*The Israeli Journal of Aquaculture – Bamidgeh • ISSN 0792-156X • IJA.74.2022.1804873, 6 pages*
 https://doi.org/10.46989/001c.39657



**Short Communication**

# Complete mitochondrial genome of *Metapenaeus affinis* (H. Milne Edwards, 1837) and *Metapenaeus ensis* (De Haan, 1844)

**Yundong Li[1, 2, 3,4], Falin Zhou[1, 2], Qibin Yang[1, 2], Song Jiang[1, 2], Jianhua Huang[1, 2], Lishi Yang[1, 2], Shigui Jiang[1, 2] ***

[1] *Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou), Guangzhou, China*

[2] *Key Laboratory of South China Sea Fishery Resources Exploitation and Utilization, Ministry of Agriculture and Rural Affairs, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou, China*

[3] *Key Laboratory of Efficient Utilization and Processing of Marine Fishery Resources of Hainan Province, Sanya Tropical Fisheries Research Institute, Sanya 572018, China*

[4] Hainan Yazhou Bay Seed Laboratory, Sanya, China

(Received Sep 07, 2022; Accepted Oct 06, 2022; Published Oct 25, 2022)

Keywords: *Metapenaeus affinis, Metapenaeus ensis,* Mitogenome, molecular phylogeny, shrimp

**Abstract**

Penaeid shrimp is one of the marine germplasm resources in tropical and subtropical regions. To better understand shrimp germplasm resources and develop cultured shrimp species, the complete mitochondrial genome of *Metapenaeus affinis* and *Metapenaeus ensis* was assembled. The length of *M. affinis* and *M. ensis* mitochondrial sequences is 15,957 and 15,943, respectively. Both mitochondrial sequences contain 13 protein coding, 22 tRNA, and two rRNA genes. The GC content of the genome was 34.23% and 34.12% in *M. affinis* and *M. ensis,* respectively. Phylogenetic analysis indicated that the *M. affinis* and *M. ensis* are closely related, and assigned to a branch of *Metapenaeus*. We assembled and published the mitochondrial genome sequences of these two species, which will provide important information for the research and utilization of shrimp germplasm resources.

* Corresponding author. Tel.: +86-20-89108338, fax: +86-20-84451442, e-mail: jiangsg@21cn.com

## Introduction

Penaeid shrimp is one of the marine germplasm resources in tropical and subtropical regions and significantly contributes to global aquaculture and fisheries (de Alaiza Garcia Madrigal et al., 2018). *Metapenaeus* is one of the most important genera of Penaeid, which makes classification difficult due to its large variety and similar morphology (Yan et al., 2019; Zhong et al., 2018). The complete mitochondrial genome will facilitate a better understanding of genome evolution and phylogeny. Here, we performed mitochondrial sequencing of the shrimp *M. affinis* and *M. ensis*. The successful assembly of the mitochondrial genomes of these two species of shrimp may provide essential reference information for the protection of shrimp resources and the development and utilization of new species in the future. Among the current world shrimp cultured species, *Penaeus vannamei* and *Penaeus monodon* are the main cultured species, especially in Asia, where the farming of these two species may account for more than 80%. However, with the intensive development of aquaculture and the diversification of aquaculture areas in recent years, developing more aquaculture species is an essential way to the orderly development of the shrimp aquaculture industry. *M. affinis* and *M. ensis*, commonly known as sand shrimp or gei wai shrimp, are popular for their meaty, nutritious quality. They are characterized by wide distribution, strong adaptability, omnivorous food, fast growth, and high commercial value. Therefore, to better use these two species, we sequenced and assembled their mitochondrial sequences to provide a reference for developing more shrimp-cultured species.

## Materials and Methods

Ethical approval: This study includes no human, animal, or endangered plant samples. The study was approved by the Animal Care and Use Committee of the South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences (No. SCSFRI96-253). Sampling and all experiments strictly complied with the guidelines and regulations established by the committee.

Shrimps *M. affinis* and *M. ensis* were captured in the South China Sea near Sanya City, Hainan province (18°21’8.68”N, 109°07’28.33”E). Shrimp samples were frozen at - 20°C and stored at the Tropical Aquaculture Research and Development Center, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Sanya, China.

Mitochondrial sequencing library construction is the standard Illumina NovaSeq sequencing experimental process. The main steps are briefly described: a) Total genomic DNA from samples was extracted using Tissue DNA Kit (Tiangen, China) following the manufacturer's protocol. b) Use the TruSeq™ Nano DNA Sample Prep Kit to build the library according to the instructions. DNA libraries (300 bp insert) were constructed with the TruSeq Nano DNA Sample Prep Kit (Illumina, CA). c) Use the Certified Low Range Ultra Agarose (Bio-Rad) kit for library recovery and purification; d) Use cBot Truseq PE Cluster Kit v3-cBot-HS kit for amplification and library enrichment on cBot solid phase support, and finally sequence the library using Illumina NovaSeq 6000 at Shanghai BIOZERON Biotechnology Co. Ltd. (Shanghai, China).

