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Morphological variations of green mussel (*Perna viridis*) in Bula, General Santos city using geometric morphometric analysis

Crystal Gayle B. Villaluz*, Jorome C. Tolete, Frenzy B. Almocera, Merhama J. Janti, Trixie Joy E. Pilar, Mark Anthony J. Torres, Elani A. Requieron

Department of Science, Mindanao State University, Philippines

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Abstract

The Philippines, being a tropical country in the Pacific, has long been culturing mussels for business and food consumption. However, they cannot determine at a glance what gender is which. This study is conducted to determine the difference of the male *P. viridis* and the female *P. viridis* through landmark based Geometric-Morphometric method. The population sample of *P. viridis* were obtained from Bula, General Santos City and was analyzed using the Rohlf's Tps series. This software helps in comparing the two landmark-defined shapes of *P. viridis*. Nine homologous points were plotted: (1) Umbo, (2) Ligament, (3) Posterior Adductor 1, (4) Posterior Adductor 2, (5) Posterior Adductor 3, (6) Posterior Adductor 4, (7) Posterior border, (8) Projection and (9) Anterior Adductor to determine the difference of shell size of male and female *P. viridis*. This study focuses on relationships between length-width and length-breadth through relative warps and on the abundance of both sexes among the specimen. The value of significance is 1 ($p > 0.05$) therefore, it is significant and there is a difference on the shell shape. The discriminant function analysis also showed that $p = 1$, therefore there is a significant difference in the shapes of both sexes. Results show that male *P. viridis* shell have a total variation of 89.56%, basing from the six relative warps, compared to that of the female *P. viridis* which is 90.65%, with a slight variation at the ligament and posterior adductor border regions. Relative warps also show that female mussels are wider and bigger than male mussels. It is suggested that the observation of the green mussel shells are done within one area only so as to prevent misleading inputs and have certainty on the report between male and female mussel shell comparison done on only one species.

***Corresponding Author:** Crystal Gayle B. Villaluz ✉ talgayle17@gmail.com

Introduction

The green mussel *Perna viridis* of the genus *Perna* is known to be native to the coastal marine waters of the Indo-pacific region, extending from the Arabian Gulf to the southern province of Guangdong and Fujian in China and southern Japan. It is of commercial importance because of rapid growth and abundance. They are indicators of pollution by heavy metal, organic chlorides and petroleum hydrocarbons. It is generally tolerant up to salinity 80 PSU, but also survives at reduced salinities. Currently, they are being extensively cultured in many Asian countries; largely because of their value as a cheap source of animal protein for human consumption. Besides being consumed as a protein rich food, they are also used as a bio monitoring agent for heavy metal contamination in various Asian countries (Monirith *et al.*, 2003).

As is typical to most of the members of the family, *P. viridis* attaches to hard surfaces by means of proteinaceous basal threads. They grow to the rates of 6-10 mm per month. (Materson, 2007). With the genus *Perna*, their karyotype revealed that *P. viridis* have 30 chromosomes instead of 28 which most species have (Ahmed, 1974).

Asian green mussels are a threat economically for they clog water systems of industrial complexes reducing their efficiency.

They tend to cover up the pumps if they accumulate in holes and areas where tubes are (CABI, 2015). On the other hand, they are also helpful to the environment. Mussels are generally filter feeders, hence, they serve as a filter in the water that sifts the toxic chemical in it. However, they pose health dangers when consumed by humans in this state because of contamination (Rajagopal, 1998). This paper is intended to help farmers of green mussels identify the genders of green mussel under the species *Perna* viridis just by looking at the shell shape differences.

Materials and methods

Study area

The study was conducted last September four (4) to five (5), 2015 in Bula, General Santos City, which is located at South-Central Mindanao that geographically lies between 0° 07" N 12°10'E and 6.117° 125.167"E. The area is rich in marine species which made it suitable for this study.

