JFLS | 2021 | Vol 6(1) | Pp 3-5

OPEN ACCESS

Journal of Fisheries and Life Sciences
International Peer-Reviewed Biannual Journal
www.fishlifesciencejournal.com

Research Article

Molecular phylogeny of a stranded Whale Shark from Tamil Nadu, India

Vaishnavi Chandramouli*, Abinaya Nadarajan, Pradeep Anbazhagan, Kanchana Rangasamy, Debasis Jana

Centre for Wildlife Forensic Sciences, Advanced Institute for Wildlife Conservation, Tamil Nadu Forest Department, Chennai-600048

ISSN: 2456-6268

ARTICLE INFO
Received: 18 October 2021
Accepted: 29 December 2021
Available online: 30 December 2021

KEYWORDS
Whale Shark,
mt DNA,
NCBI GenBank

***CORRESPONDENCE**
vaishnavi.chandramouli@gmail.com

ABSTRACT

ABSTRACT

Whale sharks (*Rhincodon typus* Smith 1828) are the sole members of the Rhincodontidae family with the largest body size among elasmobranchs. Survival of whale sharks is threatened by unregulated worldwide fishing for illegal trade of fins, meat, oil, accidental collision with boats as well as excessive coastal pollution. The species is listed under IUCN Red List of threatened species, protected globally under Appendix II of CITES and protected nationally under Schedule I of Wildlife (Protection) Act 1972. One of the greatest challenges faced by enforcement agencies is to obtain concrete evidence in wildlife forensic cases of illegal poaching and wildlife trade, especially when samples obtained do not have morphologically distinguishable features. The present study used tissue sample obtained from necropsy of whale shark for genetic analysis. Amplification of partial mitochondrial DNA regions was carried out using three gene markers, *Cytochrome b*, *12S rRNA* and *16S rRNA* to obtain nucleotide sequences for species identification, which could serve as reference sequences to aid in wildlife forensic cases. All generated sequences matched with *R. typus* sequences in NCBI-GenBank database with 98-100% sequence similarity. The sequence data generated using the three mitochondrial DNA genes, could serve as valuable additions to the limited number of *R. typus* nucleotide entries present in NCBI GenBank database

INTRODUCTION

Whale shark (*Rhincodon typus* Smith 1828) is one of the flagship species in marine conservation. It is the sole member of Rhincodontidae family and has the largest body size among elasmobranchs, with the longest recorded length of 18.8 m (Read *et al.,* 2017). Whale shark is a pelagic filter-feeder and faces decline in population due to numerous threats such as unregulated and unsustainable global-scale industrial fishing for fins, meat, oil to satisfy market demands, accidental collision with boats, accidental tangling in nets and excessive coastal pollution. These issues have awakened the need to implement conservation and management strategies to recover a healthy whale shark population globally. Whale sharks span warm-temperate as well as tropical oceans (Meekan *et al.,* 2017) and are listed as endangered under the IUCN Red List of threatened species. The species has recently emerged to have significance in ecotourism owing to its predictable occurrences in select regions across the globe. In addition to exhibiting such traits of selective existences, they are also highly migratory in nature. Major challenges to conducting genetic studies on the species include difficulty in procuring samples because of ecological complexity as well as concealed and remote nature of their movement (Hoffmayer *et al.,* 2021). Legal protection to whale sharks is conferred in India under the Wildlife (Protection) Act 1972 and the species is protected internationally through Appendix II of CITES. One of the greatest challenges faced by enforcement agencies is to obtain concrete evidence in the cases of illegal poaching and wildlife trade. From the seized consignments, samples containing biological material are obtained and mitochondrial DNA (mtDNA) specific gene amplification is performed for species identification. The mtDNA is a homologous marker for species identification as it is highly conserved among species, maternally inherited and not subjected to recombination. In this study, we obtained tissue sample from necropsy of whale shark for genetic analysis. Amplification of partial mtDNA regions was carried out using three gene markers to obtain

nucleotide sequences for species identification, which could serve as reference sequences in wildlife forensic cases.

