Phytotaxa 109 (1): 17–26 (2013)
www.mapress.com/phytotaxa/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

http://dx.doi.org/10.11646/phytotaxa.109.1.2

Molecular identification of the exotic lineage of *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae) cultivated in the tropical region of Brazil

PATRÍCIA G. ARAÚJO[1], GEORGE E. C. MIRANDA[2], MARIA B. BARROS-BARRETO[3] & MUTUE T. FUJII[4*]

[1]*Post-Graduate Program in Oceanography, Departamento de Oceanografia, Universidade Federal de Pernambuco. Av. Prof. Moraes Rego, 1235, 50670-901 Cidade Universitária, Recife, PE, Brazil.*

[2]*Departamento de Sistemática e Ecologia, Universidade Federal da Paraíba, Campus I, 58059-900 João Pessoa, PB, Brazil.*

[3]*Departamento de Botânica, Universidade Federal do Rio de Janeiro, Av. Prof. Rodolpho Rocco 211, CCS, bloco A, subsolo, sala 99, 21941-902 Rio de Janeiro, RJ, Brazil.*

[4]*Núcleo de Pesquisa em Ficologia, Instituto de Botânica, Av. Miguel Estéfano, 3687, 04301–902 São Paulo, SP, Brazil.*

[*]*Corresponding author: mutue.fujii@pq.cnpq.br. Phone: + 55 11 50676123. Fax: + 55 11 50733678*

Abstract

Kappa carrageenan is an important phycocolloid extracted from the red seaweed *Kappaphycus alvarezii*. This species has been introduced into several regions, most recently in Brazil. The remote invasive potential of this seaweed in both south and southeast waters of Brazil (subtropical regions) has been established, but introduction in the northeastern tropical area is contested due to the absence of biological information. An unknown exotic lineage of *K. alvarezii* has been illegally introduced and cultivated in an area characterized by the presence of coral reefs in Paraíba, the northeastern Brazilian coast, since the beginning of the last decade. This work focuses on the molecular identification of these samples in comparison with sequences of other strains of *K. alvarezii* and congeners available in Genbank. Maximum likelihood (ML) and Bayesian inference (BI) analyses showed strong similarities between *K. alvarezii* cultivated in the tropical waters in Brazil and those lineages cultivated in Hawaii, Venezuela, Malaysia and Tanzania that have never shown invasive behavior. However, more detailed investigations and use of environmental monitoring are recommended before commercial cultivation of this species can be authorized in tropical region of Brazil.

Key words: bioinvasive potential, carrageenophyte, *cox*2-3 spacer, cultivation

Introduction

Seaweed farming has an important role in the development of sustainable mariculture and represents an alternative livelihood for fishers (Ask & Azanza 2002, Oliveira & Paula 2003, Bindu & Levine 2011, Bixer & Porse 2011). The worldwide production of economically important macroalgae has increased significantly in recent decades, from 3.8 million tons in 1990 to 19 million tons in 2010; however, in 2008, the total value of farmed algae was ca. of US$ 4.4 billion, and its value increased to US$ 5.7 billion in 2010 (FAO 2012). Among these seaweeds, the genera *Kappaphycus* M.S.Doty in Abbott (1988: 171) and *Eucheuma* J. Agardh (1847: 16) (Rhodophyta, Solieriaceae), which are also known as eucheumatoid algae, have been cultivated worldwide as the primary source of carrageenan (Doty 1985, Areces 1995, Ask *et al*. 2003, Hayashi *et al.* 2010). *Kappaphycus alvarezii* (M.S. Doty 1985: 37) M.S. Doty ex P.C. Silva in Silva *et al.* (1996: 333) is the most cultivated seaweed in the world (Ask & Azanza 2002, Aguilan *et al.* 2003, Hayashi *et al.* 2007a, 2010, Bindu & Levine 2011, Bixler & Porse 2011). The cultivation of *K. alvarezii* began in the 1970s in the Philippines, and currently it has been introduced to more than 20 countries for mariculture purposes (Doty & Alvarez 1975, Doty 1985, Areces 1995, Ask & Azanza 2002, Ask *et al.* 2003).

Accepted by Saúl Blanco Lanza: 17 May 2013; published online in PDF: 10 June 2013

In Brazil, *Kappaphycus alvarezii* was introduced in 1995 when it was brought to São Paulo, southeast region, from the Usa Marine Biological Institute, Kochi University, Japan. All programs involved in the evaluation of the economic feasibility and the environmental risks of introducing *K. alvarezii* for the mariculture were authorized by the Governmental Agency *Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis* (IBAMA) (Paula *et al.* 1999). Experimental studies have focused on the introduction of the species and the resulting environmental risks in southeast and south Brazil (Paula *et al.* 1999, 2002, Bulboa & Paula 2005, Bulboa *et al*. 2008, Castelar *et al*. 2009, Góes & Reis 2011, 2012, Hayashi *et al.* 2011). Thus, following 18 years of research, the results showed favorable characteristics for commercial cultivation such as productivity and remote potential invasion (Paula *et al.* 1999, 2002, Bulboa & Paula 2005, Bulboa *et al*. 2008, Hayashi *et al.* 2007a, b, 2010, 2011, Castelar *et al.* 2009, Góes & Reis 2011, 2012). Based on these results, the cultivation of *K. alvarezii* in limited area of southeast Brazil was authorized by IBAMA through the Normative Instruction 185/2008. Currently, commercial scale production of *K. alvarezii* occurs only along the coast of Rio de Janeiro, a subtropical region of Brazil (Castelar *et al.* 2009, Góes & Reis 2011, 2012).

