CHAPTER VI

GENERAL CONCLUSIONS

Cultured cells and genetic variation of N. scintillans in the inner gulf of Thailand

- ESM-culture medium and *Dunaliella* were appropriated to cultured pink *Noctiluca* but in the case of this study, we must culture the much of clones (more than 30 clones/stations) for testing ISSR primers. So it vary difficult to controlled the growth of cells were enough stable cells concentration in each clones.
- These 5 ISSR primers obtained from this study could potentially be used for screening genetic variation of *N. scintillans* but there was a problem about the maintenance of *N. scintillans* culture and the quantity of extracted DNA. Therefore, this study couldn't be continue.
- There was no genetic variation in COX I and 18s rRNA gene among *N. scintillans* samples used in this study.
- The complete sequence of ITS1 region could not be obtained. This might be the result of multiple copies of this region or the problem in the primer used in this study.

APPENDICES

APPENDIX1

Primers tesed ISSR PCR

No.	primer	Sequence (5'-3')	No.	primer	Sequence (5'-3')
1	UBC809	(AG)8G	25	T8710	(CA)7YC
2	UBC811	(GA)8C	26	T8711	(CA)7YG
3	UBC827	(AC)8G	27	T8712	(GA)8AT
4	SAS1	(GIG)4C	28	T8713	(CT)8G
5	SAS3	(GAG)4C	29	T8714	(GT)6RG(CT)8T
6	814	(CT)8TG	30	T8715	(GA)6C
7	844A	(CT)8AC	31	T8716	(CA)6C
8	844B	(CT)8GC	32	T8717	(CA)6T
9	17898A	(CA)6AC	33	T8718	(GA)6T
10	17898B	(CA)6GT	34	UBC-813	(CT)8T
11	17899A	(CA)6AG	35	UBC-824	(CT)8G
12	HB12	(AC)3GC	36	UBC-845	(CT)8RG
13	HB13	(GAG)3GC	37	UBC-840	(GA)8YT
14	HB14	(CTC)3GC	38	UBC-848	(CA)8RG
15	HB15	(GIG)3GC	39	TL01	(CAG)5
16	T8701	(CT)8RA	40	TL02	(CAA)5
17	T8702	(AG)7YC	41	TL03	(GACA)4
18	T8703	(GT)6YR	42	TL04	(GATA)4
19	T8704	(GT)6AY	43	UBC812	(GA)8A
20	T8705	CAA(AG)5	44	UBC826	(AC)8C
21	T8706	GGGC(GA)8	45	UBC841	(GA)8YC
22	T8707	(GAG)4RC	46	UBC857	(AC)8YC
23	T8708	(GA)7RG	47	UBC818	(CA)8G
24	T8709	(GT)7YG	48	UBC868	(GAA)6

*Y=C/T; R=A/G

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APPENDIX2

The result from BioEdit program of COX1 forward (COX_F2) sequence of *N*. *scintillans* in the inner gulf of Thailand (1-2PB, 3-4ASL and 5-6CPY). Asterisks symbols (*) expressed that all samples appear nitrogenous base (A, C, G, and T) identity.

···· [····] ···· [····] ···· [····] ···· [····] ···· [····]

	10	0 20	30	9 40	50
1PB	TTCGGTATAA	TTAGTATTAT	TATTAGTGGA	GTTTCTCAAA	AGATTGTATT
2PB	TTCGGTATAA	TTAGTATTAT	TATTAGTGGA	GTTTCTCAAA	AGATTGTATT
3ASL	TTCGGTATAA	TTAGTATTAT	TATTAGTGGA	GTTTCTCAAA	AGATTGTATT
4ASL	TTCGGTATAA	TTAGTATTAT	TATTAGTGGA	GTTTCTCAAA	AGATTGTATT
6CPY	TTCGGTATAA	TTAGTATTAT	TATTAGTGGA	GTTTCTCAAA	AGATTGTATT
5CPY	TTCGGTATAA	TTAGTATTAT	TATTAGTGGA	GTTTCTCAAA	AGATTGTATT
Clustal Co	******	******	******	******	*****

	60	0 70	0 80	90 90	0 100
1PB	CGGGCATCAA	TCAATGATTT	TTGCTATGAG	CTGTATATGT	ATTTTAGGCT
2PB	CGGGCATCAA	TCAATGATTT	TTGCTATGAG	CTGTATATGT	ATTTTAGGCT
JASL	CGGGCATCAA	TCAATGATTT	TTGCTATGAG	CTGTATATGT	ATTTTAGGCT
4ASL	CGGGCATCAA	TCAATGATTT	TTGCTATGAG	CTGTATATGT	ATTTTAGGCT
6CPY	CGGGCATCAA	TCAATGATTT	TTGCTATGAG	CTGTATATGT	ATTTTAGGCT
5CPY	CGGGCATCAA	TCAATGATTT	TTGCTATGAG	CTGTATATGT	ATTTTAGGCT
Clustal Co	*****	******	******	******	******

