Article

Geometric-morphometric analysis of wing shape of *Lucilla sericata* Meigen (Diptera: calliphoridae) from two different environments

J. R. Pucot, **D. M. E. Guadalquiver**, **C. G. Demayo**

Mindanao State University-Iligan Institute of Technology, A. Bonifacio St., Tibanga, Iligan City, Philippines

E-mail: jayson.pucot@g.msuiit.edu.ph, donmark.guadalquiver@g.msuiit.edu.ph

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Abstract

The common green blowfly, *Lucilia sericata*, is a widespread species with forensic, medical, and veterinary importance. In this study, we use geometric morphometric as a tool to assess differences in the wing shape of female *L. sericata* occurring on two different environs. Thirty samples were collected from the two sites of Poblacion Market and Pampam Falls, Iligan City. The right wings were dissected and previously identified landmarks were digitalized using TpsDig. Morphometric analysis was conducted through Generalized Procrustes-based analysis, together with the quantification of wing area and length. Subsequent discriminant analysis was also conducted through PAST software. Results revealed intraspecific variations with >70% statistically significant discrimination. 83.33% of studied individuals were correctly classified into their corresponding group on the first discriminant function. A total of twelve significant components contributed to the differentiation of the female *L. sericata* right wings from two environs. Amongst the significant components identified, Principal Component 1 contributed the highest to the variation with 20.95% shape variability. Most variability are from the landmarks of the upper margin and middle portion of the wings extending to the branching endpoint of the media vein. The results showing variation within species were accounted as phenotypic plasticity to contrasting environmental conditions.

Keywords blowfly; discriminant analysis; intraspecific variations; landmarks; plasticity.

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1 Introduction

Recently, the effects of different types of environments on the shapes of organisms have been extensively examined. It was observed that under particular environmental conditions, organisms exhibit the capacity to adjust their phenotype to prevailing local conditions, as these changes increase their fitness (Alves et al., 2016; Altunsoy et al., 2017; Bai et al., 2019; Cabuga et al., 2017, 2018; Fraimout et al., 2018; Pajač-Zivkovic et al., 2018). This phenomenon is as much real, as unto members of order Diptera, or the true flies, where several studies have shown how different types of habitats, temperatures, food source, and the urbanization processes may have provided adaptive variations and phenotypic plasticity in their morphology particularly in their wing size and shape (Louise et al., 2015; Alves et al., 2016; Wilk-da Silva et al., 2018; Fraimout et al., 2018; Altunsoy et al., 2017).

One member of Diptera, the common green blowfly, *Lucilia sericata*, is a widespread species with forensic, medical, and veterinary importance (Heath, 1982; Singh et al., 2015; Crampton, 2019). However, as a frequent visitor to carrion, feces, and garbage, this blowfly, once they are in significant numbers, become a nuisance in the community and even threaten animal production because they can be transmitters of diseases. They caused myiasis in live stocks affecting over 80% of sheep flocks in the UK in their annual strike (Wall, 2012). They may even, though rarely, affect humans with recent studies reporting cases of myiasis in humans as well as pathogens that have been isolated from this species, indicating their contribution to transmission of vector-borne diseases (Haghi et al., 2018; Roozbehani et al., 2019).

In this study, we use geometric morphometric as a tool to assess differences in the wing shape of female *Lucilla sericata* occurring on two different environs. The wing morphology is an ideal model for qualitative or quantitative morphological studies since the wing is fairly distinctive and an important character for classification and identification of species. It is also flat and has many morphological landmarks at the points where the wing veins intersect or meet the wing margin (Sturtevant and Bier, 1995). This morphological character is vital in insect movement and epidemiology (Espra, 2015) and thus could help in the proper management and control of pest infestation of this species.

This study's objective is to investigate the variations in the wing shape of two populations of *Lucilla sericata* occurring in different environments. This is accomplished through the use of landmark-based geometric-morphometrics.

2 Materials and Methods

2.1 Specimens and data

Rotten fish meat was left for three hours to two different sites in Iligan City, namely: Poblacion wet market (Fig. 1), and Pampam falls of Brgy. Dalipuga (Fig. 2). The rotten fish served as bait to collect 30 specimens from each site using aerial nets.

