# Morphological characteristic of malaria vector *Anopheles aconitus* (Family: Culicidae) revealed by advanced light and scanning electron microscope

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**Abstract.** *Supriyomo, Soviana S, Novianto D, Musyaffa MF, Tan S, Hadi UK. 2022. Morphological characteristic of malaria vector* Anopheles aconitus *(Family: Culicidae) revealed by advanced light and scanning electron microscope. Biodiversitas 23: 3546-3552.* *Anopheles aconitus* (*An. aconitus*) is one of the primary vectors for malaria in several areas in Indonesia, especially in Java Island. Scanning electron microscope (SEM) is a powerful tool that can differentiate *Anopheles* spp. Morphology that is difficult to identify by using a light microscope only. The adult of *An. aconitus* used in this study have been reared in the laboratory. This research is conducted to better understand the *Anopheles aconitus'* external morphology, such as the head, proboscis, wings, thorax and legs, using advanced light and SEM. Results from an advanced light microscope showed that *An. aconitus* has palpus with the same length as proboscis with one narrow basal end and two broad distal ends; meanwhile, the apical half of proboscis is pale. *Anopheles aconitus* has wings with four pale spots on the costa side. Legs are uniformly dark with only very narrow, rather faint pale bands at the joining of segments. Using SEM, we found several types of sensilla trichodea: short-sharp tipped, short-blunt tipped, and long-shaped tipped in antenna, apical proboscis, maxillary palps, abdomen, and legs. Antenna is also covered with microtrichia and sensilla. This research is important for basic necessity in fully understanding and determining of mosquito bionomic due to the development of effective and efficient mosquito vector control strategies.

**Keywords:** *Anopheles aconitus*, malaria, morphology, vector

## INTRODUCTION

Malaria is a devastating and life-threatening disease caused by *Plasmodium* protozoa, especially in under five years old children and women with pregnancy in most of tropical countries (Dancan et al. 2018; Talapko et al. 2019; Boualam 2021). Malaria remains the number one killer for communicable diseases, as there were around 200 million cases in 2018 (Varo et al. 2020). Indonesia is one of the tropical countries with a high burden of malaria, as the data from 2018 showed that the national Annual Parasite Incidence (API) value was 0.84 per 1000 people distributed in 285 districts (Sitohang et al. 2018; Guntur et al. 2021). There are five species in total, *Plasmodium vivax, P. ovale, P. falciparum, P. malariae*, and *P. knowlesi* (Komayaki et al. 2018). Among these five species, *P. falciparum* and *P. vivax* give the highest threat to humans, meanwhile, *P. knowlesi*, known as zoonotic simian malaria, that always been misdiagnosed as unthreatening (Barber et al. 2017). *Plasmodium* could be transmitted from human to human by the vector female *Anopheles* mosquito's biting.

Transmission from human to human could happen based on several factors, such as vector, the Plasmodium as an agent and the host. Vector control is important as one of the tools among other interventions in decreasing malaria transmission (White et al. 2014; Coleman et al. 2017).

Vector control comprises of several tools, such as chemical indoor residual spraying (IRS), long-lasting insecticide-treated nets (LLITNs), larvicides, development of novel control strategies, and knowledge of vector behavioral ecology (Benelli and Beier 2017). IRS and ITNS are the most common strategy adopted from all aspects of vector control. Using ITNs alone leads to insecticide resistance regarding chemical component pyrethroid insecticides used in ITNs, but combining both ITNs and IRS reduces the resistance problem (Lindsay et al. 2021). This strategy is successful in decreasing malaria incidence, but not followed by decreasing malaria prevalence in general, hence must be combined with a full understanding of vector bionomics and behavioral ecology.

