Describing Variability in Wing Shapes among Three Populations of *Plesispareichei* Using Landmark-Based Geometric Morphometric Analysis

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***Abstract*—This study was conducted to describe variability in 3 populations of a coconut pest, *Plesispareichei* landmark-based geometric morphometric analysis of inner wing shapes. A total of 21 landmarks were used to represent dimensions in the left and right wings. Analysis of Variance, coordinate mapping, relative warp, Euclidean Distance Matrix and Cluster Analyses were used to analyze these landmarks. Results showed that significant variations were observed among populations. Variation in the left wing is mainly seen along the proximal landmark points but is variable in the right wing which may be an indication of asymmetry. Cluster analysis showed wing shape variations between populations indicating population differentiation in the pest. Distance was not a factor which may indicate differences in genetic structure between populations.**

***Index Terms*—Asymmetry, coconut beetle, landmarks, warp.**

I. Introduction

An emerging pest in Malaysia, *Plesispareichei* (Chapui) is considered as a potential pest of the coconut tree in Philippines [1]. The species is morphologically similar to *Brontispalongissima* (Gestro) [1], [2]. Outbreaks of *B. longissima* have been prevalent in the Philippines since it was detected in 2005 however due to similarity in appearance, outbreaks of *P. reichei* might have been misreported. Continuous high warmtropical weather and low natural enemy populations may have contributed as factors for outbreaks in many geographical areas where coconuts are planted in abundance [3]. The success of this winged insect to infest on coconuts leading to outbreaks can be attributed to the pest ability to transfer from plant to plant and from one area to another. It was therefore the objective of this study to describe population diversity by looking into variations in wing shapes which are known to be affected by environmental factors thereby affecting its feeding distribution by increasing reproductive success in an area [4].

Insect wings evolve rapidly and its development is thought to be controlled by one or a set of genes [5]-[7] and can be used to measure variation of a species across a geographic landscape [8]. We apply landmark-based geometric morphometric methods to measure the phenotypic variation in the wings [9]. Shape or reference points are reduced to a set of coordinates where statistical analyses are employed to permit comparison of shape similarities [10]. Changes in the shape and size of a structure in an organism may offer a better understanding to its development and evolution thus it is a useful tool to measure the phenotypic changes by which an organism adapts to a new environment [11]. Thus, understanding shape variations in the wings will allow a better understanding of diversity in pest populations which may contribute to the development of a better management of this pest.

II. Material and Methods

A. Collection and Identification

Adult coconut leaf beetles, *P. reichei*, were randomly collected from coconuts from three different locations in Mindanao Island, Philippines (Fig. 1). Pure ethyl alcohol was used as a preservative during transport. Identification of specimens was done according to Staines [12]. Individuals were then labeled by location and stored individually in Eppendorf tubes with 3-5 drops of pure ethyl alcohol.

B. Imaging and Landmarking of Specimens

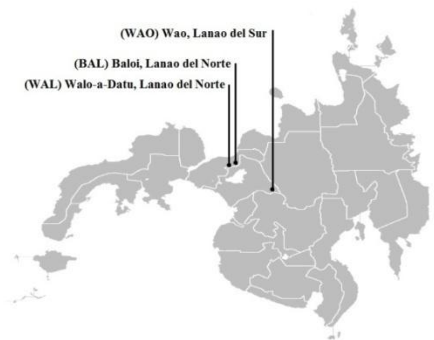

Fig. 1. Map of sampling sites in Mindanao Island, Philippines.

Images of the wings of the specimens was done by using a digital camera enhanced through a stereomicroscope. A total of 21 landmarkpoints were chosen (Fig. 2, Table I). These landmark points were chosen to represent major dimensions of the wings. These landmark points were established on the digitized images using tpsDig software v2.12 [13]. The landmark points are superimposed onto the image that generates two-dimensional coordinates. General Least

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Squares (GLS) Procrustes superimposition method was done to standardize the coordinates and to remove variation due to differences in translation, orientation and size [14], [15].

