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Relative warp analysis in determining sexual dimorphism of *Rynchophorus ferrugineus* in Malungon Sarangani Province, Phillipines

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Abstract. Many traditional ways were introduced in determining and differentiating sexes between male and female beetle. One of this is through physical differences of their snouts, but this kind of identification holds less scientific evidences. Hence, there is a need of more scientifically based aproach and one aplication that demonstrates potential methods in this discipline is known as Geometric Morphometric. Through the use of such tool, the aim of determining the sexual dimorphism of coconut snout bettle *Rynchophorous ferrugineus* using the hind wings of the species would be possible. A total of 19 landmarks from images of 120 hind wings (30 left wings; 30 ringth wings of female and and another set of 30 left wings and 30 rigth wings for male) of *R. ferrugineus* were subjected to Relative Warp (RW) analysis and Discriminant Function Analysis (DFA). The results revealed that the RW of female are RW1 = 31.11%, RW2 = 17.88%, RW3 = 10.79%, RW4 = 8.01%, RW5 = 5.27%, RW6 = 5.04%, RW7 = 4.49% and for male are RW1 = 33.87%, RW2 = 17.15%, RW3 = 9.45%, RW4 = 7.77%, RW5 = 4.81% explaining more than 5% of the variance. These results imply that females present 82.45% wing shape variation whereas males only showed 73.05% of variation. However, statistical test prove that using DFA confirmed that there were no significant differences on the wings based on the shape variations. Thus, the hind wing shape does not contribute to the selection of gender of *R. ferrugineus*.

Key Words: *Rynchophorus ferrugineus*, snout beetle, Geometric Morphometric, Discriminant Function Analysis, Malungon Sarangani, Philippines.

Introduction. Differentiating the sexes between male and female insects is quite confusing. Among groups, the manner of determination is different. Proximal mechanisms have been suggested to elucidate this dimorphism, such as in growth rate, differential mortality and the most conspicuous one is the differences in their body size (Reilly & Wainwright 1994; Ricklefs & Miles 1994). There were many different attempts that were conducted in relation to insect sexual dimorphism.

Recently, new application which is related to morphometric approaches is introduced demonstrating potential methods in this discipline has been carried out. Geometric Morphometric serves to give equally important purposes of visualization, interpretation and communication of results (Zelditch et al 2004). This application is a new field of shape analysis in which "landmark coordinates" is recorded in several dimensions to directly capture the geometry of the species (Rohlf 1993). Since insects such as coconut weevils are known to be hyperabundance as well as for possessing a well-defined exoskeleton, they are ideal for this kind of study (Adams & Funk 1997; Pretorius & Scholtz 2001).

Coconut weevils (*Rynchophorus ferrugineus*) also called snout beetles are found living in a coconut tree. This species possessed different characteristic such as having long, slender rostrum or "snout" where female uses it to penetrate palm tissues and creates access wounds in which eggs are deposited. In terms of body lengths, when they are larvae they attain lengths greater than 50 mm (2 inches). Adult attain lengths up to 35 to 40 mm (1.4-1.6 inches). Another characteristic is their color. Coloration of this species is extremely variable leaded to the erroneous classification of color-defined polymorphs (variants) as distinct species. The specimens coloration found in Sitio Napla, Malungon, Sarangani Province are predominantly black in the most typical form with orange striped which is vertically place at the dorsal head of the species (Copeland 1931). The wings of this beetle consist of two which is the inner and outer (Figure 1). Outer wings serves as a cover that protect the inner wings of the said insect.