More than 90 species of *Anopheles* mosquitoes have been identified in Indonesia, and 25 species have been confirmed as malaria vectors (Mahdalena and Wurisastuti 2020). *Anopheles aconitus,* as one of main vectors for malaria, especially in Indonesia and Malaysia, belongs to the subgenus *Cellia* (Yan et al. 2019). *An. aconitus* is one the main malaria vectors, especially in the Java island of Indonesia. It is mostly found in the wet season and is widely distributed from the coastal areas to highland areas 850-1000 m upper sea level (Stoops et al. 2009; Sugiarto et al. 2017). The main breeding site is either in natural or man-made habitat, such as terraced rice fields, shallow

irrigation canals, fish ponds, streams, and river beds (Elyazar et al. 2013; Soleimani et al. 2015). These species need warmer water (25-35°C) with concentrations of saline of 0-3 ppt, pH 6-8, and favor a place far away from inhabitation (MoH 2000). The feeding pattern of *An. aconitus* is also different depending on the habitation of the mosquitoes, as the feeding activity usually starts from 7 pm until 4 am, while the peak is at 8-9 pm (Mahdalena and Wurisa 2020). The exophagic type could be found in the highland, meanwhile, the endophagic is found mainly in the coastal area (Stoops et al. 2009).

Scanning electron microscope (SEM) has been a powerful tool recently for material characterization learning since its first commercialization 40 years ago. Even though the bionomics of *An. aconitu*s has been studied extensively, yet our knowledge of this species is still limited. In this study, we aim to investigate and describe the morphology image by using an advanced light microscope and SEM.

## MATERIALS AND METHODS

### Mosquito collection

In this research, we conducted SEM only for *An. aconitus,* due to there is difficulty in collecting and rearing another *Anopheles* species. The adult of *An. aconitus* used in this study have 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. The mosquitoes were killed by putting them into the refrigerator and stored in the freezer for 30 min before processing for this research. The eggs of *An. aconitus* were collected from female mosquitoes and stored in the refrigerator before processing by SEM.

### Mosquito identifications and characterization
*Morphological identification*

The collected mosquitoes were identified according to morphological and molecular techniques. Morphological identification of *An. aconitus* was performed using an advanced light microscope Leica M205Câ according to the morphological characteristic which is described by the Ministry of Health (2000).

*Characterization by Scanning Electron Microscope*

Morphological analysis by SEM was performed using Hitachi SU-3500 according to the manufacturer's instructions. Mosquitoes and eggs were rinsed by shaking in 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 6 h and several days. The samples were rinsed four times with Cacodylates buffer for 15 min each repetition and then continued rinsed by water (aquadest) for 15 min. 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 min and continued with 70, 85, and 95 for 20 min, respectively. The samples

were soaked twice with an alcohol concentration of 100% for 10 min. The dehydration was performed at 4°C for alcohol concentrations 50%, 70%, and 85% process, however, it was performed at room temperature for alcohol concentrations 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

The mosquitoes of *An. aconitus* used for advanced light microscope and SEM study were drawn from a laboratory colony maintained before in our insectary. Among the morphological and structural features of adult *An. aconitus* mosquito and eggs have been identified and described based on an advanced light microscope and SEM. The body of an adult *An. aconitus* can be divided into three regions: head, thorax, and abdomen, as in other mosquitoes (Figure 1A).

Prominent head, including the antenna, a pair of compound eyes, proboscis, and palpus, are described. Based an advanced light microscope showed that the compound eyes of *An. aconitus* are located dorsolaterally and composed of numerous ommatidia. The male and female of *An. aconitus* can be determined using antenna morphology, as male mosquitoes have a hairy and bushy antenna while female mosquitoes do not. The antenna of male and female *An. aconitus* mosquito comprises 15 segments with lengths of 0.2 mm and 0.3 mm, respectively. The first segment is called the scape, which is a narrow ring attached by a flexible fold of the integument to the next segment. The second part is called a pedicel, and the third part is called a flagellum, which consisting 13 segments. At each segment's junction, there is a pigmented ring on either side, there is an unpigmented area. The bristles which are hair-like structures, numerously distributed on every 13 segments of flagellum. These bristles are primary olfactory sensilla which are pointed or blunt, arising from sockets. The bristles are almost immovable on the antenna ((Figures 1B and 1C). The proboscis of both *Anopheles* female and male have as long as palpus, about 0.1 mm, with half of the proboscis being pale. Maxillary palps consist of several segments in mosquitoes. The apical palps of both male and female of *An. aconitus* are wider and rounded, a characteristic of sub-family Anophelinae. The maxillary palps have one narrow basal band and two broad distal bands. The maxillary palps are covered by scales that vary in color from silvery white to cream and yellowish to dark (Figures 1B and 1C). Thorax of *An. aconitus* is elongate, and the color of the scutal integument is brownish to gold with scattered hair (Figure 1D).

