Morphological and genetic analysis of *Gracilaria* sp. cultured in ponds and coastal waters

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**Abstract.** Seaweeds of the genus *Gracilaria* are an important aquaculture commodity, commonly cultured in brackish water ponds as well as in coastal waters. In South Sulawesi, Indonesia, the *Gracilaria* acultured in ponds has been named G. verrucosa, while *Gracilaria* cultured in coastal waters has been named G. gigas. Morphologically, these two types of *Gracilaria* do look different. Detailed examination shows that they appear to have similarities. This study aimed to examine whether these two types of *Gracilaria* are different species or not. The study used morphological characters and COI mitochondrial gene barcoding to assess the *Gracilaria* types cultured in ponds and coastal waters. Samples were collected from Ujung Baji Village in Takalar Regency, the main seaweed producing region in South Sulawesi Province, Indonesia. The results indicate that both *Gracilaria* types have similar genetic characteristics as well as shared morphological characters. Based on these analyses, it is considered that both *Gracilaria* types cultivated may be strains of the same species.

1. Introduction

Indonesia is the largest seaweed producer in the world [1], with an annual production of around 1.2 million tons dry weight [2]. For *Gracilaria* in particular, Indonesia is the second largest producer, contributing around 28% of global production [3]. This *Gracilaria* production comes from aquaculture (seaweed farming).

Economically, seaweed cultivation has several advantages, namely rapid growth, efficient cultivation systems, uncomplicated cultivation techniques, and relatively low production costs [3],
while ecologically, seaweed cultivation could contribute to mitigating threats to the environment as an absorber of carbon dioxide [4].

At present, there are three major groups of seaweed with potential for cultivation, namely Rhodophyceae (red seaweed), Chlorophyceae (green seaweed), and Phaeophyceae (brown seaweed) [3,5]. These three seaweed groups are plants that attach to a rough substrate in the intertidal or subtidal regions in the waters [6]. Seaweeds are widely used in the food, cosmetics, and medicinal industries [7–9].

Seaweeds are mainly used for the extraction of phycocolloids. Phycocolloids are polysaccharides found in seaweed cell walls [10]. Agar, carrageenan, and alginate are important phycocolloids from seaweed [8,11]. Agar is extracted from Gracilaria, carrageenan is extracted from Kappaphycus and Hypnea, and Alginate are extracted from Sargassum [12]. Amongst cultivated seaweeds, the genus Gracilaria is the largest producer of agar. Gracilaria is superior as a producer of agar because of its rapid growth and high agar content. In 2016, global agar production reached 14,500 tons with a market value of US $246 million [13].

In Indonesia, Gracilaria is currently cultivated in the sea (mariculture) and in ponds. Gracilaria cultivated in ponds is called G. verrucosa, while congeneric algae cultivated in coastal waters are called G. gigas. These two putative species of the genus Gracilaria are visually different in terms of the overall appearance of the thallus. Thalli of Gracilaria cultured in ponds typically exhibit a curly growth habit, while the thalli of Gracilaria cultured in the sea are generally much straighter. Doubts have arisen regarding the taxonomic status of these two types, because when the plants are observed in more detail, there are several morphological similarities between the two putative species, in particular in terms of branching and cystocarp characters. This raises the question of whether these two types of Gracilaria are really different species or strains of one species with different morphological phenotypes. This study aimed to examine whether these two types of Gracilaria are different species or not. The study was conducted using molecular biology methods and phylogenetic analysis as well as an examination of key morphological characters.

2. Materials and methods
Molecular phylogenetics is a branch of biomolecular studies [14] and is an approach often used in the field of biology for genome comparison and determining interspecies relationships based on the tree of life through statistical calculations of nucleotide base sequences [15]. The samples used in this study were thalli of Gracilaria seaweed cultivated in the sea and the ponds of Ujung Baji Village, Takalar Regency, South Sulawesi Province, Indonesia (S05°07'26.9" and E119°29'04.5")).

2.1. Preparation and preservation of samples
The samples were washed with distilled water and then dried [16]. Dried samples were placed in silica gel before DNA extraction [17].

