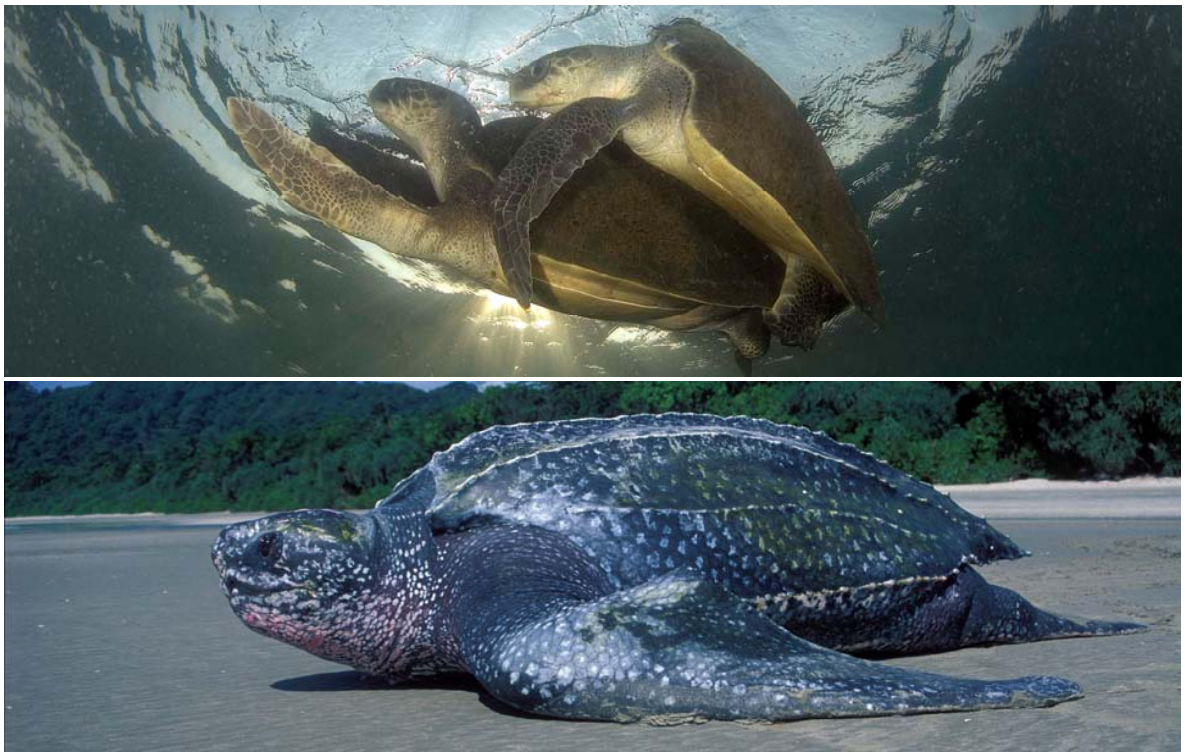
Conservation genetics of marine turtles on the mainland coast of India and offshore island

A WII Grant-in-Aid Project Report



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Project title

Conservation genetics of marine turtles on the mainland coast of India and offshore islands, using mitochondrial DNA sequencing analysis and microsatellite analysis to assess the phylogeography and population structure at various rookeries on the coast

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Cover photo: Sumer Verma (mating olive ridleys) and Kartik Shanker (leatherback turtle)

EXECUTIVE SUMMARY

There are seven species of marine turtles, of which five have global distributions, namely leatherback (*Dermochelys coriacea*), green (*Chelonia mydas*), hawksbill (*Eretmochelys imbricata*), loggerhead (*Caretta caretta*) and olive ridley turtles (*Lepidochelys olivacea*). All five are found in Indian waters. Two other species, the flatback (*Natator depressus*) and the Kemp's ridley (*Lepidochelys kempii*) are restriced to Northern Australia and the Atlantic coast of Mexico, respectively. Four species of sea turtles nest on various parts of the Indian mainland coast and offshore islands. Of these, olive ridley turtles are the most abundant with mass nesting beaches in Gahirmatha, Rushikulya and Devi River mouth in Orissa on the east coast of India. This is one of the few sites in the world where mass nesting (*arribada*) occurs, along with locations on the Pacific coast of Mexico and central America. Our earlier studies demonstrated that olive ridley turtles along the east coast of India represent one large population with no sub-structuring along the coast. More importantly, this population is most closely related to the sister species, the Kemp's ridley and represents a possible ancestral source population for contemporary global populations of olive ridleys both in the Pacific and the Atlantic oceans. The significance of these results led to a more comprehensive effort to ascertain the phylogeography and population genetics of olive ridleys and other marine turtles in Indian waters.

Olive ridley turtles from three nesting sites in Orissa and one in Tamil Nadu (sampled as part of the earlier WII funded CCMB-WII collaborative project), were analyzed using a number of microsatellite markers. The analysis revealed moderate to high level of polymorphism; all the samples exhibited heterozygosity for at least one microsatellite locus analyzed in the study. The allelic diversity data suggests that there is a considerable amount of genetic variation in the population. However, allelic diversity at microsatellite loci as well as the mitochondrial haplotypes were found randomly distributed across the samples, suggesting that there is no genetic differentiation between the populations from different nesting sites and that these represent a single large population along the east coast of India. These observations are concordant with field data that show that olive ridley turtles routinely use more than one nesting beach in Orissa.

For the microsatellite analysis, we initially used cross-species microsatellite markers. Later, novel olive ridley specific microsatellite markers were developed at CCMB using a Pre-cloning SSR-enrichment approach of genomic library construction (developed indigenously in CCMB). The efforts led to development of a number of highly informative olive-ridley specific microsatellite markers for the first time in this species.

More than 300 samples belonging to four species of marine turtles (olive ridley, leatherback, green and hawksbill turtles) were collected from different locations throughout India from 2001 to 2004. Olive ridley turtles were sampled along the east and west coasts of India, and both offshore island groups, and green turtles along the Gujarat and Tamil Nadu coasts, and Lakshadweep Islands. Hawksbill turtles were sampled opportunistically at Lakshadweep. Leatherback turtles were sampled extensively at Great Nicobar Island, at a beach which was washed away during the December 2004 tsunami. This population is one of the largest leatherback populations in the south Asian region.

The samples were processed and total genomic DNA was isolated for mitochondrial DNA sequence analysis. The analysis of the mitochondrial D-loop region of olive ridley turtles revealed that the most basal haplotype K and its derivatives, which are diagnostic of the Orissa olive ridley population, were also found in relatively high proportion in samples from locations along the Indian mainland, but these were not seen in samples from the Islands. Olive ridley samples from the Andaman and Nicobar islands, in contrast to the mainland population, comprised 2 other haplotypes and their derivatives. This is not surprising as the Andaman and Nicobar Islands constitute a different biogeographic region. Many new haplotypes were identified for olive ridleys as well as 3 other marine turtle species; leatherback haplotypes were principally similar to those found in the Indo-Pacific region in previous studies.

Mainly, these findings confirm that the east (and possibly west) coast of India represents the ancestral population of olive ridley turtles. A finer resolution is required to unravel the population genetics of other species of marine turtles in Indian waters. However, the mitochondrial DNA sequence database can serve to identify sea turtles killed in fisheries, and contribute to future studies on population genetics, stock analysis, and mixed stock analysis at foraging grounds.

INTRODUCTION

Sea turtles have been widely studied for over fifty years, but much of the work has remained restricted to the brief period in their life cycle when they come ashore to nest. In recent years, molecular genetic techniques have been used successfully to answer questions in taxonomy and ecology, which conventional field methods had failed to answer (Avise 1989; Avise 1992). Molecular sequence analysis has been used extensively to study marine turtle biology to answer questions regarding their evolution and phylogeny (Avise et al. 1992; Bowen et al. 1993; Fitzsimmons et al. 1995; Dutton et al. 1996; Naro-Maciel et al. 2008).

In particular, mitochondrial DNA analysis has been used to study the global population structure and phylogeography of different species (Bowen et al. 1992a; Bowen et al. 1994; Bowen et al. 1998; Dutton et al. 1999; Bowen and Karl 2007) as well as regional population structure and phylogeography (Bass et al. 1996; Encelada et al. 1996; Encelada et al. 1998; Shanker et al., 2004a; Bjorndal et al. 2005; Dethmers et al., 2006; Bjorndal et al. 2006; Formia et al. 2006; Bourjea et al., 2007). It has also been used to determine the origin, dispersal and migratory routes of populations (Bowen et al. 1995; Bass et al. 1998; Bolten et al. 1998; Naro-Maciel et al. 2007; Godley et al. 2010), stock analyses of feeding areas (Bowen et al. 1992b; Lahanas et al. 1998, Luke et al. 2004; Dutton et al, 2008) and to confirm the natal homing hypotheses for green turtles (*Chelonia mydas*) (Meylan et al. 1990; Allard et al. 1994; Lee et al. 2007).

