Molecular Characterization of Economically Important Penaeid Populations in South East Coast of India

P. Rajakumaran, B. Vaseeharan, V. Anita Yeshvadha

1 Crustacean Molecular Biology and Genomics Lab, Department of Animal Health and Management, Alagappa University, Karaikudi 630003, Tamilnadu, India
2 Department of Zoology and Research Centre, Scott Christian College (Autonomous), Nagercoil 629003, Tamil Nadu, India

Corresponding author email: vaseeharanb@gmail.com; Authors

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Abstract Genetic variation of wild Penaeid population of South East Coast region in India, using Randomly Amplified Polymorphic DNA (RAPD) analysis as DNA marker has been examined. Important penaeid species like Fenneropenaeus indicus, Penaeus semisulcatus, Penaeus monodon were collected at different geographical location such as Chennai, Nagapattinam, Pudukkottai, Ramanathapuram and Tuticorin, but P. monodon was not obtained from Tuticorin. For RAPD analysis, 15 numbers of primers were used, among which 3, 4, and 6 numbers of primers have worked well for F. indicus, P. semisulcatus and P. monodon respectively. The results of this study, genetic variation (6-33)% for F. indicus, (16-49)% for P. semisulcatus and (16-53)% for P. monodon. As conclusion, from the pattern of genetic diversity of these three penaeid population, it could be considered as moderate to high, and maximum three population stocks are within the collected area.

Keywords Penaeid; RAPD; DNA Marker; Polymorphism; Population stock

Introduction
Penaeid shrimps constitute one of the most important group of species for aquaculture worldwide, ranking second in overall value in 2006 (FAO 2007). Sustainable development of this industry could greatly benefit from progress in our basic knowledge of genetics, genomics, and molecular immunology of shrimp. Recently, the application of high throughput molecular tools and approaches has led to significant developments in these fields (Robalino et al., 2012).

In India P. monodon (Fabricius, 1798), F. indicus (H. Milne Edwards, 1837), Fenneropenaeus penicillatus, (Olivier, 1791) P. semisulcatus, (De Haan, 1844), and Fenneropenaeus merguiensis (De Man, 1888) are cultured, among which first two are mostly cultivable due to supplying of seed from hatcheries, traditionally which enter along the tidal water also cultured. The estimated brackish water area suitable for undertaking shrimp cultivation in India is around 11.91 lakh ha. spread over 10 states and union territories such as West Bengal, Orissa, Andhra Pradesh, Tamil Nadu, Pondicherry, Kerala, Karnataka, Goa, Maharashtra and Gujarat. Of this only around 1.356 lakh ha are under shrimp farming now. The state with high shrimp culture farms are Andhra Pradesh, Tamil Nadu, and Orissa. In the east coast the Penaeid shrimp culture practices are higher than west coast. Penaeid shrimp aquaculture provides employment opportunities to coastal rural populations and to earn valuable foreign exchange.

The over exploitation of shrimps from Indian coastal waters and the ever increasing demand for shrimps in the world market has resulted in the wide gap between the demand and supply. This has necessitated the need for exploring newer avenues for increasing shrimp production. Tamilnadu situated on the south east of Peninsular India is about 130 000 Sq.km. The length of its Coastline is about 1 050 km with its significant portion on the east coast bordering Bay of Bengal. Tamil Nadu is having the second longest coastline in the country with rich natural resources in coastal areas for coastal aqua farming. The total estimated brackish water area of Tamil Nadu is about 56 000 ha. The total area under shrimp farming is 4 455 ha. of which 3 178 ha. are creek based and 1 277 ha. are sea based. The present culture is 4 455 ha. Which is only 30 per cent of the estimated potential area of 14 880 ha. readily available for shrimp farm development. Hence
there is a wide scope for land based coastal aquaculture development in Tamil Nadu.