The *An. aconitus* have the posterior margin of the scutellum rounded. Therefore, the posterior of the thorax is covered by scutellar setae. The surface of the dorsal thorax has a lot of hair and microtrichia, while on the lateral and

below the thorax have less hair and microtrichia. There is no bristle or sensilla-like hair in the lateral and below the thorax. Scales are absent on the thorax (Figures 2A and 2B). Abdomen has dark sterna and is without scales (Figure 2C). Wing of *An. aconitus* have a length of about 3.0-4.0 mm. The wings of *An. aconitus* are covered by colored scales from dark to pale. The *An. aconitus* has four pale spots of wing costa (Figure 2D).

Mosquito has three pairs of legs. The third leg is usually important in the identification of *An. aconitus* to distinguish from another *Anopheles* species. The tarsus, which is the distal parts of mosquito legs usually used for identification. The tarsus of mosquito consists of five tarsomeres as well as the joint of tarsomeres. There were some specific features of *An. aconitus* are the white or pale color of the fifth tarsus, a narrow or small band on the joint between the tibia and tarsus (Figure 3A-D).
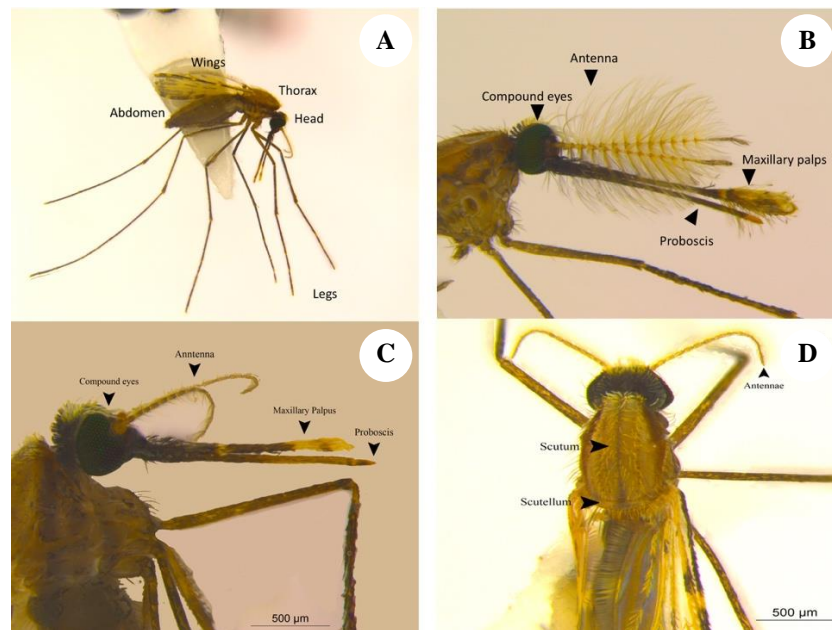


