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## MOLECULAR CHARACTERIZATION OF ENDEMIC FRESHWATER PRAWNS, *Macrobrachium lar* OF ANDAMAN

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### ABSTRACT

*Macrobrachium* genus is distributed globally across the tropical and sub-tropical regions and comprises over 200 described species. The greatest diversity of *Macrobrachium* species occurs in the Indo-Pacific region, in particular on the Indian sub-continent and through out South East Asia. *Macrobrachium* prawns inhabit a wide variety of environments from mountain streams and lowland rivers to estuaries and coastal lagoons. A number of species are also adapted to more extreme environments such as acidic rain forest streams. Majority of *Macrobrachium* sp. inhabit fresh water, many species have extended larval life cycles over several months that require estuarine or marine environments, and several complete their entire life cycle in euryhaline environments. Many species demonstrate extremely wide distributions, particularly for 'freshwater species'. *M. lar* is found from the east coast of Africa to the central Pacific islands. *M. lar*, popularly known as glass or rock/monkey prawn, is an indigenous freshwater prawn found only in streams of Andaman. In nature, the adult males are larger than females with narrow abdominal space and the male size varies from 86 mm to 112 mm with weight of 32-40 gms. The rostrum is short, upturned distally before antennal flaps. First 2-3 rostral teeth are on the carapace. The rostral teeth formula is 6-8 / 2-4 (commonly 7-8 / 2-3). The first and second pair of pereopods are chelated. Yellow spots are found both sides of abdominal segments except 3<sup>rd</sup> abdominal segments. *M. lar* stays in clear, transparent running water with rocky substrates. Dendrogram revealed that 30 different samples of *M. lar* species could be grouped into two major clusters namely I & II. The major cluster I has Bp7 which was collected from Betapur, middle Andaman located 200 km away from other locations. Major cluster II has further two clusters IIA, IIB; in this IIA having 18 sub groups from Shoal Bay, CARI stream, Burmanala and Cluster IIB has two samples from Shoal Bay and rest all from Betapur. This dendrogram revealed samples from Shoal Bay and CARI Nallah interpreting with all the locations; this may be due to anthropogenic interferences and the stocks found from both the clusters having very good genetic variation, which may be utilized for selective breeding program further.

**Key words :** *Macrobrachium lar*, freshwater prawn, RAPD, Andaman.

### INTRODUCTION

The Andaman and Nicobar Islands are located in the Bay of Bengal between 6° 45' N and 13° 41' N latitude and 92° 12' E and 93° 57' E longitude and are blessed with enormous of natural freshwater resources besides availability of large areas of brackish and open sea areas around it. The number of species in the genus *Macrobrachium* is approximately 125 worldwide, and these are widely distributed in fresh and brackish waters, mainly in subtropical and tropical areas. *Macrobrachium lar* is found from the east coast of Africa to the central Pacific islands. *M. lar*, popularly known as glass or rock/monkey prawn, is an indigenous freshwater prawn found only in streams of Andaman. In nature, the adult males

are larger than females with narrow abdominal space and the male size varies from 86 mm to 112 mm with weight of 32-40 gms (Tiwari, 1952; Sethi *et al.*, 2009). Similarly female's size varies from 66-106 mm with weight of 14-20 gms. The rostrum is short, upturned distally before antennal flaps. First 2-3 rostral teeth are on the carapace. The rostral teeth formula is 6-8 / 2-4 (commonly 7-8 / 2-3). The first and second pair of pereopods are chelated. Yellow spots are found both sides of abdominal segments except 3<sup>rd</sup> abdominal segments. It is a peculiar prawn in its habits, it can move from freshwater canals to peak of the mountains where streams originate, *M. lar* stays in clear, transparent running water with rocky substrates.

Selective breeding is a recent activity in aquaculture (fin fish and shell fish) which therefore holds great promise for genetic enhancement programmes. The use of molecular markers for enhancing selective breeding is an even newer activity, which is expected to be crucial for the development of disease resistant strains, for improved feed efficiency and product quality. Random amplified polymorphic DNA (RAPD) markers is a powerful genetic marker, useful in many areas of fish genetics and breeding. The analysis for RAPD markers is quick and simple, although results are sensitive to laboratory conditions. Polymorphic loci have been frequently applied to the analysis of genetic diversity, population genetic structure and genomic mapping. The markers have also been applied to the classification and systematic, parentage identification, germplasm conservation, and breeding programme of food fish. The aim of this work was to study the genetic variability within the population of *Macrobrachium lar* and to select the healthy brooders for breeding programs in Andaman and Nicobar islands.

