Mitochondrial genome of ‘numbfish’ Narcine timlei (Bloch & Schneider, 1801) and phylogenetic relationships among order Torpediniformes

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Abstract

Narcine timlei (Bloch & Schneider, 1801) is a medium-sized ray known to occur in inshore and offshore waters of the Indo-Pacific region, classified under the 'vulnerable' category of the IUCN Red List. This study reports, for the first time, the mitochondrial genome of *N. timlei* (average coverage ~ 60X) assembled from short Illumina reads 150 bp paired-ends reads. The mitogenome is 17,964 bp in length includes 13 protein-coding genes (PCGs), 22 tRNA genes, 2 rRNA genes. The gene order, size, and nucleotide compositions were found to be largely consistent with mitogenomic features of previously reported *Narcine* spp. However, the presence of a putative control region of 1,916 bp with three tandem repeats could be a reason for slightly larger mitogenome size than other *Narcine* spp. The phylogenetic reconstruction using concatenated PCGs (n = 13) of 9 Torpediniformes based on maximum likelihood and Bayesian inference analysis produced identical topologies. The tree showed two major clades, one clade having members of the family Narcinidae, and the second sister clade comprise of families Narkidae and Torpedinidae. Our result supports the monophyletic nature of Narcinidae based on mtDNA. The information obtained in this study will contribute to a better understanding of population genetics, phylogenetic analysis, conservation, and evolutionary biology research of *N. timlei.*

Introduction

With almost 650 species among 4 orders and 23 families, superorder Batoidea (rays) forms one of the most species-diverse groups belonging to subclass Elasmobranchii (Fricke 2021). They are phylogenetically a sister group to superorder Selachimorpha (sharks) (Naylor et al. 2012; Aschliman et al. 2012b). One of the 4 orders of Batoidea is Torpediniformes, represented by ~ 68 valid species grouped into 5 families (Eschmeyer's Catalog of Fishes). They are commonly known as electric rays, due to their ability to produce electric discharge to stun prey and for defense (Pitchers et al. 2016). Previous studies on the phylogenetic positioning of Torpediniformes suggest that they are a sister group to other orders of Batoids based on synapomorphies (Claeson 2014), ribosomal genes, and karyological structures (Rocco 2013; Rocco et al. 2007). However, molecular phylogenetic studies using different nuclear and mitochondrial genes have shown Rajiformes are sister to other orders of Batoids, and Torpediniformes are sister to order Myliobatiformes (Rocco 2013). Earlier phylogenetic studies based on morphology and/or a few molecular markers, mostly cytochrome oxidase I (COXI) and/or NADH dehydrogenase 2 (ND2) recognized 4 subfamilies within Torpediniformes, but interrelationship among them remains confusing (Aschliman et al. 2012a; Gaitán-Espitia et al. 2016). Morphological characters depicted the monophyletic nature of the families, i.e. separate clade for Platyrhinidae, Narkidae, Narcinidae, Hypnidae, and Torpedinidae (Claeson 2014). But, the molecular phylogenetics using ND2 markers suggested polyphyly of genus *Narcine* (Naylor et al. 2012).

In the last decade, mitochondrial DNA (mtDNA) has been widely sequenced for elucidating phylogenetic relationships among the taxa as it offers finer taxonomical resolution, especially among cartilaginous fishes (Gaitán-Espitia et al. 2016). The numbers of complete mitochondrial genome sequences for elasmobranchs are on the rise, mainly due to the reduced cost of sequencing and ease of bioinformatics

