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MITOGENOME REPORT

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The complete mitochondrial genome of *pseudanthias pascalus* (Jordan & Tanaka, 1927) (perciformes: serranidae)

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ABSTRACT

Pseudanthias pascalus (Jordan & Tanaka, 1927) (Perciformes: Serranidae) is a species of brightly colored saltwater fish found in tropical coastal reef communities. In this study, we reported the sequence of mitochondrial DNA from *P. pascalus*. The accession number is OP611422. The complete mitochondrial genome of *P. pascalus* was 16,863 bp in length, including 13 protein-coding genes (PCGs), 12S and 16S rRNAs, 22 tRNA genes, and one displacement loop (D-loop). Most PCGs had ATG-start codons and TAA-end codons. The A + T contents were 54.61%. Phylogenetic analysis showed that *P. pascalus* is most closely related to *Pseudanthias huchtii*. We sequenced the entire mitochondrial genome of *P. pascalus*, providing improved marker identification information for the classification of the family and species conservation. These data will be useful for relative ecological and phylogenetic studies.

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Introduction

Pseudanthias pascalus (Jordan & Tanaka, 1927) (Randall et al.1955) (Perciformes: Serranidae) is a species of bony fish in the family Serranidae (sea basses), also known as *Amethyst anthias*. It is a Marine fish that lives in coral reefs near the coast of the tropics. Depending on the individual, some have a little yellow tail fin, and gill. In the juvenile stage, its back is yellow, with a maximum length of 18 cm. The male fish's body is blue-purple, and the upper part behind the dorsal fin is red, with a yellowish-forked tail. Due to its splendid color, it can be used as an ornamental fish. This species is distributed in tropical Pacific Ocean waters northwards to the Sea of Japan, and Indonesia, extending southward to the waters of eastern Australia, including the southern part of Taiwan, and the waters of Orchid Island and Green Island. It inhabits 5 ~ 60 meters of sea surface and shady areas on reef slopes or independent reefs. The diet consists of floating crustaceans and fish eggs. Here, we sequenced the complete mitochondrial genome of *P. pascalus* and elucidated its structure, function, and phylogenetic relationships. The genetic structure and phylogenetic status of the genus *Pseudanthias* have not been investigated before, therefore the complete mitochondrial genome of *P. pascalus* was characterized for the first time in this study, which would provide useful genomic data for future phylogenetic and taxonomic classification of the Serranidae. In addition, as a coral reef fish, this study will contribute to the conservation of coral reef fish resources.

Materials and methods

P. pascalus was collected from Orchid Island (22.04°N, 121.37°E) (Figure 1) in Taiwan, China, in August 2022, the pectoral fin of the fish was cut off with sterile scissors when the fish was viable and stored in 70% alcohol to be extracted for DNA, and the specimen was stored in 70% alcohol solution at the College of Fisheries, Zhejiang Ocean University (contact: Wenli Duan, 3298602121@qq.com) under the voucher number DWL-202208-36.

Extraction and detection of genomic DNA

Total genomic DNA of muscle tissue from fins tissue was extracted using the rapid extraction kit (Beijing Aidlai Biotechnology Co., LTD.) following the manufacturer's instructions. After extraction, the genome was subjected to gel electrophoresis to detect the quality and carried out subsequent experiments.

Primer design, LA-PCR amplification, and sequencing

Primers were designed according to the mt genomic sequences of closely related species (Table S1). The long PCR (LA-PCR) amplification was performed using the standard LA Taq polymerase (Takara). The PCR products were purified using the Aid Quick Gel Extraction Kit (Aid Lab), and sequenced directly, by the dideoxynucleotide procedure, using an ABI 3730 automatic sequencer (Sanger sequencing) with the same set

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of primers. The PCR products were visualized by electrophoresis on 1.2% agarose gel, and the gel images were shown in Figures S1–S2. All obtained fragments were BLASTed to confirm that the amplicon is the target sequence.

Figure 1. The morphological characteristics of *pseudanthias pascalus*. This picture was taken by wenli duan and Chang Hu on Orchid Island, Taiwan, China.

