Chapter 2

REVIEW OF LITERATURE

SECTION- A

LOBSTERS – THE PRESENT PROFILE

2A.1. Lobsters of the world: Genera, species, classification and time of evolution

The order Decapoda Latreille, 1802 forms one of the many orders of the Class Crustacea, Brünnich 1772. There are four suborders in the Decapoda. They are Macrura Natantia (shrimps), Anomura (hermit crabs, etc.), Brachyura (crabs) and Macrura Reptantia (lobsters, crayfishes etc.). The marine lobsters were first described by Linnaeus in 1758. The marine lobsters are considered to form part of the suborder Macrura Reptantia Bouvier, 1917. In several handbooks (e.g. Bowman and Abele, 1982), the Decapoda are divided into two suborders, the Dendrobranchiata (containing the Penaeidea-penaeid and sergestid shrimps) and the Pleocyemata (containing all the other Decapoda).

The taxonomy of marine lobsters has remained fairly stable over many years and some authors, such as Burukovsky (1983) and Phillips et al. (1980) had complied lists of all the valid species of extant marine lobsters known in the world, at that time. In the monumental work of Holthuis (1991), a detailed account was provided for almost all the living marine lobster species up to 1991. Still, the discovery rate of marine lobsters remains high to this day (Chan, 2010). There are currently recognized six families, 55 genera and 248 species (with four subspecies) of living marine lobsters (Chan, 2010). The six families of extant lobsters are Enoplometopidae, Nephropidae, Glypheidae, Palinuridae, Scyllaridae and Polychelidae.

2A.1a. Family Palinuridae and Scyllaridae

The subsuborder Macrura Reptantia has four infraorders: Astacidea, Glypheidea, Achelata and Polychelida (Chan, 2010). The infraorder Achelata contains three main families, namely Palinuridae (spiny lobsters),
Scyllaridae (slipper lobsters), and Synaxidae (furry or coral lobsters) (Palero et al., 2009a). These families share a unique larval phase called phyllosoma and lack chelae on their first pair of pereiopods (Scholtz and Richter, 1995; Dixon et al., 2003). In the two most recent molecular phylogenetic analyses of Achelata and Palinuridae (Palero et al., 2009a; Tsang et al., 2009), the family Synaxidae Bate, 1881 was proven to be polyphyletic and the authors suggested to treat this family as a junior synonym of Palinuridae (Tsang et al., 2009; Chan, 2010).

The Palinuridae arose in the Atlantic-European region during early Mesozoic (250 million years ago) at a period of high sea level in the vast, warm ‘Super Tethys’ Ocean (George, 2006b). The spiny lobster family Palinuridae contain eleven genera. They are Jasus, Justitia, Linunparus, Nupalirus, Palibythus, Palinurellus, Palinurus, Palinustus, Panulirus, Projasus, Puerulus and Sagmariasus. Palinurid genera are commonly divided into two major lineages (i) spiny lobsters with a stridulating organ or Stridentes and (ii) without a stridulating organ or Silentes (Parker, 1884; George and Main, 1967). It was in the Jurassic that the family diverged into the Stridentes group, seven genera (Linunparus, Palinustus, Puerulus, Palinurus, Panulirus, Nupalirus and Justitia) of which survive today in the low-latitude equatorial zone, and the Silentes group, three genera (Jasus, Sagmariasus and Projasus) of which live today in the mid-latitude temperate zone of the Southern Ocean and one genus (Palinurellus) in the equatorial zone (George 2006b; Tsang et al., 2009). The Silentes genera (Jasus, Projasus and Sagmariasus) are shown to be the basal spiny lobsters (George 2006a; Tsang et al., 2009). As the three genera are restricted to the southern high latitudes constitute the basal lineages, Tsang et al. (2009) suggested a Southern Hemisphere origin for the Palinurid group. In the family Palinuridae, Puerulus was considered to be the oldest living genus of the Stridentes (George, 2006b). The relatively shallow water genus Panulirus was acknowledged as the most recently evolved (George and Main, 1967; Pollock, 1992; Baisre, 1994; McWilliam, 1995; George, 1997, 2006a). But Tsang et al. (2009) inferred that Panulirus diverged in the early stage of Stridentes evolution instead of being the most recently derived, as long believed. Moreover, the two deep-sea genera (generally found in depths >200 m), considered to be the most primitive, Puerulus and Linunparus (George
and Main, 1967; Baisre, 1994; George, 2006b), were relatively derived in their gene tree and they suggested that spiny lobsters invaded deep-sea habitats from the shallower water rocky reefs and then radiated.

Spiny lobsters have received great attention during recent years, including numerous studies on their ecology, phylogeography, and molecular phylogeny (Díaz et al., 2001; Patek and Oakley, 2003; Palero et al., 2008a). The spiny lobster genus *Panulirus* White, 1847, has long been of interest to evolutionary biologists because of its high level of species diversity, its wide geographic distribution, and the importance of many species to commercial fisheries. Among genera in the family Palinuridae, *Panulirus* has been the most successful in terms of species diversity; 19 species have been described to date, three of which are divided into seven recognized subspecies (Holthuis 1991; George 1997; Sarver et al., 1998; Ptacek et al., 2001). Recently Chan (2010) in his annotated checklist of lobsters revised it to 21 taxa (with three subspecies).

The slipper lobsters of the family Scyllaridae are a unique group of decapod crustaceans characterized by the flattened antennal flagellum (Spanier and Weihs, 1990) and some members with a ventrally-flattened body to a plate (Lavalli et al., 2007; Jones, 2007). However, the slipper and coral lobsters have been the subject of much less research, probably because they do not include many species of commercial interest (Holthuis, 1991; Lavalli and Spanier, 2007). Slipper lobsters are distributed world-wide throughout warm waters with a vertical range from very shallow to more than 800 m deep (Webber and Booth, 2007). Scyllarid lobsters are found in coast along continental shelf and upper slope areas across the Equator, at low latitudes, and in temperate latitudes influenced by warm water currents (Webber and Booth, 2007). The family has a mainly warm-water distribution mainly between 30˚N and 30˚S. Many large species are fished commercially (Duarte et al., 2010) although the highest taxonomic diversity is among the smaller species (Holthuis, 1991; Chan, 2010). Based on the different carapace shapes as well as the morphology of the maxilliped exopods and mandibular palp, four subfamilies were proposed by namely Arctidinae, Ibacinae, Scyllarinae, and
Theninae (Holthuis, 1985, 1991, 2002). Altogether there are four subfamilies, 20 extant genera and 89 extant species known to date in the family Scyllaridae (Arctidinae = 17 species, Ibacinae = 15 species, Theninae = 5 species, Scyllarinae = 52 species) (Yang et al., 2012).

2A.1b. Offshore shift and diversification

There are two hypotheses that postulated evolution of lobsters. They are contradictory in that the first one suggests origin of lobsters from a deep-sea ancestral stock to the shallow-water genera and the second, vice versa. It has been hypothesized that radiation in the spiny lobsters occurred when the deep-water ancestral stock of high-latitude areas invaded the shallow warm water seas in lower latitudes, with subsequent specialization and diversification (George and Main, 1967; Baisre, 1994; George, 2005, 2006b). Earlier studies based on adult similarity and a larval cladistic analysis (e.g., George and Main, 1967; Baisre, 1994; George, 2005, 2006a, b) proposed a hypothesis of deep water to shallow water evolution. Contrary to this view, Davie (1990) proposed that the ancestral form of the family initially inhabited shallower waters and then retreated into the deeper region and not the other way round. The recent studies in Palinuridae supported this view of a general onshore (shallow-water) reef origin of the spiny lobsters, which then dispersed into offshore (deeper) reefs and eventually adapted to the typical soft deep-sea bottoms (Chan et al., 2009; Tsang et al., 2009; Tsoi et al., 2011). Past researchers have suggested a deep-water origin of the Scyllaridae too based on larval and adult characteristics (e.g., George and Main, 1967; Baisre, 1994; George, 2005, 2006a, b), while recent molecular analyses suggest the opposite trend (Chan et al., 2009; Tsang et al., 2009; Yang et al., 2012).

