

MOLECULAR TAXONOMIC STUDIES OF *Gracilaria changii*
FROM VARIOUS LOCATIONS USING THE RANDOM
AMPLIFIED POLYMORPHIC DNA (RAPD) TECHNIQUE

Thesis submitted to the Institute of Postgraduate Studies
and Research, University of Malaya, Kuala Lumpur,
in fulfillment of the requirements for
the degree of Master of Philosophy

by

Gan Sook Yee

Institute of Postgraduate Studies and Research
University of Malaya
Kuala Lumpur

October 1999

Perpustakaan Universiti Malaya



A508766447

Dimikrofiksakan pada..... 21.10.2001

No. Mikrofis..... 15169

Jumlah Mikrofis..... 3

HAMSIAH BT. MOHAMAD ZAHARI
UPR UNIT REPROGRAFI
PERPUSTAKAAN UTAMA
UNIVERSITI MALAYA

ACKNOWLEDGEMENTS

My most sincere appreciation to my supervisor, Associate Professor Dr. Phang Siew Moi for her guidance, advice, support and most of all, her valuable time throughout this project. Special thanks go to Professor Tikki Pang for his assistance. Fruitful discussions on the molecular techniques with Associate Professor Thong Kwai Lin are greatly appreciated.

To the devoted staff of IPSP especially Cik Zubaidah, Puan Rodziah, Encik Gana and Ms Molly, my utmost gratitude and appreciation for their help. Very sincere thanks to Encik Wan Yusoff who helped in collecting and photographing the seaweed samples.

Special thanks to my lab-mates, Dr Chu Wan Loy, Muru, Rohani, Melor Ismail, Ching Lee, Phaik Eem, Seok Min, Jillian, Miah and Quimrul for giving me the support, encouragement and sharing wonderful moments with me. Sincere thanks to Eng Lai, Annie and Baset, who have been good listeners and great companions in the lab.

Financial support from the IRPA grant and Skim Pasca is gratefully acknowledged. Sincere gratitude is due to Dr Lum Keng Yeang from Mardi for allowing me to use the Gelcompar software. To Dr Lam Peng Fatt, Dr Isabel Meneses, Dr Mark Ragan, Dr Lawrence Liao, Dr Freshwater and Mr Julian Lim, grateful appreciation for their kind interest and advice for the development of this project.

To all my friends especially Say Bee, Lee Ang, Pauline, Elda, Chee Seng, Soo Herng, Susan, Wooi Koon, Ban Ling, Chee Yew and Kumar for their encouragement and moral support.

To my beloved parents and my two brothers, my sincere gratitude for their love and concern for me. Also, not forgetting Ling Xin who is greatly appreciated for bringing joy and happiness to the family.

Praise and Glory to God for in His faithfulness, He has walked with me all these years providing my every need and strength. My Father, being a great provider has blessed me with many blessings. May His blessing be with you.

CONTENTS

	PAGE
ACKNOWLEDGMENTS	i
CONTENTS	iii
ABSTRACT	vii
ABSTRAK	ix
LIST OF FIGURES	xi
LIST OF TABLES	xv
LIST OF APPENDICES	xvi
 CHAPTER 1: INTRODUCTION	
1.1 ECONOMIC IMPORTANCE OF SEAWEEDS	1
1.2 IMPORTANCE OF SEAWEED STUDIES	2
1.3 OBJECTIVES	5
 CHAPTER 2: LITERATURE REVIEW	
2.1 RED ALGAE (RHODOPHYTA)	6
2.2 THE GENUS <i>GRACILARIA</i>	7
2.3 ECONOMIC IMPORTANCE OF <i>GRACILARIA</i> SPECIES	9