MATERIALS AND METHODS

A putrefied whale shark carcass washed ashore the Gulf of Mannar Biosphere Reserve, Tamil Nadu, India. The State Forest Department carried out necropsy on the carcass and tissue samples were received for analysis. Tissue samples were processed for DNA extraction using Qiagen DNeasy Blood and Tissue Kit and partial fragments of three mitochondrial regions, 16S rRNA, 12S rRNA and cytochrome b were amplified using universal primers (Kocher *et al.,* 1989, Guha and Kashyap, 2006) in Eppendorf Nexus GSX1 Mastercycler. Total reaction volume was 10 µL containing 1X Taq Buffer (KAPA Biosystems, SIGMA), 0.25 mM dNTPs, 0.4 µM of both forward and reverse primer, 2.5 mM $MgCl_2$, 0.25 U Taq DNA Polymerase (KAPA Biosystems, SIGMA) and 1 µL of 10- 40 ng/µL template DNA. Thermal cycling conditions consisted of 5 min. of initial denaturation at 95 ˚C, followed by 35 cycles of 30 seconds of denaturation at 95 ˚C, annealing at 55 ˚C (for *Cytb* and *12S rRNA* primer) and 57˚C (for *16S rRNA* primer), 45 seconds of extension at 72 ˚C and final extension at 72˚C for 10 min. PCR products were analyzed on 2% agarose gels, stained using novel juice stain, visualized under UV transilluminator, purified using QIAquick gel extraction kit (Qiagen, Germany) and sequenced in ABI 3730 Genetic analyzer (Applied Biosystems, USA). Sequences generated were processed using BioEdit (Hall, 1999) tool and matched against GenBank database using BLAST (Altschul *et al.,* 1990). Most homologous sequences with threshold above 80% were downloaded from GenBank and aligned with the sequences generated from the specimen. Aligned sequences were subjected to phylogenetic analysis by reconstruction of Neighbor-joining trees (Saitou and Nei, 1987) with 500 bootstrap replicates using MEGA X (Kumar *et al.,* 2018).

RESULTS AND DISCUSSION

The sample yielded good quantity and quality of genomic DNA as well as DNA sequences. All sequences matched with *R. typus* sequences in NCBI-GenBank database with 98-100% sequence similarity. Accession numbers for the generated sequences were obtained after submitting to NCBI-GenBank database (Table 1). Neighbor joining trees clustered the specimen sequences with *R. typus*, among other shark species sequences. The sequence data generated using the three mtDNA genes used, could serve as valuable additions to the limited number of *R. typus* nucleotide entries present in NCBI GenBank database (Fig.1-3.).

Table 1. Details of *R. typus* sequences submitted to GenBank

mtDNA Region	Primer	Amplicon Size	BLAST % Similarity	GenBank Accession No.
16S rRNA	Guha and Kashyap, 2006	485	100	MZ854246
Cytochrome b	Kocher *et al.,* 1989	276	100	MZ871366
12S rRNA	Kocher *et al.,* 1989	396	98.35	MZ962421

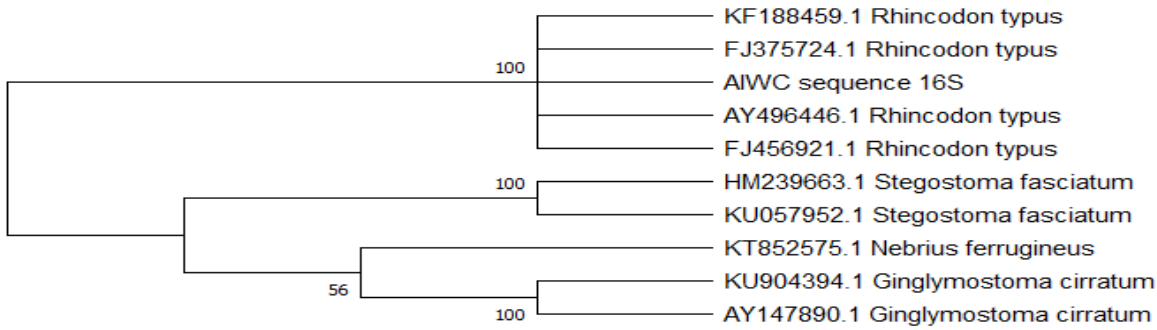

Fig.1. Phylogenetic tree reconstruction using Neighbour-joining method with 500 bootstrap replicates for *16S rRNA* sequence

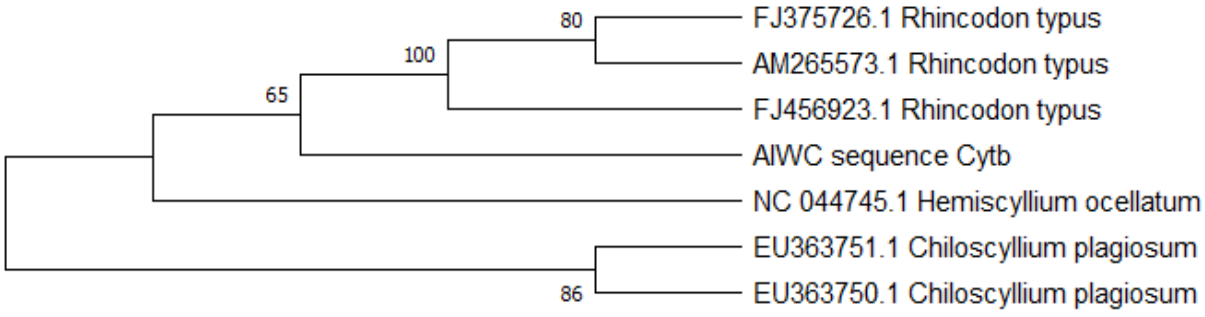

Fig.2. Phylogenetic tree reconstruction using Neighbour-joining method with 500 bootstrap replicates for *Cytb* sequence

