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Morphological characteristic of dengue vectors *Aedes aegypti* and *Ae. albopictus* (Family: Culicidae) using advanced light and scanning electron microscope

SUPRIYONO♥, SUSI SOVIANA, MUHAMMAD FALIKHUL MUSYAFFA, DIMAS NOVIANTO, UPIK KESUMAWATI HADI

Division of Parasitology and Medical Entomology, School of Veterinary Medicine and Biomedical Sciences, Institut Pertanian Bogor. Jl. Agathis, Dramaga Campus, Bogor 16680, West Java, Indonesia. Tel./Fax.: +62-251-8425503, ♥email: supriyono84@apps.ipb.ac.id

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Abstract. *Supriyono, Soviana S, Musyaffa MF, Novianto D, Hadi UK. 2023. Morphological characteristic of dengue vectors* Aedes aegypti *and* Ae. albopictus *(Family: Culicidae) using advanced light and scanning electron microscope. Biodiversitas 24: 894-900.* Dengue infection is still a major public health problem in Indonesia, with *Aedes aegypti* and *Aedes albopictus* as vectors. This study aims to determine the description characteristics of these mosquitoes using an advanced light microscope and scanning electron microscope (SEM). The adult mosquitoes of *Ae. aegypti* and *Ae. albopictus* were collected from Darmaga, Bogor, in 2021. *Ae. aegypti* has a brown to dark body color with sub median white line like a lyre-shaped on the mesonotum, dark proboscis, a white knee spot on the femora, mid femur with a longitudinal white stripe, and mesepimeron with two well-separated white scale patches. *Ae. albopictus* has dark body color, dark proboscis, white strip on the middle of mesonotum, and not separated white scale patches on the mesepimeron and mid femur without a longitudinal white stripe. SEM showed the characteristic microstructure of *Ae. aegypti* and *Ae. albopictus* did not have a significant difference. Several sensory organs, namely funiculus, microtrichia, and trichoidea, are distributed on the proboscis, palps, and antennae. Microtrichia is spread almost all over the body, especially the palps, proboscis, thorax, and abdomen. This research is important for basic information to develop effective and efficient mosquito vector control strategies.

Keywords: *Aedes aegypti, Aedes albopictus*, dengue

INTRODUCTION

Dengue fever is still a serious health problem in Indonesia. The incidence of dengue fever has an increasing trend, and in 2020 cases of dengue fever reached 108,303 cases with a morbidity or incidence rate (IR) was 40 people per 100,000 population. The number of deaths due to dengue infection in 2021 was 402 people distributed in 452 districts/cities in 34 provinces in Indonesia. Several provinces had the highest dengue cases West Java, East Java, Central Java, South Sulawesi, Jakarta, Bali, Nusa Tenggara Barat, North Sumatra, East Kalimantan, Nusa Tenggara Timur, and Riau (MoH 2021). Until now, there are four serotypes of the virus that causes dengue, DENV-1, DENV-2, DENV-3, and DENV-4. Recovery from dengue infection is believed to provide lifelong immunity to that serotype. However, cross-immunity to other serotypes after recovery is only partial and temporary. Subsequent or secondary infection by another serotype increases the risk of severe dengue fever (WHO 2022).

Mosquitoes belonging to the order Diptera, family Culicidae, are vectors for several vector-borne diseases (Camara et al. 2020; Vieira et al. 2019). *Aedes aegypti* is the main vector of Dengue fever, while *Aedes albopictus* is the secondary vector (Ong et al. 2021; Rahman et al. 2021; Saleh et al. 2021). Besides being reported as a dengue fever vector, these mosquitoes are also important in transmitting diseases to humans, including chikungunya, yellow fever, and zika (Nemg et al. 2020, Supriyono et al. 2020). *Ae. aegypti* and *Ae. albopictus* are an invasive mosquito found in tropical areas and four seasons countries. Human activities mainly caused the spread of these mosquitoes in the last three decades. The blood-sucking activity was found during the day and at night (Helmersson et al. 2019; McGregor et al. 2021; Mohd Ngesom et al. 2021). *Ae. aegypti* mosquitoes prefer to suck blood indoors (endophagic) compared to *Ae. albopictus,* which prefers the outdoors (exophagic). *Ae. albopictus* is widely found in the forests of Southeast Asia because of its zoophilic life cycle. This species adapts to be anthropogenic, requiring other food sources, namely domestic animals and humans (Paupy et al. 2009). The habitat of *Ae. aegypti* mosquito is more dominant indoors, while *Ae. albopictus* is outside. Its life cycle is closely related to human habitat, and it breeds in containers with water, tires, or other containers. *Ae. albopictus* was resting in the bushes, while *Ae. aegypti* was resting on hanging clothes and items in the room.