2A.2. Commercial importance of lobsters- catch, fishing areas, species and aquaculture

Lobsters support commercially valuable fisheries in many parts of the world and in some regions the most economically important one. The world catch of lobsters recorded in 2010 exceeded 2,79,000 MT, of which 1,88,248 MT corresponded to true lobsters (Nephropidae) 78,518 MT to spiny lobsters
(Palinuridae) and 10,310 MT to slipper lobsters (Scyllaridae) (FAO, 2010). Although the greatest number of commercial species occurs in tropical waters, the largest lobster catches come from cold-temperate regions like the northwestern Atlantic (Fishing Area 21) and the northeastern Atlantic (Fishing Area 27). Species of Nephropid lobster genera like Homarus (about 1,20,000 MT), Nephrops (66500 MT) and Palinurid genera like Jasus (about 11,679 MT) and Panulirus (about 64,000 MT) form the subject of specialized fisheries and are the basis for important industries (FAO, 2010). Overall, the global supply of lobsters from wild fisheries for all species combined appears to be at, or close to its maximum (Jeffs, 2010).

Spiny lobsters (Decapoda: Palinuridae) are one of the most commercially important types of marine animal (Phillips, 2006). They inhabit temperate and tropical seas, but most species and the highest abundances are found in the tropics (Holthuis, 1991). Spiny lobsters are captured and marketed in more than 90 countries and sustain major commercial fisheries while simultaneously supporting local, small-scale fisheries in remote coastal locations and islands. Many form the basis for specialized fisheries such as Panulirus argus in the Carribean, Panulirus cygnus in Western Australia and Jasus in New Zealand. The principal producing countries are Australia, New Zealand, South Africa, Cuba, Brazil, Mexico and the USA with over 70% of the spiny lobster catch coming from the Carribean and South-east Atlantic region and the Eastern Indian Ocean (Phillips and Kittaka, 2000). They are highly valued sea food, and the wild stocks support some of the most valuable commercial fisheries in the world’s major oceans (Booth and Phillips, 1994; Kittaka and Booth, 2000). The genus Panulirus, comprising 19 or more species, is the largest group in the family Palinuridae (George and Main, 1967; Holthuis, 1991; McWilliam, 1995), and all species are highly prized in many countries. More attention was given to the spiny and clawed lobsters as their fisheries became more profitable in the early 1990s with emphasis on potential of aquaculture. Valuable literature narrating various aspects like biology, management, fisheries ,culture etc of spiny lobsters were written by Cobb and Phillips,1980; Factor,1995; Phillips and Kittaka, 2000; Phillips, 2006).
Slipper or shovel-nosed lobsters of the family Scyllaridae are found throughout the world’s tropical and temperate oceans. Although they are often a desirable incidental catch in a commercial fishery, they are generally considered too small and scarce to warrant targeted harvesting (Nishikiori and Sekiguchi, 2001; Freitas and Santos, 2002; Vance et al., 2004). More recently, however, a few species in a few locales have become major target species for small-to-moderate scale fisheries (Coutures and Chauvet, 2003; Molina et al., 2004; Haddy et al., 2005; Radhakrishnan et al., 2007). The major species contributing to fishery were *Ibacus* (fishing area 61) and *Thenus* (fishing area 71) (FAO, 2010). In the Western and Central Pacific (fishing area 71), slipper lobsters contribute about 25-50% of the total lobster catch. Out of 89 species of extant scyllarid species, only 30 larger slipper lobster species are of commercial importance (Holthuis, 1991). The slipper lobster *Thenus* species has become focus of targeted fishery in India (Radhakrishnan et al., 2007). Other slipper lobsters that contribute to fishery are *Thenus* spp. in Australia, *Ibacus* spp in Australia, and *Scyllarides* spp. Some of the smaller species such as *Petractus rugosus* have commercial aspects in aquarium trading (Spanier and Lavalli, 2007; Kumar et al., 2009).

### 2A.3. Biology, aquaculture importance, breeding and larval dispersal of lobsters

The distinctive phyllosoma larva is the most important characteristic in the early life history of palinurids and scyllarids. Reaching 80 mm or so in total length in some species, this flat, virtually transparent, long-lived, leaf-like larva, which is often widely dispersed in the open ocean, is adapted for passive horizontal transport assisted by vertical migration. Early development in both families comprises a short-lived embryonised ‘prelarva’ (naupliosoma) in some species, a larval phase (phyllosoma), and a postlarval phase (puerulus in palinurids, nisto in scyllarids) which precedes the first juvenile stage (Phillips, 2006; Phillips and Kittaka, 2000).

The life cycle of spiny lobsters is complex and includes a long oceanic larval phase varying in length between species. Indeed, planktonic
development has in many cases been shorter in culture than in nature. Most palinurids for which there are data have estimated larval durations in nature of 6–12 months (but longer for Jasus spp.–up to 24 months), and all disperse well offshore (Phillips, 2006). The larval life of P. homarus is estimated to be 5.5–8 months (Phillips and Matsuda, 2011), but has not been successful so far anywhere in the world.

But among the Scyllaridae there is a wider range of estimated larval lifespans, from 1 month to at least 9 months. Many of the small adult scyllarid species have brief, inshore development. Warm-water species tend to have shorter larval lives than cool-water ones. Like the spiny lobster, the sand lobster, too has a complex and prolonged life cycle, though not as prolonged as in the case of the former (Kizhakudan, 2006b). The larval life estimated for Thenus species in wild is estimated to be 27–45 days (Jones, 1988; Mikami and Greenwood, 1997; Radhakrishnan et al., 2007).

There is considerable interest in the aquaculture of spiny lobsters because of their consistently high demand and price, and because of the full exploitation of the natural stocks. Rock lobster aquaculture produced 1611 MT in 2010 (FAO, 2010). Successful larval development was achieved in different parts of the world in eight species (Phillips and Matsuda, 2011). Despite success of larval rearing in some species, when the prospects for spiny lobster aquaculture were reviewed by Kittaka and Booth (1994), they stated that ‘the greatest hurdle in the commercial culture of spiny lobster is the difficulty in growing species through their larval stages’. The larval life cycle has been unsuccessful in lab due to difficulties in providing suitable diets in the later stages of development. The key bottleneck for lobster aquaculture is the hatchery nursery phase (Phillips, 2006). The hardy spiny lobsters with good growth rates for juveniles, but the long larval life extending over several months, with limited success in production of seeds, has discouraged its large-scale aquaculture (Kittaka and Booth, 2000). Problems and prospects of spiny lobster aquaculture in India were reviewed by Radhakrishnan and Vijayakumaran (2000).
The advantage in captive rearing of the sand lobster will be the relatively shorter span for larval metamorphosis as compared to the spiny lobsters (Robertson, 1968). Complete larval rearing has been successfully achieved in different parts of the world in scyllarid lobsters. The days of culture from phyllosoma to nisto ranged from 28 days (*Thenus* spp.) to 192 days (*Scyllarus arctus*) in laboratory conditions (Vijayakumaran and Radhakrishnan, 2011). The adult females of Ibacinæ and Theninæ are also relatively large (CL>70mm) and produce larger eggs that complete developments in about a month (Theninæ) or in 2-4 months (Ibacinæ). With a shorter larval life, high growth rates for juveniles, hardiness and a good market value, the slipper lobster is fast emerging as a new species of aquaculture interest (Mikami and Kuballa, 2007; Vijayakumaran and Radhakrishnan, 2011).

Except for a few species like *Panulirus cygnus*, *P. argus* etc. studies of larval transport of Palinurids integrating oceanic or coastal circulation patterns with larval distribution patterns has not been studied in detail (Phillips and Kittaka, 1994). The lengthy larval life and the often highly dispersed larval distributions have been major obstacles to research and management.

2A.4. Lobsters of the Indian Seas: Commercial importance, fishery, species distribution and abundance along the Indian coast

Though not big in volume, lobsters are one of the most valuable and highly priced crustaceans from the Indian seas. In India, lobsters form only 0.36% of the total marine crustacean landings in 2010 (CMFRI, 2011). But they were an important export commodity comprising 0.25% in quantity and 1% in value (MPEDA, 2009). India earned an approx USD 20 million through export of lobsters in 2009. Lobsters are exported as live lobster, frozen lobster tails, frozen whole cooked and frozen lobster tails. The live ones are considered superior in South-east Asian markets.

