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Wing shape variations in *Calopteryx splendens* along a latitudinal gradient using geometric morphometric analysis

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Abstract

Fluctuating asymmetry (FA) quantifies the degree of variations from a perfect symmetry and therefore expected to reflect the state of genomic and ecological stress undergone by the single species and or populations throughout its development. It identifies the extent of differences from the symmetry of left and right sides of the organisms, as both sides are expected to be identical from its genetic activity and within the environment they inhabit. In this study, geometric morphometric was utilized to measure wing shape variation in the populations of *Calopteryx splendens*. A total of 120 samples consisting of 30 males and 30 females were randomly collected each of the latitudinal gradient and subjected to Procrustes ANOVA and Principal Component Analysis (PCA) by means of Symmetry and Asymmetry in Geometric Data (SAGE) software. In the three factors analyzed: individuals, sides, and individual $\times$ sides result shows that the collected samples from the high altitude displayed high significant difference ($P < 0.0001$) in the female forewings and hindwings while male forewings and hindwings showed partly non-significant. Whereas, the collected samples from the lower altitude result shows high significant difference ($P < 0.0001$) in the female and male forewings while female and male hindwings shown partly non-significant. It implies that latitudinal gradients could influence wing shape pattern and phenotypic variability was evident between species of the same lineage. Further, utilizing geometric morphometrics is essential in identifying wing shape variations and co-variations among species relatively of the same ancestry.

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Introduction

The shape is an utmost morphological characteristic that offers a phenotypic information linking the genotype to the environment (Ricklefs and Miles, 1994). Environmental components highly affect morphological trait involving the shape thus manifesting phenotypic plasticity (Via and Lande, 1985; Schlichting and Pigliucci, 1998). The wing shape variations widely occur among insect's species and considerably one of the factors for its diversity. The wing morphology is relatively significant during food hunting and mating process (Riget *et al.*, 2008) while it is believed that the flight system and flapping kinematics are results of the same phenomenon (Demayo *et al.*, 2011). The ability of this species to develop phenotypic variation being associated with environmental adaptation (Green, 2000). Differences in wing shape is also linked with fitness and reproductive success (Kokko and Brooks, 2003; Rankin and Arnqvist, 2008). Wings variations might also attribute to geographic isolation which later results in genetic variation (Hermita *et al.*, 2013).

Also, it plays an important role in regulating the flight activity and likely the outcome of interspecific dissimilarities due to natural selection (Johansson *et al.*, 2009). As well as, study shows that modifications in wing morphology might be correlated to flight performance and sexual selection (Outomuro and Johansson, 2011).

Indeed, wing variations are primarily associated to sexual dimorphism resulting to trait specific condition (Arnqvist and Rowe, 2005; Fairbairn *et al.*, 2007). Further, wing size and shape are comparatively the primary components in which insects can respond during flight performance (Ribak *et al.*, 2009). Developmental stability of the organisms is the outcome of projected phenotype from a specific genotype towards environmental setting (Zakharov, 1992).

Moreover, to draw wing shape variations within the population of *Calopteryx splendens* fluctuating

asymmetry (FA) was utilized as an indicative tool of geometric morphometrics. This application is wide uses for defining differences among biotic elements (Klingenberg and McIntyre, 1998; Savriama *et al.*, 2012; Hermita *et al.*, 2013). FA often utilizes as a biomarker of developmental instability (Clark *et al.*, 1986; Parsons, 1992; Moller and Swaddle, 1997).

It helps to distinguished variances along bilateral symmetry. Further, it is a contributing mechanism in the field of biology in which it defines the shape variations and co-variations of organisms towards its environment (Cabuga *et al.*, 2016).

In this study, two latitudinal gradients (High & Low Altitude) were considered upon the collection of *C. splendens*, a widely spread damselfly found among regions in the Philippines. It mostly found in forests, grasslands, rice fields, waterfalls and even lakes (Kalkman, 2009). Its wings shape are the essential and fundamental basis of phenotypic variation within its population (Fraser, 1957; Hennig, 1981; Carle, 1982; Pfau, 1981; Trueman, 1996; Bechley, 2002).

The geographical location of organisms may also develop phenotypic variations due to its adaptation and ecological influences. Hence, the latitudinal gradient is a mechanism that increases species richness and morphological features (Willig *et al.*, 2003). The present study aims to identify phenotypic variations between the sexes of *C. splendens* through wings morphology by using fluctuating asymmetry.

Thus, the importance of geometric morphometric in analyzing wing shape variation is a mechanism to be utilized to further understand ecological and evolutionary history in Odonates.

Materials and methods

Description of the area

This study was conducted at Tagnote Falls, Remedios T. Romualdez, Agusan del Norte, Philippines. Geographically lies between 9°03'01.40"N and 125°39'00.15"E (Fig. 1).

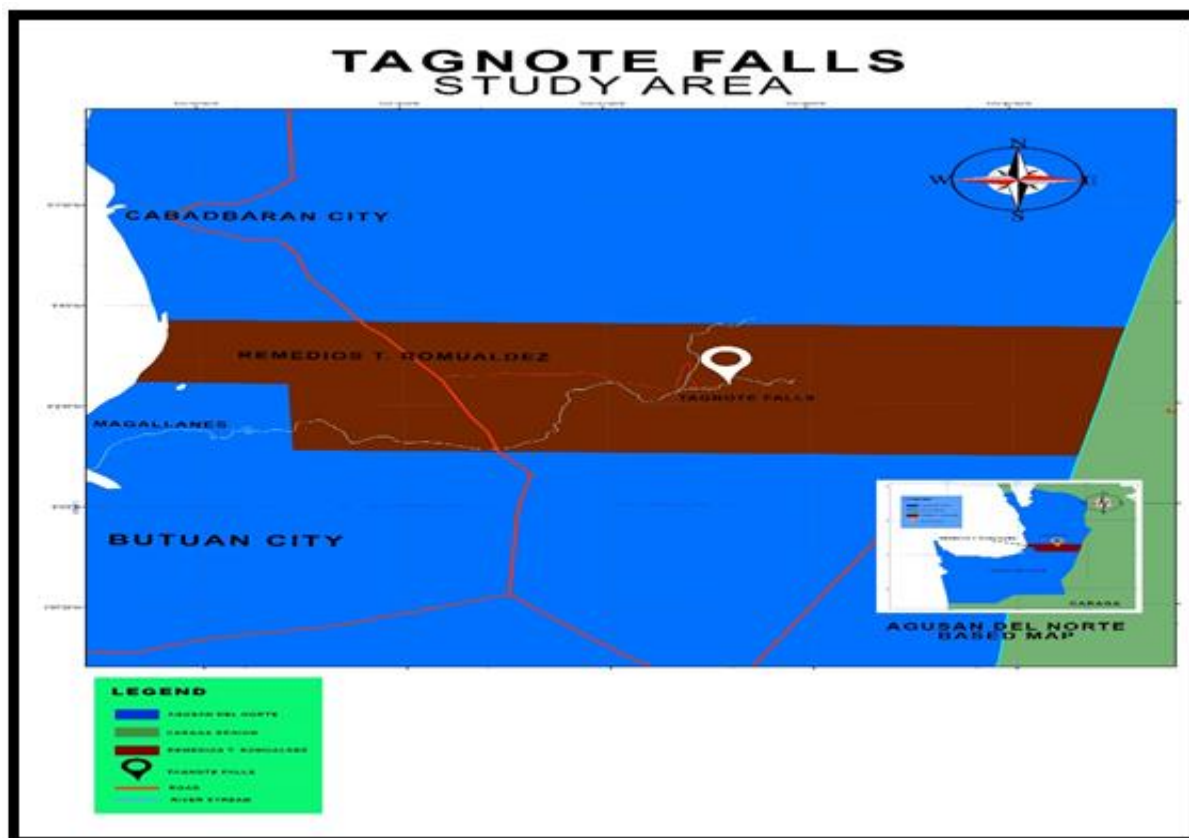


Fig. 1. Map showing the study area Tagnote, Agusan del Norte, Falls, Remedios T Romualdez Philippines.

The damselfly collection was done in the month of March and April 2017 with the aid of sweep nets for catching and proper preservation procedures were applied.

Sample collection and processing

A total of 120 individuals comprising of 30 males and 30 females was collected each of the latitudinal gradient.

The left and right forewings and hind wings of the adult *C. splendens* was first detached from the body of the insects with the use of a scalpel, dissecting needles and forceps. After which, the wings was placed properly between the two clean glass slides. Each corner of the slide was then secured with an invisible tape to prevent the slides from moving. The glass slides were labelled appropriately which includes the sex, specimen number and the place where it was collected.

The samples were then administered for image scanning to see the samples point of origin for landmarking procedures and analysis. The sex of the collected damselfly was verified by its body and wing color. Females were identified by an opaque, smoky brown rather than a transparent green while males were identified by its completely dark blue body and wing color.

Landmark selection and digitation

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 12 landmark points in forewings and hindwings respectively was used in this study (Fig. 2).

Shape analysis

The converted tps files with the anatomical landmarks were processed in symmetry and asymmetry geometric data (SAGE) (version 1.04

Marquez, 2007) software (Fig. 3) to obtain the principal component analysis which identifies the deformation grid of the individual asymmetry (Natividad *et al.*, 2015).

