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High-Resolution Microscopic Images of Mosquito Vectors (Diptera: Culicidae): Proper Identification of *Culex* and *Aedes* during Dengue Outbreak Situation in Bangladesh

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ABSTRACT

High-resolution microscopic images of the 8th abdominal segment, comb scales, pectin, tuft of hairs, saddle, head antenna, thoracic hooks and other morphological characteristics of *Culex quinquefasciatus, Aedes aegypti* and *Aedes albopictus* have been provided for the first time in Bangladesh. Mosquitoes spread deadly diseases (dengue, chikungunya, Japanese encephalitis, yellow fever, malaria, filariasis) that kill millions of people every year including Bangladesh. During dengue and dengue outbreak situations, it is very much necessary to identify the dengue-occurring mosquito vectors. Proper identification of the mosquito vectors of Bangladesh is the key factor for public health protection and disease prevention. Larval movement patterns and habitat characterization were also observed. Results obtained in this study revealed that dengue virus can also be carried by other Aedes species rather than *A. aegypti*. Results were discussed with previous study dealing with *Aedes* mosquito samples of Bangladesh. The findings will be very useful for the proper identification of dengue mosquito vectors in Bangladesh and the knowledge assists in the accuracy of mosquito control activities as well as prevention of mosquito-borne diseases.

1. Introduction

Mosquitoes are the most common insects occurring in many regions around the world, particularly in tropic and sub-tropical climate areas. There are over 40 genera and over 3,500 known species of mosquitoes. Among these genera, *Anopheles, Aedes* and *Culex* have species transmitting diseases that impact people, including malaria, dengue fever, West Nile Virus, and the Zika Virus. Mosquito vector-borne diseases like dengue, Japanese encephalitis, yellow fever, malaria and filariasis are the major public health concern in Bangladesh (Hossain *et al.* 2000). Among the mosquito vector-borne diseases Dengue fever (DF) is one of the worst systemic viral diseases that established globally both in endemic and epidemic transmission cycles and continue to increase every year (Bhatt *et al.* 2013; WHO 2023). In most cases dengue fever is self-limiting with no or mild infestation that causes lifelong immunity for that serotype (WHO- Bangladesh 2022). However, it can lead to deadly dengue shock syndrome and has the risk of fatal outcomes (Teo *et al.* 2023).

Currently, dengue fever (DF) and dengue haemorrhagic fever (DHF) are considered the most emerging disease and concern of public health problems in all the major cities in Bangladesh. This situation becomes worse in Dhaka city where more than 90% of dengue cases were noticed (Sharmin *et al.* 2015; Priyanka *et al.* 2022). Last few years dengue epidemic situation has broken down all previous records in Bangladesh due to the suspension of all kinds of mosquito surveillance under the COVID-19 pandemic and prevailed round the year (monsoon and post-monsoon) in recent years (Haider *et*

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al. 2021; Hossain *et al.* 2023). Aedes mosquitos are also the main vector of other arboviruses like chikungunya and zika (Kraemer *et al.* 2015).

Dengue case is nothing new in Bangladesh but recent deaths and casualties of public health make a great concern to the social safety net. Dengue and dengue hemorrhagic fever re-emerged in 2000 and became endemic in Bangladesh. Last 5 years, dengue outbreaks pictures are 2769 cases in 2017 to 10148 cases in 2018; in 2019, Bangladesh recorded the highest number of dengue cases 1,01,354, ever since the disease's official outbreak in 2000. That year, 179 people succumbed to the virus. Then came 2022, the year when the country recorded the most dengue deaths- 281 (DGHS 2023, The Daily Star, 20 Sep 2023). On 1st January 2024, The Business Standard presented its online version summarizing the reports of dengue cases of the Directorate General for Health Services (DGHS) from 2000-2023 in Bangladesh (Figure 1). It showed that only a minimal number of Dengue related death case (482) was recorded in twenty years from 2000-2020 period. After that, both the infection and death numbers accelerated. It reached the infection case 62,382 in 2022, among them 281 were confirmed death. But in 2023, the Dengue case broke down all of its previous records and it touched the highest number of hospitalized cases 321,179 and 1,705 deaths with 0.53% mortality rate. Among them, 24.78% deaths were reported in September 2023. Most affected areas were in Dhaka City Corporation where 35.26% affection was recorded. Other affected areas were Chittagong division (4.42%) and Barisal division (4.24%) (DGHS 2023, The Business Standard 2024).

Accurate identification is quite difficult for species level of vector mosquitoes and their larva as well.

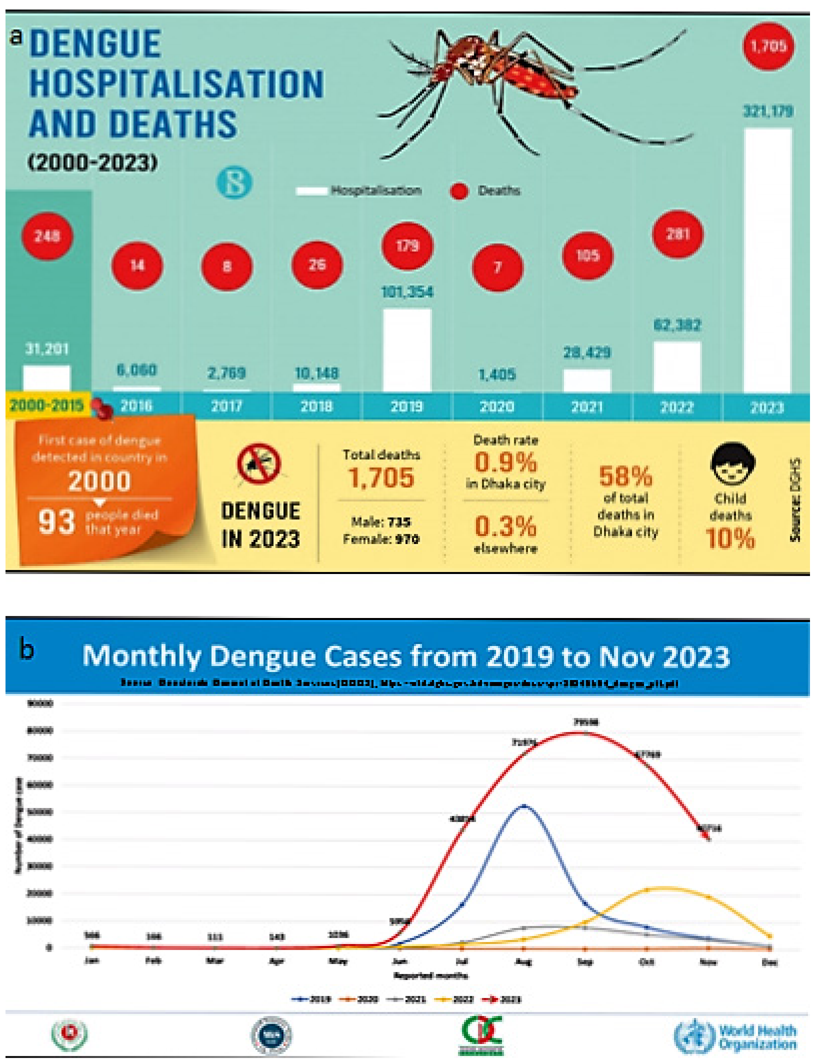


Figure 1. Dengue scenario of last twenty three years in Bangladesh. https://www.tbsnews.net/bangladesh/health/dengue-deaths-2023-double-total-toll-23-years-767546

For instance, *C. quinquefasciatus* belongs to the *C. pipiens* complex (Miller *et al.* 1996). It is very tough to identify them because members of this complex are morphologically similar (Nelms *et al.* 2013). Identification problems are further compounded as *C. quinquefasciatus* can easily be hybridized with other species, in particular with *C. pipiens* (Sanogo *et al.* 2008) and their ranges can be overlapped (Farajollahi *et al.* 2011). Similar situations have been observed in *Ae. albopictus* and *Ae. aegypti*. They share nearly identical habitats, food sources, geographical locations, and are vectors for similar types of viruses as well (Stark *et al.* 2017; Souza-Neto *et al.* 2019).

Many cases of dengue are misclassified because of the wide spectrum of disease signs and symptoms and lack of effective case definitions. Therefore, it is highly probable that dengue cases may be substantially under-reported in Bangladesh given the weak surveillance of mosquito species and healthcare system. Dhaka is the densely populated capital city in Bangladesh, it has a population (approx. 16 million) and had the highest number of dengue cases between 2012 and 2019 (WHO 2023). However, a knowledge gap remains in distinguishing larval morphological characters, despite extensive research on the effectiveness of traditional chemical and biological insecticides against mosquitoes (Begum *et al.* 2015). No research has been reported on the morphological study of *C. quinquefasciatus, Ae. albopictus* or *Ae. aegypti* in Bangladesh. So, mosquito larva spot identification is still intriguing subject. It is very crucial to know their habitat characterization and behaviors as well as mosquito surveillance programs before implementing any control activities. Through these backdrops, the present study had been undertaken to identify the larva and adult mosquitos following the established morphological characterization providing high-resolution microscopic images for the first time in Bangladesh. The current rise in the number of dengue cases in Bangladesh is due to the lack of detailed fundamental information on mosquito biology, morphology and behavior (collected from the field). Other causes are widespread mosquito vectors, invasive mosquitoes from the neighboring countries- India and Myanmar, rapid and irregular urban development, international travels, and the lack of effective control measures (DGHS 2023). Moreover, last few years dengue epidemic situation has broken down all previous records in Bangladesh due to the interruption of all kinds of mosquito surveillance during the COVID-19 pandemic. Hence, considering the continuous dengue outbreak situation, prediction of the epidemiological warning and its control requires fundamental information about the species composition of the corresponding mosquito vectors. A more accurate species-level identification of mosquitoes is essential.

In Bangladesh, much research has been carried out focusing on the effectiveness of chemical and biological insecticides against mosquitoes (Begum *et al.* 2015), but research data on the accurate morphological identification of *C. quinquefasciatus, Ae. albopictus* and *Ae. Aegypti* are inadequate and unsatisfactory at the field level control measures. So, the purpose of the study is to provide information on mosquito larval habitat, their movement pattern and spot identification during dengue out-break situation which are very much essential for mosquito surveillance programs before implementation of any control activities. In the present study, high-resolution microscopic images of field-collected mosquitoes have been provided for the first time in Bangladesh, which could provide useful information for species-level identification. Results can be used for the operative prevention and control of mosquito and mosquito-borne diseases in Dhaka city as well as the other infected areas of Bangladesh.

2. Materials and Methods

2.1. Sampling Sites

Unplanned and non-scientific development disrupts natural mosquito breeding zones, such as those found in city sewerage areas. These developments are now exported in the villages where semi-urbane areas are being built. Therefore, mosquitos and mosquito-borne diseases are common in both cities and villages in most parts of Bangladesh. A year long (January to December 2023) mosquito larva sampling program was conducted from the breeding sites of six districts of Bangladesh. Sampling sites were the Jagannath University (JnU) campus in Old Dhaka and Atomic Energy Research Establishment (AERE) campus (Dhaka district), Parulia (Satkhira district), Darsana (Chuadanga district), Baparipara Eidgha (Narshingdi district), Bilsha Bazar (Natore district) and National Academy for Computer Training and Research (NACTAR) adjacent area (Bogura district) (Figure 2 and Table 1).

