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Article

Sexual dimorphism in the carapace of mud crab (*Scylla serrate,* Forsskål, 1775) in Magallanes, Agusandel Norte using Geometric Morphometric Analysis

C. J. Presilda[1], **M.A. Salcedo**[2], **M. J. Moreno**[2], **J. Cogenera**[2], **R.A. Japitana**[2], **J. H. Jumawan**[2], **J. C. Jumawan**[2], **J. Presilda**[2], **C.J.R. Presilda**[2], **E.A. Requieron**[3], **M.A.J. Torres**[4]

[1]Graduate School, Biology Department, College of Arts and Science, Caraga State University – Main Campus, Ampayon, Butuan City, Philippines 8600

[2]Department of Education, Tubay District II, Doña Telesfora Elementary School, Tubay, Agusan del Norte, Philippines 8600

[3]Science Department, College of Natural Sciences and Mathematics, Mindanao States University – General Santos City Campus, 9500 Fatima, General Santos City, Philippines

[4]Department of Biological Sciences, College of Science and Mathematics, Mindanao State University - Iligan Institute of Technology, Iligan City, Philippines

E-mail: christianjamespresilda@gmail.com

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Abstract

The study was conducted mainly to describe and analyse the sexual dimorphism using carapace shape in both sexes of *S. serrata* collected from Magallanes, Agusandel Norte using geometric morphometrics as a tool. On the other hand, Landmark-based methods utilize biological equivalences on the homologous structures of an organism. It aims to note the position of important landmarks, amputating irrelevant data as to differences in size, rotation, and locations of the biomarker using Thin Plate Splice (TPS). This method was employed to discriminate differences between male and female and to describe various variations that occur in the form of biomarkers in response to its situations. A total of 60 individuals (30 males and 30 females) were subjected to relative warp analysis. Land-mark analysis was obtained using TPS series with 33 landmarks generated for each samples and loaded into tpsrelww32 to assess the distinction in the body shape between male and female *S. serrata.* Sexual dimorphism of *S. serrata* from Magallanes, Agusan del Norte can be seen between the two sexes, based on the MANOVA test that results to a relative significant difference that is presented and further visualized in the Canonical Variation Analysis (CVA) that produces scatter plot along the two canonical axes (X and Y axes), producing a separation of the two sexes.

Keywords sexual dimorphism; geometric morphometics; mud crab (*Scylla serrate*); Magallanes; Agusandel Norte.

