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An investigation on the molecular genetic profile of Indian marine penaeid prawn, *Penaeus monodon* Fabricius, 1798

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Abstract
The random amplified polymorphic DNA (RAPD) technique was used to examine the genetic variability in east and west coast penaeid prawn, *P. monodon* from two wild natural stocks (Chennai and Kochi). Initially, 20 oligonucleotide primers (OPA 1- 20, Bangalore Genei) were screened and only OPA-2 & 4 were selected to amplify DNA samples from these two sites. A total of 16 fractions for OPA-2 and 15 fractions for OPA-4, ranging in size from 0.0025 to 1.9 kb were scored. The unique RAPD fragments (markers) were detected by primer OPA-2 in Kochi population (0.1, 0.2, 0.8, 0.9, 0.95, 1.3, 1.9 kb) and OPA-4 from Chennai were revealed two unique fragments each in Kochi (0.80, 1.6 kb) and Chennai (0.0025, 0.95 kb) indicates that these two populations may be distinct genetic stocks. This data may provide baseline information on the genetic variation from one site each of east and west coast of Indian penaeid prawn, *P. monodon* sample populations for future management of the resource.

Keywords: Indian marine penaeid prawn - *Penaeus monodon*, genetic variation, DNA, RAPD, primer, genetic stocks.

Introduction
Penaeid prawns provide the basis for large aquaculture industries worldwide (Fast and Lester, 1992) [9]. These industries have developed rapidly over the last 30 years and are dominated by the five species (*Penaeus monodon* Fabricius, 1798 (60% of world production); *P. vannamei* Boone, 1931 (27%); *P.chinensis* Osbeck,1765 (8%); *P. stylirostris* Stimpson, 1874 (3%); *P. japonicus* Bate, 1888 (< 1%); several other species account for the remaining 2% of production, principal among which are *P. penicillatus* Alcock,1905; *P. merguiensis* De Man, 1888; *P. indicus* H. Milne Edwards, 1837; *Metapenaeus ensisde* Hann, 1844 (Rosenberry, 1997) [37]. These have proved particularly useful out of the 20 or so that were investigated originally for their aquaculture potential. Many more penaeid species have been subject to fishing for a long time (Rothlisberg and Staples, 1990) [38] and most of the published genetic studies on penaeid prawns have been aimed at determining stock structure for fisheries (Williams *et al*., 1990; Tassnakajon *et al*.., 1997, 1998; Benzie, 2000; Bindhu Paul, 2000; Vincent Terrence Rebello, 2003, 2015) [43, 39, 2, 4, 41,42].
Information on stock structure is of considerable use for aquaculture purposes. Wild populations remain the dominant source of broodstock for some of the most important aquaculture species, and sound management of the wild resource is therefore as vital for the aquaculture industry as for wild fisheries. These studies also provide useful information on the genetic diversity available in nature and for the future planning of the source of broodstock for closed-cycle breeding programme (Hedgecock and Malecha, 1991) [16]. This can also help in planning propagation assisted rehabilitation programme for endangered fish species or stocks and monitoring the impact on natural genepool. Penaeid shrimp fisheries and hatcheries have undergone an accelerated development during the past three decades (Rosenberry, 1997) [37]. Farming of this species is increased tremendously, leading to the production of at least 1,42,070 tons annually in India.
Earlier works using Allozymes failed to reveal significant geographic structure in wild populations. However, it was noted that penaeids, like many decapods, have very little

allozyme variation. The possibility that the lack of spatial differentiation among wild populations observed using allozyme techniques was simply a result of the low resolution of the technique led to interest in using potentially more variable markers, such as mtDNA, microsatellites and random amplified polymorphic DNA (RAPD). The growing interest in aquaculture of prawns also led to the development of highly variable DNA markers for use in paternity testing, confirmation of progeny identity and for tracking families in culture systems (Benzie *et al*., 1993) [3]. Recent work has shown that shrimp populations can have considerable genetic structure and this has implications for the management of these species and aquaculture development.

