Mitochondrial genome of 'spotted numbfish' *Narcine timlei* (Bloch & Schneider, 1801) and phylogenetic relationships among order Torpediniformes

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Abstract

In this study, we report, for the first time, mitochondrial genome of *Narcine timlei* (Bloch & Schneider, 1801) and its phylogenetic relationships within the order Torpediniformes. *Narcine timlei* is a medium-sized ray that occurs in nearshore waters of the Indo-Pacific, classified as a 'vulnerable' category on the IUCN Red List. The mitogenome is assembled from short Illumina reads (150 bp paired-end reads). It is 17,964 bp long and includes 13 protein-coding genes (PCGs), 22 tRNA genes, and 2 rRNA genes. The gene order, size, and nucleotide composition are largely consistent with mitogenomic characteristics of previously reported other *Narcine* spp. The slightly larger mitogenome length of *N. timlei* than other *Narcine* spp. may be due to the presence of a putative control region of 1,916 bp with three tandem repeats. Phylogenetic reconstruction using concatenated PCGs (n=13) of 9 Torpediniformes based on maximum likelihood and Bayesian inference analysis revealed identical topologies. The tree showed two main clades, one clade containing members of the family Narcinidae, and the second sister clade consisting of the 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 the population genetics, phylogenetic analysis, conservation, and evolutionary biology research of *N. timlei.*

Introduction

With nearly 650 species in 4 orders and 23 families, the superorder Batoidea (rays) forms one of the most speciose groups of the subclass Elasmobranchii (Fricke, 2021). Phylogenetically, they are sister to superorder Selachimorpha (sharks) (Naylor et al., 2012; Aschliman et al., 2012b). One of the 4 orders of Batoidea is Torpediniformes, which is grouped into 5 families with approximately 68 valid species (Eschmeyer's Catalog of Fishes). They are commonly referred as electric rays, due to their ability to generate electrical discharges to stun prey and to defend themselves (Pitchers et al., 2016). Electric rays play important role in benthic ecosystem as they are the predators, feed on the diverse invertebrates and small fishes, however their contribution in benthic dynamic is least known (Moazzam and Osmany, 2021;

Barria et al., 2015). 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 that the Rajiformes are related to other orders of Batoids, and Torpediniformes are related to the order Myliobatiformes (Rocco 2013). Previous phylogenetic studies based on a few molecular markers, mostly cytochrome oxidase I (COXI) and/or NADH dehydrogenase 2 (ND2) recognized 4 subfamilies within Torpediniformes, but the relationships among them remain confusing (Aschliman et al., 2012a; Gaitán-Espitia et al., 2016). Morphological characters indicate that the families are monophyletic i.e., separate clade for Platyrhinidae, Narkidae, Narcinidae, Hypnidae, and Torpedinidae (Claeson 2014). However, molecular phylogenetics using ND2 markers suggests polyphyly of the genus *Narcine* (Naylor et al., 2012).

In the last decade, mitochondrial DNA (mtDNA) has been widely sequenced to elucidate phylogenetic relationships among taxa because it provides finer taxonomic resolution, especially in cartilaginous fishes (Gaitán-Espitia et al., 2016). The number of complete mitochondrial genome sequences for elasmobranchs is increasing, mainly due to the reduced cost of sequencing and ease of bioinformatic data analysis, which improves our phylogenetic understanding of fishes (Amaral et al., 2018; Kousteni et al., 2021). Based on the NCBI database (checked on 20th Jan 2022), more than 175 complete or partial mtDNA are available for elasmobranchs. However, mtDNA is still scarcely available for fishes of the order Torpediniformes. Complete mtDNA has been only reported from 5 of the 68 valid species 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). Gaitán-Espitia et al. 2016 established the complete phylogeny of Torpediniformes based on 6 mitogenomes, suggesting that the individual orders of Batoidea formed separate clade i.e., monophyletic in nature, and that Torpediniformes belongs to a group that includes the order Myliobatiformes, Pristiformes, and Rajiformes. They also showed genus *Narcine* is monophyletic, contradicting the earlier report of polyphyly. The recent mtDNA phylogeny encompassing all elasmobranchs suggests that Torpediniformes and Rajiformes form a sister clade, albeit with low support node values (da Cunha et al., 2017; Amaral et al., 2018; Kousteni et al., 2021). It is worth noting that these recent mtDNA phylogeny studies had one or few representatives of the order Torpediniformes. Therefore, it is important to generate mtDNA for more species of Torpediniformes to clarify their phylogenetic position.

