# Sexual Dimorphism in the Size and Shape of the Wings of the Damselfly *Pseudagrion pilidorsum pilidorsum*

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**Abstract**

Patterns of distribution of damselflies differ by sex, with males clumped in or near water bodies and the females usually found away from the water body. Previous studies on other species of damselflies have shown that these differences are reflected as differences in the sizes of the wings between the two sexes. In this study, differences in the size and shapes of the wings between the two sexes of the damselfly *Pseudagrion pilidorsum pilidorsum* was assessed using image analysis and landmark-based analysis. To do this, images of the wings were scanned at uniform dpi. Then, the Cartesian coordinates of twenty-five landmarks from around the wing were extracted using an image analysis and processing software. These coordinates were then subjected to two different analyses. Euclidean Distance Matrix Algorithm was applied on the data to generate linear distances between the landmarks. The resultant interlandmark distances were used to compare the sizes of the wings between the sexes.

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On the other hand, the raw coordinate data was also Procrustes-fitted to generate shape variables. These shape variables were analyzed using Relative Warps analysis to determine sex-differences in the shapes of the wings. Results showed statistically significant differences in the size of the wings between males and females. Also, tests for significant differences of the Relative scores between the sexes revealed dimorphism in wing shapes related to differences in sex. The results of this study are discussed in relation to differences in the flight ecologies of the males and females of this species.

Keywords: EDMA, Procrustes-fitting, relative warps

## Introduction

Damselflies are remarkable groups of organisms that exhibit sexual dimorphism in terms of body coloration. As with other species of organisms, the males are more colorful than their female counterparts. Observations from the field also reveal that members of the opposite sexes differ in their distribution along a given landscape. The field of Functional Morphology dictates that such disparity in the flight ranges between the two sexes could also reflect differences in their flight capabilities. In order to explore such possibility, the flight morphologies between the two sexes of *P. pilidorsum pilidorsum* were compared (Figure 1). Specifically, this study was conducted to determine possible differences in the shapes of the wings between the two sexes of this species of damselfly using tools of geometric morphometrics – relative warp analysis (RWA) and euclidean distance matrix analysis (EDMA).

Relative warp analysis (RWA) (Bookstein 1991, Rohlf, 1993) was used to investigate variation in nonaffine shape components. The method is used for the analysis of within population morphometric variation based on landmark data. The method of relative warp analysis consists of fitting an interpolating function (the thin-plate spline of Bookstein 1989) to the x,y coordinates of the landmark for each specimen in a sample. Variation among the specimen within a sample is described in terms of variance in the parameters of the fitted functions. This is expressed relative to a bending energy matrix based on the coordinates of the landmarks of a reference configuration. The reference is often the mean configuration of landmarks after some appropriate alignment of specimens. The relative warps are used to describe the major trends of wing shape variation among specimens within a sample as deformations in shape (non-uniform shape variation). It is simply a principal component analysis (PCA) of the partial warp scores. It was used for both fore and hind wings. The first axis of a PCA is often interpreted as representing general size.

EDMA calculates all the possible Euclidean distance between the selected landmarks on a single object, and then compares the two objects by calculating a matrix of ratios of corresponding linear distances measured on each object. This geometric morphometric method provides an objective measurement of shape differences by localizing the sites of major variations by suggesting which of the landmarks are more involved in the form differences (Corner and Richtsmeier 1991, Lele and Richtsmeier 1991, 1992).