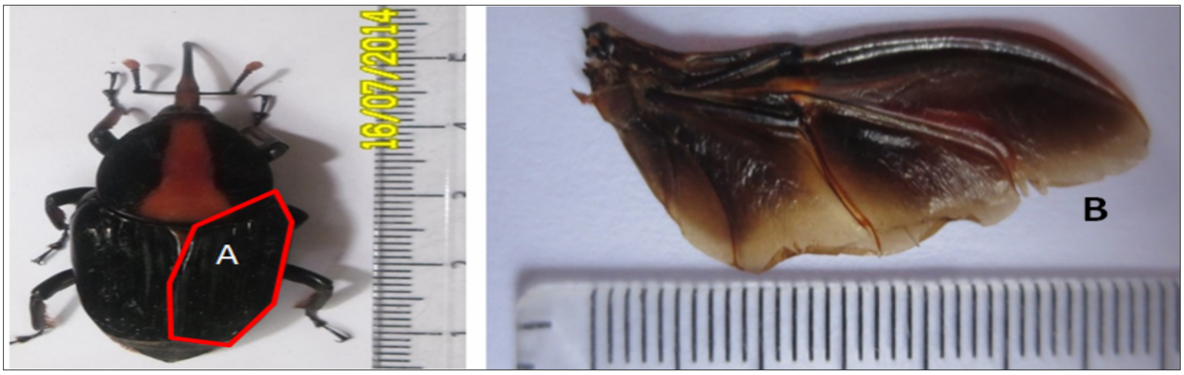


Figure 1. A. Outer wing; B. Inner wing of *Rynchophorous ferrugineus*.

Few studies made use of wings to identify the sexual dimorphism, with such reason this study aims to determine the sexual dimorphism of the *R. ferrugineus* by the use of its wings with the application of geometric morphometrics (Wootton 2000). In addition, this study will analyze the shape of the wings of the coconut beetle found in Sitio Napla, Malungon, Sarangani Province and know whether there are differences between the wings of male and female.

Material and Method

Sampling area. A total of 60 *R. ferrugineus* comprising 30 male and 30 female were collected from the coconut trees found in Sitio Napla, Malungon, Sarangani Province, Philippines on July 2014. *R. ferrugineus* were collected through the help of some men and was then placed in a closed jar. The map shown in Figure 2 illustrates the area of this study.

Identification and separation by sex. Gender of this species was identified, physically both sexes were relatively alike but through their snout, classification of their gender was successfully determined. Female *R. ferrugineus* differ from male because it has a little bump and it has a bit hair on its snout compared to male *R. ferrugineus*.

Taking of digital images. After identifying the gender of the collected specimens, the researcher carefully remove the left and right wings of both female and male *R. ferrugineus* for imaging purposes (Figure 3). Images are captured by using Canon digital camera (14 megapixels). Digital images taken will then be altered to TPS setup files using tpsDig2 database (version 2.12, Rohlf 2008).

Figure 2. Map of the Philippines (a) showing the Philippines; (b) enhanced map showing Mindanao; (c) aerial view of Malungon (www.googlemap.com).

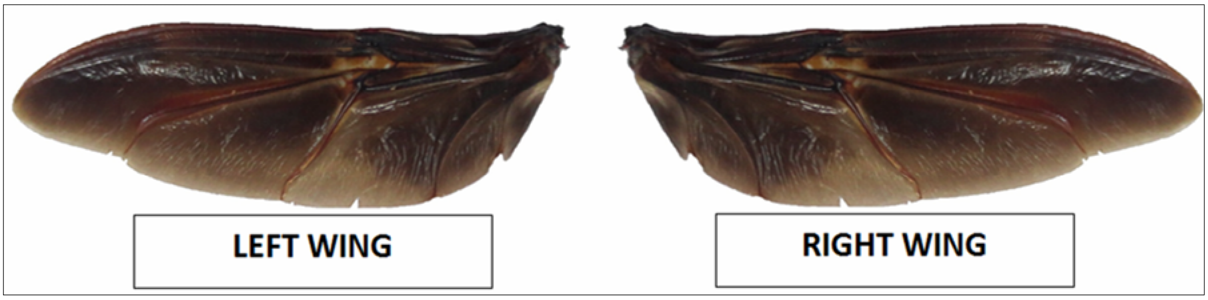


Figure 3. Photograph of left and right wings of *Rynchoporous ferrugineus*.

Landmarking. Homologous point's analyses of the left and right wings of *R. ferrugineus* will be acquired by means of thin-plate spline (TPS) order to highlight the curves and points of *R. ferrugineus* wings. Through the use of software tpsDig2, selection of landmarks will provide homogenous outline of body shape of the said species. A total of 19 landmarks will be determined for best representation of the external shape of the wings; the landmarks are placed in different parts of the hind wing as shown in Figure 4. For further analysis, X and Y coordinates of the landmarks will then be attained.