Fig. 1. (A) Map of the Philippines; (B) Map of General Santos City; (C) Sampling site: Bula, General Santos City *Species accumulations*

The specimens were fished out of water by certified samplers and fishermen of General Santos. A total of 100 *Perna viridis*, 48 and 52 male and female respectively, were collected and became a sample of this study and were analyzed for body shape differences.

There are specific ways in classifying female (Fig. 2 – a, b, and c) and Male (Fig. 3 – a, b, and c) *P. viridis*. The internal morphology of the mantel can reveal the gender base on the different coloration of the male and female gonad. The color of the reproductive tissue of green mussels varies considerably, male gonadal tissue are usually milky to creamy white than in females. The findings in the samples are that the male *P. viridis* had white flesh while female *P. viridis* had yellow-orange to dark-orange flesh. An intermediate coloration or a color similar to the mature male gonad could be found when the animals are immature or the females are at developing or spent stage (Arshad *et al.,* 2012). Nikon Coolpix s2600 was used to capture the left valve of the shell, ventral view and dorsal view.

Landmarking

In the location of the morphology of specimens, there are 9 homologous landmarks (Valladares *et al.*, 2010) digitized on the specimen using free access programs from the Rohlf'sTps series (http://life.bio.sunysb.edu/morph) which is the tpsDig version 2.12. The location of the landmarks and the anatomical descriptions of each are presented in Fig. 4.

Results and discussion

There were six relative warps on male samples which resulted to a total variation of 89.56%. From the gathered data, RW1 is 34.34% referring to the broadening of the distance between the umbo and the ligament area and the crowd bending of the posterior adductor muscles area. RW2 showed 21.22 % variance which referred to shortened distance between the umbo and the ligament area, and the longer distance between the umbo and the posterior adductor border. RW3 is 12.14% and describes the variation of the distances in the ligament to the umbo, the posterior adductor border to the projection. RW4 displays a 10.19% variance in the bending of anterior adductor close to the umbo and a slight variance in both ligament and posterior adductor border. RW5 showed 6.46% where its significant variation is located at the ligament, anterior adductor and at the posterior adductor border regions. RW6 showed 5.21% describing the significant variation on the ligament and posterior adductor border regions. Six relative warps were also gathered as a result of the female samples with a total variation of 90.65%.

From the data, RW1 is 35.98%where there is a noticeable variation in the regions of the ligament, posterior adductors 1, 2, 3, and 4, and posterior adductor border with respect to the height of the shell. RW2 is 18.00% with a noticeable variation in its ligament, posterior adductors and posterior adductor border the posterior adductors. RW3 gave a 17.26% that showed a variation in the ligament and posterior adductor border. RW4 is 9.83% and it stated a difference between the ligament and the posterior adductor border regions. RW5 is 6.00% showing a slight variance in its ligament and posterior adductors region. RW6 is 3.58% describing the single significant variation on the ligament regions.

The thin plate spline (TPS) is technique that creates models of shapes described by landmark configurations as unified deformations. As such, the TPS is not a shape ordination method at all. Rather, it's a graphical tool that can be used to compare any two landmark-defined shapes (MacLeod, 2009). These programs provide various types of statistical analyses using partial warp scores as shape variables and/or expressing the results of a morphometric analysis as a shape deformation.

The analyses of relative warps were performed using the Tpsrelw program from Rohlf's TPS software. All programs of the "TPS" series used in this study work are freeware (http://life.bio.sunysb.edu/morph).

Table 1. Report summary of relative warps in particular based on the overall shape and percentage variance of male and female *Perna viridis* species.

