RELATIVE WARP ANALYSIS ON INTRA-SPECIFIC VARIATIONS IN THE FORE- AND HINDWINGS OF SELECTED POPULATIONS OF MALE *Neurothemis terminata terminata* (Ris, 1911)

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Abstract

Landmark-based geometric morphometric analyses were conducted on 115 male individuals of *Neurothemis terminata terminata* (Ris, 1911) were collected from selected areas of Northern Mindanao, Philippines to determine intra-specific geographical variation based on their fore-wing and hindwing shapes. The landmark data were superimposed by generalized Procrutes Analysis Superimposition method. To illustrate wing shape diversity, landmark data was subjected to relative warp analysis and the resulting scores were subjected to Multivariate Analysis of Variance (MANOVA) and Canonical Variate Analysis (CVA). The results display significant variations on the wings of the male *N.terminata terminata* collected from different sites. The first extracted relative warp accounted for 16.49- 16.77% of the variation in the shapes of forewing and 15.47-28.78% in the variation in the shapes of hind wing. The results suggest that each population represents discrete panmictic units due to geographic barriers of migration and the territorial behaviour of male dragonflies.

Keywords: *Geometric morphometrics, Procrustes Analysis, MANOVA, panmictic unit, fore-wing and hindwing shape*

Introduction

The dragonfly *Neurothemis terminata terminata* (Ris, 1911) belongs to Family Libellulidae classified based on the wing venation of its forewings and hind wings. Being a cosmopolitan species, it is widely distributed in the Philippines. The males can be distinctly recognized from other male *Neurothemis sp.* by its wing tip pattern where the red area of its hind wing is straight and perpendicular to the wing margin (Needham and Gyger, 1937). Since male dragonflies are basically territorial, geographical variations in their wing veins might be found (Corbet, 1962). It is in this context that the study was conducted.

Most geographic variation is the result of adaptation to local environments, which in turn reflects some degree of genetic divergence between the separated populations. According to Klingenberg (1992) for a species to be of evolutionary or taxonomic importance, its geographic variation has to be genetically determined at least to a large extent. Demayo, *et. al* (2011) stated that there exist a localized variation in the wing shape variation among the species *Neurothemis* dragonflies but it did not include if there is intra-specific geographical variation of *Neurothemis* dragonflies. This study was conducted to search for additional information on intra-specific geographical variations among the male *N. terminata terminata* in selected areas of Northern Mindanao, Philippines utilizing landmark-based geometric morphometric methods (Klingenberg, 2003). The method provides a way of separating the shape and size components of biological form to determine trends in character evolution and to know what might be the causes of these variations and how these variations will affect the individual (Dryden & Mardia 1998).

Methodology

The samples of dragonflies used in this study were collected from selected areas in Northern Mindanao, Philippines specifically in Brgy. Rupagan, Bacolod, Lanao del Norte, Brgy. Tacub, Kauswagan, Lanao del Norte and Brgy. Poblacion, Manticao, Misamis Oriental (Figure 1).

Figure 1: Map showing the sampling areas

Sample Collection and Identification. The collection of samples was done using sweep nets in open rice fields in the said sampling sites. A total of thirty one (31) individuals from Rupagan, Bacolod, thirty two (32) from Tacub, Kauswagan and fifty two (52) from Poblacion, Manticao. The wings were removed and placed in a glass slides, scanned in jpeg format and later in tps format for geometric morphometric analysis.

Geometric Morphometric Analysis. There were 29 and 35 landmark points in fore- and hindwings respectively that were used in this study (Fig. 2) and digitized using tpsDig2 (Rohlf, 2005). These landmarks were at the intersection of wing veins or at wing edge and considered as Type I landmark and thus can capture the general wing shape (Bookstein, 1991).

