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# Genetic differentiation of black tiger shrimp *Penaeus monodon* Fabricius (Crustacea: Decapoda) in Andaman and Nicobar islands

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ABSTRACT

Black tiger shrimp *Penaeus monodon* from 19 locations of Andaman and Nicobar Islands were studied for genetic differentiation and variability using allozyme electrophoretic analysis. Thirty two presumed loci were consistently expressed from 25 enzymes and a general protein, and these were also temporaly stable during the period 2002-2005. The percentage of polymorphic loci, mean number of alleles, mean effective number of alleles, observed homozygosity, expected homozygosity, observed heterozygosity, expected heterozygosity, average heterozygosity, gene flow (Nm), $F_{is}$, $F_{it}$, $F_{st}$, Rare alleles, monomorphic loci, diagnostic loci, genetic identity, genetic distance and establishment of breeding boundaries were documented in all tested populations, and between the population of Andaman group and Nicobar group of islands. Colonization of *P. monodon* population due to the decrease of genetic diversity has been formed from north Andaman to south Andaman. As a result, two insular populations *i.e*., A (Andaman group of islands) and B (Nicobar group of islands) were differentiated.

Keywords: Alleles, Allozyme, Genetic differentiation, Heterozygosity, *Penaeus monodon*

*Penaeus monodon* has a broad geographic range in tropical and subtropical coastal waters from Africa through India, China to south-east Asia to Australia (Grey *et al.,* 1983). The increase in commercial fishing of *P. monodon* has stimulated interest in its stock identification, distribution and biology. Among the available techniques for molecular genetics studies, the allozyme electrophoresis data still presents the most reliable and powerful method for the identification of genetically distinct group of fishes (Ridgeway, 2005). Though many reports emphasized that penaeid prawns have low levels of genetic variation in comparison to other organisms (Nelson and Hedgecock, 1980), population differentiation in several penaeid species has been reported (Lester, 1979; Mulley and Latter, 1981; Benzie *et al.,* 1992; Klinbunga *et al*., 1999; Klinbunga *et al*., 2001; Tassanakajon *et al*., 2004).

Samples (10-20 individuals per collection per site) of both sexes and tissues of tiger prawn *P. monodon* (130 to 160 mm total length) were collected from trawlers at 19 sites in Andaman and Nicobar islands. Specimens were frozen immediately in dry ice, transported to the laboratory and stored at – 80 °C until further analysis. The muscle from the first abdominal segment, hepatopancreas and eyes along with stalk (100 mg each) were thawed and ground with 1000 µl of double distilled water and the homogenate was centrifuged at 10,000 rpm for 20 minutes at 4 ℃. Drops of supernatant were frozen in liquid nitrogen to form beads

and stored at -80 °C until electrophoretic analysis. The supernatants after thawing, were subjected to polyacrylamide gel electrophoresis (PAGE) with a 30 minutes pre-run at 20 V and final electrophoresis at 3°C (100 V, 90 and 180 minutes) with different buffer systems. The enzymes and proteins were visualized by using standard histochemical stain recipes (Table 1). The locus and allelic nomenclature (Allendorf and Utter, 1979) were labeled (Shaklee *et al.,* 1990) according to their migration distance relative to that of the most common allele which was assigned a value of 100. The temporal changes in allelic frequency of all the tested populations (19 sites) were carried out between the years 2002-2005 to assess the stable polymorphic loci which were considered to assess the genetic differentiation of the candidate species. The POPGEN 32 package and PHYLIP Version 3.5 were used for the analysis.

Out of the 41 enzymes studied, only 25 enzymes and the general protein loci consistently expressed polymorphism. Thirty two loci were polymorphic (Table 1) and none of these loci showed significant deviations from Hardy-Weinberg. The enzyme systems such as aconitase 4.2.1.3 (ACO), adinosine deaminase 3.5.4.4 (ADA), creatin kinase 2.7.3.2 (CK), diaphorase 1.6.4.3 (DIA), β-galactosidase 3.2.1.23. (GAL), glyoxalase - I 4.4.1.5 (GLO), glutamate dehydrogenase 1.4.12 (GLD), guanine deaminase 3.5.4.3 (GDA), fumarase 4.2.1.2

