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Scanning electron microscopic (Sem) studies on fourth instar larva and pupa of *Anopheles (Cellia) stephensi* Liston (Anophelinae: Culicidae)

Jagbir Singh Kirti and Shipali

Abstract

Anopheles (Cellia) stephensi Liston is a major vector species of malaria in Indian subcontinent. Taxonomists have worked on its various morphological aspects and immature stages to explore additional and new taxonomic attributes. Scanning electron microscopic (SEM) studies have been conducted on the fourth instar larva and pupa of *An. stephensi* to find additional taxonomic features for the first time from Punjab state.

Keywords: *Anopheles stephensi*, morphology, pupa, larva, scanning electron microscopy.

1. Introduction

Anopheles (Cellia) stephensi Liston is the most important malaria vector to large number of human population. It has been well said by taxonomist that it is a metropolitan malaria species. This species can be frequently found infected in nature i.e. both in gut and gland infections. The members of this species exploit all types of lentic aquatic habitats for breeding and breeds under natural conditions in pools, rivers, stream beds, drains and irrigation channels [4]. The larvae have the power to sink deeply and remain long period without reappearing at the surface. The species has adapted to human environment & use to live in wells in rural areas and now a days it breeds in water tanks on different floors of vertical buildings. Taxonomists have been exploring different and new taxonomic attributes on various immature stages and adult mosquito species. The morphology of larvae and pupa is not only useful for taxonomic purposes but also for interpretation of possible affinities among different genera and for host-parasite relationships of epidemiological value. Information on the biology and behavior of the vector species and its immature stages is essential to effectively control the transmission of mosquito borne diseases [1]. The larval and pupal stage of *Anopheles stephensi* were also described by Puri (1931) [12] and Christophers (1933) [4] but with the help of scanning electron microscopy it is done for the first time. In such studies, it is sometimes desirable to be able to identify the vector species in its immature stages.

Nobody has worked on larval and pupal stages of this vector species with the help of SEM studies. SEM studies have been conducted on last instar of larval and pupal stages of this species. The taxonomy of larval mosquitoes has been worked out widely but pupal stage is "neglected stage" for many mosquito collectors and often ignored in mosquito taxonomic studies [9].

2. Material and methods

For SEM studies, protocol given by Chaudhary and Gupta (2004) [3] followed. Larvae were collected from rice fields. Out of these, some larvae were killed in boiling water and remaining stored in 70% ethanol whereas, some were reared in the laboratory. The specific identification was made from the reared adult mosquitoes. The pupal exuviae were preserved in 70% ethyl alcohol immediately after emergence of adults to avoid rolling or breakage. For SEM studies, both larval and pupal exuviae were dehydrated in graded series of alcohol and only larvae not pupal exuviae were passed through Critical Dry Point and 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.

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For each larvae and pupal exuviae 5-10 specimens were examined. The terminology of various structures has been adopted from Harbach & Knight (1980) [7].

3. Results and Discussions

(A) Morphology of Larva

i. Mouthparts

Mouthparts of larvae mainly consists of Lateral Palatal Brushes (LPB), Anteromedian Palatal Brushes (APBr), Mandibular Brushes (MnB) and Mandibular Combs (MnC), Maxillary Brushes (MxB) and Mentum (Mt), Antennal Prominence (APr), Antennal Socket (AS), Dorsal apotome (DAp), Dorsomentum (DM), Frontal Ecdysial Line (FEL), Hypostomal Suture (HyS), Median Labral Plate (MLP), Mandible (Mn), Maxilla (Mx), Paraclypeal Lobe (PL), Ventromentum (Vm) (Fig. A & B).

ii. Antenna

Antenna (A) short and straight. Three types of sensilla namely Sensilla Basiconica (SB), Sensilla Trichodea Blunt (STB) and Sensilla Trichodea Pointed (STP) (Fig. C).

iii. Nuttall & Shipley's Organ (NSG)

It's a bilobed membranous structure borne dorsally on each side of pro-thorax, holds the thorax to water surface during feeding [7,8]. It is present only in Anopheline larvae (Fig. D).

iv. Mentum (Mt)

Mentum with four teeth on either side of median tooth, the first of these are very short and rounded, the first three more or less equidistant [4]. In the present study, mentum with three teeth on either side of median tooth (Fig. E & F).

