"IDENTIFICATION OF THE POPULATION GENETIC STRUCTURE OF *CARCHARHINUS LONGIMANUS* (OCEANIC WHITE TIP SHARK OR BROWN MILBERT'S SHARK) USING MITOCHONDRIAL DNA MARKERS."

By

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CERTIFICATE

Certified that this thesis entitled **"Identification of the population genetic structure of *Carcharhinus longimanus* (Oceanic white tip shark or Brown milbert's shark) using mitochondrial DNA markers**" is a record of research work done independently by **Ms. Sreelekshmi S.** (2014-09-123) under my guidance and supervision and that this has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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List of Abbreviations

AFLP	Amplified Fragment Length Polymorphism
CBOL	Consortium of Barcode of Life
CCRF	Code of Conduct for Responsible Fisheries.
CITES	Convention on International Trade on Endangered Species.
CMFRI	Central Marine Fisheries Research Institute
COI	Cytochrome Oxidase I
D –Loop	Displacement Loop
DNA	Deoxyribo nucleic acid
EEZ	Exclusive Economic Zone
EST	Expressed Sequence Tags
FAO	Food and Agricultural Organization
FISH-BOL	FISH- Barcode of Life
IBOL	International Barcode of Life
IPOA	International Plan of Action
ITS	Internal Transcribed Spacer
IUCN	International Union for Conservation of Nation
MtDNA	Mitochondrial DNA
RAPD	Random Amplified Polymorphic DNA
RFLP	Random Fragment Length Polymorphism
RuBisCo	Ribulose-1,5-bisphosphate carboxylase oxygenase
SNP	Single Nucleotide Polymorphism
VNTRs	Variable Number of Tandem Repeats

INTRODUCTION

1. INTRODUCTION

Regardless of an overall dissemination, developing proper protection plans and some pervasive human interest in sharks, shockingly very little we focus on the life history of elasmobranchs. Little data is accessible about the breeding behaviour and population genetic structure of the elasmobranch species. Among these, sharks generally displays moderate developmental pattern by producing few young ones and displaying a long inter-birth intervals. This creates a developing concern with respect to decrease of the shark species (Manire & Gruber 1990; Musick *et al.*, 2000). Even though they are the biggest gatherings in fisheries they are incredibly helpless against overexploitation and have low population flexibility to over angling. The International Union for Conservation of Nature (IUCN) assessed the current status of most taxa and records the situation over a thousand types of elasmobranchs in fisheries (Camhi *et al.*, 1998, IUCN). The most surpassing migratory sharks are among the species with mostly elevated protection concerns (Dulvy *et al.*, 2008).

In recent times, *Carcharhinus longimanus* (oceanic whitetip sharks) have been focused in the conservation studies in the oceanic regions after serious decreases in the wealth. Oceanic whitetip sharks are the tropical pelagic predators which are inadequately examined in contrast with numerous other enormous sharks. Truly assembled with *C. falciformis* and *Prionace glauca*, *C. longimanus* was found as a common pelagic maritime shark. A few investigations have indicated considerable population decreases in the case of oceanic whitetip sharks, in all likelihood identified with mortality related with the worldwide shark finning. This species is currently recorded as **"Critically Endangered"** in the Northwest Atlantic and **"Vulnerable"** throughout the series, all-inclusive by IUCN Red-list data. This develops a worldwide enthusiasm for improving the protection of this species, including an

ineffective proposition by the United States by adding them to Appendix II of the Convention on International Trade in Endangered Species (CITES) in 2010. Many programs and plans are being initiated to recover and protect the groups through sustainable management plans (Sembiring *et al.*, 2015). However, Several International organizations have banned the landings of these species in worldwide. Even though this shark is widely distributed along the Indian Ocean region, knowledge regarding its intra-specific diversity and population structure is scanty. The genetic stock structure of Atlantic populations of *C. longimanus* has been studied extensively and two genetically distinct populations have been recorded (Camargo *et al.*, 2016).

Genetic markers have been developed for deriving the population hereditary structure in the huge versatile marine life forms (Baker *et al.*, 1990; Amos *et al.*, 1993; Berube *et al.*, 1998), yet several data related to the genetic stock structure information regarding shark populations have been not available properly. Both the nuclear and mitochondrial markers have been used for the genetic structure analysis among them the mtDNA markers, the mitochondrial control region (D loop) examination is usually utilized technique to discover the hereditary stock divergence and fluctuation among species in their particular spatial locales (Clarke *et al.*, 2016). In demographic analysis, estimation of the haplotypic and nucleotype diversity of the populations, genetic divergences in the population and species population size through mismatch distribution analysis which may give information about the possible presence of species subpopulation and its present condition in those regions.

Overexploitation of these species is due to the absence of legitimate execution of the laws in few areas of Indian Ocean. This will inquire concerning current situation of the species and its loss of evolutionary lineages in these areas. Globally sharks are in danger due to their inherent vulnerabilities like long gestation time and reduced number of offsprings

coupled with over fishing and habitat degradation. Our study also corroborated the findings of shark decline, as decline in genetic diversity is an indicator of decrease in resilience capacity. The present study calls for restrictions on its fishery so that populations will get sufficient time to replenish and consequently their resilience is ensured in the face of changing oceans by sustaining the species from the point of extinction. Accordingly, this study helps to produce some management plans and preservation strategies to protect the distinguished species stocks in those areas with the following objectives:

1. To identify the population genetic structure of *Carcharhinus longimanus* (oceanic white tip shark) using mitochondrial DNA markers.
2. To identify patterns of intra-specific genetic diversity, gene flow and connectivity among oceanic white tip sharks of Indian Ocean.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1Biodiversity; Marine world

Biodiversity is normally a proportion of variety at the hereditary, species, and biological system level. Terrestrial biodiversity is typically closer to the equator, which has a warm atmosphere and high productivity. Biodiversity isn't appropriated equitably on Earth, and is most extravagant in the tropics. The fear of diminution of biodiversity are unevenly distributed and may cause detrimental effects to the ecosystem and all our living organisms, so prioritization is very much needed to slow down the biodiversity loss. (Mittermeier and Fonseca, 2006).The dispersal of biodiversity over the Earth can be depicted similarly as a reasonably unassuming number of expansive scale spatial examples. Despite the fact that these patterns are progressively very much reported to understand why they exist and comprises an intellectual challenges to scientists and bio geographers. Biogeographic classification is necessary for developing the ecologically delegate protected areas. Among them Marine spaces are still terribly underrepresented in the worldwide ensured zones to organize heavy protection (nearly 0.5% surface area of the entire ocean is presently as protected, (Chape *et al.*, 2005). Bio-geographers adds some critical and powerful tools to scaling up of the marine ecosystem preservation strategies and the key thought aims to ensure a full scope protection to the biodiversity around the world especially the marine world as in the species and genus level or even in higher taxa (Spalding *et.al.*, 2007).

2.2 Marine Life; Fishes

There are descriptions of an estimated 33,059 valid species of fish

known from around the world (Eschmeyer and Fong, 2014). They live in all conceivable aquatic habitats and exhibit huge diversity in of size, shape, biology and habitat (Asia, 1997). Accurate identification of both adult and larvae of fishes is very important for various reasons including food security, conservation and environmental controls. There are clearly defined criteria for morphological identification of fishes that help to identify both larval and adult fishes (Taylor, 2016). There is also a need to develop strategies for the identification of eggs and larvae (Rago, 1984).

Correct identification of fishes and their larval stages are important for various fields of research such as migration studies, phylogenetic analysis and prevention of illegal trade. Traditionally, for fish identification, morphological characters such as body shape, pigmentation, and measurements are used. But these characters are not enough to identify every species accurately especially rare or cryptic species (Carr *et al.*, 1999; Gharrett *et al.*, 2001; Hebert *et al.*, 2003; Spies *et al.*, 2006) and the eggs and larvae (Pegg *et al.*, 2006; Richardson *et al.*, 2007; Saitoh *et al.*, 2009). Morphological identification relies on specific features in adult fishes that may have not been developed in larval fishes (Strauss and Bond, 2016). There is a chance to misidentify larval stages upon using the same taxonomic key that of adults (Ko *et al.*, 2013). Species identification based on morphology creates an inadequacy for samples like fish fins or products do not bear intact morphological characters (Sotelo *et al.*, 1992). With these shortcomings, there is a need for an alternative method for identification of fishes (Ebert, 2009).

Among the cartilaginous fishes, sharks are an evolutionarily conservative group comprising of approximately 250 species. Most typical representatives of the class Chondrichthyes, subclass Elasmobranchii are the most ancient and have a successful lineage in the light of vertebrate evolution (Compagno, 1984). They play a crucial role in maintaining healthy marine environment (Id and Azri, 2019; Chen *et al.*, 2015). Sharks comprise a

significant predator group in marine biological system and assumes a significant job in vitality trade inside the most astounding trophic levels (Heithaus, 2016). Chondrosteans are on the verge of high rate of extinction when compared with any other vertebrate strata and only a third of the total number of species is considered safe (Dulvy *et al.*, 2014).

2.2.1 Sharks

Sharks are the most undermined gathering of vertebrates around the world (Grace, 2014). India is one among the real shark angling nations on the planet and by and by stands at second position alongside Indonesia (Vivekanandan, 2015). Evaluated shark arrivals in India during 2017 were 19,777 tons (http://www.cmfri.org.in/2017). Sharks comprise a noteworthy extent of high esteemed fishes in both household and global market (Vivekanandan, 2015). The decent variety of sharks in Indian waters has been a subject of immense examination. Day (1889) detailed 69 types of Chondrosteans, 52 species by Misra (1952), 41 species by Compagno (1984) and Talwar and 76 species by Kacker (1984). Raje *et al.,* (2002) announced 114 types of Elasmobranches while Venkitaramanan *et al.,* (2003) included 72 species in field ID hand book on sharks. Akhilesh *et al.*, (2013) revealed the presence of at any rate 157 legitimate types of sharks or. Froese and Pauly (2015) revealed 119 shark species from Indian waters and according to CMFRI, there are 160 types of sharks in Indian waters (Annual report C.M.F.R.I, 2015). The effects of unsustainable angling on sharks has been well-revealed all around and studies have showed up all through the latest many years various immense transient species usually got in gigantic scale pelagic marine fisheries are rapidly declining. IUCN assesses the protection status of most taxa, starting at now records over a thousand kinds of elasmobranchs.. The uncommonly transient sharks are among the species with most striking security concerns (Camargo *et al.*, 2016)

2.2.2 Carcarhinidae

Carcarhinidae is one among the largest and the most important of shark families. They are the dominant group of sharks found in tropical waters in terms of biodiversity and biomass richness, spotted in continental shelves and offshore. They are also present in subtropical and warm temperate warm and temperate, seas (Ebert and Dasvid, 2013). Many species among the genus Carcharhinus are quite similar to each other, which make it difficult for the researchers to distinguish one from the other. Field identification of a wide variety of closely related Carcharnids is often difficult (Camhi *et al.*, 2009).

A lot of morphological and non-morphological analyses were carried out to determine the relationship between different species of the same genus (Dosay-akbulut, 2008). For example, Lavery (1992) and Nayler (1992) studied the interrelationship of Carcharhinids using allozyme electrophoresis. Nuclear and mitochondrial phylogenetic analyses conducted by Iglésias *et al.*, (2005) showed paraphyly of Carcharhinidae. Phylogenetic analysis by Dosay-akbulut (2008) using ribosomal ITS1-2 region agreed that the Blue Shark (genus *Prionace*) belonged to the genus *Carcharhinus* instead of *Prionace.* Carcharhiniformes were the order with almost 270 species and commonly named as the ground sharks. Within this Carcharhiniforms, the species named *Carcharhinus longimanus* within the Carcharhinus genus were included in the IUCN red list.

2.2.3 *Carcharhinus longimanus*

Carcharhinus longimanus is probably the most migratory species among the sharks in various oceanic regions (Camargo *et al.*, 2016). They commonly names as oceanic whitetip sharks or Brown Milbert sharks with relatively large size. These species distributed worldwidly and formerly the most abundant oceanic pelagic shark in tropical and warm temperate areas at 18-28°C. They were also found in shallow water (37m) off oceanic islands or

where the continental shelf is very narrow, but is usually reported between the surface and depth of the at least 152m over water deeper than 184m (Compagno, 2013). Mainly feeds on oceanic bony fishes and Cephalopods, also stingrays, sea birds, turtles, marine mammal carrion and garbage. They were placental viviparous organisms by reproducing one to 15 pups per litter after about one year gestation. Information regarding the stock structure of the oceanic white tip shark in the Indian Ocean is not available. They are observed to undertake long distance movement ranging from the Mozambique Channel to the Somali Basin and the Southern Indian Ocean. They are highly migratory in nature (Bass *et al.,* 1973, White 2007, Romanov & Romanova 2009, Coelho *et al*., 2009, Filamalter *et al*., 2012).

Large stocky grey or brownish shark, underneath white, Vast rounded first dorsal fin long paddle-like pectoral fins with noticeable white mottled tips. Juveniles have black tips on some fins and black patches or saddles on the caudal peduncle. Caudal peduncle region is pigmented dark (Backus *et. al.,* 1956) Snout bluntly rounded, Small eyes, upper teeth triangular, inter-dorsal ridge present (Garrick, 1982), inconspicuous keels were found. Currently IUCN considering these species as **Vulnerable** globally (assessed in 2006) and **Critically Endangered** Western North and Central Atlantic, where long term declines up to 99% and recent declines of 60-70% are reported. A 90% decline is reported in Central Pacific Ocean (https://www.iucn.org/). Hence these species requires the conservation plans to sustain their population and to implement the conservation plan the data related to their stock information were required.

2.3 Concept of Stock structure

Shaklee *et al.* (1990) defined stock as "separately grouped population of some related people within animal groups which are genetically diverged from such population" (Booke, 1981; Dizon *et al*., 1997). Morphological

contrasts among gatherings of hereditarily homogeneous fish from various territories may basically reflect diverse natural conditions.

Grant *et al.* (1999) pointed to the significance of monitoring the stocks and their functional limits which has turned into a fundamental part of fishery the species preservation. Limited data on a particular interbreeding population i.e. of a misused or abused species won't help the administration arrangements to accomplish long term preservation objectives. It is very important to gather more relevant data about the populations stock in order to maintain proper management plans for conserving those species from the fear of extinction.

2.4 Population genetics

The study Population genetics always focuses about the statistic and developmental components which influences the genetic makeup of populations (Hartl, 2000; Ewens, 2001). Somebody defines population as a group of individuals residing on a particular region at a same time with same behaviour. Most of the population units get diminished due to the various difficulties in the adaptive nature of the individuals to the changing nature of environment and the resulted in the diminishing of the species as well as biodiversity. It is very important to focus on this diversity management process for safeguarding the organisms from the fear of extinction. Loss of hereditary decent variety inside the population may be related with inbreeding process in populations, which thus results in diminished wellness and eventually endangers the population perseverance (Bonin *et al.*, 2007). Ongoing examinations brought up that intraspecific hereditary decent variety was additionally appeared to support species extravagance and add to environment working and recuperation (Bonin *et al.*, 2007). By monitoring the type of fishes, we can discriminate the fishes among their species, population and individual levels and more over the identifying hybrids are also possible

by performing the population studies and phylo-geographic history and stock detail analysis. Hereby we can figure out the reasons of fish exploitation by analysing the stock structure, estimating the size of the populations and the mixed populations (Wirgin and Waldman, 1999; Huey *et al.*, 2006; Ilves and Taylor, 2009).

Genetic level variation can be divided into two groups however disengaged segments that must be evaluated independently and in an unexpected way (Bonin *et al.*, 2007). The first is the chosen (or useful) assorted variety emerging legitimately from versatile advancement i.e. directly adapted and evolved because of regular determination and second is the unbiased legacy of the population coming about because of the impacts of unbiased transformative powers for example, hereditary float, change, or movement among the individuals.

Characteristic determination works in various ways and it can wipe out hereditary variety or look after it. So as to see all the organizing of population in nature we need to take in to thought every one of these perspectives and break down them by taking a gander at variety of particular alleles at characterized loci known as atomic or hereditary markers (Allendorf *et al.*, 1987). Even though there are so many methods and techniques which are used in the stock structure analysis and the studies but all of them may get affected by some environmental factors (Clayton, 1981) so, molecular markers related studies will always provide perfect a way to genetic stock structure identification studies.

2.5 Molecular markers

The vast improvement in the DNA-based genetic markers provides a wide range achievement in the genetic studies of an organism by retrieving its

evolutionary lineages. As indicated by Liu and Cordes (2004), that the various biochemical, morphological and molecular markers were involved in those studies. The morphological markers only distinguish the organisms on the basis of the meristamatic features while the biochemical markers like allozymes focuses on the presence of any particular proteins. Not only these types of the ancient markers but the molecular markers like RAPD, RFLP, AFLP, SNP, EST, Micro and minisatellites were used for the investigations in finding the lineages.

Molecular markers are fundamentally of two types'protein and DNA markers. The DNA markers were further classified as nuclear DNA and mitochondrial DNA (mtDNA) (Park and Moran, 1994)were the nuclear DNA markers are Random Amplified Polymorphic DNA (RAPDs), Amplified Fragment Length Polymorphisms (AFLPs), Variable Number of Tandem Repeats loci (VNTRs like minisatellites and microsatellites) and Single Nucleotide Polymorphisms (SNPs) which are bi-parently acquired. While, mitochondrial DNA markers were the maternally acquired and non-recombining nature with the powerful hereditary population estimation nature (Ferguson and Danzmann, 1998). Investigations in several vertebrate species studies revealed that the stock difference always accumulates more quickly and widely in mitochondrial region than in nuclear DNA (Clawson, 1985).

The principle inquiries at the start of any genomic research are that what kind of marker is appropriate for the study and its efficiency in retrieving the information. As indicated by Carvalho and Hauser (1998) all ways to deal with portray population structure utilizing the genetic markers are based on the rule that migratory behaviour and mating behaviours among population may decide the degree of a typical genetic stock and in this manner their uprightness; despite the fact that there are numerous kinds of genetic markers accessible for this (Park and Moran, 1994).

Mitochondrial DNA (mtDNA), which always proves as an efficient marker in the studies than the nuclear markers. It has been utilized as a sub-atomic marker and demonstrated to be an amazing asset for explaining population structures and evaluating phylogenetic connections in different gatherings of species (Howard and Berlocher, 1998; Avise, 2000).

2.5.1 Mitochondrial DNA

One of the greatest challenges in genome research lies on the selection/identification of the marker types which was suitable for the species of interest and for the study being carried out. Some times, a combination of multiple molecular markers are utilized (Gopalakrishnan, 2009). Mitochondria contain own genetic material and protein synthetic machinery. Besides the packaged DNA in nucleus, mitochondria also contain another form of DNA called mitochondrial DNA (mtDNA). Mitochondrial DNA occurs in 10^{2}-10^{4} copies per cell. This enables higher recovery of mtDNA in extraction experiment (Hubert, 2008). Mitochondrial DNA resembles bacterial DNA due to its endo-symbiotic origin. Each cell has about 50-100 mtDNA molecules (Stevens, 1981). In case of animals, the mitochondrial DNA is a double helical circular molecule with a size range of 15-20 kb. Only, 5% of the total RNA and polypeptides required by the mitochondrion is encoded by mtDNA. Mitochondria are semi- autonomous and are able to code for some of their polypeptides (Becker *et al.*, 2007). Out of 37 genes, 13 genes encode for synthesis of respiratory enzymes involved in the oxidative phosphorylation pathway of cell metabolism. The remaining genes codes for tRNAs and rRNAs. In genetic investigations, it is considered as a single locus because of non-mendelian inheritance (Avise, 1994).

