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Skull shape variation of lesser musky fruit bat (*Ptenochirus minor*) in Lumakil, Polomolok, South Cotabato, Philippines

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Abstract. This study determines whether sexes of *Ptenochirus minor* can be determined base don skull shape variation using Relative Warp Analysis. Bat skulls were processed, cleaned and photographed. Digital images were taken and processed in TPS software extracting scores. Using a land-mark positioning method, the origin and evolutionary occurrence that affects the environmental adaptations of the bats were identified. Relative Warp Analysis was used to determine the shape variations regardless of the age, size and maturity. Using statistical analysis, results revealed insignificant differences because the score is higher than the accepted value.

Key Words: landmark, TPS software, geometric morphometrics, relative warp analysis.

Introduction. Evolutionary adaptation of a particular species is the result of the competition among individuals in response to its ever-changing environment. Species features like skull has an adaptive significance that may determine the species adaptation to various conditions. Physiological structures including the skull shape of the bats will be assessed to determine the evolution patterns due to their availability niches in bats including lesser musky fruit bat.

The lesser musky fruit bat (*Ptenochirus minor)* belongs to the suborder of Megachiroptera, the large herbivorous fruit bats. This species is said to be endemic in the Philippines (Ong et al 2008). They do not occur at urban and agricultural areas and apparently coexisting at upper elevations and sometimes they are misidentified with *Ptenochirus jagori* (Rickart et al 1993). Bats represented an important biocontrol agent for humans because they consume many insects and therefore prevent crop destruction, but their population declined due to destruction of lowland forest (Heaney et al 1998).

P. minor distribution are said to be rampant in the provinces of Luzon to the faunal region of Mindanao, it is also said to be terrestrial-common and widespread in a range of suitable habitats with the elevation of 1,000 m above sea level (Chiroptera Specialist Group 1996). It is common in primary lowland and mountain forest and sometimes present in moss forest, and can also be common in lightly degraded secondary forest (Heaney et al 1989). In a 2002, mist net survey on Mount Apo, Mindanao, *P. minor* was found to be uncommon in lower elevation mossy-mountain forest that have been subjected to habitat alteration. Due to misidentification of this species, this study provides curiosity to the researchers if indeed there is a significant difference among their skulls. In studying shape morphometrics, there is considerable interest in the evolution of morphological traits, and morphometric studies in combination with the multivariate theory of quantitative genetics. This can provide a detailed understanding of the variation and evolutionary potential of these traits (Klingeberg 2003). The geometric-morphometrics methods are the best available alternative to obtain size and shape components from any biological form or image (Bookstein 1991). Geometrical morphometrics consists of analyzing data representing particular points defined by some rule (landmarks) (Klingeberg 2003) as well as the geometric region on bats skull. Relative warps analysis (Bookstein 1991) method is performed using the software tpsRelw (Rohlf 1997). Relative warps analysis corresponds to a Principal Components analysis of the covariance matrix of the partial warp scores, which are different scales of a thin-plate spline transformation of landmarks (Frieß 2003). Bookstein gives a detailed presentation of the mathematical basis that can be applied for the analysis of the different variations in terms of skull shape of *P. minor*, a native bat found at Lumakil, Polomolok, South Cotabato. Shapes of bat skulls represented by zygomatic breadth divided by condylocanine length, vary between being as wide as they are long to being only a third of the skull length (Chiroptera Specialist Group 1996). It is not surprising that bat skulls can be short and wide or long and narrow, but it is of considerable evolutionary interest that the shortest, widest skull and the longest, narrowest skulls are formed in the same family. Thus, shape variations are identified provided that the skull components are complete.

Due to its beneficial characters and high adaptability in many terrestrial habitats, the researchers aim to provide a detailed presentation of the mathematical basis that can be applied intra variation of skull shape of *P. minor* to evaluate the reliability of Relative Warp Analysis as a tool evidences on their evolutionary pattern. This study wishes to validate the use of truss network system for discrimination of bat species as a tool to locate variations.