In the northeastern Brazilian coast, some seaweed species harvested from the native population are exploited commercially by fishing communities (Oliveira & Miranda 1998, Bezerra & Marinho-Soriano 2010). The seaweed species of greatest interest are *Gracilaria birdiae* E.M. Plastino & E.C. Oliveira (2002: 390) and *Hydropuntia caudata* (J. Agardh 1852: 598) C.F.D. Gurgel & S. Fredericq (2004: 155), and consequently, the natural populations of these species are reduced to a few individuals who have lost the ability to recover (Miranda *et al*. 2012).

In the state of Paraíba, the cultivation initiatives of *Gracilaria birdiae* and *Hydropuntia caudata* have been developed since 1983 (Oliveira & Miranda 1998). However, native seaweed production has not been economically attractive due to low growth rates of the algae and the difficulty to find seedlings. In the early 2000s, *K. alvarezii* was irregularly introduced for mariculture purposes in this area only once, and since then the seaweeds are been cultivated illegally at small scale by local fishermen (Lucena *et al*. 2007). The introduction started up with approximately 100 kg of the seaweeds without quarantine procedure and environmental monitoring (pers. comm. Marivaldo Bastos, local fisherman). The exact origin of this material is unknown.

It is known that the introduction of exotic species can cause serious environmental damage, including the loss of native biodiversity (Schaffelke *et al.* 2006, Anderson 2007, Pickering *et al.* 2007). *Kappaphycus* species such as *K. alvarezii* and *K. striatum* (F. Schmitz 1895: 151, 152) M.S. Doty ex P.C. Silva in Silva *et al.* (1996: 334) and *Eucheuma* spp. have been reported in bioinvasion events in Hawaii (Smith *et al*. 2002, Conklin & Smith 2005), India (Bagla 2008, Chandrasekaran *et al.* 2008, Kamalakannan *et al*. 2010) and Venezuela (Barrios 2005, Barrios *et al.* 2007). In addition, recently Halling *et al.* (2013) observed *K. alvarezii*, which was introduced from Asia for mariculture purposes, growing on coral reefs in Zanzibar.

The taxonomy of eucheumatoid species is difficult due to strong morphological plasticity, causing the misapplication of commercial and scientific names (Doty 1985, 1988). Zuccarello *et al.* (2006), Conklin *et al.* (2009) and Tan *et al.* (2012) have highlighted the need for more detailed studies to confirm the identity of target seaweeds and to access genotype information before seaweed strains can be used for mariculture purposes. Molecular approaches have been widely used to certify the identification of strains with high commercial value and low bioinvasion potential. Molecular markers such as the mitochondrial-encoded *cox*1 gene, a portion of the nuclear 28S rRNA gene (LSU), the small subunit rRNA gene (SSU), the Universal Plastid Amplicon (UPA) and the large subunit of the chloroplast-encoded gene (*rbc*L) are more suitable for identification and phylogenetic analysis (Lluisma & Ragen 1995, Fredericq *et al.* 1999, Conklin *et al.* 2009, Tan *et al*. 2012), whereas for inter- and intra-specific analysis, the mitochondrial segments of the *cox*2-3 spacer, the plastidial *rbc*L-*rbc*S spacer, and the nuclear-encoded ribosomal internal transcribed spacer (ITS) are more efficient due to the higher rate of change (Zuccarello *et al*. 2006, Conklin *et al*. 2009, Zhao & He 2011, Tan *et al.* 2012, 2013, Halling *et al.* 2013).

The northeastern region of Brazil is a priority area for conservation worldwide due to the presence of coral reefs. However, the demand for production of *Kappaphycus alvarezii* in Paraíba has superseded the need for environmental monitoring actions and conservation. Therefore, it is necessary to determine the biological and ecological aspects of this species to evaluate the environmental risks of its introduction into the tropical region. The present study aimed to identify the lineage of *K. alvarezii* introduced into the northeastern coast of Brazil using molecular markers, and comparing its sequences with others cultivated worldwide.

Materials and methods

Kappaphycus alvarezii samples were collected in October 2010 in Pitimbu beach, Paraíba, the tropical region of Brazil (07° 30' 10.5" S, 34°47' 53.5" W) (Fig. 1), as floating seaweeds, which were produced by cultivation in the same region. Nine drift individuals were collected along the beach near the raft cultivation: three individuals of each pigment variant: red, green, and brown. The voucher specimens were deposited in the Herbarium of the Instituto de Botânica, São Paulo, Brazil (SP 400964).

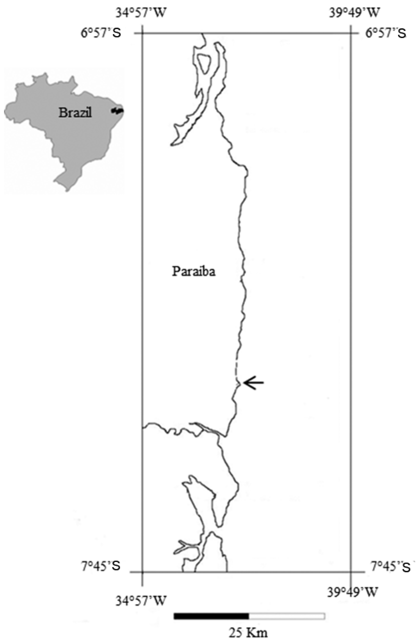

FIGURE 1. Map showing Paraíba State, northeastern Brazilian coast and *Kappaphycus alvarezii* collection site (arrow).

