Journal of King Saud University – Science 34 (2022) 101909

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

Complete sequence and characterization of the *Mobula tarapacana* (Sicklefin Devilray) mitochondrial genome and its phylogenetic implications

Kumar Chandrasekaran [a], Inbakandan Dhinakarasamy [a,*], Sridhar Jayavel [b], Thirugnanasambandam Rajendran [a], Subashni Bhoopathy [a], Dharani Gopal [c], Kirubagaran Ramalingam [c], Seyed Ajmal Khan [d]

[a] *Centre for Ocean Research (DST – FIST Sponsored Centre), MoES – Earth Science & Technology Cell, Sathyabama Institute of Science and Technology, Chennai 600 119, Tamil Nadu, India*
[b] *Department of Biotechnology (DDE), Madurai Kamaraj University, Madurai 625 021, Tamil Nadu, India*
[c] *Marine Biotechnology Division, National Institute of Ocean Technology, Ministry of Earth Sciences, Govt. of India, Chennai 600100, India*
[d] *Faculty of Marine Sciences, CAS in Marine Biology, Annamalai University, Parangipettai 608 502, Tamil Nadu, India*

ARTICLE INFO

Article history:
Received 6 August 2021
Revised 1 December 2021
Accepted 9 February 2022
Available online 15 February 2022

Keywords:
Mobula tarapacana
Mitogenome
Sequencing
Annotation
Phylogeny

ABSTRACT

Mitochondrial genome sequences provide excellent insight into the study of molecular phylogeny and evolution. *Mobula tarapacana* (Sicklefin devil ray) has an olive-green to brown colored dorsal surface and darkly colored ventral markings. Due to their grey ventral stripes, fisherman and divers sometimes mistake these species for manta rays. To avoid the misidentification during bycatch and to increase the scientific knowledge of this resource as per the SDG Target 14.A, the complete mitogenome sequence of Sicklefin devil ray was sequenced, annotated, and submitted to the GenBank (Accession: MH669414.1). The mitogenome of *M. tarapacana* is a closed circular dsDNA of 5, 686 bp in size, which transcribes into 13 protein-coding genes (PCGs), 22 tRNAs, and 2 rRNAs. The percentage of protein-coding genes in the genome was 60.30%. Each protein-coding gene is initiated with an ATG codon excluding COX1 and ATP8 that begin with GTG and CCT respectively. Leu (L) and ser (S) were the most common abundant amino acids in the PCGs. The phylogenetic relationship and evolutionary distances were constructed by maximum likelihood approach using MEGA X software. The reported mitogenome was closely related to *Mobula kuhlii* (MKU MG10, KM364987) and *Mobula eregoodootenkee* (NC 025954).

1. Introduction

Ray species are extensively available elasmobranchs in tropical and warm water regions. In which, the *Myliobatidae* family is a larger, unique, and highly non-stationary group that consists of 2 genera known as *Mobula* and *Manta* rays. These ray species are known for their feeding habit, i.e., they are filter-feeding planktivores and piscivores (mostly feed on fishes). These species are migratory and are crossing boundaries from oligotrophic regions to subtropical regions and travel across many deep oceans in their life span. It is most usually seen in the oceanic environment, though it may also be present in the neritic zone (Compagno, 1999; Couturier et al., 2012).

M. tarapacana is morphologically identified by having a long head with shorter horns, shorter cephalic region and vertically elongated spiracle. The dorsal fins are strongly curved and are in uniform olive green colour. There is a missing white spot and a stinging spine behind the dorsal fin and the tails are shorter than the disc width. These species were repeatedly reported on the Asia-pacific, Indian and Atlantic coastline (Marshall, 2009). Most importantly, *M. tarapacana* and *M. japanica* species are reported throughout the year in the India and Sri Lanka coastal regions (Fernando & Stevens 2011).

Studies about *M. tarapacana* population dynamics, ecology and life history are very rare because of their migratory behavior. This

* Corresponding authors.
E-mail address: inbakandan@sathyabama.ac.in (I. Dhinakarasamy).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2022.101909
1018-3647/

species will grow up to 3700 mm wingspan, the males are maturing at 2340 – 2522 mm wingspan and information about maturity of a female is uncertain (White et al. 2006). Currently, the Mobulid populations and the catching have declined all over the region (Raje et al. 2007).

The primary cause of vulnerability and decline of ray species is due to increased targeted and by-catch fishery. Mobulid species captured and utilized especially for gill plates that are applied in pharma and food products in several countries. This species was captured using gill, purse-seine, trawl nets and long-lines, which makes it more vulnerable. Mobulidae exhibit some conservatory characters, which led to the overexploitation of the species. It takes 5 to 6 years to attain sexual maturity and reproduction. It has a longer gestation period and a low fecundity value (Couturier et al., 2012). Due to these reasons, the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) advised that the entire Mobulids could be enlisted in Appendix II. Recently, the IUCN declared *Mobula tarapacana* sicklefin devil ray (Phillipi, 1892) as an "endangered" list (Marshall et al., 2019).

The identification of *Mobula* and *Manta* species are uncertain by the cause of their taxonomy. The similarities of species generate ambiguity in identification (Couturier et al., 2012). To avoid the misidentification during the bycatch and to increase the scientific knowledge of this resource as per the SDG Target 14.A, the complete mitogenome sequence information of *M. tarapacana* was studied in an attempt to aid to draw a distinction.

The ecological features and life stories of the Mobulidae reveal that they are exposed to extinction. The evolution history of Mobulidae can be accomplished by molecular phylogenetic inferences and a complete taxonomic sampling. However, this can be