The fishery and genetic resources of India is enormously rich and diverse (Jhingran, 1984) [19]. From time immemorial, these fisheries resources have been subjected to worldwide commercial exploitation. Uncontrolled commercial exploitation of a resource may lead to its over-exploitation or even its total loss as a fishery. Almost all these fishery resources are being exploited and managed under the traditional concept that each fishery is supported by wild populations having homogeneous characteristics. To prevent over exploitation of some of these valuable sea fishery resources, many maritime nations have forced to introduce fishing regulatory measures like, ban on trawling, reduction in fishing efforts, gear restrictions and seasonal restrictions on fishing the species suspected as being overexploited or threatened or even endangered - typical example is the commercially very valuable penaeid prawn, *P. monodon* Fabricius, 1798 of South India, popularly known as the jumbo tiger prawn. The species *P. monodon* for the present investigation ranks foremost in its fishery and aquaculture importance in India (George, 1994, 1997) [11, 12] and overseas. Along the east coast, *P. monodon* contributes to nearly 3-3.5% of the total trawl landings at Chennai and 0.6-0.8% of the landings at Kakinada. When compared to the east coast, contribution of the same species in the trawl operations is poor along the west coast (0.10 to 0.45 %).

In view of the modern concept of the units of fisheries management (Lester, 1980; Altukhov, 1981) [23, 1], it is essential to clearly know that whether all the wild populations of *P. monodon* being exploited in India are morphologically, biochemical genetically or molecular genetically homogeneous or not. Since, it is an ideal species for commercial aquaculture and selective breeding programme (Lester and Pante, 1992) [24], the questions of its stock structure become more meaningful and significant. Though, detailed information on the biology and fishery of *P. monodon* of South India is available (Kemp, 1915; Panikkar and Aiyar, 1939; Hall, 1962; Rao, 1967; Rao *et al*., 1993) [20, 34, 14, 35, 36], the important questions mentioned above were remaining almost unanswered. To achieve these objectives, an independent and refined method of molecular genetics based on random amplified polymorphic DNA (RAPD) analysis (Hallerman and Beckman, 1988; Ovenden, 1990; Jayashankar and Dharmalingam, 1997; Tassanakajon *et al*., 1997, 1998; Muneer *et al*., 2005, 2009, 2010; Muniyandi *et al*., 2006; Gopalakrishnan *et al*., 2009; Divya *et al*., 2010) [15, 33, 18, 39, 40, 27-29, 30, 13, 8] was applied to study and detect individual variations within each population samples of the species.

Material and Methods

Samples of *Penaeus monodon* were collected from two geographically isolated (east and west) coasts of India (Kochi and Chennai). The wild specimens (100-300 mm) were collected from these two coasts and were transported to the laboratory and stored in deep freezer (4ºC) until used further experiments. The muscle tissues were selected for the present study.

Total DNA was extracted from the muscle tissues following the procedure of Maniatis *et al*. (1982) [26] and Garcia and after minor modifications. Samples were treated with SDS, proteinase K and genomic DNA isolated using phenol: chloroform: isoamyl alcohol extraction and precipitation in ice-cold absolute ethanol. The precipitated DNA was pelleted by centrifugation at 10,000 rpm for 15 minutes. After a wash with 70% ethanol, the DNA was air dried and re-suspended in 100 µl TE buffer (10mM Tris, 1 mM EDTA, pH 8.0). Concentration and purity of extracted DNA was determined spectrophotometrically at 260nm and 280 nm. Samples showing the 1 OD equivalent to 50 µ/ml and purity (determined by the ratio of 260 nm and 280 nm) 1.8 alone were taken for further analysis.

Ten decamer primers, kits OPA 1-10 (60% G + C contents; Operon Technologies Inc., Almeda, USA) were tested on 10 individuals each form Chennai and Kochi. All the primers produced amplifications with repeatable, sharp and clearly stained fragments were finally selected to analyse specimens of *P. monodon*. However, only OPA-2 and OPA-4 revealed polymorphic DNA profiles in the Chennai and Kochi samples tested.

