# Charybdis by Sapto Andriyono

**Submission date:** 10-Jan-2023 01:17PM (UTC+0800)

**Submission ID:** 1990588023

File name: me\_of\_two\_spot\_swimming\_crab\_Charybdis\_bimaculata\_Miers\_1886.pdf (867.51K)

Word count: 1390 Character count: 7719



#### Mitochondrial DNA Part B



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ISSN: (Print) 2380-2359 (Online) Journal homepage: https://www.tandfonline.com/loi/tmdn20

## Characterization of complete mitochondrial genome of two-spot swimming crab *Charybdis bimaculata* (Miers, 1886)

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To cite this article: Nack-Keun Kim, Sapto Andriyono, Ah Ran Kim, Chung II Lee & Hyun-Woo Kim (2018) Characterization of complete mitochondrial genome of two-spot swimming crab *Charybdis bimaculata* (Miers, 1886), Mitochondrial DNA Part B, 3:2, 900-901, DOI: 10.1080/23802359.2018.1501292

To link to this article: <a href="https://doi.org/10.1080/23802359.2018.1501292">https://doi.org/10.1080/23802359.2018.1501292</a>

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#### MITOGENOME ANNOUNCEMENT



#### Characterization of complete mitochondrial genome of two-spot swimming crab Charybdis bimaculata (Miers, 1886)

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The two-spot swimming crab Charybdis bimaculata (Miers, 1886) is an important decapod species in the benthic ecosystem of Korean waters. In this study, we determined its complete mitochondrial genome by the combination of NGS analysis using MiSeq platform and PCR-based cloning method. The circular mitochondrial genome of C. bimaculata was 15,714bp in length in which the standard set of 13 protein-coding genes, 22 tRNA genes, and 2 rRNA genes were encoded. Phylogenic analysis showed that C.bimaculata is most closely related to Charybdis feriata. The complete mitogenome sequence information of C. bimaculata would provide useful data for the conservation of their population in the Pacific ocean.

#### ARTICLE HISTORY

Received 30 May 2018 Accepted 8 July 2018

#### KEYWORDS

Next generation sequencing; Charybdis bimaculata; mitogenome; decapod; phylogeny

Charybdis bimaculata (Miers, 1886) is a small portunid crab, which is mainly caught in Korea, China, and Japan (Rho et al. 2005; Gomez et al. 2008; Narita et al. 2008; Kwak et al. 2014). Although it is widely distributed and plays an important role in the benthic ecosystem (Kume et al. 1999; Yamaguchi and Taniuchi 2000), ecological study of C. bimaculata has been limited mainly due to its lack of genetic information. We here determined the complete mitochondrial genome of C. bimaculata using next generation sequencing (NGS) platform.

The specimen was collected from Jindo Island (34°22'42.62"N and 126°8'14.71"E), morphological identification was determined by National Institute of Fisheries Science, Korea. The specimen or its DNA was stored at Pukyong National University. Two mitochondrial DNA fragments were amplified by two degenerated primer sets targeting COI-ND5 and ND5-COI regions, respectively, and D-loop region was amplified by a sequence-specific primer set. Three PCR products were then pooled together in equal concentration and fragmented into smaller fragments (~350 bp) by Covaris® M220 Focused-Ultrasonicator<sup>TM</sup> (Covaris Inc., U.S.A). Library was constructed by TruSeg® RNA library preparation kit V2 (Illumina, USA). After confirming the quality and the quantity using 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, U.S.A), DNA sequencing was performed by MiSeq sequencer (2  $\times$  300 bp ends) (Illumina, U.S.A).

The complete mitochondrial genome of C. bimaculata (GenBank Number: MG489891) was 15,714 bp which encodes 13 proteins, 22 tRNAs, 2 rRNAs and a putative control region (D-loop). Among them, 12 genes were initiated by the start codon ATN (ATG and ATT) with an exception of ATP8 gene, which begins with GTG. The canonical stop codons (TAA or TAG) were shown in nine genes (ATP8, ATP6, ND2, ND3, ND4, ND4L, ND5, and ND6), whereas incomplete stop codons (T-or TA-) were found in the other four (COI, COII, COIII, and Cyt b) genes. Twenty one tRNA were predicted to be folded into a typical clover-leaf secondary structures by ARWEN (Laslett and Canbäck 2007) except for tRNA<sup>Ser</sup>, which was predicted without DHC arm.

The phylogenetic position of C. bimaculata within portunid crabs was analyzed using minimum evolutionary (ME) methods (Figure 1). As the result of phylogenetic tree which was constructed with the full mitochondrial genome sequences from 12 portunids and one Geryonid as out group, all four Charybdis species including C. bimaculata, C. feriata (Ma et al. 2015), C. natator (Yang et al. 2017), and C. japonica (Liu and Cui 2010) were clustered together. C. bimaculata was most closely related to C. feriata showing 86% sequence identity (Figure 1). This information would be useful for extending our knowledge about evolutional relationship of Portunidae species.

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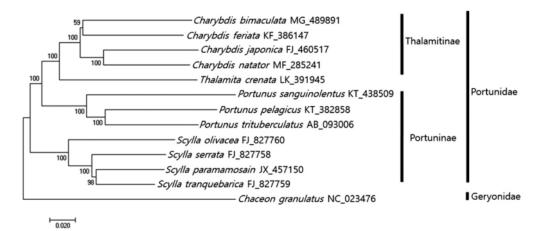


Figure 1. Phylogenetic tree of Charybdis bimaculata with other Portunid crabs. Phlyogenetic tree of Charybdis bimaculata was constructed with mitochondrial genomes of its relative species using MEGA7 software with Minimum Evolution (ME) algorithm with 1000 boothstrap replications. Chaceon granulatus in Geryonidae was adopted as the out group member. GenBank Accession numbers were shown followed by each scientific name.

#### Disclosure statement

No potential conflict of interest was reported by the authors.

#### Funding

This research was a part of the project titled 'Walleye Pollock stock management based on Marine Information & Communication Technology', funded by the Ministry of Oceans and Fisheries, Korea. We also grateful for Indonesian Endowment Fund for Education (LPDP) scholarship under Beasiswa Unggulan Dosen Indonesia-Luar Negeri (BUDI-LN) batch I 2016.

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