Article

Asymmetry in the shape of the carapace of *Scylla serrata* (Forsskål, 1755) collected from Lingayen Gulf in Luzon, Philippines

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Abstract

The nature of organisms is influenced by the conditions of the environment. The shape changes occurring in the body of organisms could be attributed to its response to environmental perturbations. This study was therefore conducted to describe the changes in carapace shape in both sexes of *S. serrata* collected from different areas in the Lingayen Gulf using geometric morphometrics as a tool. Variations particularly the level of symmetry of its carapace shape, Symmetry and Asymmetry in Geometric Data (SAGE) software was used to analyze a total of 58 landmarks to obtain the biological shape information of 130 crab individuals. Procrustes ANOVA was employed to assess intra-population variability, directional and fluctuating asymmetry within sexes. Results showed sexual dimorphism in the carapace. Individual and directional asymmetry was also observed within sexes. However, fluctuating asymmetry was also detected in the anterolateral teeth, posterolateral margin and posterior border of the carapace indicating observed asymmetry that maybe attributed to both genetic and the interplay of genotype x environmental interaction suggesting that FA existence can be due to developmental instability as a consequence of the effect of the ecological health of the Lingayen Gulf.

Keywords crabs; geometric morphometrics; landmarks; asymmetry.

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1 Introduction

Lingayen Gulf is among the water bodies in the Philippines with intermingling issues such as increasing conflicts of resource use and water quality deterioration (Guarin, 1991). The issues include pollution from various sources including increased nutrient loading associated with domestic, industrial, agriculture and aquaculture activities (San Diego-McGlone et al., 2008; McManus and Chua, 1990), heavy metal pollution, sedimentation resulting from logging and mining operations and explorations (Guarin, 1991). Included also are the toxic chemicals used in aquarium fish collection as well as biocides used in aquaculture and oil contamination from marine vessels (McManus and Chua, 1990). These stressors may have affected the conditions of organisms in the gulf, especially that of *Scylla serrata* which is one of the most important species of marine organisms that is economically important to the fishing communities in the gulf. This species is considered a good model to evaluate the nature of population of the species in the gulf area because it is commonly being collected, reared and harvested ranging from their habitats such as the brackish waters of the mangrove areas, muddy and sandy substrates, littoral zone to pure saline waters of the open sea (Baylon et al., 2001; Walton et al., 2006; Le Vay et al., 2007; Demopoulos et al., 2008).

Since the shape of the carapace of the crabs is regarded as a bioindicator of environmental conditions and community change (Brown et al., 2004; Ng and de Forges, 2007; Demopoulos et al., 2007; FAO, 2008; Ewel et al., 2009), it was used in the current study for the assessment of population variability. Dimorphism was first assessed to be able to determine if the level of asymmetry is sex-dependent and could be a factor to be considered in the asymmetry analysis. It is also argued in this study that the level of environmental stress can affect the shape of the carapace of *S. serrata.* Thus, random deviations between values of right and left traits of normally bilaterally symmetrical organisms known as fluctuating asymmetry (FA) (De Block, 2008) can be attributed to the effects of environmental stress. FA is a vital morphometrics tool that measures the small, random deviations from the ideal morphology due to its capability of providing absolute difference between the left and right side of a bilaterally symmetrical organism (Moller & Swaddle 1997; Palmer & Strobeck 2003a). It is argued that these random deviations signal the existence of genetic or environmental stressors which include temperature extremes in particular, audiogenic stress, humidity, protein deprivation and exposure to pollutants (Hardersen, 2000; Hosken et al., 2000; Hoffmann et al., 2005; Lezcano et al., 2015; Natividad et al., 2015; Chang et al., 2007).

2 Study Area and Methodology

Sixty-five male and sixty-five female individuals of *S. serrata* were collected from Lingayen Gulf (Fig. 1) and transported using boxes to DA BFAR-NIFTDC in Binloc, Dagupan for morphometric analysis. Male and female sexes were determined based on their external morphology with males having a slender abdominal flap while females have wider abdominal flap (Fig. 2).Identification was based on the taxonomy proposed by Keenan et al (1998) based on morphometrics and genetics studies. Carapace length and carapace width were measured using a plastic Vernier caliper. Body weight was taken using a 1kg capacity top loading balance. The image of the samples was scanned using an hp G2410 flatbed scanner with an optical scanning resolution of 1200 dpi. The scanned images were digitized using tpsDig2 program (version 2.0, Rohlf, 2004) and were saved as a TPS file.