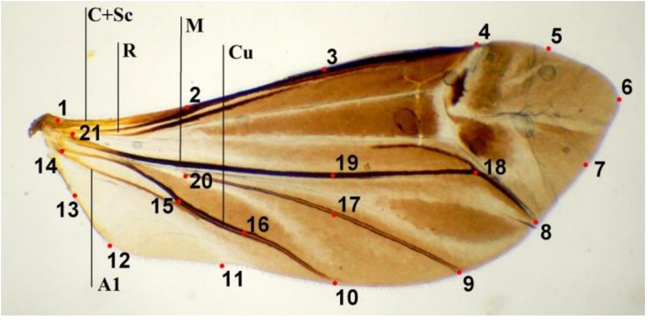

Fig. 2. *P. reichei* (Chapui) wing showing veins used as indicators of the landmark points. *Legend: C = Costa, Sc = Subcosta, R= Radius, M = Media, A = Anal.*

TABLE I: DESCRIPTIONS OF ANATOMICAL LANDMARK POINTS ON THE WING OF *PLESISPA REICHEI* (CHAPUI)

Landmark no. (LM)	Description	Landmark no. (LM)	Description
1	Proximal end of C+Sc	12	Distal end of proximal lateral margin
2	Distal end of C+Sc	13	Midpoint of proximal lateral margin
3	Midpoint of LM 2 and4	14	Proximal end of A1
4	Distal end of R	15	Distal end of A1
5	Midpoint of LM4 and 6	16	Midpoint of LM10 and 15
6	Distal point of wing	17	Midpoint of LM 9 and 20
7	Midpoint of LM 6 and 8	18	Curve of M
8	Distal end M	19	Midpoint of LM 18 and 20 on M
9	Distal end of wing fold	20	Proximal end of wing fold
10	Distal end of Cu	21	Proximal end of M
11	Midpoint of 10 and 12		

C. *Image Analysis*

Relative warp analysis using tpsRelW [16], following the algorithms developed by Bookstein [14] were used to describe variations in the wings within the populations based from a consensus or mean shape. Thin plate splines were then used to visualize the transformation of the wing by using the same software. Box plots were also generated to show the distribution of the populations away from the mean shape using PAST software 1.91 [17].

Euclidean Distance Matrix Analysis (EDMA) was done using PAST software 1.91 [17]. EDMA is a coordinate free approach in determining the correspondence between landmark points to further evaluates the changes between landmark points [18]. Principal Component Analysis (PCA) inherent in the EDMA method reduces data to determine which components retain the highest degree of the variation in the point to point analysis [19].

D. *Statistical Analysis*

The mean differences in wing centroid size among populations were determined and subjected to Analysis of Variance (ANOVA) and Tukey's test to show significant interactions between the populations using PaST software 1.91 [17]. A heatmap was also generated by using the same program to illustrate points of contraction and expansion. Cluster Analysis was used to compare similarities among populations based on centroid size.

III. RESULTS AND DISCUSSION

ANOVA results showed significant differences across the three populations of in the right wing *P. reichei* (Table II-Table III). Significant differences were also found in the left wing except between BAL and WAO populations (Table IV-Table V).

TABLE II: RESULTS OF ANOVA TEST FOR SIGNIFICANT VARIATION IN THE CENTROID SIZES OF THE RIGHT WING OF *P. REICHEI*

	Sum of squares	Df	Mean Squares	F	P(same)
Between groups	1.18×10^{06}	2	589425	14.99	1.36×10^{-06}
Within groups	5.19×10^{06}	132	39324.4		
Total	6.37×10^{06}	134			

TABLE III: TUKEY'S PAIRWISE COMPARISONS OF THE RIGHT WING BETWEEN THREE POPULATIONS OF *P. REICHEI*

	BAL	WAL	WAO
BAL		$\underline{2.18 \times 10^{-05}}$	$\underline{0.01}$
WAL	7.73		$\underline{0.04}$
WAO	4.29	3.44	

TABLE IV: RESULTS OF ANOVA TEST FOR SIGNIFICANT VARIATION IN THE CENTROID SIZES OF THE LEFT WING BETWEEN THREE POPULATIONS OF *P. REICHEI*

	Sum of squares	Df	Mean Squares	F	P(same)
Between groups	1.02×10^{06}	2	509379	16.36	4.51×10^{-07}
Within groups	4.11×10^{06}	132	31141.7		
Total	5.13×10^{06}	134			

TABLE V: TUKEY'S PAIRWISE COMPARISONS OF THE LEFT WING BETWEEN THREE POPULATIONS OF *P. REICHEI*

	BAL	WAL	WAO
BAL		$\underline{0}$	0.07
WAL	4.94		$\underline{2.18 \times 10^{-05}}$
WAO	3.08	8.02	

Relative warp analysis which compares wing morphology across the three populations shows three relative warps that were significant in both the left and right wings (Table VI and Table VII, Fig. 3 and Fig. 4). Variation between populations are also graphically shown in the inset box plots.