2.2. DNA extraction
DNA extraction was carried out using a modified CTAB extraction procedure [17]. The first step in the extraction process was grinding about 1 cm of each dried sample to fine powder using a mill (Retsch, type MM200). The ground samples were then placed in a microfuge tube containing 500µL CTAB extraction buffer (2% CTAB, 0.1M Tris-HCl (pH 8.0), 1.4M NaCl, 20mM EDTA, 1% PEG 8000), to which 50µg RNAase, and 80g Proteinase K were then added (Promega, Madison, USA). The process of DNA extraction was accelerated by inserting the microfuge containing the sample into a beaker containing water maintained at a temperature at 60°C for 30 minutes using a hotplate. To further speed up the extraction, the microfuge was occasionally removed from the beaker and turned back and forth (gently shaken) for a few seconds and then replaced.

To separate the DNA from the liquid solvents, 500 µl of chloroform was added to the microfuge, which was then centrifuged at a speed of 14,000 rpm for 10 minutes. The supernatant in the microfuge (approximately 400 µl) was taken and transferred to a new microfuge. Next, 400 µl of chloroform was
added and the microfuge was shaken, and then placed in a centrifuge for 5 minutes at 14,000 rpm. Again, the supernatant (approximately 300 µl) was taken and placed into a new microfuge to which 300 µl of isopropanol was added, and the microfuge was shaken, then left to stand (incubate) for 30 minutes at room temperature before being centrifuged for 10 minutes at 12,000 rpm to precipitate the DNA. The isopropanol supernatant was slowly discharged over a tissue. After adding 300 µl ethanol to the DNA, the tube was centrifuged for 5 minutes. The ethanol was then slowly and carefully poured out and the microfuge tube now containing a DNA pellet was reversed and left for 15 minutes until the ethanol odour faded (due to evaporation) before adding 50µL of 0.1 X TE Buffer X [17]. The microfuge containing the DNA extract was then stored in a - 20°C freezer until the DNA isolation process was carried out.

2.3. DNA isolation
A segment of the COI gene (DNA barcode) was isolated from the DNA extract and replicated using Polymorphic Polymerase Chain Reaction (PCR). The PCR process used microfuges containing 25 µL solutions with the following composition: Master Mix 12.5 µl, primer GazR1 (reverse) 1 µl, primer GazF1 (forward) 1 µl, Nuclease-free water 9.5 µl, and extracted DNA 1 µl.

The PCR unit was programmed for 35 cycles consisting of Segment 1 (Pre-denaturation) 94°C for 4 minutes, Segment 2 (Denaturation) 94°C for 30 seconds, Segment 3 (Annealing) 55°C for 1 minute, and segment 4 (Elongation) 72°C for 1 minute.

After segment 4 was complete, 12 µl of PCR product was taken for grading using electrophoresis with 1.5% agarose gel media with ethidium bromide dye. The DNA ladder and DNA PCR product (with dye) were injected into wells in the agarose gel, and subjected to electrophoresis at 50 volts for 30 minutes to stimulate the migration of the DNA ladder and DNA PCR product. The migration of the DNA ladder and DNA PCR product were photographed using a Polaroid MP-4 camera via UV transilluminator (312nm) for direct observation, as well as Polaroid 665 and 667 cameras for instant film packages.

2.4. DNA sequencing
The PCR product was sequenced using the Sanger sequencing method using the services of a genetics company, First Base Malaysia. The Sanger method is a DNA sequencing method with the principle of using deoxyribonucleotide triphosphates (ddNTPs) as a stopper of DNA synthesis in a random position. Sequence data from the COI gene of Gracilaria sp. was returned in the form of a chromatogram which was visualized using Finch TV to determine the quality of sequences. Sequence analysis was implemented in MEGA version 6 [18]. The initial and final parts of the base sequences were trimmed by approximately 50 bp and any incorrect or unclear nucleotide readings were corrected based on the level of accuracy of the data. Forward (primary) and reverse (complement) sequencing results were then combined after applying Primer Reversions to the reverse sequence.

2.5. Species identification
On order to identify the species of the two Gracilaria types based on, the BLAST (Basic Local Alignment Search Tool) process was implemented for each of the nucleotide base sequences obtained. BLAST compares the nucleotide sequence input to the sequences stored in the BOLD (Barcode of Life Database) DNA sequence database which can be accessed at www.boldsystems.org and NCBI GenBank by accessing www.blast.ncbi.nlm.nih.gov. The most similar GenBank sequences in each database were characterized by the parameters Max Score and Total Score Total, Query Coverage approaches 100%, E-value approaches 0, and Identity approaches 100%.
3. Results

3.1. Isolation of the COI Gene segment (DNA barcode)
Amplification of the COI gene in *Gracilaria* sp. used Gaz F1 and Gaz R1 primers amplified with a length of about 704 bp using 10,000 bp DNA ladder as a comparison (Figure 1).