2.1 Landmark selection and digitization.

A total of 58 landmarks (equivalent to 58 X and 58 Y Cartesian coordinates) were established in order to represent the external shape of the dorsal carapace of the crabs (Fig. 3). Selection of landmarks considered both evolutionary and functional significance based on and modified from the digitized landmarks in crab morphometrics used by Sanchez et al. (2013). The software tpsDig2 was used to digitize the landmarks in order to provide a comprehensive summary of the variation and symmetry of the dorsal carapace of the crabs. The description of the landmarks was reflected in Table 1. Plotting of landmarks was done in triplicate to minimize errors and/or biases. The X and Y coordinates of these landmarks on the images were used to analyze fluctuating asymmetry.

Fig. 1 Map of the Philippines showing the location of Lingayen Gulf (source: googlemaps).

Fig. 2 The abdominal flap of the female and male *S. serrata* (Phelan and Grubert, 2007).

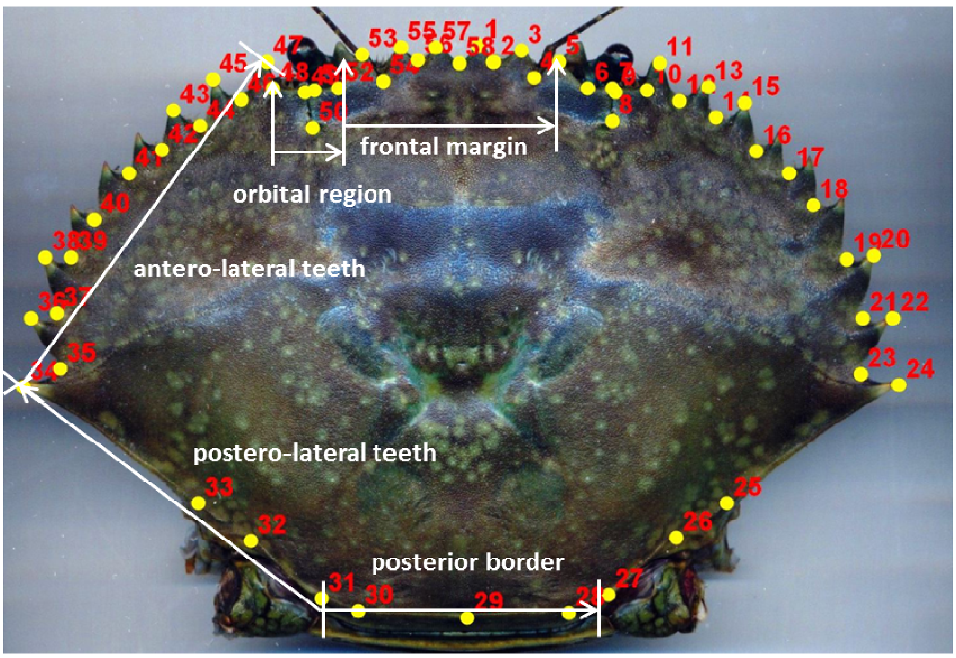

Fig. 3 Location of landmarks in the dorsal carapace of *S. serrata*.

2.2 Shape analysis

The x and y coordinates generated from landmark selection and digitization were used as baseline data in analyzing fluctuating asymmetry of *S. serrata*. The pairs of landmark coordinates of the TPS version were analyzed using Symmetry and Asymmetry in Geometric Data (SAGE) software (version 1.04, Marquez, 2007) to identify geometric data of object with emphasis on its asymmetry generating symmetrized data sets, residuals from symmetric components, in addition to shape configuration of each component of variation (symmetric, asymmetric, and error) as well as expected covariance matrices (Natividad et al., 2015). For the calculation and quantification of the residual asymmetry, a triplicate analysis with Procrustes ANOVA using 99 permutations was done. Variation between sides which is the measure of directional asymmetry were generated in these analyses as well as the percentage (%) fluctuating asymmetry which were then compared between the sexes (Table 1).