Table 1. List of polymorphic enzymes and electrophoretic conditions employed for population genetic studies in *P. monodon*

| Enzymes | EC No. | Polymorphic Loci | Tissue | Buffer systems |
|---|---|---|---|---|
| Aspartate aminotransferase | 2.6.1.1. | *AAT-1\** | M/E/H | TC/TG/ TME |
| Acid phosphatase | 3.1.3.2. | *ACP-1\** | M//H | PC |
| Alcohol dehydrogenase | 1.1.1.1. | *ADH-2\** | M/E/H | TG/ TBE |
| Adenylate kinase | 2.7.4.3. | *AK- 3\** | M/E/H | TG |
| Alkaline phosphatase | 3.1-3.1. | *AKP-2\** | M | TG/TVB/ TBE |
| Aldolase | 4.1.2.13. | *ALD-2\** | M/E/H | TG |
| Aldehyde oxidase | 1.23.1. | *AO-1\** | M/E/H | TG/TCL |
| Esterase | 3.1.1.- | *EST-1\*,2\*,3\** | H/M | TG/ TCL/ LiOH |
| Glycerol-3-phosphate dehydrogenase | 1.1.1.8. | *G3 PDH-2\** | M/E | TG/ TME |
| Glucose 6 phosphate dehydrogenase | 1.1.1.49. | *G6PDH-2\** | M/E/H | TG /TCL |
| Glucose-6-phosphate isomerase | 5.3.1.9. | *GPI-3\** | M/E | TCL /TVB |
| Hexokinase | 2.7.1.1. | *HK-2\** | M /H | TC/ TBE/ TCB |
| Isocitrate dehydrogenase | 1.1.1..42. | *IDH-3\** | M/E/H | TG/ TRIC |
| Leucine aminopeptidase | 3.4.1.1.1. | *LAP-2\** | M/E/H | TG/TCB/ TGC |
| Malatate dehydrogenase | 1.1.1.37. | *MDH-1\** | M | TM / TME |
| Malatate dehydrogenase | 1.1.1.40. | *MDH P-1\*3\** | M | CAEA/ TME |
| Malic enzyme NAD+ | 1.1.1.39. | *ME-3\** | M/H | TG/TM/ TBE |
| Manos 6 phosphate isomerse | 5.3.1.8. | *MPI-2\** | M/H | TVB/TC |
| Octanol dehydrogenase | 1.1.1.73. | *ODH-2\** | E/H | TG/TC/ TGC |
| Phosphogluconate dehydrogenase | 1.1.1.44. | *PGDH-2\** | M/H | TG/ TRIC |
| Phosphoglucomutase | 5.4.2.2. | *PGM-1\*2\** | M/E | TCE / TMgP |
| Sorbitol dehydrogenase | 1.1.1.14. | *SDH-1\** | M/H | TG/TC/TCE |
| Superoxide dismutase | 1.15.1.1. | *SOD-1\** | M/E | TG/ TBE |
| Tetrasolium reductase | | *TR-1\** | M/H/E | TG/ TVB/TCL |
| Xanthin dehydrogenase | 1.2.1.37. | *XDH-2\** | M/E | TG/TCE/TC |
| General protein | 3.1.1.X | *PT-2\*3\*, 6\** | M/H/E | TG/TCE/TC |
| Total presumed loci = 32 | | | | |

(FUM), malic enzyme NADP + 1.1.1.40 (MEP), peptidase (PEP), phospho gluconate dehydrogenase 1.1.1.43 (PGD), phosphohexose isomerase 5.3.1.9 (PHI), purine nucleoside phosphorylase 2.4.2.1 (NP) and pyruvatekinase 2.7.1.40. (PK) were not scorable because of either inadequate staining or interrupted expression of alleles and were not considered for further analysis.

The genetic variability of *P. monodon* are presented in Table 2. In all tested populations of *P. monodon*, the lowest percentage of polymorphism was observed from south Andaman (31.25%) and the highest value was from Nicobar group of islands (53.12%). The trend of polymorphism in Nicobar group of islands decreased from Car Nicobar to Campbell Bay whereas in the case of Andaman group of islands, it increased from south to north Andaman. A similar pattern of reducing genetic diversity was displayed in the mean number of alleles per locus. In addition to these, genetic identity and genetic distance, temporal unstable loci, monomorphic loci, diagonostic loci, rare alleles and establishment of breeding boundaries were documented.

