CHAPTER 4

RESULTS

Morphological studies

The general morphologies of *Spirogyra* are characterized by coiled chloroplast, light green color. The cell is cylindrical. Apical cells were tapering, with rounded tip and thick cell wall. Five different morphological characters of 36 *Spirogyra* specimens collected from some water resources of Thailand were included as bellows. The arrangement of spiral chloroplast and granules of Pattern 1 and 5 were highly condensed and compacted while Patterns 2, 3 and 4 were relatively scattered as indicated in Figure 4-1.

Pattern 1 condensed and slightly compacted spiral chloroplast.

Pattern 2 short cell with scattered spiral chloroplast.

Pattern 3 long cell with less spiral chloroplast.

Pattern 4 short cell with less spiral chloroplast.

Pattern 5 long cell with condensed and compacted spiral chloroplast.

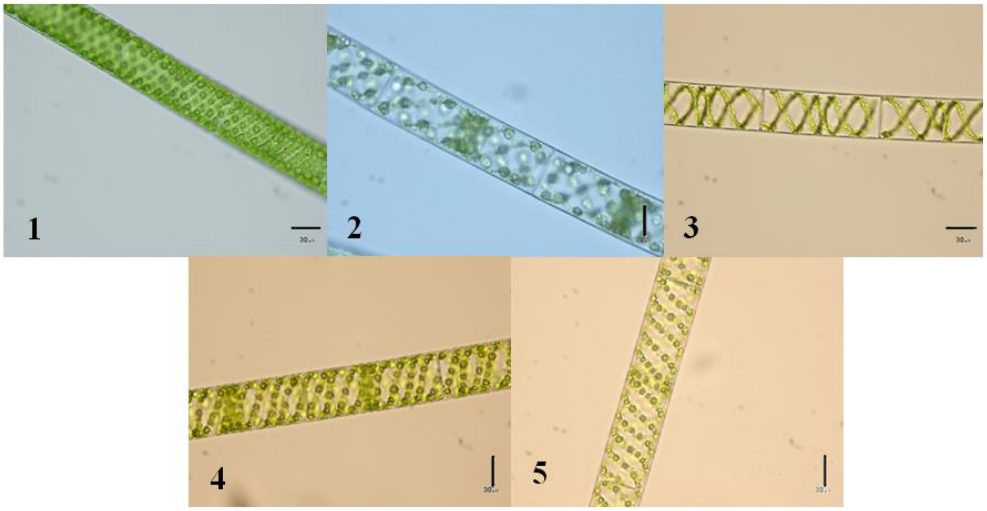

Figure 4-1. Five different morphological characters of *Spirogyra* specimens collected from Thailand (1): condensed and slightly compacted spiral chloroplast (2): short cell with scattered spiral chloroplast (3): long cell with less spiral chloroplast (4): short cell with less spiral chloroplast (5): long cell with condensed and compacted spiral chloroplast. (scale bar = 30 μm)

The arrangement of spiral chloroplast and granules of group number 1 and 5 were highly condensed and compacted while groups 2, 3 and 4 were relatively scattered as indicated in figure 4-1. Morphological characters were significant different by revealing 5 morphological traits ($p < 0.05$) among all specimens. The major criterion classified was used with numbers and arrangement of spiral chloroplast.

The descriptive of each Patterns of *Spirogyra* was described as shown in Table 4-1 and 4-2.

Table 4-1. Morphological characters descriptions of *Spirogyra ellipsospora* and *S. neglecta*

Description	*S. ellipsospora*		*S. neglecta*	
	Thiamdao, 2011	**Kim *et al*., 2004**	**Thiamdao, 2011**	**Nordhusano, 1849**
Vegetative cell width (µm)	85.5-112	137-150	41	52-60
Vegetative cell length (µm)	152-240	208-604	218	162-185
L/W ratio	1.4-2.8	1.4-4.2	5.3	3.1
Vegetative cell number of chloroplasts	2-6	5-8	3	3-4
Shape of zoospore	Ellipsoid more or less pointed ends	Ellipsoid with pointed ends	Oval or even round	Oval
Zoospore width	67-72	107-127	58	54-62
Zoospore length	82-90	146-217	81	162-187
L/W ratio zoospore	1-1.3	1.3-1.9	1.4	3.1
Shape of pyrenoids	Circular	Disc-shaped		

Table 4-2. Morphological characters descriptions of *Spirogyra* 5 Patterns from Thailand.

Details	Pattern 1 (*S.ellipsospora*)	Pattern 2	Pattern 3 (*S. neglecta*)	Pattern 4	Pattern 5
Vegetative cell width (µm)	45-90	40-55	40-60	41-50	40-60
Vegetative cell length(µm)	110-225	85-180	95-188	122-161	95-188
L/W ratio	2.4-2.5	2.13-3.27	2.38-3.13	2.97-3.22	2.35-3.13
Vegetative cell number of chloroplasts	2-3	2	3	2	4-5
Shape of zoospore	ellipsoid	-	-	-	-
Zoospore width	55-70	-	-	-	-
Zoospore length	80-90	-	-	-	-
L/W ratio zoospore	1.3-1.4	-	-	-	-
Shape of pyrenoids	Disc-shaped				

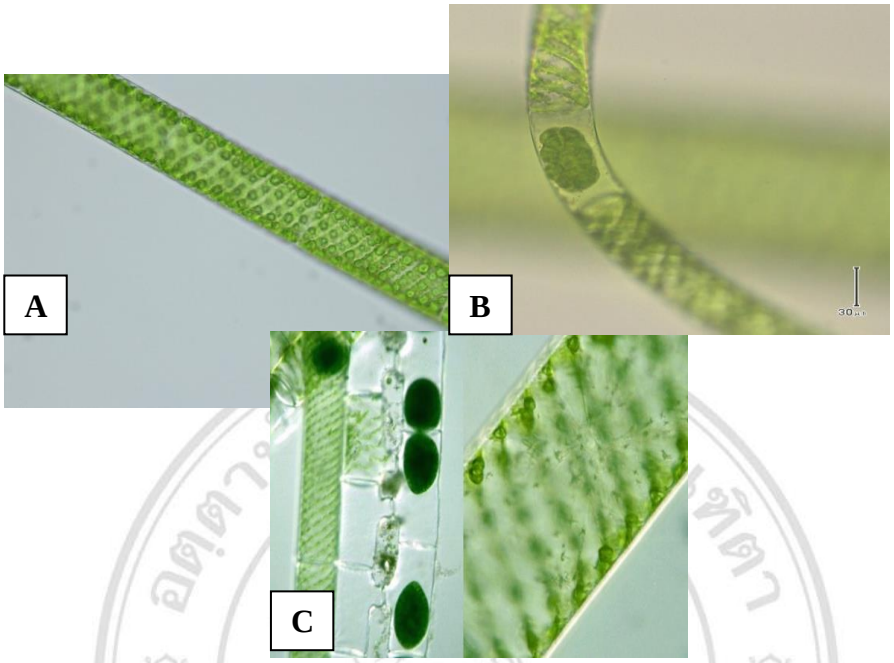

Figure 4-2. Morphological structure of *Spirogyra ellipsospora* (Pattern 1)

A. Vegetative cells with disc-shaped pyrenoids B. Zoospore and C. reference morphology (http://protist.i.hosei.ac.jp/pdb/images/Chlorophyta/Spiropgyra/group_A/sp_0b.html) (scale bar = 30 μm).

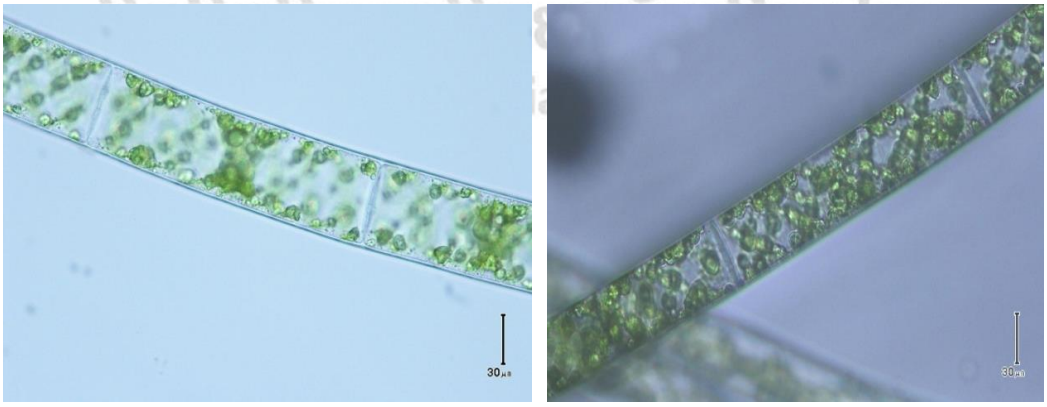

Figure 4-3. Morphological vegetative cell structure of *Spirogyra* (Pattern 2) (scale bar = 30 μm).

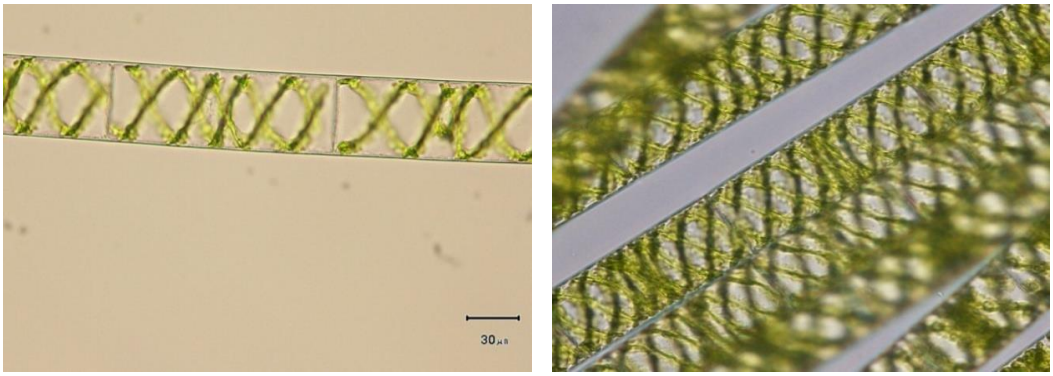

Figure 4-4. Morphological vegetative cell structure of *Spirogyra neglecta* (Pattern 3) (scale bar = 30 μm).

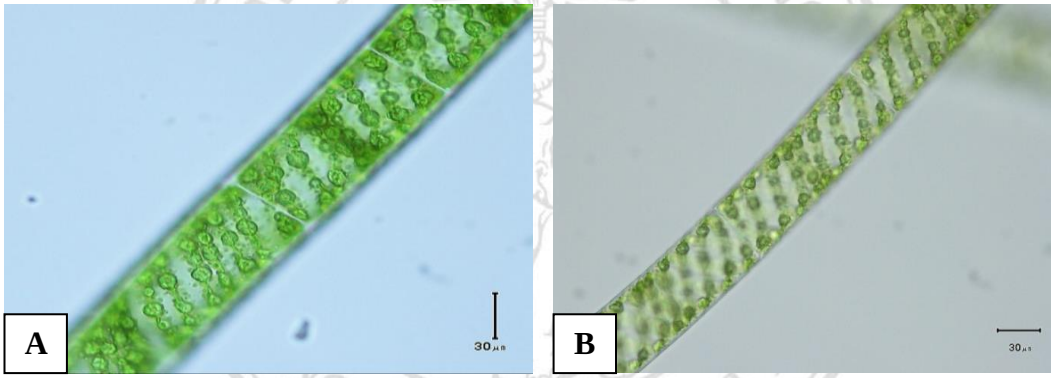

Figure 4-5. Morphological vegetative cell structure of *Spirogyra* A. Pattern 4 B. Pattern 5 (scale bar = 30 μm)

Scanning Electron Microscope (SEM) observation

Ultrastructure with SEM

Five Patterns of *Spirogyra* sp. were observed by using SEM (Figure 4-6). The surfaces of all Patterns are smooth and not branching (Figure 4-6A). For the topology of SEM, morphological differences were mainly observed in cell dimension, cell length and width (cell border) were observed prominently while the arrangement inside the cells were not clearly seen. It could be pointed in only light grayish-black lines of spiral chloroplast but inconstant due to somewhat disappeared. SEM micrograph demonstrated the different of each character as described by basing on light microscope. Pattern 2 and 4 were appeared short cell with less spiral chloroplast (Figure 4-6B). Pattern 3 was shown long cell with less spiral chloroplast (Figure 4-6C). Patterns 1 and 5 were shown long cell with condensed and compacted (Figure 4-6D).

SEM micrographs of *Cladophora* sp. (Figure 4-7) were used for compare with *Spirogyra.* Branch filaments were observed (Figure 4-7A, B). The surface is roughly minute-longitudinal fibers (Figure 4-7C).

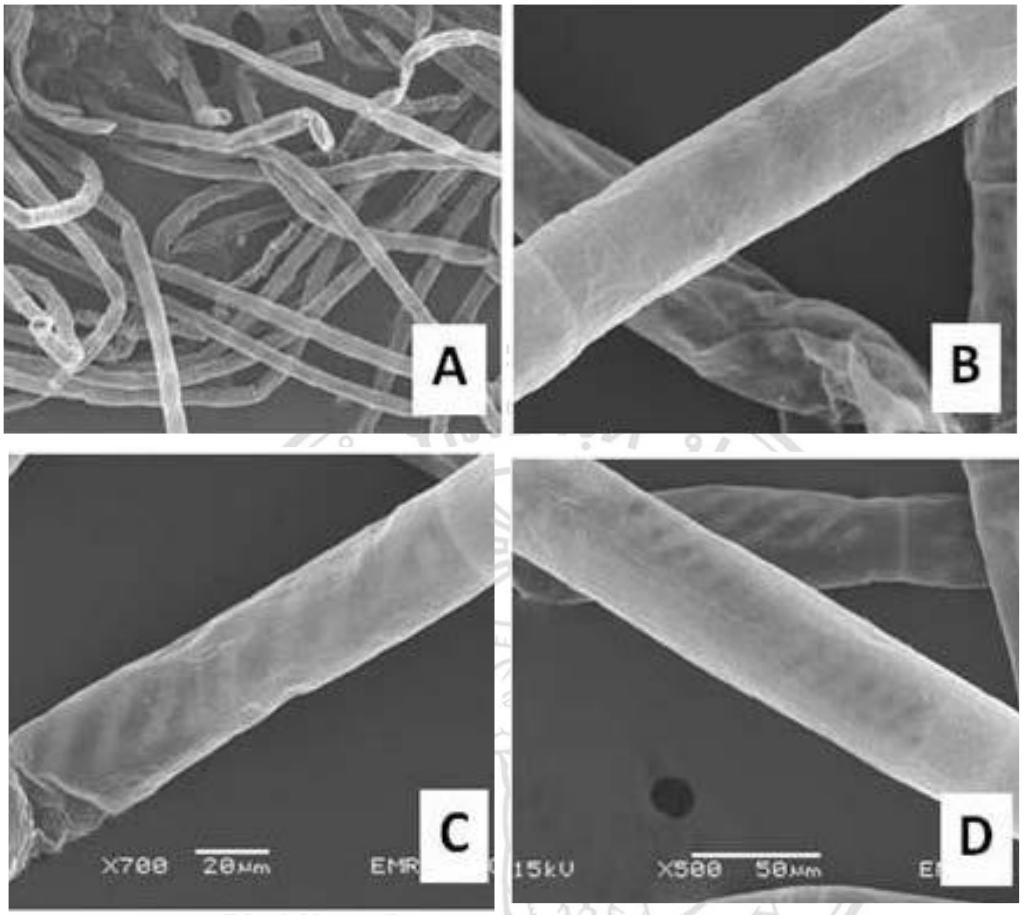

Figure 4-6. By based on light microscope of SEM micrograph of *Sprirogyra* specimen demonstrate the different of each character as described (A): over view landscape, (B): short cell with less spiral chloroplast (Pattern 2 and 4), (C): long cell with less spiral chloroplast (Pattern 3), (D): long cell with condensed and compacted spiral chloroplast (Pattern 5 and 1) (scale bar = 20 µm)

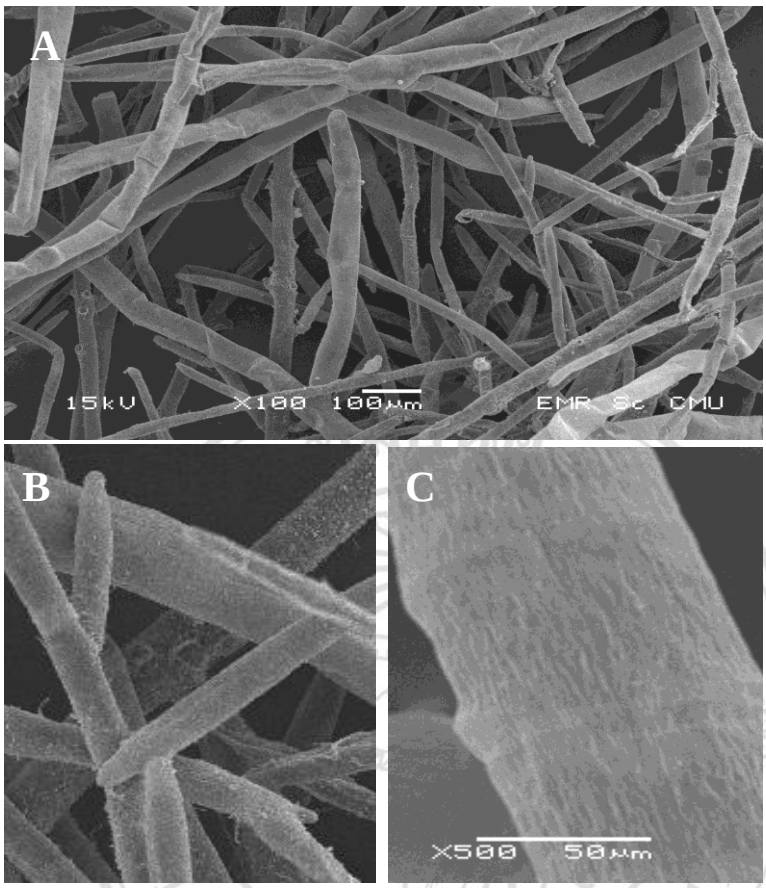

Figure 4-7. SEM micrographs of *Cladophora* sp. (A): abundance filaments of *Cladophora* sp. (scale bar = 100 µm) (B): branching filament (scale bar = 100 µm) (C): surface roughly minute-longitudinal fibers (scale bar = 50 µm).

Distribution of *Spirogyra*

The present geographic distribution of *Spirogyra* specimens in Thailand now included all region of Thailand. This result indicated that the morphological Pattern 1 and character 3 are widely distribution in Thailand including Northern, Northeastern, Central and Southern. While morphological Pattern 4 found

only in Northern and Northeastern part of Thailand (Mae Hong Son, Uttaradit, Loei and Udon Thani provinces). The morphological character 5 of *Spirogyra* population was found only in 3 provinces form this study (Nan, Nakhon Phanom and Saraburi). Finally, morphological character 4 was found in all region of Thailand except Northeastern (Figure 4-8 – 4-13).

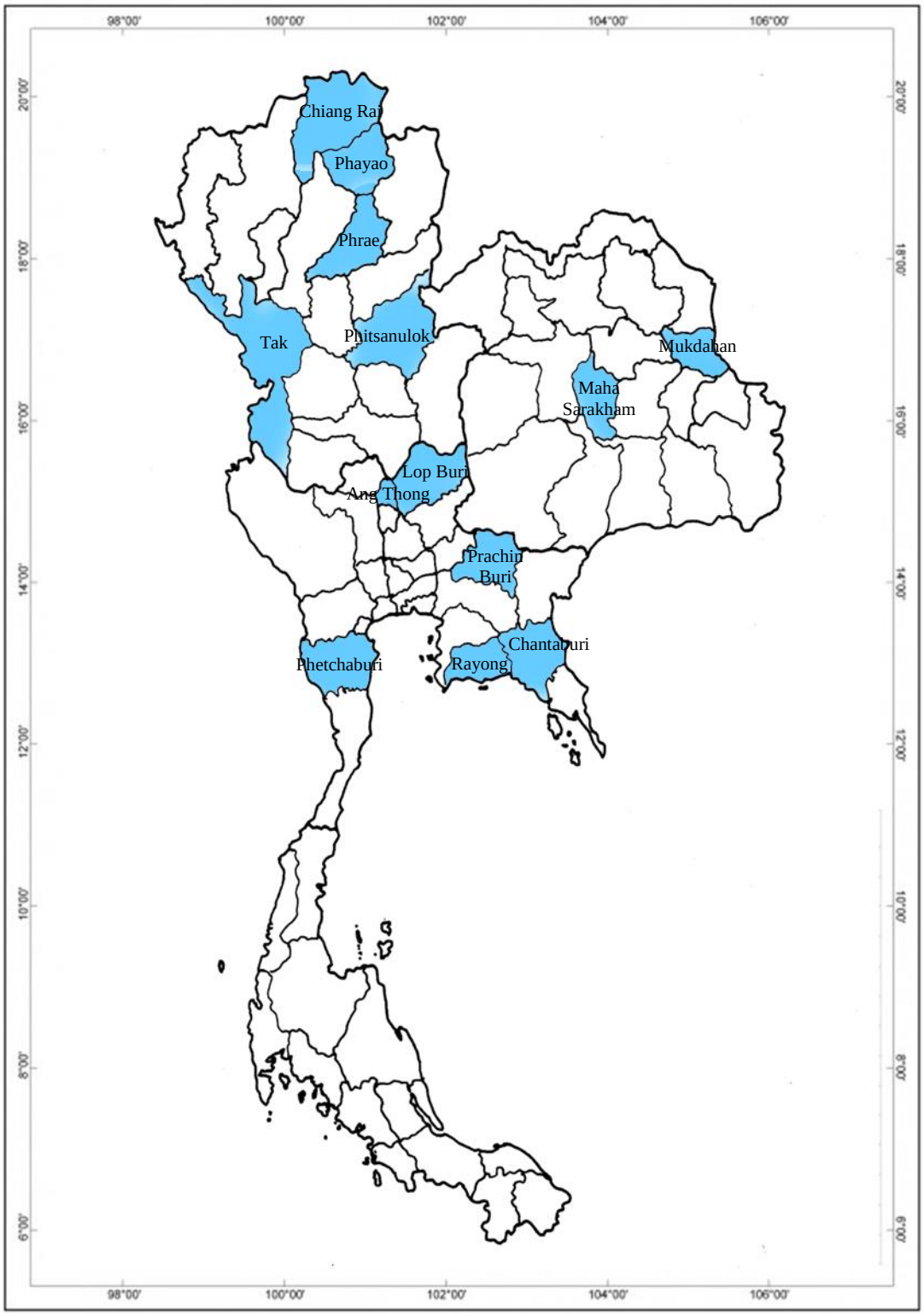

Figure 4-8. Distribution of morphological Pattern 1 of *Spirogyra* specimens collected from Thailand (The geographic coordinates as described in Table 3-1).