Morphometrics. Variations in the body shape were then deliberated by means of Relative Warp (RW) analysis which generates new variables in the form of RW scores. Using PAST software, the RW scores were then subjected to Discriminant Function Analysis (DFA). Paleontological Statistics (PAST, 2.17) software will be used to analyse the similarity and differences of the left and right wings or the fluctuating asymmetry variability of *R. ferrugineus* living in the environment of Sitio Napla (Hammer et al 2001). Box plots were also generated by means of PAST (Hammer & Harper 2001) from the RWs of the *R. ferrugineus* hind wing. These box plots are a potent display for equating distributions. They deliver a compact view of where the data are centered and how they are distributed over the range of the variable. Kruskal-Wallis test was utilized with regards to the assessment whether there are or no significant differences among the left and right wing shape of the species.

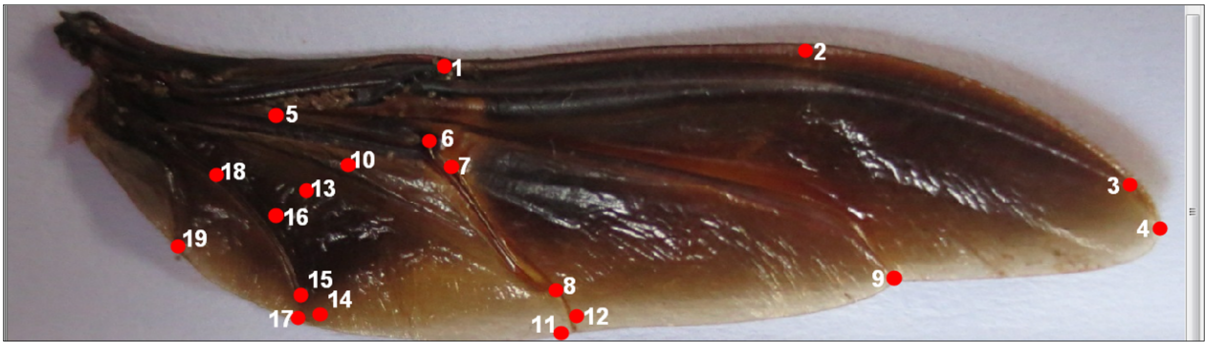


Figure 4. Homologous points of the female and male wing of *Rynchoporous ferrugineus.* (1) Marginal Joint, (2) Radius Anterior, (3) 2 and 3 Posterior branch of radius, (4) Radius Posterior, (5) Radius, (6) Radial Sector, (7) Radial-Media crossvein, (8) 1 Anterior branch of cubitus, (9) 1 Posterior branch of media, (10) Posterior branch of cubitus, (11) Posterior margin, (12) 3 Posterior branch of media, (13) Left side of Cubitus Posterior, (14) Right side of Anal vein, (15) Below Anal vein, (16) Anterior branch of cubitus, (17) 1 Branch of Anal vein, (18) 2 Branch of Anal vein, (19) Across Intervenal part of the wing. Homologous point description is based on Ha et al (2013) and Geller-Grimm (2003).

Results and Discussion. Through the RW analysis, the summary of the patterns of shape variation is displayed. It displays the frequency histograms of the RW scores as well as the grid plots of the homologous points showing the absence of shape variation along the RW axes of male and female species (Figure 5). RW analysis protrudes the negative and positive sides for the relationship of the variation. The uppermost Figure 5 portrays the mean shape of the species. The qualitative shape interpretations were summarized on Table 1. It comprises relative warp using 5% and more variations.

The result of RW analysis (Table 1) shows total shape disparities in the marginal joint, radius anterior, posterior branch of media and posterior margin of male and female *R. ferrugineus* wings. In relative warp of female, variations in the posterior margin is evident only in RWs 4 and 1. Differences in marginal joint, radius posterior, radius anterior and posterior branch of media were observed in all RWs (RW1 to RW7). While in male *R. ferrugineus* RWs, differences in posterior margin were evident in RWs 3 and 5. Variations in 2 and 3 posterior branch of radius and branch of anal vein were observed in all RWs (RW1 to RW5).