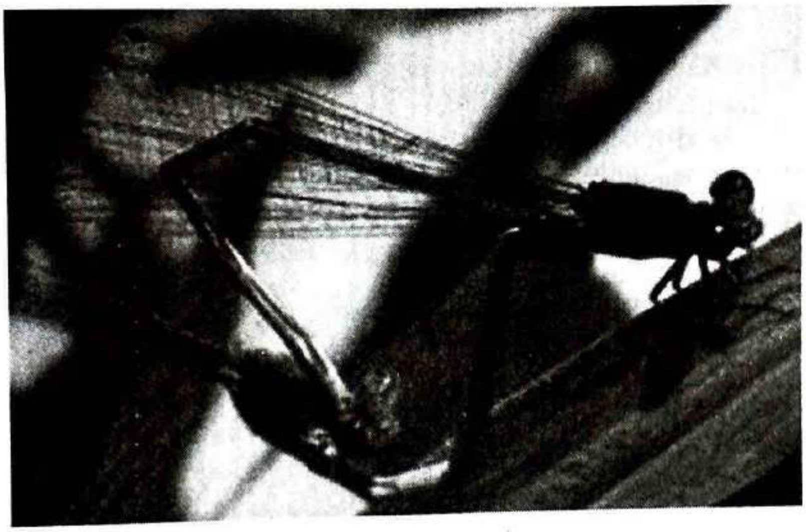

**Figure 1.** Damselflies mate in tandem and usually copulate while in flight. Shown in the picture are two *P. pilidorsum pilidorsum* individuals in copula. Male members of this species are red in color while their female counterparts are brown in color.

## Methodology

The damselflies used in this study were collected from Manticao, Misamis Oriental (Figure 2). The presence of hairy structures in the first two abdominal segments of males was used as basis for identifying the sex of the individuals. These structures are called hamules and are used as storage of sperms. The wings of the damselflies were cut and kept in glass slides. Then, the wings were scanned using a high resolution scanner (Figure 3). This was done because geometric morphometrics are usually applied to two-dimensional data such as the scanned images of the wings. Then, landmarks were placed on the wings following the points of intersection of the major veins and the peripheral boundaries of the wings. The relative positions of the homologous landmarks on the fore- and hind wings are shown in figure 4. The $x$ and $y$ coordinates of these landmarks were collected using an image analysis software. These coordinates were then subjected to Procrustes fitting, a generalized least squares fitting algorithm that eliminates size components of biological forms and retaining only information regarding the shapes of the wings. These shape variables were then used for subsequent tests such as Relative Warps Analysis (RWA), which is an ordination method that plots damselflies with similar wing shapes closed together in a scatter plot. Test for significant differences in the shapes of the wings was also done using the Relative Warp scores computed from the Procrustes-fitted values. Sexual Size dimorphism was also tested using the same landmark set used in the shape analysis. This was done using another geometric morphometric approach which is the Euclidean Distance Matrix Algorithm (EDMA). This algorithm computes the linear distances between any two of the landmarks included (Figure 5). The computed interlandmark distances were then subjected to Principal Component Analysis and other statistical tests to explore possible differences in the sizes of the wings between the two sexes.

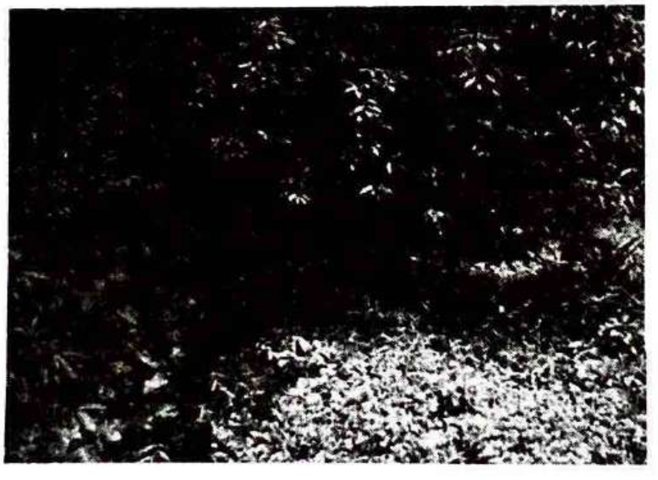

**Figure 2.** Picture of the sampling site in Manticao, Misamis Oriental. Damselflies thrive where water bodies can be found. Males are usually found in or near water bodies, while their female counterparts are usually seen away from the water body.

