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Morphological and genetic diversity levels of *Penaeus monodon* (Fabricius, 1798) populations in three coastal regions of 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 178 individuals representing three populations (eastern, western and southern) and subjected to principal component analyses (PCA) to determine the morphological variation among three populations. Two principal components (PC) derived from PCA analysis with the accumulated variance for the two PC represented 75% of the total. The plot against first and the second principal components scores revealed great morphological similarity among three populations. For genetic analysis, a total of 600 bp DNA fragment was amplified from mtC gene region and 26 haplotypes were produced for three populations from the total of 63 samples examined. Among three populations, mean haplotype diversity (h) ranged from 0.35 (southern) to 0.92 (eastern) and nucleotide diversity (π) ranged from 0.005 (southern) to 0.025 (western). 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 ($F_{st} = 0.2111$, $p < 0.05$). Genetic diversity levels of Sri Lankan *P. monodon* populations were compared with published data for this species from other geographic locations.

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

Introduction

The black tiger shrimp *Penaeus 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 broodstocks to seed farmed shrimp populations. Therefore, the origin of broodstocks and their genetic composition are significant issues that require attention and extent of genetic diversity that are present in wild stocks need to be adequately documented. This helps to identify stocks that may carry unique genetic attributes and prioritise conservation efforts (Ferguson, 1995).

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 (Sugama *et al.*, 2002), randomly amplified polymorphic DNA (Klinbunga *et al.*, 1998; 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 al.,* 2012, Khedkar *et al.*, 2013) as well as 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. The objectives of the current study were to determine diversity levels among three *P. monodon* populations using morphological and mitochondrial DNA sequencing data and and also to compare genetic diversity levels of the three Sri Lankan populations with reported data for this species from other geographic locations.

Materials and methods

Samples were collected from three populations of *P. monodon*; southern, western and eastern regions

(Fig. 1) of Sri Lanka during 2010 - 2012. Other samples were randomly collected from the markets. Initially eighty samples were collected from each population. Samples were stored in ice or frozen immediately after capture and transported to Research Laboratory of the Department of Zoology, University of Ruhuna, Matara, Sri Lanka.

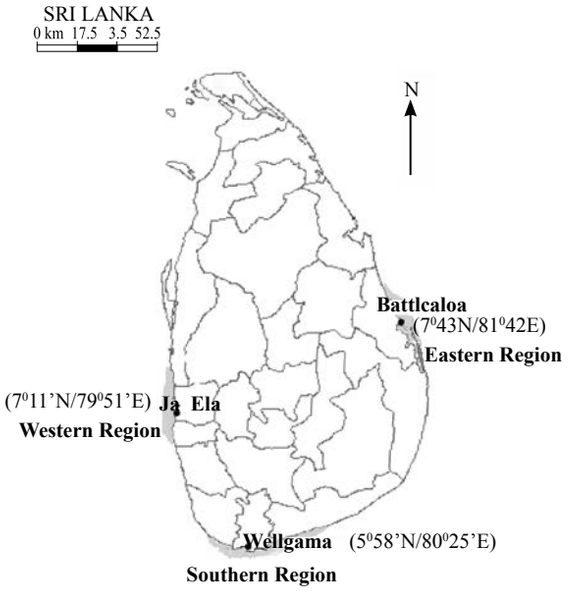

Fig. 1. Map showing sampling locations

Collection of morphometric data

For morphometric analysis, undamaged samples were selected from the initial collection of the three populations as follows: eastern - 66, western - 54 and southern - 58. Eighteen landmarks determining 40 length measurements (morphometric parameters) on the shrimp body were measured using vernier caliper, thread and ruler (Fig. 2.). Characters with missing data and data that skewed from normality test (even after log transformation) were omitted from further analysis. Finally, 37 morphometric parameters were examined and computed for analysis (Fig. 2). For normality, all morphometric data were log transformed and standardised using regression and residual analysis method. The effect of size on body proportions was removed by regression of the log of each measurement on the log of standard length for each population. The regressions for each population were then averaged to produce a common-within groups regression (Thorpe, 1976), and the residuals were calculated. Statistical analyses were performed based on residuals. Standardisation 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, principal component analysis (PCA) was performed to determine the difference among three populations according to morphological characters. All analyses were performed using SPSS (V. 16.0) or MINITAB (V. 13.0) statistical packages.

