Describing Wing Shape among Culex Quinquefasciatus Say (Diptera: Culicidae) Detected Positive and Negative for Filaria Using Relative Warp Analysis

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***Abstract*—This study examined populations of Culex quinquefasciatus in filariasis-endemic communities of Misamis Oriental, Philippines. *C.* Quinquefasciatus, has been of interest to public health since it was identified as a vector of filariasis in the Philippines in 1995. In surveillance and control programs, problems in identification is encountered as it requires some specialized skill and a well preserved set of specimen which is sometimes difficult to acquire. It is for these reasons that alternative method of identification and discrimination of Culex individuals has been desired to resolve problems related with vector species diversification and identification. In this study, geometric morphometric approach was used to test the hypothesis that there exists some variation in the wing shape pattern of *C.* Quinquefasciatus identified as positive and negative of filaria based on the landmarks' position on the wings. To illustrate ordination of the shapes' consensus, the mean shape of the two populations was measured by a relative warp ordination plot using the software tpsRelw version 1.46. Results of the relative warp analysis showed significant variation between the two populations. The four extracted significant relative warps account for a total of 68.54% variation in wing geometry pattern. Variations were observed in the wing apex and base. These variations may have genetic basis or maybe mere reflections of phenotypic plasticity brought about by the changing environmental conditions.**

***Index Terms*—filaria, geometric morphometrics, Culex, relative warps**

I. Introduction

Culex quinquefasciatus Say (Diptera: Culicudae) is a member of the Culex pipiens complex. It is the most common mosquito in urban and rural communities in the tropical and subtropical regions worldwide. It is commonly known as the southern house mosquito, a medium-sized brown mosquito that exists throughout the tropics and the lower latitudes of temperate regions. This night time-active, opportunistic blood feeder is a vector of many pathogens, several of which affect humans. For years they are considered of great medical and veterinary importance and regarded as important vectors of filariasis. Outside the U.S., Culex quinquefasciatus is responsible for transmitting the filarial nematode, Wuchereria bancrofti (Tropical Africa and Southeast Asia), Chikungunya virus (CHIKv) (Africa, India, and Asia), and Rift Valley fever virus (RVF) (Africa) [1]. Wuchereria bancrofti is a filarial nematode that can cause lymphatic filariasis. Currently, worldwide there are approximately 120 million cases of lymphatic filariasis [2]-[4]. The mosquito picks up the microfilaria from an infected vertebrate. The nematode develops inside the mosquito, and is passed on to another vertebrate [1]. It is the primary vector of St. Louis encephalitis virus (SLEv) and the West Nile virus (WNv). In the Philippines, a local study in 1995 identified *Culex* mosquitoes as the vector with the highest rate of filarial infection. In different studies worldwide however, it was reported that the vectorial status of *C.* quinquefasciatus is complex and changing [5], [6]. It has been reported that variation among natural populations of *C.* quinquefasciatus is associated with different vectorial capacities [7]. Unlike malaria and dengue, filariasis can be transmitted by mosquitoes belonging to different genera. It is thus important to establish whether these vectors are sibling or distinct species in order to come up with target-specific and sustainable vector control.

The use of geometric morphometric analysis to study wing venation has been useful because the intersections

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of the wing veins provide many well-defined landmarks and that the metric properties of the wing provide precise quantitative information for the identification of species complexes [8]-[21]. Klingenberg in 2002 [22], used geometric morphometrics to study within-species variations [23], and in describing and identification of species at the individual level [24]-[27]. Since the morphology of insects is under genetic and environmental influences, variation in morphometric traits may provide relevant information on the many aspects of insect biology which may be important in the study of vector control.

This study identified existing populations of *C. quinquefasciatus* in filariasis-endemic communities of Misamis Oriental, Philippines. Since mosquito control program in the Philippines is faced with the challenge of accurately identifying all mosquito vectors of filariasis, this study aimed to determine variations in wing geometry among individuals of *C. quinquefasciatus* detected positive and negative of filaria using landmark-based geometric morphometrics. Several studies revealed that variations in wing shape can affect the ability to occupy habitats successfully [28]-[30], to prevail in predator-prey interactions, and to reproduce successfully [30]-[32]. Thus, variation in wing geometry may provide relevant information on the biology of mosquito vectors which could be of great importance in their detection and control.

