



# Relative Warp Analysis of the Forewings of Male Dragonfly *Neurothemis ramburii*

Myrtle Ayn E. Penaredondo and Cesar G. Demayo\*

*Department of Biological Sciences, College of Science and Mathematics of Mindanao State University Iligan Institute of Technology, Philippines*

\*Corresponding author: [cgdemayo@gmail.com](mailto:cgdemayo@gmail.com)

**Abstract.** This study was conducted to determine variations in the forewings of *N. ramburii* collected from 3 selected rice agroecosystem in Lanao del Norte. Since the quantitative description, analysis and interpretation of shape variation in biology have become a fundamental area of research; the geometric method of morphometrics was used in this study aimed at assessing its variations. For the methodology, anatomical landmarks were assigned to both left and right forewing. Data were appended and file were linked in tps util and the ordination of the shapes' consensus was illustrated and consensus shape data (mean shape) of the populations was measured by a relative warp ordinations plot using tpsRelw 1.36. Relative warp scores were then subjected to Multivariate Analysis of Variance (MANOVA), Canonical Variate Analysis (CVA) and Kruskal Wallis test using PAST software version 2.0. Visualization of variations was done using histograms and boxplots. Results showed significant morphological variation in the forewings.

**Keywords.** Forewings; Geographic variation; Geometric morphometric

**MSC.** 92C37; 92C40

**Received:** July 12, 2016

**Accepted:** December 21, 2016

Copyright © 2017 Myrtle Ayn E. Penaredondo and Cesar G. Demayo. *This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.*

## 1. Introduction

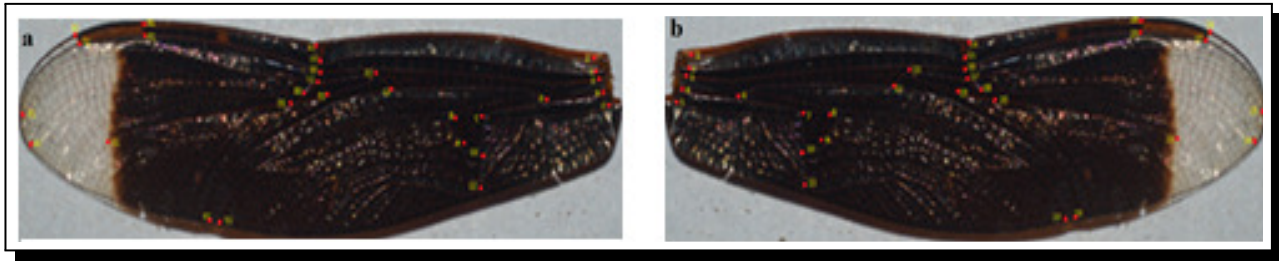
A rice field as an ecosystem is potentially valuable for many beneficial species such as the dragonflies which are considered as natural enemies by predating on rice pests [1]. Their efficiency as a predator lies on their wings which help them move at ease in mid-air to look

for prey. The efficient flight performance of a dragonfly is considerably influenced by its flight morphology, hence it is somehow expected that differences in wing topology and wing venational characters may perhaps be seen. It is of interest to study their wings because morphometric variations can explain population differentiation [2]. In this study, we evaluate if there exist morphometric differentiation in the wings of a cosmopolitan dragonfly *Neurothemis ramburii* from managed rice agroecosystem. We used an advanced tool in shape analysis known as geometric morphometrics, a tool which helped distinguish quantitatively the nature of variations in biological structures [3].

## 2. Methodology

The specimens were collected from ricefields in different farm villages located in three municipalities of Kapatagan, Panoloon and Katipunan Sapad in Lanao del Norte, Philippines. Opportunistic sampling of male dragonflies was done by handpicking and the use of sweep nets. Males were used in this study since they are not cosmopolitan unlike the females who migrates. *N. ramburii* males are distinct in terms of its wing tip pattern where, the red area of the hind wing is arched not straight [4]. Males of this species have comprehensively pigmented red-brown wings with network like venation. Wings were carefully detached from the synthorax, mounted in slides and subjected for image acquisition. A DSLR (Nikon D5100) camera was used to capture the image of the specimens. It was mounted on a tripod so as to make the acquisition of image stable and focused. Only the forewings of the insect were used in the comparison of wing shape variations between populations.