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1 Introduction

In many studies, morphometrics plays an important role in giving quantitative descriptions of different organisms. Its main goal is to study how shapes vary and their covariance with other variables. Morphometrics can be used in describing the form of certain objects thus it is widely used in describing diverse organisms. For many years, traditional morphometrics is used in determining biological aspects of an organism (Hermita et al., 2013). Many innovative tools developed in imaging, statistics, and computer science, studies of variations have become highly quantitative nowadays (Requieron et al., 2012). Geometric morphometrics (GM) on the other hand, is a widely used technique that is an excellent alternative to traditional morphometrics in obtaining and comparisons of size and shape component of any biological image or form (Requieron et al., 2012). Instead of using linear measurement, counts, ratios, data in GM recorded as coordinates of landmark points in which morphological points of specimens that are biomarker (Santos and Quilang, 2012). Morphometrics now made easier by landmark-based analysis using Thin Plate Spline (TPS) (Requieron et al, 2012). Landmark-based geometric morphometric is a method that is used to describe morphological shape variations within the objects (Hermita et al., 2013). It utilizes biological equivalences between the structures, matching or “homologous” landmarks. It will provide information the sexual dimorphism of the organism that is being essential in understanding the ecology, behavior, and life history of the species, as well as morphological comparisons within and between sexes and individuals. The greater the morphological differences occur between sexes the greater competition of the same sex to attract members of the opposite sex ensuring the continuity of the species (Requieron et al., 2012). Using TPS, it aims to record the position of certain important landmarks, removing irrelevant information such as differences in size, rotation, and locations of the biomarker. These methods used to discriminate the differences between the male and the female sex and it describes the different variations that occur in the biomarker in response to its environment (Requieron et al., 2012). On the other hand, Magallanes, Agusan del Norte lays in 9°01′N 125°31′E, with a total land area of 44.31 square kilometres (17.11 sq mi) constituting 1.62% of the 2,730.24-square-kilometre- (1,054.15 sq mi) total area of Agusan del Norte with an elevation of 2 feet (0.61 m) below sea level. The topography of its land is typically a flat and rolling, surrounded by mountains. Swamps embodied most of the situation that is situated at the opening of the two major rivers in the province, the Agusan and Baug Rivers. The center of the town was kept protected by dikes since it is considered as a river delta of the two major rivers. In addition, it has two plywood manufacturing firms: EMCO Plywood Corporation (situated at Brgy. Sto. Rosario) and Philippine Softwood Products Incorporated (PSPI and one safety matches manufacturing firm: JAKA Equities Corporation), sited at Brgy. Marcos, Magallanes, Agusandel Norte. The three manufacturing plants are all on the main street and operating near the Baug River and Agusan River (PSGC Interactive, 2016). Furthermore, Mud Crab (*Scylla serrate*), belongs to genus Scylla, Family Portunidae, Order Decapoda, Class Crustacea and Phylum Arthropoda. Its outer shell varies from a deep, mottled green to very dark brown in color. Juvenile (20 – 99 mm or 0.8 – 3.9 in carapace width) inhabits in mangrove zone settling during low tide, while subadults (100 – 149 mm or 3.9 – 5.9 in carapace width) migrate in intertidal zone to feed at high tide and retreated to subtidal waters at low tide. Adults (150 mm or 5.9 in and larger) were settled and captured mainly below the tide mark, with small numbers captured in the intertidal zone at high tide. As the mangrove floods, the crab becomes active and feed omnivorously on dead fish, crustaceans and molluscs (Nirmale et al., 2012). Mud crabs are highly cannibalistic in nature; crabs which undergo molting were typically attacked by the hard-shelled crabs and ingest them. Female crabs can give birth to a million offspring which can grow up to 3.5 kg (7.7 lb) and have a shell width of up to 24 cm (9.4 in) wide. They are commonly found on estuaries and mangroves of Africa, Australia, and Asia. (Hill et al., 1982). It has both ecological and economic importance to the aquatic environment and to the coastal fishing villages. The shape of the carapace in crabs generally used to describe populations, species, ecological (Sanchez et al, 2013), ecological (Sardà et al., 2005; Giri and Loy, 2008) and fishery – related concerns (Cadrin, 2000; Chang and Hsu, 2004). The shape in crabs is estimated through curvatures or pairwise comparison of linear measurements (Brian et al, 2006). However, allometric changes in morphological shape as shown in many studies (Cheverud, 1982; Huber, 1985; Klingenberg, 2011; Spivak and Schubart, 2003; Botello and Alvarez, 2006; Costa and Soares-Gomes, 2008) indicate that these are important sources of variation and have to result in many cases of junior synonymy (Santana and Tavares, 2010; Osawa and McLaughlin, 2010) and misidentification (Sanchez et al, 2013). Thus, this study was conducted mainly to describe and analyse the sexual dimorphism using carapace shape in both sexes of *S. serrata* collected from Magallanes, Agusandel Norte using geometric morphometrics as a tool.

2 Materials and Methods

2.1 Study area

The study was conducted in Magallanes, Agusandel Norte. The geographic coordinates of Magallanes are 9°01′N 125°31′Ewith a total land area of 44.31 square kilometers (17.11 sq mi) constituting 1.62% of the 2,730.24-square-kilometre- (1,054.15 sq mi) total area of Agusan del Norte. The specimen was collected on May 2018.

2.2 Processing of samples

Thirty male and thirty female samples of *S. serrata*were collected and analyzed. Samples were placed in flat styro for capturing image. Digital image of the carapace of each sample was tri-replicated to determine the digitize error prior to the asymmetry analysis. Using tpsUtil program, captured images were then converted to TPS format and digitized using tpsDig2 program. Sexes were determined based on their external morphology with males having a slender abdominal flap while females have a wider abdominal flap (Gonzales et al, 2017).

