Article

Geometric morphometric analysis describing sexual dimorphism in housefly, *Musca domestica* Linn. (Diptera: Muscidae)

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Abstract
Houseflies (*Musca domestica*) (Diptera: Muscidae) are medically important insect species because they serve as vectors of pathogens. The study generally described the sexual dimorphism of *M. domestica* based on their wing size and wing shape. This study examined 25 males and 25 females of F_1 offspring from wild-caught *M. domestica* parents. The wings were digitized and 17 landmarks were obtained, scaled, translated, and rotated in General Procrustes Analysis. The wing size (centroid size) of male *M. domestica* species was significantly larger compared to females ($t = -2.38$, $df = 48$, $p = 0.0200$). Principal Component Analysis (PCA) and Relative Warp Analysis (RWA) revealed that 29.72% of shape variation from the original data was attributed to a narrow wing shape, and 14.60% toa broad wing shape. Discriminant Function Analysis (DFA) successfully distinguished female and male species based on wing shape, in which males have narrower wings compared to females, indicating the occurrence of sexual dimorphism in wings.

Keywords evolution; landmarks; morphology; Philippines; wing shape.

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1 Introduction

Houseflies, *Musca domestica* (Diptera: Muscidae), are "nuisance" species and one of the economically and medically important insect species of the order Diptera. Houseflies exist in people's homes, poultry, cattle, supermarkets, dumpsites, inside malls, and other establishments where humans inhabit the area. Furthermore, *M. domestica* also serves as vectors of different pathogens and helminths (Iqbal et al., 2014; Khoso et al., 2015; Tardelli et al., 2004).

Sexual dimorphism is evident in almost all animals, especially in insects (Laporte, et al., 2018). It has been renowned as a major factor in identifying phenotypic differences between species of the same taxon. Describing the sexual dimorphism in insects provides information about the behavior, evolution, selection, and

overall fitness of an organism (Cabuga et al., 2017). In houseflies, sexual dimorphism in wings is poorly understood due to similarities in wing morphological structures. Likewise, sexual dimorphism in *M. domestica* was based on the morphological characteristics such as the distance of compound eye, arrangement of bristles (Chaetotaxy), number of spiracles, and extraction of genitalia. Sex differentiation based on these traditional methods is prone to misinterpretation. On the contrary, molecular methods provide accurate information to describe sexual dimorphism. Nonetheless, detecting variation among species and between sexes at the molecular level is expensive and requires rigorous training. Thus, Geometric Morphometric (GM) analysis provides an alternative approach that is complementary to molecular methods at the lower cost.

There were no published data about sexual dimorphism based on wings of laboratory-reared *M. domestica* using GM analysis, especially in Davao region, Philippines.Previous studies on these species in relation to GM analysis were based on morphometric variations related to geographical locations (Alves and Belo, 2002), fluctuating asymmetry (Floate and Fox, 2000; Ludoski et al., 2014), and morphometric and genetic characterization (Pastor et al., 2014). Furthermore, the abovementioned studies were mostly focused on wild-caught species. Thus, this study focused on describing the sexual dimorphism based on wing size and wing shape of laboratory-reared *M. domestica* using landmark-based geometric morphometrics. Furthermore, the present investigation also determined the wing centroid size and wing shape variation of *M. domesetica.*

2 Methods

2.1 Description of the study area

The wild-caught parents of laboratory-reared houseflies were collected from Panabo City Public Market located at Barangay Santo Niño, Panabo City, Davao del Norte, Philippines (70 18’ 22.14” N and 1250 41’ 01.41” E). The area was selected because houseflies were attracted to this type of environment where all of their possible food sources were available. Furthermore, the presence of these houseflies might contribute to food contamination if food handling is not practiced correctly (Khoso et al., 2015).

The collections site has stalls for fish, fruits, vegetables, and livestock. Moreover, the site was allocated with garbage bins which can be plausible for breeding sites of wild-type houseflies. Houseflies at larval and mature stages were observed in poultry farms, garbage, slaughterhouses, and fish markets (Iqbal et al., 2014). Among the Muscidae family, *M. domestica* is the most abundant fly species found in fresh markets (Khoso et al., 2015). The adult houseflies were collected by placing the breeding confinement with chicken manure bait on the different areas of the sampling site where houseflies usually thrived. The number of houseflies collected was large enough to ensure a higher chance of mating and oviposition rate. Furthermore, an insect net was utilized to obtain a large number of specimens (Alves and Belo, 2002; Ludoski et al., 2014; Espra et al., 2015).