	Variation	Male	Variation	Female
RW1	34.34%	The significant variation is located at the ligament RW1 region. For the negative extreme, there was a downward bending of the ligament from the frame of reference and a slight bending to the left of the posterior adductor border. The positive extreme showed the further elongation of the distance between the umbo and the ligament as it protrudes basing from the reference point.	35.98%	The significant variation is located at the ligament, posterior adductor border and its height. The negative extreme displayed almost the same warp as the reference but only thinner, its ligament region has bend loser to the umbo, also it had a more centered posterior adductor border making it look thinner. The positive extreme showed almost no significant variation other than the lengthening of the distance between the umbo and the ligament region making the warp bigger.
RW2	21.22%	The significant variation is located at the ligament RW2 and posterior adductor border region. The negative extreme showed a little bending downwards of the ligament region and the posterior adductor border is slightly located higher than the reference point. The positive extreme also showed a slight elongation of the distance between the umbo and the ligament with respect to the reference point but nonetheless, its shape is almost the same as the normal reference shape.	18.00%	The significant variation is located at the ligament and posterior adductor border. The negative extreme displayed a thinner warp due to the inward bend of the ligament region and the centered posterior adductor border. The positive extreme displayed a significant variation at the ligament region where it extended upward thus, making the warp look bigger.
RW3	12.14%	The significant variation is located at the ligament. RW3 The negative extreme displays a slight enlargement of the shape because of the downward protrusion of the ligament region with respect to the reference point while the positive extreme showed almost the same shape size as the reference except for the slight length difference of the ligament from the umbo region.	17.26%	The significant variation is located at the ligament region, the posterior adductor border region and the posterior adductor group. The negative extreme is thinner than the reference warp and had its ligament region bended inward; its posterior adductor border is closer to the projection; and its posterior adductors 1, 2, 3, and 4 are closely packed compared to the original warp. The positive extreme showed longer distance between the umbo and the ligament region and a higher posterior adductor border point making it look taller and bigger than the original warp.
RW4	10.19%	The significant region is located at both the RW4 ligament, posterior adductor border region and a slight variance at the anterior adductor. The positive extreme showed the bending of the posterior adductor border to the left, a slight downward bend of the ligament region and the closeness of the anterior adductor to the umbo. The negative extreme has a slight variance on the length of the distance between the ligament region and the umbo as it elongated.	9.83%	The significant variation is located at the ligament region, posterior adductor group region and the posterior adductor border region. The negative extreme showed a slight variation to the ligament region by its posterior adductor border being centered, its posterior adductor group being closely packed and its ligament border leaning inward towards the umbo making it look thinner. The positive extreme shows the same elongation of the distance between the umbo and the ligament making it bigger.
RW5	6.46%	The significant variation lies at the ligament, RW5 posterior adductors 1, 2, 3, and 4 and posterior adductor border. The negative extreme shows a downward bending of the ligament region and a constriction of the group of posterior adductors region. The positive region showed a significant variance in the length distance of the umbo to the ligament region as it protrudes out of the reference point.	6.00%	The significant variation still lies among the ligament, posterior adductor, and the posterior adductor border. The negative extreme shows a thinner shell warp due to the inward lean of the ligament region and the compact gathering of the posterior adductors. The positive extreme showed a very little difference in the whole warp but its slight elongation of ligament region.
RW6	5.21%	The significant variation is seen on the ligament, RW6 posterior adductor border, and the anterior adductor. The negative extreme appears to have a lengthened ligament region from the umbo with respect to its reference point, a more compact group of posterior adductor and a higher posterior adductor border. The positive extreme showed a downward bend of the ligament region and a shorter posterior adductor border.	3.58%	The significant variation lies between the ligament and the posterior adductor alone. The negative extreme still looked thinner than the reference and its possession of compact posterior adductors made it look small than usual. The positive extreme had a very slight elongation of the ligament region.
	89.56%	The total variation on male *Pernaviridis*.	90.65%	The total variation on female *Pernaviridis*.

