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## Genetic Characterization of Portunid Crabs *Scylla serrata* and *Scylla tranquebarica* from the West Coast of India Using Random Amplified Polymorphic DNA Markers

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### Abstract

Crabs of the genus *Scylla* (Decapoda: Portunidae) are commercially important and widely distributed throughout the Indian and Pacific Oceans. These crabs have an extended planktonic larval phase in their life cycle which suggests high dispersal potential and the possibility of extensive gene flow between conspecific populations on a geographic mesoscale (tens to hundreds of kilometers). Hence, genetic differentiation studies were carried out for *Scylla serrata* and *S. tranquebarica* along the west coast of India using RAPD markers. Twenty five wild specimens of each species were collected from a mangrove area of Ratnagiri district on the west coast of India. Three primers OPE-1, OPE-3 and OPE-4 produced clear and reproducible profiles and were selected for analyzing 20 individuals, each of *S. serrata* and *S. tranquebarica*. Primer OPE-4 gave a maximum percentage of 95.23% of polymorphic bands in *S. serrata* and OPE-3 showed 91% polymorphic bands in *S. tranquebarica*. Using primers OPE-1 and OPE-3, the two species were clearly clustered into separate groups. The average genetic similarity at the intra-specific level was calculated as 0.64 and 0.73 in *S. serrata* and *S. tranquebarica*, respectively, while the interspecies similarity was 0.56. The average polymorphism over all three primers were 83.33 and 63.88% in *S. serrata* and *S. tranquebarica*, respectively. A

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band of 438 bp produced with OPE-1 is diagnostic between the two species being specific to *S. serrata*.

## Introduction

The crabs (*Scylla* spp.) belonging to the family Portunidae are important crustaceans from an aquaculture point of view. Among the 700 littoral crab species, those of the genus *Scylla* are commercially important, growing to a maximum size ranging from 0.7 to 2.4 kg, and inhabiting brackish waters, such as mangrove areas and estuaries, throughout the Pacific and Indian Oceans, from Tahiti, Australia, and Japan to Southern Africa (Chahpgar 1947; Hill 1975; Sakai 1976; Dai & Yang 1991). The taxonomy of the *Scylla* crabs has become a subject of great importance for carcinologists ever since the revision of the genus by Estampador (1949). He classified mud crabs into three species and one variety, namely *S. serrata*, *S. oceanica*, *S. tranquebarica* and *S. serrata* var. *paramamosain*, using specimens collected in the Philippines based on their external morphology (colour of carapace and legs, anterolateral teeth of carapace and outer spines of cheliped carpus) and gametogenesis. Serene (1952) also recognized the existence of four forms in Vietnam in accordance with the findings of Estampador (1949). Studies have also been made on the genetic variability in the *Scylla* crabs along with morphometric analysis, suggesting the existence of three species, *S. serrata*, *S. tranquebarica* and *S. oceanica* (Fuseya & Watanabe 1996; Fuseya 1998).

In India, *Scylla serrata* is widely distributed on both the east and west coasts but *S. tranquebarica* is least abundant on these coasts as compared with *S. serrata*. Vartak et al. (2002) first recorded the occurrence of *Scylla tranquebarica* on the west coast of India. The life history of crabs (*Scylla* spp.) comprises a planktonic larval stage that lasts for 3-4 weeks (Ong 1964; Brick 1974) in which larval dispersal is wide through passive transport by ocean currents. Hence, gene flow can be assumed to be high in both species, as previously described for other marine species with planktonic larvae (Palumbi 1992). For the first time in the present study, genetic variation from both species, collected from the west coast of India, is analyzed using RAPD (random amplified polymorphic DNA) markers.

## Material and Methods

### Source of samples

Twenty-five wild individuals of *S. serrata* and *S. tranquebarica* were collected from coastal villages of the Ratnagiri district of Maharashtra state of India (Fig. 1). All the specimens were transported in bamboo baskets to the laboratory and kept in masonry tanks for 2-3 days.

After acclimatization in the laboratory, crabs were removed by hand net from the tank and the haemolymph was collected from each specimen by inserting a syringe into the sinus at the base of one of the walking legs. The crabs were released back after sampling.

