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MITOGENOME ANNOUNCEMENT

Complete mitochondrial genome of *Mastigias papua* (Scyphozoa: Rhizostomeae: Mastigiidae) based on next-generation sequencing and phylogenetic analysis

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ABSTRACT

We assembled the complete mitochondrial genome (mitogenome) of *Mastigias papua* (Scyphozoa: Rhizostomeae: Mastigiidae) by the data generated from the next-generation sequencing platform. The complete mitogenome of *M. papua* was 16,560 bp in length, containing 14 protein-coding genes, two transfer RNA genes, and two ribosomal RNA genes. The base compositions were A 30.65%, C 15.16%, G 16.34%, and T 37.86%, with a gene arrangement similar to the mitogenomes derived from other representatives of Scyphozoa. Based on the 13 common protein-coding genes of 16 species within Scyphozoa, we constructed the phylogenetic tree and found that *M. papua* has a close relationship with *Cassiopea andromeda* and *Cassiopea xamachana*. All these species belong to an order of jellyfish Rhizostomeae, which have similar morphological characteristics. This is agreement with the conclusion we got by the phylogenetic relationship analysis using molecular data. This research has practical implications for advancing understanding of the phylogenetic relationships, taxonomic classifications, and phylogeography within Scyphozoa.

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KEYWORDS
Mastigias papua; mitochondrial genome; next-generation sequencing; phylogenetic analysis

1. Introduction

Jellyfish, which belong to the phylum Cnidaria, utilize nematocysts for both prey capture and defense (Jouiaei et al. 2015). These specialized structures release a cocktail of toxins from their capsule matrix, effectively paralyzing the tissues of their targets, including marine animals and humans venturing into water environments (Watrous and Thompson 1981; Birsa et al. 2010; Bayha and Graham 2014). The remarkable ability of jellyfish to undergo rapid population growth can lead to the occurrence of severe acidification and the development of hypoxic/anoxic conditions in the local environments, thereby exerting considerable impact on the surrounding ecosystem (Dawson and Hamner 2009; Condon et al. 2011; Bayha and Graham 2014; Meredith et al. 2016). *Mastigias papua* (Lesson 1830) (Mastigiidae; Rhizostomeae; Scyphozoa; Cnidaria), a well-known representative jellyfish species originally described from West Papua, Indonesia (Stiasny 1921), holds particular interest among cnidarian biologists (Dawson and Hamner 2003; Bayha and Graham 2011; Swift et al. 2016). Despite its recent redescription as an endemic species in the tropical western Pacific islands and the designation of a neotype based on comprehensive morphological and molecular analyses (Souza and Dawson 2018), the complete mitochondrial genome (mitogenome) of this species has yet to be sequenced.

2. Materials and methods

To accomplish the comprehensive sequencing, assembly, and annotation of the complete mitogenome of *M. papua*, a single specimen was acquired from a jellyfish breeder in Xiamen, China (118°04′E, 24°26′N). The specimen was subsequently deposited at the Laboratory of the Institute of Basic Translational Medicine, Xi'an Medical University (Dr Wangxiao Xia, xiawangxiao@foxmail.com) under voucher number jellyfish202301 (Figure 1). Species identification was established based on morphological features and the cytochrome c oxidase subunit I (*COI*) gene sequence, which matched the species sequence in GenBank (accession number KU901455.1). Genomic DNA of the specimen was extracted from tissue obtained from the umbrella region using a Qiagen Blood & Cell Culture DNA Mini Kit (Crawley, UK).

For Illumina HiSeq sequencing, a short paired-end library was constructed using the NEB DNA Library Rapid Prep Kit (NEB, Ipswich, MA) according to the manufacturer's protocol as follows: (1) the purified DNA was random fragmented by enzymatic

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shearing; (2) end repair reaction; (3) 5′ and 3′ adapters were ligated; (4) cleanup of adaptor-ligated DNA without size selection; (5) PCR enrichment of adaptor-ligated DNA; (6) cleanup of PCR reaction. The sequencing was performed on the NovaSeq6000 (Illumina) platform by the company (Biomarker technologies, Beijing, China). A short paired-end library was then constructed and subjected to sequencing using the Illumina NovaSeq 6000 platform. The mitogenome was assembled using MitoZ (v2.4) software with default parameters (Meng et al. 2019). The mitochondrial genes were subsequently predicted and annotated using the MITOS Web Server (Bernt et al. 2013) and tRNAscan-SE Search Server (Chan and Lowe 2019). The complete assembly and annotation results of the *M. papua* mitogenome are publicly accessible at GenBank (accession number OQ695499).