The statistical reports showed the results for MANOVA having Wilks' lambda = 1 with a p = 1, Pillai trace = 6.745E-08 with a p = 1, and its Eigenvalue 1 = 6.745E-08 and Eigenvalue 2 = 3.218E-16. The Kruskal-Wallis test showed the H (chi^2) and Hc (tie corrected) equal to 0.1029, and its p equal to 0.9998. These proved the body shape variations within *Perna viridis* with a significant variation determined by the p value (p=1) which is greater than 0.05 level of significance (p>0.05).

The tables below further provide results and discussions among the comparison of the relative warps gathered.

Table 2. Mann-Whitney pairwise comparisons, Bonferroni corrected/uncorrected table shown under the Kruskal-Wallis test.

	RW1	RW2	RW3	RW4	RW5	RW6
RW1		0.832	0.8379	0.8846	0.8778	0.8524
RW2	1		0.9921	0.9634	0.9279	0.919
RW3	1	1		0.9565	0.8534	0.87
RW4	1	1	1		0.8476	0.8974
RW5	1	1	1	1		0.7434
RW6	1	1	1	1	1	

Moreover, the Discriminant Function Analysis (DFA) graph (Table 3) summarized the extent of variation between the male and female *P. viridis* species. This manifests the sexual dimorphism between the sexes. Differences in the body shape of the sexes varies in the functions.

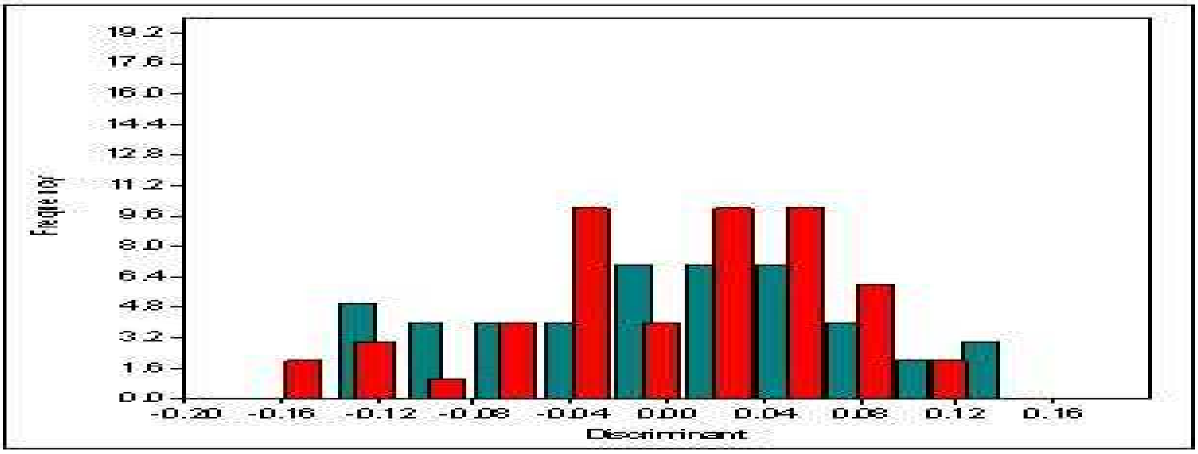

Table 3. Comparison of Discriminant Function Analysis (DFA) between male and female *P. viridis*.

Although the *Perna viridis* species are not easily distinguished externally as male and female, the table above represents the summary of the variations in the morphology of the sample species through the use of Relative Warp Scores and its equivalent frequency histograms within two sexes.

