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MITOGENOME REPORT

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# Complete mitochondrial genome of *Inimicus didactylus* (Pallas, 1769) and its phylogenetic analysis

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**ABSTRACT**

*Inimicus didactylus* is a venomous fish belonging to the family Synanceiidae, which is closely related to the true stonefishes (Synanceiidae, *Synanceia spp.*). Here, we employed high-throughput sequencing technology to assemble and annotate the complete mitochondrial genome of *I. didactylus*. The mitochondrial genome, with a length of 16,670 base pairs, exhibits A-T bias in its base composition (58.19%) and consists of 13 protein-coding genes, 22 tRNA genes, and 2 rRNA genes. Phylogenetic analysis indicates a close relationship between *I. didactylus* and *I. japonicus*. This study provides a genomic resource that enhances ecology and evolution researches on Synanceiidae species.

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Mitochondrial genome; phylogenetic analysis; *Inimicus didactylus*; Synanceiidae

## Introduction

*Inimicus didactylus* (Pallas, 1769), referred to as the demon stinger or devil stinger, is widely distributed throughout the eastern Indian and western Pacific oceans. This species is typically found in lagoons and coral reefs along the coasts of southeastern China, Australia, Thailand, and eastern India (Kodeeswaran et al. 2020). The genus *Inimicus* comprises a total of 10 documented species, all of which share a relatively short pelvic fin base, a quadrangular depression on the top of the head behind the eyes, and two free rays on the lower part of the pectoral fin (fishbase and WORM, accessed October 24, 2024). In China, four common species belonging to the genus *Inimicus* have been identified: *I. japonicus*, *I. sinensis*, *I. didactylus* and *I. brachyrhynchus*.

Those species can be distinguished based on body coloration and the patterns of their pectoral and caudal fins. However, body color can exhibit significant variations even within species and may become obscured by debris, complicating accurate species identification (Munro 1967; Delloro et al. 2020). Consequently, use of additional molecular markers, beyond morphological characteristics, is warranted for precise identification.

Unfortunately, among the 10 species of *Inimicus*, only *I. japonicus* has complete mitochondrial data available in the National Center for Biotechnology Information (NCBI, accessed January 30, 2025). The mitochondrial *cox1* gene is a widely utilized molecular marker for species identification (Bingpeng et al. 2018). Currently, there are 16 DNA barcodes of the *cox1* region for *I. didactylus* available in the NCBI database, with lengths ranging from 474 bp to 698 bp. However, this range is insufficient to provide robust molecular evidence for accurate species identification. Therefore, we assembled the complete mitochondrial genome (mitogenome) of *I. didactylus* and conducted a phylogenetic analysis to gain a better understanding of the relationships of *I. didactylus* in relation to other species within the Scorpaenoidei.

## Materials and methods

The freshly specimen of *I. didactylus* (Figure 1) was obtained from a local fisherman off the coast of Hainan, China (20.03°N, 110.33°E), in December 2023. Species identification was primarily based on the following morphological characteristics: a robust body without scales; two free fin rays in the pectoral fins, with the anterior half of the inner side black and marked with white stripes; the head length approximately 1.4 times the length of the second dorsal spine; and deep clefts in the fin membranes behind the fourth dorsal spine, extending nearly to the base. And then, the muscles and fins were dissected, rinsed with autoclaved artificial seawater, and rapidly frozen in liquid nitrogen. The samples were preserved at −80 °C in the Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou) under the voucher number GYMU0009 (contact person: Xuanjin

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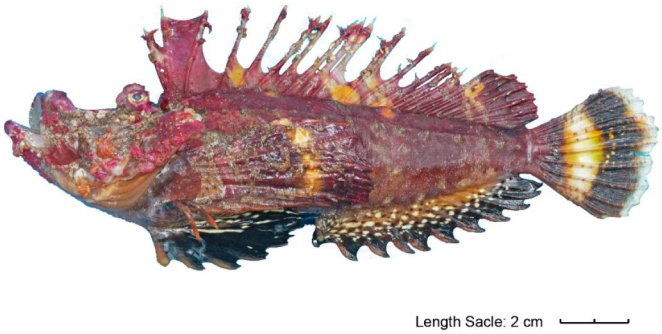


**Figure 1.** Photograph of *Inimicus didactylus*, captured by Qian Li.

Luo, email: luoxuanjin@gmlab.ac.cn) for future research applications.

