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Chiropteran Composition and Morphometric Analysis of Ptenochirus Jagori in Caraga State University, Caraga Region, Philippines

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Abstract

Due to potential and present ecological alteration in roosting sites and food source areas in neighboring vicinity of Caraga State University in Caraga Region, Philippines, unsettled progression of bats are documented through wing variation analysis and fluctuating asymmetry (FA). Twenty-one (21) collected samples of Ptenochirus jagori (Greater Musky Fruit Bat) yields asymmetry of 90.6396%, affecting landmarks of the elbow (landmark 1), carpals (landmark 2), metacarpals (landmark 3, 4, and 10), and phalanges' fingers (landmarks 5, 6, 11, and 12). Wing variation (landmark-based) analysis suggested significant skewness on both elbows and metacarpals of wings. Wider shape deformation is on the right-wing, and a narrower one was analyzed on the left. Nonetheless, even though similar alteration is present between both wings, Canonical Variation Analysis (CVA) suggests no significant difference in the deformations of histogram analysis.

Keywords: abundance, asymmetry, Ptenochirus jagori, wing variation analysis

Order Chiroptera is the second most diverse and abundant order of mammals (Hutson et al., 2001). It contains 1,100 species divided into two suborders; the echolocating Microchiroptera and non-echolocating Megachiroptera (Simmons, 2005; Murray & Kunz, 2005). Bats (Chiroptera) play an essential role in many environments for excellent pollinators and pest control by devouring insects (Jones et al., 2009). Bats are nocturnal mammals and are a unique taxonomic group with their ability to fly by using patagia modified forelimbs that support skin membranes (Teeling, 2009; de Camargo & de Oliviera, 2012).

Philippines is the world's second-largest archipelagic country with over 7,634 distinct islands. Bats are the most diverse mammal in this archipelago, with 48 microchiropteran and 25 megachiropteran species (Heaney et al., 1998). However, habitat loss has been the main threat to bats' decline in population worldwide, and it has been predicted that at the end of the 21st century, 74 % of the forest may be lost in Southeast Asia (Hutson et al., 2001; Sodhi et al., 2004). One implication is the slash and burns effects destroying vegetation cover that might lead to bat species casualties (Fenton & Rautenbach, 1998). Besides, hunting and deforestation result in threatening to over 50% of Megachiroptera (Mendoza & Mallari, 1997).

Due to anthropogenic threatening to bats, they are classified as bioindicators due to their indication of ecological changes; agricultural intensification, loss and fragmentation of forest habitats, and habitat pollution that could also affect bat population and their activities (Jones et al., 2009). Albeit, a modern technique involving the digitalization of images has been recently used to analyze the fauna's developmental instabilities by understanding its shape concerning ecological and genetic stresses. Geometric morphometrics has been used to analyze shapes about understanding the diversity caused by biological means of phenotypic transformations, especially as a measurement of developmental instability (Aroza et al., 2001).

Fluctuating asymmetry (FA) is essential because it echoes the population's condition of adjusting and mirrors individual quality. It rises under both ecological and genetic stress (Waddington, 1942). The presence of symmetry is a significant advantage of fluctuating asymmetry over other indicators of developmental instability. The fundamental hypothesis of fluctuating asymmetry analysis is that the perfect symmetry in bilaterally paired structures is affected by its phenotypic variation; thus, non-directional contrast between the sides must be the environmental origin and reflect accident development (Jose et al., 2015). Landmark-based geometric morphometrics is a substantial approach to measure biological structures, shape variation, and correlated variation with other organism factors (Webster & Sheets, 2010). Also, it undertakes the collection of dimensional coordinates of definite landmarks (Lawing & Polly, 2009).

Forested-type habitats within Caraga State University's vicinity and neighboring areas are among the flight route and food providers, with the presence of fruits to bats. Due to existing and potential human-induced stress and habitat contraction in the area, this study aims to identify the abundance and existing threat of bats to assess their ecological responses through asymmetry and wing variation analysis.