2.4	CONTROVERSIES IN THE TAXONOMY OF <i>GRACILARIA</i>	12
2.4.1	<i>Polycavernosa</i>	12
2.4.2	<i>Hydropuntia</i>	15
2.4.3	<i>Gracilariopsis</i>	16
2.4.4	Interspecific grouping	17
2.5	DEOXYRIBONUCLEIC ACID (DNA) ISOLATION AND PURIFICATION	17
2.6	MOLECULAR APPROACHES	20
2.6.1	Random Amplified Polymorphic DNA (RAPD)	24
2.6.2	Amplified fragment Polymorphism (AFLP)	26
2.6.3	DNA sequencing	28
2.7	MOLECULAR APPROACHES IN UNRAVELLING THE TAXONOMIC STUDIES OF ALGAE	28
2.7.1	Algae	29
2.7.2	<i>Gracilaria</i>	31
2.8	BIOTECHNOLOGY AND PLANT BREEDING	37

CHAPTER 3: MATERIALS AND METHODS

3.1	FIELD COLLECTION	42
3.2	DEOXYRIBONUCLEIC ACID (DNA) ISOLATION PROTOCOLS	46
3.2.1	Protocol 1	46
3.2.2	Protocol 2	48
3.3	SPECTROPHOTOMETRIC DETERMINATION	52
3.4	RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD)	52
3.4.1	PCR Parameters	52
3.4.2	Random Amplified Polymorphic DNA (RAPD)	53
3.4.3	RAPD Data Analysis	55

CHAPTER 4: RESULTS

4.1	DESCRIPTION OF <i>GRACILARIA</i> SPECIES	56
4.1.1	<i>Gracilaria changii</i> (Xia et Abbott) Abbott, Zhang et Xia	56
4.1.2	<i>Gracilaria salicornia</i> (C. Agardh) Dawson	57
4.1.3	<i>Gracilaria edulis</i> (Gmelin) Silva	59
4.2	DEOXYRIBONUCLEIC ACID (DNA) EXTRACTION AND ISOLATION	65
4.3	RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD)	68
4.3.1	Screening of Primers and Optimisation of PCR Reaction	68
4.3.2	Reproducibility	73
4.3.3	RAPD Profiles	74
4.3.4	RAPD Profiles Using Primers OPA3, OPA10, OPA11 and OPA13	74
4.4	DICE COEFFICIENT OF SIMILARITY, S_D FOR INTRASPECIES RELATIONSHIP (INTRAPOPULATIONAL STUDIES)	87
4.5	CLUSTER ANALYSIS	89
4.5.1	Intraspecific Relationship (Interpopulational Studies)	89
4.5.1.1	OPA3	90
4.5.1.2	OPA10	92
4.5.1.3	OPA11	94
4.5.1.4	OPA13	96
4.5.2	Interspecific relationship	98
4.5.2.1	OPA3	98
4.5.2.2	OPA10	100
4.5.2.3	OPA11	100
4.5.2.4	OPA13	103
4.6	SUMMARY OF RESULTS	105

CHAPTER 5: DISCUSSION

5.1	DEOXYRIBONUCLEIC ACID (DNA) EXTRACTION AND ISOLATION	107
5.2	RANDOM AMPLIFIED POLYMORPHISM DNA (RAPD)	109
5.2.1	Optimisation of PCR Reaction	109
5.2.2	Reproducibility	111
5.3	INTRASPECIFIC RELATIONSHIPS (BIOGEOGRAPHICAL ASSESSMENT)	113
5.3.1	Intrapopulational studies	114
5.3.2	Interpopulational studies	121
5.4	INTERSPECIFIC RELATIONSHIPS	126

CHAPTER 6: CONCLUSION

129

REFERENCES

132

APPENDICES

154

ABSTRACT

Malaysia which has abundant *Gracilaria changii* (Xia et Abbott) Abbott, Zhang et Xia has the potential to become a producer of good food grade agar. However, for effective utilisation of this agarophytic seaweed, Bird and Van der Meer (1993) emphasised the importance of taxonomic studies in ensuring the correct identification and recognition of this seaweed.

Molecular biology has been applied in the approach to study the phylogenetic relationship of the *Gracilaria* species. In this study, *Gracilaria changii* samples were collected from six geographical locations, namely, Phuket & Takbai (Thailand) and Ban Merbok, Morib, Carey Island & Sungai Pulai (Malaysia). Although these samples were identified as *Gracilaria changii*, some morphological differences were observed such as thallus appearance, diameter of thallus and the reproductive stage. The objective of this study is to assess the use of the Random Amplified Polymorphic DNA (RAPD) in characterising these samples. Interspecies relationship between selected *Gracilaria* species collected from Morib such as *Gracilaria changii*, *Gracilaria edulis*, and *Gracilaria salicornia* were also studied while *Sargassum oligocystum* was used as an outgroup.