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

Materials And Method

2.1 Specimen collection

The specimen of spotted numbfish *N. timlei* was collected in November 2021 during our routine survey at the Covelong Fish Landing Center (12°47’31”N; 80°15’04”E) to determine the diversity of catches and by-catch. Covelong fisher folks engage in artisanal fishing, mainly using gillnets, and bottom gillnets at depths of 0-20 m within 5-7 km of shore (Kumar et al., 2021). Collected specimens were cleaned and photographed in the field before being taken to the laboratory for detailed study of morphological and

meristic characters. Specimens were identified using standard keys and descriptions (de Carvalho. et al., 1999; Ahmad et al., 2013).

2.2 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 by 1% agarose gel electrophoresis. Quantification was performed using the Qubit™ dsDNA BR assay kit (Catalog: Q32853, Thermo Fisher Scientific) and measurements were performed in the Qubit 3.0 Fluorometer (Thermo Fisher Scientific).

After ensuring the quality of genomic DNA, whole genome sequencing libraries were prepared using the NEBNext® Ultra™ II FS DNA Library Prep Kit for Illumina (Catalog: E7805S, New England Biolabs). Briefly, 500ng of DNA was enzymatically fragmented using a fragmentation reagent by targeting 275 bp to 475 bp size. DNA fragments were subjected to end repair to convert them into blunt ends. The 3' to-5' exonuclease activity of the end-repair mixture removes the 3' overhangs and the polymerase activity fills the 5' overhangs. The fragments with the blunt-ends were adenylated by adding a single 'A' nucleotide to the 3' ends. Loop adapters were ligated to the adenylated fragments, 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) to achieve a final library size of 400-600bp. In addition, DNA was amplified by 6 PCR cycles with the addition of NEBNext Ultra II Q5 Mastermix, and “NEBNext® Multiplex Oligos for Illumina” to facilitate multiplexing during sequencing. The amplified products were then purified with 0.9X AMPure XP beads (Beckman Coulter) and the final DNA library was eluted in 15µl of 0.1X TE buffer. Library concentration was determined using Qubit 3 fluorometer and quality was assessed using the Agilent D1000 Screen Tape System. Paired end sequencing (2*150bp) was performed using Illumina NovoSeq 6000 (Illumina Inc., USA).

2.3 Mitochondrial genome assembly and annotation

A total of 16,107,264 reads were generated, and the quality of the data was checked using FastQC (Andrews, 2010) 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. Final assembled mitogenome of 17,964 bp was obtained, and subjected to BLAST homology against the NCBI nucleotide database. In addition, annotations were performed with MitoAnnotator (Iwasaki et al 2013) using the genetic code of vertebrate mitochondria. Mitogenome visualization was performed with the CGView server (Grant and Stothard 2008) using the composite fasta sequence and map file from the output of MitoAnnotator. Codon usages and relative synonymous codon usages (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 the vertebrate mitochondrial code. tRNA genes were identified using ARWEN software (Laslett

and Canbäck 2008) implemented in the MITOS Web Server (Bernt et al., 2013) and secondary structure was predicted using tRNAscan-SE v.2.0 (Chan et al., 2021). The putative control region (POR) was analyzed for the presence of repeats using the Tandem Repeat Finder v.4.09 Web Server (http://tandem.bu.edu/trf/trf.html).

2.4 Phylogenetic analysis

The phylogenetic position of *N. timlei* among other species of Torpediniformes was investigated. The assembled mitogenome of *N. timlei*, 8 other members of Torpediniformes, and *Gymnura poecilura* (Table S1) were used for mitophylogenetic analysis, performed using the MitoPhAST pipeline (Tan et al., 2015). *G. poecilura,* which belongs to the order Myliobatiformes was selected as an outgroup. The MitoPhAST pipeline extracts the nucleotide sequence for 13 PCGs from each of the 10 GenBank files of mitogenomes, aligns each gene with MAFT (Katoh and Standley 2013) and TranslatorX (Abascal et al., 2010), trims it with Gblocks (Talavera and Castresana 2007) to remove ambiguously aligned regions, and concatenates it into supermatrices with FASconCAT-G (Kück and Longo 2014). The best-fitting substitution models were selected for each partition using ProtTest (Abascal et al., 2005). The best model for the current data set was mtMAM+I+G4 for ATP6, ND5, ND3, ND4L, ND4, ATP8, and 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. The rate of invariable for ND6 was 0.431. Supermatrices along with partition information were used to perform 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 with an ultrafast bootstrap approximation for 1000 generations (Hoang et al., 2018). In addition, gene order information was also obtained for comparative analysis. We also performed phylogenetic analysis using Bayesian inference (BI) 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 100^{th} tree was sampled from the MCMC analysis, and a consensus tree was obtained after discarding the first 25% of the sampled trees. Support for the nodes in the BI tree was obtained by the posterior probability values.