2.2 Breeding confinement set-up

There were two confinements for laboratory-reared *M. domestica*, the breeding confinement, and the pupation confinement. The breeding confinement has a dimension of 30 cm x 30 cm x 30 cm (Length x Width x Height), made up of wire mesh for walls, and the flooring consisted of plywood with an ovipositional substrate installed on top (Fig. 1). Furthermore, the flooring of the enclosure was filled with a food source for the flies,which was a mixture of powdered milk and sugar, to ensure the survival of the samples in the confinement.

The ovipositional substrate (Fig. 2) was comprised of cardboard paper (16 cm x 4 cm) and had cotton wool that was soaked in milk (Nakamura et al., 2015). The pupation confinement was designed for the larvae to pupate after they were hatched from the oviposition case. The pupation confinement had dimensions 20 cm x 20 cm x 20 cm. Its flooring was filled with rearing medium which was composed of a mixture of 100 g wheat bran, 10 g dried yeast, and 240 mL of water that served as the food source for maggots to ensure successful

development (Nakamura et al., 2015).

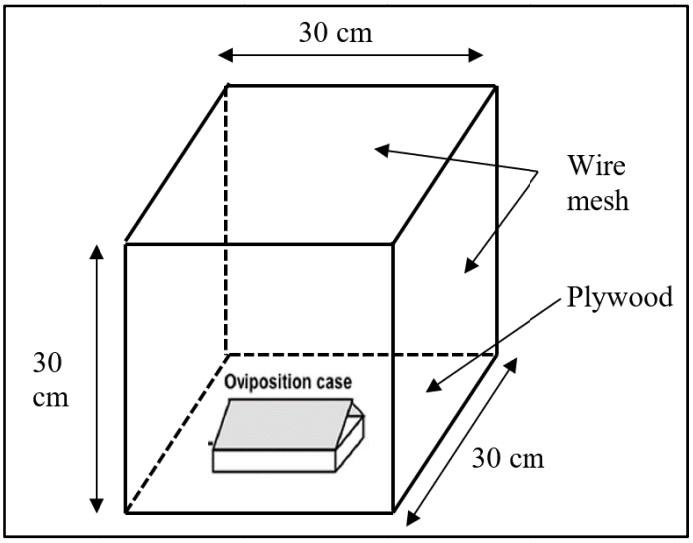

Fig. 1 Breeding case set-up.

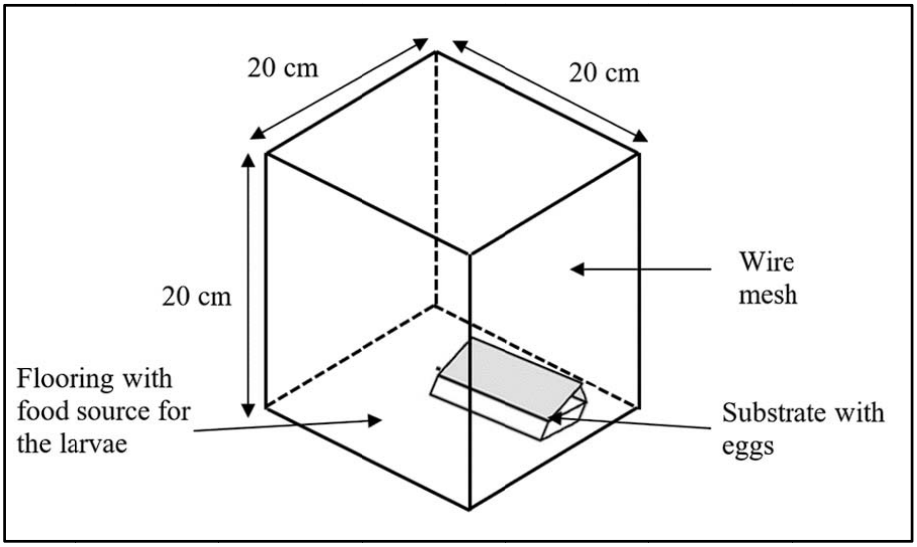

Fig. 2 Pupation case set-up.

2.3 Rearing of *M. domestica* laboratory samples

The captured adult houseflies were transferred to the breeding confinement in which they were allowed to mate and deposit their eggs on the ovipositional substrate. Once the female adults had laid their eggs, the wild-caught parents were exposed to insecticide and discarded as biological waste. The eggs deposited on the substrate were transferred into the pupation confinement with a rearing medium on its flooring.