data analysis, which is improving our phylogenetic understanding of fishes (Amaral et al. 2018; Kousteni et al. 2021). Based on the NCBI database (assessed on 20th Jan 2022), more than 175 complete or partial mtDNA are available for elasmobranchs. However, the mtDNA is still scarcely available for fishes of order Torpediniformes. Only 5 out of 68 valid species have complete mtDNA reported to date, e.g. *Narcine entemedor* (Castillo-Páez et al. 2016), N. *bancroftii, and N. brasiliensis* (Gaitán-Espitia et al. 2016), *Torpedo marmorata* (Naylor et al. 2012), *Narke japonica* (GenBank accession: MZ417389.1), and 3 species have partial mtDNA, i.e. *N. tasmaniensis, Typhlonarke aysoni*, and *Tetronarce macneilli* (Aschliman et al. 2012b). Gaitan-Espitia et al. 2016(Gaitán-Espitia et al. 2016) produced the complete phylogeny of Torpediniformes based on 6 mitogenomes suggested that the monophyletic nature of each order of Batoidea, and that the Torpediniformes was sister to a group comprised of order Myliobatiformes, Pristiformes, and Rajiformes. Further, they also showed the monophyletic nature of genus *Narcine*, contradicting the previous report of polyphyly. The most recent mtDNA phylogeny covering entire elasmobranchs suggests that the Torpediniformes and Rajiformes form a sister clade, though with low support node values (da Cunha et al. 2017; Amaral et al. 2018; Kousteni et al. 2021). It is worth mentioning that these recent mtDNA phylogeny studies had one or few representatives from the order Torpediniformes. Hence, it is important to generate mtDNA for more species of Torpediniformes to resolve their phylogenetic positions.

In the present study, for the first time, we report the mitochondrial genome sequence of *Narcine timlei* (Bloch & Schneider, 1801). This species belongs to the family Narcinidae, commonly known as numbfishes, which is a medium-sized ray with large oval/shovel-shaped discs, stout tails, and a naked body (without dermal denticles) (M.R. de Carvalho. et al. 1999; Ahmad 2013). They are known to occur in inshore and offshore waters of the Indo-Pacific region ranging from Pakistan to south China (Last et al. 2016). Their IUCN conservation status is recently changed from data deficient to ‘vulnerable’ (VanderWright), yet they are common bycatch batoids of mechanized and artisanal fisheries in the southeast coast of India (Bhagyalekshmi and Kumar 2021) (and authors’ per. obs.). We characterized the mitogenome organization of *N. timlei* and compare them with other available Torpediniformes to investigate the evolutionary relationship with the order.

Materials And Method

Specimen collection

The specimen of numbfish *N. timlei* was collected during our routine survey of the Covelong fish landing center (12°47’31”N; 80°15’04”E) for estimation of catch and by-catch diversity. Covelong fisher folks conduct artisanal fishing, mainly using gill net, bottom-gill net techniques at a depth of 0-20 m within 5-7 km from the shoreline (Kumar et al. 2021). The collected specimens were cleaned and photographed in the field before bringing to the laboratory for a detailed study of morphological and meristic characters. The specimens were identified using standard keys and descriptions of de Carvalho et al., (M.R. de Carvalho. et al. 1999; Ahmad et al. 2013).

DNA extraction, library preparation, and sequencing

Total genomic DNA was extracted using OMEGA BIO-TEK E.Z.N.A.Blood & Tissue DNA Kit, as described in (Kumar et al. 2020), and treated with RNase (Promega Corp, USA). The intactness of the DNA was checked using 1% Agarose gel electrophoresis. The quantification was done using the Qubit™ dsDNA BR assay kit (Catalog: Q32853, Thermo Fisher Scientific) and readings were taken in Qubit 3.0 Fluorometer (Thermo Fisher Scientific).

After assuring the quality of genomic DNA, the whole genome sequencing libraries were prepared using NEBNext® Ultra™ II FS DNA Library Prep Kit for Illumina (Catalog: E7805S, New England Biolabs). Briefly, 500ng DNA was enzymatically fragmented using fragmentation reagent by targeting 275 bp to 475 bp fragments size. DNA fragments were subjected to end repair to convert into blunt ends. The 3' to 5' exonuclease activity of the end-repair mix removes the 3' overhangs and polymerase activity fills in the 5' overhangs. To the blunt-ended fragments, adenylation was performed by adding a single 'A' nucleotide to the 3' ends. To the adenylated fragments, loop adapters were ligated and cleaved with the uracil-specific excision reagent (USER) enzyme. Size selection was performed according to the manufacturer's protocol with the addition of AMPure XP beads (Catalog: A63881, Beckman Coulter) for aiming a final library size of 400-600bp. Furthermore, the DNA was amplified by 6 cycles of PCR with the addition of NEBNext Ultra II Q5 master mix, and “NEBNext® Multiplex Oligos for Illumina” to facilitate multiplexing while sequencing. The amplified products were then purified using 0.9X AMPure XP beads (Beckman Coulter) and the final DNA library was eluted in 15µl of 0.1X TE buffer. The concentration of library was determined in a Qubit 3 fluorometer and quality was assessed using Agilent D1000 Screen Tape System. The paired-end (2*150bp) sequencing was performed on Illumina NovoSeq 6000 (Illumina Inc.).