Results And Discussion

3.1 Mitogenome organization

The mitogenome of *Narcine timlei* was successfully sequenced and assembled, and it was deposited in the NCBI GenBank under the accession number OM404361The 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 the 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; Gaitán-Espitia et al., 2016). The mitogenome of *N. timlei* encodes typical mitochondrial DNA genes of metazoans, including 13 protein-coding genes (PCGs) (COX1, COX2, COX3, CYTB, ND1, ND2, ND3, ND4, ND4L, ND5, ND6, ATP-6, ATP8), small and large ribosomal RNAs, and a complete set of 22 tRNAs (Table 1, Figure 1).

With the exception of the ND6 gene, all PCGs were transcribed from the heavy strands (H). These PCGs began with the common start codon ATG, with the exception of COX1, which began with the codon GTG codon. Most PCGs terminated with a complete codon (TAA/TAG/AGA), whereas incomplete termination was observed at ND4 (T). The incomplete termination at T could be extended to TAA by through polyadenylation of the 3' end of the mRNA at the posttranscriptional level, a common phenomenon in the metazoan mitogenome (Ewels et al., 2016).

The base composition of the mtDNA was in the order A (36.2%)> T (29.2%), C (22.7%), and G (11.9%), with a tendency towards A+T content. The A+T bias was also observed in all PCGs. The mtDNA showed a significant AC bias (skew_AT = 0.11, and skew_GC = -0.31), indicating a greater 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 appears to be a common pattern in most vertebrates (Boore et al., 1999). The most frequently used codons were: ATTIle (5.77%), CTALeu (5.11%), MetATA (4.43%), TTALeu (3.99%), and 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 consisted of 944 bp and was located between tRNAphe and tRNAval. 16S rRNA consisted of 1663 bp and was located between tRNAval and tRNAleu. Both rRNA genes had a positive AT skew (~0.20) and a negative GC skew (~0.1). Of the 22 transfer RNA genes identified, 8 were transcribed from the L-strand, and the remaining from the H strand. Their size ranges from 67-75 bp and exhibits a typical cloverleaf secondary structure, except for one tRNASer that contained a simple loop without a D- arm (Figure S1), similar to many metazoan mitogenomes (Satoh et al., 2016).

Gene order, size, and nucleotide composition were consistent with mitogenomic features of previously reported *Narcine* spp. (Castillo-Páez et al., 2016; Gaitán-Espitia et al., 2016) (Figure S2, Table S2).

Between the tRNA-Pro and tRNA-Phe genes, we found a putative control region (PCR) of 1916 bp, comparatively longer than in Torpediniformes which ranges between 1060 and 1328 bp. The difference could be due to the insertion and/or tandem repeats in the control regions (Kousteni et al., 2021). The base composition of the PCR was 31.1% for G, 15.2% for C, 35.2% for T, and 18.4% for T with the negative GC skew (-0.39) and positive AT skew (0.25). We found three repeats in this region, first between 270-360 bp with period size 47, second between 1403-1427 bp with period size 10, and third between 1714-1758 bp with period size 22 (Table S3 & Figure S3). The larger size of the control region might be the reason for the larger mitogenome size of *N. timlei* compared to other *Narcine* spp. (Kousteni et al., 2021).