2.2. Sample Collection

Mosquito larvae were randomly collected using the standard dipping method from different habitats (drains, sewerage lines, abandoned water bodies) mostly that are designated as *Culex* breeding sites. Indoor and outdoor

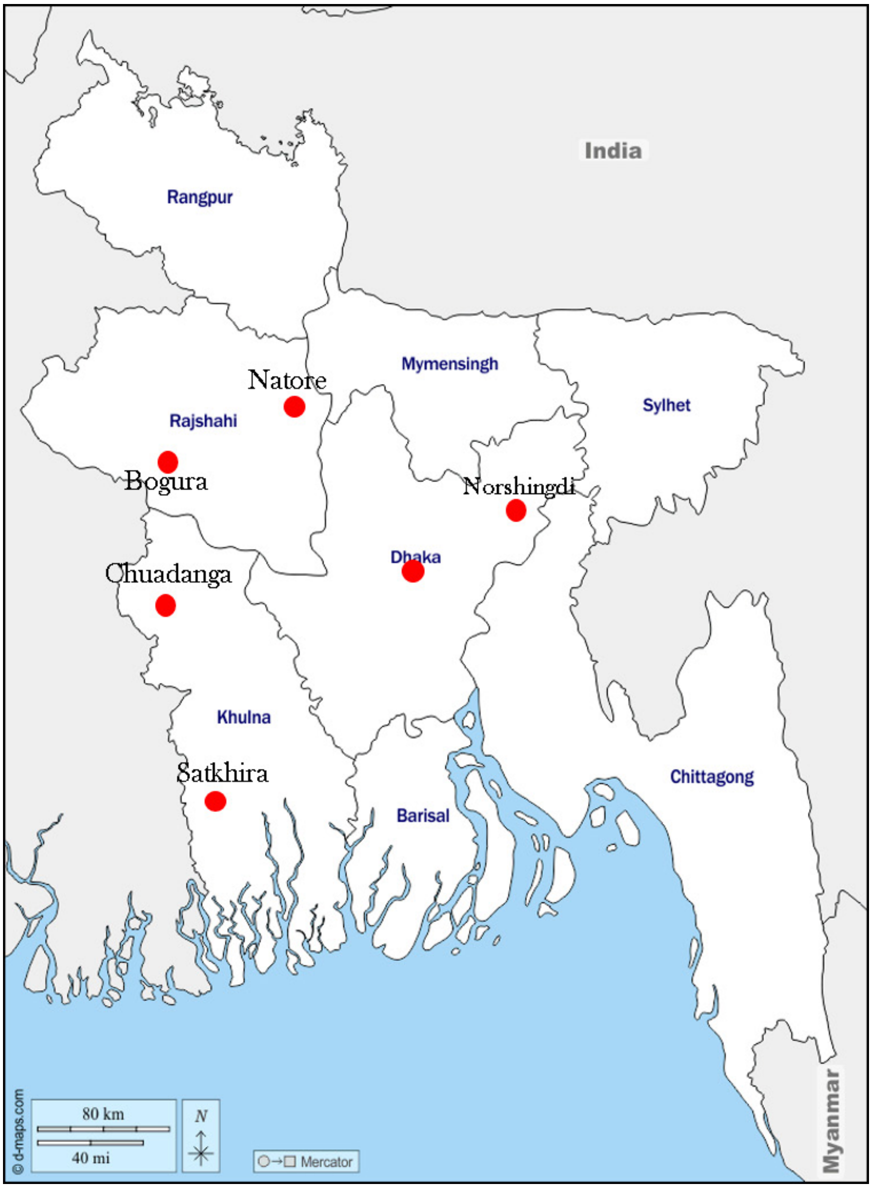


Figure 2. Administrative Map of locations of the study areas; Red circles indicate sample collection districts; Map source at https://d-maps.com/carte.php?num_car=2144&lang=en, 08/08/2023

Table 1. Location of mosquito larvae sampling sites

Location of sampling sites			
Name of sampling site	District	Latitude	Longitude
JnU	Dhaka	23°42'31.4"N	90°24'40.7"E
AERE	Dhaka	23°57'17.7"N	90°16'46.8"E
Parulia	Satkhira	22°36'47.4"N	89°00'03.5"E
Darsana	Chuadanga	23°31'15.5"N	88°47'42.7"E
Baparipara Eidgha	Narshingdi	23°55'58.4"N	90°43'02.1"E
Bilsha Bazar	Natore	24°25'01.2"N	89°16'11.8"E
NACTAR	Bogura	24°49'20.8"N	89°21'46.4"E

water reservoirs discarded plastic bottles, abandoned tires and tubes, discarded metallic buckets, trays, plastic mugs, ice-cream cups, flower tubs, etc. were checked and larval samples were collected from the common breeding sites of *Aedes* mosquitos.

Instantly the samples were preserved in a 200 ml transparent plastic container with 70% ethanol for subsequent morphological identification. Containers were labeled with the location, date of collection and type of habitats etc., and kept for further investigations. Some of sampled larvae were preserved separately to continue rest of their life cycle (from larva to adult) for identification in the adult stage. Characterization of different sampling sites is shown in Figures 3, 4 and Table 2.

Figure 3. *Aedes* mosquito breeding sites, *(A), (B), (D), (E), (J): Jagannath University sampling sites; (C), (L): Satkhira sampling sites; (G): Norshingdi sampling site; (F), (I): Darsana sampling sites; (H), (K): AERE sampling sites

2.3. Morphological Identification

Mosquito larvae were poured in a Petri dish and primarily separated with droppers and forceps observing external morphology, body shape, head size, antennal characteristics and the presence and size of the siphon. In the case of live samples, larval movement behavior is also considered for the primary differentiate of genus (*Culex* and *Aedes*). The fourth instar mosquito larva was chosen because the larva reaches its full development and the morphological identification keys depend totally on the characteristics of the larva. For example, *Aedes* larvae possess a specific comb-scale pattern, whereas other mosquito larvae have an irregular pattern in comb scale. The entomologists mostly examine the comb-scale pattern to identify an *Aedes* larva under stereo zoom microscope.

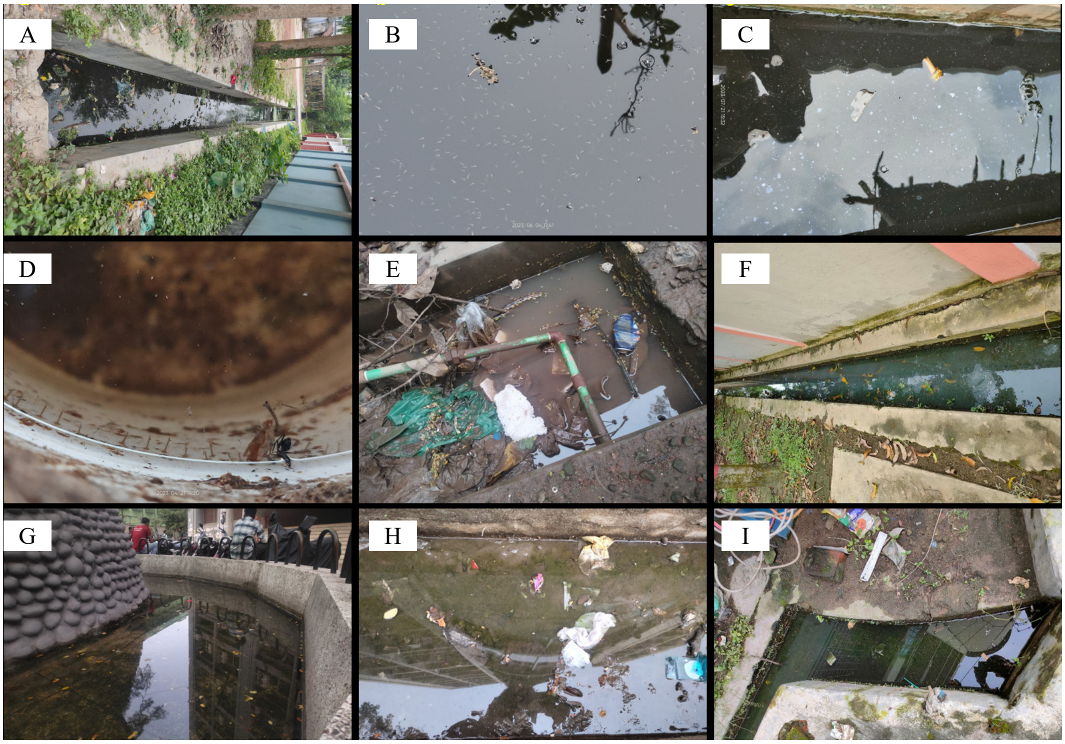


Figure 4. *Culex* mosquito breeding sites; *(A), (B): Darsana sampling sites; (C): Natore sampling site; (D):Satkhira sampling site; (E), (G): Jagannath University sampling sites; (F),(I): NACTAR sampling sites; (H): Natore sampling site

Table 2. Characteristics of mosquito larval habitats

Location	Types of breeding sites	Habitat characteristics	Identified species			Total
			Culex quinquefasciatus	*Aedes albopictus*	*Aedes aegypti*	
JnU	Plastic water bottles	Semi-shade in vegetation	00	510	00	510
	Ice-cream cups	shade in vegetation	50	295	00	345
	Clay pots	semi-shade	65	260	00	325
JnU, Darsana	Flower tubs	indoor and sunny/shade	247, 00	438, 365	00, 00	1050
	Gutters	open and sunny/semi-shade	306, 132	00, 00	00,00	438
JnU Baparipara Eidgha	Pools	sunny and semi-vegetation	157, 129	115, 00	00, 00	401
Parulia	Metallic buckets	indoor and semi-shade	00	353	00	353
	Plastic mug	indoor and semi-shade	00	35	00	35
	Paint buckets	indoor and shade	206	405	00	611
Parulia, Darsana, Bilsha Bazar	Drain	open and shade	357	00	00	705
			306	00	00	
			42	00	00	
NACTAR	Sewerage drain	semi-shade	148	00	00	148
Baparipara Eidgha	Abandon water bodies	sunny in vegetation	60	27	25	112
AERE	Tires and tubes	outdoor and shade	00	187	00	187
	Metallic trays	outdoor and semi-shade in vegetation	70	174	00	244
	Plastic cup	semi-shade	456	235	00	691
	Total		2731	3399	25	6155

JnU: Jagannath University; AERE: Atomic Energy Research Establishment; NACTAR: National Academy for Computer Training and Research

Fourth instar mosquito larvae were transferred directly into a drop of lactic acid using a fine brush and dropper. Larva was then mounted to keep the dorsal side up. A coverslip was put on the larva and in some cases thumb press slightly on the slide for preparation and removal of air bubbles. Morphological identification of the larva was performed using the following microscopes: Stemi 305 SZ with Axiocam 105 color camera (Carl Zeiss Germany), Olympus CX41 (Japan) and Zenithlab XSZ-107BN (USA). Adults were also examined with the stereo zoom microscope. Specimens were identified using the morphological characteristics according to Andreadis *et al.* (2005) and Mohamed *et al.* (2017). Experiments were conducted in the Cytology and Biocontrol Research (CBR), Radiation Entomology and Acarology Division (READ), Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Savar, Dhaka, Bangladesh.

3. Results

3.1. Physiological Observation and Larval Habitat Characterization

We collected 6155 mosquito larvae from different habitats of the sampling sites during the study period (January-December 2023). Among them, 2731 were *Culex* and 3399 were *Aedes* larvae (Table 2). Percentages of total samples are shown in Figure 5. At least 15 to 70 larvae from the collected samples in each habitat were randomly examined under the microscope. *Culex quinquefasciatus* larvae were the most abundant in semi-shade drains, sewerage lines, and abandon and debris water bodies sampled from old Dhaka, Darsana, Parulia, Bilsha Bazar and NACTAR campus adjacent area. *Ae. albopictus* and *Ae. aegypti* larvae were frequently observed in both indoor and outdoor stagnant water reservoirs in semi-shade and shady locations i.e. discarded plastic bottles, abandoned tires and tubes, discarded metallic buckets, trays, plastic mugs, ice-cream cups, curd pots, flower tubs in the sampling sites (Table 2). A mixing of *C. quinquefasciatus* and *Ae. albopictus* larvae were found in the Darsana sample where rainwater was stagnant in flower tubs for five days while only *Ae. albopictus* larvae were noticed in the plastic buckets half filled with rainwater for 4 days. The same situation was observed in Parulia sample where *Ae. albopictus* larvae gather in the storing rainwater in discarded aluminum buckets, plastic mugs and paint containers. In the AERE campus, we found 187 *Ae. albopictus* larva in 150 ml rainwater contained in a discarded vehicle tube. Almost the same larval density was noticed in stagnant rainwater in the metallic tray on this campus where we found 235 *Ae. albopictus* larva in 300 ml rainwater stored in shady place. *Ae. aegypti* larvae were identified only from the Norshingdi sample (Table 2).