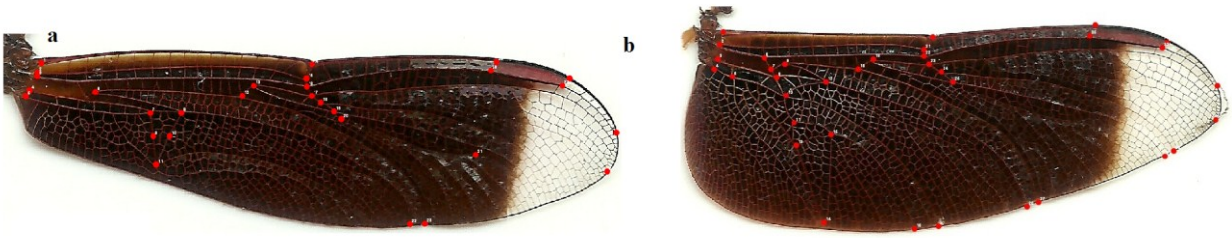

Figure 2: Right fore-wing (above) and right hindwing (below) of the samples displaying the assigned landmarks

Table 1: Description of assigned landmarks used on both left and right fore- wings respectively.

LANDMARK	ANATOMICAL DESCRIPTION	LANDMARK	ANATOMICAL DESCRIPTION
1	Proximal end of the Costa (C)	16	Distal end of the Radius (R)
2	Proximal end of the Subcosta (Sc)	17	origin of the Radial branches (R2 and R3)
3	Proximal end of the Radius + media (R + M)	18	Anterior end of the 2nd crossvein between Radial branches (R2 and R3)
4	Proximal end of the Cubitus (Cu)	19	Posterior end of the 2nd crossvein between Radial branches (R2 and R3); origin of Radial supplement (Rspl)
5	Proximal end of the 1st anal vein (A/IA)	20	Proximal end of Radial supplement (Rspl)
6	Basal end of the Arculus (Arc)	21	Distal end of Radial supplement (Rspl)
7	Proximal end of the anterior margin of the triangle (T)	22	Distal end of anterior media (MA)
8	Distal end of the anterior margin of the triangle (T)	23	Distal end of Radial branch (R4)
9	Midpoint of the triangle (T)	24	Distal end of intercalary radial vein (IR2)
10	Midpoint of the triangle (T)	25	Distal end of Radial branch (R2)
11	Posterior end of the triangle (T)	26	Antero-lateral and distal end of the pterostigma
12	origin of Radial branches (R2 and R4)	27	Postero-lateral and distal end of the pterostigma
13	origin of intercalary vein (IR3)	28	Antero-lateral and proximal end of the pterostigma
14	Nodus (N)	29	Postero-lateral and proximal end of the pterostigma
15	Distal end of the Subcosta (Sc)		

Source: Demayo, *et. al.* ,2011

The landmark data were superimposed by generalized Procrustes analysis superimposition method, the least-squares method that transforms a configuration of landmarks, superimposing it on a configuration of reference (consensus) and translating, scaling and rotating one of them so that the sum of squares of the distances between the corresponding points among configurations would be the least possible (Monteiro & Reis, 1999 as cited by Querino, *et. al,*2002) including elimination of non-shape variations such as variation in scale, location, and orientation (Rohlf and Slice, 1990). The consensus shape data of each separate population were measured by relative warps ordinations plots using tpsRelw 1.36 (Rohlf, 2003). Relative warps were characterized by its singular value and explains a given variation in shape among specimens summarizing shape differences (Adams, Slice & Rohlf, 2004). The relative warp scores were then subjected to Multivariate Analysis of Variance (MANOVA) and Canonical Variate Analysis (CVA) to test for differences in left and right fore- and hindwing shapes. Box plots and Kruskal-Wallis tests were generated using PAST (Hammer & Harper, 2007) software. Box plots provide a compact view of where the data are centered and how they are distributed over the range of the variable and the Kruskal-Wallis test was performed to analyze whether or not the species differ significantly in wing shape.

Table 2: Description of assigned landmarks used on both left and right hindwings respectively.