The lobster fishery improved from 800 MT in 1968 to 2991 MT in 1975 and attained a peak of 4075 MT in 1985. Thereafter the fishery showed a trend of decline averaging around 2200 MT for nearly 15 years, but declined to 1371 MT in 2004 (Radhakrishnan et al., 2005) and is estimated to be 1852 MT in
2011 (CMFRI, 2012). Twenty five species of lobsters have been reported so far from Indian coast (Modayil and Pillai, 2007). They are widely distributed along the entire coast of the country with maximum landings from the northwest coast (70%), followed by the southeast (16%) and southwest (14%) coasts (Radhakrishnan and Manisseri, 2003; Radhakrishnan and Thangaraja, 2008). The northwest coast comprising Gujarat and Maharashtra is particularly rich in lobster resources, contributing to nearly three quarters of the total lobster landing in India (Kagwade et al., 1991; Radhakrishnan, 1995).

Radhakrishnan and Manisseri (2003) discussed the species distribution and fishery of lobsters in Indian seas. The southeast coast except a small region is a potential lobster fishing area. *P. omatus, P. homarus* and *Thenus orientalis* are the major species exploited. Small quantities of *P. versicolor* are also landed along the Trivandrum and Chennai coasts. *P. penicillatus* and *P. longipes* are the two other species, which are not important from the fishery point of view. *Linuparus somniosus* was reported from the Andaman and Nicobar Islands, but has not been commercially exploited. In the northwest coast, 95% of lobsters are caught by trawls whereas it is traps, gill nets, trawls and trammel nets that catch most of the lobsters in southwest and southeast coasts.

Although the lobster fauna of commercial fishing grounds comprises 14 species of littoral and six species of deep sea forms, only four littoral and one deep sea form are significant in commercial fishery. Two species, the palinurid spiny lobster *Panulirus polyphagus* (Herbst) and scyllarid *Thenus orientalis* (Lund) predominate in the fishery along the northwest coast (Chhapgar and Deshmukh, 1971). The major sand lobster fishery completely collapsed by 1994 due to recruitment overfishing and there is no sign of its recovery (Deshmukh, 2001). In the southwest, *P. homarus homarus* is the dominant species in the shallow water lobster fishery (Modayil and Pillai, 2007). *Puerulus sewelli* was the only deep sea species exploited in commercial quantities from the area. However, *P. versicolor* and *P. omatus* are also landed in small quantities. Major fishing grounds for the deep sea lobster, *P. sewelli* were
located off Quilon in the southwest and off Tuticorin in the southeast coasts, at depths ranging from 150m to 400m.

The most important species that contributed to the lobster fishery in India in 2010 were the Slipper lobster, *Thenus unimaculatus* and spiny lobster, *Panulirus homarus* (CMFRI, 2011). *T. orientalis* is the only slipper lobster of commercial significance among the rich diversity of scyllarid lobsters recorded from the Indian coast (Radhkrishnan et al., 2007). They appear as by-catch in trawl fisheries and although catch rates are low, they constitute the most important component of the lobster fishery on the northwest, southwest and southeast coasts of India. In the northwest, along the Mumbai coast, the *Thenus* fishery collapsed in 1994 and has yet to recover, causing concern about the sustainability of the slipper lobster fishery (Deshmukh, 2001).

The research on lobsters in India are mainly focused on biology, breeding, larval nutrition and farming (e.g. Thangaraja and Radhakrishnan, 2012; Vijayakumaran et al., 2012; Chakraborty et al., 2010; Rao et al., 2010). The successful larval rearing was achieved for *Thenus* spp. in India other than Australia (Kizhakudan et al., 2004a). The phyllosoma larvae of *P. homarus* was successfully reared up to the eighth stage (CMFRI, 2005) in India.

**SECTION- B**

**MOLECULAR MARKERS**

2B.1. Need for genetic markers

Genetic variation enhances the capability of any species to adapt to changing environment and hence necessary for survival of a species. Patterns of genetic diversity or variation among populations can provide clues to the populations’ life histories and degree of evolutionary isolation. Population genetics can be defined as the science of how genetic variation is distributed among species, populations and individuals, and fundamentally, it is concerned
with how the evolutionary forces of mutation, selection, random genetic drift and migration affect the distribution of genetic variability (Hansen et al., 2007). Several evolutionary forces affect the amount and distribution of genetic variation among populations and thereby population differentiation (Felsenstein, 1985). Geographic distance and physical barriers enhance reproductive isolation by limiting the migration and increase genetic differentiation between populations (Ryman, 2002).

The identification of stock structure has been recognized widely as a prerequisite for sustainable management of marine fisheries (Reiss et al., 2009). Variation within and between populations and stock discrimination within exploited species are important issues for conservation programmes. Identification of non-interbreeding populations is also essential to assess the gene flow between different genetic stocks, and to monitor temporal changes in the gene pools (Carvalho and Hauser, 1994). By characterizing the distribution of genetic variation, population substructuring can be detected and the degree of connectivity among populations estimated (Nesbo et al., 2000; Ruzzante et al., 2000; Hutchinson et al., 2001). Taking into account the influence of present gene flow on the genetic structuring of the species is crucial in order to protect those populations with higher genetic diversity and greater ability to effectively be able to export individuals to other areas (Palumbi, 2004).

Many non-genetic methods of stock discrimination are available to achieve varying degrees of success in distinguishing breeding stocks. With the advent of genetic methods, stock identification based solely upon morphological and meristic differences has become rare. Instead, these data are used in conjunction with genetic data. Molecular markers provide direct assessment of pattern and distribution of genetic variation (Ferguson et al., 1995) thus helping in answering, “If the population is single unit or composed of subunits”. The powerful ability of molecular genetic markers to detect genetic variations when combined with new statistical methods having high analytical power, have revolutionized the genetic diversity studies. Various molecular markers now being used in fisheries and aquaculture provide various scientific observations which have importance in species identification, genetic variation
and population structure study in natural populations, comparison between wild
and hatchery populations, assessment of demographic bottleneck in natural
population when populations experience severe, temporary reduction in size
which influence the distribution of genetic variation within and among
populations and propagation assisted rehabilitation programmes (Chauhan and
Rajiv, 2010)

Molecular tools have become an indispensable part of innumerable
systematic and conservation-based studies (Hillis et al., 1996), providing
information across a large scale of research, ranging from differential heritage
of genes within the same individual (Avise, 2004) to population biology and
species-level relationships (Rubinoff and Sperling, 2002). For conservation
biology specifically, DNA data contribute to research as diverse as fine-scale
management of fish stocks through the assignment of individual fish to one of
several populations in the same watershed or fishery (Hansen et al., 2001;
Ruzzante et al., 2004), to cryptic and invasive species recognition, identification
of appropriate source populations for local reintroduction (Ludwig et al., 2003),
and even tracking the post harvest use of sensitive species through forensic
identification of animal parts (Shivji et al., 2002).

Molecular genetic data have become a standard tool for understanding
the evolutionary history and relationships among species (Avise, 1994). A
critical assumption for phylogenetic analyses is that gene flow among lineages
has been rare (Shaklee and Currens, 2003). The inter-specific genetic
divergence established through species specific diagnostic molecular markers
provides precise knowledge on phylogenetic relationships and also resolve
taxonomic ambiguities. Phylogenetic classification specifically attempts to show
relationships based on reconstructing the evolutionary history of groups or
unique genomic lineages.

2B.2. Molecular markers in use for population and phylogenetic
studies

A molecular marker is a gene with a known location or clear phenotypic
expression that is detected by analytical methods or an identifiable DNA
sequence that facilitates the study of inheritance of a trait or a gene (Okumus and Çiftci, 2003). In the early 1980s, the first population genetic studies based on analysis of mitochondrial DNA emerged (Avise et al., 1979). Later, with the advent of the PCR, a number of different techniques emerged, ranging from sequencing of the DNA of interest to methods analysing length polymorphisms, such as microsatellites.