**Figure 1.** *Anopheles aconitus* morphology based on advanced light microscope: A. whole body of *An. aconitus*; B. head of male *An. aconitus*; C. Head of female *An. aconitus*; D. dorsal view of thorax
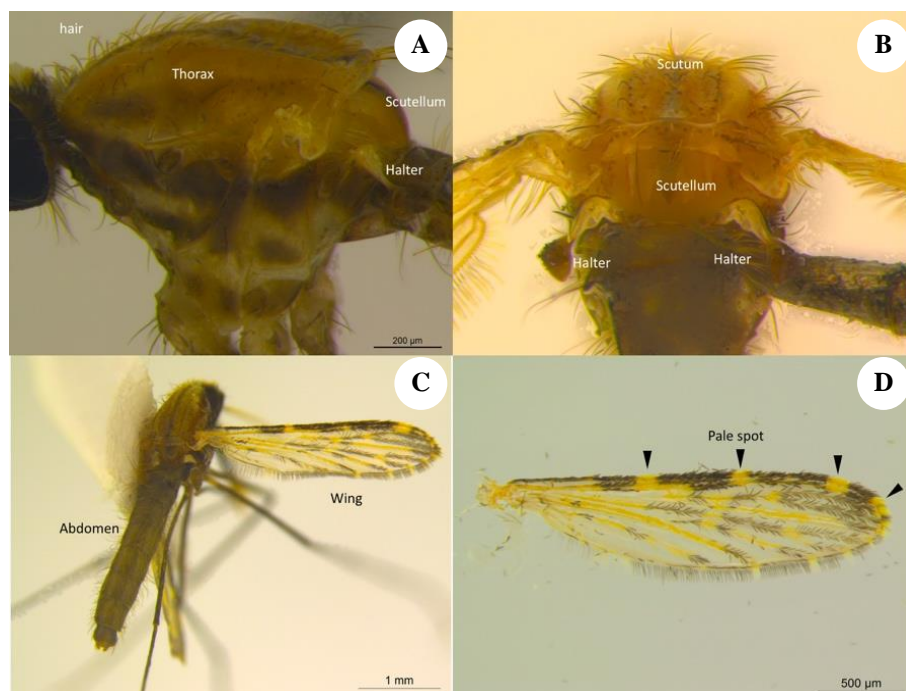


**Figure 2.** *Anopheles aconitus* morphology based on advanced light microscope: A. lateral thorax of mosquito; B. scutellum of mosquito; C. dorsal of abdomen; D. spot pale on wing of mosquito

**Figure 3.** *Anopheles aconitus* morphology based on advanced light microscope: A. front leg of mosquito; B. middle leg of mosquito; C. hind leg of mosquito; D. magnification of hind leg
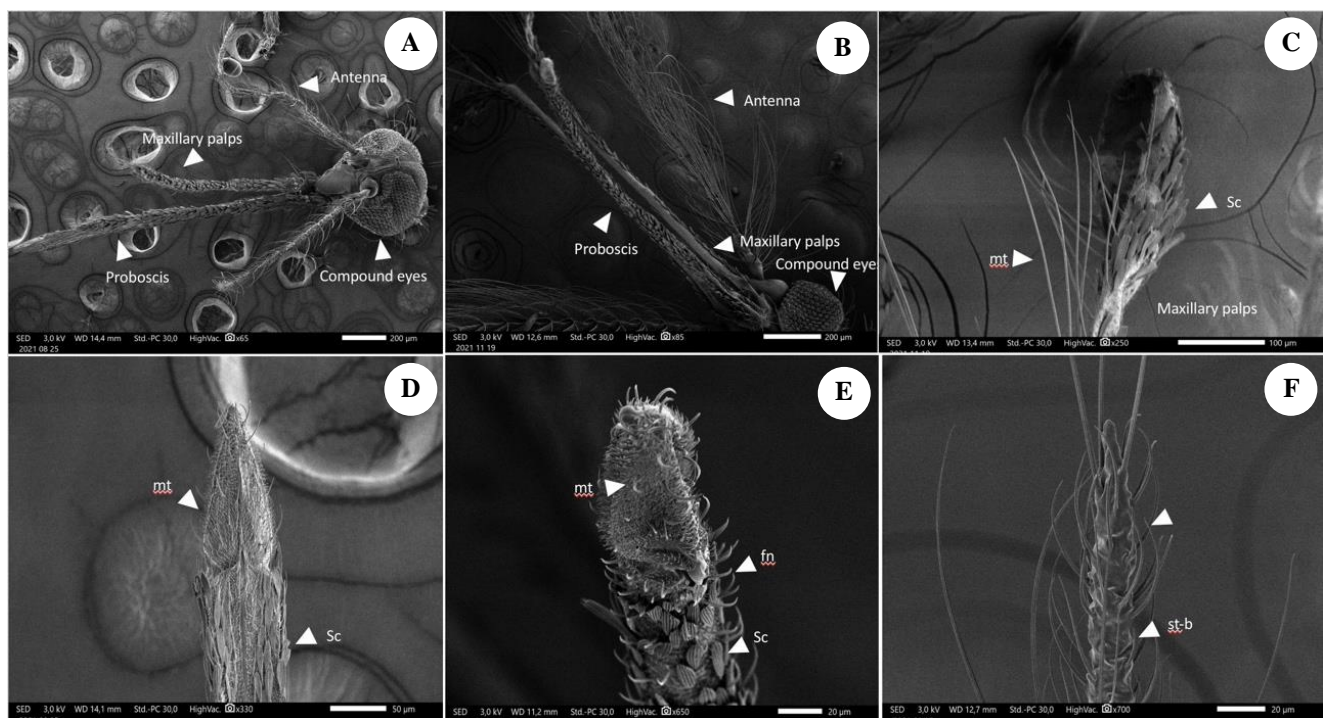