The larvae emerged after 24 hours after oviposition. There were at least three instar stages before the larvae grew into a full-sized maggot. To make sure that the moisture content inside the confinement existed, water was sprayed inside the confinement using an improvised water sprayer. Highmoisture favors the survival of the housefly larvae and will prevent them from escaping (Arroyo andCapinera, 2017). However,excessive dampness can lead to drowning of the larvae, emphasizing the need for appropriate moisture levels to prevent

such fatalities. Additionally, the confinement must be sufficiently cool to promote favorable conditions for larval development into pupae.

The entire process of pupation in housefly larvae took up to approximately six hours, which gave a rich, dark brown color. At this time, food and water supply were cut off. Adult houseflies emerged after five to seven days, however, some of the pupae did not fully develop into adults. Adult houseflies were reared until five to nine days for maturation and exposed at a temperature of 20°C in the laboratory refrigerator to be inactive. In this study, 25 males and 25 females of F_1 offspring from wild-caught parents were used for microscopy, wing dissection, and morphological characterization.

2.4 Specimen preparation

After the sorting of the male and female houseflies, the body length of each housefly was measured (in mm) using a ruler and with the aid of tpsDig software version 2.30 (Rohlf, 2015). Then, the right dorsal wing of *M. domestica* specimens was detached from its thorax with the use of forceps and stereomicroscope (NTX-5C). The wings were placed under the stereomicroscope at 20X magnification and were photographed using a digital camera. Each photo was labeled according to specimen number and sex.

2.5 Geometric Morphometrics (GM) software

The GM software used in the study was all free and did not require any license to function in the performed analyses. The GM software utilized in the present study were tpsUtil version 1.74 (Rohlf, 2015), tpsDig version 2.30 (Rohlf, 2015), R statistical programming version 3.2.5 (R Core Team, 2022), and MorphoJ version 1.06d (Klingenberg, 2011). The software tpsUtil and tpsDig were used in assigning of landmark configurations on the photos of *M. domestica* wings. For data visualization of landmark configurations, R statistical programming was utilized via packages '*geomorph'* (Adams et al., 2018), and '*shapes'* (Dryden, 2017). Under these packages, the landmark coordinates of *M. domestica* wings were aligned and superimposed using functions *gpagen*() and *procGPA*() to remove variation due to differences in scale, position, and orientation from the coordinates (Sontigun et al., 2017). Furthermore, multivariate analyses were carried out through the R program and MorphoJ software.

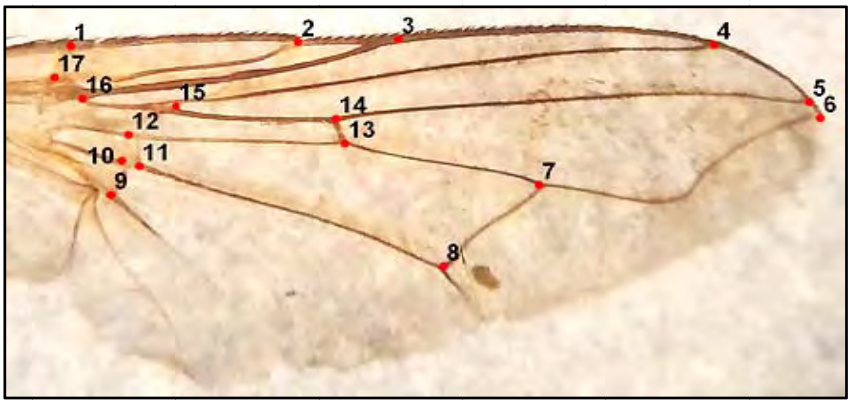

Fig. 3 Landmark points in *M. domestica* dorsal right wing.

2.6 Landmark selection and digitization

The photos were converted to tps (thin-plate spline) files by using tpsUtil software version 1.74 (Rohlf, 2015). The saved files were used for digitizing the photos to be assigned landmarks using tpsDig software version 2.30 (Rohlf, 2015). In the process of digitization, a reference length of 1 mm in the scale function was established so that the software recognized the actual size of the specimen even though each photo had different sizes. Furthermore, the digitization of all photographs was done on the same day by the same person

to avoid errors during the process. Each point selected in the digitization process corresponded to the anatomical landmarks of the specimen. The anatomical landmarks of *M. domestica* (Fig. 3) were identified based on the intersections of wing veins with wing margin, the junction of the cross vein with the major vein, and some vein branch points (Mondal et al., 2015; Espra et al., 2015; Pastor et al., 2014). The descriptions of landmark points (Table 1) were based on the wing vein labels following the accepted usage among dipterologists.