TABLE VI: VARIATION IN THE RIGHT WING BETWEEN THREE POPULATIONS OF *P. REICHEI* EXPLAINED BY THE SIGNIFICANT RELATIVE WARPS AND THE CORRESPONDING PERCENTAGE VARIANCE

Relative Warp	% Variance	Description
1	53.06	Variation along the lower and proximal margins of the wing
2	18.43	Variation along the distal margins of the wing
3	11.38	Variation along the upper margins of the wing

TABLE VII: VARIATION IN THE LEFT WING BETWEEN THREE POPULATIONS OF *P. REICHEI* EXPLAINED BY THE SIGNIFICANT RELATIVE WARPS AND THE CORRESPONDING PERCENTAGE VARIANCE

Relative Warp	% Variance	Description
1	57.49	Variation along the lower margins of the wing
2	17.22	Variation along the distal margins of the wing
3	6.88	Variation along the upper margins of the wing

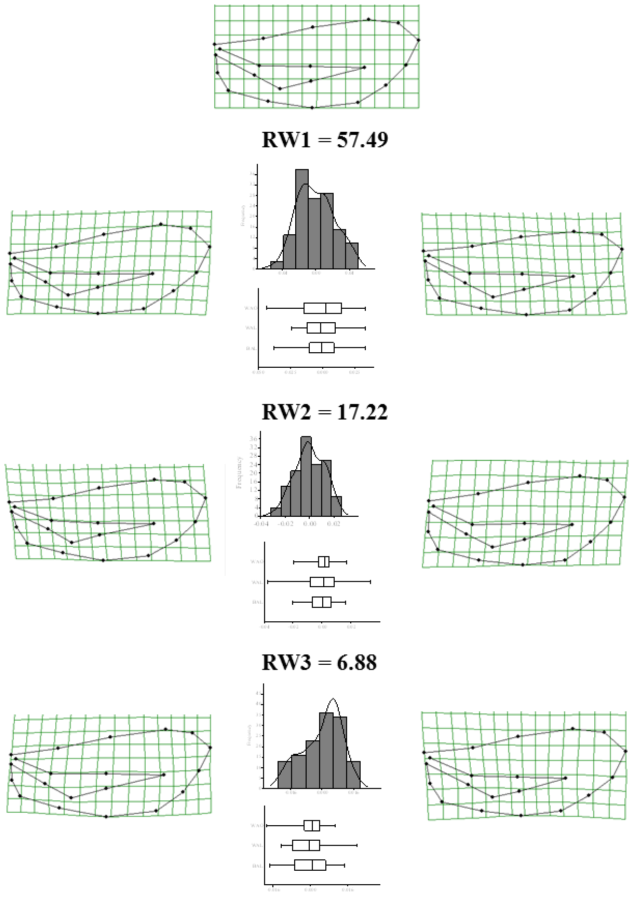

Fig. 3. Summary of the geometric morphometric relative warp analysis of the left wing showing the consensus morphology (uppermost panel) and the variation in the shapes of the pronotum among the three populations of *P. reichei*.