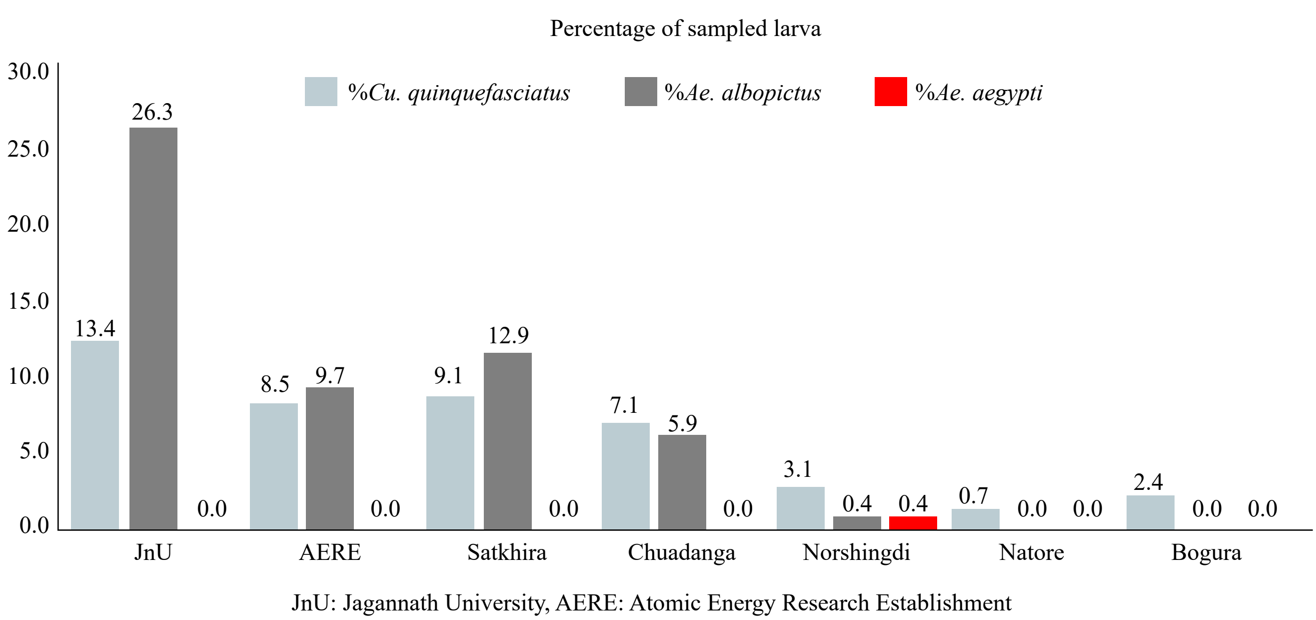


Figure 5. Percentage of mosquito larvae sampled from different sampling sites

3.2. Morphological Characteristics

3.2.1. Larva

3.2.1.1. *Culex quinquefasciatus* Larva

Culex quinquefasciatus larvae were identified collected from all selected sampling sites. The observable characteristics are as follows:

(a) Larvae lay suspended from the water surface with nearly 70-90° angle (Figure 6).
(b) The body of the larvae composed of three regions: head, thorax and abdomen (Figure 7).
(c) It followed to move over the water surface with a series of jerky movements.
(d) The anal segment had four elongated white protrusions. Morphologically it is designated as anal gills or anal papillae (Figure 8). It is considered one of the osmotic regulatory organs of the larvae. An anal brush near the base of anal gills works like a rudder at the time of swimming of the larvae.
(e) The head of the larva was sclerotized and not completely rounded (Figure 7). It slightly flattened and bears two eyes, two antennae and brush-like mouthparts. The antenna long and had a tuft of hair beyond the middle (Figure 9).
(f) Thorax appeared with the big elliptical segment and separated by a narrow neck.
(g) Lateral thoracic hairs had no hook in their base (Figure 10), which was very much prominent in Aedes species.
(h) Abdomen with 10 segments but the ninth was not distinct. It may be fused with the eighth segment.
(i) Each abdominal segment contained a hair bristle in the middle-lateral side. It is visible as the somewhat pointed part of the abdomen (Figure 7). Lack of palmate hairs on the dorsal side of its abdominal segments that is found in the Anopheles species.
(j) Air tube or siphon located in the 8^{th} abdominal segment. It allows the larvae to breathe in the aquatic environment and it spend most of their time on surface breathing. In most cases, the air tube is 3 to 5 times longer than wide (Figure 8). The air tube of this species was not pointed like the Mansonia species, and it contained four hair tufts on each side that had not decorated in a straight line. Sometimes it followed a haphazard fashion of hair tufts in the air tube, especially the 4th one.
(k) A patch of comb scales was found on the dorsal side of the 8th abdominal segment. Together it makes a half-circle structure or pyramid shaped. Terminal region of the comb scale was hairy and only visible after at least 400× zoom (Figure 11).
(l) Pecten is located with a row of closely set teeth on each side of the siphon (air tube). Number of pecten was 7-8 in *Cu. quinquefasciatus* and it started from the abdominal side. Each pecten had five long speculations. Basal speculate of pecten was comparatively short and the rest one gradually elongated (Figure 12).
(m) Saddle elongated and it formed a ring like cover of the last abdominal segment. Ventral brush was based with specialized muscles and tissues. It makes a characteristic feature of this species (Figures 13 and 25).

3.2.1.2. *Aedes albopictus* Larva

We found 55.20% *Aedes albopictus* larva from the selected sampling sites. The observable characteristics are as follows:

(a) Larvae lay suspended from the water surface at nearly 45-70° angle (Figure 6).
(b) Body of the larvae had three regions: head, thorax and abdomen like the *Culex* species (Figure 14).
(c) Eyes appeared with a black spotted on the lateral side of the head that was comparatively smaller than *Culex* species (Figure 14).
(d) The combined thoracic segment appears with an elliptical shape not too much flattened than the head (Figure 14) and it is separated by a narrow neck.
(e) Abdomen region was composed of ten rhomboid segments among them one (9^{th}) fused with the previous segment (Figure 14).
(f) Each abdominal segment contained a prominent hair bristle in the middle of its lateral side (Figure 14).
(g) Head of the larva was sclerotized and almost rounded, wider than length. It is composed of two eyes, two antennae and brush-like mouthparts.
(h) Mouthparts of this larva are modified to detritus feeding while they spend most of their time in the detritus region in the habitat. It comes to the surface layer only in the breathing time.

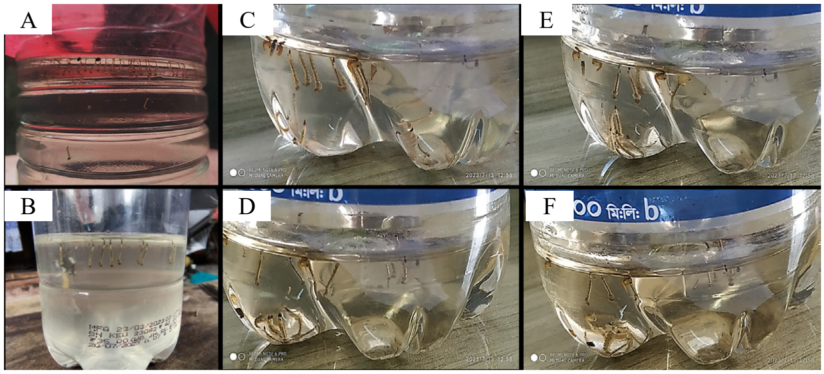


Figure 6. O_2 inhaling and hanging behavior of mosquito larva; (A) *Culex*, (B-F) *Aedes* larvae

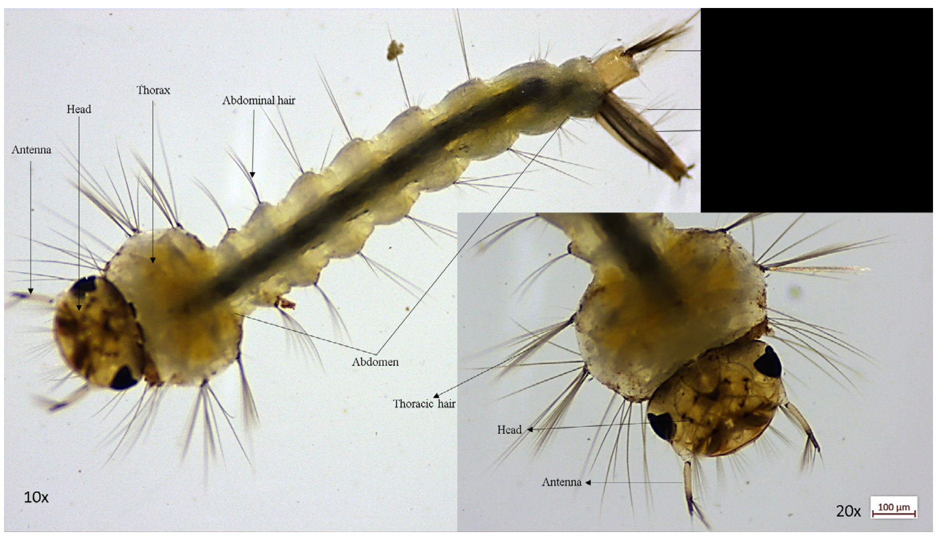


Figure 7. 4th instar Larva of *Culex quinquefasciatus*

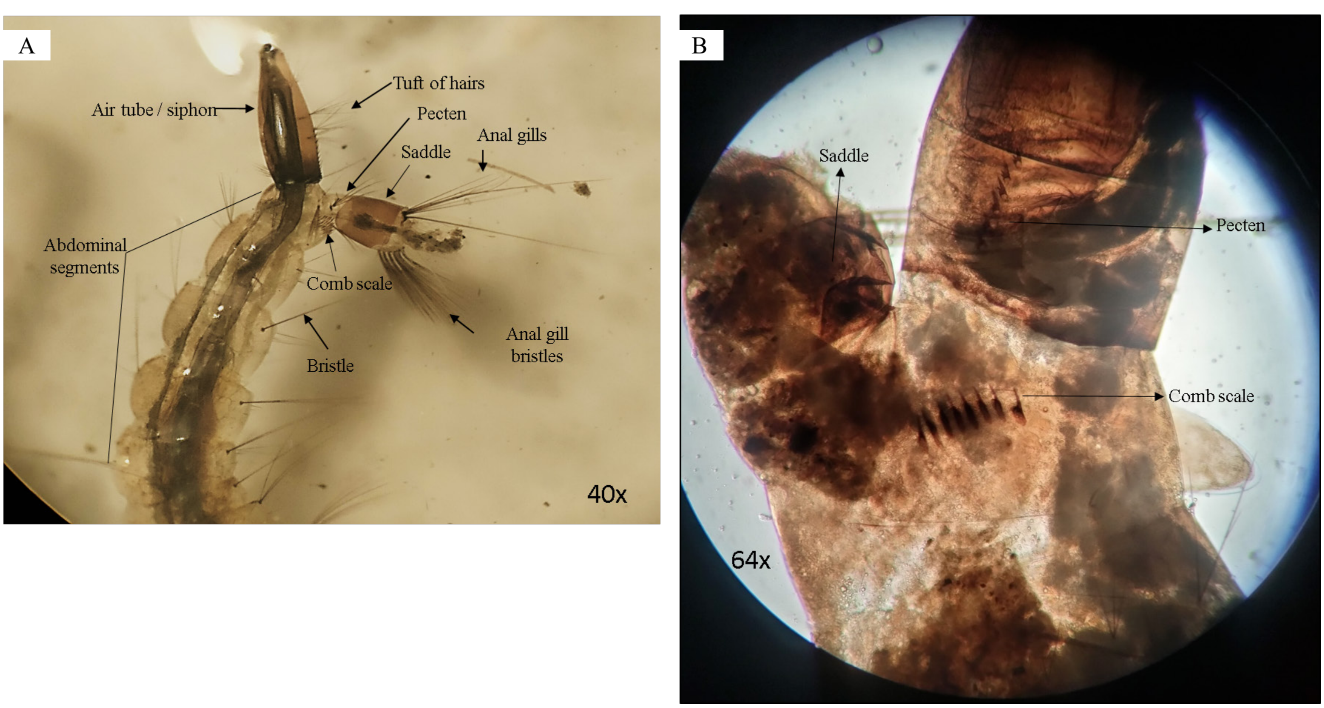


Figure 8. Posterior region of *Aedes* and *Culex* larva; (A) *Culex quinquefasciatus*, (B) *Aedes albopictus*