Polymerase Chain Reaction

PCR reactions were carried out in duplicates in the thermocycler (Perkin Elmer Geneamp PCR system, 2400) in the block and the reactions were cycled through different temperature regimes. The reaction mix contained 0.33 µl of Taq polymerase (1.0 U), 1 µl of dNTPs (300 µM), 3.3 µl of assay buffer (2mM $MgCl_2$), 2 µl of primer (10 picomoles), 17.37 µl of millipore water/ deionised DDW and 1 µl template DNA – a total of 25 µl.. It was run for 45 cycles as follows: denaturation at 92°C for 1 min, annealing at 35°C for 1 minutes 30 seconds, 72°C for 1 minute and an extension at 72°C for 7 minutes. An additional denaturation at 92°C for 3 minutes was given initially. The reaction was then cooled to 4°C. The length of the run was nearly 4 hours and 30 minutes. The presence of the PCR products were confirmed by using controls, one without primer, second without Taq DNA polymerase and the third without genomic DNA. No amplifications occurred in any of these controls. Amplification is also carried out with different concentration of template DNA in order to optimise the template DNA which give the best amplification products (1µl to 100, 30, 15 µl respectively), annealing temperatures (34, 35 and 36ºC), the number of cycles (40 & 45) and finally by the duration of each step (30 seconds to 2 minutes). Finally, the dilution of 1 µl to 100 µl with 55 ng of DNA was selected for screening of the sample. The primers, OPA-1 to OPA-10 were used for polymerase chain reactions.

PCR amplifications and electrophoresis of PCR products

Following the amplification, the reaction mixtures (loaded 7 µl product mixed with dye) were run on a 1.5% or 2 % agarose gel for one and a half hours using 1X TEB (pH 8) buffer. The gel was stained in ethidium bromide for 20 minutes. After completion of the run, the gels were washed in double distilled water and viewed under UV and captured using Image Master VDS. The alleles were designated

according to the PCR product size in relation to the molecular marker (λ DNA with E.coRI/Hind III double digest). All the OPA primers used amplified the isolated DNA samples. However only OPA-2 and OPA-4 revealed DNA variations in the samples. The sequences of the primers : TGCCGAGCTG – OPA-2 and AATCGGGCTG - OPA - 4.

The RAPD-PCR technique can produce non-reproducible amplification product (Callejas and Ochando, 2001; Yap and Nelson, 1996) [5, 46]. Reactions were therefore performed following a strict protocol with standardized conditions. Also, all amplification reactions were carried out at-least thrice in order to make sure consistency and repeatability of fingerprints generated using selected RAPD primers.

For the analysis, RAPD fragments were treated as independent and unweighted characters and a binary matrix was produced whereby the presence or absence of each DNA fragment for each sample was recorded 1 or 0, respectively. Faint or poorly amplified fragments were excluded from the analysis as were very high (above 2000bp) or low (below 300bp) molecular weight. Mathematical formulae used were based on a few assumptions. First, all RAPD fragments scored were 2-allele system i.e., presence (dominant +/+ and +/-) and absence (recessive -/-) of bands. Second, fragments that migrated at the same position, had the same molecular weight and stained to the same intensity were homologous bands from difference loci did not migrate. A third assumption is that both the populations confirmed to the Hardy-Weinberg equilibrium, p2 + 2pq + q2 = 1, with frequencies p (dominant, band present) and q (recessive, band absent) (Clark and Milligan, 1993; Lynch and Milligan, 1994) [6, 25]. From the binary matrix, the total number of RAPD fragments, species-specific diagnostic markers and polymorphic bands were calculated for each primer and for all primers. The 'species-specific diagnostic markers' are defined as those RAPD bands that are exclusive to *Penaeus monodon*.

