

The first mitochondrial genome of *Megabalanus tintinnabulum* (Sessilia: Balanidae) from China: phylogeny within Cirripedia based on mitochondrial genes

Meiping Feng^{a,b,c}, Shiquan Lin^a, Chunsheng Wang^{a,d}, Dong Sun^a, Yadong Zhou^a, Yuanxin Bi^c and Kaida Xu^c

^aKey Laboratory of Marine Ecosystem and Biogeochemistry, State Oceanic Administration & Second Institute of Oceanography, Ministry of Natural Resources, Hangzhou, PR China; ^bCollege of Marine Ecology and Environment, Shanghai Ocean University, Shanghai, PR China; ^cMarine and Fisheries Research Institute, Zhejiang Ocean University, Zhoushan, PR China; ^dSchool of Oceanography, Shanghai Jiao Tong University, Shanghai, PR China

ABSTRACT

Here we present the complete mitochondrial genome of *Megabalanus tintinnabulum*. The genome is 15,107 bp in length with a 67.35% AT content. It contains 13 protein-coding genes (PCGs), 2 rRNAs genes, and 22 tRNAs. Both rRNAs are encoded on the light strand, as in the other crustacean and barnacle mitochondrial genomes. Besides five tRNAs are encoded on the light strand (nad1, trnV, trnL1, trnC, trnQ, and trnK). Only one PCG is encoded on the light strand (nad1), whereas the other 12 PCGs are located on the heavy strand, which is consistent with *M. ajax*. Phylogenetic analysis based on mitochondrial PCGs shows that *M. tintinnabulum* is clustered with *M. ajax* into a branch (BP = 100), and the group with *M. volcano* with high support. This study contributes to further phylogenetic analysis within Cirripedia.

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Megabalanus tintinnabulum (Linnaeus, 1758) (Crustacea: Maxillopoda: Balanidae) is a large acorn barnacle, barrel shaped or narrowly conical, and commonly encountered in the shallow waters of both east and west coasts of India (Thiyagarajan et al. 1997). It is of tropical origin and spreads to other parts of the world attached to the hulls of ships. *Megabalanus* differs from other subgenera of the genus *Balanus* da Costa, 1778, by the possession of well-developed radii permeated by pore parallel to the basis (Beach 1972).

As of 4 September 2019, GenBank contained 31 mitochondrial genomes of Cirripedia, including 15 families, and 2 species belong to *Megabalanus*: *M. ajax* and *M. volcano*. Here we present the first complete mitochondrial genome of the *M. tintinnabulum*.

Specimens of *M. tintinnabulum* were collected from Yushan Island (28.88°N, 122.26°E), Ningbo City, Zhejiang Province, China. The muscle tissue isolated from the fresh specimen was immediately preserved in 95% ethanol and kept in -80°C in Key Laboratory of Marine Ecosystem and Biogeochemistry, State Oceanic Administration, Second Institute of Oceanography, Ministry of Natural Resources (Barnacle MT-N25). DNA was extracted with QIAamp Tissue Kit (QIAGEN, Hilden, Germany) and mitochondrial DNA was amplified with a DNA REPLI-g Mitochondrial DNA Kit (QIAGEN, Hilden, Germany) as directed by the manufacturer.

Library construction and sequencing were performed by Biozeron (Biozeron, Shanghai, China) using the Illumina HiSeq 4000 sequencing platform (Illumina, San Diego, CA).

The mitochondrial genome of *M. tintinnabulum* is a circular molecule which is 15,107 bp (GenBank accession number: MN 481499) in length. It contains 13 protein-coding genes (PCGs), 2 rRNAs genes, and 22 tRNAs. Total AT content of *M. tintinnabulum* is 67.35%. Both rRNAs are encoded on the light strand, as in the other crustacean and barnacle mitochondrial genomes (Shen et al. 2010, 2011, 2014; Tsang et al. 2015). Besides five tRNAs are encoded on the light strand (nad1, trnV, trnL1, trnC, trnQ, and trnK). Only one PCG is encoded on the light strand (nad1), whereas the other 12 PCGs are located on the heavy strand, which is consistent with *M. ajax* (Shen et al. 2014).

Until now three species of *Megabalanus* genus have been reported including *M. tintinnabulum*. PCGs of *M. tintinnabulum* mitochondrial genomes started with ATN. However, PCGs of the other two *Megabalanus* species have initiation codons other than 'ATN' (Shen et al. 2014). Furthermore, variation of initiation codon usage is observed between the three *Megabalanus* species. The *cox1* gene of *M. ajax*, *M. volcano* and *M. tintinnabulum* start with CTT, TTG, and ATT, respectively. *M. tintinnabulum* has ATA in nd3 and ATC in atp8, which is the same as *M. ajax*, whereas *M. volcano* uses

more mitochondrial genomic data of undetermined taxa and further analysis are required to reveal phylogeny and evolution of barnacles.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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