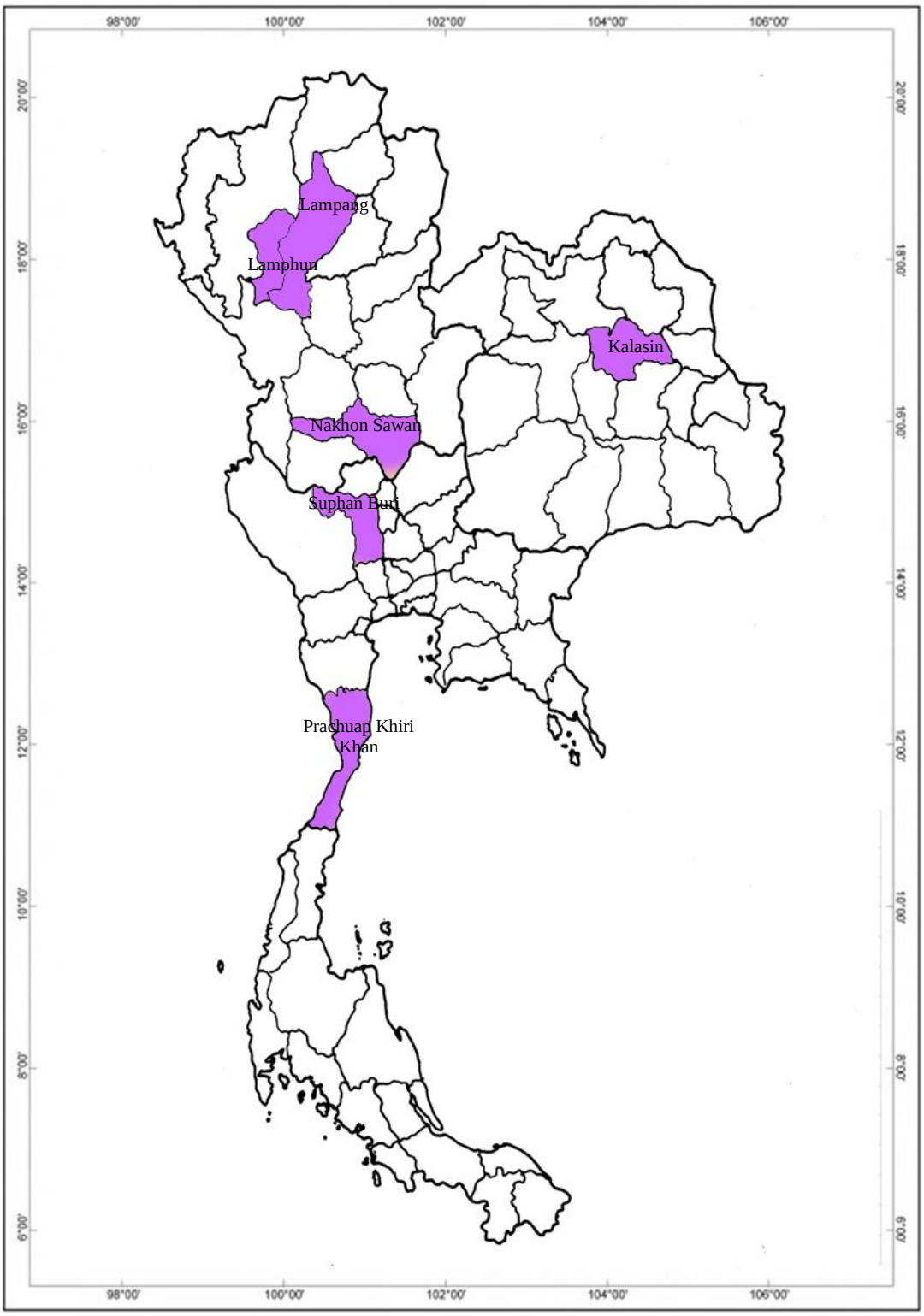

Figure 4-9. Distribution of morphological Pattern 2 of *Spirogyra* specimens collected from Thailand (The geographic coordinates as described in Table 3-1).

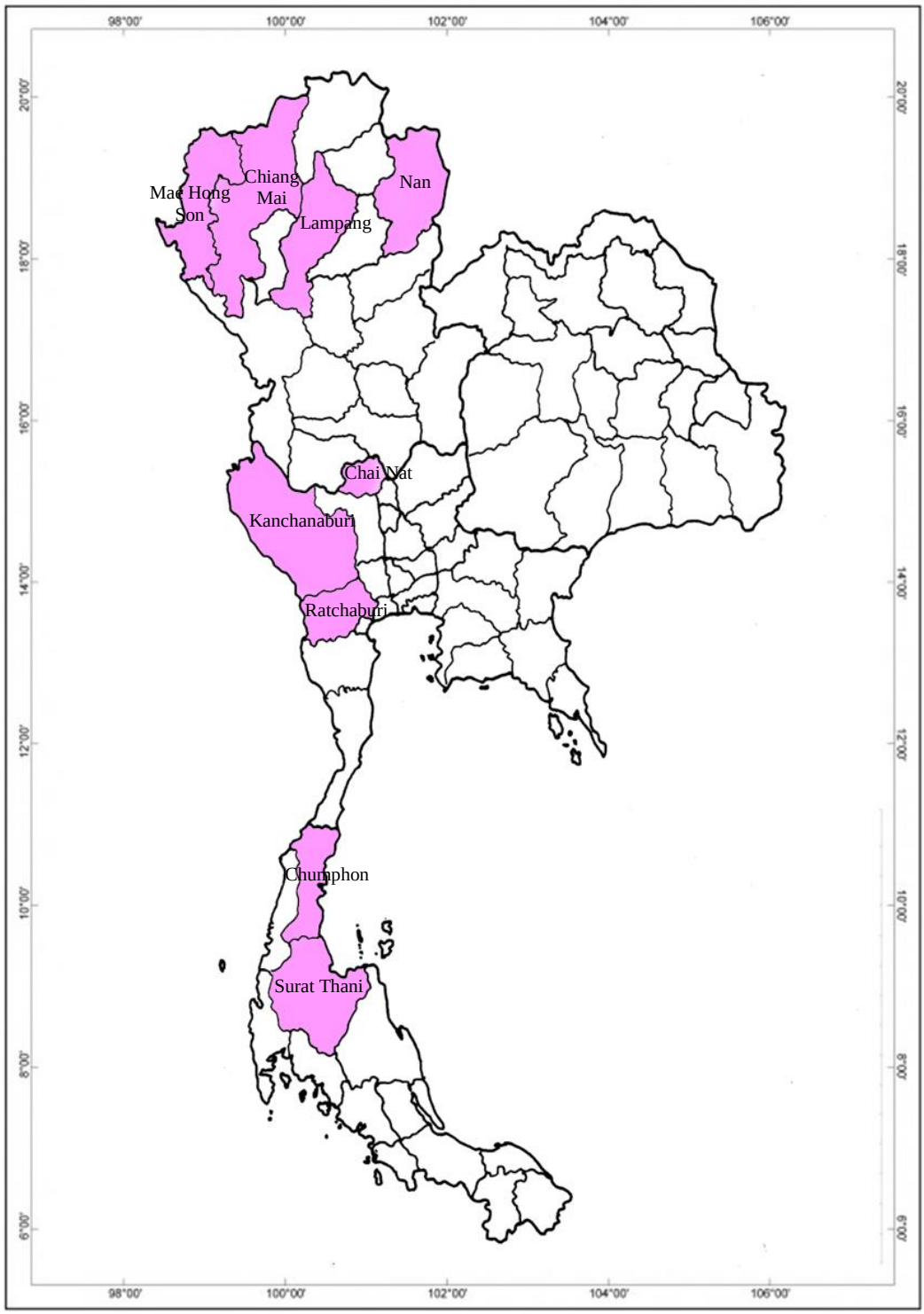

Figure 4-10. Distribution of morphological Pattern 3 of *Spirogyra* specimens collected from Thailand (The geographic coordinates as described in Table 3-1).

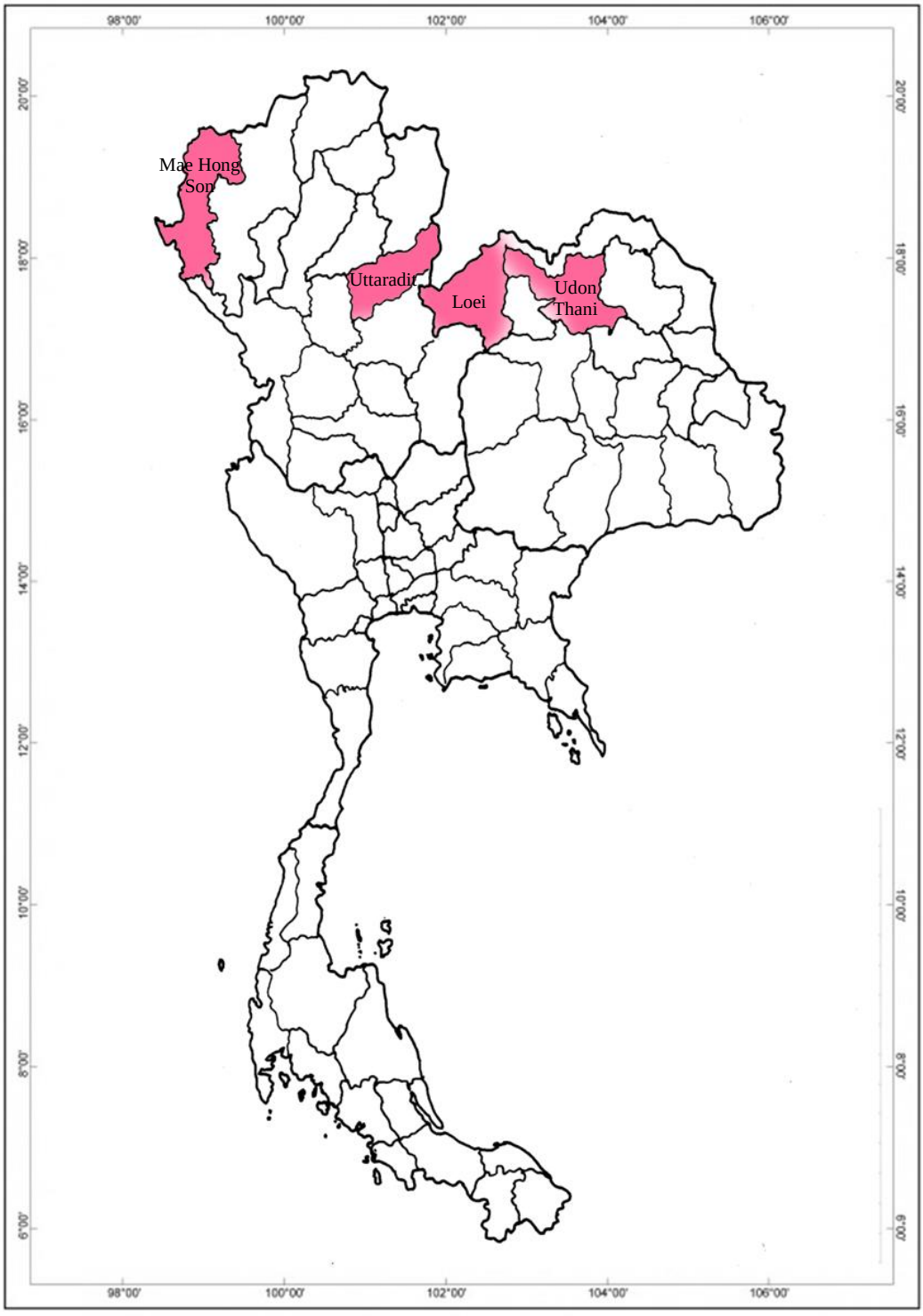

Figure 4-11. Distribution of morphological Pattern 4 of *Spirogyra* specimens collected from Thailand (The geographic coordinates as described in Table 3-1).

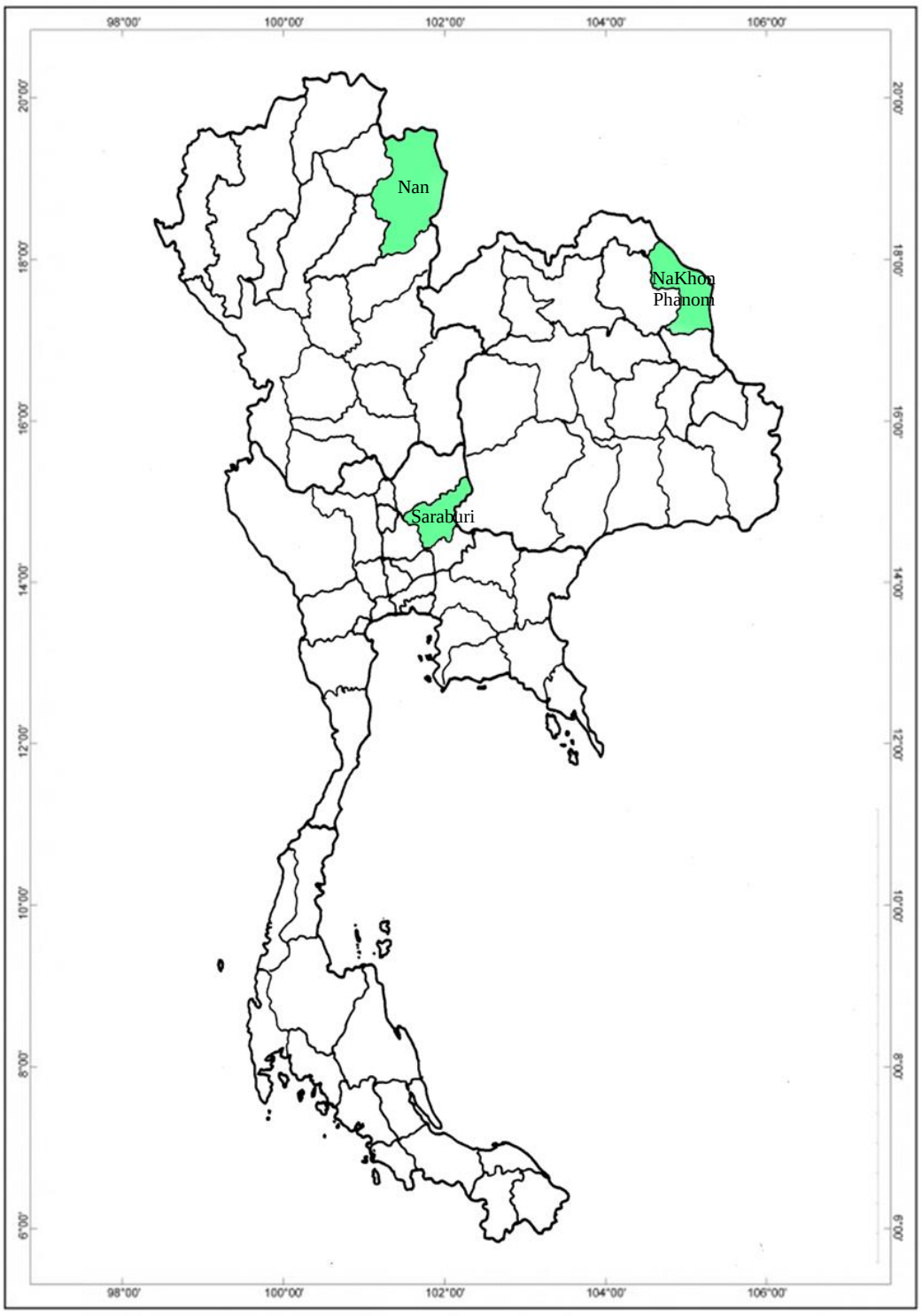

Figure 4-12. Distribution of morphological Pattern 5 of *Spirogyra* specimens collected from Thailand (The geographic coordinates as described in Table 3-1).

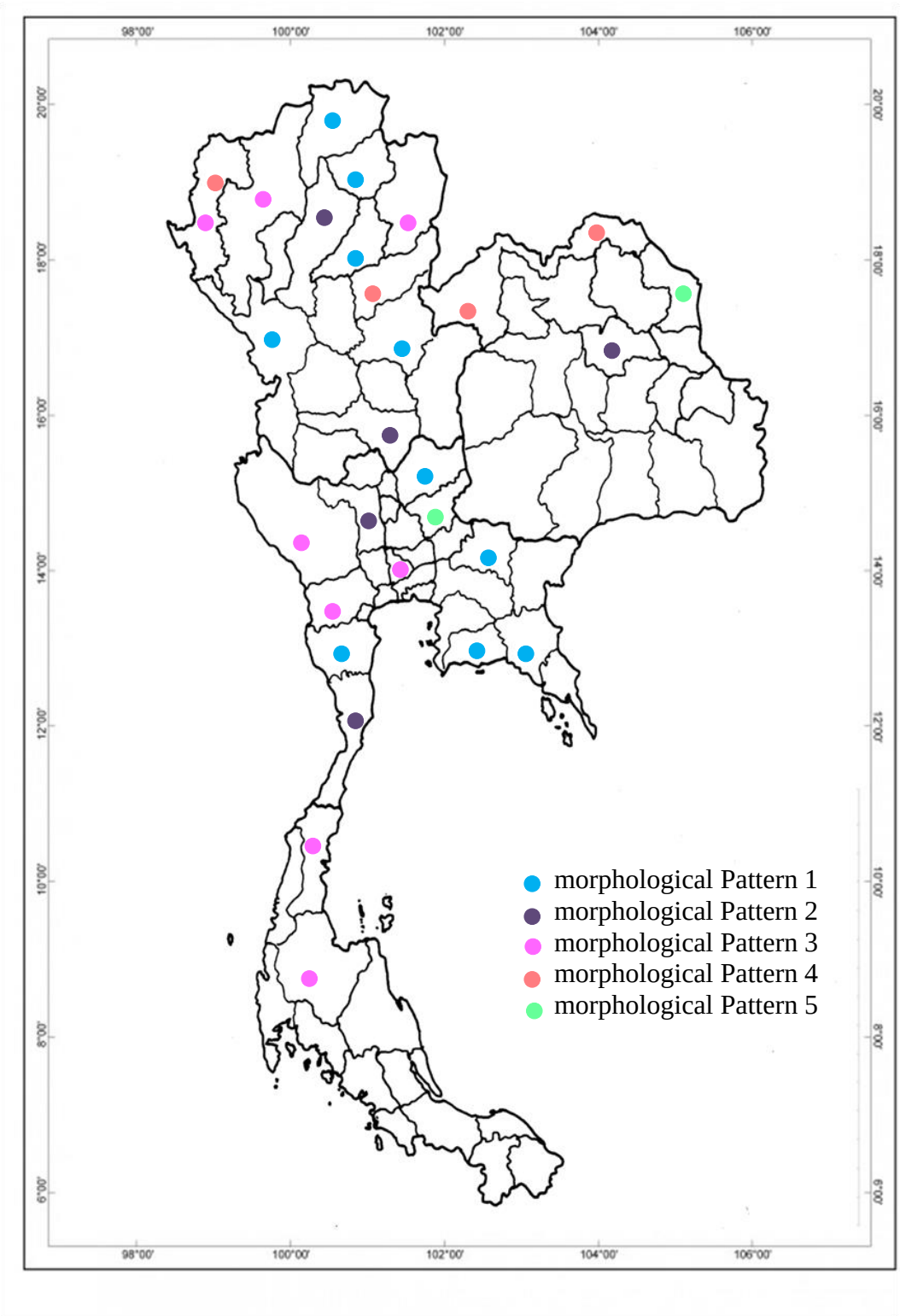

Figure 4-13. Overview of distribution of *Spirogyra* specimens collected from Thailand (The geographic coordinates as described in Table 3-1).

Correlation of biological and ecological parameters

All specimens of *Spirogyra* from each sampling sites were examined biological and ecological parameters. The analysis of biological and ecological parameters was investigated as shown in Table 4-1. The pH, conductivity, TDS, salinity, and DO ranging from 4.09 – 9.04, 113 - 752 µs, 63 – 671 ppm, 0.1 - 0.8, and 5.5 - 11.2 mg/l, respectively. While biological parameters from each *Spirogyra* specimens were comprised cell width, cell length, number of spiral chloroplast and number of chloroplast granules, its ranging from 40 - 90 µm, 85 - 263 µm, 5.5 - 16 spiral, and 50 -240 granules, respectively.

For the study of correlation coefficient analysis, the result shown that the conductivity showed significantly related with the number of chloroplast granule with relative values (r) = 0.571 (p < 0.01). While DO negative related with the number of chloroplast spiral with relative values (r) = - 0.443 (p <0.01) (Figure 4-14).

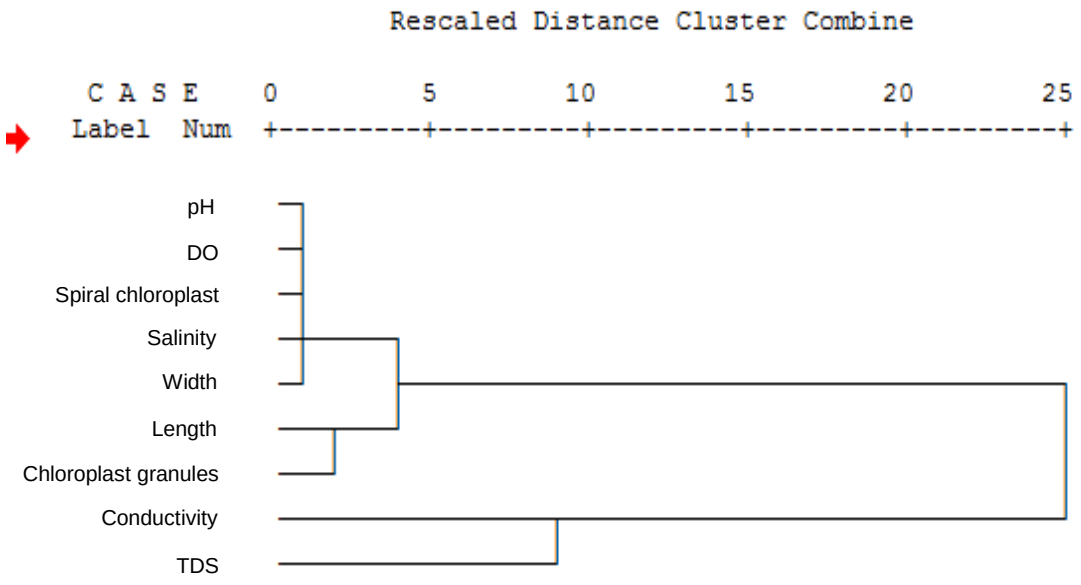

Figure 4-14. Dendrogram from hierarchical cluster analysis demonstrated correlation of each parameters

Table 4-3. The range of ecological and biological parameters were investigated.