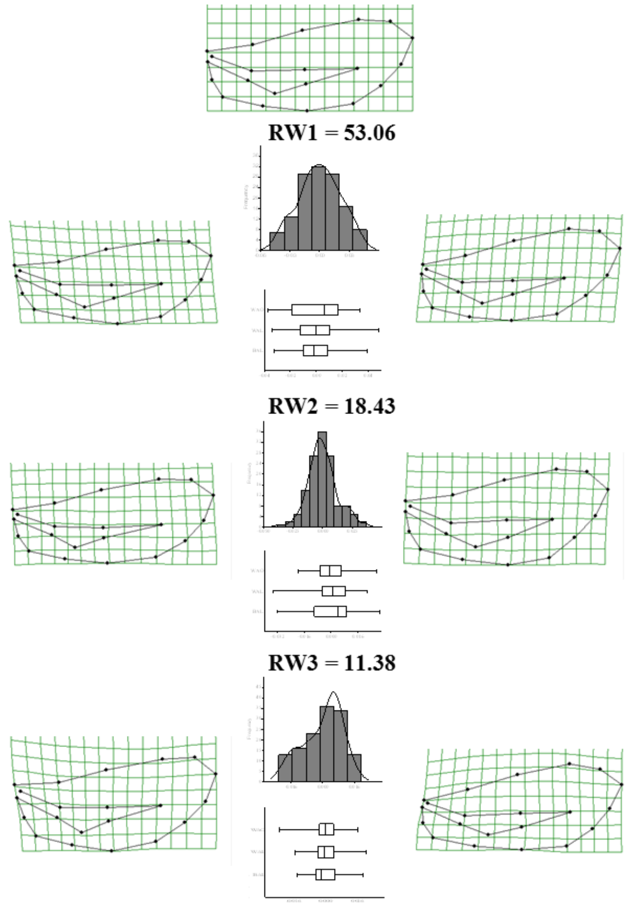

Fig. 4. Summary of the geometric morphometric relative warp analysis of the right wing showing the consensus morphology (uppermost panel) and the variation in the shapes of the pronotum among the three populations of *P. reichei*.

The first relative warp of the left wing (RW1=57.49) shows a variation along the lower and distal margin of the wing with a bimodal distribution by which a portion of all three populations contributes to a positively oriented lesser peak. The same is observed in the second relative warp (RW2=17.22). The last significant warp (RW3=6.88) shows also a bimodal distribution but the lesser peak is negatively oriented.

The right wing relative warp analysis shows that most of the population (RW1=53.06) is explained by a variation along the lower margin of the wing with a bimodal distribution with the lesser peak negatively oriented contributed mainly by WAO. The second relative warp (RW=18.43) has a trimodal distribution with the two lesser peaks oriented positively. The third significant relative warp shows a bimodal distribution with the lesser peak oriented negatively.

Coordinate maps of the landmark points in the insect wings were generated to visualize the coordinates across the populations to compare points of variation in the landmarks with a corresponding heatmap of expansion and contraction landmark points (Fig. 4 and Fig. 5). Contraction and expansion points in the right wing are seen on the proximal and distal ends of the wings whereas the left wing has a relatively neutral margin with the exception of contraction points in the distal ends. Euclidean Distance Matrix Analysis (EDMA) and Principal Component Analysis (PCA) further evaluate the distances between two landmark points to determine the landmark points that contribute to the changes in wing morphology.

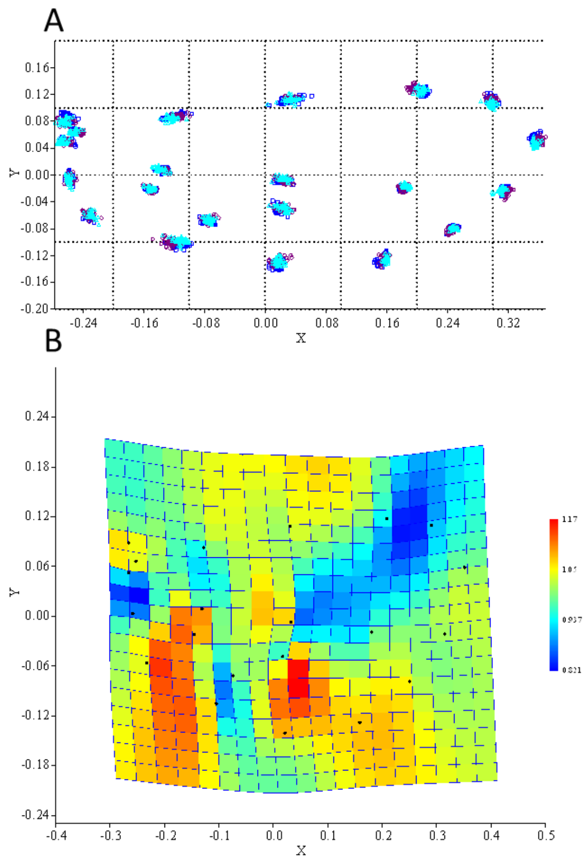

Fig. 5. Plot of landmark points of the right wing of *P. reichei* across populations (A) and (B) points of expansion (blue) and contraction (red) in the wing across three populations.

