MITOCHONDRIAL DNA PART B: RESOURCES
2024, VOL. 9, NO. 1, 75–78
https://doi.org/10.1080/23802359.2023.2301011

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MITOGENOME REPORT

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The complete mitochondrial genome of *Siganus virgatus* Valenciennes (Siganidae: Acanthuriformes)

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ABSTRACT

Siganus virgatus Valenciennes 1835 is an essential species for examining reef ecosystems; however, its mitochondrial genome has not been studied. In this research, the mitogenome of *S. virgatus* was sequenced and characterized. The results revealed a circular genome of 16,505 bp that was composed of A (28.1%), C (31.3%), G (14%), and T nucleotides (26.6%). The genome contained 13 protein-coding genes, 22 transfer RNA genes, and two ribosomal RNA genes. Most genes of the mitogenome were transcribed on the heavy strand (H-strand), whereas *ND6* and eight tRNA genes (including *tRNA-Ala*, *-Asn*, *-Cys*, *-Gln*, *-Glu*, *-Ser* (1), *-Pro*, and *-Tyr*) were transcribed on the light strand (L-strand). Comparative analysis revealed a high degree of conservation of gene content and order among the *Siganus* mitogenomes. Phylogenetic analysis inferred from whole mitogenomes exhibited a close relationship between *S. virgatus* and *S. guttatus*. The newly completed mitogenome of *S. virgatus* provides essential genomic data for further studies on population genetics and the evolution of the *Siganus* genus and the Siganidae family.

ARTICLE HISTORY
Received 16 June 2023
Accepted 27 December 2023

KEYWORDS
Mitogenome; phylogenetic relationship; Siganidae; rabbitfishes

Introduction

Siganus virgatus Valenciennes 1835, commonly known as the barhead spinefoot, is a member of the Siganidae family (rabbitfishes) (Woodland 1990). The species is commonly found in coastal waters of southern India, Southeast Asia, and northern Australia (Carpenter et al. 2018). *S. virgatus* is a herbivorous fish that has a significant impact on coral reef ecosystems (Plass-Johnson et al. 2015; Bauman et al. 2017; Müller et al. 2021; Seah et al. 2021). Previously, mitochondrial genomes (mitogenomes) of various *Siganus* species have been reported (Oh et al. 2007; Wang et al. 2015; Yan, Wang, Yang 2016; Yan, Wang, Yang, et al. 2016; Shi et al. 2018). These mitogenomes have offered valuable data for studying phylogenetics and developing molecular markers for *Siganus* species (Iwamoto et al. 2009; Ravago-Gotanco et al. 2010; Hashem et al. 2022). However, the mitochondrial genome of *S. virgatus* remains to be reported. In the current study, the complete mitogenome of *S. virgatus* was sequenced and characterized using the next-generation sequencing method. The mitogenome of *S. virgatus* provides valuable information for further studies on genomics, species identification, and phylogenetics of *Siganus* genus.

Materials and methods

S. virgatus specimen (complete dead organism) was collected in the ocean region belonging to Con Dao Island, Ba Ria – Vung Tau province, Vietnam (106°37′57″E and 8°41′08″N) (Figure 1). The IUCN Red List of Threatened Species classified *S. virgatus* as a species of 'Least Concern' (Carpenter et al. 2018). Therefore, there is no specific collection permit for this species in Vietnam. The samples were preserved in absolute ethanol at −20 °C and deposited to the Faculty of Biology, Vietnam National University, Hanoi (determined by Dr. Thanh-Nam Nguyen, email nguyenthanhnam@hus.edu.vn) under specimen number NTT-102022-CD3 (Figure 1).

Figure 1. Left lateral view of *S. virgatus*. The photo was taken on 22 December 2021, at the Center for Life Science Research, University of Science, Vietnam National University, Hanoi by the author – Thanh-Nam Nguyen.

The total genomic DNA was extracted from the muscle tissue of the organism using a salt-out protocol

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/23802359.2023.2301011.

