Article

Landmark based geometric morphometric analysis describing sexual dimorphism in wings of *Neurothemis terminata* (Ris, 1911) from Mt. Hilong-Hilong, Philippines

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Abstract

Landmark-based geometric morphometric analysis describing sexual dimorphism in wings of *Neurothemis terminata* (Ris, 1911) from Mt. Hilong-Hilong, Remedios Trinidad Romualdez, Agusan del Norte, Philippines was applied in selected female and male populations. A total of 30 females and 30 males were collected and subjected to landmark- based analysis. To demonstrate the variation in wing morphology, landmark data was employed to relative warp analysis and the resulting scores were analyzed using Multivariate Analysis of Variance (MANOVA), Principal Component Analysis (PCA) and Canonical Variate Analysis (CVA). The result shows significant differences (P<0.05) between the appended female and male populations. The obtained result indicates that each sexes of *N. terminata* displays morphological difference in wings which may be attributed to the sexual selection, flight performance and flapping kinematics.

Keywords sexual dimorphism; geometric morphometrics; *Neurothemis terminata*; Mt. Hilong-Hilong.

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1 Introduction

Sexual dimorphism (SD) is defined as the condition where males and females of the same species are morphologically distinct. It has been recognized as a primary factor for defining phenotypic differences between species belonging to the same taxa and known to be an effective mechanism for comparing sexes of organisms in the aspects of morphology (Benitez et al, 2011; Albutra et al, 2011). The importance of sexual dimorphism advances for the identification of male and female species that significantly differs in appearances (i.e. coloration, body shape and size) (Cox and Calsbeek, 2010). Furthermore, it is believed that sexual dimorphism is said to be adaptive and manifesting the variation of sex-specific traits (Fairbairn et al. 2007). Alongside, SD often exists to a large group of organisms that could be distinguished through its phenotypic traits (Arnqvist and Rowe, 2005; Fairbairn et al, 2007). Moreover, sexual dimorphism has been associated with the over-all fitness of organism within its population and this suggests sexual selection and survival (Kokko and Brooks 2003; Rankin and Arnqvist 2008).The outcome of SD is an essential part of evolutionary biology as it helps to explain the relationship between two different organisms belonging to the same lineage. Indeed, sexual dimorphism (SD) gives knowledge for many entomologists to discriminate the sexes of species and become the ultimate advantage in the field of ecology, life history and behavior (Albutra et al, 2011). Similarly, SD represents the correlation of two dissimilar species but within the same ancestry. Nonetheless, the essential function of sexual dimorphism proposes to understand the evolutionary and ecological biology of organisms that implies diversity (Berns, 2013).

In quantifying shape variation and co-variation in the wing morphology of *Neurothemis terminata* (Ris 1911), geometric morphometrics (GM) was applied in order to distinguish male from female individuals. As such, morphometrics is the numeral application to explain the biological shape, shape dissimilarity and co-variation of biotic components (Webster and Sheets, 2010). This technique represents a consistency with the distinction and similarity of species morphology. The purpose of morphometric method pays developments in research field like evolutionary biology, developmental biology and systematics (Webster and Sheets, 2010). Moreover, geometric morphometrics (GM) consists of outline and landmark-based methods that are efficient for describing morphological variation which allows to draw indicative graphics of morphological changes or variations, suggesting a prompt copy of outline and the three-dimensional localization of shape variation (Webster and Sheets, 2010). It also helps to draw significant differences and shared features of the same sexes (Cabuga et al, 2016). Furthermore, the significance of geometric morphometrics on describing shape has driven advanced means of biometric analysis to quantify shape variations (Rohlf and Marcus, 1993; Bookstein, 1996). It was continuously employed as an analytic instrument in order to define the shape and size features from any biological entities (Rohlf, 1993; Bookstein, 1991). Indeed, using landmark-based method was efficient mechanism to illustrate the landmarks relative in the shape of an organisms (Ibañez et al, 2007). Thus, in GM shape is demarcated as any geometric figures that stay when the results of translation, rotation, and scaling are detached from an object (Benítez, 2011). Therefore, several studies uses geometric morphometric as an effective mechanism to differentiate morphological variations specifically wing morphology (Benitez et al, 2011; Kiyoshi and Hikida, 2012). Thus, the importance of wings in insect systematics describes its morphological variations, evolution and diversity (Tabugo et al, 2014).

Odonata is considerably the utmost bioindicator of sexual dimorphism (Benitez et al, 2011). The wings variation widely known to be enormous and prevalent among its population (Lyons, 1999). Its wings alone are the important source of phenotypic variation thus implicating the relationships among other species relative to its order (Fraser, 1957; Hennig, 1981; Pfau 1981; Carle, 1982; Trueman 1996; Bechley, 2002). Similarly, numerous studies had been utilizing wing morphology to illustrate variation among the species related to the same taxa (Baylac et al, 2003). Furthermore, study shows that insects are group of diverse organisms and its variation may be due to its flight system (Dudley, 2000).This study utilizes wing morphology to illustrate variation *Neurothemis terminata*, Libellulidae, Odonata which is a cosmopolitan species and widely distributed in the Philippines. It is commonly found in forests, grasslands, rice fields and even occurs in man-made habitats (Kalkman, 2009). This study aims to identify sexual dimorphism between the sexes of *N. terminata* through wings morphology by using landmark-based analyses.

2 Materials and Methods

2.1 Study area

This study was conducted at Mt. Hilong-Hilong, RTR, Agusan del Norte, Philippines. Geographically it lies between $9^0$05’22.15”N and $125^0$41’31.13”E. The dragonfly collection was done in the month of September and October 2016 with the aid of sweep nets for catching. The proper preservation procedures utilizing the killing jar as a small scale fumigation for killing the collected samples.