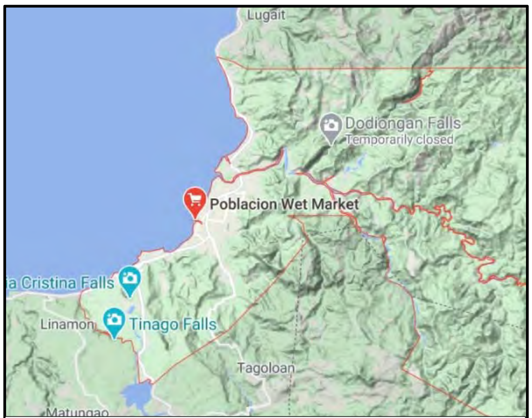

Fig. 1 Map showing Poblacion wet market in Iligan City (site 1).

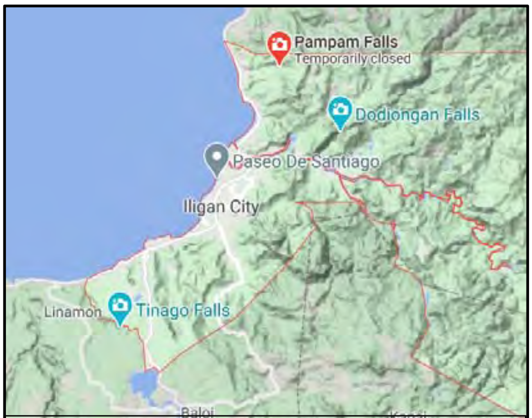

Fig. 2 Map showing Pampam falls in Brgy. Dalipuga, Iligan City (site 2).

The species were identified using taxonomic keys. Only the right wings of female blowflies were utilized and dissected and mounted on microscope slides. The wings were captured using an Olympus DP27 microscope digital camera under Olympus Light microscope BX53. The landmarks were identified following the method and descriptions used by Espra et al. (2015) and are digitized using TpsDig ver.2 (Rohlf, 2004), as shown in Table 1 and Fig. 3.

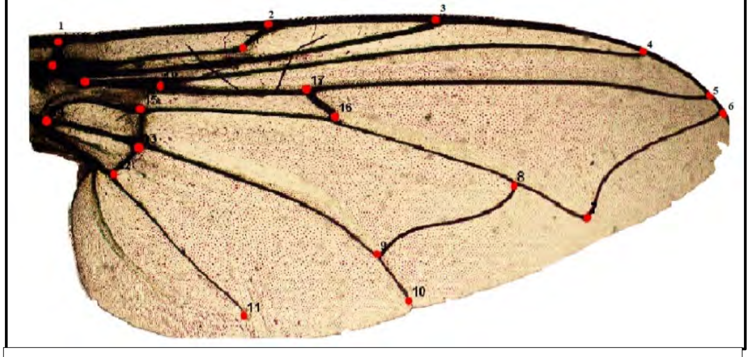

Fig. 3 Localization of the twenty-one landmarks digitized on female *Lucilla sericata* right wings, according to Espra et al. (2015).

Table 1 The description of assigned landmarks on *Lucilla sericata* wings.

Landmark	Description of the Landmark
1	Humeral cross vein
2	Subcostal vein
3	Anterior branch of radius R1 vein
4	R2+3 vein, the distal end of Radius
5	R4+5 vein, the distal end of Radius
6	Branching endpoint of Media vein
7	Curve point of Media vein
8	Intersection between Media vein and bm-cu vein
9	Intersection between bm-cu vein and CuA1 vein
10	Branching point of CuA1 vein
11	Branching point of A1+CuA2 vein
12	Intersection between A1+CuA2 vein and CuA2 vein
13	Intersection between CuA2 vein and bm-cu vein
14	Point of origin of A1+CuA2 vein and Median vein
15	Intersection between bm-cu and Median vein
16	Intersection between Median vein and r-m vein
17	Intersection between r-m vein and R4+5 vein
18	Branching point of vein Rs vein
19	Intersection of Rs vein and R1 vein
20	Intersection between humeral cross vein and R1 vein
21	Subcosta vein

Legend: Anal veins (A); Cubitus Anterior (CuA) vein; Radius (R) vein.