The comparison of mitochondrial and nuclear gene polymorphisms have helped to identify male biased gene-flow in green turtle populations (Fitzsimmons et al. 1997a, 1997b; Roberts et al. 2004) and the study of microsatellites has been widely used in the analysis of multiple paternity in sea turtle species (Fitzsimmons 1998; Kichler et al. 1999; Zbinden et al. 2007).

Several studies have looked specifically at the conservation implications of genetic data (Chassin-Noria et al. 2004; Shanker et al. 2004a; Bowen et al. 2005; Rivalan et al. 2006). It is in this context that conservation genetic studies were initiated on marine turtles in India. The study species and their distributions are described briefly below.

Olive ridley turtles

Olive ridley turtles (*Lepidochelys olivacea*) are a widely distributed species, and are unique (along with the Kemps ridley, *Lepidochelys kempi*) in the phenomenon of mass nesting. While Kemps ridleys consist of a single nesting population (Carr 1963; Marquez 1994), olive ridleys are considered the most abundant of the sea turtles (Reichart 1993). Nesting aggregates of over 100,000 females have been reported from Pacific Mexico, Pacific Costa Rica and Orissa on the east coast of India (Marquez et al. 1976; Pandav et al. 1994). Molecular genetic studies have shown that olive ridleys on the east coast of India are ancestral to populations in the Atlantic and Pacific Oceans (Shanker et al. 2004a). Despite their abundance and wide distributions, many populations have been greatly depleted by human activities (Ross 1982; Pandav et al. 1998).

Along the Indian coast, the major mass nesting beaches of olive ridleys are Gahirmatha, Devi mouth and Rushikulya on the Orissa coast, of which only Gahirmatha is legally protected (Pandav et al., 1998). However, sporadic nesting has been reported throughout the east coast of India as well as on the west coast and the Andaman and Nicobar Islands (Kar & Bhaskar 1982). All populations are threatened by coastal development, habitat destruction and fishery related mortality, particularly in Orissa, where over 100,000 turtles have been counted dead in the last decade (Shanker & Choudhury 2006). Much of this mortality is attributed to drowning in trawl fishing nets (Pandav 2000) Little is known about the population structure of these marine turtles, nor till recently was there information on the migratory routes or origin of these large nesting populations.

Leatherback turtles

There have been many sightings and strandings of leatherback turtles on the east and west coasts of India, but little, if any nesting is known to occur on these beaches (Shanker and Choudhury 2006). Though once reported to nest in small numbers on the Indian mainland coast, no confirmed nests have been recorded in the last four decades or so. A small number of leatherback turtles nest in southern Sri Lanka (Ekanayake et al.

2002), representing the only significant nesting population in the South Asian region.

The Andaman and Nicobar Islands have the best nesting beaches for leatherback turtles in the region (Andrews and Shanker 2002). Though many populations in the Andamans have declined in the last few decades (Andrews et al. 2006a), there are small nesting populations at West Bay and South Bay of Little Andaman Island which are currently being monitored.

There largest nesting populations occur in the Nicobar group across several nesting beaches on both Great and Little Nicobar Islands (Andrews et al. 2006a). These include Galathea on the east coast of Great Nicobar and Alexandria and Dagmar on the West Coast of Great Nicobar, which received 400 to 500 nests per season before the December 2004 tsunami (Andrews et al. 2006a). Similarly, beaches in Little Nicobar likely received more than a hundred nests each (Manish Chandi pers. Comm.). All the beaches in the Nicobar were destroyed by the December 2004 tsunami (Andrews et al. 2006b), but the beaches have been gradually forming again, and nesting has been reported (Naveen Namboothri, pers. comm.).

Hawksbill and green turtles

A few juvenile hawksbill and green turtles have been sighted on the mainland coast of India. Hawksbill nesting occurs primarily in the Andaman Islands (Bhaskar 1996) and foraging areas occur in the Lakshadweep as well as Andaman and Nicobar Islands (Tripathy et al. 2006; Andrews et al. 2006a). Green turtles nest in Gujarat (Sunderraj et al. 2006), Lakshadweep (Tripathy et al. 2006) and Andaman and Nicobar Islands (Andrews et al. 2006a), and forage extensively in both island groups. In particular, large numbers of green turtles are known to forage in the lagoons of some islands in the Lakshadweep (Tripathy et al. 2006; Lal et al. 2010). In these islands, green turtle densities have been found to substantially alter seagrass meadows, leading to conflict with local fisher communities (Lal et al. 2010). Few, if any, loggerheads are seen in Indian waters, and many records may represent misidentification (Frazier 1985; Tripathy 2005).

Threats

Sea turtles are affected by a variety of anthropogenic threats including direct threats such as fishery related mortality, depredation of eggs by humans and animals, predation of hatchlings by feral animals, and take of adults for consumption in a few areas (see Shanker and Choudhury, 2006 for an account of threats in different parts of the country). Sea turtle populations are also affected by a range of indirect threats such as coastal development and exotic plantations, climate change and pollution. Coastal development can affect sea turtle populations by destroying nesting habitat, beach lighting which disorients hatchlings (Karnad et al. 2009) and by changing thermal profiles. Since hatchling sex is determined by temperature (Mrosovsky and Pieau 1991), changes in thermal profiles due to development or climate change can significantly impact populations. On the east coast of India, fishery related mortality has been a significant threat over the last two decades with over 100,000 – 150,000 dead turtles washed ashore during the last 15 years of documented record (Shanker et al, 2004b; Wright and Mohanty, 2006; B. Mohanty, unpubl. Data).

Molecular genetic studies

It is necessary to understand the population structure and behavioural ecology of these turtles in order to frame appropriate conservation strategies and devise plans for the management of populations. Molecular genetic analysis can often complement information obtained by intensive long-term field studies to achieve the objectives of effective conservation and management. Our earlier studies have suggested that the olive ridleys along the east coast of India represent: a) one large population with non-significant sub-structuring along the coast, and more importantly, b) a possible ancestral source population for re-colonization of contemporary global populations of olive ridleys both in the Pacific and the Atlantic oceans. The significance of these results justified a more comprehensive effort to ascertain the phylogeography and population genetics of olive ridley turtles and other marine turtle species in Indian waters. To this end, this study was initiated with samples from all along the Indian coastline including the Lakshadweep, and Andaman and Nicobar Islands.

OBJECTIVES

The objectives of the study were:

- Study of the phylogeography of marine turtles in Indian waters using mitochondrial DNA sequencing analysis
- Study of the population genetics of the olive ridley turtles the mainland coast of India and offshore islands using microsatellite analysis
- Study of multiple paternity of olive ridley turtles on the east coast using microsatellite analysis

METHODS

Study Area

Olive ridleys are found all along the Indian coastline, but nest mainly along the east coast of India and on the Andaman and Nicobar islands. While nesting is sporadic along most of the coast, there are at least three major mass nesting beaches in Orissa on the east coast, i.e., Gahirmatha (located near Dhamra, 21°N - 87° E, part of the Bhitarkanika Wildlife sanctuary at the mouth of the river Maipura), Devi river Mouth (20°N - 86°E, located north of Puri) and Rushikulya (19°N - 85°E, southern most mass nesting rookery). In addition, sporadic nesting occurs along the east coast in Andhra Pradesh, Tamil Nadu, as well as in the Andaman and Nicobar islands. Lower density nesting occurs along the entire west coast of India, as well as the Lakshadweep Islands.

For the study, the samples comprised the samples collected under the earlier WII supported CCMB-WII collaborative study (concluded in 2000), and a large number of new samples that were collected from the mainland coast of India (Tamil Nadu, Andhra Pradesh, Gujarat), as well as the offshore islands (of Andamans & Nicobars, and Lakshadweep).

More than 300 samples belonging to four species of marine turtles (olive ridley, leatherback, green and hawksbill turtles) were collected from different locations throughout India from 2001 to 2004. Olive ridley turtles were sampled along the east west coasts of India, and both offshore island groups, and green turtles along the Gujarat and Tamil Nadu coasts on the mainland, and Lakshadweep Islands. Hawksbill turtles were sampled opportunistically at Lakshadweep. Leatherback turtles were sampled extensively at Great Nicobar Island, at a beach which was washed away during the December 2004 tsunami. This population is one of the largest leatherback populations in the south Asian region. Details of the samples analysed in the study (belonging to 4 marine turtle species), alongwith the locations in Indian waters from where the same were collected are shown in Figure-1 and Table-1, 2.