Population genetic have proven valuable for estimating stock boundaries and genetic variability of wild shrimp population for fisheries (Benzie, 2000). Genetic distances between any pair of individuals of same group are smaller than those between pair of specimen any two geographical region (Aubert and Lightner, 2000). Species are often composed of discrete breeding units (population or stock) which are not reproductively isolated from each other, may have limited opportunity to exchange genetic material because of geographic distance, barrier to migration or spawning asynchrony. Low level of gene flow stocks may result, over time, in their genetic divergence and species that are subdivided into morphological and genetically distinct stock are said to be structured. This information will help to the development of selective breeding programs applying quantitative genetic to better the performance of domesticated shrimp stock in aquaculture. Shrimp forming industry in south east Asia, two fundamental problems have risen leading to great economic loss, those are viral diseases, that affect shrimp health resulting in large economic losses in affected countries and effects of domestication on genetic diversity levels in cultured lines. Genetic diversity likely impacted during the domestication process, besides, as result of culture stock they were exposed to repeated bottlenecks as they were developed in culture. This has led to an increasing in consanguinity over time. Since the existence of natural population subdivision may imply adaption to local conditions, genetic assessment of the degree of population structuring and gene flow among natural population are practical ways of helping to preserve existing biodiversity and maintaining valuable adaptive population.

The genetic diversity in the east coast appears to be more than the west coast of India. This could be due to a large fluctuation in the physico-chemical parameters in the east coast because of several river drainage in the east coast (Kumar et al., 2007). Metapenaeus dobsoni (Miers, 1878) populations from the west coast of India state Kerala and Maharashtra clustered together, but Tamil Nadu formed a separate group as east coast of India (Lakra et al., 2010). Distribution of zoeae larvae of these Penaeid species are generally very coastal, as spawning occur in 5 m-90 m depth, The life cycle of this species can be completed in captivity, diminishing the dependence on wild caught broodstock of these species and allowing to commercially breed juveniles to sustain production or to develop selective breeding programs. Before attempting such commercial operation, it is important to obtain data on genetic diversity of wild stocks for successful broodstock and genetic resources management since viable shrimp hatcheries coexist in many of the shrimp farming regions of the south east coast of Indian where collection of larvae and breeding stocks from the wild. It is susceptible to fluctuations in the availability of wild resources as any capture fishery activity. Therefore knowledge about genetic diversity and population differentiation is imperative to assess genetic variability in the wild population for proper conservation.

Nuclear DNA markers have allowed rapid progress in aquaculture investigations of genetic variability and inbreeding, parentage assignments, and species and strain identification and in the construction of high-resolution genetic linkage maps for aquaculture species (Liu and Cordes, 2004). Nuclear DNA markers main attractiveness is abundance in the genome, Mendalian inheritances, and potential to detect high polymorphism. The deleterious effects of inbreeding are to be avoided; crossing organism from genetically different strains is of critical importance. This can be done most effectively if knowledge about genetic similarity or differences between strains is available, especially when pedigree information is lacking (Ferguson, 1994). The limitation of mitochondrial DNA studies is that the genes are linked and act as a single locus and nuclear gene could serve as additional non linked genetic marker of population genetic studies. These nuclear markers would differ from mitochondrial marker in their rates of evolution and biparental mode of inheritance (France et al., 1999).

Random amplification of polymorphic DNA is a simple and easy molecular method to estimate genetic diversity in penaeid shrimp, fish and shell fish (Zhuang et al., 2001a, 2001b; Mishra et al., 2009, Lakra et al., 2010). It has been successfully employed to determine genetic diversity in Litopenaeus vannamei, (Boone, 1931) (Garcia and Benzie, 1995), and P. monodon, (Tassanakajon et al., 1998) for population studies of Penaeid species. In Thailand
found Banana prawns *F. mrguiensis* and *F. indicus* are clearly distinguished by RAPD marker (Phongdara et al., 1999). Due to variation in RAPD profile and difficulties with reproducibility, however many genetic researcher were made because RAPD-PCR is a quick and reliable method for identifying genetic diversity and similarity among various organism (Tassanakajon et al., 1997). Mishra et al. (2009) recommended that RAPD technique is very advantageous as a preliminary tool for studying the population structure of Penaeid. RAPD technique designed to detect sequence changes within the priming site as well as differences in length, through insertion or deletion, between them. Mutation at the priming site will affect primer annealing and amplification of particular segment (Caetano-Anolles and Bassam, 1993), whereas deletion or insertion between primering site will alter the length of amplified fragment, both instances will result in changes in the RAPD profile produced by a given primer, and significance of these differences can be used to determine the extent of population structure. RAPD fragment represent a combination of product amplified from coding and non-coding loci, estimator of genetic diversity extracted from them tend to be more informative of the overall genetic variation in the species than those derived from coding loci alone. The population separated by thousands of kilometers can be genetically more similar than others over very short distances and differences between species in level of genetic variation, and genotype distribution might be related to life history types (Mulley and Latter, 1980; De La Rosa-Velea et al., 2000).