Table 1 Anatomical landmarks on the carapace of *S. serrata* (modified from Sanchez et al., 2013).

Landmark No.	Location in the carapace	Code	Landmark No.	Location in the carapace	Code
1-5	Frontal Margin (right)	FMR	32-33	Postero-lateral region (left)	PLRL
6&10	Frontal margin orbital region (right)	FMOR	34	9th antero-lateral tooth (left)	ALTL9
7-9	Slit in the orbital region (right)	SOR	35	In between 8th and 9th antero-lateral tooth (left)	B89AL
11	1st antero-lateral tooth (right)	ALTR1	36	8th antero-lateral tooth (left)	ALTL8
12	In between 1st and 2nd antero-lateral tooth (right)	B12AR	37	In between 7th and 8th antero-lateral tooth (left)	B78AL
13	2nd antero-lateral tooth (right)	ALTR2	38	7th antero-lateral tooth (left)	ALTL7
14	In between 2nd and 3rd antero-lateral tooth (right)	B23AR	39	In between 6th and 7th antero-lateral tooth (left)	B67AL
15	3rd antero-lateral tooth (right)	ALTR3	40	In between 5th and 6th antero-lateral tooth (left)	B56AL
16	In between 3rd and 4th antero-lateral tooth (right)	B34AR	41	In between 4th and 5th antero-lateral tooth (left)	B45AL
17	In between 4th and 5th antero-lateral tooth (right)	B45AR	42	In between 3rd and 4th antero-lateral tooth (left)	B34AL
18	In between 5th and 6th antero-lateral tooth (right)	B56AR	43	3rd antero-lateral tooth (left)	ALTL3
19	In between 6th and 7th antero-lateral tooth (right)	B67AR	44	In between 2nd and 3rd antero-lateral tooth (left)	B23AL
20	7th antero-lateral tooth (right)	ALTR7	45	2nd antero-lateral tooth (right)	ALTL2
21	In between 7th and 8th antero-lateral tooth (right)	B78AR	46	In between 1st and 2nd antero-lateral tooth (right)	B12AL
22	8th antero-lateral tooth (right)	ALTR8	47	1st antero-lateral tooth (left)	ALTL1
23	In between 8th and 9th antero-lateral tooth (right)	B89AR	49-51	Slit in the orbital region (left)	SOL
24	9th antero-lateral tooth (right)	ALTR9	48&52	Frontal margin-orbital region (left)	FMOL
25-26	Postero-lateral region (right)	PLRR	53-57	Frontal Margin (left)	FML
27-31	Posterior base of the abdomen (right)	PBA	58	Center of the frontal margin	CFM

Comparisons between sexes and individual symmetry were analyzed using the Paleontological Statistics (PAST) software (Hammer, et al., 2001). Relevant statistical representations such as histogram, box plot and scattered plot were generated using this software (Natividad et al., 2015).

3 Results and Discussion

Canonical variate analysis of the carapace shapes of the 2 sexes of *S. serrata* shows both male and female sexes of *S. serrata* were different (Fig. 4, Table 2). Examination of symmetry within sexes show variation among individuals indicating genotypic differences between individual crabs (size and shape variation).. Variations on the left and right sides were significantly different within sexes indicating directional asymmetry in the two sexes. However, the individual x side interaction was also significant indicating the differences in the shapes between the left and right sides of the crabs can be attributed to the failure of the effect of individual crab carapaces to be the same from side to side (Table 3). This indicates fluctuating asymmetry in the carapace in both sexes of *S. serrata*.