	110	0 120	0 130	0 140	0 150
1PB	CTATTGTCTG	GGGACATCAT	ATGTACACTG	TTGGTCTTGA	AACAGATACA
2PB	CTATTGTCTG	GGGACATCAT	ATGTACACTG	TTGGTCTTGA	AACAGATACA
JASL	CTATTGTCTG	GGGACATCAT	ATGTACACTG	TTGGTCTTGA	AACAGATACA
4ASL	CTATTGTCTG	GGGACATCAT	ATGTACACTG	TTGGTCTTGA	AACAGATACA
6CPY	CTATTGTCTG	GGGACATCAT	ATGTACACTG	TTGGTCTTGA	AACAGATACA
5CPY	CTATTGTCTG	GGGACATCAT	ATGTACACTG	TTGGTCTTGA	AACAGATACA
Clustal Co	******	******	*****	******	******

Continued

	160	0 170	0 180) 190	200
1PB	CGTGCTTATT	TTACAGGTGT	ААСТАТТТТА	ATTTCATTAC	CAACAGGAAC
2PB	CGTGCTTATT	TTACAGGTGT	AACTATTTTA	ATTTCATTAC	CAACAGGAAC
JASL	CGTGCTTATT	TTACAGGTGT	AACTATTTTA	ATTTCATTAC	CAACAGGAAC
4ASL	CGTGCTTATT	TTACAGGTGT	AACTATTTTA	ATTTCATTAC	CAACAGGAAC
6CPY	CGTGCTTATT	TTACAGGTGT	AACTATTTTA	ATTTCATTAC	CAACAGGAAC
5CPY	CGTGCTTATT	TTACAGGTGT	AACTATTTTA	ATTTCATTAC	CAACAGGAAC
Clustal Co	*******	*****	*****	******	*****

	210	0 220	0 230	0 240	250
1PB	AAAAATCTTT	AATTGGATTA	GTACATACCT	CGGTAATTCT	TTATTACTCC
2PB	AAAAATCTTT	AATTGGATTA	GTACATACCT	CGGTAATTCT	TTATTACTCC
JASL	AAAAATCTTT	AATTGGATTA	GTACATACCT	CGGTAATTCT	TTATTACTCC
4ASL	AAAAATCTTT	AATTGGATTA	GTACATACCT	CGGTAATTCT	TTATTACTCC
6CPY	AAAAATCTTT	AATTGGATTA	GTACATACCT	CGGTAATTCT	TTATTACTCC
5CPY	AAAAATCTTT	AATTGGATTA	GTACATACCT	CGGTAATTCT	TTATTACTCC
Clustal Co	*****	******	******	******	*****

	260	270	280	290	300
1PB	ATATGAGGAC	TTCTTCAGCA	CTTTTTGCGT	CGCTTTTCCT	TTTAATGTTT
2PB	ATATGAGGAC	TTCTTCAGCA	CTTTTTGCGT	CGCTTTTCCT	TTTAATGTTT
JASL	ATATGAGGAC	TTCTTCAGCA	CTTTTTGCGT	CGCTTTTTCCT	TTTAATGTTT
4ASL	ATATGAGGAC	TTCTTCAGCA	CTTTTTGCGT	CGCTTTTCCT	TTTAATGTTT
6CPY	ATATGAGGAC	TTCTTCAGCA	CTTTTTGCGT	CGCTTTTCCT	TTTAATGTTT
5CPY	ATATGAGGAC	TTCTTCAGCA	CTTTTTGCGT	CGCTTTTCCT	TTTAATGTTT
Clustal Co	*******	******	*****	*****	****

Continued

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	310	320	0 330	0 340	350
1PB	ACAATTGGAG	GTTCTTCAGG	TGTTATACTT	GGAAATGCTG	CTGTTGACCT
2PB	ACAATTGGAG	GTTCTTCAGG	TGTTATACTT	GGAAATGCTG	CTGTTGACCT
JASL	ACAATTGGAG	GTTCTTCAGG	TGTTATACTT	GGAAATGCTG	CTGTTGACCT
4ASL	ACAATTGGAG	GTTCTTCAGG	TGTTATACTT	GGAAATGCTG	CTGTTGACCT
6CPY	ACAATTGGAG	GTTCTTCAGG	TGTTATACTT	GGAAATGCTG	CTGTTGACCT
5CPY	ACAATTGGAG	GTTCTTCAGG	TGTTATACTT	GGAAATGCTG	CTGTTGACCT
Clustal Co	******	******	******	*****	*****

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2	6	n	
2	o	U	

1PB	TGCATTACAT	GATACA
2PB	TGCATTACAT	GATACA
JASL	TGCATTACAT	GATACA
4ASL	TGCATTACAT	GATACA
6CPY	TGCATTACAT	GATACA
5CPY	TGCATTACAT	GATACA
Clustal Co	*******	*****

APPENDIX3

The result from BioEdit program of ITS forward (ITS_F1) sequence of *N. scintillans* sample in the inner gulf of Thailand (1-2PB, 3-4ASL and 5-6CPY) and outing group from Philippine (11-12MB) and Indonesia (13-14ID).Asterisks symbols (*) expressed that all samples appear nitrogenous base (A, C, G, and T) identity. Green label showed the area of nucleotide sequences of samples were compared with *Pfiesteria-like dinoflagellate* position.