Molecular analyses: DNA was extracted from approximately 40 mg of dried samples processed in the Precellys homogenizer using the DNeasy@ Plant Mini Kit (Qiagen, Valencia, EUA). Polymerase chain reaction (PCR) was performed using the mitochondrial *cox*2-3 spacer. The primer sequence was the same as that used by Zuccarello *et al.* (1999), and the products were analyzed by electrophoresis in 0.7% agarose to confirm the product size. The PCR products were purified with MicroSpinTM S-300 HR columns (GE Healthcare Life Sciences, Piscataway, USA). Sequencing was performed with the BigDye Terminator Cycle Sequencing Reaction Kit (Applied Biosystems, NJ, USA) on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). The primers used for sequencing were the same as the used for PCR amplification.

Phylogenetic analyses: all sequences were aligned using BioEdit 7.1.3.0 (Hall 1999) and deposited in the NCBI Genbank, and *Kappaphycus* and *Eucheuma* sequences from other sites were selected for comparison with the sequences from this study (Table 1).

TABLE 1. Samples used in molecular analysis.

Genbank accession n◦	Species name	Locality	Reference
KC122263	*Kappaphycus alvarezii* (IBT 708)	Paraíba, Brazil	This study
KC122262	*K. alvarezii* (IBT 713)	Paraíba, Brazil	This study
KC122264	*K. alvarezii* (IBT 729)	Paraíba, Brazil	This study
JQ713902	*K. alvarezii*	Tanzania	Halling *et al.*(2013)
FJ554862	*K. alvarezii*	Hawaii	Conklin *et al.*(2009)
AY687436	*K. alvarezii*	Zanzibar	Zuccarello *et al.*(2006)
AY687432	*K. alvarezii*	Hawaii	Zuccarello *et al.* (2006)
AY687433	*K. alvarezii*	Hawaii	Zuccarello *et al.* (2006)
AY687427	*K. alvarezii*	Venezuela	Zuccarello *et al.* (2006)
AY687430	*K. alvarezii*	Madagascar	Zuccarello *et al.* (2006)
JN234759	*K alvarezii*	Malaysia	Unpublished
JN234760	*K alvarezii*	Malaysia	Unpublished
JN234761	*K alvarezii*	Malaysia	Unpublished
FJ554860	*K. striatum*	Hawaii	Conklin *et al.* (2009)
AY687431	*K. striatum*	Indonesia	Zuccarello *et al.* (2006)
AY687435	*K. striatum*	Indonesia	Zuccarello *et al.* (2006)
AY687434	*K. striatum*	Philippines	Zuccarello *et al.* (2006)
JN234763	*K. striatum*	Malaysia	Unpublished
JN234764	*K. striatum*	Malaysia	Unpublished
AY687426	*K. cottonii*	Philippines	Zuccarello *et al.* (2006)
FJ554861	*Kappaphycus* sp.	Hawaii	Conklin *et al*. (2009)
JQ713903	*Eucheuma denticulatum* (N.L. Burman 1768: 28) F.S. Collins & Hervey (1917: 106, 108)	Tanzania	Halling *et al*. (2013)
FJ554859	*E. denticulatum*	Hawaii	Conklin *et al.* (2009)
FJ561733	*E. denticulatum*	Hawaii	Conklin *et al.* (2009)
JN234756	*E. denticulatum*	Malaysia	Unpublished
AY687437	*E. denticulatum*	Indonesia	Zuccarello *et al.* (2006)
AY687425	*Eucheuma* sp.	Hawaii	Zuccarello *et al.* (2006)
AY687424	*Eucheuma* sp.	Tanzania	Zuccarello *et al.* (2006)
AY687423	*E. platycladum* (F. Schmitz 1895: 139, 152-153)	Tanzania	Zuccarello *et al.* (2006)
AY687422	*E. platycladum*	Kenya	Zuccarello *et al.* (2006)
AY687428	*E. denticulatum*	Madagascar	Zuccarello *et al.* (2006)
AY687421	*E. isiforme* (C. Agardh 1822: 271) J. Agardh (1847: 16)	Florida	Zuccarello *et al.* (2006)
AY687420	*E. isiforme*	Florida	Zuccarello *et al.* (2006)
AY687417	*Betaphycus philippinensis*	Philippines	Zuccarello *et al.* (2006)

Phylogenetic relationships were inferred with the Maximum likelihood (ML) method using MEGA 5.05 (Tamura *et al.* 2011). Gaps or missing data were treated as complete deletions, and bootstrap was performed with 1,000 replicates. MrBayes v.3.0 beta 4 (Huelsenbeck & Ronquist 2001) was used to calculate the Bayesian inference (BI). The evolution model was selected based on an ML ratio test with MEGA 5.05 using the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC). The evolution model used was the general-time-reversible model of nucleotide substitution with invariant sites and gamma distributed rates for variable sites (GTR+I+G) (Tamura *et al.* 2011). BI analysis was performed using the Markov chain Monte Carlo method, sampling one tree every 10 generations for 1,000,000 generations, starting with a random tree. *Betaphycus philippinensis* M.S. Doty (1995: 237) was used as an outgroup sample.

Results

The final sequences including the *cox*2–3 spacer contained 398–399 nucleotides. The phylogenetic tree topologies of the Maximum likelihood (ML) and the Bayesian inference (BI) methods were not identical; however, in both analyses *Kappaphycus* and *Eucheuma* samples formed distinct clusters (Fig. 2).

