Assessment of Four Molecular Markers as Potential DNA Barcodes for Red Algae *Kappaphycus* Doty and *Eucheuma* J. Agardh (Solieriaceae, Rhodophyta)

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**Abstract**

DNA barcoding has been a major advancement in the field of taxonomy, seeing much effort put into the barcoding of wide taxa of organisms, macro and microalgae included. The mitochondrial-encoded *cox1* and plastid-encoded *rbcL* has been proposed as potential DNA barcodes for rhodophytes, but are yet to be tested on the commercially important carrageenophytes *Kappaphycus* and *Eucheuma*. This study gauges the effectiveness of four markers, namely the mitochondrial *cox1*, *cox2*, *cox2-3* spacer and the plastid *rbcL* in DNA barcoding on selected *Kappaphycus* and *Eucheuma* from Southeast Asia. Marker assessments were performed using established distance and tree-based identification criteria from earlier studies. Barcoding patterns on a larger scale were simulated by empirically testing on the commonly used *cox2-3* spacer. The phylogeny of these rhodophytes was also briefly described. In this study, the *cox2* marker which satisfies the prerequisites of DNA barcodes was found to exhibit moderately high interspecific divergences with no intraspecific variations, thus a promising marker for the DNA barcoding of *Kappaphycus* and *Eucheuma*. However, the already extensively used *cox2-3* spacer was deemed to be in overall more appropriate as a DNA barcode for these two genera. On a wider scale, *cox1* and *rbcL* were still better DNA barcodes across the rhodophyte taxa when practicality and cost-efficiency were taken into account. The phylogeny of *Kappaphycus* and *Eucheuma* were generally similar to those earlier reported. Still, the application of DNA barcoding has demonstrated our relatively poor taxonomic comprehension of these seaweeds, thus suggesting more in-depth efforts in taxonomic restructuring as well as establishment.

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**Introduction**

The introduction of DNA barcodes by Herbert and co-workers [1,2,3] has brought about large impacts on the advancement of systematic biology; where short, easily amplified regions of DNA exhibiting large variation among species, yet sufficiently variable within species, are constantly used for species delineation, identification as well as archiving with reference to known, established species [4,5,6]. The Barcode of Life Data System (BOLD) is notably the largest initiative in establishing a worldwide DNA barcode library, signifying its importance and popularity for the scientific community [7,8,9]. The usefulness of DNA barcoding is evident when dealing with taxa displaying phenotypic plasticity throughout diphasic or triphasic life cycles as well as taxa involving cryptic species. These phenomena are generally predominant in marine macroalgae, thereby enticing the application of DNA barcoding, as reported in numerous studies encompassing the order Gelidiales [10], Gigartinales, [11,12,13], Gracilariales [14,15], Laminariales [16], and Fucales [17]. Studies on DNA barcoding over broader taxa of rhodophytes have also been reported with promising results [18,19,20].

The rhodophytes *Kappaphycus* and *Eucheuma*, commercially known as “cottonii” and “spinosum”, respectively are widely established as lucrative sources of carrageenan, with Indonesia and the Philippines being the largest carrageenophyte producers worldwide [21]. Despite being extensively farmed, the morphologically diverse nature of *Kappaphycus* and *Eucheuma* still poses difficulties in species identification [22,23,24,25,26,27,28], even leading to the cultivation of mixed populations that inevitably reduces overall yield [29]. These have resulted in the subsequent employment of molecular phylogenetic studies which all share one main objective – to infer and understand the phylogenetic relationships between *Kappaphycus* and *Eucheuma* congeners. As of now, various molecular markers have been introduced for the molecular taxonomy of these carrageenophytes, namely the mitochondrial-encoded partial *cox1* and *cox2-3* spacer, nuclear-encoded ribosomal Internal Transcribed Spacer (ITS) and 28S large subunit (LSU), plastid-encoded *rbcL*, RuBisCO spacer and the 23S Universal Plastid Amplicon (UPA) [23,28,30,31]. However, the suitability of these genetic markers as DNA barcodes are to date, unassessed.