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WING GEOMETRY ANALYSIS TO SEPARATE SPECIES AND SEXES OF AEDES AEGYPTI AND AE. ALBOPICTUS AS DENGUE VECTORS IN SAMUT SONGKHRAM PROVINCE, THAILAND

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Abstract- In Thailand, dengue fever is a major public health problem. Vector control is an important strategy for control of the dengue epidemic, but information on the biology, ecology and behavior of Aedes aegypti in the area is required. Ae. aegypti and Ae. albopictus are similar in appearance but can be identified by the pattern on the thorax. However, there is a problem distinguishing between the two species of Aedes in the field, caused by damage or other factors, leading to the pattern on the thorax disappearing. Therefore, the landmark-based method of geometric morphometrics (GM) was applied to identify Aedes mosquitoes. Larvae of Ae. aegypti and Ae. albopictus were collected in the most infected sub-districts of Samut Songkhram Province by Ovitrap from August to November 2016. The results showed that GM can be an alternative or complementary technique for the identification of Aedes mosquitoes. Statistically significant differences were noted in the size of Aedes mosquito ($p<0.05$). All sexes and species of Aedes mosquito are statistically different in shape, as assessed by non-parametric methods (1000 runs) ($p< 0.05$). The results of this study showed that GM can be used for the identification of Aedes mosquitoes, leading to suitable dengue planning to control mosquito vectors.

Keywords- Wing geometry, Aedes aegypti, Ae. albopictus, Samutsongkhram, Thailand

I. INTRODUCTION

Dengue Fever (DF) is a public health problem worldwide, especially in tropical and sub-tropical countries[1]. DF is caused by dengue virus, which is carried by Aedes aegypti, while Ae. albopictus, which is closely related to Ae. Aegypti, is a carrier of Chikungunya disease. In Thailand, DF is a major public health problem. The ratio of DF infection in Thailand is 219.46 per 100,000 people according to the report of the Bureau of Vector Borne Disease. Samut Songkhram province has one of the highest morbidity rates of all DF outbreak areas. In 2015, the patient ratio was 349.7 per 100,000 people and the highest numbers of cases were in August and September, with 106 and 111 individuals, respectively. The data indicate that this province is a high epidemic area of DF, which must be resolved urgently[2]. Vector control is an important strategy for control of the dengue epidemic, but information on the biology, ecology and behavior of Ae. aegypti in the area is needed. Identification of mosquitoes by morphology is a gold standard, but there are some limitations causing confusion or misidentification with species of similar morphology. Ae. aegypti and Ae. albopictus are similar in appearance but can be identified by the pattern on the thorax of the mosquito. However, there is a problem with distinguishing between the two species of Aedes mosquito in the field, caused by damage to the thorax, causing the pattern to disappear[3]. This is an important problem in identifying DF vectors, which leads to the failure of Mosquito preparation Only the right wing of Aedes aegypti and Ae. albopictus were analyzed. The right dengue surveillance data. Molecular techniques provide a way to solve these problems by detecting DNA, but they are expensive and require highly skilled personnel. Therefore, new alternative techniques are needed to solve these problems.

Geometric morphometrics (GM) is a modern technique to study shape and size. The advantage of this method is that it is inexpensive and fast[4]. There are reports of identification of morphologically similar species, sibling species or members of mosquito complexes[5]. Landmark based GM was applied in this study to identify species and sexes of Ae. aegypti and Ae. albopictus in Samut Songkhram province as a DF epidemic area, Thailand. The results of this research will provide a guideline for the use of GM in the identification of dengue vectors, leading to suitable DF control.

II. MATERIALS AND METHODS

Mosquito collection

Larvae of Aedes aegypti and Ae. albopictus were collected from the most infected sub-districts of Samut Songkhram including Ladyai, Suanluang and Jonpluak sub-districts. Ae. aegypti larvae were collected from dense household and population areas in each sub-district of DF outbreak areas by Ovitrap from August to November 2016. Ten ovitraps were set around houses in each sub-district. After that, field collected larvae were reared in the laboratory of the College of Allied Health Sciences. When adults emerged, they were identified using Illustrated Keys to the Mosquitoes of Thailand.

wings were dissected and mounted on microscope slides using Hoyer solution. All of the sample wings were photographed using a Nikon DS-Ri1 SIGHT digital camera connected to a Nikon AZ 100 M stereo-microscope (Nikon Corp., Tokyo, Japan). Then, the images were analyzed by the GM CLIC Program.