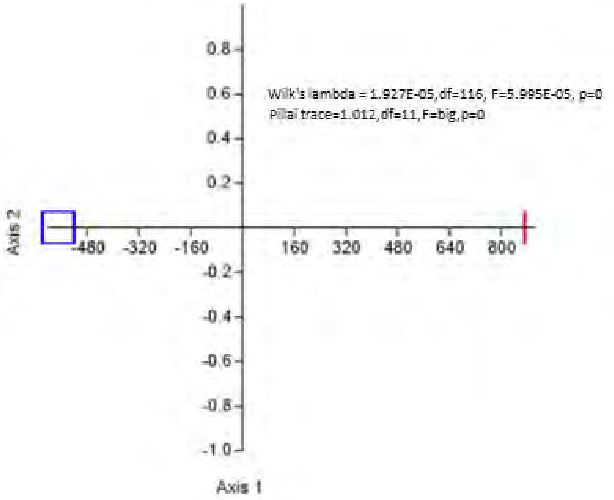

Fig. 4 Canonical variate analysis of the carapace shape, showing the differences between the two sexes computed for whole investigated samples (blue: male; red: female).

Table 2 Discriminant analysis between the shapes of the carapace between the two sexes of *S. serrata*.

	Male	Female	Total
Male	152	0	152
Female	0	99	99
% correct classification = 100%			

Principal Component Analysis was also measured to visualize the covariance shape change for each principal component and to see the general direction and magnitude of the fluctuation for each landmark (Table 4). In the female *S. serrata*, PCA-implied deformations for individual variation (symmetry) were observed for several landmarks. For the first 3 PC's, PC1 indicates asymmetry in almost all the landmarks defining the shape (except those found in the frontal margin), such as the upper anterolateral teeth (landmarks 11-15, 41-47), orbital region (8-10,48-50), posterolateral margin (25, 26,33) and posterior border (27-31); PC 2 on landmarks 22,24,34,36; and PC3 on landmarks 26 and 32 (Fig. 5). In males, the PCA-implied deformations are almost similar with females with slight differences (Table 4, Fig. 6). Both sexes show individual symmetry is higher than the values of fluctuating asymmetry although the female crabs have higher FA values than the males. These findings indicate the occurrence of bilateral asymmetry in both sexes of the crabs. The asymmetry can be argued to be the result of developmental instability or primarily in response of the crabs to environmental stress.

Table 3 Procrustes two-way, mixed model ANOVA results of carapace symmetry in the male and female *S. serrata*.

Effect	Sum of Squares	Degrees of Freedom	Mean of Squares	F-value	P-value
Female					
Individuals	0.11669	1792	6.5116e-005	10.4864	<0.0001**
Sides	0.0014011	56	2.5019e-005	4.0291	<0.0001**
Individuals x sides	0.011128	1792	6.2096e-006	13.5439	<0.0001**
Measurement Error	0.0033891	7392	4.5848e-007		
Male					
Individuals	0.14869	2800	5.3103e-005	9.019	<0.0001**
Sides	0.0012543	56	2.2398-005	3.804	<0.0001**
Individuals x sides	0.016486	2800	5.8879e-006	11.0515	<0.0001**
Measurement Error	0.0060864	11424	5.3277e-007		

** highly significant ($p < 0.001$)

Table 4 Principal component scores reflecting symmetry and asymmetry score values as well as the summary of the affected landmarks.

PC	Individual (symmetry)	Sides (directional symmetry)	Interaction (fluctuating asymmetry)	Landmarks
Female				
PC1	59.7774%	100%	25.1913%	8,9,10,11,12,13,14,15,25,26, 27,28,29,30,31,33,41,42,43, 44,45,46,47,48,49,50
PC2	12.0080%		16.9962%	22,24,34,36
PC3	7.5098%		12.2442%	26, 32
Male				
PC1	47.0749%	100%	28.6612%	7,10,11,12,13,14,15,16,19,25, 28,29,30, 32,33,42,43,44, 45, 46,47,48,52
PC2	14.2178%		16.0217%	20,38
PC3	8.9575%		8.4983%	6,52