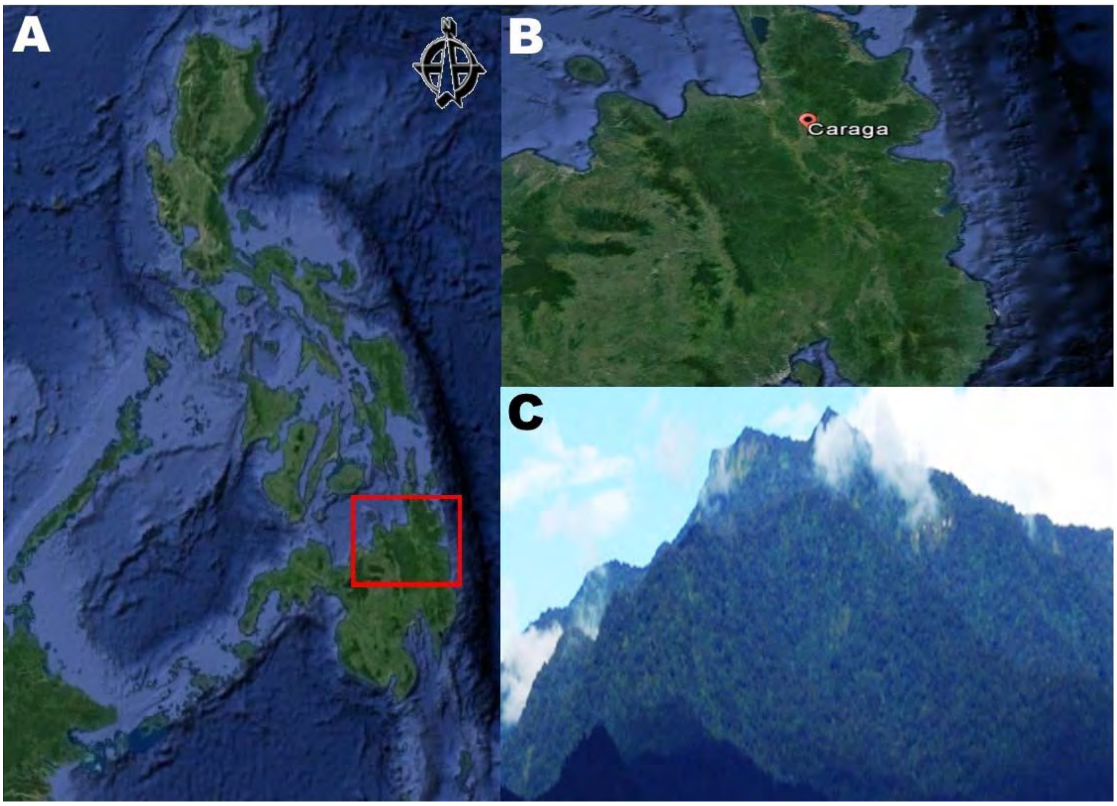

Fig. 1 Map showing **A**. Philippines **B**. Caraga Region **C**. Mt. Hilong-Hilong, RTR, Agusan del Norte, Philippines.

2.2 Relative warp analysis of *Neurothemis terminata*

2.2.1 Sample processing

Sixty individuals (30 males and 30 females) were collected in the study area. The left and right forewings and hindwings of the adult *N. terminata* were detached from the body of the insect with the use of a scalpel, dissecting needles and forceps. The wings were placed properly between two clean glass slides. Each corner of the slide were then fastened with an invisible tape to prevent the slides from moving. The slides were labeled properly which includes the specimen number, sex and the location where it was collected. The samples were then processed for image scanning to see the samples point of origin for landmarking processes and analyses. The sex of the collected dragonflies was determined by its body and wing color. Females were identified with a yellow body and wing color while males were identified with its red body and wing color.

2.2.2 Landmark selection and digitization

Digital images were sorted accordingly into sexes and converted to tps files using tpsUtil. Landmarking of the samples were digitized using the tpsDig version 2 (Rohlf, 2004). A total of 29 and 35 landmark points in forewings and hindwings respectively were used in this study (Table 1 and 2). Anatomical landmark points (Fig. 2 and 3) shows right lateral view to represent the external wing shape of the samples for male and female *N. terminata*.

2.2.3 Shape analysis

The converted tps files with the anatomical landmarks were processed in tpsRelw to get the relative warp analysis and to obtain X and Y coordinates for further analyses. Histograms were generated which serves as presentations for comparing patterns of sexual dimorphism. The generated results of the relative warp scores from the dragonfly shapes were computed and analyzed using PAST software. This software provides valuable information on the distribution of the data from the mean over the range of the variable. Collected coordinates were then subjected to MANOVA, Canonical Variance Analysis (CVA) and Principal Component Analysis (PCA) using PAST software (Hammer et al., 2009).

Table 1 Description of assigned landmarks used on both left and right forewings respectively (adopted from Demayo et al, 2011).

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
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)
		24	Distal end of intercalary radial vein (IR2)
		25	Distal end of Radial branch (R2)
9	Midpoint of the triangle (T)	**26**	Antero-lateral and distal end of the pterostigma
10	Midpoint of the triangle (T)	**27**	Postero-lateral and distal end of the pterostigma
11	Posterior end of the triangle (T)	**28**	Antero-lateral and proximal end of the pterostigma
12	Origin of Radial branches (R2 and R4)	**29**	Postero-lateral and proximal end of the pterostigma
13	Origin of intercalary vein (IR3)		
14	Nodus (N)		
15	Distal end of the Subcosta (Sc)		

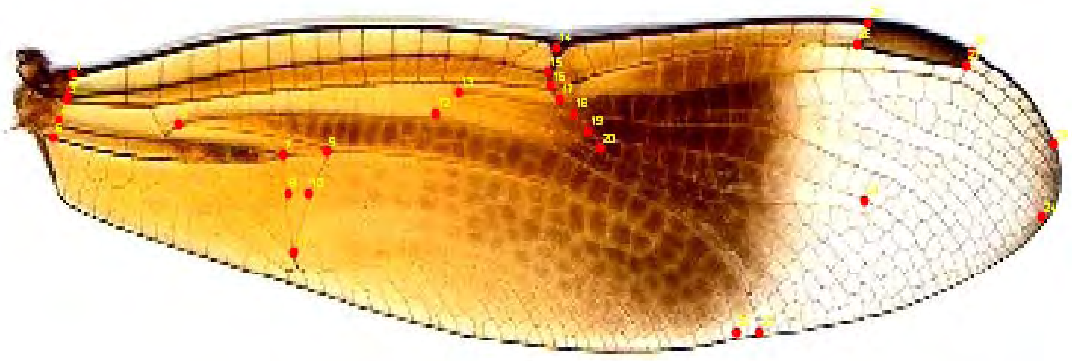

Fig. 2 Right forewing of female *N. terminata* showing the assigned landmark.

Table 2 Description of assigned landmarks used on both left and right hindwings respectively (adopted from Demayo et al, 2011).