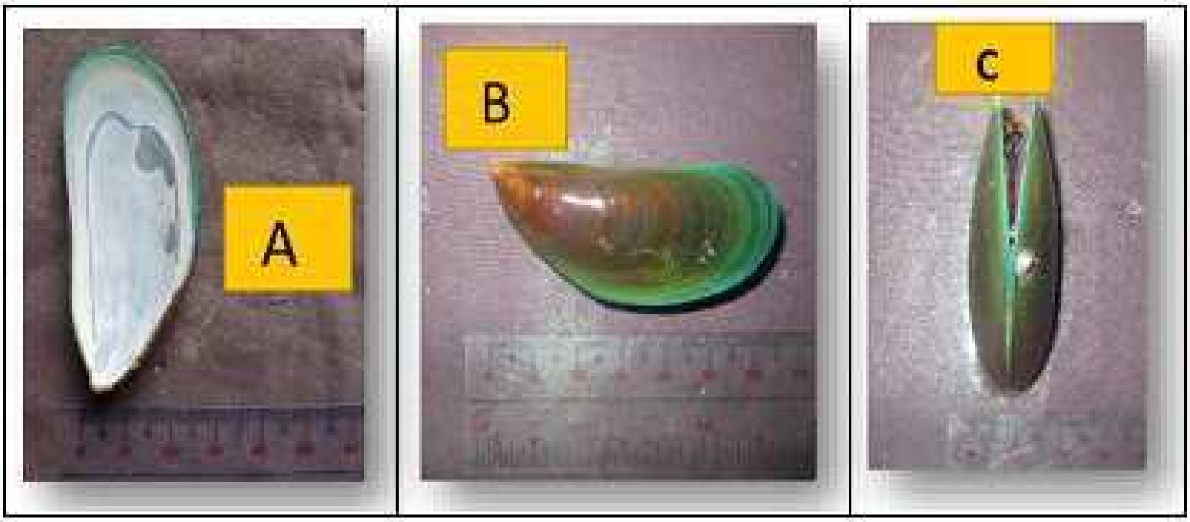

Fig. 2. *Perna viridis*. A – Male, showing its (A) Inside of shell from left valve, (B) Horizontal ventral view, and (C) Vertical dorsal view. (Image obtained by Nikon Coolpix s2600).

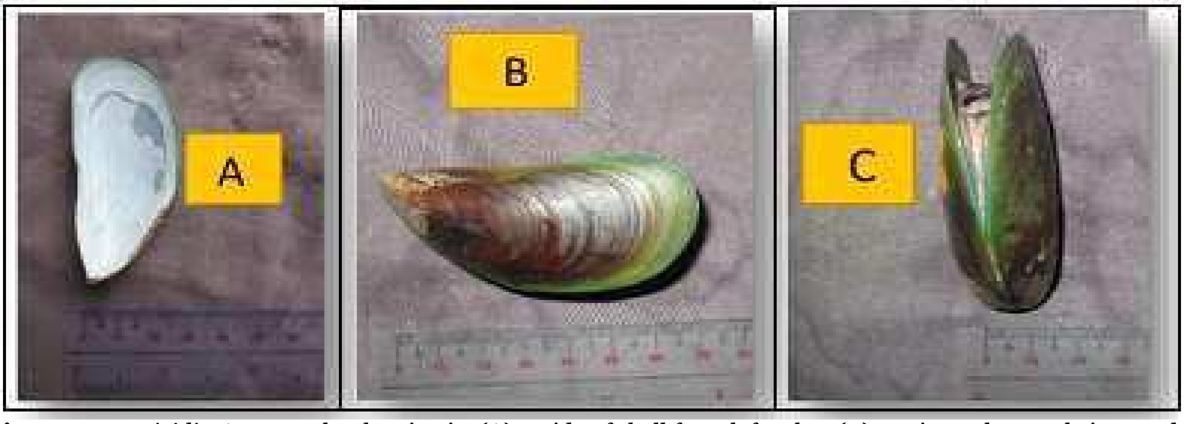

Fig. 3. *Perna viridis*. A – Female, showing its (A) Inside of shell from left valve, (B) Horizontal ventral view, and (C) Vertical dorsal view. (Image obtained by Nikon Coolpix s2600).

Based on the acquired results, male *P. viridis* appear to have smaller shell size and a wider shell width as shown by the distance of their ligament region to its umbo, whereas the female *P. viridis* appear to have larger shell size as shown by the distance in its posterior adductor border to its umbo region.