The high incidence and spread of dengue fever require a correct and effective vector control strategy, which should be based on the results of vector entomology studies. Vector control carried out in the community every year in Indonesia still uses chemical control or insecticides, which is still the best choice compared to other controls in decreasing the spread of dengue fever. Mosquito vector control of breaking the virus transmission (Weeratunga et al. 2017; Roiz et al. 2018; Santos et al. 2020; Suman et al. 2018).

Accurate identification of mosquitoes can be used to determine their behavior, ecology, importance in disease transmission, and control strategy. Ecological behavior includes feeding location, biting behavior, geographical distribution, and capacity as a vector (Weeraratne et al. 2017). These two mosquitoes' shapes and morphological characteristics are generally very similar. Both mosquitoes have a dominant dark color with white patches on the body and legs. The macroscopic of these mosquitoes can be seen clearly using an advanced stereo microscope, while the microscopic characteristic can be used in a Scanning electron microscope (SEM). SEM provides a detailed microscopic analysis of each part of the mosquito's body (Supriyono et al. 2022). This research was conducted to provide the basic information related to the identification and morphological characteristics of both *Ae. aegypti* and *Ae. albopictus* to increase understanding in developing appropriate control strategies.

MATERIALS AND METHODS

Mosquito collection

The adult of *Ae. aegypti* used in this study has been reared in the laboratory of Medical Entomology, Faculty of Veterinary Medicine, IPB University, Bogor, and reared at 27±2°C and 75±5% RH. However, *Ae. albopictus* were collected from the Darmaga campus in November 2021 using an aspirator and sweeping net. The mosquitoes were chosen and killed by putting them into the refrigerator and storing them in freezer for 30 minutes before processing for this research.

Habitat and larvae identification

The mosquito larvae of *Ae. aegypti* and *Ae. albopictus* were collected in the Darmaga campus of IPB. The type of mosquito habitats in the environment was observed. The collected larvae were reared in the laboratory. The larvae were reared in a tray filled with water and fed with pellets. Larvae that have turned into pupae were separated and put in cages. Adult mosquitoes that hatched were given sugar as a source of energy. Furthermore, the screened mosquitoes and larvae were used for identification using an advanced light microscope and/ or SEM.

Mosquito identifications and characterization

Morphological identification

The collected mosquitoes were identified according to morphological techniques. Morphological identification of *Ae. aegypti* and *Ae. albopictus* were performed using an advanced light microscope Leica M205Câ according to the characteristic morphological key described by the Ministry of Health (2000). Then, the morphological pictures were taken based on the specific characteristic of these mosquitoes.

Characterization by Scanning Electron Microscope

Morphological analysis by SEM was performed using Hitachi SU-3500 according to the manufacturer's instructions described before (Supriyono et al. 2022). Mosquitoes were rinsed by shaking in a normal saline solution to remove surface artifacts. Before fixation, mosquitoes were soaked with 2.5% glutaraldehyde for 24 h. Specimens were fixed with tannic acid 2% for six hours and several days. The samples were rinsed four times with Cacodylates buffer for 15 minutes for each repetition and then continued rinsed with water for 15 minutes. The mosquitoes were dehydrated following increasing alcohol concentrations: 50%, 70%, 85%, 95%, and 100%. The samples were soaked for times with an alcohol concentration of 50% for 15 minutes and continued with 70, 85, and 95 for 20 minutes, respectively. The samples were soaked twice with an alcohol concentration of 100% for 10 min. The dehydration was performed at 4ºC for alcohol concentrations of 50%, 70%, and 85%. However, it was performed at room temperature for alcohol concentrations of 95% and 100% process. Finally, the samples were soaked twice in Butano and were frozen. The specimens were dried using a freeze drier/vacuum drier, attached to double-stick tape on aluminum stubs, and coated with gold in an E-1010 ion coater.