Material and Method

Sampling site. Sampling site is located in a particular area of Purok Islam, Baranggay Lumakil, Polomolok, South Cotabato, Philippines. Polomolok is one of the ten municipalities that make up the province of South Cotabato. It is situated 17 km north of General Santos City and 15 kilometres south of the Municipality of Tupi (southcotabato.gov.ph, 2003). It is located in the southernmost part of Mindanao Island, with a geographical coordinates of 6.13º North latitude and 125.3º East longitudes and covering total area of 340 km^2 (Figure 1).

Figure 1. Map of the Philippines (left); Map of South Cotabato, Municipality of Polomolok (center); Map of Brgy. Lumakil (right) (www.googlemaps.com).

Bat specimens. A total of 60 *P. minor* Pteropudidae (30 male, 30 female) were collected on 10th and 11th of August 2013 from an abandoned building in Polomolok (Figure 2). By the use of hand-held mist-nets measuring 58.7 cm long and 33 mm mesh size and a mist net trap (called "bobo" by native settlers) that measure 54 cm high, 155 cm long and 33 mm mesh size. Captured bats were mercifully subjected to chloroform for photography and identification purposes. Sex determinant gonads for both bat's sexes was morphologically observed (penis for males and vagina for females). Male bats were separated from female through the use of separated marked container. Bats in same sex were boiled together for five minutes to soften and loosen the tissues to remove them from the skull easily. Purely cleaned bat's skulls were placed into a 70% isopropyl alcohol solution for one day to disinfect and to suppress it from smelling. Skulls were then washed with detergent soap carefully and rinsed with tap water and bleached under the direct sunlight until it was dry. The left half dorsal view of the bat samples was used in the analysis to avoid the effect of bilateral asymmetry. Images of the bat samples were taken using Sony Alpha digital camera with 55 mm Micro lens at 7-8X magnification.

Figure 2. Digital image of *Ptenochirus minor* male (left) and female (right) (Yoshiyuki 1979).

To avoid the effect of bilateral asymmetry in examining and analyzing the whole species of *P. minor Pteropodidae*, 11 landmarks from the left half dorsal view of the skull were plotted (Figure 3). These landmarks are (1) center of posterior curvature of internasal opening, (2) proximal extremity of premaxilla, (3) point of maximum lateral curvature of maxilla at center of upper canine, (4) center of infraorbital foramen, (5) point of the maximum curvature (width) of the infraorbital plate, (6) point of postorbital constriction inside the orbit, (7) point where internal portion of the posterior zygoma contacts the cranium, (8) most posterior internal curvature of zygomatic arch, (9) most lateral point of the mastoid process, (10) most posterior point of interparietal, and (11) center of coronal suture. (Bogdanowicz & Owen 1996).

Figure 3. Land-marks positions on the half dorsal view (Klingeberg 2003).

Landmark selection and digitization. In processing the specimens, bat skulls were laid with a ruler placed below the side to calibrate the measurement starting the zero calibration on the posterior interparietal. Skulls were then photographed using a Sony Alpha digital camera with 55 mm Micro lens at 7-8X magnification. Photograph files were converted to tps files using tps utility. All images were then digitized of landmark points using the software tpsDig2 version (Requieron et al 2012). The data obtained after digitizing was analyzed using relative warp analysis and discriminant function analysis.