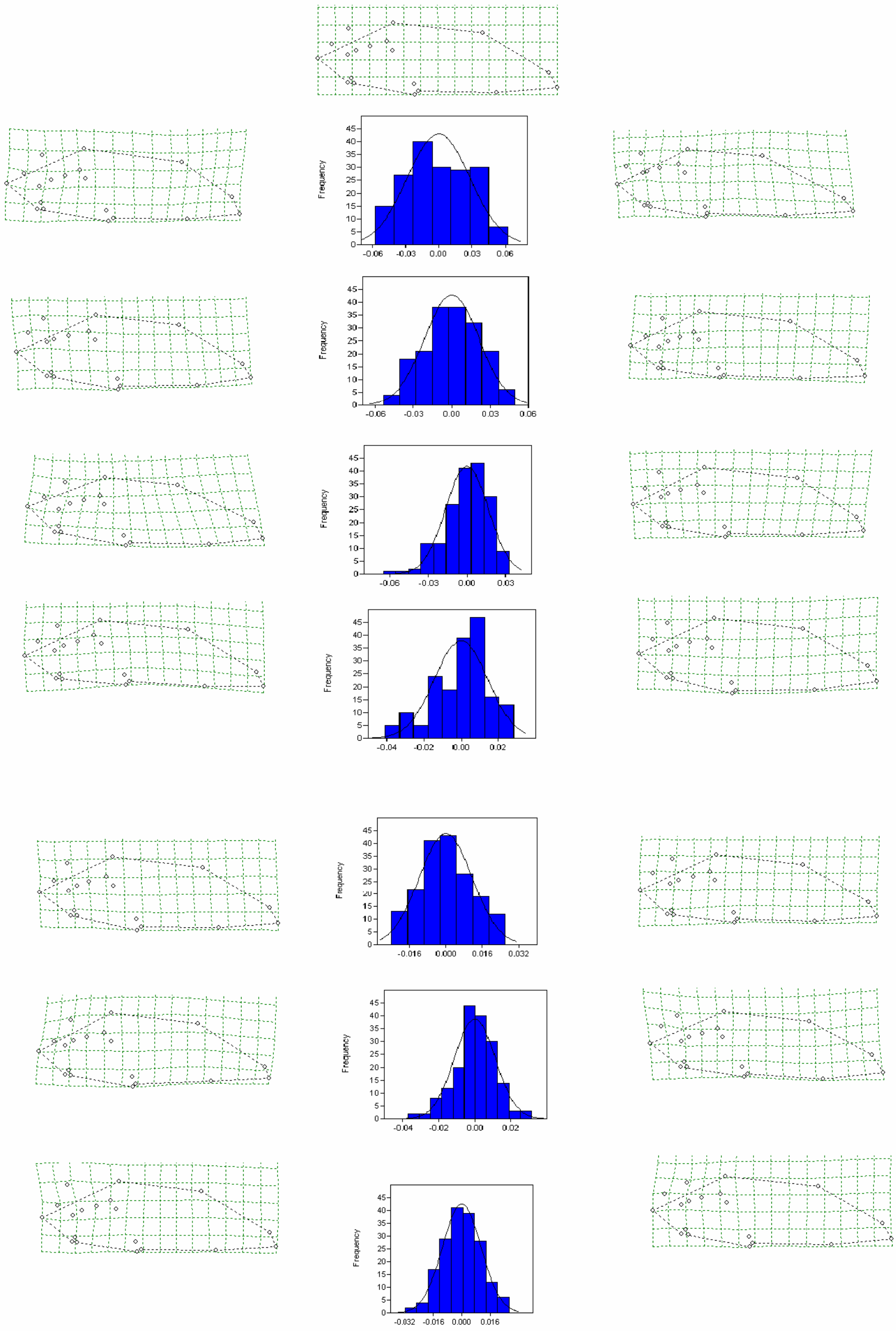
Both male and female *R. ferrugineus* wings were commonly different in the hind wing depth, specifically on their radius posterior, posterior margin, posterior branch of media, branch of anal vein, and across intervenal parts of the wing. RWs that show these differences in female are RW2, RW3 and RW4. In male *R. ferrugineus*, RWs 1 and 3 show these variations. As a whole, male and female wings differ from each other depending on the size of *R. ferrugineus* body. Also the shape variations in wings of both male and female *R. ferrugineus* could be explained by their sexual selection as it serves as their mechanism to fly.

The differences between the morphology of male and female *R. ferrugineus* was determined through DFA (Figure 6). DFA was used in determination to distinguish if the hind wing shape characteristics of *R. ferrugineus* give to the probable sexual dimorphism of the species. To make the variability statistics significant, the value of probability must be < 0.05. The result of DFA displays reveals $p = 1$.