Materials and Methods

Study Site

Bat collection was conducted within the forested-type vicinity of Caraga State University, Caraga Region, Philippines. It has geographical coordinate of 8.9560° of North and 125.5968° of South. Roosting sites in the area are not present for bat species, but it is a route and a site for food. Hence, collected bats are potentially roosting and nests at neighboring Barangays of the sampling site. *Ptenochirus jagori* species was subjected for wing variation asymmetry and analysis because of its abundance in the area as evident to the total count of collected species.

Bat Collection

Five six-meter mist nets were strategically distributed in the three sampling sites, all in a forested-type sampling site, in March 2019. The mist nets were set at 1600 H before the bats are active and periodically checked after eight (8) to ten (10) hours within two (2) to three (3) days of the occurrence to obtain at least 100 hours of sampling. Captured species were preserved in bird bags, and pictures were taken with different angles for documentation, verification, and validation.

Morphometric Measurements and Analysis

Six (6) morphometric measurements were measured for species identification and classification following the standard morphological characteristics of Ingle and Heaney (1992); head body (HB, from snout to tail length), forearm (FA, length of the elbow to carpal), length of the tibia (TIB), ear length (E), tale vent length and hindfoot (HF) length. Individual species were marked with nail polish to avoid repetition of samples from site and were released immediately. Through direct observation of the study sites, none of these were roosting sites for chiropteran species, which may be related to less than thirty (30) individuals of *Ptenochirus jagori* measured and analyzed.

Species Composition and Relative Abundance

Bats species' composition (SC) and relative abundance (RA) were determined and computed for analyzing the exact composition of bats within the sampling area.

Processing of Bat Samples

Digital imaging was done for all Ptenochirus jagori samples, both the left and right-wing using a Lumix GF6 digital single-lens reflex (DSLR) camera. Specimens were uniformly photographed so as the magnification, distance, and three replicates to reduce measurement error. Also, proper labeling of specimens were done. After the in-site processing, each fruit bat was fed with sugar syrup for energy build-up before they are released.

Digitization and Landmark Selection

The .jpeg form of images (photographed in three replicates) was converted as thin-plate spline (TPS) files using the tpsUtil2 program v1.44 (Rohlf, 2009). Inputting photographed wings (jpeg) into tpsUtil software and importing it to .tps files is a primary element for landmark selection using another software. It can be maneuvered through setting input and output directory and operation to desired file importation. Using (TPS) series tpsDig program v2.12 (Rohlf, 2008), the imported .tps files can now be assigned with anatomical landmarks to provide a standardized outline of body morphology.

Results and Discussion

Bat Species Composition

Thirty-one (31) bat individuals belonging to four species from Family Pteropodidae, were collected from the selected forested-type sampling areas of Caraga State University in Caraga Region. Among the collection, *Ptenochirus jagori* has the highest relative abundance of 67.74%, followed by the Common Short-nosed Fruit Bat, Cynopterus brachyotis, having an abundance of 25.80%.

On the other hand, *Macroglossus minimus* and *Eonycteris spelaea* have the lowest number of collected samples having one individual in every species.

Table 1. Bat species collected in Caraga State University, Caraga Region, Philippines.

Family	Scientific Name	Common Name	Total Number of Individuals
Pteropodidae	*Ptenochirus jagori*	Greater Musky Fruit Bat	21
	Cynopterus brachyotis	Common Short-nosed Fruit Bat	8
	Eonycteris spelaea	Common Nectar Bat	1
	Macroglossus minimus	Dagger-toothed Long-nosed Fruit Bat	1
		Total	**31**

Due to the frugivorous and nectarivorous dietary behaviour (Luft et al., 2003), food sources are among the high attribution to the bat species' presence in the area. In direct observations and documentation, the abundance of fruit trees within the sampling sites such as Cacao *(Theobroma cacao)*, Caimito *(Chrysophyllum caimito)*, Baluno *(Mangifera caesia)*, Santol *(Sandoricum koetjape)*, Guyabano *(Annona muricata)*, Macopa *(Syzygium samarangense)*, Mango *(Mangifera indica)* and Mabolo *(Diospyros blancoi)* can be a good chiropteran food source.