**Figure 4.** Scanning electron microscope of *Anopheles aconitus:* A. head of female mosquito; B. head of male mosquito; C. the apical of maxillary palp; D. the apical of proboscis (e) dorsal of proboscis (f) antenna. Note: mt: microthrichia; sc: scale; fn: funiculus

Based on the SEM observation, the surface of the palps of both males and females are covered by scales. The scales have a size of about 0.01 mm. The palps of both sexes are composed of the same types of structures. These are microtrichia (mt), cuticular projections and scales (sc), and flattened hairs. The proboscis is covered by fewer scales, and the apical of the proboscis consists of microtrichia and some funiculus (fn) or sensilla. The scale has a size of about 0.01 mm several sensilla are found on the proboscis. The antenna is covered by microtrichia and several types of sensilla trichodea. The types of sensilla trichodea are short-sharp tipped (st-a), short-blunt tipped (st-b), and long-shaped tipped (st-c) (Figure 4A-F).
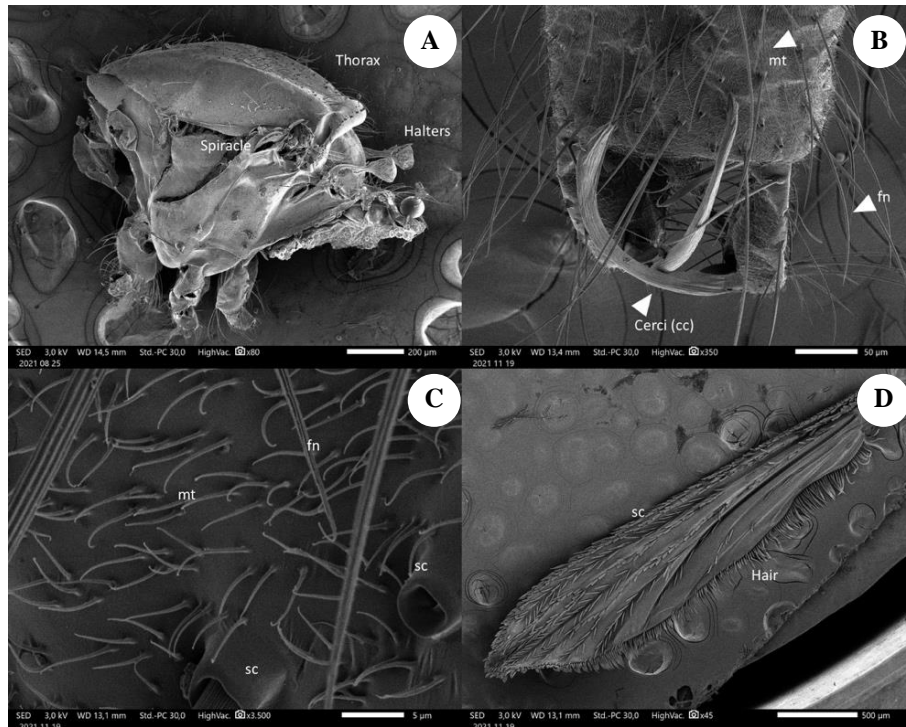
**Figure 5.** Scanning electron microscope of *Anopheles aconitus:* A. lateral thorax of mosquito; B. apical of abdomen and cerci; C. magnification of abdomen; D. wing of mosquito
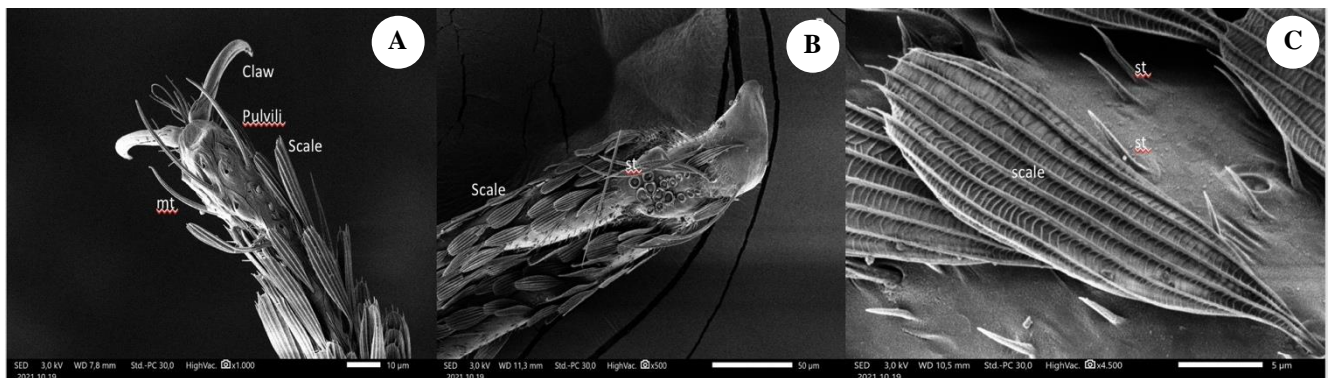