In this study, two DNA extraction protocols were carried out: the cetyltrimethylammonium (CTAB) and phenol combination adapted from Sinnappah (1994) and a modification of this protocol (with additional steps of

isolating from the residue and a reextraction step). The modified protocol was found to produce higher purity of DNA with better yield.

Of the 20 primers screened, four primers: OPA3 (AGTCAGCCAC), OPA10 (GTGATCGCAG), OPA11 (CAATCGCCGT) and OPA13 (CAGCACCCAC) were selected to generate polymorphic band patterns for all the samples under an optimised temperature. The RAPD data analysis was carried out using the Gelcompar Software 4.1 which calculated the matrix of similarities based on the Dice coefficient (S_D) and dendograms were generated using the unweighted pair group method using arithmetic averages (UPGMA). All the four primers showed that there were great intrapopulational variations among the *Gracilaria changii* as well as interpopulational variations. Overall, these primers were unable to form a relationship at the geographical scales studied as there is high intrapopulational variation. There is a possibility of developing individual fingerprints with these primers. However, for larger geographical distance, primer OPA13 can separate the Malaysian *Gracilaria changii* from the *Gracilaria changii* collected from Thailand.

In the interspecies study, OPA13 can separate *Gracilaria changii* from both *Gracilaria salicornia* and *Gracilaria edulis* while OPA11 showed that there is a possibility of hybridisation between *Gracilaria changii* and *Gracilaria salicornia* or between *Gracilaria changii* and *Gracilaria edulis*. Both OPA11 and OPA13 separate *Gracilaria* samples from *Sargassum* samples.

ABSTRAK

Malaysia dengan sumber *Gracilaria changii* (Xia et Abbott) Abbott, Zhang et Xia mempunyai keupayaan menjadi pengeluar agar gred pemakanan. Walau bagaimanapun, untuk penggunaan berkesan rumpair laut ‘agarophytic’, Bird dan Van der Meer (1993) menekan kepentingan kajian taksonomi untuk memastikan ketepatan dalam mengenalpastikan rumpair laut ini.

Biologi molekul telah digunakan untuk mengkaji perhubungan filogenetik *Gracilaria* spesis. Dalam kajian ini, sampel-sampel *Gracilaria changii* dikumpul dari enam kawasan geografi iaitu Phuket & Takbai (Negara Thai) dan Ban Merbok, Morib, Pulau Carey & Sungai Pulai (Malaysia). Walaupun sampel-sampel ini dikenalpasti sebagai *Gracilaria changii*, terdapat perbezaan morfologi seperti rupabentuk tallus, diameter tallus dan tahap pembiakan. Objektif kajian ini adalah mengkaji penggunaan ‘Random Amplified Polymorphic DNA’ (RAPD) dalam mengenalpasti sampel-sampel ini. Perhubungan interspesis antara pilihan *Gracilaria* spesis yang dikumpul dari Morib seperti *Gracilaria changii*, *Gracilaria edulis* and *Gracilaria salicornia* juga dikaji dan *Sargassum oligocystum* diguna sebagai bandingan (outgroup).

Dalam kajian ini, dua protokol pengekstrakan DNA telah dijalankan: kombinasi cetyltrimethylammonium (CTAB) dan fenol diaplifikasi dari Sinnappah (1994) dan modifikasi protokol ini (dengan penambahan langkah – langkah pengekstrakan dari sisa dan pengulangan pengekstrakan). Protokol

yang dimodifikasi itu menghasilkan DNA yang mempunyai ketulenan yang tinggi dengan hasil yang lebih baik.