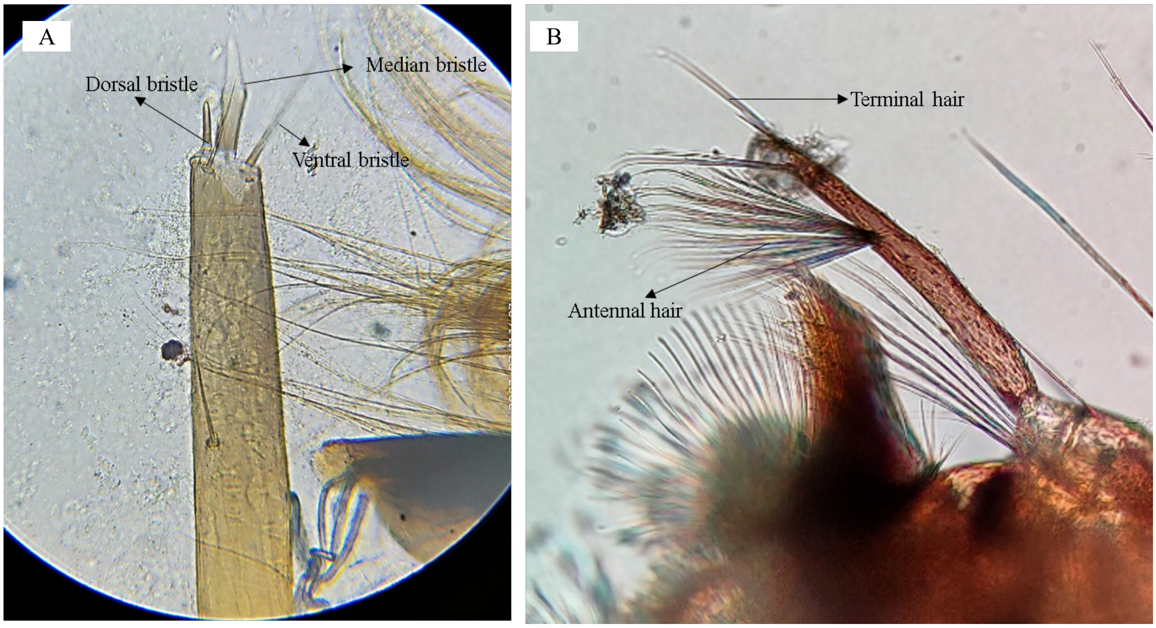


Figure 9. Antenna of *Aedes* and *Culex* larva; (A) *Aedes albopictus*; (B) *Culex quinquefasciatus*; 640×

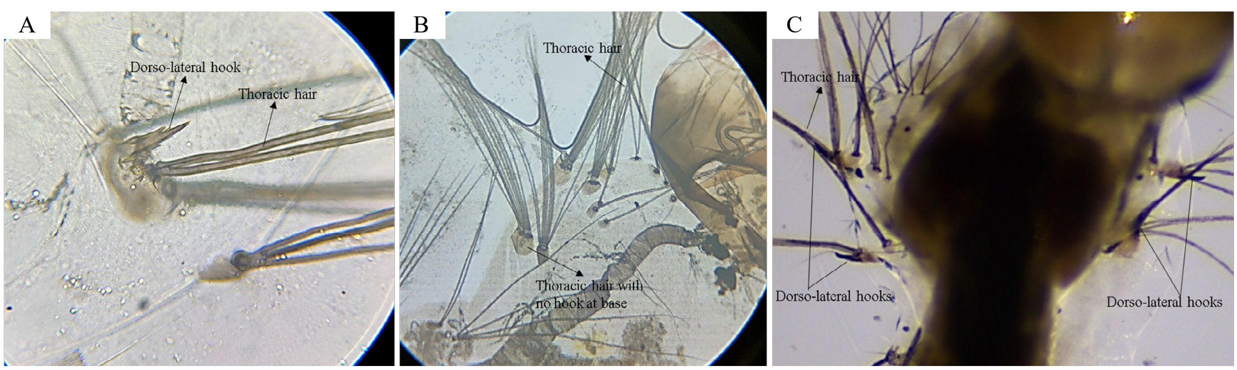


Figure 10. Thoracic hooks of *Aedes* and *Culex* larva; (A) *Aedes albopictus*, (B) *Culex quinquefasciatus*, (C) *Aedes aegypti*; 640×

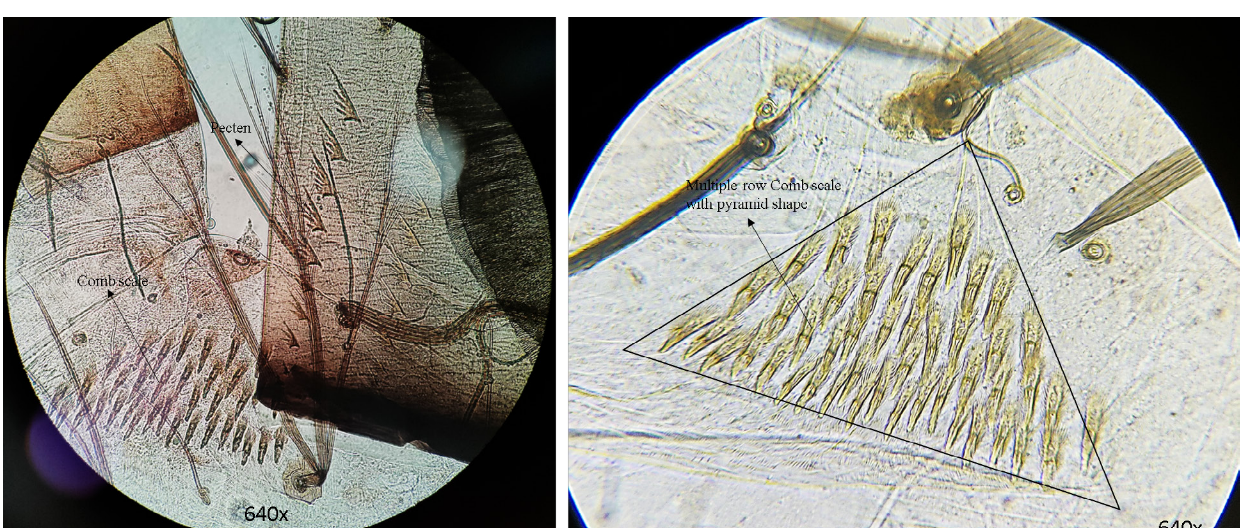


Figure 11. Comb scales of *Culex quinquefasciatus* larva; multiple row comb scale with pyramid shape

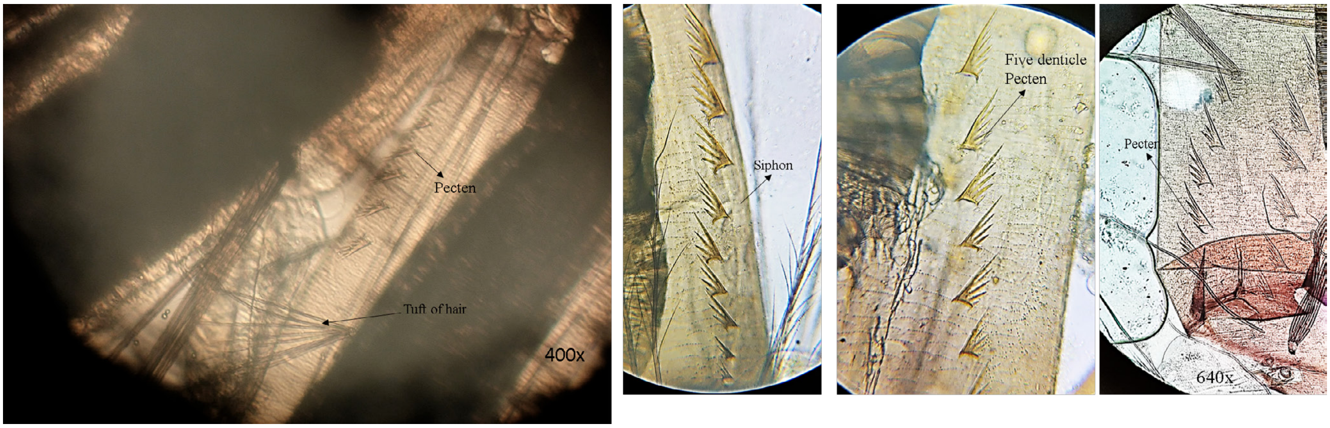


Figure 12. Pecten of *Culex quinquefasciatus* larva

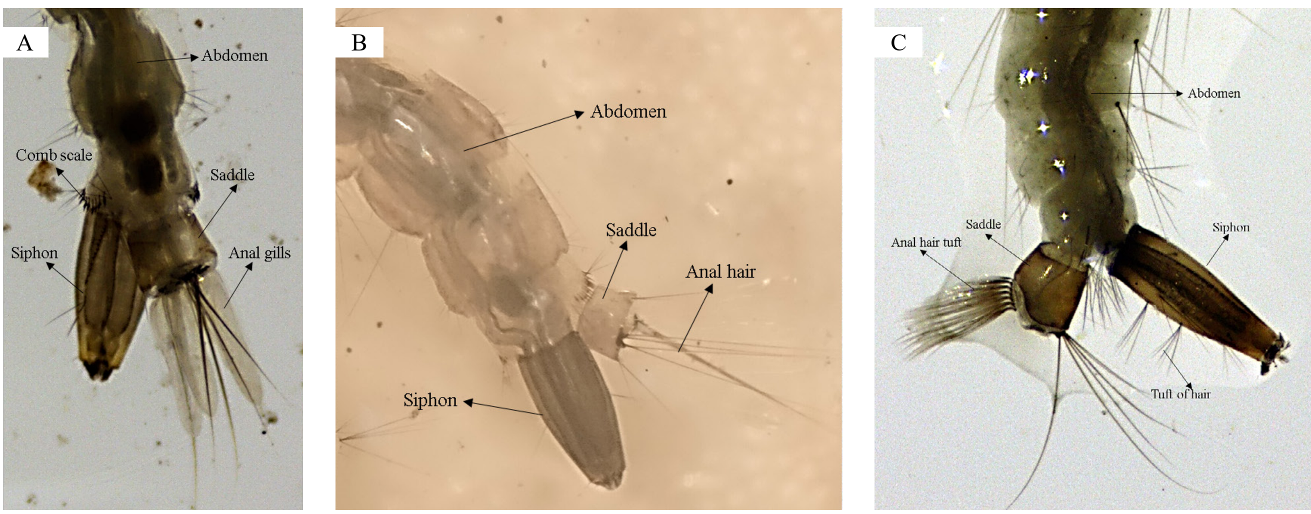


Figure 13. Siphon and saddle of *Aedes* and *Culex* larva; (A) *Aedes aegypti*; (B) *Aedes albopictus*; (C) *Culex quinquefasciatus*; 40×

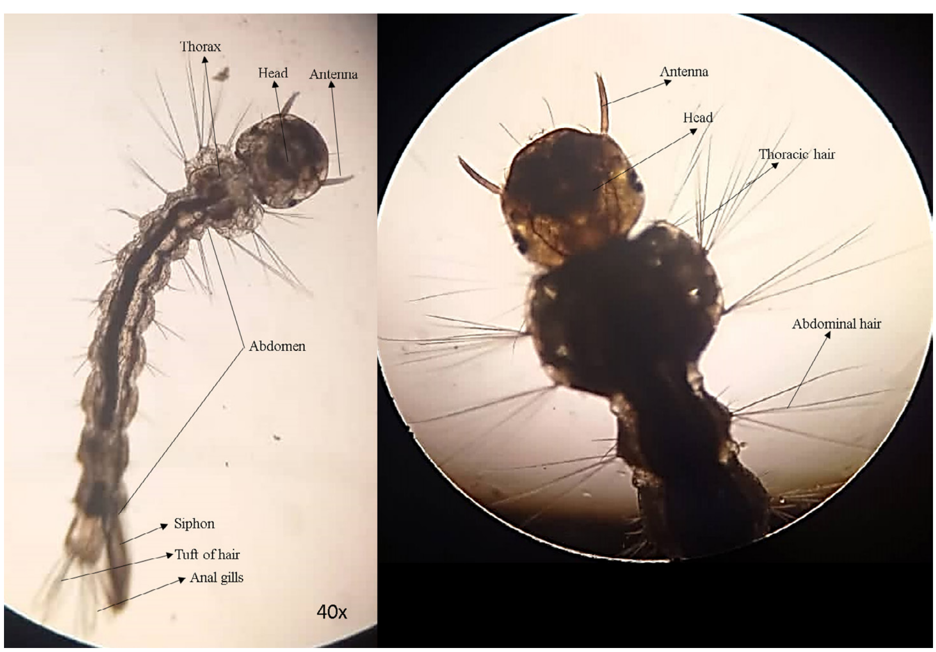


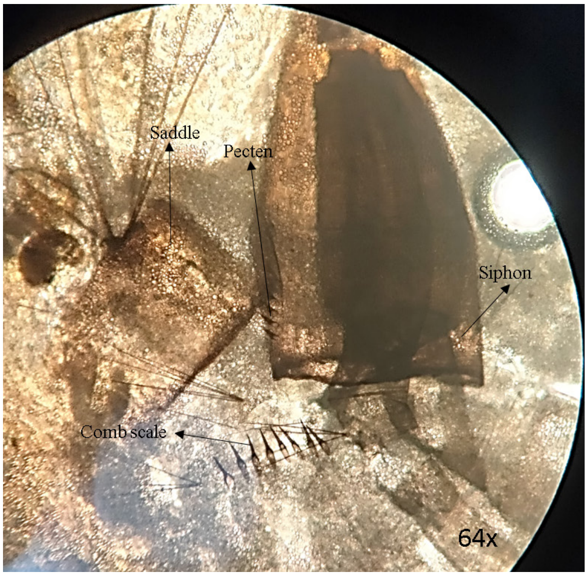
Figure 14. 4th instar Larva of *Aedes albopictus*