Molecular markers can be classified into type I and type II markers. Type I markers (e.g. Allozymes) are associated with genes of known function, while type II markers are associated with anonymous genomic regions (O'Brien, 1991). The significance of type I markers is becoming extremely important for aquaculture genetics. Sequence conservation within genes are high, allowing type I markers to serve as anchor points for genomic segments to be compared among species. Expressed Sequence Tags (ESTs), are type I markers that are considered as new generation markers. RAPD markers are type II markers because RAPD bands are amplified from anonymous genomic regions via the polymerase chain reaction (PCR). Microsatellite markers are also type II markers unless they are associated with genes of known function. In general, type II markers such as RAPDs, microsatellites, and AFLPs are considered non-coding and therefore selectively neutral. Such markers have found widespread use in population genetic studies to characterize genetic divergence within and among the populations or species (Brown and Epifanio, 2003). Usefulness of molecular markers can be measured based on their polymorphic information content (PIC, Botstein et al., 1980). PIC refers to the value of a marker for detecting polymorphism in a population.

Several marker types are highly popular in aquaculture genetics which have been subjected to a number of reviews (Liu and Cordes, 2004; Chauhan and Rajiv, 2010 etc.). The most recent approaches to gathering data relevant to fisheries and aquaculture come from direct assessments of nuclear DNA (nDNA) sequence variation (Brown and Epifanio, 2003) which exhibits the greatest variability of all genetic markers. The nuclear DNA markers include random amplified polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), Variable number tandem repeats (VNTRs), Single
Nucleotide Polymorphism (SNP) and nuclear ribosomal DNA markers. RAPD and RFLP are multiple Arbitrary Primer Markers or “anonymous nucDNA markers” used to detect anonymous, or arbitrary, sequences by “multiple arbitrary amplicon profiling”. Main applications in fisheries and aquaculture are phylogenetics and phylogeography, population genetic structure, conservation of biodiversity and effective population size, hybridization and stocking impacts, inbreeding, domestication, quantitative traits, and studies of kinship and behavioural patterns.

2B.2.1. Random Amplified Polymorphic DNA (RAPD)

RAPD procedures first developed in 1990 (Welsh and McClelland, 1990; Williams et al., 1990), utilizes random 10-base oligonucleotides as primers to amplify anonymous segments of nuclear DNA via PCR. RAPD markers are the amplified products of less functional part of the genome that do not strongly respond to selection on the phenotypic level. Such DNA regions may accumulate more nucleotide mutations with potential to assess inter-population genetic differentiation (Mamuris et al., 2002). RAPD can detect high levels of DNA polymorphisms (Williams et al., 1990; Welsh and McClelland, 1990). Because the primers are short and relatively low annealing temperatures (often 36-40 °C) are used, the likelihood of amplifying multiple products is great, with each product (presumably) representing a different locus. Because most of the nuclear genome in vertebrates is non-coding, it is presumed that most of the amplified loci will be selectively neutral. Genetic variation and divergence within and between the taxa of interest are assessed by the presence or absence of each product, which is dictated by changes in the DNA sequence at each locus. The potential power is relatively high for detection of polymorphism; typically, 5-20 bands can be produced using a given primer pair, and multiple sets of random primers can be used to scan the entire genome for differential RAPD bands. Because each band is considered a bi-allelic locus (presence or absence of an amplified product), PIC values for RAPDs fall below those for microsatellites and SNPs, and RAPDs may not be as informative as AFLPs because fewer loci are generated simultaneously. RAPD markers are inherited as Mendelian markers in a dominant fashion and scored as present/absent.
Analysis follows the assumption that populations under study follow Hardy-Weinberg expectations.

RAPDs have all the advantages of a PCR-based marker, with the added benefit that primers are commercially available and do not require prior knowledge of the target DNA sequence or gene organization and a large number of loci and individuals can be screened. The method is simple, rapid and cheap, it has high polymorphism, only a small amount of DNA (~20ng) is required no need for molecular hybridization and most importantly, no prior knowledge of the genetic make-up of the organism in question is required (Hadrys et al., 1992). RAPDs have gained considerable attention particularly in population genetics, species and subspecies identification (Bardakci and Skibinski, 1994), phylogenetics, linkage group identification, chromosome and genome mapping, analysis of interspecific gene flow and hybrid speciation, analysis of mixed genome samples (Hadrys et al., 1992), breeding analysis and as a potential source for single-locus genetic fingerprints (Brown and Epifanio, 2003).

The main drawback with RAPDs is that the resulting pattern of bands is very sensitive to variations in reaction conditions, DNA quality, and the PCR temperature profile (Liu and Cordes, 2004). RAPD markers are subject to low reproducibility due to the low annealing temperature used in the PCR amplification. Extensive standardization is required to get reproducible results. Even if the researcher is able to control the major parameters, other drawbacks of RAPD will remain: homozygous and heterozygous states cannot be differentiated and the patterns are very sensitive to slight changes in amplification conditions, giving problems of reproducibility (Ferguson et al., 1995). Some concerns about the reproducibility of RAPD analysis both within a laboratory and among laboratories remain unresolved. It is suggested that if the overall temperature profiles (especially the annealing temperature) inside the tubes are identical among the laboratories, then RAPD fragments are likely to be reproducible (Penner et al., 1993). Also difficulty is there to determine whether bands represent different loci or alternative alleles of a single locus, so that the number of loci under study can be erroneously assessed. This is
especially true if the RAPD is caused by deletion or insertion within the locus rather than at the primer binding sites. Other shortcomings of this type of marker include the difficulty of demonstrating Mendelian inheritance of the loci. The presence of paralogous PCR product (different DNA regions which have the same lengths and thus appear to be a single locus) limits the use of this marker. These difficulties have limited the application of this marker in fisheries science (Wirgin and Waldman, 1994).

2B.2.1a. RAPD technique in decapod crustacean genetics

RAPD analysis has been used to evaluate genetic diversity for species, subspecies and population/stock identification, taxonomic identity, and systemic details in a wide variety of decapod crustaceans.

Even though RAPD fingerprinting has been used in population genetics of a wide variety of crustaceans, comparatively few investigations are carried out with this marker in the case of lobsters. Population genetic studies in American lobster *Homarus americanus* (Harding *et al*., 1997) and European lobster, *Homarus gammarus* (Ulrich *et al*., 2001) were carried out using RAPD PCR. The technique was used for tissue discrimination of American lobster and European (Hughes and Beaumont, 2004) and species comparisons of Korean slipper lobster (*Ibacus ciliatus*) with Indian Ocean deep sea lobster *Puerulus sewelli* (Park *et al*., 2005). These are the few published works in lobsters with this genetic marker.

RAPD technique was used in the genetic studies of a variety of crustaceans like amphipod *Gammarus locusta* (Costa *et al*., 2004), *Caprella* spp. (Cabezas *et al*., 2010), *Argulus* sp. (Sahoo *et al*., 2011) etc. RAPD-PCR was employed to detect the DNA polymorphism to obtain molecular markers to enable the identification, to assess the phylogenetic relationship and to explore intra and interspecific variation in *Macrobrachium* spp. (Guerra *et al*., 2010). This technique has been used to estimate genetic diversity in penaeid shrimps like *Penaeus monodon* (Garcia and Benzie, 1995; Tassanakajon *et al*., 1998), *Metapenaeus ensis* and *Penaeus japonicus* (Meruane *et al*., 1997; Song *et al*., 1999), *Penaeus chinensis* (Shi *et al*., 1999; Zhuang *et al*., 2001), *Penaeus*
Review of Literature

*stylirostris* (Aubert and Lightner, 2000), *Litopenaeus vannamei* (Freitas et al., 2007), *Metapenaeus dobsoni* (Mishra et al., 2009), *Penaeus semisulcatus* (Niamaimandi et al., 2010), *M. affinis* (Lakra et al., 2010), *Fenneropenaeus indicus* (Rezvani Gilkolaei et al., 2011), atyid shrimp *C. cantonensis* (Yam and Dudgeon, 2005), sergestid shrimp *Acetes japonicus* (Aziz et al., 2010), *Pandalus borealis* (Martinez et al., 2006) and brine shrimp *Artemia* (Sun et al., 1999).