Previously many genetic diversity studies using allozyme analysis has been used to determine the levels of variation and degree of genetic subdivision for several shrimp species (Lester and Pante, 1992; De la Rosa-Velez et al., 2000; Gracia-Machado et al., 2001; Gusmao et al., 2005), and mitochondrial DNA restriction fragment length polymorphism (Benzie et al., 1993; Klinbunga et al., 1999, 2001; Gracia-Machado et al., 2001). Genetic diversity and geographic differentiation of the *P. monodon* mitochondrial COI sequences (Kumar et al., 2007; Khamnammong et al., 2009). Randomly amplified polymorphic DNAs (Aubert and Lithtner, 2000; Mishra et al., 2009; Lakra, 2010; Rezvani et al., 2011) and microsatellite (Wolfus et al., 1997; Brooker et al., 2000; Supungal et al., 2000; Maggioni et al., 2003; Robainas-Barcia and Garcia-Machado, 2012; Mandal et al., 2012). In all studies, the major Penaeid were *L. vannamei, P. monodon*, and *Masupenaeus japonicus* (Bate, 1888), but in the present study, the most available and economically important Penaeid populations were the candidate species. Previously no report was available on the molecular characterization of *Fenneropenaeus indicus, Penaeus semisulcatus, Penaeus monodon* of the south east coast region of India, and hence an attempt has been made to reveal the genetic diversity of three Penaeid populations in south east coast of India.

**1 Results**

Among 15 numbers of primers used for this study, 3, 4, and 6 number of primers, worked well for *F. indicus, P. semisulcatus* and *P. monodon* respectively. The primer sequences for this study (Table 1). Among the primer RM 03, 04, 05, 07, 11, 14, 15, 17 used for present study the RM 03, 04, 07 primers amplified the DNA product of three species taken for genetic analysis. DNA bands size produced 1000 to 10000 bp. For this 1000Kbp maker were used as reference and polymorphic profile for *F. indicus, P. semisulcatus*, and *P. monodon* shown as Figure 1, Figure 2, and Figure 3 respectively. The total number of bands, number of polymorphic bands, number of monomorphic bands and percentage of polymorphic bands for all species collected from all location (Table 2) and genetic diversity value (Table 3) were counted.

**Table 1 Primer used for present study**

| S.No | Primer name | Sequence           |
|------|-------------|--------------------|
| 1    | RM03        | 5′ AAT CGG GCT G 3′|
| 2    | RM04        | 5′ GAA ACG GGT G 3′|
| 3    | RM05        | 5′ GTG ACG TAG G 3′|
| 4    | RM07        | 5′ CAA TCG CCG T 3′|
| 5    | RM011       | 5′ TGC CTA AGA C 3′|
| 6    | RM014       | 5′ GTC TTA CCC T 3′|
| 7    | RM015       | 5′ TCC CCC GAC C 3′|
| 8    | RM017       | 5′ TCC CTC GTG C 3′|

**Figure 1 RAPD profile for *F. indicus***
As for *F. indicus*, the 3 primers totally generated 108 bands; and the percentage of polymorphism was calculated for all located samples. The number of polymorphic bands and the percentage of polymorphism for Chennai, Nagapattinam, Pudukkottai, Ramanathapuram and Tuticorin samples are 9 (33%), 8 (26%), 3 (19%), 3 (15%) and 2 (12%) respectively. High genetic variation was observed between Chennai and Tuticorin samples. High genetic distance value was observed in Chennai sample than in sample collected from other location. On the basis of UPGMA dendrogram genetic similarity coefficient value showed three clades for five geographical areas. Tuticorin-Ramanathapuram occupied the first clade, Pudukkottai-second and Chennai-Nagapattinam third respectively (Figure 4).

