


The complete mitochondrial genome sequence of the *Metschnikowia bicuspidata* (Metschnikoff, 1884), an emerging pathogen of ‘milky disease’ in Chinese mitten crab

Jiannan Liu, Jieying Yu, Senting Pu, Xinran Shi, Yihao Li, Xiaoran Zhao and Shigen Ye 

Aquatic Animal Hospital of Dalian Ocean University, Dalian Ocean University, Dalian, China

ABSTRACT

The outbreak of milky disease of Chinese mitten crab caused by *M. bicuspidata* seriously restricted the development of the crab industry. In this study, the mitochondrial genome sequence of *M. bicuspidata* was assembled, annotated, and further analyzed. The results indicated that the complete mitochondrial genome of *M. bicuspidata* was 75,095 bp, which contained two rRNAs, 23 tRNAs, and 13 protein-coding genes. The phylogenetic tree of 13 yeasts based on the complete mitochondrial genome was constructed which showed that *M. bicuspidata* (accession number OK514652) and *M. bicuspidata* (accession number MW147605.1) were clustered in a clade. To sum up, our research results would further provide essential data for the systematics and evolution study of *M. bicuspidata*.

ARTICLE HISTORY

Received 9 January 2022
Accepted 12 May 2022

KEYWORDS



Metschnikowia bicuspidata;
mitochondrial genome;
Eriocheir sinensis

Chinese mitten crab (*Eriocheir sinensis*) belongs to the phylum Arthropoda, class Crustacea, order Decapoda, family Grapsidae, and genus *Eriocheir*. It is an important economic crab in China (Fu et al. 2017). In 2018, the total production of *E. sinensis* reached 692,723 tonnes in China, with an output value of 9,386,536 thousand dollars (<http://www.fao.org/fishery/statistics>, 2018). At present, the breeding areas of *E. sinensis* in China are mainly concentrated in Jiangsu, Hubei, Anhui, and Liaoning provinces. However, with the continuous expansion of the scale and density of crab farming, the disease has been one of the most important limitations of the sustainable development of the industry (Sui et al. 2012; Ding et al. 2015, 2016; Bao et al. 2021). In 2019, a pathogenic yeast was isolated from diseased Chinese mitten crab in many farms of Panjin City, Liaoning Province, China (121°25′–122°31′E, 40°39′–41°27′N), which was identified as *Metschnikowia bicuspidata* (Metschnikoff, 1884) belongs to family Metschnikowiaceae, genus *Metschnikowia* (Ma et al. 2020, 2022; Bao et al. 2021). However, the mitochondrial genome sequence of *M. bicuspidata* was still in the blank.

The specimen of *M. bicuspidata* (Number: 2EJM001) was stored in the Aquatic Animal Hospital of Dalian Ocean University, Dalian Ocean University, Dalian, China. We constructed the library with an average length of 350 bp using the NexteraXT DNA Libraries Preparation Kit (Illumina, San Diego, CA, USA) after the libraries were sequenced on Illumina Novaseq 6000 platform. The raw data totaled 2.84 G, and the clean data totaled 2.83 G after quality control processing and yielding a 1231-fold depth of coverage of the mitochondrial genome. The GC content of the clean data

was 46.5%, the Q20 value was 97.90%, and the Q30 value was 93.96% which indicated that the quality of the mitochondrial genome sequencing and assembly results was very high. High-quality reads were assembled into the mitochondrial genome using *de novo* assembler SPAdes v.3.11.0 software (Bankevich et al. 2012). Finally, we annotated the assembled complete mitochondria genome by MITOS (Bernt et al. 2013). The sequence of *M. bicuspidata* mitochondrial genome was deposited in GenBank (accession number OK514652). Phylogenetic relationships between *M. bicuspidata* and other 12 yeasts were analyzed according to the whole mitochondrial genome from GenBank using Maximum Likelihood methods by MEGA 7.0.