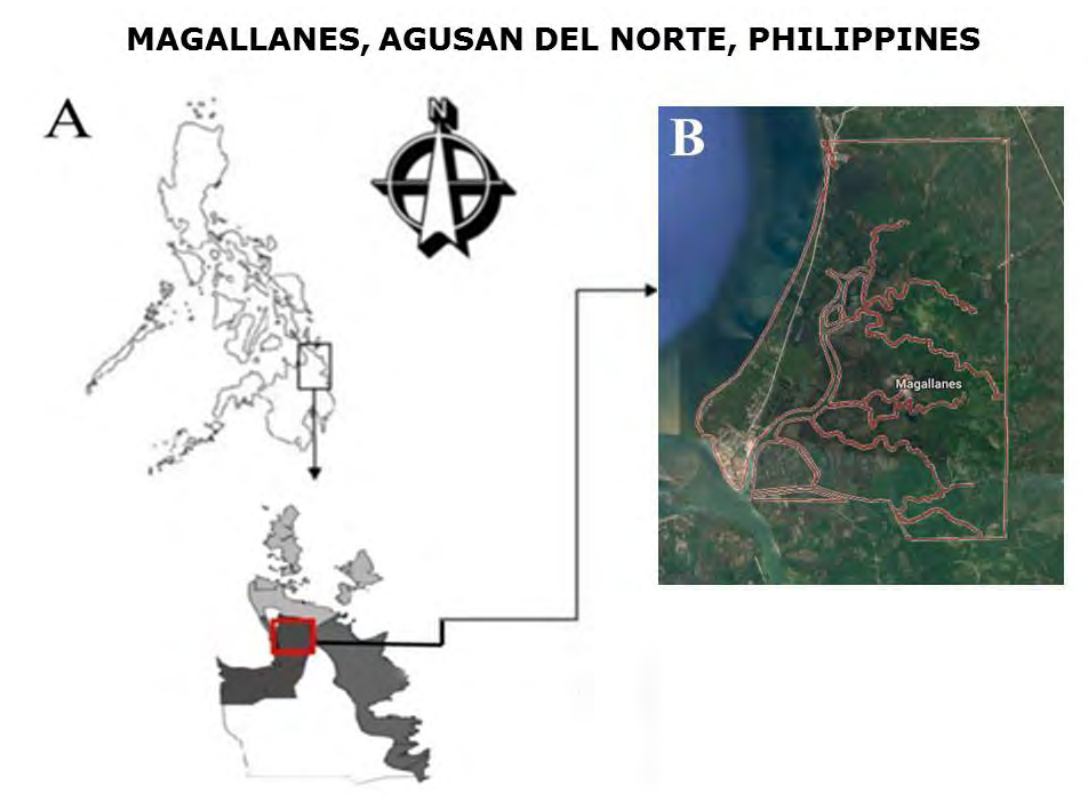


Fig. 1 A. Map of the Philippines showing the map of Mindanao, B. Magallanes, Agusan del Norte.

2.3 Landmark selection and digitization

Using thin-plate spline (TPS) series landmark analysis was obtained to incorporate all the curving part of the specimens' images. Standard forms of digitized landmarks in fish morphometrics were applied. A total of landmarks were used to obtain a homogenous outline in the carapace shape of the specimen as shown in Figure 2 and described in Table1 (Natividad et al.,2015; Jumawan et al.,2016).

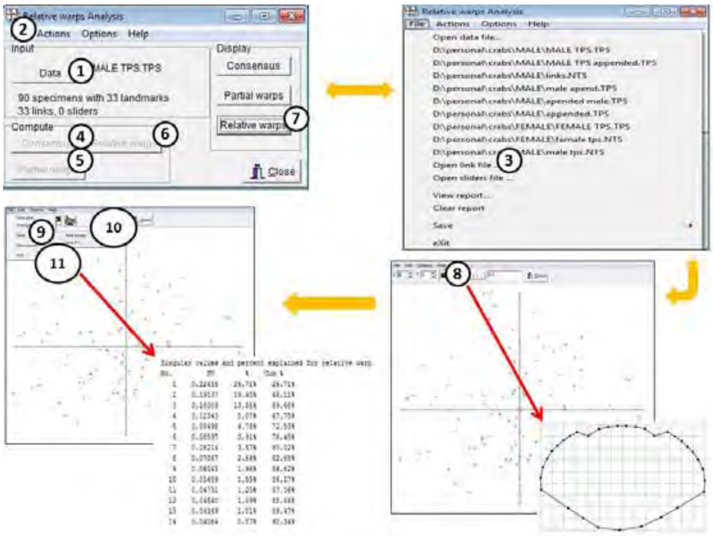
Table 1 Landmarks used to digitize the images of the carapace of *S. serrata* samples collected (modified from Sanchez et al., 2013, Gonzales et al., 2017).

COORDINATES	LOCATIONS
1 – 2	Orbital Region (Left)
3 – 11	1st –9thAntero-lateral teeth (Left)
12 – 13	Postero-lateral region (Left)
14 – 16	Posterior base of the abdomen
17 – 18	Postero-lateral region (Right)
19 – 27	9th – 1st Antero-lateral teeth (Right)
28 – 29	Orbital Region (Right)
30 – 33	Frontal Margin

Fig. 2 Landmark points of the carapace of *S. serrate* through digitations using tpsDig2 from TPS program.