The *Kappaphycus* samples were included in distinct clades with *K. cottonii* (A. Weber-van Bosse 1913: 115) M.S. Doty ex P.C. Silva in Silva *et al.* (1996: 333) from the Philippines the most distinct. Other samples formed three main clades: *K. alvarezii* (A1 and A2), *Kappaphycus* spp. from Hawaii (B) and *K. striatum* (C), with high support (ML = 99%) (Fig. 2).

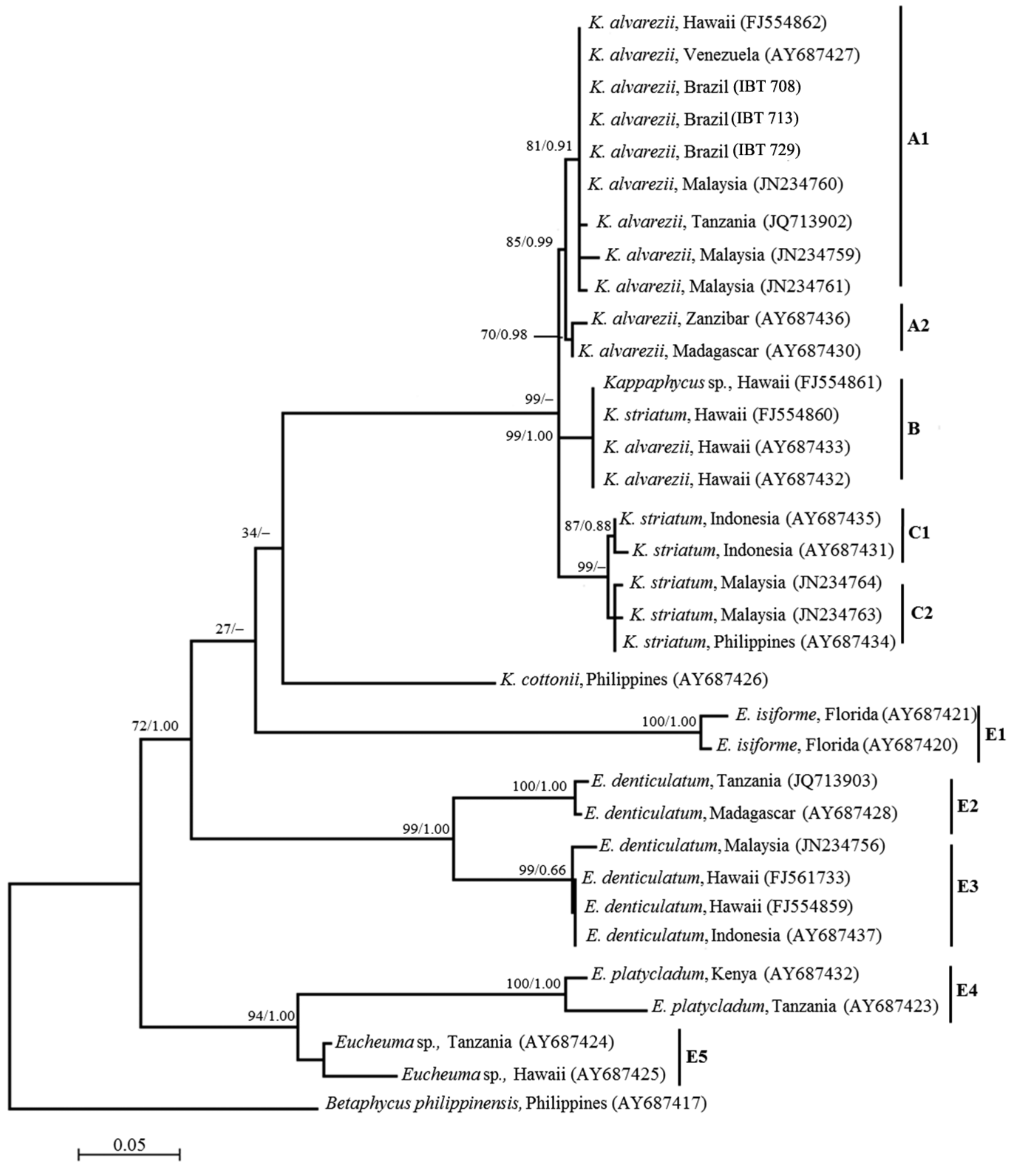

FIGURE 2. Maximum likelihood analyses of the *cox*2-3 spacer sequences based on GTR+I+G model for *Kappaphycus alvarezii* samples from northeastern Brazilian coast and sequences from Genbank accessions. The scale bar indicates substitutions per site.

The sequences of *Kappaphycus alvarezii* from northeastern Brazil grouped with those samples cultivated in Hawaii, Venezuela, Tanzania and Malaysia, forming subclade A1 with moderate support (ML = 81%, BI = 0.91). The sequence from Zanzibar and Madagascar grouped as subclade A2 with moderate support (ML = 70%, BI = 0.98).

The *Kappaphycus* spp. from Hawaii formed distinct clade (B) with high support (ML = 99%, BI = 1.00), confirming the phylogenetic relationship reported by Zuccarello *et al.* (2006) and Conklin *et al.* (2009). *Kappaphycus striatum* was divided in two clades (C1 and C2) with high support (ML = 99%). The clade C1 included species from Indonesia, and clade C2 those from Malaysia and the Philippines.

Additionally, the species of *Eucheuma* were distributed into five clades, forming distinct groups according to the species.

