

Tiger Prawn (*Penaeus monodon*) broodstock of Andaman Molecular Characterisation by RAPD Technique

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Abstract

Random amplified polymorphic DNA (RAPD) analysis was used to examine genetic variation in Tiger prawn, *Penaeus monodon*. Specimens of this prawn were collected from two different geographically separated locations in the Andaman Sea. A total of 30 samples of *P. monodon* individuals were investigated using nine random primers. The PCR amplification of template DNA produced a total of 26 scorable RAPD bands, having molecular weight ranging from approximately 500 to 5,000 bp of the nine primers tested, the maximum number of loci amplified was 5 with primer 3 and primer 4 followed by primer 1 which recorded 4 distinct scorable bands. Out of the total number of 26



Broodstock of Tiger prawn, *Penaeus monodon*, collected from Shrimp Hatchery, Andaman & Nicobar Islands, used for seed production.



Tiger prawn, *Penaeus monodon* collected from Betapur, Middle Andaman & Nicobar Islands.

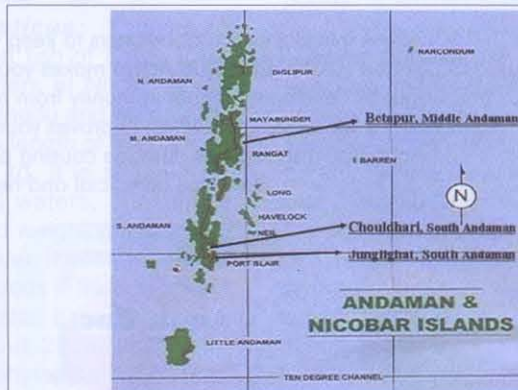


Fig.1: Map of Andaman showing the two different geographically isolated areas from where the samples were collected



RAPD patterns obtain from 3 different primers on 5 samples of Tiger Prawn

MW : 500 bp Molecular weight marker

S1-S5 : Samples of Tiger Prawn

Lane 1: Molecular weight marker, Lane2-6 : Primer 1,
Lane 7-11 : Primer 2, Lane 12-16 : Primer 3

Fig 2. RAPD fingerprints pattern obtained from 3 different primers with the samples of Tiger prawn, *P. monodon*.



RAPD patterns obtain from 3 different primer on 6 samples of Tiger Prawn

MW : 500 bp Molecular weight marker

S1-S6 : Samples of Tiger Prawn

Lane 1: Molecular weight marker, Lane 2-7 : Primer 3,
Lane 8-13 : Primer 4, Lane 14-16 : Primer 5

Fig 3. RAPD fingerprints patterns obtained from 3 different primers with the samples of Tiger prawn, *P. monodon*.



loci scored, 16 were polymorphic (61.5%). All the primers had higher G+C content of 60-80 percent. After all possible pair-wise matching genetic similarities were calculated between the six different samples of Tiger Prawn (*P. monodon*) collected from the two geographically isolated areas of the Andamans and the dendrogram obtained by the UPGMA clustering method. These revealed the genetic variation within the species. The dendrogram of the 6 different samples collected from the two geographically matching areas was constructed using 9 RAPD primers. These revealed genetic diversity within the species ranging from 52% to 77% similarity among themselves. Moreover, sample R3 collected from Betapur, Middle Andaman is genetically 48% different from the rest of the samples, even from the samples collected from the same location.

Introduction

The Andaman and Nicobar Islands are located in the Bay of Bengal, of the eastern coast of India. The Andaman and Nicobar Islands comprise a chain of more than 350 major Islands. It lies in Bay of Bengal between latitudes 6' and 14'N and longitudes 92' and 94' E. The total land area is about 8,249 sq.km with a coastline of 1,962 km having a wide range of flora and fauna of tropical rain forest in both land and sea. This Bay Island is bestowed with large tracts of brackishwater bays, lagoons, tidal mud flats and mangrove swamps, which have potential for mariculture, brackishwater aquaculture and freshwater fish culture with available finfish and shellfish resources. There are about 35,000 ha of mangrove areas in the islands, which are subjected to regular tidal inundation. In the post tsunami scenario, in south Andaman alone, due to the subduction of the land by about 1.25 m, the level of submergence due to tidal influence has also increased. A survey conducted reveals that approximately 4,000 ha areas of agricultural farmlands have been submerged, out of which 630.12 ha of area are found suitable for coastal aquaculture. However, as coastal marshy wetlands are of acid sulphate nature, there have been a lot of apprehensions among the entrepreneurs, scientists, planners and administrators regarding technical viability and success of these ventures. As per the available technology at present, these acidic soils can be rapidly reclaimed with low cost technique. A feasibility study conducted along the coastal marshy wetlands of

South Andaman explored this possibility. The ecology and scope for fisheries in mangrove areas of Andaman and Nicobar islands were explored in detail. The reduction in fish catch during 2004-2005 as revealed in the basic statistics of Andaman & Nicobar Administration makes this study imperative as an alternate source of fish/shrimp production through coastal aquaculture.