3.2 Phylogenetic reconstruction

MtDNA sequences are considered to have enough phylogenetic information to reveal relationships in fishes because they show small, stable changes over a long period of time and are better than the phylogeny of single-gene or two concatenated genes (Li et al., 2022). We 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. (2021) took 3 species of 2 families and Amaral et al. 2018 took 1 species of Torpediniformes in elasmobranch mtDNA phylogeny. The most complete mtDNA phylogeny of Torpediniformes to date was established by Gaitán-Espitia et al. (2016) with 6 species from 3 families. In the present study, phylogenetic reconstruction using ML and BI analyses revealed identical topologies with similar branch lengths. We obtained two main clades; one clade consisted of Narcinidae, while the second clade consisted of Narkidae and Torpedinidae (Figure 2). Within the family Narcinidae, *N. tasmaniensis* diverged early from other species in the geological time scale. In addition, *N. timlei* branched off and formed a separate subclade containing *N. enetmedor, N. brasiliensis,* and *N. bancroftii.* The nodes and internodes of the Narcinidae clade were supported by high bootstrap and posterior probability values. Our result supports the monophyletic hypothesis of the family Narcinidae based on mitogenome (VanderWright et al., 2021), in contrast to previous studies that used the ND2 gene phylogeny and suggested polyphyly of Narcinidae (Abascal et al., 2010). The earlier studies suggest that the Narcinidae are monophyletic only with the inclusion of Narkidae (Claeson 2014). It has also been suggested that some narkids are derived members of the Narcinidae based on comparative anatomy (da Cunha et al., 2017) and some genera such as *Narcine* are sister to Torpedinidae and Hypnidae, while genus *Discopyge* is sister to *Benthobatis* and *Typhlonarke* (Moreira and de Carvalho 2021). The inclusion of *Narke japonica* in the phylogenetic tree suggests that *N. japonica* branched early from *Typhlonarke ayosni*, a sister genus of Torpedinidae, although nodal support for these branches is lower (<50%). Excluding *N. japonica*, Torpedo has been reported to split 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 changed with inclusion of additional species. Therefore, it is necessary to obtain the complete mtDNA of more species to achieve a more accurate phylogenetic resolution within the order.

Declarations

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Data Availability Statement

Mitogenome generated in the present study is submitted to NCBI GenBank under the accession number OM404361.

Conflict of Interest Statement

The authors declare that they have no conflict of interest regarding the publication of this article.

Preprint:

A preprint has previously been published on Research Square (Kumar & Prakash, 2022, reference no.1804785).

References

Abascal, F., Zardoya, R., & Posada, D. (2005). ProtTest: selection of best-fit models of protein evolution. *Bioinformatics 21 (9):*2104-2105.

Abascal, F., Zardoya, R., & Telford, M. J. (2010). TranslatorX: multiple alignment of nucleotide sequences guided by amino acid translations. *Nucleic Acids Research* 38 (suppl_2):W7-W13.

Ahmad, A., Lim, A. P. K., Fahmi, & Dharmadi (2013). Field Guide to Look-alike Sharks and Rays Species of the Southeast Asian Region. SEAFDEC/MFRDMD/SP/22:107.

Amaral, C. R., Pereira, F., Silva, D. A., Amorim, A., & de Carvalho, E. F. (2018). The mitogenomic phylogeny of the Elasmobranchii (Chondrichthyes). *Mitochondrial DNA Part A 29* (6):867-878.

Andrews, S. (2010). FastQC: a quality control tool for high throughput sequence data.

Aschliman, N. C., Claeson, K. M., & McEachran, J. D. (2012a). Phylogeny of batoidea. *Biology of Sharks and their Relatives* 2:57-96

Aschliman, N. C., Nishida, M., Miya, M., Inoue, J. G., Rosana, K. M., & Naylor, G. J. (2012). Body plan convergence in the evolution of skates and rays (Chondrichthyes: Batoidea). *Molecular Phylogenetics and Evolution*, *63*(1), 28-42.

Barria, C., Coll, M., Navarro, J. (2015). Unravelling the ecological role and trophic relationships of uncommon and threatened elasmobranchs in the western Mediterranean Sea. *Marine Ecology Progress Series*, 539, 225-240.

Bhagyalekshmi, V., & Kumar, A. B. (2021). Bycatch of non-commercial batoids in the trawl fishery of south India: Status and conservation prerequisites. *Regional Studies in Marine Science*, *44*, 101738.

Carvalho, M. D., Compagno, L. J. V., & Last, P. R. (1999). Narcinidae. Numbfishes. *FAO identification guide for fishery purposes. The living marine resources of the Western Central Pacific, FAO, Rome*, 1433-1442..