**Figure 6.** Scanning electron microscope of *Anopheles aconitus:* A. the apical of tarsus of hind leg; B. the dorsal of femur of hind leg; C. the scale and sensilla trichodea were short-sharp tipped (st)

Examination by SEM showed the dorsal mosquito thorax is covered with hair, which is curved with a sharp or narrowly rounded apex. A small area of the thorax was covered by scales, and the form of scales was similar to the other body part. The abdomen is largely devoid of scales and covered by microtrichia. On the apical of the abdomen, there was a pair of curved cerci (cc). Wings of *An. aconitus* covered by scales (Figure 5A-D)

Based on SEM, we observed there are a lot of scales on the legs distributed on each segment of the legs. The scales are leaf-like structures. There is some sensilla forms sensilla trichodea (st), the sensilla located near the joint, and the apical of tarsus or near the claws. Besides, microtrichia is also distributed on each segment of legs but is less dominant (Figure 6A-C). The characteristic of *An. aconitus* eggs similarly with another *Anopheles* with a boat shape. The egg has a length 0.01 mm and is broader in the anterior. The color of eggs varies from brown to dark. Based on SEM, the exochorion is covered by granular layers (Figure 7A-D).
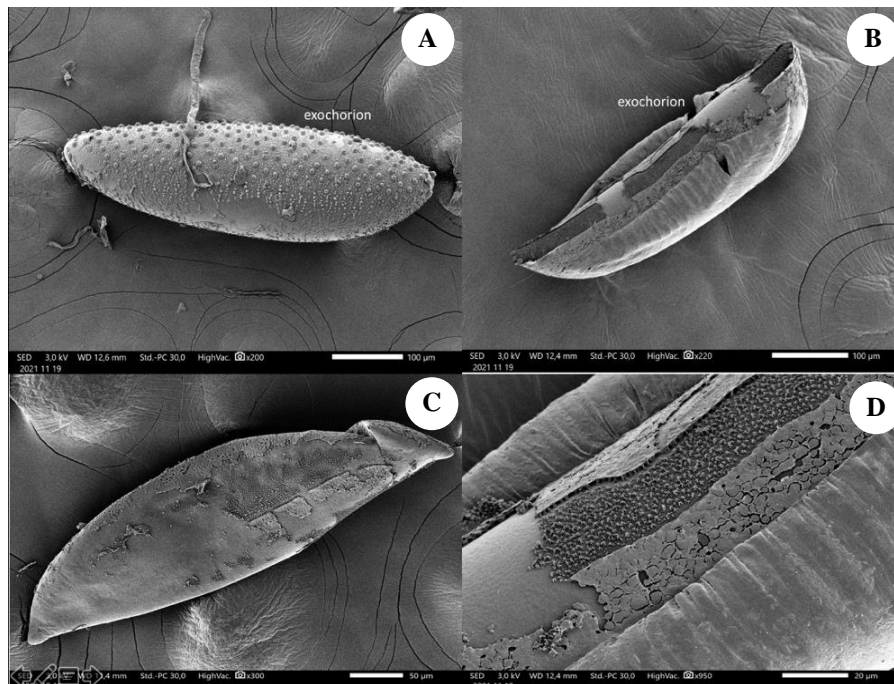
**Figure 7.** Scanning electron microscope of egg of *Anopheles aconitus*

