Entomology and Applied Science Letters
Volume 5, Issue 3, Page No: 41-47

Available Online at: www.easletters.com

ISSN No: 2349-2864

Shapes of the Abdomen Between Sexes in Two Geographically Isolated Populations of *Ranina ranina* Using Landmark-Based Geometric Morphometrics and Multivariate Statistics

Dulce Amor Matondo[1]*, Cesar G. Demayo[2]

[1] *Department of Natural and Biological Sciences, Western Mindanao State University, Zamboanga City, Philippines*
[2] *Department of Biological Sciences, College of Science and Mathematics, MSU-Iligan Institute of Technology, Iligan City 9200 Philippines*

ABSTRACT

This study was conducted to describe and compare the shapes of the abdomen between sexes in two geographically isolated populations of Ranina ranina *using landmark-based geometric morphometrics and multivariate statistics. The results showed that asymmetry was common among the sampled crabs; however, PCA-implied deformations revealed higher fluctuating asymmetry levels among female crabs collected from Siargao compared to those from Sacol. Although further studies are still warranted, the results could spell possible differences in the crab's response to different environmental realities between Siargao and Sacol. A glaring result also from this research was the apparent preponderance of asymmetrical structures in nature and the rarity of the symmetry.*

Keywords: Fluctuating asymmetry, Canonical variate analysis (CVA), PCA, deformation, Geometric morphometrics.

HOW TO CITE THIS ARTICLE: Dulce Amor Matondo*, Cesar G. Demayo, Shapes of the Abdomen Between Sexes in Two Geographically Isolated Populations of *Ranina ranina* Using Landmark-Based Geometric Morphometrics and Multivariate Statistics, Entomol Appl Sci Lett, 2018, 5 (3): 41-47 .

Corresponding author: Dulce Amor Matondo
E-mail ✉ cgdemayo @ gmail .com
Received: 08/04/2018
Accepted: 22/07/2018

INTRODUCTION

The distribution of the marine crab *Ranina ranina* just like other crab species has been arguably largely dependent upon aquatic currents, which often flow in divergent directions [1] (Figure 1). Just like other commercially important marine organisms, planktonic stages of crabs' drift in these currents as they complete their development [2]. In most cases, these crabs utilize these aquatic currents to reach new areas and colonize varied marine habitats. Sometimes, these aquatic currents are disrupted by geological barriers such as landforms that seem to also effect varying degrees of population differentiations [1]. Consequently, the landforms and other barriers confer geographic isolation, enabling organisms to develop unique ecological adaptations as a result of genetic divergence and separate histories of mutation and gene flow. In most cases, differential selection pressures in geographically isolated populations have given rise to different phenotypes that promote their survival, growth and ability to reproduce in a given ecological condition [3].

Aside from the possibility of producing varied adaptations, selection pressures might also give rise to intra-individual differences such as that of fluctuating asymmetry. By definition, phenotypic differences in the form of asymmetry in morphological structures have been believed to be secondary characteristics developed by organisms in response to stressors in their environments [4]. The carapace and other bilateral traits in crabs have been said to be not immune to such asymmetry, as populations of these organisms have also been prone to the environmental stressors be it genetic or environmental perturbations [4,5,6,7].

Since asymmetry in shapes has been often associated with handedness or constant use, these small deviations in the shapes of bilateral traits have been deemed to be the result of the genome to protect the organisms against stressors during development [4,8,9,10,11].