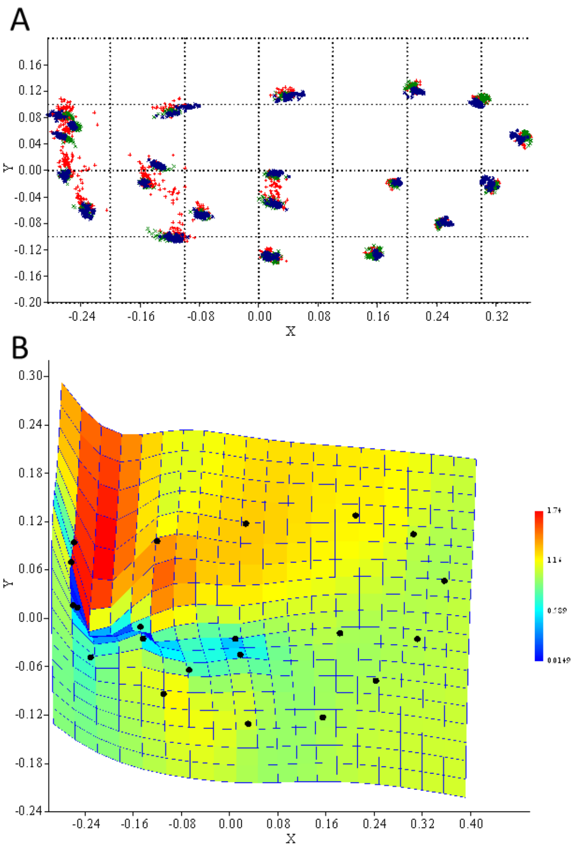

Fig. 6. Plot of landmark points of the left wing of *P. reichei* across populations (A) and (B) points of expansion (blue) and contraction (red) in the wing across three populations.

TABLE VIII: INTER-LANDMARK (LM) DISTANCES WITH THE TOP 10% PCA SCORES FOR THE SIGNIFICANT COMPONENTS OF THE PROCRUSTES TRANSFORMED LANDMARK COORDINATES OF THE LEFT WING OF *PLESISPA REICHEI* (CHAPUI) FROM THREE LOCATIONS IN MINDANAO

PC1 %var=37.39%		PC2 %var=20.26%		PC3 %var=10.50		PC4 %var=5.42	
LM	Value	LM	Value	LM	value	LM	value
12-21	0.3105	2-21	0.1849	13-21	0.1823	3-20	0.2291
11-21	0.2634	1-2	0.1762	11-19	0.1657	3-21	0.1884
10-21	0.2205	1-21	0.1667	12-20	0.1628	2-21	0.182
11-20	0.2196	2-14	0.1473	5-11	0.1579	3-12	0.1485
10-20	0.2018	1-3	0.1461	12-21	0.1547	5-20	0.1436
16-21	0.1995	1-20	0.1398	11-18	0.1469	3-15	0.1397
16-20	0.1882	2-20	0.1308	8-11	0.1459	2-12	0.134
9-21	0.1847	3-21	0.1284	6-11	0.1451	6-20	0.1326
9-20	0.1735	1-11	0.1189	4-11	0.143	3-11	0.1264
15-21	0.158	11-14	0.1188	2-13	0.1364	3-16	0.1216
17-21	0.1434	3-14	0.1173	13-20	0.1352	5-19	0.119
8-21	0.1296	2-13	0.1172	7-11	0.1351	3-13	0.1183
17-20	0.1292	1-19	0.1168	9-11	0.1341	2-20	0.1163
8-20	0.1288	14-20	0.1165	3-11	0.1289	2-13	0.1159
13-21	0.1225	1-17	0.1103	17-19	0.1148	4-20	0.1094
19-21	0.1104	1-4	0.1095	2-12	0.1145	6-19	0.1084
18-20	0.1049	1-9	0.1044	10-19	0.1131	5-17	0.1041
18-21	0.1034	3-20	0.1038	1-2	0.1041	3-19	0.1036
19-20	0.1031	14-21	0.1028	11-17	0.1014	2-14	0.1031
2-14	0.0711	2-12	0.1018	3-13	0.0958	3-14	0.1011
7-20	0.0666	1-8	0.099	2-14	0.0952	6-17	0.1008