There are many advantages of using mtDNA such as high copy number, rare combination (Sangthong and Jondeung 2003), haploid mode of inheritance absence of introns inside coding exon sequences (Rokas and

Holland, 2000) and lack of Indels in protein coding sequences. Park and Moran (1995) considered whole mtDNA as a single locus with multiple alleles that showed rapid evolution (Avise, 1994). Technical advantages of mtDNA include requirement of only a small amount of fresh, frozen or alcohol stored tissue to amplify the genes (Bineesh *et al.*, 2017). This makes mtDNA a versatile tool for the population study (Gold *et al.*, 1993). Gold *et al.*, (1993) examined mtDNA variation in 478 *Sciaenops ocellatu.* Major disadvantages of mtDNA in population study are the lack of information on male population and homogeneity of population based on allelic frequency due to the maternal inheritance pattern. However it is widely used in phylogenetic studies among various groups of species (Avise, 1994).

Mitochondrial genome of vertebrate is 15-20 kb in length in different organisms. It comprises of 40 genes coding for 2 rRNAs, 22 tRNAs, and 13 proteins which were essential in respiration process (Hartl and Clark, 1997). It also has a non-coding D-loop region which is responsible for replication otherwise called as the control region. The control region is A-T rich and is the fastest evolving region in the entire mtDNA because of high substitution rate. It is the most divergent molecule because, the mtDNA do not have repair enzymes for errors in replication and for damage of DNA (Brown *et al.*, 1979). Partial mtDNA sequences like 16s rRNA and COI are more suitable than other sequences to study the phylogenetic relationship between the families of different eukaryotes especially fishes (Barucca *et al.*, 2004). Mitochondrial DNA was used as a tool in the phylogenetic evaluation of various group of fishes comprising of the class Actinoptergii (Cypriniformes and Perciformes) and Sagoptergii (Zardoya and Meyer 1996) as revealed by the results (Lio *et al.*, 1998; Rasmussen and Arnasson, 1999).

Cytochrome b is a widely used protein coding molecular marker in the mtDNA to study species specific pattern in many animals, which shows a slow rate of evolution (Guan *et al.*, 1993). It has been used to study phylogenetic

relationship between different taxa (Meyar *et al.*, 1990). Johns and Avise (1998) demonstrated that closely related vertebrate species showed more than 2 % divergence at mitochondrial gene cytb. It also has excellent use in molecular taxonomy, population genetics and phylogenetics (Irwin *et al.*, 1991). It is an important gene coding for the transmembrane protein involved in the respiratory chain of cellular metabolism (Martin *et al.*, 1990).

The mt-cyt b gene has been used in the study of phylogenetics of anemone fishes of Persion Gulf by Ghorashi *et al.*, (2008). The phylogenetic performance of cyt b is comparable to that of CO1 of mtDNA (Zardoya and Meyer, 1996). It contains both slowly and rapidly evolving codon positions as well as more conservative and variable regions and domain. The phylogenetic utility of mt-cytb was studied at various taxonomic levels (Irwin *et al.*, 1991; Moritz *et al.*, 1992; Da Silva and Paton, 1993; Graybeal, 1993; Lamb and Lydeard, 1994; Moore and De-Filippis, 1997and Nunn and Stanley, 1998). Hsu *et al.*, (2017) surveyed 636 bp of mtDNA cyt b from 99 individuals of *Trichiurus lepturus* collected from 8 locations in Taiwan. Biochemical and molecular based analysis including mt-cyt b gene was carried out in the investigation of phylogenetic relationship diversity in *Cyprinus carpio* by Kohlmann *et al.*, (2003).

2.5.2 Cytochrome c oxidase 1 subunit (COI) and The concept of DNA barcoding

Mitochondrial gene cytochrome c oxidase I serve as the core of a global bio-identification of animals. It is supposed to be evolving faster than 16S rRNA of mtDNA and used widely in identification of animals especially fishes (Steinke *et al.*, 2005). This gene is conservative among metazoans (Jacobs *et al.*, 1988; Folmer *et al.*, 1994). The sequence diversity among various groups of closely related. In animal kingdom was examined by using mt-COI gene and concluded that species level diagnosis can be obtained through COI analysis (Hebert *et al.*, 2003). Protein coding COI gene is highly

conserved and has been sequenced in various vertebrate and invertebrate lineages (Brown, 1985; Bermingham and Lessios, 1993; Santos *et al.*, 2003; Munasinghe *et al.*, 2004; Vinson *et al.*, 2004; Ward *et al.*, 2005; An *et al.*, 2005; Whiteman *et al.*, 2004; Shander and Willassen, 2005). The total length of COI in vertebrates is about 1545bp and a region of about 655bp long near the start of the COI reading frame was named as 'barcode' region. This is a well characterized sequence near the 5' end of COI gene (Folmer *et al.*, 1994).

DNA barcoding using COI on animal species was studied by Hebert *et al.*, 2003. It also aims to employ standardized protocols. The methodology is simple and may be applied to a wide variety of organisms for solving taxonomic ambiguities (Iglésias *et al.*, 2005). Sequence and specimen data is stored and made available in Barcode of Life (BOLD) system (Ratnasingham and Hebert 2007).

In March, 2003, Consortium of The Barcode of Life (COBOL) was started and since then has been promoting the use of standardized and universal sequence for identification of species. The short sequence used for standardized identification of organism has gained attention under the terms of DNA barcoding or DNA taxonomy (Floyd *et al.*, 2002; Hebert *et al.*, 2003 and Tautz *et al.*, 2003). It is a powerful tool for the accurate identification of species (Newmaster *et al.*, 2006). In addition to species identification, it also reveals the evolutionary history of life on earth and aid phylogenetic analysis of organisms (Barucca *et al.*, 2004). It is used when the traditional method of taxonomy produced unsatisfactory results *viz.*, identification of eggs, immature forms and analysis of gut content or excreta to determine the food webs (Lijo, 2009).

Brown *et al.* (2003) for the first time described new species from holotype using DNA barcoding. Thereafter, many similar cases of describing new species from different holotype and paratype were recorded (Burns *et al.*, 2007; Yassin *et al.*, 2008; Adamski 2009). Bartlett and Davidson (1991) used

mtDNA sequencing for fish identification and reveled that cytochrome b sequence could discriminate for species of tuna.

Aquilino *et al.* (2011) for the first time, employed DNA barcoding in fishes of Taal Lake of Philippines covering118 individuals belong to 21 genera, 17 families and 19 orders. Bineesh *et al.*, (2017) studied about 528 specimens of 111 Chondrostean species and 34 families from Indian EEZ and barcoded 655 bp mitochondrial COI regions. They confirmed the potential of DNA barcoding for accurate identification of sharks, rays and their products from Indian waters.

Harvey *et al.* (2009) examined over 1000 DNA barcodes representing nearly 20% of all known Elasmobranchs and demonstrated that a character based nucleotide diagnostic approach to barcode identification is feasible. Ward and Homes *et al.* (2007) examined mt-COI barcodes of 388 species including the sub class Holocephalii, Elasmobrancii and Actinoptergii and concluded that major efficiency of mt-COI nucleotide sequence analysis for the identification of species comes from the codon degeneracy and the highly variable nature of the position of third codon of amino acid. They also revealed that CO1 amino acid diversity is less and does not possess enough power in resolving status of species.

Ward *et al.* (2005) performed important studies of fish DNA barcoding. They generated 754 sequences from Osteichthyes and Teleosts. Laskar *et al.,* (2019) generated mt-COI sequences from morphologically identified fishes from the river Diphlu in North East India to cover endemic species and to resolve the prevailing taxonomic keys.

However, the DNA might be altered by various processing methods like canning and heating even though it is more thermo-stable and resistant than proteins. It is also possible to amplify DNA fragments by polymerase chain reaction (PCR). DNA can be retrieved from any substrate because DNA is present in almost all cells of an organism (Lockley and Bardsley, 2000).The

substitution and mislabeling of fresh or processed seafood considered as a universal problem. These practices have been increasing as there is no standard system for seafood labeling (Cawthorn *et al.*, 2015). Arduro *et al.*, (2010) endorsed DNA barcoding as a successive tool in detecting food adulteration and to prevent the problems of mislabelling. They investigated the commercial landing at Amazon River, using DNA barcoding. Botti and Giuffra (2010) examined 17 processed fish species of family Scrombridae including economically important tunas and mackerels using cytochrome b sequence and reported an additional species of fish in the product as against the 'contents' of the label. Dhar and Ghosh, (2015) scrutinised 128 full length CO1 barcode sequence of traded samples and they investigated through combined approach of morphology to identify 128 ornamental fish specimens that were exported from North East India. They found that around 33% of traded samples belonged to the threatened group.

Holmes *et al.* (2009) resolved the problem associated with sharks owing to shortage of specimens. They conducted DNA barcoding of dried and removed fins of sharks. They identified 20 species of sharks from 211 fin parts. Many of the species are enlisted in the IUCN Red list including one as critically endangered. Sembiring *et al.,* (2015) scrutinized barcodes of 600-654 bp of mitochondrial COI gene from the unknown shark fins collected from Indonesian fish markets. The main findings of study revealed that, 80% of the total species identified are either considered as "endangered", "nearly threatened" or "vulnerable".

Wong *et al.* (2011) studied 9 cat fish species from United States coming under families Ictaluridae, Clarridae and Pangassidae with COI sequence and developed protocol and consensus barcode, which are valuable resources in the present world. Sarmiento-Camacho and Valdez-Moreno (2018) identified fresh fillets of shark *via* DNA barcoding.

Rasmussen *et al.* (2009) investigated the essentiality of DNA

barcoding in identifying seven commercially important salmon and trout species from North America. Several shorter barcode regions called "mini-barcodes" were identified *in silico* that can differentiate all eight different species thereby serving as a potential tool for identification of heavily processed fish products. Shokralla *et al.* (2015) established a mini-barcode system for all fish species used in fish processing. The study conducted by Asis *et al.*, (2016) established the importance of DNA barcoding in case of illegal trade. Chuang *et al.,* (2016) barcoded the processed fish products like shark fins and identified 23, 24 and 14 species from 231 fish landings, 316 fin products and 113 detained samples respectively. Leyva-Cruz *et al.,* (2016) conducted barcoding for the identification of eggs of pelagic fishes. He and his co-workers identified 42 taxa, 35 genera and 24 families. Sultana *et al.,* (2018) developed a mini-barcode to discriminate fish species in raw and processed products. Study conducted by Yan *et al.,* (2016) employed a DNA barcoding approach to authenticate different fish species imported *via* a single port of China. They came to a conclusion that performance of DNA barcoding as a practical demonstration in the prevention of fraud in international trade.

In any case, DNA barcoding has advanced technique of scientific categorization but struggle still stays in the choice of a standard marker for this even with in vertebrates. Both COI and 16S (unaligned) rRNA qualities could be utilized as the methods for viable and increasingly exact recognizable proof of species can be recommended as the solution. The preservation of the hereditary inconstancy is one of the fundamental goals in administrative projects aiding the recuperation of imperilled species. All things considered, the sequencing of the mitochondrial DNA control loop (D-circle) is a standout amongst the most normally connected strategy to population hereditary investigations of vertebrates, including sharks (Camargo *et al.,* 2016).

Hereditary i.e., genetic qualities have turned into a significant device in species preservation and conservation. A few investigations of this issue have

been directed on both marine and freshwater fish species, particularly regarding those matters to abuse of the resources. Sub-atomic procedures can give profitable information about phylogeny, structure and endemicity of target species. The collected information can hereby use for the analysis of inter population analysis, gene flow, migration, recruitment, shifts, reproductive strategies, depletion of any particular population and the behaviour of the fishes species.

As in the case of huge maritime sharks which are liable to angling weight for all intents and purposes all through its range. It is gotten in huge numbers as a by-catch in pelagic fisheries, with other pelagic fishes.. Its enormous blades are very prized in global exchange despite the fact that the body is regularly disposed of. Fishery weight is probably going to persevere if not increment in future. Outside of the regions itemized beneath, this species is under comparative angling weight from various pelagic fisheries, there are no information to recommend that decreases would and have not have moreover happened in these regions, given there are comparable fisheries all through the range.

Accordingly, a preparatory worldwide appraisal of Vulnerable is viewed as proper for the maritime white tip sharks. Endeavours are in progress to improve the gathering of information from certain areas and successful preservation and the board of this species will require universal understandings about the current size of the species in a particular region (www.iucn.in).

Camargo *et al.* (2016) developed a report regarding the *C. longimanus* in the east-west Atlantic Ocean and those data were used for the implementation of management plans of the species. The present study also focuses on the gathering of information of these species in the Indian Oceanic regions which helps for managing the species.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present study was carried out at the Marine Biotechnology Division, ICAR- Central Marine Fisheries Research Institute Kochi, Ernakulam during the year 2018-2019. Details regarding the experimental materials used and procedures followed in the study are elaborated in this chapter.

3. 1 Sample collection

150 Samples of *C. longimanus* (oceanic whitetip shark) were collected from the 5 identified sites of Indian Ocean comprising the regions of Kerala and Tamil Nadu as the fisheries of this shark exist only in these regions. Samples were then identified by morphological characters (FAO., 1983) and the dichotomous keys of Compagno (1984) and Grace (2014).The sample collection details were listed in Table 1 and the morphological characters were listed in Table 2.

Table1 Details of *C. longimanus sample* collected from Eastern Arabian Sea.

SI No	Landing centers were the samples collected	Site code	Month of sample collection
1	Cochin (Kerala)	CFH	Feb 2019 May 2019
2	Mangalore (Karnataka)	MFH	Dec 2018
3	Kollam (Kerala)	QPC	Nov 2018
4	Tamil Nadu	TFH	Oct 2018
5	Lakshadweep	LPC	Nov 2018 Dec 2018

Table 2 Morphological features of *C. longimanus*

1. Body shape	Not kite like
2. Pelvic fin	Present
3. No. of Gills	Five
4. Dorsal Fins	Two
5. Mouth position	Back along underside of head
6. Head shape	Not expanded
7. Caudal size	Varying size
8. Length of dorsal fin	Less than total body length
9. Caudal fin length	Less than total body length
10. Anal fin	Present

3.2 Mitochondrial Marker Analysis

3.2.1 Glass ware and other materials

Mortar and pestle, 2 ml Eppendorf tubes, micropipette tips and PCR tubes were autoclaved and used. 2 ml tube stand, micropipettes, measuring cylinder, bottles, petriplates, tube holders, sterilized blades, labels, and weighing pot are the other materials needed for molecular work.

3.2.2 Instruments

The equipments *viz.*, autoclave (Hirayama), electronic weighing balance (Afcoset), vortexer (Labnet), spinner (Rivotek), water bath (Memmert), microwave oven (IFB), cooling centrifuge (Eppendorf), NanoDrop™ spectrophotomete, deep freezer (-20 °C (Vestfrost), -80 °C (New Brunswick Scientific)), refrigerator (Whirlpool), electrophoresis apparatus (Cleaver Scientific), gel documentation system (Syngene), PCR machine (Proflex), and distilled water unit (ELGA) were used for the study.

3.2.3 DNA Extraction

Whole genomic DNA was isolated using standard "Phenol – Chloroform method " (Sambrook & Russel 2001).The procedure listed below;

For the DNA extraction, one piece of tissue (approx. of 1 mg) either from the fin clip or any muscular parts of the samples was excised or stored in the 95% alcohol. Approximately, 10 mg of collected tissue samples were taken and minced well (without any contamination). Later transferred to an eppendorf tube and labelled properly. To the Tube, 400µl cell lysis buffer, 100µl SDS (10%), 10µl proteinase K (1mg/ml) were added to the tube and placed in a water bath at 55°C for incubation by intermittent shaking at around 15 minutes for 2 hours. After incubation, added 500µL of phenol-chloroform-isoamyl alcohol (25:24:1) to the tube which contains a clear lysate and vortexed for 2 minutes. The sample was taken for centrifugation at 10,000 rpm for 10 minutes in a pre-cooled axis (4°C). The top aqueous layer was evacuated and transferred the samples in another eppendorf tube using a pipette. Add 500µL of Chloroform-isoamylalcohol (24:1) to the new tube and vortexed for 1 minute. The sample was again centrifuged at 10,000rpm for 10 minutes (4°C).The top aqueous layer was again transfered to another Eppendorf tube. Add 500µl of isopropanol to final tube and kept for precipitation at -20°C for 2 hours. The isopropanol added tube was centrifuged at 10,000rpm for 10 minutes at 4°C. Supernatant was removed from the tube without disturbing the pellet found at the tube bottom. The found pellet was washed with 70% ethanol and centrifuged at 10,000rpm for 10 minutes at 4°C.The supernatant was decanted without disturbing the pellet (Repeat ethanol washing steps for 3 times). After washing, the pellets get air dried and suspended in 50µl of 1x TE buffer and stored at - 20°C.

After extraction of the genomic DNA, the quantification and quality analysis was carried out using agarose gel electrophoresis.

3.2.4 Analysis of extracted DNA

The extracted DNA was quantified using the Agarose gel electrophoresis and Nano drop spectrophotometer methods. Agarose gel electrophoresis is mainly used to analyse the DNA molecules on the basis of their molecular size. 0.8 gm of agarose in 45 ml TBE buffer (pH-8) was heated using a microwave oven until agarose gets dissolved and becomes a clear solution. After the solution got cooled, Ethidium bromide (0.5µl- 10 mg/ml) was added (to visualize DNA bands in the gel documentation system).The solution was poured into a sample comb inserted casting tray, without forming air bubble and allowed to solidify at room temperature. The comb was removed once the gel gets solidified. The gel was detached from the tray and was inserted horizontally into the buffer containing electrophoretic chamber. 3µl of DNA sample was mixed with 3µl of gel loading dye (6X). Then 6µl of mixture (3µl DNA+3µl loading dye) was loaded into the wells on the gel.100 bp DNA ladder (New England Biolabs) was used along with the samples. Constant voltage (90V) was applied across the electrodes using power pack/supply unit. The current flow was confirmed by observing bubbles coming off the positive and negative electrodes. The distance at which DNA has migrated in the gel was judged by monitoring the migration of tracking dye. To visualize the DNA, a gel documentation system (with an ultraviolet transilluminator and digital camera) (Vibler) and the image was recorded with 'Bio vision' software (software package for imaging, analysis, and data basing 2-D electrophoresis gels).

The quantification was carried out using the Nano drop spectrophotometric method which always provides a wide range of accuracy and reproducibility. Through this method, the DNA extracted quality was analysed at OD 260 absorbance level and the purity was checked with the OD 260/280 ratio value. The major advantage of this method was the analysis requires only 1 µl sample.

3.2.5 Primer Screening

3.2.5.1 Primer for barcode segment of cytochrome oxidase I.

The primers developed by Ward *et al.*, 2005 were used to amplify the 650bp barcode segment of COI gene. The analysis was performed from the randomly chosen samples of each location collected for the study.

The details of primers used are given in the Table 3.

Table. 3. Details of Primer (Ward *et al.*, 2005) used in COI amplification.

SI No	Primer name	Primer sequence	Annealing Temperature
1	Ward F1	5'-TCAACCAACCACCACAAAGACATTGGCAC-3'	53°C
2	Ward R1	5'-TAGACTTCCTCTGGGTGGCCAAAGAATCA-3'	

3.2.5.2 Primer for D-loop region of mitochondrial DNA.

A partial sequence of D loop/control region of mitochondrial DNA was amplified from the collected samples. The primers used for PCR amplification were designed using the prime3 plus software based on complete mitogenome sequence of *C. longimanus*.

PCR conditions for the new primers were optimized by performing gradient PCR. Details of the primers are given in the Table 4.

Table.4. Details of Primer (D- loop primer) used in Population Genetic Analysis.