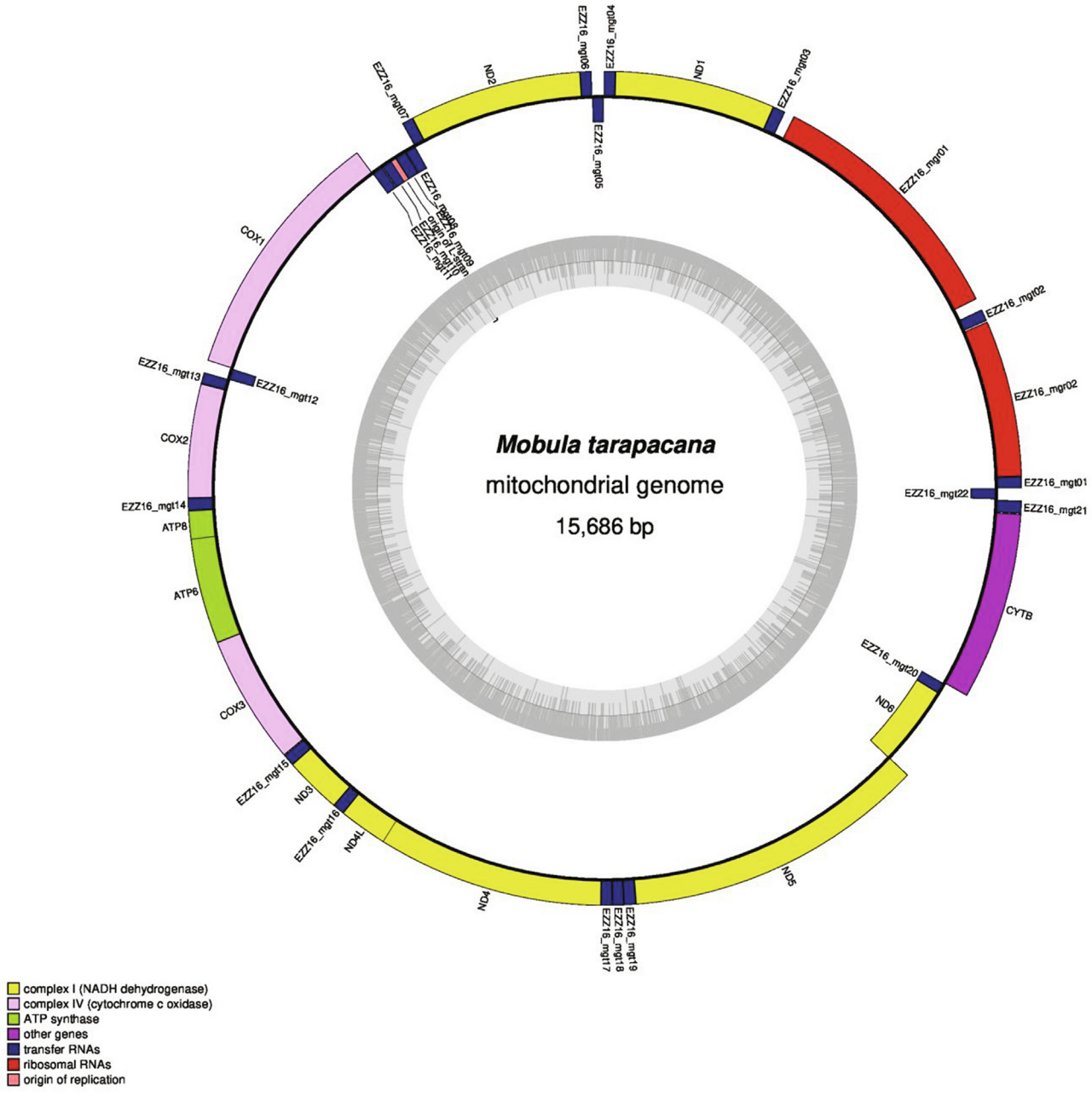

Fig. 1. Map of the *Mobula tarapacana* mitochondrial genome. The innermost circle of the images represents GC% per every 5 bp of the mitogenome; the darker lines are, the higher their GC%.

achieved through mitochondrial genome sequencing of the species (Aschliman, 2011). Mitogenome sequencing using NGS platforms would delimit the species boundaries among fishes and may give full insights into the evolutionary dynamics taking place (Miya and Nishida, 2015).

The analysis of the relatively conserved protein-coding genes revealed species and genus level interlinks with much higher resolution than the rRNA genes. To evaluate the relationship among closely related species, the whole mitogenome analysis is necessary, with which we can predict much accurate interspecies variation and evolution (Shao and Barker, 2006).

The present analysis affirms the whole mt genome sequence of *Mobula tarapacana* that frequently caught on the southeast coast of Tamil Nadu, India. These sequencing results give clear evidence of the phylogeny and evolutionary pattern of *M. tarapacana.*

2. Materials and methods

2.1. Sample collection and mitochondrial DNA extraction

Mobula tarapacana tissue sample was collected from Nagapattinam (10°45′04'' N and 79° 50′46'' E) fish landing centre, Tamil Nadu, India. The collected tissue sample was preserved in liquid nitrogen and carried to the laboratory. The mtDNA was extracted using QIAamp DNA Micro Kit (Cat.No: 56304, Germany) followed by manufacturer's instructions. The purity of DNA was analysed using NanoDrop 2000 spectrophotometer (Thermo fisher scientific, USA). Also, the quantity of the DNA was examined by Qubit fluorometer (Thermo Fisher Scientific, USA).

2.2. Construction of 2 × 150 NextSeq500 shotgun library

The pure DNA was extracted using TruSeq Nano DNA Library Prep Kit (Illumina, San Diego, California, USA) of the paired-end sequencing library. DNA fragmentation was carried out using Covaris M220 with the mean fragment dissemination of 350 bp. Covaris dissolution results in dsDNA fragments with 3′ or 5′ overhangs. The fragmented overhangs are subjected to blunt ends with the help of an end-repair mix. The 3′ overhangs were removed during the 3′ to 5′ exonuclease activity and also polymerase activity of the 5′ to 3′ overhangs occupies the 5′ overhangs followed by adapter association with the fragments. This scenario provides a low rate of fragment formation. Size selection of the fragments was done using AMPure XP beads. PCR amplification with Index primer was carried out to the size-selected fragments. The ends of the DNA fragments were tied up with the indexing adapters as they were prepared for assimilation onto a flow cell.

2.3. Cluster generation and sequencing

NextSeq500 was loaded with PE Illumina libraries for cluster formation and further sequencing. The Qubit Concentration and

Table 1
Nucleotide compositions of the mitochondrial genome (mtDNA) in different Mobula species. Note: The A + T biases of whole mitogenome, protein -coding genes, tRNA, rRNA and control regions were calculated by AT-skew = (A − T)/ (A + T) and GC-skew = (G − C)/ (G + C), respectively.

Species	Size (bp)	A%	T%	G%	C%	A + T%	AT Skewness	GC Skewness
Whole mitogenome								
M. *tarapacana*	15659.00	30.88	29.86	13.32	25.95	60.74	0.02	−0.32
M. *eregoodootenkee*	15715.00	30.26	28.57	13.79	27.38	58.83	0.03	−0.33
M. *thurstoni*	17609.00	30.69	29.10	13.69	26.51	59.80	0.03	−0.32
M. *mobular*	18913.00	32.92	29.72	12.63	24.73	62.63	0.05	−0.32
M. *japanica*	18853.00	32.73	29.77	12.79	24.71	62.50	0.05	−0.32
M. *munkiana*	15677.00	30.54	28.50	13.47	27.49	59.04	0.03	−0.34
M. *kuhlii*	15710.00	30.29	28.57	13.77	27.37	58.86	0.03	−0.33
Protein-coding genes								
M. *tarapacana*	11,236	28.47	31.83	13.12	26.58	60.30	−0.06	−0.34
M. *eregoodootenkee*	11,439	27.90	30.26	13.55	28.30	58.15	−0.04	−0.35
M. *thurstoni*	11,439	27.85	30.38	13.60	28.17	58.23	−0.04	−0.35
M. *mobular*	11,440	28.66	31.14	12.95	27.25	59.80	−0.04	−0.36
M. *japanica*	11,119	28.51	31.28	13.22	26.99	59.79	−0.05	−0.34
M. *munkiana*	11,403	28.07	30.40	13.25	28.27	58.48	−0.04	−0.36
M. *kuhlii*	11,435	27.87	30.27	13.55	28.32	58.14	−0.04	−0.35
tRNA								
M. *tarapacana*	1552	32.41	30.35	19.72	17.53	62.76	0.03	0.06
M. *eregoodootenkee*	1554	31.79	29.67	20.27	18.28	61.45	0.03	0.05
M. *thurstoni*	1557	32.11	29.99	20.10	17.79	62.11	0.03	0.06
M. *mobular*	1556	32.46	29.88	19.79	17.87	62.34	0.04	0.05
M. *japanica*	1617	32.16	29.68	20.41	17.75	61.84	0.04	0.07
M. *munkiana*	1480	32.23	30.41	19.86	17.50	62.64	0.03	0.06
M. *kuhlii*	1554	31.85	29.79	20.21	18.15	61.65	0.03	0.05
rRNA								
M. *tarapacana*	2661	34.87	26.46	17.06	21.61	61.33	0.14	−0.12
M. *eregoodootenkee*	2664	34.65	25.86	17.27	22.22	60.51	0.15	−0.13
M. *thurstoni*	2671	34.74	25.91	17.26	22.09	60.65	0.15	−0.12
M. *mobular*	2663	34.89	25.46	17.27	22.38	60.35	0.16	−0.13
M. *japanica*	2660	34.89	25.49	17.26	22.37	60.38	0.16	−0.13
M. *munkiana*	2665	34.82	25.14	17.30	22.74	59.96	0.16	−0.14
M. *kuhlii*	2664	34.68	25.19	17.23	22.15	59.87	0.16	−0.12
Control region								
M. tarapacana	ND	ND	ND	ND	ND	ND	ND	ND
M. eregoodootenkee	ND	ND	ND	ND	ND	ND	ND	ND
M. thurstoni	1884	34.08	32.38	12.74	20.81	66.45	0.03	−0.24
M. mobular	3199	43.11	32.14	9.22	15.54	75.24	0.15	−0.26
M. japanica	3164	42.54	32.24	9.83	15.39	74.78	0.14	−0.22
M. munkiana	ND	ND	ND	ND	ND	ND	ND	ND
M. kuhlii	ND	ND	ND	ND	ND	ND	ND	ND

the Mean peak value of Agilent TapeStation 4200 were also loaded with other library inputs. Forward and backward direction sequencing was possible through paired-end sequencing on the NextSeq500 platform. In the paired-end flow cell, samples were tied together with the oligonucleotide adapters using kit reagents. These oligos were responsible for selective cleavages on the forward strands and re-synthesis of the complimentary strand throughout the process. The backward sequencing was done in a counter direction and the opposite end of the fragment with the reverse strand of the previous sequence.