Mitochondrial genome assembly and annotation

A total of 16,107,264 reads were generated, and the quality of the data was checked using FastQC (Bioinformatics 2011) and MultiQC (Ewels et al. 2016). Low-quality reads (Phred score <30), and adapter sequences were removed using fastp (Chen et al. 2018). After quality filtering, the reads were assembled into contigs using Megahit v.1.1.3 (Li et al. 2015) with kmer sizes 21, 49, 77, 105, 133, 141. Contigs of less than 200bp were removed from the assembly. The size of the final assembled genome was 4, 43, 74, 290 bp with the largest contig of 17,964 bp. The assembled contig was annotated by BLAST homology against the NCBI nucleotide database. Further, annotations were conducted on the MITOS web server (Bernt et al. 2013) and MitoZ (Meng et al. 2019) using vertebrate mitochondria genetic code. Genome visualization was conducted with the CGView server (Grant and Stothard 2008) using assembled fasta sequence and map file from the output of MITOS. Codon usages and relative synonymous codon usage (RSCU) for each protein-coding gene (PCGs) were predicted in the Codon Usage web server (http://www.bioinformatics.org/sms2/codon_usage.html) and MEGA X (Kumar et al. 2018) using vertebrate mitochondrial code. tRNA genes were identified in the software ARWEN (Laslett and Canbäck 2008) implemented in the MITOS web server and secondary structure was predicted using tRNAscan-SE

v.2.0 (Chan et al. 2021). The putative control region (POR) was examined for the presence of repeats with the Tandem Repeat Finder v.4.09 webserver (http://tandem.bu.edu/trf/trf.html).

Phylogenetic analysis

The phylogenetic position of *N. timlei* among other species of Torpediniformes was examined. The assembled mitogenome of *N. timlei*, 8 other members of Torpediniformes, and *Gymura poecilura* (Table S1) were used for mitophylogenetic analysis conducted using the MitoPhAST pipeline (Tan et al. 2015). *G. poecilura* belonging to the order Myliobatiformes was chosen as an outgroup. The MitoPhAST pipeline extracts nucleotide sequence for 13 PCG from each of 10 mitogenomes GenBank file, aligns each gene using MAFT (Katoh and Standley 2013) and TranslatorX (Abascal et al. 2010), trims with Gblocks (Talavera and Castresana 2007) to remove ambiguously aligned regions, and concatenate with FASconCAT-G (Kück and Longo 2014) into super matrices. The best-fitting substitution models were selected using ProtTest (Abascal et al. 2005) for each partition. The best model for the current dataset was mtMAM+I+G4 for ATP6, ND5, ND3, ND4L, ND4, ATP8, ND2; mtMAM+I+G4 for COX1, COX2, COX3, ND1, CYTB; and mtZOA+I for ND6. The Rate Gamma and Rate Invariable for ATP6, ND5, ND3, ND4L, ND4, ATP8, and ND2 were 0.823 and 0.246 respectively; for COX1, COX2, COX3, ND1, CYTB was 0.640, and 0.412 respectively. Rate Invariable for ND6 was 0.431. Super matrices along with partition information are used to perform a maximum likelihood (ML) phylogenetic analysis by IQ-TREE (Nguyen et al. 2015). The robustness of the ML tree was analyzed by reiterating the observed data using an ultrafast bootstrap approximation for 1000 generations (Hoang et al. 2018). Further, gene order information was also obtained for comparative analysis. We also conducted Bayesian inference (BI) phylogenetic analyses in Mrbayes (Huelsenbeck and Ronquist 2001). The analysis was performed for 1, 00,000 generations (as the standard deviation of split frequencies of <0.005 was achieved), every 100th tree was sampled from MCMC analysis, and a consensus tree was obtained after discarding the first 25% of sampled trees. Support for nodes in the BI tree was obtained by posterior probability values.