Geometric morphometric

Data collection and analyses were performed using the various modules of the CLIC version 97 (Collecting Landmarks for Identification and Characterization), which is freely available at http://mome-clic.com. Fourteen landmarks were digitized (Fig.2). For these selected landmarks, there is a selection criterion that must be clearly visible in order to prevent mistakes when plotting. The landmark-based GM analyzed the size and shape of Aedes mosquitoes.

For wing size estimation, the size was measured by estimating the centroid size (CS), defined as the square root of the sum of the squared distances between the center of the configuration of landmarks and each individual landmark. Statistical difference of the centroid size of the sex and species of mosquito wings was analyzed by non- parametric permutation tests (1000 cycles).

For wing shape evaluation, shape variables were measured and analyzed by Principle Components (PCs) of the "partial wrap" scores calculated after generalized procrustes analysis of raw coordinates. Similar to wing size estimation, the statistical difference of the centroid size of the sex and species mosquito wing shape was analyzed by non-parametric permutation tests (1000 cycles). A neighbor-joining (NJ) tree was generated and calculated based on Procrustes distances between Ae. aegypti and Ae. albopictus.

III.RESULTS

By applying the GM technique, we analyzed a total of 240 samples, comprised of 113 female and 123 male mosquitoes. According to species of Aedes mosquito, 220 analyzed samples were Ae. aegypti and 20 samples were Ae. albopictus (Table 1).

Table 1. Number of Aedes mosquito used for analysis.

Sex	Species of Aedes mosquito		
	Ae. aegypti	Ae. albopictus	Total
Female	103	14	117
Male	117	6	123
Total	220	20	240

Size variation

Size variation in the wings of Ae. aegypti and Ae. albopictus was analyzed from the centroid size average of the wings. The results showed that females of both Ae. aegypti and Ae. albopictus had higher mean sizes than males. Female Ae. aegypti had the largest wing size (2.02±0.17), followed by female Ae. Albopictus (1.97±0.12), male Ae. aegypti (1.58±0.16) and male Ae. albopictus (1.57±0.14), respectively (Table 2). The statistically significant differences of the size of Aedes mosquito found that most species were different ($p<0.05$), while female and male Ae. aegypti were not statistically different ($p=0.30$) and nor were female and male Ae. albopictus ($p=0.97$) (Table 4).

Shape variation

After superimposition of the mean landmark configuration of the wings of the male and female Ae. aegypti and Ae. albopictus, polygons as connected mean landmark positions were demonstrated. The polygons of female and male Ae. aegypti and Ae. albopictus visualized the wing shape of each species, which were overlapping and unclear (Fig. 1). The factor maps of Aedes mosquito classified by sex and species from landmark-based discriminant analysis by partial wrap show the overlap between female and male Ae. aegypti and female Ae. aegypti and female Ae. albopictus (Fig. 2 A,C). There were no overlaps including female and male Ae. albopictus or male Ae. aegypti and male Ae. albopictus (Fig. 2 B,D).

Table 2. Means of wing centroid size of Ae. aegypti and Ae. Albopictus.

Sex	Species	n	Means±SD (mm.)	Range (Min - Max)
Female	Ae. aegypti	103	2.02±0.17	1.48 – 3.00
	Ae. albopictus	14	1.97±0.12	1.78 - 2.10
Male	Ae. aegypti	117	1.58±0.16	1.20 – 2.25
	Ae. albopictus	6	1.57±0.14	1.35 – 1.76