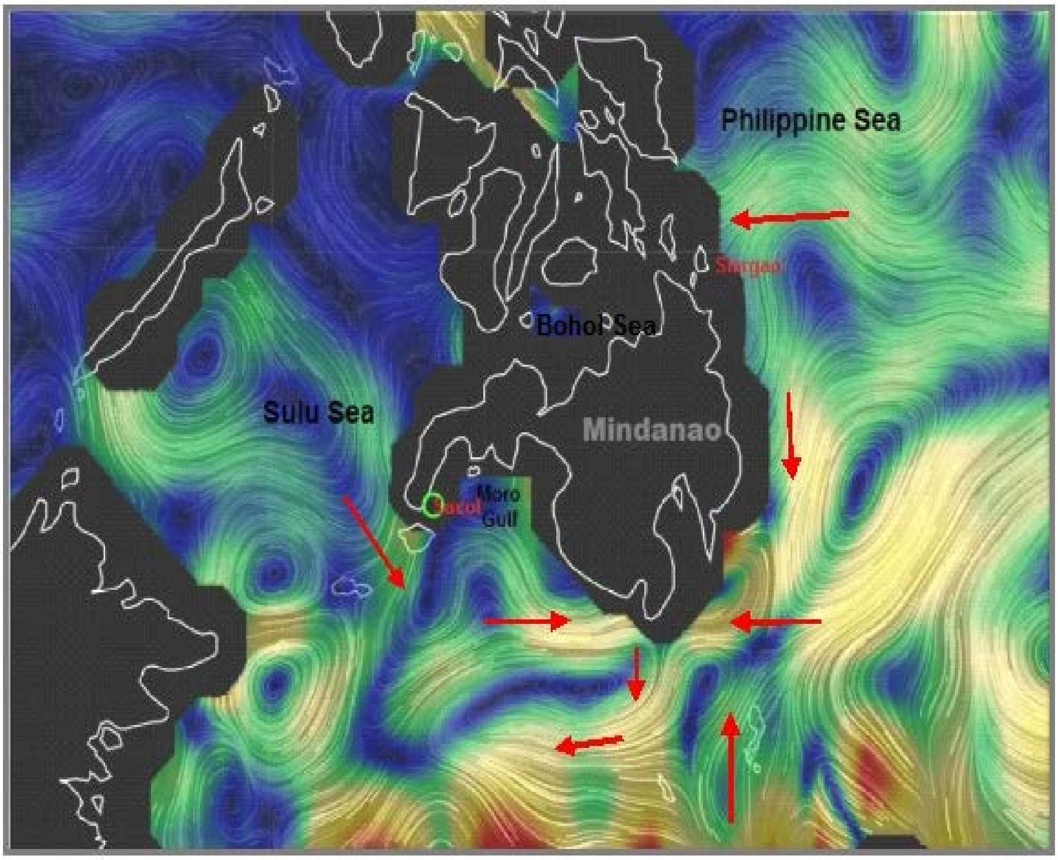

Fig. 1. Direction of water current flow along the sample sites as indicated by the red arrows [12].

This study was exploratory in nature as it attempted to describe variations in one morphological structure in crabs – the abdomen – in at least two levels of analyses. First, the shapes of the abdomen were compared between the two populations of *R. ranina* found in Siargao and Siacol. Second, the abdominal structure was scored for differences in shapes between its left and right sides, otherwise known as fluctuating asymmetry. The reasons for choosing the abdomen as the character and the main structure of analysis stemmed from the fact that it has already been present even in the first instar larval stage of the crab [13]. Function-wise, the abdomen is posteriorly attached to the carapace, which makes it an important platform for the eggs to be adhered to in ovigerous females. Another value of the abdomen has been for *R. ranina*, this structure extends away from the crab's ventral side resembling like a tail, which many have argued play a role in the digging activities of the organism [14].

METHODOLOGY

Collection and preparation of samples. Samples of *R. ranina* were collected from two different sites, namely from the coastal waters of Sacol Island in Zamboanga City and in Siargao Island in Surigao (Fig. 2). Crab samples were procured from commissioned crab fishers in the two sampling areas. Sex determination was done based on the shape of the abdomen. There were 51 female and 40 male samples collected from coastal waters of Sacol Island, while 53 female and 57 male samples were collected from the coastal water of Siargao Island. The abdomens were carefully detached, cleaned, dried and placed inside labeled zippered bags for imaging.

