Phycologia Volume 55 (4), 371–377 Published 6 May 2016

Complete mitochondrial genome, genetic diversity and molecular phylogeny of *Gracilaria salicornia* (Rhodophyta: Gracilariaceae)

Sze-Looi Song[1*], Hoi-Sen Yong[1,2], Phaik-Eem Lim[3], Poh-Kheng Ng[2,3] and Siew-Moi Phang[3]

[1]*Chancellory High Impact Research, University of Malaya, 50603 Kuala Lumpur, Malaysia*
[2]*Institute of Biological Sciences, University of Malaya, 50603 Kuala Lumpur, Malaysia*
[3]*Institute of Ocean and Earth Sciences, University of Malaya, 50603 Kuala Lumpur, Malaysia*

Abstract: *Gracilaria salicornia* is an agarophyte that has been used as food and as a source of agar. We sequenced the complete mitogenome of the species from Malaysia and compared it with the previously described sequence from Hawaii. It had a total length of 25,915 bp, comprising 50 genes [25 protein-coding genes (PCGs), 2 rRNA genes and 23 tRNA genes]. It was larger than the Hawaiian sequence (25,272 bp) and possessed three additional tRNAs (*trnY*, *trnR*, *trnS1*). Both *G. salicornia* sequences had the same start and stop codons for all the PCGs. The genetic distance between the peninsular Malaysian and Hawaiian samples was 0.44% based on 19 PCGs, 0.15% based on 2 rRNA genes and 0.38% based on 19 PCGs + 2 rRNA genes. The phylogram of *G. salicornia* within Gracilariaceae based on 19 PCGs was congruent with that based on 19 PCGs + 2 rRNA genes.

Key Words: *Gracilaria salicornia*, Malaysia, Mitochondrial genome, Phylogeny, Rhodophyta

INTRODUCTION

The knobbly agar-agar seaweed *Gracilaria salicornia* (C.Agardh) E.Y.Dawson is a member of the Gracilariaceae (Dawson 1954). It is distributed throughout Asia and the Indian Ocean (Yang *et al*. 2013b). It is an alien invasive species in Hawaii (Fukunaga *et al*. 2014). This red alga has been used for food and as a source of agar (Huynh & Nguyen 1998; Lewmanomont 1998). The appreciable quantities of protein and crude fibre as well as low total lipid content make it a potential valuable food source (Tabarsa *et al*. 2012). In addition, the extract of *G. salicornia* possesses antioxidant activity and appreciable antimicrobial activity (Vijayavel & Martinez 2010; Rasooli *et al*. 2015).

The mitochondrial cytochrome *c* oxidase subunit I (*cox1*) gene has been applied for DNA barcoding of *G. salicornia* from Southeast Asia (Yang *et al*. 2013b). Random Amplified Polymorphic DNA analysis (RAPD) has been used to investigate molecular characteristics of two morphological variants of *G. salicornia* found in peninsular Malaysia (Lim *et al*. 2001). The population genetic structure of *G. salicornia* and its red algal parasite, *Gracilaria babae* (H.Yamamoto) P.K.Ng, P.E.Lim & S.M.Phang, in Malaysia was investigated using the plastid *rbcL*, mitochondrial *cox1* and nuclear internal transcribed spacer (ITS) sequences (Ng *et al*. 2015a).

The genus *Gracilaria* is represented globally by 183 species (currently accepted taxonomically, Guiry & Guiry 2015). To date, only three whole mitochondrial genomes (mitogenomes) for *Gracilaria* are available in the National Center for Biotechnology Information (NCBI) GenBank. The complete mitogenome of *G. salicornia* from Hawaii, USA, was studied based on the Illumina platform (Campbell *et al*. 2014). We report the complete mitogenome sequence of *G. salicornia* from peninsular Malaysia and consider its genetic diversity and phylogeny.

* Corresponding author (looi511@hotmail.com).
DOI: 10.2216/15-128.1

MATERIAL AND METHODS

Gracilaria salicornia was collected from Pantai Dickson, Negeri Sembilan, peninsular Malaysia on 6 April 2015. Samples were washed with seawater, followed by distilled water and ultra high quality water. Total DNA from a sporophytic specimen of *G. salicornia* was prepared using i-genomic Plant DNA Extraction Mini Kit (iNtRON Biotechnology Inc., South Korea) following the manufacturer's instructions with minor modification. Final DNA was eluted using elution buffer instead of Tris-EDTA buffer to avoid interference of EDTA with the enzyme. The specimen was confirmed to be *G. salicornia* based on ITS nucleotide sequencing.

The purified DNA was quantified using Qubit dsDNA HS Assay Kit (Life Technologies, Carlsbad, California) and normalized to 50 ng. A library was prepared using the Nextera DNA Sample Preparation Kit (Illumina, San Diego, California) following the manufacturer's protocols. Size estimation of the library was performed on a 2100 Bioanalyzer using the High Sensitivity DNA Analysis Kit (Agilent Technologies, Santa Clara, California), and the library was quantified with a Qubit 2.0 Fluorometer (Life Technologies). The library was normalized to 1.5 pM and sequenced using the NextSeq 500 Desktop Sequencer (2 × 150 bp paired-end reads) (Illumina).

Raw sequences were extracted from the Illumina NextSeq 500 system in FASTQ format, and the quality of sequences was evaluated using the FastQC software. The ambiguous nucleotides and reads with an average quality value (lower than Q20) were excluded from further analysis. *De novo* assembly was performed using the CLC Genomic Workbench v. 8.0.1 (Qiagen, Hilden, Germany). Contigs greater