2.4. Sequence assembly and annotation

The high-quality pair-end reads acquired from Illumina NextSeq 500 platform were reference-based assembled by SeqMan NGen (DNASTAR Inc., Madison, WI, USA). Sequence alignment was described by NCBI nucleotide database with blastn tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). PCR amplification was done with the help of standard method (Sun et al., 2016). The characteristic of the assembled mitogenome of *M. tarapacana* was assessed by MitoAnnotator (http://mitofish.aori.u-tokyo.ac.jp/annotation/input.html) (Iwasaki et al., 2013).

2.5. Mitogenome analysis

The structural modification of the identified transfer RNAs (tRNAs) was predicted using the tRNAmod platform (https://webs.iiitd.edu.in/raghava/trnamod/index.html). OGDRAW 1.2 platform was used for generating the circular mitochondrial genome of *M. tarapacana* (https://chlorobox.mpimp-golm.mpg.de/OGDraw.html) (Lohse et al., 2013). MEGA 6.0 software was used in the analysis of nucleotide composition and codon usage bias (Tamura et al., 2013). The bioinformatic tool DnaSp 5.10.01 were used to calculate the ratio of synonymous and non-synonymous codon usage between the protein coding genes of different species (Rozas, 2009). The following formula AT-skew = (A − T)/ (A + T) and GC-skew = (G − C)/ (G + C) were used for calculating the A + T composition of whole Mitogenome of protein-coding genes (PCGs), tRNA, rRNA and control regions. (Junqueira et al., 2004).

2.6. Phylogenetic analysis

The phylogenetic analysis was performed on the *Mobula* species were studied using Maximum Likelihood methods, with the dataset of 21 species containing the nucleic acid and concatenated amino acid sequences. Bootstrap values were calculated using 1,000 replicates to assess node support. Evolutionary analyses were done using MEGA X (Kumar et al., 2018).

3. Results and discussion

3.1. Deciphering the mitochondrial genome

An isolated mitochondrial DNA was sequenced with Illumina NextSeq500 platform. Sequencing has resulted in 7,800,506 reads, with a minimum read length of 300 bp. The high-quality data of 2.3 GB was generated and processed to decode the mitochondrial

Table 2
Sequence characteristics of Mobula tarapacana mitochondrial genome. + and – correspond to the H and L strands, respectively.

Locus_name	One letter code	From	To	Size	Strand	No of amino acids	Anti-codon	Inferred initiation codon	Inferred termination codon	GC_percent (%)	Intergenic nucleotides*
tRNA-Phe	F	1	69	69	H		GAA			31.9	0
12S-rRNA		70	1032	963	H					40.8	0
tRNA-Val	V	1033	1104	72	H		TAC			40.3	0
16S-rRNA		1105	2805	1701	H					37.5	0
tRNA-Leu	L	2806	2880	75	H		TAG			41.3	1
ND1		2882	3856	975	H	324		ATG	TAA	40.3	1
tRNA-Ile	I	3858	3927	70	H		GAT			40.0	2
tRNA-Gln	Q	3930	4001	72	L		TTG			34.7	0
tRNA-Met	M	4002	4070	69	H		CAT			39.1	0
ND2		4071	5116	1046	H	348		ATG	TA	39.2	0
tRNA-Trp	W	5117	5186	70	H		TCA			28.6	1
tRNA-Ala	A	5188	5256	69	L		TGC			36.2	1
tRNA-Asn	N	5258	5330	73	L		GTT			37.0	34
tRNA-Cys	C	5365	5432	68	L		GCA			41.2	5
tRNA-Tyr	Y	5438	5507	70	L		GTA			47.1	1
COXI		5509	7065	1557	H	518		GTG	TAA	39.2	4
tRNA-Ser	S	7070	7140	71	L		GCT			40.8	0
tRNA-Asp	D	7141	7208	68	H		GTC			36.8	2
COXII		7211	7901	691	H	230		ATG	TTT	39.2	0
tRNA-Lys	K	7902	7976	75	H		TTT			38.7	144
ATP6		8121	8755	635	H	211		ATG	TCT	37.7	0
ATP8		8756	8788	33	H	10		CCT	CTA	41.9	0
COXIII		8789	9574	786	H	261		ATG	TAA	42.9	4
tRNA-Gly	G	9579	9650	72	H		TCC			27.8	0
ND3		9651	9999	349	H	116		ATG	AAT	39.5	0
tRNA-Arg	R	10000	10,070	71	H		TCG			31.0	0
ND4L		10071	10,367	297	H	98		ATG	TAA	45.5	−5
ND4		10361	11,741	1381	H	460		ATG	CCT	39.6	0
tRNA-His	H	11742	11,810	69	H		GTG			18.8	1
tRNA-Ser	S	11812	11,878	67	H		GCT			37.9	1
tRNA-Leu	L	11880	11,951	72	H		TAG			39.4	1
ND5		11953	13,794	1842	H	613		ATG	TAA	40.2	−2
ND6		13791	14,312	522	L	173		ATG	TAG	39.8	0
tRNA-Glu	E	14313	14,381	69	L		TTC			33.3	6
Cytb		14388	15,530	1143	H	380		ATG	TAA	40.5	3
tRNA-Thr	T	15534	15,606	73	H		TGT			54.8	10
tRNA-Pro	P	15617	15,686	70	L		TGG			41.4	0

genome. The sequencing analysis has identified the circular mitochondrial genome of *Mobula tarapacana* with a size of 15,686 bp, and submitted it to GenBank (accession number MH669414.1). An online tool MitoAnnotator were used for annotate the *M. tarapacana* mitogenome (Iwasaki et al., 2013). In total of 37 genes were annotated which includes 13 protein-coding genes, 22 tRNA genes, and 2 rRNA genes, respectively (Fig. 1). However, the similar mitogenome pattern has been reported in other *Mobula* species like *Mobula thurstoni*, *Mobula mobular*, *Mobula japonica*. These results are in strongly supported with fish mitogenome (Miya and Nishida, 1999).

The nucleotide composition of *M. tarapacana* was observed and A + T content was found to be 60.74% of the whole mitogenome. The A + T content of PCGs, tRNA, and rRNA constitutes 60.30%, 62.76%, and 61.33% of the whole mitogenome respectively. The positive AT skewness (0.02) was observed in the whole mitogenome which is lower than other *Mobula* species representing the presence of higher adenine (As) compared with thymine (Ts). The negative GC skewness (-0.32) represents the presence of more cytosine (Cs) compared with guanine (Gs), which is similar to other *Mobula* species (Table 1). Previous studies reported that the base composition bias was playing the significant part in the transcription and replication process (Chang and Clayton, 1986).

Table 3
Codon usage of *Mobula tarapacana* mitochondrial protein-coding genes.

Amino acid	Codon	Number	Frequency	(%) RSCU	Amino acid	Codon	Number	Frequency	(%) RSCU
Ala	GCC	66	1.27	1.36	Thr	ACT	117	2.25	1.25
	GCA	53	1.02	1.09		CAT	105	2.02	1.07
	GCT	62	1.19	1.28	Ile	ATT	165	3.17	1.28
	GCG	13	0.25	0.27		ATC	92	1.77	0.72
Arg	CGA	37	0.71	1.36	Leu	TTA	169	3.25	1.56
	CGT	23	0.44	0.84		CTA	128	2.46	1.18
	CGC	34	0.65	1.25		CTT	139	2.67	1.28
	CGG	15	0.29	0.55		CTC	112	2.15	1.03
ASN	AAT	147	2.82	1.1		TTG	62	1.19	0.57
	AAC	121	2.33	0.9		CTG	41	0.79	0.38
ASP	GAT	47	0.90	1.03	Lys	AAA	195	3.75	1.45
	GAC	44	0.85	0.97		AAG	74	1.42	0.55
CYS	TGT	41	0.79	1.05	Met	ATA	125	2.40	1.37
	TGC	37	0.71	0.95		ATG	57	1.10	0.63
Gln	CAA	121	2.33	1.39	Phe	TTT	138	2.65	1.05
	CAG	53	1.02	0.61		TTC	125	2.40	0.95
	GAA	63	1.21	1.17	Pro	CCA	120	2.31	1.12
	GAG	45	0.86	0.83		CCC	98	1.88	0.91
Gly	GGA	47	0.90	1.31		CCT	177	3.40	1.65
	GGC	31	0.60	0.87		CCG	34	0.65	0.32
	GGT	48	0.92	1.34	Ser	TCA	109	2.09	1.22
	GGG	17	0.33	0.48		TCT	138	2.65	1.54
His		92	1.77	0.93		TCC	119	2.29	1.33
	AGC	84	1.61	0.94		TCG	33	0.63	0.37
	AGT	55	1.06	0.61	Trp	TGA	86	1.65	1.3
	ACG	29	0.56	0.31		TGG	46	0.88	0.7
Stp*	TAA	153	2.94	1.72	Tyr	TAT	137	2.63	1.08
	AGA	60	1.15	0.67		TAC	116	2.23	0.92
	AGG	65	1.25	0.73	Val	GTA	44	0.85	1.28
	TAG	78	1.50	0.88		GTT	44	0.85	1.28
Thr	ACA	111	2.13	1.18		GTC	29	0.56	0.85
	ACC	118	2.27	1.26		GTG	20	0.38	0.58