RESULTS AND DISCUSSION

Results

This study found several mosquito breeding habitats for *Ae. aegypti* and *Ae. albopictus*. *Ae. aegypti* was found in several habitats including containers, buckets, and gutters inside buildings. While the *Ae. albopictus* was found in outdoor containers and bromeliad flowers (Figure 1.A-F). Furthermore, the larvae of *Ae. albopictus* were kept in the laboratory until they hatched into adults. Therefore, in this study, several larvae were used for morphological observations.

Some of the morphological characteristics of the larvae of both mosquitoes were observed on the head, antennae, median brush, lateral brush, thorax, abdomen, siphon, and anal papillae. The larvae have a pair of antennae that are straight, wide at the base, and narrow towards the tip. The compound eyes are prominent laterally. A pair of mouth brushes at the anterolateral margin emerging from the clypeal/labral area. The larvae have a narrow, cylindrical, webbed neck connecting the head and thorax. The thorax is round and consists of the pro, meso, and meta thorax. In the meso and metathoracic regions, a pair of large spine-like processes is seen dorsally. In addition, lateral hairs arise from the lateral sides of the pro, meso, and meta-thoracic segments. The larval abdomen consists of eight segments, long, transparent and cylindrical. Lateral tufts of hair emerge from the abdominal segments in varying numbers on each segment. Abdomen segment VIII of the larva has a row of comb spines and a respiratory tract or siphon. At the end of the siphon are the respiratory openings, the spiracles, which are surrounded by peri-spiracular lobes. The siphon has a row of pecten teeth on either side laterally and has basal denticles. The end or terminal part of the abdomen contains the anus. The anal segment has a ventral brush with five pairs of setae of variable size and four equal-sized anal papillae. The anal papillae are transparent and boat-shaped (Figure 1). Differences between *Ae. aegypti* and *Ae. albopictus* among them is on its thorn or spine comb. *Ae. aegypti* has a sharp and curved comb spine at the end having apical and subapical denticles, whereas in *Ae. albopictus* has no subapical denticles (Figure 1.G-H).

Several male and female mosquitoes were used for observations using an advanced light stereo microscope and SEM. Moreover, based on morphological observations using an advanced light stereo microscope, it was shown that *Ae. aegypti* and *Ae. albopictus* have some morphological similarities. The body is dark brown to black in color. The size of the male mosquito is relatively smaller than the female, which is around 4-5 mm and 4-6 mm. The body is patterned black and white on the thorax, abdomen, and legs. The antennae of male and female *Aedes* mosquitoes have 13 segments or flagella, 1-1.5 mm long, with hair or funiculus in each segment. Besides, each segment is separated by a pigmented ring. The antennae of male mosquitoes have denser hair (plumose) compared to females with sparse hair (pilose). The proboscis of these mosquitoes has a dark color without white bands. The scutellum consists of three parts. The maxillary palps have four segments which are shorter than the proboscis. The dorsal part of the thorax is filled with bristle hairs, whereas on the lateral part of the thorax, there is not much hair. There were white bristle hairs at the end of the thorax.

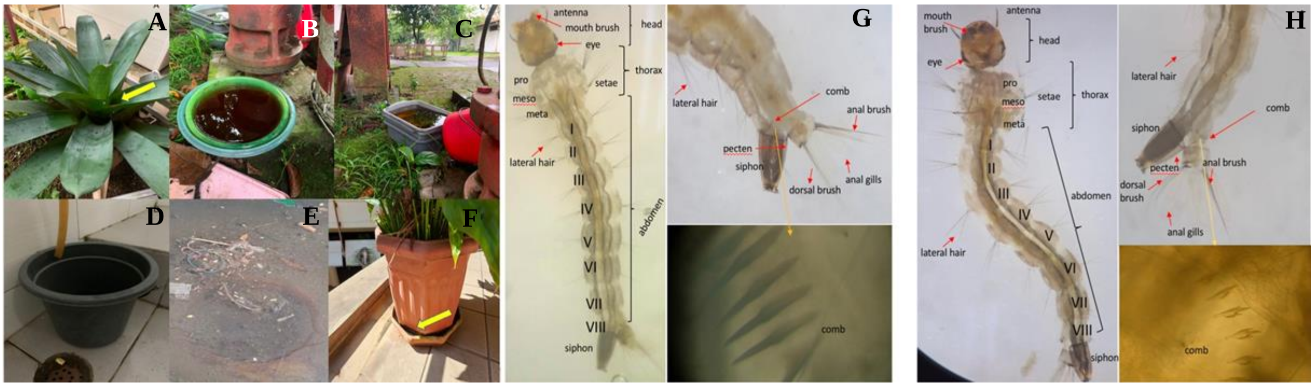

Figure 1. Several habitats of *Ae. albopictus* (A-C), *Ae. aegypti* (D-F) and morphological characteristic of *Ae. albopictus* (G) and *Ae. aegypti* (H) larva

