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Availability of Morphologically Similar, Genetically Diverge Penaeus Monodon Populations in Sri Lanka

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Abstract

Morphological and genetic variations of three *Penaeus monodon* populations in Sri Lanka were determined using data from truss network method and partial amplification of mitochondrial control (mtC) gene region respectively. Total of 37 morphometric characters were collected from 168 individual representing three populations (eastern, western and southern) and subjected to Principle Component Analyses (PCA) to determine the morphological variation among three populations. Two principal components derived from PCA analysis with the accumulated variance for the two PC represented as 75% of the total. The plot against first and the second principal components scores revealed great morphological similarity among three populations.

For genetic analysis, total of 600 bp DNA fragment was amplified from mtC gene region and twenty five haplotypes were produced for three populations from the total of 63 samples examined. Mean haplotype diversity (h) and nucleotide diversity (π) among three populations were ranged from 0.35 (southern) to 0.92 (eastern) and from 0.5% (southern) to 2.5% (western) respectively. Analysis of molecular variance (AMOVA) indicated within population variation as 78.89% and among population variation as 21.11% indicating high intra-population diversity. The overall F_{st} value indicated significant genetic structure among three populations (F_{st} = 0.2111, P = 0.00 < 0.05). The possible reasons for the occurrence of morphologically similar but genetically diverged populations in Sri Lankan waters are discussed.

Keywords: Penaeus monodon, Morphology, Truss network, Genetic variation, Mitochondrial control gene region.

1. Introduction

The black tiger shrimp *P. monodon* is one of the most important marine crustacean species and is subjected to intense fishery exploitation and aquaculture practices worldwide (Hulata, 2001). Exploitation of shrimp fisheries may deplete the wild stocks to an unsustainable level. Moreover, the tiger shrimp aquaculture industry still relies on wild-caught brood stocks to seed farmed shrimp populations. Therefore, the origin of brood stocks and their genetic composition are significant issues that require attention. Due to the inbreeding depression effects, release of hatchery stocks with limited genetic variation may be detrimental to the viability of natural populations (Ferguson, 1995). Thus, Wild stocks can provide an immediate resource for addressing genetic diversity problems in cultured stocks, and consequently require conservation. To this end, the patterns and extent of genetic diversity that are present in wild stocks need to be adequately documented so that we can identify which stocks may carry unique genetic attributes and prioritize conservation efforts.

In Sri Lanka, early 90's was the period where the prawn farming came to the climax stage. In late 90's outburst of white spot disease made a considerable damaged to the shrimp culturing industry which earn considerable amount of foreign exchange to the county (Wijegoonawardena and Siriwardena, 1996). As indicated, major reasons for this outbreak are pollution and the maintenance of the hatcheries in a very low standard in terms of the quality (Jory, 2000). However, it need to be highlighted that lack of genetic diversity within the stock may be another important issue to spread this disease rapidly among prawn farms.

Both, morphological and genetic information have been used in identification of stock structure of marine organisms. Morphological traits have been used since last two decades to discriminate populations and to determine stock structure of *P. monodon* (Chandra et al., 1997; Daud, 1995; Rebello, 2003**;** Natarajan et al., 2011; Sun et al., 2012).

To date, the population genetic data of *P. monodon* have been based on several markers such as, allozymes (Klinbunga et al., 1998; Klinbunga et al., 1999; Sugama et al., 2002), randomly amplified polymorphic DNA (Tassanakajon et al., 1998), microsatellites (Brooker et al., 2000; Supungul et al., 2000; Xu et al., 2001; You et al., 2008), mitochondrial DNA (mtDNA) fragment length polymorphism (Benzie et al.; 2002) and sequence data (Kumar et al., 2007; You et al, 2008; Waqairatu et a.,l 2012) and nuclear DNA data (Duda and Palumbi 1999).

Although *P. monodon* was one of the major sources of income in aquaculture sector in Sri Lanka, studies on population structure of *P. monodon* in Sri Lankan waters have not been reported so far. It is important to conduct aquaculture programs with the knowledge of phenotypic and genotypic background of the cultured species. Thus, it is necessary to screen the diversity of the wild populations before collecting brood stocks for culturing programs. Therefore, this study was conducted to determine diversity among three *P. monodon* populations using morphological and mitochondrial DNA sequencing data.

2. Materials and Methods

Samples were collected from three populations of *P. monodon*; Southern, Western and Eastern regions (Figure 1) during 2010 - 2011. Approximately 100 samples were collected from each population. Samples were stored on iced or frozen immediately after capture and transported to Research Laboratory of the Department of Zoology, University of Ruhuna, Matara, Sri Lanka.