Results And Discussion

Mitogenome organization

We successfully sequenced and assembled the mitogenome of *Narcine timlei*, for the first time. The mitogenome was deposited in the NCBI GenBank under the accession number OM404361. The size of the assembled mitogenome was 17,964 bp, which is the expected size range for Batoids (Kousteni et al. 2021). However, the size is slightly longer than previously published mitogenome of other *Narcine* spp. (Table S2) for e.g. 17081 bp in *N. entemedor*, 16971 bp in *N. bancroftii*, 16997 bp in *N. brasiliensis* (Castillo-Pa´ez et al., 2014; Gaitan-Espitia et al., 2016). The mitogenome of *N. timlei* encodes typical metazoan mitochondrial DNA genes, including 13 protein-coding genes (PCG) (COX1, COX2, COX3, CYTB, ND1, ND2, ND3, ND4, ND4L, ND5, ND6, ATP-6, ATP8), small and large ribosomal RNAs, and a full set of 22 tRNAs (Table 1, Fig. 1). All the PCGs were transcribed from heavy (H) strands, except for ND6 genes.

These PCGs started with the usual start codon ATG except for COX1 which starts with the GTG codon. Most of the PCGs were terminated by a complete codon (TAA/TAG/AGA), while incomplete termination was found for ND4 (T). The incomplete termination at T could be extended to TAA through polyadenylation of 3'end of mRNA at the post-transcriptional level, a common phenomenon in metazoans mitogenome (Ewels et al. 2016).

The base composition of the mtDNA was in the order of A (36.2%)> T (29.2%), C (22.7%), and G (11.9%), with a bias towards A+T content. The A+T bias was also observed in all PCGs. The mtDNA showed a clear AC bias (skew_AT = 0.11, and skew_GC = -0.31), indicating a higher abundance of A than T and C than G (Table 2). Similar skewness was also found in the complete genome of other *Narcine* spp. (Table S2) indicating a common pattern in this genus.

The A+T bias increases the AT-rich codons in codon usage, which seems a common pattern in most of the vertebrates (Boore et al., 1999). The most frequently used codons were: ATTIle (5.77%), CTALeu (5.11%), MetATA (4.43%), TTALeu (3.99%), ThrACA (3.99%), followed by others (Table 3).

The two ribosomal RNAs (large, 16S rRNA and small, 12S rRNA) were transcribed from the H-strand. 12S rRNA was composed of 944 bp and positioned between tRNAphe and tRNAval. 16S rRNA was composed of 1663 bp and positioned between tRNAval and tRNAleu. Both rRNA genes displayed a positive AT skew (~0.20) and a negative GC skew (~0.1). Among the 22 identified transfer RNA genes, 8 were transcribed from the L-strand and the rest others from the H strand. Their size ranges from 67-75 bp and has a typical cloverleaf secondary structure except for one tRNASer which contained a simple loop without a D arm (Fig. S1), similar to many metazoan mitogenomes (Satoh et al. 2016).

The gene order, size, and nucleotide compositions were found to be consistent with mitogenomic features of previously reported *Narcine* spp. (Castillo-Páez et al. 2016; Gaitán-Espitia et al. 2016) (Fig. S2, Table S2). Between tRNA-Pro and tRNA-Phe genes, we found a putative control region of 1916 bp, comparatively longer than Torpediniformes which ranges between 1060 and 1328 bp. The difference could be due to the insertion and/or tandem repeats in the control regions (VanderWright et al., 2021). We found three repeats in this region, first between 270-360 bp of period size 47, second between 1403-1427 bp of period size 10, and third between 1714-1758 bp of period size 22 (Table S3). The larger size of the control region could be the reason for the larger mitogenome size of *N. timlei* than other *Narcine* spp. (Kousteni et al. 2021).

Phylogenetic reconstruction

MtDNA sequences are considered to have enough phylogenetic information to reveal relationships in fishes as they offer small, stable changes over a long period and they are better than single-gene or two concatenated genes phylogeny (Li et al. 2022). We have used mtDNA of 9 species representing 3 families of order Torpediniformes, which is by far the most for any mtDNA phylogenetic studies on Torpediniformes. Kousteni et al. (Kousteni et al. 2021) took 3 species of 2 families and Amaral et al. (Pitchers et al. 2016) took 1 species in elucidating elasmobranch mtDNA phylogeny. The previous most

complete mtDNA phylogeny of Torpediniformes was made using 6 species belonging to 3 families by Gaitan-Espitia et al. (VanderWright et al., 2021).