SI No	Primer name	Primer sequence	Annealing Temperature
1	CRSF	Primer (Forward): **5' CTCCCAAAGCCAAGATTCTG 3'**	56°C
2	CRSR	Primer (Reverse): **5'GGCTTAGCAAGATGTCTTGGG3'**	

The genomic DNA extracted from the randomly chosen samples were used for the initial primer screening for the barcode segment region and rest of the samples were used for the screening of the D Loop region. The composition of the PCR reaction mixture was listed below in Table 5.

Table 5. Details of PCR reaction mix used for PCR amplification of CO1 and D-loop

PCR Reaction mixture components	**Vol/conc**
PCR buffer with $MgCl_2$ (Sigma-Aldrich)[10X]	2.5µl (1.5 mM)
Water	19.8µl
dNTP Mix (200 µM)[Sigma-Aldrich]	0.5µl (200mM of each dNTPs)
Forward Primer (F) (100mM)	0.5µl
Reverse Primer (R) (100mM	0.5µl
Taq polymerase (Sigma-Aldrich)	0.25µl (0.05 units/µl)
Template DNA	1 µl (20ng)

3.2.6 PCR reaction conditions for COI barcode amplification.

The PCR program was set with an initial denaturation temperature at**94°C for 4 minutes** and subsequent denaturation of 30 cycles at **94°C for 30 seconds**, annealing at **53°C for 30 seconds** and extension at **72°C for 45 seconds** and final extension at **72°C for 7minutes**.

3.2.7 PCR reaction condition for D-loop region amplification.

The PCR program was set with an initial denaturation temperature of **94°C for 4 minutes** and subsequent denaturation of 32 cycles at **94°C for 30 seconds,** annealing temperature at **56°C for 30 seconds** and extension at **72°C for 80 seconds** and final extension at **72°C for 7 minutes.**

PCR products were electrophoresed on 1.2% ethidium bromide stained agarose gel in 1X TBE buffer (composition/preparation). Electrophoresis was done at constant voltage (90 V) for 30 minutes.

The amplicons were then illuminated using UV gel documentation system and further used for the study.

3.3 Phylogenetic Analysis.

The partial CO1 gene sequences obtained were aligned and combined with the CO1 gene sequences of the same genus, class, order were *Narcine bancrofti* as out group which was listed in Table 6.

A phylogenetic tree using the Maximum Likilihood method in MEGA software using the above dataset.

Table 6. Details showing the gene sequences used in phylogenetic analysis with their accession number.

SI No	Species Name	Accession No
1.	*Carcharhinus brevipinna*	FJ519070.1
2.	*Carcharhinus plumbeus*	KJ740750.1
3.	*Carcharhinus amblyrhynchos*	KX713065.1
4.	*Carcharhinus albimarginatus*	MF508660.1
5.	*Carcharhinus falciformis*	KU497489.1
6.	*Carcharhinus signatus*	MG787978.1
7.	*Carcharhinus cautus*	FJ519071.1
8.	*Carcharhinus maclotii*	HQ530173.1
9.	*Carcharhinus fitzroyinsis*	KX982222.1
10.	*Carcharhinus leiodon*	JN034903.1
11.	*Carcharhinus limbatus*	AY766127.1
12.	*Carcharhinus tilstoni*	GQ227283.1
13.	*Carcharhinus longimanus*	KX789509.1
14.	*Carcharhinus dussumieri*	GQ227288.1
15.	*Carcharhinus porosus*	MH911149.1
16.	*Carcharhinus leucas*	MH488888.1
17.	*Carcharhinus sealei*	MH243171.1
18.	*Carcharhinus sorrah*	KF819774.1
19.	*Carcharhinus altimus*	JN313266.1
20.	*Carcharhinus brachyurus*	MH719957.1
21.	*Carcharhinus acronotus*	KM657088.1
22.	*Carcharhinus isodon*	KU255141.1
23.	*Carcharhinus amboinensis*	NC_026696.1

24.	*Carcharhinus perezii*	MH719962.1
25.	*Carcharhinus galapagensis*	MG241881.1
26.	*Carcharhinus obscurus*	KU661494.1
27.	*Rhizoprionodon oligolinx*	MH243154.1
28.	*Rhizoprionodon acutus*	MH243140.1
29.	*Rhizoprionodon taylori*	EU399000.1
30.	*Rhizoprionodon lalandii*	HM446242.1
31.	*Rhizoprionodon terraenov*	HM991198.1
32.	*Glyphis garricki*	EU818709.1
33.	*Glyphis glyphis*	EU818708.1
34.	*Glyphis gangeticus*	MH244900.1
35.	*Negaprion brevirostris*	AF457185.1
36.	*Galeocerdo cuvier*	KX858829.1
37.	*Sardinellalon giceps*	KJ888390.1
38.	*Prionace glauca*	EU427559.1
39.	*Protophormia terraenovae*	KM861203.1
40.	*Scoliodon lanticaudus*	KF927964.1
41.	*Scoliodon macrorhyrichos*	KF927964.1
42.	*Loxodonm acrorhinus*	NC_029843.1
43.	*Heterodontus francisci*	S51944.1

The genetic distance was calculated between the CO1 sequence of *C. longimanus* and the NCBI sequences using MEGA.

3.4 Population genetic analysis.

D-loop sequence data set of *C. longimanus* was prepared by aligning the sequences obtained from 150 specimens in Clustal-W on MEGA software. We estimated several parameters like nucleotype diversity (Nei, 1987), haplotype diversity (Nei, 1987), total number of synonymous and non-synonymous mutations were estimated using DnaSP (Rozas *et al.*, 2003).

To understand the demographic history of the species, we used a step-wise expansion model (demographic and spatial) with a parametric bootstrapping method to compare expected mismatch with observed mismatch distribution. It was then confirmed by estimating the sum of squared deviations (SSD), Harpending raggednes index (Hri), Tajima's D and Fu's Fs. Arlequin was used for carrying out AMOVA analysis and estimation of pairwise Φst (Φ_{ST}) (Tajima, 1983), in order to understand the demographic history *C. longimanus* populations, pairwise mismatch distribution was conducted in DnaSP. Changes in the effective population size through time were estimated using Bayesian skyline analysis as implemented in BEAST v1.7.5. Convergence was tested by running the analysis for 10000000 chains under the GTR model for above data set with a strict clock model and coalescent skyline. All parameters were automatically optimized and the skyline plot was generated by Tracer v1.6. A haplotype network tree was generated for the D-loop data using POPart program (http://popart. otago.ac.nz) using using median joining networks (Bandelt *et al.*, 1999). Accompanied by the above generated information from the D loop dataset of Indian samples, we put together a comparative analysis of the species from Indian Ocean with the information available from Atlantic Ocean. We utilized the haplotypes of *C. longimanus* D-loop sequences from Atlantic Ocean (Camargo *et al.*, 2016) was listed in Table no 7.

In addition, the above D-loop data we prepared an additional dataset containing D-loop sequence from this study and sequences characterized from

Atlantic Ocean by aligning them using Clustal W. then we estimate the pairwise Φst between the Indian Ocean populations and Atlantic Ocean population using Arlequin Version 3.5.1.2 (Schneider *et al.*, 2005).

Table7. Details showing the haplotypes of Atlantic Ocean (Camargo *et al.*, 2016) with accession number.

SI No	Haplotypes from the Atlantic Ocean (Camerago *et al.*,2016)	Accession no:
1.	***Carcharhinus longimanus*** haplotype 12 D-loop, partial sequence. mitochondrial	KT160329.1
2.	***Carcharhinus longimanus*** haplotype 11 D-loop, partial sequence. mitochondrial	KT160328.1
3.	***Carcharhinus longimanus*** haplotype 10 D-loop, partial sequence. mitochondrial	KT160327.1
4.	***Carcharhinus longimanus*** haplotype 9 D-loop, partial sequence. mitochondrial	KT160326.1
5.	***Carcharhinus longimanus*** haplotype 8 D-loop, partial sequence. mitochondrial	KT160325.1
6.	***Carcharhinus longimanus*** haplotype 7 D-loop, partial sequence. mitochondrial	KT160324.1
7.	***Carcharhinus longimanus*** haplotype 6 D-loop, partial sequence. mitochondrial	KT160323.1
8.	***Carcharhinus longimanus*** haplotype 5 D-loop, partial sequence. mitochondrial	KT160322.1
9.	***Carcharhinus longimanus*** haplotype 4 D-loop, partial sequence. mitochondrial	KT160321.1
10	***Carcharhinus longimanus*** haplotype 3 D-loop, partial sequence. mitochondrial	KT160320.1
11	***Carcharhinus longimanus*** haplotype 2 D-loop, partial sequence. mitochondrial	KT160319.1
12	***Carcharhinus longimanus*** haplotype 1 D-loop, partial sequence. mitochondrial	KT160318.1

RESULTS

4. RESULT

The study entitled "Identification of population genetic structure studies of *Carcharhinus longimanus* using mitochondrial markers from Indian Ocean" was carried out at the Marine Biotechnology Division, ICAR-Central Marine Fisheries Research Institute, Kochi Ernakulam during 2018-2019.

The collected 150 specimens of *C. longimanus* were characterized based on mitochondrial markers. The results are depicted in this chapter.

4.1 Sample collection and Identification

The collected specimens were confirmed as Carcharhinidae and the species by the paddle shaped and white pattern coloration in the pectoral fin region. The sample collection details were listed in Table 8.

Table 8. Sample details collected from different locations.

Sample No:	Sample Name	Sample collected Location	Sex	Collection period	W/L
1	CLK1	Kochi (Kerala)	F	Oct 2018	15Kg/121cm
2	CLK2	Kochi (Kerala)	M	Oct 2018	21Kg/212cm
3	CLK3	Kochi (Kerala)	F	Oct 2018	23Kg/182 cm
4	CLK4	Kochi (Kerala)	F	Oct 2018	5Kg/46cm
5	CLK5	Kochi (Kerala)	F	Oct 2018	15Kg/121cm
6	CLK6	Kochi (Kerala)	M	Oct 2018	21Kg/212cm
7	CLK7	Kochi (Kerala)	M	Oct 2018	23Kg/182 cm
8	CLK8	Kochi (Kerala)	M	Oct 2018	5Kg/46cm
9	CLK9	Kochi (Kerala)	M	Oct 2018	28kg/212cm
10	CLK10	Kochi (Kerala)	M	Oct 2018	19Kg/46 cm
11	CLK11	Kochi (Kerala)	F	Oct 2018	22kg/111cm

12	CLK12	Kochi (Kerala)	M	Oct 2018	56kg/100cm
13	CLK13	Kochi (Kerala)	F	Oct 2018	26kg/152cm
14	CLK14	Kochi (Kerala)	M	Oct 2018	33kg/111cm
15	CLK15	Kochi (Kerala)	F	Oct 2018	67kg/121cm
16	CLK16	Kochi (Kerala)	M	Oct 2018	22kg/123cm
17	CLK17	Kochi (Kerala)	M	Oct 2018	12kg/53cm
18	CLK18	Kochi (Kerala)	M	Oct 2018	72kg/154cm
19	CLK19	Kochi (Kerala)	M	Oct 2018	33kg/138cm
20	CLK20	Kochi (Kerala)	M	Oct 2018	59kg/156cm
21	CLK21	Kochi (Kerala)	M	Oct 2018	20kg/121cm
22	CLK22	Kochi (Kerala)	M	Oct 2018	32kg/111cm
23	CLK23	Kochi (Kerala)	M	Oct 2018	54kg/222cm
24	CLK24	Kochi (Kerala)	M	Oct 2018	23Kg/182 cm
25	CLK25	Kochi (Kerala)	M	Oct 2018	5Kg/46cm
26	CLK26	Kochi (Kerala)	M	Oct 2018	28kg/212cm
27	CLK27	Kochi (Kerala)	M	Oct 2018	26kg/152cm
28	CLK28	Kochi (Kerala)	M	Nov 2018	33kg/111cm
29	CLK29	Kochi (Kerala)	M	Nov 2018	67kg/121cm
30	CLK30	Kochi (Kerala)	M	Nov 2018	22kg/123cm
31	CLK31	Kochi (Kerala)	M	Nov 2018	26kg/156cm
32	CLK32	Kochi (Kerala)	M	Nov 2018	15Kg/121cm
33	CLK33	Kochi (Kerala)	F	Nov 2018	21Kg/212cm
34	CLK34	Kochi (Kerala)	M	Nov 2018	23Kg/182 cm
35	CLK35	Kochi (Kerala)	F	Nov 2018	5Kg/46cm
36	CLK36	Kochi (Kerala)	M	Nov 2018	28kg/212cm
37	CLK37	Kochi (Kerala)	F	Nov 2018	19Kg/46 cm

38	CLK38	Kochi (Kerala)	M	Nov 2018	22kg/111cm
39	CLK39	Kochi (Kerala)	F	Nov 2018	50kg/152cm
40	CLK40	Kochi (Kerala)	M	Nov 2018	20 kg/50cm
41	CLK41	Kochi (Kerala)	F	Nov 2018	15Kg/121cm
42	CLK42	Kochi (Kerala)	F	Nov 2018	26kg/152cm
43	CLK43	Kochi (Kerala)	F	Nov 2018	33kg/111cm
44	CLK44	Kochi (Kerala)	F	Nov 2018	67kg/121cm
45	CLK45	Kochi (Kerala)	F	Nov 2018	20kg/123cm
46	CLK46	Kochi (Kerala)	F	Jan 2019	26kg/156cm
47	CLK47	Kochi (Kerala)	F	Jan 2019	15Kg/121cm
48	CLK48	Kochi (Kerala)	F	Jan 2019	21Kg/210cm
49	CLK49	Kochi (Kerala)	F	Jan 2019	23Kg/182 cm
50	CLK50	Kochi (Kerala)	F	Jan 2019	26kg/158cm
51	CLK51	Kochi (Kerala)	F	Jan 2019	67kg/120cm
52	CLK52	Kochi (Kerala)	F	Jan 2019	22kg/123cm
53	CLK53	Kochi (Kerala)	F	Jan 2019	12kg/53cm
54	CLK54	Kochi (Kerala)	F	Jan 2019	72kg/154cm
55	CLK55	Kochi (Kerala)	F	Jan 2019	67kg/121cm
56	CLK56	Kochi (Kerala)	F	Jan 2019	15Kg/121cm
57	CLK57	Kochi (Kerala)	F	Jan 2019	21Kg/212cm
58	CLK58	Kochi (Kerala)	F	Jan 2019	23Kg/182 cm
59	CLK59	Kochi (Kerala)	F	Jan 2019	5Kg/46cm
60	CLK60	Kochi (Kerala)	F	Jan 2019	26kg/152cm
61	CLK61	Kochi (Kerala)	F	Jan 2019	21Kg/210cm
62	CLK62	Kochi (Kerala)	F	Jan 2019	67kg/120cm
63	CLK63	Kochi (Kerala)	F	Jan 2019	28kg/217cm

64	CLK64	Kochi (Kerala)	F	Jan 2019	26kg/152cm
65	CLK65	Kochi (Kerala)	F	Jan 2019	21Kg/210cm
66	CLK66	Kochi (Kerala)	M	Jan 2019	22kg/123cm
67	CLK67	Kochi (Kerala)	F	Jan 2019	67kg/121cm
68	CLK68	Kochi (Kerala)	M	Jan 2019	21Kg/210cm
69	CLK69	Kochi (Kerala)	M	Jan 2019	15Kg/121cm
70	CLK70	Kochi (Kerala)	F	Jan 2019	21Kg/212cm
71	CLK71	Kochi (Kerala)	F	Jan 2019	23Kg/182 cm
72	CLK72	Kochi (Kerala)	M	Jan 2019	5Kg/46cm
73	CLK73	Kochi (Kerala)	M	Jan 2019	20kg/123cm
74	CLK74	Kochi (Kerala)	M	Jan 2019	26kg/156cm
75	CLK75	Kochi (Kerala)	M	Jan 2019	15Kg/121cm
76	CLK76	Kochi (Kerala)	M	Jan 2019	21Kg/210cm
77	CLK77	Kochi (Kerala)	M	Jan 2019	23Kg/182 cm
78	CLK78	Kochi (Kerala)	M	Jan 2019	26kg/158cm
79	CLK79	Kochi (Kerala)	M	Jan 2019	67kg/120cm
80	CLK80	Kochi (Kerala)	M	Jan 2019	22kg/123cm
81	CLK81	Kochi (Kerala)	M	Jan 2019	28kg/217cm
82	CLK82	Kochi (Kerala)	M	Jan 2019	19Kg/46 cm
83	CLK83	Kochi (Kerala)	M	Jan 2019	22kg/111cm
84	CLK84	Kochi (Kerala)	M	Jan 2019	50kg/159cm
85	CLK85	Kochi (Kerala)	M	Jan 2019	20 kg/50cm
86	CLK86	Kochi (Kerala)	M	Jan 2019	28kg/212cm
87	CLK87	Kochi (Kerala)	M	Jan 2019	22kg/123cm
88	CLK88	Kochi (Kerala)	M	Jan 2019	26kg/156cm
89	CLK89	Kochi (Kerala)	M	Jan 2019	15Kg/121cm

90	CLK90	Kochi (Kerala)	M	Jan 2019	22kg/123cm
91	CLK91	Kochi (Kerala)	M	Jan 2019	12kg/53cm
92	CLK92	Kochi (Kerala)	M	Jan 2019	72kg/154cm
93	CLK93	Kochi (Kerala)	M	Jan 2019	33kg/138cm
94	CLK94	Kochi (Kerala)	M	Jan 2019	59kg/156cm
95	CLK95	Kochi (Kerala)	M	Jan 2019	20kg/121cm
96	CLK96	Kochi (Kerala)	M	Jan 2019	32kg/111cm
97	CLK97	Kochi (Kerala)	M	Jan 2019	54kg/222cm
98	CLK98	Kochi (Kerala)	M	Jan 2019	56kg/100cm
99	CLK99	Kochi (Kerala)	M	Jan 2019	21Kg/210cm
100	CLK100	Kochi (Kerala)	M	Jan 2019	67kg/120cm
101	CLK101	Kochi (Kerala)	M	Jan 2019	22kg/125cm
102	CLK102	Kochi (Kerala)	M	Jan 2019	26kg/156cm
103	CLK103	Kochi (Kerala)	M	Jan 2019	15Kg/121cm
104	CLK104	Kochi (Kerala)	M	Jan 2019	21Kg/212cm
105	CLK105	Kochi (Kerala)	M	Jan 2019	23Kg/182 cm
106	CLK106	Kochi (Kerala)	M	Jan 2019	22kg/123cm
107	CLK107	Kochi (Kerala)	M	Jan 2019	19Kg/46 cm
108	CLK108	Kochi (Kerala)	M	Jan 2019	28kg/212cm
109	CLK109	Kochi (Kerala)	M	Jan 2019	12kg/53cm
110	CLK110	Kochi (Kerala)	M	Jan 2019	59kg/156cm
111	CLK11	Kochi (Kerala)	M	Jan 2019	32kg/111cm
112	CLK112	Kochi (Kerala)	M	Jan 2019	19Kg/49cm
113	CLK113	Kochi (Kerala)	M	Jan 2019	56kg/100cm
114	CLK114	Kochi (Kerala)	M	Jan 2019	19Kg/48 cm
115	CLK115	Kochi (Kerala)	M	Mar 2019	22kg/111cm

116	CLK116	Kochi (Kerala)	M	Mar 2019	50kg/152cm
117	CLK117	Kochi (Kerala)	M	Mar 2019	20 kg/50cm
118	CLK118	Kochi (Kerala)	M	Mar 2019	52kg/95cm
119	CLK119	Kochi (Kerala)	M	Mar 2019	4 kg/31cm
120	CLK120	Kochi (Kerala)	M	Mar 2019	56kg/100cm
121	CLK121	Kochi (Kerala)	M	Mar 2019	26kg/152cm
122	CLK122	Kochi (Kerala)	M	Mar 2019	19Kg/46 cm
123	CLK123	Kochi (Kerala)	M	Mar 2019	22kg/111cm
124	CLK124	Kochi (Kerala)	M	Mar 2019	50kg/159cm
125	CLK125	Kochi (Kerala)	M	Mar 2019	19Kg/40cm
126	CLK126	Kochi (Kerala)	M	Mar 2019	15Kg/121cm
127	CLM127	Mangalore	M	Nov 2018	21Kg/212cm
128	CLM128	Mangalore	M	Nov 2018	23Kg/182 cm
129	CLT129	Tamil Nadu	M	Nov 2018	5Kg/46cm
130	CLT130	Tamil Nadu	M	Nov 2018	28kg/212cm
131	CLQ131	Kollam (Kerala)	M	Feb 2019	19Kg/46 cm
132	CLQ132	Kollam (Kerala)	F	Feb 2019	28kg/212cm
133	CLQ133	Kollam (Kerala)	M	Feb 2019	26kg/152cm
134	CLQ134	Kollam (Kerala)	F	Feb 2019	33kg/111cm
135	CLQ135	Kollam (Kerala)	M	Feb 2019	67kg/121cm
136	CLQ136	Kollam (Kerala)	F	Feb 2019	22kg/123cm
137	CLQ137	Kollam (Kerala)	M	Feb 2019	26kg/156cm
138	CLQ138	Kollam (Kerala)	F	Feb 2019	15Kg/121cm
139	CLQ139	Kollam (Kerala)	M	Feb 2019	21Kg/212cm
140	CLL140	Lakshadweep	F	Feb 2019	23Kg/182 cm
141	CLL141	Lakshadweep	M	Mar 2019	5Kg/46cm

142	CLL142	Lakshadweep	F	Mar 2019	19Kg/46 cm
143	CLL143	Lakshadweep	M	Mar 2019	22kg/111cm
144	CLL144	Lakshadweep	F	Mar 2019	50kg/152cm
145	CLL145	Lakshadweep	M	Mar 2019	20 kg/50cm
146	CLL146	Lakshadweep	F	Mar 2019	52kg/95cm
147	CLL147	Lakshadweep	M	Mar 2019	4 kg/31cm
148	CLL148	Lakshadweep	F	Mar 2019	56kg/100cm
149	CLL149	Lakshadweep	M	Mar 2019	26kg/152cm
150	CLL150	Lakshadweep	F	Mar 2019	33kg/111cm

4.2 Molecular Data Analysis

Whole genomic DNA of 150 specimens was extracted using "Phenol-chloroform method" (Sambrook & Russel 2001).