Antara 20 primer yang dikaji, empat primer: OPA3 (AGTCAGCCAC), OPA10 (GTGATCGCAG), OPA11 (CAATCGCCGT) dan OPA13 (CAGCACCCAC) dipilih untuk menghasilkan corak jalur yang berlainan untuk kesemua sampel-sampel pada suhu yang dioptimakan. Data RAPD analisis dijalankan dengan menggunakan ‘Gelcompar Software 4.1’ di mana matriks persamaan dikira berdasarkan ‘Dice coefficient’ (S_D) dan dendrogram-dendrogram dihasilkan dengan menggunakan ‘unweighted pair group method using arithmetic averages’ (UPGMA). Kesemua empat primer-primer ini menunjukkan perbezaan intrapopulasi yang besar antara *Gracilaria changii* selain daripada perbezaan interpopulasi. Primer-primer ini tidak dapat membentukkan hubungan pada skala geografi yang dikaji. Terdapat kemungkinan besar untuk menghasilkan ‘fingerprints’ untuk individu sampel dengan primer-primer ini. Walau bagaimanapun, untuk jarak geografi yang lebih besar, primer OPA13 boleh memisahkan *Gracilaria changii* dari Thailand daripada *Gracilaria changii* dari Malaysia.

Dalam kajian interspesis, OPA13 memisahkan *Gracilaria changii* daripada kedua-dua *Gracilaria salicornia* dan *Gracilaria edulis* sementara OPA11 menunjukkan kemungkinan penghibridasian antara *Gracilaria changii* dan *Gracilaria salicornia* serta antara *Gracilaria changii* dan *Gracilaria edulis*. Kedua-dua OPA11 dan OPA13 memisahkan sampel-sampel *Gracilaria* daripada sampel-sampel *Sargassum*.

FIGURES

	PAGE
Figure 1: Carey Island (Sample collection site in Malaysia-mangrove)	42
Figure 2: Sungai Pulai (Sample collection site in Malaysia-mangrove and estuary)	43
Figure 3: Phuket (Sample collection site in a mangrove in Thailand)	43
Figure 4: Map of Peninsular Malaysia and Southern Thailand showing collection site of <i>Gracilaria</i> species	45
Figure 5: Flow-chart of Protocol 2	51
Figure 6: PCR Parameters	55
Figure 7: Photograph showing species of <i>Gracilaria changii</i> collected from Morib	57
Figure 8: Photograph showing species of <i>Gracilaria salicornia</i> collected from Morib	58
Figure 9: Photograph showing species of <i>Gracilaria edulis</i> collected from Morib	59
Figure 10: Photograph showing <i>Gracilaria changii</i> from Phuket, Thailand	60
Figure 11: Photograph showing <i>Gracilaria changii</i> from Ban Merbok, Malaysia	61
Figure 12: Photograph showing <i>Gracilaria changii</i> from Morib, Malaysia	61
Figure 13: Photograph showing <i>Gracilaria changii</i> from Carey Island, Malaysia	62
Figure 14: Photograph showing <i>Gracilaria changii</i> from Sungai Pulai, Malaysia	62
Figure 15: Photograph showing <i>Gracilaria changii</i> from Takbai, Thailand	63
Figure 16: Genomic DNA isolated from representatives of <i>Gracilaria changii</i> using protocol 2 on 0.8% agarose gel, M, High DNA mass ladder.	66
Figure 17: RAPD bands patterns generated for the different DNA templates of <i>Gracilaria changii</i> (C1, C2, C3, C4, C5) with primer OPA3 (a), OPA10 (b), OPA11 (c) and OPA13 (d) at 33°C. M, 1 kb DNA ladder.	69