Castillo-Páez, A., del Río-Portilla, M. A., & Rocha-Olivares, A. (2016). The complete mitochondrial genome of the giant electric ray, *Narcine entemedor* (Elasmobranchii: Torpediniformes). *Mitochondrial DNA Part A*, *27*(3), 1760-1762.

Chan, P. P., Lin, B. Y., Mak, A. J., & Lowe, T. M. (2021). tRNAscan-SE 2.0: improved detection and functional classification of transfer RNA genes. *Nucleic Acids Research*, *49*(16), 9077-9096.

Chen, S., Zhou, Y., Chen, Y., & Gu, J. (2018). fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*, *34*(17), i884-i890.

Claeson, K. M. (2014). The impacts of comparative anatomy of electric rays (Batoidea: Torpediniformes) on their systematic hypotheses. *Journal of Morphology*, *275*(6), 597-612.

da Cunha, D. B., da Silva Rodrigues-Filho, L. F., & de Luna Sales, J. B. (2017). A review of the mitogenomic phylogeny of the Chondrichthyes. *Chondrichthyes Multidiscip. Approach*.

Ewels, P., Magnusson, M., Lundin, S., & Käller, M. (2016). MultiQC: summarize analysis results for multiple tools and samples in a single report. *Bioinformatics*, *32*(19), 3047-3048.

Fricke R, Eschmeyer, W. N. & Fong, J. D. (2021) Species by Family/Subfamily (http://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp). Electronic version accessed 26/12/2021.

Gaitán-Espitia, J. D., Solano-Iguaran, J. J., Tejada-Martinez, D., & Quintero-Galvis, J. F. (2016). Mitogenomics of electric rays: evolutionary considerations within Torpediniformes (Batoidea; Chondrichthyes). *Zoological Journal of the Linnean Society*, *178*(2), 257-266.

Grant, J. R., & Stothard, P. (2008). The CGView Server: a comparative genomics tool for circular genomes. *Nucleic Acids Research*, *36*(suppl_2), W181-W184.

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. *Molecular Biology And Evolution*, 30, 2531-2540.

Hoang, D. T., Chernomor, O., Von Haeseler, A., Minh, B. Q., & Vinh, L. S. (2018). UFBoot2: improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution*, *35*(2), 518-522.

Huelsenbeck, J. P., & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, *17*(8), 754-755.

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, *30*(4), 772-780.

Kousteni, V., Mazzoleni, S., Vasileiadou, K., & Rovatsos, M. (2021). Complete mitochondrial DNA genome of nine species of sharks and rays and their phylogenetic placement among modern elasmobranchs. *Genes*, *12*(3), 324.

Kück, P., & Longo, G. C. (2014). FASconCAT-G: extensive functions for multiple sequence alignment preparations concerning phylogenetic studies. *Frontiers in zoology*, *11*(1), 1-8.

Kumar, A., Adhavan, D., & Prakash, S. (2020). DNA barcoding revealed first record of the 'fine spotted *whipray'Himantura tutul* (Myliobatoidei: Dasyatidae) in the Indian coastal waters. *Journal of Applied Ichthyology*, *36*(4), 515-518.

Kumar, A., Vinuganesh, A., & Prakash, S. (2021). An assessment of marine and coastal diversity of Covelong, Chennai, India. *Regional Studies in Marine Science*, *48*, 102034.

Kumar, A., Prakash, S. (2022). Mitochondrial genome of 'numbfish' *Narcine timlei* (Bloch & amp; Schneider, 1801) and phylogenetic relationships among order Torpediniformes, 30 June 2022, Pre-print (Version 1) available at Research Square https://doi.org/10.21203/rs.3.rs-1804785/v1.

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*(6), 1547.

Laslett, D., & Canbäck, B. (2008). ARWEN: a program to detect tRNA genes in metazoan mitochondrial nucleotide sequences. *Bioinformatics*, *24*(2), 172-175.

Last, P., Naylor, G., Séret, B., White, W., de Carvalho, M., & Stehmann, M. (Eds.). (2016). *Rays of the World*. CSIRO publishing.,

Li, D., Liu, C. M., Luo, R., Sadakane, K., & Lam, T. W. (2015). MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. *Bioinformatics*, *31*(10), 1674-1676.

Li, W., Qiu, N., & Du, H. (2022). Complete mitochondrial genome of Rhodeuscyanorostris (Teleostei, Cyprinidae): characterization and phylogenetic analysis. *ZooKeys*, *1081*, 111.