The software Trimmomatic v0.39 (Bolger et al., 2014) and SPAdes v3.14.1 (HYPERLINK "http://bioinf.spbau.ru/spades) were used for sequencing data quality control and mitochondrial genome assembly. The mitochondrion genes were annotated using the online MITOS tool, using default parameters to predict protein-coding genes, transfer RNA (tRNA) genes, and ribosome RNA (rRNA) genes. The position of each coding gene was determined using BLAST searches against ref mitochondrial genes. Manual corrections of genes for start/stop codons were performed in SnapGene Viewer by referencing the ref mitochondrial genome. The circular genome map was drawn using the CGview tool.

## Results

The complete mitogenome of *M. affinis* and *M. ensis* were uploaded in the database with GenBank accession numbers ON599338 and ON599337. The complete length of *M. affinis* and *M. ensis* mitochondrial sequences is 15,957 and 15,943, respectively (**Figure 1**). Both mitochondrial sequences contain 13 protein coding, 22 tRNA, and two rRNA genes. The overall guanine-cytosine (GC) content of the genome was 34.23% and 34.12% in *M. affinis* and *M. ensis,* respectively. The base composition and gene distribution of mitochondria are statistically summarized, and the coding genes, rRNAs and tRNAs are summarized in genome coordinate order, showing information such as gene length, gene interval length, codons, etc., which can intuitively display the genome composition. (**Table 1**).

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at [https://www.ncbi.nlm.nih.gov/nuccore/ON599338.1/] and [https://www.ncbi.nlm.nih.gov/nuccore/ON599337.1/] under the accession no. ON599338 and ON599337. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA875204, SRR21360528, SRR21360529, SAMN30606721, SAMN30606722. A specimen was deposited at South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences under the voucher number nhsh202100865.