Though loci such as *AK-1\*, MPI-1\*, PGM-3\*, GPI-2\*, ACP-2\*, ADH-3\*, AK-2\*, AOK-2\*, AO-3\*, SDH-2\*,* *XDH-1\*, PROT-7\*, AOK-2\*, AO-3\*, LDH-2\*, LAP-1\*, MDHP-2\*, PGM-3\** showed significant polymorphisms (p<0.05), these loci have not been considered as these were not consistently expressed (temporal unstable loci). The loci such as *AAT-2\*, ACP-3\*, ADH-1\*, AKP-1\*, AO-2\*, ALD-1\*, AAT-3\*, AOK-1\*, G3PDH-1\*&3\*, G6PDH-1\*,GPI-1\*, HK-1\*, IDH-1,2\*, LDH-1, LAP-3\*, ME-1,2\*,ODH-1\*, PGDH-1\*, PROT-1\*,4\*,5\*&8\** were consistently monomorphic. The expression of the diagnostic loci and alleles: *EST-3\*, G3PDH-2\*, GPI-3\*, IDH-3\*, MDHP-1\** from Andaman waters and *AAT-1\*, ADH-2\*, G6PDH-2\*, LAP-2\*, MDH-1\*, ME-3\*, MPI-2\*, PGDH-2\* PGM-1\*,2\*, SDH-1\*, SOD-1\*, TR-1\** from Nicobar group of islands were obtained. The number of rare alleles also reduced from Nicobar to Andaman group of islands with an average of 12 in the Nicobar populations, 8 in the south Andaman populations and 4 in the middle and north Andaman populations. The frequencies of rare alleles occurring in the Nicobar group populations were higher than those occurring in the Andaman islands.

Heterozygosity in penaeid relatives is lower than in other animal groups (Nelson and Hedgecock, 1980). The

Table 2. Population differentiation of *P. monodon* from Andaman and Nicobar islands

| | Major Locations | | | |
| --- | --- | --- | --- | --- |
| | North Andaman | Middle Andaman | South Andaman | Nicobar |
| Percentage of polymorphic loci | 46.88 | 40.62 | 31.25 | 53.12 |
| Mean number of alleles | 1.7812 | 1.5312 | 1.500 | 2.1250 |
| Mean effective number of alleles | 1.1246 | 1.0759 | 1.1376 | 1.2962 |
| Observed homozygosity | 0.9108 | 0.9505 | 0.9146 | 0.8112 |
| Expected homozygosity | 0.9065 | 0.9400 | 0.9069 | 0.8197 |
| Observed heterozygosity | 0.0892 | 0.0495 | 0.0854 | 0.1888 |
| Expected heterozygosity | 0.0935 | 0.0600 | 0.0931 | 0.1803 |
| Average heterozygosity | 0.0859 | 0.0553 | 0.0884 | 0.1750 |
| Gene flow (Nm) | 2.3474 | 2.9079 | 5.1632 | 6.1228 |
| Mean $F_{is}$ | -0.0575 | 0.1042 | 0.0365 | -0.0935 |
| Mean $F_{it}$ | 0.0443 | 0.1751 | 0.0810 | -0.0506 |
| Mean $F_{st}$ | 0.0962 | 0.0792 | 0.0462 | 0.0392 |