	11				
	10	0 2	0 3	0 4	0 50
5CPY_ITS_F					
6CPY_ITS_F					
12MB_ITS_F					
13ID_ITS_F					
3ASL_ITS_F					
11MB_ITS_F					
1PB_ITS_F					
14ID_ITS_F					
2PB_ITS_F					
4ASL_ITS_F					
Pfiesteria	TCAACCTGGT	GATCCTGCCA	GTAGTCATAT	GCTTGTCTCA	AAGATTAAGC
Clustal					
	1 1				
	••••				
	•••••				
5CPY_ITS_F			0 80		0 100
5CPY_ITS_F 6CPY_ITS_F			0 80) 91	0 100
			0 80) 91	0 100
6CPY_ITS_F			0 80) 91	0 100
6CPY_ITS_F 12MB_ITS_F			0 80) 91	0 100
6CPY_ITS_F 12MB_ITS_F 13ID_ITS_F			0 80) 91	0 100
6CPY_ITS_F 12MB_ITS_F 13ID_ITS_F 3ASL_ITS_F			0 80) 91	0 100
6CPY_ITS_F 12MB_ITS_F 13ID_ITS_F 3ASL_ITS_F 11MB_ITS_F			0 80) 91	0 100
6CPY_ITS_F 12MB_ITS_F 13ID_ITS_F 3ASL_ITS_F 11MB_ITS_F 1PB_ITS_F			0 80) 91	0 100
6CPY_ITS_F 12MB_ITS_F 13ID_ITS_F 3ASL_ITS_F 11MB_ITS_F 1PB_ITS_F 14ID_ITS_F			0 80) 91	0 100
6CPY_ITS_F 12MB_ITS_F 13ID_ITS_F 3ASL_ITS_F 11MB_ITS_F 1PB_ITS_F 14ID_ITS_F 2PB_ITS_F) 9) 	
6CPY_ITS_F 12MB_ITS_F 13ID_ITS_F 3ASL_ITS_F 11MB_ITS_F 1PB_ITS_F 14ID_ITS_F 2PB_ITS_F 4ASL_ITS_F			0 80) 9) 	

		1			.1
	11	0 12	0 13	30 14	0 150
5CPY_ITS_F					
6CPY_ITS_F					
12MB_ITS_F					
13ID_ITS_F					
3ASL_ITS_F					
11MB_ITS_F					
1PB_ITS_F					
14ID_ITS_F					
2PB_ITS_F					
4ASL_ITS_F					
Pfiesteria	TTAAAACAGT	TATAGTTTAT	TTGATGGTCA	TTCCTACATG	GATAACTGTG
Clustal					
	16				
5CPY_ITS_F					
6CPY_ITS_F					
12MB_ITS_F					
13ID_ITS_F					
3ASL_ITS_F					
11MB_ITS_F					
1PB_ITS_F					
14ID_ITS_F					
2PB_ITS_F					
4ASL_ITS_F					
Pfiesteria	GTAATTCTAG	AGCTAATACA	TGCACCCAAA	CCCGACTCCG	TGGAAGGGTT
Clustal					
	210				
5CPY_ITS F					. 200
6CPY_ITS_F					
12MB ITS F					
13ID_ITS F					
3ASL_ITS_F					
11MB_ITS F					
1PB_ITS F					
14ID_ITS F					
2PB_ITS F					
4ASL ITS F					
Pfiesteria	ርሞርሞሞካልሞሞል	CTTACACAAC	CARCORRECC		
Clustal	GIGITIATIA	GIIACAGAAC	CAACCCAGGC	TTGCCTGGTC	TTGTGGGGGAT
Continued					

	26	0 27	0 28	0 29	0 300
5CPY_ITS_F					
6CPY_ITS_F					
12MB_ITS_F					
13ID_ITS_F					
3ASL_ITS_F					
11MB_ITS_F					
1PB_ITS_F					
14ID_ITS_F					
2PB_ITS_F					
4ASL_ITS_F					
Pfiesteria	TCATAATAAC	CGAACGAATC	GCATGCCTTC	GGCGGCGATG	GATCATTTAA
Clustal					
				•••••	1
	31	0 32	0 33	0 34	0 350
5CPY_ITS_F					
6CPY_ITS_F					
12MB_ITS_F					
13ID_ITS_F					
3ASL_ITS_F					
11MB_ITS_F					
1PB_ITS_F					
14ID_ITS_F					
2PB_ITS_F					
4ASL_ITS_F					
Pfiesteria	GTTTCTGACC	TATCAGCTTT	CGACGGTAGG	ATATAGGCCT	ACCGTGGCGA
Clustal					
	36	0 37	0 38	0 39	0 400
5CPY_ITS_F					
6CPY_ITS_F					
12MB_ITS_F					
13ID_ITS_F					
3ASL_ITS_F					
11MB_ITS_F					
1PB_ITS_F					
14ID_ITS_F					
2PB_ITS_F					
4ASL_ITS_F					
Pfiesteria	CGACGGGTAA	CGGAGAATTA	GGGTTCGATT	CCGGAGAGGG	AGCCTGAGAA
Clustal					