In the present study, phylogenetic reconstruction using ML and BI analyses produced identical topologies with similar branch lengths. We obtained two major clades; one clade consisted of Narcinidae, while the second clade consisted of Narkidae and Torpedinidae (Fig. 2). Among the family Narcinidae, *N. tasmaniensis* diverged early in the geological time scale from other species. Further, *N. timlei* was branched off and formed a sister subclade to *N. enetmedor, N. brasiliensis,* and *N. bancroftii.* The nodes and internodes of the Narcinidae clade were supported by high bootstrap and the posterior probability values. Our result supports the monophyletic hypothesis of family Narcinidae based on mitogenome (VanderWright et al., 2021) unlike previous studies using ND2 gene phylogeny which suggested polyphyly in Narcinidae (Abascal et al. 2010). The previous studies suggest that Narcinidae is monophyletic only with the inclusion of Narkidae (Claeson 2014). It was also suggested that few narkids are derived members of the Narcinidae based on the comparative anatomy (da Cunha et al. 2017) and some genera such as *Narcine* are sister to Torpedinidae and Hypnidae, whereas genus *Discopyge* is sister to *Benthobatis* and *Typhlonarke* (Moreira and de Carvalho 2021). The inclusion of *Narke japonica* in the phylogenetic tree indicates that *N. japonica* branched off early from *Typhlonarke ayosni* which is sister to Torpedinidae, though the node support for these branches is smaller (<50%). Without *N. japonica*, it was reported that *Torpedo* splits early from other families (Aschliman et al. 2012a; Gaitán-Espitia et al. 2016). Our analysis suggests that tree topologies and interrelationships among the members of the order Torpediniformes have been changing with the addition of more species. Hence, it is required to obtain complete mtDNA of more species to achieve a more accurate phylogenetic resolution within the order.

Declarations

Author contribution

AK and SP designed the study, AK performed the data analysis and wrote original draft which was subsequently corrected by SP and both author agreed upon final version.

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.

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Tables

Table 1 Mitochondrial genome of *Narcine timlei*: arrangements and annotation

Gene name	Gene product	Start	Stop	Length (bp)	Strand
tRNA	tRNA-Pro	298	367	70	-
tRNA	tRNA-Phe	2283	2352	70	+
rRNA	12S ribosomal RNA	2353	3297	945	+
tRNA	tRNA-Val	3297	3368	72	+
rRNA	16S ribosomal RNA	3369	5032	1664	+
tRNA	tRNA-Leu	5032	5107	76	+
CDS	NADH dehydrogenase subunit 1	5108	6083	976	+
tRNA	tRNA-Ile	6083	6151	69	+
tRNA	tRNA-Gln	6148	6219	72	-
tRNA	tRNA-Met	6219	6288	70	+
CDS	NADH dehydrogenase subunit 2	6288	7335	1048	+
tRNA	tRNA-Trp	7333	7402	70	+
tRNA	tRNA-Ala	7404	7473	70	-
tRNA	tRNA-Asn	7474	7547	74	-
tRNA	tRNA-Cys	7578	7643	66	-
tRNA	tRNA-Tyr	7643	7712	70	-
CDS	cytochrome c oxidase subunit I	7713	9270	1558	+
tRNA	tRNA-Ser	9270	9341	72	-
tRNA	tRNA-Asp	9341	9410	70	+
CDS	cytochrome c oxidase subunit II	9412	10111	700	+
tRNA	tRNA-Lys	10103	10176	74	+
CDS	ATP synthase F0 subunit 8	10177	10345	169	+
CDS	ATP synthase F0 subunit 6	10335	11019	685	+
CDS	cytochrome c oxidase subunit III	11019	11805	787	+
tRNA	tRNA-Gly	11804	11875	72	+
CDS	NADH dehydrogenase subunit	11875	12226	352	+
tRNA	tRNA-Arg	12226	12298	73	+
CDS	NADH dehydrogenase subunit 4L	12298	12595	298	+
CDS	NADH dehydrogenase subunit 4	12588	13966	1379	+
tRNA	tRNA-His	13969	14038	70	+
tRNA	tRNA-Ser	14038	14105	68	+
tRNA	tRNA-Leu	14105	14177	73	+
CDS	NADH dehydrogenase subunit 5	14177	16004	1828	+
CDS	NADH dehydrogenase subunit 6	15988	16504	517	-
tRNA	tRNA-Glu	16504	16574	71	-
CDS	cytochrome b	16576	17719	1144	+
tRNA	tRNA-Thr	17719	17789	71	+