Shape analysis. Using advance software, the images were standardized in order to remove the biases in size, shape and rotation through the use of thin-plate spline function “tps” to fit the differences in the position of landmarks in an organism relation to their position in one another. The whole species were digitized using 11 homologous points, tpsDig2 version 2 that will extract x and y coordinates of the landmarks on the images. Procrustes superimposition method was used to address the variation of y and x coordinates of images by removing biases in size, shape and rotation. This method translates the centroid of the shapes to (0.0) (Bookstein 1991; Requieron et al 2012). Warping landmark coordinates showed depicted skull variations among specimen and differences in skull shape between sexes determined by the spline shape differences. The relative warp scores for both sexes were recorded and converted into MS Excel worksheet. This was used in analyzing intraspecific variation in skulls using Paleontological Statistics Analysis (PAST) software (Frieß 2003; Requieron et al 2012). This relative warp scores were used to determine values that will show multivariate shape data sets.

Discriminate Function Analysis (DFA) was used as defiant for the sexual dimorphism of musky fruit bat. P value was shown by the application of Hotelling’s t-squarred that analyze the equality of the means of the compared groups. Multivariate Analysis (MANOVA) was used for the evaluation of these relative warp scores. The significant values were obtained with the application of PAST software. The scores obtained were subjected to Microsoft excel and posted into PAST software and statistically, the significant scores were collected at significant value of $P>0.05$.

Results and Discussion

Variations and differences. The application of relative warp analysis showed an observable variations and differences between sexes of *P. minor*. The use of TPSrelww32 determines the median, the x and y coordinates. The variances between skulls are obviously observable. Females exhibit variations in the centre posterior of the mouth (RW1, RW2, RW3, RW4 and RW5). The comparison between the x and y coordinates and the located positive and negative hemisphere of the skull differs from one another starting from RW1 until RW5. *P. minor* females show differences in premaxilla of mouth region (RW2, RW3, RW4, and RW5), posterior zygoma (RW1, RW4), and infraorbital for arm (RW2, RW4, RW5). Males exhibited variations from premaxilla on mouth region (RW1, RW2, RW3, RW4, and RW5) to infraorbital foramen on eye region (RW1, RW2, RW3, and RW5). Results that were tabulated from relative warp analysis through determining consensus, partial warp and relative warp showed variations or sexual dimorphism among individuals.

Figure 4 depicts morphometric analysis of skull showing the variations amongst shape of female *P. minor* from Lumakil, Polomolok South Cotabato, Philippines. This is based on first 5 and 6 relative warp analysis determining at least 5% of variance. RW1 = 28.82%, RW2 = 23.91%, RW3 = 16.91%, RW4 = 11.83%, RW5 = 5.39%.