RAPD was used for analysing populations of blue swimming crab *Portunus pelagicus* (Klinbunga et al., 2010) and mud crab *Scylla* spp. (Klinbunga et al., 2000). RAPD variation was surveyed in the freshwater crayfish *Cherax destructor* (Nguyen et al., 2005), *Cherax quadricarinatus* (Macaranas et al., 1995) and Spanish white-clawed crayfish *Austropotamobius pallipes* (Gouin et al., 2001, 2003; Beroiz et al., 2008).

### 2B.2.2. Allozyme studies in decapod crustaceans

Allozyme electrophoresis denotes the technique for identifying genetic variation at the level of enzymes, which are directly encoded by DNA. Allozymes are co-dominant Mendelian characters that are passed from parent to offspring in a predictable manner. They can be used to quantify genetic variation and distinguish among genetic units at the levels of populations, species, and higher taxonomic designations. Disadvantages associated with this type I marker include occasional heterozygote deficiencies due to null (enzymatically inactive) alleles and sensitive to the amount as well as quality of tissue samples. In addition, some changes in DNA sequence are masked at the protein level, reducing the level of detectable variation (Liu and Cordes, 2004).

The amount and pattern of genetic variation and stock structure was assessed by alozyme loci analysis for *Homarus americanus* (Shaklee, 1983; Kornfield and Moran 1990), European lobster *H. gammarus* (Tam and Kornfield, 1996; Jørstad and Farestveit, 1999; Jørstad et al., 2005), *Panulirus marginatus* (Shaklee and Samollows, 1984; Seeb et al., 1990), and Norway lobster *Nephrops norwegicus* (Maltagliati et al., 1998; Stamatis et al., 2006).
Allozyme analysis has been the most commonly used method to determine the levels of variation and genetic structuring for commercially important shrimps species (Garcia-Machado et al., 2001; Barcia et al., 2005; Zitari-Chatti et al., 2008) and crab species (Gomez-Uchida et al., 2003).

2B.2.3. Mitochondrial DNA (mtDNA)

Properties and phylogenetic utilities of the mtDNA

A small portion of (<%1) of the DNA of eukaryotic cells is non-nuclear; it is located within organelles in the cytoplasm called mitochondria. The mtDNA molecule exists in a high copy-number in the mitochondria of cells and has a circular structure (Fig. 2). In decapod crustaceans, the usual size ranges from 14 to 18 kb. Metazoan mtDNAs ordinarily contain 36 or 37 genes as shown in Fig. 2; two for ribosomal RNAs (16SrRNA and 12SrRNA), 22 for tRNAs and 12 or 13 subunits of multimeric proteins of the inner mitochondrial membrane (cytochrome oxidase I-III [COI-III], ATP synthase 6 and 8, NADH dehydrogenase 1-6 and 4L [ND1-6, ND4L], and cytochrome b apoenzyme [Cyt b]). In addition, there is usually at least one sequence of variable length that does not encode any gene (e.g. control region or A+T rich region).

The major features of mtDNA: a) It is in general maternally inherited haploid single molecule; b) the entire genome is transcribed as a unit except for the approximately 1-kb control region (D-loop), where replication and transcription of the molecule is initiated; c) not subject to recombination and provides homologous markers; d) mainly selectively neutral and occurs in multiple copies in each cell; e) replication is continuous, unidirectional and symmetrical without any apparent editing or repair mechanism; and f) optimal size, with no introns present (Billington, 2003).

Studies of vertebrate species generally have shown that sequence divergence accumulates more rapidly in mitochondrial than in nuclear DNA (Brown, 1985). This has been attributed to a faster mutation rate in mtDNA that may result from a lack of recombination during replication, hence low efficiency
of DNA repair mechanisms (Wilson et al., 1985). The mtDNA polymorphism has been used for genetic stock structure analysis because of its rapid evolutionary rate (Avise, 1994). In most species the highly variable mitochondrial DNA (mtDNA) is a good marker for detecting possible genetic differentiation. They are haploid and maternally inherited and consequently are one quarter the effective population size of nuclear genes (Moritz et al., 1987, Birky et al., 1989), thus allowing population level studies and systematic studies among recently diverged taxa. MtDNA evolves at a rate faster than single-copy genes in nuclear DNA which makes this molecule extremely useful for phylogenetic analyses (Brown et al., 1979). Possibly the most important reason to use mitochondrial genes is the availability of universal mtDNA primer sets that have minimized laboratory time in the initial setting up of a project. The high copy number of mitochondria in tissues makes them relatively easy to isolate. Also, there are already extensive sets of nucleotide sequences from these genes in GenBank.

MtDNA has proven useful for identifying major evolutionary lineages (Bernatchez et al., 1992). MtDNA genes have been found to vary considerably
among closely related species, making phylogenetic estimates of recent species radiations possible (Shaw, 2002). Neutral mtDNA markers can provide information about past events, while giving a picture of the overall gene flow between populations (Grant and Waples, 2000). The high mutation rate of mtDNA makes it a useful tool for differentiating between closely related species (Brown et al., 1979), a tool that is especially important when significant variations occur between species, but not within species (Blair et al., 2006; Chow et al., 2006a). MtDNA provided interesting insights into the demographic history of marine populations ('phylogeography'- Avise, 1992; Grant and Waples 2000; Grant and Bowen, 2006).

Different parts of the mitochondrial genome are known to evolve at different rates (Meyer, 1993). Due to the high rate of substitution occurring in the third codon positions (wobble positions) of protein coding genes, the DNA sequences of protein coding genes have frequently been used for species level or population level phylogeny (Navajas et al., 1996). Like nuclear DNA, the genome includes coding and non-coding regions and later evolves much faster than coding regions of DNA (Avise, 1994). The D-loop region of the mtDNA is practically the only noncoding region in the entire mtDNA of vertebrates. The control region of mtDNA, the unassigned region, is hypervariable and there exist variations even between individuals. Thus, this region has been mainly used for phylogenetic studies among species, subspecies, or populations (Zhang and Hewitt, 1997). In general, non-coding segments like the D-loop exhibit elevated levels of variation relative to coding sequences such as the cytochrome b gene, presumably due to reduced functional constraints and relaxed selection pressure. 12SrDNA, however, is highly conserved like the nuclear SSU rDNA, which has been employed to illustrate phylogeny of higher categorical levels such as in phyla or subphyla (Ballard et al., 1992). 16SrDNA is usually used for phylogenetic studies at mid-categorical levels such as in families or rare genera since it is more variable than 12SrDNA (Hwang and Kim, 1999). Compared to the nuclear rDNA, it is more difficult to design universal primers for amplifying specific regions in mtDNA due to a high variability. That is why only a few mitochondrial genes such as 12SrDNA, 16S rDNA, Cytb, ND1 and COI have been employed in phylogenetic studies. In
general, 12S and 16SrDNAs are the most conserved regions among the mitochondrial genes ((Hwang and Kim, 1999). COI is the most conserved among 3 cytochrome oxidase coding genes, and also ND1 among the seven NADH dehydrogenase coding genes. Cyt b is more conserved than ND1 but less than COI (Hwang and Kim, 1999). It implies that the frequency used as gene regions in phylogenetic studies is closely related with the degree of the gene conservation.

Mitochondrial DNA analysis has proven a powerful tool for assessing intraspecific phylogenetic patterns in many animal species (Avise, 1994). Smaller fragments of the mitochondrial genome (D-loop region) have also been targeted by probing or PCR and findings have indicated that it may be best to concentrate on the ‘slow evolving’ coding sequences for species comparisons, and to use the ‘fast evolving’ non-coding regions for population investigations. MtDNA variation can resolve relationships of species that have diverged as long as 8-10 million years before present. Afterwards, sequence divergence is too slow to allow sufficient resolution of divergence times.