The Tiger Prawn, *Penaeus monodon* brooders available are presumed to be free of viral diseases in these Bay Islands. This prawn is one of the most commercially important cultured species of the globe. It accounts for 46% of total world population of cultured marine shrimps. The main farming areas of *P. monodon* are located in various tropical countries, particularly in Southeast Asian region. Thailand has become the world's leading producer of *P. monodon* since 1991, with an average production of at least 200,000 mt per year (Tassanakajon *et al.*, 1997).

Random Amplified Polymorphic DNA, (RAPD) fingerprinting is a simple and rapid molecular technique for generating genetic markers without prior knowledge of genomic DNA sequences (Williams *et al.*, 1990). In this approach, arbitrary oligonucleotide primers are used to amplify random segments of genomic DNA by polymerase chain reaction (PCR). A single (usually 10 mer with GC content more than 50%) was utilised to amplify random segments of genomic DNA using PCR technology. It has been successfully employed to determine genetic diversity in *P. vannamei* (Garcia *et al.*, 1994) and *P. monodon* (Tassanakajon *et al.*, 1997) for population genetic studies of penaeid species.

Banana prawns found in Thailand consist of two species, *Penaeus merguensis* and *Penaeus indicus*, which are very similar in morphology. DNA patterns obtained from random amplified polymorphic DNA were compared between *P. merguensis* and *P. indicus*. Several species-specific DNA fragments were isolated and sequenced. Three pairs of primer were designed for the PCR reactions, generating single- and multi-locus profiles which could be used as genetic markers to differentiate between the two species. At least 7 haplotypes were obtained with type 1-2 found only in *P. indicus*, whereas haplotype 3-5 were found in *P. merguensis* and haplotype 6-7 occurred in samples with confused morphology,

possibly hybrids (Amornrat Phongdara *et al.*, 1999).

RAPD analysis was used to amplify the genome of black tiger prawns (*Penaeus monodon*) to detect DNA markers and assess the utility of the RAPD method of investigating genetic variation in wild *P. monodon* in Thailand (Tassanakajon *et al.*, 1997). RAPD patterns were selected for the analysis of three geographically different samples of Thailand *P. monodon*. A monte Carlo analysis illustrated geographic heterogeneity in genotype frequencies within the species (Tassanakajon *et al.*, 1998). From the above back ground information the present research work was planned for Tiger shrimp (*Penaeus monodon*) of Andamans with the aim and objective to assess the genetic variability of broodstock of Tiger Prawn, *P. monodon* in Andaman and determining of the level of genetic diversity within the geographically isolated species population of Tiger prawn in Andamans Sea.

Materials and methods

Collection of Sample: Totally 30 samples of Tiger Prawn broodstocks were collected from two different geographical locations (Chouldari, South Andaman: CH1 to CH3 & Betapur, Middle Andaman: R1 to R3) of Andaman (Figure.1). Pleopod muscles of each prawn were collected and kept in absolute ethanol during transportation. Specimens were stored at -80°C until required.

Isolation of Genomic DNA and RAPD-PCR: Isolation of genomic DNA was done with Phenol: Chloroform: Isoamyl alcohol method (Dinesh *et al.*, 1995) and DNA amplification was done using Gradient Thermal cycler (DNA Engine). PCR amplification was carried out in a 25 µl reaction volume containing 1/10th volume of 10x Buffer (10mM Tris. HCl, pH 8.0; 50mM KCl; 2mM MgCl₂ and 0.001% gelatin), 20 pmol of primer, 25 ng of Template DNA, 100 µM of dNTP (dATP, dCTP, dGTP and dTTP) and 1 unit of *Taq* DNA polymerase. The PCR reaction was carried out at 94°C for 4 minutes initially, followed by 35 cycles consisting of 1 min at 94°C, 1 min at ~36°C and 2 min at 72°C. The amplified production was electrophoretically analysed in 1.5% agarose gel.

