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Systematics and genetic variation in commercial *Kappaphycus* and *Eucheuma* (Solieriaceae, Rhodophyta)

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Abstract

The systematics and taxonomy of *Kappaphycus* and *Eucheuma* (Solieriaceae) is confused and difficult due to morphological plasticity, lack of adequate characters to identify species and commercial names of convenience. These taxa are geographically widely dispersed through cultivation. Commercial, wild and herbarium sources were analysed; molecular markers provided insights into taxonomy and genetic variation, and where sources of genetic variation may be located. The mitochondrial *cox*2-3 and plastidal RuBisCo spacers were sequenced. There is a clear genetic distinction between *K. alvarezii* ("cottonii") and *K. striatum* ("sacol") samples. *Kappaphycus alvarezii* from Hawaii and some samples from Africa are also genetically distinct. Our data also show that all currently cultivated *K. alvarezii* from all over the world have a similar mitochondrial haplotype. Within *Eucheuma denticulatum* ("spinosum") most African samples are again genetically distinct. Our data also suggest that currently cultivated *E. denticulatum* may have been "domesticated" several times, whereas this is not evident for the cultivated *K. alvarezii*. The present markers used do not distinguish all the morpho-types known in cultivation (e.g. var. *tambalang*, "giant" type) but do suggest that these markers may be useful to assess introductions and species identification in samples.

Introduction

The seaweeds most commonly cultivated for the carrageenan industry belong to the genera *Kappaphycus* Doty and *Eucheuma* J. Agardh. These crops are almost entirely farmed and are usually referred to by the commercial names "cottonii", "sacol" and "spinosum". The formal taxonomy of these taxa has for a long time been in confusion due to misapplication of commercial and scientific names, the known general paucity of adequate morphological characters and the morphological plasticity of seaweeds. Much of the taxonomic confusion was addressed by the pioneering work of Maxwell Doty (Doty, 1985, 1988; Doty & Norris, 1985). Even in the detailed work of Doty, variability in the presence or absence of diagnostic morphological characters within taxa was noted, especially in non-ideal specimens (i.e. non-reproductive specimens and specimens lacking typical attachment structures) and this was addressed by the caveat that descriptive paragraphs must carry the preamble "there is a tendency. . ." (Doty, 1988, p. 166).

Doty (1988), however, formally recognized certain species of *Eucheuma* as *Kappaphycus*, mostly based on their production of κ-carrageenan, and this generic circumscription has been supported, for the most part, by molecular studies (Fredericq et al., 1999; Aguilan et al., 2003). Nevertheless, questions remain as to the taxonomic identity of commercially produced strains. *Kappaphycus alvarezii* (Doty) Doty *ex* P. Silva is the most-grown commercial κ-carrageenan producer and many varieties and local strains are known (www.surialink.com). One of the commercially used 'strains' of *Kappaphycus* is the so-called "sacol"

variety, but its scientific name is still unresolved. While it was originally considered to be *K. striatum* (Schmitz) Doty *ex* P. Silva (Trono, 1997), recent molecular investigations suggested that it could be a form of *K. cottonii* (Weber-van Bosse) Doty *ex* P. Silva (Aguilan et al., 2003). *Kappaphycus cottonii* is morphologically quite distinct from either *K. alvarezii* or *K. striatum* as it mostly forms prostrate branches. Culture studies have shown that many of the characters used to separate *Kappaphycus* species (e.g. habit, decumbent versus dichotomous) are found to segregate in tetraspore progeny (de Paula et al., 1999) from single plants, and it is likely that the identification of individual specimens based on morphology is unreliable.

Molecular markers have proven useful in not only elucidating red algal systematics but also in discovering genetic variation within red algal species. Commonly used intraspecific markers are the nuclear-encoded internal transcribed spacers of the ribosomal cistrons (ITS 1 and 2, e.g. Marston & Villalard-Bohnsack, 2002), the plastid-encoded RuBisCo spacer (e.g. Zuccarello et al., 2002) and the mitochondrial-encoded *cox2-3* spacer (Zuccarello & West, 2003), although these markers have their limitations, such as uniparental inheritance and limited variation (i.e. they do not reflect all the genetic variation found within groups). Studies using the RuBisCo spacer have shown that even single base pair changes could indicate reproductively isolated cryptic species (Zuccarello & West, 2003), while there is more variation within species at the *cox2-3* spacer region, due to its higher mutation rate (Zuccarello & West, 2002).

This work aimed to: (1) determine the levels of genetic variation in commercially grown species of *Kappaphycus* and *Eucheuma*; (2) clarify some of the taxonomic confusion in commercial strains and wild strains of *Kappaphycus* and *Eucheuma*; (3) determine which geographic regions contain samples with ecologically superior genotypes or with genetic variation that is potentially useful to the industry.

Materials and methods

Samples were for the most part obtained from commercial supplies. Thalli were selected from the corners of representative bales delivered from suppliers for industrial extraction of carrageenan. Samples were placed in silica gel until DNA extraction. Although drying and storage methods may have differed, nearly all samples were adequate for DNA extraction and amplification. Often exact provenance (specific region, farm) of the samples was unknown. Other samples were collected (Hawaii and Indonesia) and dried immediately in silica gel. Hawaiian samples collected at Kane'ohe Bay spanned the range of morphologies at this site where material is introduced and invasive, and contained many cystocarpic or tetrasporic specimens. Other samples were removed from herbarium sheets and processed as below. All samples used are listed in Table 1.

DNA extractions were performed using a modified CTAB extraction procedure. Dried samples (approx. 1 cm tip) were pulverized using a shaking mill (Retsch, type MM200) and then placed in a microfuge tube containing 500 μL of CTAB extraction buffer (2% CTAB, 0.1 M Tris-HCl (pH 8.0), 1.4 M NaCl, 20 mM EDTA, 1% PEG 8000) plus 50μg RNAse A and 80 g Proteinase K (Promega, Madison, USA). Samples were then placed at 60°C for 30 min, mixing occasionally. Two extractions using an equal volume of chloroform:isoamyl alcohol (24:1), mixing, and spinning for 10 min at 12,000 g were preformed. DNA was precipitated with an equal volume of 100% isopropanol, the tube inverted and placed at room temperature for 30 min. The sample was spun for 30 min at 12,000 g and decanted and then washed in 70% ethanol, air-dried and 50 μL of 0.1 X TE buffer was added.

Amplification of the plastid-encoded RuBisCo spacer followed procedures outlined in Zuccarello et al. (1999b). Amplification of the mitochondrial-encoded *cox2-3* spacer and sequencing followed procedures outlined in Zuccarello et al. (1999a). Sequences were aligned by eye. Phylogenetic relationships were inferred with PAUP*4.0b10 (Swofford, 2002). Data sets from different genomic regions were tested for incongruence using the partition homogeneity test (PHT) (Farris et al., 1994) as implemented in PAUP* (1000 replicates, 5 random additions, 100 trees per addition saved). A combined dataset was subjected to maximum-parsimony (MP) analysis, using the heuristic search option, 500 random sequence additions, 100 trees per addition saved, TBR branch swapping, unordered and unweighted characters, and gaps treated as missing data. The program Modeltest version 3.06 (Posada & Crandall, 1998) was used to find the model of sequence evolution that best fits each data set by a hierarchical likelihood ratio test ($\alpha = 0.01$). When the best sequence evolution model had been determined, maximum-likelihood searches were performed in PAUP* using the estimated parameters (substitution model, gamma distribution, proportion of invariable