Type of parameters	Parameters	Value
Biological	Cell width	40 - 90 μm
	Cell length	85 - 263 μm
	Number of chloroplast sprirals	5.5 - 16
	Number of pyrenoids	50 -240
Ecological	pH	4.09 – 9.04
	Conductivity	113 - 752 μs
	TDS	63 – 671 ppm
	DO	5.5 - 11.2 mg/l
	Salinity	0.1-0.8

Molecular studies

ISSR-PCR

The conditions of PCR amplification such as the concentration of DNA template, *Taq* DNA polymerase, $MgCl_2$, and annealing temperature are very crucial for molecular analysis. The genomic DNA concentration 50 ng/μl was found to be optimal for PCR amplification.

The ten ISSR primers including UBC 809, UBC 826, UBC 835, UBC 808, UBC 825, UBC 827, UBC 864, UBC 857, UBC 880 and UBC 807 preliminarily screened for totally 36 *Spirogyra* specimens generated 111 PCR fragments with sizes ranging from 130 to 2850 base pair (bp). The number of polymorphic bands generated varied between 3 and 16 bands, with an average of 12 bands per one primer. Of them,

five RAPD primers (O 15, O 16, V 14, V 15 and B 01) were found generating highly polymorphic and reproducible band (see Appendix A). They were then selected to investigate the relationship of each locality of *Spirogyra*.

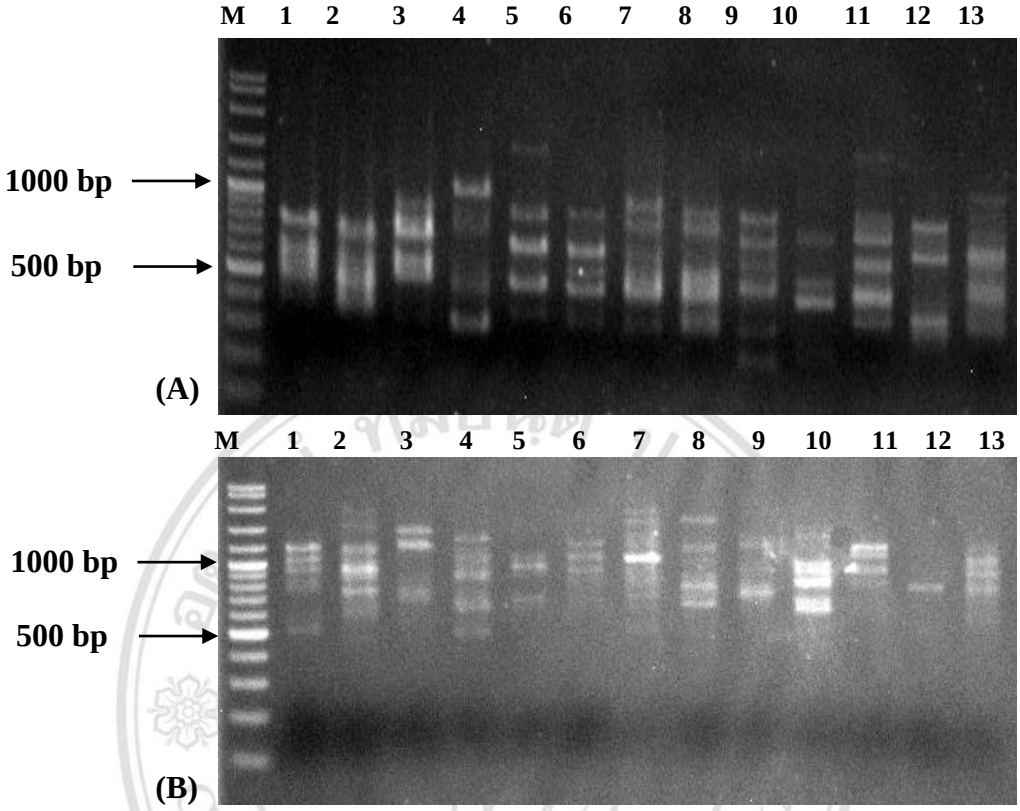

Figure 4-15. ISSR-PCR profiles of *Spirogyra* form northern Thailand generated by (A) UBC 835 and (B) UBC 826 primer**.** (Lane M: 100 bp DNA marker, lane 1: Lamphun, lane 2: Lampang-1, lane 3: Lampang-2, lane 4: Chiang Mai, lane 5: Phrae, lane 6: Mae Hong Son-1, lane 7: Mae Hong Son-2, lane 8: Nan-1, lane 9: Nan-2, lane 10: Phayao, lane 11: Chiang Rai, lane 12: Uttaradit, lane 13: Tak)

The UBC 835 primer can be generated the PCR fragment ranging from 200 - 1500 bp, while UBC 826 primer can be generated the PCR fragment ranging from 500 -1500 bp. The number of polymorphic fragment from UBC 835 primer and UBC 826 primer generated 16 and 9 fragments, respectively.

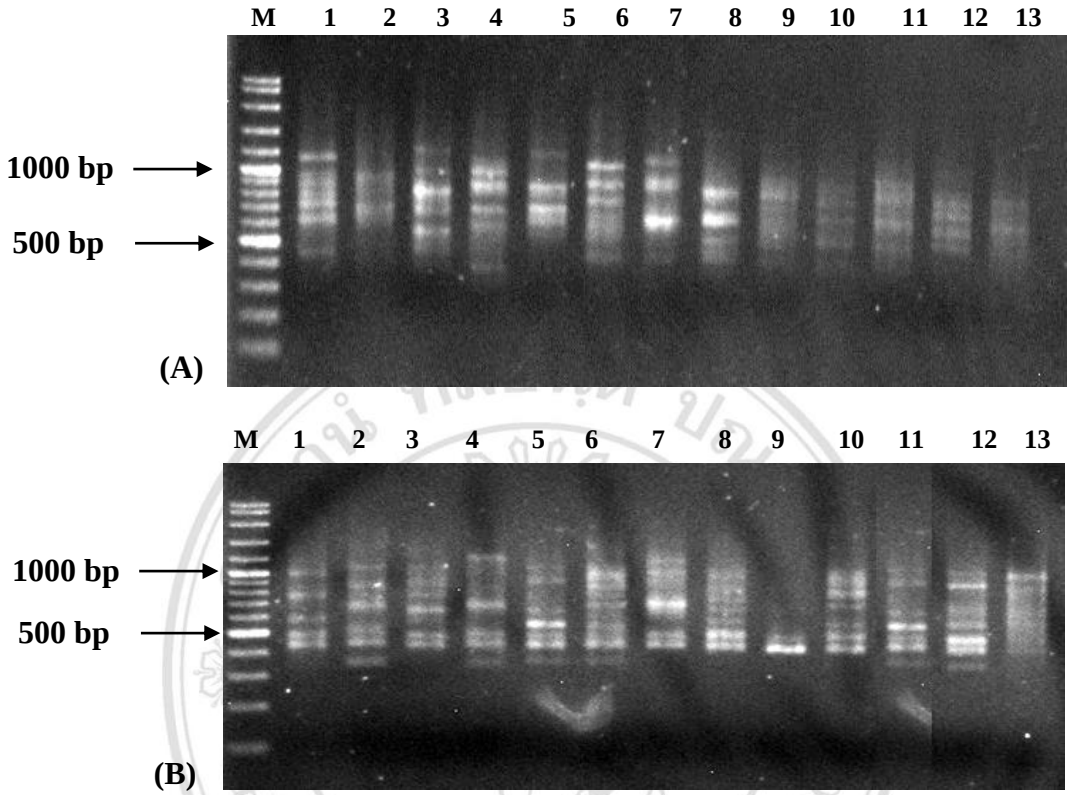

Figure 4-16. ISSR-PCR profiles of *Spirogyra* from northern Thailand generated by (A) UBC 809 and (B) UBC 808 primer**.** (Lane M: 100 bp DNA marker, lane 1: Lamphun, lane 2: Lampang-1, lane 3: Lampang-2, lane 4: Chiang Mai, lane 5: Phrae, lane 6: Mae Hong Son-1, lane 7: Mae Hong Son-2, lane 8: Nan-1, lane 9: Nan-2, lane 10: Phayao, lane 11: Chiang Rai, lane 12: Uttaradit, lane 13: Tak)

The UBC 809 primer can be generated the PCR fragment ranging from 500 - 1000 bp, while UBC 808 primer can be generated the PCR fragment ranging from 400 -1200 bp. The number of polymorphic fragment from UBC 809 primer and UBC 808 primer generated 7 and 9 fragments, respectively.

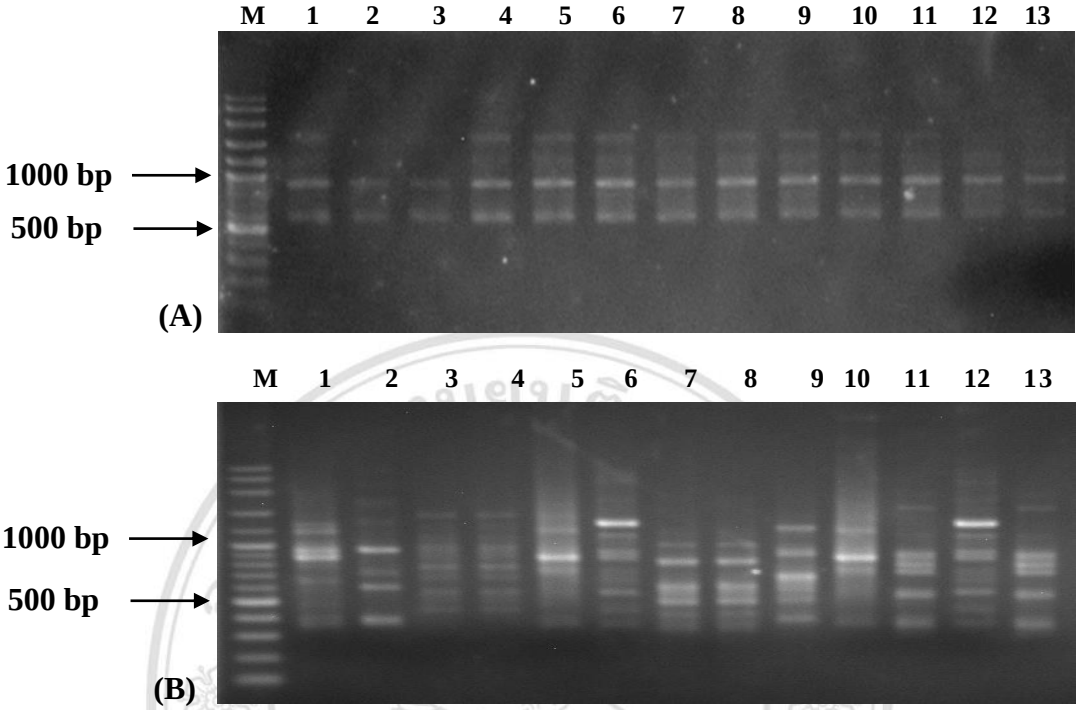

Figure 4-17. ISSR-PCR profiles of *Spirogyra* from northern Thailand generated by (A) UBC 825 and (B) UBC 827 primer**.** (Lane M: 100 bp DNA marker, lane 1: Lamphun, lane 2: Lampang-1, lane 3: Lampang-2, lane 4: Chiang Mai, lane 5: Phrae, lane 6: Mae Hong Son-1, lane 7: Mae Hong Son-2, lane 8: Nan-1, lane 9: Nan-2, lane 10: Phayao, lane 11: Chiang Rai, lane 12: Uttaradit, lane 13: Tak)

The UBC 825 primer can be generated the PCR fragment ranging from 600 - 1750 bp, while UBC 827 primer can be generated the PCR fragment ranging from 500 -1500 bp. The number of polymorphic fragment from UBC 825 primer and UBC 827 primer generated 3 and 7 fragments, respectively.

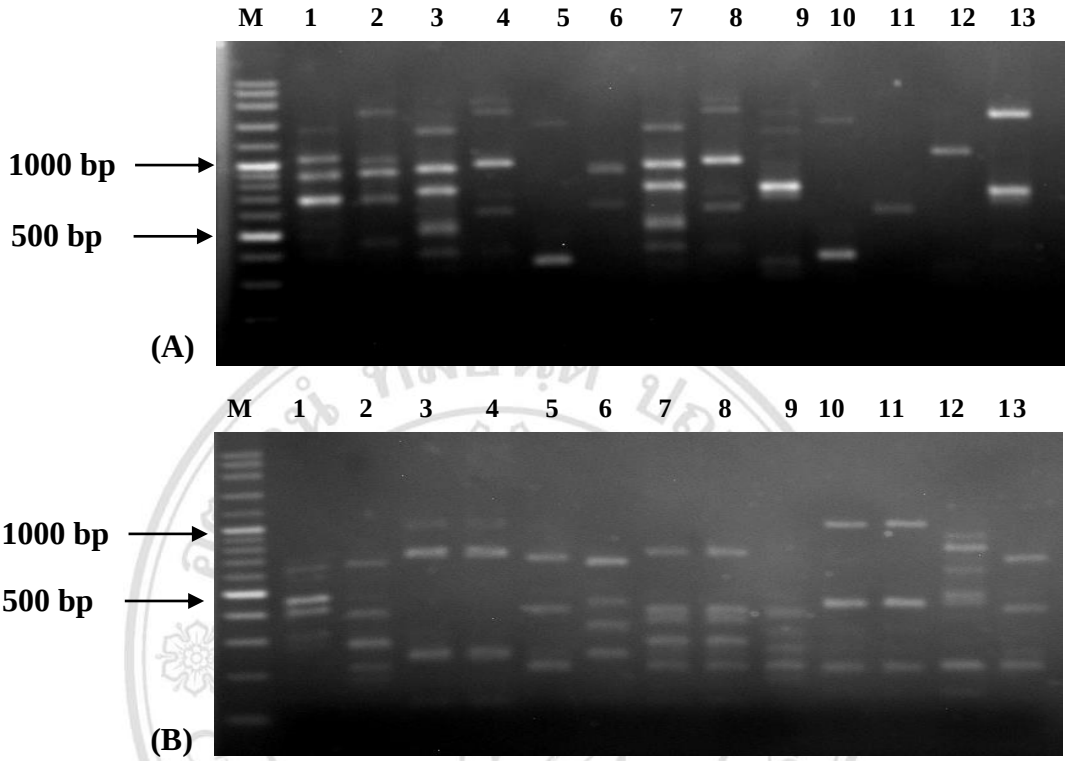

Figure 4-18. ISSR-PCR profiles of *Spirogyra* from northern Thailand generated by (A) UBC 864 and (B) UBC 807 primer**.** (Lane M: 100 bp DNA marker, lane 1: Lamphun, lane 2: Lampang-1, lane 3: Lampang-2, lane 4: Chiang Mai, lane 5: Phrae, lane 6: Mae Hong Son-1, lane 7: Mae Hong Son-2, lane 8: Nan-1, lane 9: Nan-2, lane 10: Phayao, lane 11: Chiang Rai, lane 12: Uttaradit, lane 13: Tak)

The UBC 864 primer can be generated the PCR fragment ranging from 300 - 2000 bp, while UBC 807 primer can be generated the PCR fragment ranging from 150 - 1000 bp. The number of polymorphic fragment from UBC 864 primer and UBC 807 primer generated 9 and 11 fragments, respectively.

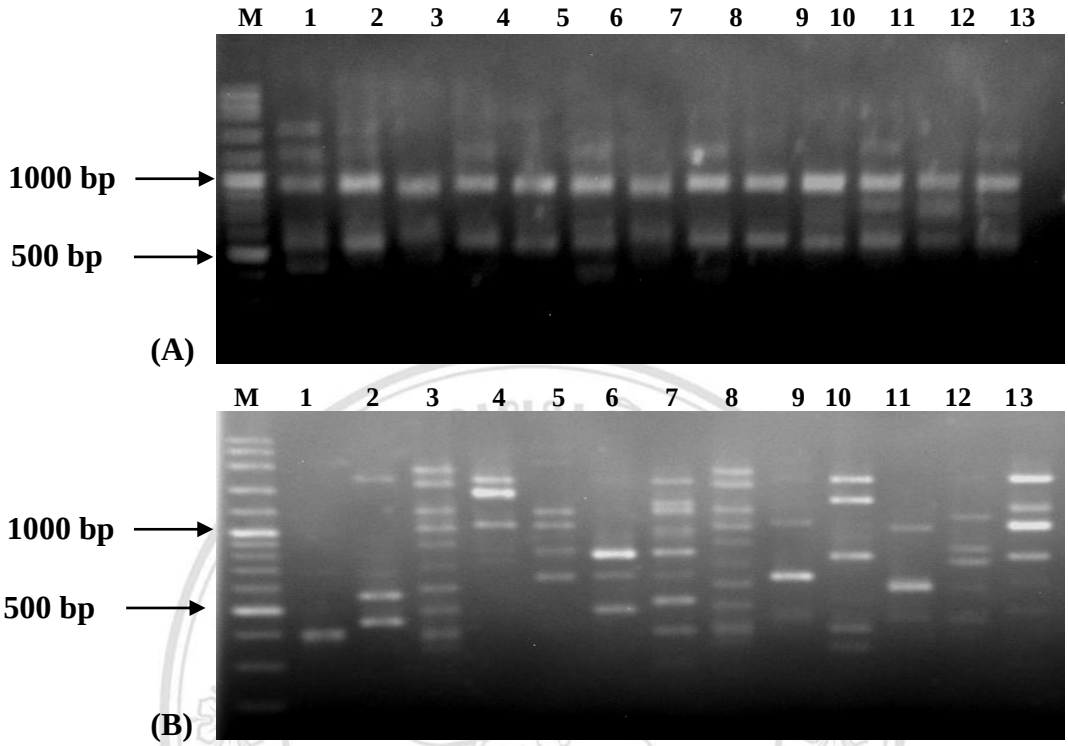

Figure 4-19. ISSR-PCR profiles of *Spirogyra* from northern Thailand generated by (A) UBC 857 and (B) UBC 880 primer**.** (Lane M: 100 bp DNA marker, lane 1: Lamphun, lane 2: Lampang-1, lane 3: Lampang-2, lane 4: Chiang Mai, lane 5: Phrae, lane 6: Mae Hong Son-1, lane 7: Mae Hong Son-2, lane 8: Nan-1, lane 9: Nan-2, lane 10: Phayao, lane 11: Chiang Rai, lane 12: Uttaradit, lane 13: Tak)

The UBC 857 primer can be generated the PCR fragment ranging from 400 - 1700 bp, while UBC 880 primer can be generated the PCR fragment ranging from 400 - 1700 bp. The number of polymorphic fragment from UBC 857 primer and UBC 880 primer generated 5 and 9 fragments, respectively.

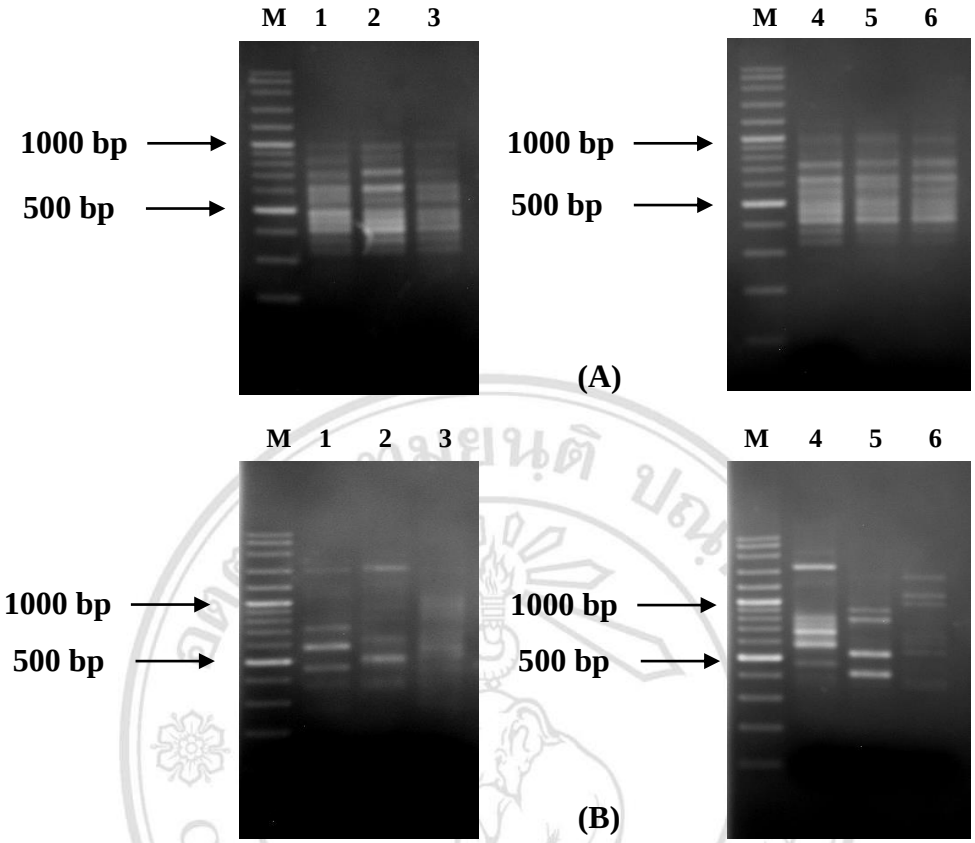

Figure 4-20. ISSR-PCR profiles of *Spirogyra* from southern and eastern Thailand generated by (A) UBC 835 and (B) UBC 826 primer**.** (Lane M: 100 bp DNA marker, lane 1: Prachuap Kriri Khan, lane 2: Surat Thani , lane 3: Chumphon, lane 4: Rayong, lane 5: Chanthaburi, lane 6: Chon Buri)

The UBC 835 primer can be generated the PCR fragment ranging from 230 - 1000 bp, while UBC 826 primer can be generated the PCR fragment ranging from 400 -1500 bp. The number of polymorphic fragment from UBC 835 primer and UBC 826 primer generated 7 and 12 fragments, respectively.

