HISTO-MORPHOLOGY OF THE LARVAE OF *Aedes aeygypti* (L.) (DIPTERA: CULICIDAE)

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Abstract

The external morphology fo the fourth instar larvae of *Aedes aegypti* (L.) (Dipter: Culicidae) was observed under under UV light in bright field condition at 20X under a Nikon optiphot florescent microscope. Histological studies of the brain, alimentary canal, and the internal cellular structures of the larvae were also observed using a Huma Scope Classic–110 GmbH microscope at 40X. *Ae. aegypti* larvae were identified by the presence of a single siphon and a single rowed comb in the tail region as observed in the florescent microscope. Histological evidence of the Corpora cardiaca-Corpora allata (CC-CA) complex was observed. Six of the outpockets of epithelium or gastric caeca were observed. Circular muscle, longitudinal muscle, brush border and peritrophic membrane were also identified.

Key words: *Aedes aegypti*, CC-CA complex, brush border, peritrophic membrane.

INTRODUCTION

Aedes aegypti is the most widely distributed mosquito in the world. In recent years, dengue a viral disease transmitted by infected female *Ae. aegypti* caused serious health problem in Bangladesh. Dengue fever was first reported in Dhaka as a 'Dacca fever' in 1964. After that some sporadic dengue incidence were also recorded. In year the year 2000, dengue appeared in Dhaka city as an 'outbreak' when 93 deaths were recorded (Hossain *et al*. 2000). People only can control dengue fever by controlling *Ae. aegypti*. In order to know the proper controlling method of any insect it must be known their full internal and external structure. Although many research have been conducted on the effectiveness chemical and biological insecticides against mosquito in Bangladesh (Ameen *et al*. 1982, Ahmed *et al*. 1986 and Begum *et al*. 2006, 2012, 2015), but no research has been reported in the study of the morphology of *Ae. aegypti* in Bangladesh. Morphological study is important not only for the insect control, but also for different functional aspects like insect physiology or endocrinology.

In its life cycle *Ae. aegypti* has four larval instars and a pupal stage; all are aquatic, whereas the adults are aerial. Most of the identification key of *Ae. aegypti* is based on the adult and 4th instar larval characteristics (Bar and Andrew 2013). The external morphology of the larvae of *Ae. aegypti* including head, neck, thorax, abdomen, mouth brush, palatum, preclypeal spines, mentum, compound eye, antenna, comb spines, siphon tube, pectin teeth and anal papilla were described by various researchers (Sevice 1996, Nelson 1986, Clements 1992). Internal morphology of the foregut, midgut and hindgut regions of mosquito was described in Zhaung *et al*. 1999. The present research describes the external structure of the head, thorax, abdomen and tail of the 4th instar larvae of *Ae. aegypti* and the histological structures of its respective larval stage.

MATERIAL AND METHODS

Rearing of Ae. aegypti

Ae. aegypti was reared in the laboratory of the Department of Zoology, University of Dhaka in an ambient environmental condition at 28±6°C and 70-80% RH. The adult mosquitoes were kept in a rearing cage made of steel frame (size: 30×30×30 cm), covered with mesh net. The larvae were kept in a water plastic bowl (7cm in diameter) covered with a piece of fine mesh net and they larvae were fed with cereals and adult female mosquitoes were fed with pigeon blood meal. Adult males were supplied