Discussion

Our results showed that the northeastern Brazilian specimens of *Kappaphycus alvarezii* is part of the worldwide cultivated subclade A strain, as predicted by Zuccarello *et al.* (2006) and Conklin *et al.* (2009). These strains have been considered having low invasion potential, since they are biologically limited because they were not found to produce viable spores, while it could reproduce by vegetative fragmentation (Zuccarello *et al.* 2006, Conklin *et al.* 2009). In southeast region of Brazil, Paula *et al.* (1999) observed tetraspore production from *K. alvarezii* in laboratory experiments only once. Although tetraspores of *K. alvarezii* germinated, they had low survival rates, suggesting that the strain studied is almost sterile (Bulboa & Paula 2005, Bulboa *et al.* 2008). According to Paula *et al.* (1999) the high mortality of tetraspore progeny of *K. alvarezii* cultivated in southeastern Brazil could be a consequence of their hybrid origin or clonal propagation, which may cause a reduced reproductive potential and abortive spore formation.

Previous studies demonstrated that *K. alvarezii* has remote invasion potential in southern and southeastern Brazil (Paula *et al.* 1999, 2002, Bulboa & Paula 2005, Bulboa *et al.* 2008, Castelar *et al.* 2009, Hayashi *et al.* 2011). Reproductive structures in cultivated *K. alvarezii* are rare (Doty 1985, Areces 1995, Castelar *et al*. 2009; Hayashi *et al*. 2011), and even the spores produced in the laboratory are not all viable (Bulboa *et al.* 2008). Moreover, in the field, the drift algae fragments are most likely affected by herbivory or are killed by environmental factors such as temperature and water transparency (Bulboa & Paula 2005, Castelar *et al.* 2009, Hayashi *et al.* 2011). However, these results are not applicable for northeastern Brazilian coast, which has different environmental conditions, such as water temperature, transparency and nutrients (Castelar *et al.* 2009), as well as a reef formation. Therefore, further studies are desirable to evaluate the potential risk of invasion according to local environmental conditions and to highlight the need for monitoring programs in these regions.

Furthermore, our samples had a close relationship with those cultivated lineages from Hawaii, Venezuela, Malaysia, and Tanzania (A1), suggesting the similarity of these strains. This information can support the identification of the origin for *K. alvarezii* strains introduced and cultivated in northeastern Brazil. Moreover, the accurate identification of this strain will help us to support the future management and monitoring program, including ecological studies as well as improving the seaweed farming.

The phylogenetic analysis indicated that the subclades A1 and A2 corresponded to the cultivated species of *Kappaphycus* (Zuccarello *et al.* 2006, Conklin *et al*. 2009, Zhao & He 2011, Halling *et al.* 2013, Tan *et al.* 2012, 2013) where our samples were assembled. The clade B was formed by a different *Kappaphycus* species. Some authors dispute the identification inconsistency of this material; however, this clade represents a single wild seaweed haplotype from Hawaii and expresses invasive characteristics only in Hawaii (Conklin & Smith 2005, Zuccarello *et al*. 2006, Conklin *et al.* 2009). *Kappaphycus cottonii* was shown to be a highly divergent sister taxon to the all other *Kappaphycus* samples, and more studies are needed to investigate the taxonomy of this species (Zuccarello *et al*. 2006, Tan *et al*. 2013).

The mitochondrial *cox*2-3 spacer was effective for the identification and comparison of our strain of *K. alvarezii* with other available strains of this species as predicted in previous studies, which highlighted the usefulness of this marker for the phylogenetic analysis of *Kappaphycus* and *Eucheuma* (Zuccarello *et al*. 1999, 2006, Conklin *et al*. 2009, Halling *et al*. 2013, Tan *et al*. 2012, 2013). This effectiveness is due to the relatively short length of the sequences, contributing for simpler amplification and sequencing which provides better resolution and more variation within the *cox*2-3 spacer region (Zuccarello *et al*. 1999, 2006, Conklin *et al*. 2009, Tan *et al*. 2012). However, Tan *et al*. (2012) considered *cox*2-3 spacer to be more appropriate to DNA barcode in these two genera. Zhao & He (2011) demonstrated the efficacy of the ITS marker to determine inter- and intra-specific analyses, arguing that such a marker has more accurate molecular protocol for the classification of eucheumatoid algae as compared to others, despite few *Kappaphycus* and *Eucheuma* have been sequenced with ITS marker up to date.

This study represents the first investigation towards the knowledge on *Kappaphycus alvarezii* cultivated in the northeastern Brazilian coast. The results obtained in the present study confirmed the identification and similarity of the northeastern Brazilian strain with those cultivated worldwide, which have never shown invasive behavior. However, more detailed investigations and use of environmental monitoring are recommended before commercial cultivation of this species can be authorized in tropical region of Brazil

Acknowledgements

This study was partially supported by the Ministério da Pesca e Aquicultura (MPA) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). The first author thanks the Coordenação de Aperfeiçoamento de Nível Superior (CAPES) for a Doctoral Fellowship and housing allowances at the Instituto de Botânica, São Paulo, during the development of the part of a Doctoral thesis. M.T. Fujii thanks CNPq for a Research Productivity Fellowship (Proc. 301438/2009-9). Beneficiary of the financial support from CAPES – Brazil. We are grateful to the anonymous reviewers and associate editor of Phytotaxa, SBL, whose suggestions improved significantly this manuscript.

References

Abbott, I.A. (1988) *Taxonomy of economic seaweeds with special reference to Pacific and Caribbean species*. California Sea Grant College Program, La Jolla, California, 237 pp.

Agardh, C.A. (1822) *Species algarum rite cognitae, cum synonymis, differentiis specificis et descriptionibus succinctis. Volumen primum pars posterior*. Ex officina Berlingiana, Lund, 229 pp.