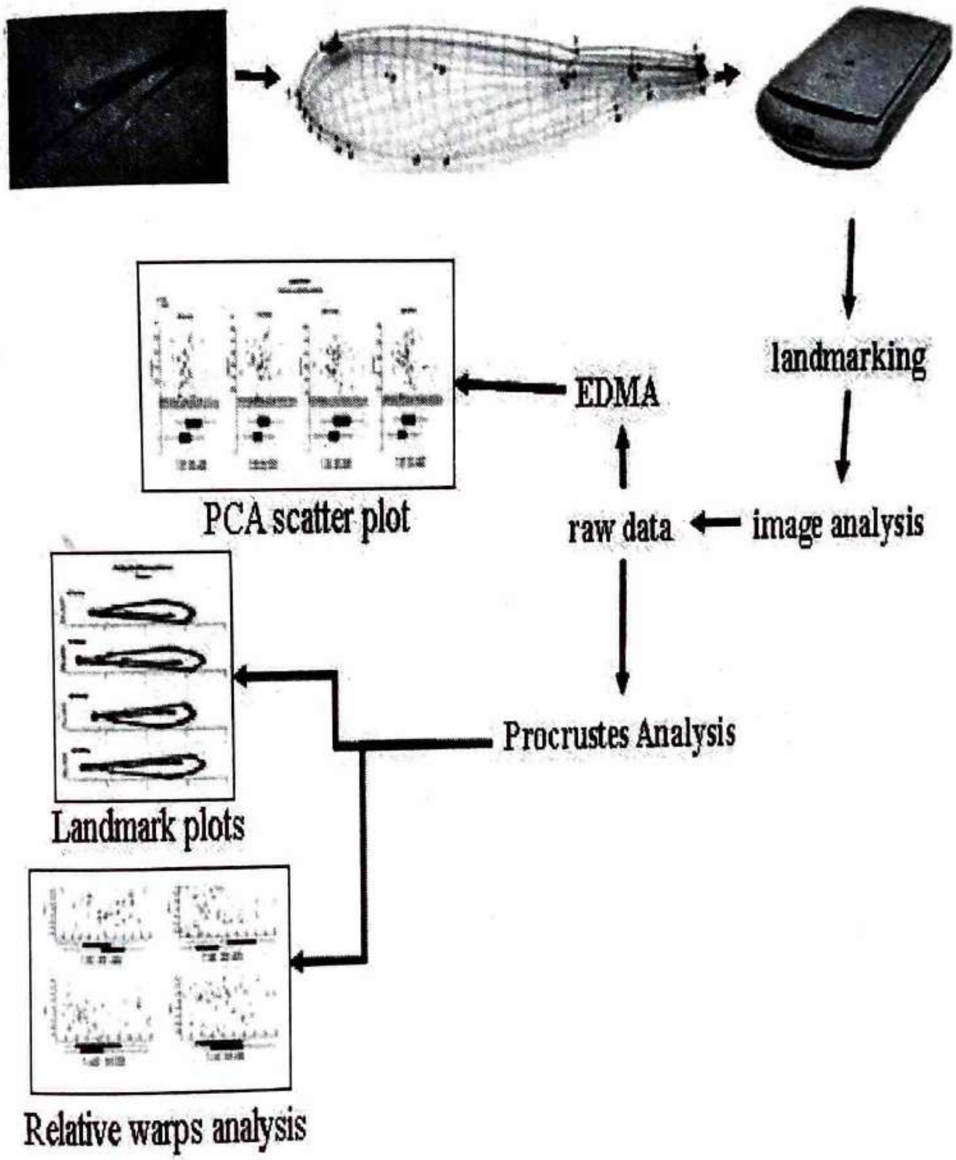


**Figure 3.** Analyses flow chart.

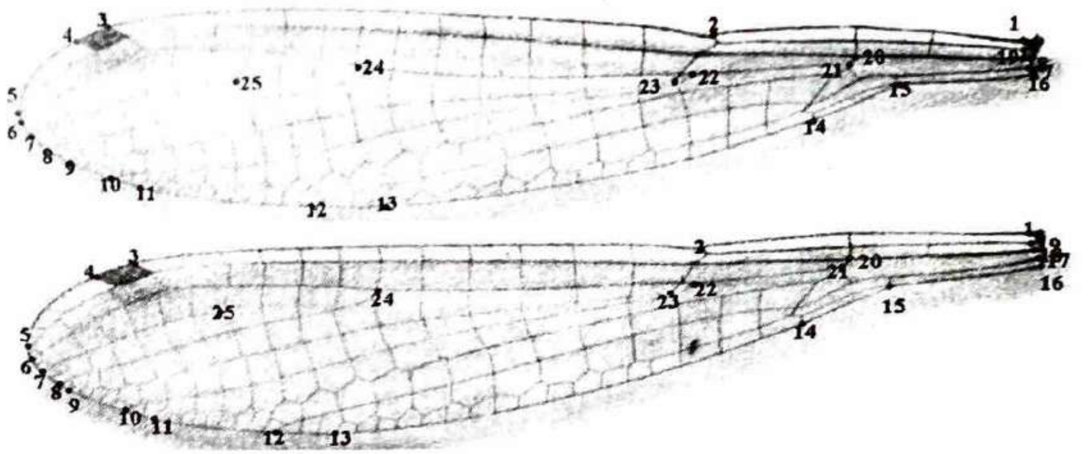

**Figure 4.** Relative positions of the twenty-five landmarks on the fore and hind wings of the damselflies.

**Figure 5.** Linear distances determined using Euclidean Distance Matrix Algorithm. EDMA is a computer based application that automatically determines the distances between any two landmarks without the hassle of using tools such as Vernier Caliper, thereby reducing the amount of measurement error.

## Results and Discussion

Studies of shapes using the tools of geometric morphometrics has revolutionized biometric analysis (Rohlf & Marcus 1993, Bookstein 1996,Monteiro & Reis 1999). In this study, the use of geometric morphometrics to analyze wing shape differences among *P. pilidorsum pilidorsum* based on anatomic landmarks defined by Cartesian coordinates (x and y) were not altered with changes in scale, translation and rotation (Rohlf 1996) These coordinates were primarily used to compare between the fore- and hindwings of both male and female damselflies after removing the effects of size, position, and orientation, allowing to singly evaluate the differences in shape.

The result presented in figure 6 shows the effect of the Procrustes fitting algorithm on the landmark data where size and rotational translation were removed leaving only the shape residuals. Landmark plots of the Procrustes-fitted residuals generated for the two sexes were compared and the results reveal differences in the shapes of the forewings between the sexes (Figure 7). The result of the Relative Warp Analysis is concordant with the results revealed using the landmark plots (Figure 8). Also, the observed differences in shapes of the wings are found to be statistically following a T-test of the Relative Warp scores ($P < 0.05$). Sexual size dimorphism was also tested using the interlandmark distances as variables. PCA of these values reveal that the females have bigger wings than their male counterparts as shown in the accompanying box-and-whisker plots (Figure 9). This study revealed statistically significant differences in the shapes and sizes of the wings between the two sexes of the damselfly *P. pilidorsum pilidorsum*. The observed differences could possibly be explained in the light of possible differences in the flight capabilities of the two sexes, thus explaining the differences in their flight ranges. The results imply that size, shape and symmetry of the wings of the damselflies are fundamental features of form and function and that any observed differences could reflect possible disparities in flight capabilities of the two sexes of damselflies.