2.4 Shape analysis

The tri-replicated images that is converted to Thin-plate Spline (TPS) format was then subjected to relative warp analysis using tpsrelww32 to test the discrimination in the carapace of both male and female samples of *S. serrate* (Dorado et al., 2012; Solis et al., 2015). RW scores were then subjected to Multivariate Analysis of Variance (MANOVA) and Canonical Variate Analysis (CVA) using Paleontological Statistics (PAST) software (Hammer et al., 2001).



90 specimens with 33 landmarks
33 links, 0 sliders

Fig. 3 Overall schematic diagram of landmark-based analysis using relative warp analysis.

3 Results and Discussion

The deformation grids together with the histograms of the relative warps (RW) scores reveals dimorphic traits along with the extremely positive and negative warps in the samples from Magallanes (Fig. 4). Consensus of the mean body shape from the population was shown in the uppermost portion. The following table (Table 2) contains the descriptions of the change of each of the warps with their variances.

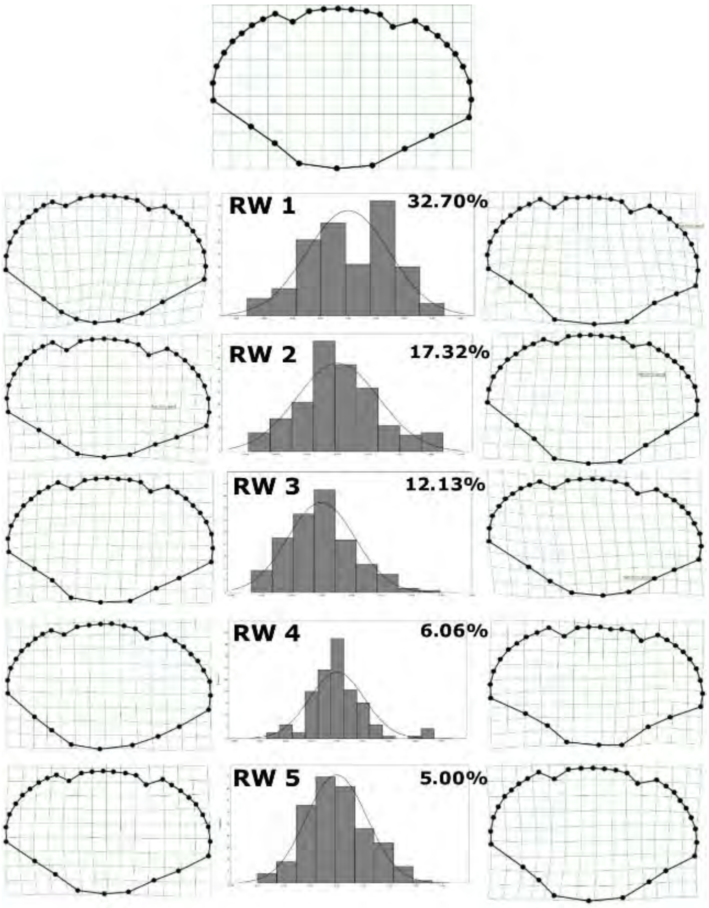


Fig. 4 Summary of the geometric analysis showing the consensus morphology (uppermost) and the variation in the carapace of *S. serata.*

Table 2 Variations observes in the carapace of *S. serrata* from Magallanes, Agusandel Norte.