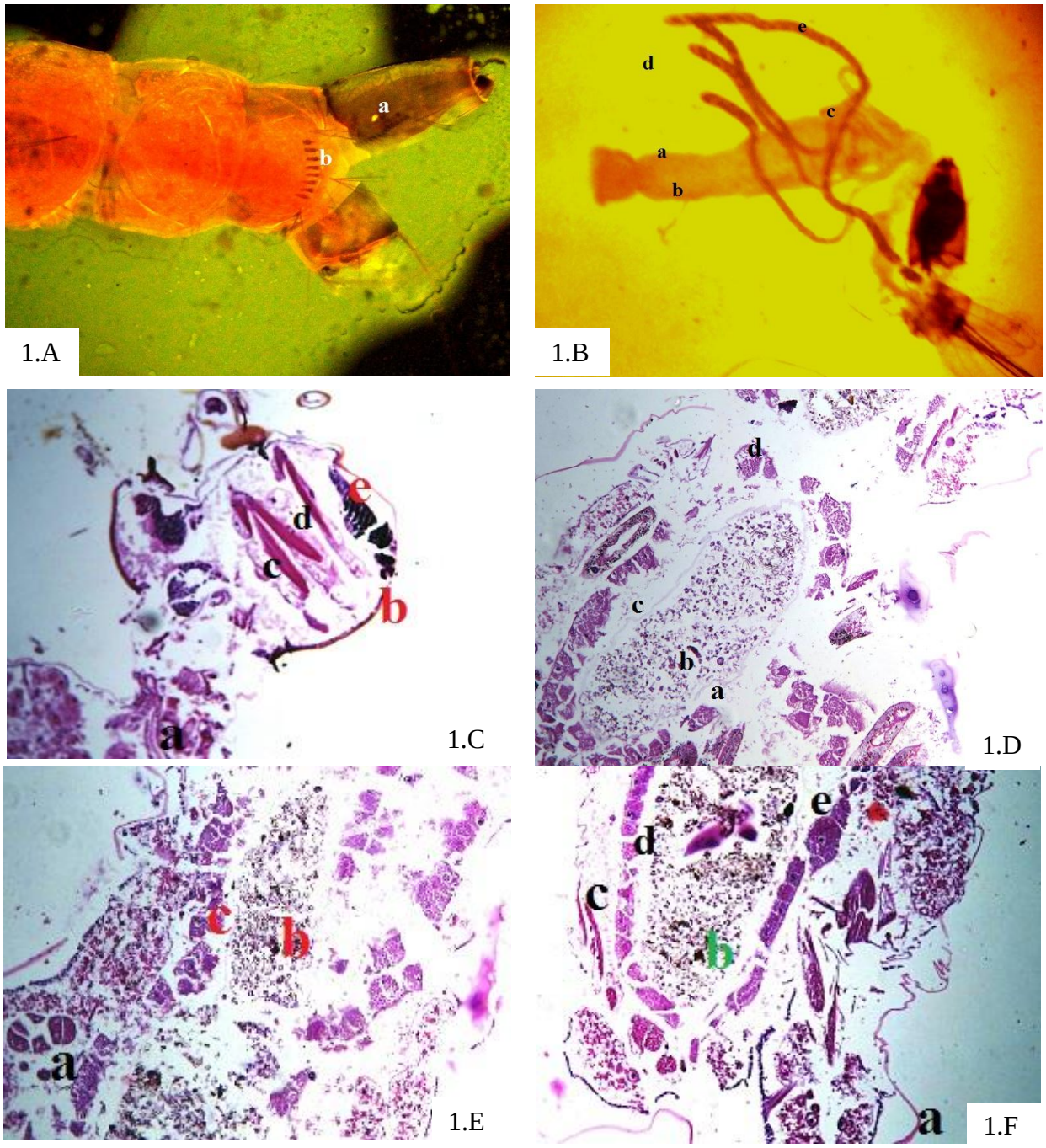

Fig. 1. A. Tail region of third instar larvae of *Aedes aegypti* (a. siphon, b. single lined comb); B. Alimentary canal and associated organs of fourth instar larvae (a. gastric caeca, b. anterior midgut, c. posterior midgut, d. foregut, e. Malpighian tubules); C. Longitudinal section of head capsule and foregut of the fourth instar larvae (a. foregut, b. head capsule, c. CC-CA complex, d. brain, e. compound eye); D. Longitudinal section of the foregut and the anterior midgut of the fourth instar larvae (a. peritrophic membrane, b. midgut contents, c. ectoperitrophic space, d. gastric caeca); E. Longitudinal section of the posterior midgut of the fourth instar larvae (a. circular muscle, b. midgut contents, c. brush border); F. Longitudinal section of the posterior midgut with tail and siphon of the fourth instar larvae (a. siphon, b. gut contents, c. longitudinal muscle, d. brush border, e. peritrophic membrane).

with sugar solution soaked in wads of cotton wool. Lengths and widths of the alimentary canal of the 4th instar larvae were also measured.

Histological slides and whole mount preparation

Histological slides of the 4th instar larvae of *Ae. aegypti* were prepared by longitudinal sectioning the tissues of the whole larval body. Ethanol, Myer's albumin and Xyline were used as fixatives. Serial longitudinal sections of the tissues were cut at 0.3µm thickness with the help of a rotary microtome machine (model 08–260–02, ERMA INC, Japan). The tissue sections were stained with eosin and Heidenhein's haematoxylene in the laboratory condition. Whole mount preparation was the same as the preparation of histological slides, except the use of rehydration and Heidenhein's haematoxylene.