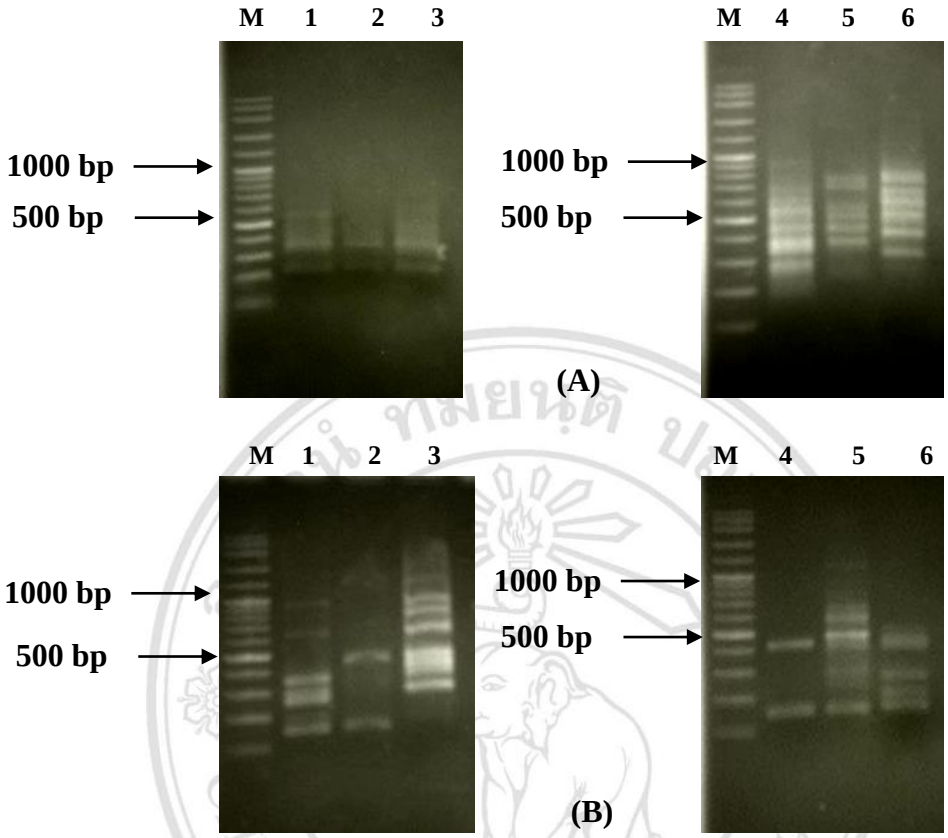

Figure 4-21. ISSR-PCR profiles of *Spirogyra* from southern and eastern Thailand generated by (A) UBC 809 and (B) UBC 808 primer**.** (Lane M: 100 bp DNA marker, lane 1: Prachuap Khiri Khan, lane 2: Surat Thani , lane 3: Chumphon, lane 4: Rayong, lane 5: Chanthaburi, lane 6: Chon Buri).

The UBC 809 primer can be generated the PCR fragment ranging from 200 - 900 bp, while UBC 808 primer can be generated the PCR fragment ranging from 150 -1200 bp. The number of polymorphic fragment from UBC 809 primer and UBC 808 primer generated 12 and 15 fragments, respectively.

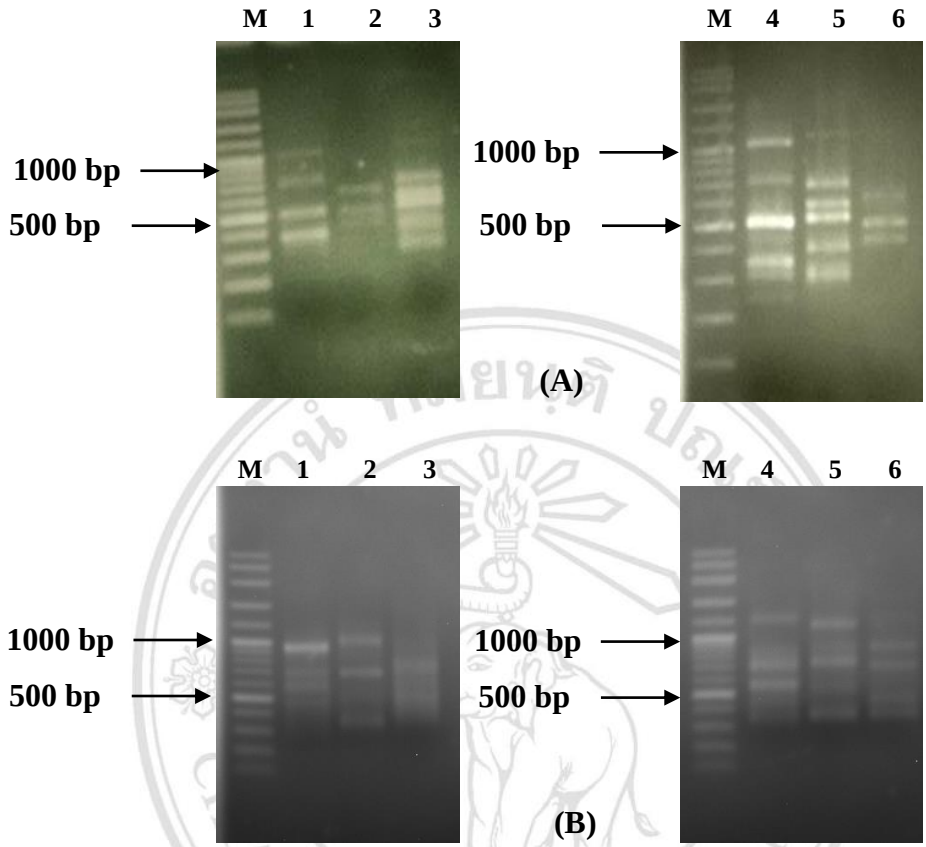

Figure 4-22. ISSR-PCR profiles of *Spirogyra* from southern and eastern Thailand generated by (A) UBC 825 and (B) UBC 827 primer**.** (Lane M: 100 bp DNA marker, lane 1: Prachuap Khiri Khan, lane 2: Surat Thani, lane 3: Chumphon, lane 4: Rayong, lane 5: Chanthaburi, lane 6: Chon Buri)

The UBC 825 primer can be generated the PCR fragment ranging from 250 - 1000 bp, while UBC 827 primer can be generated the PCR fragment ranging from 300 - 1200 bp. The number of polymorphic fragment from UBC 825 primer and UBC 827 primer generated 10 and 6 fragments, respectively.

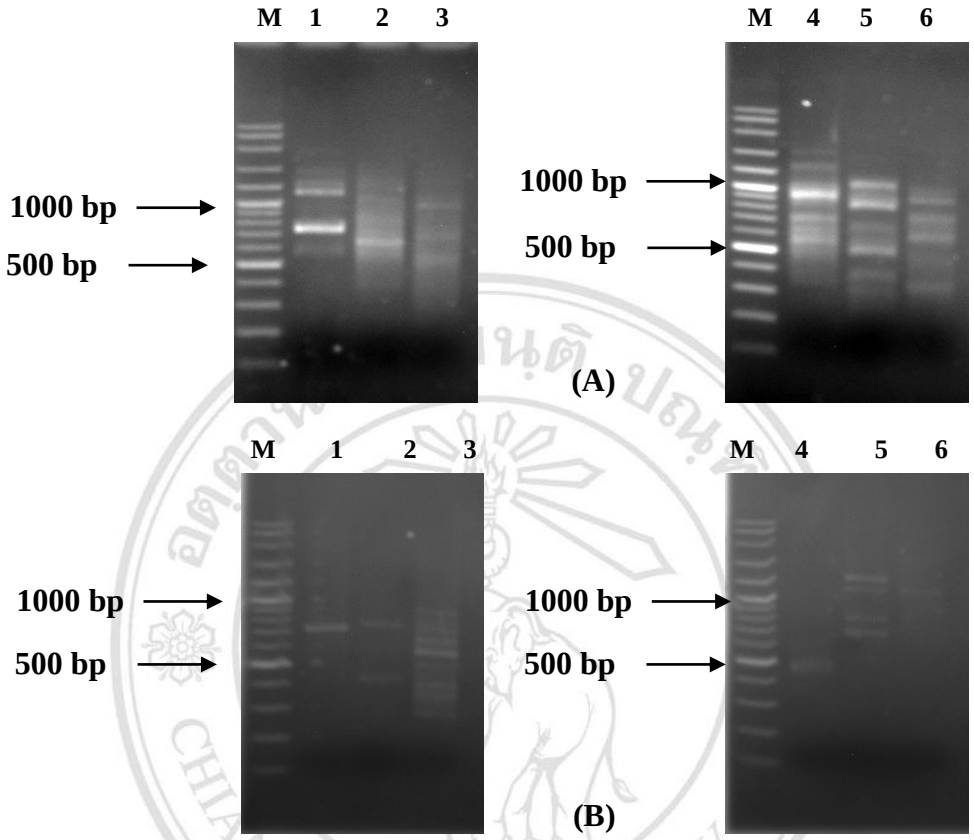

Figure 4-23. ISSR-PCR profiles of *Spirogyra* from southern and eastern Thailand generated by (A) UBC 864 and (B) UBC 884 primer**.** (Lane M: 100 bp DNA marker, lane 1: Prachuap Khiri Khan, lane 2: Surat Thani , lane 3: Chumphon, lane 4: Rayong, lane 5: Chanthaburi, lane 6: Chon Buri)

The UBC 864 primer can be generated the PCR fragment ranging from 300 - 1500 bp, while UBC 844 primer can be generated the PCR fragment ranging from 300 - 1050 bp. The number of polymorphic fragment from UBC 864 primer and UBC 844 primer generated 11 and 10 fragments, respectively.

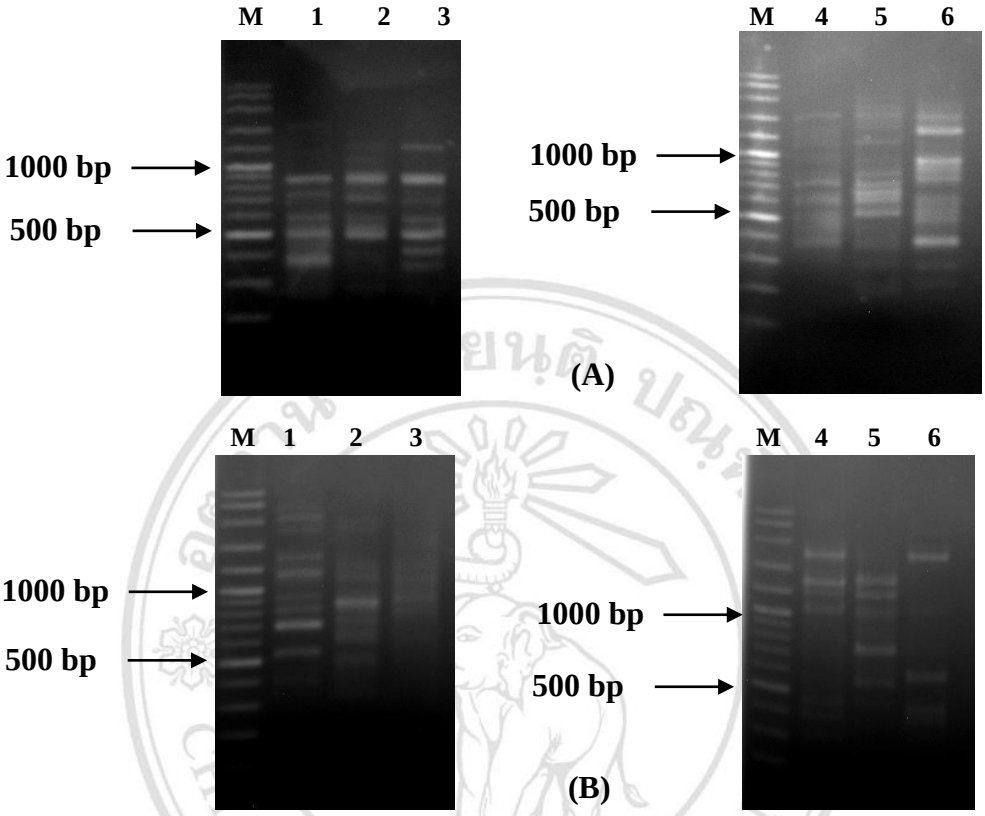

Figure 4-24. ISSR-PCR profiles of *Spirogyra* from southern and eastern Thailand generated by (A) UBC 857 and (B) UBC 880 primer. (Lane M: 100 bp DNA marker, lane 1: Prachuap Khiri Khan, lane 2: Surat Thani , lane 3: Chumphon, lane 4: Rayong, lane 5: Chanthaburi, lane 6: Chon Buri)

The UBC 857 primer can be generated the PCR fragment ranging from 250 - 1500 bp, while UBC 880 primer can be generated the PCR fragment ranging from 250 - 2700 bp. The number of polymorphic fragment from UBC 857 primer and UBC 880 primer generated 8 and 12 fragments, respectively.

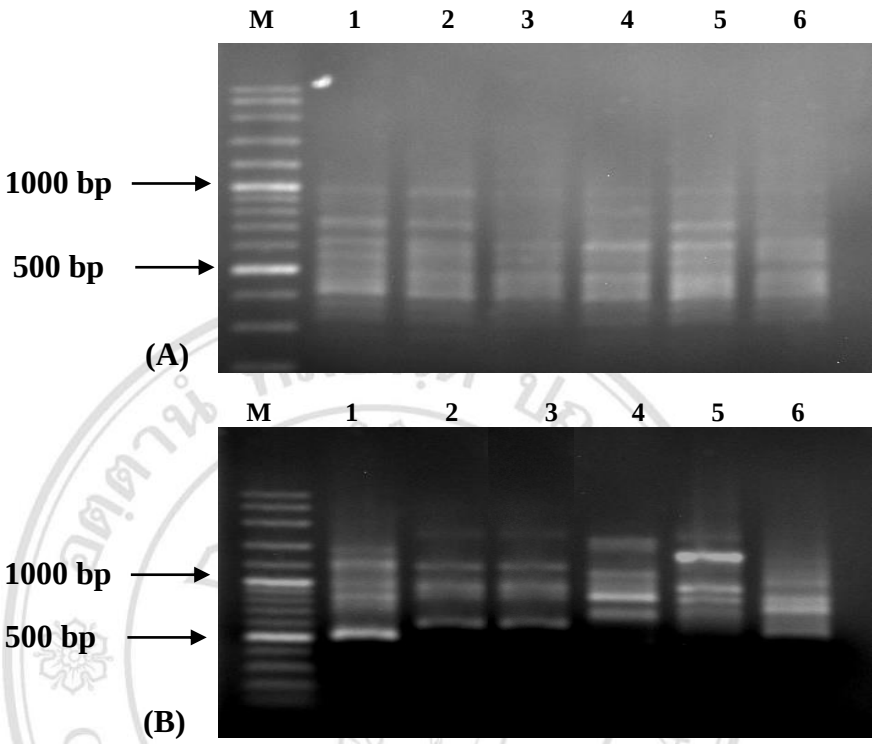

Figure 4-25. ISSR-PCR profiles of *Spirogyra* from northeastern Thailand generated by (A) UBC 835 and (B) UBC 826 primer**.** (Lane M: 100 bp DNA marker, lane 1: Kalasin, lane 2: Maha Sarakham, lane 3: Mukdahan, lane 4: Loei , lane 5: Nakhon Phanom, lane 6: Udon Thani)

The UBC 835 primer can be generated the PCR fragment ranging from 300 - 1000 bp, while UBC 826 primer can be generated the PCR fragment ranging from 550 - 1700 bp. The number of polymorphic fragment from UBC 835 primer and UBC 826 primer generated 5 and 7 fragments, respectively.

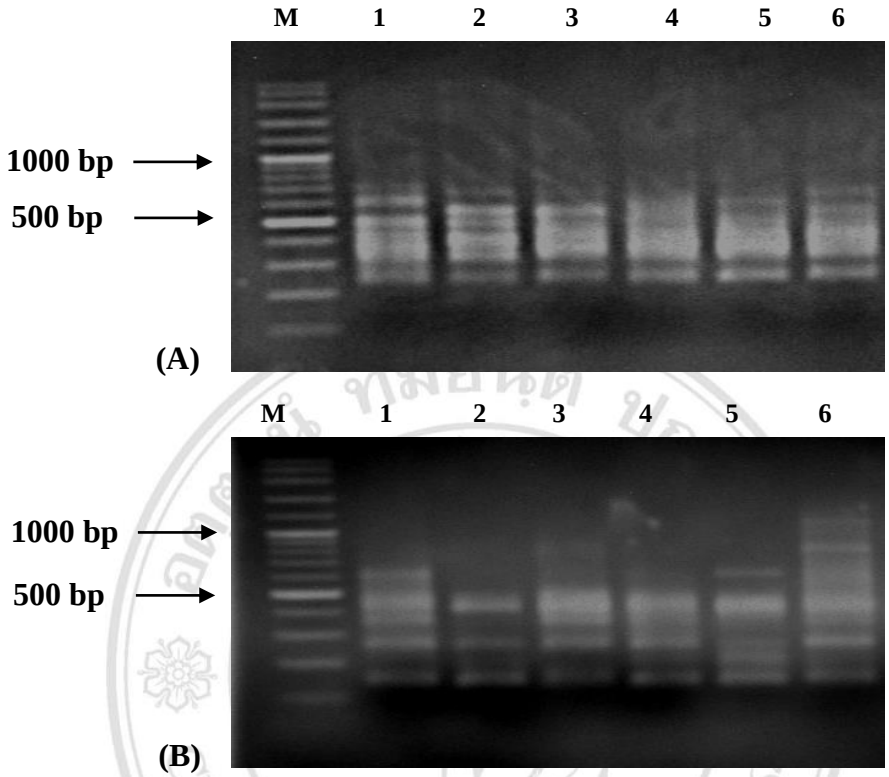

Figure 4-26. ISSR-PCR profiles of *Spirogyra* from northeastern Thailand generated by (A) UBC 809 and (B) UBC 808 primer. (Lane M: 100 bp DNA marker, lane 1: Kalasin, lane 2: Maha Sarakham, lane 3: Mukdahan, lane 4: Loei, lane 5: Nakhon Phanom, lane 6: Udon Thani)

The UBC 809 primer can be generated the PCR fragment ranging from 230 - 700 bp, while UBC 808 primer can be generated the PCR fragment ranging from 130 - 1500 bp. The number of polymorphic fragment from UBC 809 primer and UBC 808 primer generated 6 and 9 fragments, respectively.

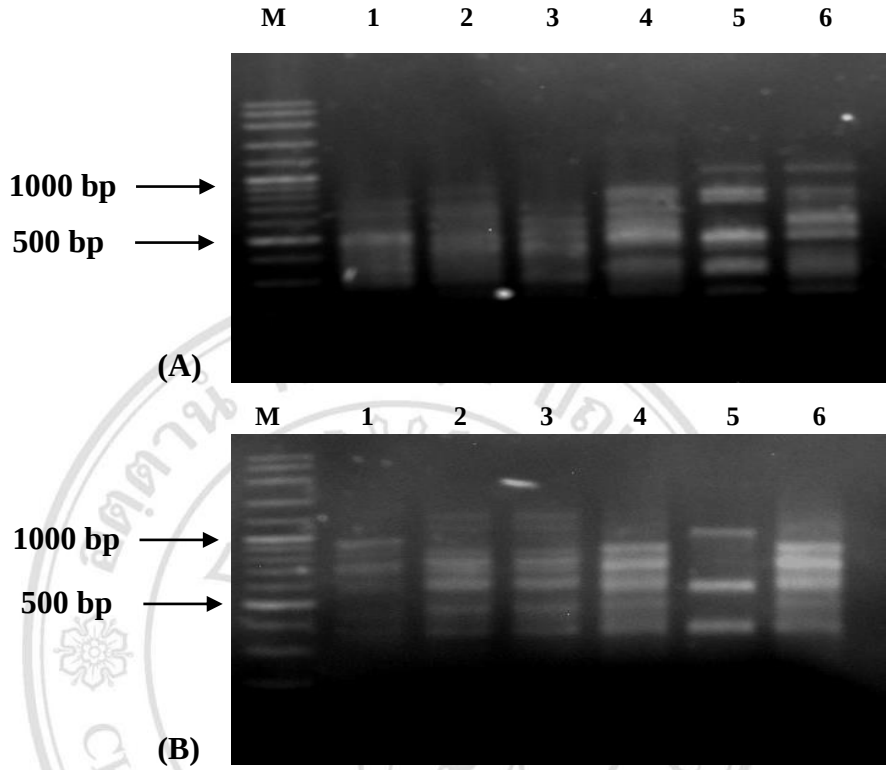

Figure 4-27. ISSR-PCR profiles of *Spirogyra* from northeast Thailand generated by (A) UBC 825 and (B) UBC 827 primer. (Lane M: 100 bp DNA marker, lane 1: Kalasin, lane 2: Maha Sarakham, lane 3: Mukdahan, lane 4: Loei, lane 5: Nakhon Phanom, lane 6: Udon Thani)

The UBC 825 primer can be generated the PCR fragment ranging from 250 - 1200 bp, while UBC 827 primer can be generated the PCR fragment ranging from 350 - 1300 bp. The number of polymorphic fragment from UBC 825 primer and UBC 827 primer generated 6 and 7 fragments, respectively.