This software provides valuable information on the distribution of the data from the mean over the range of the variable. The collected coordinates were then subjected to Procrustes ANOVA that helps to draw the significant difference of the factors considered.

These factors were individuals, sides, and interaction of the individuals and sides of *C. splendens*.

The level of significance was confirmed at $P < 0.0001$. The differences between the side and measure of directional asymmetry also show. The percentage (%) of FA were compared and obtained between the sexes of the samples (Natividad *et al.*, 2015).

Results and discussion

Procrustes ANOVA was employed to determine the intraspecific wing shape variations of *C. splendens*. The analysis to compare the left-right symmetry and size shape presented in (Table 2, 3, 4 & 5).

Table 1. Anatomical landmark points used to define wing shapes in *C. splendens*. adopted from (Tillyard and Fraser, 1940).

Coordinates	Locations/Nomenclature
1	anterior end of the Arculus (Arc)
2	Posterior end of nuchal spine
3	Anterior insertion of dorsal fin
4	Posterior insertion of dorsal fin
5	Dorsal insertion of caudal fin
6	Midpoint of lateral line
7	Ventral insertion of caudal fin
8	Posterior insertion of anal fin
9	Anterior insertion of anal fin
10	Dorsal base of pelvic fin
11	Ventral end of lower jaw articulation
12	Posterior end of the premaxilla
13	Anterior margin through midline of orbit
14	Posterior margin through midline of orbit
15	Dorsal end of operculum
16	Dorsal base of pectoral fin

Table 2. Procrustes ANOVA results for the forewings of *C. splendens* interms of sexes (High Altitude).

Factors	SS	DF	MS	F	P-VALUE
Female					
Individuals	0.5898	580	0.001	2.1441	0.0001**
Sides	0.0341	20	0.0017	3.5906	0.0001**
Individual × Sides	0.2751	580	0.0005	5.3796	0.0001**
Measurement Error	0.2116	2400	0.0001	--	--
Male					
Individuals	1.1091	580	0.0019	1.045	0.298 ^{ns}
Sides	0.1201	20	0.006	3.2829	0.0001**
Individual × Sides	1.0613	580	0.0018	7.2706	0.0001**
Measurement Error	0.604	2400	0.003	--	--

Note: Individual – symmetry, Sides - directional asymmetry, individual x sides interaction – fluctuating asymmetry, ** ($P < 0.0001$)-statistically significant, ns – statistically insignificant ($P > 0.05$): significance was tested with 99 permutations.

There were three factors: the individuals, side, and individual $\times$ sides were considered in the analysis and was applied to both male and female samples.

The obtained results indicated FA for the three factors measured and similarly among female and male populations ($P < 0.0001$).

It was observed that there was a high significant difference among the individual damselfly associated to the other individual samples. The so-called individual is the symmetry and individual by sides reflecting fluctuating asymmetry. While the sides also displayed highly significant difference indicating FA in the left-right sides of the populations.

Table 3. Procrustes ANOVA results for the hind wings of *C. splendens* interms of sexes (High Altitude).

Factors	SS	DF	MS	F	P-Value
Female					
Individuals	0.8318	580	0.0014	2.1257	0.0001**
Sides	0.0892	20	0.0045	6.6139	0.0001**
Individual $\times$ Sides	0.3913	580	0.0007	3.2701	0.0001**
Measurement Error	0.4951	2400	0.0002	--	--
Male					
Individuals	1.6547	580	0.0029	0.823	0.9904 ^{ns}
Sides	0.0607	20	0.003	0.8755	0.6191 ^{ns}
Individual $\times$ Sides	2.0106	580	0.0035	17.6495	0.0001**
Measurement Error	0.4714	2400	0.0002	--	--

Note: Individual – symmetry, Sides - directional asymmetry, individual $\times$ sides interaction – fluctuating asymmetry, **($P < 0.0001$) statistically significant, ns – statistically insignificant ($P > 0.05$): significance was tested with 99 permutations.

Table 4. Procrustes ANOVA results for the forewings of *C. splendens* interms of sexes (Low Altitude).

Factors	SS	DF	MS	F	P-Value
Female					
Individuals	0.4062	580	0.0007	1.7517	0.0001**
Sides	0.038	20	0.0019	4.7563	0.0001**
Individual $\times$ Sides	0.2139	580	0.0004	5.3796	0.0001**
Measurement Error	0.1603	2400	0.0001	--	--
Male					
Individuals	0.3254	580	0.0006	2.1109	0.0001**
Sides	0.044	20	0.0022	0.0022	0.0001**
Individual $\times$ Sides	0.1542	580	0.0003	3.1893	0.0001**
Measurement Error	0.2	2400	0.0001	--	--

Note: Individual – symmetry, Sides - directional asymmetry, individual $\times$ sides interaction – fluctuating asymmetry, **($P < 0.0001$)-statistically significant, ns – statistically insignificant ($P > 0.05$): significance was tested with 99 permutations.

It is the differences between the two sides denoting directional asymmetry (Galbo and Tabugo, 2014).

On the other hand, the error term is the measurement and is by chance effect (Samuels *et al.*, 1991; Palmer and Strobeck, 1986, 2003; Carpentero and Tabugo, 2014). Thus, FA was detected in the three factors both in female and male damselflies.

The observed variations among the collected samples might be attributed to its environmental preferences. Hence, ecological causation also develops phenotypic plasticity among species of the same taxa. Accordingly, high levels of asymmetry could be attributed towards environmental stress; since species are most adaptable to ecological alterations (Sadeghi *et al.*, 2009; Yuto *et al.*, 2016).

Table 5. Procrustes ANOVA results for the hind wings of *C. splendens* interms of sexes (Low Altitude).

Factors	SS	DF	MS	F	P-Value
Female					
Individuals	0.713	580	0.0012	1.1437	0.0531 ^{ns}
Sides	0.0465	20	0.0023	2.165	0.0025 ^{ns}
Individual × Sides	0.6235	580	0.0011	11.3811	0.0001 ^{**}
Measurement Error	0.2267	2400	0.001	--	--
Male					
Individuals	0.2904	580	0.0005	1.1164	0.0926 ^{ns}
Sides	0.1014	20	0.0051	11.3069	0.0001 ^{**}
Individual × Sides	0.2601	580	0.0004	8.0823	0.0001 ^{**}
Measurement Error	0.1332	2400	0.0001	--	--

Note: Individual – symmetry, Sides - directional asymmetry, individual x sides interaction – fluctuating asymmetry, ^{**}($P < 0.0001$)-statistically significant, ^{ns} – statistically insignificant ($P > 0.05$): significance was tested with 99 permutations

Table 6. Principal component scores showing the values of symmetry and asymmetry scores with the summary of the affected landmarks of forewings between sexes of *C. splendens* (High Altitude).

PCA	Individual (Symmetry)	Sides (Directional Asymmetry)	Interaction (Fluctuating Asymmetry)	Affected Landmarks
Female				
PC1	55.2018%	100%	55.4913%	1,3,4,5,6,7,9,10,11
PC2	20.6031%		16.2597%	1,2,3,4,5,8,12
PC3	8.3872%		12.5196%	1,2,4,7,8,11,12
PC4	6.0017%		5.7609%	1,2,3,4,5,6,7
	90.1931%		90.0315%	
Male				
PC1	68.4979%	100%	61.8754%	1,2,3,4,5,6,7,8,9,10,11,12
PC2	10.5307%		14.3351%	1,3,4,5,6,9,11,12
PC3	6.2422%		8.7132%	1,2,3,4,5,8,9,10,12
	85.2708%		84.9237%	

Table 7. Principal component scores showing the values of symmetry and asymmetry scores with the summary of the affected landmarks of hind wings between sexes of *C. splendens* (High Altitude).

PCA	Individual (Symmetry)	Sides (Directional Asymmetry)	Interaction (Fluctuating Asymmetry)	Affected Landmarks
Female				
PC1	65.9541%	100%	49.8903%	1,2,3,4,5,6,7,8,9,10,11,12
PC2	13.4226%		18.127%	1,2,3,5,6,7,9,10,12
PC3	6.1627%		10.3615%	1,3,4,5,6,8,9,10,11
PC4	5.0209%		8.0131%	1,3,4,5,7,8,9,10,12
	90.5603%		86.3919%	
Male				
PC1	84.7305%	100%	86.494%	1,2,3,4,5,6,7,8,9,10,11,12
PC2	5.567%		5.588%	1,2,3,4,5,6,7,8,9,10,11
	90.2975%		92.082%	

The occurrence of non-directional dissimilarities among the sides might be related to environmental requirements implying developmental variations (Valen, 1962; Palmer 1994; Gangestad and Thornhill, 1997).

While, it is assumed that environment plays a major role for species phenotypic changes; thus abiotic factors i.e. climate, elevation, geographic isolation also proposes morphological differences.

Moreover, Eco geographical isolation constitutes body size modification and allows to shift dispersal ability between island species (Lomolino *et al.*, 2005; Whittaker and Fernández-Palacios, 2007).

The study shows that different wing shape and body size within the normal population signifies adaptation to island heterogeneity (Lee and Lin, 2011).

Table 8. Principal component scores showing the values of symmetry and asymmetry scores with the summary of the affected landmarks of forewings between sexes of *C. splendens* (Low Altitude).