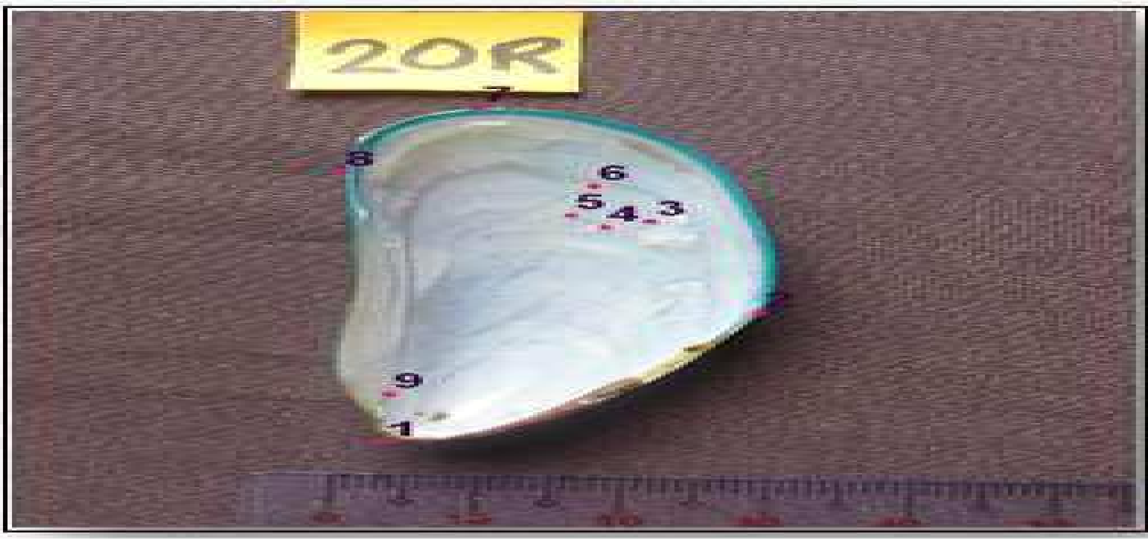

Fig. 4. Map of the 9 homologous landmarks of the left valve of *Perna viridis*. (1) Umbo, (2) Ligament, (3) Posterior Adductor 1, (4) Posterior Adductor 2, (5) Posterior Adductor 3, (6) Posterior Adductor 4, (7) Posterior Adductor border, (8) Projection and (9) Anterior Adductor. Base figure from Valladares *et al.* (2010).

Classification of *Perna viridis* are as follows:

Kingdom: Animalia
Phylum: Mollusca
Class: Bivalvia
Subclass: Lamellibranchia
Order: Mytiloida
Family: Mytilidae
Genus: Perna
Species: viridis (Linnaeus 1758).

Among the Family of Mytilidae, *P. viridis* is sometimes mistaken with the *Perna indica* due to their similar shell structure. However, the green mussel has greener shell coating than that of the reddish to greenish shell coating of the *P. indica*. By knowing this, it will ensure that this study is based solely on the results gathered among the green mussels, *P. viridis*.