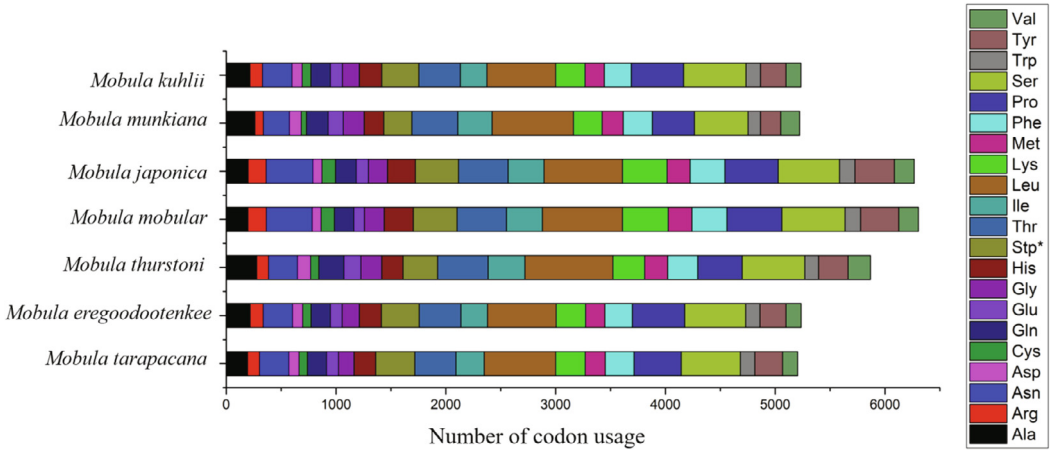

Fig. 2. Comparison of codon usage within the mitochondrial genome of members of the Mobula species *(Mobula tarapacana, Mobula eregoodootenkee, Mobula thurstoni, Mobula mobular, Mobula japonica, Mobula munkiana and Mobula kuhlii).*

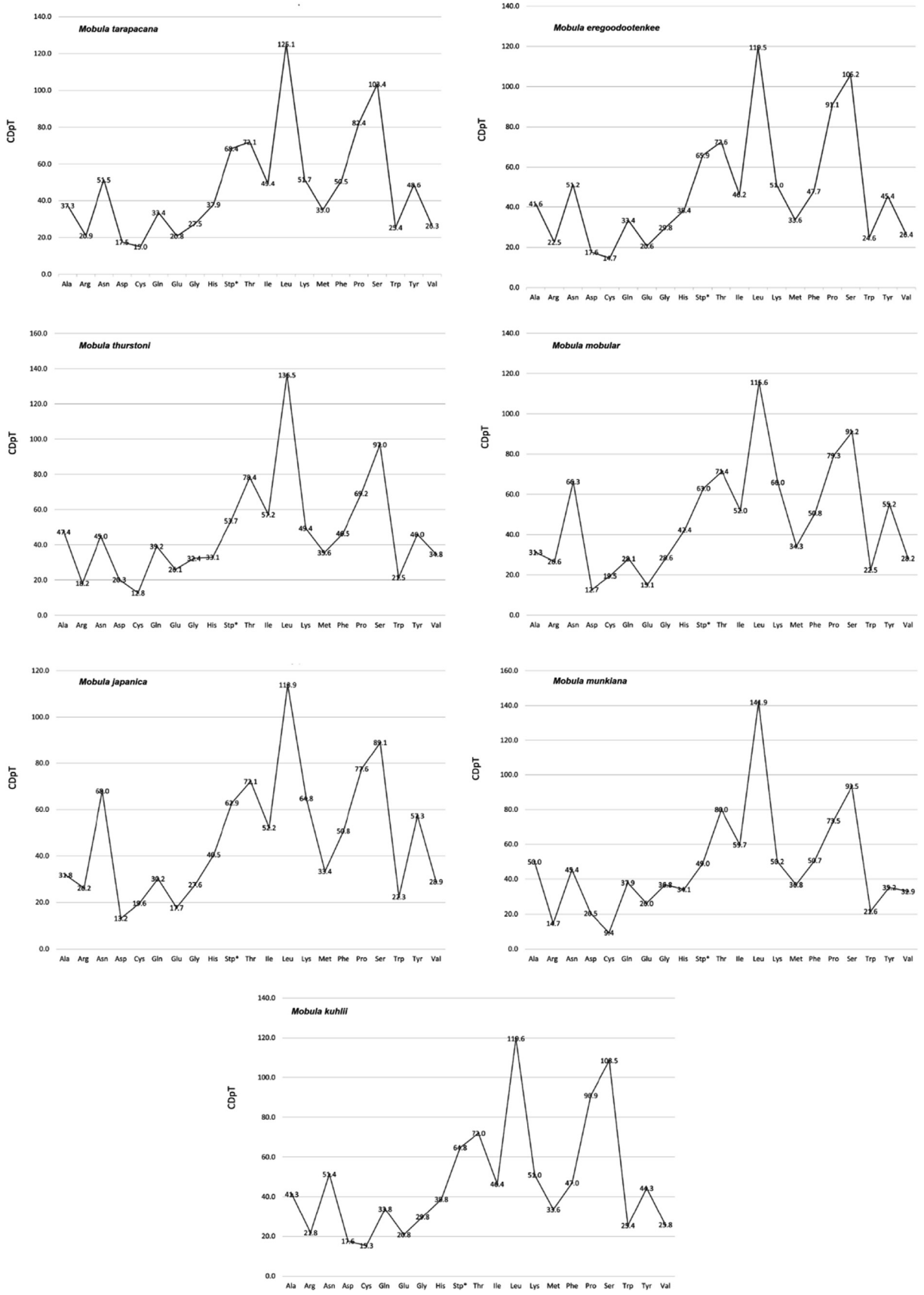

Fig. 3. Codon distribution in members of seven species in the *Mobula*. CDspT = codons per thousand codons.

The bases of 2 gene regions overlapped with their neighbouring genes, from 2 −5 bp in size. The predominant overlapping region observed among ND4L and ND4 with a size of 5 bp. The intergenic spacer nucleotides were observed in 18 places ranging from 1 – 144 bp and the longest spacing sequence of 144 bp was detected between tRNA- Lys and ATP6 (Table 2).

3.2. Protein-coding genes

There are 13 protein-coding genes obtained from the mitogenome of *M. tarapacana* with the length of 11, 236 bp, which was shorter than other *Mobula* species except for *Mobula japanica*, though it covers 60.30% of A + T content. The start codon ATG was used as a canonical putative initiative for all PCGs, except COX1 and ATP8 where they use GTG and CCT as a start codon respectively. These features of initial start and stop codons are generally ascertained in all elasmobranchs (Liu et al., 2013). This was common among all vertebrate mitogenomes (Yue et al., 2016). Among 13 PCGs, 12 of them were determined by heavy strand (ND1, ND2, COXI, COXII, ATP6, ATP8, COXIII, ND3, ND4L, ND4, ND6, Cytb) and ND6 was stated by light strand. The protein-coding genes ND1, COXI, Cytb, COXIII use TAA as termination codon, whereas ND3, ND4L, ND5 use AAT as a termination codon. Likewise, COXII, ATP6, ATP8, ND4, and ND6 use TTT, TCT, CTA, CCT, and TAG respectively. ND2 was encoded by an incomplete termination codon TA (Table 2). The same decoding pattern was observed in the vertebrate mitogenome and extended to a complete TAA termination codon via post-transcription polyadenylation (Pindaro et al., 2016).