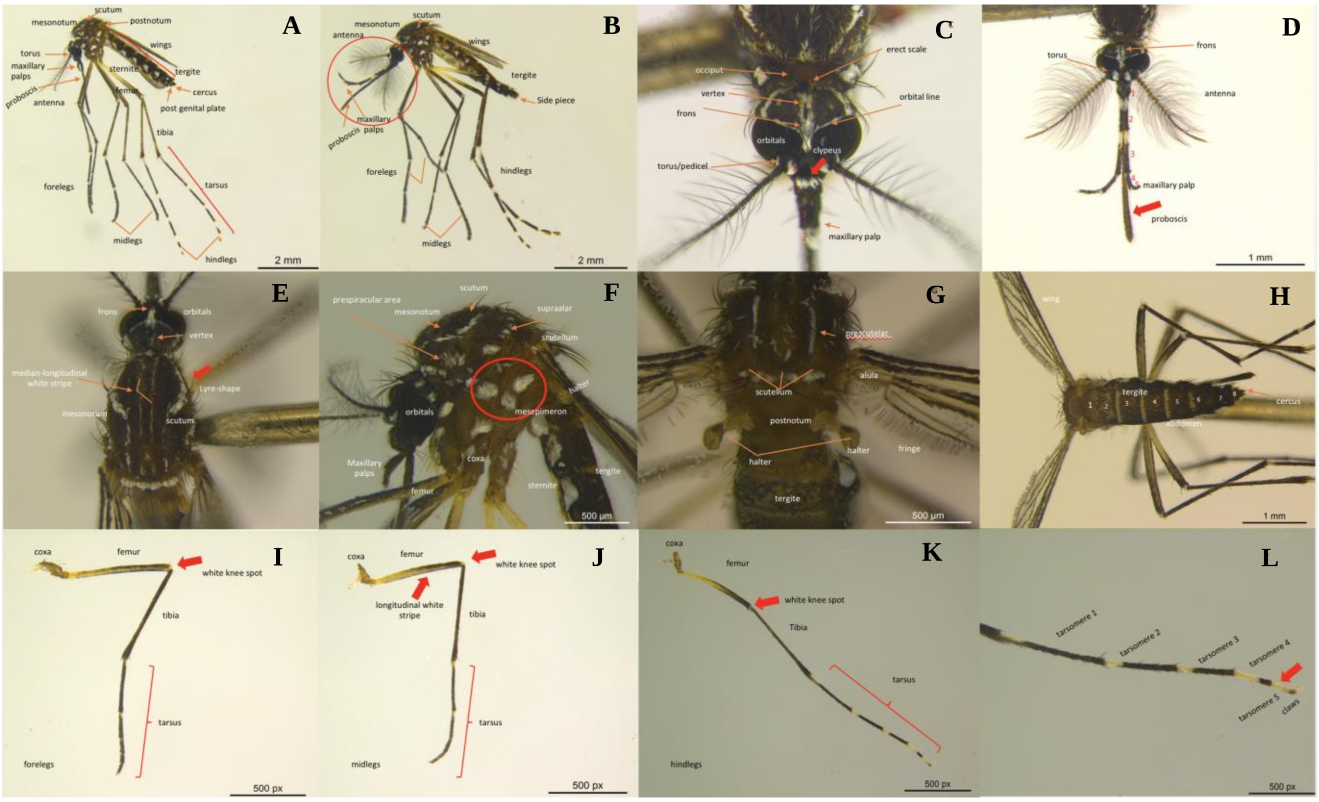

Figure 2. Adult of *Ae. aegypti*: female (A), male (B), head and antenna (C-D), dorsal, lateral and posterior thorax (E-G), abdomen (I), foreleg, midleg, and hindleg (I-K) and hind leg tarsus (L). The big arrow indicates the specific characteristic of the mosquito