As for *P. semisulcatus*, 4 primers totally formed 132 bands; the number of polymorphic bands and percentage of polymorphism for all geographical sample produced were Chennai 4 (16%), Nagapattinam 4 (16%),
Figure 4 Phylogeography of *F. indicus*

Pudukkottai 5 (17%) Ramanathapuram 3 (17%) and Tuticorin 9 (49%). Genetic distance value was high in Tuticorin sample than the other samples. Upgma dendrogram produced 3 clades, in which Tuticorin occupied the first clade, Pudukkottai-Ramanathapuram second and Chennai-Nagapattinam third respectively (Figure 5).

Figure 5 Phylogeography of *P. semisulcatus*

For *P. monodon*, 6 primers produced 126 bands, Chennai sample showed 15 polymorphic bands (51%) followed by Nagapattinam 12 (35%), Pudukkottai11 11 (30%) and Ramanathapuram 6 (16%). The genetic similarity value was less in Chennai sample than the other location sample. UPMGA dendrogram showed three clades for four geographical areas such Chennai in first clade Nagapattinam second clade and Pudukkottai-Ramanathapuram third clade (Figure 6).

Figure 6 Phylogeography of *P. monodon*

### 2 Discussion

The understanding of population genetic structure is of primary importance for the management and conservation of genetic resources in exploited marine organism (Hillis et al., 1996). Benzie (2000) revealed little genetic variation over long distances than short distances for many Penaeid species. These limits vary widely with species, habitats, local ocean conditions, or historical events, and they may produce sufficient chances for genetic variation (Palumbi and Baker, 1994). Molecular genetic marker has a wide range of applications in aquaculture research and for improvement of aquaculture stocks (Hallerman and Beckmann, 1988., Poompuang and Hallerman, 1997; Liu and Cordes, 2004; Chistiakov et al., 2006). Nuclear DNA marker only provides high resolution to resolve the evolutionary relationship and genetic diversity of the shrimp (Benzie, 2000; Dall, 2007; Vaseeharan et al., 2012). Breeders will want to design future matings to avoid inbreeding. Culturists may want to know whether the offspring maintain the genetic characters of the wild or founder population. In particular, they may want to know whether genetic variability was maintained in terms of number of polymorphic loci, number of alleles per locus, and heterozygosity. Marker assisted selection like growth rate; diseases resistant often have high enough heritability to be improved using classical selective breeding. The use of molecular marker to address genetic identification and discrimination of aquaculture stock, monitoring of inbreeding or other changes in the genetic composition of the stocks that may result from such phenomena as breeding programmes, founder events and genetic drift thereby assisting selective breeding programmes.

In the present study, *F. indicus* species population, high number of polymorphic bands and percentage of polymorphism producing in Chennai, followed by Nagapattinam, Pudukkottai, Ramanathapuram, and Tuticorin sample was observed, indicating that among *F. indicus* population genetic diversity increases from Tuticorin to Chennai and also *F. indicus* population are highly diverged towards northern direction of south east coast of India. And phylogenetic relationship based on genetic similarity, Chennai and Nagapattinam comes under one clade, Pudukkottai as separate clade, Ramanathapuram–Tuticorin in another clade. From the above relationship it is evident that *F. indicus*, even though polymorphism there in all population tested, these populations could be considered three distinct stocks
those are Chennai-Nagapattinam, Pudukkottai, and Ramanathapuram-Tuticorin. As analyzing *F. indicus* at reared condition using RAPD marker, concluding RAPD marker is enough to assess genetic variation for the establishment of marker-assisted selective breeding program in Penaeid (Rezvani et al., 2011).