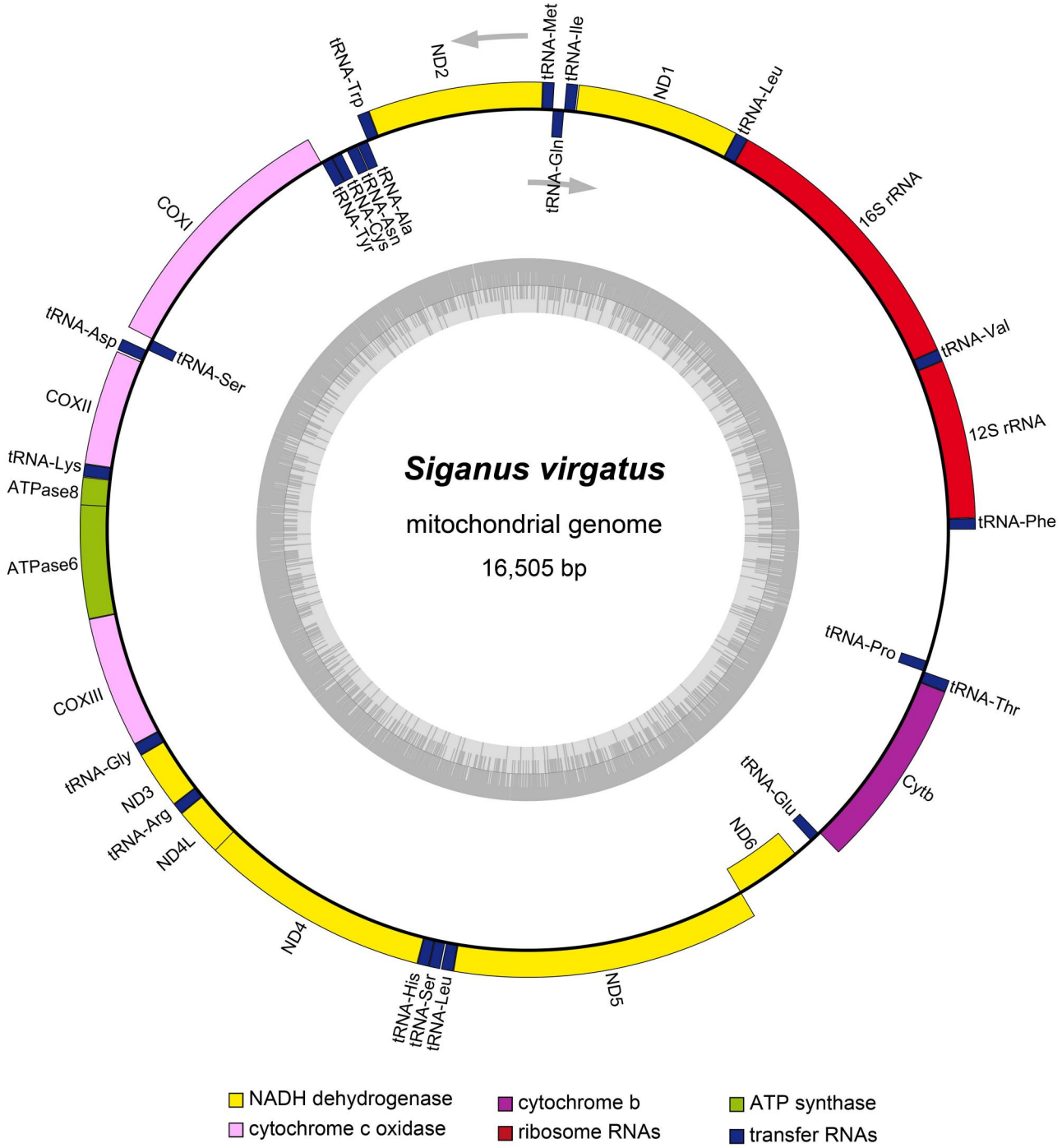

Figure 2. The mitogenome map of *S. virgatus*. Genes in the inner circle (H-strand) are oriented clockwise, while the orientation of the genes in the outer circle (L-strand) is counterclockwise. The dark gray area within the inner gray circle represents the GC content, and the grayish area corresponds to the AT content. The colored blocks represent various functional groups, which are labeled in the lower part of the figure.

