Complete mitochondrial genome of *Tetraclita squamosa squamosa* (Sessilia: Tetraclitidae) from China and phylogeny within Cirripedia

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**ABSTRACT**

Here we present the complete mitochondrial genome of *Tetraclita squamosa squamosa*, which is 15,191 bp in length with 67.20% AT content. It contains 13 protein-coding genes, 2 ribosomal-RNA genes and 22 transfer-RNA genes. All PCGs except nad4l in *T. squamosa squamosa* start with ATN, and terminated with a complete stop codon, except nad3. Phylogenetic analysis based on mitochondrial PCGs shows that *T. squamosa squamosa* is clustered with *T. serrata* into a branch (BP = 100). Our result is consistent with previous reports that genus *Tetraclita* and family *Tetraclitidae* are not monophyletic. This study contributes to further phylogenetic analysis within Cirripedia.

The acorn barnacle *Tetraclita* is a common space occupier in the intertidal zone of tropical and subtropical waters worldwide, and has at least 12 subspecies for the high intraspecific morphological variation (Darwin 1854; Newman and Ross 1976). *Tetraclita squamosa squamosa* (Crustacea: Sessilia: Tetraclitidae) is one of the common major acorn barnacles in Chinese waters (Yan et al. 2012). *Tetraclita squamosa squamosa* was originally described as *Balanus squamosa* by Bruguière 1789, and was separated from another species *Tetraclita japonica* by allozyme electrophoresis and DNA analysis (Yamaguchi 1987; Hasegawa et al. 1996; Chan 2001; Chan et al. 2007). It has been used to monitor the bioavailability of metals in the coastal waters (Rainbow and Phillips 1993; Blackmore 1996, 1999; Blackmore and Chan 1998; Blackmore et al. 1998). Here, we present the first complete mitochondrial genome of the species *T. squamosa squamosa*.

Specimens of *T. squamosa squamosa* were collected from Daya Bay (114.60°N, 22.55°E) in the South China Sea. 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-04). 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 *T. squamosa squamosa* is 15,191 bp in length with a 67.20% AT content (GenBank Accession number: MT232759). It contains 13 protein-coding genes, two ribosomal-RNA genes, and 22 transfer-RNA genes. The length of coding sequences is 10,958 bp (72.13%), and it is shorter than *Tesseractopora rosea* which was the lowest among the available mitochondrial genomes of *Tetraclitidae* in previous report (Cai et al. 2018). Both rRNAs are encoded on the light strand, as in the other crustacean and barnacle mitochondrial genomes. Four PCGs are encoded on the light strand (nd1, nd4, nd4L, and nd5), while the other nine PCGs are located on the heavy strand, which was the same as those of *Tetraclita rufoincta* (Song et al. 2017). Besides two rRNAs and seven tRNAs are encoded on the light strand. Twelve PCGs in *T. squamosa squamosa* start with ATN, while nad4l was initiated with GTG. Most of the PCGs terminated with a complete stop codon (TAA or TAG), but one PCG (nad3) had incomplete stop codons (T).