Quantity of DNA was estimated through the Nano drop spectrophotometric method. Quantity of the isolated DNA ranged from 188 to 640ng/µl and the average 260/280 values ranged from 1.5 to 2.02.

The extracted DNA was separated using Agarose gel electrophoresis method and the image was shown in Plate 1.

4.3 Mitochondrial Marker Analysis

Isolated DNA was subjected to the PCR reactions with selected primers and after the amplification the PCR product was separated using 1.2% Agarose gel electrophoresis.

The amplification of the COI and D-loop region resulted to a product size of 650 base pairs (shown in Plate 2) and 1300 base pairs (shown in Plate 3).

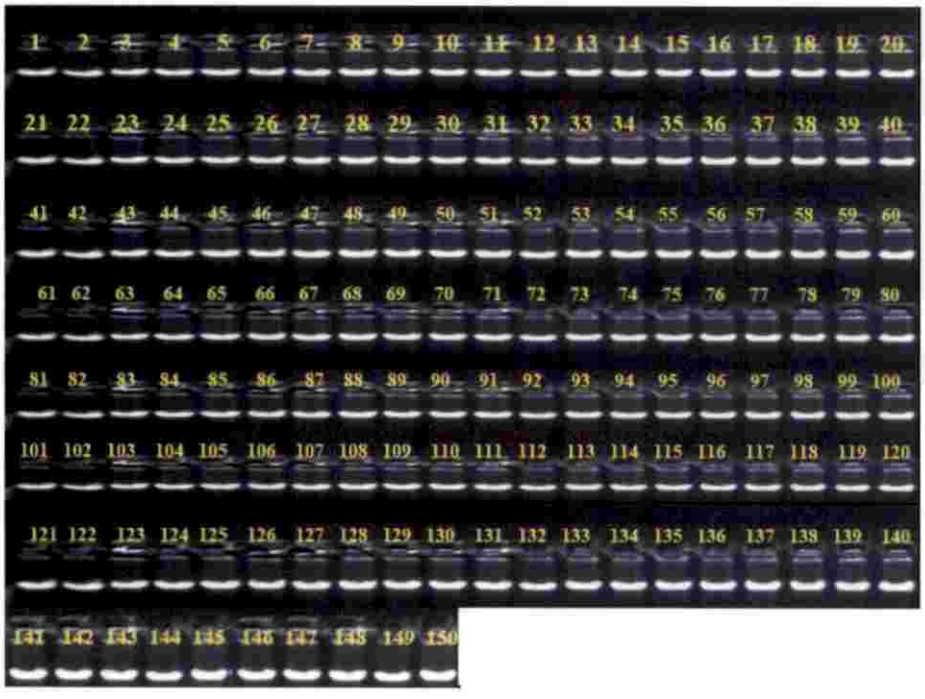

Plate 1: 0.8% Agarose gel image of the genomic DNA isolated from *C. longimanus.*

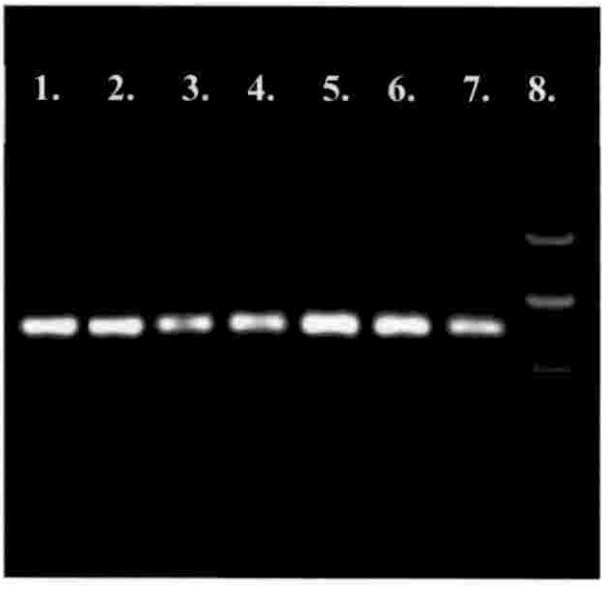

Plate 2: 1.2% Gel image showing the PCR product of COI (Lane 1-7 shows *C. longimanus* samples and Lane 8 – 100 mb Ladder

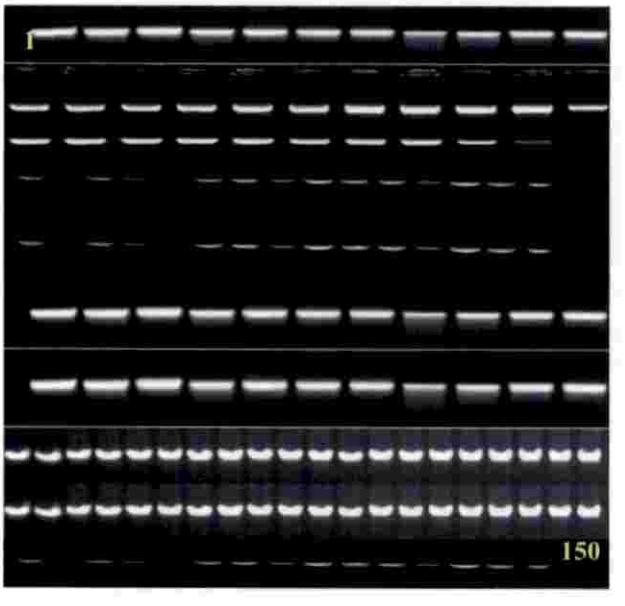

Plate 3: Gel image showing the PCR product of D loop region

[1 -150: PCR products of D-loop regions]

3.4 Phylogenetic Analysis

The evolutionary history was inferred by using the Maximum Likelihood method and Kimura 2-parameter model (Kimura, 1980). The tree with the highest log likelihood (-5887.30) is shown in figure 4.

The phylogenetic tree generated with the COI sequences from the collected samples (represented as *Carcharhinus longimanus* 1,2,3,4,5) and the Gen bank deposited sequence of the desired species (TH1) observed to be in a single clade which reveals that all collected samples belongs to the desired species.

The tree is drawn to scale with branch lengths measured in the number of substitutions per site. This analysis involved 77 nucleotide sequences. There were a total of 641 positions in the final dataset. From the phylogenetic tree, we found that the *C. longimanus* shows more similarity towards the species *Carcharhinus galapagenesis* and *Carcharhinus obscurus.*

The genetic distance calculated using the Kimura2 parameter in MEGA software. The collected sample shows a genetic distance of 0.2 with the gene bank deposited sequences which confirm that both the sequences belong to same species. The results of genetic distance calculated shown in Figure 4.

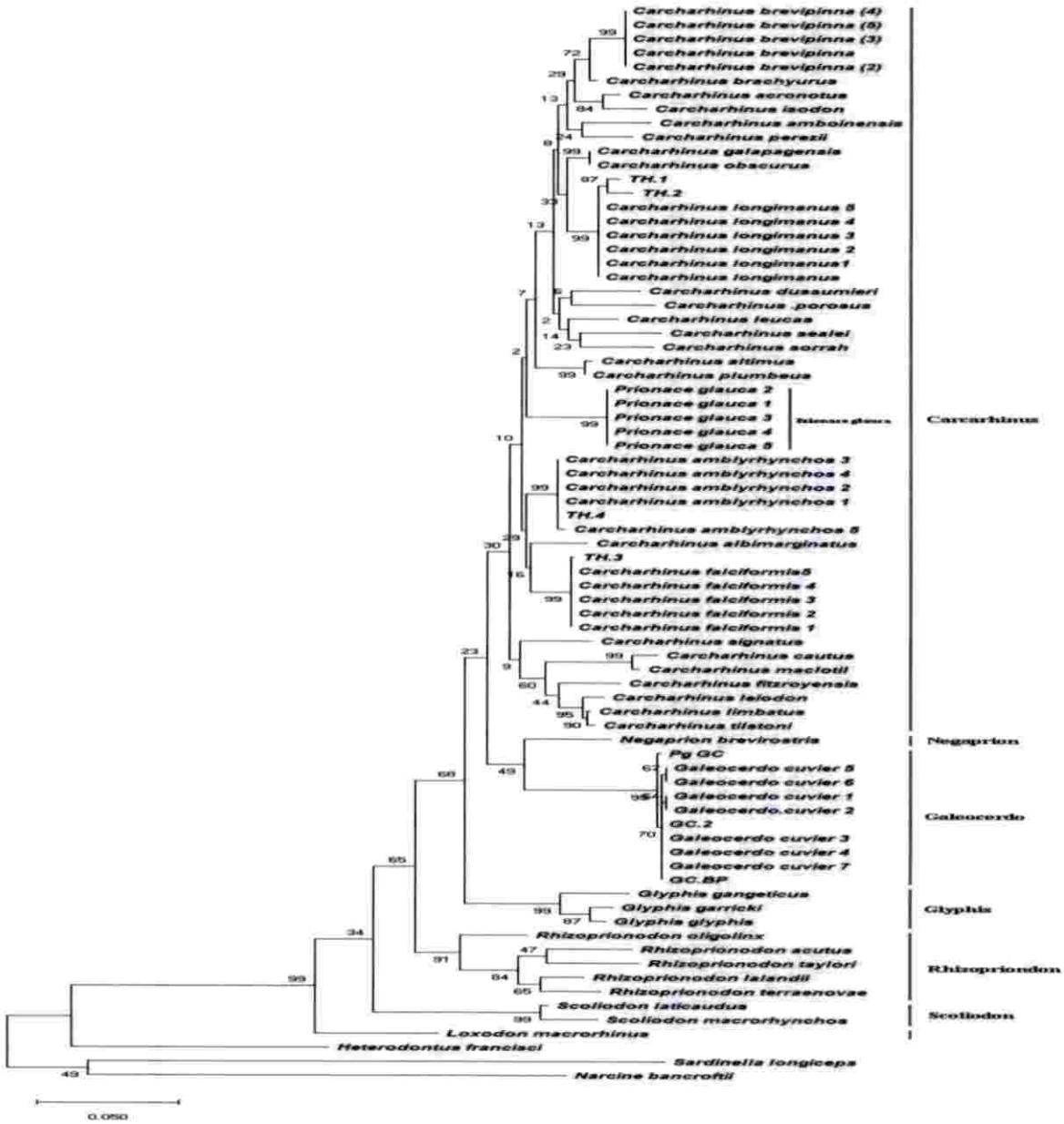

Fig. 4: Phylogenetic tree proving the collected specimens were similar to the desired samples (TH1=*Carcarhinus longimanus*, TH2= *C. longimanus*, TH3= *C. falciformis*, GC= *Galeocerdo cuvier* , Pg. GC=*Galeocerdo cuvier*)

4.5 Population genetic analysis with mitochondrial control region (D loop)

Among the 150 specimens of *C. longimanus* sequenced, we obtained sequences ranging from 720 to 980bp in length. After alignment and trimming, we got dataset with common sequences of 720 base pairs.

In the overall polymorphic analysis using the DNA sequence polymorphism (DnaSP), we found three major haplotypes. H1, H3& H5 were the most commonly found and almost representing majority of individuals with an overall haplotypic diversity (Hd) of 0.718 and nucleotypic diversity (π) of 0.00168. The basic statistical values were mentioned in Table 9.

Table 9. Sample details and the sequence characters of the Mitochondrial control regions.

SI No	Sampling locations	Total no. of samples	Total No. of haplotypes	Haplotype diversity	Nucleotype diversity
1.	Kochi	126	17	0.708	0.0016
2.	Tamil Nadu	4	4	1.00	0.003
3.	Kollam	10	6	0.88	0.002
4.	Lakshadweep	10	3	0.37	0.0005

We found 12 polymorphic sites yielding 13 haplotypes. The obtained haplotypes were in almost all regions which substantiates the interbreed of individuals among the populations which is shown in figure 6. The respective haplotypes and their sequences are shown in figure 7.

Genetic differentiation among the populations of *C. longimanus* from Indian Ocean were tested using the Φst pairwise difference comparisons in the control region sequences and the obtained Φst values are shown in Table 10.

The results reveal a non-significant statistical analysis after the Bonferroni correction with these set of genes.

Table 10. Pairwise Φst values using control regions of samples from different locations

	Kochi	Tamil Nadu	Kollam
Kochi	0.00	-	-
Tamil Nadu	0.13*	0.00	-
Kollam	-0.03	0.03	0.00

* indicates P> 0.05 at Bonferroni correction

Estimated the genetic differentiation by pairwise nucleotide difference using the F- Statistics showed a Fixation index (Φst) of 0.13 [Kochi & Tamil Nadu], 0.03 [Kochi &Kollam] and 0.03 [Tamil Nadu &Kochi]. The P values associated to these results were greater than the significant value i.e., 0.05.

AMOVA test was conducted to confirm the results of pairwise differences and hence partitioned the molecular variance as among and within the populations was listed in the Table 11.

The pairwise difference within the population and among the population shows a % of variance as 97.92% and 2.08% with an Φst value of 0.02080 and estimated p value as 0.26002+-0.0174 were the significant limit of p value as $p < 0.05$.

Table11. Details of AMOVA carried out in the mitochondrial regions.

Samples	Observed values			
Source of samples	Variance	% total	Φst	P
Among the population	0.008	2.08	0.02080	0.26 (P > 0.05)
Within the population	0.39	97.92		

Mismatch distribution analysis indicated signals of population expansion as the graph was uni-modal shown in figure 8. This findings was corroborated by negative Tajima' D values (-1.68) and the Fu-Fs test values (-16.33).

In addition to these, historical demographic studies using Bayesian skyline plot revealed a slowly expanding population historically followed by a recent decline as in Figure 9.

Shared haplotypes were found in all regions of Indian Ocean which evident that there were no specifically isolated populations of *C. longimanus* among Indian Oceanic regions. They exhibit a heavy gene flow and migration among the populations.

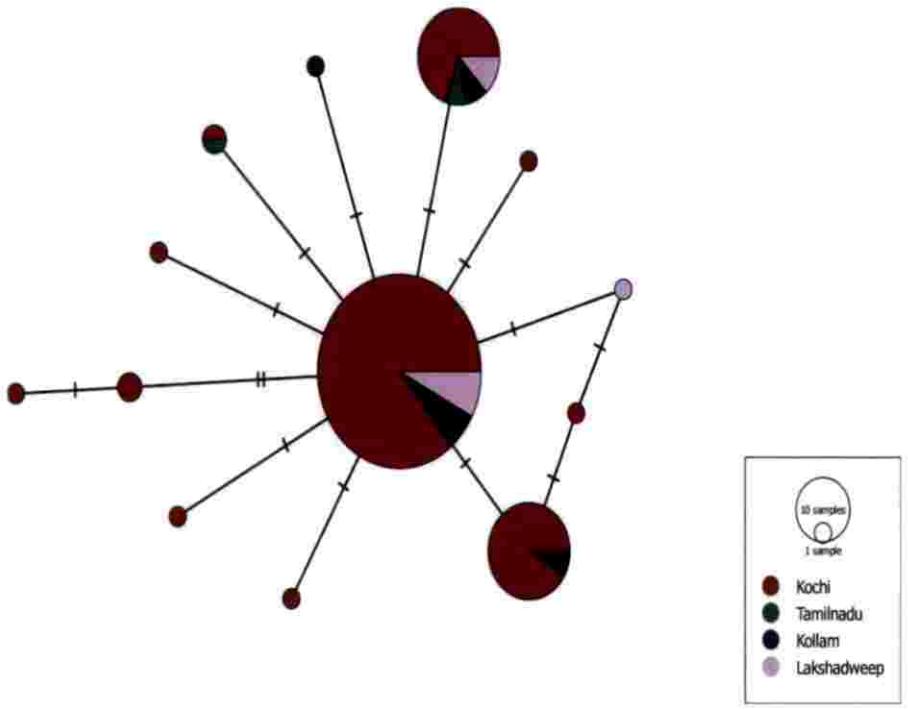

Fig. 5: Medium joining network diagram using the control regions.