Random Amplified Polymorphic DNA (RAPD) fingerprinting has been successfully employed to determine genetic diversity in *P. vannamei* and *P. monodon* (Tassanakajon *et al.*, 1997) for population genetic studies of *penaeid* species. Genetic diversity of the giant tiger shrimp, *P. monodon* was examined by RAPD and mitochondrial DNA (16S ribosomal DNA and intergenic COI-COII) polymorphism. He also studied the genetic diversity during 2000 of three mud crabs, *Scylla serrata* (Forsk.) , *S. oceanica* (Dana) and *S. tranquebarica* (Fabricius) of Thailand was examined by RAPD-PCR. Large genetic differences between species were found, whereas those between populations within each species were much lower. Random Amplified Polymorphic DNA assay has been used by to generate species specific markers in a study in four Indian major carps such as Rohu, Catla, Mrigal and Kalbasu and also to quantify the genetic variation and construct the phylogenetic dendrogram for the six *Labeo* species viz dycheilus, rohu, kalbasu, bata, fimbriatus and gonius. Randomly Amplified Polymorphic DNA was used to study the genetic diversity and assay

polymorphisms of *Macrobrachium rosenbergii* in Malaysia (See *et al.*, 2008)

## MATERIALS AND METHODS

### Isolation of DNA

Specimens of *M. lar* were collected from four (BP: Betapur, SB: Shoal Bay, CA: CARI Nallha, and BN: Burma Nallha) different locations of Andaman. A total of 30 prawn samples were collected for RAPD analysis, thirty five 10-base primer were used for amplification. Pleopod muscle of the prawns were collected and kept in absolute ethanol during transportation. Specimens were stored in  $-80^{\circ}\text{C}$  until required. DNA was extracted from fine tissue (Pleopod) following the method described by Tassanakajon *et al.* (1997).

Purity of isolated genomic DNA influences all downstream applications. Therefore, it is very important to ensure the quality and quantity of isolated DNA before it is further used. This can be performed ideally in a spectrophotometer. DNA/RNA absorbs UV lights at 260nm whereas protein at 280nm. The ratio of absorbance at 260nm to 280nm for a sample is an indicator of quality of the isolated DNA. The pure DNA should have optical density ratio (260:280) equal to 1.8. Qualitative analysis of the DNA was also performed using 0.8% agarose. 2 ml of DNA sample was loaded into the slot of 0.8% agarose gel containing 0.5% ethidium bromide. The electrophoresis was carried out until the dye migrates approximately 1-2 cm from the well. The DNA band was estimated by visually on the UV transilluminator. (Table.1). Primers used for RAPD analysis in *M. lar*

### PCR amplification and agarose gel electrophoresis:

PCR amplifications was carried out in a total reaction of 25 mL containing 1 U of Taq DNA polymerase, 200 mM dNTPs and 10 pmol of random primer, 2.5 mL of 10X Taq DNA polymerase buffer and 40 ng of genomic DNA. The final reaction mixture was placed in a thermal cycler. The PCR programme included an initial denaturation step at  $94^{\circ}\text{C}$  for 4 minutes followed by 45 cycles with  $94^{\circ}\text{C}$  for 1 minutes for

DNA denaturation, annealing at 36°C for 1 minute, extension at 72°C for 2 minutes and final extension at 72°C for 10 minutes were carried out. The samples were cooled at 4°C. The amplified DNA was mixed with 6X gel loading dye (2ml) and run on 1.5% agarose gel using 1X TAE running buffer system. The ethidium bromide (10 mg/ml) stained gel was visualized under UV transilluminator connected to the gel documentation.

## RESULTS AND DISCUSSION

Only twenty-one primers produced amplified bands. Eight primers that showed reproducible RAPD patterns. A total of 42 scorable bands, range in size

from 500 to 5000 bp. 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 analyzed 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.