(i) Their snake-like movement allows them to free drift in the detritus areas.
(j) Four transparent or white leaf-like anal gills (anal papillae) located in the last abdominal segment. Anal gills were almost equal in length. It performs the osmotic regulatory activities of the larva. Anal brush attached to the base of anal gills. It's not so dense compared to those of *Culex* species and it works as a rudder while swimming.
(k) Air tube or siphon in the 8th abdominal segment allows the larva to breathe in the aquatic environment. Thus, the larva spends most of its time on surface breathing. Air tubes as long as the anal gills as well as somewhat fat and blunt compared to those of *Culex* and other mosquito species (Figure 13). It contained one pair of hair tufts on each side.
(l) The antenna is rod-like with five spines at its terminal end: two on the ventral side, two on the dorsal side, and one flattened in the middle (Figure 9).
(m) Hairs on the head was simple, not spine type.
(n) Lateral thoracic hairs had a prominent but small hook-like spine in its base (Figure 10).
(o) A line of comb scales found on each lateral side of the 8th abdominal segment. Eight to ten straight thorn-like comb scales located in a single row. The basal region of the comb scale was hairy and only visible with at least 400× zoom (Figures 15 and 16).
(p) Pecten is located with a row of closely set teeth on each side of the siphon (air tube). Number of pecten was 9-14 in *Ae. albopictus* and it started from the abdominal side. Each pecten had five-pointed speculates. Basal speculate of pecten was comparatively short while the 5th was the longest and pointed one (Figure 17).
(q) The saddle prominent and broad that forms almost ring like a cover of the last abdominal segment. Ventral anal brush based on distinct muscles and tissues. It makes different to other Culicidae larvae (Figures 13 and 25).

3.2.1.3. *Aedes aegypti* Larva

Aedes aegypti larva was found from only one sampling site (Baparipara Eidgah, Norshingdi) and the percentage is 0.40% in the present study. The observable characteristics are as follows:

(a) The body of the larva had typical three regions: head, thorax and abdomen like other mosquito species (Figure 18).
(b) Larvae lay suspended from the water surface at nearly 45-70° angle.
(c) The head of the larva is sclerotized and almost rounded. It comprised of two eyes, two antennae and brush-like mouthparts.
(d) Mouthparts are modified to detritus feeding and they spend most of their time adjacent to detritus except surface breathing.
(e) Their snake-like, jerking movements allow them to navigate easily through detritus areas.
(f) Two prominent black dots were observed near the base of the antenna. Eyes appeared with a black spot in the lateral side of the head that is similar to that of *Ae. albopictus*.
(g) Antenna rod-like with five spines at the terminal end that follow the same characteristics of *Ae. albopictus*.
(h) The combined thoracic segment appears with an elliptical shape as flattened as the head and separated by a very narrow neck (Figure 18).
(i) Each abdominal segment contained a prominent hair bristle in the middle of its lateral side (Figure 18).
(j) Lateral thoracic hairs had distinct strong black hooks in its base (Figure 19).
(k) Abdomen region composed of ten elongated and rhomboid segments where the 9th segment merged with the previous one.
(l) The last abdominal segment has four transparent leaf-shaped anal gills (anal papillae) (Figure 20). Anal gills were almost similar in size and it works as the osmotic regulatory activities in their aquatic habitat. Prominent anal brushes are implicated to the basal region of anal gills. Numbers of hair were lighter compared to those of *Culex* species. It is modified to adapt in the detritus layer of the habitat.
(m) Air tube (siphon) attached to the 8th abdominal segment allows the larva to breathe. The length of the blunt air tube and anal gills were almost similar (Figure 20). It contained one pair of hair tufts on its ventral side.



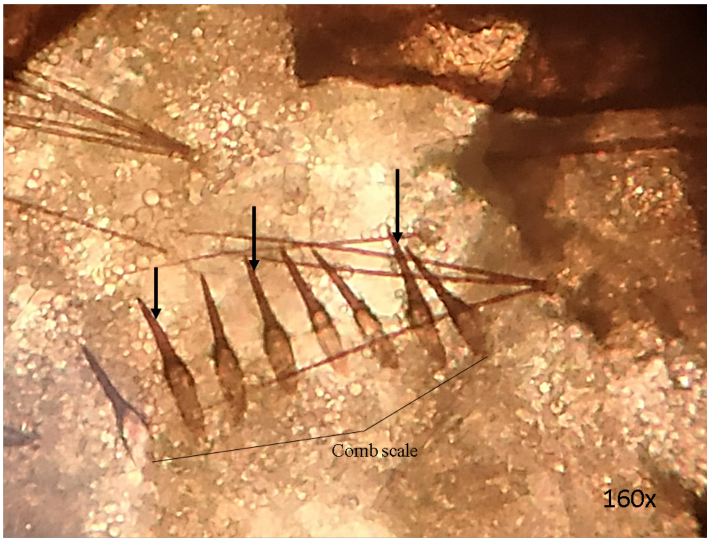


Figure 15. Comb scales of *Aedes albopictus* larva; single row comb scale with straight thorn shape

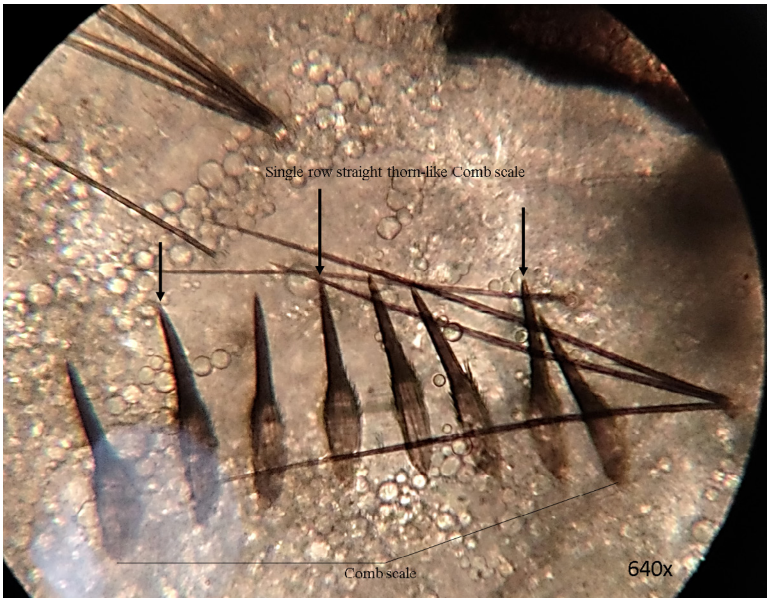


Figure 16. Comb scales of *Aedes albopictus* larva

(n) A single line of comb scales found in each lateral side of the 8th abdominal segment. Eight to ten pitchfork shaped comb scales located in each patch (Figures 21 and 22). Straight thorn-like spine (in *Ae. albopictus*) and the pitch fork spine (in *Ae. aegypti*) only visible with at least 35x zoom. Otherwise, all were similar look (single straight spine) in normal microscopic vision. Basal regions of the comb scale composed of short hair that only visible with at least 400× zoom.

(o) All pectens displayed with narrow spaces. Pecten is located with a row of closely set teeth on each side of the siphon (air tube). Number of pecten was 10-16 in *Ae. aegypti* and it started from the abdominal side. Each pecten had four pointed speculates. Basal speculate of pecten were comparatively short while the 4th one was the longest and pointed (Figures 23 and 24).