To elucidate phylogenetic relationships of *T. squamosa squamosa* with the other barnacles, phylogenetic tree (Figure 1) is constructed based on the PCGs with Maximum Likelihood using phyML ver 3.0 (http://www.atgc-montpellier.fr/phyml/). A total of 30 species with 31 mitochondrial genomes from Cirripedia have been used in the phylogenetic tree (Shen, Chan, et al. 2015, 2016; Shen, Tsang, et al. 2015, 2017; Wares 2015; Shen, Chu, et al. 2016; Shen, Tsoi, et al. 2018). Our result shows that the species of *T. squamosa squamosa* is clustered with *T. serrata* into a branch (BP = 100). Our result is consistent with previous reports that genus *Tetraclita* and family *Tetraclitidae* are not monophyletic. This study contributes to further phylogenetic analysis within Cirripedia.
This analysis involved four species belonging to genus *Tetraclita*: *T. divisa*, *T. serrata*, *T. japonica* CN/JP, and *T. rufotincta*. Result shows that *T. squamosa squamosa* is clustered with *T. serrata* into a branch (BP = 100), and the two group with *T. rosea* and *T. japonica*, with *T. rufotincta* and *T. divisa* as the most distantly related species (Figure 1). In the tree, *T. divisa* as the most distantly related species within Tetraclitidae, which was consistent with the previous results (Song et al. 2017; Cai et al. 2018). *Chelonbia testudinaria* (Coronulidae) clusters with species from Tetraclitidae, which is consistent with Song et al. (2017). Song et al. (2017) compared genome of four species from family Tetraclitidae, and found the mitochondrial gene order was highly conserved in the family. However, it has been reported that the genus *Tetraclita* does not constitute a monophyletic assemblage (Cai et al. 2018); and Tetraclitidae is not monophyletic (Tsang et al. 2015). Our result is consistent with these previous reports.

In conclusion, we decode the complete mitochondrial genome of *T. squamosa squamosa* and report the phylogenetic analysis for the first time, which will provide data for further molecular and evolutionary analysis within Tetraclitidae and Cirripedia. Further analyses are required to reveal phylogeny and evolution of barnacles.