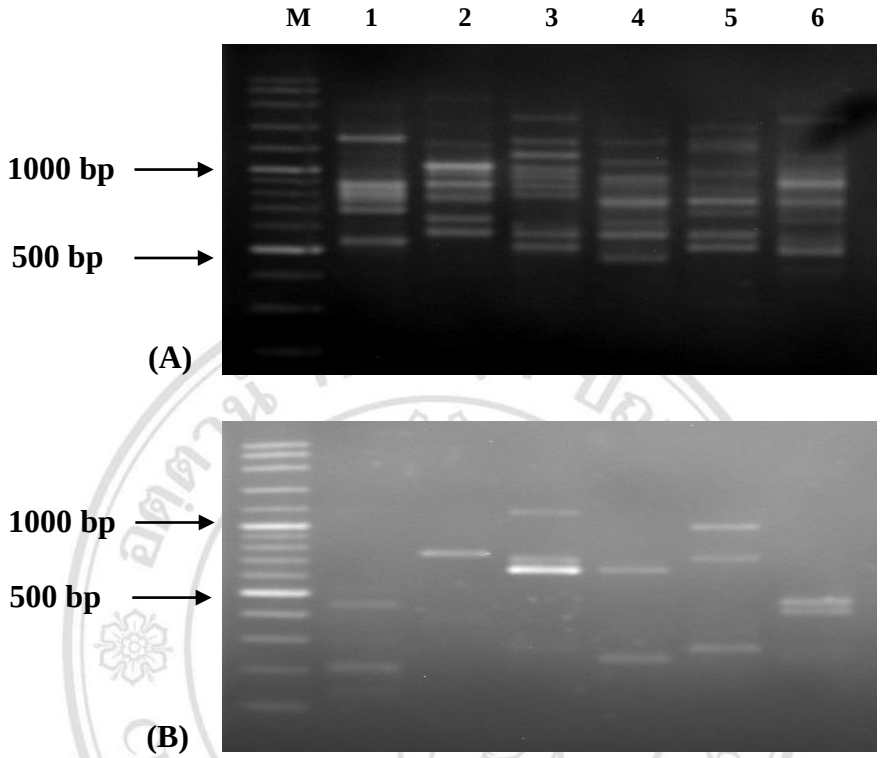

Figure 4-28. ISSR-PCR profiles of *Spirogyra* from northeastern Thailand generated by (A) UBC 864 and (B) UBC 807 primer**.** (Lane M: 100 bp DNA marker, lane 1: Kalasin, lane 2: Maha Sarakham, lane 3: Mukdahan, lane 4: Loei, lane 5: Nakhon Phanom, lane 6: Udon Thani)

The UBC 864 primer can be generated the PCR fragment ranging from 480 - 1850 bp, while UBC 844 primer can be generated the PCR fragment ranging from 150 - 1100 bp. The number of polymorphic fragment from UBC 864 primer and UBC 807 primer generated 14 and 11 fragments, respectively.

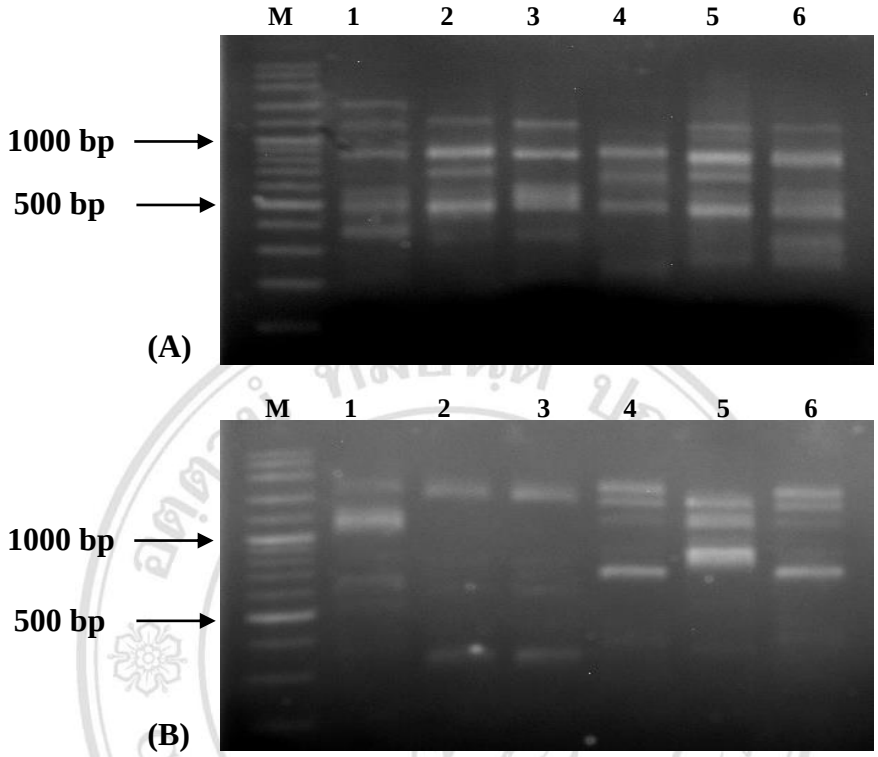

Figure 4-.29 ISSR-PCR profiles of *Spirogyra* from northeastern Thailand generated by (A) UBC 857 and (B) UBC 880 primer**.** (Lane M: 100 bp DNA marker, lane 1: Kalasin, lane 2: Maha Sarakham, lane 3: Mukdahan, lane 4: Loei, lane 5: Nakhon Phanom, lane 6: Udon Thani)

The UBC 857 primer can be generated the PCR fragment ranging from 350 - 1500 bp, while UBC 880 primer can be generated the PCR fragment ranging from 240 - 1700 bp. The number of polymorphic fragment from UBC 857 primer and UBC 880 primer generated 7 and 6 fragments, respectively.

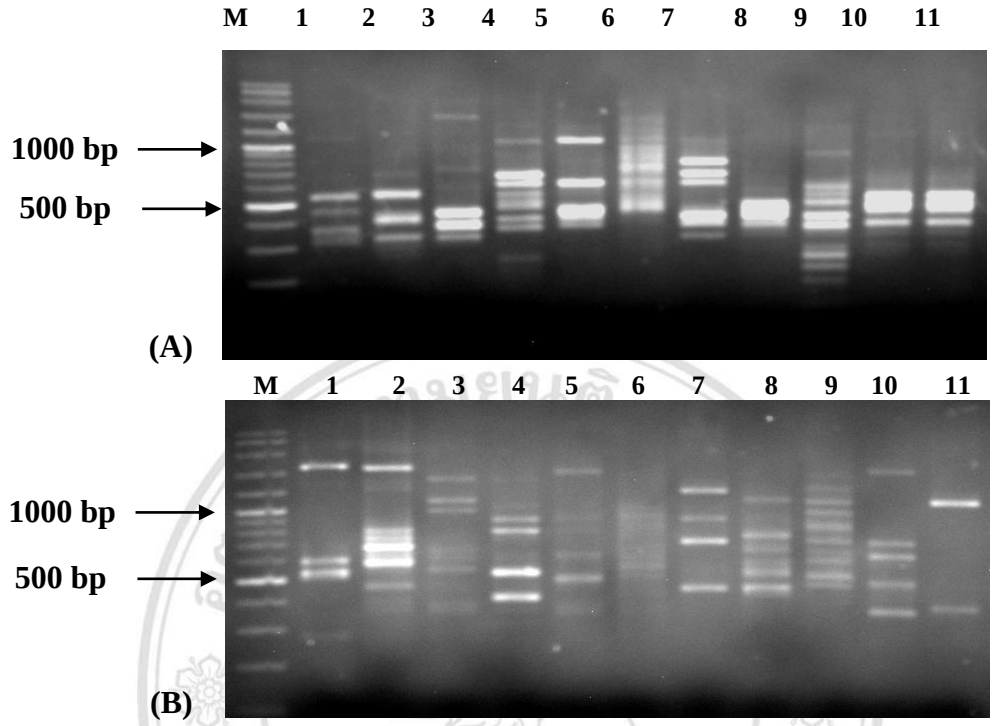

Figure 4-30. ISSR-PCR profiles of *Spirogyra* from central Thailand generated by (A) UBC 835 and (B) UBC 826 primer**.** (Lane M: 100 bp DNA marker, lane 1: Nakhon Sawan, lane 2: Suphan Buri, lane 3: Kanchanaburi, lane 4: Ratchaburi, lane 5: Phetchaburi, lane 6: Prachin Buri, lane 7: Lop Buri, lane 8: Saraburi, lane 9: Chai Nat, lane 10: Ang Thong, lane 11: Phitsanulok)

The UBC 835 primer can be generated the PCR fragment ranging from 400 - 900 bp, while UBC 826 primer can be generated the PCR fragment ranging from 300 - 2250 bp. The number of polymorphic fragment from UBC 835 primer and UBC 826 primer generated 5 and 13 fragments, respectively.

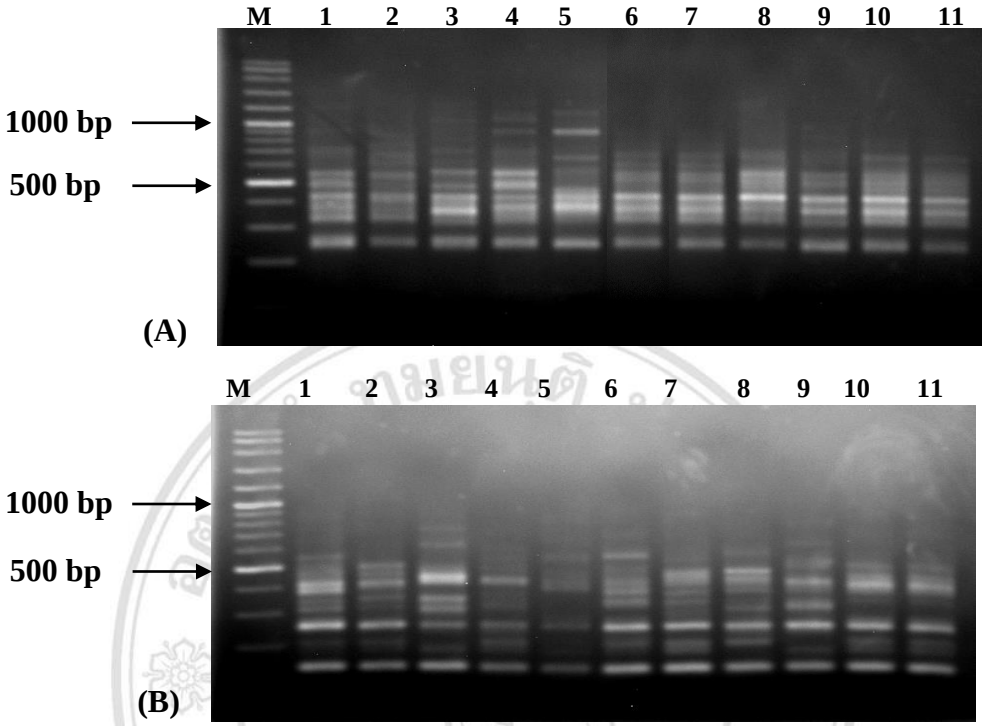

Figure 4-.31 ISSR-PCR profiles of *Spirogyra* from central Thailand generated by (A) UBC 809 and (B) UBC 808 primer**.** (Lane M: 100 bp DNA marker, lane 1: Nakhon Sawan, lane 2: Suphan Buri, lane 3: Kanchanaburi, lane 4: Ratchaburi, lane 5: Phetchaburi, lane 6: Prachin Buri, lane 7: Lop Buri, lane 8: Saraburi, lane 9: Chai Nat, lane 10: Ang Thong, lane 11: Phitsanulok)

The UBC 809 primer can be generated the PCR fragment ranging from 250 - 1150 bp, while UBC 808 primer can be generated the PCR fragment ranging from 150 - 650 bp. The number of polymorphic fragment from UBC 809 primer and UBC 808 primer generated 8 and 8 fragments, respectively.

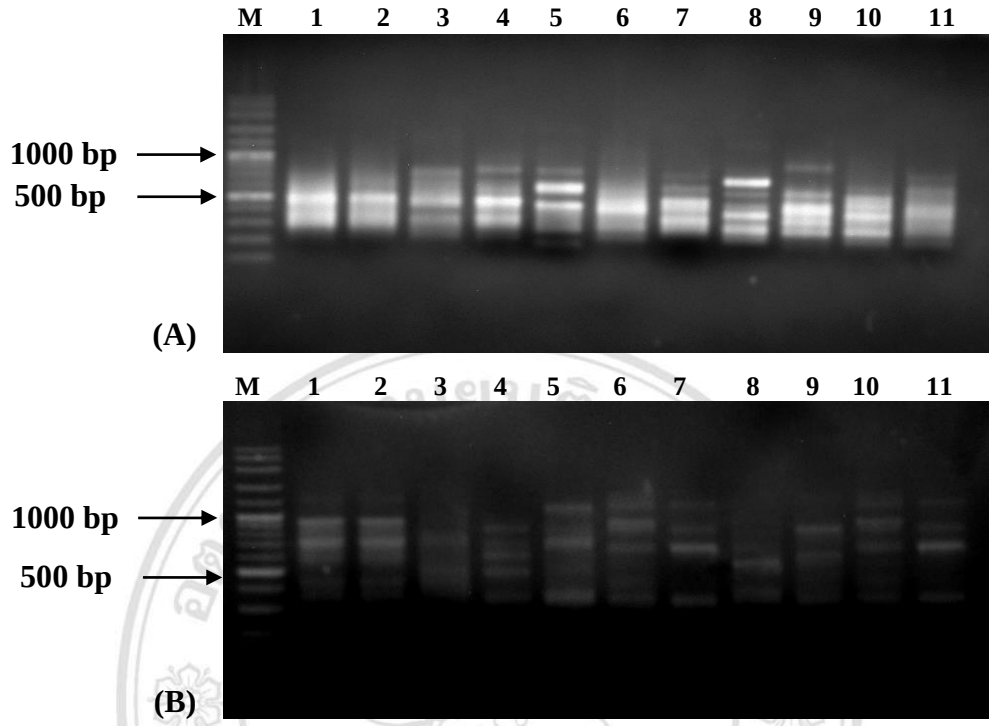

Figure 4-32. ISSR-PCR profiles of *Spirogyra* from central Thailand generated by (A) UBC 825 and (B) UBC 827 primer**.** (Lane M: 100 bp DNA marker, lane 1: Nakhon Sawan, lane 2: Suphan Buri, lane 3: Kanchanaburi, lane 4: Ratchaburi, lane 5: Phetchaburi, lane 6: Prachin Buri, lane 7: Lop Buri, lane 8: Saraburi, lane 9: Chai Nat, lane 10: Ang Thong, lane 11: Phitsanulok)

The UBC 825 primer can be generated the PCR fragment ranging from 200 - 700 bp, while UBC 827 primer can be generated the PCR fragment ranging from 300 - 1200 bp. The number of polymorphic fragment from UBC 825 primer and UBC 827 primer generated 6 and 7 fragments, respectively.

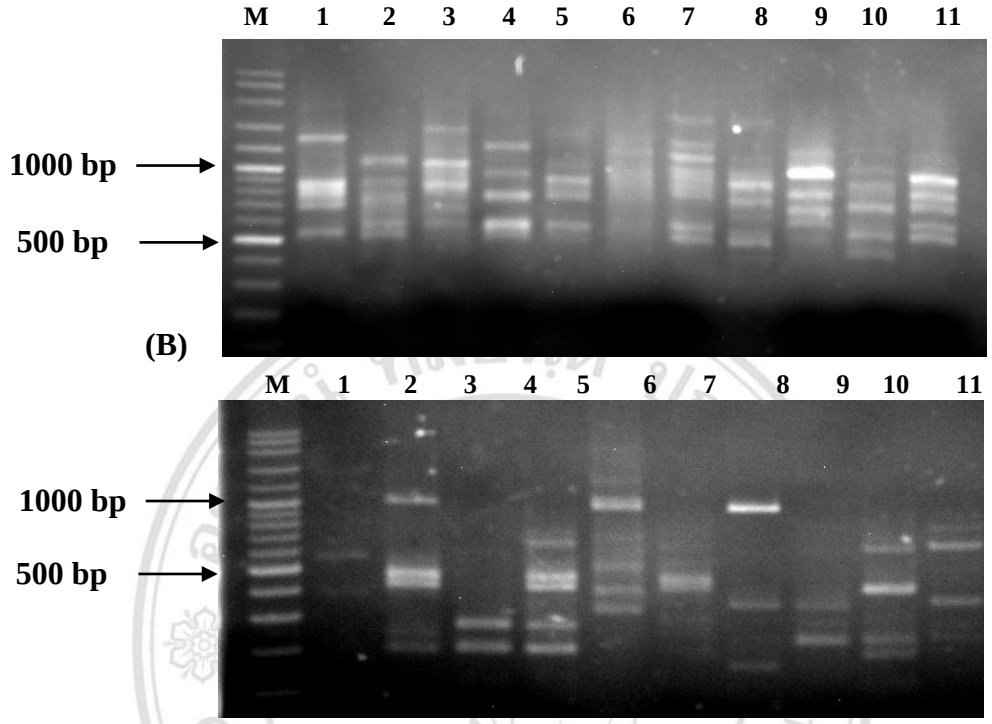

Figure 4-33. ISSR-PCR profiles of *Spirogyra* from central Thailand generated by (A) UBC 864 and (B) UBC 807 primer**.** (Lane M: 100 bp DNA marker, lane 1: Nakhon Sawan, lane 2: Suphan Buri, lane 3: Kanchanaburi, lane 4: Ratchaburi, lane 5: Phetchaburi, lane 6: Prachin Buri, lane 7: Lop Buri, lane 8: Saraburi, lane 9: Chai Nat, lane 10: Ang Thong, lane 11: Phitsanulok)

The UBC 864 primer can be generated the PCR fragment ranging from 450 - 1500 bp, while UBC 807 primer can be generated the PCR fragment ranging from 150 - 1000 bp. The number of polymorphic fragment from UBC 864 primer and UBC 807 primer generated 7 and 11 fragments, respectively.

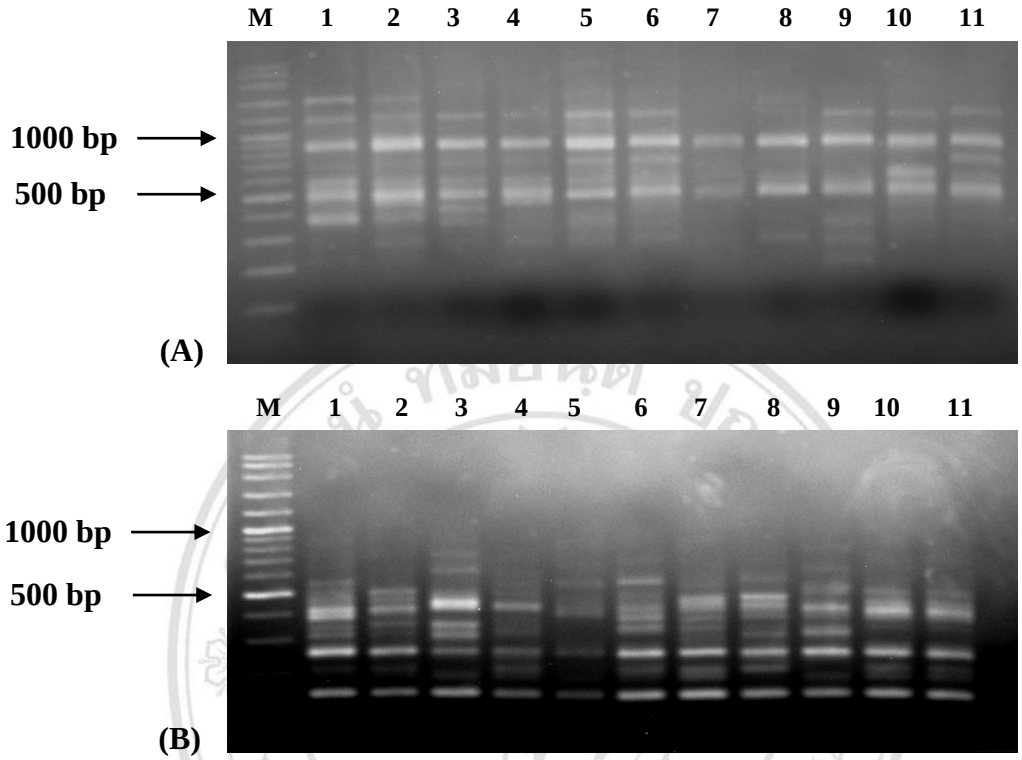

Figure 4-34. ISSR-PCR profiles of *Spirogyra* from Central Thailand generated by (A) UBC 857 and (B) UBC 880 primer. (Lane M: 100 bp DNA marker, lane 1: Nakhon Sawan, lane 2: Suphan Buri, lane 3: Kanchanaburi, lane 4: Ratchaburi, lane 5: Phetchaburi, lane 6: Prachin Buri, lane 7: Lop Buri, lane 8: Saraburi, lane 9: Chai Nat, lane 10: Ang Thong, lane 11: Phitsanulok)

The UBC 857 primer can be generated the PCR fragment ranging from 200 - 1500 bp, while UBC 880 primer can be generated the PCR fragment ranging from 150 - 700 bp. The number of polymorphic fragment from UBC 857 primer and UBC 880 primer generated 9 and 8 fragments, respectively**.**

Ten ISSR primers produced a total of 108 scorable markers. The cluster analysis of ISSR marker separated the *Spirogyra* specimens into 5 distinct clusters including cluster 1: N1-N13, cluster 2: C1-C11 and NE1- NE-6, cluster 3: S1 and E1, cluster 4: S2, S3, and E3, cluster 5: E2 (Figure 4-35).