**Table 1 :** Primers used for RAPD analysis in *Macrobrachium* lar.

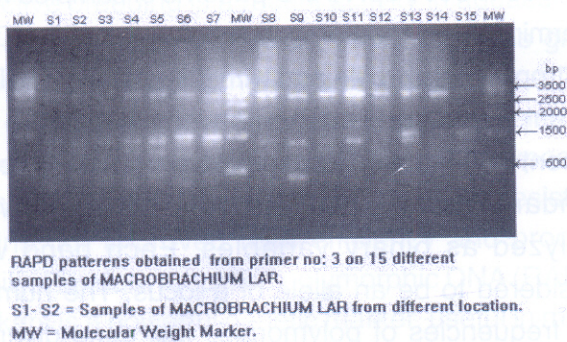
Primer No.	5' → 3' nucleotide sequence	GC content (%)	No. of bands	Polymorphic bands	Percentage of polymorphism
1	GCGCCTGGAG	80	1	1	0.
2	AACGGGCAGG	70	3	3	100
3	GGCTGCGGTA	70	4	2	50
4	GCGGAGGTCC	80	4	1	25
5	CGACGCCCTG	80	8	7	87.5
6	GCGCCTGGAG	80	6	6	100
7	AACGGGCAGG	70	6	6	100
8	GGCTGCGGTA	70	5	5	20

**Table 2 :** Four populations of *Macrobrachium* lar used for RAPD fingerprinting and their sources in Andaman.

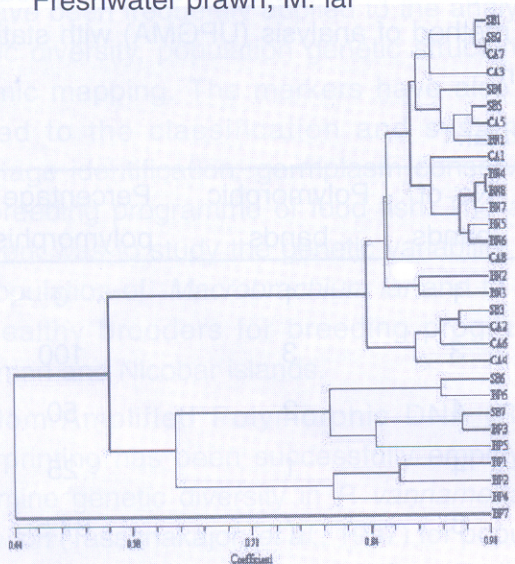
Prawn population	Collection site/source	Abbreviation
Betapur Stock	Middle Andaman	BP
Shoal Bay Stock	Middle Andaman	SB
CARI Nallha Stock	South Andaman	CA
Burma Nallha Stock	South Andaman	BN



**Fig.1 :** Freshwater rocky / monkey prawn, *M. lar* of Andaman & Nicobar Islands.



**Fig.2 :** RAPD fingerprints pattern obtain from 4 different primers with the samples of Freshwater prawn, *M. lar*



**Fig.3 :** Dendrogram of genetic similarity among the species of 4 different locations from Andaman based on UPGMA Method.(BP: Betapur, SB: Shoal Bay, CA: CARI Nallha, and BN: Burma Nallha) The UPGMA dendrogram revealed the relationship between 30 different samples of *M. lar* species could be grouped into two major clusters namely I and II. The major cluster I has BP7 which is collected from Betapur, middle Andaman, it is located 200 km away.

RAPD can be an efficient tool to differentiate geographically and genetically isolated populations, and has been used to verify the existence of locally adapted populations within a species that may have arisen either through genetic selection under different environmental conditions or as a result of genetic drift Fuchs *et al.*, (1998). Populational genetic differentiation can be driven by ecological, evolutionary and historical factors. In *Barbus neumayeri*, genetic differentiation among sampling

sites that presented different oxygen rates could represent the effects of selective pressure (Chapman *et al.*, 1999). The well-developed homing instinct of salmonid fish seems to be a decisive factor leading to strong population subdivisions (Ryman, 1983). An evolutionary unit can be identified for each tributary, with particular genetic traits possibly related to local adaptation and/or to inbreeding. In *Oncorhynchus nerka*, genetic differences were found between two populations inhabiting regions with distinct environmental conditions (Hendry *et al.*, 2000). Furthermore, some river or lake systems contain metapopulations composed of distinct breeding units (Carvalho, 1993; Hansen and Loeschcke, 1994).

In the present study, the dendrogram revealed that 30 different samples of *M. lar* species could be grouped in to two major clusters namely I & II. The major cluster I has Bp7 which was collected from Betapur, Middle Andaman located 200 km away from other locations. Major cluster II has further two clusters IIA, IIB; in this IIA having 18 sub groups from Shoal Bay, CARI stream, Burmanala and Cluster IIB has two samples from Shoal Bay and rest all from Betapur. This dendrogram revealed samples from Shoal bay and CARI Nallah interpreting with all the locations which may be due to anthropogenic interferences and the stocks found from both the clusters having very good genetic variation, which may be utilized for selective breeding programme further.

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