PCA	Individual (Symmetry)	Sides (Directional Asymmetry)	Interaction (Fluctuating Asymmetry)	Affected Landmarks
<i>Female</i>				
PC1	38.5565%	100%	60.8608%	1,2,3,7,8,9,10,11,12
PC2	34.7785%		17.4092%	1,2,3,4,5,6,9,11
PC3	6.0017%		5.7609%	1,2,3,4,5,6,7
	79.3367%		84.0307%	
<i>Male</i>				
PC1	36.494%	100%	38.2195%	1,2,3,4,5,6,7,8,9,10,11,12
PC2	31.8515%		21.4229%	1,2,4,5,11,12
PC3	11.7156%		15.769%	1,4,8,9,10,12
PC4	6.5522%		8.4153	1,4,8
	85.2708%		83.8267%	

Table 9. Principal component scores showing the values of symmetry and asymmetry scores with the summary of the affected landmarks of hindwings between sexes of *C. splendens* (Low Altitude).

PCA	Individual (Symmetry)	Sides (Directional Asymmetry)	Interaction (Fluctuating Asymmetry)	Affected Landmarks
<i>Female</i>				
PC1	76.5344%	100%	70.6971%	1,2,3,4,5,6,7,8,9,10,11,12
PC2	9.911%		9.0047%	1,2,3,4,5,9,11,12
PC3	4.652%		6.4242%	1,4,9,10,12
	91.0974%		94.1391%	
<i>Male</i>				
PC1	60.087%	100%	54.1619%	1,2,3,4,5,6,7,8,9,10,11,12
PC2	13.399%		16.7657%	1,3,4,5,6,10
PC3	8.6095%		9.4353%	1,2,4,12
PC4	5.5462%		6.3975%	1,4,6,7,8,9,12
	87.6417%		86.7604%	

In addition, insect's wing variability could also be related to the energy expense during flight maneuverability (Betts and Wootton, 1988; Grodnitsky, 1999; Dudley, 2000; Wootton and Kukulová-Peck, 2000). Nonetheless, the ability of species to inhabit islands most especially insects likely results to flightless activity due to reduced competition and predation (Mcnab, 1994).

The principal component analysis (PCA) shows the Interaction or Fluctuating Asymmetry for the collected female and male samples at higher altitude (Table 6 & 7). In the forewings, female have 90.03% percentage of FA constituting four principal component scores (PC) accounting to 90.19%. While the affected landmarks common among the four PC score were landmarks 1 (anterior end of the arculus) and 4 (posterior end of the radius).

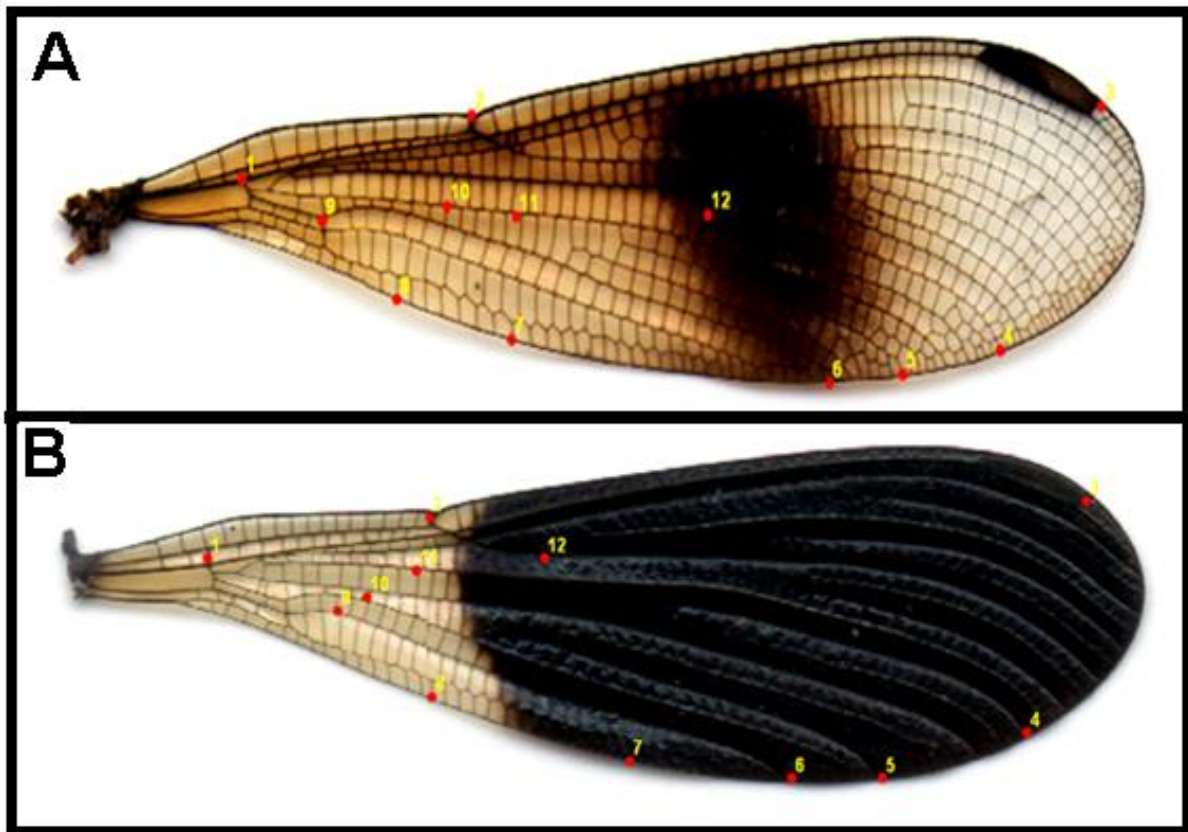


Fig. 2. Landmark points used to describe the wing shape of *C. splendens* (a) forewing (b) hindwing.

The male forewings have 84.92% of interaction (FA) constituting three principal component scores (PC) accounting to 85.27%. The affected landmarks common among the three PC score were landmarks 1, 3, 4, 5, 9 and 12. These were portion of anterior end of

the arculus, posterior intersection of the Pterostigma & Radius 1, posterior end of the radius 4, posterior end of the anterior media, anterior end of the anal vein and anterior end of the radius 4 supplementary.

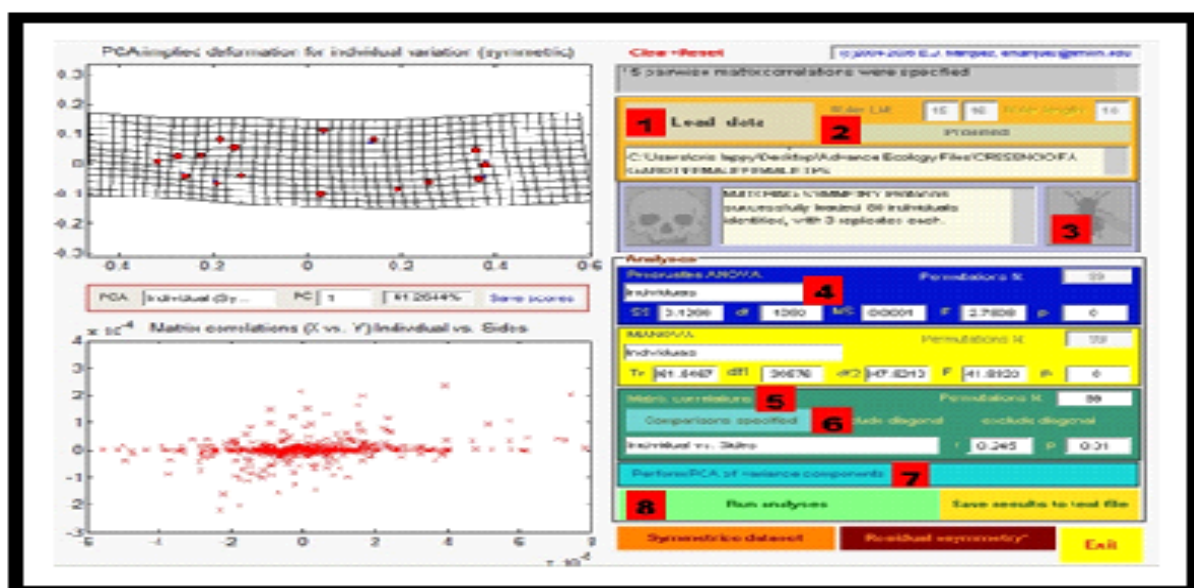


Fig. 3. Overview of the schematic flow of shape analysis using SAGE.

It was observed that female had the highest percentage of FA in the forewings when compared to male. Meanwhile, the female hind wings have 86.39% of FA constituting four principal component scores (PC) accounting to 90.56%. The common affected landmarks among the four PC score were landmarks

1,3,5,9 and 10. These were portion of anterior end of the arculus, posterior intersection of the Pterostigma & Radius 1, posterior end of the Anterior Media, anterior end of the Anal vein, anterior end of the Cubital vein supplementary.