Genomic DNA was extracted using the modified Cetyltrimethylammonium bromide (CTAB) protocol as described previously (Clarke 2009). A DNA library with an insert size of 300 base pairs (bp) was constructed using the MGIEasy FS DNA Library Prep Kit (MGI-Tech, China) and subsequently sequenced on the DNBSEQ-T7 platform (MGI Tech, China). A total of 8.46 gigabases of raw data were generated. Clean paired-end reads were obtained by processing the raw data with Fastp v0.23.2 (Chen 2023). Mitogenome assembly and annotation were performed using MitoFinder v1.4.2 (Allio

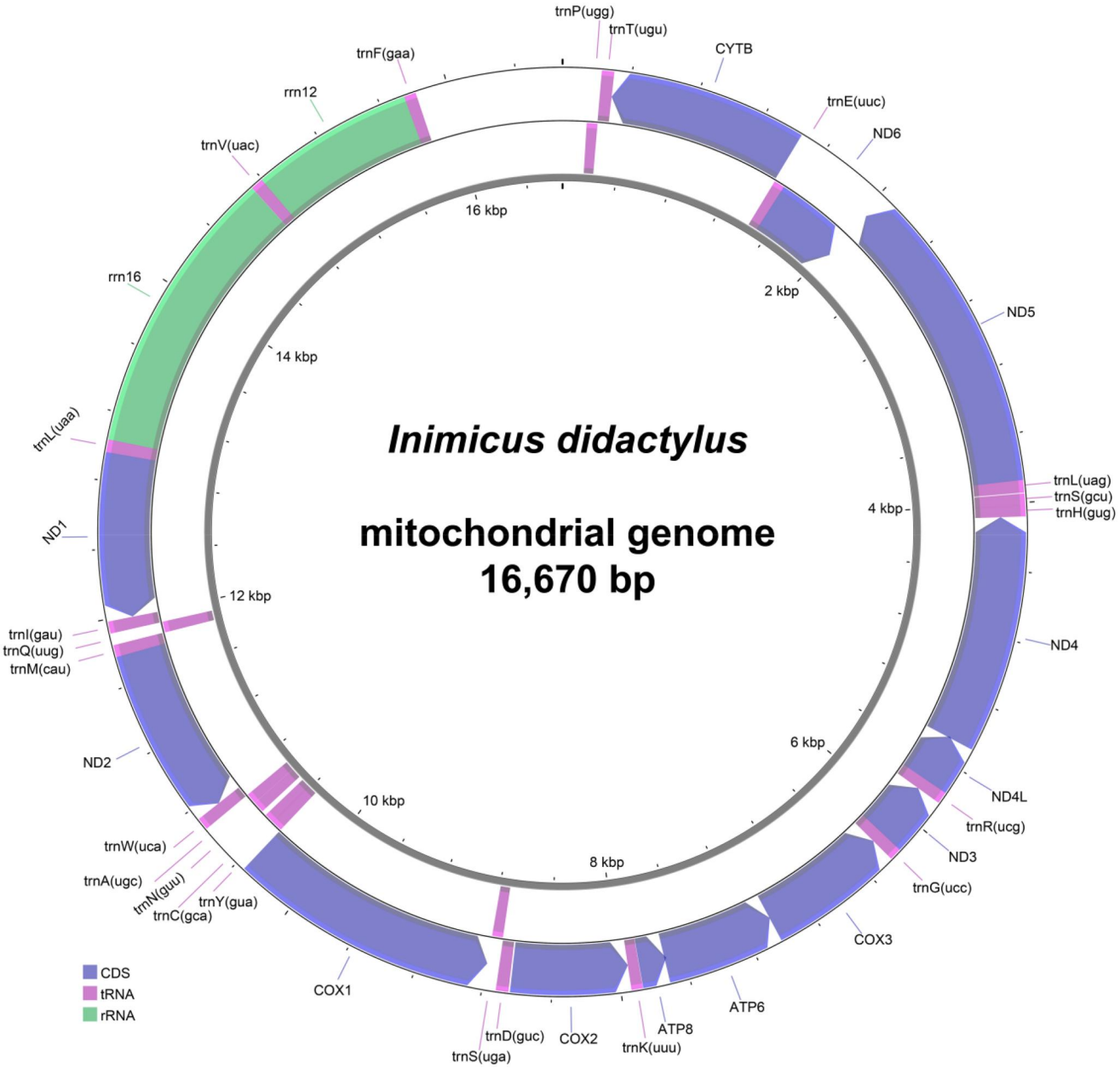


**Figure 2.** Mitogenome maps for *Inimicus didactylus*. The legend employs different colors to represent gene types: CDS (coding sequence), tRNA (transfer RNA), and rRNA (ribosomal RNA). Arrowheads indicate the direction of the genes. The innermost circle represents the length of the *Inimicus didactylus* mitogenome map, with the L-strand shown in the inner circle and the H-strand in the outer circle. PCGs located on the H-strand are displayed in a counterclockwise direction on the outer circle, while genes on the L-strand are arranged in a clockwise direction on the inner circle. The outermost labels denote the arrangement of the genes.

et al. 2020) with default parameters, utilizing *I. japonicus* (MT375601) as the reference mitogenome. The mitogenome map was visualized using Proksee Map Builder v2.0.5 (Grant et al. 2023). In addition, the sequencing depth and coverage of the mitogenome (Supplementary Figure S1) was assessed using BWA v0.7.17-r1188 (Li and Durbin 2009) and Samtools v1.16 (Danecek et al. 2021).