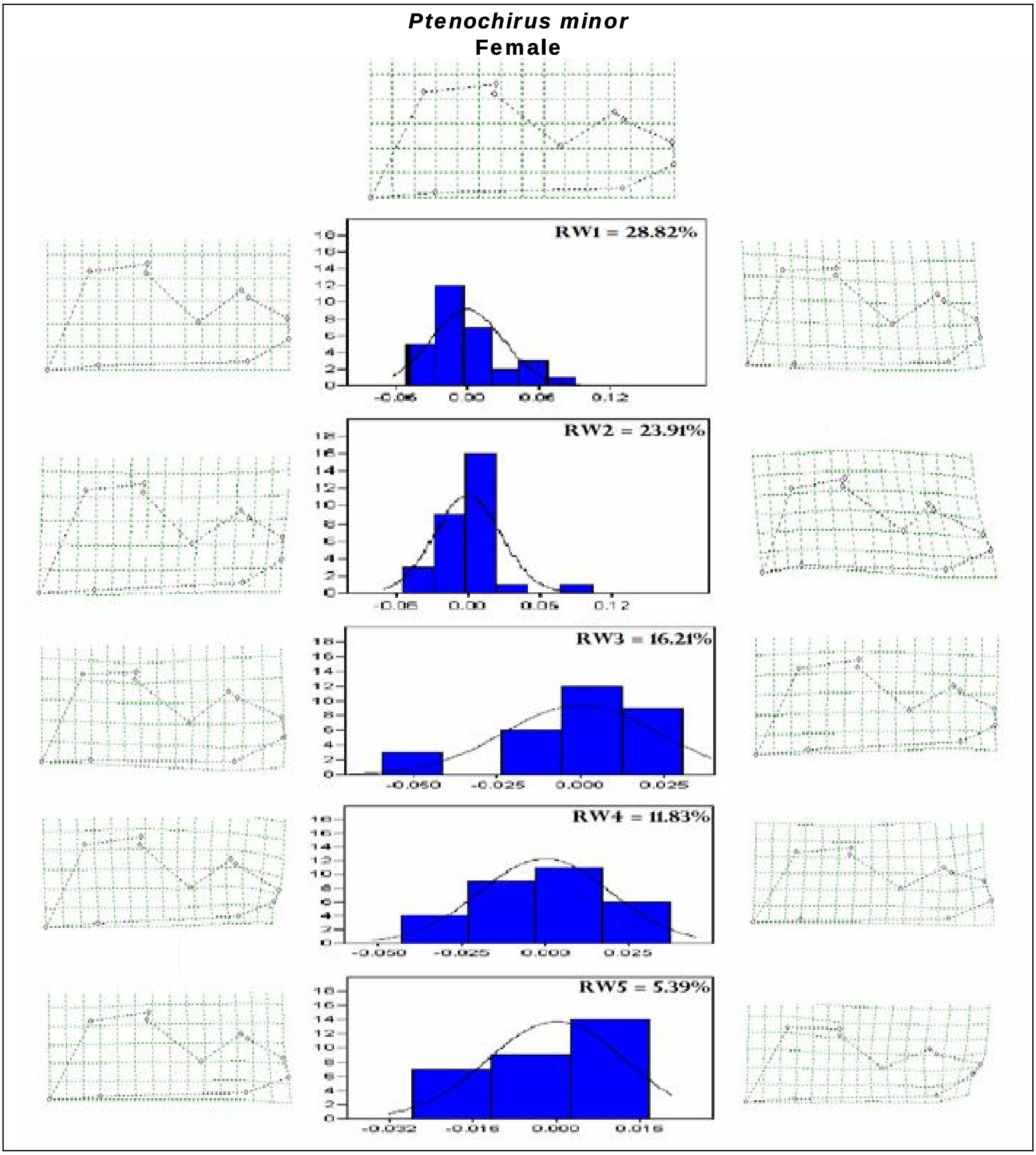

Figure 4. Summary of the geometric morphometrics in female *Ptenochirus minor* showing the relative warps and the variation in skull shapes.

Morphometric analysis of skull shows the variations among shape of male *P. minor* from Lumakil, Polomolok South Cotabato, Philippines. This is based on first 5 and 6 relative warp analysis determining at least 5% of variance. RW1 = 36.92%, RW2 = 25.69%, RW3 = 13.23%, RW4 = 6.49%, RW5 = 5.39% (Figure 5).