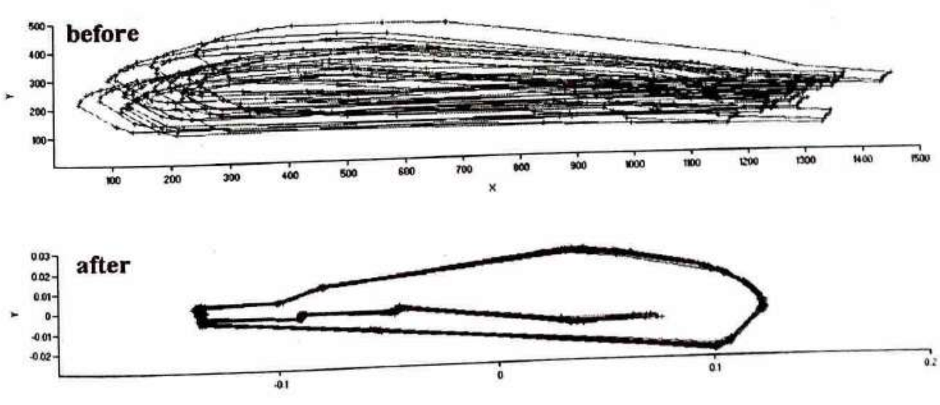


**Figure 6.** Comparison of landmark plots before and after information regarding the sizes of the wings were removed allowing for the comparison of shapes.