Sequence annotation

The mt genome was annotated roughly following the procedure described before (Zou et al. 2017; Zhang et al. 2018). First, the original mitogenomic sequences were imported into MITOS web servers to determine the approximate boundaries of genes. The exact positions of protein-coding genes (PCGs) were found by searching for ORFs (employing genetic code 2, the vertebrate mitochondrion). All tRNAs were identified using ARWEN, DOGMA, and MITOS. The precise boundaries of rrnL and rrnS were determined *via* a comparison with homologs.

To analyze the phylogenetic position of *P. pascalus* the complete sequences of mitochondrial genomes of the thirteen closest species were downloaded based on the blasting results in GenBank, twelve of these species are from the Serranidae family, and *Acrossocheilis fasciatus* was selected as the outgroup (Figure 3). For each species, we extracted the 13 PCGs and concatenated them into one sequence (Thierry-Mieg and Thierry-Mieg 2006) (Figure 2). The topology of the

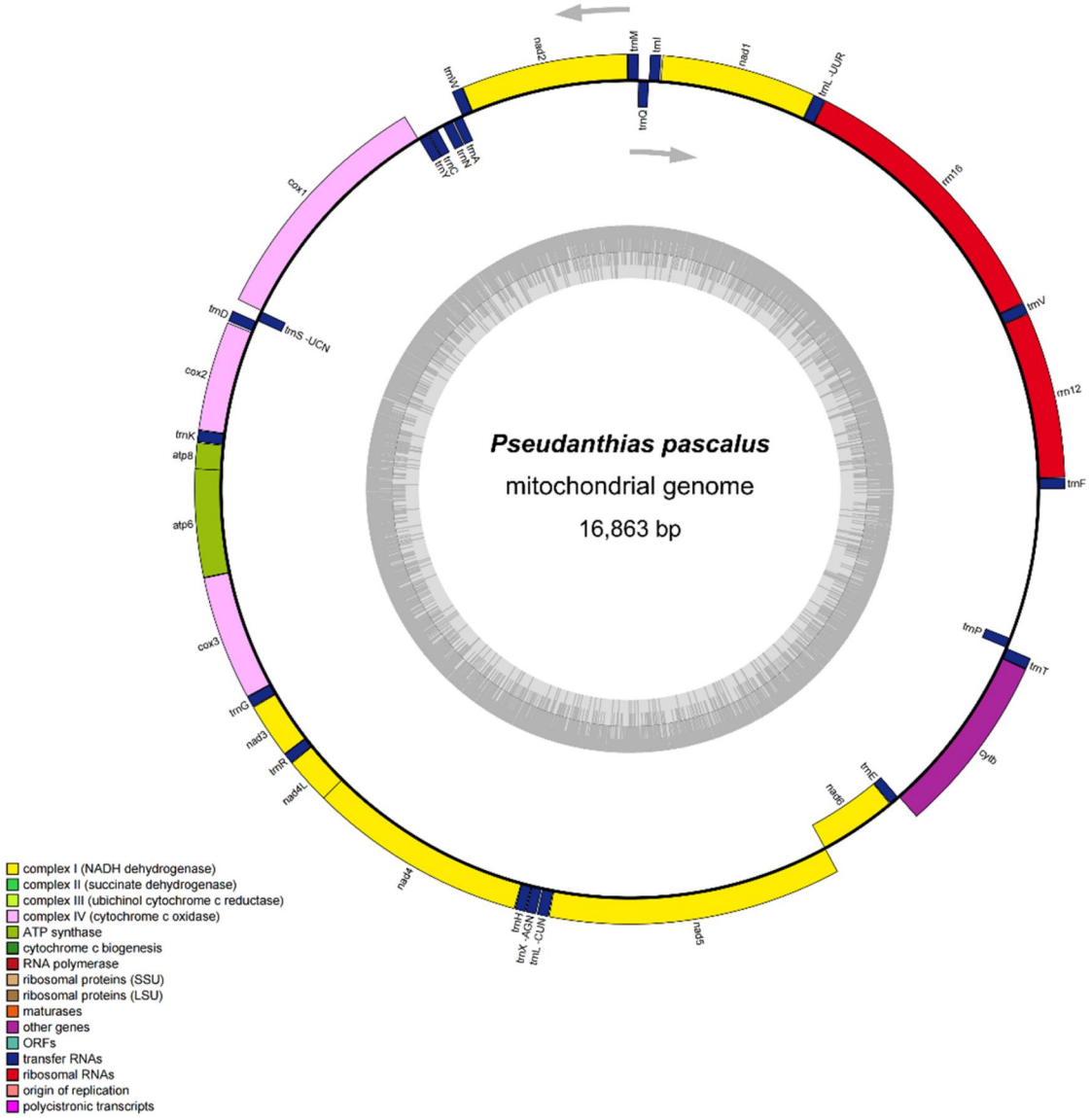

