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Research Article

Structural differentiation of *Aedes aegypti* and *Aedes albopictus* eggs using Scanning Electron Microscope

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Abstract: *Aedes aegypti* and *Aedes albopictus* are common arboviral mosquitoes causing the fatal diseases globally. Although these two vectors are bionomically similar, the differences in morphology of egg between these two mosquitoes are very limited in Myanmar. In this study, fifty eggs of each *A. aegypti* and *A. albopictus* were studied to determine any differences between these two-mosquito species morphometrically and morphologically using scanning electron microscopy (SEM). Average length and width of *A. albopictus* eggs were 509.3±34.9 µm and 157±6.8 µm respectively, and those of *A. aegypti* eggs were 555.5±42.6 µm and 141.7±30.6 µm respectively. There was no significant different in morphometric analysis of these two species, but they had different features on their egg surface morphologically. *A. albopictus* eggs were smaller and taper at the posterior end. But the micropylar disc of *A. aegypti* had incomplete circular sectors. *A. albopictus* had a circular disc without sectors. Outer chorionic cells enclosing both large central tubercle and peripheral tubercles were also different between these two species. Furthermore, the exchorionic networks in *A. albopictus* were narrow, prominent, solid wall like whereas they were interwoven, reticulated and extremely wide in *A. aegypti*. The morphological analysis of the eggs attributes of *A. aegypti* and *A. albopictus* using SEM enables the differentiation of the species and may be helpful in understanding the egg biology.

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INTRODUCTION

Aedes aegypti and *Aedes albopictus* are common arboviral mosquitoes causing the fatal diseases globally (1, 2). These two mosquitoes are day biter, anthropophilic and peridomestic habitation. These mosquito eggs can survive devoid of water for longer period that facilitate to spread over throughout the countries and regions. Vector control is very important in Dengue hemorrhagic fever and Chikungunya because of the lack of vaccines and effective treatment. These mosquito species can be differentiated by adult morphology (3) and genetics (4) and also by egg morphology and morphometry (5).

Nowadays, it is a difficult task to detect the different mosquito vectors in urban area affecting the impact assessment of the ongoing vector control programs. Therefore, ovitrap is used for providing the artificial breeding site for mosquitoes. Researchers can easily collect and study the eggs found in the container. Therefore egg-based species identification is also important in vector control programs.

Although these two vectors are bionomically similar, the differences in morphology of eggs between these two species are very limited in Myanmar. Therefore, present study was done to determine any differences between eggs of *A. aegypti* and *A. albopictus* morphometrically and morphologically at subcellular level.

MATERIALS AND METHODS

Sample Preparation

A. aegypti and *A. albopictus* mosquitoes were maintained at 27±2°C, 80 ± 10% RH, in the Medical Entomology Research Division. Females fed on mouse blood were isolated individually for oviposition. Embryonated or 24 hrs. old oviposited eggs were taken for the study.

Small strips of the filter paper substrate were cut to obtain the small number of egg batches obtaining from the oviposition substrates randomly. Eggs containing filter paper substrate were mounted to SEM stubs, using double-sided carbon tape. Then stub surfaces were sputter coated with gold. Images of the eggs were obtained using Zeiss Evo 18 scanning electron microscope. Fifty numbers of each *A. aegypti* and *A. albopictus* eggs were studied. Terminology for description of eggs morphology was adopted from (6).

Data Analysis

The lengths of mosquito eggs were measured from the anterior to posterior end. The width from the widest, 1/3 anterior and 1/3 posterior regions were determined. The length to width ratio was calculated. To determine the statistically significant differences ($p < 0.05$) between the eggs of two mosquito species, t-test was used.