Figure 18: RAPD bands patterns generated for the different DNA templates of <i>Gracilaria changii</i> (C1, C2, C3, C4, C5) with primer OPA3 (a), OPA10 (b), OPA11 (c) and OPA13 (d) at 36°C. M, 1 kb DNA ladder.	70
Figure 19: RAPD bands patterns generated for the different DNA templates of <i>Gracilaria changii</i> (C1, C2, C3, C4, C5) with primer OPA3 (a), OPA10 (b), OPA11 (c) and OPA13 (d) at 38°C. M, 1 kb DNA ladder.	71
Figure 20: RAPD bands patterns generated for the different DNA templates of <i>Gracilaria changii</i> (C1, C2, C3, C4, C5) with primer OPA3 (a), OPA10 (b), OPA11 (c) and OPA13 (d) at 40°C. M, 1 kb DNA ladder.	72
Figure 21: Assessment of reproducibility of RAPD. RAPD pattern generated using DNA from <i>Gracilaria changii</i> from Morib with OPA10. M, 1 kb plus DNA ladder.	73
Figure 22: RAPD profile for different DNA samples isolated from <i>Gracilaria changii</i> of Carey Island (N) with OPA3 (lanes 1-11). M, 1 kb plus DNA ladder	75
Figure 23: RAPD profile for different DNA samples isolated from <i>Gracilaria changii</i> of Morib (M) with OPA3 (lanes 1-20). M, 1 kb plus DNA ladder	75
Figure 24: RAPD profile for different DNA samples isolated from <i>Gracilaria changii</i> of Ban Merbok (Bm) with OPA3 (lanes 1-19). M, 1 kb plus DNA ladder	76
Figure 25: RAPD profile for different DNA samples isolated from <i>Gracilaria changii</i> of Sungai Pulai (Sp) with OPA3 (lanes 1-15). M, 1 kb plus DNA ladder	76
Figure 26: RAPD profile for different DNA samples isolated from <i>Gracilaria changii</i> of Phuket, P (lanes 1-4) and Takbai, T (lanes 5-8) with OPA3, M, 1 kb plus DNA ladder	77
Figure 27: RAPD profile for different DNA samples isolated from <i>Gracilaria changii</i> of Carey Island (N) with OPA10 (lanes 1-10). M, 1 kb plus DNA ladder	78
Figure 28: RAPD profile for different DNA samples isolated from <i>Gracilaria changii</i> of Morib (M) with OPA10 (lanes 1-15). M, 1 kb plus DNA ladder	79

Figure 29: RAPD profile for different DNA samples isolated from <i>Gracilaria changii</i> of Ban Merbok (Bm) with OPA10 (lanes 1-9). M, 1 kb plus DNA ladder	79
Figure 30: RAPD profile for different DNA samples isolated from <i>Gracilaria changii</i> of Sungai Pulai (Sp) with OPA10 (lanes 1-9). M, 1 kb plus DNA ladder	80
Figure 31: RAPD profile for different DNA samples isolated from <i>Gracilaria changii</i> of Phuket, P (lanes 1-4) and Takbai, T (lanes 5-8) with OPA10, M, 1 kb plus DNA ladder	80
Figure 32: RAPD profile for different DNA samples isolated from <i>Gracilaria changii</i> of Carey Island (N) with OPA11 (lanes 1-7). M, 1 kb plus DNA ladder	81
Figure 33: RAPD profile for different DNA samples isolated from <i>Gracilaria changii</i> of Morib (M) with OP11 (lanes 1-13). M, 1 kb plus DNA ladder	82
Figure 34: RAPD profile for different DNA samples isolated from <i>Gracilaria changii</i> of Ban Merbok (Bm) with OPA11 (lanes 1-11). M, 1 kb plus DNA ladder	82
Figure 35: RAPD profile for different DNA samples isolated from <i>Gracilaria changii</i> of Sungai Pulai (Sp) with OPA11 (lanes 1-10). M, 1 kb plus DNA ladder	83
Figure 36: RAPD profile for different DNA samples isolated from <i>Gracilaria changii</i> of Phuket, P (lanes 1-4) and Takbai, T (lanes 5-8) with OPA11, M, 1 kb plus DNA ladder	83
Figure 37: RAPD profile for different DNA samples isolated from <i>Gracilaria changii</i> of Carey Island (N) with OPA13 (lanes 1-18). M, 1 kb plus DNA ladder	84
Figure 38: RAPD profile for different DNA samples isolated from <i>Gracilaria changii</i> of Morib (M) with OPA13 (lanes 1-7). M, 1 kb plus DNA ladder	84
Figure 39: RAPD profile for different DNA samples isolated from <i>Gracilaria changii</i> of Ban Merbok (Bm) with OPA13 (lanes 1-14). M, 1 kb plus DNA ladder	85
Figure 40: RAPD profile for different DNA samples isolated from <i>Gracilaria changii</i> of Sungai Pulai (Sp) with OPA13 (lanes 1-16). M, 1 kb plus DNA ladder	85