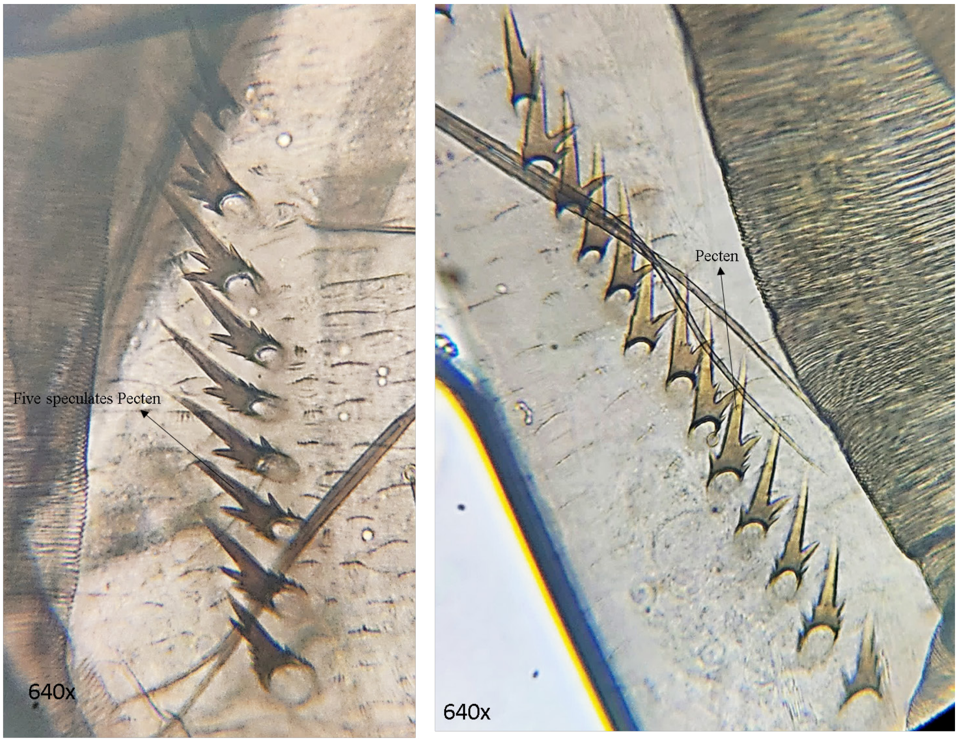


Figure 17. Pecten of *Aedes albopictus* larva

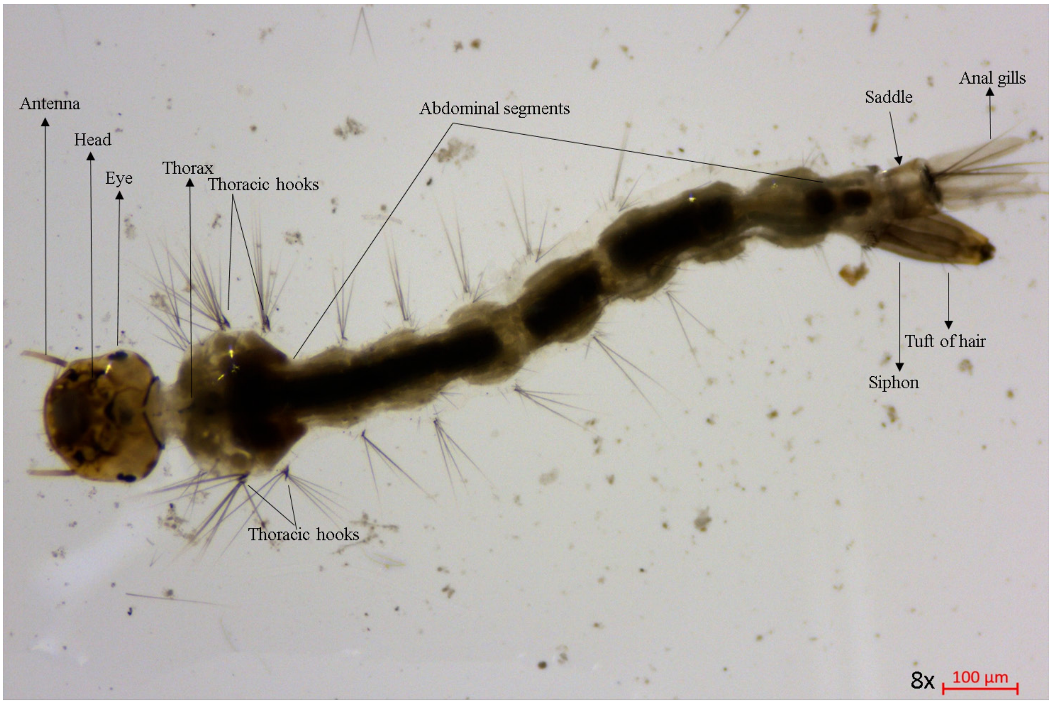


Figure 18. 4th instar Larva of *Aedes aegypti*

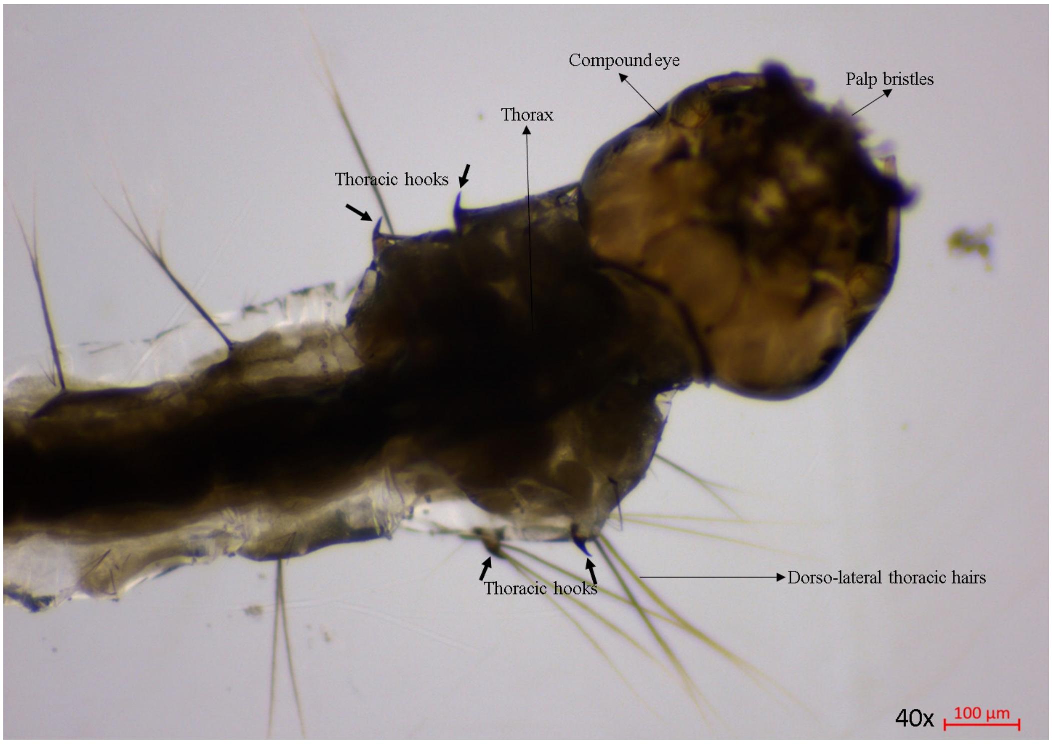


Figure 19. Head and thoracic region of *Aedes aegypti*; bold arrows indicate prominent thoracic hooks