### Extraction of genomic DNA

Approximately 100  $\mu\text{l}$  of haemolymph were immediately transferred to an Eppendorf tube containing 4 volumes of a lysis solution (100 mM Tris-HCl pH 9.0, 50 mM EDTA, and 100 mM NaCl, 200 mM sucrose, 10% sodium dodecyl sulfate (SDS), and 300  $\mu\text{g}\cdot\text{ml}^{-1}$  proteinase K). After incubation for 2 hours at 60°C, the lysate was extracted once with phenol and twice with chloroform/isoamyl alcohol. The DNA was precipitated with isopropanol, washed once with 70% ethanol and suspended in TE solution. The concentration of extracted DNA was determined spectrophotometrically and further adjusted using a mini-gel method (Maniatis et al. 1982). The DNA was stored at 4°C until required for further processing.

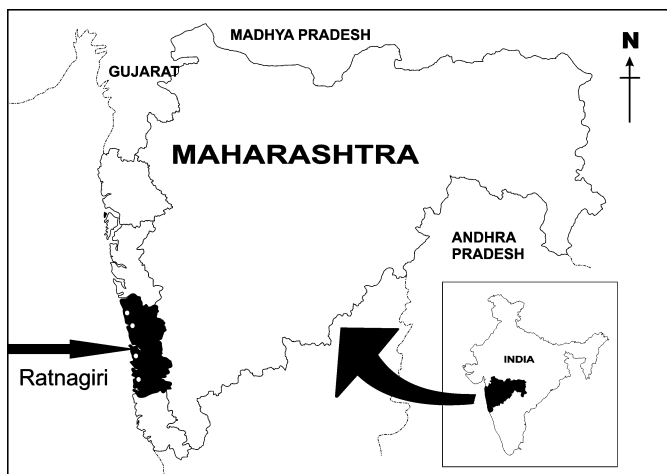


Fig.1: Sample collection site of two crab species (*Scylla serrata* and *Scylla tranquebarica*) used in this study

### ***PCR-RAPD amplification***

The genomic DNA was amplified by PCR (polymerase chain reaction) using short, randomly constructed primers. Prior to analysis, 10 primers of Kit E (E-1 to E-10, Operon Technologies Inc.) were tested with DNA from 5 individuals from each species taken at random. Three primers OPE-1 (5'-CCCAAGGTCC-3'), OPE-3 (5'-CCAGATGCAC-3') and OPE-4 (5'-GTGACATGCC-3') were chosen for further analysis on the basis of sharpness, clarity of the profiles (Puterka et al. 1993), and the existence of polymorphism. These primers were used for analyzing 20 individuals, each of *S. serrata* and *S. tranquebarica*. The amplification reactions were carried out in 25- $\mu$ l reaction volumes containing 200  $\mu$ mol $\cdot$ L<sup>-1</sup> each of dNTPs, 2 mmol $\cdot$ L<sup>-1</sup> MgCl<sub>2</sub>, 1x standard *Taq* polymerase buffer, 0.2  $\mu$ mol $\cdot$ L<sup>-1</sup> random primer, 40 ng of genomic DNA, and 0.75 U of *Taq* polymerase (Perkin-Elmer Cetus). Amplifications were performed in a Hybaid thermocycler for 35 cycles consisting of denaturation at 94°C for 1 min, annealing at 36°C for 1 min, and extension at 72°C for 2 min. The final extension was carried out at 72°C for 7 min. The resulting products were electrophoretically separated through 2.0% agarose gels, stained with ethidium bromide (Sigma). The gels were imaged using Syngene Gel Documentation System, UK. A 100 bp DNA ladder (MBI Fermentas) was used as size standard. The sizes of major bands were determined by the Syngene Gene Tool Software on the gel documentation system.

### ***Data analysis***

The bands below 3 kb were analyzed to assess genetic variations between the *Scylla* species. RAPD profiles of individuals were scored based on band presence (1) or absence (0). The index of similarity (F) between individuals from each species, i.e. intraspecific genetic similarity was calculated using the formula  $F_{xy} = 2n_{xy} / (n_x + n_y)$  (Nei & Li 1979), where  $n_{xy}$  is the number of RAPD fragments shared by the two individuals, and  $n_x$  and  $n_y$  are the numbers of RAPD fragments scored in each individual. Within species similarity (F) is calculated as the average of  $F_{xy}$  across all possible comparisons between individuals within a species. The genetic distance (d) was calculated using the formula  $d = 1 - F$  of Hillis et al. (1996). The cladograms were constructed and analysis was done with NTSYS software.

