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Describing variations in wing shapes of *Anopheles flavirostris* detected positive and negative of filaria using relative warp and Euclidean distance matrix analysis

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Abstract

Geometric morphometrics such as relative warp (RW) analysis has been increasingly used in assessing medically important mosquito species. It is used as a tool for quantifying phenotypic variation among populations. In this study however, relative warp (RW) and Euclidean Distance Matrix analysis (EDMA) were both used to describe intraspecific variation among *Anopheles flavirostris* that were detected positive and negative of filaria. Relative warp analysis generated five significant relative warps which account for most variation in the wing venation patterns in most of the landmarks near the base and apex. The posterior end of the wing is more labile as compared to the more rigid costa-radial boundary in the anterior side indicating the presence of a more tapered wing span for the identified vectors and a broader base and a wider wing tip among the non-vectors. Euclidian Distance Matrix Analysis show fifteen interlandmark distances that could explain wing shape differences between *An. flavirostris* found positive and negative of the presence of filarial parasite. Results of this study show that both RW and EDMA are useful tools in quantitatively describing shape variation in the wings of *A. flavirostris.*

Keywords: Filariasis, venation, EDMA, relative warps, interlandmark

1. Introduction

Filariasis a condition caused by filarial nematodes, was considered endemic in 45 out of 77 provinces in the Philippines [1]. It earlier reported as Bancroftian caused by *Wuchereria bancrofti* [2] and Malayan caused by *Brugia malayi* [3]. The Malayan filariasis in the Philippines is of the subperiodic type transmitted by *Mansonia bonneae* and *Mansonia uniformis* especially those reported in Palawan, Sulu, Agusan and Samar [3-7]. The 2 species of mosquito vectors earlier identified were *Aedes (Finlaya) poeciius* and *An. flavirostris*. *An. flavirostris* is among the twenty six species of Anophelenes that are regarded as major vectors of the Bancroftian filariasis and *Brugia malayi*.

In understanding host-parasite relationship, it is argued that while parasites exploit and reduce the fitness of their hosts, the damage is counterbalanced by the requirements the host survives the infection [8-10] and to remain capable of locating and feeding on the host [11]. Since not all individuals are able to vector the parasite, it is argued that there is considerable variation within vector populations in physiological and fitness responses to parasitism [11-13]. We tested this hypothesis in *An. flavirostris* specifically those detected positive and negative to the filarial parasite by examining the shape of the wings which is an important anatomical structure used in moving from one host to another to transmit the filarial parasite.

In this study, we describe the shape of the wings of *An. flavirostris* using geometric morphometrics. As an analytical tool, geometric morphometrics offer a more comprehensive approach to the study of shape through multivariate statistical analysis of anatomical landmarks of biological homology [14]. It preserves the information about the relative spatial arrangement of data throughout the analysis [15], making it possible to find and analyze shape variations in the organisms within and between populations [16]. Multivariate morphometry has also been useful for the distinction of medically important species that are difficult to identify due to overlapping morphological characters [17-18]. Several researches have used the method of geometric morphometrics to study intraspecific variation in wing pattern of different organisms such as in Diptera [19], *Apis mellifera* [20], and in different species of *Drosophila* [21-22] thus was considered in this study.

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2. Methodology

This study was conducted from January 2013 to April 2014. Adult female *An. flavirostris* were collected using mosquito nets and human bait trap from endemic communities in Misamis Oriental as identified by the Department of Health, Region X (Fig. 1). Sampling was done in the evening until early dawn for the whole month in January 2013. 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 and taxonomic keys. Only female *An. flavirostris* mosquitoes were used in this study as they are presumed to be in direct contact with human hosts. Figure 2 shows a typical female *An. flavirostris* wing with the presence of wing spots. Each identified *An. flavirostris* 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 *An. flavirostris* mosquito was recorded and noted as either positive or negative for filaria. The wings were then 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 and later used in data analysis (Fig. 2).

Fig 1: Map showing study area

In this study, eighteen anatomical landmarks of the wings (Fig. 3) of female *An. flavirostris* were utilized for geometric mophometric analysis [23]. Landmarks were tagged at the intersection of wing veins with the wing margin, intersection of cross vein with major veins and some vein branch points. Table 1 identifies the specific location of the assigned landmarks in *An. flavirostris* [24].