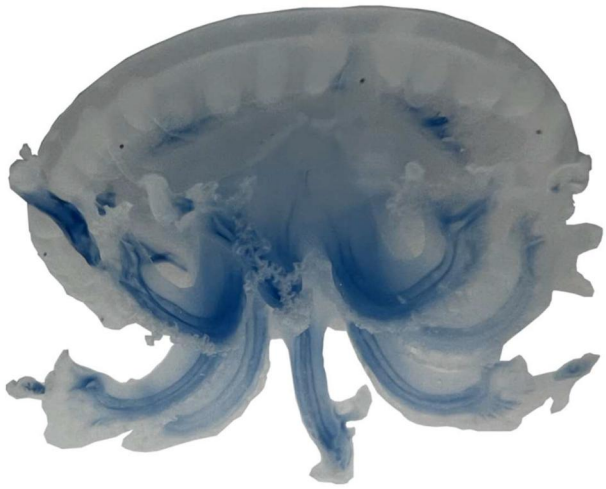

Figure 1. Reference image of *M. papua* (note umbrella is about 3 cm in diameter). Species reference pictures were taken by the author Yaowen Liu on 1 May 2023 at the biological Laboratory of the College of Veterinary Medicine, Yunnan Agricultural University, China.

3. Results and conclusions

The complete mitogenome of *M. papua* was 16 560 bp in length, containing 14 protein-coding genes, two transfer RNA (tRNA) genes ($tRNA^{Trp}$ and $tRNA^{Met}$), and two ribosomal RNA (rRNA) genes (Figure 2). Most of the protein-coding genes were located by the H-strand, except for *COX1*, *dpo*, and 16S rRNA, which were located on the L-strand. For the start codons, 12 genes started with ATG, while two genes (*ATP8* and *ND3*) started with GTG. For the stop codons, eight genes (*ATP6*, *ND2*, *ND5*, *ND3*, *ND4L*, *ND4*, *dpo*, and *CYTB*) ended with TAA, while six genes (*ND1*, *ND6*, *COX3*, *ATP8*, *COX1*, and *COX2*) ended with TAG. The base compositions were A 30.65%, C 15.16%, G 16.34%, and T 37.86%. Furthermore, the AT and GC contents were 68.50% and 31.50%, respectively, thus showing considerable AT bias. The total length of the 14 protein-coding genes was 12,892 bp, accounting for 77.85% of the complete mitogenome and encoding a total of 4284 amino acids (the TAA stop codon of *dpo* was completed by the addition of 3′A residues to the mRNA). The predicted lengths of the 12S rRNA and 16S rRNA genes were 936 bp and 1 642 bp, respectively.

To determine the phylogenetic placement of *M. papua* within Scyphozoa, we employed available mitogenome sequences and conducted sequence alignment using ClustalW (Thompson et al. 1994) in BioEdit (Hall 1999). The phylogenetic tree was constructed using the neighbor-joining (NJ) method with 10 000 bootstraps in MEGA7 (Kumar et al. 2016). The phylogenetic