mean heterozygosity for *P. monodon* from Andaman and Nicobar group of islands in the present study is similar to that of other reports (Mulley and Latter, 1980; Redfield *et al*., 1981). The low average heterozygosity in Andaman group of population may be due to local differentiation, which may also reflect the effects of isolation and increased genetic distances from the other populations (Nicobar group of islands). The loci such as *AAT-1, ADH-2, G6PDH-2, LAP-2, MDH-1, ME-3, MPI-2, ODH-2, PGM-1, PGM-2, PGDH-2, SDH-1, SOD-1* and *TR-1* were polymorphic from Nicobar group of islands and monomorphic in the Andaman group of islands. Similarly the loci *ALAT\*, GPI\*, IDHP\** were heterozygous in *P. monodon* from Gulf of Thailand whereas these loci were homozygous from the Andaman sea (Sodsuck, 1996). The variation in expression of loci may be due to the spatial structuring of a species and effect of adaptive fitness which in turn can alter the gene pool (Wright, 1978; Nelson and Hedgecock, 1980). Though the mean values of $F_{is}$, $F_{it}$ and $F_{st}$ from over all populations in Anadaman and Nicobar group of islands were lower (Table 2.) compared to other aquatic animals, inter-population differentiation were observed due to the significant contribution of high frequency alleles (location specific). The genetic variation from Nicobar group and Andaman group of islands was also confirmed with F statistics. Pair-wise comparison of gene frequencies between populations using chi-square confirmed the general pattern of variation in all tested populations Comparison revealed significant heterogeneity between Andaman and Nicobar group of islands ($p<0.01$ and $p<0.05$). Gene frequencies of all loci in these populations were also significantly different ($p<0.01$ and $p<0.05$). The stock structure of *P. monodon* showed that Andaman population was significantly different from that of the Gulf of Thailand (Tassanakajon *et al.,* 2004). In the present study, population differentiation noticed between Andaman and Nicobar group of islands can be attributed to the prevention of adult spawning migration as a result of hindrance from ten degree channel. Seasonal currents channel of the Andaman sea (Subramanian, 1977) or past biogeographical barriers occurring as a result of changes in the sea level as reported in *P. latisulcatus* from western and southern Australia than the northern populations (Mulley and Latter, 1981) influenced its larval dispersal.

The genetic diversity data of *P. monodon* revealed that it has decreased from north Andaman to middle Andaman but remarkable decrease was observed between the locations of south Andaman and Car Nicobar. The colonization of the candidate species might have been generated from Nicobar and Andaman group of islands due to the effects of currents and founder effects of the population. Loss of genetic variation was noticed in Andaman population which also had increased frequencies of rare alleles. This suggests colonization by a relatively small number of individuals and highly restricted dispersal between the northern, middle and southern region. The genetic distinction of the Nicobar population of *P. monodon* may reflect an isolated population on the periphery of the species range. Such peripheral populations often show reduced genetic diversity and shifts in gene frequency (Baker *et al*., 1990) and the genetically discrete stocks represent the best adapted genotypes for each particular area (MacLean and Evans, 1981). Thus the present study indicates that the stock of *P. monodon* from north, middle and south Andaman are of the same breeding population. Although these populations showed few genetic variations, data have not been considered as these differences were not significant. However, there exists significant genetic differentiation between the Andaman and Nicobar populations as demonstrated by the cluster analysis. (Fig. 1). It is therefore suggested that two insular population of *P. monodon* existed in the waters of Nicobar and Andaman regions which should be managed as two separate broodstocks for captive breeding in future.
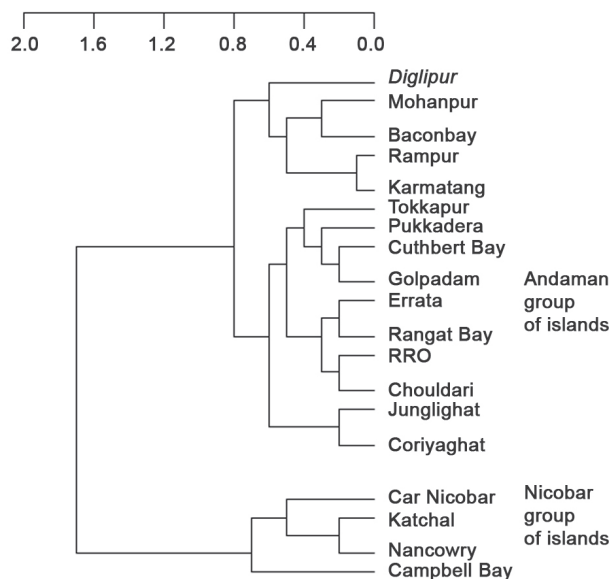
Fig. 1. Dendrogram Based Nei's (1978) showing the genetic relationships (genetic distance) among A & N population of *P. monodon*: (Method = UPGMA clustering method)

## Acknowledgements

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