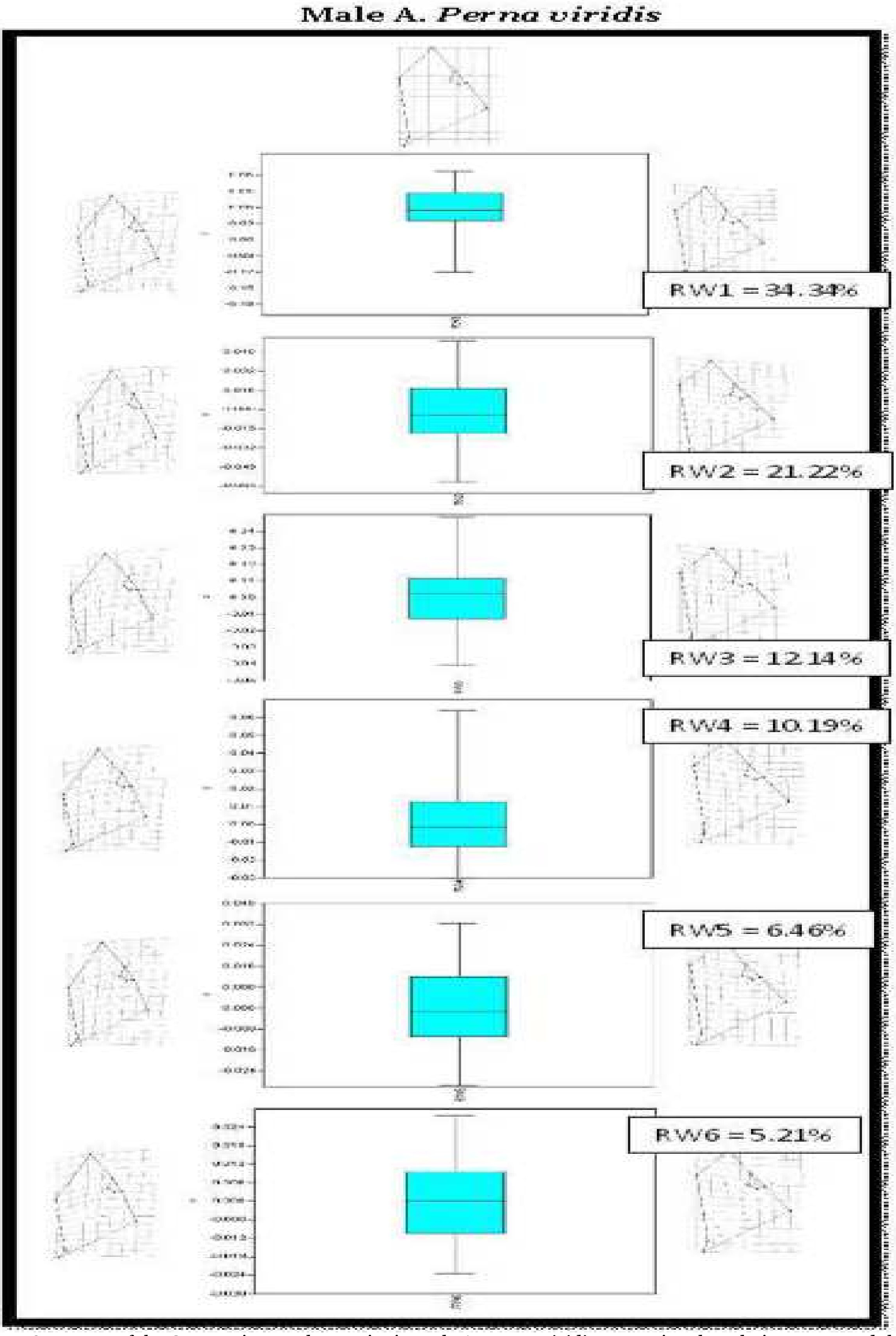

Fig. 5. Summary of the Geometric Morphometrics in Male A. *Perna viridis* presenting the relative warps and the variation in body shapes from maximum negative (left) towards maximum positive (right) as compared to the normal shape (on top).