All analytical results indicated that the complete mitochondrial genome of *M. bicuspidata* was 75,095 bp. Its nucleotide composition was as follows: A, 39.1%; C, 11.8%; G, 13.5%; T, 35.6%, with a high AT content of 74.7% which was higher than other kinds of yeast, such as *Sporobolomyces* sp and *Cystobasidium* sp (Huang et al. 2020; Liu and Wang 2020). The circular mitogenome of this yeast includes two rRNAs (16S rRNA and 12S rRNA), 23 tRNAs (tRNA-Ser, tRNA-Thr, tRNA-Val, tRNA-Cys, tRNA-Leu, tRNA-Glu, tRNA-His, tRNA-Met, tRNA-Tyr, tRNA-Asp, tRNA-Arg, tRNA-Phe, tRNA-Gly, tRNA-Asn, tRNA-Pro, tRNA-Lys, tRNA-Ile, tRNA-Gln, tRNA-Ala, and tRNA-Sec) and 13 protein-coding genes (PCGs). From the 13 PCGs of *M. bicuspidata* mitogenome, nine PCGs (*nad1*, *nad4*, *nad6*, *atp6*, *atp9*, *cob*, *cox1*, *cox2*, and *cox3*) were located on the heavy strand and four PCGs (*nad2*, *nad3*, *nad4L*, and *nad5*) on the light strand. Among these PCGs, three genes (*nad3*, *nad4L*, and *nad2*) used the start codon

CONTACT Shigen Ye  shgye@dlou.edu.cn  Aquatic Animal Hospital of Dalian Ocean University, Dalian Ocean University, Dalian, 116023, China

© 2022 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

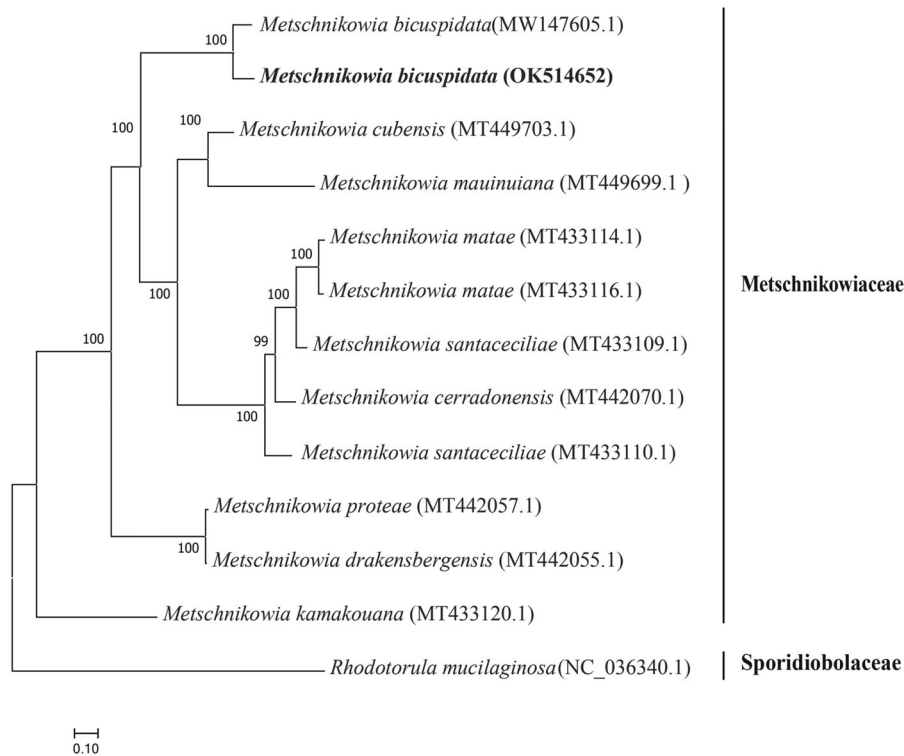


Figure 1. The phylogenetic tree was inferred using the maximum-likelihood method based on the complete mitochondrial genome of 13 yeasts. A total of 1000 bootstrap replicates were computed, and the bootstrap support values are shown at the branches.

ATT, 4 genes (*nad2*, *atp6*, *atp9*, and *cox2*) used the start codon ATA, and the other genes (*cob*, *cox1*, *cox3*, *nad1*, *nad4*, and *nad6*) used the start codon ATG. Besides, except that the stop codon of *nad2* was TAG, the stop codon of other PCGs (*cob*, *cox1*, *cox2*, *cox3*, *nad1*, *nad3*, *nad4*, *nad4L*, *nad5*, *nad6*, *atp6*, and *atp9*) was TAA.

The resulting phylogenetic tree showed that there were two main clades, Metschnikowiaceae and Sporidiobolaceae. Twelve yeasts species were clustered together within Metschnikowiaceae; *Metschnikowia bicuspidata* (accession number OK514652) and *Metschnikowia bicuspidata* (accession number MW147605.1) were clustered in a clade (Figure 1). In conclusion, the mitochondria of *M. bicuspidata* reported in this study provided more essential information about the yeast, which will help our understanding of *M. bicuspidata* better. Further, the information will do good to develop the rapid detection methods which will favor the control of the 'milky disease' in Chinese mitten crab.