## Discussion

*Anopheles aconitus* is the primary vector of malaria in Java Island (Matowo et al. 2021). In this study, we characterized one species *An. aconitus* morphology based on advanced light and SEM. The details morphological description of *An. aconitus* can be used for bank data of mosquito characteristics and to distinguish species *An. aconitus.* In general, adult *Anopheles* mosquitoes can be distinguished by scales color on wing veins, head, legs, thorax, and abdomen; proboscis long, clypeus, and wing venation (Harbach and Kitching 1998; Jourdain et al. 2018).

We showed that *An. aconitus* has unique morphological characteristics that can be used for the identification of this species. As a basis for the identification key of *Anopheles* based on morphology in Indonesia by the Ministry of Health (MoH 2000). Our observation showed the unique characteristic of *An. aconitus* using photographs to make an easier and more details description of this species. In general, the characteristics of *An. aconitus* is different from other *Anopheles* where half of the proboscis pale, four pale spots of wing costa, scutellum rounded, and white or pale color of the fifth tarsus, narrow or small band on the joint between tibia and tarsus (Lesmana et al. 2020; Sallum et al. 2020). The proboscis of both *An. aconitus* female and male as long as palpus and consists of five segments similar structures were reported in other mosquitoes (Hempolchom et al. 2017). Male mosquito has hairy and bushy antennae, while female mosquitoes are a lot less hairy. The male mosquito has fewer sensory organs, especially receptors of the olfactory system (Sallum et al. 2020). Mosquitoes usually use antenna and palps for the reception of stimuli and host finding.

Based on the SEM, we found that *An. aconitus* has various types of sensilla, bristle-like hair, or microtrichia on different body parts with distinct morphological characteristics. Microtrichia or the bristle-like hair and sensilla were dominantly found on antenna and palps. While scales are dominantly found in palps, proboscis, and legs. The scales are less on the thorax and abdomen. The sensilla are used to identify the hosts, visual cues, water vapors, heat and $CO_2$, and body odor (Mathania et al. 2020). Several non-olfactory sensilla usually find in mosquito are sensilla chaetica, sensilla coeloconica, and sensilla ampullacea. Sensilla chaetica are found at the apical sensilla coeloconica, sensilla ampullacea, found on top of antenna. The olfactory sensillum, sensilla trichodea are usually distributed on the antenna (Hempolchom et al. 2017). Some mosquito species have a clear pattern of scales and some are not. The presence of scales on the mosquito's body can be used to identify mosquito species. Scales have different colors and are generally scattered on the thorax, legs, and other body parts and dependent on the type of species. *An. aconitus* usually laid their eggs singly on the water surface or on floating objects. The eggs of most species show characteristic features by which they can be identified, for example, the character development of the air floats, development of the exo-chorion and differences in size and shape (Mathania et al. 2020).

Identification of mosquito species using external morphological characteristics is still the preferred method. General morphological features can be seen under a light microscope, but specific aspects can be used SEM as advanced tools. Morphological characteristics are very important in determining the identification of *Anopheles* mosquito species. There is difficulty in collecting and rearing another *Anopheles* species. Therefore, we

conducted research only for *An. aconitus* species. However, there is no morphological variation within *An. aconitus* species, detailed information related to the morphological characteristics of *An. aconitus* is very essential to know the complete information both macroscopically and microscopically. This information is also useful for developing the control strategy of *Anopheles*.

## ACKNOWLEDGEMENTS

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