![Figure 1. Electrophoreogram results of the amplification of the COI specimen of *Gracilaria* sp. in 1% agarose gel](image)

Notes:
1kb = DNA Ladder
Gl = *Gracilaria* sp. specimen from the sea
Gt = *Gracilaria* sp. specimen from the pond

3.2. Molecular Phylogeny
The nucleotide sequence obtained from *Gracilaria* sp. cultivated in the sea (Figure 2) was 562 bp long. Nucleotide composition was thymine 38.6%, cytosine 16.5%, adenine 27.4%, and guanine 17.5%.

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ATTGGCTCAACCAAGGAAATCAATTACTTCTTGGGAATCGATCAATGTTTATATGTTTTTATTGGTCTGAGTTTGGTTGGTTG
GAAATGTTGAGTATCAATTATGATGTGAGATCCAGATATGGCTTCCCCGTTTAAATATAATTCTTTGTTTTACCTCTCTTCAG
GGCAATTACCGAGATGAACTATATTACATCCAGCATTACATTTTATTTTATTTTATTTTAGGAAATTGATTAGTACCTATTATG
CTGGCGCGCTGTAGATCTTGCCATATTTAGTTTACATATTTCAGGAGCGTCTTCAATTCTAGGGCAATTAATTTTATTTCTACAAT
CTTGAATATGCGCAATCCAGGTAAAGTATGTATCGTATGCCTTTATTTGTATGATCTATTTTTATTACAGCATTTTACTTCTTCTAGCTGT
TTAGCAGGAGCTATACATTTGCTTTAAACAGATCGTAATTTAACACAGCATTTTTTGTACCTGCTTGGTG
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![Figure 2. Barcode nucleotide sequence of *Gracilaria* sp. cultivated in the Sea.](image)

The nucleotide sequence obtained from pond cultured *Gracilaria* sp. (Figure 3) was 638 bp long. The nucleotide composition was thymine 39.2%, cytosine 15.8%, adenine 26.5%, and guanine 18.6%.

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TTTTAGGTGGTTGTATGTCAATGCTAATTCGTATGGAATTGGCTCAACCAGGAAATCAATTACTTCTTGGGAATCGATCAATGTTTATATGGTTTTTATTGGTCTGAGTTTGGTTGGTTG
GAAATGTTGAGTATCAATTATGATGTGAGATCCAGATATGGCTTCCCCGTTTAAATATAATTCTTTGTTTTACCTCTCTTCAG
GGCAATTACCGAGATGAACTATATTACATCCAGCATTACATTTTATTTTATTTTATTTTATTTTAGGAAATTGATTAGTACCTATTATG
CTGGCGCGCTGTAGATCTTGCCATATTTAGTTTACATATTTCAGGAGCGTCTTCAATTCTAGGGCAATTAATTTTATTTTCTACAAT
CTTGAATATGCGCAATCCAGGTAAAGTATGTATCGTATGCCTTTATTTGTATGATCTATTTTTATTACAGCATTTTACTTCTTCTAGCTGT
TTAGCAGGAGCTATACATTTGCTTTAAACAGATCGTAATTTAACACAGCATTTTTTGTACCTGCTTGGTG
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![Figure 3. Barcode nucleotide sequence of *Gracilaria* sp. cultivated in ponds.](image)
The BLAST routine showed that the sequences obtained from *Gracilaria* types cultivated in the sea and in ponds were very similar to deposited sequences from *Gracilaria blodgettii*, *Gracilaria changii*, and *Gracilaria firma* (Table 1 and Table 2). The alignment result was consonant with the BLAST results, and confirmed that the two specimens of *Gracilaria* sp. (from the sea and pond) belong to the same genus. The similarity between the two specimens was 99.64%. The results of the molecular phylogenetic analysis using the BLAST routine indicate that *Gracilaria* sp. from the sea and ponds have a close kinship with *G. blodgettii*, *G. changii*, and *G. firma*.