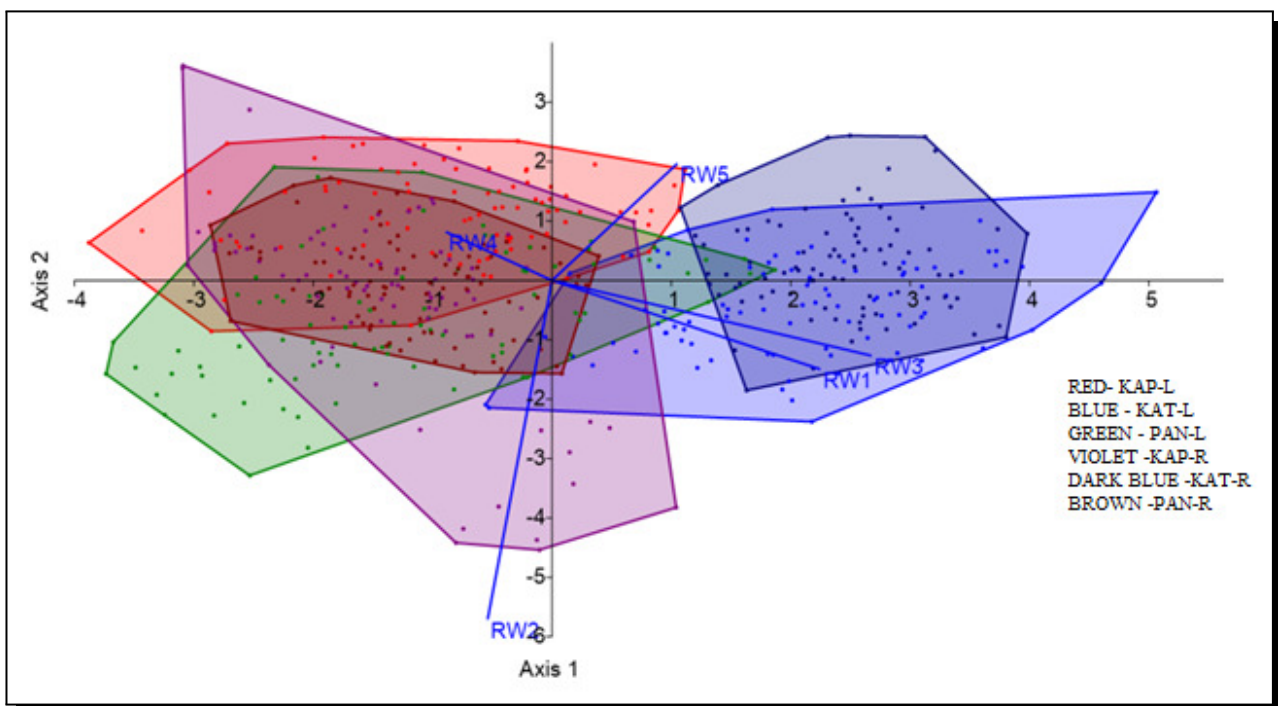
Digital images of the wings were subjected to landmark-based geometric morphometric (GM) analysis. A total of 29 landmark points was assigned to the forewing. Landmark descriptions were based on an earlier publication [5]. Image were digitized using TpsDig2 software [6]. Tps data file for the left and the right fore and hind wings were appended and Tps files were linked using TpsUtil [7]. The consensus shape data of each separate population were measured by relative warps ordinations plots using tpsRelw 1.36 [8]. Relative warps were characterized by its singular value and explains a given variation in shape among specimens summarizing shape differences. The relative warp scores were then subjected to Multivariate Analysis of Variance (MANOVA) and Canonical Variate Analysis (CVA) to test for differences in the left and right forewing shapes of the different populations. The relative warp scores obtained were used for the generation of histograms and boxplots using the Paleontological Statistics software (PAST) version 2.17 [9]. Box plots and histograms provide a compact view of where the data are centered and how they are distributed over the range of the variable and Kruskal-Wallis test which is also perform in PAST helps in analyzing whether or not the species differ significantly in wing shapes.