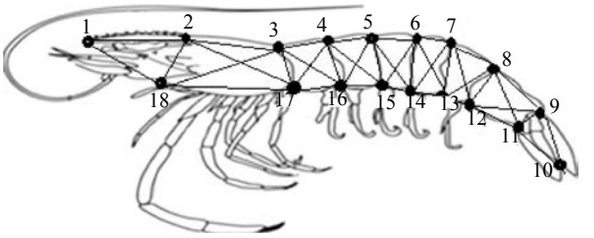

Fig. 2. Truss network system used in the current study to collect morphological data of *P. monodon*.

Collection of molecular data

Molecular analyses were conducted at two places, Molecular Genetics Laboratory at Brigham Young University (BYU), USA and 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 and Tissue (QIAGEN) extraction kit following the manufacturer's instructions. A fragment of mitochondrial control gene region was amplified using primers: 12S (forward) 5'AAGAACCAGCTAGGATAAAACTTT3' and 1R (reverse) 5'GATCAAAGAACATTCTTT AACT AC3' (Chu *et al.*, 2003). PCR reactions were conducted in 25 µL reaction mixture that contained 1× Taq polymerase buffer, 1.5 mM $MgCl_2$, 0.4 mM each dNTP, 0.2 µm of each primer, 100 ng of DNA template and 0.5U of Taq DNA polymerase. The PCR conditions were: 2 min at 94°C following 30 cycles of 30 s at 94°C, 30 s at 45°C, 1 min at 72°C and finally, 7 min at 72°C. The size and quality of PCR products were assessed by 1% agarose gel electrophoresis. PCR products were sequenced using facilities available at the sequencing centre, BUY University, USA or at Macrogen Company, Korea.

Sequence alignment was performed using Bioedit V. 07 (Hall, 2004) program. Population genetic analyses were performed using ARLEQUIN V.3.2 program (Schneider *et. al*., 2000). 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.

Results and discussion

Morphological diversity

Log transformed data for landmarks distances between 2-3, 12-13, and 11- 12 did not show normal distribution, and hence omitted from further analysis. After transformation of data into size - independent data, 37 morphometric characters were considered for analysis. Principal component analysis revealed two components and indicated that the accumulated variance for the two principal components (PC) represented 75% of the total (Table 1). First principal component closely associated to the size parameters 6-13 and 6-14 while the 2nd principal 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 (Fig. 3).

Morphological variability among different geographical populations may be attributed 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 utilised widely in stock differentiation in fisheries (Dwivedi and Dubey, 2013). 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 principal components (PC1 and PC2) grouped three populations into one cluster indicating that all three populations are morphologically homozygous. Therefore, in phenotypical point of view, we can consider these three populations as a single stock, irrespective of geographic variation. 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 and thus need to be considered as one stock.

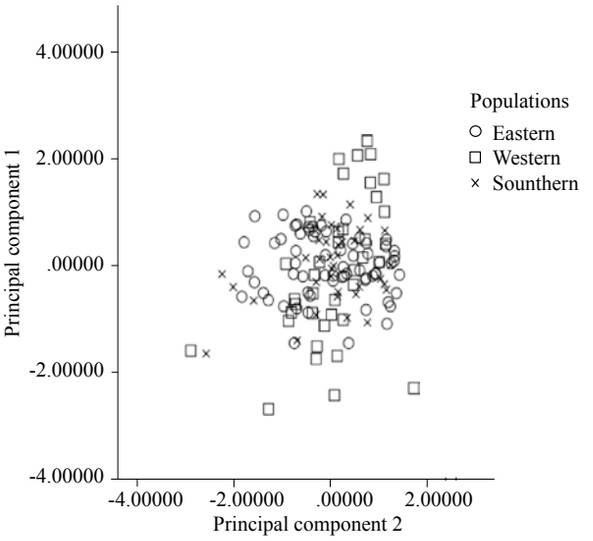

Fig. 3. Graph derived from principal component analysis (PCA) performed using morphometric data of *P. monodon*.

