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Larvae, cibarium and female genitalia of Anopheles (Cellia) vagus van der wulp (Anophelinae: Culicidae) with the aid of scanning electron microscopic (Sem) from Punjab

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Abstract
New taxonomic attributes have been found on first instar larva, cibarium and external female genitalia of *Anopheles (Cellia) vagus* Van der Wulp with the aid of Scanning Electron Microscopic (SEM) studies in India. Many new/additional attributes will strengthen the diagnosis of the present species.

Keywords: Scanning Electron Microscope, *Anopheles (Cellia) vagus*, first instar larva, cibarium, female genitalia, Punjab.

1. Introduction
Mosquitoes are unquestionably the most important vectors of various diseases. The members of genus *Anopheles* Meigen have attracted the attention of human beings since ages because they are malaria vectors. Different species of genus *Anopheles* Meigen and *Culex* Linnaeus have affected the welfare of mankind by spreading various diseases. Some of the diseases are responsible for sickening, killing and disfiguring human beings. Besides this, many species of mosquitoes have direct annoyance as nuisance with their bites during day and night time (Service, 1993). The most important disease transmitting and nuisance causing mosquitoes belong to the genera like *Anopheles* Meigen, *Culex* Linnaeus, *Aedes* Meigen, *Mansonia* Blanchard, *Haemogogus* Linnaeus, *Sabethes* Robineau-Desvoidy and *Psorophora* Robineau-Desvoidy. Various species of *Anopheles*, *Culex*, *Aedes* and *Mansonia* are important vectors of many serious diseases like Malaria, Filariasis, Yellow Fever, Dengue, Dengue Hemorrhagic Fever, Japanese Encephalitis etc. in India. However, the genus *Anopheles* Meigen has malaria transmitting species. This disease has been serious problem in our country since ancient times. It kills 6000 persons in a day in this world that means every 30 seconds a person dies due to it. The diversity in Anopheline species together with the diverse geographical, ecological and environmental conditions are responsible for abundance and wide prevalence of malaria in our country and many other parts of the world.

Anopheles (Cellia) vagus Van der Wulp is not very dominant species from Punjab state but a large number of representatives of this species were collected during collection-cum-survey tours from different localities. Christophers, 1933; Reid, 1968; Ramachandra Rao, 1984 studied this species and reported that it was not vector for human malaria parasites. This status was primarily on thousands of specimens in many blood feeding studies indicating that that Southeast Asia *Anopheles vagus* fed primarily (over 90%) on cows and water buffalos and was usually ranked the least attracted to humans of all the *Anopheles* tested (Reid, 1961, 1968; Bruce-Chwatt *et al.*, 1966; Ramachandra Rao, 1984). But some of the workers indicated that the present species may serves as secondary vector under unusual circumstances that include dense concentrations of humans in association with low numbers or absence of bovids and/ or primates (Baker *et al.*, 1987; Mahesway *et al.*, 1994; Amersinghe *et al.*, 1999; Prakash *et al.*, 2004). These areas included Thailand, Kampuchea, Bangladesh, Sri Lanka and Assam state (India) respectively.

Manguin *et al.* (2010) listed *Anopheles vagus* as one of the 19 *Anopheles* species that co-transmit *Plasmodium* and *Wuchereria bancrofti* in Asia. This species is very closely related to *Anopheles (Cellia) subpictus* Grassi, both the species can be differentiated on the basis of apical band and fore leg tarsomeres. This species breeds in a variety of places where *Anopheles subpictus* breeds but prefers muddy waters where it breeds more intensely (Nagpal & Sharma, 1995).

Taxonomists have always tried to update the diagnosis and status of various mosquito species. An effort has also been made to study first instar larva, cibarium and female genitalia. As far as their ultrastructures are concerned and many new taxonomic characters have come to light and these will be used to update status and diagnosis of present species.