Field sampling

Permits were obtained for sampling from the Ministry of Environment and Forests, and State Forest Departments. Field sampling was carried out on the mainland coast by visiting various sites in Gujarat, Tamil Nadu and Andhra Pradesh. In the Lakshadweep, sampling was carried out during field visits, and as part of an ongoing WII project on status survey of sea turtles.

For the Andaman and Nicobar Islands, a camp was established on Great Nicobar Island at Galathea beach by the Andaman and Nicobar Environmental Team, as part of a a long term monitoring programme. Samples were collected at this beach from Novenmber 2001 to March 2002. In addition, leatherback samples were also collected from Kophen Heat on the west coast of Great Nicobar Island. These nesting beaches were destroyed during the December 2004 tsunami, but may currently be recovering.

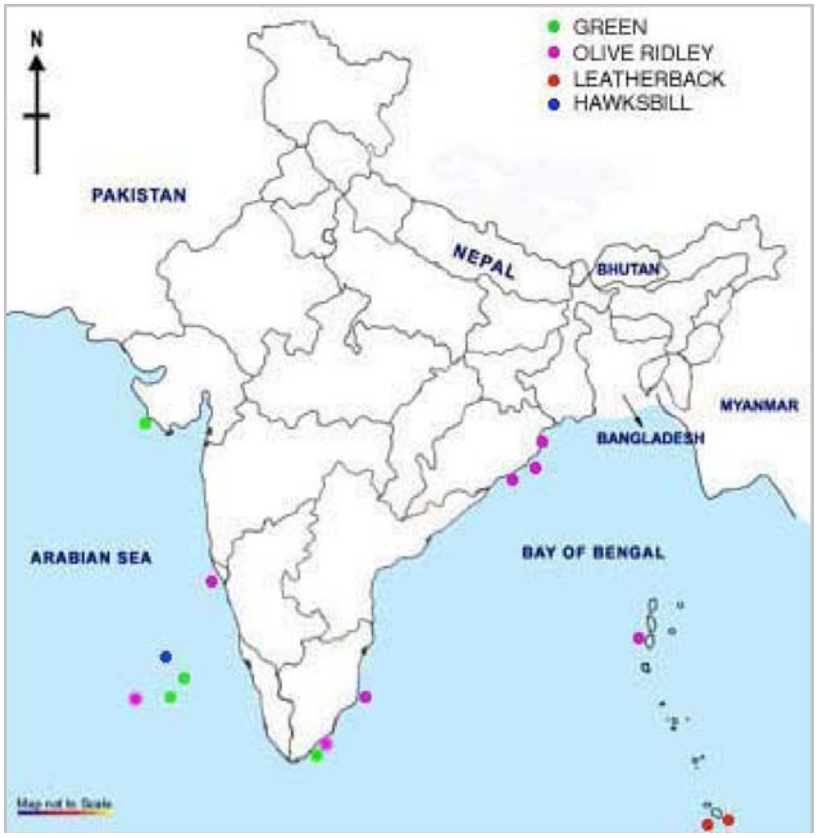


Figure 1: Map showing the locations in Indian waters from where the samples (belonging to 4 marine turtle species) were collected.

Table 1: Details of samples of olive ridley turtles used for microsatellite studies to infer population genetics/structure (collected off the east coast of Orissa, India during the earlier WII funded CCMB-WII project concluded successfully in 2000).

Sample sites	Sample type	Sample size	DNA
Gahirmatha	Mating pair - male	13	10
	Mating pair - female	12	9
Gahirmatha - Nasi 1	Nesting female	19	10
Gahirmatha -Nasi 2	Nesting female	32	25
Devi River mouth	Nesting female	14	14
Rushikulya	Nesting female	17	17
Chennai	Hatchling	51	15

Table 2: Details of samples related to four martine turtle species collected during the project period from different locations off the maincoast of India and offshore islands (used for d-loop haplotyping).

Turtle Species	Samples collected	No. of samples used for DNA isolation	No. of bad samples [PCR negative]	No. of working samples	Collection site
	November 2001 to March 2002				
Leatherback	139	139	25	114	GN
Olive ridley	71	71	1	70	GN, LK, TN
Green turtle	39	37	3	34	LK, TN, GJ
Hawksbill	8	7	2	5	LK
	January - March 2003				
Multiple species	51	51	15	36	AN, AP
	AN59b-AN 69b; AN 198-218; AN 247-266; AN 350; AP 01-04				
	JanuaryFebruary 2004				
Olive ridley	47	31 (16 of 31 hatchlings belonging to the same clutch)	13	18	Goa; Kerala
Green turtle	45	30 (26 hatchlings belonging to 2 clutches+ 4 adults)	nil	30	G; TN
Total	400	366	59	307	
AN: Andaman& Nicobar Islands; GN: GreatNicobar; LK: Lakshadweep;					
GJ: Gujarat; TN: Tamil Nadu; AP: Andhra Pradesh					

Collection and storage of samples

Details of the samples collected from the coast of Orissa (offshore Gahirmatha) are provided in the earlier project report of WII, Dehradun (Shanker et al 2000). In addition to these, new samples were collected from different locations during 2001-2004. A small aliquot of blood and/or soft tissue was collected from each animal, as detailed below.

Blood was collected from the cervical sinus of adults and hatchlings (Owens and Ruiz, 1980). In general, 0.5 to 1ml of blood was collected from adults using a 2ml disposable syringe with a 22-gauge needle. A few drops of blood were collected from hatchlings using a disposable insulin syringe (plate 2b). Blood samples were stored in lysis buffer (Bowen et al., 1992) comprimisng 100 mM Tris-HCl (ph 8.0), 100 mM EDTA (pH 8.0), 10 mM NaCl, 1-2 % SDS. The dilution of blood to buffer was 1: 1 for adults, and approximately 1: 20 for hatchlings.

Tissue samples (skin and muscle) were collected from the shoulder of adults and hatchlings and stored in 75 - 90% ethanol and/or more preferably in a NaCl saturated buffer solution SED (saturated NaCl salt, 250 mM EDTA, pH 7.5, and 20% DMSO). This solution is inexpensive, non-flammable, and nontoxic, and thus has lots of advantages over alcohol. Blood and tissue samples were brought to CCMB at room temperature, and later used for isolation of DNA and molecular analysis.

Molecular genetic analysis

All samples were processed for DNA isolation. The DNA samples from the Orissa coast, representing the three main nesting sites, were used for microsatellite analysis to elucidate the population structure using published as well as CCMB developed markers. Samples collected from all other locations were used for mitochondrial d-loop haplotyping (by amplification followed by DNA sequencing) to provide insights about the phylogeography of the marine turtles in Indian waters. In addition, to carry out the microsatellite analysis, CCMB supported the development of olive ridley specific microsatellite markers; to this end, a few olive ridley samples were also used to prepare a small-insert, partial SSR-enriched genomic library.

DNA Isolation

Standard laboratory techniques were followed (Maniatis et al., 1982). DNA was extracted from tissue samples using the proteinase-K digestion approach. For this purpose, tissue samples were transferred into a clean conical flask and washed with saline for 2-3 hours with constant stirring. Then these tissues were transferred on a clean tissue paper and all moisture was removed. This step was essential for samples that were stored in ethanol. Tissues were transferred to a petri dish, minced with a clean razor blade (to 2 mm or less in cross section), and ground in liquid nitrogen into fine powder. The powdered tissue samples were mixed with 150 µl of lysis buffer (500 mM Tris, 20 mM EDTA, 10 mM NaCl, pH 9.0) and 10 ul of 2 % SDS in 1.5-ml centrifuge tubes. To each of the homogenates, 5 µl of 20 mg/ml of proteinase K was added, and the tubes were kept for digestion at 37° C. After every six hours, the homogenates were supplemented with additional (5 µl of 20 mg/ml) protienase K to ensure complete protein digestion. Afterwards, an equal volume of phenol (Tris saturated) was added to each sample. The tubes were inverted several times to mix and then centrifuged at 10,000 rpm for 10 minutes. The aqueous phase from each sample was removed with pipette and placed in clean 1.5 ml tubes. The organic extraction of digested protein impurities was repeated twice again with equal volumes of Phenol–Chloroform-Isoamyl alcohol (25:24:1), each time separating the aqueous phase from the centrifuged extract by pipette into a clean 1.5 ml tube. A final extraction of the aqueous phase was made with one volume of chloroform and centrifuged as before. The aqueous phase from each sample was transferred to a new tube and 1/10 volume of 3 M sodium acetate (pH 5.5) solution added.