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 cross-vein between Radial branches (R2 and R3)
7	Posterior and proximal vertex of the hypertrigone (ht)	**25**	Posterior end of the 2nd cross-vein 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)		

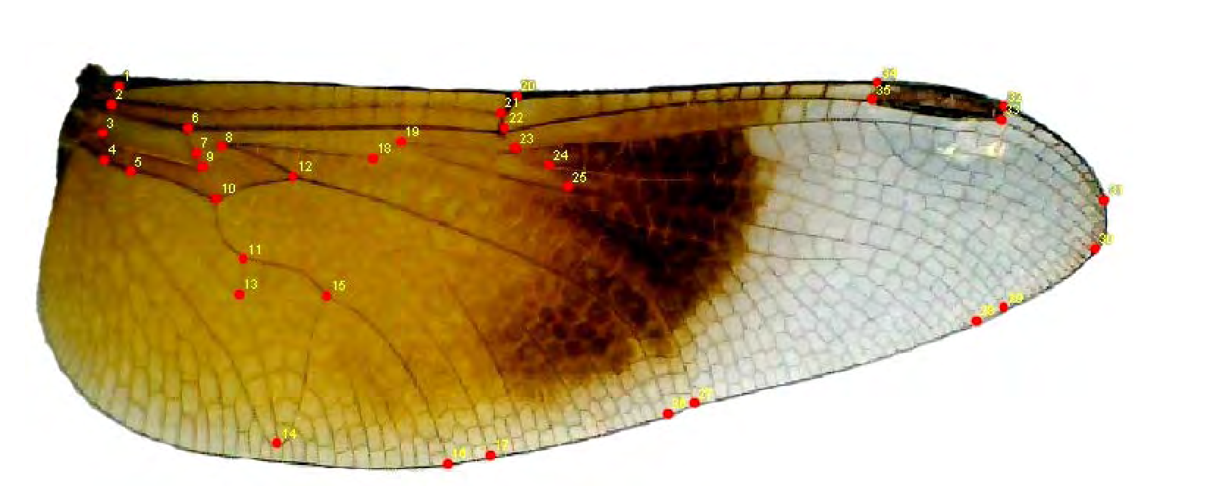

Fig. 3 Right hindwing female *N. terminata* showing the assigned landmark.

3 Results and Discussion

Multivariate Analysis of Variance (MANOVA) was utilized to show the significant wing variations both of the female and male populations. The result shows a high significant value of $P<0.05$ between the appended female and male forewings left, forewings right, hindwings left and hindwings right (Table 3).The observed differences on the wing morphology could be associated to these factors: random mating process within the individuals, greater population density, food preference, heat pressure, influence of parasite, diseases, sexual selection and some genetic components (Riget et al, 2008). Also, study shows that wing variations might be attributed to the flight system and flapping kinematics (Demayo et al, 2011). Hence, the differences in the wing morphology could be associated to natural selection (Green, 2000). Likewise, sexual selection is the primary factor affecting sexual dimorphism or SD (Navarro et al, 2009; Abouheif and Fairbairn, 1997; Hedrick and Temeles, 1989; Anderson, 1994). Moreover, SD evolves in a way that each sex, especially males can make advance in terms of reproductive aspects resulting to be attractive towards other sex or in the level to overthrow same sex competition (Stuart-Fox, 2009). Furthermore, according to (Butler et al, 2007). SD is the consequences of ecomorphological modification.

Table 3 Results of MANOVA TEST (Appended female and male populations).

Wing Shape	**Wilk's Lambda**	**df1**	**df2**	**F**	**p(same)**
Female & Male forewings left	0.1769	3	176	273	$6.004E^{-66**}$
Female & Male forewings right	0.01867	4	175	2300	$4.55E^{-150**}$
Female & Male hindwings left	0.04931	66	113	33.01	$3.572E^{-51**}$
Female & Male hindwings right	0.04255	66	23	7.842	$5.307E^{-07**}$

***** (P<0.05) highly significant;*** ns= *not significant*

Table 5 Description of the observed variations in the wings of female *N. terminata*.

	FOREWING		HINDWING	
	LEFT	RIGHT	LEFT	RIGHT
RW1	Variations observed in the anterior end of the 2nd cross-vein between radial branches (R2 and R3) The sample shows elongated and narrower wing size.	Variations observed in the regions of proximal end of the anterior margin of the Triangle (L), Distal end of the anterior margin of the triangle (L), Mid-point of the triangle (T).The triangle have narrower and elongated shape.	Variations observed in the basal and apical region of the wings. Samples have fatter size of the triangle.	Variations found in the basal region of the wings. Samples have fatter size of the triangle.
RW2	Variations observed in the basal and apical region of the wings. The samples have fatter size of the triangle.	Variations found in the apical and basal regions of the wings. Samples have elongated triangle.	Variations observed in the apical and basal region. The triangle have a wider size.	Same as RW 1
RW3	The observed variations found in the apical and basal regions. The populations have wider size of the triangle.	The differences found was in apical and basal potions of the wings. The triangle have an elongated and narrower size.	The variations observed in the apical and basal areas. The triangle have a shorter and narrower size.	Dissimilarities found in the apical and basal regions of the wings. The triangle have wider size.
RW4	The observed variations found in the apical and basal parts of the wing. The samples have wider wing size.	The variation found in the apical and basal portion of the wing. The triangle have an elongated and narrower wing size.	Same as RW3	The observed differences found in the portion of apical and basal. The samples have wider wing size of the triangle.
RW5	The variations found in the apical and basal region. The samples have fatter size of the triangle.	Dissimilarities found in in the apical and basal .portion. Samples with positive scores have elongated and narrower size of the triangle.		

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Table 6 Description of the observed variations in the wings of male *N. terminata*..

	FOREWING		HINDWING	
	LEFT	RIGHT	LEFT	RIGHT
RW1	Variations observed in the apical and basal region of the wing. The samples have elongated size of the triangle.	Observed variations found in the region of apical and basal. The samples have shortened size of the triangle.	Differences found in the basal and apical region of the wing. Samples have thinner size of the triangle.	Dissimilarities observed in the apical and basal region. Samples have wider size of the triangle.
RW2	Variations observed in the basal and apical region of the wings. The samples have fatter size of the triangle.	Variations found in the apical and basal region of the wings. Samples have elongated triangle.	Variations observed in the apical and basal region. The triangle have a wider size.	Same as RW 1
RW3	The observed variations found in the apical and basal regions. The populations have wider size of the triangle.	The differences found was in apical and basal potions of the wings. The triangle have an elongated and narrower size.	The variations observed in the apical and basal areas. The triangle have a shorter and narrower size.	Dissimilarities found in the apical and basal regions of the wings. The triangle have wider size.
RW4	The observed variations found in the apical and basal parts of the wing. The samples have elongated size of the triangle.	The variation found in the apical and basal portion of the wing. The samples have a fatter size of the triangle.	Same as RW1	The observed differences found in the portion of apical and basal. The samples have wider size of the triangle.

The observed variations in the wing morphology of female and male *N. terminata* presented in (Table 5 and 6) and were detected that there were trait specific condition occurs. These has also correlated to the speciation rate, heterogeneity and population dynamics (Kokko and Brooks, 2003; Butler et al, 2007; Rankin and Arnqvist, 2008). The dissimilarities of wing morphology might be a factor of force production which is significant mechanism during food hunting and mating process. Indeed, the distinct characteristic of an insects could be related to its flight activity and kinematics movement (Ellington, 1984). In addition, the incidence of variation in the wings of *N. terminata* shows that competition within the taxa constitutes (SD) or sexual dimorphism (Bean and Cook, 2001).