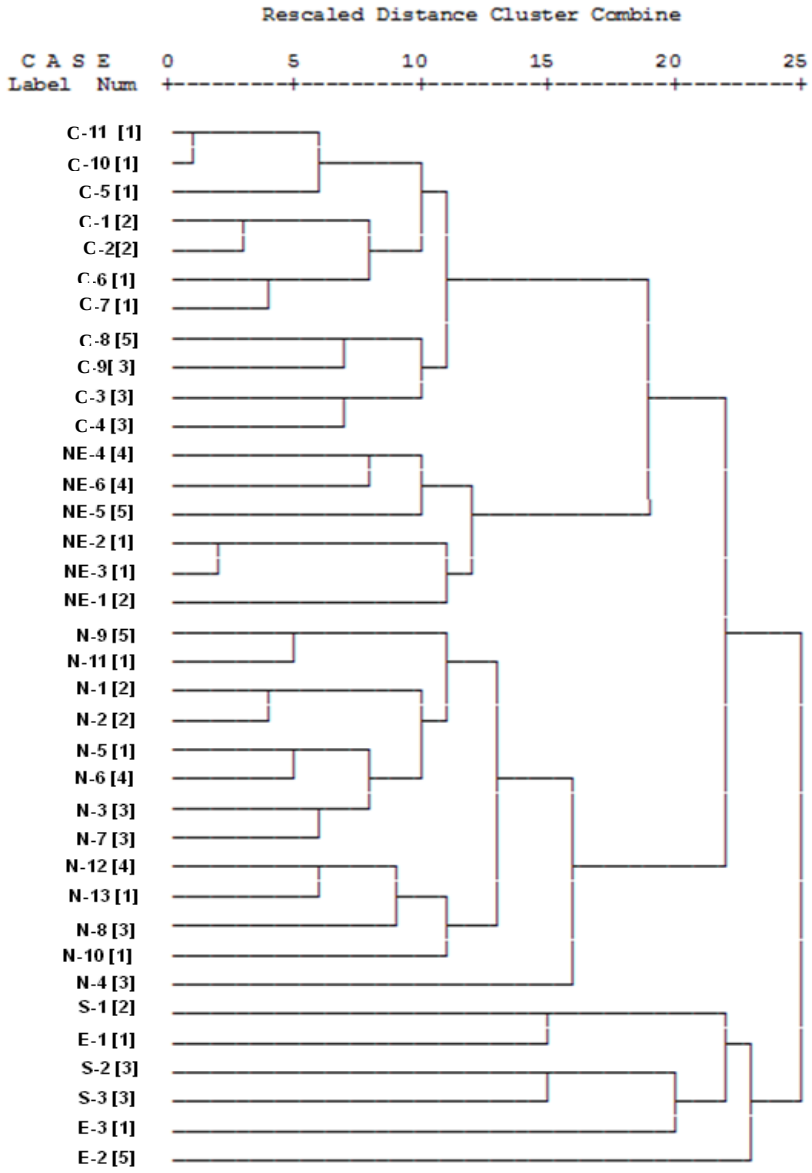

Figure 4-35. Dendrogram illustrating the cluster of *Spirogyra* using ISSR-PCR **(**The number in parentheses is morphological pattern status from each samples) (C: central, N: northern, S: southern, E: eastern, NE: northeastern).

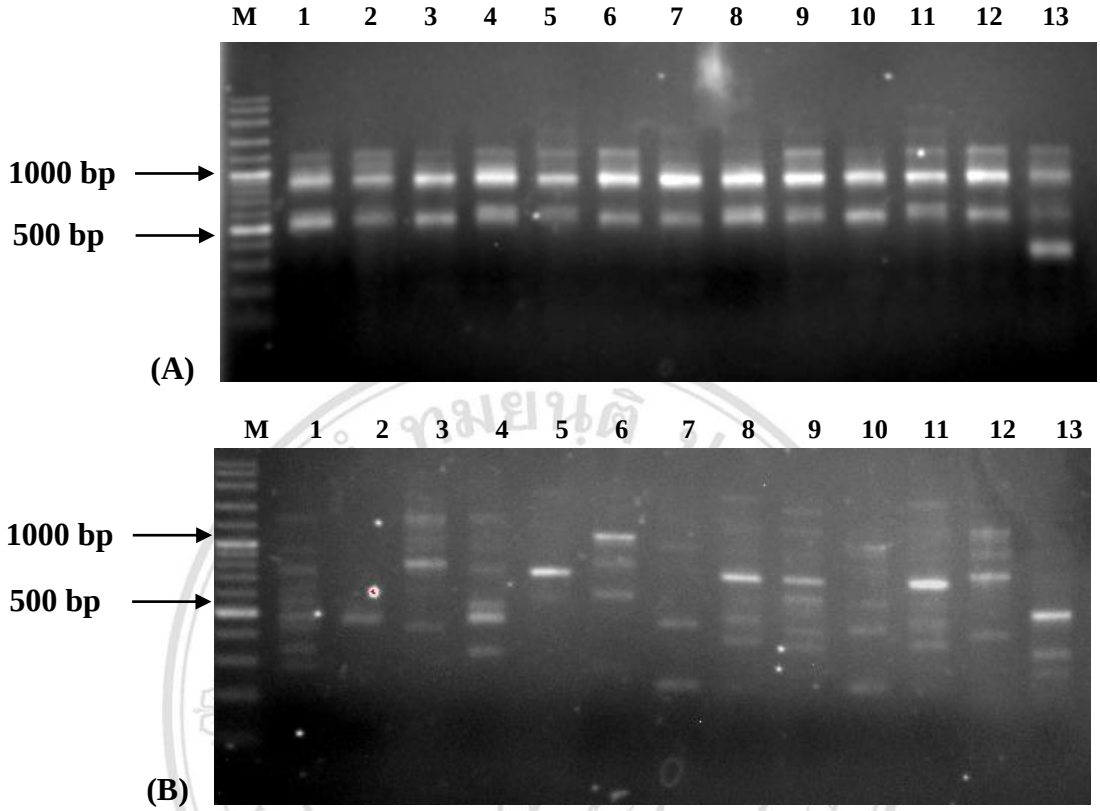

Figure 4-36. HAT-RAPD PCR profiles of *Spirogyra* from northern Thailand generated by (A) O 15 and (B) O 16 primer**.** (Lane M: 100 bp DNA marker, lane 1: Lamphun, lane 2: Lampang-1, lane 3: Lampang-2, lane 4: Chiang Mai, lane 5: Phrae, lane 6: Mae Hong Son-1, lane 7: Mae Hong Son-2, lane 8: Nan-1, lane 9: Nan-2, lane 10: Phayao, lane 11: Chiang Rai, lane 12: Uttaradit, lane 13: Tak)

The O 15 primer can be generated the PCR fragment ranging from 400 - 1200 bp, while O 16 primer can be generated the PCR fragment ranging from 220 - 1350 bp. The number of polymorphic fragment from O 15 primer and O 16 primer generated 4 and 7 fragments, respectively.

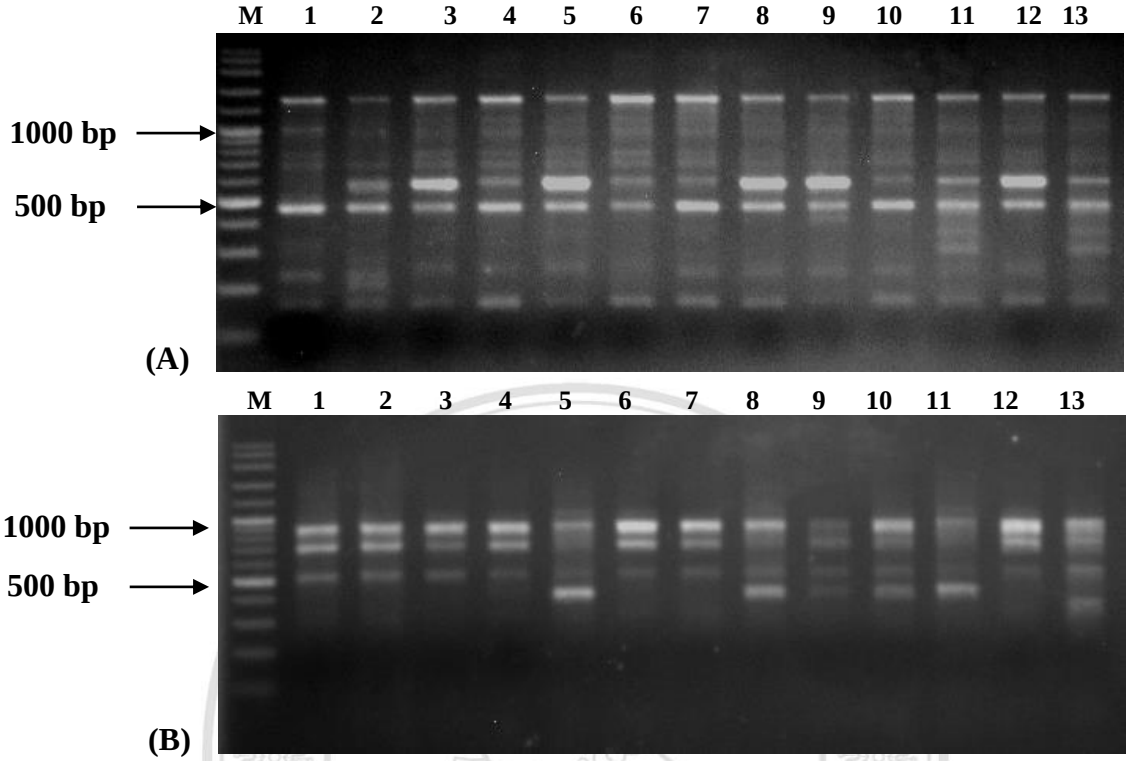

Figure 4-37. HAT-RAPD PCR profiles of *Spirogyra* from northern Thailand generated by (A) V 14 and (B) V 15 primer**.** (Lane M: 100 bp DNA marker, lane 1: Lamphun, lane 2: Lampang-1, lane 3: Lampang-2, lane 4: Chiang Mai, lane 5: Phrae, lane 6: Mae Hong Son-1, lane 7: Mae Hong Son-2, lane 8: Nan-1, lane 9: Nan-2, lane 10: Phayao, lane 11: Chiang Rai, lane 12: Uttaradit, lane 13: Tak)

The V 14 primer can be generated the PCR fragment ranging from 170 - 1300 bp, while V 15 primer can be generated the PCR fragment ranging from 320 - 900 bp. The number of polymorphic fragment from V 14 primer and V 15 primer generated 8 and 5 fragments, respectively.

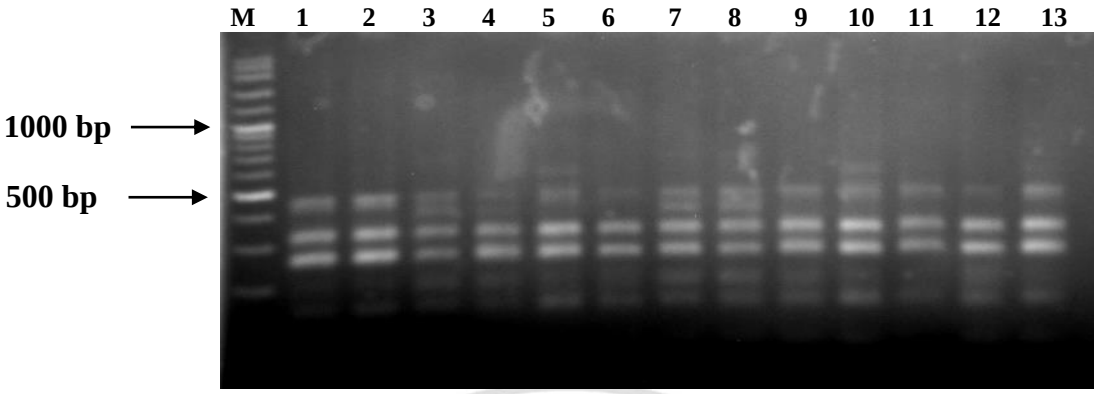

Figure 4-38. HAT-RAPD PCR profiles of *Spirogyra* from northern Thailand generated by B 01 primer**.** (Lane M: 100 bp DNA marker, lane 1: Lamphun, lane 2: Lampang-1, lane 3: Lampang-2, lane 4: Chiang Mai, lane 5: Phrae, lane 6: Mae Hon Song-1, lane 7: Mae Hon Song-2, lane 8: Nan-1, lane 9: Nan-2, lane 10: Phayao, lane 11: Chiang Rai, lane 12: Uttaradit, lane 13: Tak)

The B 01 primer can be generated the PCR fragment ranging from 160 - 550 bp. The number of polymorphic fragment from B 01 primer generated 7 fragments.

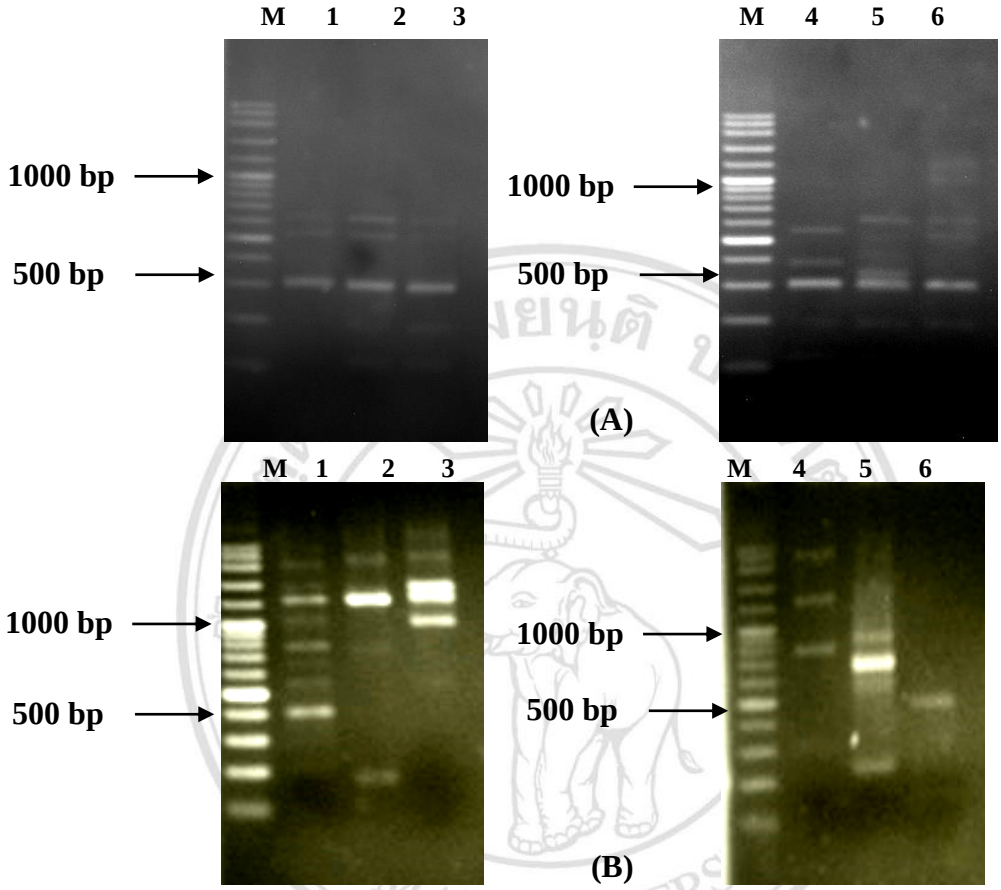

Figure 4-39. HAT-RAPD PCR profiles of *Spirogyra* from southern and eastern of Thailand generated by (A) O 15 primer (B) O 16 primer**.** (Lane M: 100 bp DNA marker, lane 1: Prachuap Khiri Khan, lane 2: Surat Thani, lane 3: Chumphon, lane 4: Rayong, lane 5: Chanthaburi, lane 6: Chon Buri)

The O 15 primer can be generated the PCR fragment ranging from 120 - 1200 bp, while O 16 primer can be generated the PCR fragment ranging from 200 - 3000 bp. The number of polymorphic fragment from O 15 primer and O 16 primer generated 9 and 16 fragments, respectively.

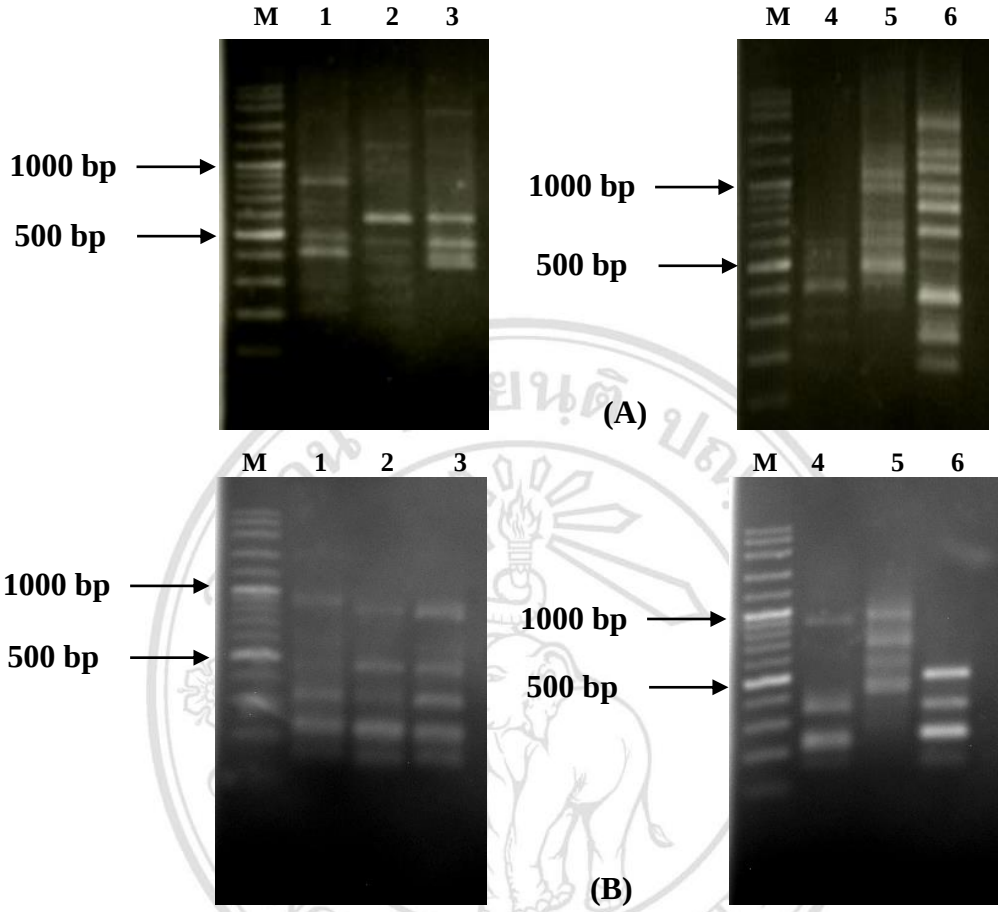

Figure 4-40. HAT-RAPD PCR profiles of *Spirogyra* from southern and eastern part of Thailand generated by (A) V14 and (B) V15 primer**.** (Lane M: 100 bp DNA marker, lane 1: Prachuap Khiri Khan, lane 2: Surat Thani, lane 3: Chumphon, lane 4: Rayong, lane 5: Chanthaburi, lane 6: Chon Buri)

The V 14 primer can be generated the PCR fragment ranging from 200 - 3000 bp, while V 15 primer can be generated the PCR fragment ranging from 150 - 800 bp. The number of polymorphic fragment from V 14 primer and V 15 primer generated 17 and 9 fragments, respectively.

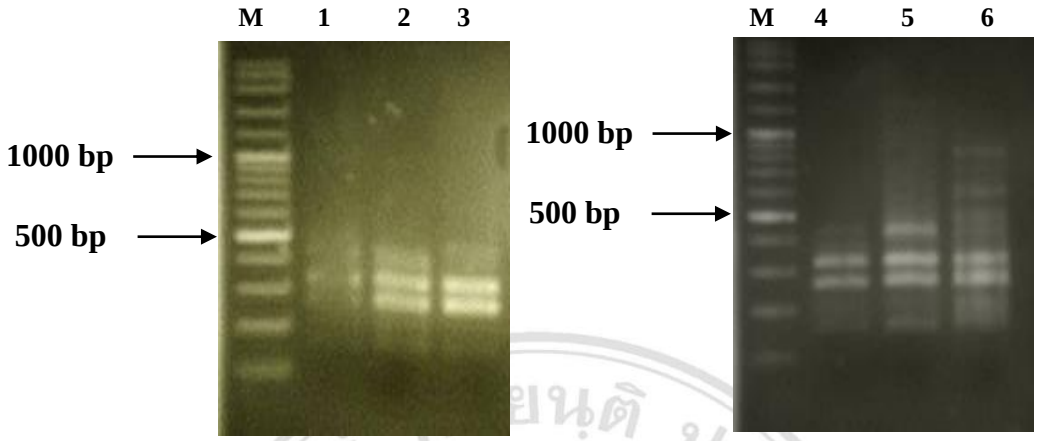

Figure 4-41. HAT-RAPD PCR profiles of *Spirogyra* from southern and eastern Thailand generated by B 01 primer**.** (Lane M: 100 bp DNA marker, lane 1: Prachuap Khiri Khan, lane 2: Surat Thani, lane 3: Chumphon, lane 4: Rayong, lane 5: Chanthaburi, lane 6: Chon Buri)

The B 01 primer can be generated the PCR fragment ranging from 150 - 900 bp. The number of polymorphic fragment from B 01 primer generated 9 fragments.

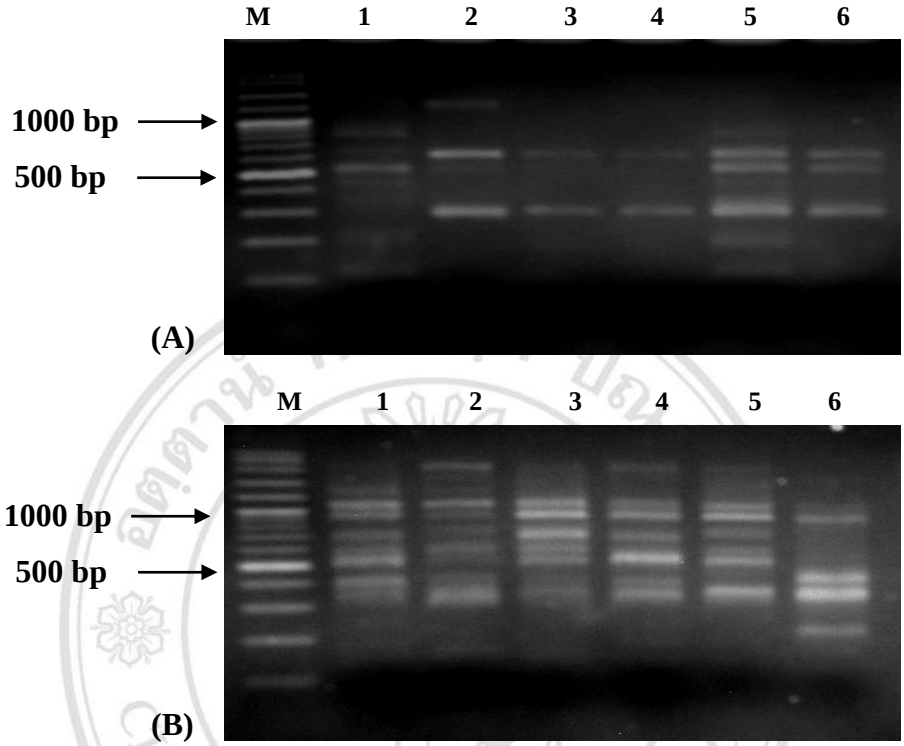

Figure 4-42. HAT-RAPD PCR profiles of *Spirogyra* from northeastern Thailand generated by (A) O 15 and (B) O 16 primer**.** (Lane M: 100 bp DNA marker, lane 1: Kalasin, lane 2: Maha Sarakham, lane 3: Mukdahan, lane 4: Loei, lane 5: Nakhon Phanom, lane 6: Udon Thani)

The O 15 primer can be generated the PCR fragment ranging from 120 - 1200 bp, while O 16 primer can be generated the PCR fragment ranging from 230 - 2500 bp. The number of polymorphic fragment from O 15 primer and O 16 primer generated 11 and 11 fragments, respectively.