## Results

A total of 57 scorable bands ranging from 172 to 1890 bp were observed from the RAPD analysis of *S. serrata* and *S. tranquebarica* using three oligonucleotide primers (OPE-1, OPE-3 and OPE-4). The numbers of amplified bands in all the samples were 16, 19, and 22 for primers OPE-1, OPE-3 and OPE-4, respectively (Figs. 2 to 4). The average percentage of polymorphic bands in *S. serrata* was relatively higher (83.83 %) than that in *S. tranquebarica* (63.88 %). A specific band of 438 bp was found only with primer OPE-1 in *Scylla serrata*. The average intraspecific similarity index was 0.64 in *S. serrata* and 0.73 in *S. tranquebarica*, whereas the genetic distance between species was 0.44.

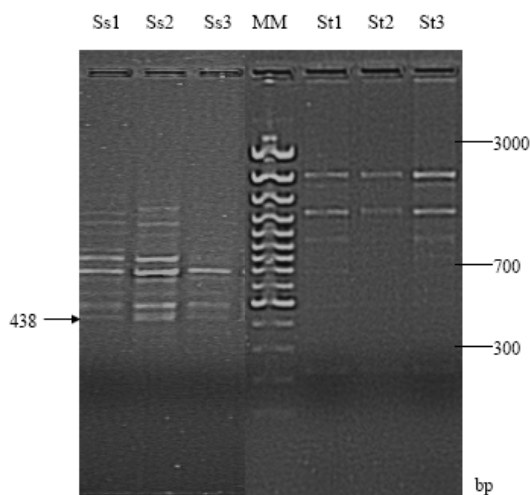


Fig. 2. RAPD profile generated by OPE-1

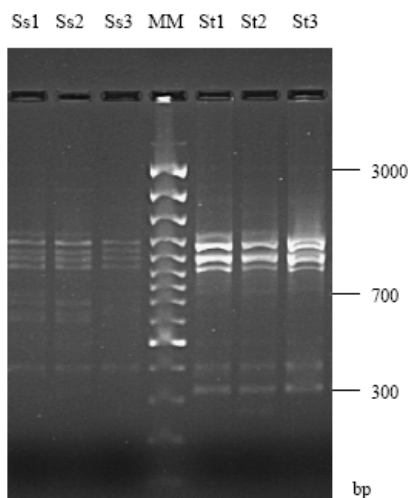


Fig. 3. RAPD profile generated by OPE-3

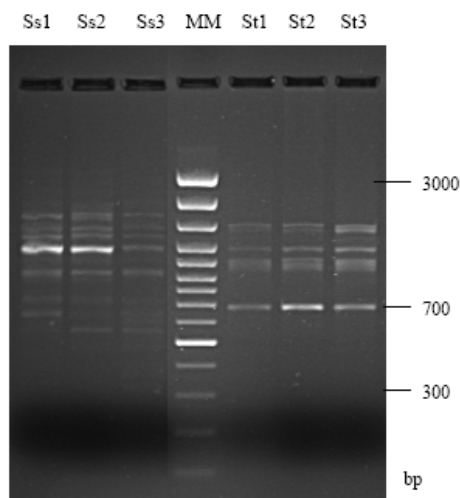


Fig. 4. RAPD profile generated by OPE-4

The cladograms constructed using primers OPE-1, OPE-3 and OPE-4 are shown in figures 5 to 7, respectively. The cladograms generated using the OPE-1 and OPE-3 primers have shown clear differentiation between the species *S. serrata* and *S. tranquebarica* from the Ratnagiri coastal region. Primers OPE-1 and OPE-3 clustered the two species into clearly separate groups. Primer OPE-1 produced a diagnostic amplicon of 438 bp, present in *S. serrata* but absent in *S. tranquebarica*. The RAPD bands of 662 and 487 bp are common in both species.

