

## EVALUATION OF BODY MORPHOLOGY AND SHAPE OF BLACK TIGER PRAWN (*PENAEUS MONODON*) BY MORPHOMETRIC ANALYSIS

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### SUMMARY

Morphometric analysis of body shape is an important tool for assessing shape variation among a wide variety of organisms. We carried out a morphometric analysis of prawns (n=8,164) by using land-marked points on images. Principal component (PCA) analysis revealed that the data were clustered in three major landmark derived distance groups, and the first principal component (PC1) as a representation of overall size of the prawn primarily related to overall length, and accounted for 94.9% of the variation, whereas PC2 explained 1.5% of the total variation mainly associated with the measure of the body thickness. The preliminary results suggest that morphometric techniques can be used to reveal shape variation in shrimp species.

### INTRODUCTION

Morphometrics is largely defined as an approach to evaluate variation and co-variation of shapes with other traits. Traditional morphometric analysis is based on univariate/multivariate analysis of variables such as distance measures, angles, and/or ratios, to capture and describe complex two- and three-dimensional shapes. Over the last two decades, morphometric analysis of body shape has gradually become an important tool for assessing and studying shape variation among a wide variety of organisms. This technique has been applied in various fields including phylogenetic analysis, species identification and classification, examining extent of phenotypic plasticity, defining and explaining of ancestral morphotype (Adams *et al.* 2013). Although morphometric approaches are successful in objectively describing complex shapes, these methods are lacking in the application of genetic analyses and genomic selection studies. Most aquaculture studies have been restricted to several fish species, only a limited number of studies are available regarding shrimp species using small number of animals (Claverie and Wainwright 2014; Adams 2013; Aktas *et al.* 2006). To address this deficit, we carried out a morphometric analysis of a large number of shrimp by using land-mark points derived from images.

Landmarks are fixed points of reference on an object that can be matched between and within samples. Derived distances between landmarks are employed to capture two- and three-dimensional shapes using sophisticated data collection tools. The first step of any geometric morphometric data analysis is the superimposition of the landmark configurations. Superimposition is the process of merging two or more landmarks data positions to remove any differences between data collection times and scales all the samples to a common unit. After superimposition, coordinates of each sample are represented by a single point in a common shape and space, and shapes are defined based on the combination of a number of landmarks. To reduce the high-dimensionality of the landmarks, ordination methods are generally employed to summarize the variation of shape space. The most commonly employed ordination technique is principal component analysis (PCA), a method that is used to perform

an eigen decomposition of the covariate matrix and evaluate the distribution of samples along each of the resulting eigenvectors or principal component (PC) axes (Slice 2007). Therefore, the resulting PC axes are linear groupings of the original data that are statistically independent of each other. Standard multi-variate statistical analysis techniques (e.g., MANOVA) can be used for exploring statistically significant shape differences among groups related to categorical variables and fixed effects (e.g., sex, geographical location, experimental treatments etc.) (Webster *et. al.* 2011). The purpose of this study was to develop an efficient morphometric approach to characterize shrimp morphometric traits and investigate factors that affect the variability of the traits across sex, environments and other treatments. The characterized morphometric shape traits will further be utilized in the *P. monodon* selection breeding program by estimating genetic parameters and genomic selection.

**MATERIALS AND METHODS**

Despite the lack of morphometric analyses in shrimp, there is a consensus on the traits that are of industry/commercial significance. These traits include; total length, body length, carapace length, length width ratios. Typically, measurements are made between one easily distinguishable landmark to another and that distance is ascribed with a trait name. The morphological measurements proposed in this study have been generated using an amalgamation of methods used in other studies as well as what measurements are possible within the limitations of the available images (Figure 1 and Table 1 and 2) (Aktas *et. al.* 2006; Hung *et. al.* 2014).

**Table 1. Lists of landmark description used in our analysis**

Landmark number	Landmark Description
1	Placed on the most anterior point of the antennal scale
2	Placed on the most anterior point of the tail
3	Placed on the most posterior point of the tail
4	Placed on the junction of the carapace and abdomen placed at the most dorsal point
5	Placed on the midway along the carapace on the ventral side of the prawn
6	Placed on the junction of the carapace and abdomen placed at the most ventral point
7	Placed dorsally on the midpoint of the first abdominal segment
8	Placed ventrally on the midpoint of the first abdominal segment
9	Placed dorsally on the midpoint of the third abdominal segment
10	Placed ventrally on the midpoint of the third abdominal segment
11	Placed dorsally on the midpoint of the last abdominal segment
12	Placed ventrally on the midpoint of the last abdominal segment



**Figure 1. Scored morphometric traits of *P. monodon***

**Table 2. Lists of traits derived from landmark data**

Trait	Landmark Coordinates	Trait Description
Total length	1-3	From the most anterior point of the antennal scale to most posterior point of the tail
Body length	1-2	From the most anterior point of the antennal scale to most anterior point of the tail
First abdominal segment height	7-8	From the dorsal midpoint to the ventral midpoint on the first abdominal segment
Third abdominal segment height	9-10	From the dorsal midpoint to the ventral midpoint on the third abdominal segment
Last abdominal segment height	11-12	From the dorsal midpoint to the ventral midpoint on the last abdominal segment

For the morphometric shape analysis, 8164 photographed specimens were used to estimate 66 morphometric distances derived from twelve landmarks (Figure 1). All the images were annotated for these twelve landmarks using ImageJ software. These unambiguous landmarks were representative of prawn size and shape from various aspect ratios. To evaluate the reliability of landmark data, repeated measurement of 300 image landmarks were done by the same operator and between two independent operators. The overall repeatability of the measurements was high ( $r = 0.98$ ).

## RESULTS AND DISCUSSION

PCA analysis revealed that the first three PCA accounted for 97.5% of the variability of the data. The data were clustered in three major landmark derived distance groups (Figure 1). The first principal component (PC1) accounted for 94.9% of the recorded variation in the landmark distance data (Table 1). PC1 was taken as a representation of overall size of the prawn, since all PC1 loadings were positives and PC1 scores were significantly correlated with d\_1\_3 (which corresponds total length). Moreover, variation in d\_1\_3 contributed the highest variability in the PCA. For instances, a comparison between the image of highest and lowest PC1 scores revealed very contrasting sized prawns (data not shown). The higher variance accounted by PC1 may be due to the presence of a wide range of size in the samples, and often observed when samples in different sizes were studied (Stauffer 1991; Claverie and Wainwright 2014). On the other hand, the second principal component (PC2) explained 1.5 % of the total variation among landmarks, which is the measure of the proportionate body width/shape of this species. For example, extremes on this axis are very thin/elongated body shape at the low end and the thick/fatty shape at the high end.

**Table 3. Variability explained by different PCs**

	PC1	PC2	PC3	PC4	PC5	PC6
Standard deviation	192.21	24.54	20.19	16.16	12.62	10.46
Proportion of Variance	0.949	0.015	0.010	0.006	0.004	0.002
Cumulative Proportion	0.949	0.964	0.975	0.981	0.985	0.988