2. Material and Methods

About 100 representatives of *Anopheles vagus* were captured from various localities of Punjab state. 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. After hatching of eggs, some of the larvae so formed were preserved in 70% ethanol (Larvae were boiled in water for some time to kill them before preservation). Larvae were dehydrated in graded series of alcohol and after air dried on filter paper, mounted on SEM specimen stubs using only a small strip of double-sided adhesive tape (Kirti & Kaur, 2011). The samples were then sputter coated with gold and scanned under JSM- 6100 scanning electron microscope at Indian Institute of Technologies (IIT) Ropar. Micrographs of about 5-10 larvae were examined from all desired directions.

For SEM studies of cibarial armature, the method given by Lee and Craig (1983) has been adopted. The head of adult female mosquitoes were snipped off from body and boiled in 10% KOH solution till their clearance. Dissected material was washed several times with water. The head was placed on a slide with a drop of water and dissection was completed with needles under the binocular microscope. Compound eyes were slowly pulled apart in order to expose cibarium that is located immediately behind the clypeus. Dissected material was washed several times with water and dehydrated by passing through ascending grades of alcohol. The specimens were placed on stubs in dorsal position after air drying on filter paper and then coated with gold.

For genitalic attributes, last three segments of both male and female specimens were dissected with the help of forceps. These were first boiled in 10% KOH for 20-25 minutes, washed with water several times, air-dried and mounted on stubs for micrographs.

Further procedure for cibarial armature and genitalia was same as explained for larvae. Taxonomic keys developed by Puri (1931), Ross & Roberts (1943), Nagpal & Sharma (1995), Amersinghe *et al.* (2002) were used for identification. The terminology given by Hara (1959), Harbach & Knight (1978 & 1980) and Lee & Craig (1983) have been used for various structures.

3. Results and Discussions

Larva:

a. **Ventral aspect of head:** Some structures of larvae head were damaged during sample preparation. So, few structures which studied are A (Antenna), LPB (Lateral palatal brush), Mx (maxilla), Lat (Lateralia), PTP (Posterior tentorial pit), HEL (hypocranial ecdysial line), Col (Collar), Mx (Maxilla).
b. **Mentum:** Mentum consists of Ventromentun (Vm) and Dorsomentum (Dm).
c. **Dorsal aspect of head:** FEL (Frontal ecdysial line), DAp (Dorsal apotome), Apr (antennal prominence), APBr (Anteromedian palatal brush), EpS (Epistomal suture).
d. **Pecten Plate:** Pecten consists of pecten plate (PP), pecten scales (PS) and PSL (Posterolateral spiracular lobe).

4. Cibarial Armature:

As in Anopheline species, length of cibarium twice its width and Anterior Hard Palate (AHP) about one third length of cibarium. Cibarium consists of two parts: Cibarial armature and cibarial sense organs.

Cibarial armature consists of two rows of teeth i.e. Rods and Cones.

6. Rods: The number of rods always species specific. In the present communication, number of rods 14-14, long, broad at base and middle, pointed at tip, fabricated ends with lateral spines on either side.

7. Cones: The number of cones same for rods i.e. 14-15.

8. Cibarial sense organs: It consists of four types of papillae namely Dorsal papillae, Campaniform papillae, Trichoid papillae, ventral papillae but the fifth type i.e. palatal papillae are not studied or absent in species under reference. Dorsal papillae, Campaniform papillae, Trichoid papillae are socketed but ventral papillae are not. Dorsal and campaniform papillae are in pairs i.e. one on either side of AHP. Three trichoid papillae in centre of cibarium but in some specimens these are two in number. 4 ventral papillae are studied. Shape of ventral papillae socketed with plug like outgrowth.

9. Female Genitalia:

It consists of cerci, Post Genital Lobe (PGL), IX-tergum, insula, cowl and sigma. Pair of cerci covered with several types of small and large setae. PGL clearly visible with pair of long and thin seta. Shape of IX-tergum almost straight in line covered with very small setae; insula also straight, band like, covered with small teeth like setae; cowl slightly upward making small curve; sigma very structure, apex pointed, protruding outwards and join to form cup shaped structure in middle.