	Absolute Position											
1	Hap 1	a	g	c	t	t	a	a	g	a	t	a	t
2	Hap 2	.	.	.	.	c	.	.	.	.	.	.	.
3	Hap 3	.	.	t	.	.	.	.	.	.	.	.	.
4	Hap 4	.	.	.	.	.	g	g	t	.	.	.	.
5	Hap 5	.	.	.	c	.	.	.	.	.	.	.	.
6	Hap 6	.	.	.	.	.	.	.	.	.	.	g	.
7	Hap 7	.	.	t	.	.	.	.	.	.	c	.	.
8	Hap 8	.	.	.	.	.	.	.	.	.	c	.	.
9	Hap 9	.	.	.	.	.	.	.	.	g	.	.	.
10	Hap 10	.	.	.	.	.	g	g	.	.	.	.	.
11	Hap 11	.	.	.	.	.	.	.	.	.	.	.	a
12	Hap 12	.	a	.	.	.	.	.	.	.	.	.	.
13	Hap 13	c	.	.	.	.	.	.	.	.	.	.	-

Fig. 6 Haplotypes with their respective polymorphic sites

4.5.1Population size analysis graphical representations

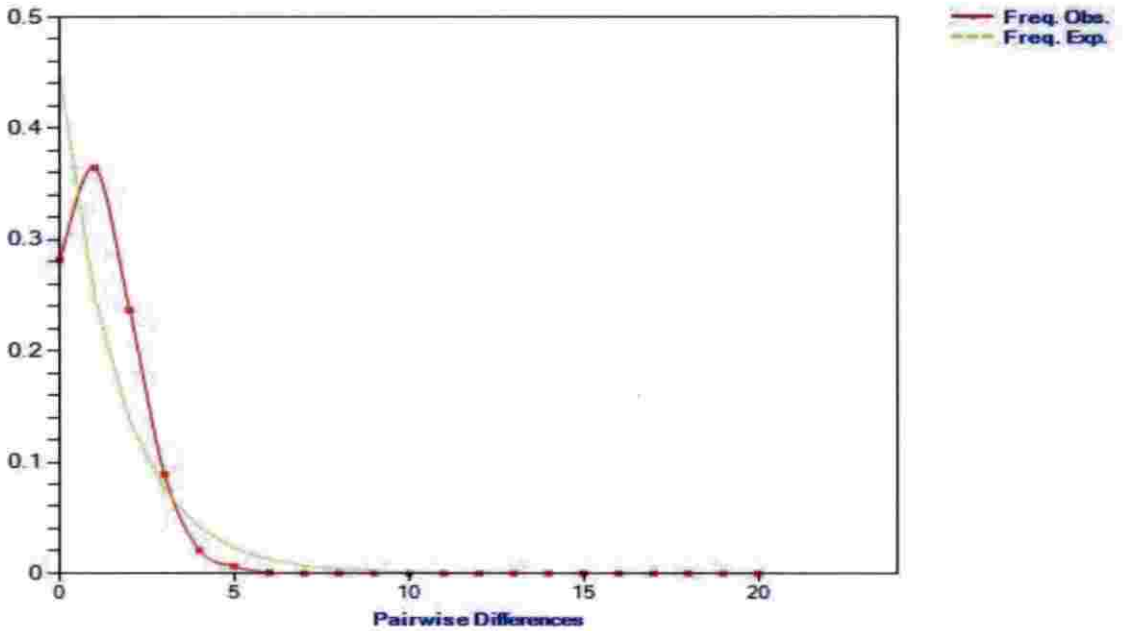

Fig.7: The mismatch distribution analysis generated a unimodal functional graph.

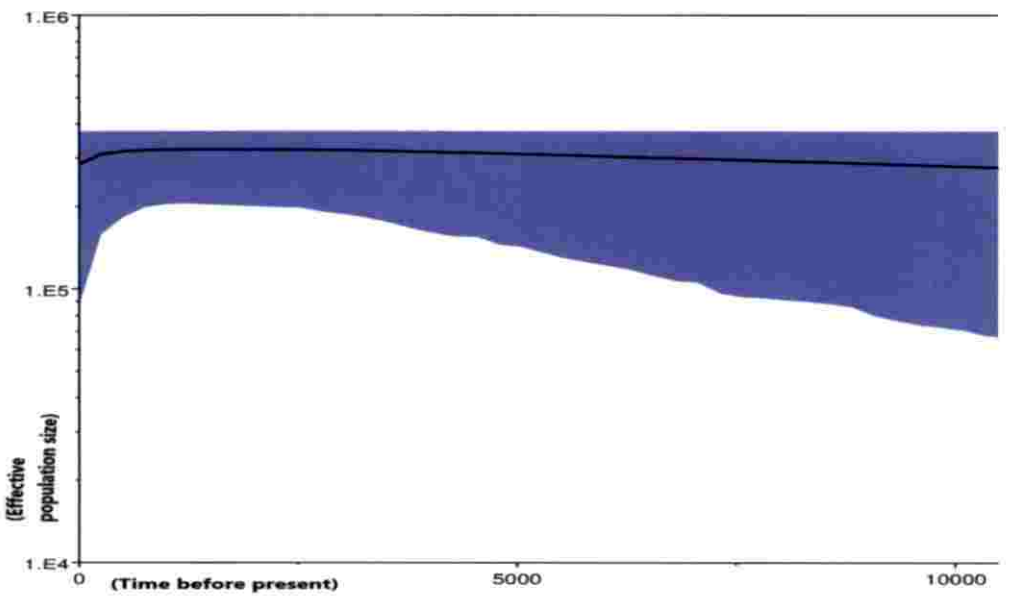

Fig. 8: Population size determination using the Bayesian skyline plot analysis method.

4.5.1 Comparison between Atlantic Ocean haplotypes to understand global genetic structure of *C. longimanus*.

In a previous study by Camargo *et al.*, (2015), they obtained 12 haplotypes with 9 polymorphic sites from various regions of East- West Atlantic Ocean and Indian Ocean. 4 haplotypes from the 9 samples were found in the previous study while the present study viewed only 13 haplotypes from the 150 specimens collected from various regions of Indian Ocean. In accordance to the study by Camargo *et al.*, 2016, the pairwise Φst value was estimated among the populations of inter oceanic regions which show non-significant genetic differentiation as listed in Table 12.

Table 12. Pairwise Φst values of samples using control regions from different locations of Indian Ocean and the deposited haplotypes from Atlantic Ocean.

	Kochi	Tamil Nadu	Kollam	Lakshadweep	West Atlantic Ocean	East Atlantic Ocean	Indian S1
Kochi	0.000	-	-	-	-	-	-
Tamil Nadu	0.1320*	0.000	-	-	-	-	-
Kollam	-0.0325	0.031*	0.000	-	-	-	-
Lakshadweep	0.132*	0.333*	0.031	0.000	-	-	-
West Atlantic Ocean	0.183*	0.022	0.030	0.022	0.000	-	-
East Atlantic Ocean	-0.030	0.066*	0.113*	0.066*	0.065*	0.000	-
Indian S1	0.409*	0.055*	0.209*	0.055*	0.105*	0.054*	0.000

* indicates P> 0.05 at Bonferroni correction

Three major haplotypes were observed from Indian Ocean in the present study H1, H3 & H5 and the other independent haplotypes which had diverged from the majorly found groups with least nucleotide differences.

In the study given by Camargo *et al.*, in various regions of Atlantic Ocean, they observed 4 major groups and some diverged haplotype groups. The lower genetic differences observed between individuals collected from different regions. A comparative statistical parsimony haplotype network tree was also generated with the haplotypes of both the Indian Ocean and Atlantic Ocean regions as shown in Figure 10.

Many haplotypes were shared between Indian and Atlantic oceans whereas some unique haplotypes were observed in West Atlantic and the Indian Ocean. The minor haplotypes were diverged from the major ones usually by a single nucleotide difference. The minor haplotypes were diverged from the major ones usually by a single nucleotide difference. The respective network diagram was shown in Figure 11.

Global Φst was significant with the $P<0.05$ ($P=0.009$) which may be due to the partitions observed by Camargo between East and West Atlantic Ocean. The Φst values were not significant ($P>0.05$) which substantiates the migration of individuals between inter oceanic regions. Comparisons of Indian Ocean and Atlantic Ocean sequences (Camargo *et al.*, 2016) also revealed absence of subpopulation structure between Indian and East Atlantic ocean samples with insignificant pairwise Φst value ($P>0.05$). On the contrary, significant pairwise Φst value ($p<0.05$) was observed between Indian and West Atlantic Ocean samples.

AMOVA test was conducted to confirm the results of pairwise differences and hence partitioned the molecular variance as among and within the populations was shown in Table 13.The pairwise difference within the population and among the population shows a % of variance as 74.04% and 5.5% with an Φst value of 0.09 and estimated p value as 0.007 were the significant limit of p value as $p < 0.05$.

The findings were further corroborated by AMOVA analysis as significant Φst values were observed in one, two and three gene pool comparisons due to the differentiation of West Atlantic Ocean samples from all the other samples. Within the Atlantic Ocean significant genetic differentiation was observed between East and West Atlantic Ocean by Camargo *et al.*, (2016) which may be the reason for significant global Øst in the present study in comparisons with NCBI data.

The results show some moderate genetic differentiation among the populations. Due to the heavy migration and interbreeding reveal that the entire population belongs to a single stock. Only a small isolation is there in between the populations of East-West Atlantic Regions. In the haplotype network diagram, there are four major haplotype groups separated by one or two mutations.

The haplotypes were shared between Indian Ocean, West Atlantic and East Atlantic Ocean. A star like phylogeny indicates signals of population expansion which had happened historically. Along the haplotypes obtained from the study, we only observed the nucleotide substitutions.

The different groupings of the haplotypes found in this examination, were stored in Gene-Bank with Accession numbers shown in Table 13.

Table13. Details showing the AMOVA carried out in the haplotypes from Indian and Atlantic Ocean.

Structure tested	Observed partition			
	Variance	% total	Ø statistics	P (after Bonferroni correction)
1.One gene pool (Indian Ocean, East Atlantic , West Atlantic)				
Among populations	**0.04**	**9.6**	$Ø_{ST}$:0.09	**0.007**
Within populations	**0.45**	**90.4**		
2. Two gene pools (Indian Ocean) (East Atlantic, West Atlantic)				
Among group	**1**	**1.3**		
Within group	**5**	**4.2**	$Ø_{ST}$:0.094	**0.009**
Within populations	**162**	**74.3**		
3. Three gene pools (Indian Ocean) (East Atlantic) (West Atlantic)				
Among group	**10.47**	**0.056**	$Ø_{ST}$:0.15	**0.008**
Within group	**4.62**	**0.024**	$Ø_{Sc}$:0.05	**0.131**
Within populations	**84.91**	**0.456**	$Ø_{CT}$:0.10	**0.239**

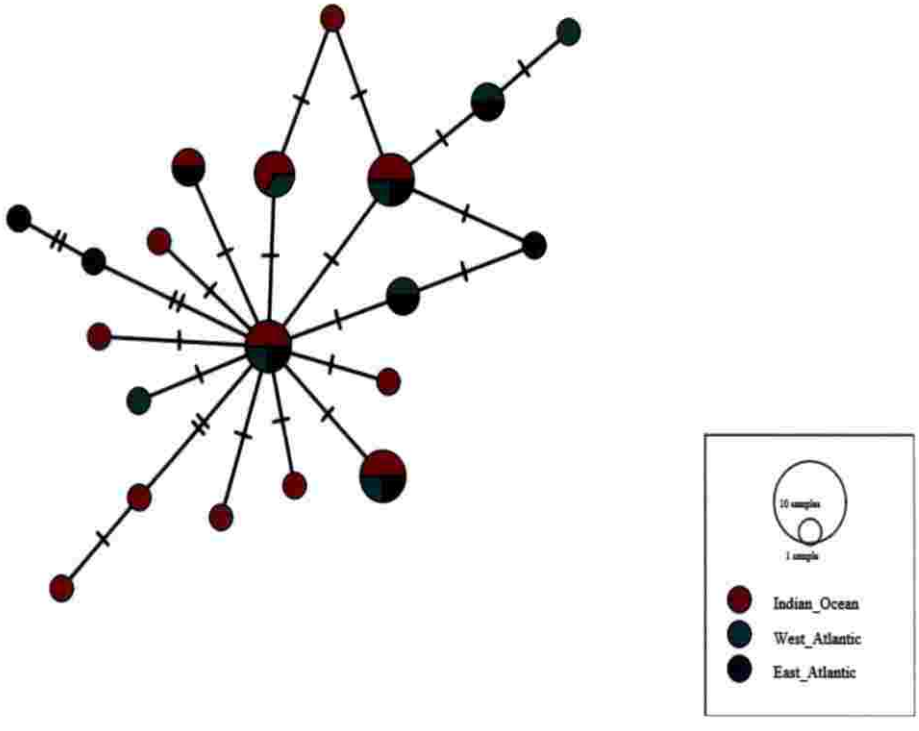

Fig. 9: Medium joining network diagram using the haplotype sequences of Atlantic Ocean along with the samples from Indian Ocean.

DISCUSSION

5. DISCUSSION

Studies on population genetic structure of *C. longimanus* from Indian Ocean region indicated lack of significant genetic differentiation when partial control region sequences were analysed pointing towards substantial gene flow and migration within Indian Ocean. Comparisons across oceanic basins, mainly between Indian and Atlantic oceans (based on sequences deposited in NCBI, GenBank) also indicated lack of significant sub-structuring indicating the potential of these sharks for inter-oceanic migrations.

A gradual expansion in effective population size starting from the Pleistocene epoch until the late Holocene followed by a recent decline was evident in Bayesian skyline plot which calls for efficient management measures to protect this species from further decline and extinction. The reasons for the recent decline may be habitat degradation and over exploitation. Haplotype network diagram also corroborated the findings of lack genetic differentiation as shared haplotypes were present on all locations of Indian and Atlantic Ocean.

The oceanic white tip shark (*Carcharhinus longimanus*) is one of the most basic top predators in open waters of every tropical sea of the world (Nakano, 2008). Regardless of its overall dispersion and regular appearance in most oceans in tropical zones, little consideration has been paid to the life history of oceanic white tip sharks. Since Bigelow and Schroeder (1948) called attention to that "incredibly little is known about its habitat, taking into account that it is one among the Carcharhinidae family that has been perceived the longest," just a bunch of papers have concentrated on the studies about this shark. Studies by Backus *et al.*, (1956) in the western North Atlantic and Strasburg (1958) in the eastern Pacific Ocean mainly concentrate on the population structuring, distribution, biological nature and its reproductory behaviour. The oceanic white tip shark is a tropical, epipelagic shark seen

from the surface to the depth of 152 m only. It has an unmistakable inclination for open sea waters (Backus *et al.*, 1956; Strasburg, 1958; Compagno, 1984). In spite of the fact that it tends to be found in waters somewhere in the range of 15°C and 28°C, it is also found in waters with temperatures above 28°C. It is one of the most plenteous maritime sharks. The information regarding the migratory behaviour of these sharks is very little. Backus *et al.*, 1956 reported the movement of these sharks towards the Gulf of Mexico regions during the winter times and return when the temperature changes.

These sharks are the major predators of the open waters mainly feeding on teleosts and cephalopods (Backus *et al.*, 1956). They belong to the viviparous group with embryonic placental development (Seki *et al.*, 2008) which signifies that they follow philopatry in shark behaviour and that may got affected with the habitat degradation. Shared haplotypes were observed between the populations mainly due to the heavy migratory behaviour of the species between the populations of different regions. Haplotypes mixing were evident in all the locations of Indian oceans and the Atlantic regions. In a previous study by Camargo *et al.*, 2016, significant genetic divergence was observed between the populations of East and West Atlantic Oceanic regions which were attributed to difference in oceanic parameters between the two regions, while in our study we got non-significant genetic differentiation between the populations. So within Indian Ocean *C. longimanus* can be assumed to move freely. Bayesian skyline plot indicated a recent decline in populations of *C. longimanus* which may be due to habitat alterations, overfishing and climate change. Sharks are characterized by a life history of slow growth, late maturity, and low fecundity. They are extremely vulnerable to overexploitation and have low population resilience to over fishing. Sharks have been increasingly exploited in recent years (Bineesh *et al.*, 2017). Over fishing led to exploitation of oceanic and coastal sharks (Naylor, 1992). Generally, sharks are caught by trawling, long lining, gill netting *etc.* Shark finning practices use shark resources and speed up the crumple of shark

population. Even though shark finning has been banned in many countries, illegal shark finning seems to continue at an alarming rates (Heithaus *et al.*, 2001; Pank *et al.*, 2001). Many programms are being initiated to recover and protect this group through sustainable management plans (Sembiring *et al.*, 2015).

Studies on population genetic structure of *C. longimanus* from Indian Ocean region indicated lack of significant genetic differentiation when partial control region sequences were analysed pointing towards substantial gene flow and migration within Indian Ocean. Comparisons across oceanic basins, mainly between Indian and Atlantic oceans (based on sequences deposited in NCBI, GenBank) also indicated lack of significant substructuring between Indian Ocean and East Atlantic oceans indicating the potential of these sharks for inter-oceanic migrations. Whilst, significant genetic differentiation was observed between Indian ocean and West Atlantic ocean which may be due to absence of gene flow between these regions. A gradual expansion in effective population size starting from the Pleistocene epoch until the late Holocene followed by a recent decline was evident in Bayesian skyline plot which calls for efficient management measures to protect this species from further decline and extinction. The reasons for the recent decline may be habitat degradation and over exploitation. Haplotype network diagram also corroborated the findings of lack of genetic differentiation within Indian Ocean as well as between Indian and East Atlantic oceans as shared haplotypes were present.

The lack of significant genetic differentiation within Indian Ocean and between Indian and East Atlantic Oceans indicated the capacity of oceanic white tip sharks for long distance migration and mixing. Shared haplotypes were observed between the populations mainly due to the heavy migratory behaviour of the species between the populations of different regions. Camargo *et al.*, 2016 observed significant genetic differentiation between East and West Atlantic regions in control region sequences which was attributed to

natal homing or Philopatry as mitochondrial genes are maternally inherited. In spite of that, a lack of significant structuring pattern was evident between East Atlantic and Indian Ocean region even though only 9 samples were analysed. The present study using large number of samples from the Indian Ocean region corroborated these findings which indicate that sharks move between these regions.

The recent decline in effective population size in Bayesian skyline plots is a cause for concern and it calls for urgent management and conservation measures. The reasons for this decline may be habitat alterations, overfishing and climate change. The oceanic white tip shark (*Carcharhinus longimanus*) is one of the most basic top predators in open waters of every tropical sea of the world (Nakano *et al.,* 1996; Bonfil *et al.*, 2008). Population structuring, distribution, biology and reproductive behaviour of these sharks from western North Atlantic (Backus *et al.*, 1956) and Eastern pacific (Strasburg *et al.*, 1958) have been studied. The oceanic white tip shark is considered as a tropical, epipelagic shark occurring from the surface to a depth of approximately 150 m. It has an unmistakable inclination for open sea waters (Matsunaga *et al.*, 2005). Even though the preferred range of temperature is between 15°C and 28°C, it is also found in waters with temperatures above 28°C. They feed mainly on teleosts and cephalopods (Backus *et al.*, 1956) and are viviparous with embryonic placental development (Seki *et al.*, 1998). Life history traits like viviparity make them vulnerable to overfishing. International Union of Conservation of Nature (IUCN) has characterized the greater part (58%) of this species as "threatened" for extinction. In addition to targeted fishing practices like angling, hook and line fishing, sharks are also landed as by catch in major gears like trawls, gill nets and purse seines. So in order to prevent shark population decline, it is also important to reduce by catch by devising some by catch reduction devices which can selectively remove some of the fished sharks. Shark finning practices also speed up the crumple of shark population. Even though shark

finning has been banned in many countries including India, illegal shark finning seems to continue at an alarming rate (Pank *et al.*, 2001; Greig *et al.*, 2005).

In demographic analysis studies to estimate the current scenario of the population size in the targeted regions which is very much important to sustain that current population size by implementing any measures of conservation. The present examination demonstrates a chart with declining population of these species in the Indian Coast which as of now uncovers an overwhelming gene flow within the individuals taking all things together over oceanic locales. For whatever length of time that the specimen *Carcharhinus longimanus* is by all accounts a "vulnerable" species by the IUCN red list (Sharks are considered as top predators and excessive fishing will upset the predator prey relationships or in other words the "trophic relationships". This subsequently impacts the marine ecosystems adversely. It is difficult to predict the impact of excessive shark removals on oceanic ecosystems due to the complex nature of marine ecosystems (Ferretti *et al.*, 2010).