Figure 2. The circular-mapping mitochondrial genome of *pseudanthias pascalus*. Gene names on the outside line indicated that these genes were located on the H-strand, whereas the others were located on the L-strand. Color codes for different genes were listed on the map.

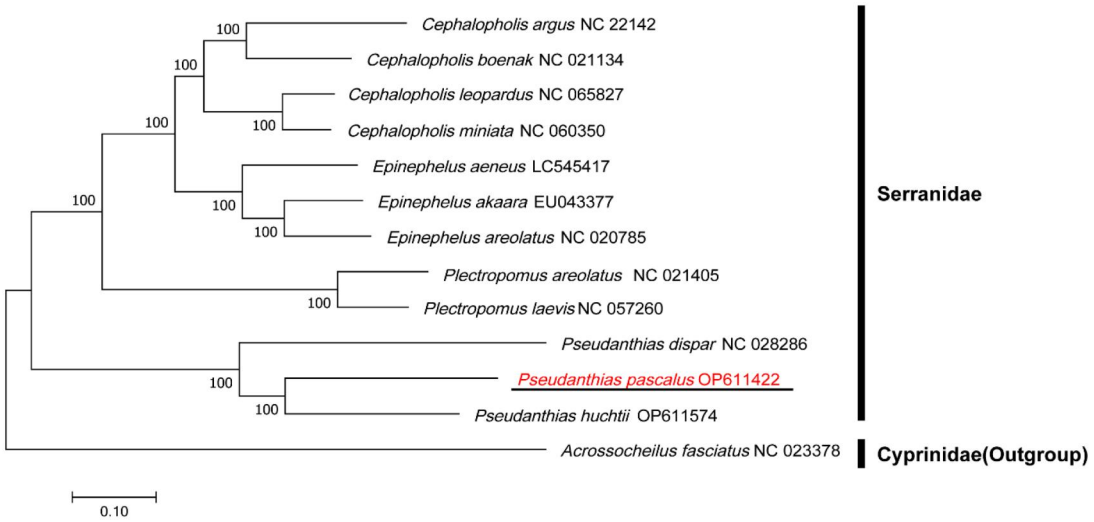

Figure 3. The maximum-likelihood (ML) phylogenetic tree of *Pseudanthias pascalus* and other 12 species of fish. The following sequences were used: *Cephalopholis argus* NC022142, *Cephalopholis boenak* NC021134, *Cephalopholis leopardus* NC065827, *Cephalopholis miniata* NC060350, *Epinephelus aeneus* LC545417, *Epinephelus akaara* EU043377, *Epinephelus areolatus* NC020785, *Plectropomus areolatus* NC021405, *Plectropomus laevis* NC057260, *Pseudanthias dispar* NC028286, *Pseudanthias huchtii* OP611574 and *Acrossocheilis fasciatus* NC023378. Phylogenetic reconstruction was done from a concatenated matrix of 13 protein-coding mitochondrial genes and two ribosomal RNA genes. Alphanumeric terms indicated the GenBank accession numbers.

