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Scanning electron microscopy (sem) studies to explore new taxonomic features on the egg of *anopheles (cellia) annularis* van der wulp

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Abstract
The egg surface structures *anopheles (cellia) annularis* van der wulp have been studied and illustrated with the aid of scanning electron microscope (sem). Many additional and new taxonomic features have come to light, which have been discussed in detail.

Keywords: scanning electron microscope, *anopheles annularis*, egg.

1. Introduction
The adult representatives of species *anopheles (cellia) annularis* van der wulp were collected during the recent collection-cum-survey tours conducted in the state of punjab. a good number of species complexes of genus *anopheles* have been sorted out on the basis of egg structures and other immature stages. A*nopheles annularis* belongs to subgenus *cellia* having two sibling species, is most abundant species from punjab state. This species is a secondary vector of malaria in india, breeds in still water with abundant vegetation in tanks, ponds, borrowpits, ricefields, wells, lakes [1]. But in present studies, not even single specimen has been collected from above said habitats. During these surveys eggs of this species were collected from the field as well as from the rearing of species in the laboratory. Studies on egg ornamentation of various species have been conducted by [2], [3], [4] and [5] from different parts of the country. However, there is no information available on Ultra structural studies of this present species. Scanning electron microscopic (sem) studies have been conducted on the egg of this species in order to explore additional taxonomic features. Various workers like [6, 7, 8, 9, 10, 11, 12, 13, 14] have studies egg and various stages of the species *an. annularis*. However, sem studies have been conducted for the first time on egg of this species and many new taxonomic attributes have come to light.

2. Materials and methods
The representatives of *anopheles annularis* were captured from cattle sheds. The blood fed females were reared in laboratory and shifted to test tube containing some fresh water and after 2-3 days eggs were laid by female. These eggs were preserved in 70% ethanol whereas, some were reared upto adult stage. For sem studies, protocol given by [15] followed. Eggs were dehydrated in graded series of alcohol and mounted on sem specimen stubs using only a small strip of double-sided adhesive tape [16]. The samples were then sputter coated with gold and scanned under jsm- 6100 scanning electron microscope. Micrographs of about 5-10 eggs were examined from all desired directions. The terminology given by [17] has been used for various structures.

3. Results and discussion
The eggs laid singly, are black in color and boat shaped in ventral and dorsal view and ends rather pointed. Beck broader at the anterior end and narrows considerably towards posterior end, more rounded than anterior end. Floats are located slightly towards posterior end and cover about 45% of the deck on ventral view. The external coating of the eggs presented an extremely regular exochorion.

3.1 Ventral (upper) surface (fig. 1)
Beck continuous along length of the egg. Entire deck is covered with irregular tubercles of different shape at all regions. Small tubercles are scattered in between the larger ones.

3.2 Posterior end (fig. 1)
Posterior end is broader as compare to anterior end. Number of lobed tubercles on this region is near about 5-6.

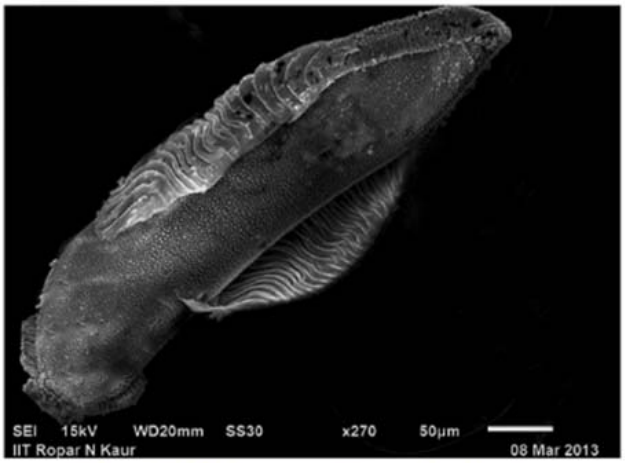

Fig. 1 Whole egg (Dorsal view)

3.3 Dorsal surface (fig. 2 & 3)
Chorionic cells covering the dorsal surface are mushroom shaped convex structures interconnected by bridges of nearly same width (same as in *anopheles stephensi* liston, studied by [15]). Floats cover the lateral sides and number of ridges on float is species specific. In the species under reference, number of ridges about 17-18.