The predominant amino acids predicted in the protein-coding genes (PCGs) of *M. tarapacana* were leucine, proline, and serine in the range of 12.51%, 8.24%, and 10.34% respectively (Table 3). The number of codon usage within the PCGs of *M. tarapacana* was compared with other *Mobula* species, and it revealed that leucine, serine, and proline were higher in all species (Figs. 2 & 3).

3.3. Transfer RNAs (tRNA) and ribosomal RNAs (rRNA)

The tRNA act as a vital role in adaptor molecule during the protein synthesis. The tRNAs of *M. tarapacana* was 1552 bp in length with 62.76% of A + T content, which includes 32.41% A, 30.35% T, 19.72% G and 17.53% C. AT skewness of tRNA of *M. tarapacana* was 0.03 whereas the GC skewness was 0.06 which was similar with some *Mobula* species. The rRNA of the *M. tarapacana* mitochondrial genome was 2661 bp in length and covers 61.33% of A + T content. Here the As (34.87%) are higher than Ts (26.46%) resulting in a positive AT skewness of 0.14 which was lower than other *Mobula* species. Similarly, Gs (17.06%) was lower than Cs (21.61%) resulting in a negative GC skewness of 0.12 which is similar to *M. thurstoni* and *M. kuhlii* (Table 1). There are 22 tRNA molecules predicted in *M. tarapacana* mitochondrial genome which is about 70–80 nucleotides long and exhibiting a characteristic cloverleaf structure. The Post-transcriptional modifications carried out in rRNA, mRNA, and tRNA molecules leads to a certain stability,

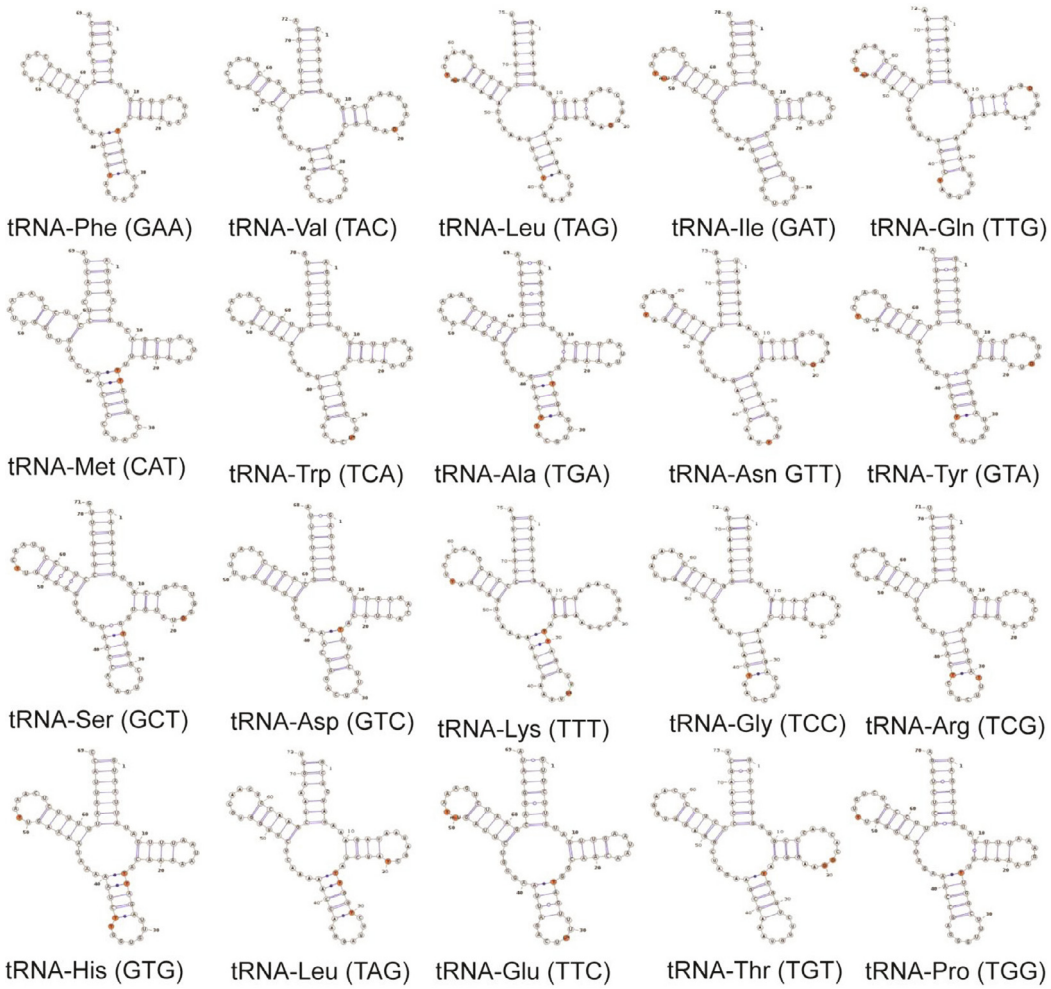

Fig. 4. Schematic representation of tRNA modification in mitogenome of *M. tarapacana*.

translational efficiency, and fidelity of tRNA. The tRNAmod is a tool that is used to identify the modifications carried out in the tRNA structure. Using this tool on *M. tarapacana*, it was observed that 20 tRNAs were showing the possibility of modifications in their structure, rather 2 of them were not undergoing any modifications (Fig. 4). Codon-anticodon interactions were highly influenced based on the position of modification, which is usually near the wobble position. This property was well preserved in eukaryotes that resulted in regulation of translational efficiency, frameshifting, and maintenance (Ranjan and Rodnina, 2016; Tuorto and Lyko, 2016). The stability of tRNA depends on the changes occurring within the central tRNA structure, which may result in tRNA degradation and pool discrepancy (Lorenz et al., 2017).

3.4. Codon usage bias and the control region

The control region of *M. tarapacana, M. eregoodootenkee, M. munkiana*, and *M. kuhlii* were not defined, while a few *Mobula* species have the control region information of their mitochondrial genome (Table 2). The previous finding revealed, the mitochondrial DNA act as a tool for studying various classes of fish including teleost and elasmobranch. They were focused on the control region (CR) as a marker for studying intraspecific variations. The control region exhibits variation in many vertebrates comprising teleosts (Aquadro & Greenberg, 1983), Humans (Lee et al., 1995), and birds (Wenink et al., 1994). Martin et al. (1992) reported the evolutionary rates of elasmobranchs in mitochondrial genes are minimum