Genetic variability within *Penaeus monodon* from Kochi and Chennai were estimated from the percentage of polymorphic RAPD loci (%P) and average gene diversity using POPGENE Version 1.31 (Yeh *et al*., 1999) [47]. Pairs of RAPD loci were compared within each species to test for linkage disequilibrium. The %P values were calculated using the criterion for polymorphism of which, the frequency of the most common allele was <0.95. Average gene diversity index (φ statistics) also known as average heterozygosity (H) (Nei, 1978, 1987; Khoo *et al*., 2002) [31, 21] is a measurement of genetic variation for randomly mating populations and is analogous to Wright's (1951) F_{ST} statistics (fixation index). H is defined as the mean of heterozygosity (h) for all loci. It is given as h = 1-mΣ i=1x2i where xi is the population frequency of the i th allele at a particular locus and m is the number of alleles.

Pair-wise genetic similarity or identity index (SI) among *Penaeus monodon* were computed by POPGENE into genetic distance (GD) according to Hillis and Moritz's (1990) [17] formula. GD = 1 – SI. Genetic similarity index is given as SI $=2N_{AB}/(N_{A} = N_{B})$ where NB is the number of bands shared in common by individuals of *P. monodon*. Intraspecies mean genetic distance measures among the individuals were compared within *P. monodon*. The statistical significance of the intraspecies genetic measure within the species was evaluated using student's t-test. Cluster analysis was performed and dendrogram plotted based on pair wise genetic distance estimated using the unweighted pair-group method with arithmetic mean (UPGMA) based on Nei (1978) [31], modified from NEIGHBOR procedure of PHYLIP version 3.5c, using POPGENE Version 1.31 (Yeh *et al*., 1999) [47]. To test the candidate level of each branch of UPGMA based dendrogram, the binary data matrix was bootstrapped 1000 times, using Win Boot (Yap and Nelson, 1996) [46]. Bootstrap values between 75 and 95 were considered significance and above 95 highly significant (Lehmann *et al*., 2000) [22]

Results and Discussion

RAPD profiles of total DNA/Polymerase chain reaction

To detect random amplified polymorphic DNA (RAPD), ten primers (Operon) that are known to amplify the DNA were used. The primers OPA 1-20 amplified the total DNA of the species. However, only OPA-2 and OPA-4 revealed polymorphic DNA profiles in the Chennai and Kochi samples tested. Hence, samples were analysed using only these two primers. The RAPD profiles revealed by these two primers are described below.

1. OPA - 2

Specimens from Chennai and Kochi were screened with OPA 2 (Fig. 1, 2). A total of 16 bands of different sizes were detected in the species (Table 1). Thus OPA 2 produced 16 RAPD fragments from Kochi and Chennai. The kilobases of the OPA-2 fragments considered for comparison varied from 0.1 to 1.9 kb (0.1, 0.125, 0.2, 0.56, 0.85, 0.87, 0.9, 0.95, 1.0, 1.1, 1.15, 1.2, 1.3, 1.4, 1.5, 1.6, 1.9 kb). The significant aspects of the results are that the number of fragments present in the two samples of the species is significantly different. Seven RAPD fragments (0.1, 0.2, 0.8, 0.9, 0.95, 1.3, 1.9 Kb) produced by this primer were unique to Kochi population where as no fragments were unique to Chennai population.

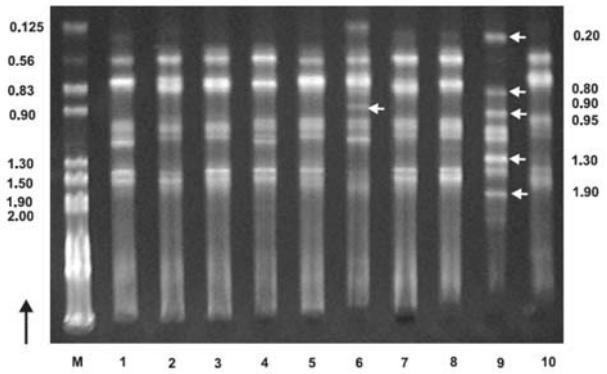

Fig.1 RAPD (OPA-2) banding pattern of *P. monodon* of South India

Columns 1 to 5 belong to specimens of Chennai and 6 to 10 are that of Kochi.