LANDMARK	ANATOMICAL DESCRIPTION	LANDMARK	ANATOMICAL DESCRIPTION
1	Proximal end of the Costa (c)	19	origin of the intercalary radial vein (IR3)
2	Proximal end of the Radius + media (R + M)	20	Nodus (N)
3	Proximal end of the media (m)	21	Distal end of the subcosta (sc)
4	Proximal end of the Cubitus (Cu)	22	Distal end of the radius (R)
5	Posterior end of the anal crossing (Ac)	23	origin of the Radial branches (R2 and R3)
6	Basal end of the Arculus (Arc)	24	Anterior end of the 2nd crossvein between Radial branches (R2 and R3)
7	posterior and proximal vertex of the hypertrigone (ht)	25	Posterior end of the 2nd crossvein between Radial branches (R2 and R3); origin of Radial supplement (Rspl)
8	anterior and proximal vertex of the subtrigone (ht)	26	Distal end of the Anterior media (AM)
9	anterior and proximal vertex of the hypertrigone (ht)	27	Distal end of Radial branch (R4)
10	posterior and proximal vertex of the subtrigone (t)	28	Distal end of the Intercalary Radial vein (IR3)
11	(Cu2 + A2)	29	Distal end of Radial branch (R3)
12	Distal vertex of the subtrigone (t)	30	Distal end of intercalary radial vein (IR2)
13	Anal supplement (Aspl)	31	Distal end of Radial branch (R2)
14	Basal end of the Anal vein (A3)	32	Antero-lateral and distal end of the pterostigma
15	Second branch of cubital vein(Cu2)	33	Postero-lateral and distal end of the pterostigma
16	Distal end of the cubito-anal vein (Cu2)	34	Antero-lateral and proximal end of the pterostigma
17	Distal end of the posterior cubital vein (Cu1)	35	Postero-lateral and proximal end of the pterostigma
18	Origin of Radial branch (R4)		

Source: Demayo, *et. al.* ,2011

Results and Discussion

Relative warp analysis showed significant variations on the wings of the male species of *N. terminata terminate* (Fig. 3). The first extracted relative warp accounted for 16.49- 16.77% of the variation in the shapes of forewing (Fig. 3A,B)and 15.47-28.78% in the variation in the shapes of hind wing (Fig. 3C,D). Multivariate Analysis of Variance (MANOVA) also revealed significant differences in geographically based on the dragonfly's wing shapes (Table 3).

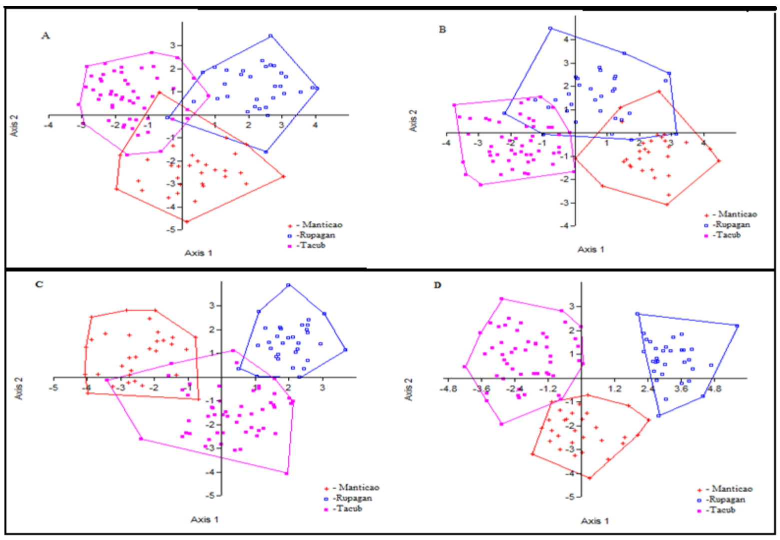

Figure 3: Distribution of the three populations along the first two canonical variate showing the differences fore-wing shapes of male *N. terminata terminata* (A) left fore-wing (B) right fore-wing and in the hindwing shapes of male *N. terminata terminata* (C) left hindwing (D) right hindwing.

Table 3. Results of Multivariate Analysis of Variance (MANOVA).