		ii	1	1	.1
	41	0 42	0 43	0 44	0 450
5CPY_ITS_F					
6CPY_ITS_F					
12MB_ITS_F					
13ID_ITS_F					
3ASL_ITS_F					
11MB_ITS_F					
1PB_ITS_F					
14ID_ITS_F					
2PB_ITS_F					
4ASL_ITS_F					
Pfiesteria	ACGGCTACCA	CATCTAAGGA	AGGCAGCAGG	CGCGCAAATT	ACCCAATCCT
Clustal					
	•••• ••••				1
	46				
5CPY_ITS_F					
6CPY_ITS_F					
12MB_ITS_F					
13ID_ITS_F					
3ASL_ITS_F					
11MB_ITS_F					
1PB_ITS_F					
14ID_ITS_F					
2PB_ITS_F					
4ASL_ITS_F					
Pfiesteria	GACACAGGGA	GGTAGTGACA	AGAAATAACA	ATACAGGGCA	TCCATGTCTT
Clustal					
		•••••	••••1••••1		11
	510	520) 530	540	0 550
5CPY_ITS_F					
6CPY_ITS_F					
12MB_ITS_F					
13ID_ITS_F					
3ASL_ITS_F					
11MB_ITS_F					
1PB_ITS_F					
14ID_ITS_F					
2PB_ITS_F					
4ASL_ITS_F					
Pfiesteria	GTAATTGGAA	TGAGTAGAAT	TTAAATCCCT	TTATGAGTAT	CAATTGGAGG
Clustal					
Continue					

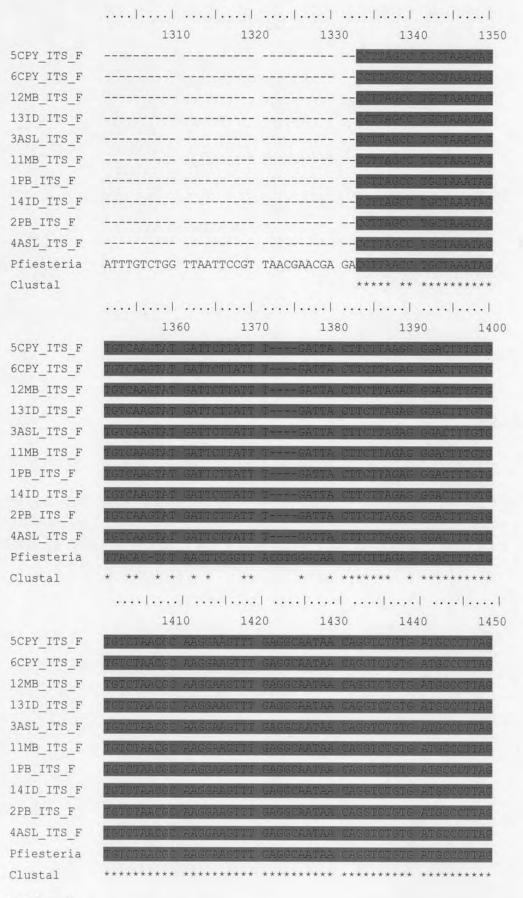
	••••1••••1		1		
	560	570	580	590	600
5CPY_ITS_F					
6CPY_ITS_F					
12MB_ITS_F					
13ID_ITS_F					
3ASL_ITS_F					
11MB_ITS_F					
1PB_ITS_F					
14ID_ITS_F					
2PB_ITS_F					
4ASL_ITS_F					
Pfiesteria	GCAAGTCTGG	TGCCAGCAGC	CGCGGTAATT	CCAGCTCCAA	TAGCGTATAT
Clustal					
					11
	610	620	630	640	0 650
5CPY_ITS_F					
6CPY_ITS_F					
12MB_ITS_F					
13ID_ITS_F					
3ASL_ITS_F					
11MB_ITS_F					
1PB_ITS_F					
14ID_ITS_F					
2PB_ITS_F					
4ASL_ITS_F					
Pfiesteria	TAAAGTTGTT	GCGGTTAAAA	AGCTCGTAGT	TGGATTTCTG	CCGAGGACGA
Clustal					
			11		11
	66	0 670	0 680	0 69	0 700
5CPY_ITS_F					
6CPY_ITS_F					
12MB_ITS_F					
13ID_ITS_F					
3ASL_ITS_F					
11MB_ITS_F					
1PB_ITS_F					
14ID_ITS_F					
2PB_ITS_F					
4ASL_ITS_F					
Pfiesteria	CCGGTCCGCC	CACTGGGTGT	GCATCTGGCT	CGGCCTGGGC	ATCTTCTTGG
Clustal					
Continued					