**Disclosure statement**

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References

Blackmore G. 1996. Biomonitoring of heavy metal pollution in Hong Kong coastal waters, using barnacles. Asian Mar Biol. 13:1–13.
Blackmore G. 1999. Temporal and spatial biomonitoring of heavy metals in Hong Kong coastal waters using Tetractia squamosa. Environ Pollut. 106(3):273–283.
Blackmore G, Chan HM. 1998. Heavy metal concentrations in barnacles (Tetraclita squamosa) in Hong Kong: a revisit. In: Morton B, editor, The marine biology of the South China Sea III. Proceedings of the Third International Conference on the Marine Biology of the South China Sea, Hong Kong. 1996. Hong Kong (China): Hong Kong University Press; p. 397–410.
Blackmore G, Morton B, Huang ZG. 1998. Heavy metals in Balanus amphitrite and Tetractia squamosa (Crustacea: Cirripedia) collected from the coastal waters of Xiamen, China. Mar Pollut Bull. 36(1):32–40.
Cai YF, Shen X, Zhou L, Chu KH, Chan B. 2018. Mitochondrial genome of Tesseropora rosea: molecular evidence for non-monophyly of the genus Tesserotia. Mitochondrial DNA Part B. 3(1):92–94.
Chan BKK, Tsang LM, Chu KH. 2007. Morphological and genetic differentiation of the acorn barnacle Tetractia squamosa (Crustacea, Cirripedia) in East Asia and description of a new species of Tetractia. Zool Scripta. 36(1):79–91.
Chan B. 2001. Studies on Tetractia squamosa and Tetractia japonica (Cirripedia: Thoracica). I. adult morphology. J Crustacean Biol. 21(3):616–630.
Darwin C. 1854. A monograph of sub-class Cirripedia: Volume II, The Balanidae. In: Barrett P, Freeman RB, editors. The works of Charles Darwin. Vol. 12. Washington Square (NY): New York University Press; p. 1–436.
Feng MP, Lin SQ, Wang CS, et al. 2019. The first mitochondrial genome of Megabalanus tintinnabulum (Sessilia: Balanidae) from China: phylogeny within Cirripedia based on mitochondrial genes. Mitochondrial DNA Part B. 4(2):4016–4018. DOI: 10.1080/23802359.2019.1688104
Ge T, Song J, Ji N, Cai Y, Chen P, Zhao H, Shen X. 2019. The first mitochondrial genome of Tetractia japonica (Crustacea: Sessilia) from China: phylogeny within Cirripedia based on mitochondrial genes. Mitochondrial DNA Part B. 4(1):2008–2010.
Hasegawa T, Yamaguchi T, Kojima S, Ohta S. 1996. Phylogenetic analysis among three species of intertidal barnacles of the genus Tetractia (Cirripedia: Balanomorpha) by nucleotide sequences of a mitochondrial gene. Benthos Res. 51(2):33–39.
Kim RO, Chan BKK, Hou BK, Ju SJ, Kim SJ. 2019. Complete mitochondrial genome of the deep-water epibiotic stalked barnacle, Glyptelasma annandalei (Cirripedia, Lepadiformes, Poecilasmatidae). Mitochondrial DNA Part B. 4(1):99–100.
Newman WA, Ross A. 1976. Revision of the balanomorph barnacles; including a catalogue of the species. Memoir 9, San Diego Society of Natural History. 1–108.
Rainbow PS, Phillips D. 1993. Cosmopolitan biomonitors of trace metals. Mar Pollut Bull. 26(11):593–601.
Shen X, Chan BKK, Tsang LM. 2015. The complete mitochondrial genome of common fouling barnacle Amphibalanus amphitrite (Darwin, 1854) (Sessilia: Balanidae) reveals gene rearrangements compared to pan-crustacean ground pattern. Mitochondrial DNA. 26(5):773–774.
Shen X, Chan BKK, Tsang LM. 2016. The mitochondrial genome of Nobia grandis Sowerby, 1839 (Cirripedia: Sessilia): the first report from the coral-inhabiting barnacles family Pyrgomatidae. Mitochondrial DNA A DNA Mapp Seq Anal. 27(1):339–341.
Shen X, Chu KH, Chan BKK, Tsang LM. 2016. The complete mitochondrial genome of the fire coral-inhabiting barnacle Megabalanus ajax (Sessilia: Balanidae); gene rearrangements and atypical gene content. Mitochondria DNA. 27(2):1173–1174.
Shen X, Tsang LM, Chu KH, Achituv Y, Chan B. 2015. Mitochondrial genome of the intertidal acorn barnacle Tetractita serrata Darwin, 1854 (Crustacea: Sessilia): gene order comparison and phylogenetic consideration within Sessilia. Mar Genomics. 22:63–69.
Shen X, Tsang LM, Chu KH, Chan B. 2017. A unique duplication of gene cluster (S2–C–Y) in Epopelia plicata (Crustacea) mitochondrial genome and phylogeny within Cirripedia. Mitochondrial DNA A DNA Mapp Seq Anal. 28(2):285–287.
Shen X, Tsoi KH, Cheang CC. 2016. The model barnacle Balanus balanus Linnaeus, 1758 (Crustacea: Maxillopoda: Sessilia) mitochondrial genome and gene rearrangements within the family Balanidae. Mitochondrial DNA A DNA Mapp Seq Anal. 27(3):2112–2114.
Song J, Shen X, Chu KH, Chan B. 2017. Mitochondrial genome of the acorn barnacle Tetractita rubintecta Pilsbry, 1916: highly conserved gene order in Tetractitaidae. Mitochondrial DNA Part B. 2(2):936–937.
Tsang LM, Chu KH, Achituv Y, Chan B. 2015. Molecular phylogeny of the acorn barnacle family Tetraclitidae (Cirripedia: Balanomorpha: Tetracitoidea): validity of shell morphology and arthropodal characteristics in the systematics of tetractid barnacles. Mol Phylogenet Evol. 82:324–329.
Wares JP. 2015. Mitochondrial evolution across lineages of the vampire barnacle Notochthamalus scabrosus. Mitochondrial DNA. 26(1):7–10.
Yamaguchi T. 1987. Changes in the barnacle fauna since the Miocene in Hong Kong coastal waters, using barnacles. Asian Mar Biol. 13:1–13.
Yan T, Li ZF, Hu YF, Li XX, Cao WH, Luo WJ, Cheng ZQ. 2012. [A review on the balanomorph barnacles in the coastal waters of China]. Acta Ecologica Sinica. 32(16):5230–5241. in Chinese with English abstract.