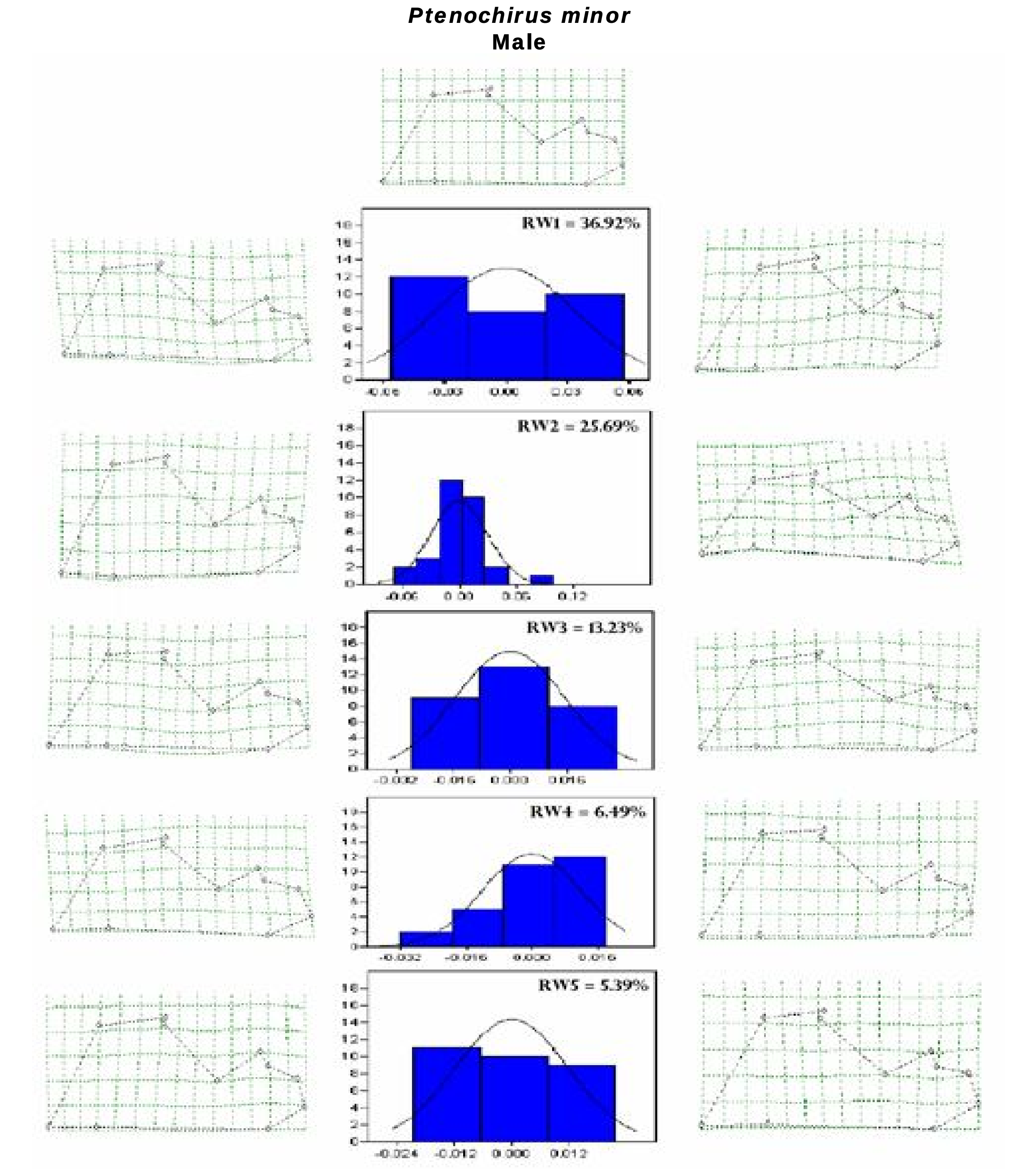

Figure 5. Summary of the geometric morphometrics in male *Ptenochirus minor* showing the relative warps and the variation in skull shapes.

Variation in the skull shapes of males and females evolved from high to low (Table 1). The highest values were located in RW1 with the value of 36.92% in males from positive extreme to posterior point of the interparietal. The lowest values were located in RW1 in males and females with the value of 5.39%. In males, variations are from extreme mastoid to uppercenter canine. While in the females, variations are from posterior orbital to proximal extremity.