Wing Shape	Wilk's Lambda	Pillai Trace	P- value*	
			Wilk's Lambda	Pillai Trace
Left Fore-wing	0.0857	1.414	$1.846E^{-07}$	$1.034E^{-07}$
Right Fore-wing	0.1224	1.255	$9.294E^{-05}$	$4.282E^{-04}$
Left Hindwing	0.08793	1.399	$6.137E^{-05}$	$5.493E^{-05}$
Right Hindwing	0.06005	1.479	$1.368E^{-07}$	$5.84E^{-07}$

*P-value significant at α=0.05

The differences among the three male dragonfly populations indicate that each population is a discrete panmictic unit showing no opportunity for interbreeding or colonization from other local populations (Whitesel, *et. al.*, 2004) which could either be due to geographical barriers or due to the territorial behaviour of the dragonfly (Corbet, 1962). Panmictic population is a population where all individuals are potential partners and assumes no mating restrictions, neither genetic nor behavioural, upon the population (Gayon and Cobb, 1998).

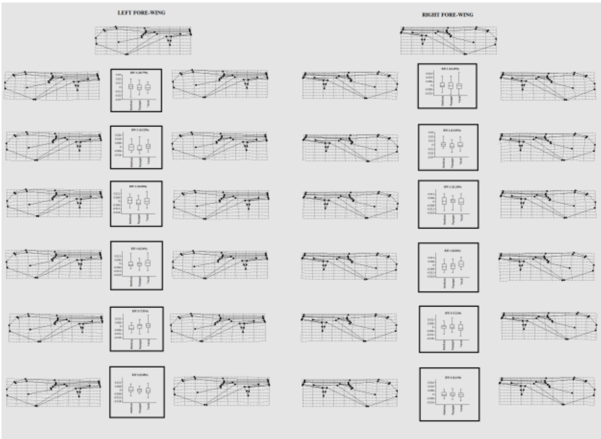

Figure 4. Relative warp box plot showing left and right fore-wing variations.