Column **M** is the pattern of the Marker **(Lambda DNA Eco R1, Hind III Double Digest) (Size: 0.125 to 2.0 kb)**

→ **Shows unique bands in specimens from Kochi.**

2. OPA - 4

Specimens from Kochi and Chennai were screened with OPA 4 (Fig. 3, 4). The total number of fragments or the bands produced OPA 4 was 15 (Table 1). A total of 15 fractions were produced by OPA 4 from Kochi and Chennai populations. The sizes of the fragments were 0.0025, 0.003, 0.005, 0.009, 0.1, 0.125, 0.3 0.56, 0.8, 0.9, 0.95, 1.0, 1.1, 1.4

and 1.6 Kb size in Kochi and Chennai populations. OPA 4 revealed two unique fragments each in both the sample populations. The two unique fragments in Kochi population (0.80, 1.6 Kb) whereas, the two unique fragments (0.0025, 0.95 Kb) were seen in Chennai.

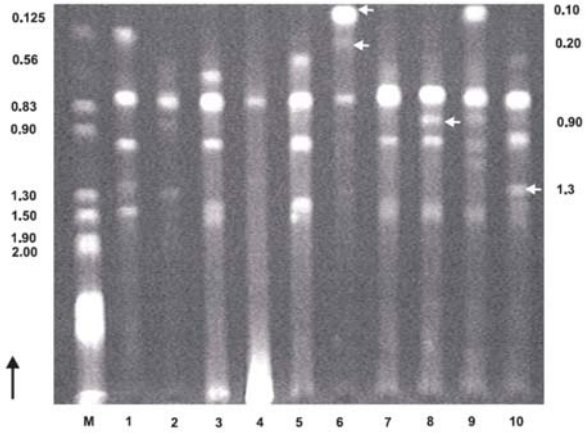

Fig. 2 RAPD (OPA - 2) banding pattern of *P. monodon* of South India

Columns 1 to 5 belong to specimens of Chennai and 6 to 10 are that of Kochi.

Column **M** is the pattern of the Marker
(Lambda DNA Eco R1. Hind III Double Digest)
(Size: 0.125 to 2.0 kb)

→ Shows unique bands in specimens from Kochi.

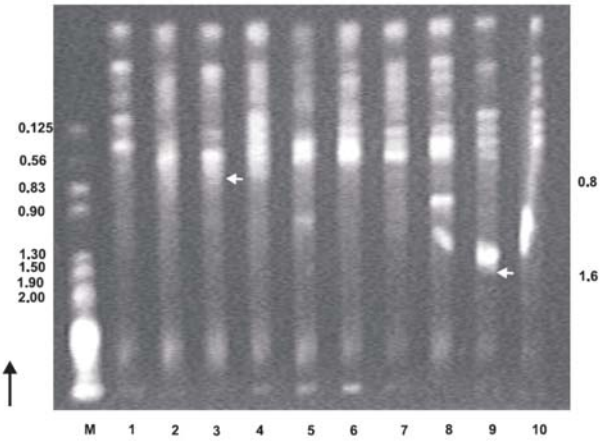

Fig. 3 RAPD (OPA - 4) banding pattern of *P. monodon* of South India

Columns 1 to 10 belong to specimens of Kochi

Column **M** is the pattern of the Marker
(Lambda DNA Eco R1. Hind III Double Digest)
(Size: 0.125 to 2.0 kb)

→ Shows unique bands in specimens from Kochi.

The number of bands was counted from the photographs and band sharing indices between the individuals were estimated using Nei's formula (Nei, 1978, 1987) [31, 32] for each primer. These index values were averaged over the primers in order to find out the mean genetic similarities among the individuals within the populations and between the populations. The similarity values in the total population ranged between 0 and 1. The mean percentage similarity among the individuals of Kochi population for OPA 2 and 4 was 74%, whereas that of Chennai population was 69%. A mean percent similarity of 78% was observed between the Kochi and Chennai populations for the primers (Table 3). This indicate the large changes at the base pair level, reflecting the difference in the population structures of east-west coasts.

