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# Scanning Electron Microscopic Study on Cibarial Attributes on Threes Species of Culex vishnui Subgroup Complex (Diptera: Culicidae) from Punjab

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ABSTRACT: The Scanning Electron Microscopic study on Cibarium of three species of *Culex vishnui* subgroup Complex includes *Culex (Culex) tritaeniorhynchus* Giles, *Culex (Culex) pseudovishnui* Colless and *Culex (Culex) vishnui* Theobald, all of which are commonly reported throughout Punjab. The description of Cibarium includes characterization of cibarial armature such as Lateral Flanges, cibarial teeth on cibarialbar and sensory papillae: ventral, palatal, larger dorsal, smaller dorsal.

**Keywords:** Culex, Cibarium, Cibarial armature, sensory papillae, SEM.

#### INTRODUCTION

Culex vishnui subgroup complex has attracted great attention because of its importance as main potential vector of Japanese encephalitis virus in many areas of South Asia including India. The various adult species of this subgenus complex are very similar to show considerable morphological variations and are often impossible to identify at species level. Moreover, this complex shares the same habitat, breeding sites, feeding habits, biting habits as well on man and cattles. The immense similarity in morphology and bionomics of this complex pose a serious problem to attempt accurate species identification. The purpose of this paper is to support authentic and reliable identification of closely related adult species by taking detailed description of an additional internal taxonomic attribute "Cibarium".

In the head region, ventral to the clypeus, a dorsoventrally flat structure is located called cibarium (Lee and Craig 1983) which is attached to the pharyngeal pump at the proximal end of the proboscis. The structure is composed of two parts: Cibarial armature and four types of sense organs called sensory papillae. In the field of taxonomy, a large number of morphological variations is noticed within the different taxon levels that makes it as a distinct morphological feature. Previous workers mentioned these variations as a particular important character for the identification of many species in their research papers (Chen 1972; Sirivanakarn 1975, 1978; Forattini and Sallum 1992; Kirti and Shipali 2014; Kirti et al., 2015a, 2015b, 2015c, 2015d; Kirti and Sadeura 2016).

## MATERIAL AND METHODS

For collection of adult mosquitoes, several visits were conducted throughout the state of Punjab during dawn and dusk times. All the Specimens were preservation properly in collection boxes (Becker et al., 2010). For Identification, keys suggested by Barraud (1934); Sirivanakarn (1976); Reuben et al. (1994) was followed. For classification and nomenclature Harbach (2007) and terminology given by Chen (1972) was For scanning electron microscopy, methodology suggested by Lee and Craig (1983) was considered for preparation of materials with certain modifications. Dissection was performed to expose Cibarium in head region. Boiled in 10% KOH solution. Several washings were given with distilled water. Then, dehydration of the exposed Cibarium was done by passing the material in grades of alcohol (30%, 50%, 70%, 90%, 100%). The material exposed in filter paper for air drying and was mounted on stubs. Sputter coating of gold was done for scanning in the presence of technical expert. Images were captured under JSM-6610LV Scanning Electron Microscope at Indian Institute of Technology (IIT), Ropar, Punjab.

### RESULT AND DISCUSSION

#### 1. Culex (Culex) vishnui Theobald

**CIBARIUM:** Length of cibarium ranges 221.42µm and width approximately half of its length. The length of AHPnearly one third the length of a complete structure (Fig. 1).

Cibarial Armature (CA): LF thick, stout, ends curved outwards, average width between the ends ranges 70.71  $\pm$  1.00µm; CB thin, smooth, not clearly distinct, convex from centre, otherwise concave from sides, on which 28-30 CT located (Fig. 2); CT long, in a single row, ranges 18.55  $\pm$  1.06µm length, each CT is wide at the base, and pointed at the apex, comparatively longer (rod shaped) from convex aspect of CB, well developed near to the LF (Fig. 2 & 3).

### Sensory organs:

- Ventral Papillae (VP): 4, linearly placed, with a small outgrowth of length ranges 1.89  $\pm$  0.20  $\mu$ m; socket diameter ranges 2.57  $\pm$  0.83 $\mu$ m (Fig. 2 & 4)
- Palatal Papillae (PPa): 4, forms a cluster, apart from the edges of AHP; socket diameter ranges 2.48  $\pm$  0.29  $\mu m$  (Fig. 5 & 6)
- Larger Dorsal Papillae (LDP): 4, form quadrangle, two on each side of AHP; socket diameter ranges  $1.88 \pm 0.29 \mu m$  (Fig. 5).
- Smaller Dorsal Papillae (SDP): 6, three on each side, linearly and equally placed on both sides of AHP; socket diameter ranges  $1.54 \pm 0.51 \mu m$  (Fig. 5).

# Culex (Culex) vishnui Theobald

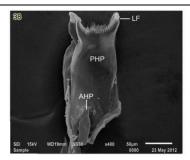


Fig. 1. Cibarium.

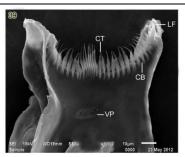


Fig. 2. Cibarial armature.