Larva of *Anopheles* (*Cellia*) *vagus* Doenitz

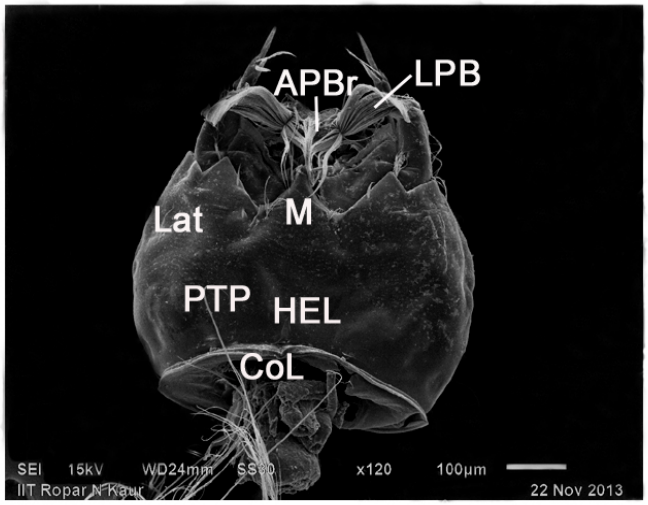

Fig. 1 Head of larva

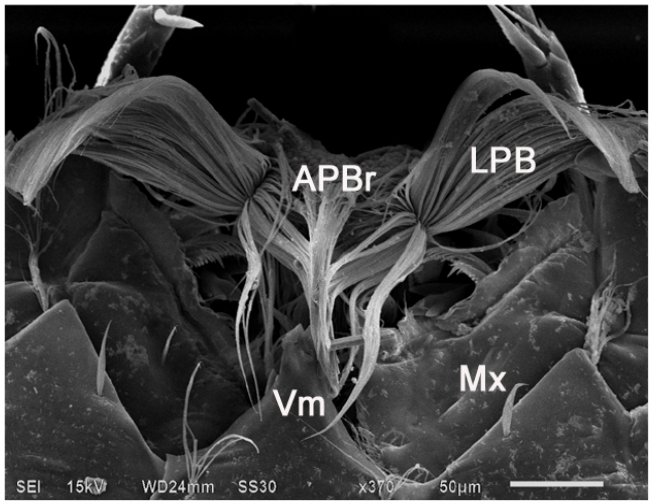

Fig. 2 Magnified view

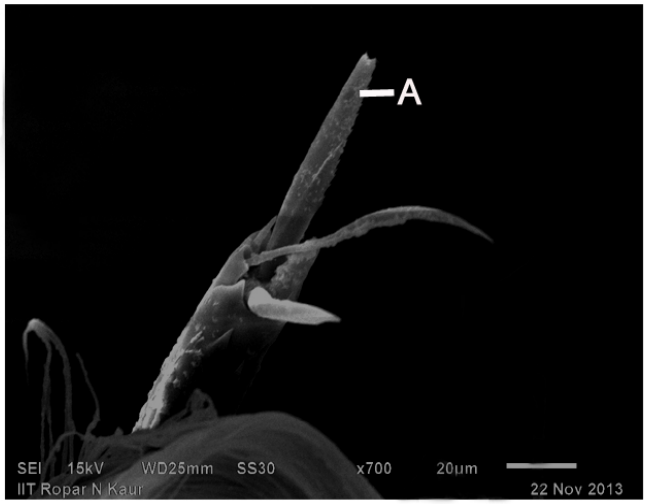

Fig. 3 Antenna

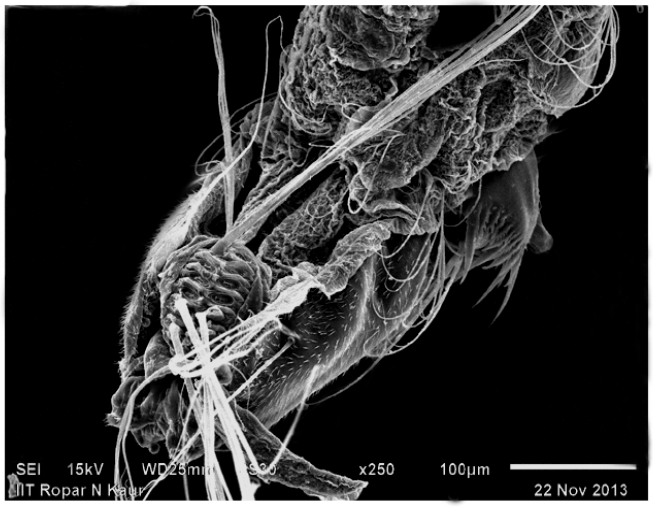

Fig. 4 Grid

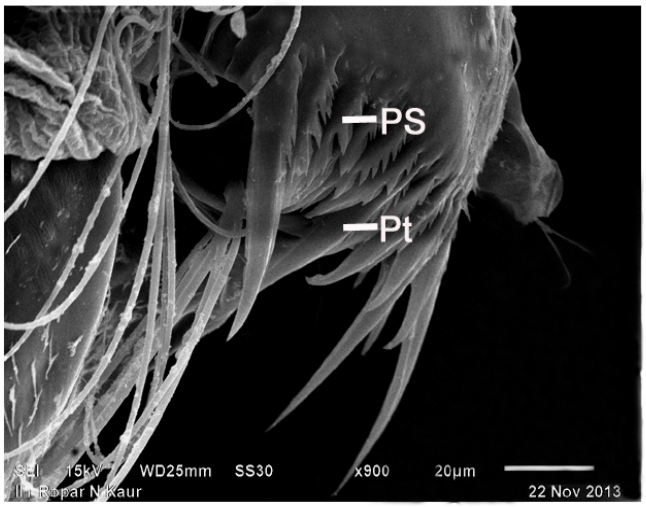

Fig. 5 Pectum

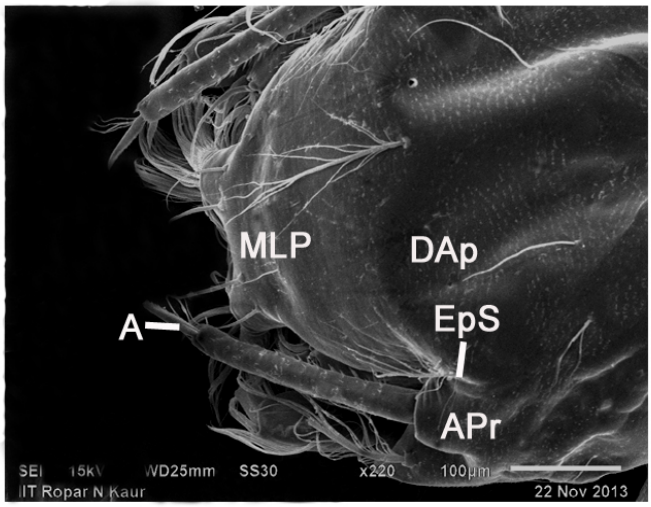