n = Number of mosquito

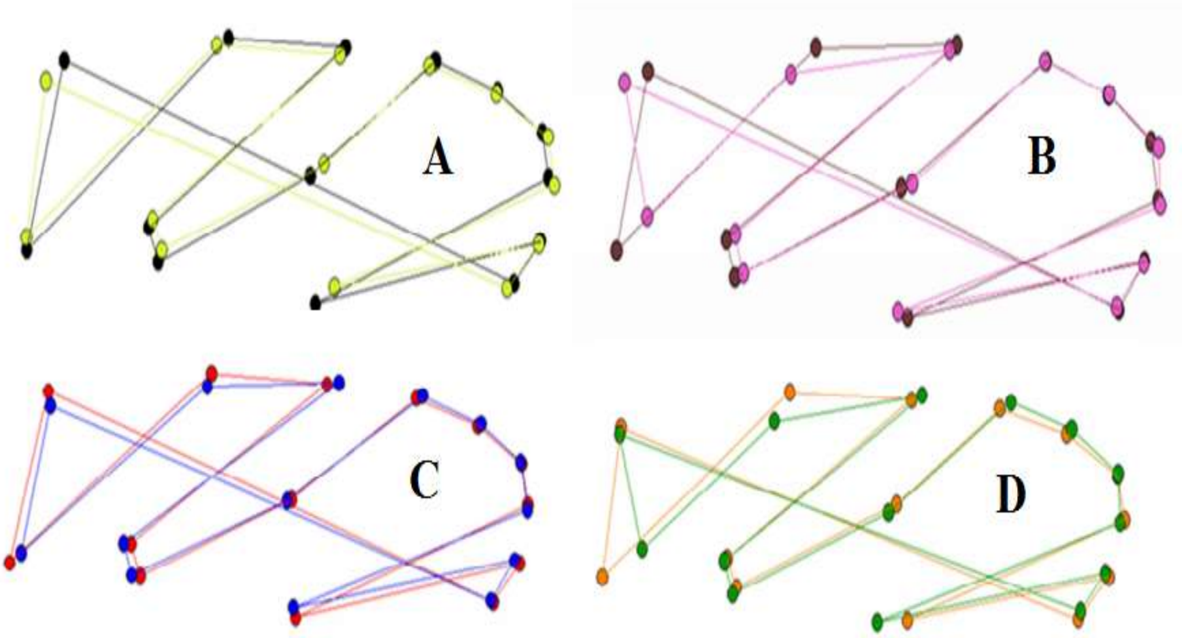

Fig.1 Superposition of the mean landmark configurations of Aedes mosquito classified by sex and species; A = female (black) and male (yellow) Ae. aegypti, B = female (black) and male (pink) Ae. albopictus, C = female Ae. aegypti (red) and female Ae. Albopictus (blue), and D = male Ae. aegypti (green) and male Ae. albopictus (orange),