Similar to the present investigation, studies on catch rate of oceanic white tips in the Northwest Atlantic and Gulf of Mexico also proposed a solid pattern of declining population. In the Northwest Atlantic, pelagic long line catch rates for *C. longimanus* demonstrated a 70% decline from 1992 to 2000 in spite of the fact that such patterns are more difficult to decipher for maritime shark species on the grounds that their environments vary widely (Baum *et al.*, 2003). In the previous period, the oceanic whitetip was the most well-known shark, representing 61% of every single snared shark. International Union of Conservation of Nature (IUCN) has characterized the greater part (58%) of this species as "threatened" for extinction. In addition to targeted fishing practices like angling and hook and line fishing, sharks are also landed as by-catch in major gears like trawls, gill nets and purse seines. So in order to prevent shark population decline, it is also important to reduce

by-catch by devising some by-catch reduction devices which can selectively remove some of the fished sharks. In addition to the reduced genetic variability among the species in the areas and the current declining population size might be focused to the extinction of the species. These low genetic flow rates found to a sensational hazard to the adapting capability of the species and prompting a flimsier capacity of the species which react to natural changes, and consequently could advance elimination of certain ancestries. So as to anticipate further population decreases, we recommend that worldwide management of all haplotypes through global participation, and especially for the two particular populations of oceanic white tips distinguished in both the Indian and Atlantic regions.

Recent reports indicate that catch rate of this species has declined by around 99%. In spite of contrasts in the operational arrangements, the authors inferred that this species is in peril of extinction (Ramon *et al.*, 2009).Habitat degradation, pollution, overexploitation and restricted migration may somewhat *lead* to the species population decline.

The present study was the first attempt to understand the dynamics of oceanic white tip shark, *Carcharhinus longimanus* in the Indian Ocean as well as its relationship with populations of Atlantic Ocean. Vital insights were gained from this study indicating lack of significant substructuring and its capability to migrate across large expanses of Open Ocean. The capability to migrate may provide it with some buffering against habitat loss and climate change, but excessive fishing is a danger to its populations. Globally sharks are in danger due to their inherent vulnerabilities like long gestation time and reduced number of offsprings coupled with over fishing. Our study also corroborated the findings of shark decline, as decline in genetic diversity is an indicator of decrease in resilience capacity. The present study calls for restrictions on its fishery so that populations will get sufficient time to

replenish and consequently their resilience is ensured in the face of changing oceans.

Globally sharks are in danger due to their inherent vulnerabilities like long gestation time and reduced number of off springs coupled with over fishing. Our study also corroborated the findings of shark decline, as decline in genetic diversity is an indicator of decrease in resilience capacity. The present study calls for restrictions on its fishery so that populations will get sufficient time to replenish and consequently their resilience is ensured in the face of changing oceans.

SUMMARY

6. SUMMARY

Carcharhinus longimanus is probably the most migratory species among the sharks in various oceanic regions (Camargo *et al.*, 2016). It belongs to the Carcharhinidae family of sharks with a world-wide importance. The International Union for Conservation of Nature (IUCN), 'Red list of Threatened Species' showed almost 30% of all sharks as "threatened" or "near threatened" with a near extinction risk (Dulvy *et al.*, 2014). IUCN considering the oceanic white tip sharks as Vulnerable globally (assessed in 2006) Critically Endangered Western North and Central Atlantic, where long term declines up to 99% and recent declines of 60-70% are reported. A 90% decline is reported in Central Pacific Ocean.

Information regarding the stock structure of the oceanic white tip shark in the Indian Ocean is not available. They are observed to undertake long distance movement ranging from the Mozambique Channel to the Somali Basin and the Southern Indian Ocean. They are highly migratory in nature. To recognize geological distributions and fundamental genetic attributes of detached population is a fundamental requirement for the logical management and protection of species in a particular region. Despite the fact that the Oceanic White tip sharks are the larger sharks it feels as difficult for analysing the population structure in the interoceanic scales using the mitochondrial markers due to its high migratory nature. In the Indian Ocean, the absence of structure might be the after effect of the mix of high capability of movement and the absence of compelling hindrances in the regions.

Here we provide evidence by saying that all the specimens collected from the different regions belongs to a similar stock due to the heavy migratory nature. In spite of their exceedingly transient nature, obstructions to the quality progression of oceanic white tips in the Atlantic outcome in two

hereditarily unmistakable and demographically free population (Camargo *et al.*,2016). This structure ought to be joined into evaluations and checking of this species. The elements that confine gene flow in the Atlantic might be present in between the east Atlantic and parts of the Indian Ocean, as there gives off an impression of being network between these all locales. In addition to the reduced genetic variability among the species in the areas and the current declining population size might be focused to the extinction of the species. These low genetic flow rates found to a sensational hazard to the adapting capability of the species and prompting a flimsier capacity of the species which react to natural changes, and consequently could advance elimination of certain ancestries. So as to anticipate further population decreases, we recommend that worldwide management of all haplotypes through global participation, and especially for the two particular populations of oceanic white tips distinguished in both the Indian and Atlantic regions.

The outcomes together with the rate of gene flow, shared incessant haplotypes, and comparable genetically assorted variety and population parameters among population gatherings support the idea of an absence or incredibly weak genetic separation of the Indo-Atlantic shark collection that is scarcely recognizable at the degree of haplotype recurrence. Here, we found a result in AMOVA as the entire population while considering all as one population shows a non-significant P value which shows the absence of subdivisions in the group. While comparing the Indian and Atlantic species in AMOVA resulted significance in the entirely grouped model and at the same time the subgroup shows non-significance.

Finally, we concluded that the entire populations of oceanic white tip sharks in the interoceanic regions were belongs to a similar group with low genetic differentiation among the population and hence we can say that the all belongs to a single stock.

REFERENCES

7. REFERENCES

Achanta, G., Sasaki, R., Feng, L., Carew, J. S., Lu, W., Pelicano, H., Keating, M. J. And Huang, P. 2005. Novel role of p53 in maintaining mitochondrial genetic stability through interaction with DNA Pol γ. *The EMBO J. 24*(19):3482-3492.

Akhilesh, K. V., Purushottama, G. B., and Thakurdas, S. J. 2013. Catch composition, reproductive biology and diet of the bramble shark Echinorhinus brucus (Squaliformes: Echinorhinidae) from the south-eastern Arabian Sea. *J. of Fish Biol.* 83(5):1112–1127.

Alam, M., Robert, A., Read T., and Alistair D.M. 2014. The complete mitochondrial genome sequence of the world's largest fish, the whale shark (Rhincodon typus), and its comparison with those of related shark species. Gene. Elsevier. 539(1): 44–49.

Almerón-Souza, F., Sperb, C., Castilho, C. L., Figueiredo, P., Gonçalves, L. T., Machado, R., Oliveira, L. R., Valiati, V. H., and Fagundes, N. 2018. Molecular identification of shark meat from local markets in Southern Brazil based on DNA barcoding: Evidence for mislabeling and trade of endangered species. *Frontiers in Genet.* 9:1–12.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D. J. 1990. Basic local alignment search tool. *J. Mol. Biol.* 6:5-7.

Alves, M.J., Coelho, H., Collares-Pereira, M.J. and Coelho, M.M. 2001. Mitochondrial DNA variation in the highly endangered cyprinid fish Anaecypris hispanica. Importance for Conserv. Heredity. 87:463-473.

Amaral, C. R. L., Pereira, F., Silva, D. A., Amorim, A., & de, Carvalho, E. F. 2018. The mitogenomic phylogeny of the Elasmobranchii (Chondrichthyes). Mitochondrial DNA Part A: DNA Mapping, Sequencing, and Analysis. Informa UK Ltd., 29(6):867–878.

Ambili, T. 2013. Morphological and Molecular Characterization of Genus Tor from Southern Western Ghats, INDIA. Bharathiar University, Coimbatore.

Amos, B., Schlotterer, C. and Tautz, D. 1993. Social structure of pilot whales revealed by analytical DNA profiling. *Sci. 260*(5108):670-672.

Anna, F. 2015. DNA barcoding of freshwater fishes in Matang, Malaysia. Bioinformatics Engineering, Program Uppsala University School of Engineering. 59:45.

Aquilino, V. L., Tango, Jazzlyn., Fontanilla, Ian., Pagulayan, K.C., Roberto C, Basiao., Zubaida U, Ong., Perry, Quilang., Jonas P. 2011. DNA barcoding of the ichthyofauna of Taal Lake, Philippines. *Mol. Ecol. Resour.* 11(4): 612–619.

Asis, A. M. J. M., Lacsamana, J. K. M. and Santos, M. D. 2016. Illegal trade of regulated and protected aquatic species in the Philippines detected by DNA barcoding, Mitochondrial DNA. 27(1):659–666.

Avise, J. 2012. Molecular markers, natural history and evolution.

Avise, J. C. 1994. Molecular Markers, Natural History and Evolution. Springer US.

Avise, J. C. 2000. Phylogeography : the history and formation of species. Harvard University Press.

Avise, J. C. 2000. *Phylogeography: the history and formation of species*. Harvard university press.

Backus, R. H., Springer, S. and Arnold Jr, E. L. 1956. A contribution to the natural history of the white-tip shark, Pterolamiopslongimanus (Poey). *Deep Sea Res. 3*(3):178-188.

Bailey, R. M. 2006. Distribution of the American Cyprinid Fish Hybognathus hankinsoni with Comments on Its Original Description. Copeia. 1954(4):289.

Balanyá, J., Oller, J.M., Huey, R.B., Gilchrist, G.W. and Serra, L. 2006. Global genetic change tracks global climate warming in Drosophila subobscura. *Sci. 313*(5794):1773-1775.

Bandelt, H. J., Forster, P. and Röhl, A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. and Evol. 16*(1):37-48.

Barman, A. S., Singh, M. and Pandey, P. K. 2018. DNA barcoding and genetic diversity analyses of fishes of Kaladan River of Indo-Myanmar biodiversity hotspot, Mitochondrial DNA Part A: DNA Mapping, Sequencing, and Analysis. Informa UK Ltd. 29(3):367–378.

Bartlett, S. E., & Davidson, W. S. 1991. Identification of Thunnus tuna species by the polymerase chain reaction and direct sequence analysis of their mitochondrial cytochrome b genes. Canadian *J. of Fish. and Aquatic Sci.* 48:309–317.

Barucca, M., Olmo, E., Schiaparelli, S. and Canapa, A. 2004. Molecular phylogeny of the family Pectinidae (Mollusca: Bivalvia) based on mitochondrial 16S and 12S rRNA genes. *Mol. phylogenetics and Evol. 31*(1):89-95.

Barucca, Marco., Olmo, Ettore., Schiaparelli, Stefano., Canapa, Adriana. 2004. Molecular phylogeny of the family Pectinidae (Mollusca : Bivalvia) based on mitochondrial 16S and 12S rRNA genes. 31:89–95.

Basheer, V. S., Mohitha, C., Vineesh, Divya, P. R., Gopalakrishnan, A., Jena, J. K. 2015. Molecular phylogenetics of three species of the genus Rastrelliger using mitochondrial DNA markers. *Mol. Biol.* 42(4):873–879.

Bass, A. J. 1973. Sharks of the east coast of southern Africa. I. The genus Carcharhinus (Carcharhinidae). *Invest Rep. Oceanogr Res. 33*:1-168.

Bass, A. J. 1973. Sharks of the east coast of southern Africa. I. The genus Carcharhinus (Carcharhinidae). *Invest Rep Oceanogr Res Inst. 33*:1-168.

Baum, J. K., Myers, R. A., Kehler, D. G., Worm, B., Harley, S. J. and Doherty, P. A. 2003. Collapse and conservation of shark populations in the Northwest Atlantic. *Sci. 299*(5605):389-392.

Baum, Julia K. Myers., Ransom, A., Kehler, Daniel, G., Worm, Boris., Harley, Shelton J., Doherty, Penny A. 2003. Collapse and conservation of shark populations in the Northwest Atlantic. *Sci.* 12(2):22-32.

Baum, Julia K. Myers., Ransom, A., Kehler, Daniel, G., Worm, Boris., Harley, Shelton J., Doherty, Penny A. 2003. Collapse and conservation of shark populations in the Northwest Atlantic', *Sci.* 299(5605): 389–392.

Beerkircher, L. R., Cortés, E. and Shivji, M. 2002. Characteristics of shark bycatch observed on pelagic longlines off the southeastern United States, 1992–2000. *Marine Fish. 64*(4):40-49.

Begg, G.A. and Waldman, J.R. 1999.An holistic approach to fish stock identification. *Fish. Res. 43*(1-3):35-44.

Bendezu, I.F., John, W.S. and Brian, F. C. 2005. Identification of Mytilus spp. and Pecten maximus in Irish Waters by Standard PCR of the 18S rDNA Gene and Multiplex PCR of the 16S rDNA Gene. *Marine Biotechnol.* 7(6): 687-696.

Bernard, A. M., Feldheim, K. A., Heithaus, M. R., Wintner, S. P., Wetherbee, B. M. and Shivji, M. S. 2016. Global population genetic dynamics of a highly migratory, apex predator shark. *Mol. Ecol. 25*(21):5312-5329.

Bérubé, M., Aguilar, A., Dendanto, D., Larsen, F., Notarbartolo Di Sciara, G., Sears, R., Sigurjónsson, J., and Palsbøll, P.J. 1998. Population genetic structure of North Atlantic, Mediterranean Sea and Sea of Cortez fin whales, *Balaeno pteraphysalus* (Linnaeus 1758): analysis of mitochondrial and nuclear loci. *Mol. Ecol. 7*(5):585-599.

Bhattacharya, Manojit., Sharma, Ashish Ranjan., Patra, Bidhan Chandra., Sharma, Garima., Seo, Eun-min Nam, Ju-suk ., Chakraborty,

Chiranjib., Lee, Sang-soo. 2015. DNA barcoding to fishes : current status and future directions, Mitochondrial DNA. Informa UK Ltd. 8(2):1–9.

Bigelow, H. B. and Schroeder, W. C. 1953. *Fishes of the Gulf of Maine.* 53:588

Bigelow, H. B., 6. W. C. Schroeder. 1948. Sharks. *Fishes of the Western North Atlantic, Part One, HB Bigelow, WC Schroeder, and IP Farfante(eds.). Mem. Sears Found. Mar. Res.* 1:1-12.

Bineesh, K. K., Gopalakrishnan, A., Akhilesh, K. V., Sajeela, K. A., Abdussamad, E. M., Pillai, N. G. K., Basheer, V. S., Jena, J. K. and Ward, R. D. 2017. DNA barcoding reveals species composition of sharks and rays in the Indian commercial fishery. *Mitochondrial Dna Part A. 28*(4):458-472.

Bineesh, K. K., Gopalakrishnan, A., Akhilesh, K. V., Sajeela, K. A., Abdussamad, E. M., Pillai, N. G. K., Basheer, V. S., Jena, J. K. and Ward, R. D. 2017. DNA barcoding reveals species composition of sharks and rays in the Indian commercial fishery. *Mitochondrial Dna Part A. 28*(4):458-472.

Bleeker. 1856. *Carcharhinus amblyrhynchos*, Blacktail reef shark : fisheries, gamefish. https://www.fishbase.se/summary/861, 20 July 2019.

Bone, Q., Marshall, N. B., & Blaxter, J. H. S. L. B. 1995. Biology of fishes. 2:4-6.

Bonfil, R., Clarke, S. and Nakano, H. 2008. The biology and ecology of the oceanic whitetip shark, *Carcharhinus longimanus. Sharks of the Open Ocean: Biology, Fish. and Conserv.* 5(8):128-139.

Bonin, A., Ehrich, D. and Manel, S. 2007. Statistical analysis of amplified fragment length polymorphism data: a toolbox for molecular ecologists and evolutionists. *Mol. Ecol. 16*(18):3737-3758.

Bonin, A., Ehrich, D. and Manel, S. 2007. Statistical analysis of amplified fragment length polymorphism data: a toolbox for molecular ecologists and evolutionists. *Mol. Ecol. 16*(18):3737-3758.

Botti, S. and Giuffra, E. 2010. Oligonucleotide indexing of DNA barcodes: Identification of tuna and other scombrid species in food products. *BMC Biotechnol.* 10:8-15.

Bowen, B. W., Kamezaki, N., Limpus, C. J., Hughes, G. R., Meylan, A. B. and Avise, J. C. 1994. Global phylogeography of the loggerhead turtle (Carettacaretta) as indicated by mitochondrial DNA haplotypes. *Evol. 48*(6):1820-1828.

Brooks, T. M., Mittermeier, R. A., da Fonseca, G. A., Gerlach, J., Hoffmann, M., Lamoreux, J. F., Mittermeier, C. G., Pilgrim, J. D. and Rodrigues, A. S. 2006. Global biodiversity conservation priorities. *Sci. 313*(5783):58-61.

Brown, J. W., Miller, S. E., & Horak, M. 2003. Description of a new species of Xenothictis Meyrick (Lepidoptera: Tortricidae: Archipini). 105(4): 1043-1055.

Brown, W. M., George, M. and Wilson, A. C. 1979. Rapid evolution of animal mitochondrial DNA. 76(4):1967–1971.

Buhay, J. E. 2009. Sequences are becoming problematic in molecular systematic and barcoding studies. *J. of Crustacean Biol.* 29(1):96-110.

Burt, A., and Carter, D. 1996. Molecular markers reveal cryptic sex in the human pathogen *Coccidioides immitis*. National Acad. *Sci.*https://www.pnas.org/content/93/2/770.

Camargo, S. M., Coelho, R., Chapman, D., Howey-Jordan, L., Brooks, E. J., Fernando, D., Mendes, N. J., Hazin, F. H., Oliveira, C., Santos, M. N. and Foresti, F. 2016. Structure and genetic variability of the oceanic whitetip shark, *Carcharhinus longimanus*, determined using mitochondrial DNA. *PloS one. 11*(5):20-39.

Camhi, M. 1998. *Sharks and their relatives: ecology and conservation* (No. 20). IUCN.

Camhi, M., Pikitch, E. K. 2008. Sharks of the Open Ocean: *Biol. Fish. and Conserv.* 5(1):8-12.

Cao, Y., Waddell, P. J., Okada, Norihiro., Hasegawa, Masami. 1998. The Complete Mitochondrial DNA Sequence of the Shark Mustelus manazo : Evaluating Rooting Contradictions to Living Bony Vertebrates. 8(2):1637–1646.

Carr, S. M., Kivlichan,, P. 1999. Molecular systematics of gadid fishes: implications for the biogeographic origins of Pacific species. *Canadian J. of Zool.* 77(1): 19–26.

Carvalho, G. R. and Hauser, L. 1995. Molecular genetics and the stock concept in fisheries. *Mol. Genet. In fish.* 5(2):55-79.

Carvalho, G. R. and Hauser, L. 2004. Molecular genetics and the stock concept in fisheries. *Fish Biol. and Fish.* 4(3):326–350.

Cawthorn, D., Andrew, H. and Witthuhn, R. C. 2012. DNA barcoding reveals a high incidence of fish species misrepresentation and substitution on the South African market. FRIN. *Elsevier.* 46(1):30–40.

Chakraborty, M. and Ghosh, S. K. 2014. An assessment of the DNA barcodes of Indian freshwater fishes. 537:20–28.

Chape, S., Harrison, J., Spalding, M. and Lysenko, I. 2005. Measuring the extent and effectiveness of protected areas as an indicator for meeting global biodiversity targets. *Philosophical Transactions of the Royal Society B: Biological Sci. 360*(1454):4-10.

Chen, H. and Kishino, H. 2015. Global pattern of phylogenetic species composition of shark and its conservation priority. 2(5):22-28.