Figure 41: RAPD profile for different DNA samples isolated from <i>Gracilaria changii</i> of Phuket, P (lanes 1-4) and Takbai, T (lanes 5-7) with OPA13, M, 1 kb plus DNA ladder	86
Figure 42: Dendrogram generated with the dice similarity coefficient (S_D) of <i>Gracilaria changii</i> from different locations with primer OPA3, where M, Morib, N, Carey Island, Bm, Ban Merbok, Sp, Sungai Pulai, P, Phuket and T, Takbai.	91
Figure 43: Dendrogram generated with the dice similarity coefficient (S_D) of <i>Gracilaria changii</i> from different locations with primer OPA10, where M, Morib, N, Carey Island, Bm, Ban Merbok, Sp, Sungai Pulai, P, Phuket and T, Takbai.	93
Figure 44: Dendrogram generated with the dice similarity coefficient (S_D) of <i>Gracilaria changii</i> from different locations with primer OPA11, where M, Morib, N, Carey Island, Bm, Ban Merbok, Sp, Sungai Pulai, P, Phuket and T, Takbai.	95
Figure 45: Dendrogram generated with the dice similarity coefficient (S_D) of <i>Gracilaria changii</i> from different locations with primer OPA13, where M, Morib, N, Carey Island, Bm, Ban Merbok, Sp, Sungai Pulai, P, Phuket and T, Takbai.	97
Figure 46: Dendrogram generated for <i>Gracilaria changii</i> (M), <i>Gracilaria salicornia</i> (S), <i>Gracilaria edulis</i> (E) and <i>Sargassum</i> (Sar) using OPA3	99
Figure 47: Dendrogram generated for <i>Gracilaria changii</i> (M), <i>Gracilaria salicornia</i> (S), <i>Gracilaria edulis</i> (E) and <i>Sargassum</i> (Sar) using OPA10	101
Figure 48: Dendrogram generated for <i>Gracilaria changii</i> (M), <i>Gracilaria salicornia</i> (S), <i>Gracilaria edulis</i> (E) and <i>Sargassum</i> (Sar) using OPA11	102
Figure 49: Dendrogram generated for <i>Gracilaria changii</i> (M), <i>Gracilaria salicornia</i> (S), <i>Gracilaria edulis</i> (E) and <i>Sargassum</i> (Sar) using OPA13	104

TABLES

	PAGE
Table 1: Morphological Differences between <i>PolycaVERNOSA</i> and <i>Gracilaria</i>	14
Table 2: The list of samples collected from different places around Malaysia and Thailand	44
Table 3: List of random primers from the OPA kit	54
Table 4: Description of the <i>Gracilaria changii</i> collected from different locations	64
Table 5: Comparison of the purity and quantity of DNA obtained from <i>Gracilaria</i> samples isolated using Protocol 1 and Protocol 2	65
Table 6: The quality and quantity of DNA isolated from <i>Gracilaria changii</i> collected from different locations using Protocol 2	67
Table 7: Dice Similarity Coefficient (S_D) obtained for each population of <i>Gracilaria changii</i> collected from different locations using primers OPA3, OPA10, OPA11 and OPA13.	88

APPENDICES

	PAGE
Appendix 1 : Dice similarity coefficient, S_D obtained for <i>Gracilaria changii</i> samples of Carey Island with primer OPA3	154
Appendix 2 : Dice similarity coefficient, S_D obtained for <i>Gracilaria changii</i> samples of Carey Island with primer OPA10	155
Appendix 3 : Dice similarity coefficient, S_D obtained for <i>Gracilaria changii</i> samples of Carey Island with primer OPA11	156
Appendix 4 : Dice similarity coefficient, S_D obtained for <i>Gracilaria changii</i> samples of Carey Island with primer OPA13	157
Appendix 5 : Dice similarity coefficient, S_D obtained for <i>Gracilaria changii</i> samples of Morib with primer OPA3	158
Appendix 6 : Dice similarity coefficient, S_D obtained for <i>Gracilaria changii</i> samples of Morib with primer OPA10	159
Appendix 7 : Dice similarity coefficient, S_D obtained for <i>Gracilaria changii</i> samples of Morib with primer OPA11	160
Appendix 8 : Dice similarity coefficient, S_D obtained for <i>Gracilaria changii</i> samples of Morib with primer OPA13	161
Appendix 9 : Dice similarity coefficient, S_D obtained for <i>Gracilaria changii</i> samples of Ban Merbok with primer OPA3	162
Appendix 10: Dice similarity coefficient, S_D obtained for <i>Gracilaria changii</i> samples of Ban Merbok with primer OPA10	163
Appendix 11: Dice similarity coefficient, S_D obtained for <i>Gracilaria changii</i> samples of Ban Merbok with primer OPA11	164