To further investigate the direct phylogenetic context of *I. didactylus*, we downloaded mitogenomes of 28 species within the Scorpaenoide from NCBI, which included all available congeneric species of *I. didactylus*, with *Anguilla japonica* as the outgroup (Kim et al. 2020; Li et al. 2020). The 13 protein-coding genes (PCGs) were extracted from 29 species and aligned using MUSCLE v3.8.31 with default parameter (Edgar 2022). Inconsistent aligned sites were trimmed by TRIMAl v1 (Capella-Gutiérrez et al. 2009). Then, the PCGs were concatenated using our custom Python scripts (Supplementary material). A phylogeny tree was reconstructed using the Maximum Likelihood (ML) method implemented in IQ-TREE v1.6.12 (Nguyen et al. 2015; Wei et al. 2024), with 5,000 ultrafast bootstrap replicates. The resulting phylogenetic tree was visualized using iTOL (Letunic and Bork 2024).

## Results

The mitogenome of *Inimicus didactylus* (PQ773495) is 16,670 bp long and exhibits an archetypal circular structure characteristic of vertebrate mitogenomes. The nucleotide composition is 28.73% A, 29.46% T, 16.17% C and 25.64% G. The mitogenome contains 13 PCGs with a total length of 11,442 bp, which constitutes 69.47% of the entire genome and the gene order is visible in the Figure 2. Notably, only the *nd6* gene and eight *tRNA* genes are encoded by the light strand (L-strand), while the remaining twelve PCGs are encoded by the heavy strand (H-strand). Adjacent pairs of PCGs exhibit overlaps in base pairs: *atp8-atp6* (10 bp), *atp6-cox3* (1 bp), *nd4l-nd4* (7 bp), and *nd5-nd6* (4 bp). These overlaps can be categorized into two primary patterns: one occurring between genes on the same strand (*nd4l-nd4,atp8-atp6* and *atp6-cox3*) and the other between genes on opposite