The DNA was precipitated from the purified aqueous phase by adding two volumes of absolute alcohol and by keeping at - 20 °C overnight. Each sample was centrifuged at 5,000 rpm for 10 minutes and the supernatant carefully poured off, leaving a DNA pellet. The pellets were washed twice with 80 % ethanol and air-dried. The pellets were resuspended in 0.1 x TE solution (10 mM Tris-HCl, 0.1 mM EDTA, pH 7.2). The DNA samples thus prepared were treated with RNase (final concentration of 20 µg/ml) at room temperature for 1 hour; DNA was once again extracted with equal volume of chloroform Isoamyl alcohol (24:1) followed by centrifugation at 10000 rpm for 10 minutes. The

aqueous phase of the solution was collected carefully and directly stored at – 20 °C as stock DNA solution. The stock DNA was manually quantified on a 0.8 % agarose gel. Depending on the quantity of the stock, further dilutions were carried out for a working stock (with DNA concentration in the range of 5 ng/ul) and stored at 4 °C till further use.

In order to isolate DNA from blood samples, blood was washed out of the storage vials with an equal volume of lysis buffer and SDS (final concentration of 1-2 %) into a glass homogenizer. The homogenate was incubated at 37 °C for 4 h in presence of 150 µg/ml of protenase K and 2% SDS. After incubation, lysate was extracted first with tris-HCl saturated phenol, followed by two extractions with phenol: chloroform: isoamyl alcohol (25: 24: 1), and finally with chloroform. The separated aqueous phase was then collected and the DNA was precipitated with 1/10 vol of 3M sodium acetate (pH 5.5) and 0.8 - 1.0 vol of ice-chilled iso-propanol or 2.0 vol of ethanol. Precipitated DNA was then washed twice with 80% ethanol, air-dried and finally dissolved in TE buffer (10mM Tris-HCl, 1mM EDTA, pH 7.2).

Development of new olive-ridley specific microsatellite markers

For the above, an SSR-enriched genomic library was constructed following a modified protocol based on the methods of Bloor et al. (2001) and Edwards et al. (2000). Approximately 10 mg of total genomic DNA of olive ridley, isolated from blood, was digested with RsaI and HaeIII restriction endonucleases and fractionated on a 1.5 % agarose gel. Fragments of 0.5 - 1.5 kb were gel-eluted and ligated with MluI adaptors (Edwards et al. 2000). The adaptor-ligated SSR-rich DNA fragments were selected by hybridization to biotinylated oligonucleotides [(GA)15, (CA)15, (AGA)10, (CAA)10] and capturing with streptavidin conjugated magnetic beads (Dynabeads, DYNAL, Netherlands). SSR-enriched DNA fragments were cloned into pMOS vector and transformed into competent *E. coli* DH5a cells. Plasmids were isolated from 50 random clones using alkaline-lysis method (Sambrook et al. 1989). Cloned inserts were amplified and sequenced using M13 universal primers and Bigdye terminator sequencing kit on ABI-PRISM 3700 automated DNA sequencer (Applied Biosystems, USA) for both strands. Sequences having SSR motifs were identified and primers were designed and synthesized from sequences

that had >18 bp long repeat regions, using the program GENETOOL version 1.0 (http://www.doubletwist.com) and DNA/RNA Synthesizer-394 (Applied Biosystems, USA). All primer pairs were used to standardize the PCR-amplification conditions and the utility of the working pairs as genetic microsatellite markers was tested on a panel of olive ridley turtle samples and a few on the three other marine turtle species (leatherback, green and hawksbill turtles).

Microsatellite analysis

A total of 22 unmapped microsatellite loci, eight from the published literature and fourteen newly developed markers, were employed for ascertaining the allelic diversity in the populations samples representing the nesting sites on the Orissa coast. All the primers were synthesized CCMB on the DNA oligosynthesizer-384 (Applied Biosystems, USA). For the fluorescence-based detection of SSR polymorphisms, the 5'end of each of the forward primers was labelled with one of the flurophore FAM, TERT or HEX (Applied Biosystems, USA).

The microsatellite allelic diversity was detected by cyclic amplifications (PCR) of target motifs using SSR-specific primer-pairs on the thermocycler model PE9600 (Perkin Elmer, USA). Each PCR reaction was carried out using 5 ng of template DNA in 15 µL volume, and it comprised: 1 x PCR-buffer-II, 100 mM dNTP (equimolar mixture of the four deoxy¬nucleotides), 2 mM MgCl2, 2 pM of each of the forward and reverse primers and 1 U of AmpliTaq Gold polymerase (Perkin-Elmer, USA). The thremocycling conditions were: 95 °C/ 10 min, 94 °C/ 1 min, 55 °C/ 45 sec, 72 °C/ 2 min, for 30 cycles, and a final extension at 72 °C for 5 min. From the amplified product, 1 µL for each sample was saved in a separate tube for the GeneScan analysis and the rest was mixed with the loading dye and electrophoresed on 2.5 percent tris-acetate agarose gels to check for the success of amplification and also to visualize the SSLP at a gross level. When found satisfactory, the saved samples were treated with de-ionized formamide, loading dye containing the fluorescent internal molecular marker standard TAMRA 500 (PE-AB, USA) and loaded on to six percent denaturing long-ranger polyacrylamide gel as per the instructions in the

documentation of the GeneScan ABI Prism 370 (Applied Biosystems, USA). The microsattelite electrophoretograms were acquired and analyzed by the GeneScan software version 3.1 (Applied Biosystems, USA) and the size of the individual bands determined using the Genotyper version 2.1 (Applied Biosystems, USA).

The intra- and inter-location genetic variability coefficients were calculated over all the primer-pairs, based on the presence/absence (1/0) matrix. The resulting similarity matrix was used for phenetic clustering using the 'unweighted pair group based on arithmetic averages (UPGMA)' method, as well as, the principal component analysis (PCA) using the software NTSYS-pc version 2.02j (Applied Biostatics Inc., USA) and PHYLIP version 3.6 (Felsenstein 1989). The principal component analysis (PCA) was performed using the EIGEN option of ORDINATION routine (NTSYS-pc) and graphed employing the Statistica V 4.0 software. The matrix based on size of the alleles across the samples was subjected to genetic analysis using the software ARLEQUIN ver3.01 (http://cmpg.unibe.ch/software/arlequin3; Schneider et al. 2000) that implements a model-based clustering method for inferring population genetics/structure using genotypic data. Arlequin can be used to extract information on genetic and demographic features of a population (F-statistics, observed/expected heterozygosity, deviations from Hardy-Weinberg Equilibrium, Linkage Disequilibrium, Gene flow etc.).

Mitochondrial D-loop haplotyping/ DNA sequencing analysis

Approximately 350 bp sequence of the mitochondrial d-loop region was amplified and then sequenced using turtle specific primers (Allard 1994; Norman et al., 1994) for all the samples. In each case, the target mitochondrial sequence was PCR amplified using standard protocols on PE9600 thermocycler. The PCR reactions were set using approximately 5 ng of template genomic DNA in 20 µl reaction volume containing: 2 pico-moles of each primer, 150 µM dNTP, 1.5 mM MgCl2, 0.1 M KCl, 20 mM Tris-HCl, and 0.5 to 1.0 U of AmpliTaq Gold polymerase (Perkin Elmer). In general, PCR profile comprised an initial denaturation of 5 min at 95°C, followed by 35 cycles of: 94°C for 1 min., 50°C for 1 min., 72°C for 1 min.; and 72°C for 7 min. The PCR products were quantified on 1.5% agarose gel.

The amplified PCR products (~100 ng) were sequenced for both strands using the fluorescence-labelled BigDye Terminator ready reaction chemistry (Perkin Elmer) on the ABI-3700 Automated DNA sequencer. In order to be confident about the sequences, about 10 % of the samples were sequenced more then 3 to 4 times. Sequencing PCR cycle conditions were: 30 cycles of 96°C for 10 sec, 50°C for 5 sec and 60°C for 4 min. Extended products were purified by alcohol precipitation followed by washing with 70 % ethanol. The processed samples were then dissolved in loading dye and sequenced using automated DNA sequencer ABI Prism3700. Sequences were edited using the auto-assembler software package.

The EMBL-Genbank database (http://www.ncbi.nlm.nih.gov) was searched for reference turtle d-loop sequences for use in comparative analysis. Initially, the sequences of the samples analyzed in the study, were aligned using the CLUSTAL-X program (Thompson et al., 1994) alongwith the retrieved reference d-loop turtle sequences. The alignment was checked manually for gaps, and flushed at the ends to avoid missing information for any compared reference entries. The resulting alignment were used to infer phylogenetic relationships using the neighbor-joining method (Saitou and Nei, 1987) with analytical routines available in the software packages PHYLIP 3.6 (Felsenstein, 1989) (http://evolution.genetics.washington.edy/phylip.html) and/or MEGA2 (Kumar et al., 2001), and median-joining network trees using the program NETWORK (http://www.fluxus-engineering.com/netin_ie2.htm).