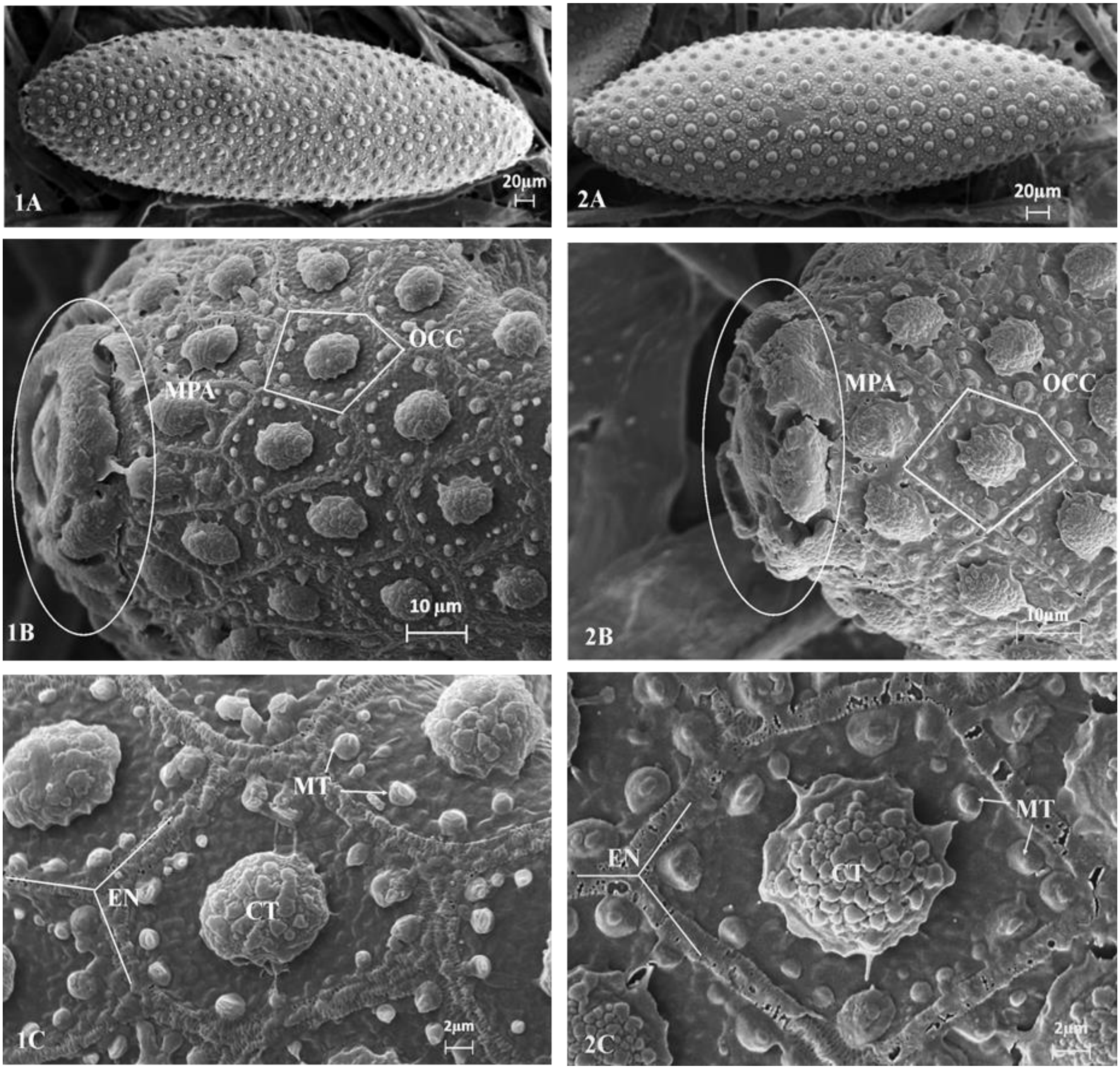

Fig. 1: Scanning electron micrographs of eggs of *A. aegypti* (1A-C) and *A. albopictus* (2A-C). Figure C represent the detail structure of outer chorionic cell (OCC) with central tubercle (CT), minute tubercles (MT) and exochorionic network (EN), respectively. MPA: Micropylar apparatus, MT: Minute tubercles.