2.1. Collection of Morphometric Data

For morphometric analysis total of 168 individuals (Eastern= 66, Western = 54, Southern = 58) were randomly selected from each population and eighteen landmarks determining 40 length measurements (morphometric parameters) on the prawn body were measured using vernier caliper, thread and ruler as illustrated in the Figure 2. Characters with missing data and data that skewed from normality test (even after log transformed) were omitted from further analysis. Finally, 37 morphometric parameters were examined and computed for analysis (Figure 2). For normality, all morphometric data were log transformed and standardized using regression and residual analysis method (Thrope, 1976). Standardization of size related data is important in this analysis as measured parameters could be biased among populations due to growth stages and the sampling technique. To conduct multivariate analysis, Principle Component Analysis (PCA) was performed to determine the difference among three populations according to morphological characters. All analysis was performed using SPSS (V. 16.0) or MINITAB (V. 13.0) Statistical packages.

2.2. Collection of molecular data

Molecular analyses were conducted at two places, Molecular Genetics Laboratory at Brigham Young University (BYU), USA or Molecular Unit at the University of Colombo, Sri Lanka. Approximately 20 – 25 samples were collected from each population for genetic analysis. DNA extraction was carried out using DNeasy Blood & Tissue (QIAGEN) extraction kit following the manufacturer's instructions. A fragment of mitochondrial control gene region was amplified using primers: 12S (forward) 5'AAGAACCAGCTAGGATAAAACTTT 3' and 1R (reverse) 5'GATCAAAGAACATTCTTTAACTAC 3'(Chu et al., 2003). PCR reactions were conducted in 25µL reaction mixture contained 1× Taq polymerase buffer, 1.5mM $MgCl_2$, 0.4mM each dNTP,

0.2µm of each primer, 100ng of DNA template and 0.5U of Taq DNA polymerase. The PCR conditions were: 2min at 94°C following 30 cycles of 30s at 94°C, 30s at 45°C, 1min at 72°C and finally, 7min at 72°C. The size and quality of PCR products were assessed using 1% agarose gel electrophoresis. PCR products were sequenced using facilities available at the sequencing centre, BUY University, USA or at Macrogen company, Korea.

2.3. Data analysis

Sequence alignment was performed using Bioedit V. 07 (Hall, 2004) program. Population genetic analyses were performed using ARLEQUIN (Schneider et al., 2000) program. Haplotype diversity (h) and nucleotide diversity (π) were calculated. Analysis of molecular variance (AMOVA) (Excoffier, 1992) was performed to estimate the variation contributing to the differentiations among populations and among individuals. For each level, the sum of the squared deviation, the mean squared deviation and the variance component were calculated, and the variance component was then expressed as a percentage. The significance of variance among populations was tested by 1000 random permutation test against the null hypothesis that all individuals belonged to the same population.

3. Results

3.1. Morphological Diversity

Log transformed data for landmarks distances between 2-3, 12 – 13, and 11- 12 did not show normal distribution, thus omitted from the further analysis. After transformation of data into size - independent data, 37 morphometric characters were considered for analysis. Principle component analysis revealed two components and indicated that the accumulated variance for the two principal components (PC) represented 75% of the total. (Table. I). First principle Component closely associated to the size parameters 6-13 and 6-14 while the 2nd Principle Component better discriminates for 14-15, and 9-10. The plot against first and the second principal components showed an overlap of populations, which indicates that there is a great resemblance among three populations (Figure 3).

3.2. Genetic Diversity

Approximately 600 bp fragment of the mtDNA control region was amplified for 63 individuals of *P. monodon*. Twenty five haplotypes were resulted from three populations and sequences were deposited in the gen bank under accession numbers KF639857 - KF639882. Number of haplotypes and nucleotide diversity levels for each population are given in the Table 2. Only one haplotype was common among southern and western populations and all other haplotypes were specific to each population. Frequency of haplotype distribution among three populations is given in the Table 3. Mean haplotype diversity (h) among three populations was ranged from 0.35 (Southern) to 0.92 (Eastern) (Table 4). The nucleotide diversity (π) among three populations was ranged from 0.5% (Southern) to 2.5% (Western). The overall F_{st} value indicated significant genetic structure among populations (F_{st} = 0.2111, P = 0.00 < 0.05) (Table 5).

4. Discussion

Morphological variability among different geographical populations may be attributed due to distinct genetic structure and environmental conditions. Therefore, animals with the same morphometric characters are often assured to constitute a stock and that has been utilized widely in stock differentiation in fisheries industry (Dwivedi and Dubey, 2013).