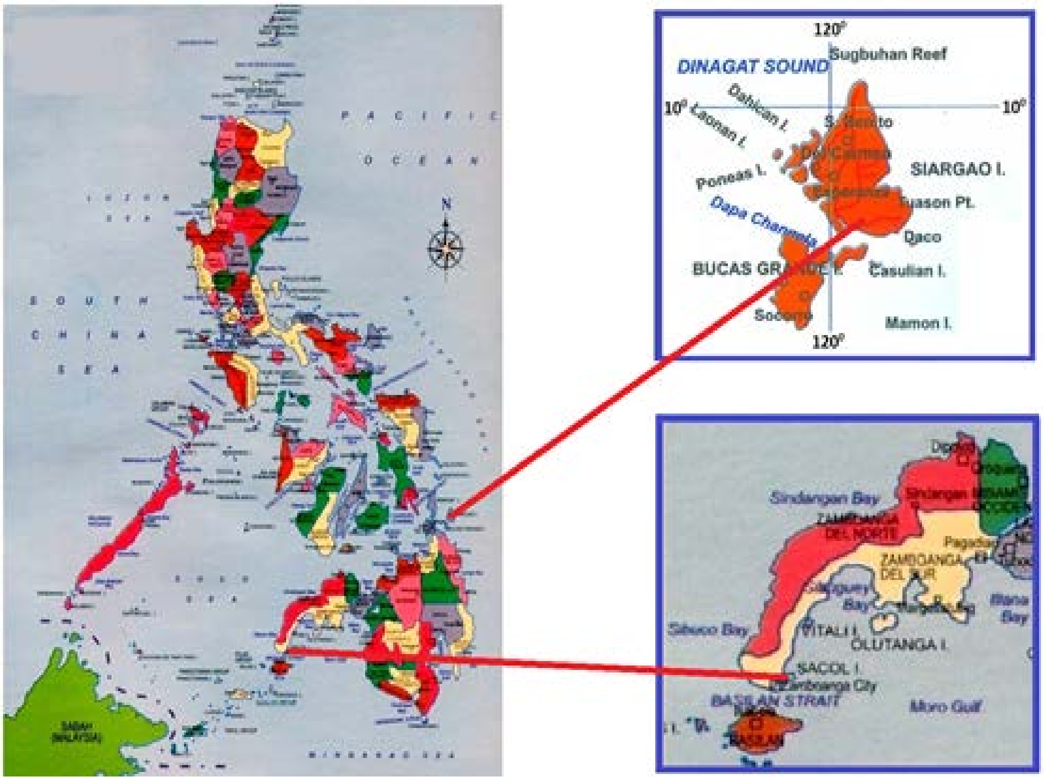

Fig. 2. Location map of Sacol and Siargao Islands

Imaging and Digitization of landmark data. Photographs of the male and female crab abdomens were done using a Fujifilm camera model Fine Fix S2000HD S2100HD in macro mode, mounted in a tripod to obtain standardized images in tri-replicates. To generate landmark data from the crab abdomen, 106 landmark points were assigned on the dorsal aspect of the abdomen (Fig. 3). Using the TPSdig2 software version 2.16 [15], each sample was digitized thrice on different days by the same individual, to address inter-rater digitization error. The software then produced the x and y Cartesian coordinates for each landmark point, which were later subjected to Generalized Procrustes Analysis (GPA) to extricate size from the shape components of the landmark configurations.

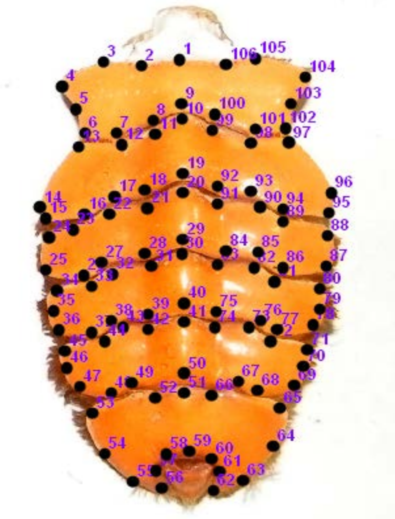

Fig. 3. Assignment of anatomical landmarks along the dorsal aspect of the abdomen.