The RW or relative warp analysis showed significant differences on the wing shape of *N. terminata*. Likewise the histogram were also displayed to compare patterns of sexual dimorphism among the female and male populations (Fig. 4 and 5). In female samples, the left forewings generate four relative warp (RW) scores accounted to 75.11% while the right forewings generate five RW scores accounted to 63.28%. On the other hand, the left hindwings generate five relative warp (RW) scores accounted to 71.75% and the right hindwings generate four RW scores accounted to 71.73%. In male samples, the left forewings generate five relative warp (RW) scores accounted to 67.92% and the right forewings generates five RW scores accounted 68.34%. While the left hindwings generate four RW scores accounted to 64.10% and the right hindwings generate four RW score accounted to 63.45%.The observed differences of the wing morphology of the dragonfly could be attributed to the geographical and territorial behaviour specifically in male populations (Corbet, 1962).

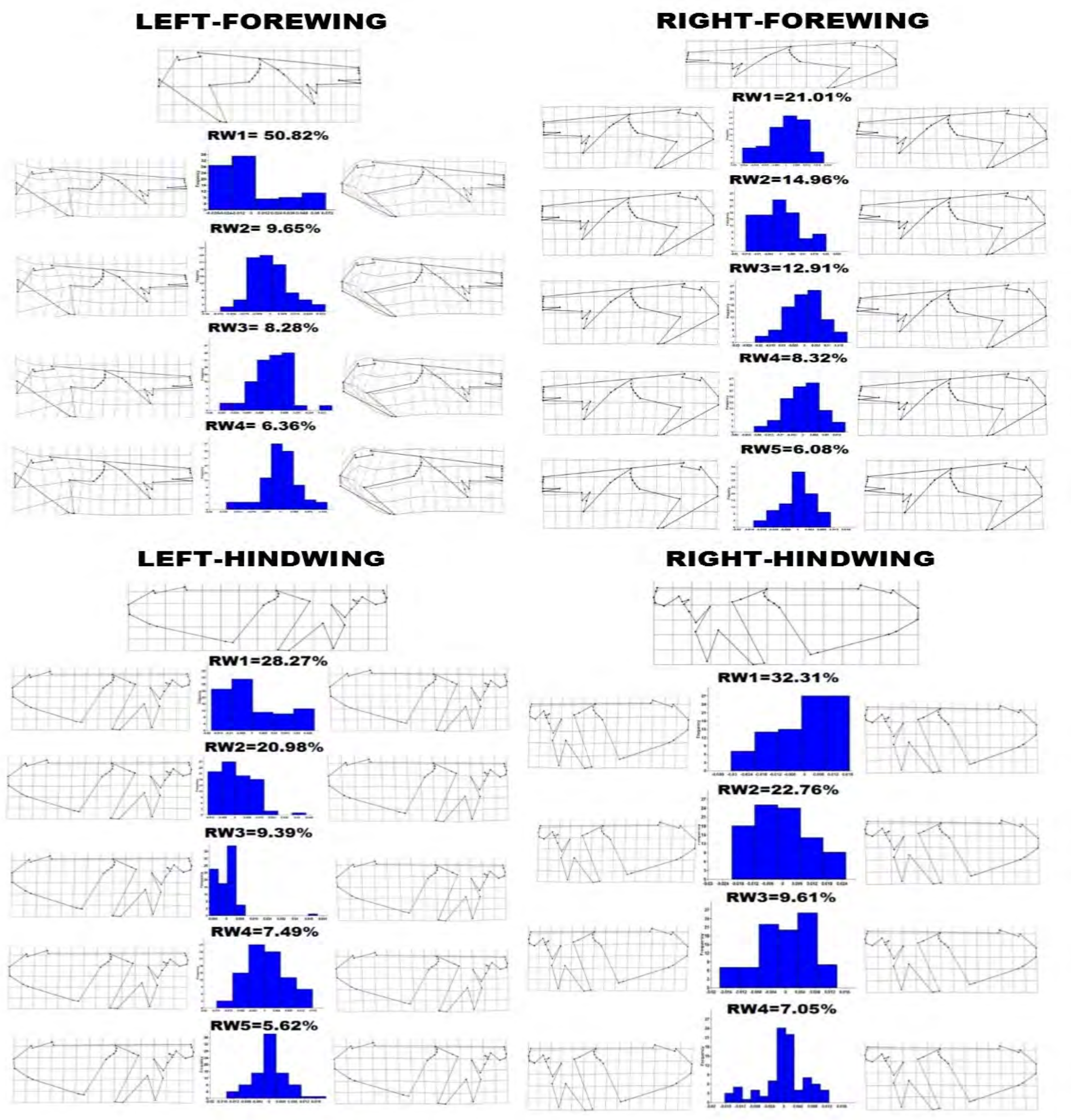

Fig. 4 Relative warp box plot showing variations in the left and right fore-wings and hind-wings of female *N. terminata*.