It was found out that one factor that affects the shape of both front and hind wings of insects is migration (Johansson et al 2009). This discovery is related with a theory predicting that a higher aspect ratio shape allows faster and energetically more efficient flight (Norberg 1990). Comparing the hind wings of both female and male of *R. ferrugineus*, we found no significant variations. This is in concurrence with the very limited migration of *R. ferrugineus* and also of their environment.

Consequently, sexual dimorphism contributes to the differentiation of both male and female in the species *R. ferrugineus* in Sitio Napla, Malungon, Sarangani Province. This serves as a powerful force in support of its morphological discrepancies. Moreover, one factor which also provides a capability to differentiate the physical characteristics as well as habits of both male and female in the species *R. ferrugineus* is the environment.

A. FEMALE WARP



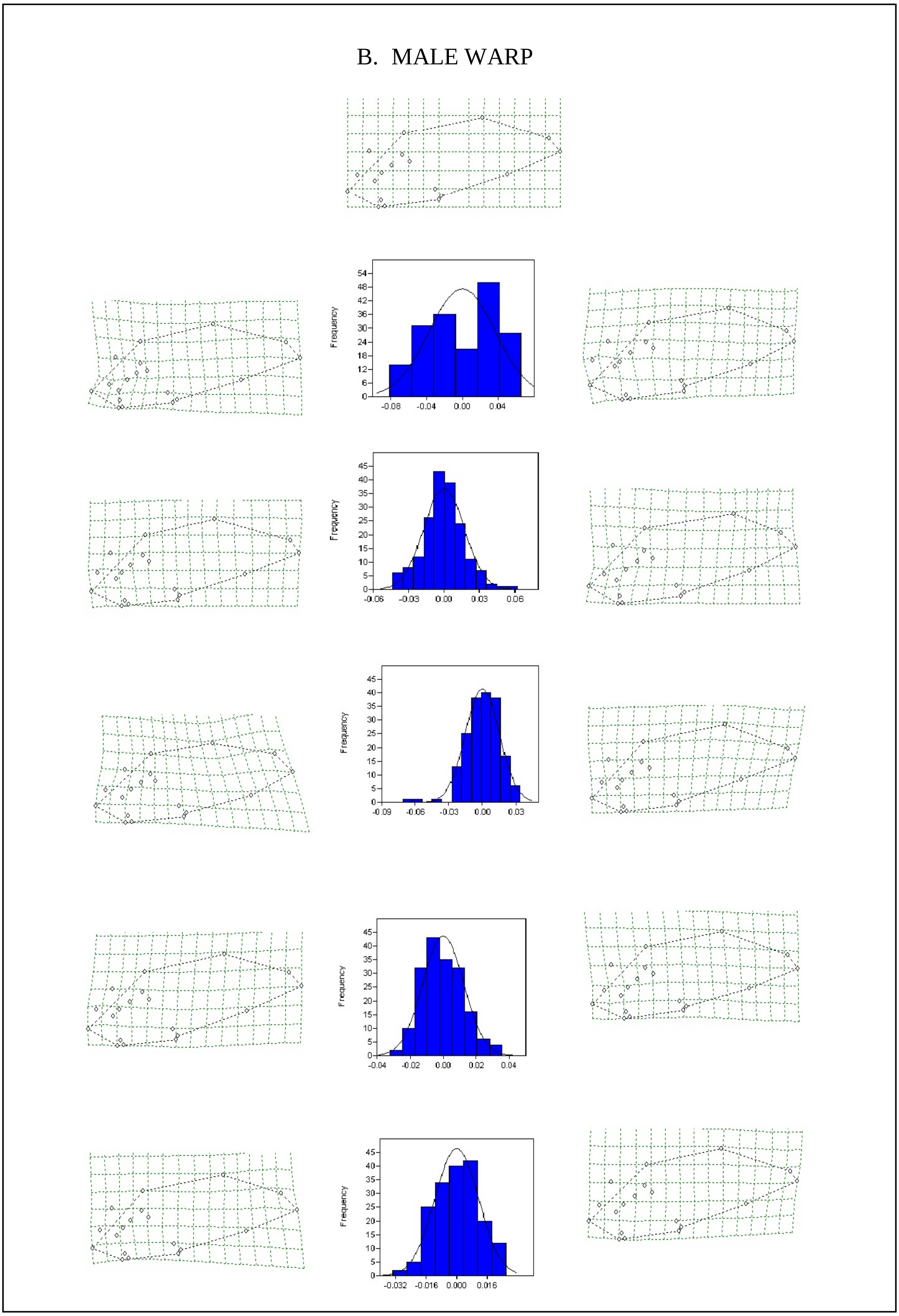


Figure 5. Geometric morphometric analysis showing consensus morphology and variations in wings shape by histogram and box plot of (A) female and (B) male of *R. ferrugineus*, made by the first 7 and 5 RWs respectively explaining more than 5% of the variance.