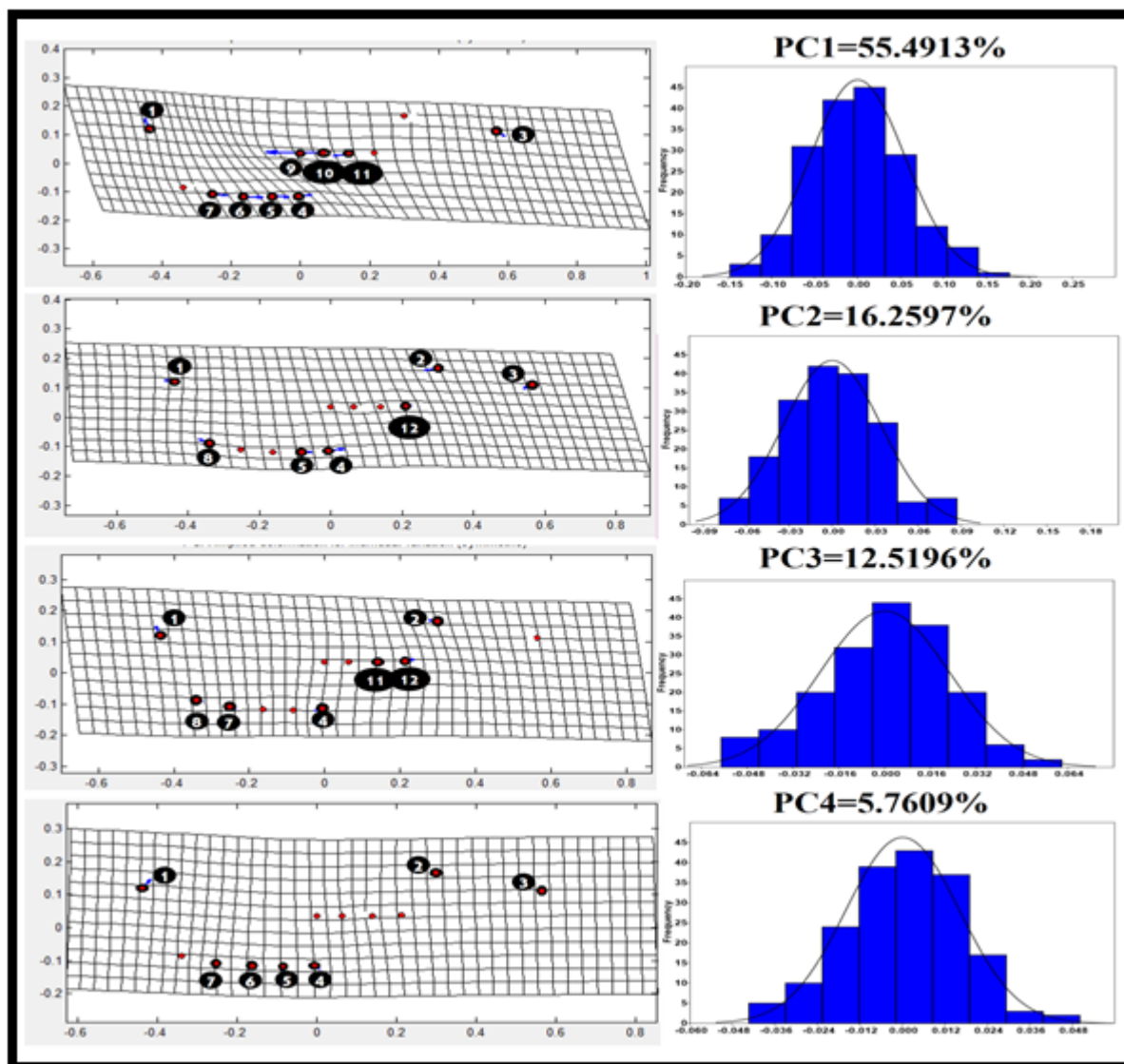


Fig. 4. Principal Component (PC) implied deformation grid and histogram of individual (Fluctuating Asymmetry) of female-forewings collected at high altitude.

The male hindwings have 92.08% of FA constituting only two principal component scores (PC) accounting to 90.30%. The common affected landmarks among the two PC score were 1,2,3,4,5,6,7,8,9,10 and 11. These were the anterior and posterior regions of the damselflies wings. It was observed that male

displayed highest percentage of fluctuating asymmetry (FA) in the hindwings when compared to female. The affected landmarks of the female and male forewings and hindwings were shown in deformation grid and histogram of the values displayed asymmetry in the wing form (Fig. 4, 5, 6, & 7).

On the other hand, the principal component analysis (PCA) for the collected female and male samples at lower altitude presented in (Table 8 & 9). In the forewings, female have 84.03% of FA constituting three principal component scores (PC) accounting to 79.34%. The common affected landmarks among

the three PC score were 1, 2, and 3. These were the portion of anterior end of the arculus, the Nodus and posterior intersection of the Pterostigma & Radius 1. While male forewings have 83.83% of FA constituting four principal component scores (PC) accounting to 85.27%.

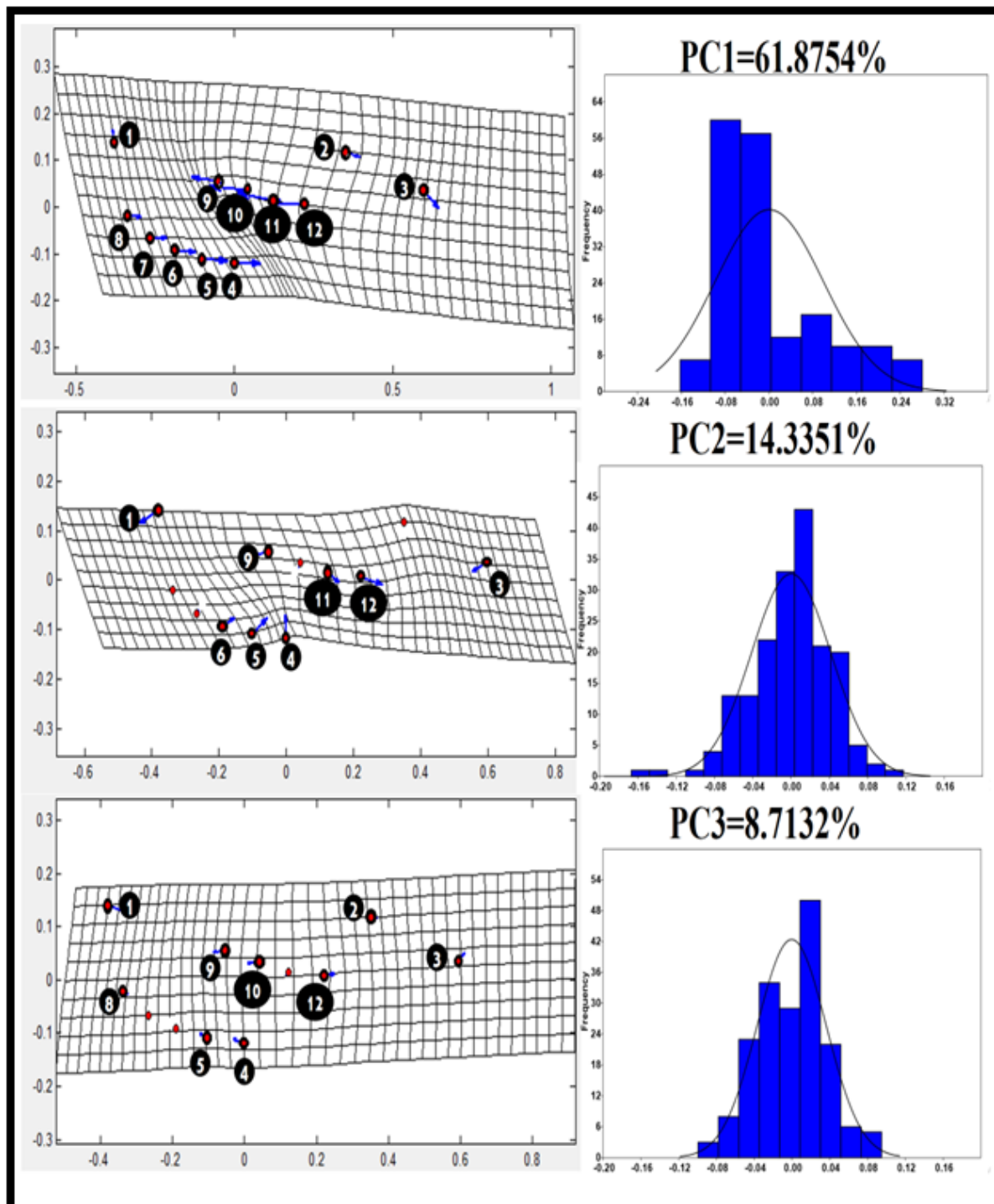


Fig. 5. Principal Component (PC) implied deformation grid and histogram of individual (Fluctuating Asymmetry) of male-forewings collected at high altitude.

The common affected landmarks among the four PC score were 1 (anterior end of the arculus) and 4 (posterior end of the Radius 4). Whereas, the female hindwings have (94.14%) of FA constituting three principal component scores (PC) accounting to 92%.

The common affected landmarks 1, 4 and 9. These were the portion of anterior end of the arculus, posterior end of the Radius 4 and anterior end of the anal vein.

