The first complete mitochondrial genome of the endangered spotted eagle ray *Aetobatus ocellatus*

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Abstract

The complete mitochondrial genome of the endangered spotted eagle ray *Aetobatus ocellatus* was determined for the first time using Next Generation Sequencing (NGS) reads mined from the Sequence Read Archive (SRA) of the GenBank database (BioSample SAMN31811701, collection site: Bohol Island, Philippines). The spotted eagle ray mitogenome (GenBank accession pending) has 20,217 bp in length displaying a typical vertebrate mitogenome organization (13 protein-coding genes, 22 tRNA genes, 2 rRNA genes, and a control region). A phylogenetic analysis based on the nucleotide sequence information of 13 protein-coding genes from different related species confirmed the species status of *A. ocellatus* as an Indo-West Pacific species, which was formerly described as *A. narinari* whose current accepted distribution is restricted to the Atlantic Ocean.

Keywords: Aetobatidae, mitogenome, phylogenetics, Next Generation Sequencing

69 Eagle rays of the genus *Aetobatus* (Myliobatiformes: Aetobatidae) suffer from strong
70 overfishing and slow recovery from overexploitation due to their low regeneration rate, slow
71 growth, and late maturity (Yamaguchi et al. 2021), which has led to a continued population
72 decline throughout their distribution ranges (Dulvy et al. 2021; Finucci et al. 2024) and
73 subsequent categorization of all of its members as Endangered or Vulnerable within the IUCN
74 red list (IUCN 2024). The classification of Aetobatidae species is particularly susceptible to
75 taxonomic confusion due to the interspecific overlap of their morphological features (White et
76 al. 2013) and during the last decade a number of taxonomic changes were introduced with the
77 aid of genetic markers, including the resurrection of old synonyms and description of new
78 species (White 2010; White and Moore 2013; White et al. 2013).
79 Herein, I report the first complete mitochondrial genome of the endangered spotted eagle ray
80 *Aetobatus ocellatus*, which has only recently been resurrected as the valid name for the Indo-
81 West Pacific member of the *narinari* complex (White et al. 2010). The *A. ocellatus* sample was
82 collected in Bohol Island, Philippines (BioSample: SAMN31811701) and sequenced by the
83 Smithsonian National Museum of Natural History in their "NOAA Genome Skimming of
84 Marine animals" project (BioProject accession: PRJNA720393). Paired-end FASTQ genomic
85 reads (2.2 Gbp within 14.6 million reads, Platform: Illumina NovaSeq 6000) were downloaded
86 from the public GenBank SRA repository (SRA accession: SRS15852991), trimmed with
87 BBDuk (14.5 million reads remained), and merged with BBMerge (4.1 million reads remained)
88 implemented in Geneious Prime v. 2021.1.1 (Biomatters Ltd., Auckland, New Zealand).
89 Unmerged (22,239) and merged (16,553) reads were independently assembled against the only
90 complete available mitogenome reference sequence of *Aetobatus* prior to this study (*A.*
91 *flagellum*, 20,201 bp, GenBank accession NC_022837) using the "Map to Reference" tool in
92 Geneious Prime. Protein-coding (PC), transport RNA (tRNA), and ribosomal RNA (rRNA)
93 genes were annotated using the "Annotate and Predict" feature of Geneious Prime by

comparing the novel *A. ocellatus* mitogenome to other complete mitogenomes of closely related species. The position of tRNAs was verified using tRNAscan-SE (Chan and Lowe 2019).

The 20,217 bp consensus mitogenome of *A. ocellatus* (GenBank accession pending) has a typical fish mitogenome organization (Winn et al. 2024) with 13 PC, two rRNA, 22 tRNA genes, and one putative control region, showing an overall nucleotide composition of A: 31.4%, C: 27.1%, G: 15.5%, and T: 26%. All PCGs use the typical ATG start codon except for the *COI* which utilizes the alternative GTG start codon.