Agardh, J.G. (1847) Nyaalgerfrån Mexico. Öfversigtaf Kongl. [*Svenska*] *Vetenskaps-Akademiens Förhandlingar* 4: 5–17.

Agardh, J.G. (1852) *Species genera et ordines algarum, seu descriptiones succinctae specierum, generum et ordinum, quibus algarum regnum constituitur. Volumis secundi: Algas florideas complectens. Part 2, fasc. 2*. C.W.K. Gleerup, Lund, 142 pp.

Aguilan, J.T., Broom, J.E., Hemmingson, J.A., Dayrit, F.M., Montaño, M.N.E., Dancel, M.C.A., Niñonuevo, M.R. & Furneaux, R.H. (2003) Structural analysis of carrageenan from farmed varieties of Philippine seaweed. *Botanica Marina* 46: 179–192.
http://dx.doi.org/10.1515/BOT.2003.018

Anderson, L.W.J. (2007) Control of invasive seaweeds. *Botanica Marina* 50: 418–437.

Areces, A.J. (1995) Cultivo comercial de carragenofitas del gênero *Kappaphycus alvarezii* Doty. *In*: Alveal, K., Ferrario, M.E., Oliveira, E.C. & Sar, E. (eds.) *Manual de Métodos Ficológicos*. Universidad de Concepción, Concepcion, pp. 529–549.

Ask, E.I. & Azanza, R.V. (2002) Advances in cultivation technology of commercial eucheumatoid species: a review with suggestions for future research. *Aquaculture* 206: 257–277.
http://dx.doi.org/10.1016/S0044-8486(01)00724-4

Ask, E.I., Batibasaga, A., Zertuche-Gonzalez, J.A. & San, D. (2003) Three decades of *Kappaphycus alvarezii*

(Rhodophyta) introduction to non-endemic locations. *In*: Chapman, A.R., Anderson, O.R.J., Vreeland, V.J. & Davison, I.R. (eds.) *Proceedings of the 17th International Seaweed Symposium.Cape Town 2001*. Oxford University Press, Oxford, pp. 49–57.

Bagla, P. (2008) Seaweed Invader Elicits Angst in India. *Science* 320: 1271.

Barrios, J.E. (2005) Dispersión del alga exótica *Kappaphycus alvarezii* (Gigartinales: Rhodophyta) en la región nororiental de Venezuela. *Boletín del Instituto Oceanográfico del Venezuela* 44: 29–34.

Barrios, J., Bolaños, J. & Lopez, R. (2007) Blanqueamiento de arrecifes coralinos por la invasión de *Kappaphycus alvarezii* (Rhodophyta) en Isla Cubagua, Estado Nueva Esparta, Venezuela. *Boletín del Instituto Oceanográfico del Venezuela* 46: 147–152.

Bezerra, A.F. & Marinho-Soriano, E. (2010) Cultivation of the red seaweed *Gracilaria birdiae* (Gracilariales, Rhodophyta) in tropical waters of northeast Brazil. *Biomassa & Bioenergia* 34: 1813–1817. http://dx.doi.org/10.1016/j.biombioe.2010.07.016

Bindu, M.S. & Levine, I.A. (2011) The commercial red seaweed *Kappaphycus alvarezii* – an overview on farming and environment. *Journal of Applied Phycology* 23: 789–796. http://dx.doi.org/10.1007/s10811-010-9570-2

Bixler, H.J. & Porse, H. (2011) A decade of change in the seaweed hydrocolloids industry. *Journal of Applied Phycology* 23: 321–335. http://dx.doi.org/10.1007/s10811-010-9529-3

Bulboa, C.R. & Paula, E.J. (2005) Introduction of non-native species of *Kappaphycus alvarezii* (Rhodophyta, Gigartinales) in subtropical waters: comparative analyses of growth rates of *Kappaphycus alvarezii* and *Kappaphycus striatum in vitro* and in the sea in southeastern Brazil. *Phycological Research* 53: 183–188. http://dx.doi.org/10.1111/j.1440-183.2005.00385.x

Bulboa, C.R., Paula, E.J. & Chow, F. (2008) Germination and survival of tetraspores of *Kappaphycus alvarezii* (Solieriaceae, Rhodophyta) introduced in subtropical waters of Brazil. *Phycological Research* 56: 39–45.

Burman, N.L. (1768) *Flora indica*: *cui accedit series zoophytorum indicorum, nec non prodromus florae capensis.* Apud Cornelium Haek & Johannem Schreuderum, Leiden & Amsterdam, 242 pp.

Castelar, B., Reis, R.P., Moura, A.L. & Kirk, R. (2009) Invasive potential of *Kappaphycus alvarezii* off the south coast of Rio de Janeiro State, Brazil: a contribution to environmentally secure cultivation in the tropics. *Botanica Marina* 52: 283–289.

Chandrasekaran, S., Nagendran, N.A., Pandiaraja, D., Krishnankutty, N. & Kamalakannan, B. (2008) Bioinvasion of *Kappaphycus alvarezii* on corals in the Gulf of Mannar, India. *Current Science* 94: 1167–1172.

Collins, F.S. & Hervey, A.B. (1917) The algae of Bermuda. *Proceedings of the American Academy of Arts and Sciences* 53: 1–195.