RESULTS

Isolation of DNA

All the new samples were processed for DNA isolation, and DNA was extracted from 366 samples. Fifty-nine of these samples were found to be of poor quality, as they either did not any amplify or resulted in poor quality/erratic results (Table-2). Therefore, these were not considered for further analysis. The remaining 309 samples, along with 83 samples from nesting populations on the Orissa coast (collected as part of the previous WII-CCMB study), were used for further molecular genetic analysis.

Development of new olive ridley specific microsatellite markers

The small-insert partial SSR-enriched genomic DNA library of the olive ridley turtle comprised ~350 recombinant clones. The amplification/sequencing of about 100 randomly selected clones revealed a large number of SSR-positive non-redundant sequences of which 24 sequences with >18 bp long repeat regions were used to design the primer pairs. Fourteen of these 24 primer pairs were validated as highly polymorphic and informative microsatellite markers useful for genetic studies on olive ridley turtles as well as other marine turtle species tested in the study. The details of six of these new markers – locus designation, repeat motifs, primer sequences, allele attributes, PIC estimates and Genebank accession numbers – are summarized in Table 3. Cross-species amplification is shown in Table 4. Interestingly, some markers such as OR-3 that were monomorphic in olive ridley turtles were found to be highly polymorphic in other sea turtle species.

In this study, these fourteen markers were used, along with eight other cross-species markers from published literature, to ascertain the genetic diversity across 83 individuals representing different nesting populations of olive ridley turtles along east coast of India to gain insights about their population structure.

Microsatellite analysis of olive ridley populations on the east coast of Orissa

The conservation of olive ridley turtles (*Lepidochelys olivacea*) on the east coast of India, particularly Orissa, where the major mass nesting sites are found, has been a matter of great concern due to large scale mortality of sea turtles, and the possible decline of these populations (Shanker et al, 2004b; Shanker and Choudhury 2006).

As part of this project, the genetic structure of the turtle populations along the east coast of India was studied. Olive ridley sea turtles were sampled as part of our earlier collaborative project from four major nesting sites, three in Orissa and one in Tamil Nadu. These were analysed using a total of 22 markers that comprised fourteen newly developed olive ridley specific markers, and eight cross-species microsatellite markers (Table-5).

Out of the 22 markers tested, ten markers (five newly developed olive ridley specific markers and five cross-species markers) were found to be monomorphic/fixed in the analysed samples. A moderate to very high level of polymorphism was observed for the remaining 12 markers among the 80+ individuals from four sites. All the samples exhibited heterozygosity for at least one microsatellite locus analysed in the study.

For the polymorphic markers, the number of alleles varied from 3 to 35. A maximum of 35 alleles was observed for the marker Ei8, followed by 18- 25 alleles for the new markers OR-1, OR-4, OR-11, and OR-14. A minimum of four alleles was seen for the cross-species marker KLK-315. Representative profiles of microsatellite allelic diversity/ distribution pattern observed across olive ridley samples from the four nesting populations are shown for one such marker in Figure 2.

The observed microsatellite diversity was further analysed to ascertain different genetic estimates of population differentiation. Significantly, all such estimates including population average pair-wise differences (Table 6), genetic distance coefficients (Table 7), and Hardy-Weinberg Equilibrium (Table 8) show that the allelic diversity at microsatellite loci was randomly distributed across the samples. These results strongly suggest that there is no genetic differentiation between the populations from different nesting sites.

Microsatellite analysis for ascertaining multiple paternity

We also attempted microsatellite analysis of some samples to address the issue of multiple paternity. To this end, samples of related hatchlings from 5 different clutches belonging to olive ridley, green and leatherback turtles were analysed using the six most polymorphic microsatellite markers. Data analysis has not been completed.

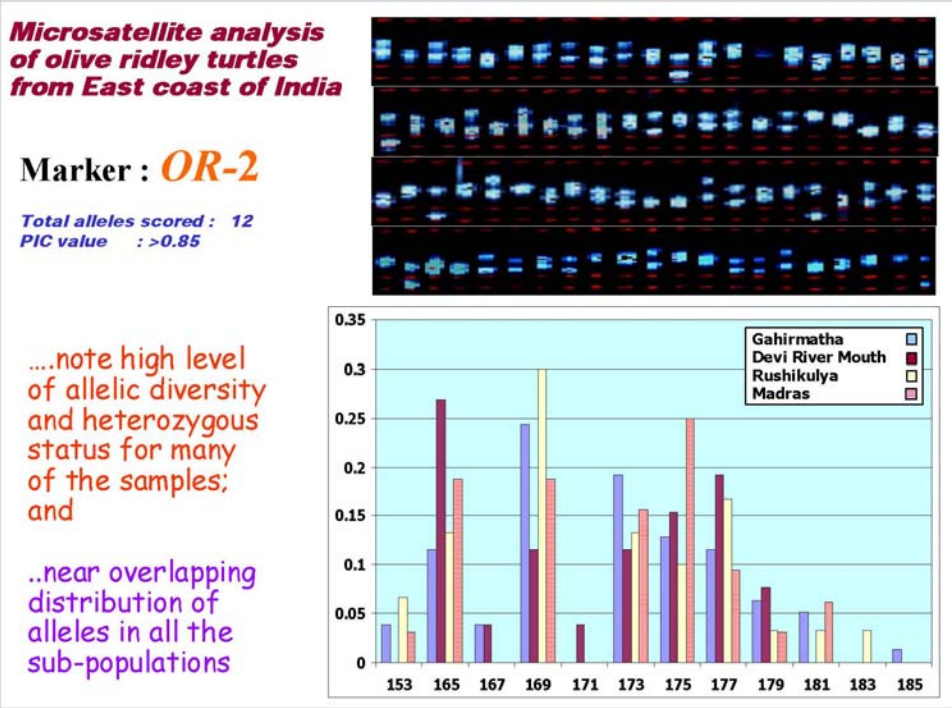


Figure 2: Microsatellite allelic diversity seen at the locus OR-2 for olive ridley samples from the four nesting sites off east coast of India

Table 3: Details of the microsatellite markers developed in the study

Locus	Repeat motif	Primer sequence(5' - 3')	Taga	Ta (°C)	Size range (bp)b	N	NA	Ho	He	PIC	GenBank Accession
OR-1	(CAAA)16	F: CCCCTTGTGTTCTGAAATCCTATGA R: CAGGCATAGGGAAAAATCAGAGGTA	FAM	55	150-202 [160, 162]	83	24	0.71	0.94	0.92	AY325422
OR-2	(GT)8GCC(GT)5	F: GCTCCTGCATCACTATTTCCTGTT R: TGCTGCCCCCACACCCTCTG	FAM	55	153-185 [169]	83	12	0.86	0.85	0.84	AY325423
OR-3*	(TC)9(AC)6GC(AC)2	F: TTGTTTTATTTTTATTGGTCATTTCAG R: GCACCTTTTCACGTTGTCCACATGT	FAM	55	146	83	1	0	0		AY325424
OR-4	(TG)9..(TG)23	F: AGGCACACTAACAGAGAACTTGG R: GGGACCCTAAAATACCACAAGACA	HEX	52	123-172 [128, 157]	83	18	0.72	0.91	0.92	AY325425
OR-7	(GT)6(GA)7	F: GGGTTAGATATAGGAGGTGCTTGATGT R: TCAGGATTAGCCAACAAGAGCAAAA	FAM	55	185-219 [187]	83	16	0.68	0.85	0.84	AY325427
OR-8	(TC)23	F: GCACTGGTGGGAAAATATTGTTGT R: GCTGGGCTAATAAAATGTTGTGCA	FAM	55	148-166 [148, 154]	83	8	0.93	0.78	0.76	AY325428

[a] :Flourescencelabel at 5`-end; Ta:Locus specific annealing temperature; Size range - Figures in paraenthesis are the most frequent allele(s); N: Number of samples analyzed; NA : Number of alleles; Ho: Observedheterozygosity; He:Expectedheterozygosity

*** :** Loci was foundto be polymorphic with Green turtle, Leatherback and Hawksbill turtle species

Table 4: Cross-species amplification status of olive ridley turtle specific microsatellite markers developed in the study.

