



## Assessment of nine markers for phylogeny, species and haplotype identification of *Kappaphycus* species and *Euचेuma denticulatum* (Solieriaceae, Rhodophyta)

Ji Tan <sup>a</sup>, Pui-Ling Tan <sup>b</sup>, Sze-Wan Poong <sup>b</sup>, Janina Brakel <sup>c,d,e</sup>, Cecilia Rad Menendez <sup>c,f</sup>, Eka Sunarwidhi Prasedya<sup>g,h</sup>, Alison R. Sherwood <sup>i</sup>, Flower E. Msuya <sup>j</sup>, Claire Gachon <sup>c,k</sup>, Juliet Brodie <sup>l</sup>, Azhar Kassim<sup>m</sup> and Phaik-Eem Lim <sup>b</sup>

<sup>a</sup>Department of Agricultural and Food Science, Faculty of Science, Universiti Tunku Abdul Rahman, Kampar 31900, Malaysia; <sup>b</sup>Institute of Ocean and Earth Sciences, Universiti Malaya, Kuala Lumpur 50603, Malaysia; <sup>c</sup>Scottish Association for Marine Science, Scottish Marine Institute, Oban PA37 1QA, UK; <sup>d</sup>Helmholtz Institute for Functional Marine Biodiversity (HIFMB), University of Oldenburg Germany, Ammerländer Heerstrasse 231, Oldenburg 26129, Germany; <sup>e</sup>Alfred-Wegener-Institute, Helmholtz Center for Polar and Marine Research, Am Handelshafen 12, Bremerhaven 27570, Germany; <sup>f</sup>Culture Collection of Algae and Protozoa, Scottish Marine Institute, Oban PA37 1QA, UK; <sup>g</sup>Bioscience and Biotechnology Research Centre, Faculty of Mathematics and Natural Sciences, University of Mataram, Mataram 83115, Indonesia; <sup>h</sup>Department of Biology, Faculty of Mathematics and Natural Sciences, University of Mataram 83115, Indonesia; <sup>i</sup>School of Life Sciences, University of Hawaii, 3190 Maile Way, Honolulu, HI, USA; <sup>j</sup>Zanzibar Seaweed Cluster Initiative, Zanzibar 3794, Tanzania; <sup>k</sup>Unité Molécules de Communication et Adaptation des Micro-organismes, UMR 7245, Muséum National d'Histoire Naturelle, CNRS, Paris 75005, France; <sup>l</sup>Research, Natural History Museum, London SW7 5BD, UK; <sup>m</sup>Department of Fisheries Sabah, Ministry of Agriculture, Fisheries and Food Industries Sabah, Kota Kinabalu 88624, Malaysia

### ABSTRACT

Molecular studies have contributed to the taxonomy of carrageenan-producing *Kappaphycus* spp. and *Euचेuma denticulatum*. However, unresolved species complexes and the lack of standardization in the use of genetic markers impede the identification of specimens and the delineation of a robust taxonomic framework. Here, nine molecular markers (*cox1*, *cox2*–3 spacer, *cox2*, *cox3*, COB, ITS, *psbA*, UPA and *rbcl*) were used to generate a multilocus phylogeny for 113 fresh euचेumatoid samples and four herbarium specimens. Analyses of species delineation and genetic distances confirmed the monophyly of currently accepted taxa. These analyses suggest that clades previously reported as *K. striatus* KS1 and KS2 are conspecific, and that *E. denticulatum* EDA 'spinosum' and EDB 'endong/cacing' are also conspecific. The results also unveiled possible new taxa from Hawaii and Indonesia. Each molecular marker and combinations thereof were assessed with regard to species identification, ease of amplification and sequencing, and haplotype characterization. All genetic markers recorded at least 94% success in the amplification and sequencing of fresh specimens, with *cox1* being the most phylogenetically informative. Automatic partitioning, phylogenetic and tree-based assessments showed *cox1*, *cox2*–3 spacer, *cox2* and *rbcl* were able to correctly identify species while *cox1*+*rbcl*, COB+*rbcl*, *cox2*+*rbcl* or *cox1*+COB+*rbcl* trees best represented the phylogeny with consistently high nodal support. Among individual markers, *cox1* identified the greatest number of haplotypes, while UPA, partial *rbcl* (750 bp), ITS, *cox3* and *cox2*–3 spacer were able to retrieve information from herbarium specimens of 12–16 years of age. These molecular results provide a basis for a database essential for the taxonomic framework, cultivar development and germplasm conservation of euचेumatoids.

### HIGHLIGHTS

- Mitochondria *cox1*, *cox2*–3 spacer, *cox2* and plastid *rbcl* can be used for species identification and *cox1* for haplotype detection of euचेumatoids.
- *cox1*+*rbcl*, COB+*rbcl*, *cox2*+*rbcl* or *cox1*+COB+*rbcl* are the most cost-effective molecular markers for phylogenetic inference.
- The most comprehensive up to date multilocus phylogeny of *Kappaphycus* spp. and *Euचेuma denticulatum* is presented.

**ARTICLE HISTORY** Received 29 January 2024; Revised 10 August 2024; Accepted 19 August 2024

**KEYWORDS** Euचेumatoids; herbarium; molecular markers; multilocus phylogeny; taxonomy

### Introduction

The use of genetic markers has enabled rapid developments in phycological research related to taxonomy, evolutionary history, population genetics, conservation, DNA barcoding, genetic selection and breeding (Bartolo *et al.*, 2020; McCoy *et al.*, 2020; Brakel *et al.*, 2021). Genetic resources from these studies are invaluable for germplasm conservation and strain improvement, especially in the face of challenges such as climate change, ocean acidification, anthropogenic pollution as well as pests and diseases (Ward *et al.*, 2020, 2022; Yang *et al.*,

2021). Whilst studies that characterize and evaluate genetic resources are more prevalent in the brown algae, e.g. *Undaria pinnatifida* (Avia *et al.*, 2017; Brakel *et al.*, 2021; Shan & Pang, 2021), they are gaining momentum in the red algae (Yang *et al.*, 2016; van Beveren *et al.*, 2022; Borg *et al.*, 2023).

The euचेumatoids *Kappaphycus* and *Euचेuma denticulatum* are commercially important red seaweeds that produce carrageenan, a valuable phycocolloid increasingly used in the food and cosmetic industries (Campo *et al.*, 2009). These seaweeds are mostly cultivated in

**CONTACT** Phaik-Eem Lim phaikem@um.edu.my; Sze-Wan Poong szewan@um.edu.my

© 2024 British Phycological Society