Fig. 6 Dorsal aspect of head

Cibarial armature of *Anopheles* (*Cellia*) *vagus* Doenitz

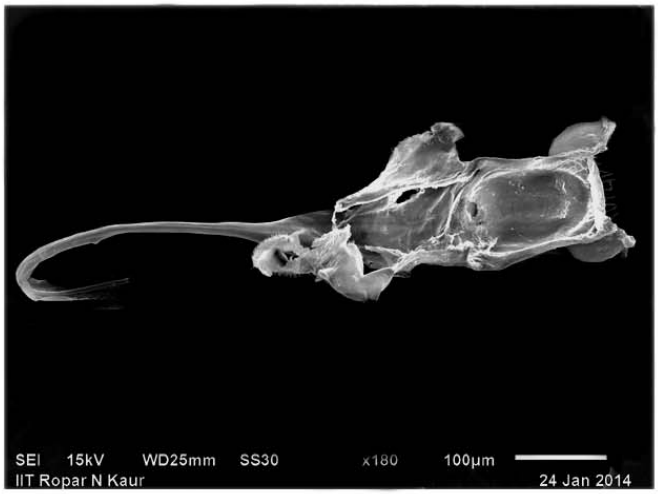

Fig.7 Cibarial armature

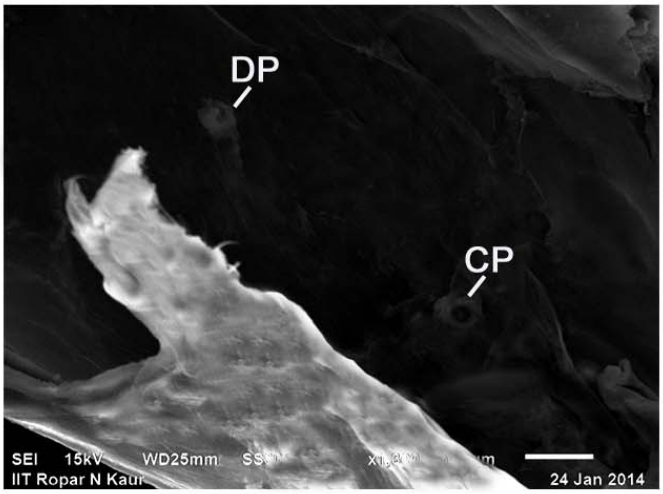

Fig. 8 Dorsal and companiform papillae

Fig. 9 Cibarial armature showing trichoid papilla

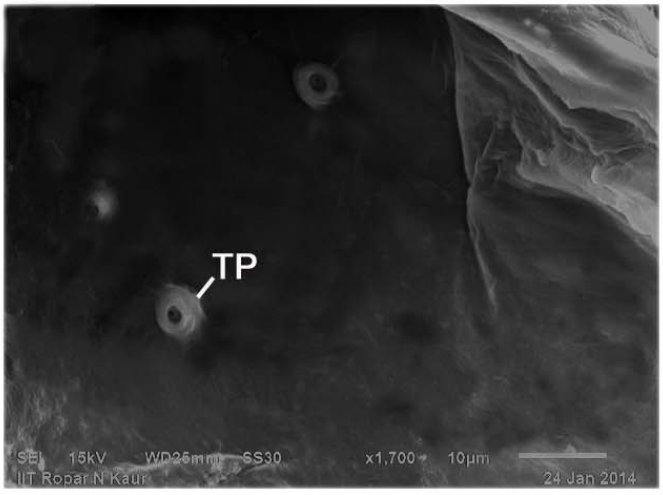

Fig. 10 Trichoid papillae (Magnified)