Contrary to *F. indicus*, in *P. semisulcatus* population from the Tuticorin samples have high genetic variation than other location, and the Ramanathapuram and Pudukkottai samples more diverged than Nagapattinam and Chennai samples. This result says the *P. semisulcatus* population diversity high towards southern direction of south east coast of India. The phylogenetic analysis produced 3 distinct clade such Tuticorin, Ramanathapuram-Pudukkottai, and Chennai-Nagapattinam, form these observation asserted that 3 stock population in *P. semisulcatus* those are Chennai-Nagapattinam, Pudukkottai-Ramanathapuram, and Tuticorin. The population genetic structure of *P. semisulcatus* in different geographical area of worldwide is very limited number of work only there. Niamaimandi et al.’s (2010) study was the first RAPD study of the population of *P. semisulcatus* in Busher (Persian Gulf), which were collected from Halailechan and Daylam, using 9 primers. Percentage of polymorphism is 14.8 %, and this indicated that populations around Bushehr are not well structured, which is consistent with a previous PCR–RFLP analysis by Rezvani et al. (2001) and Rezvani (2002). But in present study, the percentage of polymorphism is (16-49)% which shows substantial genetic diversity south east coast of India than Iran.

As for *P. monodon* populations the pattern of number and percentage of polymorphism and phylogenetic relationship as those of *F. indicus*. The absence of availability of *P. monodon* in Tuticorin during studied period indicate may be due to this animal is seasonal. The four populations could be split up into 3 stock such as Chennai, Nagapattinam and Pudukkottai-Ramanathapuram. These findings also implicit that the *P. monodon* highly diverged toward north direction of South East Coast of India as in the case of *F. indicus*. The RAPD analysis of broodstocks of *P. monodon* from three different locales (or localities) of Thailand such as Satun-Trang, Trat, and Angsila, revealed, the percentages of polymorphic bands were 48% and 45% in Satun-Trang and Trat, respectively, suggesting a high genetic variability of the two samples to be used in selective breeding programs. Only 25% polymorphic bands were found in the Angsila sample (Tassanakajon et al., 1998). Gracia et al. (1994) reported a higher genetic variability in *L. vannamei* populations as polymorphic bands ranged from 39 to 77%. In *P. monodon* 32% of the RAPD banding patterns differences between Kochi and Chennai in India (Vincent, 2003), but in 35% differences in the present investigation.

In the present study, the genetic diversity of all Penaeid populations tested is moderate to high and diversity based on the closer geographic distribution such as Chennai, Nagapattinam, Pudukkottai, Ramanathapuram and Tuticorin. Maggioni et al. (2003) analyzed the population structure of *Litopenaeus schmitti* (Burkenroad, 1936) occurring only in Brazil and reported that six microsatellite loci were observed across eight geographic locations and influenced by environmental factors that exist across relatively small geographic scales. The geographic differentiation have been observed in Penaeid (Aubert and Lightner, 2000; De La Rosa –Velez et al., 2000; Gracia et al., 2001; Xu et al., 2001). Phylogeographical subdivision of genetically distinct varieties is not uncommon in *Penaeus* species such as *Farfantepenaeus subtilis* (Perez Farfante, 1967) in South America (Gusmao et al., 2000), *F. merguiensis* in Thailand (Hualkasin et al., 2003) and *P. monodon* in Thailand (Klinbunga et al., 1999; Supungul et al., 2000) and in the Indo west Pacific region (Duda and Palumbi, 1999; Benzie et al., 2002).

The South East Coast is endowed with varied landscape such as sandy beaches, beach ridges, backwaters, estuaries, intertidal mud and sand flats, dunes, cliffs, beach rocks, deltas, lagoons, mangrove forests and coral reef ecosystems. The coast has been constantly undergoing physical changes in the geological past and at present. Many rivers bring considerable sediments, which affect shore processes significantly. The seas having algae, coral and other organisms accumulate in varying nature of landforms and their disposition along huge quantities to form reefs, which sometimes extend the beaches and inland represents the successive phase considerable distance offshore, and also the position of transgression and regression of sea level. Along development of these features are related to the stand of the eastern coast of peninsular India, narrow belts of sand the sea during
their formation. The Gulf of Mannar is a protected area of India consisting of 21 small islands (islets) and adjacent coral reefs in the Gulf of Mannar in the Indian Ocean islands that lie along the 140 km stretch between Tuticorin and Rameswaram (Ramathapuram district). These topographic features can be clearly traced by integrating the landform serve as ecosystems, which have components of distribution different terrestrial, marine and atmospheric processes. Jackson et al. (2001) reported that the marine larvae may move as much as 100 km between the offshore inshore habitats. Many marine species including the Penaeid, are thought to have a high dispersal capacity throughout their geographic range during their larval planktonic stage caused by oceanographic current, and also the habitat of coastal species are rarely continuous, heterogeneous genetic diversity could be generated by some environmental factor like water current and physical barrier and biological factors like reproductive behavior (Feral, 2002). The gravid P. monodon females spawn in the open sea. Benthic post larvae are found along the coast or in mangrove swamps and other estuarine areas wild fry become juveniles and adults in estuarine areas but return to the sea for spawning. As for F. indicus, the adult spawn offshore, maintain till at post larval stage, The Post larvae migrate using water current towards estuary (with estuarine juveniles) become juvenile which likes mud or sandy mud at depths of 2 to 90 m. after mature coming towards offshore. Life history of P. semisulcatus adult spawn in off shore, larvae migrate towards grass and algal bed near sea shore. Then juvenile P. semisulcatus migrate to offshore to become adult. F. indicus and P. monodon somewhat same adapted life cycle rather than P. semisulcatus. These attribute could be the reason why P. semisulcatus different geographical pattern of diversity against P. monodon and F. indicus.