RW (%)	POSITIVE	NEGATIVE
RW 1 (32.70)	Differences in the carapace of *S.serrata* can be observed in the orbital region (Left), 1st – 9thantero-lateral teeth (Left), postero-lateral region (Left), postero-lateral region (Right), 9th – 1stantero-lateral teeth (Right), orbital region (Right) and in frontal margin	Variations observed are associated with the postero-lateral region (Left), posterior base of the abdomen, postero-lateral region (Right), and in 9th – 1stantero-lateral teeth (Right)
RW 2 (17.32)	Deviations in the orbital region (Left), 1st – 9thantero-lateral teeth (Left), postero-lateral region (Left), posterior base of the abdomen, postero-lateral region (Right), 9th – 1stantero-lateral teeth (Right), and in the orbital region (Right) was observed	Observable carapace differences relating to the orbital region (Left), 7th – 9thantero-lateral teeth (Left), postero-lateral region (Left), posterior base of the abdomen, postero-lateral region (Right), 9th – 1stantero-lateral teeth (Right), and in orbital region (Right)
RW 3 (12.13)	Fluctuations of orbital region (Left), 1st – 9thantero-lateral teeth (Left), postero-lateral region (Left), posterior base of the abdomen, postero-lateral region (Right), 9th – 1stantero-lateral teeth (Right), orbital region (Right) and frontal margin was observed	Curvature in the orbital region (Left), postero-lateral region (Left), postero-lateral region (Right), and in 9th – 1stantero-lateral teeth (Right) was observed
RW 4 (6.06)	Variations observed on the orbital region (Left),3rd – 6thantero-lateral teeth (Left), postero-lateral region (Left), posterior base of the abdomen, postero-lateral region (Right), and frontal margin	Carapace differences in individuals relating to orbital region (Left), 1st – 9thantero-lateral teeth (Left), postero-lateral region (Left), posterior base of the abdomen, 9th – 1stantero-lateral teeth (Right), orbital region (Right) and frontal margin were observed
RW 5 (5.00)	Distortion was observed in 1st – 9thantero-lateral teeth (Left), postero-lateral region (Left), postero-lateral region (Right), 9th – 1stantero-lateral teeth (Right), and in orbital region (Right)	Deviations can be observed in orbital region (Left), 1st – 9thantero-lateral teeth (Left), posterior base of the abdomen, 9th – 1stantero-lateral teeth (Right), orbital region (Right) and frontal margin

Table 2 describes the variations observed in the carapace of *S. serrata.*These includes, narrowing and broading of orbital region (Left), 1st – 9thantero-lateral teeth (Left), postero-lateral region (Left), posterior base of the abdomen,postero-lateral region (Right), 9th – 1stantero-lateral teeth (Right), orbital region (Right) and in frontal margin. Variations between right and left traits in the carapace of *S. serrate* can be associated to the effects of environmental stressors (De Block, 2008). Random signals may signal to the occurrence of genetic and environmental stressors that may include temperature extremes, audiogenic stresses, humidity, protein deprivation and exposure to different kinds of pollution present in the area (Hardersen, 2000; Hosken et al., 2000; Hoffmann et al., 2005; Lezcano et al., 2015; Natividad et al., 2015; Chang et al., 2007).

Table 3 Results for MANOVA test in the carapace between sexes of *S. serrata*.

Wilks' lambda	df1	df2	f-value	p-value
0.2625	5	174	97.77	1.16E – 48

The results in MANOVA test (Table 3) suggests that there is a significant difference between sexes of *S. serrata*given that p-value (P<0.0001**). These differences can be visualized in the scatter plots of specimens that reveal the significant difference between sexes of *S. serrata* that will be presented in Fig. 5 and 6.

