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Using thin-plate spline grids in modeling sex differences in the shapes of the apical disc in the sea urchin (*Tripneustes gratilla*)

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Abstract

Variations in the shapes of the apical discs between sexes in the sea urchin *Tripneustes gratilla* were modeled through the use of Thin-plate spline (TPS) deformation grids.The analysis was done in two levels. First, the consensus or average morphologies of each sex were established and compared. Second, patterns of shape variation were analyzed through a careful study of a series of TPS grids when data was subjected to the geometric morphometric method of Relative Warp Analysis (RWA). The results show that differences in the shapes of apical disc were almost impossible to detect when only the consensus shapes were compared. Variations were much more obvious when the TPS of the top seven relative warps were analyzed. Among the local variations observed was the presence of two shape classes among the females. The males on the other hand assumed a unimodal distribution which is suggestive of it belonging to a single shape class. An important recurrent theme is the apparent asymmetry in the shapes of the lateral margins of the apical disc in both sexes.

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Introduction

Understanding the relationship between structure and function has long been a favorite topic of functional morphology. Also, objective descriptions and measurements of biological structures have been the basis of taxonomic classification of organisms (Benitez, 2013). It is within this context that the concept of Geometric Morphometrics (GM) was born. Compared to the more traditional linear morphometrics, GM works by separating the shape from the size components of biological variation. Also, instead of taking a series of measurements representing the distance between two points, GM preserves the geometric properties of biological shape by looking at the locations of the points or landmarks. With this method, the results of the analysis are not just number but shapes and thin-plate spline deformation grids that are visually appealing and intuitive (Webster and Sheets, 2010; Fruciano, 2011). Today, GM is already widely used in testing hypothesis on the evolution of biological diversity (Adams *et al.*, 2002).

In this study, the thin-plate spline deformation grids were used to model variations in the shapes of the apical disc between sexes in the sea urchin *Tripneustes gratilla* Linnaeus 1758. Sexual dimorphism in this disc has been difficult to detect using the linear morphometrics. Aside from being externally indistinguishable, males and females of this species are said to differ only in the lengths of the genital papillae with the former having a longer one compared to the latter (Tahara *et al.*, 1958).

Materials and methods

Collection of sea urchins

A total of one hundred and twenty (120; 60M and 60 F) sea urchins were collected from Layag-Layag, Talon-Talon, Zamboanga City in July of 2014 (Fig. 1).

Fig. 1. Sample of T. gratilla collected from the sampling site.

The location of the sampling site is shown in figure 2. Only those with test diameters more than 7.0 cmwere collected. This is to ensure that the collected samples are already in their sexual maturity (Junio-Meñez, 2000). The sexes of the sea urchins were identified after the samples were induced to spawn. Males exude a whitish material while that of the females is yellowish in color (Fig. 3).

Removal of spines and image processing

Spines surrounding the apical disc were removed and slimes were brushed out to expose it clearly. Right after removal of spines and brushing out of slime, all 120 *T. gratilla* samples were photographed at a uniform focusing distance (fd) of 0.45m using Canon ef 50mm f/1.8 lens mounted on Canon EOS 1100D, all 120 samples were 5x zoom in crop.

Landmark assignments

A total of 35 landmarks equivalent to 70 X and Y Cartesian coordinates were digitize from around the apical disc using the software tpsDig ver. 2.15 software (Rohlf F.J. 2008) (Fig. 4). These landmarks represent points from the anterior, posterior, and left and right lateral sides of the disc. Detailed descriptions of these points are presented in Table 1.

Thin plate spline grids

Relative warp analysis was performed using the tpsRelw version 1.46 (Rohlf F.J. 2008) to generatethe thin-plate spline deformation grids. Also, the relative warp scores were analyzed further using histograms and discriminant function analysis to determine divergence in the shapes of the apical discs between sexes.

Fig. 2. Map of the Philippine showing the study site of Layag-layag, Talon-Talon, Zamboanga City.

Results and discussion

Results showed no apparent differences in the apical discs when only the consensus morphologies were compared (Fig. 5). Further analyses using vector plots however revealed some variations in the directions of the arrows which is indicative of the presence of sexual dimorphism (Fig. 6). For example, differences in the direction of the vectors are apparent in the posterior part of the apical disc.