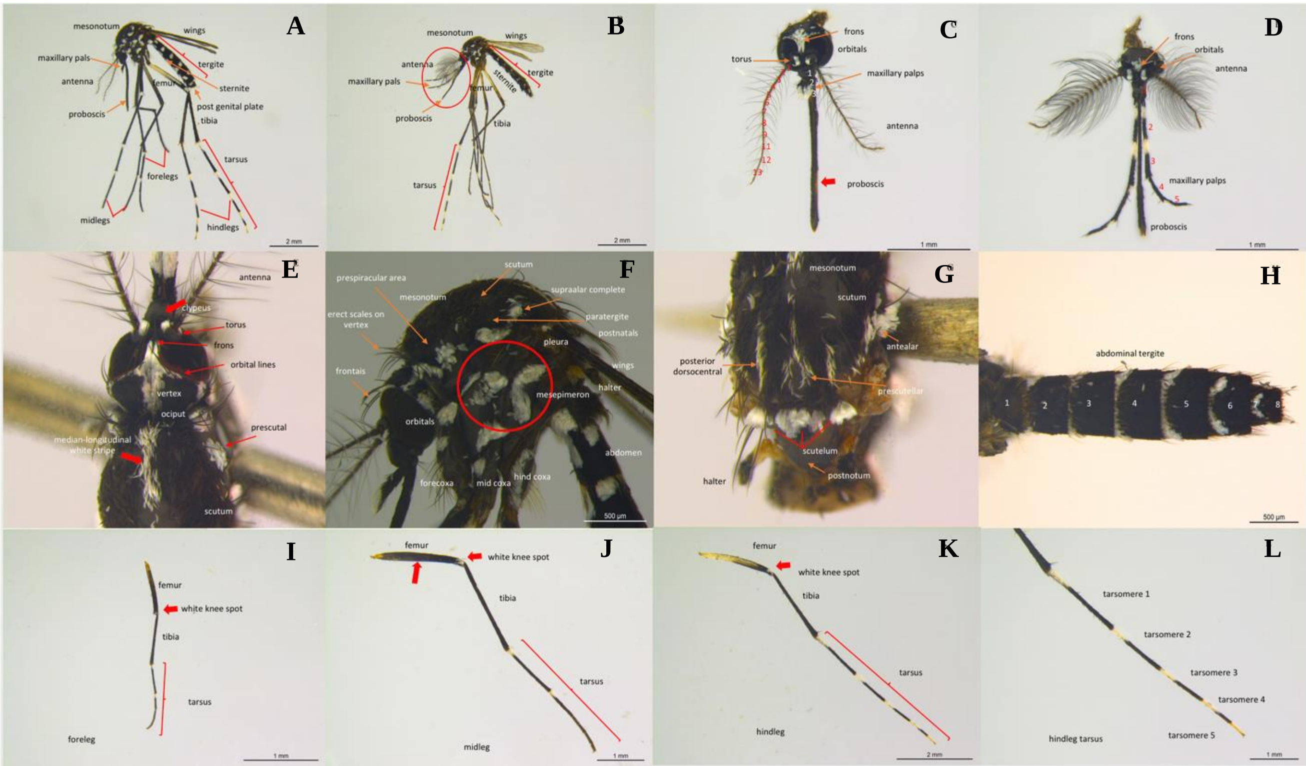

Figure 3. Adult of *Ae. albopictus*: female (A), male (B), head of female (C), head of male (D), mesonotum, lateral thorax and posterior thorax (E-G), dorsal abdomen (H), fore leg, mid leg and hind leg (I-K), tarsus of hind leg (L). The big arrow indicates the specific characteristic of the mosquito