2.2 Morphometric analysis

The digitized landmarks were subjected to Generalized Procrustes-based analysis (GPA). This is used to standardized the size of the structure and optimize their rotation and translation, thus effectively showing variations in the entire wing shapes.

Quantification of wing variables was also done as follow: (1) wing area as the area of the polyline defined by landmarks 1 to 6 continuing to the edges of the upper portion of the wing to the point of origin of landmark 11 to landmark 14, crossing to landmark 21 and ending in landmark 1. (2) Wing length as the distance between landmarks 1 and 6. To statistically test the effect of the two environments on the female *Lucilla sericata* right wings, flight-related traits were tested using ANOVA.

Subsequently, PAST software (Hammer et al. 2001) was used for discriminant analysis (DA) to provide insights on the quantitative differences of the wings from the two different populations. The software was also used to show the box & whiskers plot of the relative warps scores from the two populations.

3 Results and Discussion

After subjecting the data obtained from the superimposition method to relative warp analysis, wing variations from the two populations are highly evident, as shown in Figs 4 and 5. The relative warp analysis visualized via box and whiskers plot (Fig. 4) showed that the right wings of female *L. sericata* sampled from the two different environments are concentrated towards the mean shape. Figs 6-10 also visualized shape variability via projections of the mean shapes and the (-) and (+) deviation from the mean shapes. The first relative warp axis (RW1) shown in Fig. 6 explains 20.14% of the variation observed in the subcostal vein to the anterior and distal end of the radius. The second relative warp axis (RW2), on the other hand, account for 13.37% variations in the media vein to the cubitus anterior vein. The third relative warp axis (RW3) shown in Fig. 8 is attributed to the variability in the humeral cross vein to the anal veins, which accounts for 11.94% variation. Lastly, the fourth and fifth relative warp axis (RW4 & RW5) accounts for 8.89% and 8.17% variability in the humeral cross vein to the subcostal vein to the distal end of the radius, and variability in the humeral cross vein to the curve point media vein, respectively. Moreover, the discriminant analysis (Fig. 5) showed intraspecific variations with >70% statistically significant discrimination. A total of 83.33% of studied individuals were correctly classified into their corresponding group on the first discriminant function.

The proportional variation of the Principal Component Analysis (PCA) reveals the number of significant components with their corresponding eigenvalues and percent contribution to variations shown in Table 2. Twelve significant components with eigenvalues above Jolliffe cut off score contributed to the differentiation of the variability of the female *L. sericata* right wings. Amongst the significant components identified, Principal Component (PC1) contributed the highest with 20.95% shape variability, while components 2 and 3 yields % shape variations of 14.87% and 12.28%, respectively. The rest showed <10% shape variances. The possible variables that could contribute to the differentiation of the population of *L. sericata* are shown in appendices 1-12 and justified by bar graphs shown in Figs 11-16.

Based on table PC1-12, it can be observed that most variables belong to the landmarks of the upper margin and middle portion of the wings extending to the branching endpoint of the media vein. The variability in the specified landmarks is responsible for the length of the wings of female *L. sericata* from two different populations. After subjecting wing length as a dependent variable in ANOVA, the result showed no statistical difference in the wing length of the two populations with a p-value of 0.274. However, based on actual computations, the mean length of the wings of female *L. sericata* from Poblacion Market tends to be longer and wider with mean length and mean area value of 2143.9 and 5433.56 respectively, compared to that of samples coming from Pampam falls with slightly shorter mean length value of 2101.5 and mean area of 5273.94. These variations in sizes between populations are said to be associated with alterations in shape (Jirakanjanakit et al., 2008) and may have resulted from various environmental and ecological factors affecting wing evolution, as hypothesized by Motoki et al. (2012).