Table 1 Landmark description of *M. domestica* wing veins.

Landmark points	Wing vein character
1	Humeral cross vein
2	Subcostal vein
3	Distal end of first longitudinal vein
4	Distal end of second longitudinal vein
5	Distal end of third longitudinal vein
6	Distal end of fourth longitudinal vein
7	Intersection between posterior cross vein and fourth longitudinal vein
8	Intersection between posterior cross vein and fifth longitudinal vein
9	Intersection between third basal vein and sixth longitudinal vein
10	Intersection between distal end of second and third basal cell
11	Proximal origin of fifth longitudinal vein
12	Intersection between the distal end of basal cell and proximal origin of fourth longitudinal vein
13	Intersection between anterior cross vein and fourth longitudinal vein
14	Intersection between anterior cross vein and third longitudinal vein
15	Intersection between the proximal origin of second and third longitudinal vein
16	Proximal origin of first longitudinal vein
17	Intersection between humeral cross vein and proximal origin of subcostal vein

2.7 Wing size analysis

Wing size or centroid size is the general measure of *M. domestica* wing size, which is the square root of the sum of the squared distances of all landmarks from the center of each configuration (Alves and Belo, 2002; Sontigun et al., 2017). The centroid size of male and female *M. domestica* wings were obtained from the Procrustes superimposition in gpagen function under *'geomorph'* package in R (Adams et al., 2018). Moreover, the wing centroid size according to sex was described using the mean, standard deviation, and maximum and minimum values. In addition, the centroid size of the male and female specimens was compared using the t-test. Scatterplot and boxplot were utilized to visualize the descriptive statistics of wing size according to the sex of *M. domestica*. The data were analyzed using R statistical programming software (R Core Team, 2022).

2.8 Analysis of wing shape variation

The landmark points from the digitization process were converted into two-dimensional coordinates and these configurations were scaled, translated, and rotated in Procrustes superimposition through General Procrustes Analysis (GPA) (Mitteroeckerand Gunz, 2009). The GPA is an important technique because it removes the variation in digitizing the location, orientation, and scale of each specimen. Furthermore, the GPA superimposed these specimens in a common coordinate system (plots) or tangent space (Adams et al., 2004). The scores (points) on the axes of the coordinate system were treated as multivariate data that represented the

shape variables and were used in multivariate analyses (Adams et al., 2004).

The plots of landmark positions after Procrustes superimposition were useful to assess the wing shape variation of the data. However, these are not suitable for a complete examination of the variation in the data because Procrustes superimposition cannot show covariation among landmarks and therefore, hide a fundamental aspect of the variation in the morphometric data (Klingernberg, 2013). Hence, the superimposed coordinates obtained from Procrustes superimposition were analyzed using multivariate analyses. The wing shape variation of *M. domestica* individuals was determined using Principal Component Analysis (PCA). The PCA is one of the ordination methods that simplify descriptions of shape variation among individuals (Pepinelli et al., 2013).

Furthermore, the visualization of shape variation between sex of *M. domestica* specimens was performed utilizing *'ggplot2'* package (Wickham, 2009) in R, in which the first two PC scores were plotted (PC 1 on the X-axis, PC 2 on the Y-axis). Following the method of Alves and colleagues (2016), the first two PC scores were selected and analyzed through Relative Warp Analysis (RWA) in MorphoJ software version 1.06d (Klingenberg, 2011). The analysis was done to visualize the wing shape change from the average wing shape configuration. Furthermore, the effect of wing size in each PC was estimated through multivariate regression using MorphoJ, in which the dependent variable was the PC score, and the independent variable was the centroid size (Sontigun et al., 2017).