Table 1

Summary of shape descriptions showing variation between male and female *R. ferrugineus*

	% Variation	*Female*	*% Variation*	*Male*
1	31.11%	RW1 shows total shape variations. Differences in the radius anterior R1, posterior branch of radius R2+3, radius posterior R4 were notable.	33.87%	RW1 displays variations focused on the lower portion of the hind wing particularly in the posterior branch of media. Total shape difference is observed in the positive side.
2	17.88%	RW2 reveals variations in the depth of the hind wing especially in the posterior margin. This difference could be clearly seen on the positive side.	17.15%	RW2 shows differences in the radius anterior found at the upper part of the hind wing. Also differences in the posterior margin of positive and negative side were observed.
3	10.79%	RW3 displays variances in the branch of anal vein A1 and across intervenal part of the hind wing as well as on the marginal joint.	9.45%	RW3 illustrates figure variations in the upper portion of the hind wing particularly at the radius anterior and posterior branch of radius R2+3 as well as the posterior margin located at the lower part of the hind wing.
4	8.01%	RW4 implies shape variations in the posterior margin of the hind wing. The wing depth between positive and negative side also varies.	7.77%	RW4 indicates differences in the radius anterior found at the upper portion of the hind wing.
5	5.27%	RW5 indicates differences in the radius anterior, posterior margin and in the branch of anal vein.	4.81%	RW5 exhibits shape variations. Negative side mostly exhibits variations such as in the radius anterior and posterior margin.
6	5.04%	RW6 illustrates shape variations. Hind wing shape differences were mostly observed on the upper and lower portion particularly in the marginal joint, posterior margin, posterior branch of media and posterior branch of radius R2+3.		
7	4.49%	RW7 portraits differences in the upper portion of the hind wing especially on the radius anterior of the wing.		

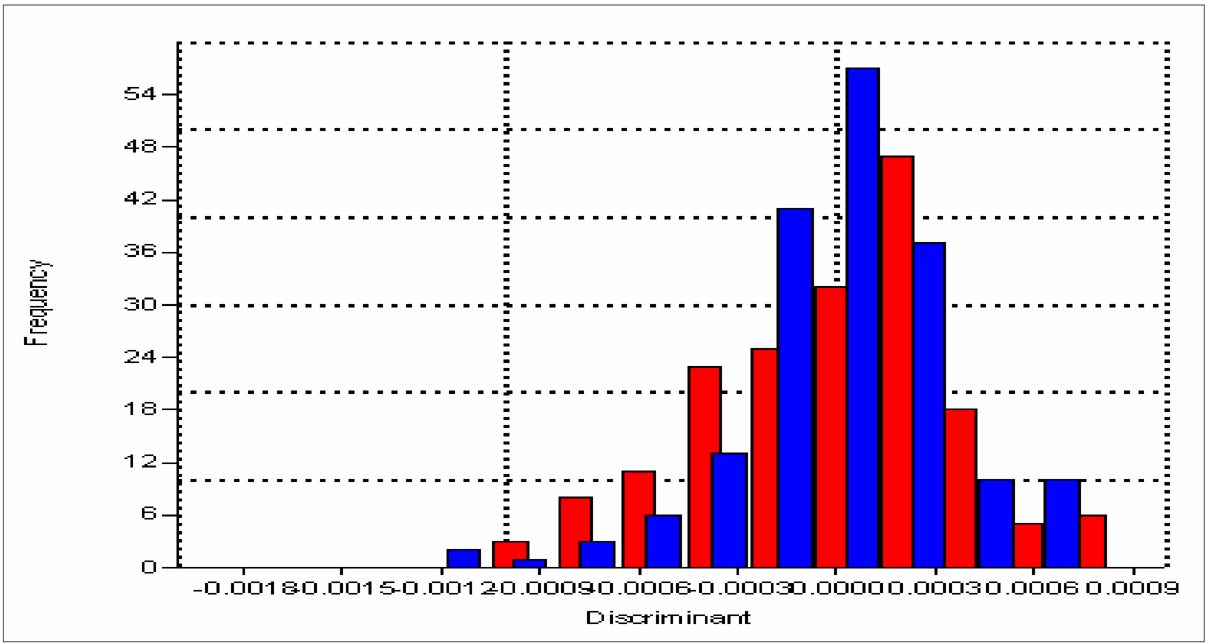


Figure 6. Discriminant/Hotelling's graph of the frequency and distribution of RW scores of the wing shape variation between male and female *R. ferrugineus* with the difference between sexes being highly significant ($p < 0.05$).