Fluctuating Asymmetry

FA analysis was examined as a biomarker of stress and an indicator of developmental instability in investigating the left and right wings of P. jagori. Three significant principal components with a total variation of 90.64% were accounted for asymmetry scores (Table 2.). Common affected landmarks are documented throughout the statistical analysis of both wings. Affected landmarks are documented from the elbow (landmark 1), carpal (landmark 2), metacarpals (landmarks 3, 4, and 10), phalanges of the third finger (landmarks 5 and 6), and phalanges of the fifth finger (landmarks 11 and 12).

Environment and genetic factors assume a considerable job in swerving the standard's phenotype (Palmer, 1994). FA has been used in estimating ecological stress and developmental instability; thus, FA is impartially possible as a bioindicator of stress in P. jagori (Jose et al., 2015). Nevertheless, the results showed a high percentage of FA. Human habitats change, noise, and disturbing activities are the potential factors that may unsettle their development.

Table 2. Principal component scores showing the values of symmetry and asymmetry scores with the summary of the affected landmarks.

PCA	Individual (Symmetry)	Sides (Directional Symmetry)	Interaction (Fluctuat-ing Asymmetry	Affected andmarks
PC1	75.9089%	100%	61.1989%	1,2,3,4,5,6,7,8,9,10,11,12
PC2	10.2919%		21.7883%	1,2,3,4,5,6,7,10,11,12
PC3	4.9333%		7.6524%	1,2,3,4,5,6,8,10,11,12

Landmark-based Analysis

Deformation of grids in the body shapes were analyzed through the frequency of histogram of relative warp (RW). Asymmetrical traits were yielding along the extremely positive and negative warps in the samples from the different disturbance levels. Tables 3 & 4 consists of the detailed description and the shape variation analysis in each warp. Relative Warp Analysis (RWA) for the right-wing exhibits that the total variation analyzed by the Relative Warp was 83.89% (Table 3), also its consensus morphology best illustrates the proportion of the wing (Figure 1). RW1 explained well the differences of the shape at 61.35 %, strived after by RW2 with 11.84% and to end, RW3 with 10.70%.

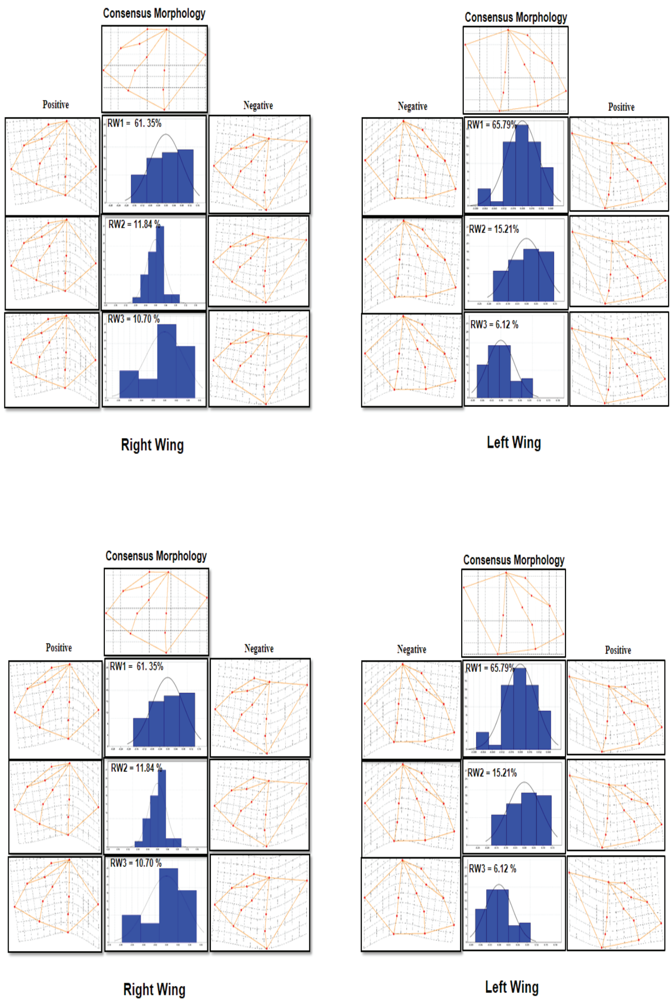

Figure 1. Summary of the Geometric Morphometric Analysis of the right wing and left-wing shapes showing the consensus morphology (topmost) and the variation in the body shape on P. jagori from neighbouring Barangays surrounding Caraga State University, Caraga Region, Philippines.