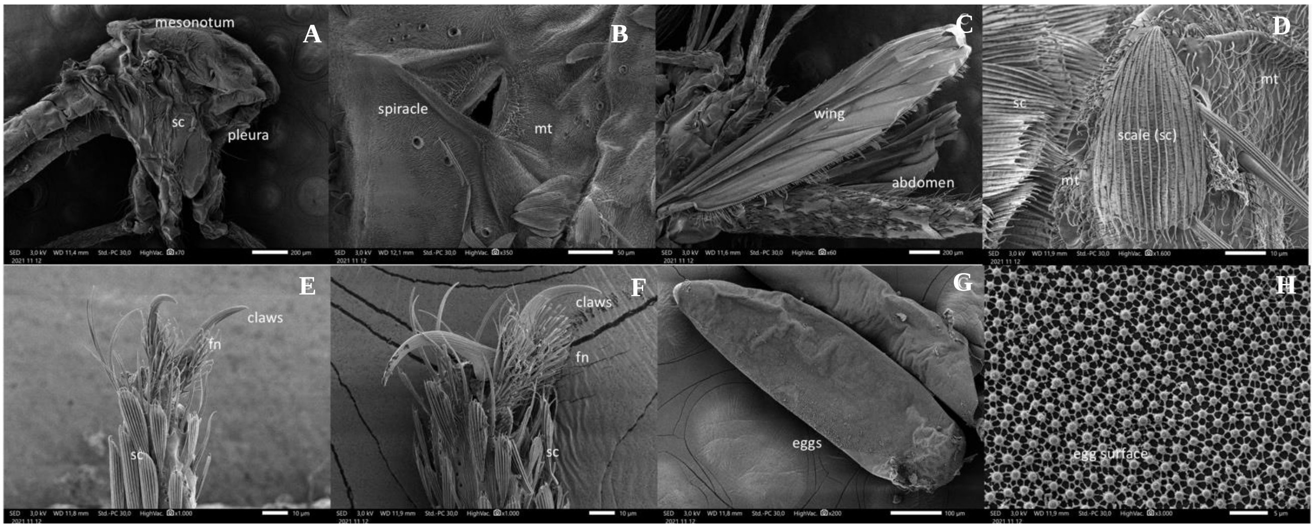

Figure 4. Scanning electron microscope of *Ae. aegypti*. lateral thorax (A), metathoracic spiracle (B), wing (C), body scale (D), tarsomere/claw (E-F), the eggs of *Ae. aegypti* and the surface (G-H). Note: leaf-shaped scales (sc); funiculus (fn); microtrichia (mt)

The two mosquitoes have different morphological characteristics, *Ae. aegypti* has a specific characteristic on the dorsal thorax or mesonotum: white lines like a lyre-shaped arch, white knee-spot on the femora, anterior portion of mid-femur with a longitudinal white stripe, mesepimeron with two well-separated white scale patches, and clypeus with white scale patches (Figure 2A-L). The dorsal part of the abdomen has a white line on each segment. Like mosquitoes in general, this mosquito has three pairs of legs. The first pair of legs is shorter than the second pair, while the third pair is the longest. The tibia of the third pair has no white stripes. The tarsus of the hind legs has white stripes, especially on the 5th tarsomere which has a white dominant (Figure 2A-L).

Aedes albopictus has a narrow median-longitudinal white stripe on the scutum. Mesepimeron with not separated white scale patches or forming V-shaped white patch, anterior portion of mid-femur without longitudinal white stripe, and clypeus without white scale patches. The size of the proboscis is slightly shorter than the femur of the forelegs. There are white scales on the posterior mesonotum or supra alar. The dorsal abdomen or tergite has white scales with slightly curved edges on each segment. The hind legs have dominant white color in the 5th tarsomere. *Ae. albopictus* also has three parts of the scutellum (Figure 3A-L).

In this study, several organs of both mosquito *Ae. aegypti* and *Ae. albopictus* were observed using SEM were head, antennae, maxillary palps, thorax, abdomen, and eggs. Based on SEM observations of *Ae. aegypti* and *Ae. albopictus* have no significant difference within all parts of the body. Each segment of the mosquito antenna *Ae. albopictus* and *Ae. aegypti* have hair, funiculus, or bristle, which are sensory organs.

The proboscis has a similar shape that is pointed and covered by leaf-shaped scales (sc), bristle, funiculus (fn), and microtrichia (mt). The maxillary palps are blunt at the ends and covered with scales all over the surface, and there are several funiculi, bristles, and microtrichia. Bristles and scales measure about 200 μm and 0.01 mm, respectively. The antennae have many microtrichia with shorter sizes, funiculus, and sensilla trichodea. Whereas in males, it is dominated by long funiculi. Several types of sensilla were found in the proboscis short-sharp tipped (st-a), short-blunt tipped (st-b), and long-shaped tipped (st-c). The lateral part of the thorax shows that all parts are covered by short microtrichia and do not have many scales. Mosquito spiracles are oval holes measuring about 50 μm with microtrichia at the edges. The surface of the abdomen is covered with scales and some bristle, funiculus or sensilla. At the end of the abdomen, there is a pair of cerci appear at the end of the abdomen. Egg of *Ae. aegypti* is cigar-shaped, shiny jet black with slight dorsoventral curvature, ornamented with polygonal outer chorionic cells (OCC) that covers the entire surface of the egg and are tapered at the ends (Figure 4 and 5).