Table 1. Component loadings of variables after principal component analysis (PCA) of the morphometric data of *P. monodon*

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 - 13	.573	.624
7 - 8	.552	.392
9 - 10	.495	.696
14 - 15	.479	.708
8 – 9	.474	.593
13 - 14	.469	.695
5 - 6	.430	.426
2 - 18	.409	.657
1 -2	.169	.652
10 - 11	.154	.686
Total % of variance	44.152	30.738
Cumulative % of Variance	44.152	74.890

Genetic diversity

Approximately 600 bp fragment of the mtDNA control region was amplified for 63 individuals of *P. monodon*. Twenty six haplotypes were resulted from three populations and sequences were deposited in the Genbank under accession numbers KF639857 - KF639882. Haplotype and nucleotide diversity levels for each population are given in Table 2. Only one haplotype was common among southern and western populations and all other haplotypes were specific to each population. Mean haplotype diversity (h) among three populations ranged from 0.35 (southern) to 0.92 (eastern) (Table 3). The nucleotide diversity (π) among three populations ranged from 0.005 (southern) to 0.025 (western). The overall F_{st} value indicated significant genetic structure among populations ($F_{st} = 0.2111$, $p = 0.00 < 0.05$) (Table 4).

The 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 from different geographic locations where mitochondrial control gene region produced high haplotype diversity levels (Kumar *et al.,* 2007; Zhou *et al*., 2009; You *et al.*, 2008). In contrast, southern population in the current study indicated low haplotype and nucleotide diversity levels ($h = 0.3579 +/- 0.1266$, $\pi = 0.005478 +/- 0.003295$) indicating low genetic variation within the population. These results indicate the signs of overexploitation of the population or restriction of gene flow among other populations due to isolation. Kumar *et al.* (2007) studied Indian populations of *P. monodon* 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 western Sri Lankan populations (0.90 and 0.92 respectively) were close to those levels reported from the east coast (0.93) (Kumar *et al.*, 2007) as well as Vizag and Kakinada populations in India (0.92 and 0.93) (Khedkar *et al*., 2013) and values reported by You *et al.* (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, high variation exists within population (78.89%) rather than among populations (21.11%). Pairwise F_{st} values indicated significant genetic structure among three Sri Lankan populations ($p<0.01$) (Table 3). The F_{st} values of the current study (from 0.06911 to 0.36891) are much greater than the values reported for the Indian populations which ranged from 0.053 to 0.218 (Khedkar *et al*., 2013) and other indo-pacific populations which ranged from 0.004 to 0.095 (You *et al.,* 2008) and 0.0007 to 0.098 (Zhou *et al.,* 2009). This indicate that southern Indian Ocean has more structured *P. monodon* populations than eastern Indian Oean populations. This was recently reported 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 be represented by phenotypic variation. This may be due to phenotypic plasticity that allows

Table 2. Summary of molecular diversity for the three populations of *P. monodon*.

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. Pairwise F_{st} values for the three populations of *P. monodon*.

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

*$p < 0.01$

Table 4. Results of the AMOVA test

Source of variation	d.f.	Sum of squares	Variance components	% Variation
Among populations	2	72.659	1.46057 Va	21.11
Within Population	60	329.516	5.49193 Vb	78.89
Total	62	402.175	6.96149	

Fixation Index, F_{st}: 0.21110; $p < 0.05$
Significance tests (10100 permutations)

them to respond adaptively to environmental change by modification in their physiology and behaviour which leads to changes in their morphology, reproduction or survival that mitigate the effects of environmental variations which could underestimate biodiversity values in nature (Lefebure *et al.,* 2006). The information from molecular markers can assist in identifying populations as a single unit or as comprising of several subunits (Chauhan and Rajiv, 2010). Therefore, it is important to utilise both phenotypic and genetic information in stock identification and conservation studies of wild populations.

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