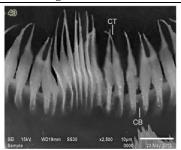


Fig. 3. Cibarialteeth.

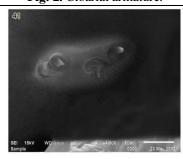


Fig. 4. Ventral papillae.

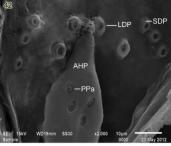


Fig. 5. Antero-dorsal membrane

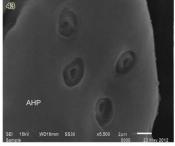


Fig. 6. Palatal papillae.

### 2. Culex (Culex) pseudovishnui Colless

Cibarial Armature (CA): LF thin, stout, ends not curved outwards, average width between the ends ranges 77.77  $\mu m$ ; CB thin, smooth, not distinct, convex from centre otherwise concave from sides, on which 32-35 CT located; CT long, in a single row, ranges 10.97  $\pm$  3.5  $\mu m$  length, each CT is a little wide at the base and pointed at the apex, comparatively smaller but well developed in shape from convex aspect of CB, well developed near to the LF(Fig. 7).

### **Sensory organs:**

• Ventral Papillae (VP): Absent

- Palatal Papillae (PPa): 4, very closely packed, forms a cluster, apart from edges of AHP; socket diameter ranges  $2.88 \pm 0.51 \mu m$  (Fig. 8 & 10).
- $\bullet$  Larger Dorsal Papillae (LDP): 4, forms a quadrangle, two on each side of AHP; socket diameter ranges 1.90  $\pm$  0.45  $\mu m$  (Fig. 8).
- Smaller Dorsal Papillae (SDP): 5, three on one side and two on another, linearly placed on both sides of AHP; socket diameter ranges  $1.45 \pm 0.36 \mu m$  (Fig. 8 & 9).

### Culex (Culex) pseudovishnui Colless

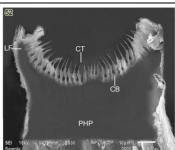


Fig. 7. Cibarial armature.

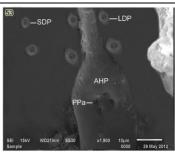


Fig. 8. Antero-dorsal membrane.



Fig. 9. Smaller Dorsal papillae.

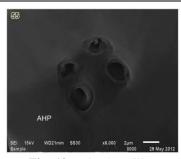


Fig. 10. Palatal papillae.

#### 3. Culex (Culex) tritaeniorhynchus Giles

Cibarial Armature (CA): LF thick, stout, ends curved outwards, average width between the ends ranges  $55.55\mu m$ ; CB thick, clearly distinct, on which 28-30 CT located; CT long, in a single row, ranges  $11.53\pm0.92\mu m$  length, each CT narrow at the base, broad and curved from centre and pointed at the apex, CT from both concave and convex aspect of CB similar and equal in length, CT located near to the LF well developed (Fig. 11&12).

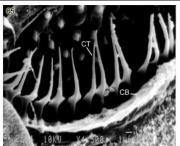
### **Sense organs:**

- Ventral Papillae (VP): Absent
- Palatal Papillae (PPa): 4, closely placed in two pairs, apart from edges of AHP; Socket diameter ranges 2.42  $\pm$  0.10 $\mu$ m (Fig. 13 & 14).
- Larger Dorsal Papillae (LDP): 4, forms quadrangle, two on each side of AHP; socket diameter ranges  $2.29 \pm 0.35 \mu m$  (Fig. 13).
- Smaller Dorsal Papillae (SDP): 6, three on each side, linearly placed on both sides of AHP; Socket diameter ranges  $1.96 \pm 0.26 \mu m$  Fig. 13).

# Culex (Culex) tritaeniorhynchus Giles



Fig. 11. Cibarial armature.



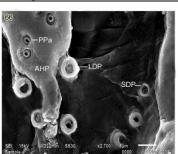


Fig. 13. Antero-dorsal membrane.

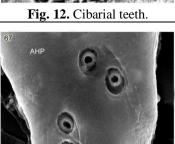


Fig. 14. Palatal papillae.

#### CONCLUSION AND FUTURE SCOPE

It is for the first time the scanning electron microscopy of cibarium have been done on the three species of *Culex vishnui* subgroup Complex: *Culex* (*Culex*) *tritaeniorhynchus* Giles, *Culex* (*Culex*) *pseudovishnui* Colless and *Culex* (*Culex*) *vishnui* Theobald. The new taxonomic characters will be helpful to solve the identification of closely related species complex very easily which are the prominent vector of Japanese Encephalitis in South Asia.

Abrreviations. AHP: Anterior Hard Palate; CA: Cibarial Armature; CB: Cibarial Bar; CT: Cibarial Teeth; LDP: Larger Dorsal Papillae; LF: Lateral Flange; PHP: Posterior Hard Palate; PPa: Palatal Papillae; SA: Shagreened Area; SDP: Smaller Dorsal Papillae; VP: Ventral Papillae.

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