetovari (Linley et al., 1996). An. culicifacies (Chaudhary et al., 2003), An. albimanus (Rodriguez et al., 1992), An. apimacula (Rodriguez et al., 1996), An. gambiae complex (Lounibos et al., 1997), An. dirus complex (Damrongphol et al., 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. apimacula (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, dependent upon the pattern of float. Firstly in the species viz., An. nunezetovari (Linley et al., 1996), An. gambiae complex (Lounibos et al., 1999), An. dirus complex (Damrongphol et al. 1989), An. culicifacies (Chaudhary 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. vestitipennies (Rodriguez et al., 1999), and An. apimacula (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. apimacula (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 differenting 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. vestitipennes, and in An. apimacula. However, in An. albimanus (Rodriguez et al., 1992; Lounibos et al., 1997), the frill 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. nunezetovari and An. apimacula 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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REFERENCES