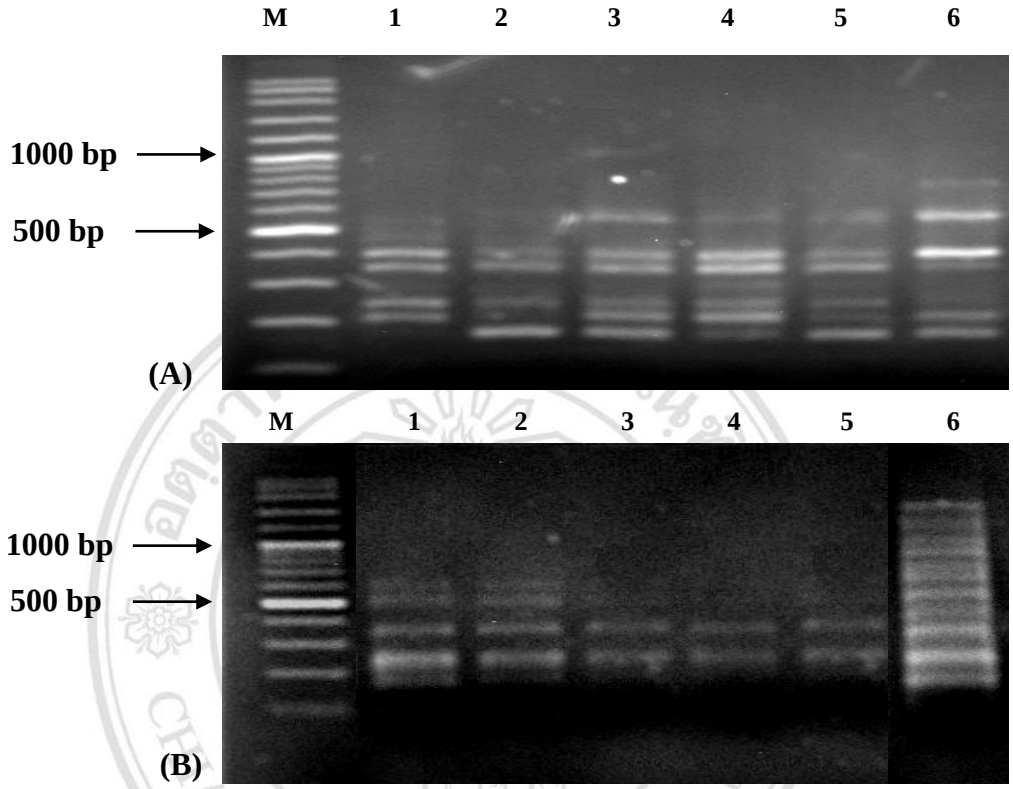

Figure 4-43. HAT-RAPD PCR profiles of *Spirogyra* from northeastern Thailand generated by (A) V 14 and (B) V 15 primer**.** (Lane M: 100 bp DNA marker, lane 1: Prachuap Khiri Khan, lane 2: Surat Thani, lane 3: Chumphon, lane 4: Rayong, lane 5: Chanthaburi, lane 6: Chon Buri)

The V 14 primer can be generated the PCR fragment ranging from 170 - 650 bp, while V 15 primer can be generated the PCR fragment ranging from 180 - 3000 bp. The number of polymorphic fragment from V 14 primer and V 15 primer generated 8 and 14 fragments, respectively.

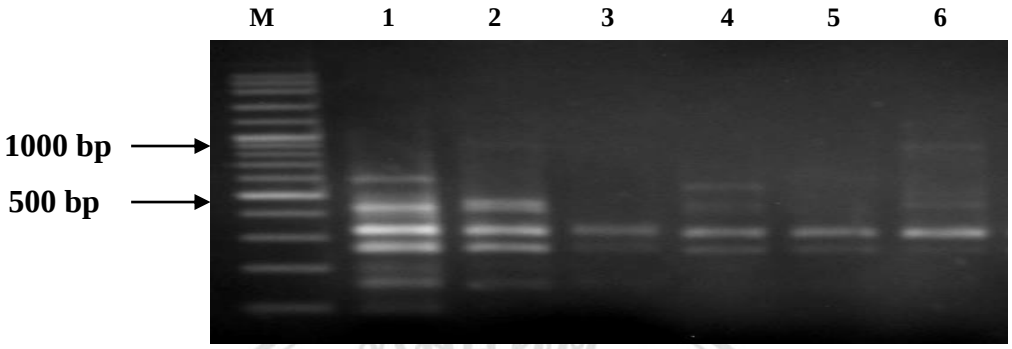

Figure 4-44. HAT-RAPD PCR profiles of *Spirogyra* from northeastern Thailand generated by B 01 primer**.** (Lane M: 100 bp DNA marker, lane 1: Kalasin, lane 2: Maha Sarakham, lane 3: Mukdahan, lane 4: Loei, lane 5: Nakhon Phanom, lane 6: Udon Thani)

The B 01 primer can be generated the PCR fragment ranging from 100 - 900 bp. The number of polymorphic fragment from B 01 primer generated 11 fragments.

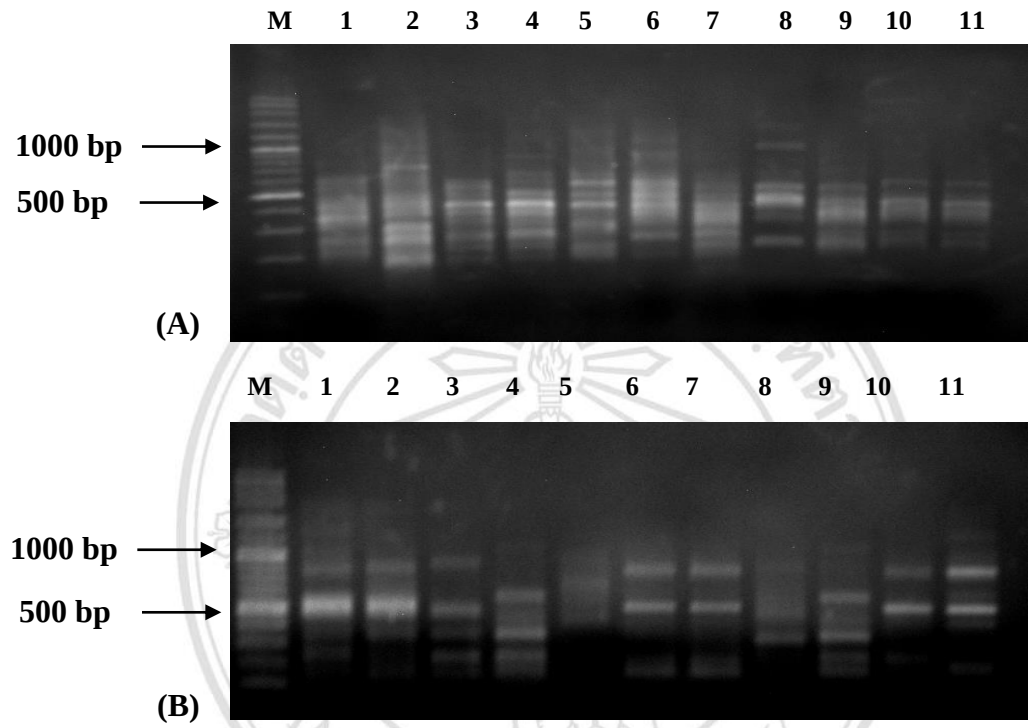

Figure 4-45. HAT-RAPD PCR profiles of *Spirogyra* from central Thailand generated by (A) O 15 and (B) O 16 primer**.** (Lane M: 100 bp DNA marker, lane 1: Kalasin, lane 2: Maha Sarakham, lane 3: Mukdahan, lane 4: Loei, lane 5: Nakhon Phanom, lane 6: Udon Thani)

The O 15 primer can be generated the PCR fragment ranging from 180 - 950 bp, while O 16 primer can be generated the PCR fragment ranging from 100 - 1500 bp. The number of polymorphic fragment from O 15 primer and O 16 primer generated 7 and 9 fragments, respectively.

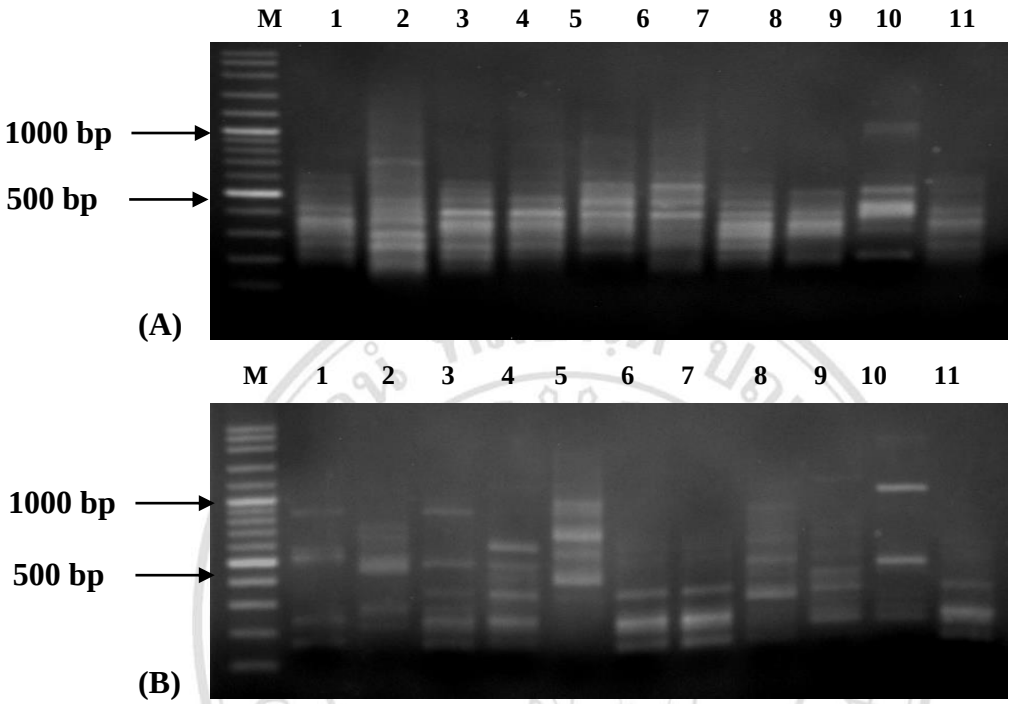

Figure 4-46. HAT-RAPD PCR profiles of *Spirogyra* from central Thailand generated by (A) V 14 and (B) V 15 primer**.** (Lane M: 100 bp DNA marker, lane 1: Prachuap Khiri Khan, lane 2: Surat Thani, lane 3: Chumphon, lane 4: Rayong, lane 5: Chanthaburi, lane 6: Chon Buri)

The V 14 primer can be generated the PCR fragment ranging from 150 - 1000 bp, while V 15 primer can be generated the PCR fragment ranging from 170 - 1200 bp. The number of polymorphic fragment from V 14 primer and V 15 primer generated 7 and 9 fragments, respectively.

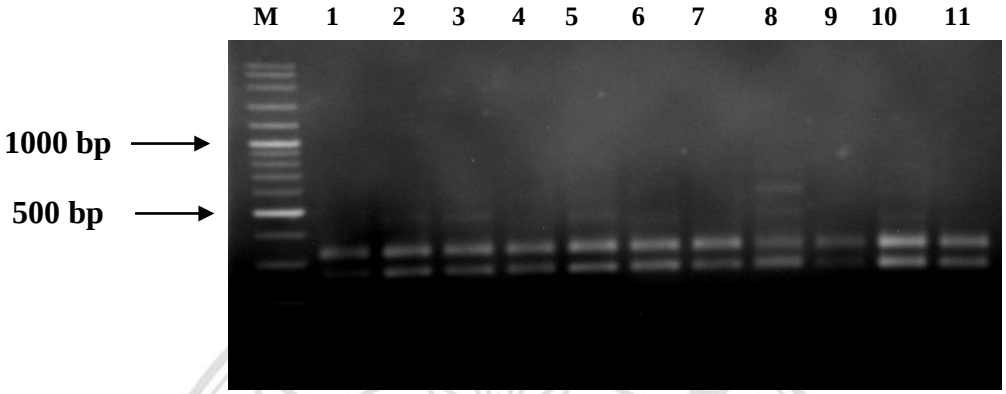

Figure 4-47. HAT-RAPD PCR profiles of *Spirogyra* from central Thailand generated by B 01 primer**.** (Lane M: 100 bp DNA marker, lane 1: Nakhon Sawan, lane 2: Suphan Buri, lane 3: Kanchanaburi, lane 4: Ratchaburi , lane 5:Phetchaburi, lane 6: Prachin Buri, lane 7: Lop Buri, lane 8: Saraburi, lane 9: Chai Nat, lane 10: Ang Thong, lane 11: Phitsanulok)

The B01 primer can be generated the PCR fragment ranging from 250 - 600 bp. The number of polymorphic fragment from B 01 primer generated 4 fragments.

Five RAPD primers produced a total of 69 scorable markers. The cluster analysis of ISSR marker separated the *Spirogyra* specimens into 5 distinct clusters including cluster 1: N1-N13 and C1-C11, cluster 2 NE1- NE-5, cluster 3: NE6, cluster 4: S1-S3 and E1, E2, cluster 5: E3 (Figure 4-48).

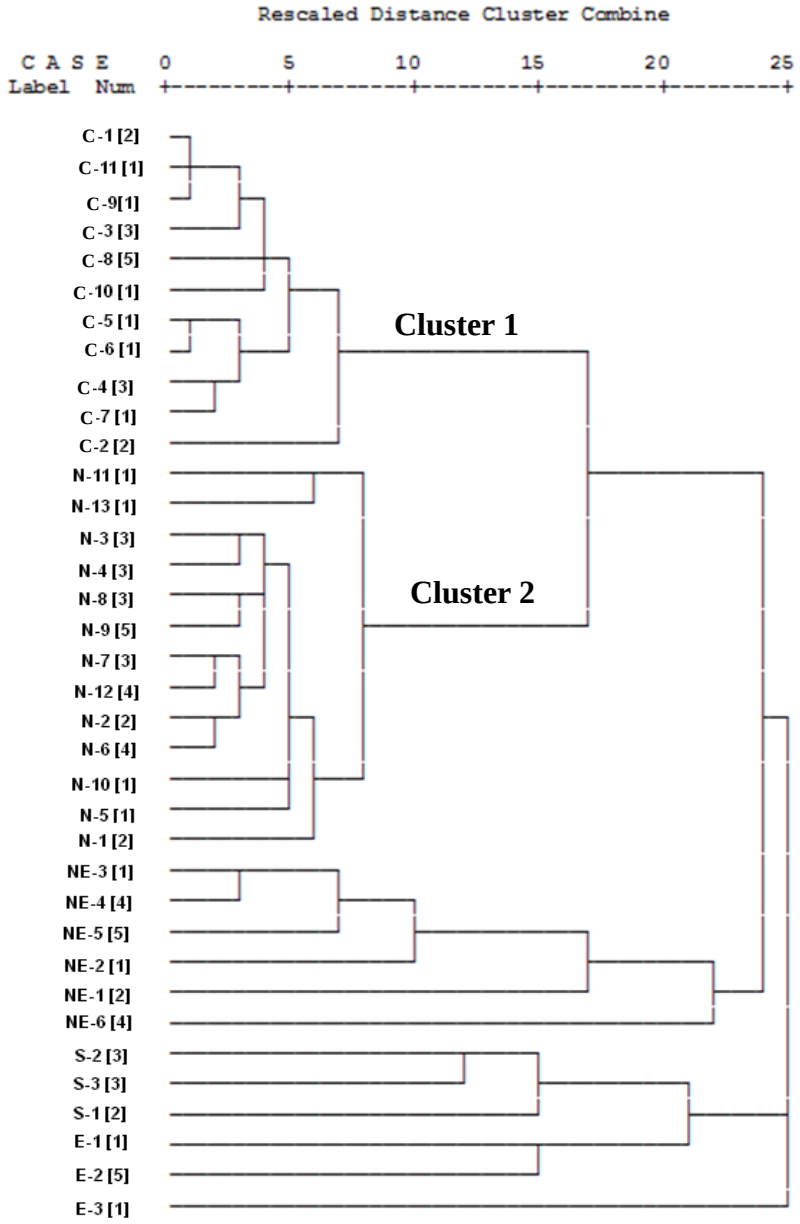

Figure 4-48. Dendrogram illustrating the cluster of *Spirogyra* using HAT-RAPD PCR (The numbers in parentheses is morphological pattern status from each samples) (C: central, N: northern, S: southern, E: eastern, NE: northeastern).

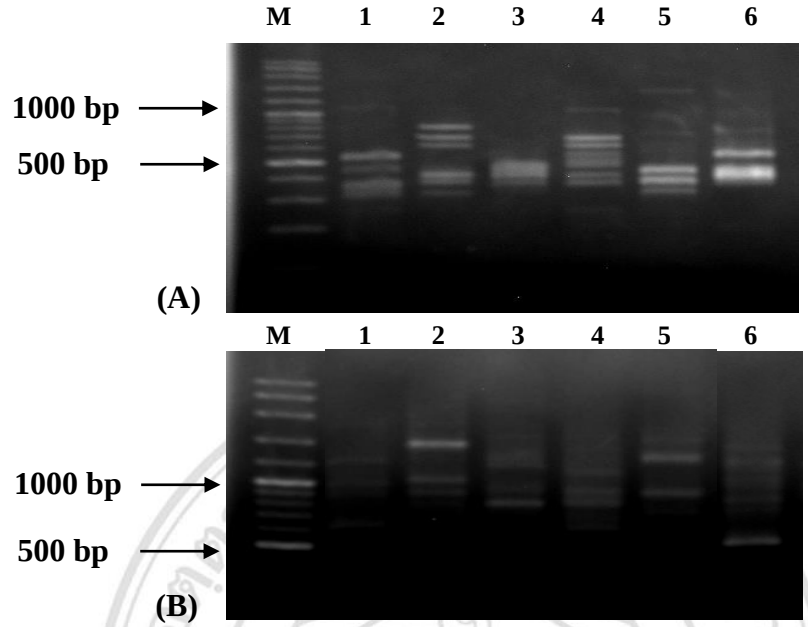

Figure 4-49. ISSR-PCR profiles of five morphological patterns of *Spirogyra* generated by (A) UBC 835 and (B) UBC 826 primer(Lane M: 100 bp DNA marker, lane 1: pattern 1, lane 2: pattern 2, lane 3: pattern 3, lane 4: pattern 4, lane 5: pattern 5, lane 6: *Cladophora* sp.)

The UBC 835 primer can be generated the PCR fragment ranging from 200 - 1500 bp, while UBC 826 primer can be generated the PCR fragment ranging from 100 - 700 bp. The number of polymorphic fragment from UBC 835 primer and UBC 826 primer generated 9 and 8 fragments, respectively.

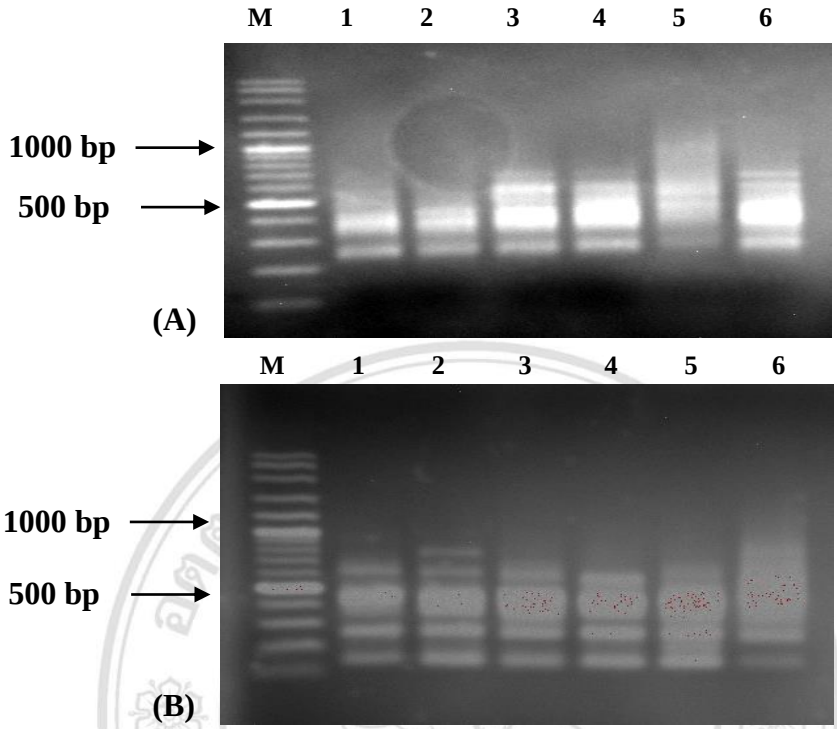

Figure 4-50. ISSR-PCR profiles of five morphological patterns of *Spirogyra* generated by (A) UBC 809 and (B) UBC 808 primer (Lane M: 100 bp DNA marker, lane 1: pattern 1, lane 2: pattern 2, lane 3: pattern 3, lane 4: pattern 4, lane 5: pattern 5, lane 6: *Cladophora* sp.).

The UBC 809 primer can be generated the PCR fragment ranging from 230 - 600 bp, while UBC 808 primer can be generated the PCR fragment ranging from 130 - 800 bp. The number of polymorphic fragment from UBC 809 primer and UBC 808 primer generated 5 and 6 fragments, respectively.