Over past few years, morphometric studies of crustacean species rely on set of traditional length measurements of the body parts. To avoid limitations of the previous method Truss Network System has been used for stock identification of different fish species (Bemvenuti, 2006; Jaffrian et al., 2010) including *Penaeus* species (Aktas et al., 2006; Rebello, 2003). Unlike the traditional method, the Truss Network System covers the entire fish in a uniform network, which increase the possibility of extracting morphometric differences, thus is much more powerful in describing morphological variation between and among species (Turan, 1999). The graph produced by plotting two Principle components (PC1 and PC2) grouped three populations into one cluster indicating that all three populations are morphologically homozygous. Therefore, in phonotypical point of view, we can consider that irrespective of geographic variation these three populations as a single stock. Similar

results have been reported from a previous study conducted for Indian *P. monodon* Populations (Rebello, 2003). The recent study conducted by Sun et al., (2012) also pointed out that populations of east coast of Indian ocean and Pacific Ocean are morphologically similar thus need to be considered as one stock.

However, genetic data collected from three populations did not support the results of morphological study. High haplotype (h) and nucleotide diversity levels (π) were observed in Eastern and Western populations (Table 2) indicating great genetic variation within the populations. Similar results have been reported in past studies for different geographic locations which mitochondrial control gene region produced high haplotype diversity levels (Kumar et al., 2007; Zhou et al., 2009; You et al., 2008). In contrast to that, Southern population of the current study indicated low haplotype and nucleotide diversity levels (h = 0.3579+/- 0.1266, π = 0.005478 +/- 0.003295) indicating low genetic variation within the population. These results indicate the signs of over exploitation of the population or restriction of gene flow among other populations due to isolation.

Results derived from the present study could be compared with studies conducted on *P. monodon* populations using mitochondrial control gene region. Kumar et al., (2007) studied on Indian populations and Zhou et al., (2009) and You et al., (2008) covered large geographic distribution from west Indian to east Pacific ocean. The haplotype diversity values derived from Eastern and Westen Sri Lankan populations (0.9 – 0.92) were close to those levels reported from the east coast populations of India (0.93) (Kumar et al., 2007) and values reported from You et al's study (2008) for Australia (0.969) to Eastern Thailand and India to Kenya (1.000). However, nucleotide diversity values derived for three Sri Lankan populations are in between (0.5% in Southern to 2.5% in Western) the values reported for Indian (1.3% in Gopalpur to 5.4% in Andaman sea) (Kumar 2007) and other populations (2.2% in Kenya to 8.1% Eastern Thailand) (You et al., 2008).

According to the results of AMOVA test, high percentage of variation was occurs due to within population variation (78.89%) and it was low for among population variation (21.11%). Pairwise F_{st} values indicated significant genetic structure among three Sri Lankan populations (Table 4) which may result due to high genetic diversity within three populations which indicated restricted of gene flow among them. The F_{st} values of the current study (from 0.06911 - 0.36891) are much more greater than the values reported for the Indian populations (Kumar et al., 2007) which was ranged from - 0.06393 to 1.6477 (Kumar et al., 2007) and other indo-pacific populations which ranged from 0.004 to 0.095 in You et al (2008) study and 0.00068 to 0.098 in Zhou et al., study (2009). This revealed that south Indian Ocean have more structured populations than eastern pacific and western Indian populations which this hypothesis was recently predicted by Waqairatu et al., (2012) reviewing the distribution pattern of *P. monodon* in a broad view using mtC data.

Finally, it can be concluded that genetic differences may not always represent by phenotypic variation. This may be due to phenotypic plasticity of fish that allows them to respond adaptively to environmental change by modification in their physiology and behavior which leads to changes in their morphology, reproduction or survival that mitigate the effects of environmental variations which could underestimate biodiversity values in nature (Lefébure et al., 2006). Therefore, it has been highlighted the importance of utility of genetic information in stock identification and conservation studies for wild population molecular markers provide direct information to identify populations as a single unit or composed of several subunits (Chauhan and Rajiv, 2010).

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Tables

Table-1. Loadings of variables for two main principle components

Parameter	PC1	PC2
6 – 13	.844	.399
6 -14	.801	.533
4 – 15	.794	.519
5 – 15	.790	.548
6 – 7	.776	.416
7 – 14	.775	.594
4 – 16	.769	.563
6 – 17	.767	.553
6 – 15	.767	.599
8 - 12	.760	.492
5 - 14	.751	.497
4 – 17	.750	.501
5 – 16	.748	.499
3 – 17	.738	.547
7 – 12	.727	.604
3 - 16	.722	.512
8 – 13	.710	.616
15 - 16	.709	.535
8 – 11	.708	.647
4 - 5	.692	.411
9 – 12	.687	.624
2 - 17	.679	.560
18 - 1	.670	.450
9 – 11	.661	.612
17 - 18	.642	.535
3 - 18	.626	.481
3 - 4	.575	.430
7 - 8	.552	.392
5 - 6	.430	.426
14 - 15	.479	.708
9 - 10	.495	.696
13 - 14	.469	.695
10 - 11	.154	.686
2 - 18	.409	.657
1 -2	.169	.652
7 - 13	.573	.624
8 – 9	.474	.593
Total % of variance	44.152	30.738
Cumulative % of Variance	44.152	74.890

Table-2. Summary of molecular diversity for three populations.