EDMA and PCA assessed the 210 possible inter-landmark measurement combinations from the 21 landmarks in the wings. Four component axes were found to be significant in explaining the variation of the left wing. The top 10% of expanding inter-landmark points were found to be between landmark points in the lower part of the wings (Table VIII) and the proximal point of the median vein (LM21) or the proximal landmark of the wing fold (LM20), with LM12-LM21 having the highest expansion, indicating an increase in the lower margins of the wing. The bottom 10% of contraction inter-landmark points was observed to be between upper margins landmarks and between the proximal and distal points in the wings (Table X), with LM1-LM21 waving the most contraction. These observations indicate that the wing become shorter from proximal to distal point but longer in the upper margin to the lower margin.

TABLE IX: INTERLANDMARK (INTER-LM) DISTANCES WITH THE TOP 10% PCA SCORES FOR THE SIGNIFICANT COMPONENTS OF THE PROCRUSTES TRANSFORMED LANDMARK COORDINATES OF THE RIGHT WING OF *P. REICHEI* FROM THREE LOCATIONS IN MINDANAO

PC1 %var=24.22%		PC2 %var=15.75%		PC3 %var=12.33		PC4 %var=6.39		PC5 %var=5.25	
LM	value	LM	value	LM	value	LM	value	LM	value
6-11	0.1975	3-20	0.2061	3-4	0.1912	1-11	0.2309	1-16	0.1574
7-11	0.1869	3-13	0.1845	2-4	0.1821	11-12	0.194	7-19	0.1502
5-11	0.1736	3-21	0.1804	7-10	0.1332	11-13	0.1844	7-17	0.1448
3-11	0.1322	3-15	0.1738	7-17	0.1293	11-21	0.164	1-20	0.142
4-7	0.1201	3-12	0.1669	4-10	0.1289	11-15	0.154	1-10	0.1417
6-10	0.1196	3-14	0.164	8-17	0.1182	11-14	0.1407	1-7	0.141
4-6	0.1194	1-3	0.1455	8-10	0.1176	2-11	0.1334	1-15	0.1361
5-9	0.1112	2-13	0.1376	4-16	0.1152	1-10	0.1312	13-16	0.1105
5-10	0.1096	2-21	0.1361	4-17	0.1127	1-12	0.1284	9-17	0.1098
6-17	0.1083	3-16	0.1305	10-19	0.1089	12-17	0.1154	1-11	0.1061
6-9	0.1081	2-14	0.1206	7-9	0.1087	1-17	0.1119	1-2	0.1025
7-10	0.1037	4-20	0.1156	2-7	0.1081	2-12	0.1074	14-16	0.1015
11-18	0.1025	2-12	0.115	6-10	0.1059	10-15	0.1063	12-16	0.1014
6-16	0.1023	4-19	0.1132	10-18	0.1037	15-17	0.1055	6-19	0.101
4-11	0.0997	11-13	0.1035	2-8	0.09844	6-18	0.1054	10-13	0.09436
5-18	0.09928	4-17	0.102	3-5	0.09765	5-18	0.1049	7-14	0.09183
6-18	0.09823	1-2	0.09781	8-9	0.09633	11-20	0.1033	14-20	0.09132
9-11	0.09699	11-12	0.09292	5-10	0.09575	2-10	0.1012	13-15	0.08559
8-11	0.09648	11-21	0.09282	6-17	0.09188	10-12	0.1001	12-20	0.08396
7-17	0.09464	4-15	0.0866	2-5	0.09128	12-19	0.0989	1-21	0.08387
5-17	0.09375	3-11	0.08533	7-19	0.09094	16-17	0.09195	10-14	0.08362

TABLE X: INTERLANDMARK (INTER-LM) DISTANCES WITH THE BOTTOM 10% PCA SCORES FOR THE SIGNIFICANT COMPONENTS OF THE PROCRUSTES TRANSFORMED LANDMARK COORDINATES OF THE LEFT WING OF *P. REICHEI* FROM THREE LOCATIONS IN MINDANAO