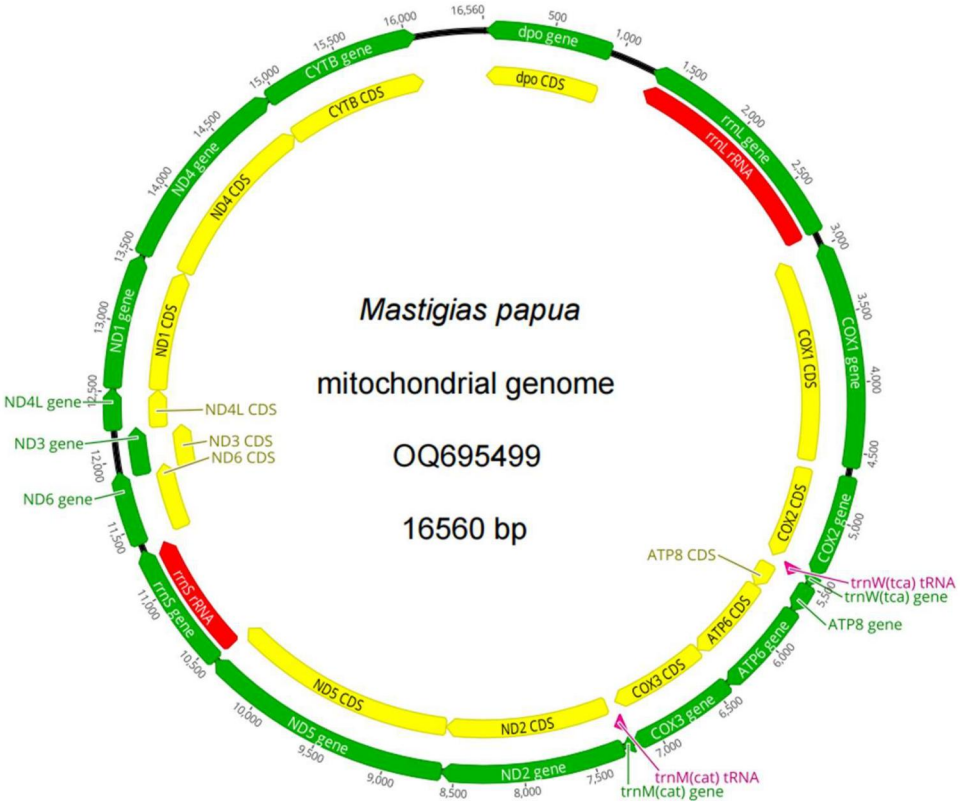

Figure 2. Circular map of *M. papua* mitogenome.