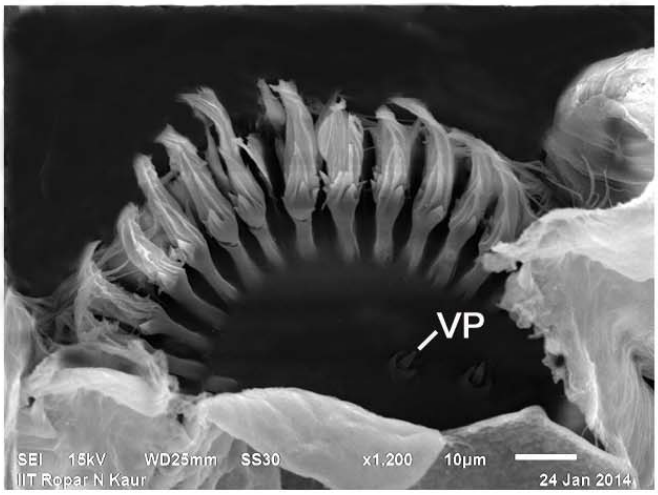

Fig. 11 Ventral papillae

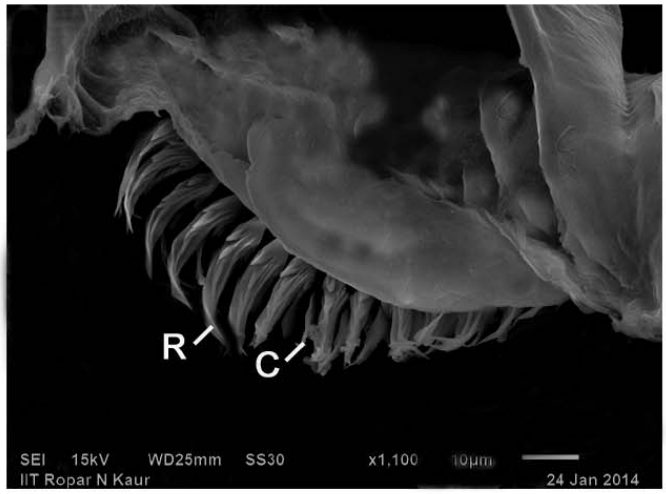

Fig. 12 Cibarial teeth (Magnified)