Table 2 Nucleotide of genes and the mitochondrial genome skew of *N. timlei*

	Size bp	A%	G%	C%	T%	G+C (%)	A+T (%)	Skew_AT	Skew_GC
t DNA	17964	36.19	11.92	22.68	29.21	34.60	65.40	0.11	-0.31
CGs	11428	33.81	11.89	23.40	30.90	35.29	64.71	0.05	-0.33
D1	975	34.26	10.15	27.28	28.31	37.44	62.56	0.10	-0.46
D2	1047	40.21	8.12	24.93	26.74	33.05	66.95	0.20	-0.51
DX1	1557	30.06	15.35	23.12	31.47	38.47	61.53	-0.02	-0.20
DX2	699	34.91	13.59	22.03	29.47	35.62	64.38	0.08	-0.24
TP8	168	43.45	6.55	20.24	29.76	26.79	73.21	0.19	-0.51
TP6	684	35.67	9.21	23.39	31.73	32.60	67.40	0.06	-0.43
DX3	786	29.90	15.27	24.55	30.28	39.82	60.18	-0.01	-0.23
D3	351	32.76	10.54	23.65	33.05	34.19	65.81	0.00	-0.38
D4L	297	32.66	9.76	26.60	30.98	36.36	63.64	0.03	-0.46
D4	1378	35.49	10.01	23.88	30.62	33.89	66.11	0.07	-0.41
D5	1827	37.38	9.85	23.54	29.23	33.39	66.61	0.12	-0.41
D6	516	18.22	25.78	6.98	49.03	32.75	67.25	-0.46	0.57
YTB	1143	32.11	11.37	25.28	31.23	36.66	63.34	0.01	-0.38

Table 3 Codon usage analysis of PCGs in the mitochondrial genome of *N. timlei*

AA	Codon	N	%	RSCU	Fraction	AA	Codon	N	%	RSCU	Fraction
Ala	GCG	0	0.000	0	0	Asn	AAT	136	3.570	1.24	0.62
	GCA	93	2.442	1.99	0.5		AAC	84	2.205	0.76	0.38
	GCT	39	1.024	0.83	0.21	Pro	CCG	10	0.263	0.18	0.04
	GCC	55	1.444	1.18	0.29		CCA	124	3.255	2.21	0.55
Cys	TGT	23	0.604	0.98	0.49		CCT	62	1.628	1.11	0.28
	TGC	24	0.630	1.02	0.51		CCC	28	0.735	0.5	0.13
Asp	GAT	30	0.788	1.09	0.55	Gln	CAG	4	0.105	0.09	0.04
	GAC	25	0.656	0.91	0.45		CAA	88	2.310	1.91	0.96
Glu	GAG	6	0.158	1.85	0.07	Arg	CGG	7	0.184	0.37	0.09
	GAA	75	1.969	0.15	0.93		CGA	33	0.866	1.76	0.44
Phe	TTT	130	3.413	1.22	0.61		CGT	11	0.289	0.59	0.15
	TTC	83	2.179	0.78	0.39		CGC	24	0.630	1.28	0.32
Gly	GGG	12	0.315	0.31	0.08	Ser	AGT	34	0.893	0.73	0.12
	GGA	84	2.205	2.2	0.55		AGC	55	1.444	1.18	0.2
	GGT	16	0.420	0.42	0.1		TCG	9	0.236	0.19	0.03
	GGC	41	1.076	1.07	0.27		TCA	106	2.783	2.27	0.38
His	CAT	73	1.917	0.99	0.5		TCT	42	1.103	0.9	0.15
	CAC	74	1.943	1.01	0.5		TCC	34	0.893	0.73	0.12
Ile	ATT	220	5.776	1.31	0.66	Thr	ACG	6	0.158	0.08	0.02
	ATC	115	3.019	0.69	0.34		ACA	152	3.991	1.94	0.49
Lys	AAG	9	0.236	0.15	0.08		ACT	87	2.284	1.11	0.28
	AAA	110	2.888	1.85	0.92		ACC	68	1.785	0.87	0.22
Leu	TTG	15	0.394	0.17	0.03	Val	GTG	4	0.105	0.13	0.03
	TTA	152	3.991	1.69	0.28		GTA	58	1.523	1.83	0.46
	CTG	16	0.420	0.18	0.03		GTT	42	1.103	1.32	0.33
	CTA	195	5.119	2.16	0.36		GTC	23	0.604	0.72	0.18
	CTT	106	2.783	1.18	0.2	Trp	TGG	37	0.971	0.56	0.28
	CTC	57	1.496	0.63	0.11		TGA	95	2.494	1.44	0.72
Met	ATG	32	0.840	0.32	0.16	Tyr	TAT	103	2.704	1.29	0.64
	ATA	170	4.463	1.68	0.84		TAC	57	1.496	0.71	0.36