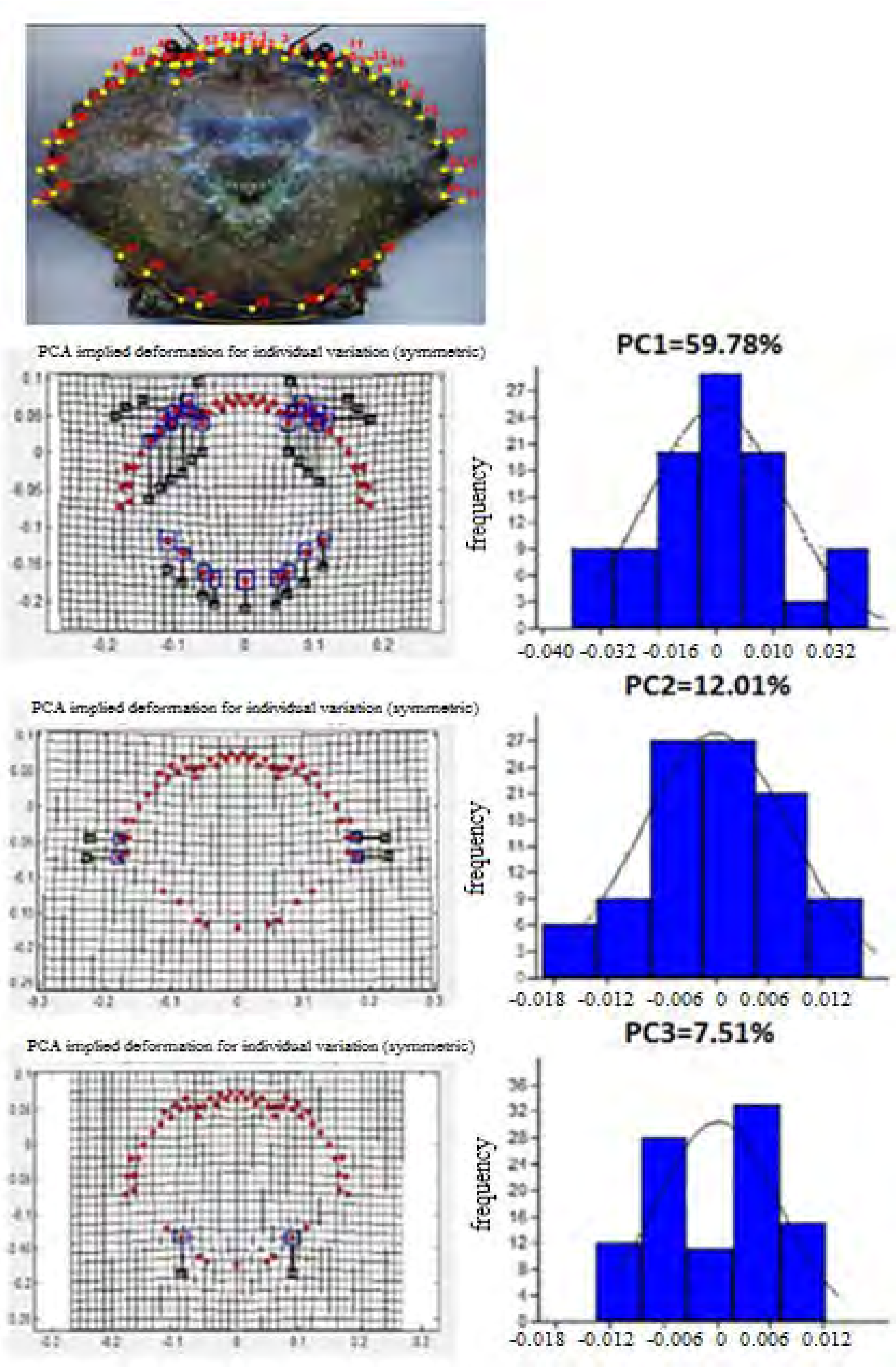

Fig. 5 Shape changes associated with first three significant PC axes for the female *S. serrata* (For each of the two PCs the line shows the shape deformation corresponding to the observed extremes of the PCA axes, (from negative to positive directions) and histogram of individual variations (symmetric) in *S. serrata*fe males.