Application of mtDNA in animals, including fishes has some major problems as well. The drawbacks of mtDNA in population genetics have been thoroughly discussed by Zhang and Hewitt (2003). The effectiveness of using mtDNA in population-genetic studies has been greatly weakened by the fact that mitochondrial pseudogenes are present in the nuclear genome of a wide range of organisms. In addition mtDNA data on their own have some important limitations. Since mtDNA represents only a single locus (Avise, 1994), we can look only through a single window of evolution. This window reflects at best only the maternal lineal history (Skibinski, 1994). The phylogenies and population structures derived from mtDNA data may not reflect those of the nuclear genome due to gender-biased migration (Birky et al., 1989) or introgression and hence could well differ from that overall of populations or species. Therefore, the inference we make on species/population history is likely to be highly biased and the need for independent, genomic molecular markers to support mtDNA analysis is clear. Second, the effective population size of mtDNA is only a fourth of that of nuclear autosomal sequences; that
means mtDNA lineages have a much faster lineage sorting rate and higher allele extinction rate (Zhang and Hewitt, 2003). In addition, mtDNA markers are subject to the same problems that exist for other DNA-based markers, such as back mutation, parallel substitution, and rate heterogeneity or mutational hot spots (Liu and Cordes, 2004). Although mtDNA loci can exhibit large numbers of alleles per loci and its PIC values higher than those for allozymes it is lower than highly variable nuclear markers such as RAPDs, microsatellites, AFLPs, and SNPs. Use of mtDNA probes and PCR amplification of selected regions have made examination of mtDNA much faster.

2B.2.4. MtDNA in crustacean population genetics

2B.2.4a. MtDNA Cytochrome Oxidase I (COI) gene

Mitochondrial markers have been favored in population genetic studies for several reasons (Wan et al., 2004, Galtier et al., 2009). Mitochondrial DNA is highly variable in natural populations because of its elevated mutation rate, which can generate some signal about population history over short time frames. Variable regions (e.g. the control region) are typically flanked by highly conserved ones (e.g. ribosomal DNA), in which PCR primers can be designed. Clearly, mtDNA is the most convenient and cheapest solution when a new species has to be genetically explored in the wild. Mitochondrial ribosomal genes 12S and 16S, mitochondrial control region (CR) and coding genes such as Cytochrome Oxidase (COI) and Cyt b have been extremely useful in population genetic and systematic studies.

Genetic diversity and population structure of marine species with long-lived larval phase like the lobsters was analyzed using partial sequences of mtDNA COI gene. Nucleotide sequence analysis of mitochondrial COI gene was used to infer population structure of spiny lobsters *Panulirus japonicus* (Inoue et al., 2007; Sekiguchi and Inoue, 2010), *Palinurus elephas* (Cannas et al., 2006; Palero et al., 2008a), *Panulirus regius* (Froufe et al., 2011), *P. argus* (Naro-Maciel et al., 2011), *P. mauritanicus* (Palero et al., 2008a) etc. Genetic connectivity of lobster *Jasus tristani* was studied using the cytochrome oxidase II (COII) gene (Von der Heyden et al., 2007). Partial nucleotide sequences of
mitochondrial DNA COI and 16SrDNA regions were used for population genetics in *P. penicillatus* (Chow et al., 2011).

The population genetics and historical demography were assessed using mitochondrial DNA sequences from portions of the cytochrome c oxidase subunit I (COI) and cytochrome b (Cyt b) genes of the swimming crab *Callinectes bellicosus* (Pfeiler et al., 2005). COI gene was used to examine the population structure in a number of crab species like *Scylla serrata* (Fratini and Vannini, 2002), *Carcinus maenas* (Roman and Palumbi, 2004), *Erimacrus isenbeckii* (Azuma et al., 2008), *Pachygrapsus crassipes* (Cassone and Boulding, 2006), *Epilobocera sinuatifrons* (Cook et al., 2008), *Portunus trituberculatus* (Liu et al., 2009), *Eriocheir* spp. (Zhao et al., 2002) and *Uca annulipes* (Silva et al., 2010).

The population genetics was examined by nucleotide sequence variation in mtDNA COI gene of in the Antarctic krill species *Euphausia crystallorophias* (Jarman et al., 2002), freshwater shrimp *Caridina zebra* (Hurwood and Hughes, 2001) and in many penaeid shrimp species like *Penaeus kerathurus* (Zitari-Chatti et al., 2009), *Fenneropenaeus chinensis* (Quan et al., 2001; Li et al., 2009), *Fenneropenaeus indicus* (De Croos and Palsson, 2010), *Metabetaeus lohena* (Russ et al., 2010) and *Penaeus monodon* (Klinbunga et al., 2001; Khamnamtong et al., 2009).

Levels and patterns of distribution of genetic diversity in crayfish populations were analyzed using mitochondrial COI gene sequences in a number of species like *Austropotamobius pallipes* (Die´Guez-Uribeondo et al., 2008, Stefani et al., 2011), *Cherax quadricarinatus* (Baker et al., 2008), *Austropotamobius italicus* (Zaccara et al., 2005; Matallanas et al., 2011) and *Euastacus* spp. (Ponniah and Hughes, 2006).

Partial sequences of mtDNA COI gene has been used for population structure investigation in a variety of invertebrate organisms like sponge (Whalan et al., 2008), planktonic copepods (Nuwer et al., 2008), may fly (Hughes et al., 2003), giant clam (Kochzius and Nuryanto, 2008), Ark Shell (Cho et al., 2007), octopus (Keskin and Atar, 2011), asteroids (Flowers and
Foltz, 2001; Waters et al., 2004), brittle star (Christensen et al., 2008) and mysids (Remerie et al., 2009) in addition to use in decapod crustaceans.

2B.2.4b. Taxonomic and phylogenetic utility of MtDNA genes

The reasons for the adoption of mtDNA as marker of choice are well-known. Experimentally, mtDNA is relatively easy to amplify because it appears in multiple copies in the cell. Mitochondrial gene content is strongly conserved across animals, with very few duplications, no intron, and very short intergenic regions (Gissi et al., 2008). Because of its relatively high substitution rate, mtDNA has been extensively used as a phylogenetic marker at recent time scales, both for tree building and molecular dating. Mitochondrial ribosomal genes such as 12SrRNA, 16SrRNA and coding genes such as COI have been extremely useful in population genetic and systematic studies in Crustaceans (Tudge and Cunningham, 2002).

2B.2.5. Nuclear DNA genes in crustacean phylogenetics

Tsang et al. (2008) utilized the nuclear protein-coding genes, phosphoenolpyruvate carboxykinase (PEPCK) and sodium–potassium ATPase a-subunit (NaK) for decapod phylogenetics. The evolutionary relationships and divergence ages were estimated for 37 penaeoid genera using nuclear protein-coding genes (Ma et al., 2009). Studies have been undertaken to reconstruct the phylogeny of Palinuridae and its allies using sequences from three nuclear protein-coding genes-phosphoenolpyruvate carboxykinase, sodium–potassium ATPase a-subunit and histone 3 (Tsang et al., 2009). RE digestion of 28S ribosomal DNA was used to differentiates adults or larvae of Panulirus argus, Panulirus guttatus, and P. laevicauda (Silberman and Walsh, 1992). The contribution of the small-subunit 18S ribosomal (r) DNA nuclear gene to crustacean phylogeny is well known and has been useful in investigating relationships across a wide variety of groups (Spears and Abele, 1998; Kim and Abele 1990; Crandall et al., 2000; Morrison et al., 2002; Perez-Losada et al., 2002; Ahyong et al., 2007). The 18s rDNA gene polymorphism of P. homarus has been studied using standard markers in five major fish landing centres of Indian peninsula (Mon et al., 2011). Partial 28S rRNA gene has been
used to construct phylogeny of four spiny lobster species in India (Suresh et al., 2012).

2B.3. DNA barcoding

Species are the principal currency of biodiversity and usually the focal taxonomic unit of conservation biology. The majority of conservation programs and legislation are focused on saving species. Dayrat (2005) clearly expressed, 'delineating species boundaries correctly - and also identifying species - are crucial to the discovery of life’s diversity because it determines whether different individual organisms are members of the same entity or not'. The DNA barcode itself consists of a 648 bp region 58-705 from the 5'-end of the cytochrome c oxidase 1 (COI) gene using the mouse mitochondrial genome as a reference. It is based on the postulate that every species will most likely have a unique DNA barcode and that genetic variation between species exceeds variation within species (Hebert et al., 2003a, b; Hebert et al., 2004).