Fig 2: *An. flavirostris* wing samples collected from Misamis Oriental

Generally, in mosquitoes the six major longitudinal veins are the costa, subcostal, radius, media, cubitus and anal vein. The rest are intersection of major wing veins and cross veins. The coordinates of the landmarks were digitized using image analysis TPSdig software [25]. TpsDig facilitates the statistical analysis of landmark data in morphometrics by making it easier to collect and maintain landmark data from digitized images [26].

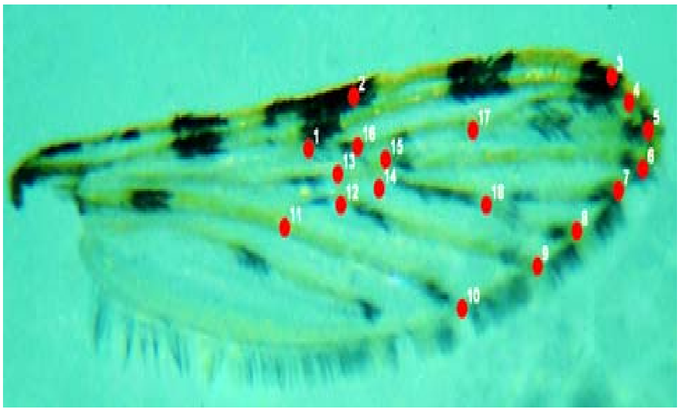

Fig 3: *An. flavirostris* wing with typical wing spots

Table 1: Description of assigned landmarks in the *An. flavirostris* wing

Landmarks	Description of tagged landmarks
1	Radius (R)
2	Costa (C)
3	Radius one (R_1)
4	Radius two (R_2)
5	Radius three(R_3)
6	Radius four-plus-five (R_{4+5})
7	Media one (M_1)
8	Media two (M_2)
9	Media three-plus-four (M_{3+4})
10	Cubitus anterior (cuA)
11	Midpoint of cuA and medio-cubital
12	medio-cubital cross vein (mcu)
13	medio-cubital and media cross vein
14	Media-one-plus-two vein(M_{1+2})
15	Cross vein between M_{1+2} and R_{2+3}
16	Radius two-plus three vein (r_{2+3})
17	Intersection point between R_2 and R_3
18	Intersection point between M_1 and M_2

These coordinates were then transferred to Microsoft Excel for organization of the data into groups. The generalized orthogonal least squares Procrustes average configuration of landmarks was computed using the generalized Procrustes Analysis (GPA) superimposition method. GPA was performed using the software tpsRelw, ver. 1.46 [26]. The relative warps RWs, which are the principal components of the covariance matrix of the partial warp scores were computed using the unit centroid size as the alignment-scaling method. Histogram and box plots were generated using PAST software [27] from the relative warps of the wing shapes. These figures provided a clear view of where the data is centered and how they are distributed over the range of the variable. Kruskal-Wallis test was used to analyze whether or not the species differ significantly with regards to their wing shape.

Euclidian Distance Matrix Analysis was used to determine localized variation in specific landmarks based on matrix of distances between landmarks (Fig. 4). Euclidian Distance Matrix Analysis (EDMA) is a coordinated-free method based on the distances between landmarks and is invariant to rotation of the original specimen [27].

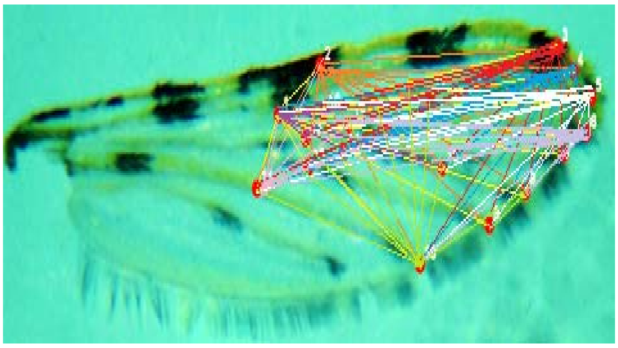

Fig 4: Matrix of distances between landmarks.