PC1 %var=37.39%		PC2 %var=20.26%		PC3 %var=10.50		PC4 %var=5.42	
LM	value	LM	value	LM	value	LM	value
3-5	-0.0436	7-19	-0.0803	9-20	-0.09452	2-5	-0.08838
6-12	-0.04885	6-20	-0.08442	3-20	-0.09572	14-20	-0.08918
4-6	-0.05065	10-11	-0.08649	10-20	-0.09655	1-18	-0.09027
7-12	-0.05072	5-18	-0.08685	4-21	-0.09743	14-17	-0.09039
2-21	-0.05222	6-18	-0.08819	2-7	-0.09952	1-20	-0.0919
6-19	-0.05267	5-19	-0.09237	7-21	-0.1028	3-8	-0.09504
3-7	-0.05606	5-17	-0.09479	6-21	-0.108	12-20	-0.09931
5-19	-0.05697	6-17	-0.1007	5-20	-0.1113	1-8	-0.1014
3-6	-0.06029	6-19	-0.1031	19-21	-0.1127	2-7	-0.1023
2-5	-0.06294	4-5	-0.1031	8-20	-0.1133	2-9	-0.1023
2-19	-0.06519	7-11	-0.1147	11-14	-0.1137	13-20	-0.1038
3-19	-0.06538	4-7	-0.1215	18-20	-0.1191	2-17	-0.1058
15-20	-0.06762	3-7	-0.1234	4-20	-0.1202	1-19	-0.1073
2-7	-0.07251	4-6	-0.125	1-21	-0.1227	2-19	-0.111
2-6	-0.07721	3-5	-0.135	11-12	-0.1238	1-17	-0.1121
13-20	-0.124	5-11	-0.1407	2-19	-0.1269	15-20	-0.1142
14-20	-0.1576	2-7	-0.1455	17-20	-0.133	3-4	-0.1294
2-20	-0.176	3-6	-0.1461	11-13	-0.1334	2-4	-0.132
1-20	-0.1862	6-11	-0.1466	6-20	-0.1369	2-18	-0.1368
14-21	-0.1945	2-5	-0.1663	7-20	-0.1417	2-8	-0.1418
1-21	-0.305	2-6	-0.1746	19-20	-0.1508	13-21	-0.1496

A similar effect is also seen in the right wing. Expansion is seen between distances in the distal landmark points of the wing to the lower margin points (Table IX), with LM6-LM11 having the longest distance. Contraction is observed in the landmark points in the right wing between points in the lower margins of the wing to the proximal landmark points (Table XI), with LM11-LM14 having the shortest distance.

Overall, the contraction and expansion of the landmark points suggest that the overall shape of the wings become shorter from proximal to distal landmark points and broader between points in the upper margins to the lower margins. Also, the proximal points become closer together suggesting a slender wing.

According to Chapman [20], narrower and petiolate bases are characteristics of slow-flying insects while those with broader bases are faster. Based on the data, the wings of *P.reichei* from the three populations are observed to be more narrow proximally, shorter in length and broader between the leading edge to the trailing edge of the wing. This suggests that wing shape contribute to a slower flight but more powerful since more force is produced to lift the body up.

TABLE XI: INTERLANDMARK (INTER-LM) DISTANCES WITH THE BOTTOM 10% PCA SCORES FOR THE SIGNIFICANT COMPONENTS OF THE PROCRUSTES TRANSFORMED LANDMARK COORDINATES OF THE RIGHT WING OF *P. REICHEI* FROM THREE LOCATIONS IN MINDANAO