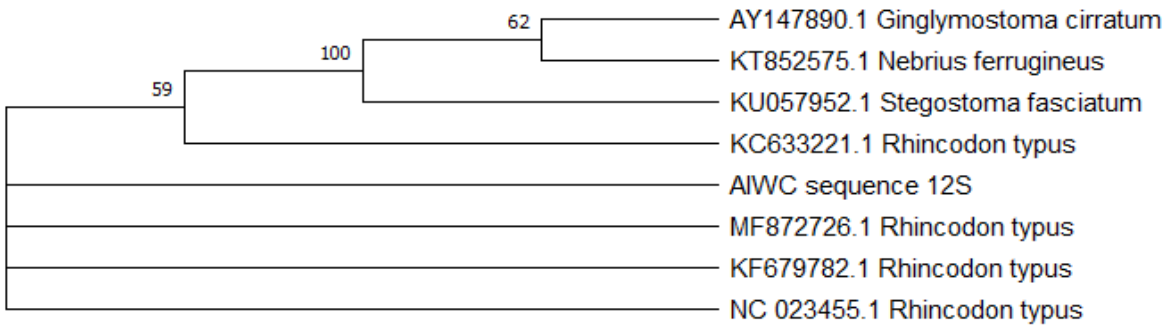

Fig.3. Phylogenetic tree reconstruction using Neighbour-joining method with 500 bootstrap replicates for *12S rRNA* sequence

CONCLUSION

The present study demonstrates the effectiveness of partial mtDNA markers in identification of shark species. The sequences generated will be of important value in cases involving illegal trade of sharks and their fins in the absence of clearly defined morphological features for identification. Further investigation using molecular data could help determine the genetic status of whale sharks inhabiting the Indian coast to identify any changes in gene flow in the population.

ACKNOWLEDGEMENT

This study was supported by the core funding of Advanced Institute for Wildlife Conservation, Tamil Nadu Forest Department, Chennai, Tamil Nadu. The authors would like to thank Principal Chief Conservator of Forests and Chief Wildlife Warden, Tamil Nadu Forest Department, Government of Tamil Nadu for providing the samples.

AUTHORS' CONTRIBUTIONS

VC- experimental design, conduct of experiments, writing of manuscript, interpretation of data, AN- interpretation of data, writing of manuscript, PA- writing and editing of the manuscript, KR and DJ- substantive editing of manuscript.

REFERENCE

Altschul S.F., Gish W., Miller W., Myers E.W., Lipman D.J. 1990. Basic local alignment search tool. *Journal of Molecular Biology*. 215:403–410.

Guha S., Kashyap V.K. 2006. Molecular identification of lizard by RAPD and FINS of mitochondrial 16S rRNA gene. Legal *Medicine* (Tokyo). 8(1):5-10.

Hall T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium series*. 41:95–98 19.

Hoffmayer E.R., McKinney J.A., Franks J.S., Hendon J.M., Driggers W.B. III, Falterman B.J., Galuardi B., Byrne M.E. 2021. Seasonal Occurrence, Horizontal Movements, and Habitat Use Patterns of Whale Sharks (*Rhincodon typus)* in the Gulf of Mexico. *Frontiers in Marine Science*. 7:598515

Kocher T.D., Thomas W.K., Meyer A., Edwards S.V., Paabo S., Villablanca F.X., Wilson A.C. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *PNAS.* 86 (16): 6196-6200. https://doi.org/10.1073/pnas.86.16.6196.

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

Meekan M., Austin C.M., Tan M.H., Wei N-W.V., Miller A., Pierce S.J., Rowat D., Stevens G., Davies T.K., Ponzo A., Gan H.M. 2017. iDNA at Sea: Recovery of Whale Shark (*Rhincodon typus*) Mitochondrial DNA Sequences from the Whale Shark Copepod (*Pandarus rhincodonicus*) Confirms Global Population Structure. *Frontiers in Marine Science*. 4:420.

Read T.D., Petit R.A. 3rd, Joseph S.J., Alam M.T., Weil M.R., Ahmad M., Bhimani R., Vuong J.S., Haase C.P., Webb D.H., Tan M., Dove A.D.M. 2017. Draft sequencing and assembly of the genome of the world's largest fish, the whale shark: *Rhincodon typus* Smith 1828. *BMC Genomics*. Jul 14;18(1):532.

Saitou N. and Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*. 4:406-425.

.