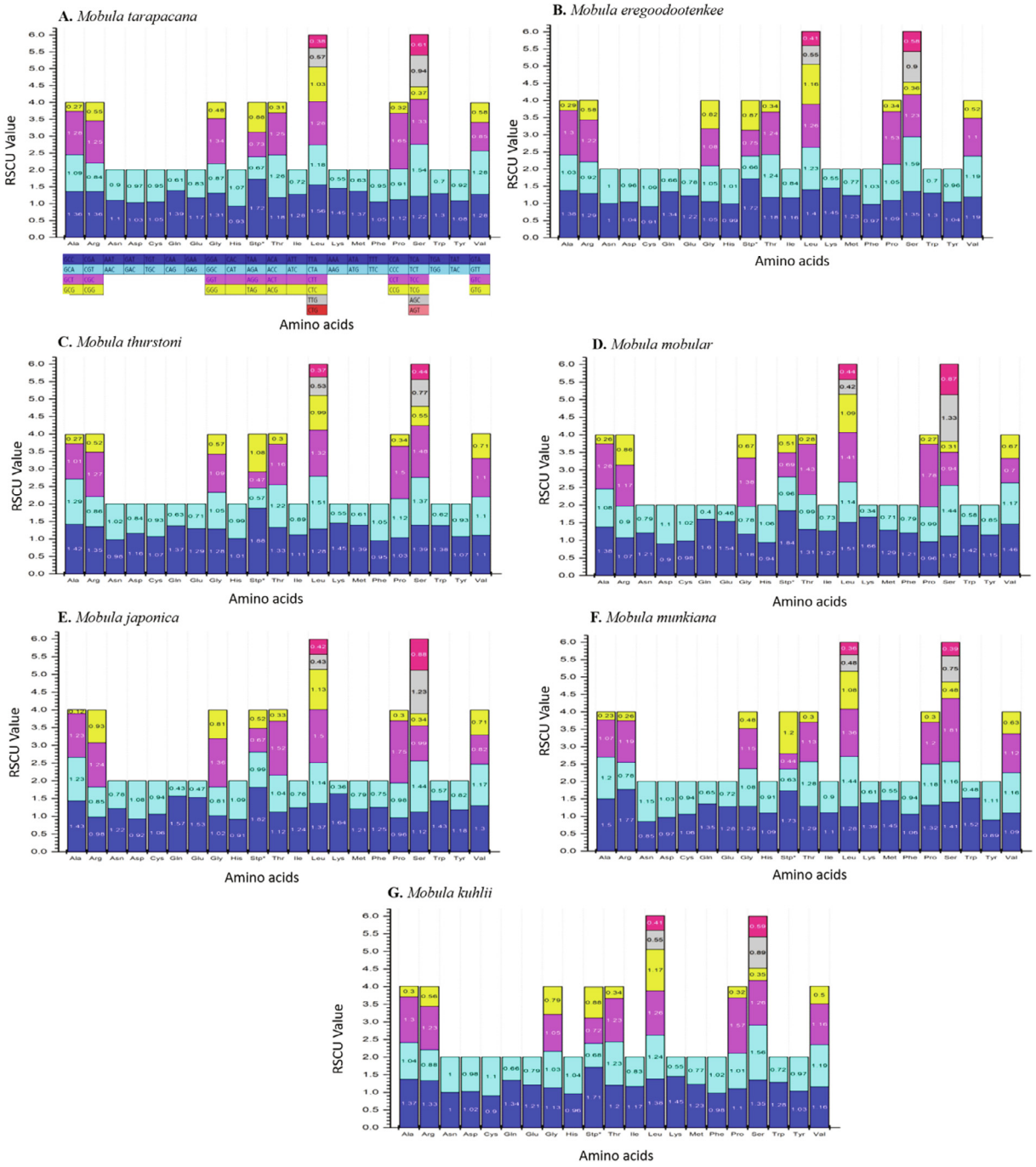

Fig. 5. Relative Synonymous Codon Usage (RSCU) of the mitochondrial genome of seven species in the *Mobula*. Codon families are plotted on the x-axis. Codons indicated above the bar are not present in the mitogenome.

compared with other vertebrates. In contrast, it is difficult to find out the difference between diverse populations with commonly used CR sequencing approaches. The present study focused on the mitogenome markers except for the control region for the phylogenetic evaluation of *M. tarapacana*. The Relative synonymous codon usage concerning amino acid utilization of the mitochondrial genome of *M. tarapacana* and their closely related species were exhibited in Table 3 and Fig. 5. Relative synonymous codon usage (RSCU) analysis of PCGs in *M. tarapacana* revealed that the amino acid codons encoding Leu, Pro, Ser, Stp, Lys, Thr, Asn, and Ile were the most recurrent ones and those encoding Asp and Lys were rare. The usage of hydrophobic amino acid codons is comparatively higher in the vertebrate mitogenome than general nuclear genome (Satoh et al., 2010). This indicates that the genomic region closer to CR is highly used, hence exhibiting high translational efficiency. This can result in a favorable translation in the vertebrate mitogenome.

3.5. The nucleotide substitution of PCGs

Two homologous PCGs of closely related species were compared to find out the evolutionary behavior of similar species. The Ka/Ks ratio was the key to evaluate this evolutionary behavior. The Value of Ka = Ma/Na, the number of nonsynonymous substitutions observed divided by the total number of nonsynonymous changes in the entire gene sequences of two species. Similarly, Ks = Ms/Ns, the number of synonymous substitutions observed divided by the total number of synonymous changes in the entire gene sequences of two species. Hence, the Ka/Ks ratio reflects the rate of evolutionary changes that occurred throughout the sequence and also reflects the selective pressure on the evolution of the organism. Ka/Ks > 1, Ka = Ks and Ka/Ks < 1 are the possible cases and correspond to positive, neutral, and negative selection respectively (Li et al., 2015). The sequence divergence of *Mobula* mtDNA (*Mobula mobular*, *Mobula tarapacana*, and *Mobula thurstoni*) was determined by Ka and Ks substitution rates. The Ka/Ks value of 13 PCGs is varying from 0.008 (COI) to 1.9 (ATPase8) (Fig. 6).

Here the nonsynonymous substitution rate is faster than the synonymous substitutions taken place. The % ratio of variable sites of MTA/MTH were maximum in ATPase6 and ATPase8 and minimum in the COI gene, signifying that ATPase6 and ATPase8 were broadly distributed and COI was selectively distributed in the mitochondrial proteins. In *M. thurstoni* and *M. mobular*, the Ka/Ks ratio was minimum in all 13 protein-coding genes compared to

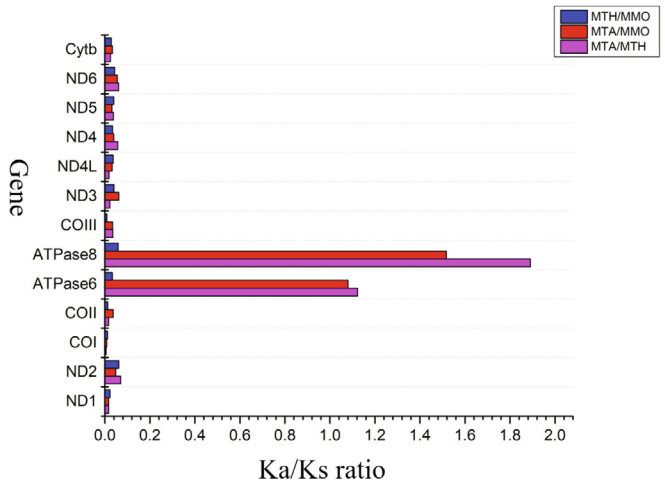

Fig. 6. Ka/Ks ratios for the 13 mitochondrial protein-coding genes among the reference *Mobula tarapacana, Mobula thurstoni* and *Mobula mobular.*

M. tarapacana, indicating that the MMO and MTH had a closer phylogenetic relationship than *M. tarapacana.*

3.6. Phylogenetic analysis

Phylogenetic investigation of mtDNA is a broadly utilized for outlining the relationship between species (Moritz, 1994) considering its quick pace of succession advancement and fast ancestry arranging comparative with the nuclear genetic material (Avise, 1989). Nevertheless, the data obtained from mtDNA could provide negligible information when small regions are studied. Taxonomy is recognized to be the foundation of the understanding of biodiversity and evolutionary behavior. Phylogenetic analysis on the other hand is used to compare and study the similarities and dissimilarities within a family. Such studies have been challenging due to poor fossil record, misidentification of organism alongside lack of mitochondrial sequence data (Hinojosa-Alvarez et al., 2015). There exist three methods to predict phylogeny, namely maximum parsimony, maximum likelihood, and distance-based phylogenetic methods. Among the three, the maximum likelihood (ML) method yields the closest match and widely accepted among vertebrate genome analysis. The lengthier the branch, causing multiple problems in the Phylogenetic tree due to the nucleotide changes leads to extensive evolutionary patterns (Broughton et al., 2001).

The phylogenetic data from the whole mitochondrial genome data analysis of 21 species and 7 genera in the order: Myliobatiformes. The Maximum likelihood phylogenies of the *Mobula* species were compatible with the same species. All the clades are in agreement with the bootstrap values and Bayesian posterior probability. There were three clades separated among the *Mobula* species. The sequence of our strain *Mobula tarapacana* CK02, MH669414 is closely related to *Mobula kuhlii* MKU MG10KM364987 and *Mobula eregoodootenkee* NC 025954. Furthermore, *Mobula mobular* KT203434 and *Mobula japonica* JX392983 formed a cluster, and *Mobula munkiana* MMU MG01KM364990 and *Mobula thurstoni* NC 037,219 clustered in a separate clade. Given that the sister relationship between *Mobula tarapacana* CK02MH669414 is closely related to *Mobula kuhlii* MKU MG10KM364987 based on nucleotide sequence (Fig. 7). Family Mobulidae comprises two genera namely *Mobula* and *Manta.* The *Mobula* genus is holds nine recognized species, whereas the *Manta* genus has two species. Based on both morphological data and molecular data these species are categorized into 3 clades. The first clade has larger *Mobulid* species like, *M. birostris, M. alfredi, M. tarapacana, M. mobular,* and *M. japanica.* The other two clades include smaller species, three each. *M. eregoodootenkee, M. kuhlii,* and *M. thrustoni* belong to the second clade, and *M. mukiana, M. hypostoma,* and *M. rochebrunei* belong to the third clade (Muller and Henle, 1841; Aschliman, 2011; Poortvliet and Hoarau, 2013; William et al., 2018).