To make reliable and consistent conservation and fisheries management decisions, accurate, unambiguous, and robust species identifications are needed. DNA barcodes are supposed to increase our ability and efficiency in identifying new species. Specifically, COI as a barcoding tool helps to identify an organism based on DNA sequence variability and assignment to a certain species previously described (Lefe`bure et al., 2006). DNA barcode sequences can be used as a DNA taxonomy tool to perform prediction and classification of potentially new species. However other genes and phylogenetic methods are required to evaluate the evolution information contained in the barcode region of COI (da Silva et al., 2011).

Mitochondrial DNA can be a powerful tool in the effort to identify species, their relationships to each other, and threatened or endangered populations with divergent haplotypes worthy of conservation attention (Moritz 1994, 2002; Avise 2004). DNA barcodes have been proposed as a fast, efficient, and inexpensive technique to catalogue all biodiversity (Hebert et al., 2003b; Stoeckle et al., 2003; Mortiz and Cicero, 2004; Hebert et al., 2004). Sequence variation within species for COI is generally much lower than
sequence variation among species, permitting most unknown samples to be sequenced and allocated correctly to species (Ward et al., 2009). DNA barcoding offers taxonomists the opportunity to greatly expand, and eventually complete, a global inventory of life’s diversity. It will make the Linnaean taxonomic system more accessible assigning specimens to known species. DNA barcoding will play an increasingly important role as a taxonomic screening tool because of its ability to rapidly reveal the genetic discontinuities that ordinarily separate distinct species (Janzen et al., 2005). Based on past results for varied animal groups, DNA barcoding will deliver species-level resolution in 95% to 97% of cases (Hebert et al., 2004; Janzen et al., 2005; Ward et al., 2005).

Despite the potential benefits of DNA barcoding to both the practitioners and users of taxonomy, it has been controversial in some scientific circles (Will and Rubinoff, 2004; Ebach and Holdredge, 2005). Barcode differences appear to accumulate quickly, making it possible to distinguish all but the youngest of sister species. It has also been suggested that, it is undesirable to rely on a single sequence for taxonomic identification (Sites and Crandall, 1997; Mallet and Willmortt, 2003; Matz and Nielsen, 2005). Thus the feasibility of using additional genes, particularly ribosomal RNA genes, as DNA barcodes has also been explored (Blaxter et al., 2004; Chu et al., 2006).

The use of mitochondrial genes encoding ribosomal (12S and 16S) DNA in broad taxonomic analyses is constrained by the prevalence of base insertions and deletions (indels) that complicate sequence alignments (Doyle and Gaut, 2000; Hebert et al., 2003a). Sequence alignment is a major obstacle that limits the effective use of rRNAs for barcode purposes. More recent evidence from the better-studied taxa such as birds and fishes suggests that in most cases barcoding will in fact permit accurate identifications (Kerr et al., 2007; Tavares and Baker, 2008; Ward et al., 2008). This identification tool can clearly give support to improve classifications and to critically examine the precision of morphological traits commonly used in taxonomy (Frézal and Leblois, 2008).
2B.3a. MtDNA genes in decapod barcoding and crustacean phylogenetics

Cytochrome Oxidase subunit I (COI or COX), was recently elected as the standardized tool for molecular taxonomy and identification (Ratnasingham and Hebert, 2007). It was used in distinguishing lobsters *Panulirus femoristriga*, *P. longipes bispinosus* and *P. longipes longipes* (Ravago and Juinio-Meñez, 2003). The genus *Thenus* was barcoded with COI and 16SrRNA genes to identify five distinct species in the previously monotypic subfamily Theninae (Burton and Davie, 2007). COI gene was amplified and sequenced to identify *Thenus* species in Thailand (Iamsuwansuk *et al*., 2012). Nucleotide sequence analysis of the mtDNA COI gene was performed to identify phyllosoma larvae of spiny lobsters of the genus *Panulirus* (Chow *et al*., 2006b). Nucleotide sequence analysis of mitochondrial 16SrDNA identified *Panulirus echinatus* phyllosoma larva in the central Atlantic which was undescribed (Konishi *et al*., 2006).

Partial sequences of mitochondrial DNA genes especially 16SrRNA and COI have proved suitable than other gene sequences to resolve the phylogenetic relationships within the family in several group of eukaryotes. Most of the molecular phylogenetic studies using mtDNA genes on spiny lobsters have focused on species-level relationships within a genus. Partial sequences of mitochondrial 12S and 16S genes were used to infer phylogenetic relationships of the recent clawed lobster genera (Chu *et al*., 2006; Tshudy *et al*., 2009). Using 16S mtDNA, Tam and Kornfield (1998) produced a tree including five nephropid lobster genera. Chu *et al.* (2006) produced a 12S mtDNA-based tree for ten clawed lobster genera using *Neoglyphea* as outgroup. The phylogenetic relationships within the family Palinuridae (among the two species of *Palinurus- Palinurus elephas* and *P. mauritanicus*, most of the species of *Panulirus* and all the species of *Jasus*) were examined using mtDNA COI gene (Cannas *et al*., 2006). The phylogenetic relationships of the extant *Linuparus* species, including the colour forms, were investigated using mitochondrial 12SrRNA and COI gene sequence analysis (Tsoi *et al*., 2011). Sequence data derived from the
mitochondrial DNA 16SrRNA and COI genes were used to determine the phylogenetic relationships among six *Palinurus* spiny lobster species (Groeneveld *et al.*, 2007) and in *Jasus* (Ovenden *et al.*, 1997). Phylogenetic relationships among all described species and four subspecies (total of 21 taxa) of the spiny lobster genus *Panulirus* White, 1847 were examined with nucleotide sequence data from portions of two mitochondrial genes, large-subunit ribosomal RNA (16S) and COI gene by Ptacek *et al.* (2001). Phylogeny of Iranian coastal lobsters was inferred from mitochondrial DNA-COI restriction fragment length polymorphism (Ardalan *et al.*, 2010).

Mitochondrial DNA structure of cray fish *Austropotamobius italicus italicus* was assessed using 16S and COI (Pedraza-Lara *et al.*, 2010). Nucleotide variation and phylogenetic relations within and between four species of freshwater crayfish of the genus *Cherax* was investigated using four fragments amplified from the 16SrRNA, 12SrRNA, Cytochrome Oxidase I (COI), and Cytochrome b (Cyt b) gene regions (Munasinghe *et al.*, 2003). Phylogenetic and phylogeographic relationships in crustaceans like copepods (Dippenar *et al.*, 2009) and crayfish genus *Austropotamobius* inferred from mitochondrial COI gene sequences (Trontelj *et al.*, 2005). The sequence analysis of mtDNA has been considered a useful tool for phylogeny and systematics among closely related crab species (Geller *et al.*, 1997; Schubart *et al.*, 2001; Tang *et al.*, 2003; Imai *et al.*, 2004; Sotelo *et al.*, 2008). The mitochondrial DNA markers including 16SrRNA and cytochrome oxidase I (COI) genes were employed to reconstruct the phylogenetic relationships among *Penaeus* spp. (Palumbi and Benzie 1991; Gusmão *et al.*, 2000; Quan *et al.*, 2004; Lavery *et al.*, 2004; Voloch *et al.*, 2005), squat lobsters (Machordom and Macpherson, 2004) and in achelate lobsters. Similarly, the mitochondrial 16SrDNA gene has been regularly used to investigate decapod relationships and phylogenetic studies in decapods (Ovenden *et al.*, 1997; Kitaura *et al.*, 1998; Crandall *et al.*, 2000; Stillman and Reeb, 2001; Parhi *et al.*, 2008).
Review of Literature

2B.4. Use of concatenated sequence data in phylogeny reconstruction in decapod crustaceans

A solid taxonomy is fundamental to all biology, and phylogenies provide a sound foundation for establishing taxonomy. The choice of marker will have a significant effect on divergence estimates obtained (Carvalho and Hauser, 1999). The highly conserved molecular markers and/or gene regions are useful for investigating phylogenetic relationships at higher categorical levels while the hypervariable molecular markers are useful for elucidating phylogenetic relationships at lower categorical levels or recently diverged branches (Hwang and Kim, 1999). Thus Because different genes may reflect different evolutionary histories (Avise 2004), use of multiple genetic markers is often necessary even for many intra- and interspecific studies to provide an accurate perspective on an organism’s evolutionary history and systematic/ taxonomic relationships (Funk and Omland 2003). Use of nuclear genes in addition to mitochondrial genes adds to the number of independent markers in a dataset, thus increasing the chances of reconstructing the true species phylogeny. In addition, a larger effective population size, and on average, a lower substitution rate (Moriyama and Powell, 1997), results in nuclear genes evolving slower than mitochondrial genes. Consequently, they may be better at resolving deeper phylogenetic nodes (Chu et al., 2009). Hence an integrated approach that uses mtDNA and nuclear DNA, usually in conjunction with morphology and ecology, is better able to access different avenues of inheritance, producing more accurate results that are essential when assessing and managing biodiversity (Rubinoff, 2006).