Conclusions. As a conclusion in this study, the environment and the sexual selection could be contributed by sexual dimorphism. These two factors have the potential to affect the variations of the shape in both male and female *R. ferrugineus*. But the results of this study focusing on the hind wings shape of *R. ferrugineus* reveal no significant difference based on the shape variations and the statistical data. Therefore, the hind wing shape does not contribute to the selection of gender of *R. ferrugineus*. In addition, this study has also proved the tremendous advantage of using Geometric Morphometrics as a powerful tool in determining the sexual dimorphism in *R. ferrugineus*.

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References

Adams D. C., Funk D. J., 1997 Morphometric inferences on sibling species and sexual dimorphism in *Neochlamisus bebbianae* leaf beetles: multivariate applications of the thin-plate spline. Systematic Biology 46(1):180-194

Copeland E. B., 1931 The coconut. London, UK: Macmillan and Co., Ltd., 233 pp.

Geller-Grimm F., 2003 Terminologie - Flügel. Available at: Robber flies of Germany: ///C:/Users/Edgar/Desktop/FA%20SYSTEMATICS/Fotoatlas%20und%20Bestimmungsschlüssel%20der%20Raubfliegen%20Deutschlands%20(Diptera%20%20Asilidae).html. Accessed: October 20, 2014.

Ha N. S., Truong Q. T., Goo N. S., Park H. C., 2013 Biomechanical properties of insect wings: the stress stiffening effects on the asymmetric bending of the *Allomyrina dichotoma* beetle's hind wing. PLoS ONE 8(12): e80689.

Hammer O., Harper D. A. T., Ryan P. D., 2001 PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4(1):9 pp.

Johansson F., Söderquist M., Bokma F., 2009 Insect wing shape evolution: independent effects of migratory and mate guarding flight on dragonfly wings. Biological Journal of the Linnean Society 97:362-372.

Norberg U. M., 1990 Vertebrate flight: mechanics, physiology, morphology, ecology and evolution. Zoophysiology, volume 27, Springer-Verlag Berlin Heidelberg ISBN-13-978-3-642-83850-7, 291 pp.
Pretorius E., Scholtz C. H., 2001 Geometric morphometrics and the analysis of higher taxa: a case study based on the metendosternite of the Scarabaeoidea (Coleoptera). Biological Journal of the Linnean Society 74(1):35-50.
Reilly S. M., Wainwright P. C., 1994 Conclusion: ecological morphology and the power of integration. In: Ecological morphology: integrative organismal biology. Wainwright P. C., Reilly S. M. (eds), University of Chicago Press, Chicago, Illinois, USA, pp. 339-354.
Ricklefs R. E., Miles D. B., 1994 Ecological and evolutionary inferences from morphology: an ecological perspective. In: Ecological morphology: integrative organismal biology. Wainwright P. C., Reilly S. M. (eds), University of Chicago Press, Chicago, Illinois, USA, pp. 13-41.
Rohlf F. J., 1993. Relative-warp analysis and example of its application to mosquito wings. In: Contributions to morphometrics. Marcus L. F., Bello E., Garcia-Valdecasas A. (eds), Museo Nacional de Ciencias Naturales, Madrid, pp. 131-159.
Rohlf J. F., 2008 tpsRelw version 1.46. Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook.
Wootton R. J., Evans K. E., Herbert R., Smith C. W., 2000 The hind wing of the desert locust (*Schistocerca gregaria* Forskål). I. Functional morphology and mode of operation. Journal of Experimental Biology 203:2921-2931.
Zelditch M. L., Swiderski D. L., Sheets H. D., Fink W. L., 2004 Geometric morphometrics for biologists: a primer. Elsevier Academic Press, New York and London.

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