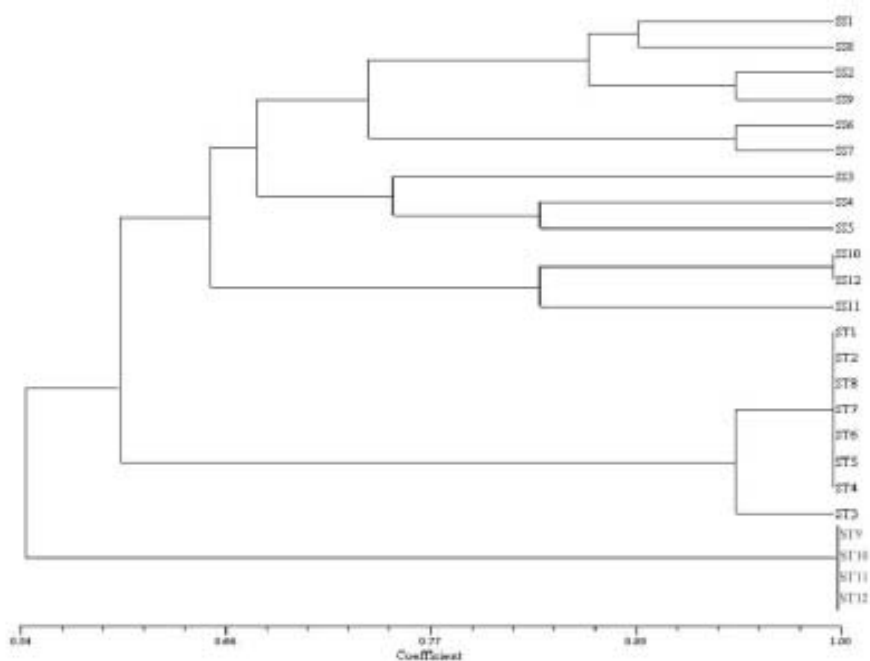


Fig. 5. Phylogenetic tree plot of *S. serrata* and *S. tranquebarica* using RAPD primer OPE-1

## Discussion

The development of powerful DNA techniques such as AFLP, RAPD and microsatellites are useful in generating large numbers of molecular markers. This has boosted the relatively fast progress in fish and crustacean linkage mapping. The RAPD method is based on the principal that the shorter the length of the primers, which are used in PCR, the greater is the chance that non-target sequences will be amplified. RAPDs have all the advantages that primers are commercially available and do not

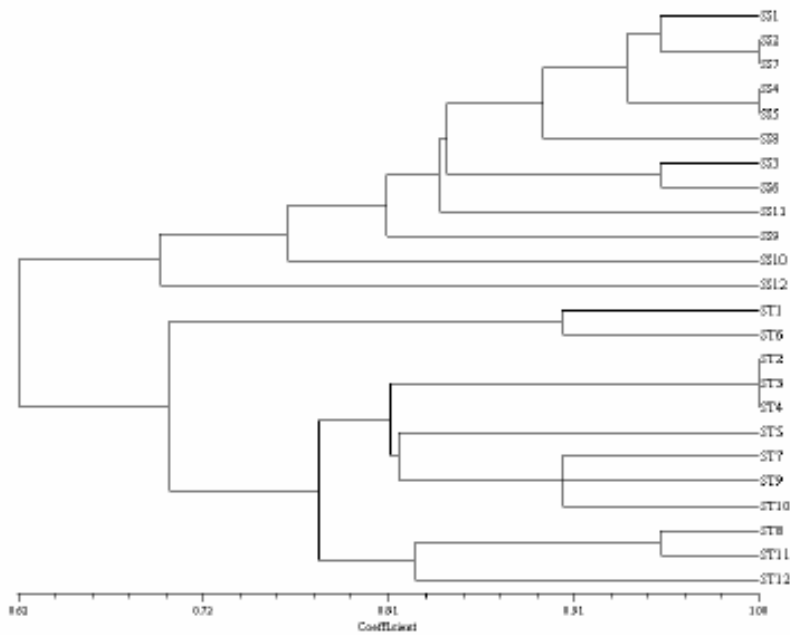


Fig. 6. Phylogenetic tree plot of *S. serrata* and *S. tranquebarica* using RAPD primer OPE-3