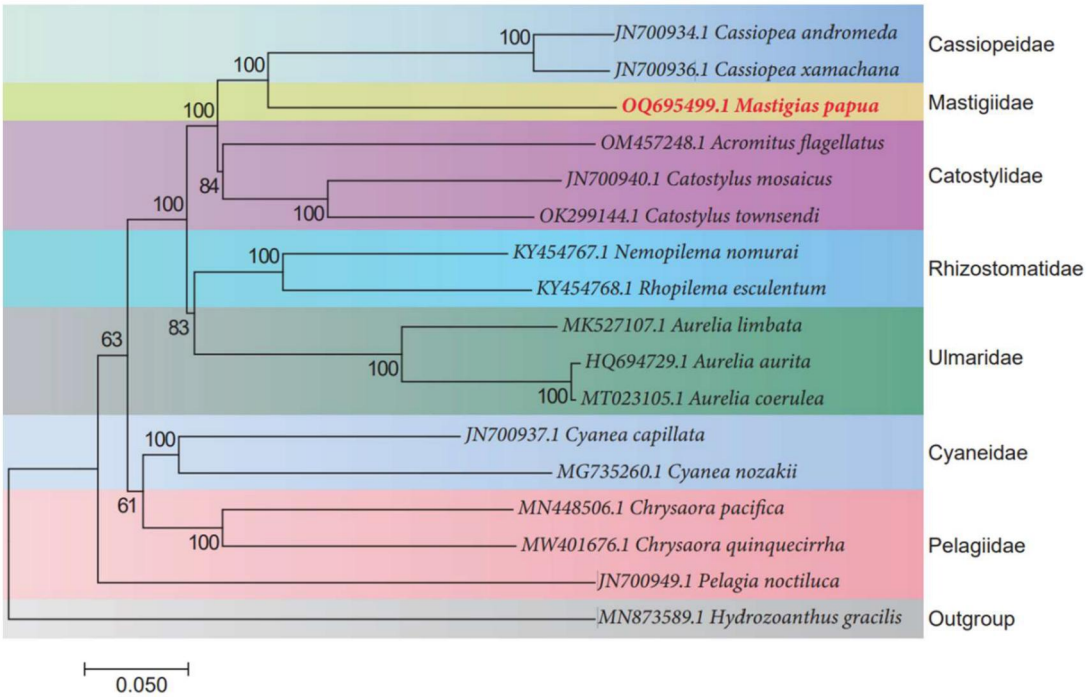

Figure 3. Phylogenetic neighbor-joining tree of 16 Scyphozoa species and *Hydrozoanthus gracilis* (outgroup) based on 13 protein-coding genes. Number at each node is bootstrap probability. Red indicates *M. papua* species from this study. The following sequences were used: *Hydrozoanthus gracilis* MN873589.1 (Poliseno et al. 2020), *Pelagia noctiluca* JN700949.1 (Kayal et al. 2012), *Chrysaora quinquecirrha* MW401676.1 (unpublished), *Chrysaora pacifica* MN448506.1 (Wang and Yin 2020), *Cyanea nozakii* MG735260.1 (Karagozlu et al. 2018), *Cyanea capillata* JN700937.1 (Kayalet al. 2012), *Aurelia coerulea* MT023105.1 (unpublished), *Aurelia aurita* HQ694729.1 (Park et al. 2012), *Aurelia limbata* MK527107.1 (Karagozlu et al. 2019), *Rhopilema esculentum* KY454768.1 (Wang and Sun 2017), *Nemopilema nomurai* KY454767.1 (Wang and Sun 2017), *Catostylus townsendi* OK299144.1 (unpublished), *Catostylus mosaicus* JN700940.1 (Kayalet al. 2012), *Acromitus flagellates* OM457248.1 (Lin et al. 2022), *Cassiopea xamachana* JN700936.1 (Kayal et al. 2012), and *Cassiopea andromeda* JN700934.1 (Kayal et al. 2012).

relationships between *M. papua* and 15 other Scyphozoa species were analyzed based on the 13 common protein-coding genes (except *dpo*), using *Hydrozoanthus gracilis* as an outgroup. Results showed that *M. papua* clustered with the *Cassiopea andromeda* and *Cassiopea xamachana* jellyfish species (Figure 3). Thus, these results indicate that the classifications derived from morphological observations are in alignment with those obtained through molecular analysis (Krapm 1961; Keith et al. 2010; Schoch et al. 2020).

4. Discussion

The worldwide population of jellyfish consists of approximately 2000 different species, including about 200 species within Scyphozoa. Here, we successfully assembled the first complete mitogenome of *M. papua*, which was 16,560 bp in length and contained14 protein-coding genes, two tRNA genes ($tRNA^{Trp}$ and $tRNA^{Met}$), and two rRNA genes (Figure 2). Most of the gene arrangement and base composition were consistent with previous reports on other Scyphozoa species (Shao et al. 2006; Stampar et al. 2013; Karagozlu et al. 2018; Wang and Yin 2020). Compared to the mitochondrial genomes of other jellyfishes, such as *A. aurita*, which contains 13 protein coding genes (Park et al. 2012), the mitochondrial genome of *M. papua* encodes one more *Dpo* (DNA polymerase gene) gene.

Although previously several studies have discussed the phylogenetic relationship between *M. papua* and other close-related species, only very limited genomic information, such as the sequences of 5.8S and partial-28S ribosomal DNA, were used (Dawson 2004; Keith et al. 2010). Our result confirms that *M. papua* (belongs to Mastigiidae) is closely related to Cassiopeidea and Catostylidae, which is consistent with previous molecular research (Dawson 2004; Keith et al. 2010). Besides, previous studies suggest that the morphology of Mastigiidae and Cassiopeidea has more similarity, and our molecular results are consistent with those of morphological observations (Krapm 1961; Keith et al. 2010). Taken together, this study not only indicates the characteristics of the *M. papua* mitochondrial genome and its phylogeny, but also provides important mitochondrial genome resources for comparative analyses of jellyfishes in the future.

Ethical approval

The samples used in this study were obtained from common jellyfish species not included in the 'List of Protected Animals in China'. Thus, our sampling did not violate any laws, rules, or regulations in China. We also confirm that all research was conducted in strict accordance with ethical guidelines and the legal requirements of the study country.

Author contributions

Wangxiao Xia and Hui Jiang contributed significantly to analysis and manuscript preparation. Wenbo Fan and Xiaomin Li were involved in critical revision of the paper for intellectual content. Xingchun Gou, Lixian Xu, and Yaowen Liu contributed to the conception and design of the study and final approval of the version to be published. All authors agree to be accountable for all aspects of the work.

Disclosure statement

No potential competing interests are declared by the authors.

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Data availability statement

The genome sequence data that support the findings of this study are available at GenBank (https://www.ncbi.nlm.nih.gov/) under accession no. OQ695499.1 and the Sequence Read Archive (SRA) BioProject under BioSample numbers PRJNA992184, SRR25183318, and SAMN36344375.

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