Figures

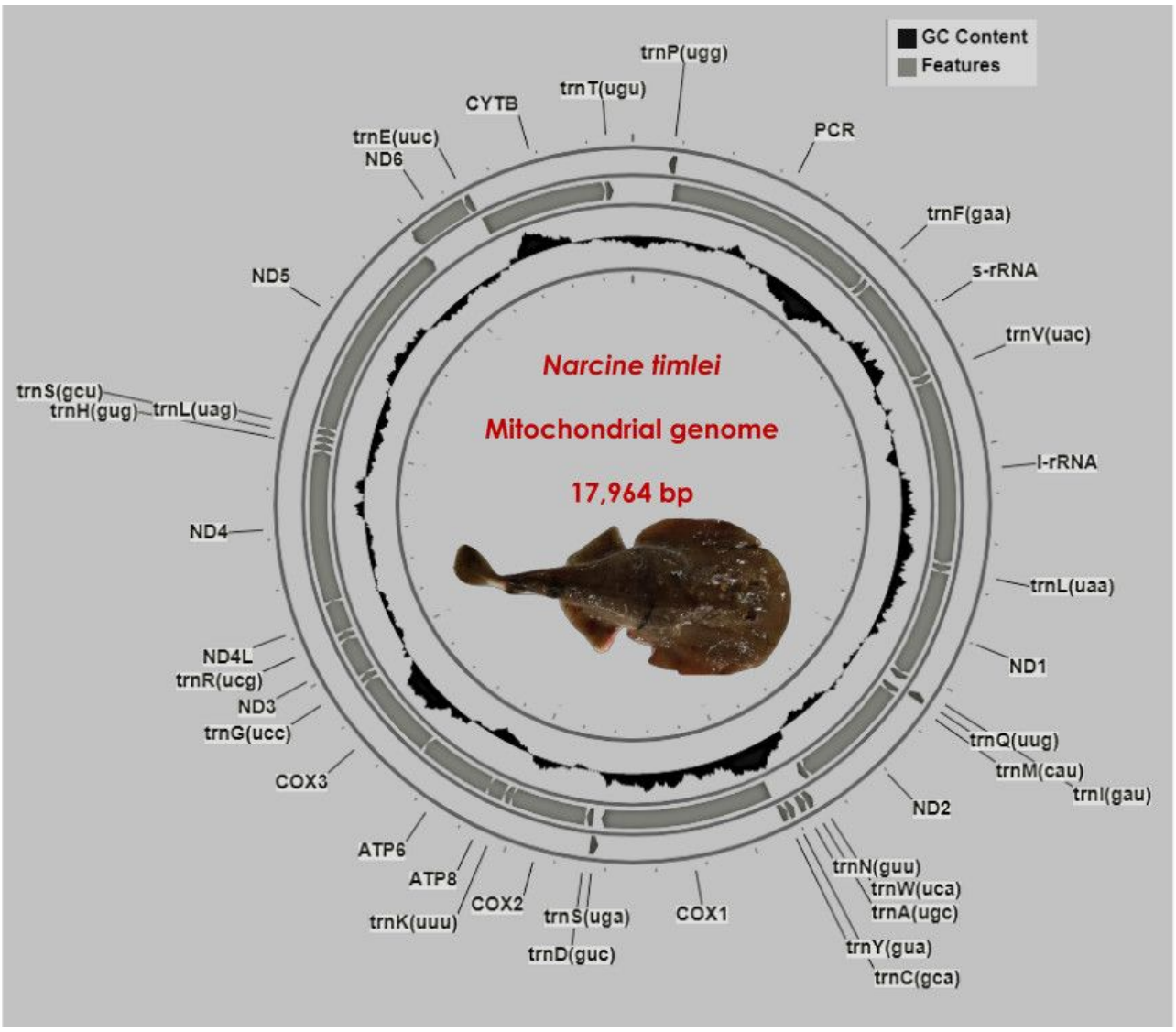

Figure 1

Schematic representation of the mitochondrial genome of . The outer most circle represents light strand having a PCG and 8 tRNAs. The second circle represents heavy strand having 12 protein-coding genes (PCGs), 14 transfer RNAs (tRNA), 2 ribosomal RNA genes, and a putative control region. The inner-circle depicts GC content along the mitogenome. Abbreviations: COX: cytochrome oxidase, CYTB: cytochrome b, ND: Nicotinamide adenine dinucleotide oxidoreductase, ATP: Adenosine Triphosphate synthase, PCR: putative control region.