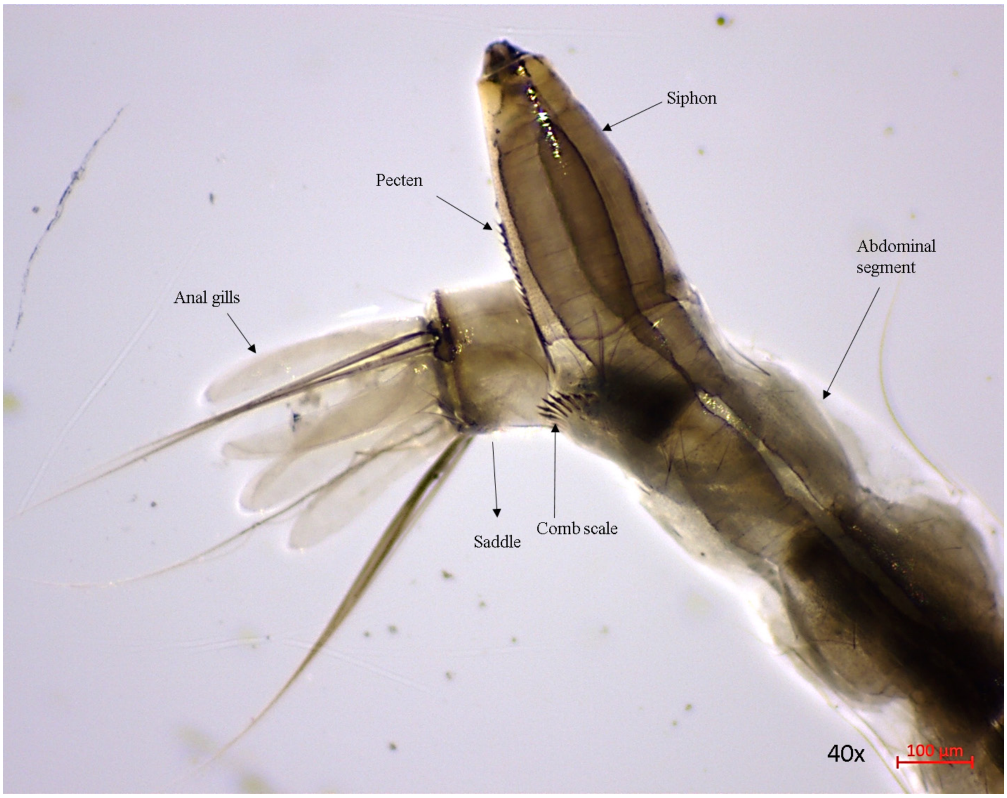


Figure 20. Posterior region of *Aedes aegypti* larva

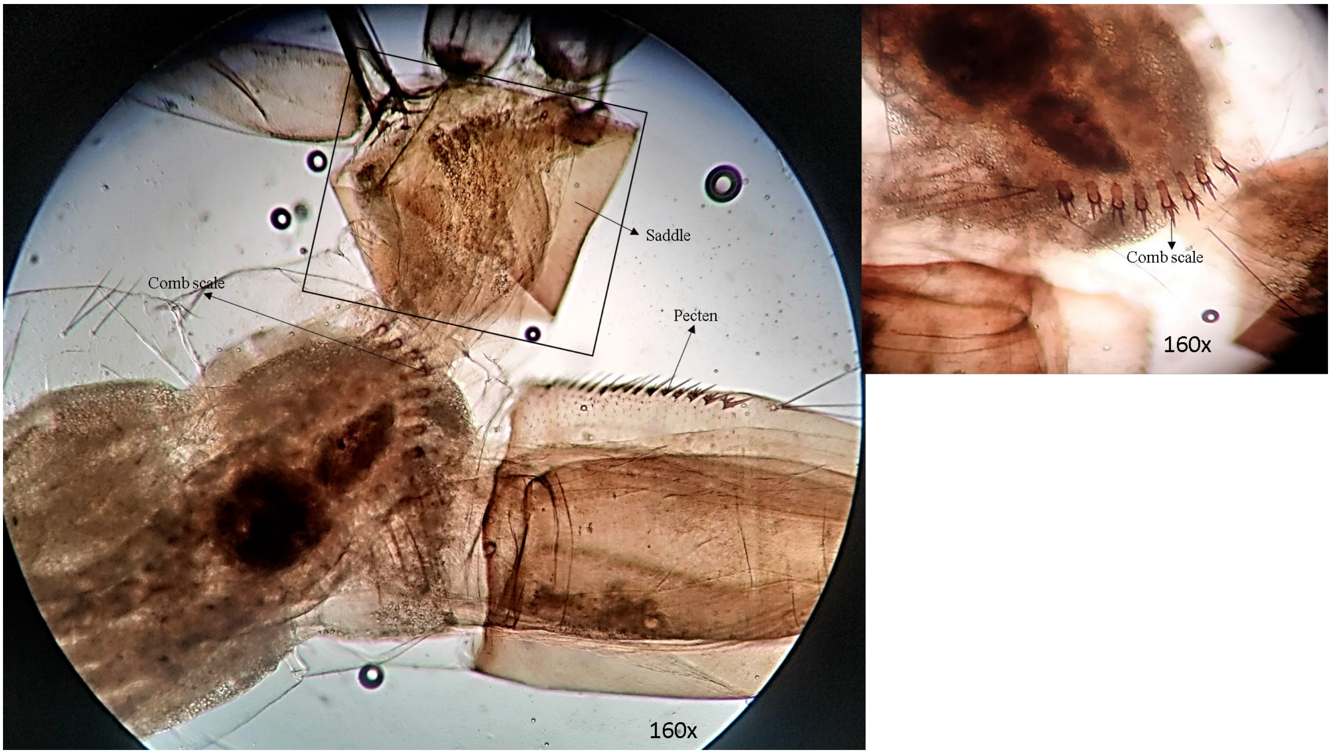


Figure 21. Saddle and Comb scales of *Aedes aegypti* larva

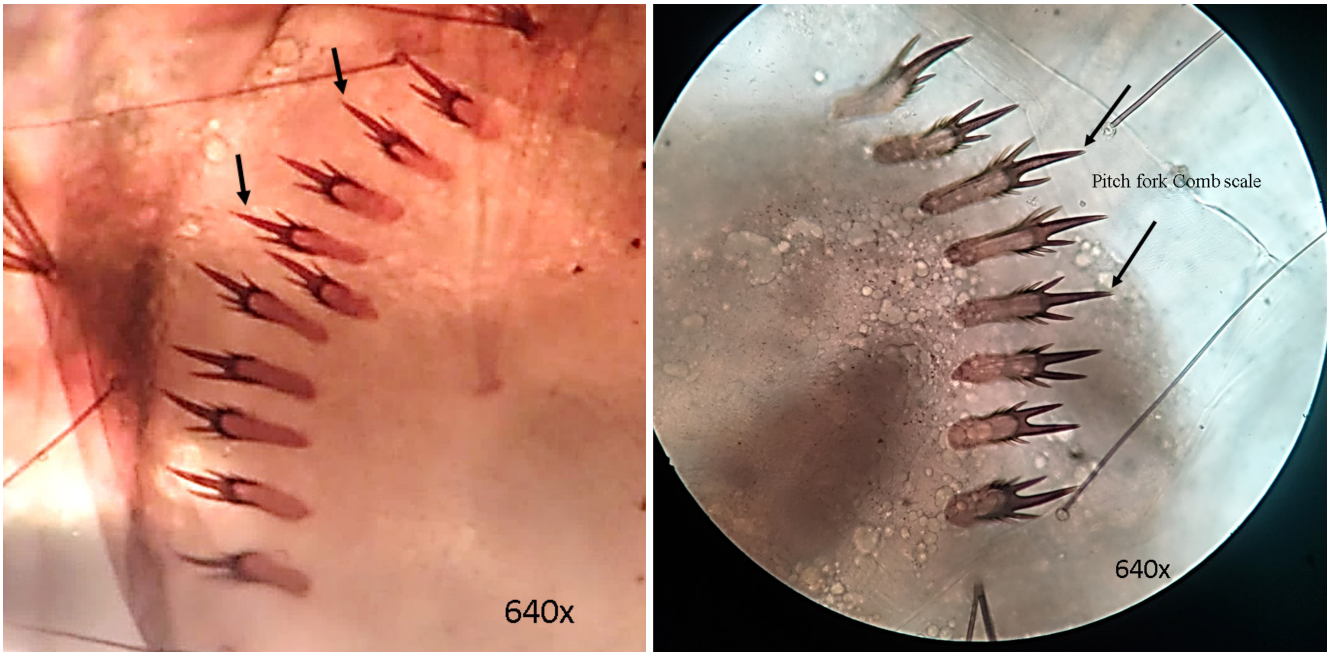


Figure 22. Pitch fork Comb scales of *Aedes aegypti* larva

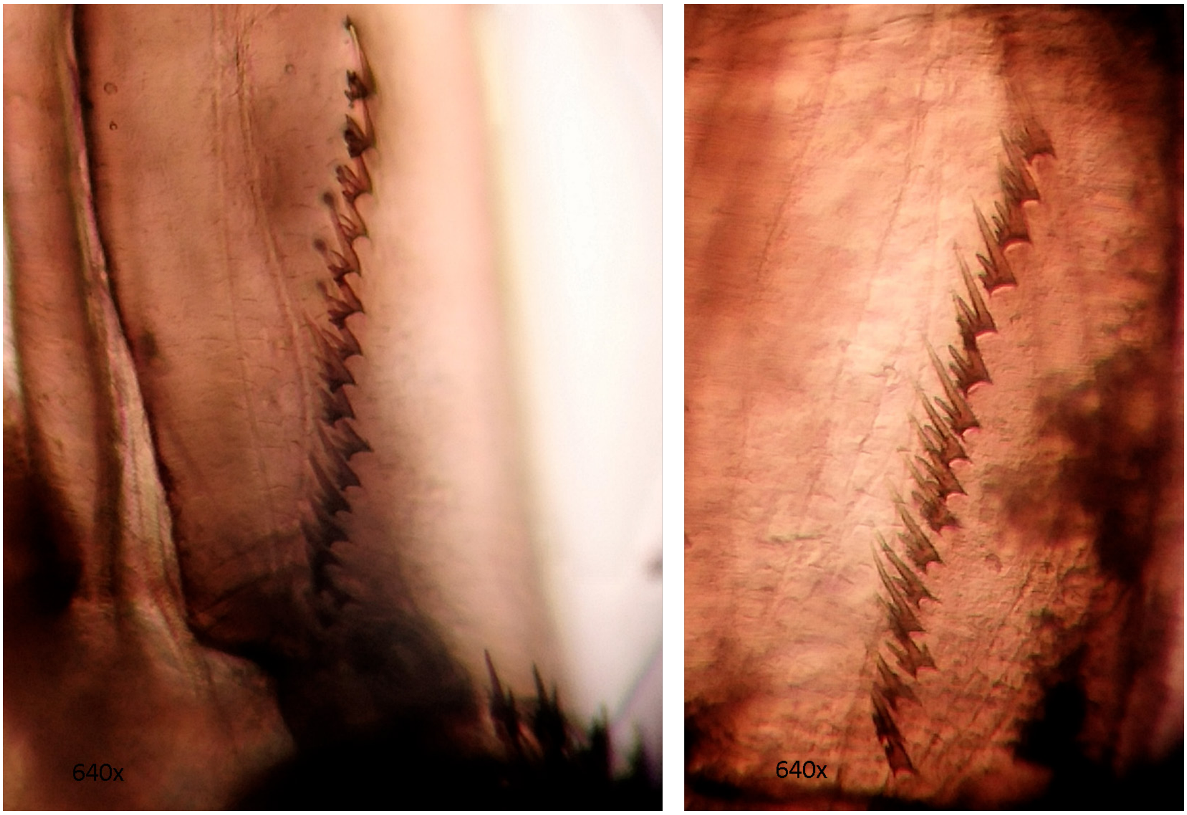


Figure 23. Pecten of *Aedes aegypti* larva

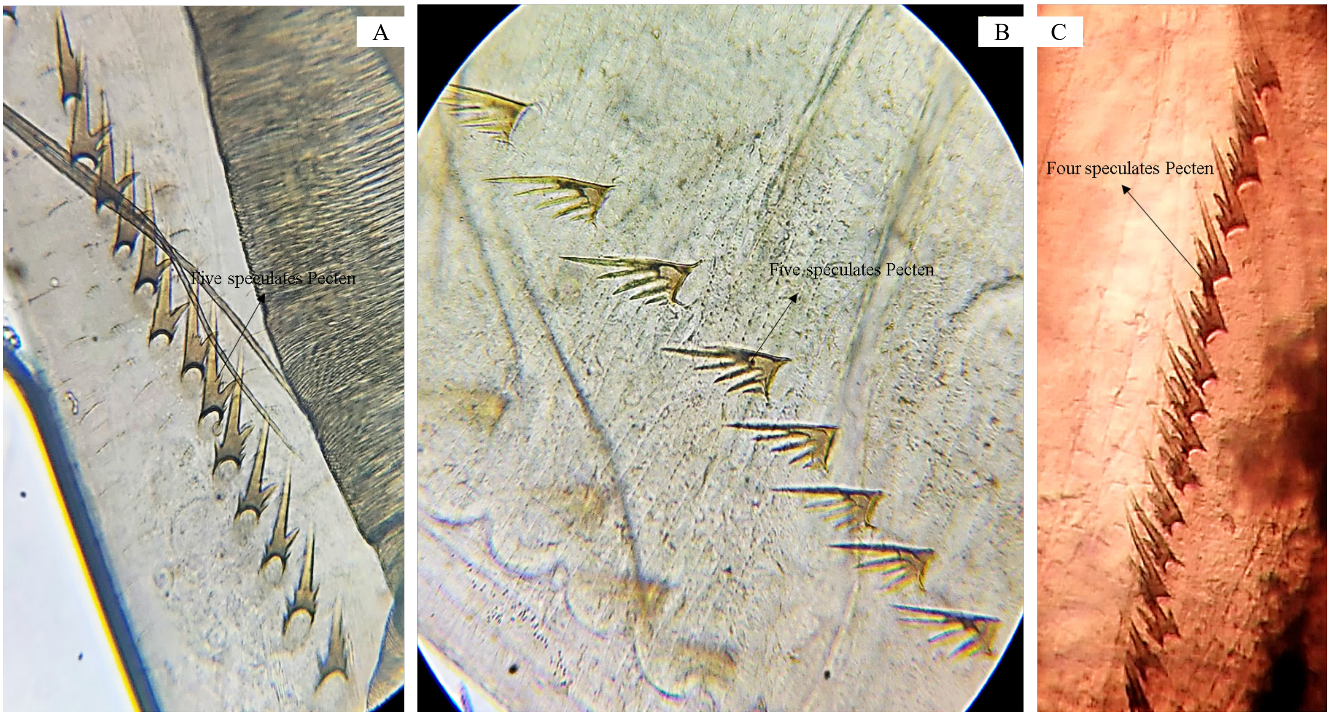


Figure 24. Pecten *Aedes* and *Culex* larva; (A) *Aedes albopictus*, (B) *Culex quinquefasciatus*, (C) *Aedes aegypti*; 640×

(p) Ventral anal brush based with distinct muscles and tissues, which make differences to other Culicidae larva.
(q) The last abdominal (Anal segment) is not completely ringed by the saddle plate and ventral hair tufts do not pierce the saddle (Figures 13 and 20). This characteristic was very specific in the Aedesgenus.

3.2.2. Adult Mosquito

Like other insects, adult mosquitos had three body regions: head, thorax and abdomen. Each of the three regions was further divided into some sub-segments that were sometimes discernible as distinct or fused. Head and thoracic segments of adult mosquitos were mostly fused and not easily distinguished with the naked eyes. Male and female mosquitos beard almost the same characteristics except the antennal shape, proboscis and genital organ.

3.2.2.1. *Culex quinquefasciatus* Adult:

The observable characteristics are as follows:
(a) *Culex* quinquefasciatus is a medium size mosquito with yellowish body colour.
(b) Females were comparatively robust and larger than male mosquitoes.
(c) Proboscis was long, pointed and modified for blood-sucking in female while in males antenna and the maxillary palps were equal in length and the palps were curved in their terminal end.
(d) The antennae are as long as the proboscis and composed of small bristles in female mosquitos but in males, long hairy bristles were found with the full length of the antennae.
(e) Small palps were found in the basal region of the proboscis in females.
(f) Scutum black with dark brown strips in its dorsal views in both sexes.
(g) Abdomen is composed of broad dark brown and comparatively narrow yellowish-white bands.
(h) Prominent yellowish bands were not found in the leg segments of male and females (Figure 26).

3.2.2.2. *Aedes albopictus* Adult

The observable characteristics are as follows:
(a) *Aedes albopictus* is a small to medium-sized mosquito.

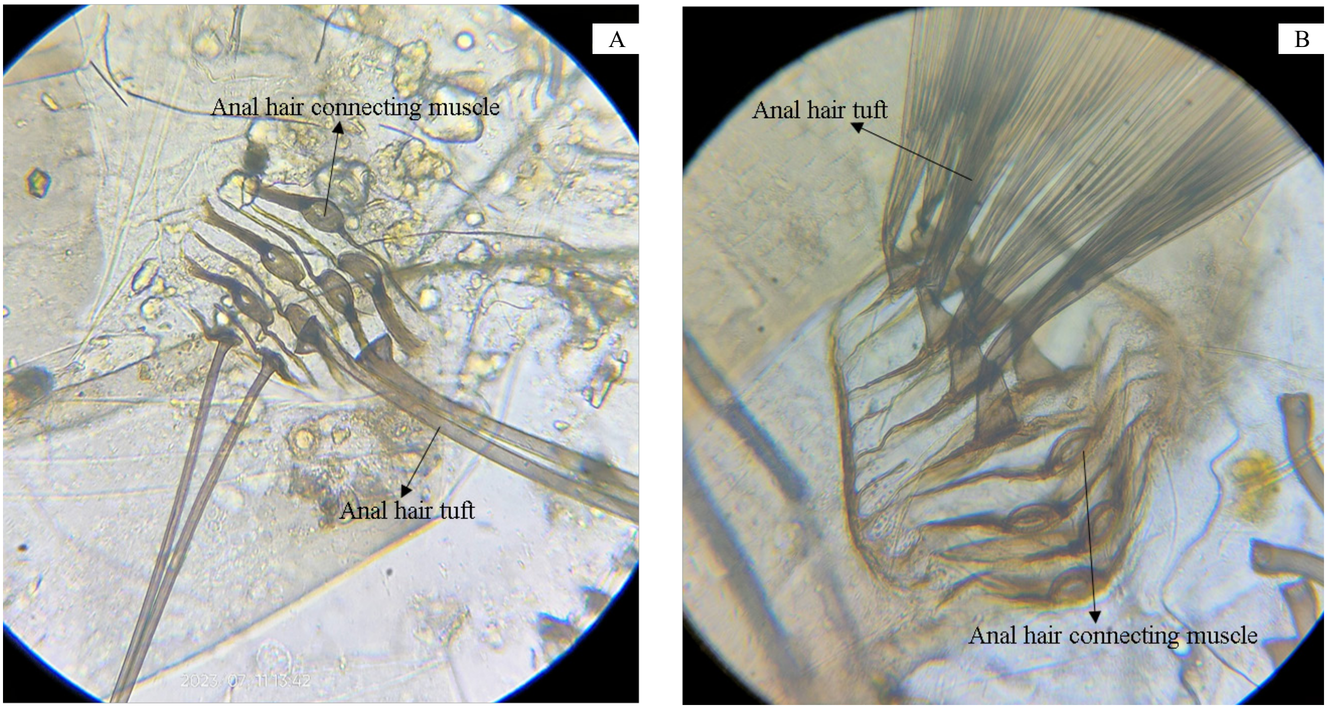


Figure 25. Anal hair tufts and their connecting muscles of *Aedes* and *Culex* larva; (A) *Aedes albopictus*; (B) *Culex quinquefasciatus*; 640×