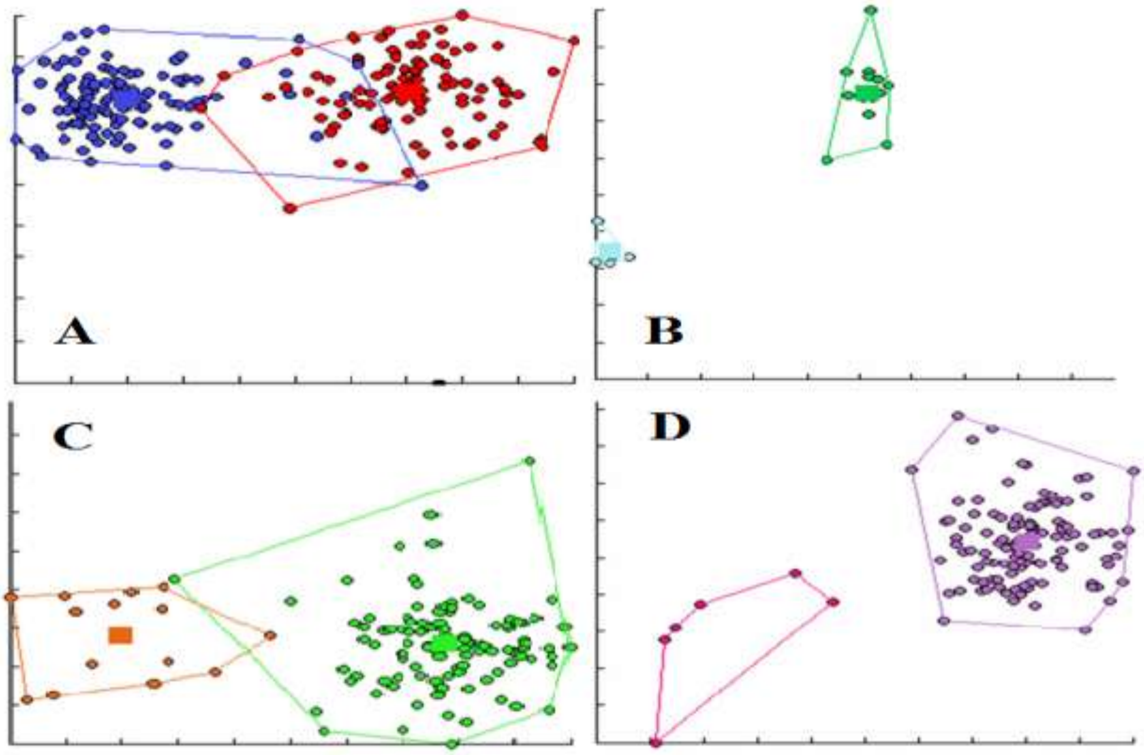

Fig. 2 Factor map from landmark-based discriminant analysis by partial wrap of Aedes mosquito classified by sex and species; A = female (red) and male (blue) Ae. aegypti, B = female (green) and male (light blue) Ae. albopictus, C = female Ae. aegypti (green) and female Ae. albopictus (orange), and D = male Ae. aegypti (purple) and male Ae. albopictus (pink).

Mahalanobis distances of female Ae. aegypti and male Ae. albopictus gave the highest value (6.98) (Table 3), showing that this group was the most different, while the lowest value of Mahalanobis distances was for female Ae. albopictus and male Ae. aegypti (1.84) (Table 3). Wing shape was significantly different for Aedes mosquitoes using non-parametric methods (1000 runs) applied to the pairwise Mahalanobis distances ($p< 0.05$) (Table 4). The neighbor-joining trees, based on Mahalanobis distances between PCs, separated each sex and each species of Aedes mosquito (Fig. 3).

Table 3. Mahalanobis distances between wing shapes of Aedes mosquito classified by sex and species.

	Females		Males	
	Ae. aegypti	Ae. albopictus	Ae. aegypti	Ae. albopictus
Females				
Ae. aegypti	0.00			
Ae. albopictus	4.00	0.00		
Males				
Ae. aegypti	3.88	1.84	0.00	
Ae. albopictus	6.98	5.89	5.16	0.00

Table 4. Statistical significance of size and shape differences of Aedes mosquito classified by sex and species by non-parametric permutation tests (1000 cycles).

	Females		Males	
	Ae. aegypti	Ae. albopictus	Ae. aegypti	Ae. albopictus
Size				
Females				
Ae. aegypti	S			
Ae. albopictus	S	S		
Males				
Ae. aegypti	0.30	S	S	
Ae. albopictus	S	0.97	S	S
Shape				
Females				
Ae. aegypti	S			
Ae. albopictus	S	S		
Males				
Ae. aegypti	S	S	S	
Ae. albopictus	S	S	S	S

* Statistically significant ($p < 0.05$)

