

Genetic variation in *Gracilaria tenuistipitata* (Rhodophyta) from northern Singapore and neighbouring countries

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Abstract. *Gracilaria tenuistipitata* Chang et Xia is a commercially important red alga species and has gradually become a model species for cultivation due to its rapid growth and high agar yield. *G. tenuistipitata* is found in Singapore but no information regarding the genetic variation of this species is available. The present study examined the genetic variability of this species collected at various localities: three from Singapore, two from Peninsular Malaysia, and one each from Thailand and Vietnam, using the mitochondrial *cox1* gene and microsatellite markers. The SSR (simple sequence repeat) marker separated the Singapore specimens into two different genotypes with *G. tenuistipitata* from Ubin Island (Singapore), Pattani (Thailand) and Quy Kim (Vietnam) in one clade while *G. tenuistipitata* from Lim Chu Kang and Pasir Ris Park (Singapore) and Peninsular Malaysia formed another clade. The mitochondrial *cox1* gene analyses showed that *G. tenuistipitata* from Singapore were grouped together with specimens from Middle Banks (Malaysia) while specimens from Batu Laut (Malaysia), Pattani (Thailand) and Quy Kim (Vietnam) were grouped in three distinct clades. These results are congruent with the TCS analysis in which five mitochondrial haplotypes (T1–T5) were displayed but no genetic variation was observed for all the specimens from Singapore and Middle Banks. This study demonstrated the low genetic diversity of *G. tenuistipitata* from Singapore despite the higher variability of *cox1* gene over the microsatellite markers.

Key words. *cox1* gene, genetic variation, *Gracilaria tenuistipitata*, SSR markers

INTRODUCTION

The second largest genus amongst the red algae, *Gracilaria* species are distributed worldwide and cultivated for hydrocolloids in various countries including China, Vietnam, the Philippines, Indonesia and the Republic of Korea (McHugh, 2003). In Malaysia, several species of *Gracilaria* (e.g., *G. changii* and *G. tenuistipitata*) are consumed as salads as well as used for agar extraction (Phang, 2006). Agar has been extensively used in the food, cosmetic and pharmaceutical industries, as well as in microbiological research. *Gracilaria tenuistipitata* var. *liui* Zhang & Xia is consumed as a sea vegetable in the Philippines, and it is also intensively cultivated in China and Taiwan for food (Haglund & Pedersen, 1993; Tseng & Xia, 1999).

The high morphological plasticity of macroalgae can be detrimental to the proper identification of commercially important seaweeds such as *Gracilaria* (Yow et al., 2011), *Kappaphycus* (Tan et al., 2013) and *Sargassum* (Dixon & Huisman, 2010; Endo et al., 2013). Their morphology varies based on the adaptive response to changing environments. This can be problematic especially for species with high

economic value. For example, members of the family Gracilariaceae (Rhodophyta) are economically important for agar production and as abalone feed. However, their taxonomic and systematic positions are generally uncertain due to the lack of distinct morphological diagnostic characters and unreliable developmental characters of the female reproductive system (Wattier et al., 1997; Iyer et al., 2005). In a study by Iyer et al. (2005), small subunit rDNA and RuBisCO spacer sequences were employed to unravel the phylogenetic relationships of several members of the Gracilariaceae from South Africa. Their results showed that these markers were able to resolve the southern African gracilarioid complex into three species: *Gracilaria gracilis*, *Gracilariopsis longissima* and *Gracilariopsis funicularis* (Iyer et al., 2005). Hence, molecular information may be essential to distinguish between different species and to overcome the limits of morpho-species (John & Maggs, 1997). In addition, genetic analyses can reveal the extent of population structuring and give an indication of subgroup distinctiveness (Beebe & Rowe, 2008). To date, several genetic marker approaches have been used in the study of seaweeds, for example, restriction fragment length polymorphisms (RFLPs; Candia et al., 1999), random amplified polymorphic DNA (RAPD; Lim et al., 2004; Sim et al., 2007), and amplified fragment length polymorphisms (AFLPs; Niwa et al., 2004; Sun et al., 2005). However, there are always limitations arising from each technique.

Microsatellite markers, or simple sequence repeats (SSRs) are abundantly found in eukaryotic genomes and have high levels of polymorphism (Powell et al., 1996). These

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