Generalized Procrustes Analysis. The x and y coordinate values generated by the relative warp analysis were subjected to Procrustes superimposition using the PAST-Paleontological

Statistics Software Package for Education and Data Analysis software [16]. Generalized Procrustes Analysis (GPA) translated and rotated each shape around a common origin (centroid) until the sum of the squared distances among them was reduced, so that the differences in size were removed [17] and the differences in shape were also subsequently minimized. The GPA ensured that only the real shape differences were being measured and the main measure of shape similarities and differences was the Procrustes distance [18]. The variance-covariance residuals produce by the GPA was used as new data in comparing shape variation between populations.

Multivariate Statistical Analyses.

The variance-covariance component of the residuals from GPA was subjected to MANOVA to test whether each group in the given population had the same multivariate mean. The method of Canonical Variate Analyses was also used to detect group differences by determining linear variation of data that could maximize the among group variation corresponding to the pooled within group variations [19].

Detecting Fluctuating Asymmetry (FA). Bilateral FA levels were measured using the "Symmetry and Asymmetry in Geometric Data" (SAGE) program, version 1.21 software [20]. The process produced the least squares Procrustes consensus of the set of landmark configurations and their re-labeled mirror images in a perfectly symmetrical shape. Any deviation from the bilaterally symmetrical Procrustes consensus was considered as FA. Procrustes ANOVA was done to assess the significance of symmetry producing the following effects: individuals (symmetry), sides (directional asymmetry; DA), individual x sides (fluctuating asymmetry; FA) with their respective measurement errors. Principal Component Analysis of the covariance matrix associated to each component of variation (symmetry, directional asymmetry, fluctuating asymmetry, and their respective error matrices) was also done to measure the components of variances and deviations from the ideal shape configuration based on the thin-plate spline to come up with deformation grids that would help visualize shape changes as landmark displacement in the deformation grids [21,22].

RESULTS AND DISCUSSION

Canonical variate analysis (CVA) of relative warp scores showed a significant variability among the populations of *R. ranina* (Table 1). The distribution of individuals in the CVA scatterplot showed significant differences between sexes and populations of the crab (Fig. 4). Discriminant analysis showed a 100% correctly classified individuals within and between the populations of the crab (Table 2). Thin plate splines of the shapes of the abdomen showed the areas where the variations could be observed (Fig. 5). It was clear from these results that abdominal shapes in *R. ranina* were sexually dimorphic. Both sexes in Sacol area showed variations which were observed along the antero-lateral aspect of the first segment where the shoulder and distal tip were elevated which could be attributed to variations in walking behavior of the crab where the movement could either be sideways or forward. Between populations, however, showed that Sacol female crabs had wider abdomens than Siargao females, and Siargao males had narrower abdomens than the Sacol crabs. More pronounced variations in shapes were observed between the points along the left lateral margin among females. The location of landmark points along the left lateral margin among Siargao females was more elevated on the dorsal aspect of the abdomens providing them with the narrower width but longer segments. Among males, the right distal tip of the first segment in the Sacol population was more elevated than those male cranbs from Siargao. Sacol males had expanded right lateral margin (segments 4,5, and 6) while Siargao male crabs were slightly compressed. The landmark points along the left lateral margin among Siargao crabs were more elevated and protruded outward making Siargao male abdomens more broader on the left aspect than that of the Sacol population.

Table 1. Results of the multivariate analysis of variance of the left and right aspect of the abdomen mean shapes within and between the two populations of *R. ranina*.