Chen, X., and Liu, M. 2014. Complete mitochondrial genome of the critically endangered speartooth shark *Glyphis glyphis* (Carcharhiniformes: Carcharhinidae), Mitochondrial DNA. 25(6):431–432.

Chen, X., and Peng, X. 2014. Complete mitochondrial genome of the spadenose shark (*Scoliodon macrorhynchos*). 1736(2):91–92.

Chen, X., Peng, Z., Cai, L., Xu, Yuziwei. 2015. The fish diversity in the upper reaches of the Salween River, Nujiang River, revealed by DNA barcoding. *Nat.* 5(3): 1–12.

Chen, X., Peng, Zaiqing., Cai, Ling., Xu, Yuziwei. 2015. Mitochondrial genome of the spot-tail shark *Carcharhinus sorrah* (Carcharhiniformes: Carcharhinidae), Mitochondrial DNA. 26(5):734–735.

Chuang, P. S., Hung, T. C., Chang, H., Huang, C. Kang., Shiao, J. Chieh., 2016. The species and origin of shark fins in Taiwan's fishing ports, markets, and customs detention: A DNA barcoding analysis. *PLoS ONE.* 11(1): 1–13.

Clawson, D. L. 1985. Harvest security and intraspecific diversity in traditional tropical agriculture. *Econ. Bot. 39*(1):56-67.

Clayton, D. A. 1982. Replication of Animal Mitochondrial DNA. 28(4):1982-1989.

Coelho, R., Fernandez-Carvalho, J., Lino, P. G. and Santos, M. N. 2012. An overview of the hooking mortality of elasmobranchs caught in a swordfish pelagic longline fishery in the Atlantic Ocean. *Aquat. Living Resour. 25*(4):311-319.

Compagno, C. 1999. Management della qualità: dagli standard al knowledge management. UTET Libreria.

Compagno, L. J. 1984. *Sharks of the world: an annotated and illustrated catalogue of shark species known to date* (No. QL 638.9. C65).

Compagno, L. J. V and Niem, V. H. (2002) 'Carcharhinidae', in Carpenter, K. E. The living marine resources of the Western Central Atlantic. FAO. 2(9) 1312–1324.

Compagno. 1984. Family details of family Carcaarhinidae. FAO Fish. 125(42):251-655.

Craig, M. T. and Hastings, P. A. 2007. A molecular phylogeny of the groupers of the subfamily Epinephelinae (Serranidae) with a revised classification of the Epinephelini. *Ichthyological Res. 54*(1):1-17.

Craig, M.T. and Hastings, P. A. 2007. A molecular phylogeny of the groupers of the subfamily Epinephelinae (Serranidae) with a revised classification of the Epinephelini. *Ichthyological Res.* 54:1–17.

D.L. and Clark, A. G. 1997. Principles of Population genetics, Sinauer Associates, Sunderland, Massachusetts, USA.

Day Francis 1889. The Fauna of British India, Including Ceylon and Burma. Fishes, Taylor and Francis, 1:500-548.

Dhar, B. and Ghosh, S. K. 2017. Mini-DNA barcode in identification of the ornamental fish: A case study from Northeast India. *Gene*, 627:248–254.

Dosay-akbulut, M. 2008. The phylogenetic relationship within the genus Carcharhinus. 331:500–509.

Dubois, A. 2003. The relationships between taxonomy and conservation biology in the century of extinctions. Comptes Rendus *Biol.* 326: 9-21.

Dulvy Nicholas K.,, Baumb J. K., Clarke, S., Compagno, L. J. V. D. 2007 Subtidal macrobenthic structure in the lower lima estuary, NW of Iberian Peninsula. *Aquat. Conserva. : Marine and Freshwater Ecosystems.* 18:432–445.

Dulvy, N. K., Baum, J. K., Clarke, S., Compagno, L. J., Cortés, E., Domingo, A., Fordham, S., Fowler, S., Francis, M. P., Gibson, C. and Martínez, J. 2008. You can swim but you can't hide: the global status and conservation of oceanic pelagic sharks and rays. *Aquat. Conserv. : Marine and Freshwater Ecosystems. 18*(5):459-482.

Dulvy, N. K., Fowler, S. L., Musick, J. A., Cavanagh, R. D., Kyne, P. M., Harrison, L. R., Carlson, J. K. 2014. Extinction risk and Conserve. of the world's sharks and rays.

Dulvy, N. K., Fowler, S. L., Musick, J. A., Cavanagh, R. D., Kyne, P. M., Harrison, L. R., Carlson, J. K., Davidson, L. N., Fordham, S.V., Francis, M. P. and Pollock, C. M. 2014. Extinction risk and conservation of the world's sharks and rays. *Elife*. *3*:590-605.

Dunn, M. R., Szabo, A., McVeagh, Margaret S., Smith, P. J., 2010. The diet of deepwater sharks and the benefits of using DNA identification of prey. Deep-Sea Research Part I, *Elsevier.* 57(7): 923–930.

Ebert D. A., Fowler S., D. M. 2015. Sharks of the World. Princeton University Press; Princeton, New Jersey. 5(4):54-63.

Ebert, D. A., Fowler, S.L. and Compagno, L. J. 2013. *Sharks of the world: a fully illustrated guide*. Wild Nature Press.

Ebert, P. D. N. H. 2009. The campaign to DNA barcode all fishes, *Fish-Biol.* 2(1):329– 356.

Eio, W., DRAFT: EXECUTIVE SUMMARY: OCEANIC WHITETIP SHARK.

Eschmeyer, W. 2014. Catalog of fishes. Electronic publication in World Wide Web.

Esposti, D.M., De Vries, S., Crimi, M., Ghelli, A., Patarnello, T., and Meyer, A. 1993. Mitochondrial cytochrome b: evolution and structure of the protein. Biochimica Estoup. 1143:243–271.

Excoffier, L., Laval, G. and Schneider, S. 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinforma.*

Farias, I. P., and Hrbek, T. 2008. Patterns of diversification in the discus fishes (Symphysodon spp. Cichlidae) of the Amazon basin. *Mol. Phylogenetics and Evol.* 49(1): 32–43.

Feldheim, K. A., Gruber, S. H. and Ashley, M.V. 2001. Population genetic structure of the lemon shark (*Negaprion brevirostris*) in the western Atlantic: DNA microsatellite variation. *Mol. Ecol. 10*(2):295-303.

Felker, P., Lopez, C., Soulier, C., Ochoa, J., Abdala, R. and Ewens, M. 2001. Genetic evaluation of Prosopisalba (algarrobo) in Argentina for cloning elite trees. *Agroforestry Systems*. *53*(1):65-76.

Ferguson, M. M. and Danzmann, R. G. 1998. Role of genetic markers in fisheries and aquaculture: useful tools or stamp collecting. *Canadian J. of Fish. and Aquat. Sci.* *55*(7):1553-1563.

Ferretti, F., Worm, B., Britten, G. L., Heithaus, M. R. and Lotze, H. K. 2010. Patterns and ecosystem consequences of shark declines in the ocean. *Ecol.* *13*(8):1055-1071.

Feutry, Pierre., Pillans, Richard D., Kyne, Peter M., Chen, Xiao. 2015. Complete mitogenomic sequence of the Critically Endangered Northern River Shark Glyphis garricki (Carcharhiniformes: Carcharhinidae), Mitochondrial DNA. 26(6):855–856.

Feutry, Pierre., Pillans, Richard D., Kyne, Peter M., Chen, Xiao. 2016. Complete mitogenome of the Graceful Shark *Carcharhinus amblyrhynchoides* (Carcharhiniformes: Carcharhinidae), Mitochondrial DNA. 27(1): 314–315.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R. 1994. DNA primers for amplifica- tion ofmitochondrial cytochrome c oxidase subunit I from diverse metazoan inverte- brates. Mol. Mar. Biol. Biotechnol. 3 (5): 294–299.

Froese, R. and Pauly, D. 2015. Fishbase. Version (02/2015) world wide web electronic publication.

Froese, R.A. and Pauly, D. 2009. FishBase: a global information system on fishes. World Wide Web electronic publication. http://fishbase. org.

Gadagkar, R. 2005. Ernst Mayr, *J. of Genet.* 5(2):24-44.

Garrick, J. A. F. 1982. Sharks of the genus Carcharhinus.

Garrick, J. A. F. 1985. Additions to a revision of the shark genus Carcharhinus: Synonymy of Aprionodon and Hypoprion, and

description of a new species of Carcharhinus (Carcharhinidae). NOAA Technical Report, NMFS 34(9):52-89.

Gene, Meta., Díaz-jaimes, Píndaro., Bayona-vásquez, Natalia J., Adams, Douglas H., Uribe-alcocer, Manuel. 2016. Complete mitochondrial DNA genome of bonnethead shark, Sphyrna tiburo , and phylogenetic relationships among main superorders of modern elasmobranchs, *MGENE.* 6(3): 48–55.

Gharrett, A. and Gray, A. 2001. Identification of rockfish (Sebastes spp.) by restriction site analysis of the mitochondrial ND-3/ND-4 and 12S/16S rRNA gene regions, Bulletin, J Heifetz - Fisherygo.galegroup.com.

Ghorashi, S.A., Fatemi, S.M., Amini, F., Houshmand, M., Salehi Tabar, R. and Hazaie, K. 2008. Phylogenetic analysis of anemone fishes of the Persian Gulf using mtDNA sequences. *African J. of Biotechnol.* 7(12): 2074-2080.

Gold, J. R. 1993. Mitochondrial DNA differentiation and population structure in red drum (*Sciaenops ocellatus*) from the Gulf of Mexico and Atlantic Ocean', *Marine Biol.: International J. on Life in Oceans and Coastal Waters.* 116(2):175–185.

Gold, L. and Tuerk, C., University Research Corp. 1993. *Methods for identifying nucleic acid ligands.*U.S. 5:149-163.

Gopalakrishnan, A., Musammilu, K. K., Basheer, V. S., John, L., Padmakumar, K. G., Lal, K. K., Mohindra, V., Punia, P., Dinesh, K., Manjebrayakath, H. and Ponniah, A. G. 2009. Low genetic differentiation in the populations of the Malabar carp Labeodussumieri as revealed by allozymes, microsatellites and RAPD. *Asian Fish. Sci. 22*(2):359-391.

Gopalakrishnan, D. A. 2018. C.M.F.R.I Annual report.

Grace, M. A. 2014. A Scientific Paper of the Fishery Bulletin Field Guide to Requiem Sharks (Elasmobranchiomorphi : Western North Atlantic, (September 2001).

Grace, M. A. 2014. Field Guide to Requiem Sharks (Elasmobranchiomorphi :Western North Atlantic. (September 2001).

Grandjean, F., Bouchon, D. and Souty-Grosset, C. 2002. Systematics of the european endangered crayfish species *Austropotamobius pallipes* (decapoda: astacidae) with a reexamination of the status of *Austropotamobius berndhauseri. J. of Crustacean Biol.* 22(3):677–681.

Grant, P.R. 1999. *Ecology and evolution of Darwin's finches. 5*(23):5-25.

Greenwood, P. H., Rosen, D. E., Weitzman, S. H., Myers. 1966. Phyletic studies of teleostean fishes, with a provisional classification of living forms. http://digitallibrary.amnh.org/handle/2246/1678.

Greig, Thomas W., Moore, M Katherine., Woodley, Cheryl M., Quattro, Joseph M. 2001. Mitochondrial gene sequences useful for species identification of western North Atlantic Ocean sharks. 9(4):516–523.

Guan, Kunliang., Farh, Lynn., Marshall, Tricia K., Deschenes, Robert J. 1993. Normal mitochondrial structure and genome maintenance in yeast requires the dynamin-like product of the MGM1 gene, *Current Genet.* 24(1–2):141–148.

Guimarães, K.L.A., de Sousa, M.P.A., Ribeiro, F.R.V., Porto, J.I.R. and Rodrigues, L.R. R. 2018. DNA barcoding of fish fauna from low order River basin streams of Tapajo. 5:1–16.

Hall, T. A. 1999. BioEdit: a user-friendly sequence alignment editer programe for Windows.

Hanner, Robert., Floyd, Robin., Bernard, Andrea., Collette, Bruce B., Shivji, Mahmood. 2015. DNA barcoding of billfishes. 22(10):27–36.

Hanner, Robert., Martı, Juan., Astarloa, De., Zhang, Junbin., Mabragan, Ezequiel. 2011. The Fish Barcode of Life (FISH-BOL), Mitochondrial DNA. Taylor & Francis. 22(1):1–2.

Hartl, D.L. and Clark, A.G. 1989. Principles of population genetics, Sinauer Assoc. *Inc, Sunderland, Massachusetts.*9(3):15-39.

Harvey, Guy., Beach, Dania. 2009. Identifying sharks with DNA barcodes : assessing the utility of a nucleotide diagnostic approach. 9:243–256.

Hebert, P. D. N., Cywinska, Alina, Ball, Shelley L., DeWaard, Jeremy R. 2003. Biological identifications through DNA barcodes, Proceedings of the Royal Society of London. Series B: *Biol. Sci.* 270(1512):313–321.

Heithaus, M. R. 2016. SHARKS AND THEIR RELATIVES. (March 2004).

Heithaus, M. R., Marshall, G. J., Buhleier, B. M. and Dill, L. M. 2001. Employing Crittercam to study habitat use and behavior of large sharks. *Mar. Ecol. Progress Series*. 209:307-310.

Hinojosa-alvarez, S., Galván-Tirado, C., Hinojosa-Alvarez, S., Diaz-Jaimes, P., MarcetHouben, M. and García-De-León, F. J. 2014. The complete mitochondrial DNA of the silky shark (*Carcharhinus falciformis*). 1736:1–2.

Holmes, B. H., Steinke, D. and Ward, R. D. 2009. Identification of shark and ray fins using DNA barcoding. Fish. Res. 95(2–3):280–288.

Howard, D. J. and Berlocher, S. H. eds. 1998. *Endless forms: species and speciation*. Oxford University Press on Demand.

Howard, D. J. and Berlocher, S. H. eds. 1998. *Endless forms: species and speciation*. Oxford University Press on Demand.

Hsu, Kui-Ching., Shih, N. J., Ni, I. Chih., Shao, Kwang-Tsao. 2007. Genetic variation in *Trichiurus lepturus* (Perciformes : Trichiuridae) in waters off Taiwan, Several species or cohort contribution.

Hubert, A. and Schäfer, R. 2008. *Magnetic domains: the analysis of magnetic microstructures. Springer Sci. & Business Media.*

Hubert, N., Torrico, J.P., Bonhomme, F. and Renno, J. F., 2008. Species polyphyly and mtDNA introgression among three Serrasalmus sister-species. *Mol. Phylogenetics and Evol.* 46(1):375–381.

Id, T. A. and Azri, A. 2019. Diversity, occurrence and conservation of sharks in the southern South China Sea. 4(3):1–21.

Iglésias, S. P., Lecointre, G. and Sellos, D. Y. 2005. Extensive paraphylies within sharks of the order Carcharhiniformes inferred from nuclear and mitochondrial genes. *Mol. Phylogenetics and Evol.* 34(3):569–583.

Ihssen, P. E., Booke, H. E., Casselman, J. M., McGlade, J. M., Payne, N. R. and Utter, F. M. 1981. Stock identification: materials and methods. *J.of Fish. And Aquat. Sci. 38*(12):1838-1855.

Ilves, K. L. and Taylor, E. B. 2009. Molecular resolution of the systematics of a problematic group of fishes (Teleostei: Osmeridae) and evidence for morphological homoplasy. *Mol. Phylogenetics and Evol.* 50:163–178.

Ilves, K.L. and Taylor, E.B. 2009. Molecular resolution of the systematics of a problematic group of fishes (Teleostei: Osmeridae) and evidence for morphological homoplasy. *Mol. phylogenetics and Evol. 50*(1):163-178.

Irwin, D.M., Kocher, T. D., and Wilson, A. C. 1991. Evolution of cytochrome b gene in mammals. *J. of Mol. Biol. and Evol.* 2:13–34.

IUCN, S.S.C. 2001. IUCN Red List categories and criteria: version 3.1. *Prepared by the IUCN Species Survival Commission*.

J,F. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evol.* 39:783-791.

Jo, H., Gim, J.A., Jeong, K.S., Kim, H.S. and Joo, G. J. 2014. Application of DNA barcoding for identification of freshwater carnivorous fish diets: Is number of prey items dependent on size class for *Micropterus* salmoides?. *Ecol. and Evol.* 4(2):219–229.

John, L. 2009. Population Genetic Structure of Indigenous and *Puntius Chalakkudiensis*, Science And Technology. KUSAT.

Kim, S., Eo, H.S., Koo, H., Choi, J.K. and Kim, W., 2010. DNA barcode-based molecular identification system for fish species. Molecules and Cells. 30(6):507–512.

Klinbunga, S., Khamnamtong, B., Puanglarp, N., Jarayabhand, P., Yoosukh, W. and Menasveta, P. 2005. Molecular Taxonomy of Cupped Oysters (Crassostrea, Saccostrea, and Striostrea) in Thailand Based on COI, 16S, and 18S rDNA Polymorphism. *Marine Biotechnol.* 7: 306–317.

Ko, H.L., Wang, Y.T., Chiu, T.S., Lee, M.A., Leu, M.Y., Chang, K.Z., Chen, W.Y. and Shao, K. T., 2013. Evaluating the Accuracy of Morphological Identification of Larval Fishes by Applying DNA Barcoding. *PLoS ONE.* 8(1):3–9.

Kumar, S., Stecher, G., Li, M., Knyaz, C.T. K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Mol. Biol. and Evol.* 35:1547-1549.

Kutik, S., Stojanovski, D., Becker, L., Becker, T., Meinecke, M., Krüger, V., Prinz, C., Meisinger, C., Guiard, B., Wagner, R. and Pfanner, N. 2008. Dissecting membrane insertion of mitochondrial β-barrel proteins. *Cell, 132*(6):1011-1024.

Lakra, W. S., Goswami, M. and Gopalakrishnan, A. 2009. Molecular identification and phylogenetic relationships of seven Indian Sciaenids (Pisces: Perciformes, Sciaenidae) based on 16S rRNA and cytochrome c oxidase subunit i mitochondrial genes. *Mol. Biol.* 36(5):831–839.

Lakra, W.S., Singh, M., Goswami, M., Gopalakrishnan, A., Lal, K.K., Mohindra, V., Sarkar, U.K., Punia, P.P., Singh, K.V., Bhatt, J. P. and Ayyappan, S. 2016. DNA barcoding Indian freshwater fishes DNA barcoding Indian freshwater fishes.

Lakra, W.S., Verma, M.S., Goswami, M., Lal, K.K., Mohindra, V., Punia, P., Gopalakrishnan, A., Singh, K.V., Ward, R. D. and Hebert, P. 2011. D N A B A R C O D I N G DNA barcoding Indian marine fishes.

Laskar, B.A., Kumar, V., Kundu, S., Darshan, A., Tyagi, K. and Chandra, K., 2019. DNA barcoding of fishes from River Diphlu within Kaziranga National Park in northeast India, Mitochondrial DNA Part A: DNA

Mapping, Sequencing, and Analysis. Informa UK Ltd. 30(1):126–134.

Leary, R. F., Allendorf, F. W., Phelps, S. R. and Knudsen, K. L. 1987. Genetic divergence and identification of seven cutthroat trout subspecies and rainbow trout. *Transactions of the American Fish. Society. 116*(4):580-587.

Leigh, J., Bryant, D. and Steel, M. 2015. PopART (Population Analysis with Reticulate Trees).

Letters, E. 2006. Global estimates of shark catches using trade records from commercial markets. 3:1115–1126.