PC1		PC2		PC3		PC4		PC5	
%var=24.22%		%var=15.75%		%var=12.33		%var=6.39		%var=5.25	
LM	value	LM	value	LM	value	LM	value	LM	value
14-19	-0.0949	3-18	-0.0955	4-5	-0.0987	4-21	-0.0737	5-16	-0.0874
1-10	-0.0950	1-19	-0.0960	3-12	-0.1001	2-3	-0.0746	5-20	-0.0895
9-21	-0.0957	2-18	-0.0960	17-21	-0.1005	8-19	-0.0841	10-18	-0.0903
18-21	-0.0967	8-11	-0.101	3-20	-0.1019	3-21	-0.0845	3-20	-0.0936
19-21	-0.0971	2-8	-0.102	2-13	-0.1047	11-16	-0.0861	8-10	-0.0948
16-21	-0.0975	3-4	-0.1026	4-7	-0.108	4-14	-0.0900	16-18	-0.1005
14-16	-0.0997	3-8	-0.1077	14-17	-0.109	8-14	-0.0911	3-17	-0.1006
10-21	-0.1006	2-5	-0.1083	11-17	-0.1116	2-18	-0.0934	4-10	-0.1007
11-13	-0.1006	7-11	-0.1084	15-17	-0.113	10-11	-0.0949	8-16/	-0.1048
10-14	-0.1023	9-11	-0.1137	1-19	-0.1161	7-11	-0.0996	17-21	-0.1085
14-17	-0.1025	6-11	-0.1142	2-21	-0.1181	9-17	-0.1006	17-19	-0.1089
1-4	-0.1026	2-17	-0.1181	12-17	-0.1247	3-14	-0.1011	4-15	-0.1121
17-21	-0.103	10-11	-0.1195	1-17	-0.1252	18-21	-0.1091	4-20	-0.1161
8-14	-0.1065	2-7	-0.125	3-21	-0.1262	18-20	-0.1099	4-16	-0.1219
8-21	-0.1092	11-19	-0.1318	13-17	-0.1273	8-17	-0.1102	2-17	-0.1375
4-14	-0.1199	2-6	-0.141	2-14	-0.1282	18-19	-0.1214	19-20	-0.1545
4-21	-0.1263	2-19	-0.1429	3-13	-0.1287	17-18	-0.1263	16-17	-0.1577
11-21	-0.1278	3-5	-0.1494	3-14	-0.1368	14-18	-0.1318	15-19	-0.1588
1-11	-0.1323	3-7	-0.1507	4-6	-0.1379	9-11	-0.163	16-19	-0.1603
2-4	-0.1361	11-17	-0.1558	1-2	-0.156	8-11	-0.1636	15-17	-0.1699
11-14	-0.1416	3-6	-0.1779	1-3	-0.166	11-18	-0.1752	17-20	-0.1753

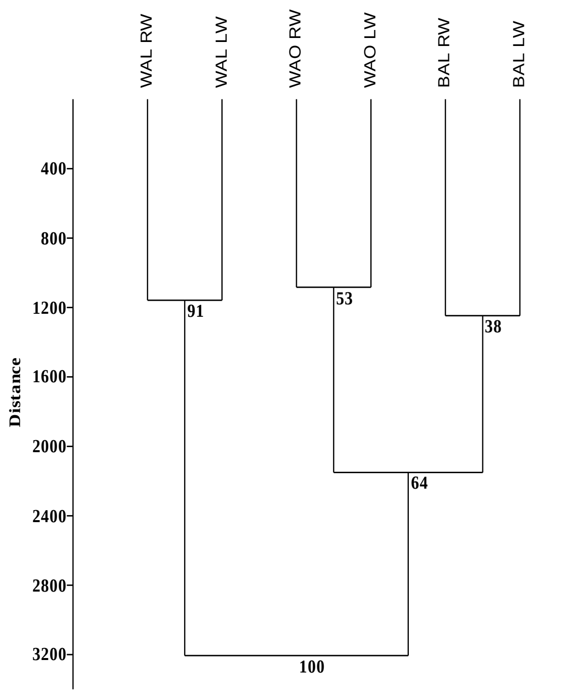

Fig. 7. Dendrogram of centroid size comparison using ward's method from three populations of *P. reichei*.

Cluster analysis of the centroid sizes of the wing morphology showed that the left and right wings were grouped according to geographic location. Each location is seen to be significantly different from each other ($p<0.5$; Fig. 7). Left and right wings were also significantly different except for BAL indicating functional asymmetry arises in the wings. Functional asymmetry is deviations in the bilateral symmetry of an organism believed to be induced by stresses in the environment. Furthermore, it shows that these environmental factors might be similar in BAL and WAO despite a large geographic distance between the two locations.

The variations observed in *P.reichei* may be attributed to the effects of latitude and altitude as has been observed in *Drosophila melanogaster* [21], [22]. Moreover these wing morphology changes including wing asymmetry may also be attributed to the diet during development [23], relative humidity and rainfall [24], [25].

IV. CONCLUSION

This study has clearly described the variations in the wings of three geographically distant populations of *P. reichei* using landmark-based geometric morphometric analysis. While differences were observed between geographical locations, distance was not a factor for the differences which may indicate differences in the genetic structure of the populations. Environmental differences may also have triggered wing development. Similar environmental cues may produce similarity in wing landmarks but distinct geographically.

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