(Chowdhury et al. 2016). The quality of the DNA sample was assessed using agarose gel electrophoresis and Qubit 4 fluorometer (Thermo Fisher Scientific, Waltham, MA). The DNA sample of high quality (concentration of at least 100 ng/μL, A260/A280 ratio ~1.7–2.0, and A260/A230 ratio ~2.0–2.2) was sequenced using MiniSeq platform (Illumina, San Diego, CA) to generate 150 bp paired-end reads. The raw data were evaluated and filtered using FastQC v0.11.9 and Trimmomatic v0.32, respectively (Andrews 2010; Bolger et al. 2014). The filtered reads were used to reconstruct the complete mitochondrial genome using NOVOPlasty 4.3.1 with the reference mitogenome of *Siganus sutor* Valenciennes 1835 (Dierckxsens et al. 2017; Shi et al. 2018). The complete mitochondrial genome (average coverage depth = 95x, Supplementary Figure S1) was then annotated utilizing the MITOS online web server (Bernt et al. 2013) and manually checked with homologous genes using Geneious Prime software. The fully annotated mitogenome of *S. virgatus* was submitted to GenBank with accession number OQ852681. The mitochondrial genome map was illustrated using the OGDRAW tool (Greiner et al. 2019).

The mitogenomes of *S. virgatus* and six members of the Siganidae family were comparatively analyzed to locate

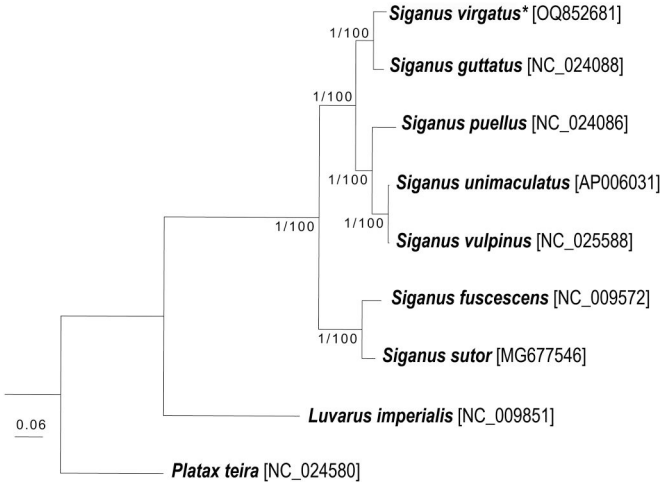

Figure 3. Phylogenetic tree of the Siganidae family inferred from 13 protein-coding regions of nine mitochondrial genomes using maximum-likelihood and Bayesian inference methods. The numbers indicate the posterior probability and bootstrap values. *The mitogenome sequenced in this study. The following sequences were used: *Siganus guttatus* (NC_024088; Yan et al. 2016b), *Siganus puellus* (NC_024086; Wang et al. 2015), *Siganus unimaculatus* (AP006031; Yagishita et al. 2009), *Siganus vulpinus* (NC_025588; Yan et al. 2016a), *Siganus fuscescens* (NC_009572; Oh et al. 2007), *Siganus sutor* (MG677546; Shi et al. 2018), *Luvarus imperialis* (NC_009851; Kerr et al. 2020), and *Platax teira* (NC_024580; Li et al. 2016).