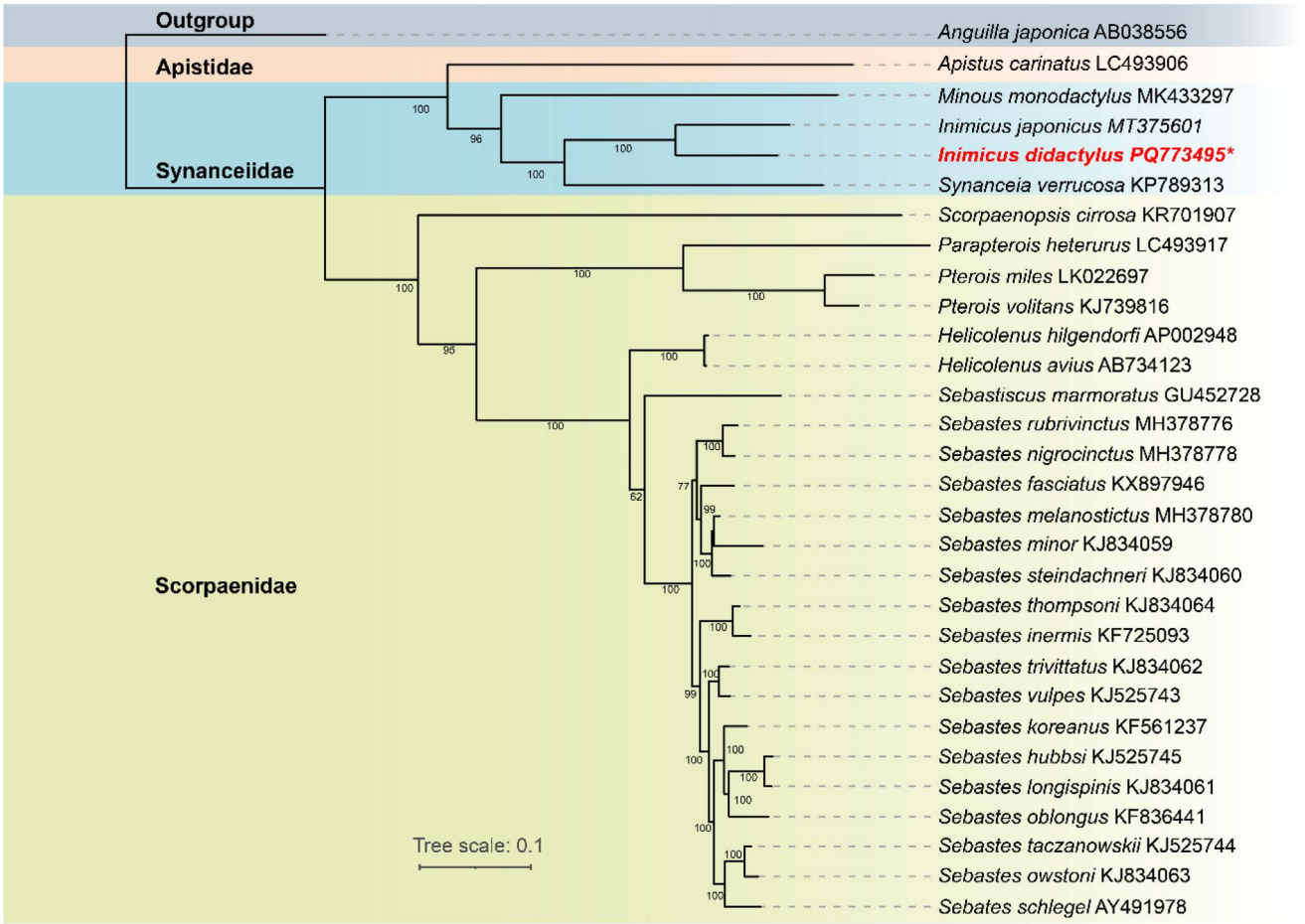


**Figure 3.** The phylogenetic tree was reconstructed based on 13 PCGs extracted from the complete mitochondrial genomes of 30 species with *Anguilla japonica* serving as the outgroup. The species *Inimicus didactylus* is highlighted in bold red. Bootstrap support values are displayed at the nodes. All sequences used in this analysis were obtained from GenBank, with their corresponding accession numbers as follows: AB038556 (Inoue et al. 2001), LC493906 (unpublished), MK433297 (Kim et al. 2019), MT375601 (Kim et al. 2020), PQ773495 (this study), KP789313 (Wang et al. 2016), KR701907 (Wu et al. 2016), LC493917 (unpublished), LK022697 (Dray et al. 2016), KJ739816 (Del Río-Portilla et al. 2016), AP002948 (Miya et al. 2001), AB734123 (unpublished), GU452728 (unpublished), MH378776 (Sandel et al. 2018), MH378778 (Sandel et al. 2018), KX897946 (unpublished), MH378780 (Sandel et al. 2018), KJ834059 (unpublished), KJ834060 (unpublished), KJ834064 (unpublished), KF725093 (Jang, Kim, and Kim 2015), KJ834062 (unpublished), KJ525743 (Jang, Kim, et al. 2016), KF561237 (Jang, Kim, Oh, et al. 2015), KJ525745 (Jang, Lee, et al. 2016), KJ834061 (Jang et al. 2016), KF836441 (Jang, Oh, Lee, et al. 2016), KJ525744 (Jang, Oh, Park, et al. 2016), KJ834063 (Oh et al. 2016), AY491978 (Kim and Lee 2004).