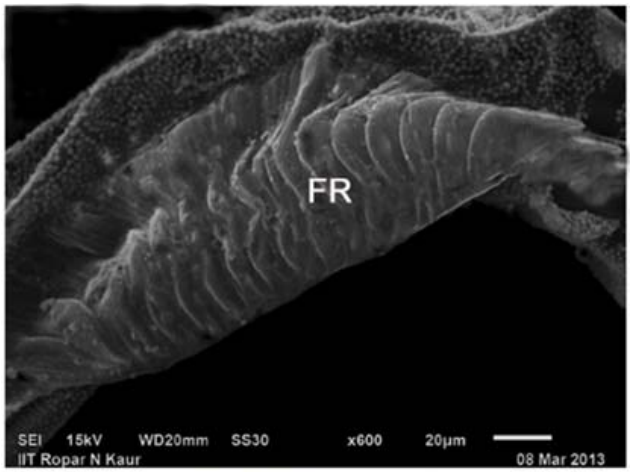

Fig. 2 Float

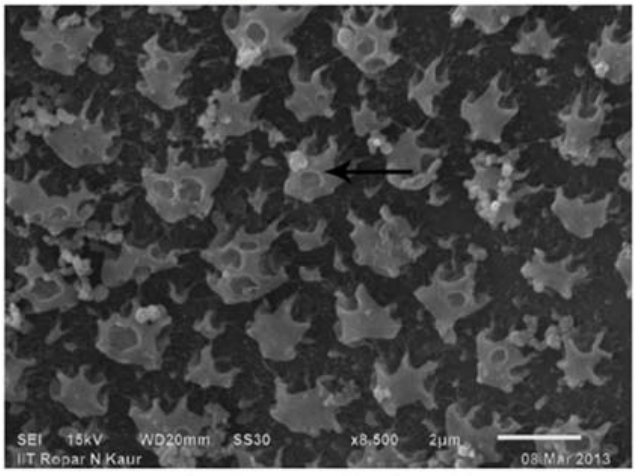

Fig. 3 Dorsal tubercles

3.4 Anterior end (fig. 4)
Lobed tubercles on anterior end are not clearly visible, but micropyle present on this region is very prominent. micropyle is separated from tubercles by frill and thin plastron.

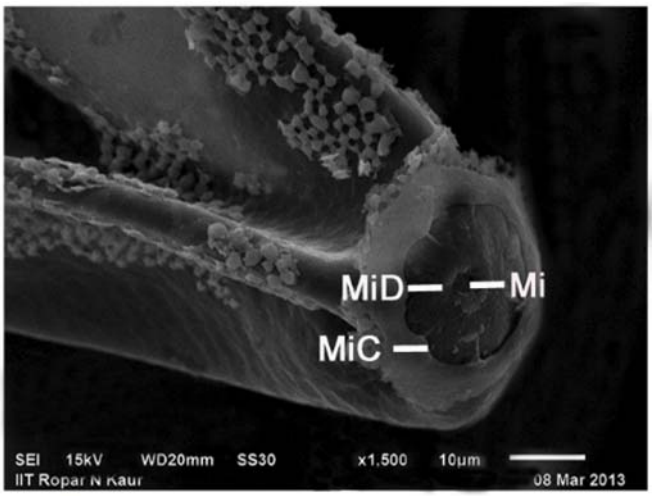

Fig. 4 Micropyle on anterior region

4. Conclusions
The ornamentation of the exochorion is an excellent parameter for making comparisons between and reveals significant differences, especially in relation to the presence of tubercles in the chorionic cells [18]. In many species, egg chorionic architecture has also become indispensible in the identification of those species which are clearly defined in early stages of development than in adult stages [15]. Therefore, the attributes viz., number and shape of tubercles, lobed tubercles and shape of ridges on float region, studied in present species with the help of scanning electron microscope will be very fruitful for authentic identification of species.

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