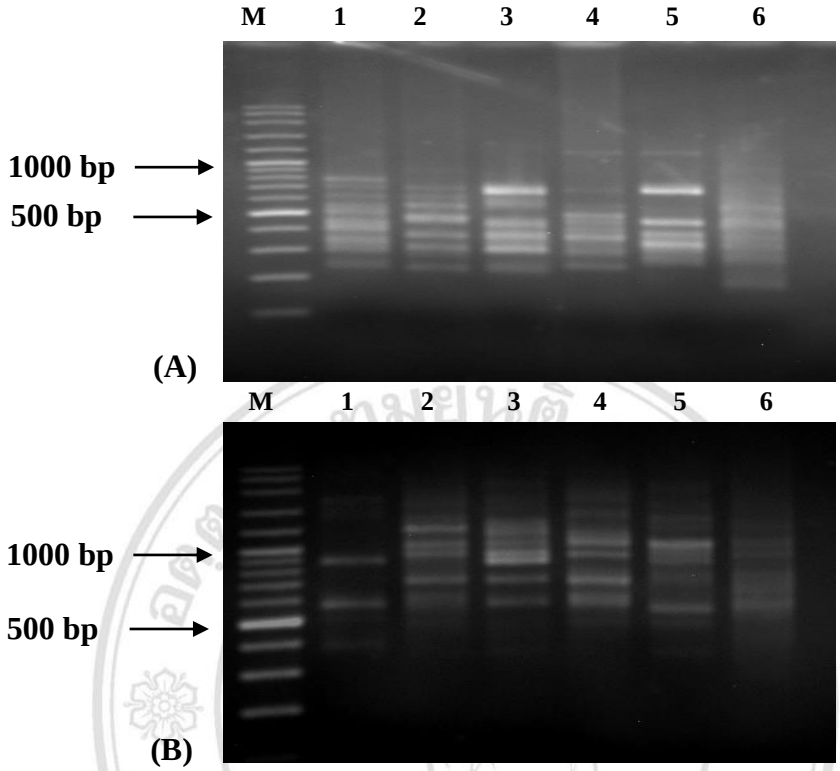

Figure 4-51. ISSR-PCR profiles of five morphological patterns of *Spirogyra* generated by (A) UBC 825 and (B) UBC 827 primer (Lane M: 100 bp DNA marker, lane 1: pattern 1, lane 2: pattern 2, lane 3: pattern 3, lane 4: pattern 4, lane 5: pattern 5, lane 6: *Cladophora* sp.).

The UBC 825 primer can be generated the PCR fragment ranging from 180 - 1100 bp, while UBC 827 primer can be generated the PCR fragment ranging from 300 - 2700 bp. The number of polymorphic fragment from UBC 825 primer and UBC 827 primer generated 11 and 9 fragments, respectively.

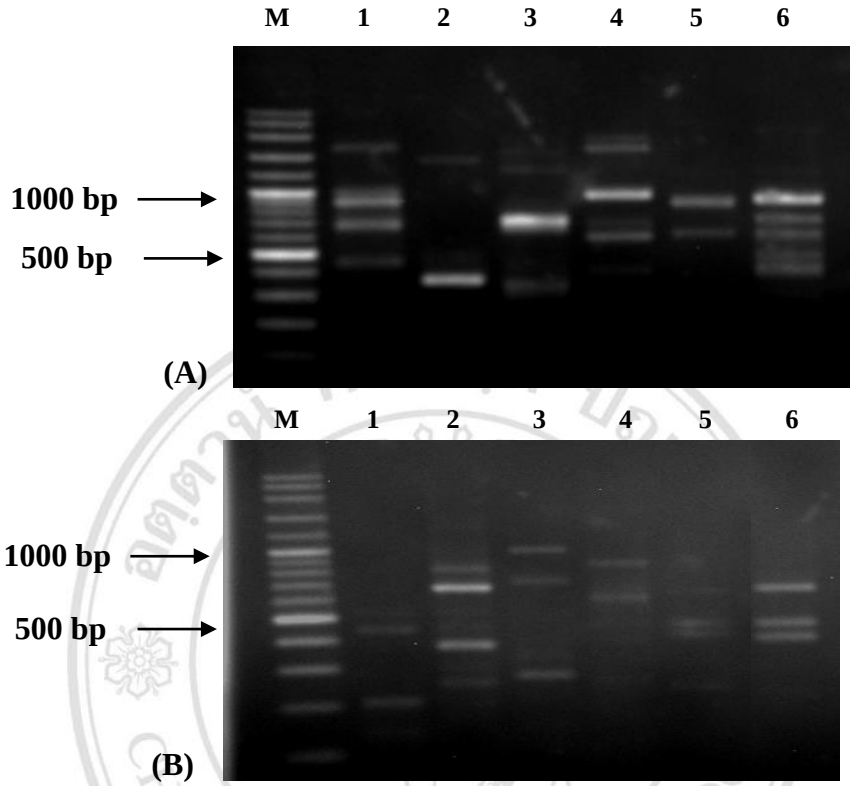

Figure 4-52. ISSR-PCR profiles of five morphological patterns of *Spirogyra* generated by (A) UBC 864 and (B) UBC 807 primer (Lane M: 100 bp DNA marker, lane 1: pattern 1, lane 2: pattern 2, lane 3: pattern 3, lane 4: pattern 4, lane 5: pattern 5, lane 6: *Cladophora* sp.).

The UBC 864 primer can be generated the PCR fragment ranging from 300 - 2700 bp, while UBC 807 primer can be generated the PCR fragment ranging from 140 - 1000 bp. The number of polymorphic fragment from UBC 864 primer and UBC 807 primer generated 9 and 12 fragments, respectively.

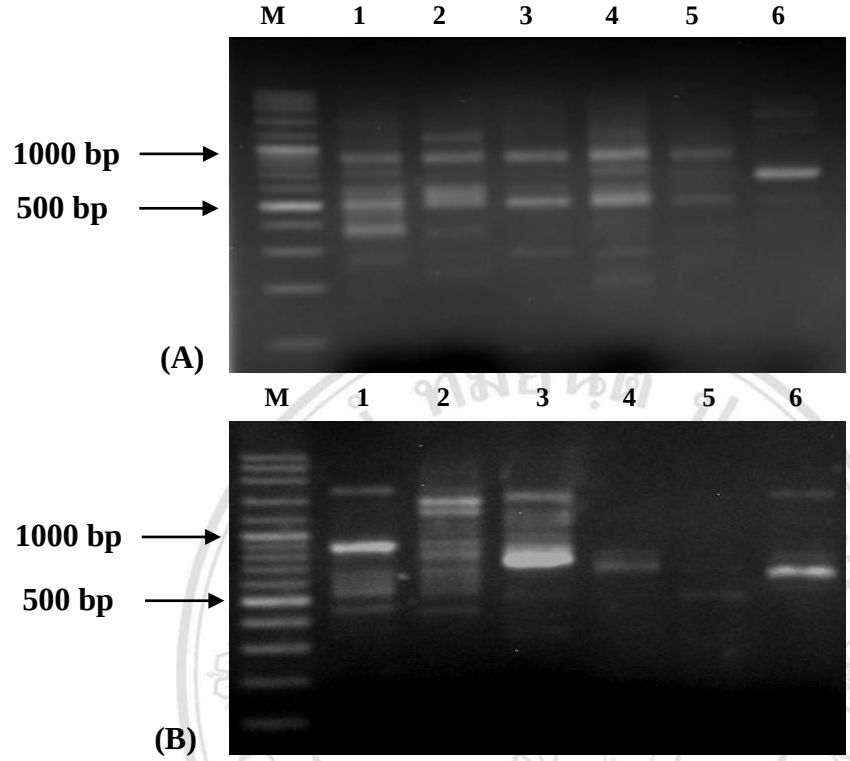

Figure 4-53. ISSR-PCR profiles of five morphological Patterns of *Spirogyra* generated by (A) UBC 857 and (B) UBC 880 primer (Lane M: 100 bp DNA marker, lane 1: pattern 1, lane 2: pattern 2, lane 3: pattern 3, lane 4: pattern 4, lane 5: pattern 5, lane 6: *Cladophora* sp.).

The UBC 857 primer can be generated the PCR fragment ranging from 200 - 1700 bp, while UBC 880 primer can be generated the PCR fragment ranging from 350 - 2850 bp. The number of polymorphic fragment from UBC 857 primer and UBC 880 primer generated 12 and 11 fragments, respectively.

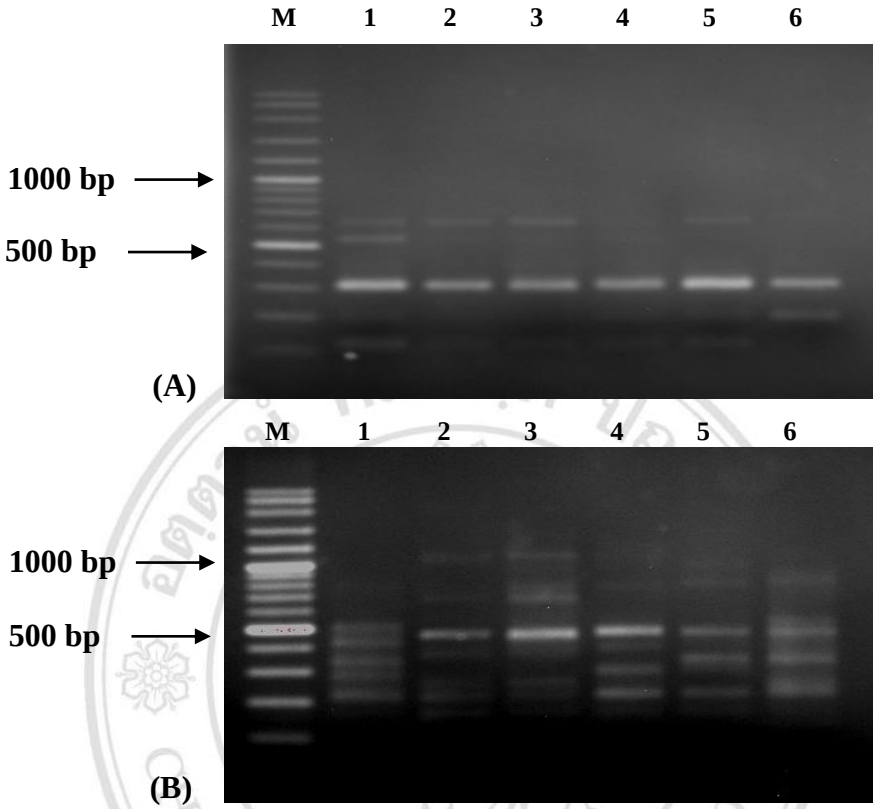

Figure 4-54. HAT-RAPD PCR profiles of five morphological patterns of *Spirogyra* generated by (A) O 15 and (B) O 16 primer**.** (Lane M: 100 bp DNA marker, lane 1: pattern 1, lane 2: pattern 2, lane 3: pattern 3, lane 4: pattern 4, lane 5: pattern 5, lane 6: *Cladophora* sp.).

The O 15 primer can be generated the PCR fragment ranging from 110 - 630 bp, while O 16 primer can be generated the PCR fragment ranging from 180 - 1050 bp. The number of polymorphic fragment from O 15 primer and O 16 primer generated 5 and 11 fragments, respectively.

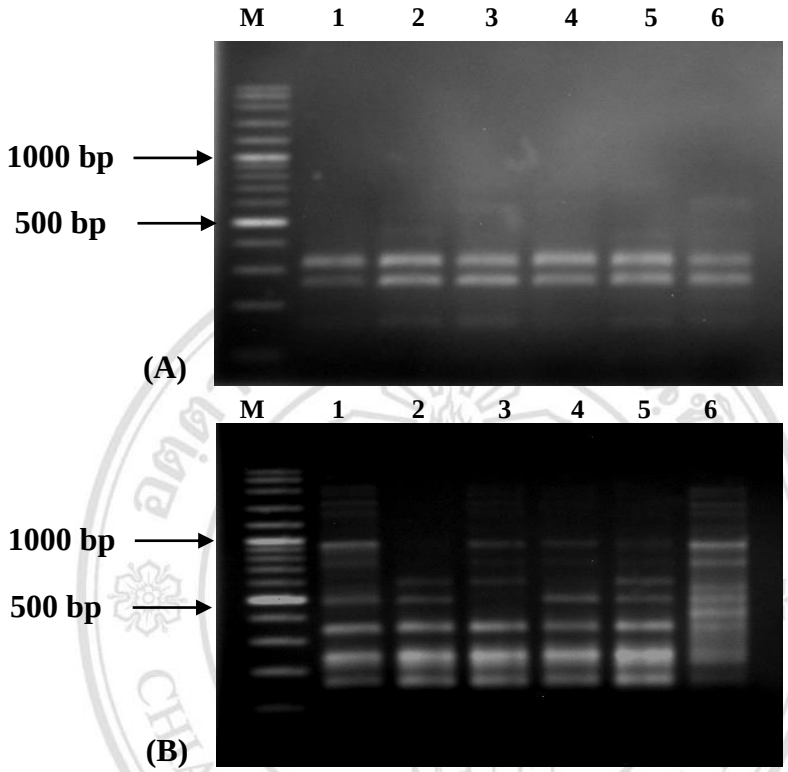

Figure 4-55. HAT-RAPD PCR profiles of five morphological patterns of *Spirogyra* generated (A) V 14 and (B) V 15 primer**.** (Lane M: 100 bp DNA marker, lane 1: pattern 1, lane 2: pattern 2, lane 3: pattern 3, lane 4: pattern 4, lane 5: pattern 5, lane 6: *Cladophora* sp.).

The V 14 primer can be generated the PCR fragment ranging from 160 - 600 bp, while V 15 primer can be generated the PCR fragment ranging from 170 - 2600 bp. The number of polymorphic fragment from V 14 primer and V 15 primer generated 4 and 11 fragments, respectively.

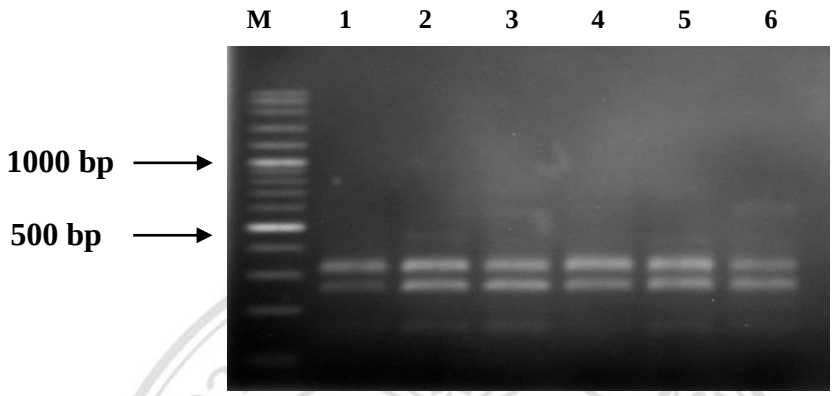

Figure 4-56. HAT-RAPD PCR profiles of five morphological patterns of *Spirogyra* generated by B 01 primer**.** (Lane M: 100 bp DNA marker, lane 1: pattern 1, lane 2: pattern 2, lane 3: pattern 3, lane 4: pattern 4, lane 5: pattern 5, lane 6: *Cladophora* sp.).

The B 01 primer can be generated the PCR fragment ranging from to 170 - 600 bp. The number of polymorphic fragment from B 01 primer generated 5 fragments.

Ten ISSR primers and 5 RAPD primers produce a total of 180 scorable markers. The cluster analysis of ISSR marker separated the 5 morphological patterns of *Spirogyra* populations with *Chadophora* sp. as out group into 4 distinct clusters including cluster 1: Pattern 4 and Pattern 5, cluster 2 Pattern 3, cluster 3: Pattern 1, cluster 4: Pattern 2, cluster 5: *Cladophora* sp. (Figure 4-57)**.**

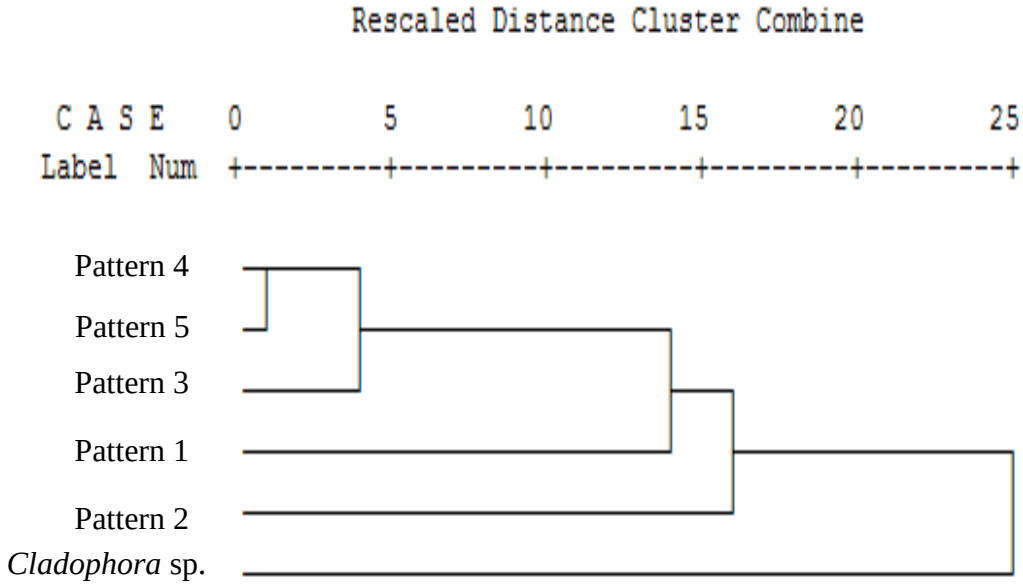

Figure 4-57. Diagram illustrating the cluster of each morphological Patterns of *Spirogyra* using ISSR and RAPD markers

Ribulose-bisphosphate carboxylase gene (*rbcL*) amplification

A pair of universal primer was used to amplified *rbcL* region as describe by Saiki *et al.* (1988). The *rbcL* gene of 5 morphological Patterns of *Spirogyra* was amplified using primers rbcl-F and rbcl-R. The amplification procedure involved an initial denaturation step at 95 ◦C for 3 min, then 40 cycle including denaturation at 95 ◦C for 1 min, primer annealing at 55 ◦C for 1 min, extension at 72 ◦C for 1 min, and final extension at 7 ◦C for 7 min, respectively.

Certain of *rbcL* nucleotide amplification would reveal the 550 - 570 bp fragments in each morphological group (Figure 4-57), and were obtained and submitted to Genbank.

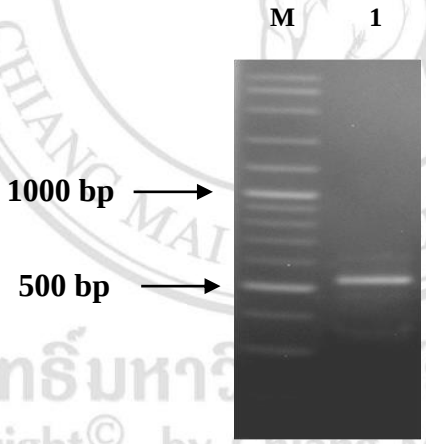

Figure 4-58. The *rbcL* product of *Spirogyra* (Lane M: 100 bp DNA marker, lane 1: DNA fragment of *rbcL* gene)

Base on *rbcL* sequences data obtained in this study, they were trimmed to provide an equivalence sequences among each morphological group. The specific DNA fragment of *rbcL* was analyzed data by BLAST (Basic Local Alignment Search

Tool) program in the NCBI (National Center for Biotechnology Information) database. Sequence data of morphological pattern 1 revealed definitive identity matches in the range of *Spirogyra ellipsospora* for consensus sequences with 2 accession numbers of *S. ellipsospora* that available on the NCBI databases.

Moreover, they were aligned using multiple alignment method (Clustal W 2.0 program). The result shown that, the maximum length of *rbcL* sequences provided in this study was 578 bp.

Phylogenetic trees were analyzed for the *rbcL* sequences data using UPGMA. The phylogram from these result are separated into 3 clade including clade A: Pattern 1, clade B: Pattern 3 and Clade C: Pattern 2, Pattern 5 and Pattern 4 (Figure 4-59.).

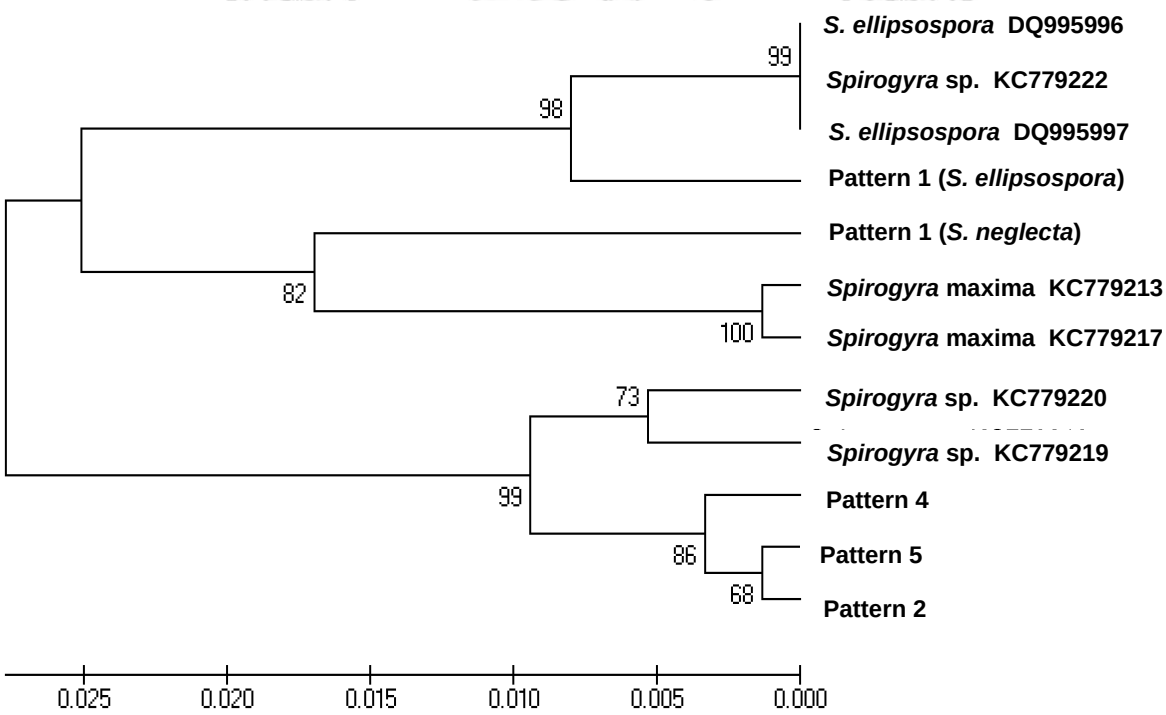

Figure 4-59. Phylogram derived from an UPGMA analysis depicting phylogenetic relationships of each morphological pattern of *Spirogyra* used in this study basing on *rbcL* sequences