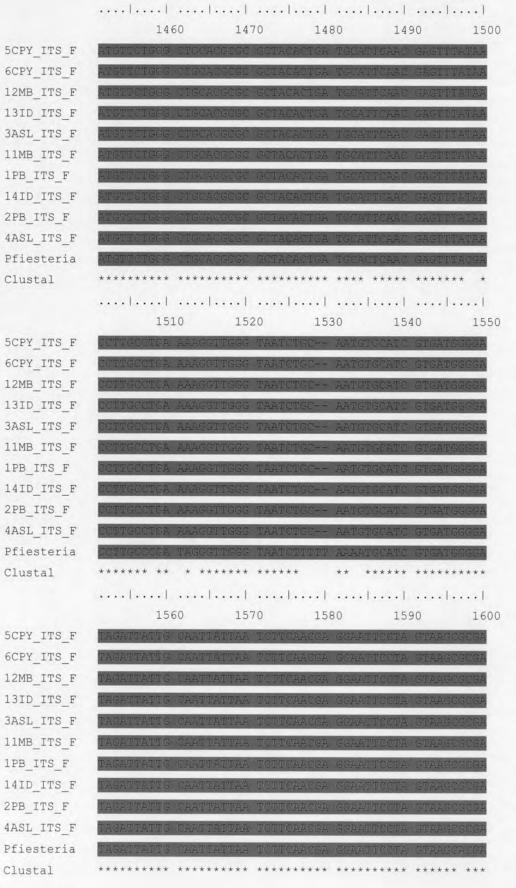
				1	11
	71	0 720	0 73	0 740	0 750
5CPY_ITS_F					
6CPY_ITS_F					
12MB_ITS_F					
13ID_ITS_F					
3ASL_ITS_F					
11MB_ITS_F					
1PB_ITS_F					
14ID_ITS_F					
2PB_ITS_F					
4ASL_ITS_F					
Pfiesteria	AGAACGTATC	TGCACTTCAC	TGTGTGGTGC	GGTATCCAGG	ACTTTTACTT
Clustal					
					11
	76	0 770	0 78	0 790	008 00
5CPY_ITS_F					
6CPY_ITS_F					
12MB_ITS_F					
13ID_ITS_F					
3ASL_ITS_F					
11MB_ITS_F					
1PB_ITS_F					
14ID_ITS_F					
2PB_ITS_F					
4ASL_ITS_F					
Pfiesteria	TGAGGAAATT	AGAGTGTTTC	AAGCAGGCAC	ACGCCTTGAA	TACGTTAGCA
Clustal					
	•••••				
	. 81	820	0 83	0 840	850
5CPY_ITS_F					
6CPY_ITS_F					
12MB_ITS_F					
13ID_ITS_F					
3ASL_ITS_F					
11MB_ITS_F					
1PB_ITS_F					
14ID_ITS_F					
2PB_ITS_F					
4ASL_ITS_F					
Pfiesteria	TGGAATAATA	AGATAGGACC	TTGGTTCTAT	TTTGTTGGTT	TCTAGAACTG
Clustal					
Continued					

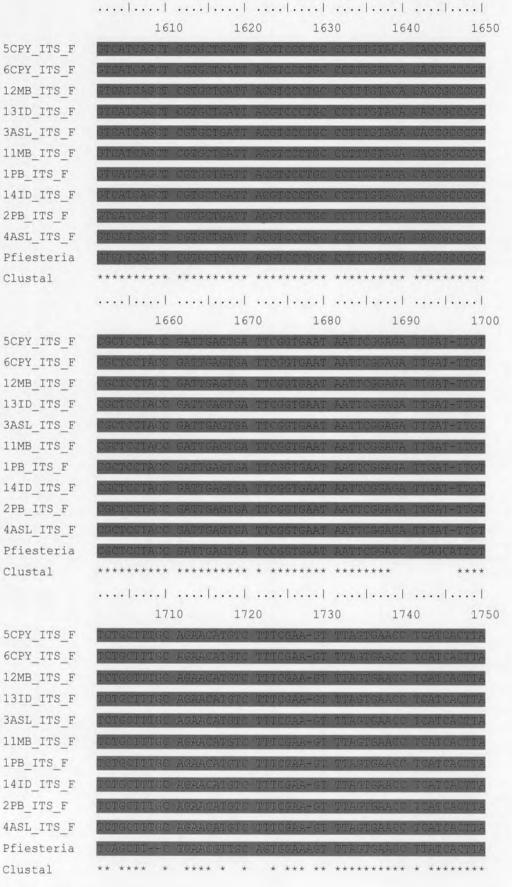
	•••• ••••	•••• ••••			
	86	0 87	0 88	0 89	0 900
5CPY_ITS_F					
6CPY_ITS_F					
12MB_ITS_F					
13ID_ITS_F					
3ASL_ITS_F					
11MB_ITS_F					
1PB_ITS_F					
14ID_ITS_F					
2PB_ITS_F					
4ASL_ITS_F					
Pfiesteria	AGGTAATGAT	TAATAGGGAT	AGTTGGGGGGC	ATTCGTATTT	AACTGTCAGA
Clustal					
	91	0 92	0 93	0 94	0 950
5CPY_ITS_F					
6CPY_ITS_F					
12MB_ITS_F					
13ID_ITS_F					
3ASL_ITS_F					
11MB_ITS_F					
1PB_ITS_F					
14ID_ITS_F					
2PB_ITS_F					
4ASL_ITS_F					
Pfiesteria	GGTGAAATTC	TTGGATTTGT	TAAAGACGGA	CTACTGCGAA	AGCATTTGCC
Clustal					
	96	970	. 98	0 990	0 1000
5CPY_ITS_F					
6CPY_ITS_F					
12MB_ITS_F					
13ID ITS F					
3ASL ITS F					
11MB ITS F					
1PB_ITS_F					
14ID_ITS F					
2PB ITS F					
4ASL ITS F					
Pfiesteria	AAGGATGTT			TTAGGGGATC	GAAGACGATC
Clustal		- UNIT TOATOA	. 101 2100000000	THOUGOAIC	UNNUACUAIC
Contraction of the second					