Location	Sample Size	Number of Haplotypes	Haplotype diversity	Nucleotide diversity
Eastern	21	12	0.9238 +/- 0.0383	0.024256 +/- 0.012647
Western	22	11	0.9004 +/- 0.0382	0.025349 +/- 0.013154
Southern	20	03	0.3579 +/- 0.1266	0.005478 +/- 0.003295

Table- 3. Frequencies and distribution of haplotypes among three populations

Haplotype (Sample No.)	Southern (20)	Eastern (21)	Western (22)
H1	16	0	2
H2	2	0	0
H3	2	0	0
H4	0	1	0
H5	0	3	0
H6	0	5	0
H7	0	1	0
H8	0	2	0
H9	0	2	0
H10	0	2	0
H11	0	1	0
H12	0	1	0
H13	0	1	0
H14	0	1	0
H15	0	1	0
H16	0	0	4
H17	0	0	5
H18	0	0	4
H19	0	0	1
H20	0	0	1
H21	0	0	1
H22	0	0	1
H23	0	0	1
H24	0	0	1
H25	0	0	1

Table- 4. Pairwise F_{st} values for three populations

	Eastern	Western	Southern
Eastern	-		
Western	0.06911*	-	
Southern	0.36891*	0.22701*	-

*P < 0.01

Table-5. Results of the AMOVA test

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	2	72.659	1.46057 Va	21.11
Within Population	61	329.516	5.49193 Vb	78.89
Total	63	402.175	6.96149	
Fixation Index F_{st}: 0.21110				
Significance tests (10100 permutations)				

P < 0.05

Figures

Figure-1. Sampling locations of the current study

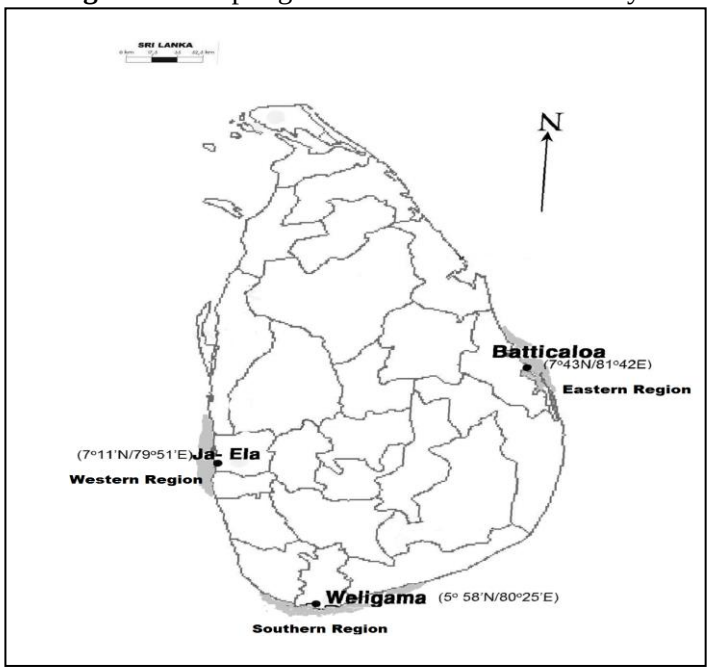

Figure-2. Truss network system used in the current study to collect morphological data.

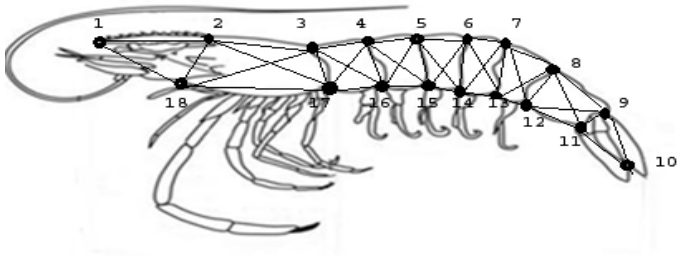

Figure-3. Graph derived from Principle Component Analysis performed using morphometric data