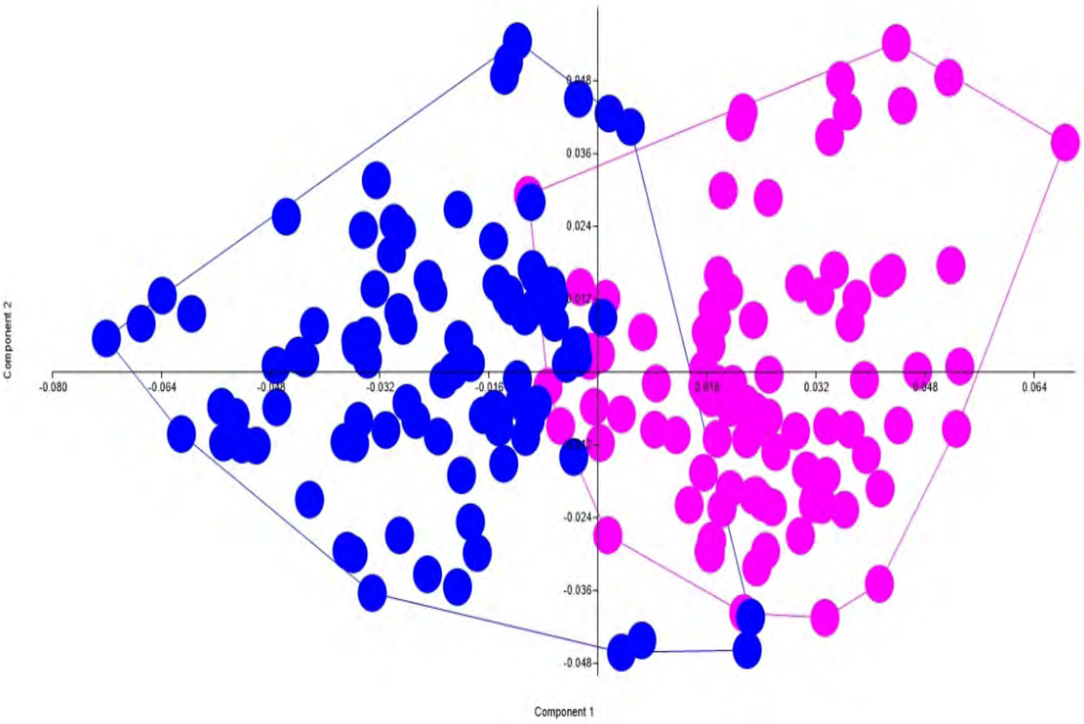


Fig. 5 Distribution of the two sexes of *S. serrata*.

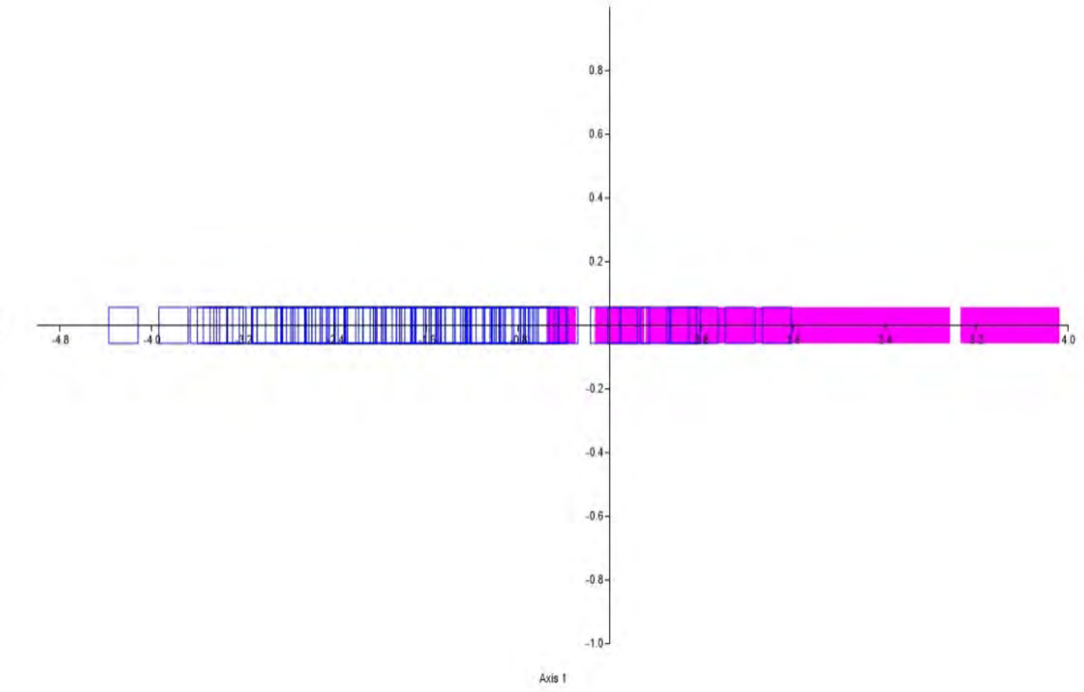


Fig. 6 Canonical Variation Analysis (CVA) plot of the relative scores of *S. serrata*.

Canonical Variation Analysis (CVA) is one application of multivariate statistics that is used to examine interrelationship between a numbers of populations simultaneously through graphical representation. Axes of variation are chosen to maximize the separation between the populations relative variation (Dorado et al., 2012). In the CVA scatter plots presented (Figure 5 & 6), both male and female points overlap around zero on the X and Y-axes. CVA produces a scatter plot of specimens along the two canonical axes, producing separation between the two sexes of each species evaluated. Thus, the result shown in CVA scatter plots (Figure 5&6) revealed a significant difference between male and female sexes of *S. serrata* based on their carapace.

4 Conclusion

The study verify the use of landmark-based analysis in discriminating between two sexes of *S. serrata*from Magallanes, Agusan del Norte an evidence in variability in the carapace of the samples. Sexual dimorphism can be seen in the samples from Magallanes, Agusan del Norte based on the MANOVA test that results to a relative significant difference between sexes of *S. serrata*that is presented and further visualized in the Canonical Variation Analysis (CVA) that produces scatter plots along the two canonical axes (X and Y axes) producing a separation of two sexes. Thus, this assumes that the samples from Magallanes experiences sexual dimorphism between the population.

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