2.9 Sexual dimorphism in wings

The analysis of sexual wing shape dimorphism was performed under MorphoJ software (Sontigun et al., 2017). Procrustes Analysis of Variance (Procrustes ANOVA) was performed to determine the significant difference between wing shape and sex. Furthermore, multivariate regression with the permutation test of 10,000 rounds was implemented to estimate the effect of wing size on the wing shape of *M. domestica* based on sex. Moreover, the residuals from the regression of Procrustes coordinates on centroid size were used to assess the sexual shape dimorphism in wings without the effect of wing size. Continually, these residuals were subjected to leave-one-out cross-validation in Discriminant Function Analysis (DFA) (Sontigun et al., 2017; Cabuga et al., 2017; Klingenberg, 2011). DFA examined the separation between two groups of observations, which were in this study, female and male *M. domestica*. Lastly, the shape difference between sexes was visualized by comparing the mean shape of each sex generated from a wireframe graph in MorphoJ software (Klingenberg, 2011). Furthermore, the Procrustes and Mahalanobis distance between two shape configurations was calculated as a measure of shape difference (Mitteroeckerand Gunz, 2009; Sontigun et al., 2017).

3 Results

3.1 Wing centroid size

The study reveals that the wing centroid size of *M. domestica* individuals (n=50) is 5.49 mm ± 0.40 mm (mean ± SD), with exact maximum and minimum values of 6.20 mm and 4.50 mm, respectively. Moreover, female houseflies (n=25) exhibited an average wing size of 5.36 ± 0.37 mm, with exact maximum and minimum values of 6.10 mm and 4.57 mm, respectively. Conversely, males (n=25) demonstrated an average wing size of 5.62 ± 0.39 mm, with exact maximum and minimum values of 6.20 mm and 4.50 mm, respectively (Fig. 4).

Moreover, t-test analysis indicates a significant difference between the wing sizes of female and male houseflies ($t = -2.38$, $df = 48$, $p = 0.02$). Therefore, in this paper, the wing size of male *M. domestica* was significantly higher compared to female *M. domestica*.

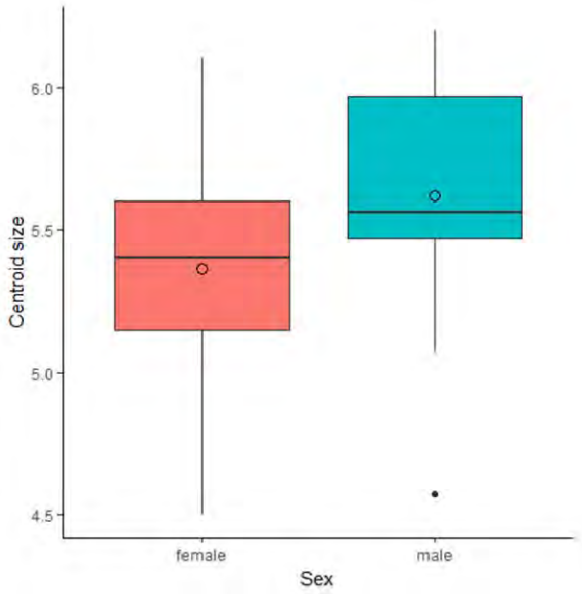

Fig. 4 Centroid size comparison of male and female *M. domestica.*

3.2 Wing shape variation

Principal Component Analysis (PCA) determined the wing shape variation of *M. domestica* individuals quantitatively. Fig. 5 shows the scatterplot in which the PCA scores from superimposed coordinates were plotted. The X and Y axes represented the shape variation (PC scores) of superimposed coordinates. Each data point represented the location of *M. domestica* individuals in the shape space. A total of 30 PC scores (shape variables) were generated from the analysis and only the first two PC scores with the highest variations were selected. The first PC axis represents 29.72 % of shape variation while the second PC represents 14.60 % of shape variation, which accounted for 44.32% of overall shape variation from the original data (Fig. 5a). In addition, the spread of female *M. domestica* individuals in shape space is more dispersed compared to males; suggesting that shape variation in female houseflies is more evident compared to males (Fig. 5b). Moreover, the distribution of male houseflies in PC 1 was highly clustered on the positive axis while in the female population, scores were clustered more on the negative axis. In PC 2, the distribution of both sexes was clustered in the positive and negative axis. Therefore, there were phenotypic distances between female and male *M. domestica* individuals. However, overlapping in the confidence ellipses of male and female shape variables specified that there were some shared traits within the individuals of *M. domestica* (Fig. 5b).