Microscopic study

All microscopic observation were performed under a Huma Scope Classic–110 GmbH microscope. A Nikon optiphot florescent microscope was also used to observe the external larval morphology under UV light in bright field condition. Photographs were taken by a Canon power shot 5200 Wi Fi camera. A Nikon UFX–II camera was also used.

RESULTS AND DISCUSSION

Service (1996) identified *Aedes* larvae by siphon and single rowed comb present in the tail region. The present research also finds the same result (Fig. 1. A-B). The fore gut, anterior mid gut and posterior mid gut were identified using a stereoscopic binocular microscope (20X). The total length of the alimentary canal was 6.88±0.5 mm. The foregut was 1.71±0.06 mm long and 1.03±0.04 mm wide. Similarly the midgut was 2.80±0.31mm long and 0.53±0.04 mm wide; and the hind gut was 2.37±0.40 mm long and 0.63±0.02 mm wide (Table 1). The Malpighian tubules were 1.2±0.03 mm long and 0.02±0.003 mm wide. Bar and Andrew (2013) found that the 4th instar larvae was 7.7202mm long. The present data also represents similarity to the results of Bar and Andrew (2013).

Table 1. Measurements of alimentary canal and its different parts of 4th instar larvae of *Aedes aegypti.*

Different parts of the AC	Number of sample examined	Length (mm)	Width (mm)
Total length of the AC	15	6.88±0.5	- -
Different parts of the fore gut			
Pharynx	17	0.44±0.05	0.28±0.04
Oesophagus	16	0.91±0.08	0.33±0.05
Crop	19	0.37±0.03	0.41±0.02
Mid intestine	17	2.80±0.31	0.53±0.04
Malpighian tubules	21	1.20±0.03	0.02±0.003
Different parts of the hind gut			
Ileum	20	1.09±0.12	0.31±0.03
Colon	17	0.49±0.05	0.19±0.01
Rectum	17	0.79±0.08	0.14±0.02

Li *et al*. (2003) isolated the CC-CA complex of *Ae. aegypti* attached to the aorta which was connected to the intact head capsule. In the present research, histological evidence of the presence of this complex was also observed (Fig. 1.C). The foregut is divided into pharynx, oesophagus and crop. The midgut is divided into four parts, the cardia, the gastric caeca, the anterior midgut and the posterior midgut (Figs. 1. D-F). The hind gut is divided into ilium, colon and rectum. Histological study revealed that gastric caeca were found in the anterior midgut (Fig. 1. D).

Zhaung *et al*. (1999) found six out pockets of epithelium which form the cavities that connect medially with the midgut lumen. The present research holds the same things true. Circular muscle, longitudinal muscle, brush border and peritrophic membrane (pm) were also identified in the histological study (Figs. 1. C-F). Insect peritrophic membrane contains the microvilli which called the brush border are very important for the pesticidal mode of action. For example, the active toxin pass through the peritrophic membrane (PM) and binds to specific receptor of the brush border membrane vesicles (BBMVs) of the midgut epithelial cell (Gill *et al.* 1992, Hofmann *et al.* 1988, Van Rie *et al.* 1989, de Maaged *et al.* 2001, Knowles and Dow 1993). Begum *et al.* (2015) identified only the digestion of the cellular layer of the mid gut by the treatment of *Bacillus thuringiensis israelensis*.

Jones and Zeve (1968) identified two types of cells in the electron micrographic study. In one type of cell there were microvilli that do not contain mitochondria and cytoplasm, and other type of cell has long thick microvilli, each of which contains a mitochondrion. The present observations were done only under a compound microscope (40X) and no mitochondrion was observed but microvilli were possibly found in the brush border.

The previous authors also identified the presence mitochondrion or microvilli in the other parts of midgut (viz. Jones and Zeve 1968, Hecker 1977). However, the mitochondrial presence in the various parts of midgut of insects may play a role in the programmed cell death during metamorphosis (Gillbart 2009). This type of cell organelle may be absent in the midgut of adult insect. Further study is required for development and metamorohosis.

It can be said that the present research describes the basic morphology of larval *Ae. aegypti*. Further research may be done using latest technological procedure to unexplor the function of different cell organelles found in insect.

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