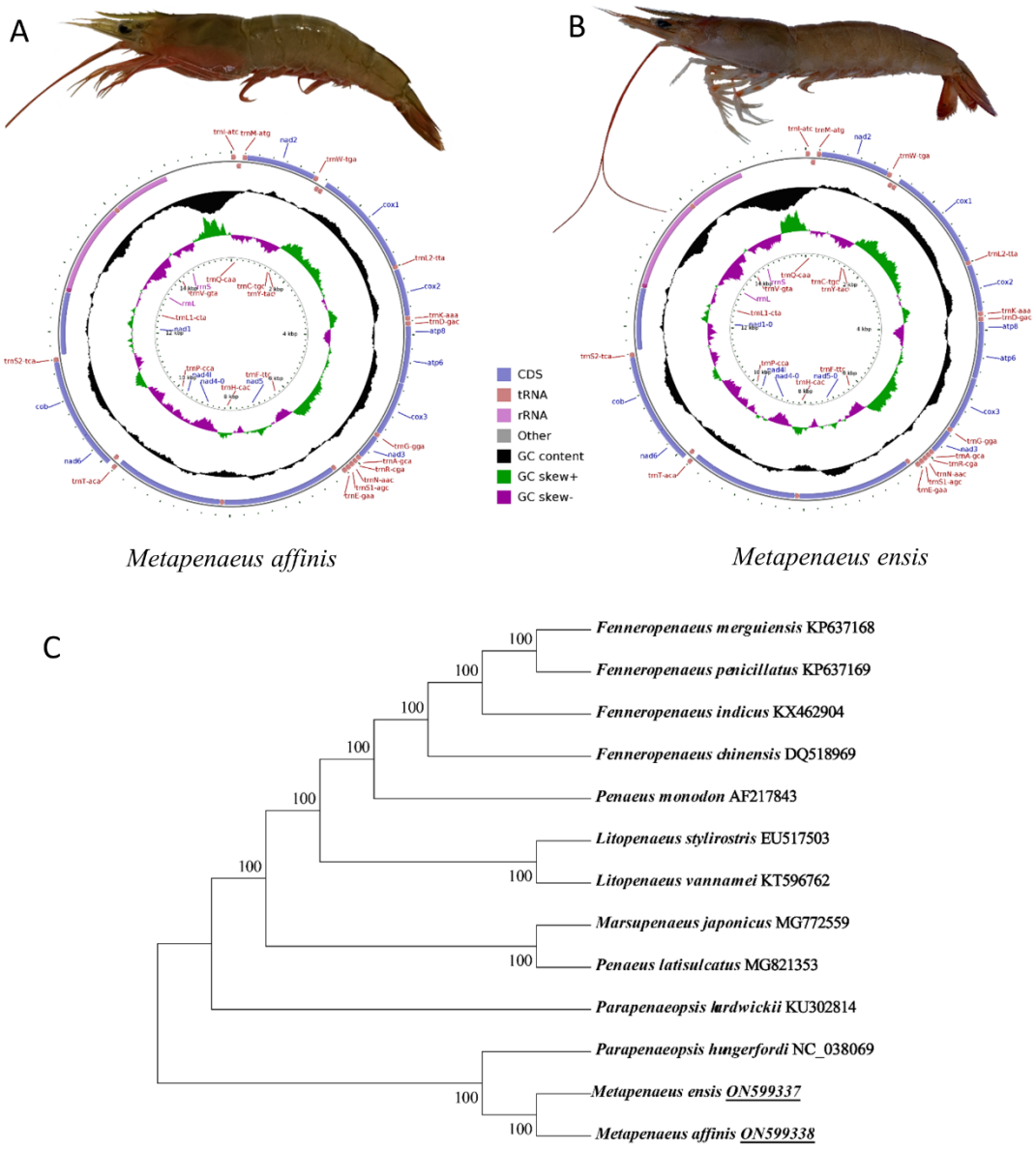


**Figure 1** Complete mitochondrial genome of *Metapenaeus affinis* and *Metapenaeus ensis*. (A) The mitochondrial genome of *Metapenaeus affinis*. (B) The mitochondrial genome of *Metapenaeus ensis*. (C) The phylogenetic tree of 13 species in the family Penaeidae. The complete mitogenomes were downloaded from GenBank, and the phylogenic tree was constructed by the maximum-likelihood method. The numbers on the branches represent the confidence in the relationship between the two species.

**Table1** Base Composition and Gene Distribution of Mitochondria genome of *Metapenaeus affinis* and

| Base Composition and Gene Distribution of Mitochondria genome | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ***M. affinis*** | **Region** | **Length(bp)** | **T/U%** | **C%** | **A%** | **G%** | **AT%** | **GC%** |
| | Genome | 15957 | 31.25 | 21.46 | 34.62 | 12.67 | 65.88 | 34.12 |
| | Protein coding genes | 11190 | 36.9 | 18.89 | 27.2 | 17.01 | 64.1 | 35.9 |
| | First position | 3729 | 33.19 | 18.74 | 26.19 | 21.88 | 59.38 | 40.62 |
| | Second position | 3729 | 41.53 | 20.83 | 22.04 | 15.6 | 63.57 | 36.43 |
| | Third position | 3729 | 35.98 | 17.1 | 33.38 | 13.54 | 69.36 | 30.64 |
| | tRNA | 1488 | 31.18 | 16.06 | 32.66 | 20.09 | 63.84 | 36.16 |
| | rRNA | 2281 | 35.99 | 10.35 | 33.23 | 20.43 | 69.22 | 30.78 |
| ***M. ensis*** | Genome | 15943 | 30.9 | 21.72 | 34.87 | 12.51 | 65.77 | 34.23 |
| | Protein coding genes | 11190 | 36.63 | 19.08 | 26.99 | 17.3 | 63.62 | 36.38 |
| | First position | 3729 | 32.49 | 19.54 | 26.35 | 21.61 | 58.85 | 41.15 |
| | Second position | 3729 | 41.8 | 20.43 | 21.53 | 16.25 | 63.32 | 36.68 |
| | Third position | 3729 | 35.6 | 17.27 | 33.08 | 14.05 | 68.69 | 31.31 |
| | tRNA | 1487 | 30.87 | 16.48 | 32.82 | 19.84 | 63.69 | 36.31 |
| | rRNA | 2288 | 36.32 | 9.75 | 33.65 | 20.28 | 69.97 | 30.03 |

*Metapenaeus ensis.*

## Discussion

The structural circle map of the mitochondrial genome was constructed by CGView (http://stothard.afns.ualberta.ca/cgview_Server/) software. The phylogenetic tree was constructed by the software MEGA 7.0 using the maximum-likelihood method, and multiple sequence alignment was ClustalW. The phylogenetic tree showed that *M. affinis* and *M. ensis* are closely related, which is also consistent with the previous research results (Ahmed et al., 2021). The appearance of *M. affinis* and *M. ensis* are very similar, which is difficult to distinguish. Here, we assembled and published the mitochondrial genome sequences of these two species, which will provide vital information for the research and utilization of shrimp germplasm resources.

Mitochondrial gene resources are essential reference data for species identification and evolutionary analysis. Shrimp is an important species of aquaculture in the world and one of the most successful species groups in Arthropoda. The mitochondrial sequences of the two species we assembled are 13 protein-coding, 22 tRNA, and two rRNA genes, consistent with previous reports. *M. affinis* and *M. ensis* are commonly cultured species in Asia. The appearance and morphology of the two kinds of shrimp are very similar, and they are often mixed cultured objects. In this study, we also assembled the mitochondria of these two new prawn species, which provides essential information for the protection, development, and utilization of species resources in the future.

## Acknowledgments

This work was supported by the Key Special Project for Introduced Talents Team of Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou) (GML2019ZD0605), Central Public-interest Scientific Institution Basal Research Fund, CAFS (2020TD30), China Agriculture Research System of MOF and MARA (CARS-48), Central Public-Interest Scientific

Institution Basal Research Fund, South China Sea Fisheries Research Institute, CAFS (2020ZD01, 2021SD13), Hainan Provincial Natural Science Foundation of China (320QN359), Guangdong Basic and Applied Basic Research Foundation (2020A1515110200), and Guangzhou Science and Technology Planning Project (202102020208).

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