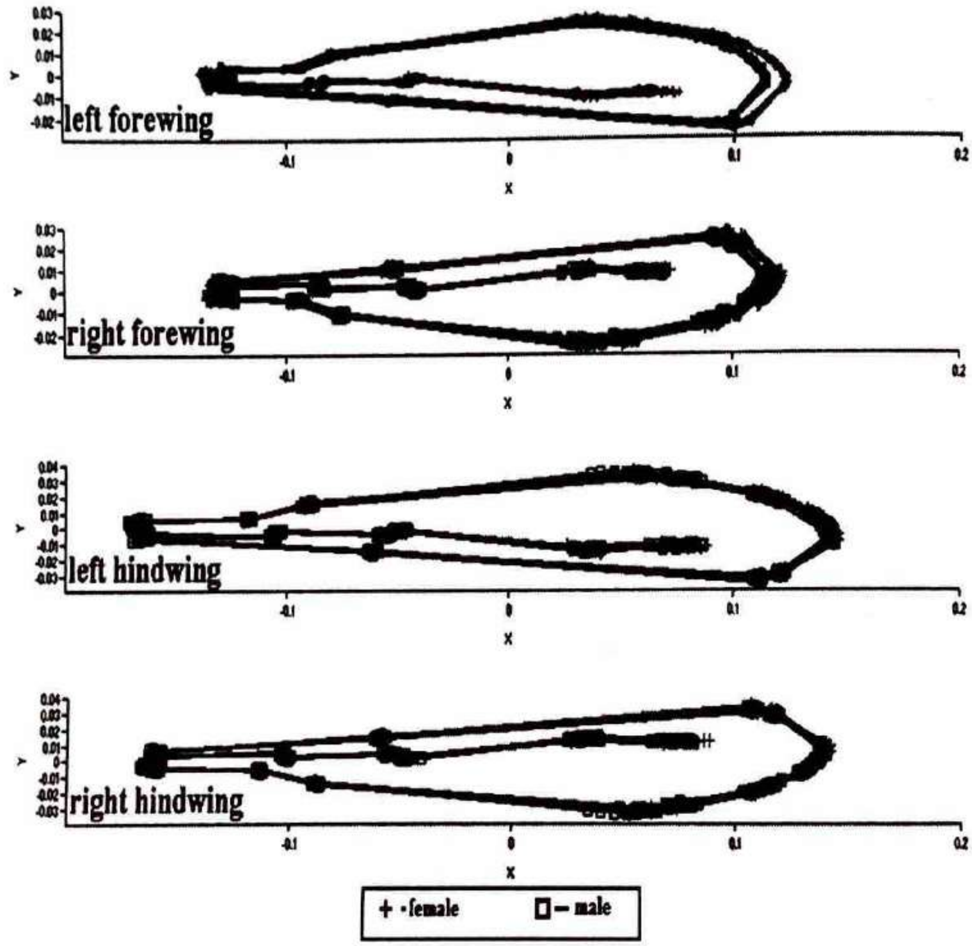


**Figure 7.** Comparison of the shapes of the wings between the two sexes using landmark plots.

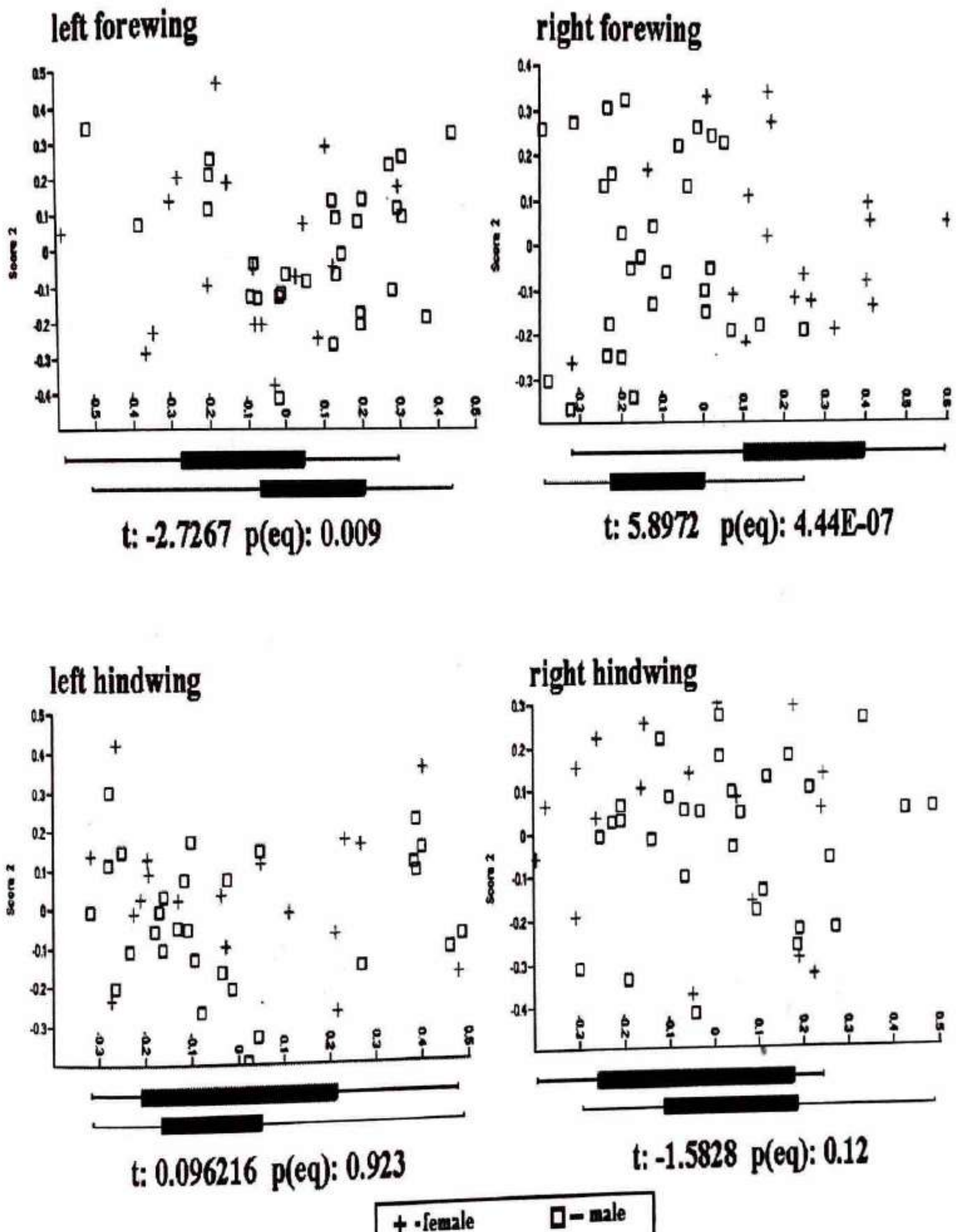


Figure 8. Results of the Relative Warps Analysis showing statistically significant differences in shapes of the forewings between the two sexes.