Conklin, E.J. & Smith, J.E. (2005) Abundance and spread of the invasive red algae, *Kappaphycus* spp., in Kane'ohe Bay, Hawaii and an experimental assessment of management options. *Biological Invasions* 7: 1029–1039. http://dx.doi.org/10.1007/s10530-004-3125-x

Conklin, K.Y., Kurihara, A. & Sherwood, A.R. (2009) A molecular method for identification of the morphologically plastic invasive algal genera *Eucheuma* and *Kappaphycus* (Rhodophyta, Gigartinales) in Hawaii. *Journal of Applied Phycology* 21: 691–699. http://dx.doi.org/10.1007/s10811-009-9404-2

Doty, M.S. (1985) *Eucheuma alvarezii* sp. nov. (Gigartinales, Rhodophyta) from Malaysia. *In*: Abbott, I.A. & Norris, J.N. (eds.) *Taxonomy of economic seaweeds: with reference to some Pacific and Caribbean species*. California Sea Grant College Program, La Jolla, California, pp. 37–45.

Doty, M.S. (1988) Prodromus ad Systematica Eucheumatoideorum: A tribe of commercial seaweeds related to *Eucheuma* (Solieriaceae, Gigartinales). *In*: Abbott, I.A. (ed.) *Taxonomy of economic seaweeds with special reference to Pacific and Caribbean species*. California Sea Grant College Program, La Jolla, California, pp. 159–207.

Doty, M.S. (1995) *Betaphycus philippinensis gen. et sp. nov.* and related species (Solieriaceae, Gigartinales). *In*: Abbott, I.A. (ed.) *Taxonomy of Economic Seaweeds*. California Sea Grant College Program, La Jolla, California, pp. 237–245.

Doty, M.S. & Alvarez, V.B. (1975) Status, problems, advances and economics of *Eucheuma* farms. *Marine Technology Society Jounal* 9: 30–35.

FAO (2012) *The State of world fisheries and aquaculture*. FAO Fisheries and Aquaculture Departament. Rome, 230 pp.

Fredericq, S., Freshwater, D.W. & Hommersand, M.H. (1999) Observations on the phylogenetic systematics and biogeography of the Solieriaceae (Gigartinales, Rhodophyta) inferred from *rbc*L sequences and morphological evidence. *Hydrobiologia* 398/399: 25–38.

Góes, H.G. & Reis, R.P. (2011) An initial comparison of tubular netting versus tie-tie methods of cultivation for *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae) on the south coast of Rio de Janeiro State, Brazil. *Journal of Applied Phycology* 23: 607–613.

Góes, H.G. & Reis, R.P. (2012) Temporal variation of the growth, carrageenan yield and quality of *Kappaphycus* *alvarezii* (Rhodophyta, Gigartinales) cultived at Sepetiba Bay, Southeastern Brazilian coast. *Journal of Applied Phycology* 24: 173–180.

Gurgel, C.F.D. & Fredericq, S. (2004) Systematics of the Gracilariaceae (Gracilariales, Rhodophyta): a critical assessment based on *rbc*L sequence analysis. *Journal of Phycology* 40: 138–159.

Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Sympsium Series* 41: 95–98.

Halling, C., Wikström, S.A., Lilliesköld-Sjöö, G., Mörk, E., Lundsør, E. & Zuccarello, G.C. (2013) Introduction of Asian strains and low genetic variation in farmed seaweeds: indications for new management pratices. *Journal of Applied Phycology* 25: 89–95.

Hayashi, L., Hurtado, A.Q., Msuya, F.E., Bleicher-Lhonneur, G. & Critchley, A.T. (2010) A review of *Kappaphycus* farming: prospects and constraints. *In*: Israel, A., Einav, R. & Seckbach, J. (eds.) *Seaweeds and their role in globally changing environments*. Springer, Berlin, pp. 255–283.

Hayashi, L., Oliveira, E.C., Bleicher-Lhonneur, G., Boulenguer, P., Pereiria, R.T.L., von Seckendorff, R., Shimoda, V.T., Leflamand, A., Vallée, P. & Critchley, A.T. (2007a) The effects of selected cultivation conditions on the carrageenan characteristics of *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae) in Ubatuba Bay, São Paulo, Brazil. *Journal of Applied Phycology* 19: 505–511.

Hayashi, L., Paula, E.J. & Chow, F. (2007b) Growth rate and carrageenan analyses in four strains of *Kappaphycus alvarezii* (Rhodophyta, Gigartinales) farmed in the subtropical waters of São Paulo State, Brazil. *Journal of Applied Phycology* 19: 393–399.
http://dx.doi.org/10.1007/s10811-006-9135-6

Hayashi, L., Santos, A.A., Faria, G.S.M., Nunes, B.G., Souza, M.S., Fonseca, A.L.D., Barreto, P.L.M., Oliveira, E.C. & Bouzon, Z.L. (2011) *Kappaphycus alvarezii* (Rhodophyta, Areschougiaceae) cultivated in subtropical waters in Southern Brazil. *Journal of Applied Phycology* 23: 337–343.

Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.

Kamalakannan, B., Jeevamani, J.J.J., Nagendran, N.A., Pandiaraja, D., Kutty, N.K. & Chandrasekaran, S. (2010) *Turbinaria* sp. as victims to *Kappaphycus alvarezii* in reefs of Gulf of Mannar, India. *Coral Reefs* 29: 1077.
http://dx.doi.org/10.1007/s00338-010-0684-4

Lluisma, A.O. & Ragen, M.A. (1995) Relationships among *Eucheuma denticulatum*, *Eucheuma isiforme* and *Kappaphycus alvarezii* (Gigartinales, Rhodophyta) based on nuclear ssu-rRNA gene sequences. *Journal of Applied Phycology* 7: 471–477.
http://dx.doi.org/10.1007/BF00003931

Lucena, L.A.F., Kanagawa, A.I., Martins, G.J.M., Targino, C.H., Miranda, G.E.C. & Horta, P.A. (2007) Levantamento da flora do infralitoral no município de Pitimbu, litoral sul do Estado da Paraíba, Brasil. *Revista Brasileira de Biocências* 5: 585–587.