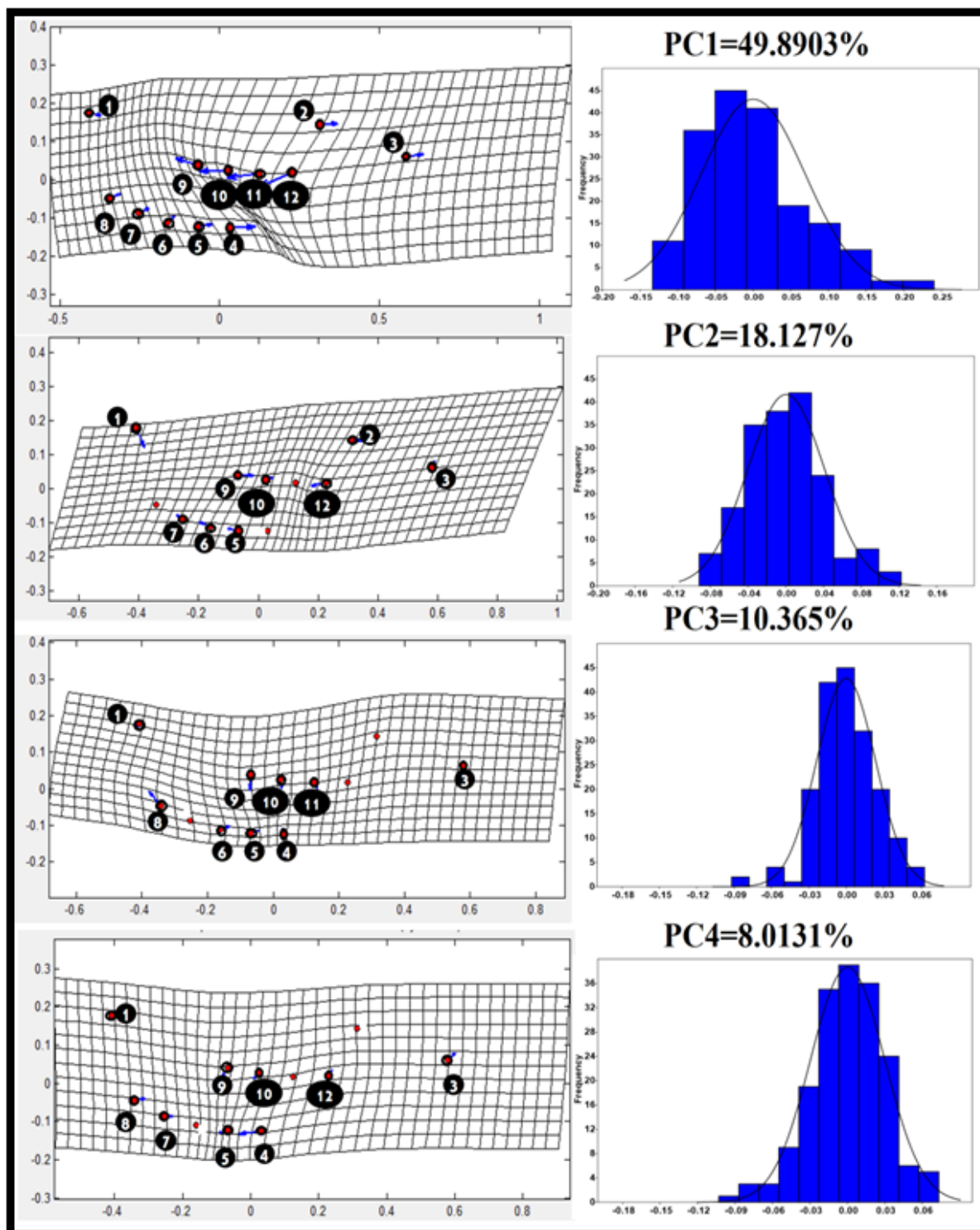


Fig. 6. Principal Component (PC) implied deformation grid and histogram of individual (Fluctuating Asymmetry) of female-hindwings collected at high altitude.

The male hindwings have 86.76% constituting four principal component scores (PC) accounting to 86.76%. The common affected landmark among the four PC score were 1 (anterior end of the arculus) and 4 (Posterior end of the Radius 4). It was observed that female had the highest percentage of FA when

compared to male hindwings. The affected landmarks of the female and male forewings and hindwings were shown in deformation grid and histogram of the values displayed asymmetry in the wing form (Fig.8, 9, 10 & 11).

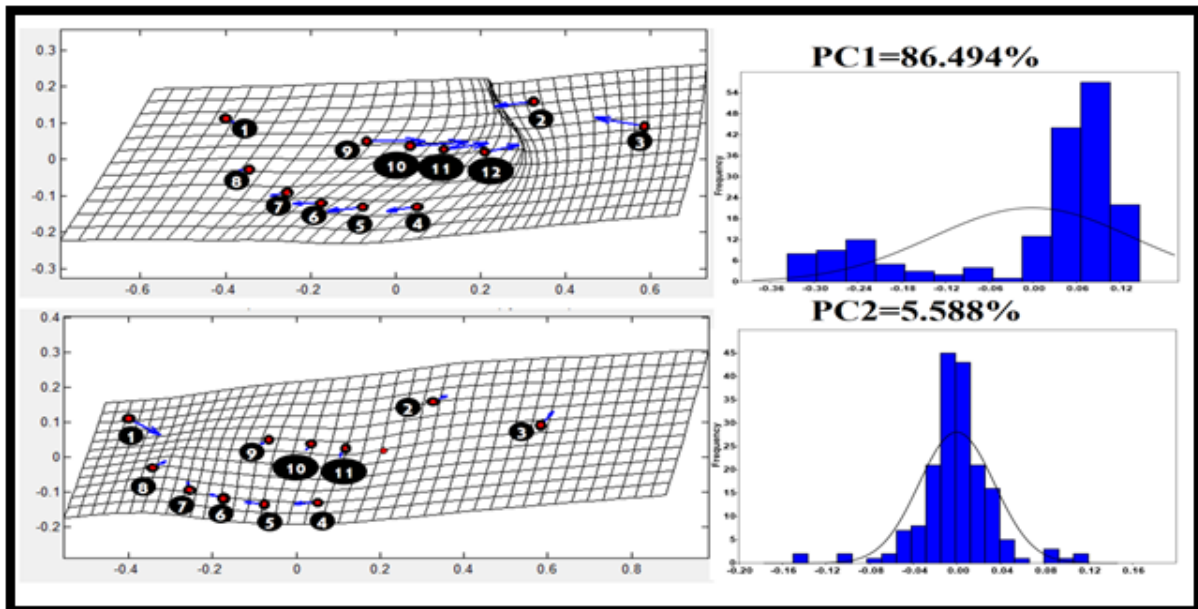


Fig. 7. Principal Component (PC) implied deformation grid and histogram of individual (Fluctuating Asymmetry) of male-hindwings collected at high altitude.

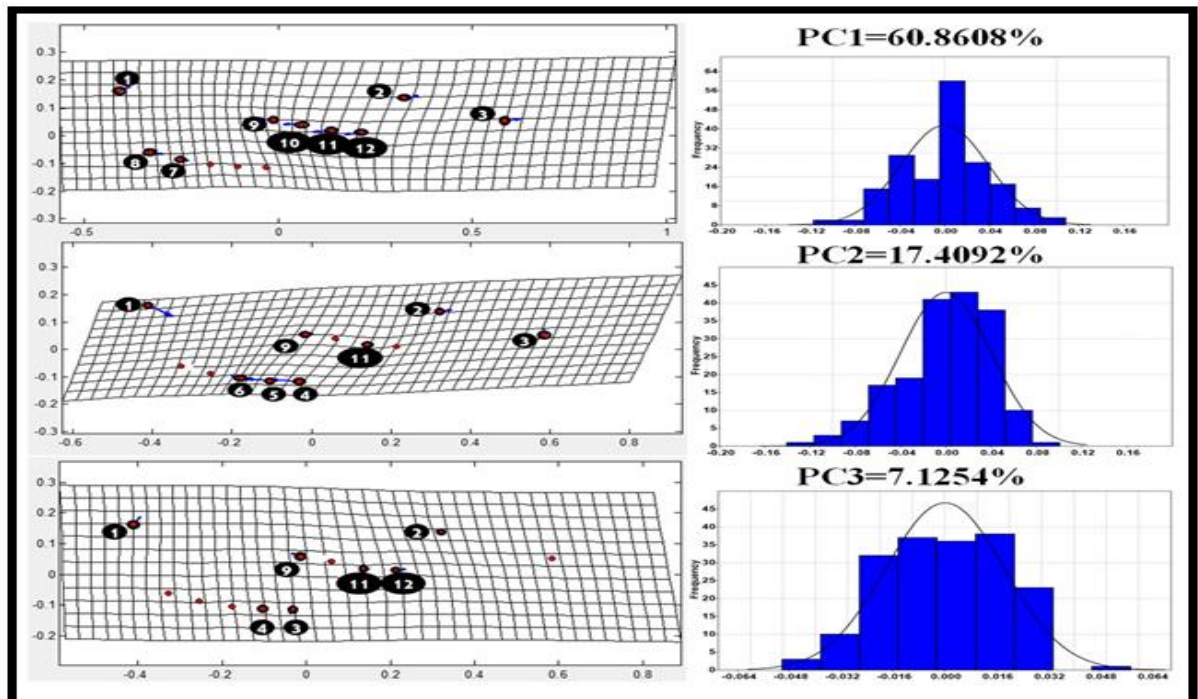


Fig. 8. Principal Component (PC) implied deformation grid and histogram of individual (Fluctuating Asymmetry) of female-forewings collected at low altitude.