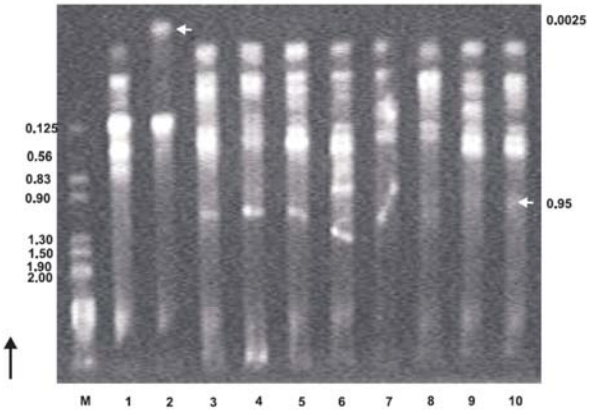

Fig. 4 RAPD (OPA- 4) banding pattern of *P. monodon* of South India

Columns 1 to 10 belong to specimens of Chennai

Column **M** is the pattern of the Marker
(Lambda DNA Eco R1. Hind III Double Digest)
(Size: 0.125 to 2.0 kb)

→ Shows unique bands in specimens from Chennai

An accurate knowledge of the natural units that sustain a fishery is of fundamental importance for its scientific exploitation, conservation and for its modern aquaculture practices through selective breeding programme. Such knowledge can be gained by accurate measurement of genetic characteristics of the sample populations of the species in question (Altukhov, 1981; Lester and Pante, 1992) [1, 24]. The topic of the present discussion here is the results of the study of the population genetic characteristics of *P. monodon* of South India (George, 1994, 1997) [11, 12]. The genetic characteristics of the species were measured by applying modern technique of molecular genetics. The significant results produced by this independent method is discussed below.

A total of sixteen RAPD kilobased fractions were produced by the primer, OPA-2 and fifteen fractions by OPA-4. However, the number of fractions present in Kochi and Chennai were significantly very different. For example, Chennai had only nine out of sixteen fractions, while Kochi had all the sixteen fractions. In other words, seven fractions (0.1, 0.2, 0.8, 0.9, 0.95, 1.3, 1.9 Kb) of the primer OPA-2 were unique to Kochi sample (Table 1). The OPA-4 primer produced a total of fifteen fractions. However, Kochi and Chennai had only 13 fractions. In other words, each sample was short of two but different fractions. For example, the fractions 0.0025 and 0.95 Kb were absent in Kochi, while the fractions 0.8 and 1.6 Kb were absent in Chennai. That is, each sample had its own two unique OPA-4 fractions (Table 1). In short, the above significant differences in the number of fractions strongly suggest that the east and west coast samples may be genetically distinct stocks. Besides, the significant differences in the number of DNA fractions between individuals of each sample also mean that level. Comparable reports of molecular genetic stock differences were reported in Melvin trout populations (Ferguson *et al*., 1995) [10] and *Labeo dussumeri* (Gopalakrishnan *et al.,* 2009) [13]. There, the unique DNA fractions (alleles) present

enabled to separate Ferox, Gillaroo and Sonaghen populations. A single RAPD fraction present in one of the two populations of *Macrobrachium borellii* was considered as genetic marker for stock identification (Amato and Corach, 1996) [7]. The recent review of the genetic structure of penaeids (Benzie, 2000; Tassanakajon, 1997, 1998; Williams *et al*., 1990) [2, 39, 40, 43] reveals that the RAPD technique is the most efficient technique for detection of natural genetic diversities in penaeid prawns, especially in *P. monodon* which fact is significant. This, thus strongly reports the present conclusion of genetic stock structure differences detected only by RAPD method and not by morphometric and biochemical genetic methods in *P. monodon* of South India.

The present finding of higher level RAPD genetic variability in Kochi and the unique RAPD fractions present in the east and west coast samples support the earlier non-genetic hypothesis based on stock assessment results (Rao *et al*., 1993) [36] that east and west coast samples of *P. monodon* are separate fishery stocks. Besides, these unique DNA fractions can be used as genetic markers to select the desired east and west coast breeders for the purpose of selective breeding programme and to monitor the level of DNA variability in the wild or cultured populations of the species. Besides, these stock-specific unique alleles can be used to detect any possible mixing of these two stocks especially during selective breeding programme or larval rearing period.