Miranda, G.E.C., Yokoya, N.S. & Fujii, M.T. (2012) Effects of temperature, salinity and irradiance on carposporeling development of *Hidropuntia caudata* (Gracilariales, Rhodophyta). *Brazilian Journal of Pharmacognosy* 22: 818–824.

Oliveira, E.C. & Miranda, G.E. (1998) Aspectos sociais e econômicos da explotação de algas marinhas no Brasil. *In*: Paula, E.J., M. Cordeiro-Marino, D.P. Santos, E.M. Plastino, M.T. Fujii & N.S. Yokoya (eds.) *Anais do Congresso Latino Americano, II Reunião Ibero-Americana e VII Reunião Brasileira de Ficologia*. Sociedade. Ficológica da América Latina e Caribe, São Paulo, pp. 149–156.

Oliveira, E.C. & Paula, E.J. (2003) Exotic seaweeds: friends or foes? *In*: Chapman, A.R., Anderson, O.R.J., Vreeland, V.J. & Davison, I.R. (eds.) *Proceedings of the 17th International Seaweed Symposium.Cape Town 2001*.Oxford University Press, Oxford, pp. 87–93.

Paula, E.J., Pereira, R.T.L. & Ohno M. (1999) Strain selection in *Kappaphycus alvarezii* var. *alvarezii* (Soliericeae, Rhodophyta) using tetrasporesprogeny. *Journal of Applied Phycology* 11: 111–121.

Paula, E.J., Pereira, R.T.L. & Ohno, M. (2002) Growth rate of carragenophyte *Kappaphycus alvarezii* (Rhodophyta, Gigartinales) introduced in subtropical waters of São Paulo State, Brazil. *Phycological Research* 50: 1–9.

Pickering, T.D., Skelton, P. & Sulu, R.J. (2007) International introductions of commercially harvested alien seaweeds. *Botanica Marina* 50: 338–350.

Plastino, E.M. & Oliveira, E.C. (2002) *Gracilaria birdiae* (Gracilariales, Rhodophyta), a new species from the tropical South American Atlantic with a terete frond and deep spermatangial conceptacles. *Phycologia* 41: 389–396.
http://dx.doi.org/10.2216/i0031-8884-41-4-389.1

Schaffelke, B., Smith, J.E. & Hewitt, C.L. (2006) Introduced macroalgae – a growing concern. *Journal of Applied Phycology* 18: 529–541.

Schmitz, F. (1895) Marine Florideen von Deutsch-Ostafrica. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 21: 137–177.

Silva, P.C., Basson, P.W. & Moe, R.L. (1996) Catalogue of the benthic marine algae of the Indian Ocean. *University of*

California Publications in Botany 79: 1–1259
Smith, J.E., Hunter, C.L. & Smith, C.M. (2002) Distribution and reproductive characteristics of nonindigenous and invasive marine algae in the Hawaiian Islands. *Pacific Science* 56: 299–315.
Tamura, K., Peterson, D., Peterson, Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: Molecular evolutionary genetics analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony methods. *Molecular Biology and Evolution* 4: 1–9.
http://dx.doi.org/10.1093/molbev/msr121
Tan, J., Lim, P.E. & Phang, S.M. (2013) Phylogenetic relationship of *Kappaphycus* Doty and *Eucheuma* J. Agardh (Solieriaceae, Rhodophyta) in Malaysia. *Journal of Applied Phycology* 25: 13–29.
http://dx.doi.org/10.1007/s10811-012-9833-1
Tan, J., Lim, P.E., Phang, S-M., Hong, D.D., Sunarpi, H. & Hurtado, A.Q. (2012) Assessment of four molecular markers as potential dna barcodes for red algae *Kappaphycus* Doty and *Eucheuma* J. Agardh (Solieriaceae, Rhodophyta) *PLoS ONE* 7: 1–15.
http://dx.doi.org/10.1371/journal.pone.0052905
Weber-van Bosse, A. (1913) Marine algae. Rhodophyceae, of the "Sealark" Expedition, collected by Mr. J. Stanley Gardiner, M.A. *Transactions of the Linnean Society of London, Second Series, Botany* 8: 105–142.
http://dx.doi.org/10.1111/j.1095-8339.1913.tb00282.x
Zhao, S. & He, P. (2011) Molecular identification based on ITS sequences for *Kappaphycus* and *Eucheuma* cultivated in China. *Chinese Journal of Oceanology and Limnology* 29: 1287–1296.
http://dx.doi.org/10.1007/s00343-011-1032-4
Zuccarello, G.C., Burger, G., West, J.A. & King, R.J. (1999) A mitochondrial marker for red algal intraspecific relationships. *Molecular Ecology* 8: 1443–1447.
http://dx.doi.org/10.1046/j.1365-294x.1999.00710.x
Zuccarello, G.C., Critchley, A.T., Smith, J.E., Sieber, V., Lhonneur, G.B. & West, J.A. (2006) Systematics and genetic variation in commercial *Kappaphycus* and *Eucheuma* (Solieriaceae, Rhodophyta). *Journal of Applied Phycology* 18: 643–651.