Generally, the collected samples of *C. splendens* in the high altitude establishes highest percentage of fluctuating asymmetry when compared to low altitude. While the common affected landmarks

between female and male were anterior end of the arcus, the Nodus and posterior end of the Radius 4 that could be detected from the two altitudes.

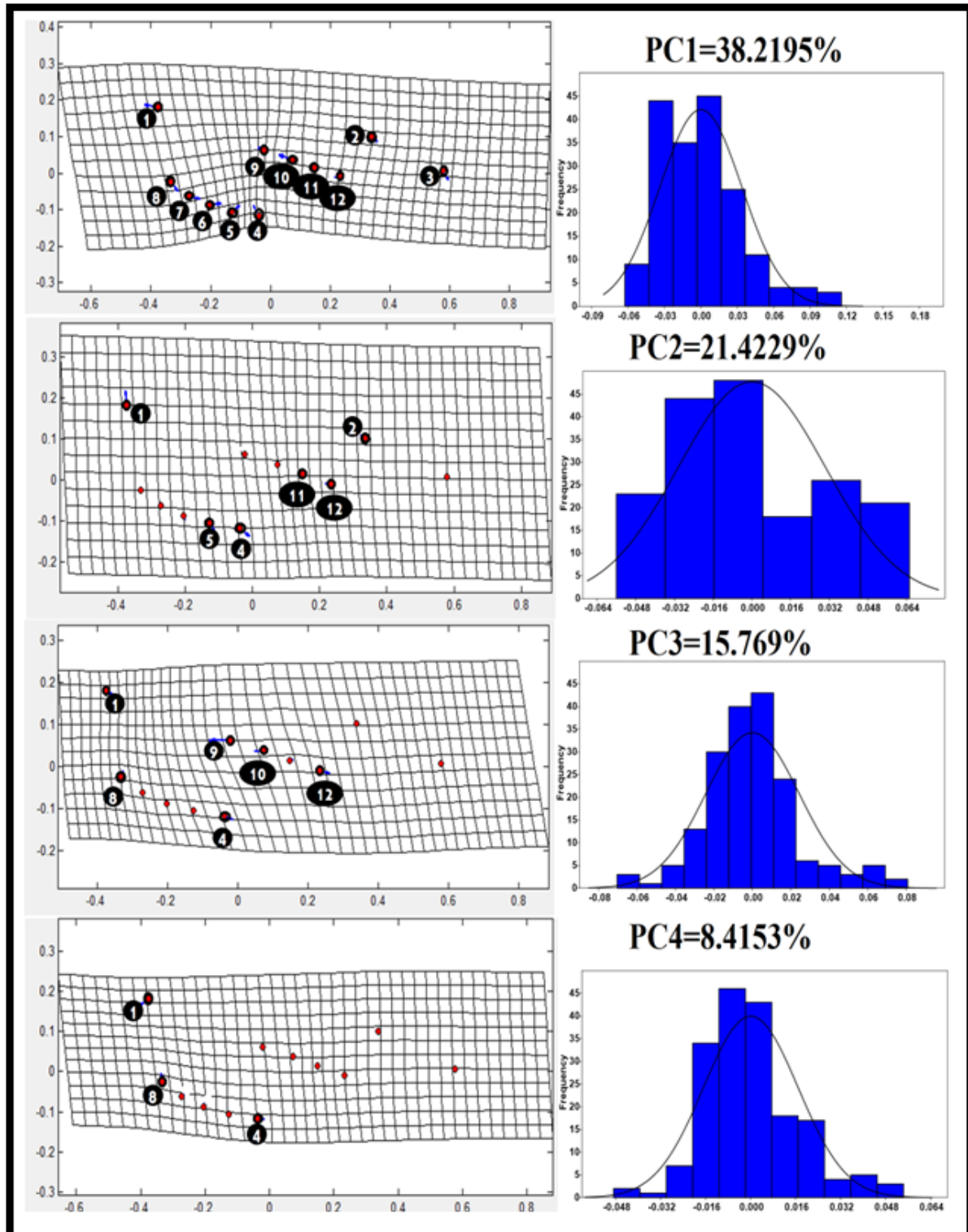


Fig. 9. Principal Component (PC) implied deformation grid and histogram of individual (Fluctuating Asymmetry) of male-forewings collected at low altitude.

It signifies that organism's inhabited great elevation requires more energy utilizing the wings to compensate toward food hunting and predation pressures (Stoks, 2001; Svensson and Friberg, 2007) and sexual causation (Outomuro and Johansson 2011) thus enhances wing shape variability. Accordingly, the wing shape dissimilarities of *Euphea* damselflies could be associated with flight

performance, dispersal capacities, and foraging skills (Hayashi, 1990). Furthermore, Odonata has been a long source of wing shape variations including wing venation pattern and this constitutes a basis of taxonomic classification (Rehn, 2003). Also, flying insects that had longer and thinner wings were estimated to be efficient during flight performance and faster kinematics (Norberg, 1989).

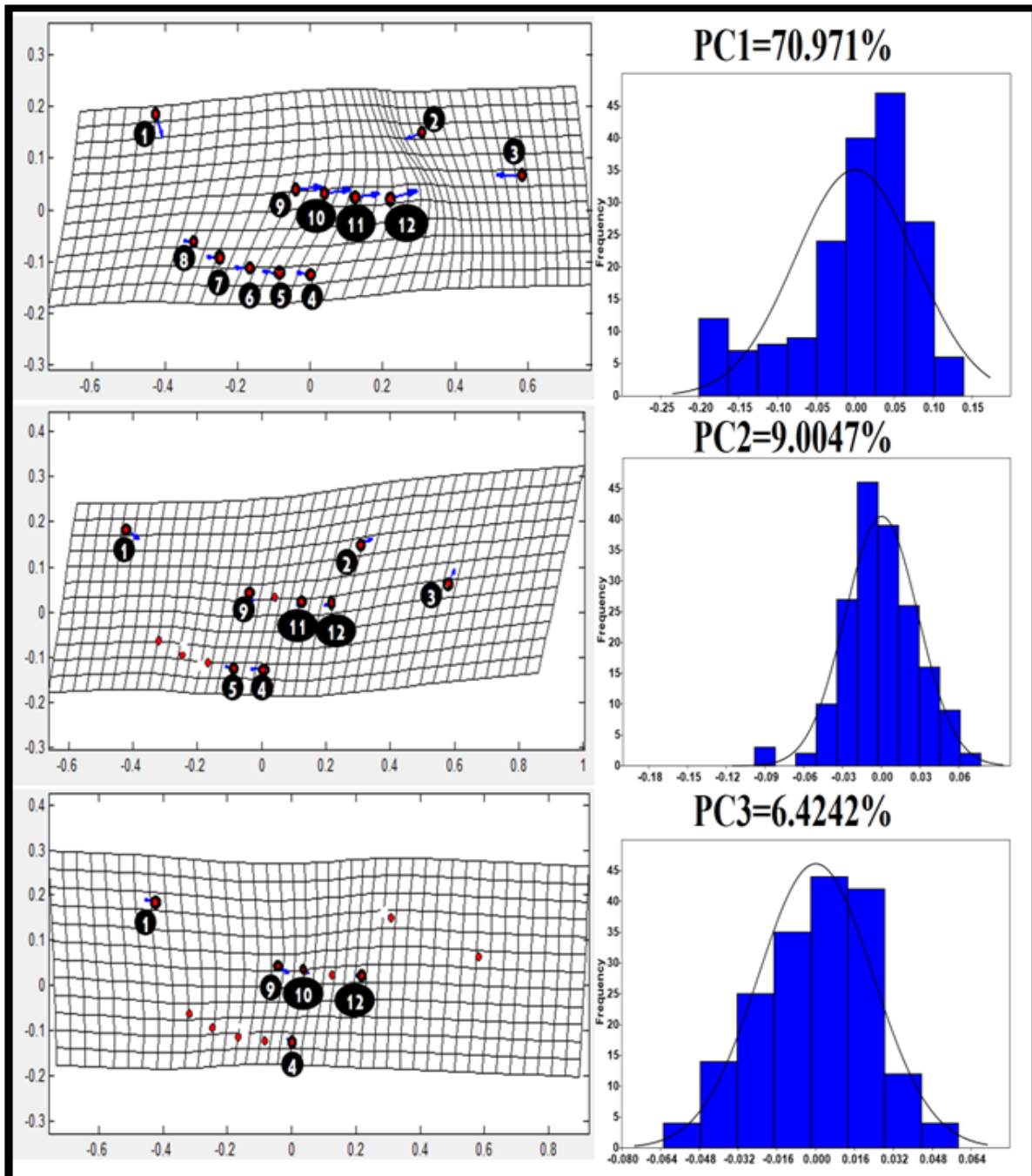


Fig. 10. Principal Component (PC) implied deformation grid and histogram of individual (Fluctuating Asymmetry) of female-hindwings collected at low altitude.

Indeed, Calopterygid damselflies exhibit trait specific environment; it indicates intraspecific wing shape variation (Abbot, 2005). Likewise, several studies also proposed that sexual selection and latitudinal gradient (Outomuro and Johansson, 2011) predation and nutrient requirements (Stoks, 2001; Svensson

and Friberg, 2007) and human induced pressures comprising landscape creation (Taylor and Merriam, 1995) suggested affecting wing shapes evolution in damselflies. Nonetheless, the results suggests that wing shape variations among and within species of the same taxa are common.