The present findings and conclusions are based on only a few specimens and a single test. Considering the sensitivity of the RAPD procedures, the reproducibility of the present results should be confirmed before drawing a final conclusion on the east-west stock diversity of *P. monodon*.

Table 1: Regional inter-specimen RAPD similarities (above the diagonal) and distances (below the diagonal) in *P. monodon* of South India

OPA 2/Kochi										
	1	2	3	4	5	6	7	8	9	10
1	--	0.27	0.92	0.92	0.71	0.86	0.80	0.77	0.86	0.77
2	0.73	--	0.25	0.25	0.35	0.47	0.56	0.50	0.35	0.63
3	0.08	0.75	--	0.86	0.67	0.80	0.63	0.57	0.67	0.57
4	0.08	0.75	0.14	--	0.67	0.93	0.63	0.63	0.8	0.71
5	0.29	0.65	0.33	0.33	--	0.88	0.94	0.93	0.75	0.67
6	0.14	0.53	0.2	0.07	0.12	--	0.71	0.67	0.63	0.67
7	0.20	0.44	0.37	0.37	0.06	0.29	--	0.88	0.71	0.50
8	0.23	0.50	0.43	0.37	0.07	0.33	0.12	--	0.67	0.43
9	0.14	0.65	0.33	0.20	0.25	0.37	0.29	0.33	--	0.40
10	0.23	0.37	0.43	0.29	0.33	0.33	0.50	0.57	0.60	--
				OPA 2/Chennai						
	1	2	3	4	5	6	7	8	9	10
1	--	0.86	1.00	0.80	0.85	0.92	0.73	0.85	0.77	0.71
2	0.14	--	0.85	0.80	1.00	0.92	0.55	0.57	0.62	0.86
3	0.00	0.15	--	0.80	0.71	0.92	0.55	0.71	0.62	0.57
4	0.20	0.20	0.20	--	0.80	1.00	0.50	0.80	0.57	0.67
5	0.15	0.00	0.29	0.20	--	0.92	0.55	0.71	0.46	0.57
6	0.08	0.08	0.08	0.00	0.08	--	0.40	0.77	0.50	0.46
7	0.27	0.45	0.45	0.50	0.45	0.60	--	0.36	0.40	0.36
8	0.15	0.43	0.29	0.20	0.29	0.23	0.64	--	0.46	0.57
9	0.23	0.38	0.38	0.43	0.54	0.50	0.60	0.54	--	0.31
10	0.29	0.14	0.43	0.33	0.43	0.54	0.64	0.43	0.69	--
				OPA 4/Kochi						
	1	2	3	4	5	6	7	8	9	10
1	--	0.80	0.88	0.94	0.94	1.00	0.94	0.88	0.88	0.80
2	0.20	--	0.71	0.75	0.75	0.82	0.67	0.67	0.67	0.71
3	0.12	0.29	--	0.95	0.95	0.80	0.86	0.78	0.78	0.71
4	0.06	0.25	0.05	--	0.88	0.84	0.90	0.82	0.82	0.88
5	0.06	0.25	0.05	0.12	--	0.74	0.80	0.82	0.82	0.75
6	0.00	0.18	0.20	0.16	0.26	--	0.86	0.89	0.89	0.82
7	0.06	0.33	0.14	0.10	0.20	0.14	--	0.84	0.84	0.67
8	0.12	0.33	0.22	0.18	0.18	0.11	0.16	--	0.88	0.67
9	0.12	0.33	0.22	0.18	0.18	0.11	0.16	0.12	--	0.67
10	0.20	0.29	0.29	0.12	0.25	0.18	0.33	0.33	0.33	--