Storage location of the specimen or its DNA

The specimen of *M. bicuspidata* (Number: 2EJM001) was stored in the Aquatic Animal Hospital of Dalian Ocean University, Dalian Ocean University, Dalian, China. And the e-mail of Shigen Ye, the person in charge of the collection, is shgye@dlo.u.edu.cn.

Acknowledgments

We thank Baoshi Guan for his kind help in mitogenome data analysis.

Author contributions

Shigen Ye: conception and design. Jiannan Liu, Jieying Yu, Yihao Li, Xinran Shi, Senting Pu, and Xiaoran Zhao: analysis and interpretation of the data. Jiannan Liu: draft the paper. Shigen Ye: revising it critically for intellectual content. Shigen Ye: the final approval of the version to be published. And all authors to agree to be accountable for all aspects of the work.

Disclosure statement

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

Funding

This work was financially supported by the Liaoning Province's Project of Selecting Best Candidates to Lead Key Researches [Grant No. 2021JH1/10400040].

ORCID

Shigen Ye  <http://orcid.org/0000-0002-9280-6604>

Data availability statement

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/OK514652>] under the accession no. OK514652. The associated BioProject accession number of the genome is PRJNA771255 [<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA771255>]. The associated BioSample accession number of the genome is SAMN22266430 [<https://www.ncbi.nlm.nih.gov/biosample/?term=SAMN22266430>]. The associated SRA is SRR16328191 [<https://www.ncbi.nlm.nih.gov/sra/SRR16328191>].

References

- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, et al. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol.* 19(5):455–477.
- Bao J, Jiang HB, Shen HB, Xing YN, Feng CC, Li XD, Chen QJ. 2021. First description of milky disease in the Chinese mitten crab *Eriocheir sinensis* caused by the yeast *Metschnikowia bicuspidata*. *Aquaculture.* 532:735984.
- Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsche G, Pütz J, Middendorf M, Stadler PF. 2013. MITOS: improved *de novo* metazoan mitochondrial genome annotation. *Mol Phylogenet Evol.* 69(2):313–319.
- Ding Z, Meng Q, Liu H, Yuan S, Zhang F, Sun M, Zhao Y, Shen M, Zhou G, Pan J, et al. 2016. First case of hepatopancreatic necrosis disease in pond-reared Chinese mitten crab, *Eriocheir sinensis*, associated with microsporidian. *J Fish Dis.* 39(9):1043–1051.
- Ding ZF, Yao YF, Zhang FX, Wan JJ, Sun ML, Liu HY, Zhou G, Tang JQ, Pan JL, Xue H, et al. 2015. The first detection of white spot syndrome virus in naturally infected cultured Chinese mitten crabs, *Eriocheir sinensis* in China. *J Virol Methods.* 220:49–54.
- Fu LL, Zhou G, Pan JL, Li YH, Lu QP, Zhou J, Li XG. 2017. Effects of Astragalus polysaccharides on antioxidant abilities and non-specific immune responses of Chinese mitten crab, *Eriocheir sinensis*. *Aquacult Int.* 25(3):1333–1343.
- Huang J, Qu H, Shen X. 2020. Characterization of the complete mitochondrial genome of biocontrol yeast *Sporobolomyces* sp. (sporidiobolales: sporidiobolaceae) with phylogenetic analysis. *Mitochondrial Part B.* 5(3):3057–3059.
- Liu Q, Wang X. 2020. Characterization and phylogenetic analysis of the complete mitochondrial genome of a basidiomycetous yeast *Cystobasidium* sp. (cystobasidiales: cystobasidiaceae). *Mitochondrial DNA B Resour.* 5(3):2449–2450.
- Ma HL, Lu XC, Liu JN, Guo SC, Zhao XR, Ye SG. 2022. *Metschnikowia bicuspidata* isolated from milky diseased *Eriocheir sinensis*: phenotypic and genetic characterization, antifungal susceptibility and challenge models. *J Fish Dis.* 45(1):41–49.
- Ma HL, Sun N, Lu XC, Liu JN, Guo SC, Zhao XR, Ye SG. 2020. Isolation and identification of pathogen in milky disease of Chinese mitten crab *Eriocheir sinensis* in Liaoning Province. *J Dalian Ocean Univ.* 35(05):714–718.
- Sui LY, Cai JL, Sun HX, Wille M, Bossier P. 2012. Effect of poly- β -hydroxybutyrate on Chinese mitten crab, *Eriocheir sinensis*, larvae challenged with pathogenic *Vibrio anguillarum*. *J Fish Dis.* 35(5):359–364.