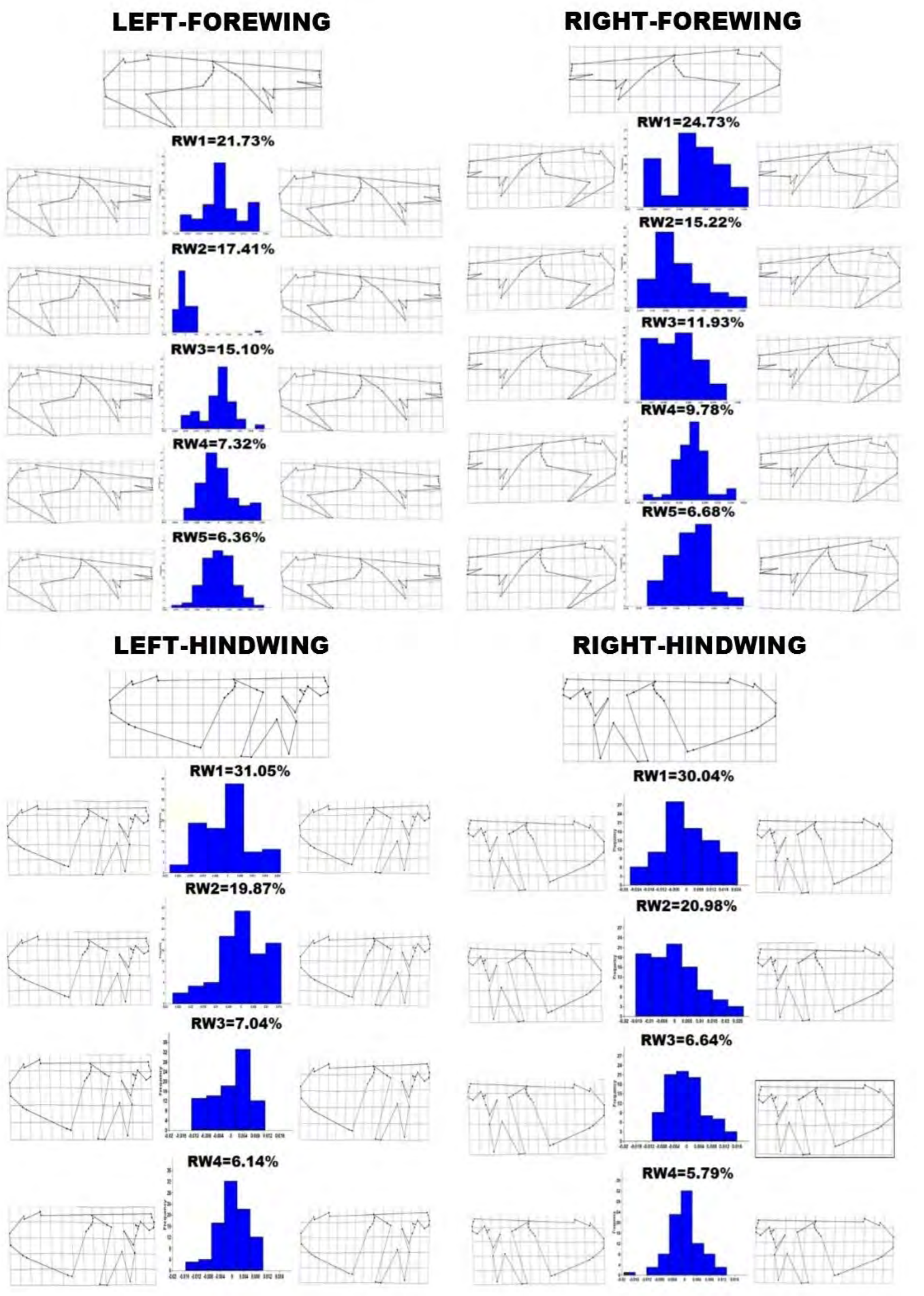

Fig. 5 Relative warp box plot showing variations in the left and right fore-wings and hind-wings of male *N. terminata*

The CVA or canonical variate analysis shown in (Fig. 6, 7, 8 and 9) where the scatterplot shows the appended female and male forewings (left & right) and hindwings (left & right) respectively. It was found out that sexes of *N. terminata* show dissimilar traits and these implies variation within its populations. The dragonfly is holometabolous insect owing to its growth can cause sexually preferred morphological characteristics (Nijhout and Emlen, 1998; Emlen & Nijhout, 2000; Emlen, 2001).Furthermore, wing shape and size also a compensatory process in which insects can counteract during flight performance (Ribak et al, 2009). Lastly, the environment also plays a key role to drive the evolution of diverse characters between the sexes of dragonflies (Berns, 2013).

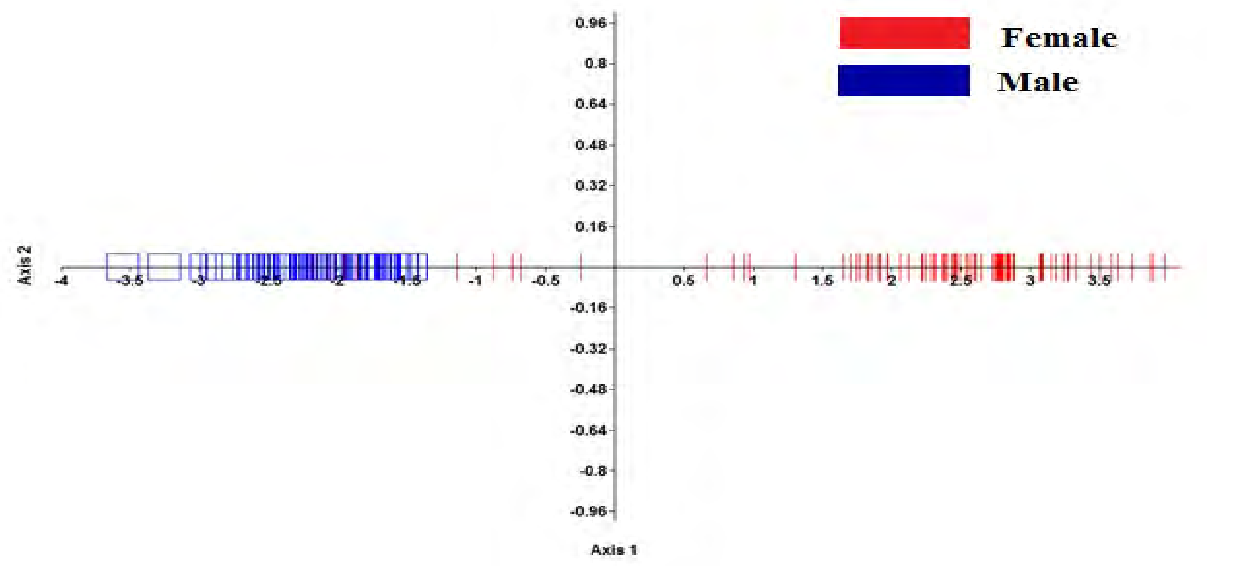

Fig. 6 CVA scatterplot showing appended female and male forewings left.