For a detailed comparison between sexes, the thin-plate spline deformation grids associated with each of the top 7 relative warps were constructed to identify localized shape differences (Fig. 7). Results revealed the presence of two shape classes among the females. However, the males only assumed a unimodal distribution which means they only belong to one shape class. Also, asymmetry between the left and right lateral sides is a recurring theme in almost all relative warps. Detailed descriptions of the meanings of the thin-plate splines are summarized in Table 2.

To determine whether the observed differences in the shape of the apical discs between sexes is statistically significant, the relative warp scores were subjected to Discriminant Function Analysis (DFA). Results revealed a divergence in the discriminant scores which is indicative of sexual dimorphism (Fig. 7). This is also supported by a percentage correct classification of 100%.

Table 1. Description of the landmarks in the apical disc of *Tripneustes gratilla*.

#	Description of landmark
1	Apex of madreporic plate
2	Landmark intermediate to apex and left base of madreporic plate
3	Left basal landmark of madreporic plate
4	Upper right corner of ocular disc I
5	Upper left corner of ocular disc I
6	Right basal landmark of genital plate I
7	Landmark intermediate to right basal landmark and apex of genital plate I
8	Apex of genital plate I
9	Landmark intermediate to apex and left basal landmark of genital plate I
10	Left basal landmark of genital plate I
11	Upper right corner of ocular disc II
12	Upper left corner ocular disc II
13	Right basal landmark of genital plate II
14	Landmark intermediate to right basal landmark and apex of genital plate II
15	Apex of genital plate II
16	Landmark intermediate to apex and left basal landmark of genital plate II
17	Left basal landmark of genital plate II
18	Upper right corner of ocular disc III
19	Upper left corner of ocular disc III
20	Right basal landmark of genital plate III
21	Landmark intermediate to right basal landmark and apex of genital plate III
22	Apex of genital plate III
23	Landmark intermediate to apex and left basal landmark of genital plate III
24	Left basal landmark of genital plate III
25	Upper right corner of ocular disc IV
26	Upper left corner of ocular disc IV
27	Right basal landmark of genital plate IV
28	Landmark intermediate to right basal landmark and apex of genital plate IV
29	Apex of genital plate IV
30	Landmark intermediate to apex and left basal landmark of genital plate IV
31	Left basal landmark of genital plate IV
32	Upper right corner of ocular disc V
33	Upper left corner of ocular disc V
34	Right basal landmark of madreporic plate
35	Landmark intermediate to apex and left basal landmark of madreporic plate

Table 2. Description of the shape changes associated with the top seven relative warps.

RW	%	Females	%	Males
1	23.5	Significant variations along the anterior region of the apical disc is observed from the negative to the positive extremes of the RW1	22.1	Slight variation from negative to positive extremes has been observed.
2	14.9	The negative extreme shows a constricted apical disc shape while the positive extreme shows an expanded apical disc shape.	14.5	A narrow apical disc can be inferred from the negative to positive extreme.
3	11	The negative extremes shows a leaning apical disc shape to the right lateral side while on the positive extreme, the apical disc leans to the left lateral side.	11.5	The apical disc shapes from the negative to positive extremes are very much similar to the mean shape.
4	7.7	A very significant distortion can be seen on the right postero-lateral region of the apical disc on the positive extreme while minimal variation is seen on the negative extreme.	7.5	Only a slight variation is observed. The positive extreme shows a slightly wider apical disc shape compared to the negative extreme.
5	4.8	Greater variations can be observed in the anterior region of the apical disc shape, positive extreme shows a narrow apical disc shape while wider on the negative extreme.	4.2	Slight variation is shown from negative to positive extremes.
6	4.6	A constricted apical disc is seen on the positive extreme while a more pronounced shape is seen on the negative extreme.	3.8	Left lateral region of the apical disc on the positive extreme shows a greater variation while less variation is seen on the negative extreme.
7	3.8	Negative extreme shows a constricted-leaning apical disc shape to the left lateral region while positive extreme shows a constricted-leaning apical disc shape to the right lateral region.	3.6	Less variation is observed from negative to positive extreme.

With appropriate statistical analysis, the results presented above indicate the presence of sexual dimorphism in *Tripneustes gratilla*. This however may not hold true for other species of sea urchins. In reality, patterns of sexual dimorphism are said to be species specific.

Fig. 3. Sexing of *T. gratilla* samples.

Taking for example the study by Abdel-Rahman *et al,* (2009), differences between sexes was only found in one out of two species of rodents examined. Also, very specific methods of geometric morphometric analysis were used to detect sexually dimorphic characters, which were otherwise difficult to detect when traditional linear morphometrics was applied. In a study by de Camargo *et al,* (2015) however, the wings of seven species of moths were found to be sexually dimorphic.