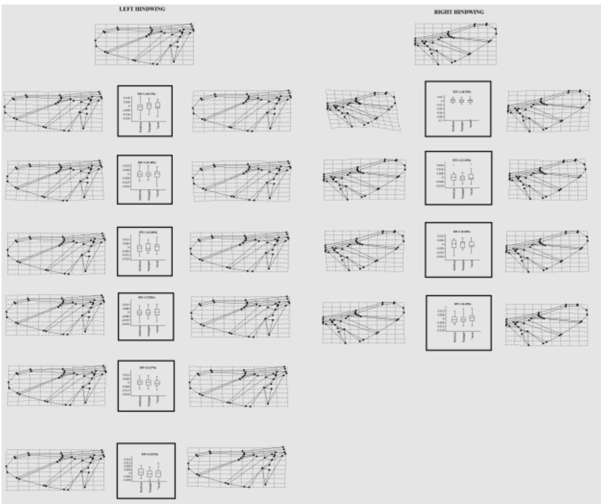

Figure 6. Relative warp box plot showing left and right hindwing variations

Table 4. Variations observed in the male *N. terminata terminata*

RW	MALE POPULATIONS			
	Fore-wing		**Hindwing**	
	Left	**Right**	**Left**	**Right**
	CV Remarks	**CV Remarks**	**CV Remarks**	**CV Remarks**
RW1	Variation found in the basal and apical region of the wing. Samples with negative scores have elongated size of the triangle, shorter length of the radial supplement, narrower pterostigma, and longer length in the region bounded between the distal end of radial branch (R4) and distal end of the intercalary radial vein (IR2). On the other hand, samples with positive scores have the exact opposite of the above mentioned remarks.	Variation found in the basal and apical region of the wing. Samples with negative scores have shortened size of the triangle, longer length of the radial supplement, narrower pterostigma. On the other hand, samples with positive scores have the exact opposite of the above mentioned remarks.	Variation found in the basal and apical region of the wing. Samples with negative scores have thinner size of the triangle, shorter length of the anal vein, and narrower pterostigma.On the other hand, samples with positive scores have the exact opposite of the above mentioned remarks.	Variation found in the basal and apical region of the wing. Samples with negative scores have all of their wing veins that tend to be shorter than that of samples with positive scores.
RW2	Variation found in the basal and apical region of the wing. Samples with negative scores have elongated size of the triangle, longer length of the radial supplement, broader pterostigma. On the other hand, samples with positive scores have the exact opposite of the above mentioned remarks.	Variation found in the basal and apical region of the wing. Samples with negative scores have shortened size of the triangle, longer length of the radial supplement, narrower pterostigma and shorter length in the region bounded between the distal end of radial branch (R4) and distal end of the intercalary radial vein (IR2).. On the other hand, samples with positive scores have the exact opposite of the above mentioned remarks.	Same as RW1	Variation found in the basal and apical region of the wing. Samples with negative scores have bigger size of the triangle, longer length of the anal vein, longer length in the radial veins and broader pterostigma.On the other hand, samples with positive scores have the exact opposite of the above mentioned remarks.
RW3	Variation found in the basal and apical region of the wing. Samples with negative scores have fatter size of the triangle, longer length of the radial supplement, narrower pterostigma, and shorter length in the region bounded between the distal end of radial branch (R4) and distal end of the intercalary radial vein (IR2). On the other hand, samples with positive scores have the exact opposite of the above mentioned remarks.	Variation found in the basal and apical region of the wing. Samples with negative scores have shortened size of the triangle, shorter length of the radial supplement, broader pterostigma and longer length in the region bounded between the distal end of radial branch (R4) and distal end of the intercalary radial vein (IR2). On the other hand, samples with positive scores have the exact opposite of the above mentioned remarks.	Variation found in the basal and apical region of the wing. Samples with negative scores have fatter size of the triangle, and broader pterostigma.On the other hand, samples with positive scores have the exact opposite of the above mentioned remarks.	Variation found in the basal and apical region of the wing. Samples with negative scores have thinner size of the triangle, longer length of the anal vein, and shorter length of the radial veins.On the other hand, samples with positive scores have the exact opposite of the above mentioned remarks.
RW4	Variation found in the basal and apical region of the wing. Samples with negative scores have elongated size of the triangle, shorter length of the radial supplement, broader pterostigma, and shorter length in the region bounded between the distal end of radial branch (R4) and distal end of the intercalary radial vein (IR2). On the other hand, samples with positive scores have the exact opposite of the above mentioned remarks.	Variation found in the basal and apical region of the wing. Samples with negative scores have fatter size of the triangle, longer length of the radial supplement, narrower pterostigma, and longer length in the region bounded between the distal end of radial branch (R4) and distal end of the intercalary radial vein (IR2). On the other hand, samples with positive scores have the exact opposite of the above mentioned remarks.	Same as RW 1	Variation found in the basal and apical region of the wing. Samples with negative scores have fatter size of the triangle, and broader pterostigma.On the other hand, samples with positive scores have the exact opposite of the above mentioned remarks.
RW5	Variation found in the basal and apical region of the wing. Samples with negative scores have thinner size of the triangle, longer length of the radial supplement, narrower pterostigma, and longer length in the region bounded between the distal end of radial branch (R4) and distal end of the intercalary radial vein (IR2). On the other hand, samples with positive scores have the exact opposite of the above mentioned remarks.	Variation found in the basal and apical region of the wing. Samples with negative scores have elongated size of the triangle, longer length of the radial supplement, broader pterostigma, and longer length in the region bounded between the distal end of radial branch (R4) and distal end of the intercalary radial vein (IR2). On the other hand, samples with positive scores have the exact opposite of the above mentioned remarks.	Same as RW3	
RW6	Variation found in the basal and apical region of the wing. Samples with negative scores have shortened size of the triangle, narrower pterostigma, and shorter length in the region bounded between the distal end of radial branch (R4) and distal end of the intercalary radial vein (IR2). On the other hand, samples with positive scores have the exact opposite of the above mentioned remarks.	Variation found in the apical region of the wing. Samples with negative scores have longer length of the radial supplement, broader pterostigma, and shorter length in the region bounded between the distal end of radial branch (R4) and distal end of the intercalary radial vein (IR2). On the other hand, samples with positive scores have the exact opposite of the above mentioned remarks.	Variation found in the basal and apical region of the wing. Samples with negative scores have fatter size of the triangle, shorter length of the anal vein, and narrower pterostigma.On the other hand, samples with positive scores have the exact opposite of the above mentioned remarks.	

Kruskal-Wallis test was also used to analyze if populations of the species differ or not significantly with regards to the wing shape. The results above show that there are significant intra-species differences among male *N. terminata terminate* (Table 5&6).