II. Methodology

Adult mosquitoes were collected using human bait trap from endemic communities of Misamis Oriental, Philippines as identified by the Department of Health, Region X. Collection of samples was done in the evening until early dawn. The adult mosquitoes trapped inside the net were collected using handheld vacuum and transferred into small portable net cages for easy transport to the laboratory. The collected mosquitoes were sorted and identified based on morphological characteristics. Only female mosquitoes were used in this study since they are the ones that transmit the parasite to humans after a blood meal. Each identified female mosquito was dissected and the mouth parts and body segments were teased apart to screen for the presence of filaria larvae using conventional microscopy.

The identified *C. quinquefasciatus* species was recorded and noted as either positive or negative for filariae. The wings were removed using a scalpel and mounted in glass slides. Wings were photographed under a stereoscope with consistent magnification, and the digital images were kept on file for use in data analysis.

In this study, eighteen landmarks of the wings of female *C. quinquefasciatus* were used for geometric morphometric analysis following the method of Rohlf and Slice (1990) [19]. Landmarks are the points at which biological structures are sampled (Fig. 2). These points produce an exact geometric description of the differences in shape of a structure. The approximated tangent space [33] enables one to perform standard multivariate statistics on a data set of homologous landmarks (or *x*,*y* co-ordinates) of taxa being compared. The techniques uses Procrustes distances to capture shape variation considered to be the most reliable method to determine geometric morphometric relationships among taxa Landmarks were tagged at the intersections of wing veins with the wing margin, intersection of cross vein with major veins and some vein branch points. In mosquitoes the six major longitudinal veins are the costa, subcosta, radius, media, cubitus and anal veins.

Figure 1. Map showing the sampling area.

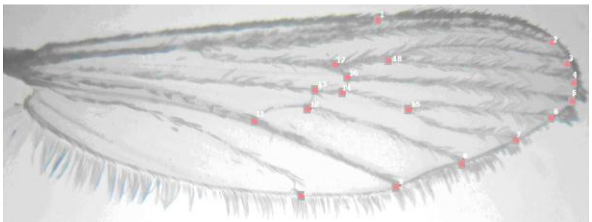

Figure 2. *C.* quinquefasciatus wing with eighteen landmarks. (1) intersection of Costa (C) and Subcosta(Sc), (2) distal end of the Radius (R), (3) radial branch 2, (4) radial branch 3, (5) distal end of radial branches 4 & 5, (6) distal end of M1 & 2, (7) distal end of cubital vein 1, (8) distal end of cubital vein 1, (9) distal end of cubital vein 2, (10) Anal vein, (11) origin of cubital 1, (12) midpoint branch of cubital 3, (13) medio-cubital cross vein, (14) midpoint branch of medial vein, (15) radio-sectoral vein, (16) radio-medial cross vein, (17) midpoint branch of radial vein, (18) origin of radius branches 2 & 3

The coordinates of the landmarks were digitized using TPSdig software [34]. Thin-plate spline of relative warp analysis was carried out using the coordinates of all aligned wings [18], [35], [36].The connections between 18 landmarks created polygons of comparative size and shape of mosquitoes' wings. The uniform component of shape variation was estimated by the Linearized Procrustes method [37]. In order to assess the local shape changes in the species, relative warp analysis was used. The reference used in this analysis is the average configuration of landmarks. The warps were computed with the alpha at zero, in order to weigh all landmarks equally [30], [35].

Geometric morphometrics utilizes powerful and comprehensive statistical procedures to analyse shape differences of a morphological feature, using either homologous landmarks or outlines of the structure [35], [38], [39]. Rohlf, [38] developed the tps series of programs which performs the statistics and visualizations of geometric morphometrics and were used in the current study.

Consensus shape data of the coordinates was measured by a relative warp ordination plot using tpsRelw 1.46. Partial and relative warps was computed and plotted. The principal warp describes shape distortions of the reference configuration. The projection of the superimposed wings on the principal warps produced the partial warp scores. These scores describe the deviations from the reference configuration. The relative warps are the principal components of variation among wings [18], [35], [36]. The coordinates was placed on a grid using thin plate spline relative warps analysis to visualize the directional and quantitative change in wing 8shape.