In both *A. aegypti* and *A. albopictus*, the total egg surface was decorated with polygonal shape outer chorionic cells that composed of large central and small peripheral tubercles apart from the micropylar apparatus region (Figure 1 and 2). Outer chorionic cells (OCC) were frequently hexagonal shape, rarely pentagonal and quadrilateral cells (Figure 1B and 2B). These OCCs were almost similar to the features that described in other studies of *Aedes* mosquito (8, 9) and *Culex* species (11). Minute tubercles were observed closely adjacent to the exochorionic network (EN) structure and large central tubercle was present at the center of chorionic cells in the eggs of both species.

RESULTS AND DISCUSSION

Eggs of *A. aegypti* and *A. albopictus* were identified as cigar shaped, glistening black in appearance. Although both eggs have narrowed ends, *A. albopictus* eggs are narrower posteriorly (Fig. 1 A and B). The micropylar apparatus was very prominent feature with central micropylar pore, micropylar disc and a wall of tooth-like tubercles in both mosquitoes. The micropylar apparatus was similar to the features of other studies (8, 9) and found to be the prominent feature for species confirmation in these Aedes mosquitoes, as in Anopheles (10) and Culex (11).

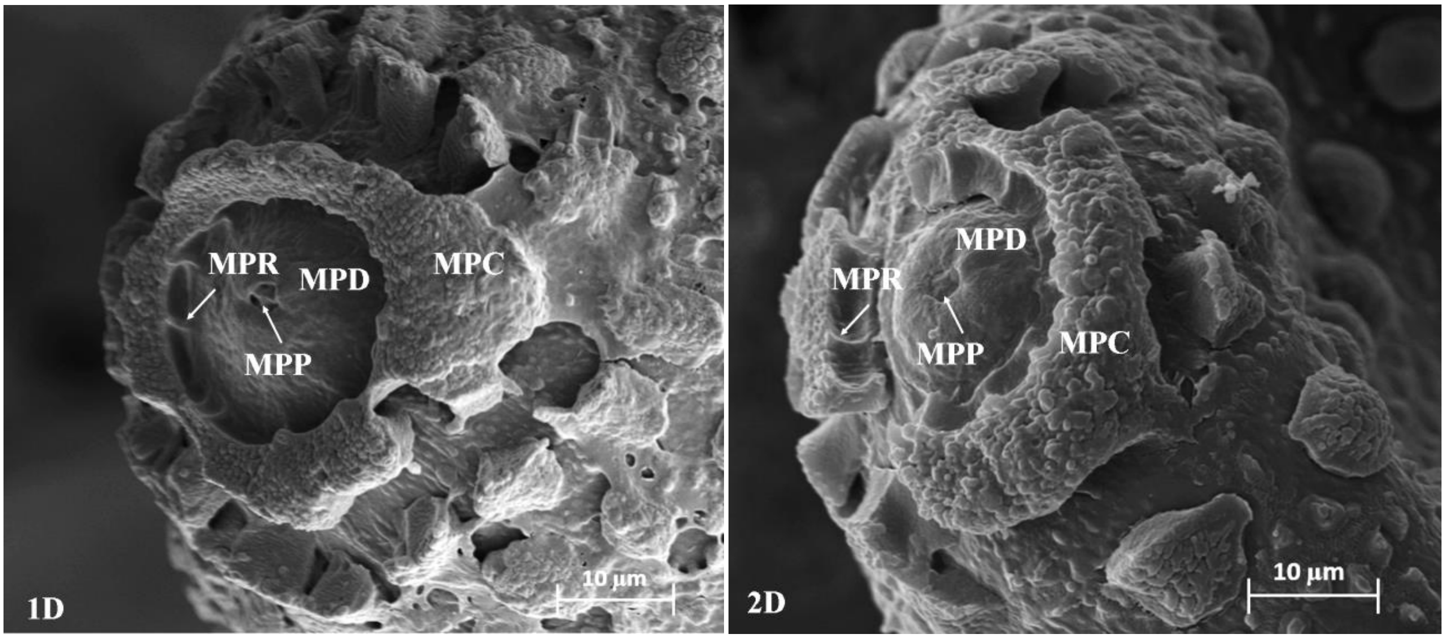

Fig. 2: Detail structure of micropylar apparatus in the eggs of *A. aegypti* (1D) and *A. albopictus* (2D). MPD: Micropylar disc, MPP: Micropylar pore, MPR: Micropylar ridges, MPC: Micropylar collar.