Moazzam1, M., & Osmany, H. B. (2021). Species composition and distribution of electric rays (class: Pisces; subclass: Elasmobranchii; order: Torpediniformes) from Pakistan. *International Journal of Biology and Biotechnology* 18 (4): 725-743, 2021.

Moreira, R. A., & de Carvalho, M. R. (2021). Phylogenetic significance of clasper morphology of electric rays (Chondrichthyes: Batoidea: Torpediniformes). *Journal of Morphology*, *282*(3), 438-448.

Naylor, G. J., Caira, J. N., Jensen, K., Rosana, K., White, W.T., Last, P. (2012**).** A DNA sequence–based approach to the identification of shark and ray species and its implications for global elasmobranch diversity and parasitology. *Bulletin of the American Museum of Natural History* 2012 (367):1-262.

Nguyen, L. T., Schmidt, H. A., Von Haeseler, A., & Minh, B. Q. (2015). IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular biology and evolution*, *32*(1), 268-274.

Pitchers, W. R., Constantinou, S. J., Losilla, M., & Gallant, J. R. (2016). Electric fish genomics: progress, prospects, and new tools for neuroethology. *Journal of Physiology-Paris*, *110*(3), 259-272.

Rocco, L. (2013). Molecular and Chromosomal Markers for Evolutionary Considerations in Torpediniformes (Chondrichthyes, Batoidea). *International Scholarly Research Notices*, *2013*.

Rocco, L., Liguori, I., Costagliola, D., Morescalchi, M. A., Tinti, F., & Stingo, V. (2007). Molecular and karyological aspects of Batoidea (Chondrichthyes, Elasmobranchi) phylogeny. *Gene*, *389*(1), 80-86.

Satoh, T. P., Miya, M., Mabuchi, K., & Nishida, M. (2016). Structure and variation of the mitochondrial genome of fishes. *BMC genomics*, *17*(1), 1-20.

Talavera, G., & Castresana, J. (2007). Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology*, *56*(4), 564-577.

Tan, M. H., Gan, H. M., Schultz, M. B., & Austin, C. M. (2015). MitoPhAST, a new automated mitogenomic phylogeny tool in the post-genomic era with a case study of 89 decapod mitogenomes including eight new freshwater crayfish mitogenomes. *Molecular Phylogenetics and Evolution*, *85*, 180-188.

VanderWright, W. J., Bin Ali, A., Bineesh, K. K., Derrick, D., Dharmadi, F. F. D., & Haque, A. B. (2021). Narcine timlei. The IUCN Red List of Threatened Species 2021: e.T161445A178201830. https://dx.doi.org/10.2305/IUCN.UK.2021-2.RLTS.T161445A178201830.en. Accessed on 20 January 2022.

Tables

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

Gene name	Gene product	start	stop	Strand
tRNA	tRNA-Phe	1	69	+
rRNA	12S rRNA	70	1014	+
tRNA	tRNA-Val	1015	1085	+
rRNA	16S rRNA	1086	2749	+
tRNA	tRNA-Leu	2750	2824	+
CDS	ND1	2826	3800	+
tRNA	tRNA-Ile	3801	3868	+
tRNA	tRNA-Gln	3866	3936	-
tRNA	tRNA-Met	3937	4005	+
CDS	ND2	4006	5050	+
tRNA	tRNA-Trp	5051	5119	+
tRNA	tRNA-Ala	5122	5190	-
tRNA	tRNA-Asn	5192	5264	-
tRNA	tRNA-Cys	5296	5360	-
tRNA	tRNA-Tyr	5361	5429	-
CDS	COI	5431	6987	+
tRNA	tRNA-Ser	6988	7058	-
tRNA	tRNA-Asp	7059	7127	+
CDS	COII	7130	7820	+
tRNA	tRNA-Lys	7821	7893	+
CDS	ATPase 8	7895	8062	+
CDS	ATPase 6	8041	8736	+
CDS	COIII	8737	9521	+
tRNA	tRNA-Gly	9522	9592	+
CDS	ND3	9593	9943	+
tRNA	tRNA-Arg	9944	10015	+
CDS	ND4L	10016	10312	+
CDS	ND4	10306	11686	+
tRNA	tRNA-His	11687	11755	+
tRNA	tRNA-Ser	11756	11822	+
tRNA	tRNA-Leu	11823	11894	+
CDS	ND5	11895	13721	+
CDS	ND6	13706	14221	-
tRNA	tRNA-Glu	14222	14291	-
CDS	Cyt b	14294	15436	+
tRNA	tRNA-Thr	15437	15506	+
tRNA	tRNA-Pro	15980	16048	-