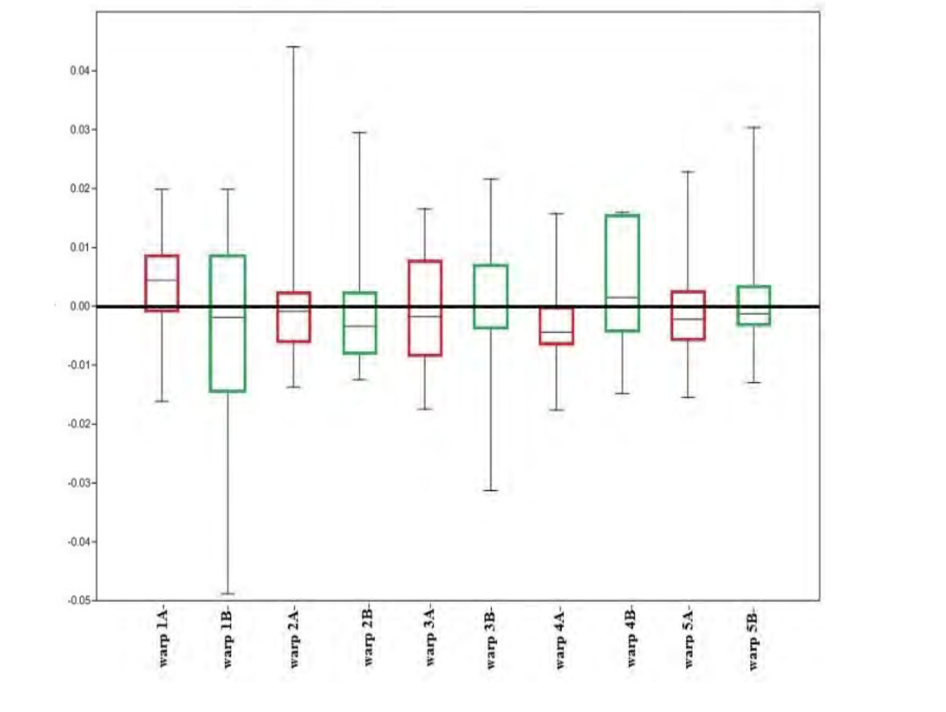

Fig. 4 Box and whiskers plot of the relative warp analysis scores. Legend: A(red)- samples from Pampam falls, Dalipuga; B(green)- samples from Poblacion market.

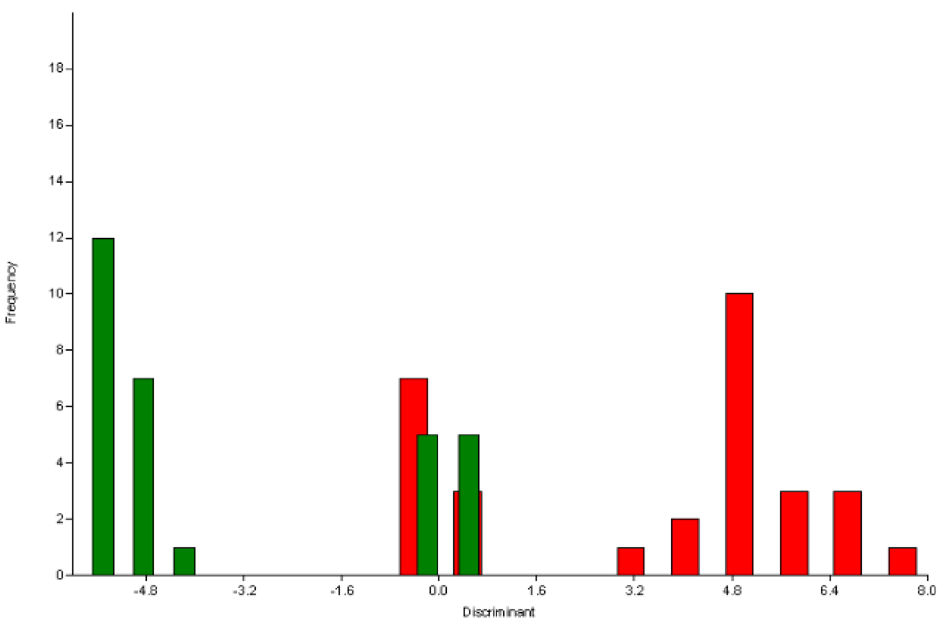

Fig. 5 Histogram of the first canonical axis after a discriminant analysis on the wing morphology of *Lucilla sericata*. Legend: A (red)- samples from Pampam falls, Dalipuga; B (green)- samples from Poblacion market.