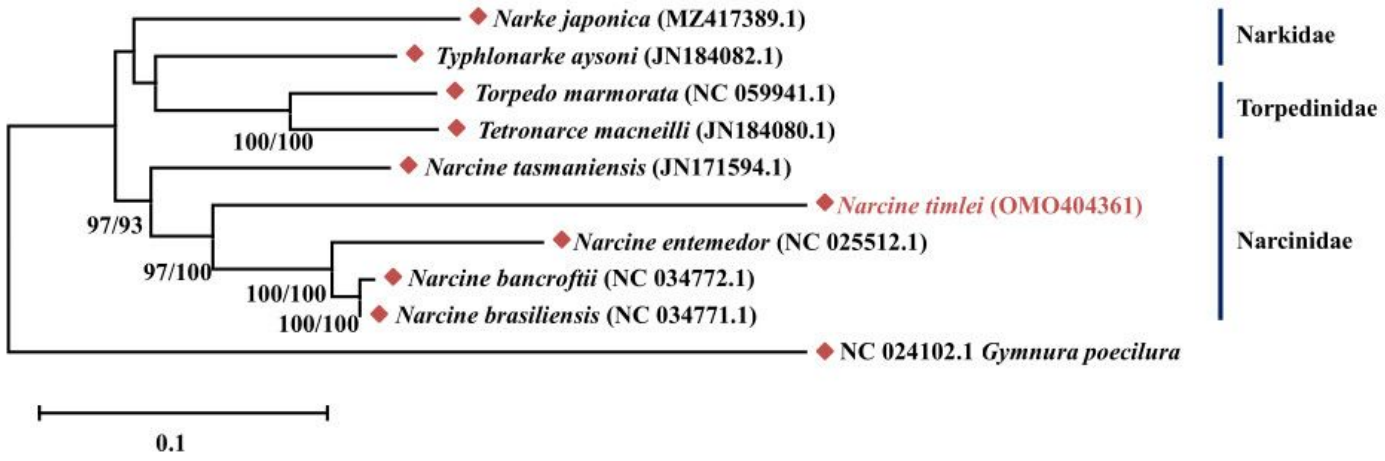

Figure 2

Phylogenetic tree obtained from ML and BI analysis based on a concatenated alignment of 13 protein coding genes for 9 species belonging to order Torpediniformes, and (Myliobatiformes) which was taken as outgroup. ML bootstrap and BI posterior probability values are represented in nodes. Values lesser than 50 is not shown.

Supplementary Files

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- FigS1.tif
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- TableS1.docx
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- TableS3.docx