Most molecular phylogenetic studies of Decapoda have relied heavily on mitochondrial DNA and nuclear ribosomal DNA markers. The former, however, exhibit rapid substitution saturation that limits their utility in resolving deep nodes, whereas the latter suffer from alignment ambiguities. These disadvantages can complicate analysis and hamper accurate recovery of phylogenetic signal (Tsang et al., 2008).
Many studies have shown an increase in resolution when multiple genes are combined in phylogenetic analyses (Ahyong and O’meally 2004; Porter et al., 2005). The nuclear and mtDNA gene sequences have been concatenated to construct phylogeny in a number of animals and also in decapods crustaceans. However, Fisher-Reid and Weins (2011) opined that lower homoplasy of nucDNA characters may outweigh the influence of the larger numbers of variable mtDNA characters and combined-data analyses need not necessarily be dominated by the more variable mtDNA data sets that may lead to widespread discordance between trees from mtDNA and nucDNA. Their results from 14 vertebrate clades showed that combined mtDNA-nucDNA data analyses are not necessarily dominated by the more variable mtDNA data sets.

The concatenated data set of 3139 bp including one mitochondrial gene (16S) and three nuclear genes (18S, 28S, H3) was used to investigate phylogeny in decapoda (Bracken et al., 2009a). The concatenated alignment containing 3398 bp, including the partial 18S, 28S and COI fragments from all four horseshoe crab species yielded an overall stable phylogeny in the combined analysis (Obst et al., 2012). A detailed phylogeny of all Euastacus species was constructed using nucleotide sequence data from the 16SrRNA, 12SrRNA, and cytochrome c oxidase subunit I (COI) mitochondrial gene regions, and from the 28S rRNA gene region of the nuclear genome. The different gene regions were then concatenated for a single data file for subsequent analyses (Shull et al., 2005).

Few workers have conducted DNA-based cladistic analyses on the clawed lobsters. Ahyong and O’Meally (2004) used 16S mtDNA along with 18S and 28S nuclear DNA data (2,500 bp total) to evaluate reptant decapod phylogeny, including six lobster genera. Porter et al. (2005) used 16S mtDNA along with 18S and 28S nuclear DNA data and the histone H3 gene (3,601 bp total) to evaluate decapod phylogeny (43 genera), including four lobster genera.

Patek and Oakley (2003) presented the first attempt to reconstruct the molecular phylogeny of palinurid genera based on morphological characters
and ribosomal DNA evidence (16S, 18S and 28S nuclear and mitochondrial ribosomal RNA gene regions). Different nuclear (18S, 28S, and H3) and mitochondrial (16S and COI) gene regions were sequenced and the concatenated sequence was used to test conflicting hypotheses of evolutionary relationships within the Achelata infraorder and solve the taxonomic disagreements in the group (Palero et al., 2009a). Partial sequences of three mitochondrial genes, the small subunit ribosomal RNA (12SrRNA), the large subunit ribosomal RNA (16SrRNA) and cytochrome c oxidase subunit I (COI) genes, and the nuclear gene histone H3 from all the 17 extant species to reconstruct the phylogeny of the species of Metanephrops (Chan et al., 2009). To ascertain phylogenetic relationships and monophyly patterns in species of the genus Palinurus mitochondrial DNA sequence data and microsatellite markers were used (Palero et al., 2009b). Yang et al. (2012) collected nucleotide sequence data from regions of five different genes (16S, 18S, COI, 28S, H3) to estimate phylogenetic relationships among 54 species of Scyllaridae.

2B.5. Present study

Commericially, lobsters are generally the most highly prized crustaceans in all parts of the world. Among them, spiny lobsters are one of the most commercially important groups of decapod crustaceans (Palero and Abelló, 2007; Follesa et al., 2007) and have received great attention during recent years, including numerous studies on their ecology, phylogeography, and molecular phylogeny (Díaz et al., 2001; Patek and Oakley, 2003; Palero et al., 2008a). However, the slipper and coral lobsters have been the subject of much less research, probably because they do not include many species of commercial interest (Holthuis, 1991; Lavalli and Spanier, 2007).

Majority of the scientific works on lobsters from the Indian seas have concentrated on fishery and stock assessment, growth and culture and breeding. Except a few works like PCR-RFLP of mtDNA COI gene for larval identification of P. homarus (Dharani et al., 2009), study using 18S rDNA gene polymorphism in P. homarus (Mon et al., 2011) and phylogeny construction of
four spiny lobster species using 28SrRNA gene (Suresh et al., 2012), no comprehensive molecular genetic works have been reported on lobsters from India.

The identification of stock structure has been recognized widely as a prerequisite for sustainable management of marine fisheries (Reiss et al., 2009). Variation within and between populations and stock discrimination within exploited species are important issues for conservation programmes. The main aim is to recognize genetic stocks within a species which are largely reproductively isolated from each other. Patterns of genetic diversity or variation among populations can provide clues to the populations’ life histories and degree of evolutionary isolation. The genetic variation can be observed using molecular markers. Population genetic structure investigated using RAPD and hypervariable region of COI gene in *P. homarus homarus* and *T. unimaculatus* which are the most dominant lobster species from the Indian Seas (CMFRI, 2011). The stock structure analysis is especially important in the present context of alarming decline in lobster landings in the country to formulate suitable conservation strategies.

Crustaceans are an interesting target for DNA barcoding because they represent one of the most diverse metazoan groups from a morphological and ecological point of view. Morphological identification of crustaceans can be difficult, time-consuming and very often requires highly trained taxonomists. Previous work on crustaceans found DNA barcoding to be a useful tool for specimen identification in both marine and freshwater species (Bucklin et al., 2007; Costa et al., 2007). Of the 17635 morphologically described freshwater and marine extant species of decapod crustaceans, only 5.4% are represented by COI barcode region sequences (da Silva et al., 2011). DNA barcoding of fish and marine life was initiated in India during 2006 and 115 species of marine fish from the Indian Ocean have been barcoded (Lakra et al., 2011). In this work, the barcodes using mtDNA COI were generated for all lobster species of commercial importance from the Indian EEZ. But closely related species may have identical or nearly identical COI sequences (Scott Harrison, 2004; Lorenz et al., 2005; Brower, 2006). It is also suggested that it is undesirable to rely on
a single sequence for taxonomic identification (Sites and Crandall, 1997; Mallet and Willmott, 2003; Matz and Nielsen, 2005). Thus in the present study, the feasibility of using partial sequences of additional genes like 16SrRNA, 12SrRNA and nuclear 18SrRNA has also been explored. These barcodes will be helpful in accurate species identification of lobster larvae too which is usually difficult by visual examination.

Recent advances in morphological and molecular phylogeny studies have created great impacts on the concepts of the evolutionary relationships of marine lobsters and other Decapoda. Some analyses of the last decades suggest that marine lobsters do not comprise a monophyletic group (Schram and Dixon, 2004; Ahyong and O’Meally, 2004; Porter et al., 2005). These results also showed that the relationships of the superfamilies and families of marine lobsters are mostly different from the previously well-established scheme of Holthuis (1991). The phylogenetic studies also have yielded significantly contrasting results (Tsang et al., 2008; Bracken et al., 2009a; Toon et al., 2009). The latest and by far the most robust phylogenetic analysis (Tsang et al., 2008) utilised newly developed molecular markers, concluding that lobsters are indeed a monophyletic group (Chan, 2010). The present work also aims to reconstruct the phylogeny of eleven species of commercially important lobsters from the Indian EEZ using molecular markers to understand evolutionary relationships.