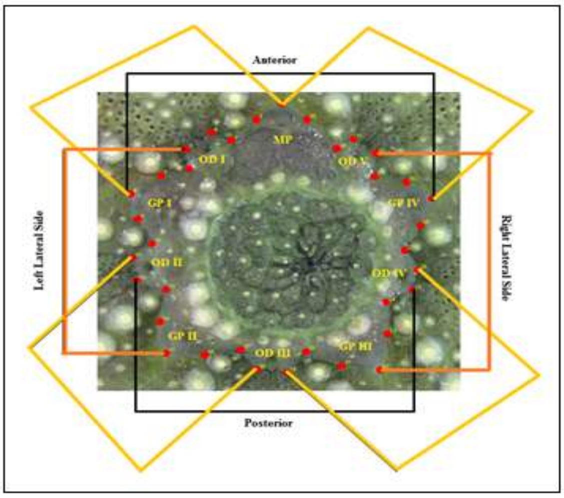

Fig. 4. Landmarks on *Tripneustes gratilla* apical discused for geometric morphometric analysis. Legend: MP= Madreporic plate, OD=Ocular disc, GP=Genital plate.

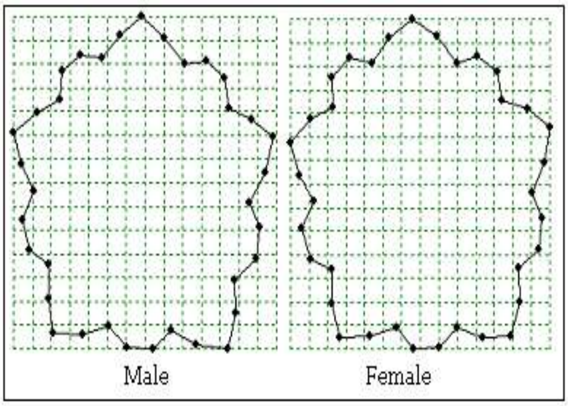

Fig. 5. Consensus topologies of the apical disc of male and female *T. gratilla.*

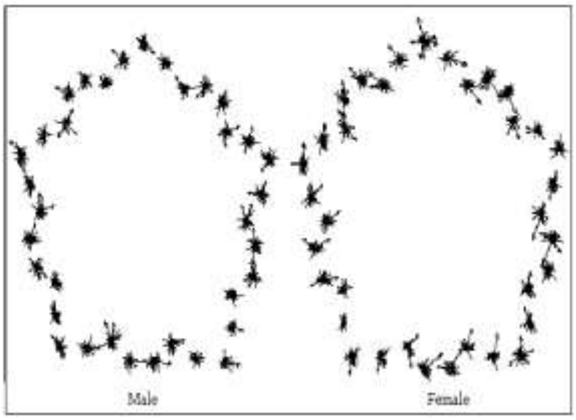

Fig. 6. Vector plot showing the relative influence of the 35 landmarks marked along the apical disc of male and female *Tripneustes gratilla*.

This result was a new finding as moths belonging to family Sphingidae often have very discreet wing differences, which is almost always not easily observed. While practical applications of sexual dimorphism studies are many, those for sea urchins might be difficult to ascertain at this moment. However, there are reasons to believe that studies on disparities in the biologic features between sexes are important in ecological studies.