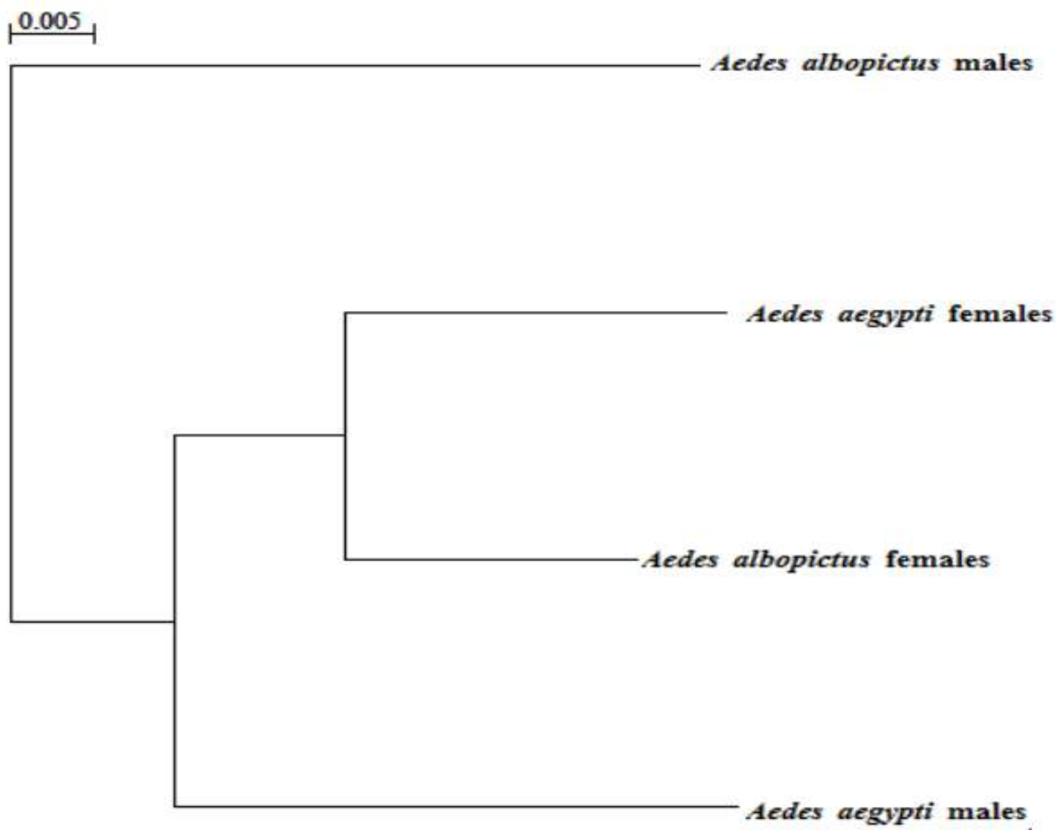

Fig. 3 Neighbor-joining trees for shape based on GM analyses of Aedes mosquito classified by sex and species.

IV. DISCUSSION

The average centroid size of female Aedes mosquito wings was higher than that of males. This was consistent with many morphometric research findings. The results showed that female Ae. aegypti and male Ae. aegypti were not statistically different (p=0.30), and neither were female Ae. albopictus and male Ae. albopictus (p=0.97) (Table 4). Usually, each species of mosquito is different in size, with many factors being involved, such as temperature, humidity and food quality or quantity[6]. Previous research has suggested that shape is more appropriate than the size of morphologically similar species and an important informative character for the study of the genetics and evolution of organisms such as mosquitoes.

Shape is suitable for mosquito identification by GM, with wing venations being a unique way to divide genera and species. Many reports showed that GM could separate species by considering the variation in size and shape separately[7]. However, not all species can be identified using this technique. We found statistically significant differences in shape for all groups of Aedes mosquito. Consistent with the research of Jaramillo-O et al., GM for species identification is able to distinguish 11 species of mosquitoes[4]; however, some species were not suitable for recognition. The results of this study showed that GM can be used for the identification of Aedes mosquitoes, leading to suitable dengue planning for the control of mosquito vectors.

CONCLUSION

The results of this research showed that GM can be an alternative for the identification of Aedes mosquito. Highlights of GM are that it is a very fast, inexpensive procedure, especially compared with genetic techniques, and it does not require more equipment than that which is already present in any entomology laboratory.

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REFERENCES

[1] Damapong P, Damapong P, Jumparwai S, Kaen K. Model development for outbreak of dengue fever. International Journal of GEOMATE. 2016; 11 (27):2777–81.
[2] Chaiphongpachara T, Pimsuka S, Saisanan W, Ayudhaya N, Author C. The application of geographic information system in dengue haemorrhagic fever risk assessment in Samut songkhram province, Thailand. International Journal of GEOMATE. 2017;12(30) :53–60.
[3] Sumruayphol S, Apiwathnasorn C, Ruangsittichai J, Sriwichai P, Attrapadung S, Samung Y, et al. DNA barcoding and wing morphometrics to distinguish three Aedes vectors in Thailand. Acta Tropica. 2016;159:1–10.
[4] Jaramillo-O N, Dujardin JP, Calle-Londoño D, Fonseca-González I. Geometric morphometrics for the taxonomy of 11 species of Anopheles (Nyssorhynchus) mosquitoes. Medical and Veterinary Entomology. 2015;29(1):26–36.
[5] Gómez GF, Márquez EJ, Gutiérrez LA, Conn JE, Correa MM. Geometric morphometric analysis of Colombian Anopheles albimanus (Diptera: Culicidae) reveals significant effect of environmental factors on wing traits and presence of a metapopulation. Acta Tropica. 2014;135(1):75–85.
[6] Ruangsittichai J, Apiwathnasorn C, Dujardin JP. Interspecific and sexual shape variation in the filariasis vectors Mansonia dives and Ma. bonneae. Infection Genetics and Evolution. 2011;11(8):2089–94.
[7] Motoki MT, Suesdek L, Bergo ES, Sallum MAM. Wing geometry of Anopheles darlingi Root (Diptera: Culicidae) in five major Brazilian ecoregions. Infection Genetics and Evolution.2012;12(6):1246–52.

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