The Bayesian phylogenetic analysis was performed using MrBayes 3.2 (Ronquist & Huelsenbeck 2003) and consisted of a concatenated matrix of 13 PCGs (11,460 bp) from 15 mitogenomes of closely related species retrieved from the GenBank database. Substitution saturation in single codon positions from each PCG was analyzed using DAMBE 5 (Xia 2013). The software jModelTest 2 (Darriba et al. 2012) was used to find the best-fit model of evolution for each PCG. Figure 1 shows the Bayesian phylogenetic tree in which all samples were grouped into 4 distinct family clades according to their taxonomy. Aetobatidae species formed a basal monophyletic clade with robust statistical support. *A. ocellatus* was clustered together with *A. narinari* into a discrete subclade (338 variable sites, genetic divergence 3.03%), reflecting their low estimated divergence age (from 2 to 5 mya, Sales et al. 2019). The genetic material analyzed herein (BioSample SAMN31811701) came from an organism collected in the Indo-West Pacific region (Bohol Island, Philippines) originally identified as *A. narinari*. However, the phylogenetic results obtained herein indicate that BioSample SAMN31811701 belongs to *A. ocellatus*, supporting its species status as an Indo-West Pacific species (White et al. 2010), while *A. narinari* is restricted to the Atlantic region (Sales et al. 2019).

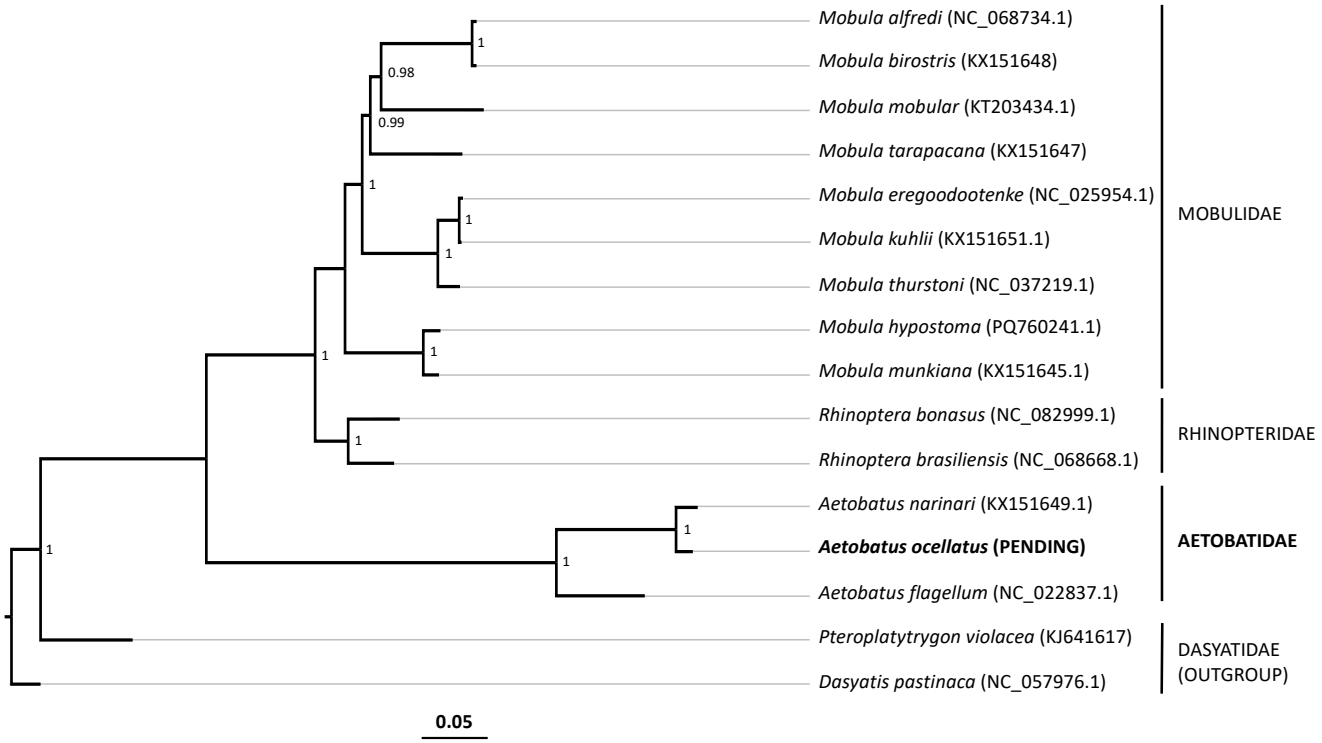

Figure 1. Bayesian phylogenetic tree inferred from 13 concatenated mitochondrial protein-coding genes of Aetobatidae, Dasyatidae, Mobulidae, and Rhinopteridae species. The sequence matrix (11,460 nt) used in the phylogenetic analysis consisted of unambiguously aligned regions of the first, second, and third codon positions. The position of *Aetobatus ocellatus* is highlighted in bold. *Pteroplatytrygon violacea* and *Dasyatis pastinaca* (Dasyatidae) were used as outgroups. Posterior probabilities are shown at each node. GenBank accession numbers for each species are shown in parentheses.

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