The genetic divergence study reveals genetically indistinguishable pairs in Mobulidae species. Cephalic lode length, tooth morphology, and bronchial filter plate were used as a key to distinguish species of Mobulidae. Morphology along with molecular data analysis shows the indistinguishable nature within a pair of species. *M. mobular* and *M. japanica* are morphologically similar except for the difference in tooth morphology, yet further detailed studies revealed the similarities of the species pair at the molecular level (Adnet et al., 2012). *M. kuhlii* and *M. eregoodootenkee* share the same evolutionary independent unit based on Phylogenetic analysis (White et al., 2017). Similarly, the molecular data reveals the similarity between *M. hypostoma* and *M. rochebrunei* with 100% bootstrap (White et al., 2017). The nuclear exon data reveals a strong link between *M. mukiana* and *M. hypostoma,* where the width of the lower tooth band is the only distinguishing morpho-

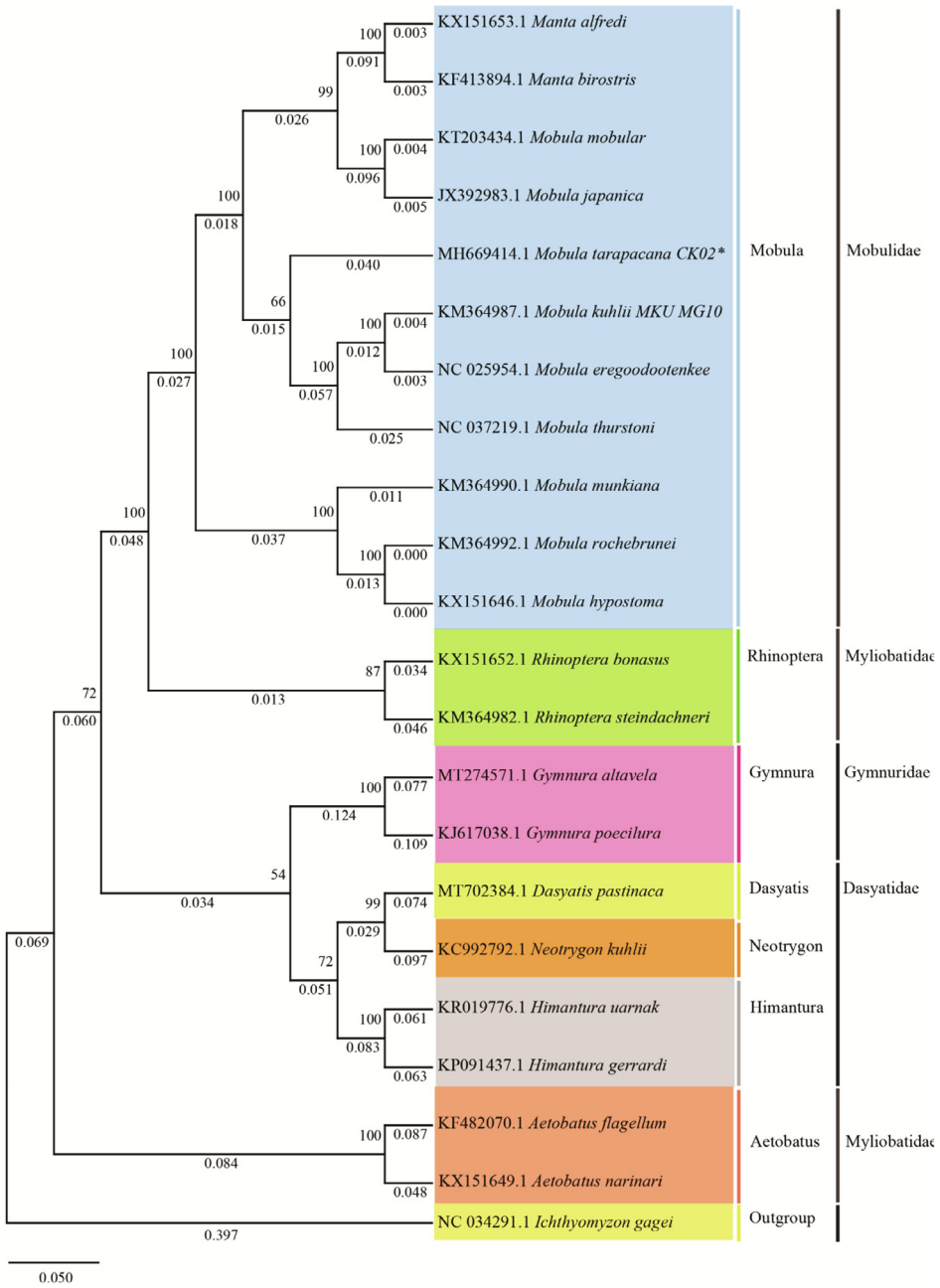

Fig. 7. Phylogenetic trees of *Mobula tarapacana* relationships from the complete genome. The concatenated amino acid datasets and evolutionary history was inferred using the maximum likelihood method. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. This analysis involved 21 amino acid sequences. Evolutionary analyses were conducted in MEGA X.

logical features present (William et al., 2018). Morphological features like pectoral fins, tail, and structure beneath and above the first dorsal fin are the key characters to distinguish between interspecies. These morphological studies revealed that these key features remain small in *M. tarapacana*, compared with *M. mobular*, *M. kuhlii* and *M. thurstoni* (Paig-Tran et al., 2013).

4. Conclusion

The sequencing of the whole mitogenome of *Mobula tarapacana* reveals the genetic identity and its divergence from closely related species. Ka and Ks substitution rates showing an important observation of selective pressure on different *Mobula* species. Among 13 PCGs, COX1 shows a low Ka/Ks ratio, while ATPase 6 and ATPase 8 show a higher Ka/Ks ratio. From this observation, we can conclude that the COX1 gene has not been evolved much (Ka/Ks < 1) therefore it could be applied as a tool for evolutionary and phylogenetic analyses. ATPase 6 and ATPase8 genes were much evolved among all PCGs, and play a unique role in interspecies evolution. *M. tarapacana* shows a much closer relationship with *M. kuhlii* and *M. eregoodootenkee*.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors are grateful to the management of the Sathyabama Institute of Science and Technology, Chennai for providing necessary facilities to carried out the research work.

Funding

There is no funding involved in this research work from other agencies.

References

Adnet, S., Cappetta, H., Guinot, G., Notarbartolo di Sciara, D., 2012. Evolutionary history of the devil rays (Chondrichthyes: Myliobatiformes) from fossil and morphological inference. Zool. J. Linnean Soc. 166, 132–159.

Aquadro, C.F., Greenberg, B.D., 1983. Human mitochondrial DNA variation and evolution: analysis of nucleotide sequences from seven individuals. Genetics 103 (2), 287–312.

Aschliman, N.C., 2011. The batoid tree of life: recovering the patterns and timing of the evolution of skates, rays and allies. Batoidea, Chondrichthyes.

Avise, J.C., 1989. Gene trees and organismal histories: a phylogenetic approach to population biology. Evolution. 43 (6), 1192–1208.

Broughton, R.E., Milam, J.E., Roe, B.A., 2001. The complete sequence of the zebrafish (Danio rerio) mitochondrial genome and evolutionary patterns in vertebrate mitochondrial DNA. Genome. Res. 11 (11), 1958–1967.

Chang, D.D., Clayton, D.A., 1986. Identification of primary transcriptional start sites of mouse mitochondrial DNA: accurate in vitro initiation of both heavy- and light-strand transcripts. Mol. Cell. Biol. 6 (5), 1446–1453.