genetic variations such as deletion, insertion, and inversion using mVISTA (Frazer et al. 2004). For phylogenetic analysis, complete mitogenomes of *Siganus* species were downloaded from the GenBank database (Supplementary Table S1). The mitogenomes of *Platax teira* Forsskål 1775 (NC_024580) and *Luvarus imperialis* Rafinesque 1810 (NC_009851) were used as outgroups. The protein-coding sequences of mitogenome were extracted and aligned using MUSCLE program (Edgar 2004). The best-fit model GTR + G + I was identified for the data matrix using jModeltest 2 (Darriba et al. 2012). The phylogenetic tree was reconstructed using maximum-likelihood (ML) by IQ-TREE with 1000 replications (Nguyen et al. 2015). The Bayesian inference (BI) method was also conducted with 1,000,000 generations using MrBayes v3.2.7 (Huelsenbeck and Ronquist 2001). In the BI analysis, the tree was sampled every 1000 generations and 25% of the resulting trees were discarded as burn-in when the split frequency was lower than 0.01. The obtaining trees were visualized and modified using FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

Results

The mitogenome of *S. virgatus* was 16,505 bp in length, comprising 13 protein-coding genes (PCGs), 22 tRNA genes, and two rRNA genes (Figure 2, Supplementary Table S2). The nucleotide composition of the genome is as follows: A (28.1%), C (31.3%), G (14%), and T (26.6%). The light strand (L-strand) contains nine genes, including *ND6*, *tRNA-Ala*, *tRNA-Asn*, *tRNA-Cys*, *tRNA-Gln*, *tRNA-Glu*, *tRNA-Ser*, *tRNA-Pro*, and *tRNA-Tyr*. The remaining genes were encoded on the heavy strand (H-strand). Except for *COXI*, which has GTG as the start codon, all the PCGs start with ATG codon (Supplementary Table S2). The stop codon TAA or TAG was found at the terminals of *ND1*, *COXI*, *ATP8*, *ND4L*, *ND5*, and *ND6*. In contrast, the incomplete stop codon T- was found in *ND2*, *COXII*, *ND3*, *ND4*, and *Cytb*. The *ATP6* and *COXIII* genes ended with the incomplete stop codon TA– (Supplementary Table S2).

The sequence alignment data revealed a high degree of conservation of gene content and order within Siganidae mitogenomes (Supplementary Figure S2). The ML and BI methods generated identical phylogenetic trees. Additionally, the monophyly of *Siganus* species was found with high support values (bootstrap = 100 and posterior probability = 1) (Figure 3). *S. virgatus* had a close relationship with *Siganus guttatus* Bloch 1787.

Discussion and conclusions

The newly completed mitogenome of *S. virgatus* showed a high level of mitogenome genome conservation with other *Siganus* species (Figure 2, Supplementary Table S2). In particular, all previously reported mitogenomes of *Siganus* species and *S. virgatus* possess 13 PCGs, 22 tRNA genes, and two rRNA genes (Oh et al. 2007; Yagishita et al. 2009; Wang et al. 2015; Yan, Wang, Yang 2016; Yan, Wang, Yang, et al. 2016; Shi et al. 2018). Previous phylogenetic analyses inferred from *COI*, *Cytb*, and *16S* rRNA revealed a close relationship between *S. virgatus* and *S. guttatus* (Borsa et al. 2007; Yan et al. 2019; Ali et al. 2021). Similarly, our current phylogenetic analysis based on 13 mitochondrial PCGs indicated a high-support clade including *S. virgatus* and *S. guttatus* (Figure 3). Our study is the first report of the *S. virgatus* mitochondrial genome, which significantly contributes to further studies in phylogeny, population genetics, and biogeography of the Siganidae family.

Author contributions

MTV and TNN conceived the study; TNN collected and determined the samples; HDN, HDKD, and MTV conducted the experiments, analyzed the data, and wrote the draft manuscript; TNN, HDKD, and MTV revised the draft manuscript. All authors read and approved the final manuscript.

Ethical approval

The material of *Siganus virgatus* in this study does not involve ethical conflicts. This species is a least-concern rank, so it does not need specific permissions or licenses to collect it in Vietnam.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

There is no funding for this research.

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Data availability statement

The data generated in this study are openly available in the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) under the accession number OQ852681. The associated BioProject, SRA, and BioSample numbers are PRJNA980936, SRR24848286, and SAMN35654606, respectively.

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