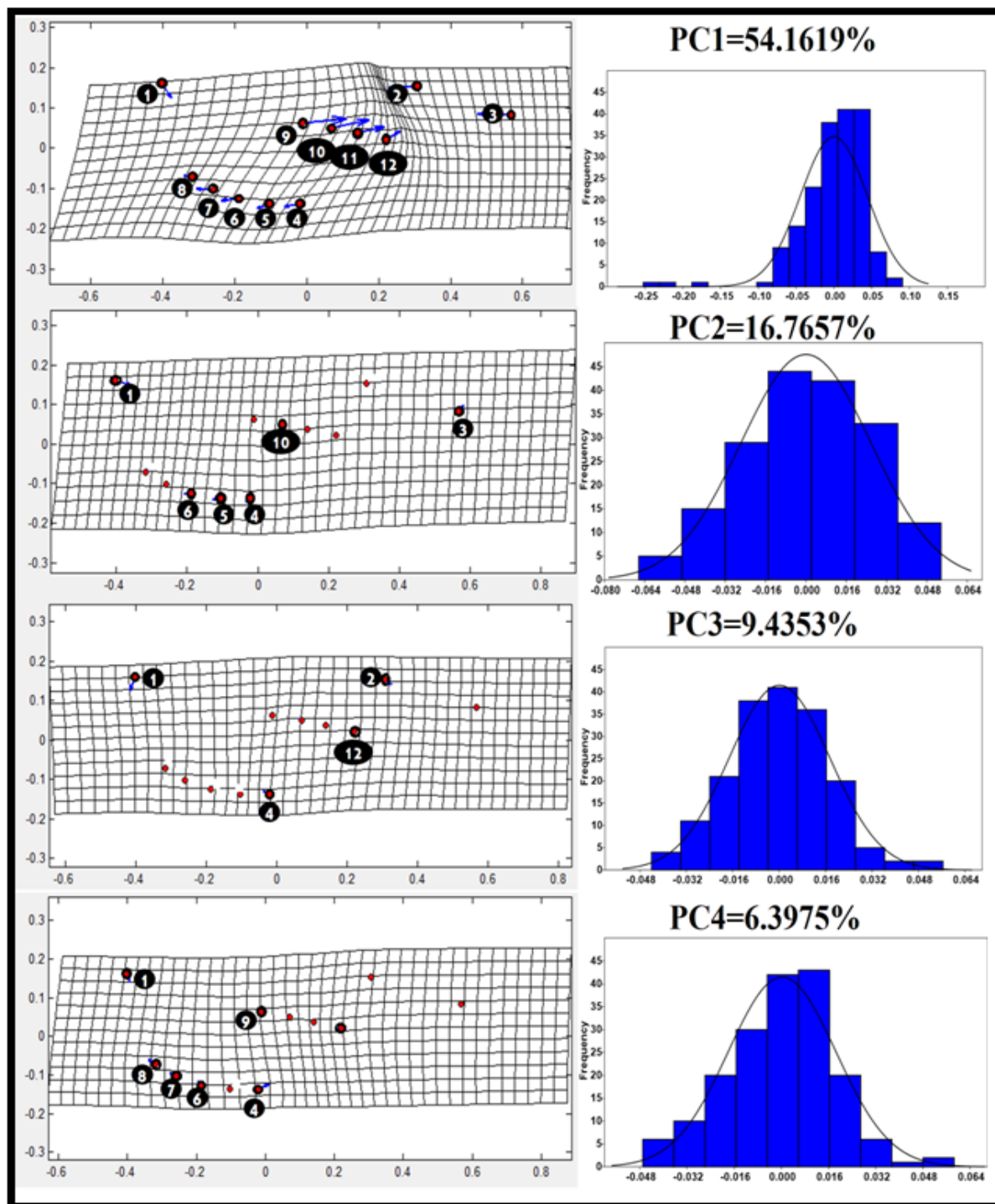


Fig. 11. Principal Component (PC) implied deformation grid and histogram of individual (Fluctuating Asymmetry) of male-hindwings collected at low altitude.

Similarly, comparative studies also performed wing shape analysis in damselfly (European *Calopteryx splendens*) to discriminate variation within its population (Sadeghi *et al.*, 2009), flight morphological differentiation (*Enallagma cyathigerum*, Bots *et al.*, 2009), development in wing shape (Johansson *et al.*, 2009); influences of elevation (high altitude & low altitude) and wing shape dissimilarity (*Calopteryx virgo meridionalis*, Outomuro and Johansson, 2011). Meanwhile, body size and wing shape also attributed to the evolutionary development such the bottleneck effect, founder effect, and genetic drift (Lomolino *et al.*, 2005; Whittaker and Fernández-Palacios, 2007). Moreover, (Mpho *et al.*, 2000) emphasize that developmental instability of the species may be due to environmental influences: climatic variations, food insufficiency, pesticides and parasitism and genetically derived conditions: novel mutants, hybridization, and inbreeding. Likewise, extreme precipitation and temperature are the outcomes of climate change; these create potential source of wing shape modification and likely to establish a threat to this family (Palmer and Raisanen, 2002; Karl and Trenberth, 2003).

The PCA implied deformation for individual variation (fluctuating asymmetry) were shown in (Fig.4, 5, 6, 7, 8, 9, 10 & 11). These illustrate the affected landmarks of individual damselflies that later causes FA levels in its wing shape. Accordingly, insect's flight performance are attributed to its fitness while complemented by the wing shape (Sadeghi *et al.*, 2009). Moreover, differentiation in the body form and wings are essential factors that could influence the capability to inhabit environments, resist predators and successfully reproduce (Gatz, 1979; Losos and Sinervo, 1989; Walker, 1997; Nagel and Schuler, 1998.) In general, the observation of wing shape dissimilarities could be associated to the interrelationship between organisms and its environment that would likely results to phenotypic variability. The wide-ranging manifestation of wing shape dissimilarities is pervasive due to geographic

variation (Mayr, 1963), and often results to an adaptation of organisms to confined environments, limiting biotic and abiotic components. Thus, the mechanism of adaptation are a probable cause of organisms to derive into sub-speciation, yet this phenomenon is difficult to understand (Mc Peek, 1990; Ricklefs and Miles, 1994).

Further, the application of Geometric Morphometrics in determining shape variation advances to illustrate the intraspecific morphological differences. The obtained data illustrated the importance of the application to draw significant wing differences in the female and male population of *C. splendens*. Indeed, insect wings provide foremost source of taxonomic classification.

Conclusion

The potential of fluctuating asymmetry (FA) in measuring developmental variability and indicator of environmental related stressor is an advancement to quantify phenotypic variances among species of the same taxa. In the three factors analyzed result shows that the collected samples within the high altitude displayed statistically significant ($P < 0.0001$) in the female forewings and hindwings when compared to male forewings and hindwings.

The collected samples within the lower altitude result show statistically significant ($P < 0.0001$) in the female and male forewings when compared to female and male hindwings. It implies that environmental causation is direct component affecting phenotypic differences of the species. Latitudinal gradients could also a factor affecting wing shape variations.

The considerable FA levels were an indication that variation of the wing size and shape among *C. splendens* is widespread and could associate with flight performance, fitness and sexual selection. Thus, using geometric morphometrics is efficient to quantify shape differences and could establish taxonomic classification within species of the same ancestry.

References

- Abbott JC.** 2005. Dragonflies and Damselflies of Texas and the South Central United States. Princeton: Princeton University Press. 344 p.
- Arnqvist G, Rowe L.** 2005. Sexual conflict. Princeton University. Press, Princeton, NJ.
- Bechley G.** 2002. Accessed 05 January, www.bechly.de/phylosys.htm.
- Bergstrom CA, Reimchen TE.** 2002. Geographical variation in asymmetry in *Gasterosteus aculeatus*. Biological Journal of the Linnean Society 77, 9-22.
- Betts CR, Wootton RJ, Biology.** 1988. Wing shape and flight behavior in butterflies (Lepidoptera: Papilionoidea and Hesperioidea): A preliminary analysis. Journal of Experimental 138, 271-288.
- Bots J, Breuker CJ, Van Kerkhove A, Van Dongen S, De Bruyn L, Van Gossum H.** 2009. Variation in flight morphology in a female polymorphic damselfly: intraspecific, intrasexual, and seasonal differences. Canadian Journal of Zoology. 87, 86-94.
- Cabuga CC Jr, Masendo CBE, Hernando BJH, Joseph CCD, Velasco JPB, Angco MKA, Ayaton MA, Obenza OLP, Jumawan JH, Jumawan JC, Requieron EA, Torres MAJ, Havana HC.** 2016. Relative warp analysis in determining morphological variation and sexual dimorphism between sexes of flathead goby (*Glossogobius giuris*). Computational Ecology and Software. 6(3):109-119.
- Carle FL.** 1982. Society International Odonatologica. (Rapid Communication.) 4(66).
- Carpentero ER, Tabugo SRM.** 2014 Determining developmental instability via fluctuating asymmetry in the shell shape of *Arctica islandica* Linn. 1767 (Ocean quahog). European Journal of Zoological Research 3(3), 1-7.
- Clarke GM, Brand GW, Whitten MJ.** 1986. Fluctuating asymmetry: A technique for measuring developmental stress caused by inbreeding. Australian Journal of Biological Sciences. 39, 145-153.
- Demayo CG, Harun SA, Torres MAJ.** 2011. Procrustes analysis of wing shape divergence among sibling species of *Neurothemis* Dragonflies, Australian Journal of Basic and Applied Sciences. 5(6), 748-759.
- Dudley R.** 2000. The Biomechanics of Insect Flight: Form, Function, Evolution. Princeton University Press.
- Fairbairn DJ, Blanckenhorn WU & Székely, T.** 2007. Sex, size and gender roles: evolutionary studies of sexual dimorphism. Oxford University Press, Oxford, UK.
- Fraser FC.** 1957. A reclassification of the order Odonata, Royal Zoological Society of New South Wales, Sydney, Australia.
- Gangestad SW, Thornhill R.** 1997. The evolutionary psychology of extrapair sex: the role of fluctuating asymmetry. Evolution and Human Behaviour. 18(2), 69-88.
- Gatz AJ.** 1979. Community organization in fishes as indicated by morphological features. Evolution. 60, 711-718.
- Green AJ.** 2000. The scaling and selection of sexually dimorphic characters: an example using the Marbled Teal. Journal of Avian Biology 31, 345-350.
- Groditsky DL.** 1999. Form and Function of Insect Wings: the Evolution of Biological Structures. Johns Hopkins University Press.
- Hammer O, Harper DAT, Ryan PD.** 2001 Past: Paleontological statistics software package for education and data analysis. Palaeontological Electronica 4(1), 1-9.