Table 3. Variation observed in the right wings of P. jagori from Caraga State University.

	Variance (%)	Positive(+)	Negative(-)
RW1	61.35%	Shows variation on the elbow, and between the metacarpals (landmarks 3 & 4) and phalanges of the third and fourth finger are shorter than the consensus morphology. The overall shape of the bat wing is narrow compared to RW1-.	Accounts the differences in the landmark of the carpal where it is shorter and narrow, and the metacarpals (landmarks 3, 4, 7 & 10) are narrow and shorter compared to RW1+. Overall shape of the wing is wider.
RW2	11.84%	Indicates the variation in the elbow, observable in the metacarpals (landmarks 4, 7 & 10) and the phalanges (landmarks 8, 9, 11 & 12) were shorter than the RW1-. In overall shape form of the bat wing is shorter.	Observes differences in the carpal and metacarpals (landmarks 3, 4, 7 & 10) are shorter, and some of the phalanges (landmarks 5, 8, 9, 11 & 12) are longer than the RW1+. The overall shape form is wider than RW1+.
RW3	10.70%	Displays a minimal upward curvature in the figure, the distance between the elbow and carpal is elongated, metacarpals (landmarks 3, 4, 7 & 10) were shorter and phalanges (landmarks 5, 9, 11 & 12) were shorter than the RW-.	Presents the variation in the figure; the carpal was lower than the RW1+, metacarpals (landmarks 4 & 7) were longer and elongated, and phalanges (landmarks 5, 9 & 12) are elongated that concludes on being wider than the RW1+.

Variation determined the differences through the histogram. RW1 and RW3 were observed to be skewed to the right (Figure 1).

Table 4. Variation observed in the left wings of P. jagori from Caraga State University.

	Variance (%)	Positive(+)	Negative(-)
RW1	65.79%	The length from the elbow to metacarpals is shorter. Landmarks (9 & 12) is wider than the RW1-. The length from the elbow to the phalanges of the fifth finger is shorter as observed. Its overall shape of the wing is wider. The overall shape of the wing is wider.	The length from the elbow to metacarpal is longer and the phalanges (landmarks 6, 9, & 12) is shorter compared to the RW1+. The length from elbow to the phalanges of the fifth finger is shorter as observed. The overall shape of the wing is narrower.
RW2	15.21%	Widening of variation was observed, however, the length of elbow to the metacarpals and the length of the elbow to the phalanges of the fifth finger were shorter as perceived.	The constrictions of points between the landmarks were observed and the distance between the elbow to phalanges (11 & 12) is longer compared to RW1+.
RW3	6.12%	Shows variations in elbow to metacarpals which is narrower and landmarks 9 & 12 and the length of elbow to phalanges of the fifth finger which is narrower as noticed.	Shows variations in elbow to metacarpals, landmarks 9 & 12 and the length of elbow to phalanges of the fifth finger which is shorter.

Precise variation in left-wing shape with a percentage of 65.79% indicates RW1, followed by RW2 with a significant rate of 15. 21%, and RW3 with 6.12%. Wider shape indicates that it has negative RW scores. Nonetheless, it shows a shorter measurement between elbows to metacarpal, meanwhile, a narrower shape indicates that it has positive RW scores. Nevertheless, it shows a longer measurement between elbow and metacarpal. Hence, variation distinctions were skewed in histogram analysis and examination. RW1, RW2, and RW3 were seen to be skewed to one side. This variation implies that the biological aggravations influenced samples. Also, possible factors such as human living space change, commotion, and irritating exercises are the potential factors that may disrupt their improvement (Jose et al., 2015). These factors are present during the chiropteran survey, and it may be a potential factor and implication to the analyzed wing variation.