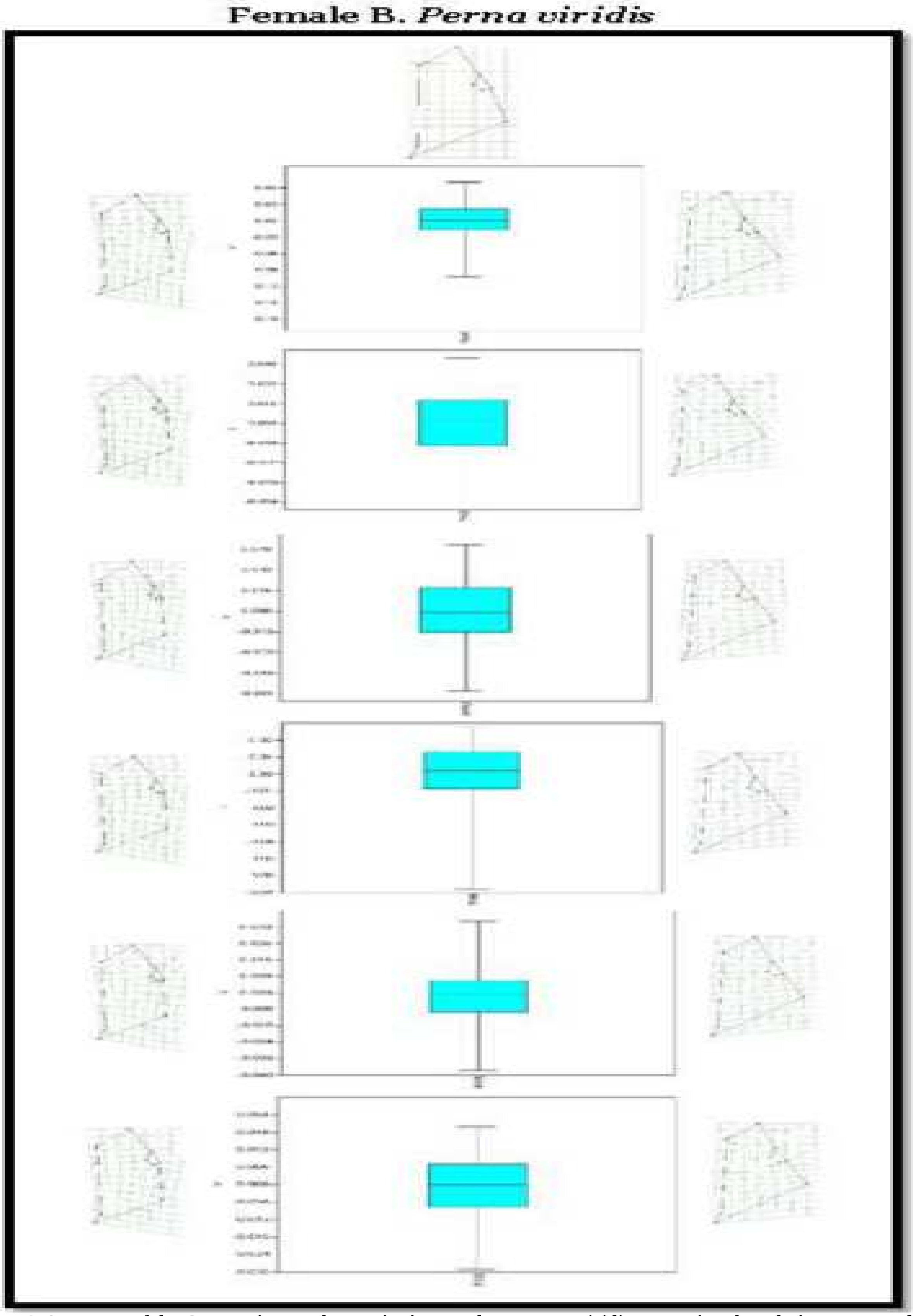

Fig. 6. Summary of the Geometric Morphometrics in Female B. *Perna viridis* presenting the relative warps and the variation in body shapes from maximum negative (left) towards maximum positive (right) as compared to the normal shape (on top).

Conclusion

As shown in the discussion and the data that were presented, the researchers therefore conclude that finding out the proper shell size to the green mussel shells through comparison is one way to determine their sexes. With the relative warps showing a significant variance of 1, this means a noticeable difference between the sexes of *Perna viridis*. This study can help the farmers culture mussels and control their produce by determining the sexes through the shells as shown in the gathered data.

Acknowledgement

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