Table 2: Base composition and skewness of the mitochondrial genome skew of *N. timlei*

	A%	G%	C%	T%	G+C (%)	A+T (%)	Skew_AT	Skew_GC
verall	36.19	11.92	22.68	29.21	34.60	65.40	0.11	-0.31
CGs	33.81	11.89	23.40	30.90	35.29	64.71	0.05	-0.33
D1	34.26	10.15	27.28	28.31	37.44	62.56	0.10	-0.46
D2	40.21	8.12	24.93	26.74	33.05	66.95	0.20	-0.51
OX1	30.06	15.35	23.12	31.47	38.47	61.53	-0.02	-0.20
OX2	34.91	13.59	22.03	29.47	35.62	64.38	0.08	-0.24
TP8	43.45	6.55	20.24	29.76	26.79	73.21	0.19	-0.51
TP6	35.67	9.21	23.39	31.73	32.60	67.40	0.06	-0.43
OX3	29.90	15.27	24.55	30.28	39.82	60.18	-0.01	-0.23
D3	32.76	10.54	23.65	33.05	34.19	65.81	0.00	-0.38
D4L	32.66	9.76	26.60	30.98	36.36	63.64	0.03	-0.46
D4	35.49	10.01	23.88	30.62	33.89	66.11	0.07	-0.41
D5	37.38	9.85	23.54	29.23	33.39	66.61	0.12	-0.41
D6	18.22	25.78	6.98	49.03	32.75	67.25	-0.46	0.57
YTB	32.11	11.37	25.28	31.23	36.66	63.34	0.01	-0.38
RNA	38.83	15.78	19.93	25.44	35.71	64.28	0.20	-0.11
RNA	31.1	15.2	35.2	18.4	50.4	49.5	0.256566	-0.39683
CR	37	11	19.8	32.2	30.8	69.2	0.069364	-0.28571