Table 1

Variations in the skull shapes of male and female *Ptenochirus minor*

	Variation	*Male*	*Variation*	*Female*
RW1	36.92%	Variations from the positive extreme in most lateral point of the mostoid the process and negative extreme in the posterior point of the interparietal.	28.82%	Variations from the center posterior to center orbital forearm. Positive extreme has in the posterior internal curvature and negative extreme in the point of center posterior.
RW2	25.69%	Variations from positive extreme the point of mostoid process to point of center posterior in the mouth portion. Negative extreme mostly from point of dorsal interparietal to center coronal suture.	23.91%	Variation from positive extreme to upward from mostoid process. Negative extreme varies in the posterior zygoma cranium from maximum curvature to infraorbital plate.
RW3	13.23%	Variations in the posterior internal in zygomatic arch. Positive extreme from center posterior to center intraorbital. The negative extreme from the posterior interparietal to point or coronal suture.	16.91%	Variations from the center posterior portion. Positive extreme from the point of maximum curvature to the proximal extremity. Negative extreme from center of coronal suture to the posterior point of the interparietal.
RW4	9.49%	Variations from positive extreme mostoid process to zygomotic arch and negative extreme from center posterior to the center of infraorbital.	11.83%	Variations from positive cranium to the posterior orbital constriction inside the orbit. Negative extreme near from the posterior point of the interparietal goes through the center of corona and center posterior.
RW5	5.39%	Variation from positive extreme to the center posterior of proximal extremity. Negative extreme from the center intraorbital to uppercenter canine.	5.39%	Variations from positive extreme in the point of posterior orbital constriction inside the orbit. Negative extreme in the point of the maximum curvature of the infraorbital plate goes through the center orbital foramen to the proximal extremity.

Statistical analysis showed insignificant results in RW1, RW2, RW3, RW4 and RW5 (Table 2). This shows no variation between male and female bats of *P. Minor.* These warps showing insignificant scores mean that sexual dimorphism do not exists between male and female musky fruit bat. On the other hand, frequency distribution of the discriminant scores of the relative warp shows a values ranges from -0.0012 to 0.0006 (Figure 6). Consequently the five warps are insignificant because the score is higher than the accepted significant score. This means that no significant difference between male and female shape variation occurs on the left half dorsal view of the skull.

Table 2

Statistical result of male and female *Ptenochirus minor* from RW1 to RW5

H (chi^2):	0.9538
Hc (tie corrected):	0.9538
p(same):	0.9167

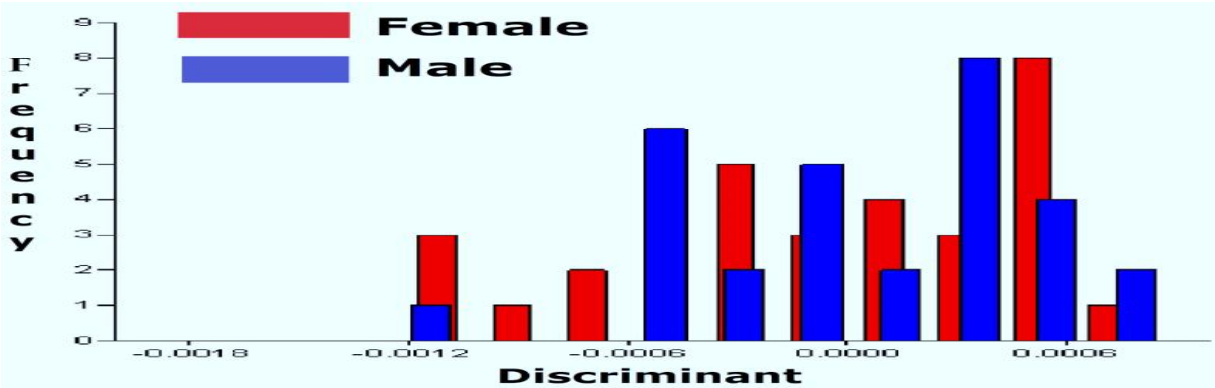

Figure 6. Frequency distribution of the discriminant scores (DFA) of the relative warp scores of highly significant scale variation between male and female *Ptenochirus minor.*

Conclusions. Based on the results, samples are qualitatively tasted under the use of statistical tools that made possible to distinguish the skull shape variation of bats in both sexes. This only proves how significant is the geometric morphometrics in studying variation and similarities of shapes of a sample.

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