Turtle Species	*Dermochelys coriacea* (Leatherback)			*Chelonia mydas* (Green Turtle)			*Eretmochelys imbricata* (Hawksbill)		
Locus	(N = 6)	NA	Ta / Mg++	(N = 6)	NA	Ta / Mg++	(N = 6)	NA	Ta / Mg++
OR-1	±		±	Monomorphic		550C / 2 mM	Monomorphic		570C / 2 mM
OR-2	Polymorphic	3	550C / 1.5 mM	Polymorphic	7	550C / 2 mM	Polymorphic	5	550C / 1.5 mM
OR-3	Monomorphic	1	550C / 1.5 mM	Polymorphic	4	550C / 2 mM	Polymorphic	3	550C / 1.5 mM
OR-4	±		±	Polymorphic	3	520C / 2 mM	Polymorphic	2	520C / 2 mM
OR-7	Monomorphic	1	570C / 2 mM	Polymorphic	3	550C / 2 mM	Polymorphic	4	570C / 1.5 mM
OR-8	Polymorphic	6	550C / 1.5 mM	Polymorphic	5	550C / 2 mM	Polymorphic	6	570C / 1.5 mM

N : Number of samples analyzed NA : Number of alleles Ta : Locus specific annealing temperature Mg^{++} : Magnesium concentration in the PCR reaction

Table 5:Details of the microsatellite markers used for ascertaining allelic diversity in olive ridleys sampled from the east coast of India off the Orissa coast.

S.No	STR marker	Source turtle Species	Repeat motif	Tm (0C)	MgCl2 (mM)	Allele Size (bp)	Polymorphism status	Source
1	OR-1	*L. olivaceae*	(CAAA)17	55	1.5	186	Polymorphic	CCMB
2	OR-2	*L. olivaceae*	(GT)8 GC (GT)5	55	1.5	165	Polymorphic	CCMB
3	OR-3	*L. olivaceae*	(TC)9 (AC)6 GC (AC)2	55	1.5	146	Monomorphic	CCMB
4	OR-4	*L. olivaceae*	(TG)9 ------ (TG)23	52	1.5	140	Polymorphic	CCMB
5	OR-6	*L. olivaceae*	(GT)5 GA (GT)7 (GA)7	55	1.5	120	Monomorphic	CCMB
6	OR-7	*L. olivaceae*	(GT)5(GA)7	55	1.5	187	Polymorphic	CCMB
7	OR-8	*L. olivaceae*	(TC)23	55	1.5	162	Polymorphic	CCMB
8	OR-9	*L. olivaceae*	(GA)16	55	2	150-174	Polymorphic	CCMB
9	OR-10	*L. olivaceae*	(GA)5GT(GA)5	55	2	160	Monomorphic	CCMB
10	OR-11	*L. olivaceae*	(GA)22	55	2	194-242	Polymorphic	CCMB
11	OR-12	*L. olivaceae*	(GT)8(GA)5GC(GA)5GC (GA)4	55	2	100	Monomorphic	CCMB
12	OR-14	*L. olivaceae*	(GT)5....(GT)14(GA)12	55	2	147-201	Polymorphic	CCMB
13	OR-16	*L. olivaceae*	(GT)5(GA)7	55	2	220-252	Polymorphic	CCMB
14	OR-17	*L. olivaceae*	(CA)7C(CA)6	55	2	234	Monomorphic	CCMB
15	Nigra32			55	1.8		Monomorphic	
16	Klk315	*Lepidochelyskempi*		55	2.5	135	Polymorphic	Kilc he r et al (1999)
17	Pe344	*Podocnemis expansa*	(AG)13	50	2	144-208	Monomorphic	Va le nzue la (2000)
18	Cc176	*Carettacaretta*		55	1.5		Polymorphic	M o o re e t a l (2002)
19	Ccm2	*Caretta caretta*		55	2		Monomorphic	M o o re e t a l (2002)
20	Cm84	*Chelonia mydas*	(CA)15	55	2.5	348-354	Polymorphic	Fitzs im m o ns e t a l (1995)
21	Cm3	*Chelonia mydas*	(CA)13	55	1.5	169-187	Polymorphic	As above
22	Ei8	*Eretmochelys imbricata*	(CA)19	55	1.5	192-254	Polymorphic	As above

Table 6: Inter-/intra- Population average pair-wise differences between the four nesting sites off the east coast of India based on the observed microsatellite diversity.

Above diagonal : **Average number of pairwise differences between populations (PiXY)**

Diagonal elements : **Average number of pairwise differences within population (PiX)**

Below diagonal : **Corrected average pairwise difference (PiXY-(PiX+PiY)/2)**

	1	2	3	4
Gahirmatha	10.06886	9.98389	10.02342	9.87247
Devi River mouth	0.04176	9.81538	9.82179	9.82091
Rushikulliya	0.12577	0.05088	9.72644	9.83750
Madras	0.01344	0.08862	0.14968	9.64919

Table 7: Inter-/intra- genetic estimates (F-statistics) for the four nesting sites off the east coast of India based on the observed microsatellite diversity

(Kimura 2-P distance estimates)

	1	2	3	4
Gahirmatha	0.00000			
Devi River Mouth	0.00384	0.00000		
Rushikulya	0.01212	-0.00521	0.00000	
Madras	0.00097	0.00909	0.01523	0.00000

FST P values

Number of permutations : 1023

	1	2	3	4
Gahirmatha	*			
Devi River Mouth	0.20721+-0.0490	*		
Rushikulya	0.00000+-0.0000	0.18018+-0.0359	*	
Madras	0.38739+-0.0408	0.09009+-0.0303	0.02703+-0.0194	*

Table 8: Inter-/intra- Population HWE estimates for the four nesting sites off the east coast of India based on the observed microsatellite diversity**.**

	Gahirmatha		Devi River		Rushikulya		Madras		Overall sites	
Locus/Site	Obs Het	Exp Het	Obs Het	Exp Het	Obs Het	Exp Het	Obs Het	Exp Het	Obs Het	Exp Het
OR-1	0.783	0.929	0.615	0.914	0.533	0.938	0.750	0.927	0.711	0.935
OR-2	0.891	0.863	0.769	0.865	1.000	0.857	0.750	0.859	0.855	0.858
OR-3	0.846	0.870	0.462	0.892	0.467	0.834	0.688	0.881	0.687	0.852
OR-4	0.729	0.920	0.846	0.923	0.666	0.935	0.687	0.933	0.732	0.927
OR-7	0.837	0.868	0.461	0.892	0.466	0.834	0.687	0.881	0.612	0.868
OR-8	0.891	0.825	1.000	0.720	1.000	0.737	0.937	0.806	0.957	0.772
OR-9	0.297	0.727	0.307	0.800	0.400	0.905	0.187	0.709	0.297	0.785
OR-11	0.918	0.947	0.916	0.956	0.666	0.928	0.187	0.935	0.671	0.941
OR-14	0.756	0.947	0.692	0.907	0.785	0.960	0.666	0.940	0.724	0.938
OR-16	0.783	0.869	0.500	0.902	0.733	0.834	0.800	0.836	0.704	0.860
Ei-8	0.810	0.921	1.000	0.929	0.667	0.855	0.750	0.827	0.807	0.893
CM-84	0.513	0.745	0.308	0.803	0.400	0.724	0.625	0.863	0.470	0.752
KLK-315	0.594	0.577	0.769	0.640	0.333	0.543	0.500	0.516	0.554	0.565

Phylogeography of marine turtles in Indian waters as revealed by d-loop haplotype analysis

In our earlier study, based on the mitochondrial DNA haplotype analysis, we showed that the olive ridley population on the east coast of India is panmictic, but distinct from all other populations including Sri Lanka (Shanker et al 2004a). About 96 % of the Indian population consisted of a distinct 'K' clade with many new haplotypes not found in any other population. Nested clade analysis and conventional analysis both supported range expansions and/or long distance colonisation from the Indian Ocean clades to other oceanic basins, which suggested that these are the ancestral source for contemporary global populations of olive ridley turtles. These data support the distinctiveness of the Indian Ocean ridleys, suggesting that conservation prioritization should be based on appropriate data and not solely on species designations (Shanker et al 2004a).

In the current study, we extended the analysis of olive ridley turtles to the rest of the Indian coast including the offshore islands. In total, 119 additional samples were used for d-loop haplotype analysis. Significantly, the analysis revealed that olive ridleys all along the mainland coast of India share the same haplotypes (K and K derivatives), whereas, in the Andaman and Nicobar islands, a large proportion of samples show the haplotype J (which is most basal, after haplotype K, to olive ridley haplotypes across other global basins) or its variants (Figure 3). Further, a large number of new haplotypes were documented, all immediate derived variants of haplotype J. Thus, our data suggest that while the east (and perhaps west) coast of India represents the ancestral population of ridleys, the diversification and dispersal of ridleys contributing to the global populations might include Indian Ocean waters including Sri Lanka and the Andaman and Nicobar Islands.