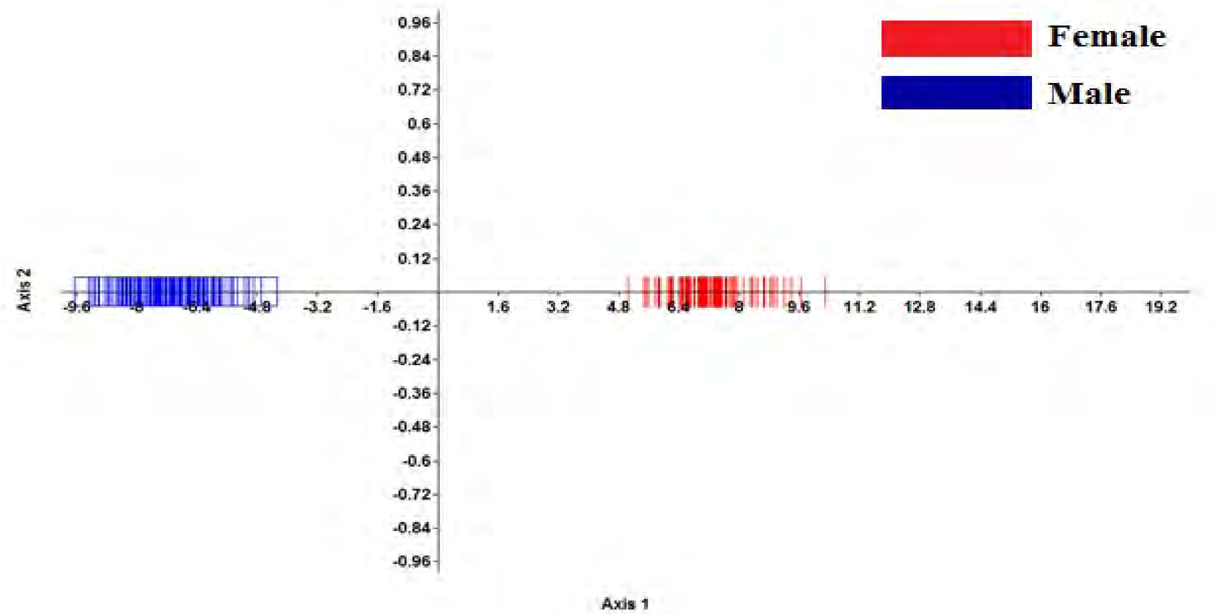

Fig. 7 CVA scatterplot showing appended female and male forewings right.

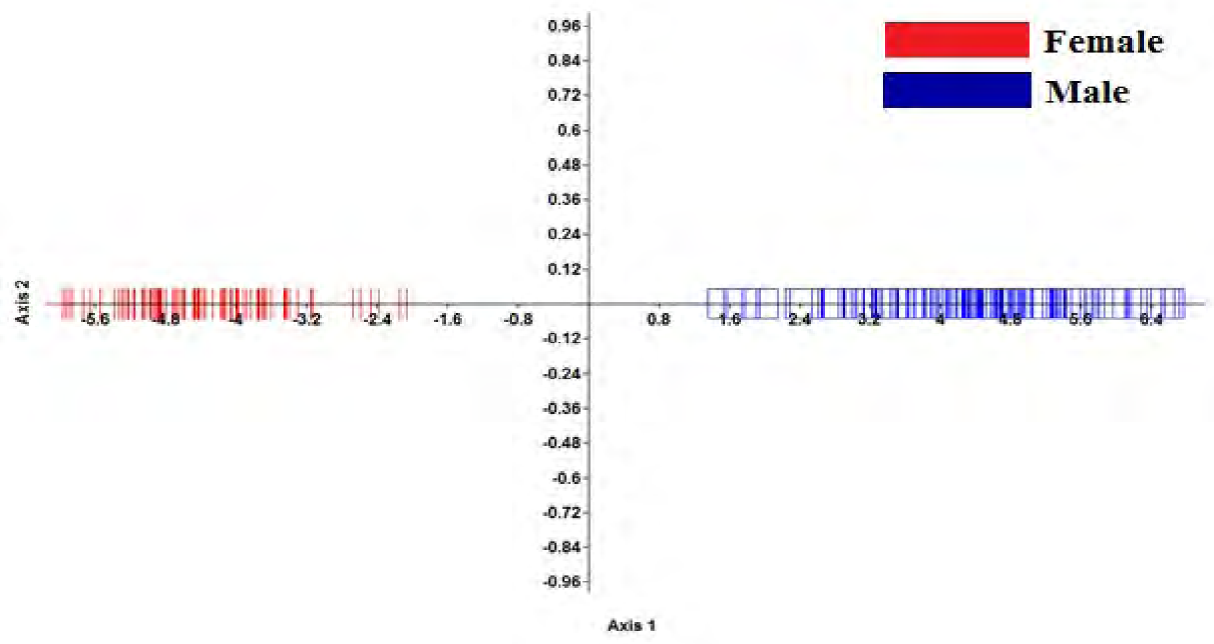

Fig. 8 CVA scatterplot showing appended female and male hindwings left

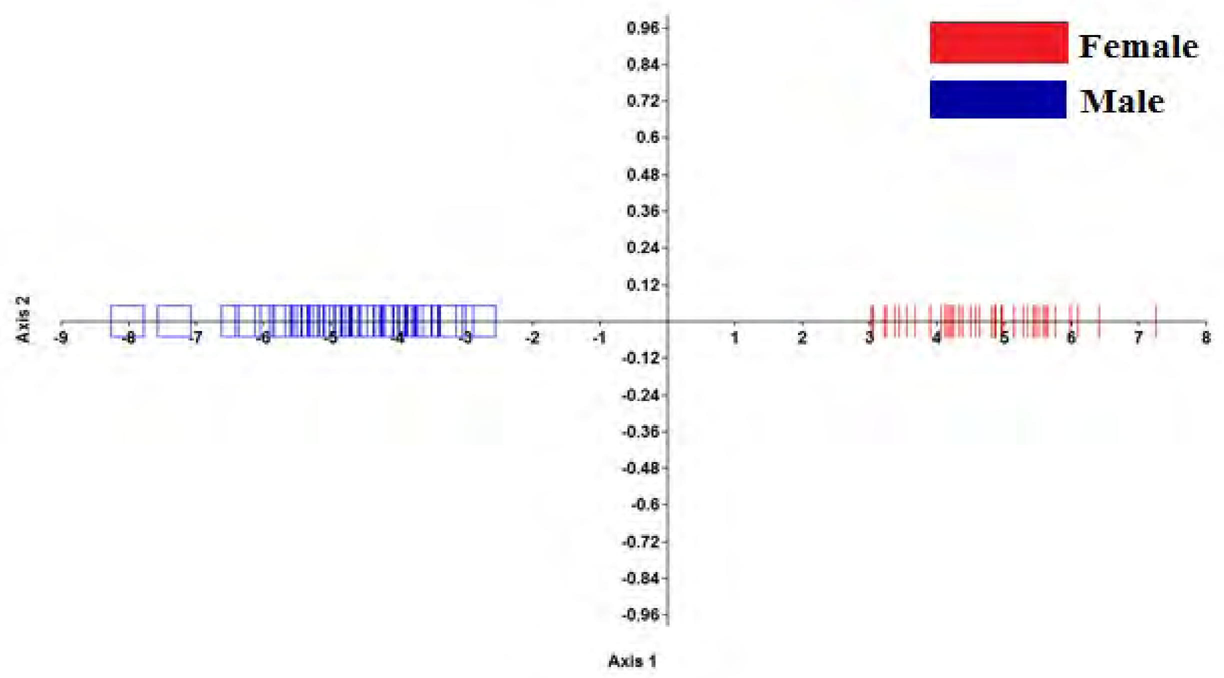

Fig. 9 CVA scatterplot showing appended female and male hindwings right.