Data analysis: RAPD patterns of samples were determined by direct comparison of the amplified DNA

electrophoresis profile and with the use of BIO 1D++ system software. Fragments were scored as 1 if present or 0 if absent, based on a molecular weight standard marker, and the data obtained were analysed as binary variables. Each band was considered to be an allele of a locus. The number and frequencies of polymorphic loci gene diversity indices and unbiased genetic distances were estimated using NTSYS 2.02 system software. Clustering was performed by the unweighted pair-group method of analysis (UPGMA) with statistical support (Sneath and Sokal, 1973).

Results & Discussion

RAPD analysis: Nine random primers were selected in our analysis for the reproducible and polymorphic DNA amplification patterns. The results obtained based on analysis of 6 different samples of Tiger Prawn, *P. monodon* collected from two different locations using 9 random primers are furnished in Table 1. The PCR amplification of template DNA produced a total of 26 scorable RAPD bands. The RAPD products generated were having molecular weight ranging from approximately 500 to 5,000 bp. The numbers of loci amplified in all 6 samples with all 9 primers are given in Table 1. The maximum number of loci amplified was 5 with primer 3 and primer 4 followed by primer 1 which recorded 4 distinct scorable bands. All the primers had higher G+C content of 60-80 percent. Out of the total number of 26 loci scored, 16 were polymorphic (61.5%) and thus informative to characterise the species of Tiger Prawn, *P. monodon* collected from two different areas for the selection of brooders.

Genetic diversity within the species of Tiger Prawn, *P. monodon* as assessed by RAPD analysis: All

possible pair-wise genetic similarities were calculated between the 6 different samples of Tiger Prawn, *P. monodon* collected from the two geographically isolated areas of the Andamans (Figure. 1) and the dendrogram obtained by the UPGMA clustering method revealed the genetic variation within the species.

The dendrogram revealed that the 6 different samples of Tiger Prawn, *P. monodon* species could be grouped into two major clusters namely A and B. The major cluster A has R3 which was collected from Betapur, Middle Andaman. The cluster B is further divided into two sub clusters namely B1 and B2. The cluster B1 has R1 and R2 which was collected from Betapur, Middle Andaman (Figure. 4). The cluster B2 is again subdivided into b1 and b2. The cluster b1 has CH3 which was collected from the Chouldhari, South Andaman. The cluster b2 has CH1 and CH2 which was collected from Chouldhari, South Andaman.

The sample CH1 and CH2 are 77% similar. While these two samples from cluster b2 are 75.5%, similar to cluster b1 having sample CH3, which was collected from the same location. The samples of cluster B1 exhibited 74% similarity with each other R1 and R2. The cluster B1 showed 67.5% similarity with the cluster B2 as they were collected from the two different geographically isolated areas.

Our results show that the sample R3 collected from Betapur, Middle Andaman is genetically 48% different from the rest of the samples, even from the samples collected from the same location. Thus, these results show that there is some variation in the genetic level which can be studied further for the identification of the healthy brooders.

Genomic mapping and genetic relatedness: Tassankajon *et al.*, 1998 examined the genetic variation in wild black tiger shrimp, *P. monodon* of five geographically separated locations in Thailand and found that 58 of 80 reproducible RAPD fragments ranging in size 200 to 2,200 bp were polymorphic. The genetic distance between population and dendrogram indicated that the Medan population was genetically different from Thai *P. monodon*. The percentage of polymorphic bands is varied from 51.5 to 57.7%. Sodsuk (1996) studied genetic polymorphisms of *P. monodon* in South East Asia using mt-DNA RFLP of four restriction enzymes (*Bam*HI, *Eco*RV, *Pvu*II and *Sac*I) with small samples sizes ($N = 9.3$ in average). His data indicated geographic homogeneity among the east and west coast populations. Klinbunga *et al.* (1999a & 2000b) determined genetic variation and population structure of *P. monodon* collected from Surat, Sataun and Trat using mt-DNA-RFLP analysis with 11 restriction endonucleases. They found high genetic diversity and significant geographic heterogeneity ($P < .0001$) between the Andaman Sea and the Gulf of Thailand samples. Govindaraju and Jayasankar (2004) found the taxonomic relationship among seven species of Groupers (Genus *Epinephelus*; Family Serranidae) as revealed by RAPD fingerprinting and the genetic polymorphism within species were the highest in *Epinephelus chlorostigma* (49.15%) and the lowest in *E. tauvina* (25.42%).