III. Results and Discussion

Fig. 3 shows the consensus morphology and variation in wing shape among C. quinquefasciatus as produced by the relative warps. The topmost figure shows the mean shape of all the wing samples obtained. Negative deviation from the mean shape is expressed as projections on the left side of the axis while positive deviation is seen on the right side.

The eighteen wing landmarks generated four significant relative warps. Relative warp 1 (RW1) accounts for 47.57% of the variation in wing shape. Variation in wing shape as seen in RW1 is observed as the movement of landmark affecting either the base of the wings or the apex. Specifically, this was noted in landmarks 1 & 10, the costal and anal vein. The bimodal curve of the histogram clearly indicates two morphologically distinct populations deviating from the mean.

The direction of variation among *C.* quinquefasciatus that are negative of filaria is towards the positive axis while those that are positive of filaria is towards the negative axis. This indicates that *C.* quinquefasciatus that are negative of the parasite have a more tapered wing compared to those that are positive of filaria. The position of landmarks 1, 2, 9 and 10 accounts for this variation. Samples in the positive axis shows the subcosta vein (Sc) binding with the costa vein (C) before the bifurcation of the radial2+3 vein (R2+3) while in the negative axis the Subcosta vein binds to vein Costa at the same point or variance. The populations are at either negative or positive axis which signifies that wings are either broad at the base and or tapered towards the tip. Relative warp 3 (RW3) accounts for 6.45% variation. Samples along a positive RW 3 axis displayed a slightly tapered wing compared to the samples in the negative axis. Relative warp 4 accounts for 5.29% of the total variance. Most of the samples are concentrated on the right axis which shows a narrower wing base. Kruskal-Wallis test showed significant differences in mean shape among the two populations of *C.* quinquefasciatus as presented in Table I.

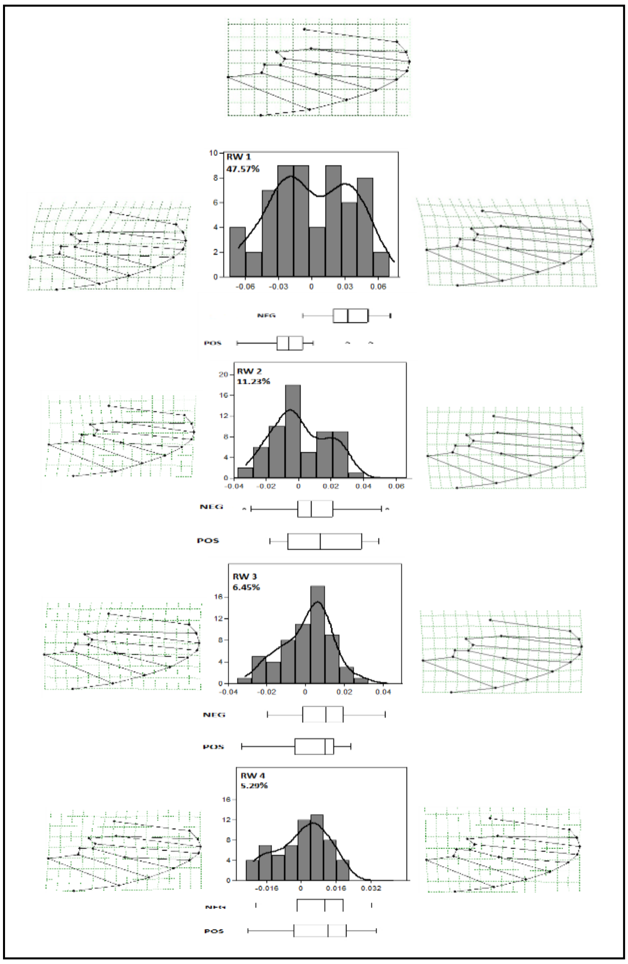

Figure 3. Relative warps histogram and boxplots showing variations in wing shape pattern of Culex quinquefasciatus

TABLE I. Variation in the Landmark's Position in the Wing Shape Pattern among Culex Quinquefasciatus as Defined by Each of the Significant Relative Warps