- Hayashi F.** 1990. Convergence of insular dwarfism in damselflies (*Euphaea*) and dobsonflies (*Protothermes*). *Freshwater Biology*. **23**, 219-231.
- Hennig W.** 1981. *Insect phylogeny*, Wiley, New York.
- Hermita JM, Gorospe JG, Torres MAJ, Lumasag JL, Demayo CG.** 2013. Fluctuating asymmetry in the body shape of the mottled spinefoot fish, *Siganus fuscescens* (Houttuyn, 1782) collected from different bays in Mindanao Island, Philippines. *Science International (Lahore)* **25(4)**, 857-861.
- Johansson F, Soderquist 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.
- Karl TR, Trenberth KE.** 2003. Modern global climate change. *Science*. **302**, 1719-1723.
- Klingenberg CP, McIntyre GS.** 1998. Geometric morphometrics of developmental instability: analysing patterns of fluctuating asymmetry with Procrustes methods. *Evolution*. **52(5)**, 1363-1375.
- Kokko H, Brooks R.** 2003. Sexy to die for? Sexual selection and the risk of extinction. *Annual Zoologica Fennici*, **40**, 207-219.
- Lee YH, Lin CP.** 2012. Morphometric and genetic differentiation of two sibling gossamer-wing damselflies, *Euphaea formosa* and *E. yayeyamana*, and adaptive trait divergence in subtropical East Asian islands. *Journal of Insect Science* **12(53)**, 1-17.
- Lomolino MV, Riddle BR, Brown JH.** 2005. *Biogeography*, 3rd edition. Sinauer Associates.
- Losos JB, Sinervo B.** 1989. The effect of morphology and perch size on sprint performance in *Anolis* lizards. *Journal Experimental Biology*. **145**, 23-30.
- Marquez E.** 2007. *Sage: symmetry and asymmetry in geometric data version 1.05* (compiled 09/17/08). Available online at: www.personal.umich.edu/~emarquez/morph/
- Mayr E.** 1963. *Animal species and evolution*. Harvard University. Press, Cambridge.
- Mc Nab BK.** 1994. Energy conservation and the evolution of flight lessness in birds. *The American Naturalist* **144**, 628-642.
- Mcpeek MA.** 1990. Behavioral differences between *Enallagma* species (Odonata) influencing differential vulnerability to predators. *Ecology* **71**, 1714-1726.
- Nagel L, Schluter D.** 1998. Body size, natural selection, and speciation in sticklebacks. *Evolution* **52**, 209-218.
- Natividad EC, Dalundong AO, Ecot J, Requieron EA, Jumawan JH, Torres MJ.** 2015. Ecological health status of Goby (*Glossogobius celebius*) using fluctuating asymmetry in Lake Sebu, South Cotabato, Philippines. *AACL Bioflux*. **8(3)**, 323-331.
- Norberg UM.** 1989. *Vertebrate Flight, Mechanics, Physiology, Morphology, Ecology and Evolution*. Berlin: **322**, Springer-Verlag.
- Outomuro D, Johansson F.** 2011. The effects of latitude, body size, and sexual selection on wing shape in a damselfly. *Biological Journal of the Linnean Society*, **102**, 263-274. www.dx.doi.org/10.1111/j.1095-8312.2010.01591.x
- Palmer AR, Strobeck C.** 1986. Fluctuating asymmetry - measurement, analysis, patterns. *Annual Review Ecology and Systematics* **17**, 391-421.
- Palmer TN, Raisen J.** 2002. Quantifying the risk of extreme seasonal precipitation events in a changing climate. *Nature* **415**, 512-514.

- Palmer AR, Strobeck C.** 2003. Fluctuating asymmetry analyses revisited. In *Developmental instability: Causes and consequences*. Polak M. (Ed), Oxford University Press, New York. 1-77.
- Pfau HK.** 1991. *Advances Odonatology*. **5**, 109-141.
- Rankin DJ, Arnqvist G.** 2008. Sexual dimorphism is associated with population fitness in the seed beetle *Callosobruchus maculatus*. *Evolution*. **62**, 622-630.
- Rehn AC.** 2003. Phylogenetic analysis of higher-level relationships of Odonata. *Systematics Entomology* **28**, 181-239.
- Ribak G, Pitts ML, Wilkinson GS, Swallow JG.** 2009. Wing shape, wing size, and sexual dimorphism in eye-span in stalk-eyed flies (Diopsidae). *Biological Journal of the Linnean Society* **98**, 860-871.
- Riget FF, Bechshoft TG, Wiig O, Soone C.** 2008. Fluctuating asymmetry in metric traits; a practical example of calculating asymmetry, measurement error and repeatability. *Annual Zoologica Fennici*. **15**, 32-38.
- Sadeghi S, Adriaens D, Dumont HJ.** 2009. Geometric morphometric analysis of wing shape variation in ten European populations of *Calopteryx splendens* (Harris, 1782) (Zygoptera: Odonata). *Odonatologica* **38**, 343-360.
- Samuels ML, Casella G, McCabe GP.** 1991. Interpreting blocks and random factors: Rejoinder. *Journal of the American Statistical Association* **86**, 798-808.
- Savriama Y, Gomez JM, Perfectti F, Klingenberg CP.** 2012. Geometric morphometrics of corolla shape: dissecting components of symmetric and asymmetric variation in *Erysimum mediohispanicum* (Brassicaceae). *New Phytologist* **196**, 945-954.
- Schlichting CD, Pigliucci M.** 1998. Phenotypic evolution: A reaction norm perspective. *Phenotypic evolution: A reaction norm perspective*. 387.
- Stoks R.** 2001. Food stress and predator-induced stress shape developmental performance in a damselfly. *Oecologia* **127**, 222-229.
- Svensson EI, Friberg M.** 2007. Selective predation on wing morphology in sympatric damselflies. *The American Naturalist*. **170**, 101-112.
- Taylor PD, Merriam G.** 1995. Wing morphology of a forest damselfly is related to landscape structure. *Oikos* **73**, 43-48.
- Tillyard RJ, Fraser FC.** 1940. A reclassification of the order Odonata based on some new interpretations of the venation of the dragonfly wing. *Australian Zoologist* **9**, 124-396.
- Trueman WH.** 1996. *Odonatologica*, **2**, 559-72.
- Van Valen L.** 1962 A study of fluctuating asymmetry. *Evolution*. **16**:125-142.
- Via S, Lande R.** 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution* **39**, 505-522.
- Walker JA.** 1997. Ecological morphology of lacustrine three spine stickleback *Gasterosteus aculeatus* L. (Gastersteidae) body shape. *Biological Journal of Linnean Society*. **61**, 3-50.
- Whittaker RJ, Fernández-Palacios JM.** 2007. *Island Biogeography: Ecology, Evolution and Conservation*. Oxford University Press.
- Willig MR, Kaufmann DM, Stevens RD.** 2003. Latitudinal gradients of biodiversity: pattern, process, scale and synthesis. *Annual Review Ecological Evolution of Systematics*. **34**, 273-309.

Wootton RJ, Kukalová-Peck J. 2000. Flight adaptations in Palaeozoic Palaeoptera (Insecta). *Biological Reviews* **75**, 129-167.

Yuto CMM, Lumogdang L, Tabugo SRM. 2016. Fluctuating asymmetry as an Indicator of Ecological Stress in *Rhinocypha colorata* (Odonata: Chlorocyphidae) in Iligan City, Mindanao, Philippines. *Entomology and Applied Science Letters*. **3(1)**, 13-20.

Zakharov VM. 1992. Population phenogenetics. Analysis of developmental stability in natural population. *Acta Zoologica Fennica*. **191**, 7-30.