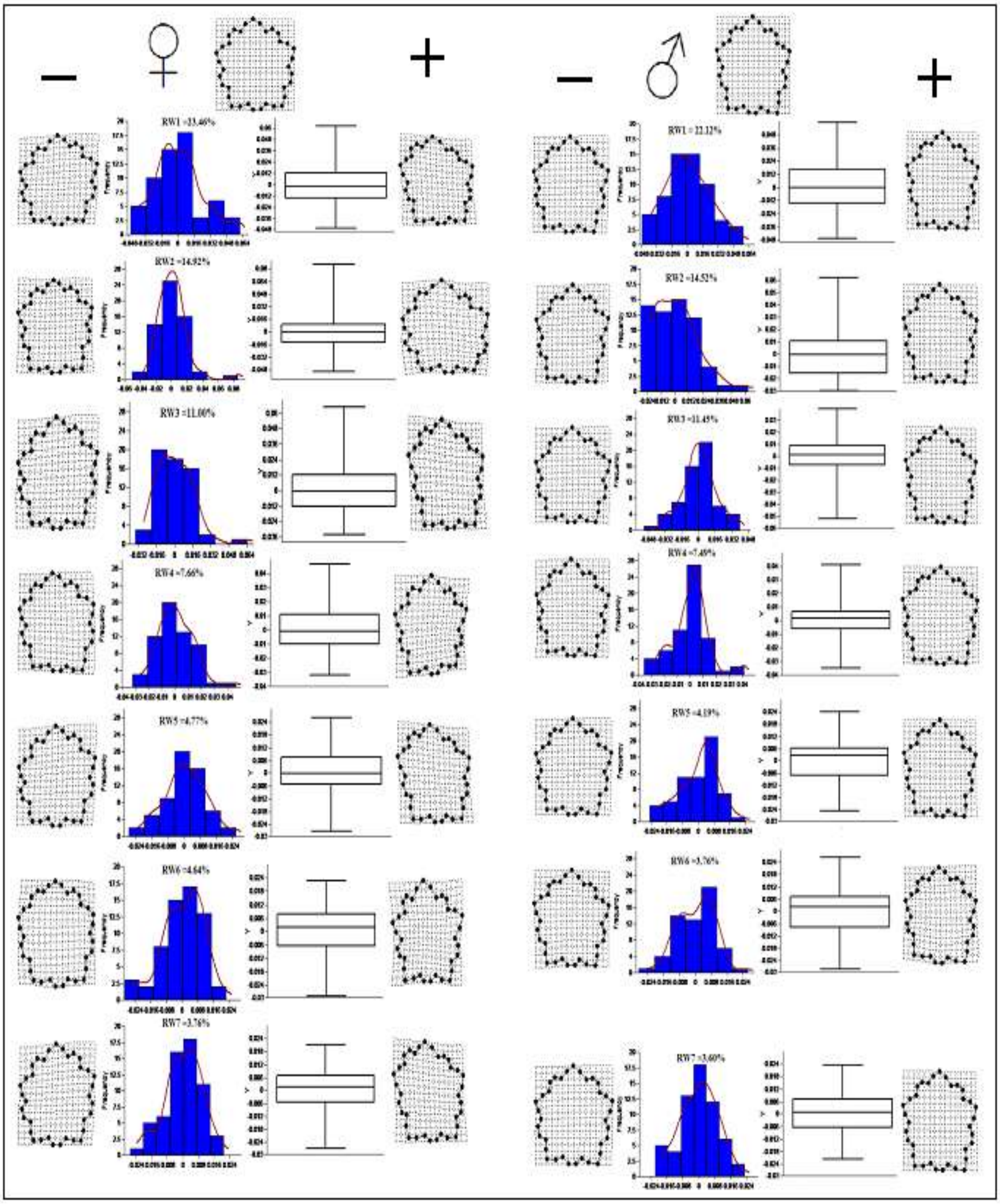

Fig. 7. Summary of the geometric morphometric analysis showing the consensus morphology of the apical disc and the variation among female and male populationof *T. gratilla* found in Layag-layag, Talon-talon Zamboanga City.

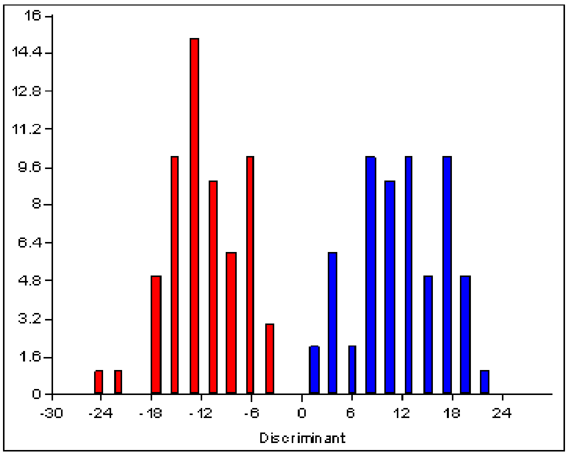

Fig. 7. Disparity in the distribution of the discriminant scores between male and female *T. gratilla* indicating sexual dimorphism in this species of sea urchin.

Conclusion

The results of this study undermines the utility of the method of thin-plate spline grids in visualizing shape differences in biological structures such as the apical disc of *T. gratilla*. Previous studies using linear morphometrics have failed to distinguish size differences between males and females. Also, patterns of local and global (apical-wide) shape variation can easily be identified through the deformation grids which offer opportunities for a detailed and objective description of the structure.

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