In our study, the genetic variation including all species in the range of 12-53%, and genetic similarity with close geographical population, these observation matched with previous studies on population of Penaeid M. dobsoni genetic diversity 22.3% to 40.9 % (Mishra et al., 2009), and M. affinis (31.10–57.20)% (Lakra et al., 2010) samples collected from Indian coast and showed clear differences between west and east coast population and genetic diversity justified by the closer geographic distribution. Zhuang et al. (2001a, b) and Song et al. (1999) reported that two wild Kuruma prawn populations from Taiwan Strait and Xiamen water area had average heterozygosities of 0.214 and 0.245 respectively. In our study, the genetic diversity for F. indicus, P. semisulcatus and P. monodon 0.275, 0.447 and 0.329 respectively.

Benzie (2000) reviewed Penaeid prawn and concluded that estimated variability from DNA-based markers showed high level of diversity in natural population than that inferred from allozyme. The results of P. monodon populations differentiation are genetically significant between Chumphon and Trat while using RAPD, whereas these results did not show differentiation between these samples while using mtDNA polymorphism. From these findings, it is concluded that under the presumption of selective neutrality of this mitochondrial marker, biased female gene flow between Trat and Chumphon P. monodon may exist and be responsible for an anomalous differentiation pattern (Klinbunga et al., 2001). RAPD markers are used to differentiate between wild and cultured shrimp populations and families useful in breeding (Vaseeharan et al., 2012).

The studies on genetics indicated a very good heritability of productive traits for Penaeid shrimp, and this could open a good outlook to detect related genes for marker-assisted selections in future selective breeding programs of shrimp (Hoa, 2009). One of the domestication effects is the loss of rare alleles. The changes in allelic diversity after bottlenecks may be more striking than changes in heterozygosity (Allendorf and Ryman, 1987). A stick correlation between loss in genetic variation and shrimp production performance that would thereby reduce their diversity that is adaptive potential, relative to their wild counter parts is well documented (Dumas and Ramos, 1999; Sbordoni et al., 1986; Harris et al., 1990; Xu et al., 2001; Zhuang et al., 2001a, 2001b). A species that has little or no genetic diversity will produce offspring that are susceptible to diseases and have reduced biologic fitness and increased chances of species extinction. In the present study, the penaeid population have moderate to high genetic variation and maximum three stock there, interbreeding among these stocks which help to aquaculture industry which prevail in these site to prevent the deleterious effect of bottleneck. Therefore, in penaeid population, detection of the genetic variation using different molecular
markers is useful to geneticists for genetic improvement program of those economically valuable species.