Similarly, d-loop analysis was carried out for 62 samples of green turtles collected from three locations (Tamil Nadu, Gujarat, Lakshadweep), 131 samples of leatherback turtles (Figure 4) and four samples of hawksbill turtle collected from the Lakshadweep Islands. The preliminary analysis of these data revealed a few new unreported haplotypes, but no significant differences from populations known in adjoining global waters. All leatherback haplotypes were either global haplotypes or were previously found in the Indo-Pacific region.

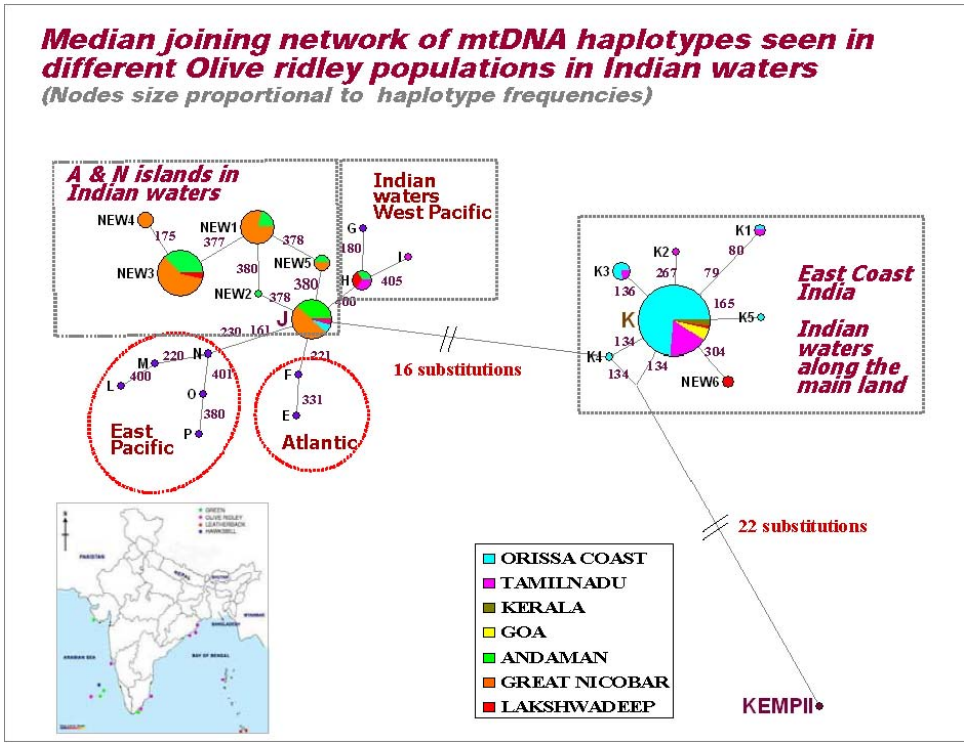
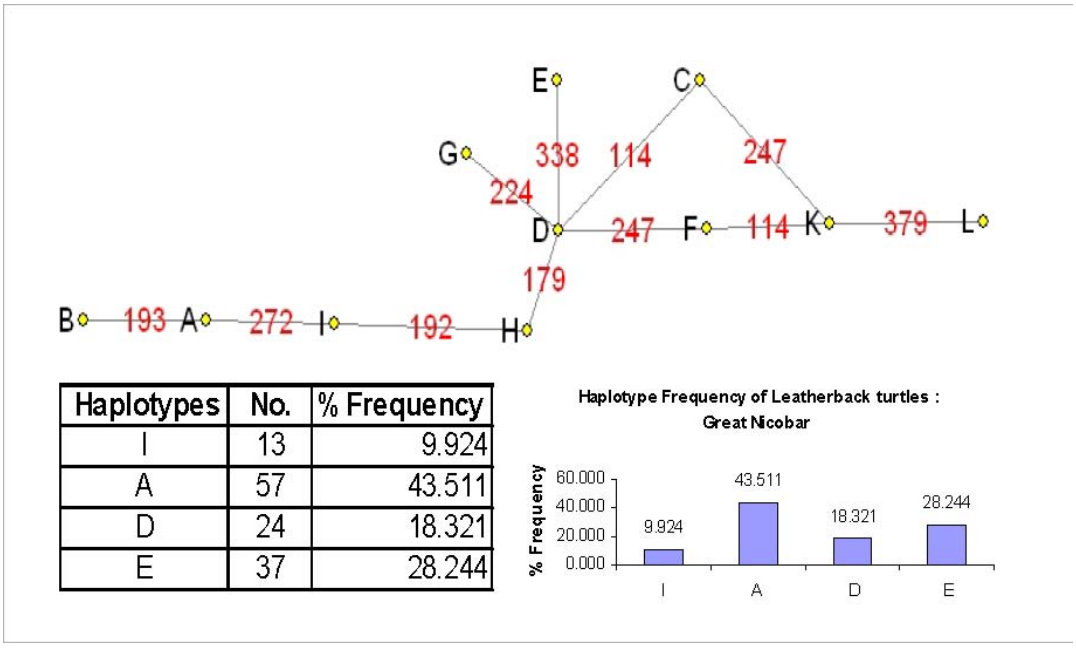


Figure 3: Median-joining network of mitochondrial d-loop haplotypes seen in olive ridley populations in Indian waters.



Haplotypes	No.	% Frequency
I	13	9.924
A	57	43.511
D	24	18.321
E	37	28.244

Figure 4: Median-joining network, and frequency distribution of mitochondrial d-loop haplotypes seen in leatherback turtles in Indian waters.

DISCUSSION

Olive ridley turtles

While olive ridley turtles nest on both coasts of the Indian mainland, the larger nesting populations are on the east coast, with major mass nesting sites in Orissa. Since the late 1990s, mass nesting has occurred primarily at Gahirmatha and Rushikulya (Shanker et al., 2004b). Results from both mitochondrial DNA as well as microsatellite analysis showed no population structure suggesting that these probably represent a single large population along the east coast of India. These results provide support to field data that olive ridley turtles routinely use more than one nesting beach in Orissa. Moreover, the results suggest that natal homing may not be as precise or significant as it is in some other sea turtle species and populations. The latter inference has important implications in the management of ridley populations on the Indian coast and elsewhere.

However, the results also show that the populations along the east and west coasts are not differentiated. This bears further enquiry as the two coasts represent different marine systems with different current patterns, and interchange between these two areas (but not Sri Lanka) presents an interesting biogoegraphic challenge.

Recent results from satellite telemetry studies of olive ridley turtles on the Orissa coast show that these turtles primarily use the offshore waters of Orissa, while some migrate southwards to feed in the Gulf of Mannar region and offshore waters of Sri Lanka (WII, unpubl. Data). Hence, the populations on the east coast appear to represent an ecological and management unit for conservation. The current data show that the Andaman and Nicobar ridley populations are distinct and need to managed as a separate unit, likely along with other populations of olive ridleys in Southeast Asia.

Given that the threats along the east coast are very distinct (mainly fisheries related mortality and coastal development), strategies can be framed for this population as a management unit. On the other hand, the Andaman and Nicobar populations need to be managed for loss of nesting beaches and possible mortality in feeding areas.

Leatherback turtles

The leatherback turtle populations in the Andaman and Nicobar Islands are the most significant in the region. The leatherback nesting season commences in the islands in November each year and goes on till April of the following year. Prior to the 2004 tsunami, the total number of nests in Galathea beach for each of the 4 seasons recorded was 424 in 2000-2001, 297 in 2001-02, 164 in 2002-03 and 592 in 2003-04 (K. Shanker et al. in prep.; Madras Crocodile Bank Trust, unpubl. Data). Since 2007, monitoring has been carried out in Little Andaman Island by the Andaman Nicobar Environmental Team and the Centre for Ecological Sciences, Indian Institute of Science, Bangalore (Swaminathan et al. 2011). While nesting is fairly low on South Bay, results from briefy surveys in previous years and monitoring in 2011 reveal that about 100 nests are laid each season on West Nau (Swaminathan et al. 2011). Satellite tracking of three adult females showed that the turtles moved in a southeasterly direction along the coast of Sumatra and towards Australian waters (K. Shanker et al. unpubl. Data). A brief survey of Great Nicobar Island showed that the nesting beach at Galathea had formed again, and over 200 tracks were counted (N. Namboothri unpubl. Data).

The leatherback turtle populations on Little Andaman and Little and Great Nicobar Islands are clearly significant populations for the region. While they do not show genetic divergence from the Indo-Pacific clade, they represent a large part of the Indian Ocean populations. Located on remote beaches, some of these populations may be easiest to conserve and manage in the long-term, if appropriate policy is designed and implemented.

Green and Hawksbill turtles

Few samples of hawksbill turtles were collected, and were not sufficient to gain insights into the population genetics or phylogeography of the species. Green turtle haplotypes from Gujarat were similar to those from the northern Indian Ocean region, and those from Lakshadweep were insufficient to carry out a mixed stock analysis.