Canonical Variation Analysis

CVA plot suggests no significant relation of both the right and left-wing of the collected *Ptenochirus jagori* species in the area. These may have been an implication of the species' adaptation to ecological disturbances, while the concentration of environmental alteration inside and neighboring areas of Caraga State University is evident. These may be accounted to the area as the only food source for chiropteran species and not as a roosting site. These can be visualized in the MANOVA/CVA plot that proved to have a little relation, as shown in Figure 2.

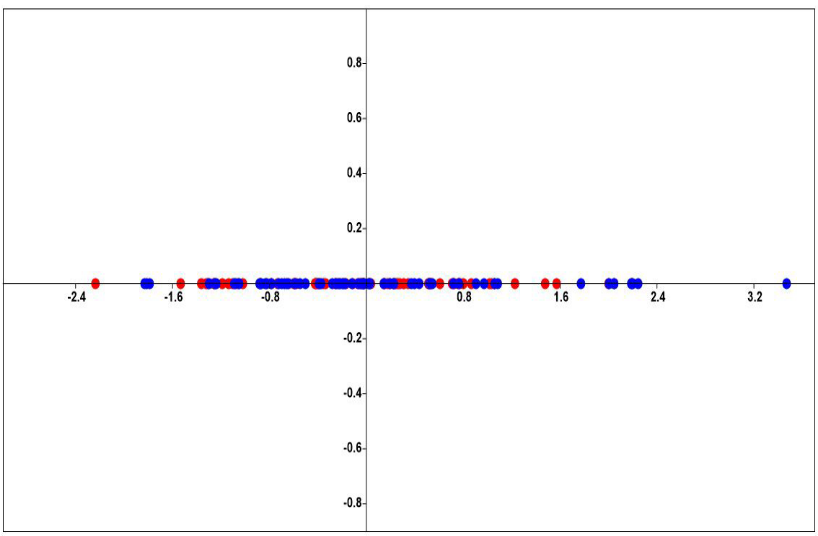

Figure 2. Canonical Variation Analysis (CVA) plot in the relative scores of both right and left wing of Ptenochirus jagori.

Structure and proportions of the wings of chiropterans are described as their powered flight's functional significance. Aerodynamical relationships of chiropteran species were applied and considered on mollosids and vespertilionids varying in their flight areas (Swartz et al., 2003). Geometric similarity on different species of bats was documented in the study of Thollesson & Norberg in 1991; only the morphological difference across wings of different bat species proportionally affects their flight and maneuverability for food capturing.

Summary of Findings

As among the route and food provider to chiropteran species, it is important to examine an in-depth relational analysis of human-induced stress and habitat contraction with bat species in the neighboring roosting sites in Caraga State University in Caraga Region, Philippines. Thus, environmental degradation's ecological impacts on wing variation analysis of the collected twenty-one (21) Ptenochirus jagori species are the study's objective.

Wing landmarks yielded significant FA values (90.64%) in the anatomical points of P. jagori based on Procrustes Analysis of Variance (ANOVA) of Symmetry and Asymmetry in Geometric Data (SAGE) program. Principal Component Analysis (PCA) distinguished the total variation of 90.64% affecting the elbow (landmark 1), carpal (landmark 2), and metacarpals (landmarks 3, 4, and 10), also phalanges of the third (landmarks 5 and 6) and fifth finger (landmarks 11 and 12).

Landmark-based geometric morphometric variation suggested common skewness to both the right and left-wing. Asymmetrical traits were yielding along both positive and negative warps in the samples from different disturbance levels. As also suggested by the RWA results, the total wing variation elucidated was 83.89% for the right-wing and 87.12% for the left-wing. Nonetheless, Canonical Variation Analysis (CVA) was suggested to have no significant relation of both wings, with P (same) and Wilks' Lambda of 1.

Results of wing variation analysis of collected P. jagori species vividly suggested different disturbances on chiropteran species. Human living space change, forested-type areas subjected to sizeable concrete construction, and agricultural expansion on the neighboring areas of the study site are impartially the possible and potential factors that may unsettle their progression.

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