**Figure 1.** Designated landmarks of the left and right forewing

### 3. Results and Discussion

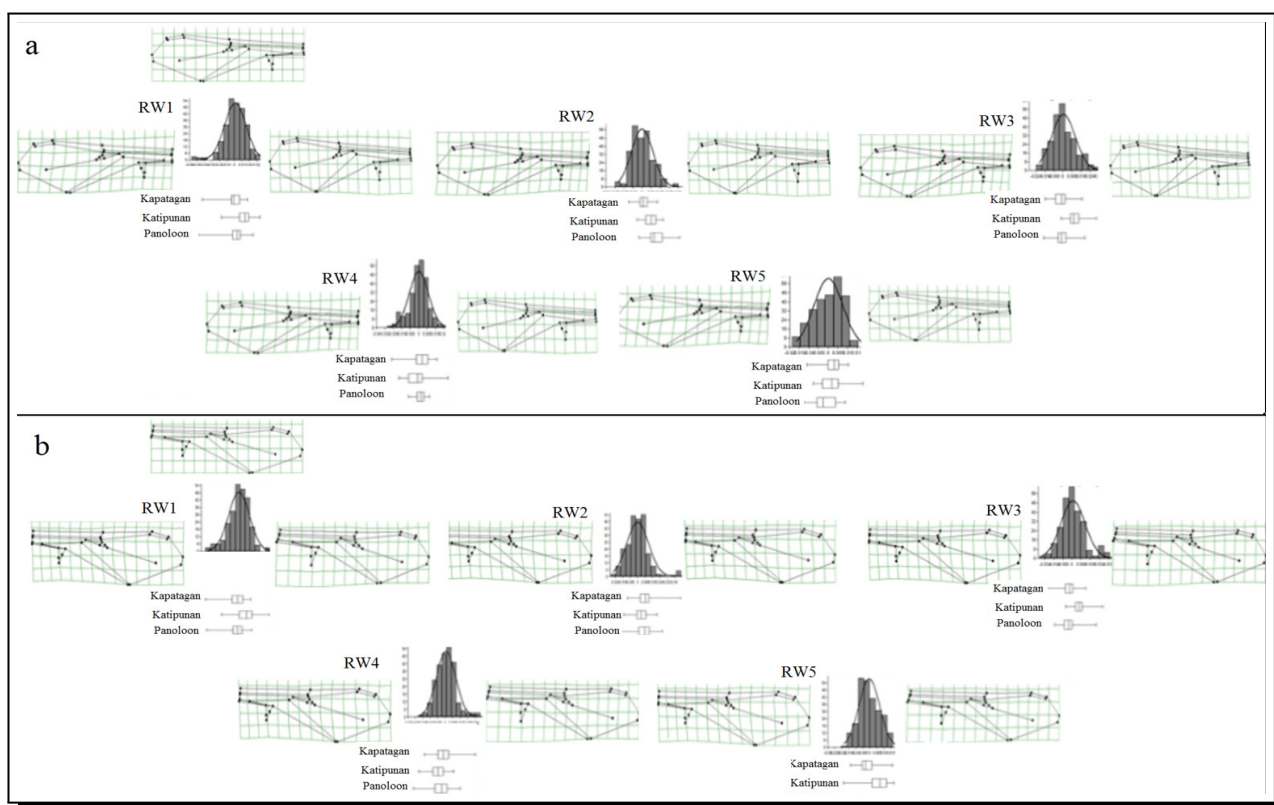
Canonical variate analysis of the relative warp (RW) scores show significant variations between populations of *N. ramburii* (Wilk's lambda 0.434;  $F=24.41$ ,  $P=5.84-79$ , Figure 2). Comparison between populations show significant differentiation between all populations (Table 1). However, only the Kapatagan and Katipunan left forewings were having an acceptable correct classification (Table 2). It can therefore be argued that the variations between the forewings of *N. ramburii* are due to the variations observed within shapes of the forewings within the populations. The nature of variations in shapes are shown in Figure 3.



**Figure 2.** CVA showing the differences fore-wing shapes of male *N. ramburii* (Legend: KAP-L (Kapatagan left forewing (FW)); KAT-L (Katipunan-Left FW); PAN-L (Panoloan-Left FW); KAP-R (Kapatagan-: violet-Katipunan-right FW), KAT-R (Katipunan right FW); PAN-R (Panoloan right FW)).

**Table 1.** Comparison of variations in forewing shapes of *N. ramburii*.

	KAT-L	PAN-L	KAP-R	KAT-R	PAN-R
KAP-L	1.10E-48	7.39E-21	1.85E-15	1.18E-48	1.81E-11
KAT-L	-	5.32E-48	1.11E-49	3.51E-05	1.33E-45
PAN-L		-	0.046709	7.32E-54	0.000884
KAP-R			-	1.89E-54	0.021034
KAT-R				-	2.33E-50



**Figure 3.** Mean and variant shapes of the left (a) and right (b) forewings of *N. ramburii*

**Table 2.** Classification of *N. ramburii* populations based on the shapes of the forewing.

	KAP-L	KAT-L	PAN-L	KAP-R	KAT-R	PAN-R
KAP-L	<b>71.91%</b>	2.25%	1.125	1.57%	2.25%	6.74%
KAT-L	0	<b>55.56%</b>	4.44%	1.11%	<b>36.67%</b>	2.22%
PAN-L	15.12%	3.49%	<b>45.35%</b>	20.93%	5.81	9.30%
KAP-R	18.39%	1.15%	22.99%	<b>36.78%</b>	0	20.69%
KAT-R	0	26.44%	0	0	<b>73.56%</b>	0
PAN-R	17.86%	0	22.62%	25%	0	<b>34.52%</b>