Larvae of *Anopheles (Cellia) stephensi* Liston

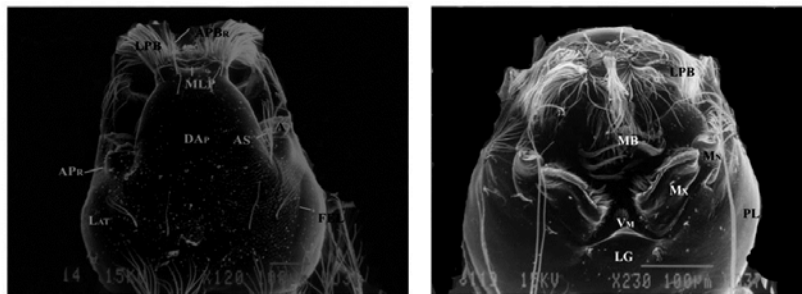


Fig. A & B. Dorsal & Ventral aspects of Head

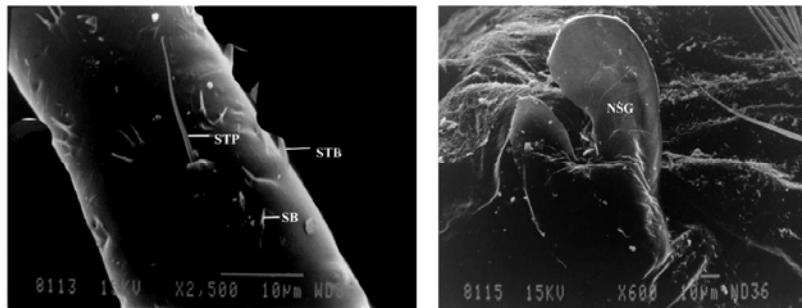


Fig. C. Antenna showing sensillae

Fig. D. Nuttall and Shipley's organ

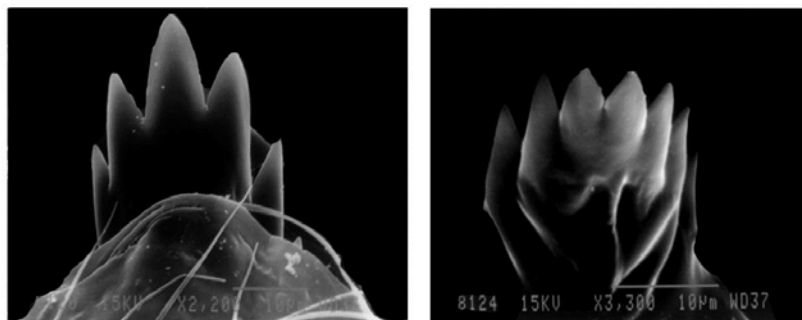


Fig. E-F. Mentum and associated structures

v. Segment X

It consists of Grid (G), Anal Papilla (APP), Saddle (Sa), Ventral Brush (VB). Grid is a network of sclerotized ridges which bear the cratal setae of the ventral brush with Transverse Grid Bar (TGB) at the base of individual seta, sometimes with Lateral Grid Bar (LGB) which may be

confluent with the ventro-posterior margin of saddle [11] (Fig. G-I).

vi. Spiracular apparatus

It is five lobed valvular structure encompassing the post abdominal spiracles, structurally comprising parts of embryonic abdominal segments VIII & IX. 7 (Fig. J).

vii. Pecten (PT)

It borne on the posterior margin of the pecten plate [7]. Pecten with 3-5 long and 8-11 short processes, all serrated on basal

half [4]. In the present study, 4 long and 9 short dentate-serrated processes are present (Fig. K).

Larvae of *Anopheles (Cellia) stephensi* Liston



Fig. G & H. Terminal segment IX (Dorsal & Ventral aspects)

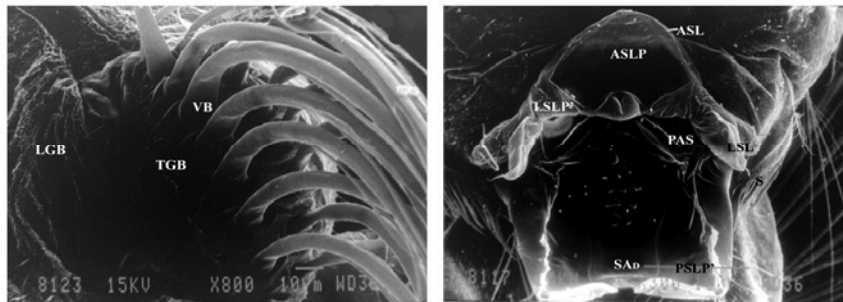


Fig. I. Grid

Fig. J. Spiracular apparatus

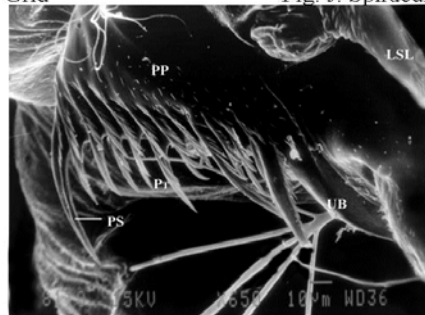


Fig. K. Pecten and Pecten plate

(B) Morphology of Pupa

As emphasize above, the pupal stage of various species has been neglected by earlier workers. The significance of pupal stage in *Anopheles* was highlighted by Reid (1963) [13] and Harrison & Peyton (1984) [9] described importance of structure of pupal trumpet in mosquito species. In the present study, trumpet and terminal abdominal characters have been studied in detail with the help of SEM and highlighted in present communication.

i. Trumpet

Trumpet (T) is a paired, usually movable, dorsolateral appendage of the cephalothorax containing the mesothoracic spiracle. Apex of trumpet is cut off obliquely to form Pinna (Pi) and the rest is known as Meatus (Mea). Two types of trumpet are known in mosquito pupae i.e. angusticorn and laticorn [7]. In case of *An. stephensi*, the trumpet is of angusticorn type i.e. trumpets having the longest axis vertical, more or less in line with stem. It is narrow funnel shaped when closed, with a split (meatal cleft) down one side, open widely

to provide passage for water [14]. In addition to this trumpet possesses a network of specialized spicules which covers the atrial wall, sometimes forming a perforated plate known as Filter Apparatus (FA) at its apex (Fig. L).