### Table 1. NCBI GenBank top 3 BLAST results for *Gracilaria* cultivated in the sea.

| Description | Max score | Total score | Query cover | E-Value | Identity | Accession |
|-------------|-----------|-------------|-------------|---------|----------|-----------|
| *G. blodgettii* isolate 31-3-2R cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial | 1033 | 1033 | 100% | 0.0 | 99.82% | JQ407591.1 |
| *G. changii* mitochondrion, complete genome. | 1027 | 1027 | 100% | 0.0 | 99.64% | KY009863.1 |
| *G. firma* isolate G18 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial | 1027 | 1027 | 100% | 0.0 | 99.64% | KY315285.1 |

### Table 2. NCBI GenBank top 3 BLAST results for *Gracilaria* cultivated in ponds.

| Description | Max score | Total score | Query cover | E-Value | Identity | Accession |
|-------------|-----------|-------------|-------------|---------|----------|-----------|
| *G. blodgettii* isolate 31-3-2R cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial | 1168 | 1168 | 100% | 0.0 | 100.00% | JQ407591.1 |
| *G. changii* mitochondrion, complete genome. | 1162 | 1162 | 100% | 0.0 | 99.84% | KY009863.1 |
| *G. changii* isolate GC2 mitochondrion, complete genome | 1162 | 1162 | 100% | 0.0 | 99.84% | KX980031.1 |

### 3.3. Morphological characteristics

Morphological and anatomical examination of the *Gracilaria* sp. samples cultivated in the sea and ponds show morphological characters similar to those of *G. changii* [19]. Analysis of the rbcL and cox1 gene sequences also supports *G. firma* and *G. changii* as one species [19]. Detailed morpho-anatomical observations and comparisons of species descriptions in literature show that the reproductive anatomy of *G. firma* is largely in accordance with *G. changii* [20]. *G. firma* and *G. changii* are characterized by branches that are restricted at the base and taper to an acute or blunt apex [19]. Morphology of *Gracilaria* sp. which is maintained in the sea and ponds is very similar to the morphology of *G. changii* (Figure 4, 5, 6, 7, and 8).
Figure 4. Comparison of the thallus of *Gracilaria* samples cultivated in the sea and ponds with *Gracilaria changii*. A: *Gracilaria* cultivated in the sea (Present Study); B: *Gracilaria* cultivated in the pond (Present Study); and C: *Gracilaria changii* [20,21].

Figure 5. Comparison of the branching pattern of *Gracilaria* samples cultivated in the sea and ponds with *Gracilaria changii*. A: *Gracilaria* cultivated in the sea (Present Study); B: *Gracilaria* cultivated in the pond (Present Study); and C: *Gracilaria changii* [20,21].

Figure 6. Comparison of the cell size gradation of *Gracilaria* samples cultivated in the sea and ponds with *Gracilaria changii*. A: *Gracilaria* cultivated in the sea (Present Study); B: *Gracilaria* cultivated in the pond (Present Study); and C: *Gracilaria changii* [20,21].

Figure 7. Cystocarp shape on a *Gracilaria* sample cultivated in the sea (Present Study).
Figure 8. Gonimoblasts of: A. *Gracilaria* sample cultivated in the sea (Present Study; Gonimoblasts of *Gracilaria* cultivated in ponds were similar); and B. *Gracilaria changii* [20,21].

This study found that the longitudinal section of cystocarps of *Gracilaria* sp. cultivated in the sea and ponds more closely resembled published cystocarp longitudinal sections of *G. changii* than cystocarp longitudinal sections of *G. blodgettii* (Table 3). Therefore, based on molecular data and morphological observations, *Gracilaria* sp. cultivated in the sea and ponds were similar to *G. changii*.

### Table 3. Comparison between *Gracilaria changii*, *G. blodgettii*, and *G. firma* [20].

| Species       | Vegetative character | Reproductive character | Reference |
|---------------|----------------------|------------------------|-----------|
| *G. blodgettii* | Constricted          | Present                | Large cells | Textoritype | [22]     |
| *G. changii*   | Constricted          | Present                | Large cells | Polycavernosa type | [21]     |
| *G. firma*     | Constricted          | Present                | Small cells | Verrucosatype  | [23]     |

4. Discussion

Molecular analysis based on the COI gene sequence showed that the sequences from specimens of *Gracilaria* sp. cultivated in the sea and in ponds are very similar to sequences deposited as *G. blodgettii*, *G. changii*, and *G. firma* (Table 1 and Table 2). There is considerable controversy over the taxonomy of the genus *Gracilaria* and related genera [24,25]. It is not unlikely that the two types in this study may belong to the same species, which may be synonymous with one or more of the other nominal species in this genus, e.g. *G. changii* or *G. firma*.