The results of this study show the existence of variations in forewing shapes within populations of *N. ramburii* which contributed significantly to the differences between populations. This may explain the argument that geographic variation in Odonata is prevalent for they are generally wide ranging because of its dispersive characteristic [10]. Territorial and predatory flight is a common flight behaviour in Odonata [11–13]. The observed variations in forewings, apical, anal, basal veins, intercalary supplement, triangle, and pterostigma may indicate differences in dragonflies' flight performance [14, 15]. In addition, power output is positively related to flight morphology, indicating that variations could be due to an effort to be of the best quality of a male dragonfly. Hence, males of this species of dragonfly with a greater flight performance achieve better mating and success in predation [16].

## 4. Conclusion

The results of the current study showing variations in the shapes of the forewings of the different populations of *N. ramburii* using relative warp analysis may indicate differences in territorial and predatory flight performance of the insect.

### Competing Interests

The authors declare that they have no competing interests.

### Authors' Contributions

All the authors contributed significantly in writing this article. The authors read and approved the final manuscript.

## References

- [1] P.L. Miller, *Dragonflies*, Naturalists' Handbooks 7, 2nd ed., Richmond, Slough (1995).
- [2] A. Tofilski, Drawing, a program for numerical description of insect wings, *J. Insect Sci.* 4, 17 (2004).
- [3] F.L. Bookstein, *Morphometric tools for landmark data: geometry and biology*, Cambridge Univ. Press, New York, p. 435 (1991).
- [4] C.G. Demayo, S.A. Harun and M.A.J. Torres, Procrustes Analysis of Wing Shape Divergence among Sibling Species of *Neurothemis* Dragonflies, *Australian Journal of Basic and Applied Sciences* 5(6), 748 – 759 (2011).
- [5] S.R. Tabugo, M.A.J. Torres, J.G. Gorospe, R.F. Amparado and C.G. Demayo, Integrative approach in describing *Neurothemis* species using correlation analysis based on distances, *European Journal of Zoological Research* 3(1) 19–3 (2014).
- [6] F.J. Rohlf, *TpsDig, version 1.39*, Stony Brook, Department of Ecology and Evolution, State University of New York at Stony Brook (2004).
- [7] F.J. Rohlf, *TpsUtil, version 1.58*, Stony Brook, Department of Ecology and Evolution, State University of New York at Stony Brook (2013).

- [8] F.J. Rohlf, *TpsRelw, version 1.36*, Department of Ecology and Evolution, State University of New York, Stony Brook (2003).
- [9] O. Hammer, D.A.T. Harper and P.D. Ryan, PAST: Paleontological statistics software package for education and data, Analysis, *Paleontologia Electronica* **4**(1) (2001).
- [10] E. Mayr, *Animal Species and Evolution*, Harvard Univ. Press, Cambridge/MA (1963).
- [11] D.J. Kemp and G. Wilkund, Fighting without weaponry: a review of male-male contest competition in butterflies, *Behav. Ecol. Sociobiol.* **49**, 429 – 442 (2001).
- [12] J.H. Marden and J.K. Waage, Escalated damselfly territorial contests are energetic wars of attrition, *Anim. Behavior* **39**, 954 – 959 (1990).
- [13] M.A. Serrano-Meneses, A. Córdoba-Aguilar, M. Azpilicueta-Amorín, E. González-Soriano and T. Székely, Sexual selection, sexual size dimorphism and Rensch's rule in Odonata, *J. Evol. Biol.* **21**, 1259 – 1273 (2008).
- [14] V. Debat, M. Bégin, H. Legout and J.R. David, Allometric and nonallometric components of *Drosophila* wing shape respond differently to developmental temperature, *Evolution* **57**, 2773 – 2784 (2003).
- [15] F. Johansson, M. Söderquist and F. Bokma, Insect wing shape evolution: independent effects of migratory and mate guarding flight on dragonfly wings, *Biological Journal of the Linnean Society* **97**, 362 – 372 (2009).
- [16] J.H. Marden, Dragonfly flight performance: a model system for biomechanics, physiological genetics, and animal competitive behavior, in: *Dragonflies: Model Organisms for Ecological and Evolutionary Research*, A. Córdoba-Aguilar (editor), Oxford University Press (2008).