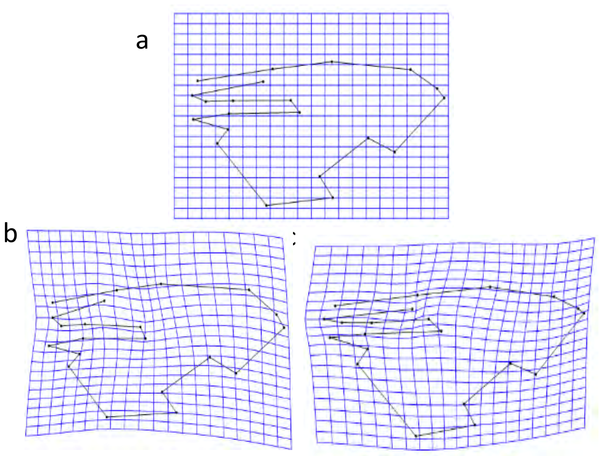

Fig. 6 Visual presentation of Relative Warp 1 (RW1). a- Mean shape, b- negative deviations of the mean shape, c- positive deviations of the mean shape

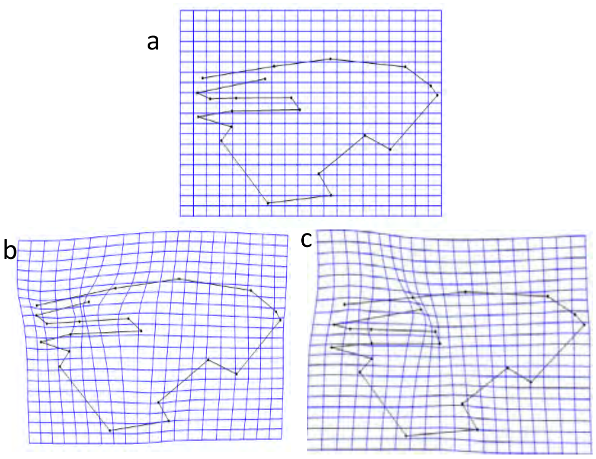

Fig. 7 Visual presentation of Relative Warp 2 (RW2). a- mean shape, b- negative deviations of the mean shape, c- positive deviations of the mean shape

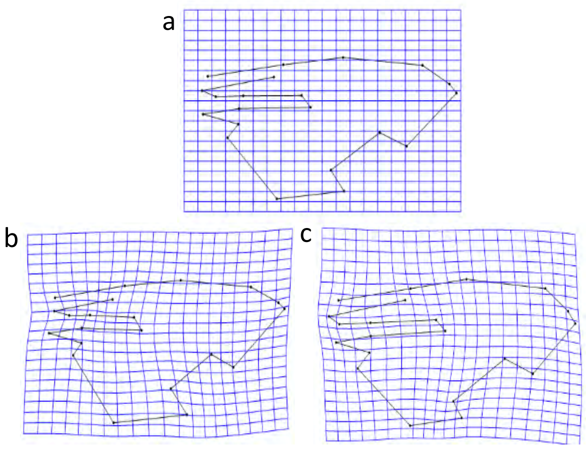

Fig. 8 Visual presentation of Relative Warp 3 (RW3). a- mean shape, b- negative deviations of the mean shape, c- positive deviations of the mean shape