Genitalia of *Anopheles* (*Cellia*) *vagus* Doenitz

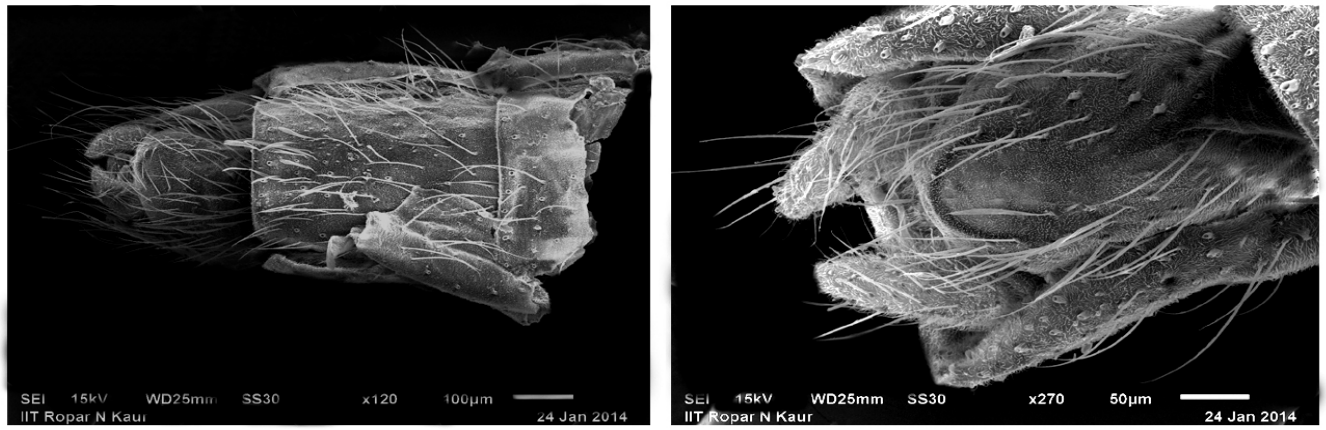

Fig. 13 & 14 Female genitalia

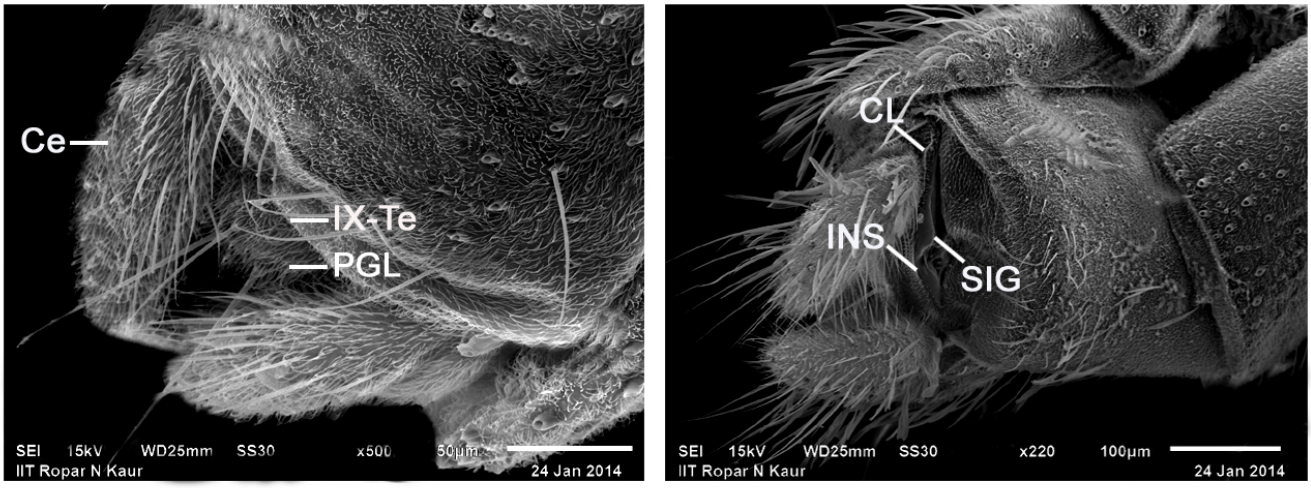

Fig. 15 & 16 Genitalia showing different structures

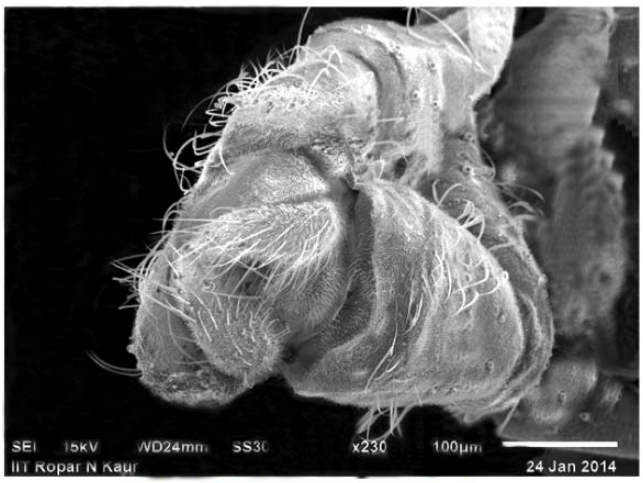

Fig. 17 Dorso-caudal view

10. Conclusions

Genus *Anopheles* Meigen is known for the existence of species complexes consisting of different sibling species.

The identification becomes very difficult in sorting out these species. Additional taxonomic attributes i.e. characters on immature stages and ultrastructures on mouthparts and genitalia have found to be useful which were studied by various workers. Very little information is available on the cibarium and different species of *Anopheles* in India. This structure has been studied in detail in the present species with the aid of SEM. cIbariala rmature and cibarium have taxonomic significance. Similarly, many structures on female genitalia like IX-tergum, post genital lobe, insula and cowl are of taxonomic importance. All these additional taxonomic attributes will be incorporated in the diagnosis of present species.

11. Abbreviations

A (Antenna), AHP (Anterior Hard Palate), APBr (Anteromedian palatal brush), Apr (antennal prominence), C (Cones), Ce (Cerci), CL (Cowl), Col (Collar), CP (Campaniform papillae), DAp (Dorsal apotome), Dorsomentum (Dm), DP (Dorsal papillae), EpS (Epistomal suture), FEL (Frontal ecdysial line), HEL (hypocranial ecdysial line), INS (Insula), IX-Te (IX-tergum), Lat (Lateralia), LPB (Lateral palatal brush), Mx (maxilla), Mx (Maxilla), Pecten plate (PP), pecten scales (PS), PGL (Post genital lobe), PSL (Posterolateral spiracular lobe), PTP (Posterior tentorial pit), R (Rods), SIG (Sigma), TP (Trichoid papillae), Ventromentun (Vm), VP (Ventral papillae).

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