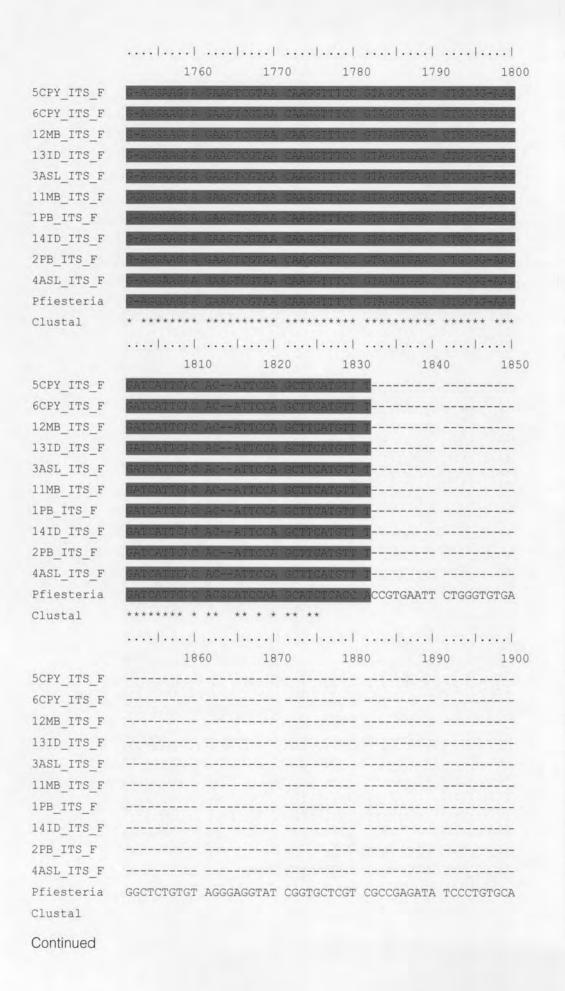
····· [·····] ·····] ·····] ·····] ·····] ·····] ·····] ·····] 1020 1030 1010 1040 1050 5CPY ITS F 6CPY ITS F 12MB ITS F 13ID_ITS_F 3ASL ITS F 11MB_ITS_F 1PB ITS F 14ID_ITS_F 2PB ITS F 4ASL ITS F _____ Pfiesteria AGTATACCGT CCTAGTCTTA ACCATAAACC ATGCCGACTA GAGATTGGAG Clustal ····· [·····] ·····] ·····] ·····] ·····] ·····] ·····] 1060 1070 1080 1090 1100 5CPY ITS F 6CPY ITS F 12MB ITS F 13ID_ITS_F 3ASL ITS F 11MB ITS F 1PB ITS F 14ID ITS F 2PB ITS F 4ASL ITS F Pfiesteria GTCGTTATCT TTACGACTCC TTCAGCACCT TATGAGAAAT CAAAGTCTTT Clustal 1110 1120 1130 1140 1150 5CPY ITS F 6CPY ITS F 12MB ITS F 13ID ITS F 3ASL ITS F 11MB ITS F 1PB ITS F 14ID ITS F 2PB ITS F 4ASL ITS F GGGTTCCGGG GGGAGTATGG TCGCAAGGCT GAAACTTAAA GGAATTGACG Pfiesteria Clustal

1170 1180 1190 1160 1200 5CPY ITS F 6CPY ITS F 12MB ITS F _____ 13ID ITS F 3ASL ITS F ------11MB ITS F 1PB ITS F 14ID ITS F ------ ------- ------- --------2PB ITS F 4ASL ITS F ----- ------ ------ ------- -------Pfiesteria GAAGGGCACC ACCAGGAGTG GAGCCTGCGG CTTAATTTGA CTCAACACGG Clustal 1210 1220 1230 1240 1250 5CPY ITS F 6CPY ITS F 12MB ITS F 13ID ITS F **3ASL ITS F** ---- ---11MB ITS F 1PB ITS F 14ID ITS F 2PB ITS F 4ASL ITS F Pfiesteria GGAAACTTAC CAGGTCCAGA CATAGTAAGG ATTGACAGAT TGATAGCTCT Clustal 1260 1270 1280 1290 1300 5CPY ITS F ----- ----- ------ ----------6CPY ITS F 12MB ITS F ----- ------ ------ ------13ID ITS F 3ASL_ITS_F 11MB ITS F ----- ------ ------- --------1PB ITS F 14ID_ITS_F ------ ------ ------- -------2PB ITS F ----- ------- ------- ------- -------4ASL ITS F Pfiesteria TTCTTGATTC TATGGGTGGT GGTGCATGGC CGTTCTTAGT TGGTGGAGTG Clustal









	19	10 19	20 19	30 19	40 1950
5CPY_ITS_F					
6CPY_ITS_F					
12MB_ITS_F					
13ID_ITS_F					
3ASL_ITS_F					
11MB_ITS_F					
1PB_ITS_F					
14ID_ITS_F					
2PB_ITS_F					
4ASL_ITS_F					
Pfiesteria	GCAGGCTCGA	GGGCGGCTCA	GTGCAGGATG	GGTAGCATTT	GCTTACCTCC
Clustal					
	19	60 19	70 19	80 19	90 2000
5CPY_ITS_F					
6CPY_ITS_F					
12MB_ITS_F					
13ID_ITS_F					
3ASL_ITS_F					
11MB_ITS_F					
1PB_ITS_F					
14ID_ITS_F					
2PB_ITS_F					
4ASL_ITS_F					
Pfiesteria	TTTCTGTGTT	GGTCGCCACC	CATTTCCCTA	CCATATTTAC	CGTCTCTTCC
Clustal					
	20			30 20	
5CPY_ITS_F					
6CPY_ITS_F					
12MB_ITS_F					
13ID_ITS_F					
3ASL_ITS_F					
11MB_ITS_F					
1PB ITS F					
14ID ITS F					
2PB_ITS F					
4ASL_ITS F					
Pfiesteria	ATACTATCCC	TTTGCTCGGG	CTGTGGGGGTG	CTGTGTGTGTTC	GCACACTCAT
Clustal			0101000010	0101010110	GUACACICAI
Continued					