Table 1: Comparative dimensions of the whole egg of *Aedes albopictus* and *Aedes aegypti.*

Attributes	*A. aegypti*	*A. albopictus*	Level of significance
Egg length (μm)	555.5±42.6	509.3±34.9	0.85
Egg width (μm)	141.7±30.6	157±6.8	0.78
Egg width at 1/3 ant end (μm)	130.5±30.1	146.1±2.0	0.62
Egg width at 1/3 post end (μm)	126.3±17.7	130.2±4.5	0.94
Ratio Egg length & width	3.9±1.2	3.2±0.1	1.2

All the values are in mean ± standard deviation.

Furthermore, the EN in *A. albopictus* was narrow, prominent, solid wall like whereas they were interwoven, reticulated and extremely wide in *A. aegypti* (Figure 1C and 2C). *A. albopictus* had a circular micropylar disc without sectors. But the micropylar disc of *A. aegypti* had incomplete sectors with distinct ridges (Figure 1D and 2D).

Average length and width of *A. aegypti* eggs were 555.5±42.6 μm and 141.7±30.6 μm respectively, and those of *A. albopictus* eggs were 509.3±34.9 μm and 157±6.8 μm respectively. Egg width at anterior 1/3 end of *A. aegypti* and *A. albopictus* were 130.5±30.1 μm and 146.1±2.0 μm, and Egg width at posterior 1/3 end of *A. aegypti* and *A. albopictus* were 126.3±17.7 μm and 130.2±4.5 μm respectively. Length and width ratio was not much difference between the eggs of *A. aegypti* and *A. albopictus* two species (3.9±1.2 vs 3.2±0.1). The eggs of *A. aegypti* were longer and broader than those of *A. albopictus* (Table 1).

This study demonstrated the fine structure of the eggs of *A. aegypti* and *A. albopictus*, similar to in other studies, but smaller than the reported sizes (4, 5). The eggs of *A. aegypti* were larger size with broader reticulated EN compared to the eggs of *A. albopictus*, in which they were smaller with a thinner solid EN. Thus *A. albopictus* has the strong egg shell protecting the eggs against crushing from drying and other problems, which suggested that eggs of *A. albopictus* may provide the effective colonization in both natural and artificial breeding sites compare to those of *A. aegypti*. It has been documented that *A. albopictus* has occupied most regions of the world in contrast to *A. aegypti* which has suitable oviposition sites in urban centers (7).

Although there was no significant different between these eggs of two Aedes species morphometrically, they had different features on their egg surface morphologically.

CONCLUSION

Present study identified the subcellular structure of the eggs of *A. aegypti* and *A. albopictus*. The morphological analysis of the eggs attributes of *A. aegypti* and *A. albopictus* using SEM enables the structural differentiation of the mosquito species in Species Specific Surveillance System for vector control and helpful in understanding the egg biology.

CONFLICTS OF INTEREST

The authors have reported no conflict of interest.

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Dr. Min Min Win is serving as a Research Scientist in Department of Medical Research, Yangon, Myanmar, for 16 years. She has received her M.B.B.S degree in 2002 and M. Med. Sc. (Pathology) in 2006 from Institute of Medicine (1) Yangon. She has published 6 research papers in reputed journals and delivered several oral presentations in national and international conferences. Her areas of interest are Electron Microscopy, Histopathology and Molecular pathology. She has received second best poster award in South East Asia Breast Cancer Symposium, 2018.