3 Concluding Remarks

The last decade of research and development in shrimp genomics and genetics has seen significant advancements. An abundance of sequence information from expressed genes is available in public databases, providing a first glimpse at the gene content of several Penaeid species. Researchers and aquaculture geneticists have begun to exploit these resources, especially for the identification of genetic markers, candidate disease resistance genes, and genes linked to reproduction and other aquaculture-relevant processes. A growing number of initiatives are mining the available sequence data to implement markers and generate increasingly more extensive linkage maps. The number of shrimp genes for which at least some sequence information is now known is in the thousands, rather than dozens, as was the case just a few years ago. The tools are also in place to refine the selection of candidate aquaculture-relevant genes through the characterization of two key aspects of the function of a gene: its expression and its loss-of-function phenotype. Now we are able to measure the expression of thousands of these genes simultaneously, thanks to progress in transcriptomic methodologies, although much effort is still necessary within the community to meet the analytical challenges involved. The relative lack of unified efforts and multilab resources such as EST databases, microarray platforms, and library repositories, are indicators of the need to strengthen the ties among shrimp researchers. The challenges imposed by the sheer magnitude of genomic projects will likely be met only by a unified community, with a demonstrated capacity to gain the most out of shared resources. Such an evolution of attitudes will likely make it feasible to support a full-genome sequencing project for a Penaeid shrimp in the near future (Robalino et al., 2012). With the increasing global demand for aquaculture products and the early stage of selection for most aquatic species, molecular genetics is expected to play a pivotal role in the management of breeding programs aimed at developing improved strains for the most economically important species (Vaseeharan et al., 2012). Molecular characterization of economically important penaeid population in South east coast of India will open doors genetic diversity of wild stocks for successful broodstock and genetic resources management in many of the shrimp farming regions of the south east coast of Indian where collection of larvae and breeding stocks from the wild.

4 Materials and Methods

4.1 Collection of Samples

_Fenneropenaeus indicus_ (H. Milne Edwards, 1837), _Penaeus monodon_ (Fabricius, 1798), _Penaeus semisulcatus_ De Haan (1844) were collected from landing centre of five district of East coast of Tamilnadu such Tuticorin, Ramanathapuram, Pudukkottai, Nagapattinam, and Chennai (Figure 7). In Tuticorin the _Penaeus monodon_ is not obtained. The collection period October–December 2012. The collected shrimp were kept in icebox, brought to lab, preserve in -20°C still DNA isolation.

![Figure 7 Collected site of penaeid population in South east coast of India](image)

4.2 DNA Isolation

Total genomic DNA was isolated from muscle tissue by SDS–Phenol/Chloroform method described in Williams et al. (1990) with slight modifications. Briefly, shrimp muscle (200 mg) was cut into small pieces, crushed using a sterile porcelain mortar and pestle with 1 mL of chilled TEN buffer (50 mM Tris–HCl, pH 8.0, 50 mM EDTA and 100 mM NaCl), and transferred to 2 mL Eppendorf tube. Proteinase K 8uL (300 mg/mL), sucrose 20uL (2%), and 20uL Sodium dodecyl sulfate (SDS 2%) were added to the tube. After overnight incubation at 60°C, the lysate was extracted once with Phenol and twice with Chloroform/Isoamylalcohol. DNA was precipitated
with isopropanol, washed once with 70% ethyl alcohol, and suspended in TE (Tris EDTA, pH 8.0) buffer. DNA quality and quantity were determined by agarose gel electrophoresis and biophotometer plus (Eppendorf, Germany).

4.3 RAPD-PCR Amplification and Data Analysis

Fifteen primers were used for RAPD analysis. DNA amplification reactions were performed in 200 umol/L each dNTP, 2 mmol/L MgCl₂, 19 standard Taq polymerase buffer, 0.2 umol/L random primer, 40 ng genomic DNA and 0.75 U Taq polymerase in a final volume of 200 μL. PCR conditions included initial denaturation at 94°C for 5 min, followed by 45 cycles of denaturation at 94°C for 30 seconds, annealing at 35°C for 1 min, extension at 72°C for 2 min and final extension at 72°C for 10 min. The amplified DNA was separated by electrophoresis through 2% Agarose gel containing Ethidium bromide in 1x TBE buffer at a constant 80 V.

To maintain consistency, only the repeatable major bands were scored. Molecular weights of amplified bands were estimated by comparing with known molecular weight marker (1Kbp DNA ladder, Bangalore Genie, India). DNA profiles generated for all samples were compared in a pairwise manner. RAPD bands were recorded on spread sheets as binary matrix marking alleles absent (0) and present (1). The distance matrix between species (Jaccard, 1908) calculated and subsequently the data used to construct a dendrogram using the (UPGMA) algorithm of Rohlf (1993).

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