tree was inferred using the maximum likelihood method in the program MEGA 7 The best fit substitution model (GTR + G + I) was selected using jModelTest 2.1.10 (Darriba et al. 2012) based on the Akaike information criterion (AIC). Maximum-likelihood phylogeny was generated with 1000 replications using MEGA 7 (Pattengale et al. 2010; Meier et al. 2017; Cuadra et al. 2020). The species used for tree construction were as follows *Cephalopholis argus* NC022142, *Cephalopholis boenak* NC021134, *Cephalopholis leopardus* NC065827, *Cephalopholis miniata* NC060350, *Epinephelus aeneus* LC545417, *Epinephelus akaara* EU043377, *Epinephelus areolatus* NC020785, *Plectropomus areolatus* NC021405, *Plectropomus laevis* NC057260, *Pseudanthias dispar* NC028286, *Pseudanthias huchtii* OP611574 and *Acrossocheilis fasciatus* NC023378.

Results

The complete mitochondrial genome (Figure 2) of *P. pascalus* was circular with 16,863bp in length and consisted of 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNA), two ribosomal RNA genes (rRNA) and one control region (D-loop). The overall base composition of mitogenome was A 26.96%, T 27.65%, C 27.82%, and G 17.57%. A + T content (54.61%) was a little more than the G + C content (45.39%) (Cheng et al. 2016) in common with other vertebrate mitogenomes. Among the 13 PCGs in *P. pascalus*, the initial codon of 11 PCGs was ATG, except that the COX1 gene was GTG and NAD6 gene was TAA. 8 PCGs (NAD2, COX1, ATP8, ATP6, COX3, NAD4L, NAD5, and CYTB) used TAA as the stop codon, and NAD1 used TAG as the stop codon. For each of the 15 mitogenomes, there were two different starting codon types (ATG and GTG) and three different stop codon types (TAA, TAG, and T-) (Ma et al. 2022). Our results showed that among the 13 PCGs, NAD 5 was the longest gene (1839 bp), and ATP8 was the shortest gene (165 bp). The lengths of the 12S and 16S ribosomal RNA genes were 1044 bp and 1778 bp, respectively, which were located between tRNA-Phe and tRNA-Leu and separated by the tRNA-Val gene. To determine the phylogenetic relationships in the Serranidae family, we used MEGA7 software to construct a phylogenetic tree including *P. pascalus* and other 12 fishes based on maximum likelihood (ML) (Figure 3). The phylogenetic position of *P. pascalus* was shown. Phylogenetic analysis supported the close genetic relationship between *P. pascalus* and *Pseudanthias huchtii*, indicating that the two species share a more recent common ancestor gene (Figure 3). The GenBank accession numbers of 13 fishes were given in parentheses.

Discussion and conclusion

In this study, the complete mitogenome of *P. pascalus* was assembled and analyzed. We found that the gene content and arrangement of the newly sequenced mitogenome are similar to those of other determined mitogenomes of Serranidae. The complete mitogenome of *P. pascalus* will provide important information for improving the taxonomic system and phylogenetics of Serranidae. In the ML phylogenetic tree, *P. pascalus* is grouped with *P. huchtii* and they cluster with *P. dispar*. These results will be helpful for understanding the systematics among members of the Genus *Pseudanthias* and will provide the basis for molecular identification and population genetic study of the species.

Author contributions

Wenli Duan and Zhangjie Chu designed the experiments and wrote the original manuscripts; Bo Zhao, Yuxuan Gong, Lin Jiang, and Chang Hu revised the text; Zhangjie Chu and Bo Zhao collected samples; and Wenli Duan, Bo Zhao, Yuxuan Gong, Lin Jiang, Chang Hu analyzed the data. All authors gave final approval of the version to be published and agreed to be accountable for all aspects of the work.

Disclosure statement

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

Ethical approval

Experiments were performed in accordance with the recommendations of the Ethics Committee of Zhejiang Ocean University. The sample of this study did not involve endangered or protected animals, and the experiment followed the Laboratory Animal—Guideline for Ethical Review of Animal Welfare of the National Standard of the People's Republic of China (GB/T 35892-2018).

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Data availability statement

The genome sequence data obtained in this study are openly available in GenBank of NCBI (https://www.ncbi.nlm.nih.gov/) under the accession number OP611422.

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