Leyva-Cruz, E., Vásquez-Yeomans, L., Carrillo, L. and Valdez-Moreno, M. 2016. Identifying pelagic fish eggs in the southeast Yucatan Peninsula using DNA barcodes. Genome. 59(12):1117–1129.

Li, W., Dai, X., Xu, Q., Wu, F., Gao, C. and Zhang, Y. 2016. The complete mitochondrial genome sequence of Oceanic whitetip shark, *Carcharhinus longimanus* (Carcharhiniformes: Carcharhinidae), Mitochondrial DNA. Part A, DNA mapping, sequencing, and analysis. 27(3):1775–1776.

Linnaeus. 1758. Prionace glauca, Blue shark : fisheries, gamefish. https://www.fishbase.se/summary/Prionace-glauca.

Liò, P. and Goldman, N. 1998. Models of molecular evolution and phylogeny. *Genome Res.* 8(12):1233-1244.

Liu, Z. J. and Cordes, J. F. 2004. DNA marker technologies and their applications in aquaculture genetics. *Aquac. 238*(1-4):1-37.

Lut, L. U. T., Vis, De. 1985. FAO Names: Names. 367:116–118.

M., K. 2002. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. of Mol. Evol.* 16:111-120.

M., S. N. and N. 1987.The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. and Evol.* 9(3):56-98.

Manire, C. A. and Gruber, S. H. 1990. Many sharks may be headed toward extinction. *Conserv. Biol.* 4(1):10-11.

Martin, A. P., Naylor, G. J. and Palumbi, S. R. 1992. Rates of mitochondrial DNA evolution in sharks are slow compared with mammals. *Nat.* 357(6374):153-164.

Matarese, A.C., Spies, I.B., Busby, M.S. and Orr, J. W. 2011. Early larvae of *Zesticelus profundorum* (family Cottidae) identified using DNA barcoding. Ichthyological Res. 55 58(2):170–174.

Mayden, R.L., Chen, W.J., Bart, H.L., Doosey, M.H., Simons, A.M., Tang, K.L., Wood, R.M., Agnew, M.K., Yang, L., Hirt, M.V. and Clements, M.D. 2009. Reconstructing the phylogenetic relationships of the earth's most diverse clade of freshwater fishes-order Cypriniformes (Actinopterygii: Ostariophysi): A case study using multiple nuclear loci and the mitochondrial genome. *Mol. Phylogenetics and Evol. Elsevier.* 51(3):500–514.

Mendonça, F.F., Hashimoto, D.T., De-Franco, B., Porto-Foresti, F., Gadig, O.B.F., Oliveira, C. and Foresti, F. 2010. Genetic identification of lamniform and carcharhiniform sharks using multiplex-PCR. *Conserv. Genetics Resour.* 2(1):31–35.

Meyer, C. P., Paulay, G. 2005. DNA barcoding: error rates based on comprehensive sampling. *PLoS Biol.* 3 (12).

Miloslavich, P., Klein, E., Díaz, J.M., Hernandez, C.E., Bigatti, G., Campos, L., Artigas, F., Castillo, J., Penchaszadeh, P.E., Neill, P.E. and Carranza, A. 2011. Marine Biodiversity in the Atlantic and Pacific Coasts of South America: Knowledge and Gaps. *PLoS ONE.* 9(15):69-102.

Misra.K. 1950. On a new species of Scyliorhinid fish from Andaman Sea, Bay of Bengal.Record from Zoological Survey of India. 8(2):87–90.

Mittermeier, R.A., Turner, W.R., Larsen, F.W., Brooks, T. M. and Gascon, C. 2011. Global Biodiversity Conservation: The Critical Role of

Hotspots in Biodiversity Hotspots. Berlin, Heidelberg. Springer Berlin Heidelberg.5:3–22.

Moore, A. B. M., Ward, R. D. and Peirce, R. 2012. Sharks of the persian (Arabian) gulf: A first annotated checklist (chondrichthyes: Elasmobranchii). Zootaxa. 3167: 1–16.

Moritz, C. 1994. Applications of mitochondrial DNA analysis in conservation. *Mol.Ecol.* 3(4):401–411.

Moritz, C. 1994. Applications of mitochondrial DNA analysis in conservation: a critical review. *Mol. Ecol. 3*(4):401-411.

Musick, J. A., Burgess, G., Cailliet, G., Camhi, M. and Fordham, S., 2000. Management of sharks and their relatives (Elasmobranchii). *Fish. 25*(3):9-13.

Mužinić, R. 1977. On the shoaling behaviour of sardines (Sardinapilchardus) in aquaria. *ICES J. of Mar. Sci. 37*(2):147-155.

Nair, K. P. 1988. Shark Resources of India, with Notes on Biology of a Few Species. *Fish.* 67:58-69.

Naro-Maciel, E., Stering, E. and Rao, M. 2008. Protected Areas and Biodiversity Conservation I: Reserve Planning and Design. Researchgate.Net. 2:18–48.

Naylor, G. J. P. 1992. The phylogenetic relationships among requiemand hammerhead shark % inferring phylogeny when thousands of equally most parsimonious trees result regions.5:295–318.

Nelson, J. S., Grande, T. and Wilson, M. V. H. 2006. Fishes of the world.

O'Corry Crowe, G. M., Suydam, R. S., Rosenberg, A., Frost, K. J. and Dizon, A. E. 1997. Phylogeography, population structure and dispersal patterns of the beluga whale *Delphina pterusleucas* in the western Nearctic revealed by mitochondrial DNA. *Mol. Ecol. 6*(10):955-970.

Paddack, M. J., Reynolds, J. D., Aguilar, C., Appeldoorn, R. S., Beets, J., Burkett, E. W., Chittaro, P. M., Clarke, K., Esteves, R., Fonseca, A.

C. and Forrester, G. E. 2009. Recent region-wide declines in Caribbean reef fish abundance. *Current Biol. 19*(7):590-595.

Pank, M., Stanhope, M., Natanson, L., Kohler, N. and Shivji, M. 2001. Rapid and simultaneous identification of body parts from the morphologically similar sharks Carcharhinus obscurus and *Carcharhinus plumbeus* (Carcharhinidae) using multiplex PCR. *Marine Biotechnol.* 3(3):231–240.

Pank, M., Stanhope, M., Natanson, L., Kohler, N. and Shivji, M. 2001. Rapid and simultaneous identification of body parts from the morphologically similar sharks *Carcharhinus obscurus* and *Carcharhinus plumbeus* (Carcharhinidae) using multiplex PCR. *Mar. Biotech. 3*(3):231-240.

Park, L. K. and Moran, P. 1995. Developments in molecular genetic techniques in fishsries. Molecular Genetics in Fisheries. Marine Policy. Pergamon. 36(3):746–752.

Pavan-Kumar, A., Gireesh-Babu, P., Babu, P.S., Jaiswar, A.K., Krishna, V.H., Prasasd, K.P., Chaudhari, A., Raje, S.G., Chakraborty, S.K., Krishna, G. and Lakra, W. S. 2014. Molecular phylogeny of elasmobranchs inferred from mitochondrial and nuclear markers. *Mol. Biol.* 41(1):447–457.

Pelicano, H., Feng, L., Zhou, Y., Carew, J.S., Hileman, E.O., Plunkett, W., Keating, M.J. and Huang, P. 2003. Inhibition of Mitochondrial Respiration. *J. of Biological Chem.* 278(39):37832–37839.

Petrov, D.A., Sangster, T.A., Johnston, J.S., Hartl, D.L. and Shaw, K.L. 2000. Evidence for DNA loss as a determinant of genome size. *Sci. 287*(5455):1060-1062.

Poey. 1861. *Carcharhinus longimanus,* Oceanic whitetip shark : fisheries, gamefish. Available at: https://www.fishbase.in/summary/875.

Rand, D. M. 1996. Neutrality Tests of Molecular Markers and the Connection Between DNA Polymorphism, Demography, and Conservation Biology. *Conserv. Biol.* 10(2):665–671.

Rasmussen, R. S., Morrissey, M. T. and Hebert, P. D. N. 2009. DNA barcoding of commercially important salmon and trout species (oncorhynchus and salmo) from north America. *J. of Agric. and Food Chemistry.* 57(18): 8379–8385.

Ratnasingham, S. and Hebert, P. D. N. 2013. A DNA-Based Registry for All Animal Species: The Barcode Index Number (BIN) System. *PLoS ONE.*3:29-59.

Riley, M. J., Hale, M. S., Harman, A. and Rees, R. G. 2010. Analysis of whale shark Rhincodontypus aggregations near South Ari atoll, Maldives Archipelago. *Aquat. Biol. 8*(2):145-150.

Rogers, A. R. and Harpending, H. 1992. Population growth makes waves in the distribution of pair-wise genetic differences. *Mol. Biol. and Evol. 9*(3):552-569.

Rokas, A. and Holland, P. W. 2000. Rare genomic changes as a tool for phylogenetics. *Trends in Ecol. & Evol. 15*(11):454-459.

Rokas, A., Carroll, S. B. 2005. More genes or more taxa? The relative contribution of gene number and taxon number to phylogenetic accuracy. Mol. Biol. Evol.

Romanov, E. and Romanova, N. 2009. *Size distribution and length-weight relationships for some large pelagic sharks in the Indian Ocean.* IOTC–2009–WPEB–06.

Rozas, j, Ferror mata, Sanchez Del-Barrio, J.C Guirao-Rico, Librado.p Ramos-onsins, S. G. 2017.Dna sp V6 12.3.

Rozas, J., Sánchez-DelBarrio, J. C., Messeguer, X. and Rozas, R. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinforma. 19*(18):2496-2497.

Saitou, N. and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. and Evol. 4*(4):406-425.

Sambrook, J. and Russel, D. W. 2001. Molecular cloning: a laboratory manual. 3rd Edn., New York, NY.

Sambrook, J. and Russell, D. 2001. Molecular cloning: A laboratory manual, the third edition.

Sangthong, P. and Jondeung, A. 2003. Cloning and nucleotide sequence of four tRNA genes in mitochondrial genome of Thai walking catfish (*Clariasmacrocephalus Gunther*). *Kasetsart J. Nat. Sci.* 37:33-40.

Sarmiento-Camacho, S. and Valdez-Moreno, M. 2018. DNA barcode identification of commercial fish sold in Mexican markets. *Genome.* 61(6): 457–466.

Schneider, S. and Excoffier, L. 1999. Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. *Genet. 152*(3):1079-1089.

Sebastian, W., Sukumaran, S., Zacharia, P.U. and Gopalakrishnan, A. 2018. The complete mitochondrial genome and phylogeny of Indian oil sardine, *Sardinella longiceps* and *Goldstripe Sardinella, Sardinella gibbosa* from the Indian Ocean. *Conserv. Genetics Resour.* 10(4):735–739.

Sembiring, A., Pertiwi, N. P. D., Mahardini, A., Wulandari, R., Kurniasih, E. M., Kuncoro, A.W., Cahyani, N. D., Anggoro, A.W., Ulfa, M., Madduppa, H. and Carpenter, K. E. 2015. DNA barcoding reveals targeted fisheries for endangered sharks in Indonesia. *Fish. Res. Elsevier* 164:130–134.

Shaklee, J. B., Phelps, S. R. and Salini, J. 1990. Analysis of fish stock structure and mixed-stock fisheries by the electrophoretic characterization of allelic isozymes. *Electrophoretic and isoelectric focusing techniques in Fish. Manag.* 2(4):173-196.

Shokralla, S., Hellberg, R.S., Handy, S.M., King, I. and Hajibabaei, M. 2015. A DNA Mini-Barcoding System for Authentication of Processed Fish Products. Scientific Reports. Nature Publishing Group. 5:1–11.

Slatkin, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. *Genet. 139*(1):457-462.

Snelson, F. F., Roman, B. L. and Burgess, G. H. 2008. The reproductive biology of pelagic elasmobranchs. *Sharks of the Open Ocean: Biol., Fish. and Conserv.* 8(2):24-45.

Sotelo, C. G., Pineiro, C., Gallardo, J. M., & Perez-Martin, R. I. 1992. Identification of fish species in smoked fish products by electrophoresis and isoelectric focusing. Forschung. 25:547-602.

Sparks, J. S. and Smith, W. L. 2004. Phylogeny and biogeography of cichlid fishes (Teleostei: Perciformes: Cichlidae). Cladistics. 20: 501–517.

Strasburg, D. W. 1958. Distribution, abundance, and habits of pelagic sharks in the central Pacific Ocean. *Fish. 1*(2):54-69.

Suk, S. H., Smith, S. E. and Ramon, D. A. 2009. Bioaccumulation of mercury in pelagic sharks from the northeast Pacific Ocean. *CalCOFI Rep. 50:*172-177.

Sukumaran, S. and Sumithra, T. G., 2018. Training Manual ICAR Short course on Application of advanced molecular methods in marine fishery resource management, conservation and sustainable mariculture.

Sukumaran, S., Gopalakrishnan, A., Sebastian, W., Vijayagopal, P., Nandakumar Rao, S., Raju, N., Ismail, S., Abdussamad, E. M., Asokan, P. K., Said Koya, K. P. and Rohit, P. 2016. Morphological divergence in Indian oil sardine, *Sardinella longiceps* Valenciennes. *J. of Applied Ichthyology.* 32(4): 706– 711.

Sultana, S., Ali, M. E., Hossain, M. M., Naquiah, N. and Zaidul, I. S. M. 2018. Universal mini COI barcode for the identification of fish species in processed products. *Elsevier.* 5(2):20-36.

Taguchi, M., King, J. R., Wetklo, M., Withler, R. E. and Yokawa, K. 2015. Population genetic structure and demographic history of Pacific blue sharks (*Prionace glauca*) inferred from mitochondrial DNA analysis. *Marine and Freshwater Res.* 66(3):267-300.

Tajima, F. 1983. Evolutionary relationship of DNA sequences in finite populations. *Genet. 105*(2):437-460.

Teletchea, F. 2009. Molecular identification methods of fish species : reassessment and possible applications. 2:265–293.

Templeman, W. 1981. Vertebral numbers in Atlantic cod, Gadusmorhua, of the Newfoundland and adjacent areas, and their use for delineating cod stocks. *J. Northwest Atl. Fish. Sci. 2*: 21-45.

Vences, M., Thomas, M., Van der Meijden, A., Chiari, Y. and Vieites, D. R. 2005. Comparative performance of the 16S rRNA gene in DNA barcoding of amphibians. *Frontiers in zool.* 2(1):1-5.

Victor, B. C., Hanner, R., Shivji, M., Hyde, J. and Caldow, C. 2009. Identification of the larval and juvenile stages of the Cubera Snapper. 36(8): 24–36.

Vinson, C., Gomes, G., Schneider, H. and Sampaio, I. 2004. Sciaenidae fish of the Caeté River estuary, Northern Brazil: mitochondrial DNA suggests explosive radiation for the Western Atlantic assemblage. *Genetics and Mol. Biol.* 5(3):20-28.

Wang, H. Y., Dong, C. A. and Lin, H. C. 2017. DNA barcoding of fisheries catch to reveal composition and distribution of cutlass fishes along the Taiwan coast. *Fish. Res. Elsevier* 187(1): 103–109.

Ward, R. D., Costa, F. O., Holmes, B. H. and Steinke, D. 2008. DNA barcoding of shared fish species from the North Atlantic and Australasia: minimal divergence for most taxa, but *Zeus faber* and *Lepidopus caudatus* each probably constitute two species. *Aquat. Biol.* 3(2):71–78.

Ward, Robert D. 2017. DNA barcoding reveals species composition of sharks and rays in the Indian commercial fishery, Mitochondrial DNA Part A: DNA Mapping, Sequencing, and Analysis. 28(4):458–472.

Wiley, E. O., Johnson, G. D. and Dimmick, W. W. 1998. The Phylogenetic Relationships of Lampridiform Fishes (Teleostei: Acanthomorpha), Based on a Total-Evidence Analysis of Morphological and Molecular Data. *Mol. Phylogenetics and Evol.* 10(3):417–425.

Wong, L. L., Peatman, E., Lu, J., Kucuktas, H., He, S., Zhou, C., Na-Nakorn, U. and Liu, Z. 2011. DNA barcoding of catfish: species authentication and phylogenetic assessment. *PloS one.* 6(3):121-184.

Wright, J. M. and Bentzen, P. 1995. Microsatellites: genetic markers for the future. In *Mol. Genet. in fish.* 5(2):117-121.

Yamazaki, Y., Goto, A. and Nishida, M. 2003. Mitochondrial DNA sequence divergence between two cryptic species of Lethenteron, with reference to an improved identification technique. *J. of Fish Biol.* 62(3):591–609.

Yan, S., Lai, G., Li, L., Xiao, H., Zhao, M. and Wang, M. 2016. DNA barcoding reveals mislabeling of imported fish products in Nansha new port of Guangzhou, Guangdong province, China. *Food Chem.* 202:116–119.

Yang, L., Matthes-Rosana, K. A. and Naylor, G. J. P. 2016. Complete mitochondrial genome of the blacknose shark *Carcharhinus acronotus* (Elasmobranchii: Carcharhinidae). Mitochondrial DNA. 27(1):169–170.Zardoya, R. and Meyer, A. 1996. Coelacanth, Lung fishes. *Evol.* 93(5): 5449–5454.

APPENDICES

APPENDICES

1. MEGA (MOLECULAR EVOLUTIONARY GENETICS ANLYSIS, version 6.0) SOFTWARE INSTALLATION

The archived package is available from: https://www.megasoftware.net

Unzip and extract the MEGA package in your target location.

2. DnaSP (DNA sequence polymorphism) SOFTWARE INSTALLATION

The archieved package is available from : https://en.bio-soft.net › dna › dnasp.

3. PopART (Population Analysis with Reticulate Trees) SOFTWARE INSTALLATION

The package available from: http://popart.otago.ac.nz

4. Arlequin 3.5 SOFTWARE INSTALATION

The package available from: https://en.bio-soft.net › other › arlequin

"IDENTIFICATION OF THE POPULATION GENETIC STRUCTURE OF *CARCHARHINUS LONGIMANUS* (OCEANIC WHITE TIP SHARK OR BROWN MILBERT'S SHARK) USING MITOCHONDRIAL DNA MARKERS."

By

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ABSTRACT

Even though sharks are the largest fishes in the world with their size varying size and behaviour, they were over exploited and most of them were at the fear of extinction. Among these *Carcharhinus longimanus*, an epipelagic bottomless shark considered as at the point of extinction were IUCN Red list points out this shark as a "**vulnerable**" species at global level. In order to implement the management measures for these species which require the information regarding its population in interoceanic regions. Population genetics can be characterized as the study of how hereditary variance is dispersed among the species and population on a very basic level (Hansen, 2003). Assessment of genetic makeup and variability of fish stock is important for scientific management of fishery, conservation and rejuvenation of endangered species. Mitochondrial DNA (mtDNA), which in general possess a five to ten times greater variability than single copy nuclear genes hence, served as a powerful tool for elucidating population structures studies. Among the 150 specimens of *C. longimanus* sequenced, we obtained sequences ranging from 720 base pairs were obtained 12 polymorphic sites yielding 13 haplotypes. Genetic differentiation among the populations of *C. longimanus* from Indian Ocean was revealed as a non-significant statistical analysis. Vital insights were gained from this study indicating lack of significant sub-structuring and its capability to migrate across large expanses of Open Ocean. The capability to migrate may provide it with some buffering against habitat loss and climate change, but excessive fishing is a danger to its populations. Globally sharks are in danger due to their inherent vulnerabilities like long gestation time and reduced number of offsprings coupled with over fishing. Our study also corroborated the findings of shark decline, as decline in genetic diversity is an indicator of decrease in resilience capacity. The present study calls for restrictions on its fishery so that populations will get sufficient time to replenish and consequently their resilience is ensured in the face of changing oceans