Discussion

Aedes aegypti and *Ae. albopictus* are dengue's main and secondary vectors in Indonesia, respectively (MoH 2021). In addition, these mosquitoes are also vectors of several diseases including Chikungunya and Zika (Severini et al. 2018; Vairo et al. 2019). Besides, mosquitoes are very close to human life and are often found in settlements. The behavior of *Ae. aegypti* is closed places or rooms, while *Ae. albopictus* tends to be outdoors. Therefore, the breeding habitat of *Ae. aegypti* is more often found indoors than outdoors and vice versa for *Ae. albopictus*. In addition to breeding sites, the blood-sucking activity of *Ae. aegypti* is more common indoors, while *Ae. albopictus* outdoors (Rahman et al. 2021; Saleh et al. 2021).

This research found several habitats around the campus, including buckets and containers inside and outside the room. In addition, mosquito larvae are also found in bromeliad flower plants. *Ae. albopictus* are primarily a mosquito often found in forests. However, this mosquito can adapt to species in rural, suburban and urban environments (Lien et al. 2015). Flexible physiological and ecological factors cause the high adaptation of these mosquitoes. This mosquito can survive in the tropics and four seasons, and its ecology is flexible, with many *Ae. albopictus* in the environment, ranging from tree holes, and bamboo strips to various manufactured containers (Waldock et al. 2013).

Aedes aegypti and *Ae. albopictus* have unique morphological characteristics that can be used to identify these mosquito species. This research makes it easier to identify and provide detailed information about these mosquitoes' characteristics. The microscopic picture of the two types of mosquitoes is still not fully understood until now.

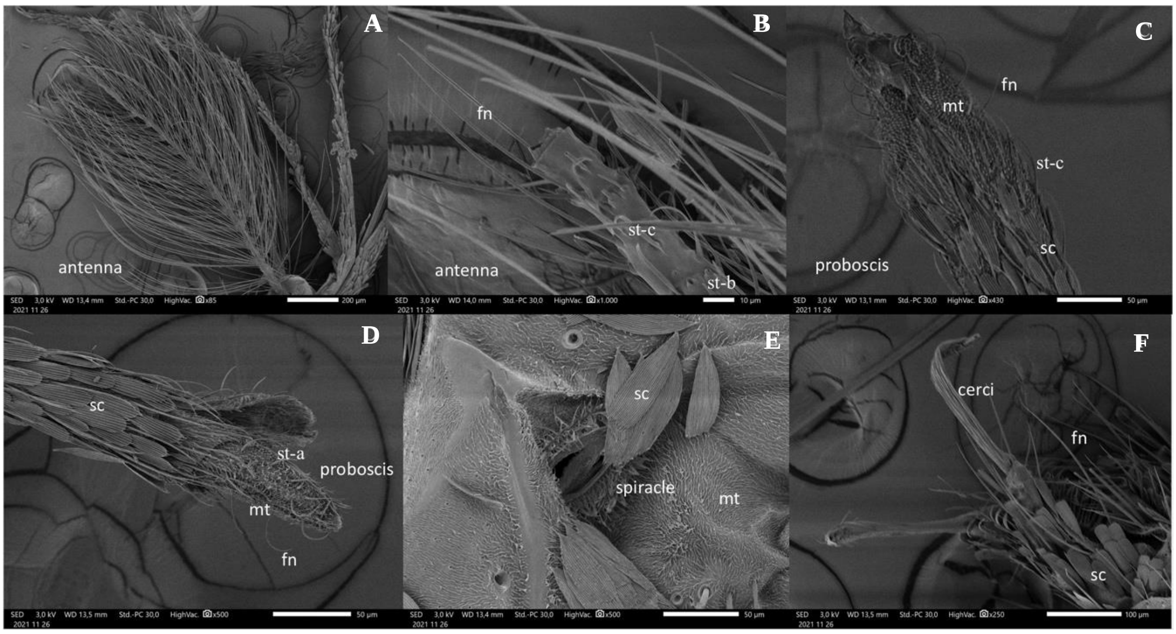

Figure 5. Scanning electron microscope of *Ae. albopictus*. antenna (A-B), proboscis (C-D), spiracle (E), the end of the abdomen (F). Note: short-sharp tipped (st-a); short-blunt tipped (st-b); long-shaped tipped (st-c)