Appendix 12: Dice similarity coefficient, S_D obtained for <i>Gracilaria changii</i> samples of Ban Merbok with primer OPA13	165
Appendix 13: Dice similarity coefficient, S_D obtained for <i>Gracilaria changii</i> samples of Sungai Pulai with primer OPA3	166
Appendix 14: Dice similarity coefficient, S_D obtained for <i>Gracilaria changii</i> samples of Sungai Pulai with primer OPA10	167
Appendix 15: Dice similarity coefficient, S_D obtained for <i>Gracilaria changii</i> samples of Sungai Pulai with primer OPA11	168
Appendix 16: Dice similarity coefficient, S_D obtained for <i>Gracilaria changii</i> samples of Sungai Pulai with primer OPA13	169
Appendix 17: Dendrogram generated with the Dice similarity coefficient, S_D of <i>Gracilaria changii</i> from Ban Merbok (Bm) with primer OPA03	170
Appendix 18: Dendrogram generated with the Dice similarity coefficient, S_D of <i>Gracilaria changii</i> from Ban Merbok (Bm) with primer OPA10	171
Appendix 19: Dendrogram generated with the Dice similarity coefficient, S_D of <i>Gracilaria changii</i> from Ban Merbok (Bm) with primer OPA11	172
Appendix 20: Dendrogram generated with the Dice similarity coefficient, S_D of <i>Gracilaria changii</i> from Ban Merbok (Bm) with primer OPA13	173
Appendix 21: Dendrogram generated with the Dice similarity coefficient, S_D of <i>Gracilaria changii</i> from Morib (M) with primer OPA03	174
Appendix 22: Dendrogram generated with the Dice similarity coefficient, S_D of <i>Gracilaria changii</i> from Morib (M) with primer OPA10	175
Appendix 23: Dendrogram generated with the Dice similarity coefficient, S_D of <i>Gracilaria changii</i> from Morib (M) with primer QPA11	176

Appendix 24: Dendrogram generated with the Dice similarity coefficient, S_D of <i>Gracilaria changii</i> from Morib (M) with primer OPA13	177
Appendix 25: Dendrogram generated with the Dice similarity coefficient, S_D of <i>Gracilaria changii</i> from Carey Island (N) with primer OPA03	178
Appendix 26: Dendrogram generated with the Dice similarity coefficient, S_D of <i>Gracilaria changii</i> from Carey Island (N) with primer OPA10	179
Appendix 27: Dendrogram generated with the Dice similarity coefficient, S_D of <i>Gracilaria changii</i> from Carey Island (N) with primer OPA11	180
Appendix 28: Dendrogram generated with the Dice similarity coefficient, S_D of <i>Gracilaria changii</i> from Carey Island (N) with primer OPA13	181
Appendix 29: Dendrogram generated with the Dice similarity coefficient, S_D of <i>Gracilaria changii</i> from Sungai Pulai (Sp) with primer OPA03	182
Appendix 30: Dendrogram generated with the Dice similarity coefficient, S_D of <i>Gracilaria changii</i> from Sungai Pulai (Sp) with primer OPA10	183
Appendix 31: Dendrogram generated with the Dice similarity coefficient, S_D of <i>Gracilaria changii</i> from Sungai Pulai (Sp) with primer OPA11	184
Appendix 32: Dendrogram generated with the Dice similarity coefficient, S_D of <i>Gracilaria changii</i> from Sungai Pulai (Sp) with primer OPA13	185