Moreover, the wireframe graph of PC 1 shows that the proximal (landmarks 1, 2, 3, 16, and 17), and posterior (landmarks 7 and 8) part were displaced toward the center of the wing. Furthermore, the distal (landmarks 4, 5, and 6) part of the wing margin showed displacement towards the tip of the wing. Overall, the displacements of the landmarks in PC 1 gave a shorted wing blade (vs mean shape); hence, the wing shape of the first PC score could be attributed to a narrow wing shape (Fig. 6a). Furthermore, the graphical reconstruction of the wing shape reveals that the proximal (landmarks 1,2,16, and 17) and posterior (landmarks 7 and 8) part of the wing margin expanded the wing blade. Likewise, the distal part of the wing (landmarks 4, 5, and 6) moves towards the base of the wing; hence, PC 2 exemplified an enlarged wing (Fig. 6b). In general, the two PC scores of shape variables suggest that the housefly population have two distinct wing shape patterns, the narrow shape (represented by PC 1), and the broad shape (represented by PC 2).

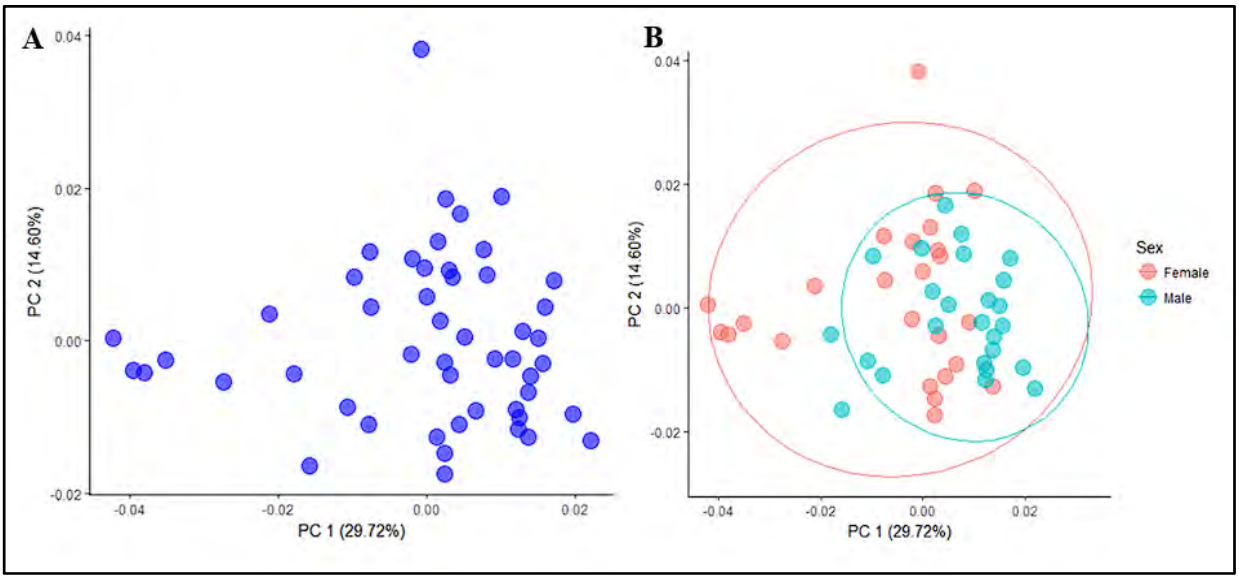

Fig. 5 Wing shape variation within *M. domestica* samples. (a) wing shape variation per individual, (b) wing shape variation according to sex.

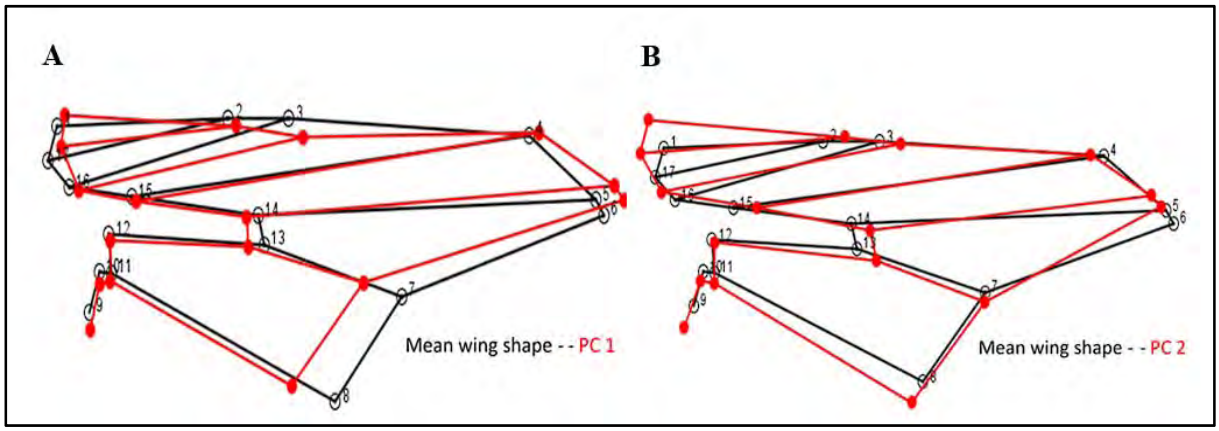

Fig. 6 Graphical reconstruction of the wing showing (a) PC1 (narrow wing shape); (b) PC2 (broad wing shape).