The PCA or principal component analysis shown in (Fig. 10, 11, 12 and 13) where the scatterplot shows appended female and male forewings (left & right) and hindwings (left & right) respectively. It was observed that there was a similarities found in the wing morphology especially in the female and male right hindwings see (Fig. 13). The overlapping condition indicates that there were shared traits within the individuals. It is interesting to know that in some cases this could happen in dragonfly species. This scenario could be used for an insects to be more environmentally adaptive, fit and increase the chance of mating process. Indeed, female dragonflies are more likely to acquire fitness rather than males and this may be an advantage for reproductive success (Charnov et al, 1981). Relatively, time and development may enhance modifications among each sex of an insects and considered to be the immediate processes to create sexual dimorphism (Roff, 1992; Fairbairn, 1990; Blanckenhorn et al, 2007). Nonetheless, the existence of SD has been related to fitness within the population (Kokko and Brooks, 2003; Rankin and Arnqvist, 2008).

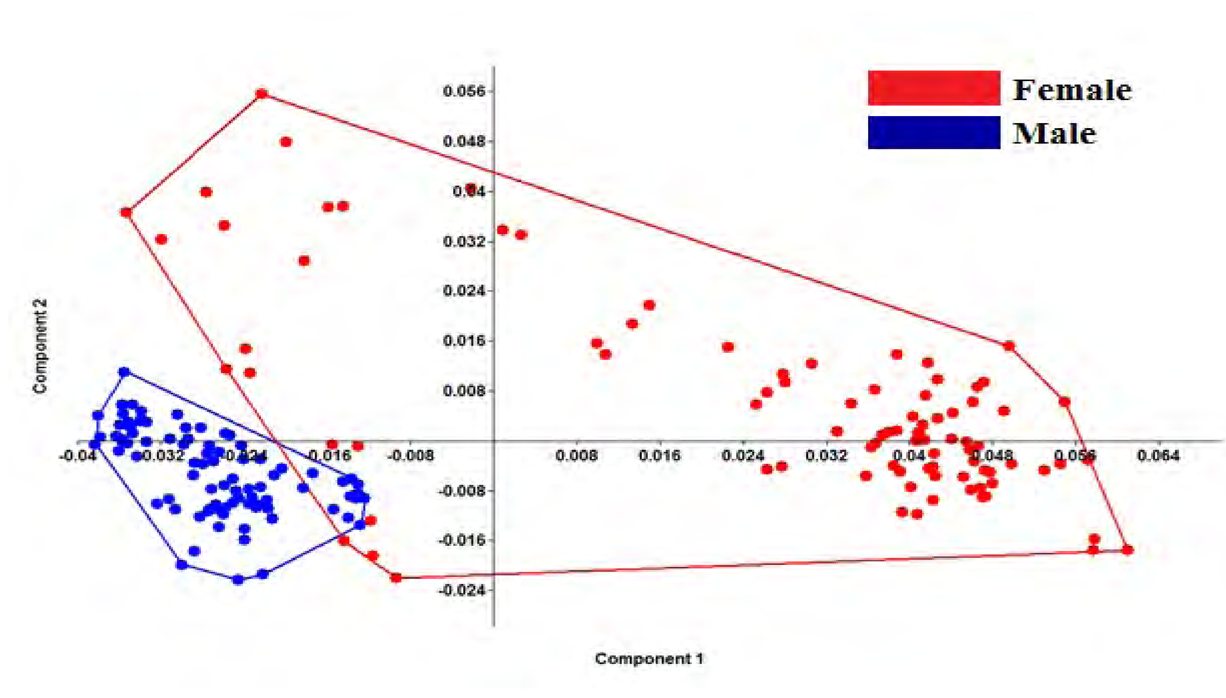

Fig. 10 PCA scatterplot showing appended female and male forewings left.

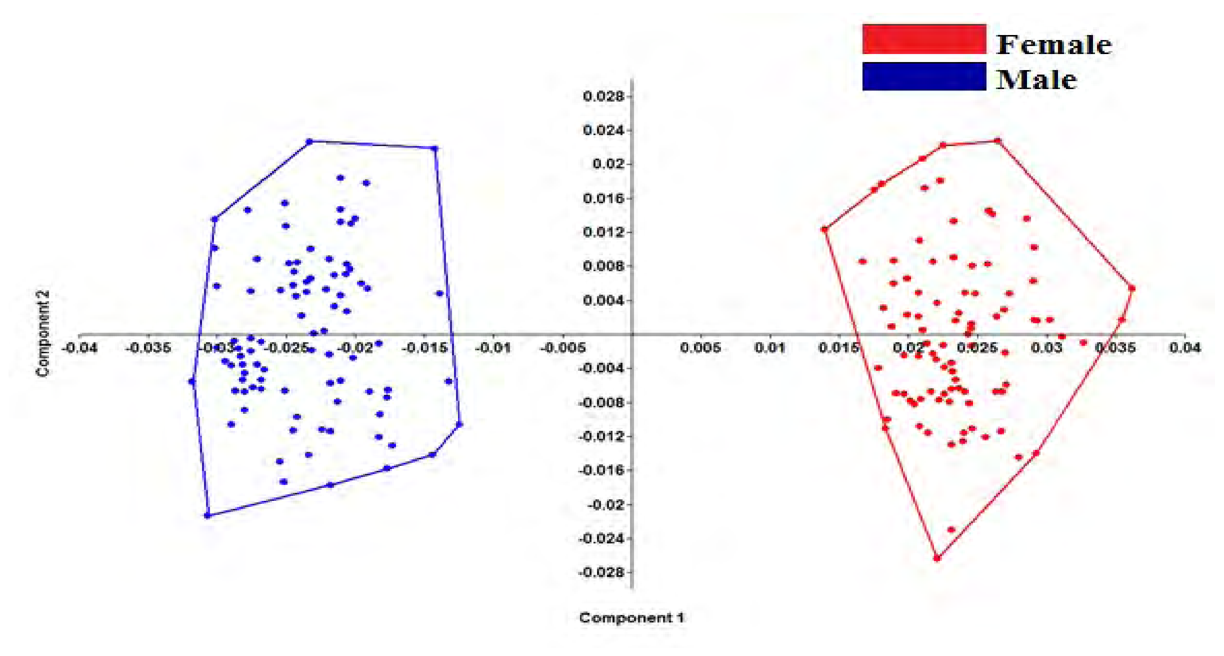

Fig. 11 PCA scatterplot showing appended female and male forewings right.