3. Results and Discussion

Geometric morphometric analysis of the eighteen landmarks in the wing of *An. flavirostris* generated five significant relative warps (Fig. 5). Box plots and the shown histogram present the direction of variation in wing venation pattern. It can be observed that the variations in shapes of the wing are generally found as a shift in most of the landmarks near the base and apex. The posterior end of the wing is more labile as compared to the more rigid costa-radial boundary in the anterior side indicating the presence of a more tapered wing span for the identified vectors and a broader base and a wider wing tip among the non-vectors. This is further substantiated by quantitatively describing the variations between the two *An. flavirostris* groups using Euclidian Distance Matrix Analysis (EDMA) where the distances between landmarks subjected to Principal Component Analysis (PCA) were computed (Table 2). Results show fifteen interlandmark distances were found to have contributed to most variation in the wing shape pattern of *An. flavirostris* (Table 3, Fig. 6). These interlandmark distances explain why *An. flavirostris* found positive of filaria have more tapered apices and narrower base while those found negative for filaria have interlandmark distances which appeared significantly shorter resulting to a wing with a more rounded apex and broader base. It is argued that the presence of a slender wing is a characteristic of mosquitoes foraging in higher altitude thus this may explain the observed wing pattern in affected *An. Flavirostris* found positive for filaria.