In our present study, out of the total number of 26 loci scored, 16 were polymorphic (61.5%) in nature. The dendrogram showed the 52-77% genetic similarity between the six different samples collected from two geographical isolated locations in Andaman and the polymorphic level was as high as those previously reported in Tiger Prawn, *P. monodon* (51.5

Table .1. RAPD Primer used for Tiger prawn, *P. monodon* species by random 10mer primers

Primers No.	Nucleotide sequence(5'→3')	G+C content (%)	Number of total bands	Polymorphic bands	Percentage of polymorphism(%)
1.	GCGCCTGGAG	80	4	2	50
2.	AACGGGCAGG	70	3	2	66.66
3.	GGCTGCGGTA	70	5	5	100
4.	GCGGAGGTCC	80	5	4	80
5.	CGACGCCCTG	80	3	0	0
6.	GCGTCGAGGG	80	1	0	0
7.	CCTGGGCTTC	70	2	1	50
8.	CCTGGGCTTG	70	0	0	0
9.	CCTGGGCTTA	60	3	2	66.66



to 57.7%) by (Tassanakajon *et al.*, 1998) , and Pacific white leg shrimp, *P. vannamei* (39-77%) (Garcia *et al.*, 1994).

Conclusion

The Andaman and Nicobar Islands are isolated and far away from the Mainland. The Tiger Prawn, *Penaeus monodon* brooders available in this Bay Islands. Their identification and molecular characterisation become important for the selection of the healthy brooders. The selection of the good and healthy brooders plays an important role in the quality of the seeds. The RAPD is an efficient low cost molecular technique, which can be applied to generate genome specific banding patterns to determine genetic diversity in *P. monodon* for population studies of penaeid species. The dendrogram of the 6 different samples collected from the 2 geographically separated areas constructed using 9 RAPD primers. It revealed genetic diversity within the species ranging from 52% to 77% similarity among themselves. Moreover, sample R3 collected from Betapur, Middle Andaman is genetically 48% different from the rest of the samples, even from the samples collected from the same location. Thus, these results show that there is some variation in the genetic level which has great importance in the monitoring and management of any shrimp breeding programme.

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Fishermen for a uniform schedule on 45 – day ban

Need to protect eco-balance stressed

Fishermen from coastal districts of Andhra Pradesh and Yanam on 15 April 2011 sought the designing and implementation of a uniform schedule of the 45-day ban on fishing along the coast, which aims at saving fish from excessive exploitation.

The fishermen narrated their problems faced because of modern methods of fishing at the 'Trans-boundary Diagnostic Analysis (TDA) Consultation Workshop' for the fishermen from Andhra Pradesh and Yanam, organised by the Bay of Bengal Large Marine Ecosystem Project (BOBLME) here. The Participants observed that though they were not venturing into the sea for fishing during the ban period (of 45 days from April 15 to May end) their counterparts from Indonesia, Malaysia, Thailand, Myanmar, Sri Lanka and Maldives located in the Indian Ocean were not stopping fishing, due to which the purpose of the ban was not being achieved

Long-term plan : Commissioner of the Fisheries Department Dr. Manmohan Singh, who inaugurated the programme, said that the objective of the BOBLME was to protect the environment and maintain the ecological balance in the Bay of Bengal. He said that a long-term plan was designed and was being implemented by the eight nations mentioned above that were on the coast of the Bay of Bengal. Referring to the ban on fishing, Dr. Singh said that the ban would be implemented from April 15 to May 31 and the government made alternative arrangements for the livelihood of the fishermen during the ban period.

Question mark: Fishermen from Visakha and from Nellore felt that there was a question mark over fishing in the near future due to the large scale industrialisation along the coast. They pointed out that the sea coast was getting polluted due to the industries and there

were no concrete steps from the government to protect the sea from the pollutants. Fishermen from Srikakulam and Krishna districts sought ban on use of ring net, as it was affecting the livelihood of small fishermen. They sought support from the government for using foreign vessels for fishing to provide training to the fishermen in alternative trades of livelihood. National coordinator of the BOBLME Dr. Vijayakumaran stressed the need for maintaining ecological balance in the Bay of Bengal. He said that the BOBLME was conducting similar workshops in different States to create awareness among the fishermen about the trans-boundary issues. District Collector M. Ravi Chandra felt that the local problems of the fishermen must be considered while making policy decisions pertaining to the sea coast and the BOBLME must focus on eradicating poverty among the fishermen and improving the literacy rate among them. ☺