						- 1
	20	60 20	70 20	80 20	90	2100
5CPY_ITS_F						
6CPY_ITS_F						
12MB_ITS_F						
13ID_ITS_F						
3ASL_ITS_F						
11MB_ITS_F						
1PB_ITS_F						
14ID_ITS_F						
2PB_ITS_F						
4ASL_ITS_F						
Pfiesteria	CTCTCATCTC	TGGCATTTCA	TTTTTCTTACA	ACTTTCAGCG	ACGAATGI	CT
Clustal						
						.1
	21	10 21	20 21	30 21	40	2150
5CPY_ITS_F						
6CPY_ITS_F						
12MB_ITS_F						
13ID_ITS_F						
3ASL_ITS_F						
11MB_ITS_F						
1PB_ITS_F						
14ID_ITS_F						
2PB_ITS_F						
4ASL_ITS_F						
Pfiesteria	CGGCTCGAAC	AACGATGAAG	GGCACAGCGA	ACTGTGATAA	GCATTGTG	AA
Clustal						
			···· ····			.1
	21					2200
5CPY_ITS_F						
6CPY_ITS_F						
12MB_ITS_F						
13ID_ITS_F						
3ASL_ITS_F						
11MB_ITS_F						
1PB_ITS_F						
14ID_ITS_F						
2PB_ITS_F						
4ASL_ITS_F						
Pfiesteria	TTGCAGATTT	CCGCGAATCA	ATAGAAACTT	GAACGTAAAA	TGCGGTTT	CG
Clustal						
Continued						

				11	1
	221	LO 22:	20 22	30 22	40 2250
5CPY_ITS_F					
6CPY_ITS_F					
12MB_ITS_F					
13ID_ITS_F					
3ASL_ITS_F					
11MB_ITS_F					
1PB_ITS_F					
14ID_ITS_F					
2PB_ITS_F					
4ASL_ITS_F					
Pfiesteria	GGATATCCCT	GAAACCATGC	CTGCTTCAGT	GTCCCAACCT	CTCCTTCCGG
Clustal					
	1				1
	226	50 22	70 22	80 225	90 2300
5CPY_ITS_F					
6CPY_ITS_F					
12MB_ITS_F					
13ID_ITS_F					
3ASL_ITS_F					
11MB_ITS_F					
1PB_ITS_F					
14ID_ITS_F					
2PB_ITS_F					
4ASL_ITS_F					
Pfiesteria	TCGAACCTTT	CGGTGACTGA	TGTATTTCGG	TGCTCCGAAG	GTTCGGTCTC
Clustal					
			11		11
	231	.0 233	20 23	30 234	10 2350
5CPY_ITS_F					
6CPY_ITS_F					
12MB_ITS_F					
13ID_ITS_F					
3ASL_ITS_F					
11MB_ITS_F					
1PB_ITS_F					
14ID_ITS_F					
2PB_ITS_F					
4ASL_ITS_F					
Pfiesteria	GGTGTCGTTG	TGTGTTAAGG	TGCTTCGGCT	CTCGTCGTCG	CCCTTGACAC
Clustal					
Continued					

	23				
5CPY_ITS_F					
6CPY_ITS_F					
12MB_ITS_F					
13ID_ITS_F					
3ASL_ITS_F					
11MB_ITS_F					
1PB_ITS_F					
14ID_ITS_F					
2PB_ITS_F					
4ASL_ITS_F					
Pfiesteria	ATCTAGGACA	TGGAGATATC	CCTGGCGGCT	CTTTGGCGAC	GAATCATGCT
Clustal					
	1				
		10 24:			
5CPY_ITS_F					
6CPY_ITS_F					
12MB_ITS_F					
13ID_ITS_F					
3ASL_ITS_F					
11MB_ITS_F					
1PB_ITS_F					
14ID_ITS_F					
2PB_ITS_F					
4ASL_ITS_F					
Pfiesteria	CACCCATGCA	TTTGCTCGCC	TCCCGTCTTG	GGGTTTTGTC	TCCCACAGTC
Clustal					
	24				
5CPY_ITS_F					
6CPY_ITS_F					
12MB ITS F					
13ID ITS F					
3ASL ITS F					
11MB_ITS F					
1PB_ITS_F					
14ID_ITS F					
2PB_ITS_F					
4ASL ITS F					
Pfiesteria					
Clustal	TICCIINGCA	CATTGTCACA	COACGATGGC	ICICAAGACA	TGAAGTCAGG
Justar					
A					