In terms of morphological traits, *Gracilaria* sp. cultivated in the sea and in ponds are both characterized by the presence of branches which taper towards the apex. Many species of *Gracilaria* sp. with branches tapering towards the apex are described from the Western Pacific, including *G. firma*, *G. changii*, and *G. fisheri*, and have been equated with *G. blodgettii* from the Western Atlantic based on lower branch morphology [23,26]. Atlantic taxa are distinguished from Pacific taxa by spermatogonial characteristics [21,22]. *G. firma*, *G. changii*, and *G. fisheri* have traditionally been distinguished by differences in talus size, the size of cell transitions from cortex to medulla, and the presence or absence of nutritive filaments in the cystocarp.

*G. firma* shows a gradual cell transition from the cortex to the medulla in young branches and sudden cell size transitions in adult cells. The type of transition in cell size from cortex to medulla, gradual or sudden, is considered consistent for *Gracilaria* species [27]. The transition of cell size in all vegetative thallus of various ages was also observed in *G. blodgettii* [22]. *Gracilaria* of the *G. changii* type are often incorrectly identified as *G. blodgettii*, due to a similar narrowing at the base of the branch. Some studies mention the difficulty of distinguishing between *G. changii* and *G. blodgettii* [28].

Morphological differences in *Gracilaria* sp. which is cultivated in the sea and ponds are thought to be caused by differences in environmental conditions and cultivation methods. When *Gracilaria* sp. is cultivated in ponds, the stocking method used allows the thalli to touch and overlap one another. This
could affect the growth and result in curly shapes as the growing thalli attempt to avoid each other and seek the sunlight. *Gracilaria* sp. cultivated in the sea is attached to long-lines and hangs down so that without external interference and being subject to the effect of gravity the thalli tend to grow with relatively straight branch morphology.

5. Conclusion

Morphological observations and molecular DNA analysis show that the *Gracilaria* types cultivated in the sea and ponds (putative species *G. gigas* and *G. verrucosa*) are both similar to other species, including the nominal species *G. changii*. Genetically, the two *Gracilaria* cultivated in different environments have a similarity rate of 99.64%. Morphologically, both *Gracilaria* have characteristics that closely resemble *G. changii*, especially the narrowing (restriction) at the branch base, branches tapered towards the tip, large thallus medullary cells and the presence of a polycavernosa type cystocarp.

References

[1] Aprillatu P D 2016 Indonesia Dinilai jadi Penghasil Rumput Laut Terbesar di Dunia
[2] MMAF 2018 Marine and Fisheries in numbers, 2018. Center for Statistics and Information Data (Jakarta: Ministry of Maritime Affairs and Fisheries)
[3] Kim J K, Yarish C, Hwang E K, Park M and Kim Y 2017 Seaweed aquaculture: cultivation technologies, challenges and its ecosystem services *Algae* 32 1–13
[4] Arbit N I S, Omar S A B, Tuwo A and Soekendarsi E 2018 Effect of Global Warming Scenarios on Carotenoid Pigments *Gracilaria changii* Int. J. Environ. Agric. Biotechnol. 3
[5] Mulyani S, Tuwo A, Syamsuddin R and Jompa J 2018 Effect of seaweed Kappaphycus alvarezii aquaculture on growth and survival of coral Acropora muricata *Aquac. Aquarium, Conserv. Legis.* 11 1792–8
[6] Kavale M G, Veeragurunathan V and Mantri V A 2018 *Hand book on Farming of Gracilaria dura* (Bhavnagar, Gujarat: CSIR-Central Salts & Marine Chemicals Research Institute)
[7] Freile-Pelegrín Y and Murano E 2005 Agars from three species of *Gracilaria* (Rhodophyta) from Yucatán Peninsula. *Bioresour. Technol.* 96 295–302
[8] Ma Z, Khalid N, Shu G, Zhao Y, Kobayashi I, Neves M A, Tuwo A and Nakajima M 2019 Fucoxanthin-Loaded Oil-in-Water Emulsion-Based Delivery Systems: Effects of Natural Emulsifiers on the Formulation, Stability, and Bioaccessibility *ACS Omega* 4 10502–10509
[9] Melanie H, Taarji N, Zhao Y, Khalid N, Neves M A, Kobayashi I, Tuwo A and Nakajima M 2019 Formulation and characterisation of O/W emulsions stabilised with modified seaweed polysaccharides *Int. J. Food Sci. Technol.*
[10] Yang Y, Chai Z, Wang Q, Chen W, He Z and Jiang S 2015 Cultivation of seaweed *Gracilaria* in Chinese coastal waters and its contribution to environmental improvements *Algal Res.* 9 236–44
[11] Syamsuddin R, Tuwo A and Aswar N 2019 Weight gain and carrageenan content of Kappaphycus alvarezii (Rhodophyta, Solierisceae) polycultured with Sargassum polycystum (Paeophyta, Sargassaceae) *IOP Conf. Ser. Earth Environ. Sci.* 253
[12] Rioux L E and Turgeon S L 2015 Seaweed carbohydrates, in Seaweed Sustainability *Food and Non Food Applications* ed B Tiwari and D Troy (Amsterdam: Elsevier) pp 141–92
[13] Porse H and Rudolph B 2017 The seaweed hydrocolloid industry: 2016 updates, requirements, and outlook *J. Appl. Phycol.* 29 2187–200
[14] Kumar S and Filipski A J 2001 Molecular Phylogeny Reconstruction *Encycl. Life Sci.*
[15] Yang Z and Rannala B 2012 Molecular phylogenetics: principles and practice *Nat. Rev. Genet.* 13 303
[16] Ho C L, Phang S M and Pang T 1995 Application of polymerase chain reaction (PCR) using random amplified polymorphic DNA (RAPD) primers in the molecular identification of selected Sargassum species (Phaeophyta, Fucales) *Eur. J. Phycol.* 30 273–80
[17] Zuccarello G C, Alan T C, Smith J, Sieber V, Lhonneur G B and West J A 2006 Systematics and genetic variation in commercial shape Kappaphycus and shape Eucheuma (Solieriaceae, Rhodophyta) J. Appl. Phycol. 18 643–51