Test Statistics			
Wilks lambda : 8.07E-05		Pillai trace: 2.715	
df 1	636	df 1	636
df 2	1165	df 2	1170
F	40.56	F	17.5
p	0	p	0

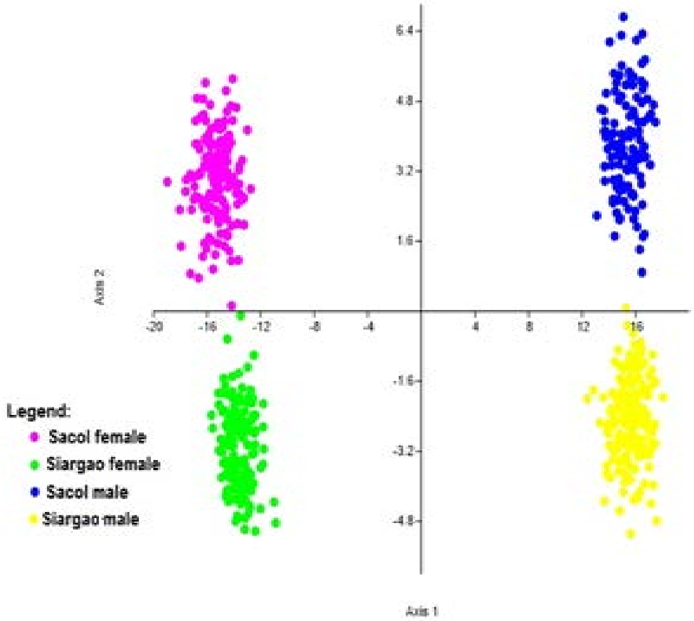

Fig. 4. A CVA scatterplot showing the varied distribution of abdominal shapes between geographically isolated populations of *R. ranina*.

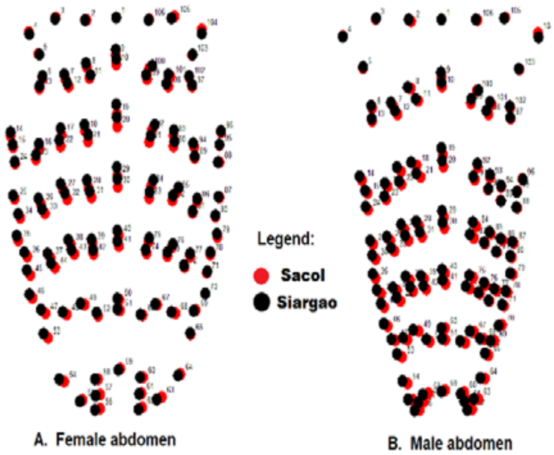

Fig. 5. Variation in abdomens' mean shapes in male and female *R. ranina* between the two populations

Table 2. Confusion matrix of abdominal shapes between males and between females *R. ranina* from the two populations

	Sacol F	Sacol M	Siargao F	Siargao M	N	% correctly Classified
Sacol F	153	0	0	0	153	100
Sacol M	0	120	0	0	120	100
Siargao F	0	0	159	0	159	100
Siargao M	0	0	0	171	171	100

To further assess the variations in symmetry in abdominal shapes in *R. ranina*, Procrustes ANOVA (Table 3) used to quantitatively ascertain the presence of FA in the abdominal shapes of *R. ranina* revealed a significant individual x side interaction indicating the variations between individuals due to the differences between sides of the abdomens. This indicated FA in the abdomens within and between sexes of the crab. It could be seen from the result that both male and female abdomens in the two populations were manifesting a fluctuating asymmetry. Male crabs from Siargao showed the highest FA (F=1.54) while among crabs from Sacol, both sexes showed a relatively similar FA in abdominal shapes indicating that the two sexes might have developed similar responses to similar ecological stress. The abdomen *R. ranina* was not only for digestive purposes but also for reproductive function [23]. In females, this was by holding the eggs while the male abdomens enclosed the gonopod responsible for reproductive activities. Since *R. ranina* abdomen was very exposed when compared to other crab species being hyperextended at the posterior margin and open to water movement [23], it could be argued that the ecological environment might have led to the manifestation of random slight deviations in abdominal shapes [24,25,26].