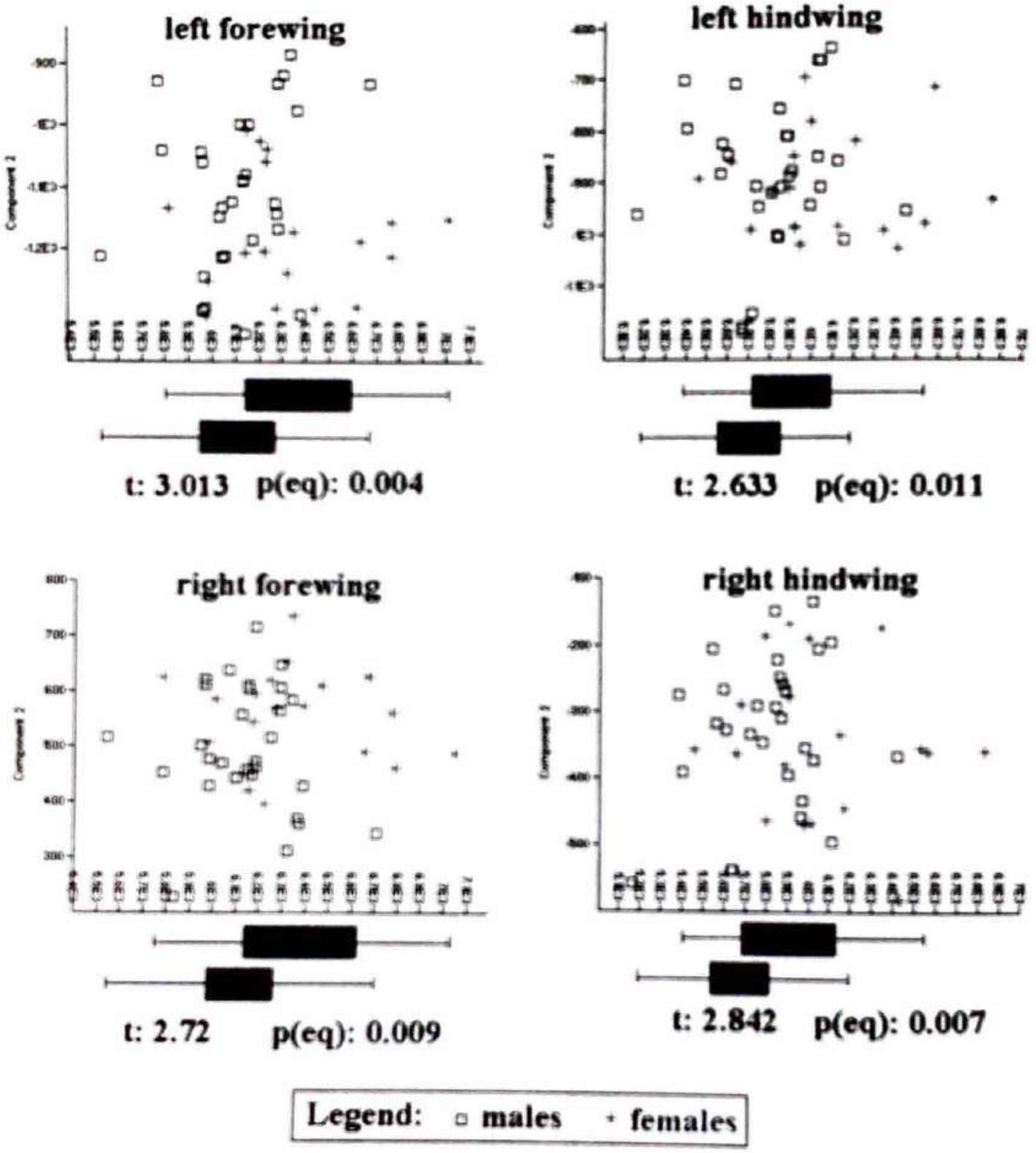


**Figure 9.** Results of the PCA analysis of the interlandmark distances generated using Euclidean distances matrix algorithm showing statistically significant differences in the sizes of the wings between the two sexes.

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