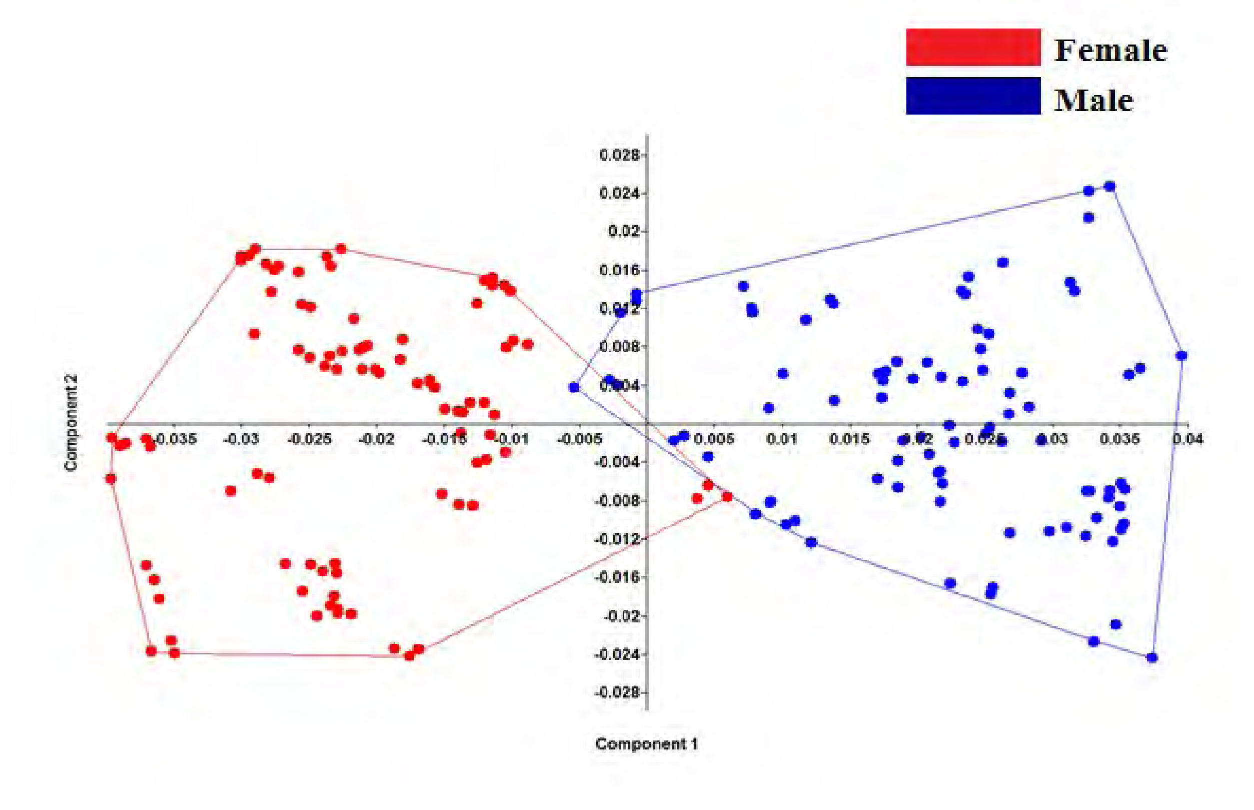

Fig. 12 PCA scatterplot showing appended female and male hindwing left.

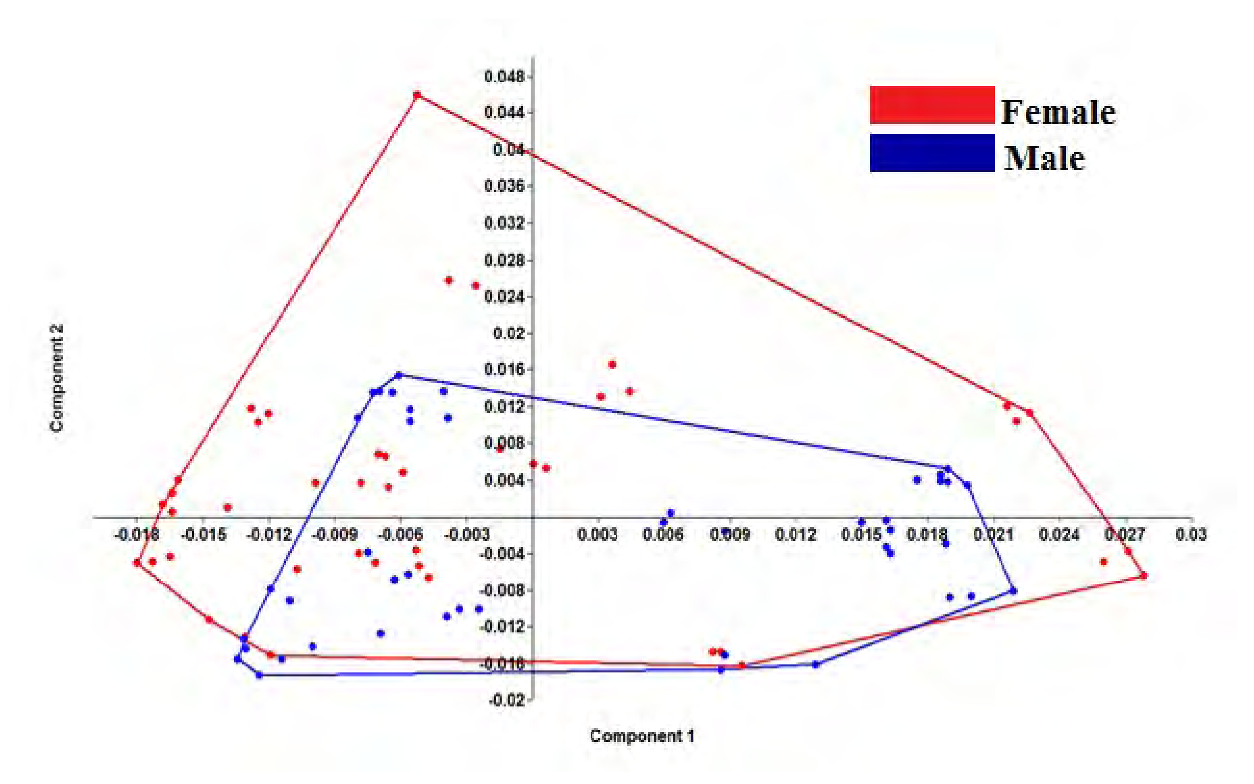

Fig. 13 PCA scatterplot showing appended female and male hindwing right.

4 Conclusion

Landmark-based geometric morphometric was employed to describe sexual dimorphism in wings of *N. terminata*. The result shows significant differences of (P<0.05) between the appended female and male populations. The wing variations among the selected samples likely to associate in the sexual selection, flight performance and flapping kinematics. The obtained results suggested that the wing shape of the dragonfly *N. terminata* indicates distinction and this could be related to fitness and environmental causation. This study also shows the advancement of using geometric morphometric to draw shape variations among the species of dragonflies.

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