Compagno, L.J.V., 1999. An overview of chondrichthyan systematics and biodiversity in Southern Africa. Trans. R. Soc. S. Afr. 54 (1), 75–120. https://doi.org/10.1080/00359199909520406.

Couturier, L.I.E., Marshall, A.D., Jaine, F.R.A., Kashiwagi, T., Pierce, S.J., Townsend, K. A., Weeks, S.J., Bennett, M.B., Richardson, A.J., 2012. Biology, ecology and conservation of the Mobulidae. J. Fish. Biol. 80 (5), 1075–1119.

Fernando, D., Stevens, G., 2011. A study of Sri Lanka's manta and mobula ray fishery. The Manta Trust 29.

Hinojosa-Alvarez, S., Díaz-Jaimes, P., Marcet-Houben, M., Gabaldón, T., 2015. The complete mitochondrial genome of the Giant Manta ray, Manta birostris. Mitochondr. DNA. 26 (5), 787–788.

Iwasaki, W., Fukunaga, T., Isagozawa, R., Yamada, K., Maeda, Y., Satoh, T.P., Sado, T., Mabuchi, K., Takeshima, H., Miya, M., Nishida, M., 2013. MitoFish and MitoAnnotator: a mitochondrial genome database of fish with an accurate and automatic annotation pipeline. Mol. Biol. Evol. 30 (11), 2531–2540.

Junqueira, A.C., Lessinger, A.C., Torres, T.T., da Silva, F.R., Vettore, A.L., Arruda, P., Azeredo Espin, A.M., 2004. The mitochondrial genome of the blowfly Chrysomya chloropyga (Diptera: Calliphoridae). Gene. 15, 7–15. https://doi.org/10.1016/j.gene.2004.06.031. PMID: 15363841.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., Battistuzzi, F.U., 2018. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Mol. Biol. Evol. 35 (6), 1547–1549.

Lee, W.-J., Conroy, J., Howell, W.H., Kocher, T.D., 1995. Structure and evolution of teleost mitochondrial control regions. J Mol Evol 41 (1), 54–66.

Li, X.J., Huang, Y., Lei, F.M., 2015. Comparative mitochondrial genomics and phylogenetic relationships of the Crossoptilon species (Phasianidae, Galliformes). BMC. Genomics. 16, 42.

Liu, G.-H., Chen, F., Chen, Y.-Z., Song, H.-Q., Lin, R.-Q., Zhou, D.-H., Zhu, X.-Q., 2013. Complete mitochondrial genome sequence data provides genetic evidence that the brown dog tick Rhipicephalus sanguineus (Acari: Ixodidae) represents a species complex. Int. J. Biol. Sci. 9 (4), 361–369.

Lohse, M., Drechsel, O., Kahlau, S., Bock, R., 2013. Organellar Genome DRAW a suite of tools for generating physical maps of plastid and mitochondrial genomes and visualizing expression data sets. Nucleic. Acids. Res. 41 (W1), W575–W581.

Lorenz, C., Lünse, C., Mörl, M., 2017. tRNA modifications: Impact on structure and thermal adaptation. Biomolecules. 7 (4), 35. https://doi.org/10.3390/biom7020035.

Marshall, A., Barreto, R., Bigman, J.S., Carlson, J., Fernando, D., Fordham, S., Francis, M.P., Herman, K., Jabado, R.W., Liu, K.M., Pardo, S.A., Rigby, C.L., Romanov, E. & Walls, R.H.L. 2019. Mobula tarapacana. The IUCN Red List of Threatened Species 2019: e.T60199A124451161.

Marshall, A.D., 2009. Biology and population ecology of Manta birostris in southern Mozambique. The University of Queensland, Brisbane, Australia. PhD Thesis.

Martin, A.P., Naylor, G.J.P., Palumbi, S.R., 1992. Rates of mitochondrial DNA evolution in sharks are slow compared with mammals. Nature 357 (6374), 153–155.

Miya, M., Nishida, M., 2015. The mitogenomic contributions to molecular phylogenetics and evolution of fishes: a 15-year retrospect. Ichthyol. Res. 62 (1), 29–71.

Miya, M., Nishida, M., 1999. Organization of the mitochondrial genome of a deep-sea fish, Gonostoma gracile (Teleostei: Stomiiformes): first example of transfer RNA gene rearrangements in bony fishes. Mar. Biotechnol. 1 (5), 416–426.

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

Muller, J., Henle, J., 1841. Systematische beschreibung der plagiostomen. Verlag von Veit & Co. 934 Berlin.

Paig-Tran, E.W.M., Kleinteich, T., Summers, A.P., 2013. The filter pads and filtration mechanisms of the devil rays: variation at macro and microscopic scales. J. Morphol. 274 (9), 1026–1043.

Pindaro, D.J., Natalia, J., Vasquez, B., Douglas, H., Adams, A., M.u.,, 2016. Complete mitochondrial DNA genome of bonnethead shark, Sphyrna tiburo, and phylogenetic relationships among main superorders of modern elasmobranchs, Meta. Gene. 7, 48–55.

Poortvliet, M., Hoarau, G., 2013. The complete mitochondrial genome of the Spinetail Devilray, *Mobula japanica*. Mitochondri. DNA. 24 (1), 28–30.

Raje, S.G., Sivakami, Mohan Raj, S. Manoj Kumar, G., Raju, P.P. A., Joshi. K.K., 2007. An Atlas on the Elasmobranch fishery resources of India.CMFRI. Sp. Publ., 95, 253.

Ranjan, N., Rodnina, M.V., 2016. tRNA wobble modifications and protein homeostasis. Translation 4 (1), e1143076. https://doi.org/10.1080/21690731.2016.1143076.

Rozas, J., 2009. DNA sequence polymorphism analysis using DnaSP. In: Posada, D. (Ed.), Bioinformatics for DNA Sequence Analysis Methods in Molecular Biology Series vol. 537. Humana Press, NJ, USA, pp. 337–350.

Satoh, T.P., Sato, Y., Masuyama, N., Miya, M., Nishida, M., 2010. Transfer RNA gene arrangement and codon usage in vertebrate mitochondrial genomes: a new insight into gene order conservation. BMC. Genomics. 11 (1). https://doi.org/10.1186/1471-2164-11-479.

Shao, R., Barker, S.C., 2006. Mitochondrial genomes of parasitic arthropods: implications for studies of population genetics and evolution. Parasitology. 134 (2), 153–167.

Sun, Y.-X., Wang, L., Wei, G.-Q., Qian, C., Dai, L.-S., Sun, Y.u., Abbas, M.N., Zhu, B.-J., Liu, C.-L., 2016. Characterization of the Complete Mitochondrial Genome of Leucoma salicis (Lepidoptera: Lymantriidae) and Comparison with Other Lepidopteran Insects. Sci. Rep. 6 (1). https://doi.org/10.1038/srep39153.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30 (12), 2725–2729.

Tuorto, F., Lyko, F., 2016. Genome recoding by tRNA modifications. Open Biol. 6 (12), 160287. https://doi.org/10.1098/rsob.160287.

Yue, X., Shi, J., Zou, Y., Wang, B., 2016. The complete mitochondrial genome sequence of Schizothorax lissolabiatus (Cypriniformes: Cyprinidae). Mitochondrial DNA Part. A 27 (4), 2450–2452.

Wenink, P.W., Baker, A.J., Tilanus, M., 1994. Mitochondrial control-region sequences in two shorebird species, the Turnstone and the Dunlin, and their utility in population genetic studies. Mol Biol Evol 11 (1), 22–31.

White, W.T., Clark, T.B., Smith, W.D., Bizzarro, J.J., 2006. Mobula japanica. In IUCN Red List of Threatened Species. Version 2013.2, IUCN: Gland: Switzerland.

White, W.T., Corrigan, S., Yang, L., Henderson, A.C., Bazinet, A.L., Swofford, D.L., Naylor, J.P., 2017. Phylogeny of the Manta and Devil Rays (Chondricthyes: Mobulidae), with an update's taxonomic arrangement for the family. Zool. J. Linn. Soc. 20, 1–26.

White, W.T., Corrigan, S., Yang, L., Henderson, A.C., Bazinet, A.L., Swofford, D.L., Naylor, G.J.P., 2018. Phylogeny of the manta and devilrays (Chondrichthyes: mobulidae), with an updated taxonomic arrangement for the family. Zool. J. Linn. Soc. 182 (1), 50–75.