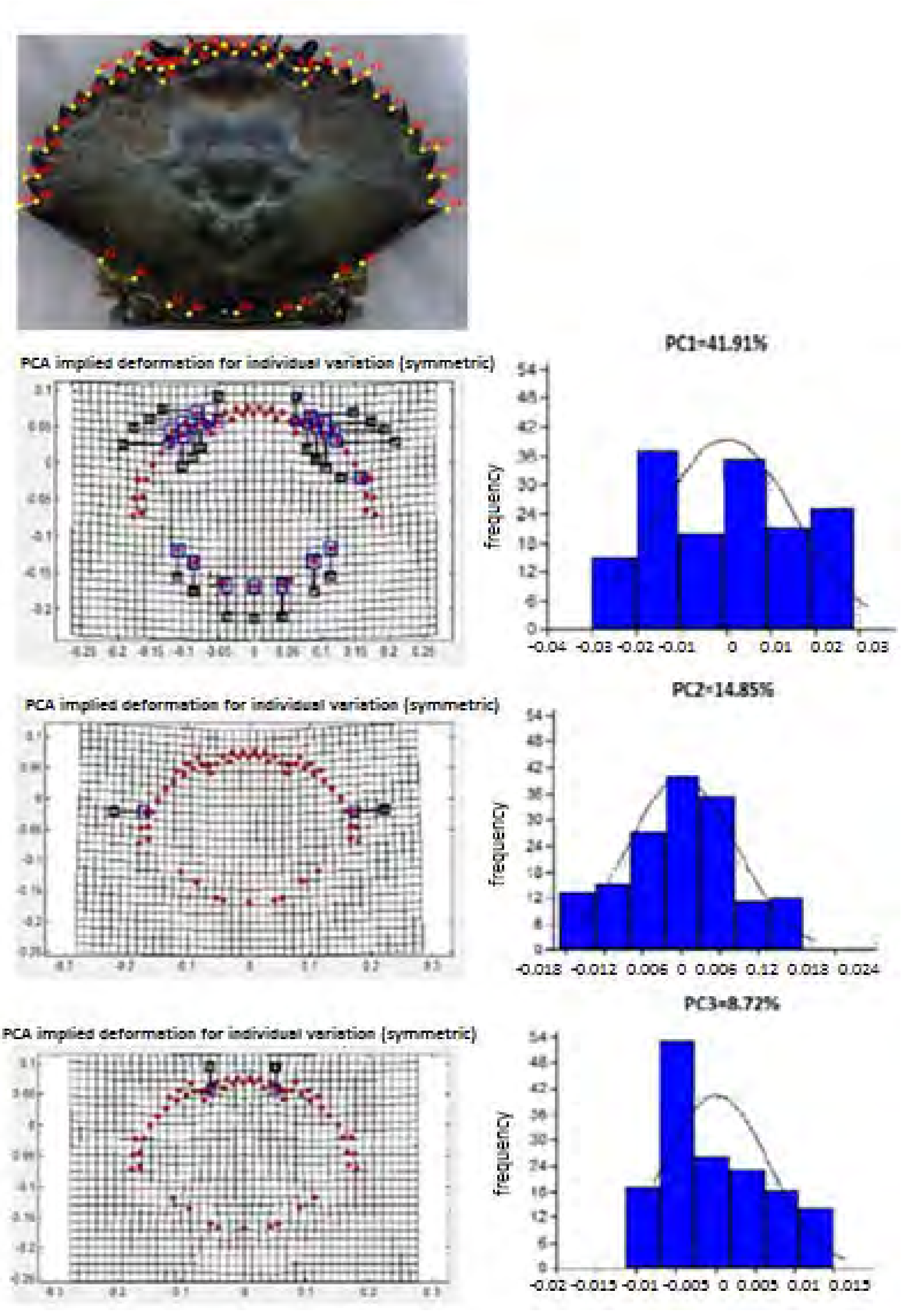

Fig. 6 Shape changes associated with first four significant PC axes in male *S. serrata* (For each of the two PCs the line shows the shape deformation corresponding to the observed extremes of the PCA axes, from negative to positive directions) and histogram of individual variations (symmetric) in *S. serrata* males.

4 Conclusion

This study shows fluctuating asymmetry in the shape of the carapace in both sexes of the crab *S. serrata*as shown by the high FA values resulting from the variations in left and right sides of the carapace. Between sexes, females have higher FA which means they are more affected by the stressed conditions in the gulf.

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