[18] Tamura K, Stecher G, Peterson D, Filipski A and Kumar S 2013 MEGA6: molecular evolutionary genetics analysis version 6.0 Mol. Biol. Evol. 30 2725–9

[19] Ng P K, Lin S M, Lim P E, Hurtado A Q, Phang S M, Yow Y Y and Sun Z 2017 Genetic and morphological analyses of Gracilaria firma and G. changii (Gracilariaceae, Rhodophyta), the commercially important agarophytes in western Pacific PLoS One 12

[20] Phooprong, Ogawa S, Nanba H and Nobuyoshi 2006 Morphological and photosynthetic studies on Gracilarian species from Trat Province, eastern Thailand Coast. Mar. Sci. 30 226–32

[21] Bangmei X and Abbott I A 1987 New species of Polycavernosa Chang & Xia (Gracilariaceae, Rhodophyta) from the western Pacific Phycologia 26 405–18

[22] Fredericq S and Norris J M 1992 Studies on cylindrical species of western Atlantic Gracilaria (Gracilariales, Rhodophyta): G. cylindrica Borgesen and G. blodgettii Harvey. Taxonomy of economic seaweeds, with reference to some Pacific and western Atlantic species Conference: Taxonomy of Economic Seaweeds, with reference to some Pacific and Western Atlantic species (San Diego: University of California) pp 211–31

[23] Chang C and Xia B 1976 Taxonomic studies on Gracilaria from China. Stud Mar Sin. 11 91–163

[24] Gurgel C F D, Norris J N, Schmidt W E, Le H N and Fredericq S 2018 Systematics of the Gracilariales (Rhodophyta) including new subfamilies, tribes, subgenera, and two new genera, Agarophyton gen. nov. and Crassa gen. nov. Phytotaxa 374 1–23

[25] Lyra G M, Costa E S, de Jesus P B, de Maltos J C, Calres T A, Oliveira M C, Oliveira E C, Xi Z, Nunes J M and Davis C C 2015 Phylogeny of Gracilariaceae (Rhodophyta): evidence from plastid and mitochondrial nucleotide sequences J. Phycol 51 356–66

[26] Doty M, Santos G and Sin O K 1983 Agar from Gracilaria cylindrica Aquat. Bot. 15 299–306

[27] Witherell A, Millar A and Kraft G 1994 Taxonomic studies of the genus Gracilaria (Gracilariales, Rhodophyta) from Australia Aust. Syst. Bot. 7 281–352

[28] Lewmanomont K and Phang S M 2016 Gracilaria changii (PROSEA)