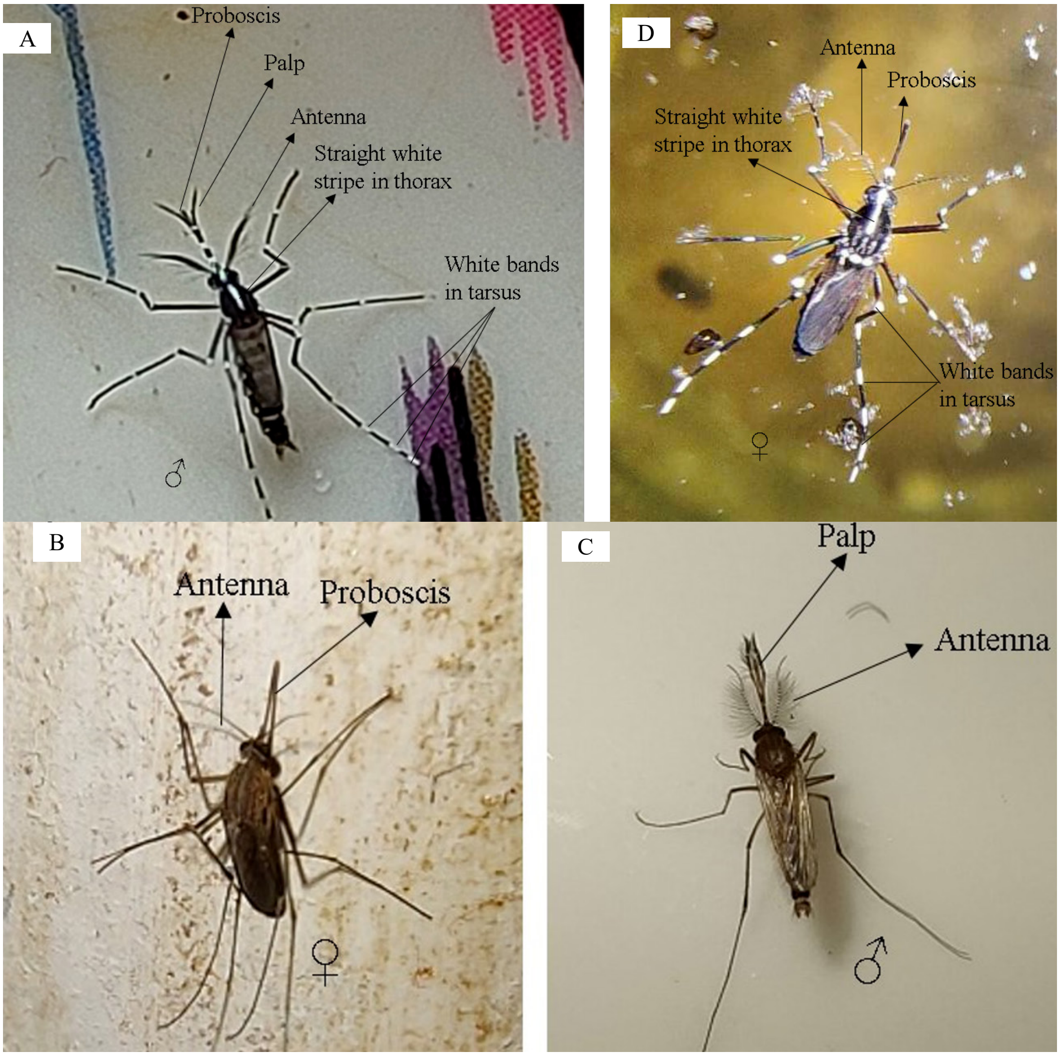


Figure 26. Adult *Aedes* and *Culex* mosquito; (A, D) *Aedes albopictus* and (B, C) *Culex quinquefasciatus*

(b) They were easily recognized from other mosquito species by their narrow and typically black body, and unique patterns of light white and dark black scales on the abdomen and thorax.
(c) Legs were decorated with white and alternating black bands in both sexes.
(d) Females are comparatively larger than males in body size. Prominent three white spots are located in the first abdominal segment in its dorsal side in both sexes.
(e) Scutum dark black and had a prominent white band in the middle in both male and female mosquitos.
(f) The proboscis was long, pointed and had a white stripe at the basal part of the female. It is modified for blood-sucking.
(g) Palps were short in females while it was as long as the proboscis in male. Palps in male mosquitos were slightly curved and decorated with black and white stripes.
(h) The antenna almost as long as the proboscis in females while it is shorter than the proboscis in male.
(i) A short bristle composed of the whole length of the antenna in females but it incorporates long and hairy bristles in male *Ae. albopictus*.
(j) Three ocelli-like apical white dots were observed in between two compound eyes.
(k) The abdomen composed of broad white and black bands. A line of white dots was noticed on each lateral side of male and female mosquitos (Figure 26).

3.2.2.3. *Aedes aegypti* Adult

The observable characteristics are as follows:

(a) Adult Aedes aegypti is a small to medium size mosquito.
(b) They were easily recognized from other mosquito species by their narrow and typically brownish body, and unique patterns of white and brownish-black scales on their abdomen and thorax.
(c) The legs of both sexes were well-visible and composed of yellowish black and white bands.
(d) Females were comparatively larger than males.
(e) Three white spots were found in a first abdominal segment on the dorsal side in males and female.
(f) Scutum brownish-black and had two longitudinal white stripes in the middle including a violin/lyre-shaped stripe in each side of both male and female mosquitos.
(g) Proboscis long pointed and has a white band at the basal part of female.
(h) Palps were short in females but as long as the proboscis in case of males. Palps in male mosquitos were slightly curved in its terminal end and had brown and white strips.
(i) Antenna almost as long as the proboscis in females while shorter than proboscis in male.
(j) A short bristle composed of a whole length of antennae in females and in males, it picked up of long and hairy bristle that form oak leaf shaped.
(k) Three white dots like ocelli and a white stripe are found in between two compound eyes.
(l) The lateral side of abdomen composed of white dots with broad white and brownish-black bands in its dorsal view in both sexes.

4. Discussion

Mosquitoes (*C. quinquefasciatus, Ae. albopictus, Ae. aegypti*) are important vectors of various diseases i.e. urban yellow fever, dengue, chikungunya, Zika, leishmaniasis and so on (Souza-Neto *et al.* 2019). So, vector control is considered the most effective way to minimize infestation of those diseases or prevent arbovirus outbreaks (Achee *et al.* 2015). In most cases preventive measures have been taken by applying chemical insecticides or biological control agents to eliminate adult mosquitoes or their immature stages (eggs, larvae and pupae) in breeding sites (Baldacchino *et al.* 2015). But in most cases, it is found that *Aedes* spp. eggs are not sensitive to conventional chemical insecticides and remain viable for an extended period (Diniz *et al.* 2015; Farnesi *et al.* 2015). Some other species i.e. *Ae. polynesiensis, Ae. scutellaris* also work as vectors of dengue and each mosquito has a different ecology, behavior pattern and geographical distribution (WHO 2017). Identification of mosquitoes at the species level is crucial for implementing effective preventive measures.

Thus, we conducted the present study and identified three types of mosquitos (*C. quinquefasciatus, Ae. albopictus* and *Ae. aegypti*) at the species level by presenting their high-resolution microscopic images for the first time in Bangladesh. Selected characteristics are almost similar and follow the key analysis by Pratt (1966) for *Culex* and *Aedes* species. *Ae. albopictus* is commonly referred to as one of the forest species, but now it is habituated to a variety of environmental situations including indoor breeding equipment (Hawley 1988). They can breed in artificial containers in human dwellings and their eggs become viable in dry conditions for many months which aggravated dengue transmission (WHO 2017). The current dengue situation in Bangladesh and the result of our present findings follow it. Absent of *Ae. aegypti* in most of our sampling sites (except Norshingdi) indicates that *Ae. albopictus* may be another vector of the Dengue fever virus in Bangladesh at present. It follows the study of Mitchell *et al.* (1993), where they found *Ae. albopictus* is one of the strong vectors of dengue, LaCrosse encephalitis and eastern equine encephalitis.

In case of larval habitat analysis, Roberts (2014) noticed that *C. quinquefasciatus* inhabited highly polluted water where their predators were normally absent. It supported the present finding. We sampled most of the *C. quinquefasciatus* from dirty water and sewerage drains. Juliano *et al.* (2004) monitored that container-dwelling mosquitoes inhabit both man-made (discarded automobile tires, cemetery vases) and natural (tree holes, bamboo internodes) vessels. Within these habitats, multiple mosquito species are present simultaneously. We observed the same situation in our Darsana sample where *C. quinquefasciatus* and *Ae. albopictus* shared the same habitat (indoor flower tub) (Figure 3I). Significant differences also observed in the behavior of *C. quinquefasciatus* and *Ae. Albopictus*. *Culex quinquefasciatus* tended to occupy the top of containers while *Ae. albopictus* spent most of their time thrashing in the middle or at the bottom. This is because *Culex* species are filter feeders while the *Aedes* species are configured as detritus feeder (Figure 6). Same behavioral patterns had been described by Yee *et al.* (2004). They observed that *Culex* mosquitos were commonly filtering the water column and the container-dwelling genus. *Aedes* primarily browse on container and detritus surfaces. It supports the present findings. Mathews *et al.* 2017 analyzed the morphological characteristics of *C. quinquefasciatus* larva compared with the studies of Snell (2005). They found four pairs of ventrolateral hair tufts in the anal siphon that are not aligned in a single line. Those mosquitos have 8-12 pecten teeth on both sides of the siphon. We observed the same siphon characters in *C. quinquefasciatus*. The lateral comb patch contained 30-40 comb hairs in the 8^{th} abdominal segment which is very much similar to our present study of *Culex* species. Saddle, anal gills and antennae also follow the same characteristic features of the described *C. quinquefasciatus* of Mathews *et al.* (2017) and our *Culex* species.

Andreadis *et al.* (2005) formulated the taxonomic identifying keys of the mosquitoes of Conneticut where they described that *Ae. albopictus* have comb scales with long, pointed, unfringed median spine. It is arranged in a straight single lateral row. Anal saddle was almost complete, and the ventral brush of the anal segment contained four pairs of setae. It reconciles our findings of the morphological characteristics of *Ae. albopictus* larva. Hayley 1988 studied the morphology of the adult Asian tiger mosquito, *Ae. albopictus*. He described several distinct characteristics that aid in their easy identification. These include bold black shiny scales and silver-white scales on the palps and tarsi. The scutum is black with a distinctive white stripe running from the dorsal surface of the head to the thorax. Males have plumose antennae and mouthparts adapted for nectar feeding. Females have thread-like antennae with short bristles, which are as long as the proboscis.

The above characteristics are completely similar of our present findings both in male and female *Ae. albopictus* and it makes different forms *Ae. aegypti*, while *Ae. aegypti* has a pair of longitudinal white stripes and violin/lyre-shaped markings on its scutum. Yellowish black body color with black and white banded legs configured it easily differentiate from *Ae. albopictus*. We found the same characteristic features of *Ae. aegypti* in the present findings and that of Bar and Andrew (2013).

The present investigation shows that morphologically *Ae. albopictus* larva has rod-like antenna with five terminal bristles, dorso-lateral thoracic bristle with small basal hook, rhomboid abdominal segments, single line comb scales with thorn-like structure in each comb scale, distinct five toothed pectens in siphon. These completely follow the morphological structure of *Ae. albopictus* described by Andreadis *et al.* (2005). We found the above-mentioned characters in *Ae. albopictus* and are differentiated from those of *Ae. aegypti*. Dorso-lateral thoracic bristle of *Ae. aegypti* have comparatively big basal hook. Single-line comb scales with pitch fork-shaped thorn and four-toothed pecten make it easy to differentiate from *Ae. albopictus*. We noticed a vis-à-vis result with Priyanka *et al.* (2022). They noticed only *Ae. aegypti* (not any *Ae. albopictus*) in Dhaka city while we found only *Ae. albopictus* (not any *Ae. aegypti*). Moreover, they did not provide any microscopic images of the reported *Aedes* mosquito larvae and followed the keys of Barraud (1933), Bram (1967) and Puri (1931). In these three papers, Bram (1967) was on the Genus *Culex*; Puri (1931) was on the larvae of Anopheline mosquitoes and Barraud (1933) article was not available. So, the *Aedes* mosquito larvae identification by Priyanka *et al.* (2022) was arbitrary and inconsistent.

In conclusion, mosquito-transmitted dengue fever has become a major public health burden in Bangladesh in recent years, particularly in 2023. Usually, *Aedes aegypti* is considered a known vector for dengue, but during the survey, this species was rare in Dhaka city. It was found only in Baparipara Eidgha (Narshingdi district) area, which is more than 50 km away from Dhaka city. Considering the situation, we presented here for the first time, high-resolution microscopic images of the major morphological characteristics of the commonly occurring mosquito larvae (*C. quinquefasciatus, Ae. albopictus* and *Ae. aegypti*) that are very much essential for accurate identification. Their larval habitat characterization and larval movement pattern were also investigated. So, now it is necessary to examine whether *Ae. aegypti* is the only vector for dengue transmission or there are some other mosquito species/or species complex. The information provided here will serve as key factors for the implementation of effective mosquito control strategies and it may also decrease the quantity of insecticides/pesticides sprayed in the environment.

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