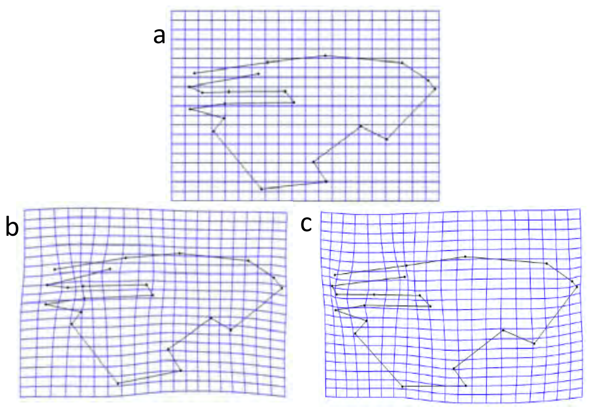

Fig. 9 Visual presentation of Relative Warp 4 (RW4). a- Mean shape, b- negative deviations of the mean shape, c- positive deviations of the mean shape

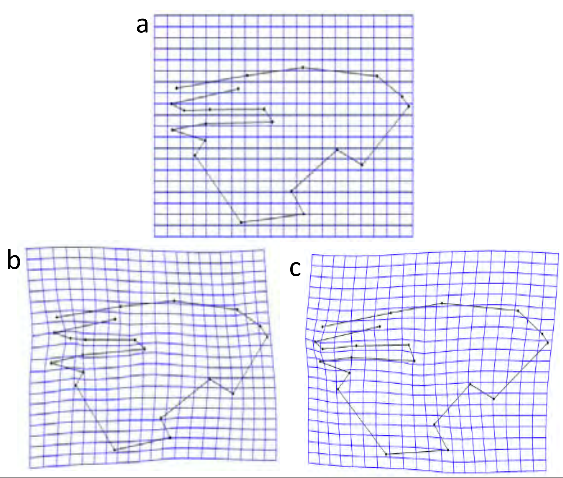

Fig. 10 Visual presentation of Relative Warp 5 (RW5). a- mean shape, b- negative deviations of the mean shape, c- positive deviations of the mean shape.

Table 2 The proportion of variation associated with PCA loading values for the significant components of the landmark coordinates of female *Lucilla sericata* right wings.

PRINCIPAL COMPONENT	Eigenvalue	% variance
1	0.000251605	20.95
2	0.000178628	14.874
3	0.000147491	12.281
4	0.000109912	9.1519
5	0.0000932	7.7614
6	0.0000790	6.5763
7	0.0000566	4.7116
8	0.0000513	4.2709
9	0.0000349	2.9052
10	0.00003.6	2.63
11	0.0000252	2.0993
12	0.0000232	1.9331
Jolliffe cut-off:	0.00002002	