Based on observations using an advanced light microscope, *Ae. aegypti* could be distinguished from *Ae. albopictus* of several distinct characteristics. *Ae. aegypti* has curved white scales on both dorsal edges of the scutum, the anterior portion of mid-femur with a longitudinal white stripe, and mesepimeron with two well-separated white scale patches. However, *Ae. albopictus* has white stripe scales on the median scutum and mesepimeron with no separated white scale patches or forming V-shaped and anterior portion of mid-femur without the longitudinal white stripe. In general, the morphological characteristics of the two mosquitoes can be easily distinguished by looking at these characteristics. This characteristic is related to the previous study by the Ministry of Health (2000), Diouf et al. (2020), Rueda (2004), and Kumar et al. (2022).

Several morphological variations commonly occur in the mosquito population. The size and color of mosquitoes often differ between populations, depending on environmental conditions and nutrients mosquitoes obtained during development. Several morphological variations in *Ae. aegypti* can also occur on the fifth tarsus. Generally, the fifth tarsus is predominantly white, but some *Ae. aegypti* has $1/3^{rd}$ to $2/3^{rd}$ of the 5^{th} segment of hind tarsi is dark (Diouf et al. 2020; Kumar et al. 2022). In addition, variations also occur in the pale color of the abdominal tergite in both male and female mosquitoes. According to McClelland (1974), variations can occur in the scales on the abdominal tergite in *Ae. aegypti* range from darkest to the palest color. The color variations in *Ae. aegypti* are strongly influenced by their natural habitat, artificial breeding places, environment, and rainfall (Kumar et al. 2022).

Various morphometric and morphological studies on egg chorionic cells have demonstrated their importance in species identification (Linley 1989). Eggs of *Ae. aegypti* and *Ae. albopictus* were found to be cigar-shaped, shiny jet black with a slight dorsoventral curvature. The eggs of both species are tapered at the end, but the eggs of *Ae. albopictus* are more tapered posteriorly. In addition, the eggs of *Ae. aegypti* were significantly longer and wider than those of *Ae. albopictus* (Bar and Andrew 2013). A study by Suman et al. (2011) showed that morphometrically (the eggs of this species) were 48.48% significantly different from 33 attributes. Those attributes include egg dimensions, micropyle apparatus, outer chorionic cell (OCC) dimensions and density, tubercles, and exo-chorionic tissue width.

In general, observations using SEM showed the characteristic microstructure of *Ae. aegypti* and *Ae. albopictus* did not have a significant difference. Several sensory organs, namely funiculus, microtrichia, and trichodea, are distributed on the proboscis, maxillary palps, and antennae. Microtrichia is a sensory organ spread almost all over the body, especially the maxillary palps, proboscis, thorax and abdomen. Scales are predominant on the maxillary palps, proboscis, and feet. While on the lateral thorax, and antennae, scales are rarely found. The sensory organ in the antenna is the olfactory sensilla, namely sensilla trichodea, which plays an important role in knowing environmental changes and detecting surrounding objects, including the host. This sensilla also functions to help mosquitoes to get a host, water, temperature, and CO_2. At the same time, some non-olfactory sensilla are scattered in several parts of the body, including the thorax and abdomen, namely sensilla chaetica, sensilla coeloconica, and sensilla ampullaceal (Hempolchom et al. 2017).

Identification of mosquitoes based on morphology is still the gold standard. This identification can use an advanced light microscope to provide clearer pictures, while SEM can be used to find out the microscopic structure. Generally, the microscopic picture based on SEM of the two types of mosquitoes is almost the same and difficult to distinguish. Even though there are differences based on macroscopic, sometimes on a micro level, there is not necessarily a significant difference. This information is very important, to know the morphological characteristics of the two types of mosquitoes as a basis for determining the appropriate control of dengue vector mosquitoes. Detailed microscopic images are very important to obtain complete information as the basis for developing *Aedes* mosquito control strategies.

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