Table 5. Results of the Kruskal-Wallis test for significant differences in mean shapes of forewing among the *N. terminata terminata*. Upper matrix of the test represents for the right forewing and the lower matrix of the test represent the left forewing.

		Manticao	Rupagan	Tacub
RW 1	Manticao		0.4875	0.221
	Rupagan	0.2836		0.5837
	Tacub	0.3762	0.7788	
RW 2	Manticao		**0.03143**	0.4263
	Rupagan	0.4373		0.1152
	Tacub	0.1215	**0.002635**	
RW 3	Manticao		0.4662	0.7812
	Rupagan	**0.03198**		0.2384
	Tacub	0.5916	0.05777	
RW 4	Manticao		0.6353	**0.002632**
	Rupagan	0.6353		**0.00878**
	Tacub	0.142	0.1507	
RW 5	Manticao		0.869	0.165
	Rupagan	**0.01907**		0.1862
	Tacub	**0.000622**	0.3176	
RW 6	Manticao		0.7259	0.3612
	Rupagan	0.6501		0.6287
	Tacub	0.3417	0.4473	

Table 6. Results of the Kruskal-Wallis test for significant differences in mean shapes of hindwing among the *N. terminata terminata* dragonflies. Upper matrix of the test represents for the right hindwing and the lower matrix of the test represent the left hindwing.

RW 1		Manticao	Rupagan	Tacub
	Manticao		0.3189	0.6412
	Rupagan	**0.03854**		0.6684
	Tacub	0.2853	0.2937	
RW 2	Manticao		0.6158	0.7241
	Rupagan	0.6353		0.3791
	Tacub	0.6178	0.8394	
RW 3	Manticao		0.9288	0.3562
	Rupagan	0.4789		0.2958
	Tacub	0.2666	0.6921	
RW4	Manticao		0.6062	0.132
	Rupagan	0.7259		0.2771
	Tacub	0.3712	0.5773	
RW 5	Manticao			
	Rupagan	0.8907		
	Tacub	0.2874	0.5072	
RW 6	Manticao			
	Rupagan	0.1394		
	Tacub	0.186	0.9963	

Differences in the shapes of the pterostigma and triangle were observed on the left and right fore-wings and hindwings among the three populations of male *N. terminata terminata.* Some veins, such as radial and anal veins, were observed and showed differences based on their size and length. The pterostigma can greatly affect the flight performance of dragonflies by influencing the degree of deformation of the wings under forced vibration. This deformation of dragonfly wings can be modulated through the inertial effect of pterostigma and changing flapping kinematics by the dragonflies (Chang, *et al*., 2010 as cited by Demayo, *et.al,* 2011).

The triangle provides strong wing framework and adapts the wings for rapid sculling forward motion (Needham, 1899). Thus, the differences in the shapes of the pterostigma and triangle observed among the male *N. terminata terminata* might reflect differences in their flight performance. The differences among the three populations may be connected to random mating of individuals in the populations including increased population density, stress, sexual selection, nutritional stress, heat stress, diseases and parasitic stress, and other genetic factors (Riget *et al*, 2008).

Summary and Conclusion

Geometric morphometric analyses of 29 and 35 landmarks from the fore- and hind wings were analysed using statistical tools such as the MANOVA, CVA, and Kruskall-Wallis test and the results suggest that there are significant geographical wing variations among the three populations of male *N. terminata terminata* maybe due to the territorial behaviour among male dragonfly. It showed localized variation in the shape of the pterostigma and triangle among the species. The pterostigma affects the flight performance by influencing the degree of deformation of the wings under forced vibration and triangle provides strong wing framework for rapid sculling forward motion of dragonflies. Variations in the distance between the distal end of the radial planate supplement and the distal margin of the wings bounded by the end points of the intercalary vein and the radial branch in the fore-wings and length of the anal vein and shape in the region bounded between the anal supplement (Aspl), basal end of the anal vein (A3) and second branch of cubital vein (Cu2) in the hindwings were also observed.

The study also shows the utility of the advanced methods in geometric morphometrics in describing variations in shapes. The method can be further used in describing other morphological parts of the dragonfly body for taxonomic purposes.

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