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

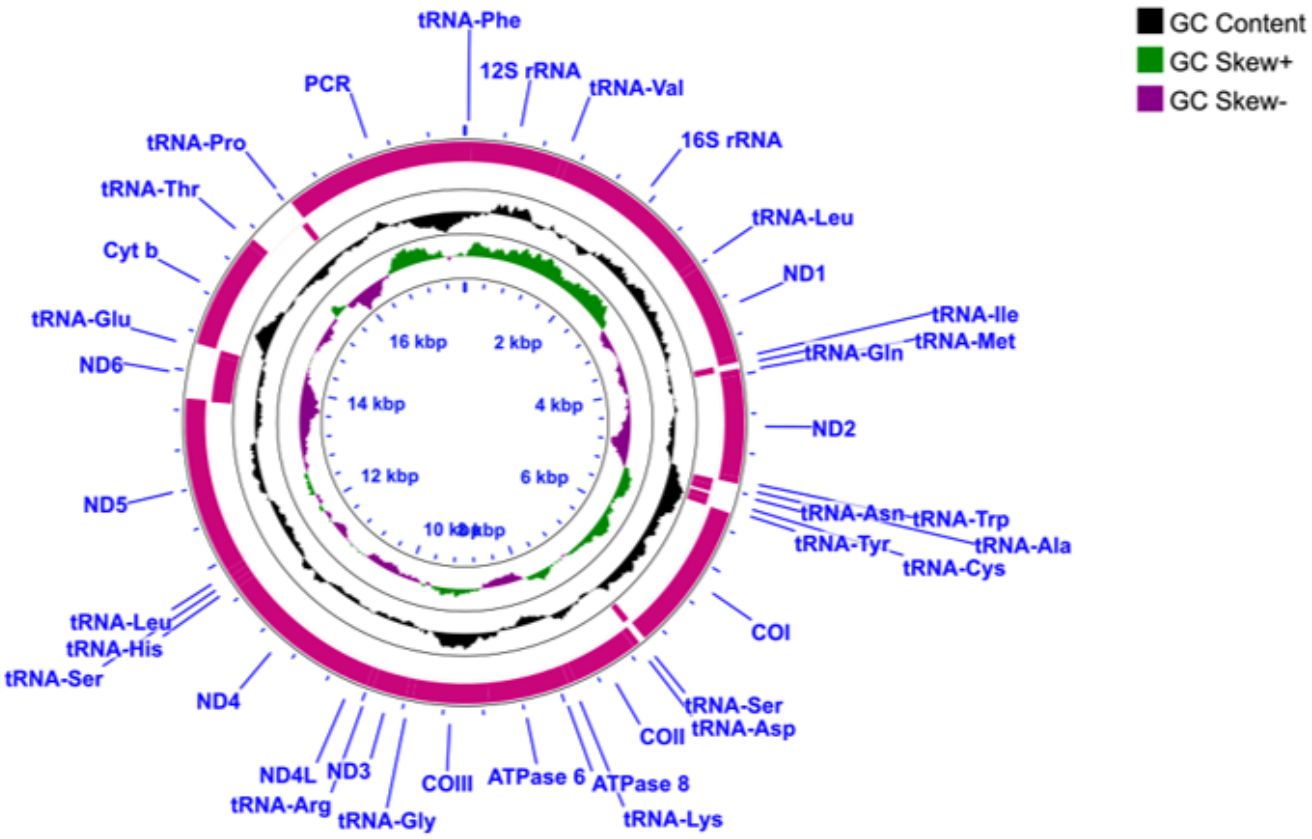

Figure 1

Schematic representation of the mitochondrial genome of *Narcine timlei*. The outermost circle represents a heavy strand having 12 protein-coding genes (PCGs), 14 transfer RNAs (tRNA), 2 ribosomal RNA genes, and a putative control region. The second circle represents a light strand having a PCG and 8 tRNAs. 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.

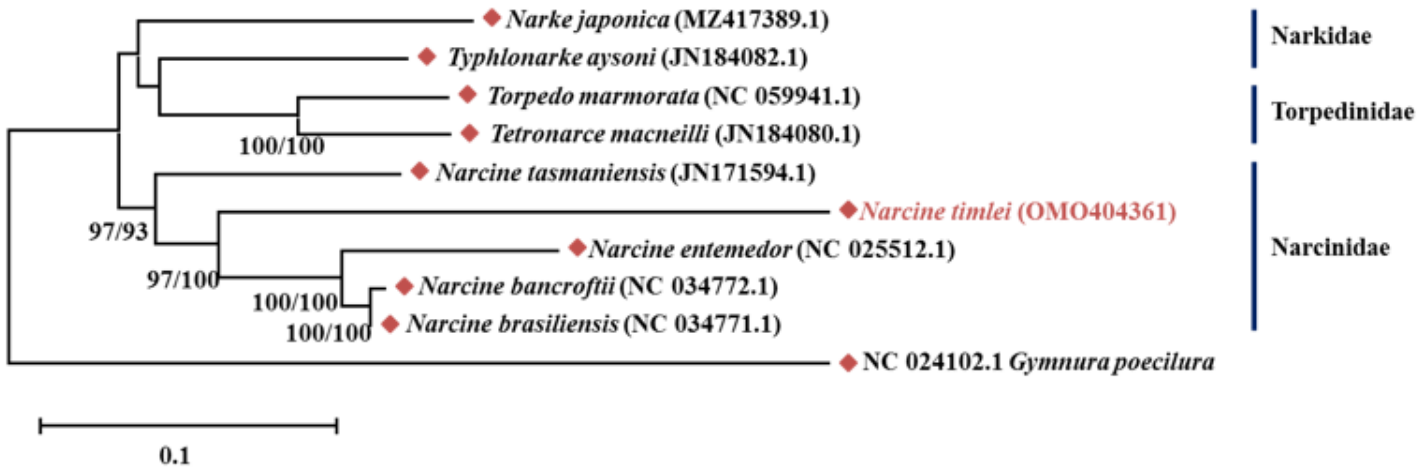

Figure 2

Phylogenetic tree obtained from Maximum Likelihood (ML)and Bayesian Inference (BI) analysis based on a concatenated alignment of 13 protein-coding genes for 9 species belonging to order Torpediniformes, and Gymnura *poecilura* (Myliobatiformes) which was taken as outgroup. ML bootstrap and BI posterior probability values are represented in nodes.

Supplementary Files

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