				OPA 4/Chennai						
	1	2	3	4	5	6	7	8	9	10
1	--	0.88	0.80	0.86	0.82	0.82	0.71	0.82	0.36	0.88
2	0.12	--	0.80	0.71	0.82	0.82	0.71	0.82	0.36	0.75
3	0.20	0.20	--	0.77	0.75	0.75	0.63	0.75	0.40	0.67
4	0.14	0.29	0.23	--	0.80	0.80	0.80	0.67	0.44	0.86
5	0.18	0.18	0.25	0.20	--	0.78	0.89	0.89	0.33	0.82
6	0.18	0.18	0.25	0.20	0.22	--	0.89	0.89	0.33	0.71
7	0.29	0.29	0.37	0.20	0.11	0.11	--	0.78	0.33	0.71
8	0.18	0.18	0.25	0.33	0.11	0.11	0.22	--	0.33	0.71
9	0.64	0.64	0.6	0.56	0.67	0.67	0.67	0.67	--	0.36
10	0.12	0.25	0.33	0.14	0.18	0.29	0.29	0.29	0.64	--

Table 2: Inter regional RAPD similarities in *P. monodon* of South India

				OPA 2/Kochi-Chennai						
	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10
C1	0.92	0.38	0.86	0.86	0.93	0.93	0.63	0.86	0.80	0.57
C2	0.76	0.38	0.86	0.86	0.93	0.93	0.75	0.86	0.80	0.57
C3	0.76	0.38	1.00	1.00	0.93	0.93	0.75	0.86	0.80	0.57
C4	0.86	0.35	0.80	0.93	0.75	1.00	0.71	0.80	0.86	0.53
C5	0.76	0.38	0.86	0.860	0.80	0.93	0.63	0.86	0.67	0.71
C6	1.00	0.40	0.92	0.92	1.00	0.71	0.67	0.92	0.86	0.62
C7	0.60	0.46	0.55	0.55	0.50	0.50	0.62	0.55	0.67	0.72
C8	0.77	0.38	0.71	0.71	0.67	0.80	0.63	0.86	0.67	0.71
C9	0.67	0.40	0.62	0.62	0.57	0.57	0.67	0.62	0.71	0.62
C10	0.62	0.38	0.57	0.57	0.67	0.67	0.75	0.71	0.67	0.86
				OPA 4/Kochi-Chennai						
	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10
C1	0.88	0.67	0.78	0.82	0.82	0.89	0.84	0.75	0.88	0.80
C2	0.88	0.67	0.78	0.82	0.94	0.89	0.95	0.75	0.88	0.80
C3	0.93	0.71	0.82	0.88	0.88	0.82	0.78	0.80	0.80	0.86
C4	0.86	0.77	0.88	0.80	0.93	0.75	0.71	0.71	0.86	0.77
C5	0.94	0.75	0.95	0.780	0.77	0.95	0.90	0.82	0.82	0.75
C6	0.94	0.75	0.95	0.89	0.89	0.84	0.80	0.82	0.82	0.75
C7	0.94	0.75	0.84	0.89	0.89	0.95	0.70	0.82	0.94	0.75
C8	0.94	0.75	0.95	0.89	0.89	0.95	0.80	0.75	0.94	0.75
C9	0.73	0.60	0.46	0.50	0.50	0.46	0.43	0.55	0.73	0.60
C10	0.88	0.80	0.78	0.82	0.82	0.89	0.74	0.88	0.88	0.80

Table 3: Average of inter-regional RAPD similarities in *P. monodon of South India*

OPA 2/OPA 4	Kochi	Chennai	Average
K1/C1	0.92	0.88	0.90
K2/C2	0.38	0.67	0.53
K3/C3	1.00	0.82	0.91
K4/C4	0.93	0.80	0.87
K5/C5	0.80	0.77	0.79
K6/C6	0.71	0.84	0.78
K7/C7	0.62	0.70	0.66
K8/C8	0.86	0.75	0.81
K9/C9	0.71	0.73	0.72
K10/C10	0.86	0.80	0.83
	0.71	0.78	0.78

1. Primer OPA 2/4 - 'S' values of individuals of Kochi = 0.74 (74%)
2. Primer OPA 2/4 - 'S' values of individuals of Chennai = 0.69 (69%)
3. Primer OPA 2/4 - 'S' values of individuals of Kochi/Chennai = 0.78 (78%)

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