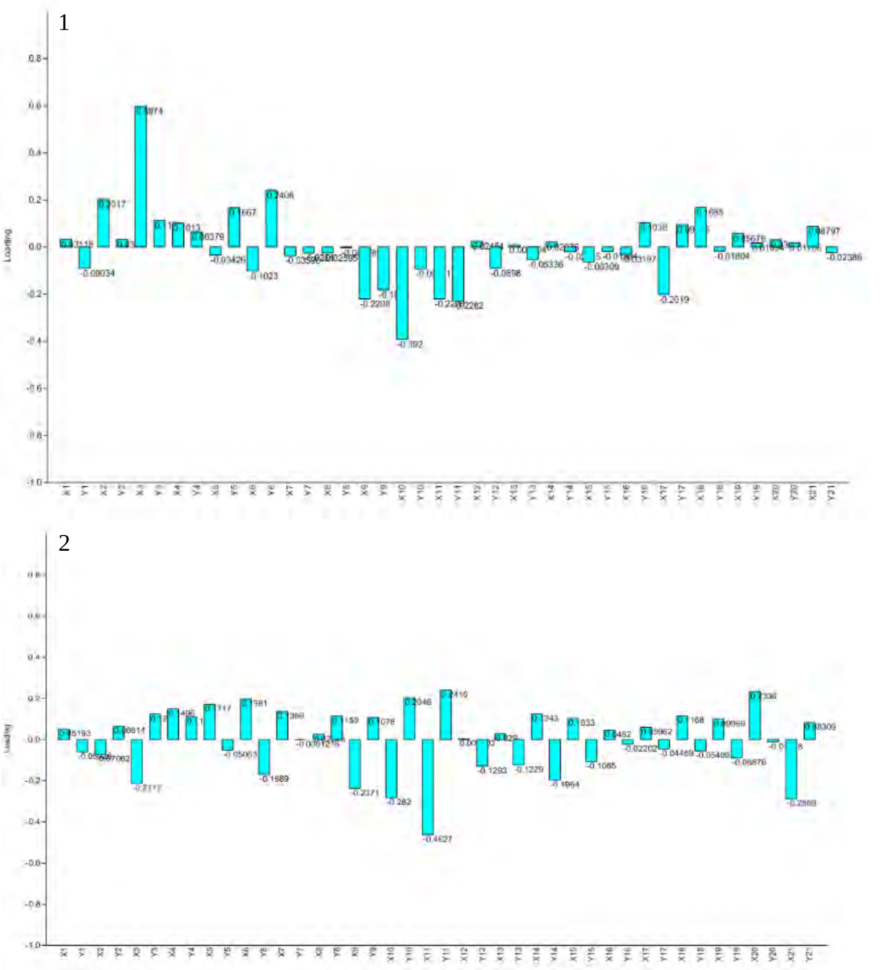

Fig. 11 PCA loadings 1 and 2 of the significant components that contributes to the variation corresponding to the significant principal component of the proscrustes.