Table 3. Results of the Procrustes ANOVA on deviancies from the mean landmark configuration of the abdomen shape of the entire *R. ranina* populations from Sacol and Siargao populations.

EFFECT	SS	DF	MS	F	P	Remark
	Sacol Females					
Individuals	0.19099	5200	3.67E-05	3.0445	0	significant
Sides (DA)	0.053209	104	0.000511163	42.42	0	significant
Individuals x Sides (FA)	0.062731	5200	1.21E-05	1.4121	0	significant
Measurement error	0.18125	21216	8.54E-06			
	Sacol Males					
Individuals	0.12491	4056	3.08E-05	3.0457	0	significant
Sides(DA)	0.092488	104	0.000889	87.95	0	significant
Individuals x Sides(FA)	0.041012	4056	1.01E-05	1.4287	0	significant
Measurement error	0.11777	16640	7.08E-06			
	Siargao Females					
Individuals	0.15499	5408	2.887E-05	2.7679	0	significant
Sides(DA)	0.065067	104	0.000626	60.425	0	significant
Individuals x Sides(FA)	0.055995	5408	1.04E-05	1.409	0	significant
Measurement error	0.16202	22048	7.3487e-06			
	Siargao Males					
Individuals	0.14937	5824	2.56E-05	2.0731	0	significant
Sides (DA)	0.11635	104	0.001119	90.433	0	significant
Individuals x Sides (FA)	0.072049	5824	1.24E-05	1.5431	0	significant
Measurement error	0.1901	23712	8.02E-06			

DA = directional asymmetry; FA= fluctuating asymmetry; ns-not significant; hs-highly significant

CONCLUSION

The results of the study showed differences in abdominal shapes between sexes and geographically isolated populations in *R. ranina*. While the differences between sexes could be attributed to differences in reproductive functions of the abdomens, further investigation of variations in shapes showed the existence of FA which could be attributed to the differences in the crab's capacity in responding to the environmental stressors during the course of their development.

ACKNOWLEDGMENT

The senior author would like to acknowledge the Philippine Commission on Higher Education (CHED) for the scholarship grant and the Climate Change Group of the PRISM of MSU-IIT for the support.

REFERENCES

1. Hampton K, Hopkins M, McNamara J. Thurman C. Intraspecific variation in carapace morphology among fiddler crabs (Genus *Uca*) from the atlantic coast of Brazil. Aquatic Biology Vol. 20. 2014; 53-67.
2. Mann KH, Lazier JRN. Dynamics of Marine Ecosystems. Blackwell Scientific Publications, 2nd Edition. 2006.
3. Darwin C. On the origin of species by means of natural selection, or the Murray, preservation of favored races in the struggle for life. Murray, London. 1859.
4. McLaughlin P, Lemaitre R, Tudge C. Carcinization in the Anomura – fact or fiction? II. Evidence from larval, megalopal and early juvenile

morphology. Contributions to Zoology, 2004;73(3):

