

Assessing the use of mitochondrial *cox1* gene and *cox2-3* spacer for genetic diversity study of Malaysian *Gracilaria changii* (Gracilariaceae, Rhodophyta) from Peninsular Malaysia

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Abstract Advances in DNA-based genetic markers provide the essential tools in measurement of genetic diversity relating to the evolution, biogeography, and systematics of red algae by exploiting genetic variation in the entire genome of organisms. The understanding of genetic diversity in *Gracilaria changii* (Gracilariaceae, Rhodophyta) will provide valuable information for conservation, plant breeding management, and strain selection for cultivation. However, information of intraspecific genetic variation is still rudimentary. In this study, two mitochondrial encoded markers, cytochrome oxidase subunit 1 (*cox1*) and intergenic spacer between the cytochrome oxidase subunits 2 and 3 (*cox2-3* spacer) were used to investigate genetic diversity in 40 individuals of *G. changii* collected from 11 different geographically distinct populations from Peninsular Malaysia. Seven distinct mitochondrial haplotypes were identified with the *cox1* gene and three mitochondrial haplotypes with the *cox2-3* spacer. Intra-specific nucleotide differences ranged from 0 to 6 bp for the *cox1* and 0–4 bp for the *cox2-3* spacer, respectively. This is the first report comparing the suitability of mitochondrial markers of the *cox1* gene and the *cox2-3* spacer for genetic diversity studies on *G. changii*. The present study showed that the *cox1* gene is a potential molecular marker to infer intraspecific genetic variation in red macroalgae. The *cox1* marker is more variable compared to the *cox2-3* spacer and revealed genetic

variation and phylogeographic structure for this ecologically and economically important species.

Keywords *Gracilaria changii* · Rhodophyta · *cox1* gene · *cox2-3* spacer · Genetic diversity · Phylogeography

Introduction

The red algae of *Gracilaria changii* (Xia and Abbott) Abbott, Zhang and Xia is a potential agarophytic species for commercialization in Malaysia as it has high yields of good quality agar with high gel strength for production of food grade agar and agarose (Phang 1994; Phang et al. 1996). Due to potential commercial value in Malaysia, *G. changii* has been the subject of interest among researchers in various aspects: random amplified polymorphism DNA (RAPD) molecular markers for differentiation of different life stages (Sim et al. 2007), tissue and protoplast culture (Yeong et al. 2008), functional genomics (Teo et al. 2007; Ho et al. 2009; Teo et al. 2009; Siow et al. 2012), and genetic transformation (Gan et al. 2003).

Anthropogenic activities leading to pollution, coastal degradation, climate change, transoceanic introduction of alien species and invasive species, including natural seaweed populations, have impacted diversity of the seaweed genetic resources. In addition, ecological factors and natural evolutionary processes also influence a range of population, community, and ecosystem responses that may generate changes in allele sequences or genotype frequency in seaweeds (Fussmann et al. 2007). Regular sampling at various coastal areas in Peninsular Malaysia has indicated that the wild populations of *G. changii* are decreasing. To date, there is no commercial-scale cultivation of *G. changii* in Malaysia, except for experimental culture in a shrimp farm using the monofilament method (Phang 1998). Information on intraspecific genetic diversity of this economically important species is scarce (e.g. Yow et al.

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