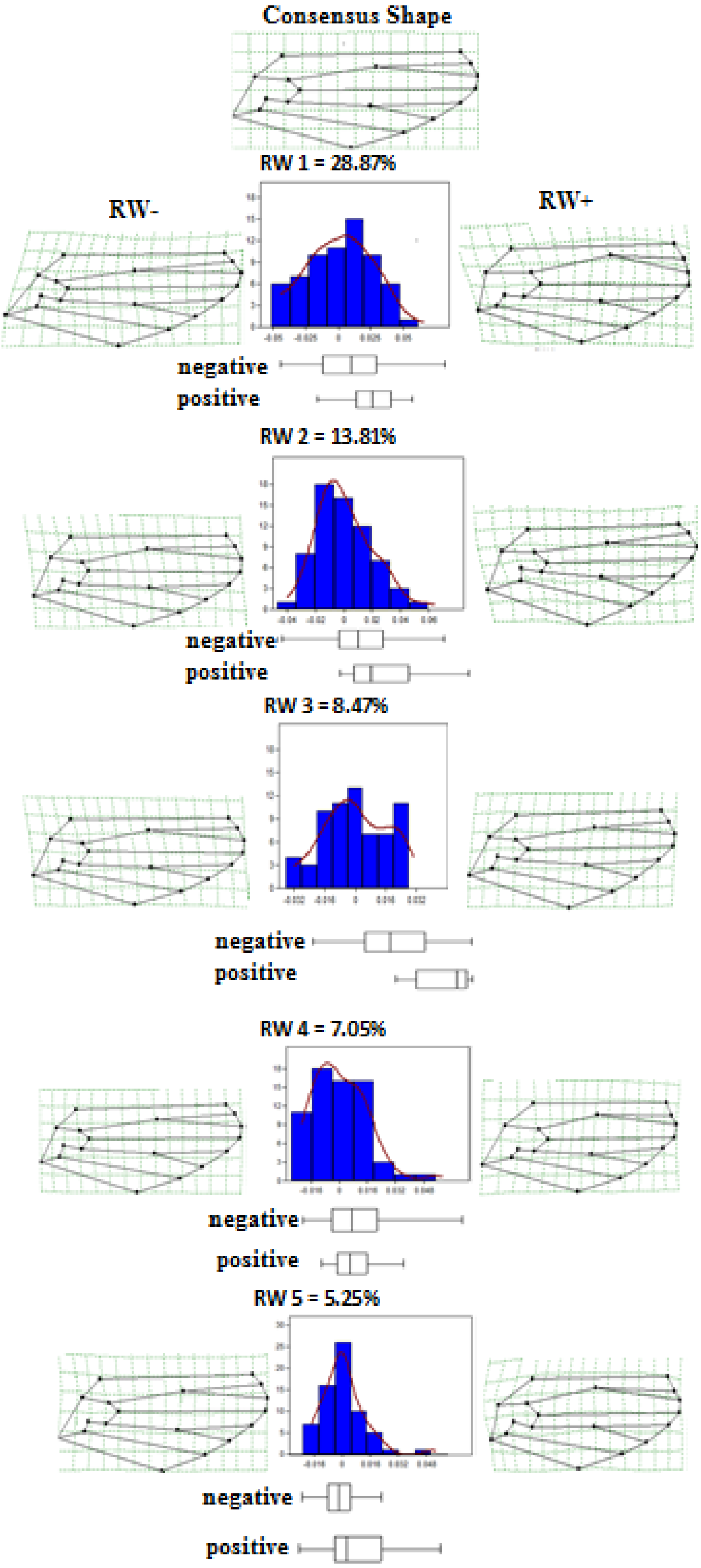

Fig 5: Significant relative warps showing the variation in shapes of the wing of *An. flavirostris*

Table 2: EDMA proportion of variation with the most significant components of the wing shape of *An. flavirostris.*

Principal Component	Eigenvalue	% Variance
1	89578.2	69.258
2	10530.3	8.1416

Jolliffee cut-off= 591.75

Table 3: Interlandmark distances of the first PCA loading values for the significant component of the EDMA landmark coordinates

Interlandmark Distances	Values
1-7	0.1490
1-6	0.1462
7-12	0.1446
6-12	0.1434
1-4	0.1418
4-12	0.1413
5-12	0.1392
7-11	0.1389
6-11	0.1379
3-12	0.1365
4-11	0.1360
1-3	0.1358
5-11	0.1338
7-13	0.1332
3-11	0.1312

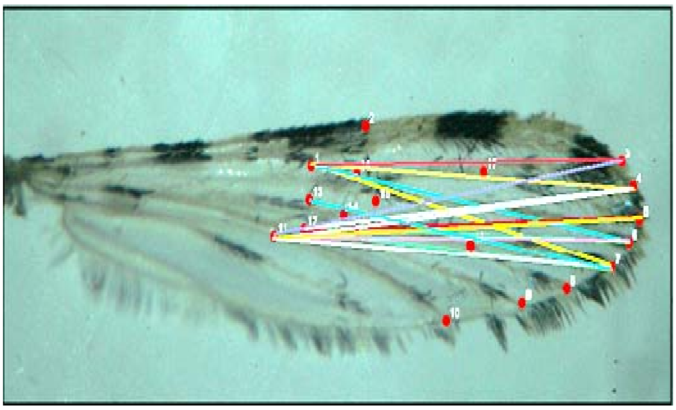

Fig 6: EDMA interlandmark distances of wing shape pattern of *An. flavirostris*.

Morphological shape changes observed in the wing of *An. flavirostris* brought about by changes in vein pattern are significant as these may affect flight performance in the mosquito. Since veins increase the mechanical rigidity of the wing, direct flight muscles attached to the wing base contribute to the twisting of the wings during flight [8]. Shape is an output of a cascade of genes and in nature, genetic drift which maybe brought about by several factors parasitic infection included may have contributed to significant differences in morphological shapes among conspecific populations [28]. Several studies revealed significant shape differences among mosquitoes between different geographic areas [19, 29] thus the morphological variations observed in the *An. flavirostris* wing as shown in this study may be attributed to the migration of the affected individuals to different environments and their subsequent adaptation [29]. Other sources of variations such as wing size could have also an impact on the mosquito wing vein pattern. While the size of a mosquito has a genetic basis, environmental factors such as temperature, nutrition, larval density and salinity may also affect the size of individuals [30]. Variation in wing geometry between populations may provide insights into population structure, ecology and species complex. Variation in morphology could also be influenced by environmental effects, genetic mutations and developmental perturbations; buffering mechanisms include canalization, phenotypic plasticity, and developmental stability [31].

4. Conclusion

It can be seen from this study that the use of geometric morphometrics (GM) such as relative warp (RW) and Euclidean distance matrix analysis (EDMA) is very useful in describing shape changes in the wings of *An. flavirostris* tested positive and negative for filaria. The tools of GM as shown in this study is an alternative tool to quantitatively describe variations not only within populations but might also provide insights into population structure and ecology of species.

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