Further, Procrustes Analysis of Variance (Procrustes ANOVA) revealed a significant difference between the wing shape variables and sex of laboratory-reared *M. domestica* (F = 3.76, df =30, p = 0.0001). Hence, the wing shape of *M. domestica* is significantly distinct between male and female populations. Furthermore, the performed multivariate regression estimated the effect of wing size on the wing shape between sex. The regression of Procrustes coordinates (wing shape) on the centroid size (wing size) pooled within sex showed that wing allometry explained 3.60% of total shape variation. However, the association between wing size and wing shape showed no significant relationship (p=0.681). Therefore, the wing shape difference between sex was independent of the wing size of *M. domestica* individuals.

3.3 Sexual dimorphism in wing shape

Discriminant Function Analysis (DFA) correctly allocated 100% of the specimens to both female and male houseflies. The reliability of the analysis was assessed by leave-one-out cross-validation and correctly assigned

22 out of 25 (88%) female *M. domestica* while in males, 23 out of 25 (92%) was allocated correctly (Fig. 7). The Procrustes distance between female and male species was 0.0150 while the Mahalanobis distance was 6.517. Furthermore, Procrustes (p=0.0012) and Mahalanobis (p=0.00012) distances revealed a significant difference between female and male species; thus, sexual dimorphism in wing shape was exhibited in *M. domestica.*

The graphical reconstruction of the wing displayed the displacement on the distal-posterior (landmarks 7 and 8), and proximal wing base (landmarks 15, 16, and 17) regions towards the center of the male wing relative to the female wing. Also, the distal part of the male wing (landmarks 4, 5 and 6) extended towards the tip of the wing (vs female wing shape). Altogether, data revealed that males have narrower wings compared to females. Furthermore, the wing shape difference between females and males was highly evident in the center and distal-posterior portion of the wings specifically on landmarks 13 and 14, and landmarks 7 and 8 respectively (Fig. 8).

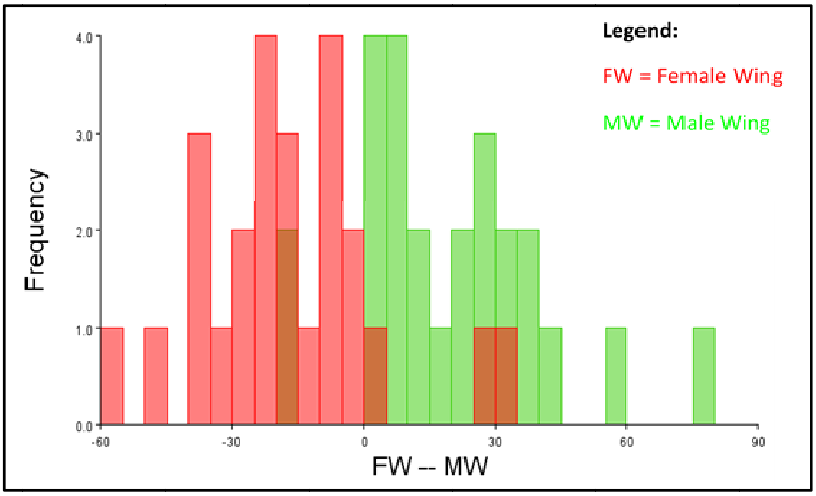

Fig. 7 Cross validation of male and female *M. domestica*.