strands (*nd5-nd6*). Furthermore, all PCGs begin with ATG, except for *cox1*, which starts with GTG. Regarding stop codons, TAA were used in nine genes (*nd1*, *nd2*, *cox1*, *cox3*, *atp6*, *atp8*, *nd4l*, *nd5*, *nd6*), while AGA was employed in two genes (*cox2*, *nd4*). Additionally, an incomplete stop codon, represented by a single T, is found in the *cytb* and *nd3* genes. This incomplete stop codon is subsequently completed to TAA through the addition of a poly-A tail during RNA processing.

The phylogenetic tree showed high support values at the majority of nodes (Figure 3). The tree topology indicates that *I. didactylus* shares the closest relationship with *I. japonicus*, exhibiting a high sequence similarity of 87.73% based on the 13 PCGs.

## Discussion and conclusion

In this study, we present the first complete mitogenome of *I. didactylus*. Consistent with the base pair composition of mitochondrial genomes in other vertebrates, the AT content of this species is 58.19%, which is higher than the GC content (Asakawa et al. 1991). Furthermore, the gene order of the mitogenome of *I. didactylus* is comparable to that of other vertebrate species, with most PCGs are encoded by the H strand, while the *nd6* gene and eight *tRNA* genes are located on the light L strand (Pereira 2000; Satoh et al. 2016; Sun et al. 2016; Song et al. 2019; Montaña-Lozano et al. 2022; Alvarenga et al. 2024). The *I. didactylus* contains four pairs of directly adjacent protein-coding genes, all of which display overlapping regions. The occurrence of overlapping adjacent protein-coding genes is a relatively common phenomenon in fish mitochondrial genomes (Satoh et al.), as observed in species such as *Minous monodactyius* (Kim et al. 2019), *I. japonicus* (Kim et al. 2020), *Synanceia verrucosa* (Wang et al. 2016), and *Anguilla japonica* (Inoue et al. 2001).

The phylogenetic tree, which included additional species from the Scorpaenoidei, further supported the validity of the assembled mitogenome of *I. didactylus* based on the analysis of 13 PCGs. The observed phylogenetic relationships were consistent with morphological taxonomy at the family level, with all species of Synanceiidae clustering into a single clade that formed a sister group to Scorpaenidae (Shinohara and Imamura 2007), exhibiting a topology analogous to that of the phylogenetic trees reconstructed in references (Kim et al. 2020; Li et al. 2020). The well-assembled mitogenome presented in this study will not only serve as a valuable genetic resource for future research and applications involving *I. didactylus*, but also contribute to ecological and evolutionary studies of Synanceiidae species.

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QL, HL and ZP collected the samples. WY and SW conceived and designed the experiments. QL performed data analysis and drafted the initial manuscript. WY and SW critically revised the manuscript. SW secured funding and provided research resources. WY and SW served as corresponding authors, with SW primarily responsible for editorial correspondence. All authors contributed to manuscript editing, approved the final version, and agreed to its submission.

## Author contributions

CRediT: **Qian Li**: Visualization, Writing – original draft; **Hao Li**: Resources; **Zhaojie Peng**: Resources; **Wenhua Yu**: Supervision; **Shichao Wei**: Project administration.

## Disclosure statement

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

## Ethical approval

The data collection of this fish species was conducted with the permission of the Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou).

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## Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI under the accession: PQ773495, with secondary accessions: PQ483949 and PQ723780. The associated BioProject, Bio-Sample and SRA numbers are PRJNA1170921, SAMN44309270 and SRR31023621, respectively.

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