Relative Warp	*Culex quinquefasciatus* (+ for filaria)	*Culex quinquefasciatus* (- for filaria)
RW 1	The apex of the wing is wider and the base broader. The landmark points at the base are more stretched to the left.	The apex of the wing is more tapered. The distance between landmarks 1 & 2 is longer creating a more elongated tip
RW 2	The span of the wing is broader at the base. The apex is also wider.	The span of the wing is narrower. The apex is a bit stretched and elongated
RW 3	The base is broader. The apex is wider.	The base is broad. Over all shape closely resembles the mean
RW 4	Wing is more tapered in the apex with broader base. Distance of landmarks 1 & 2 is stretched	Wing is broader both in the apex and base.

The direction of variation among *C.* quinquefasciatus that are negative of filaria is towards the positive axis while those that are positive of filaria is towards the negative axis. This indicates that *C.* quinquefasciatus that are negative of the parasite have a more tapered wing compared to those that are positive of filaria.

TABLE II. KRUSKAL-WALLIS TEST

Relative Warp	P-Value
1	1.5797^{-08}
2	0.3145
3	0.717
4	0.8475

Multivariate analysis of variance (MANOVA) on relative warp scores showed significant variation between the two populations in the variances with the F=15.19, and p value less than 0.05 (<0.05). This is true to relative warp 1.

The CVA scatter plot in Fig. 4 shows the distribution of the two populations of *C.* quinquefasciatus based on landmark analysis of wing shape pattern. The right-most group are *C.* quinquefasciatus that are negative of filaria characterized by tapered wing apices. In the left-most side are those that are positive of filaria with broader wing base and rounded apices. Morphological variation may be attributed to migration of vector species to different environments and their subsequent adaptation that would sometimes interfere with correct morphological distinction [26]. In nature, genetic drift is the main force that produces significant differences in shape [23].

In this study wing venation pattern among *C.* quinquefasciatus population are relatively the same, but slight variations in shape were described by relative warps specifically affecting the costal and anal veins. Generally, mosquito populations that forage in higher altitude have slender wings while lower altitude populations show broader wings by comparison. Results of this study suggest that *C.* quinquefasciatus positive of filaria are confined in the lower altitude as they are characterized by broader wings. It might be possible that the presence of parasite within the mosquito is one constraint on flying at higher altitude as it becomes heavier. Other reasons for the observed differences in wing geometry among *C.* quinquefasciatus, positive and negative of filaria are yet to be explored. In mosquitoes phenotypic variation is influenced by an assortment of environmental factors such as genetic and environmental variables that include temperature, altitude, nutritional factors at the immature stages and host population distribution [40]. Wing morphology can also respond to environmental cues during the developmental period, when genes that need to be activated during wing development can be altered by environmental conditions [41]. Results in this study have demonstrated that geometric morphometrics using relative warp analysis can be used to quantify phenotypic variation in *C.* quinquefasciatus wings detected as positive and negative of filarial. These variations maybe mere reflections of phenotypic plasticity brought about by varied environmental conditions. It is however recommended that further studies should be pursued to evaluate if these variations may have genetic basis or maybe parasite-induced.

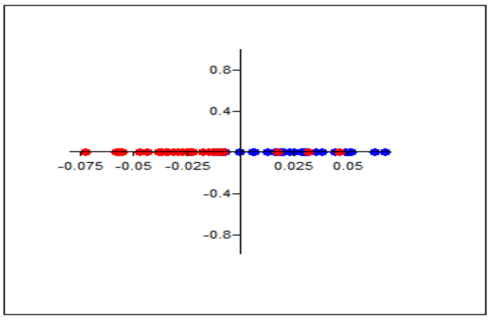

Figure 4. CVA scatter plot of relative warps showing mean shape variation in wings of *C.* quinquefasciatus

IV. CONCLUSION

Geometric morphometrics can be used as a tool for quantifying phenotypic variation among mosquito vectors of filaria. The correct identification of vector species is an essential issue for entomological surveillance in order to understand the epidemiology of arthropod borne disease. In this study we were able to describe differences in wing pattern among populations of *C.* quinquefasciatus that are positive and negative of filaria by means of geometric morphometric techniques.

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