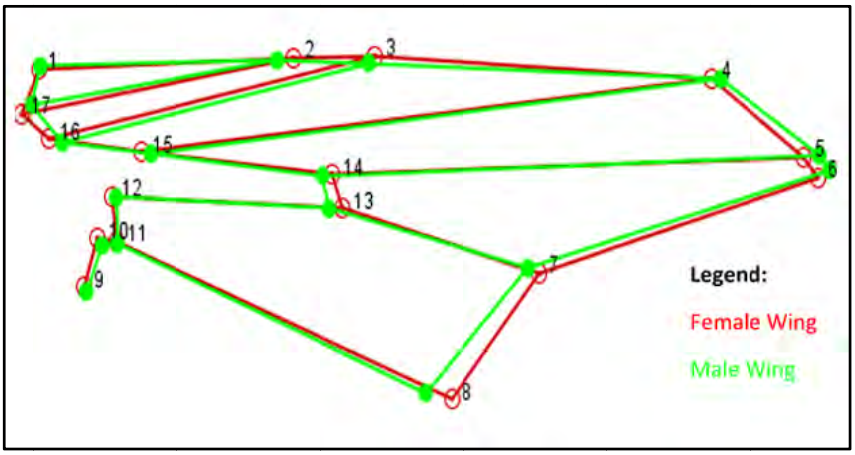

Fig. 8 Graphical reconstruction of the wing showing shape difference between male and female *M. domestica.*

4 Discussion

The study aimed to determine the sexual dimorphism of *M. domestica* wings using the techniques of Geometric Morphometrics. In wing centroid size, results show that male houseflies have high centroid size compared to females. According to Ludoski and colleagues (2014), the centroid size in laboratory-reared *M. domestica* was significantly different according to sex (existence of wing sexual size dimorphism); however, their study did not determine which sex had larger wing size. Furthermore, data from Pastor et al. (2014) showed a significant difference between the wing size of three different strains of *M. domestica*, where two out of three strains had larger wings in females, while one strain showed males had larger wings compared to females. However, female houseflies may increase their wing size for adaptation in the selection of the best breeding sites with less expenditure of energy (Alves and Belo, 2002). In addition, Alves and Belo (2002) revealed that wing size significantly increases with high latitude in female *M. domestica*. Also, wing size is affected by environmental factors such as temperature, relative humidity, and food availability (Changbunjong et al., 2016).

Wing shape data suggests that sexual dimorphism in *M. domestica* is apparent on the distal-posterior and proximal base regions of wing, and shape difference is highly evident in the center and distal-posterior portion of the wings. Therefore, these morphological traits can be used to distinguish male and female *M. domestica*. Likewise, these wing morphological characters may be important for insect dispersion, migration, and sexual selection (Espra et al., 2015). The distal-posterior region of the wings suggested to be important in aerodynamic performance and courtship song among dipteran species (Francuski et al., 2009). Similar results were reported in two species of blow flies (*Cochliomyia hominivorax,* and *C. macellaria*), in which sexual dimorphism showed that male wings were narrower compared to females (Sontigun et al., 2017). Similarly, wing shape difference was noted between male and female blowflies, *Lucilia sericata* (Diptera: Calliphoridae) on the distal part of the wing margin and wing base (Espra et al., 2015). Likewise, wing shape dimorphism was significantly different between sexes of three species of *Stomoxys* (Changbunjong et al., 2016).The wing shape difference between sexes was correlated with elevation, precipitation, and temperature, and therefore wing shape results from the plastic response from local environmental conditions (Alves et al., 2016). The morphological differences between sex of species can make an advantage in terms of reproductive aspects, which either result in being attractive to the other sex or overthrowing the same sex in a competition (Cabuga et al., 2017). Also, sexual dimorphism can affect various functions during an organism's lifetime such as feeding, mating, and parental care (Laporte et al., 2018). In fishes, a narrower shape is typically found in habitats requiring greater swimming activities (Laporte et al., 2018). Therefore, the narrow wing shape of male *M. domestica* species might be required for flight performance especially during maneuvering for selecting a potential mate in a limited confinement.

Similarly, wing size and wing shape have different genetic properties the former has low heritability while the latter is less sensitive to environmental changes and highly heritable (Francuski et al., 2009). The observed wing shape differences between sexes could be due to random mating, population density, food preference, heat pressure, flight system, and flight kinematics (Cabuga et al., 2017). Similarly, the existing wing shape sexual dimorphism could be attributed to sexual selection among individual species (Cabuga et al., 2017).

5 Conclusion

The study found that wing size variation existed in the laboratory-reared *M. domestica* which indicated the presence of wing sexual size dimorphism. Furthermore, the wing size of *M. domestica* revealed that the male wing size was significantly larger compared to females. Furthermore, wing shape change showed that male *M. domestica* have narrower wings compared to females, which is vital for their reproduction.

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