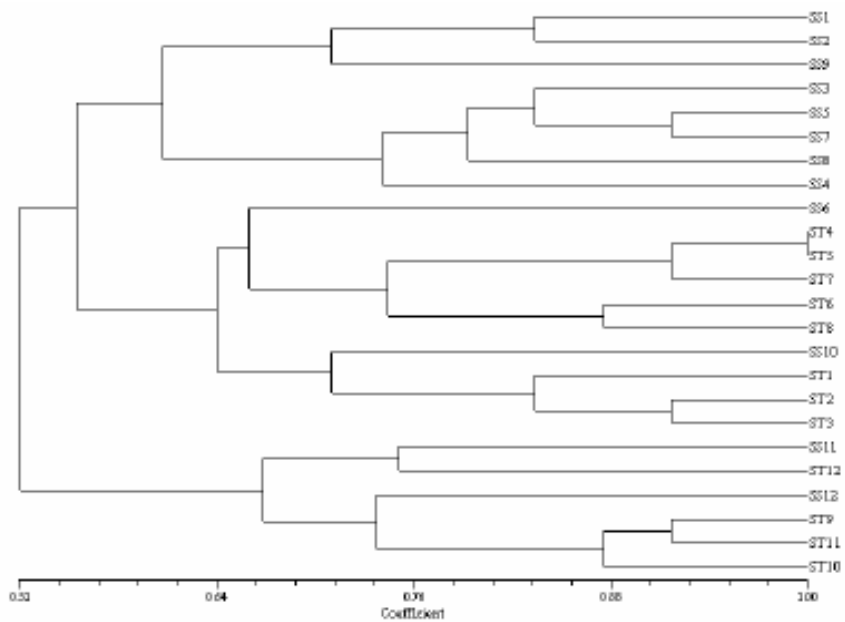


Fig. 7. Phylogenetic tree plot of *S. serrata* and *S. tranquebarica* using RAPD primer OPE-4

require prior knowledge of the target DNA sequence or gene organization. Studies on the genetic diversity of crabs (*Scylla* spp.) from Asia have been conducted by [Klinbunga et al. \(2000\)](#) and [Sara & Marco \(2002\)](#). In an earlier study, [Fuseya & Watanabe \(1996\)](#) initially classified the crabs into three species: *S. serrata*, *S. tranquebarica* and *S. oceanica*. They used starch gel electrophoresis to analyze genetic variation at 17 allozymic loci in these three species. In the current study, the genetic analysis of crabs of the genus *Scylla* from India has been carried out for the first time using RAPD markers. This approach has been used for species identification in fishes ([Partis & Wells 1996](#)), mollusks and crustaceans ([Crossland et al. 1993](#); [Tassanakajon et al. 1998](#); [Klinbunga et al. 2000](#)).

The development and applications of DNA marker technologies have already been used in other areas such as molecular systematics, population genetics, evolutionary biology, molecular ecology, conservation genetics, and seafood safety ([Liu & Cordes 2004](#)). In the present study, three primers namely OPE-1, OPE-3 and OPE-4 used for the genetic characterization of crabs were found to be useful in obtaining DNA variation. The RAPD markers developed in this study can be useful for the identification of the crab seed of the two *Scylla* species. This information was scarce for the crab (*Scylla* spp.) populations in India. Also, these genetic markers can be used for assessing growth performance of both the species in mixed aquaculture.

[Klinbunga et al. \(2000\)](#) also used three primers to study the genetic diversity of mud crabs in Thailand. [Liu et al. \(1994\)](#) suggested that six to seven primers were sufficient to assess genetic variability within and among populations of highly polymorphic species. [Tassanakajon et al. \(1997; 1998\)](#) used six polymorphic primers to document population diversity of *P. monodon* in Thailand. In the present study, the RAPD profiles generated by the three primers revealed a high level of polymorphism in two *Scylla* species collected from Ratnagiri (83.33 and 63.88%). [Klinbunga \(1996\)](#) and [Klinbunga et al. \(1999\)](#) reported percentages of polymorphic bands of 46.7 and 61.4% in black tiger prawn from two geographic locations. Studies by [Garcia et al. \(1994\)](#) on genetic variation in *P. vannamei* using the RAPD technique also indicated a high degree of genetic variability among populations (percentages of polymorphic bands ranging from 39 to 77).



## Conclusion

Genetic identification of species or strains is very essential in aquaculture. In our study, we assessed DNA variation in two species of *Scylla* crabs from India using PCR-RAPD markers. This approach is useful not only for characterizing different species but also for determining relationships among them. In addition, it can be of great value in selective breeding programs and also for the assessment of growth performance in crab aquaculture. Interestingly, the band of 438 bp produced by OPE-1, which is present only in *S. serrata*, can be used as a species-specific marker. The similarity values at interspecies level suggested that the level of gene flow is high in these species.

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