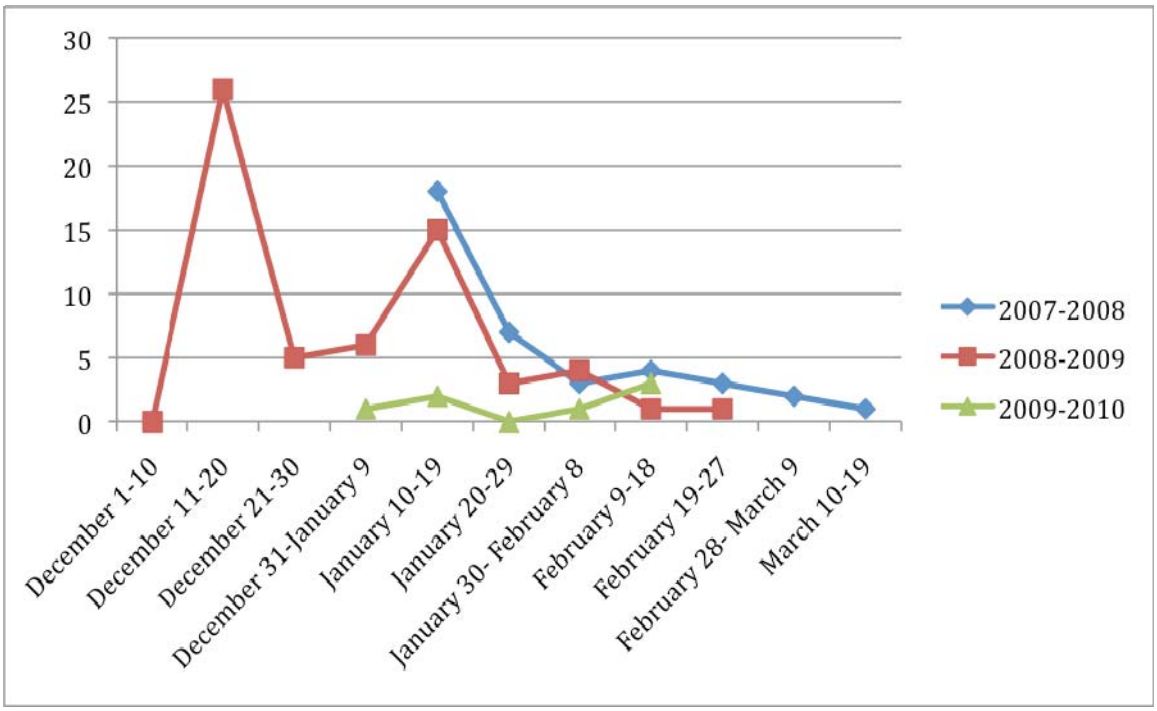


Figure 5a. Leatherback turtle nesting patterns (December-March) in Little Andaman Island, Andaman and Nicobar Islands

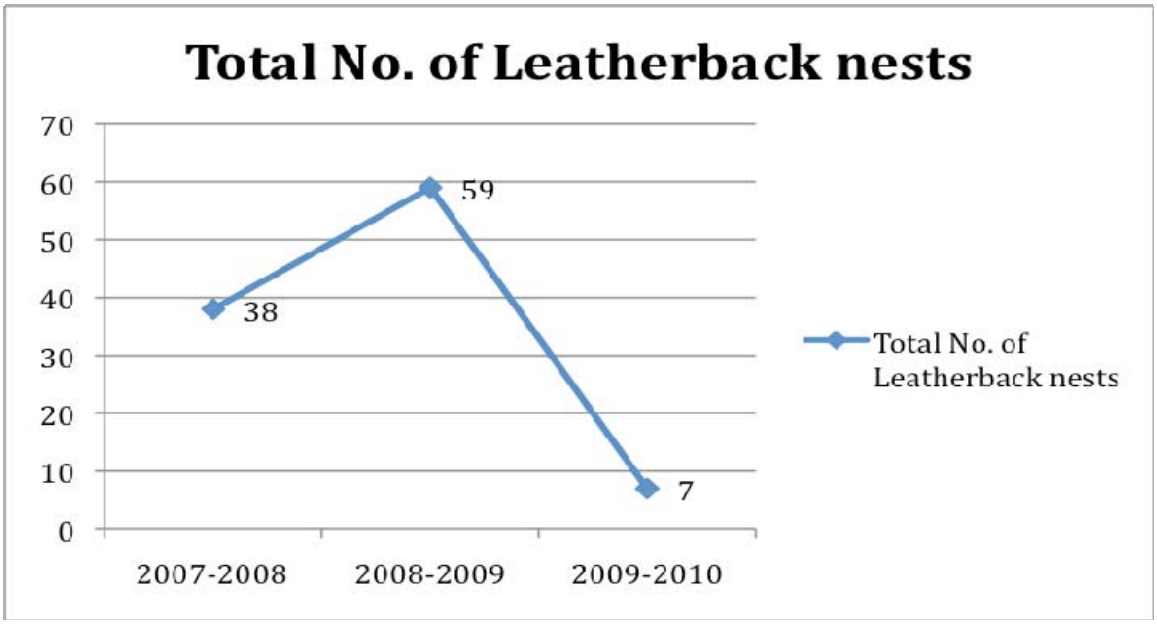


Fig. 5b. Nesting trend of leatherback turtles over the three years of observation at South Bay, Little Andaman Island, Andaman and Nicobar Islands

CONSERVATION IMPLICATIONS AND RECOMMENDATIONS

1. The conservation of olive ridley turtles on the east coast of India, particularly Orissa, is one of the key conservation challenges. In recent years, nesting appears to be shifting from Gahirmatha to Rushikulya, This needs to be monitored both for research and management, particularly because of the climate change and possibly related beach erosion and other coastal geomorphological changes. Since these sites have been shown to be part of the same large nesting populations, it is possible that nesting beaches act as patches in a metapopulation with periodic extinctions and colonisations. In such a framework, it is critical that a large number of patches must remain suitable for possible future colonization. What this means for sea turtles is that beaches that are currently not used (for nesting or mass nesting eg. Devi) must not be abandoned to coastal development for these represent future nesting sites for ridley turtles.

Samples should also be collected from feeding populations in the Gulf of Mannar and Sri Lanka to confirm if these populations are derived from the east coast of India alone or whether there are other nesting beaches from which they derive. This would help frame management strategies as well as as trans-boundary initiatives for conservation.

2. Leatherback turtles in the Andaman and Nicobar Islands are not genetically distinct, but represent a significant part of the Indian Ocean population. Since the beaches in the Nicobars have formed after the tsunami, it is necessary to undertake a comparison of the current and pre-tsunami nesting population. This can be carried out with a more detailed population genetic analysis of pre-tsunami samples with current samples.

Detailed population genetic analysis with new methods and markers needs to be carried out to identify unique molecular markers for these populations, in order to connect them to areas where they may feed or are threatened by fisheries interactions. Given that leatherback turtles may forage in distant locations, where fishing on the high seas occurs, this information could valuable for management in the future.

3. Lakshadweep is a significant foraging area for green turtles. These populations have a signficant impact on the sea grass meadows in some islands, and modify these ecosystems substantially. Local fishers believe that these turtle deplete the sea grass leading to a reduction in their fish catch. Large densities of green turtles also result in direct impacts such as the damage of fishing nets. Over the years, this has evolved into conflict between turtles and fisher communities.

A mixed stock analysis needs to be carried out to determine the nesting beaches that this foraging population is derived from. This would provide insights into the increase in green turtle populations on some islands and whether these increases are caused by internal movement or by recruitment from nesting beaches.

4. Future conservation genetic studies can address ecological and evolutionary questions including finer resolution population genetic analysis and mixed stock analysis. A range of behavioural questions relating to dispersal, homing, migration and multiple paternity can also be addressed. These questions can now be addressed using a range of new tools such as mitogenomics and single nucleotide polymorphisms (SNPs).

With an increasing emphasis on development, as well as the ever pervasive threat of climate change and its impacts on both rising sea levels (and consequent impact on nesting habitat) as well as its impact on incubation temperatures, sea turtle populations are going to need carefully designed conservation and management plans. While much of the anthropogenic impact may need to be addressed by social change and policy, a knowledge of stocks, connectivity, migratory routes, and movement can only improve decision making. Tools such as genetics and satellite telemetry also have the capacity to provide information in a form that is appealing and can attract public interest and participation in conservation. A combination of scientific techniques (field observations, tagging, genetics, stable isotope analysis and satellite telemetry) is required to fully understand the biology of these animals and provide the knowledge and involvement that is required for their long term conservation.

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