(C) Terminal abdominal segments

i. Genital lobe (GL)

It's a rounded projection lying ventral to the Proctiger (Pr) in female, usually small, inconspicuous and shaped like inverted U-shape (Fig. M & N) whereas in males, large and partially bilobed representing fused gonocoxopodites.

ii. Paddle (Pa)

Movable or immovable paired appendages of abdominal segment IX; appearing to arise from caudolateral part of abdominal segment VIII (Knight, 1971). In case of present species, paddle is broad, external Buttress (Bu) moderately strong and distinct; distal margin indistinct; Midrib (Mr) lightly darkened (Fig.N).

Pupa of *Anopheles (Cellia) stephensi* Liston

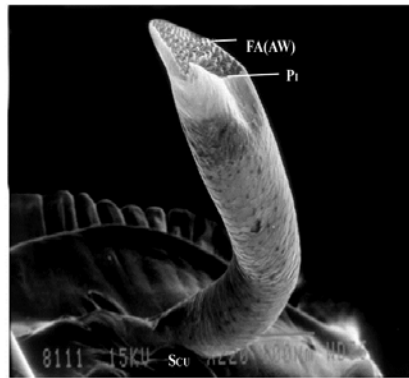


Fig. L Mesoposterior aspect of trumpet

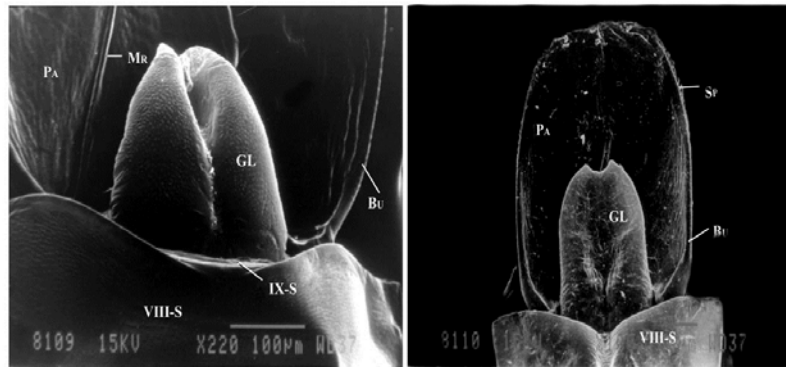


Fig. M & N Ventral aspect of terminal abdominal structures

4. Conclusion

Anopheles stephensi is major vector species responsible for urban malaria throughout India. Taxonomists and biologists have always tried to update its diagnosis by studying its different taxonomic attributes. Some of the eminent workers like Harrison & Peyton (1984) [9], Senevet (1930) [15], Christophers (1933) [4], Baisas (1936) [2] and Crawford (1938) [5] have conducted taxonomic studies on the present species. However, there is a still big gap in our knowledge as far as, taxonomic information on its immature stages is concerned. An effort has been made to study the fourth instar larva and pupal stage if this vector species with the help of Scanning electron microscope. The additional taxonomic attributes like number of teeth on mentum, short and long processes on Pecten and shape of trumpet are some of the additional taxonomic attribute which certainly prove useful in distinguishing and discriminating the larva and pupa of other allied species of *An. stephensi*.

5. Abbreviations

Anal papillae (APP), Antenna (A), Antennal socket (AS), Antennal Prominence (APr), Anteromedian Palatal Brushes (APBr), Buttress (Bu), Dorsal apotome (DAp), Dorsomentum (DM), Filter apparatus (FA), Frontal Ecdysial Line (FEL), Genital lobe (GL), Grid (G), Hypostomal Suture (HyS), Lateral Palatal Brushes (LPB), Longitudinal grid bar (LGB), Mandible (Mn), Mandibular Combs (MnC), Mandibular

Brushes (MnB), Maxilla (Mx), Maxillary Brushes (MxB), Meatus (Mea), Median Labral Plate (MLP), Mentum (Mt), Midrib (Mr), Nuttall & Shipley's Organ (NSG), Paddle (Pa), Paraclypeal Lobe (PL), Pinna (Pi), Proctiger (Pr), Saddle (Sa), Sensilla basiconica (SB), Sensilla trichodea blunt (STB), Sensilla trichodea pointed (STP), Transverse grid bar (TGB), Trumpet (T), Ventral brush (VB), Ventomentum (Vm).

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