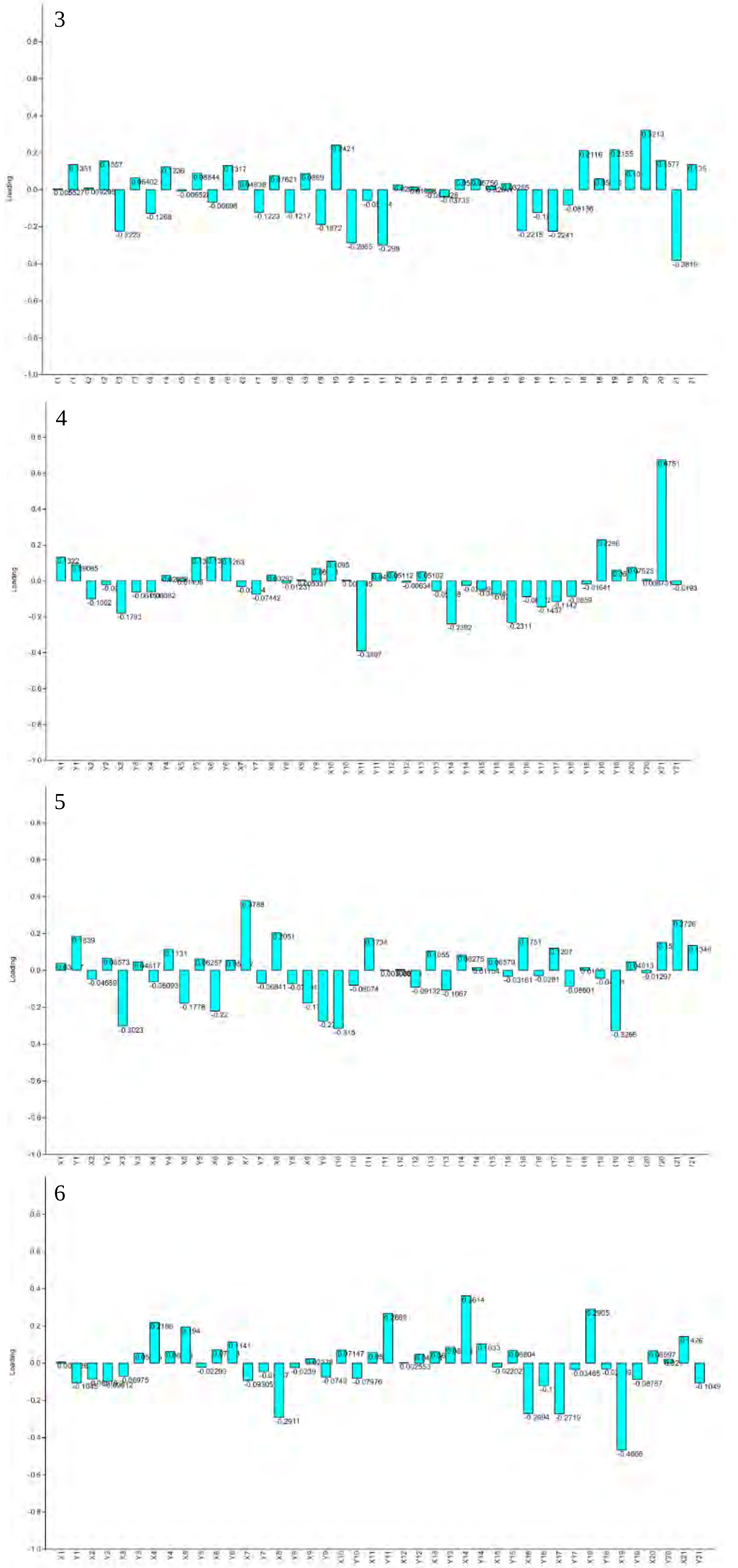

Fig. 12 PCA loadings 3,4,5, & 6 of the significant components that contributes to the variation corresponding to the significant principal component of the proscrustes fitted landmark coordinates of *L. sericata* right wings.

The effect of the environment is a probable factor affecting the observed variation inwing shape and size of *L. sericata* in this study. This is supported by Alves et al. (2016) in his study where they found that the wing shape of flies is influenced by environmental variabilities such as elevation, precipitation, and temperature. This was also true in the case of Altunsoy et al. (2017), where the wing shape of flies was discriminated into two groups in terms of altitude, precipitation, and wind. The wet market in Brgy. Poblacion is situated in a coastal area with low elevation, and generally has a warmer temperature (37^0C at 10 AM on a sunny day). On the other hand, Pampam falls situated 115 m a.s.l. and also have natural spring area surrounded by an agroecosystem with patches of forest and grassland that generally has a cooler environment (33^0C at 10 AM on a sunny day). This relative difference in temperature and elevation could be accounted for the difference in the wing shape of *Lucilla sericata* in this study. Moreover, external factors such as the degree of disturbance and urbanization also contribute variability in wing shapes and sizes. The wet market area is marked by the mass presence of garbage and rotting meat. The area thus prospers abundance of the blowfly populations since blowflies lay their eggs in such places (Lewis et al., 2019), so it is considered ideal for blowfly populations to thrive.In contrast, the second sampling area of Pampam falls, in Dalipuga, Iligan City is a natural spring area relatively far from anthropogenic activities. In this area, a lower abundance of blowflies was observed, and it took a while to attract individuals to the fish carcass used as bait. Less carrion and dung can be observed in this area; thus, Blowflies may have lesser food source and oviposition area and might have to rely on plant sources for food (Stutz, 2006) (Imasheva et al., 1999). The two areas thus may have imposed different developmental conditions on *Lucilla sericata*. Accordingly, poor rearing conditions and nutritional stress affect the morphology of flies (Imasheva et al., 1999). One study found out that wing size and wing shape of flies are affected by larval density and the nature of the developmental substrate (Baleba et al., 2019). As emphasize in one study, poor environmental conditions may cause ecological stress and developmental instability resulting to fluctuating asymmetry (Uba et al., 2019). Areas with different levels of urbanization have different features and selective pressures affecting differently the phenotype of organisms as well as the population structure of the species (Wilk-da-Silva et al., 2018).

These contrasting factors in the two areas imply how wing shape differences in *Lucilla sericata* resulted from a plasticity response to local environmental conditions. As mentioned by Relyea (2004), plasticity arises because environmental variability induces developmental changes, which alter the expression and connection between traits. As a developmental response, plastic traits enables species to cope with environmental variability enabling a fitness optimization to these conditions (Ghalambor et al., 2007). Both abiotic and biotic factors significantly influence *Lucilla sericata* and its population dynamics and, in turn, may prolong their metamorphic stages, survival, and rate of multiplication (Khaliq, 2014).The geometric –morphometric analysis in this study showed clearly that there is differences and proper discrimination between populations of the same species occurring in different environ. Understanding these matters helps in the proper control of the *Lucilla sericata* population, especially as wings are important in insect dispersion, migration, and sexual selection (Espra et al., 2015).

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