5. Oppenheimer JM. Asymmetry revisited. Am. Zool. 1974;14: 867-879.
6. Przibram H. Die "Heterochelie" bei decapoden Crustaceen (zugleich: Experimentelle Studien über Regeneration. III). Arch. Entwick. 1905;. 19: 181-247.
7. Emmel VA. The experimental control of asymmetry at different stages in the development of the lobster. J. Exp. Zool. 1908; 5: 471-484.
8. Ludwig W. "Das Rechts Link-Problems in Tirreich und biem Menshen". Springer, Verlag, Berlin. 1932.
9. Waddington, CH. The Strategy of the Genes A Discussion of Some Aspects of Theoretical Biology. George Allen and Unwin, London. 1957.
10. Zakharov VM. Population phenogenetics: Analysis of developmental stability in natural populations. Acta Zoologica Fennica 1992;191:7-30.
11. Swaddle JP. Fluctuating asymmetry, animal behavior and evolution. Advances in the Study of Behavior, 2003; 32: 169-205.
12. Minagawa M. Relative growth and sexual dimorphism in the red frog *Ranina ranina* (Decapoda:Raninidae). Nippon Sulsan Gakkaishi 1993; 59(12): 2025-2030.
13. Faulks Z. The locomotor toolbox of the spanner crab, *Ranina ranina* (Brachyura, Raninidae). Crustaceana 2006; 79 (2): 143-155
14. Rohlf, F. J. tpsDig. Stony Brook, NY Department of Ecology and Evolution, State University of New York. 2010.
15. Hammer OH, Harper DAT, Ryan PD. PAST: Paleontological Statistics Software Package for Education and Data Analysis. Paleontologia Electronica, 2001; 4(1): 9. http;//paleoelectronica.org/2001_1/past/issue1.01.htm
16. Webster M, Sheets HD. A practical introduction to landmark-based geometric morphometrics. Pp. 163-188 in J. Alroy and G. Hunt (eds.). 2010.
17. Polly PD. Euclidean distance matrix analysis. Department of Geological Sciences, Indiana University. 2012.
18. Responte A, Torres MA, Gorospe J, Tabugo SR, Manting MM, Demayo CG. Describing Variations in the Carapace Shape of the Red-Clawed Crab. Environmental Biology, 2015; 9(19): 137-145
19. Marquez E. Sage: Symmetry and Asymmetry in Geometric Data Version 1.21 (compiled 03/1114) Mammals Division. University of Michigan Museum of Zoology http://www-personal.umich. edu/~emarquez/morph/© The Mathworks, Inc. (2012-2014);1984-2013.
20. Albarra´n-Lara AL, Mendoza-Cuenca L, Valencia-Avalos S, Gonzalez-Rodrı´guez, A, Oyama K. Leaf Fluctuating Asymmetry Increases with Hybridization and Introgression Between *Quercus Magnoliifolia* and *Quercus Resinosa* (Fagaceae) Through an Altitudinal Gradient in Mexico. Int. J. Plant Sci. 2010; 171(3):310–322.
21. Pojas RG, Tabugo SRM 2015. Fluctuating asymmetry of parasite infested and non-infested *Sardinella* sp. from Misamis Oriental, from Misamis Oriental, Philippines. AACL Bioflux 2015; 8(1):7-14.
22. Matondo P, Demayo CG. Morphological description of the red frog crab *Ranina ranina* Linnaeus, 1758 (Brachyura: Raninidae) from South Western Mindanao, Philippines. Journal of Entomology and Zoology Studies 2015; 3 (2): 251-256.
23. Castrence-Gonzales R. Asymmetry in the shape of the carapace of *Scylla serrata* (Forsskal, 1755) collected from Lingayen Gulf in Luzon, Philippines Proceedings of the International Academy of Ecology and Environmental Sciences, 2017, 7(3): 55-66.
24. Hermita JM, Gorospe JG, Torres MAJ, Lumasag JL, Demayo CG. Fluctuating asymmetry in the body shape of the mottled spinefoot fish, *Siganus fuscescens* (Houttuyn, 1782) collected from different bays in Mindanao Island, Philipp. Science International (Lahore) 25 (4), 857-86.
25. Genotiva DGP, Tabugo SRM, Manting MME, Gorospe JG, Sabado EM, Asymmetry Analysis of *Brontispa longissima* Gestro, 1885 (Coleoptera: Chrysomelidae) Metasternum Using Symmetry and Asymmetry on Geometric Data (SAGE). International Journal of Bioscience, Biochemistry and Bioinformatics 4 (5), 412.