	25.	10 25.	20 25.	30 25.	40 2550
5CPY_ITS_F					
6CPY_ITS_F					
12MB_ITS_F					
13ID_ITS_F					
3ASL_ITS_F					
11MB_ITS_F					
1PB_ITS_F					
14ID_ITS_F					
2PB_ITS_F					
4ASL_ITS_F					
Pfiesteria	TCAGCAAACC	CGCTGAATTT	AAGCATATAA	GTAAGCGGAG	GAAAAGAAAC
Clustal					

	256	50 25	70 25	30 259	90
5CPY_ITS_F					
6CPY_ITS_F					
12MB_ITS_F					
13ID_ITS_F					
3ASL_ITS_F					
11MB_ITS_F					
1PB_ITS_F					
14ID_ITS_F					
2PB_ITS_F					
4ASL_ITS_F					
Pfiesteria	TAAATAGGAT	TCCCTTAGTA	ATGGCGAATG	AACAGGGAAA	TCAC
Clustal					

APPENDIX4

The result from BioEdit program of ITS reverse (ITS_R2) sequence of N. scintillans sample in the inner gulf of Thailand (1-2PB, 3-4ASL and 5-6CPY) and outing group from Philippine (11-12MB) and Indonesia (13-14ID). Asterisks symbols (*) expressed that all samples appear nitrogenous base (A, C, G, and T) identity. Blue label showed the area of nucleotide sequences of samples were compared with *Pfiesteria-like dinoflagellate* position.

	10) 2	0 3	0 4	0 50
5CPY_ITS_R					
6CPY_ITS_R					
3ASL_ITS_R					
Pfiesteria	TCAACCTGGT	GATCCTGCCA	GTAGTCATAT	GCTTGTCTCA	AAGATTAAGC
Clustal					
	1				
	60) 7	0 8	0 9	0 100
5CPY_ITS_R					
6CPY_ITS_R					
3ASL_ITS_R					
Pfiesteria	CATGCATGTC	TCAGCATAAA	TTCCTGCACG	GTAAGGCTGC	GAATGGCTCA
Clustal					
	•••• ••••	•••••1•••••1	•••• ••••		
	110	120	0 13	0 140	0 150
5CPY_ITS_R					
6CPY_ITS_R					
3ASL_ITS_R					
Pfiesteria	TTAAAACAGT	TATAGTTTAT	TTGATGGTCA	TTCCTACATG	GATAACTGTG
Clustal					
	160	170) 180	0 190	200
5CPY_ITS_R					
6CPY_ITS_R					
3ASL_ITS_R					
	GTAATTCTAG	AGCTAATACA	TGCACCCAAA	CCCGACTCCG	TGGAAGGGTT
Clustal					
Oraliand					

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	260	27	0 28	0 29	0 300		
5CPY_ITS_R							
6CPY_ITS_R							
3ASL_ITS_R							
Pfiesteria	TCATAATAAC	CGAACGAATC	GCATGCCTTC	GGCGGCGATG	GATCATTTAA		
Clustal							

	•••• ••••	•••• ••••	1		
	310	320	330	0 340	0 350
5CPY_ITS_R					
6CPY_ITS_R					
3ASL_ITS_R					
Pfiesteria	GTTTCTGACC	TATCAGCTTT	CGACGGTAGG	ATATAGGCCT	ACCGTGGCGA
Clustal					

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	360	371	38	0 390	0 400			
5CPY_ITS_R								
6CPY_ITS_R								
3ASL_ITS_R								
Pfiesteria	CGACGGGTAA	CGGAGAATTA	GGGTTCGATT	CCGGAGAGGG	AGCCTGAGAA			
Clustal								

				1	
	.410) 420	9 430	9 440	o 450
5CPY_ITS_R					
6CPY_ITS_R					
3ASL_ITS_R					
Pfiesteria	ACGGCTACCA	CATCTAAGGA	AGGCAGCAGG	CGCGCAAATT	ACCCAATCCT
Clustal					

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	460) 47(480	0 490	500		
5CPY_ITS_R							
6CPY_ITS_R							
3ASL_ITS_R							
Pfiesteria	GACACAGGGA	GGTAGTGACA	AGAAATAACA	ATACAGGGCA	TCCATGTCTT		
Clustal							

				!	1
	510	520	530	540	550
5CPY_ITS_R					
6CPY_ITS_R					
3ASL_ITS_R					
Pfiesteria	GTAATTGGAA	TGAGTAGAAT	TTAAATCCCT	TTATGAGTAT	CAATTGGAGG
Clustal					

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	560	570	0 580	590	600		
5CPY_ITS_R							
6CPY_ITS_R							
3ASL_ITS_R							
Pfiesteria	GCAAGTCTGG	TGCCAGCAGC	CGCGGTAATT	CCAGCTCCAA	TAGCGTATAT		
Clustal							

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	610	620	630	640	650	
5CPY_ITS_R						
6CPY_ITS_R						
3ASL_ITS_R						
Pfiesteria	TAAAGTTGTT	GCGGTTAAAA	AGCTCGTAGT	TGGATTTCTG	CCGAGGACGA	
Clustal						

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	660	670	680	690	700	
5CPY_ITS_R						
6CPY_ITS_R						
3ASL_ITS_R						
Pfiesteria	CCGGTCCGCC	CACTGGGTGT	GCATCTGGCT	CGGCCTGGGC	ATCTTCTTGG	
Clustal						

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	710) 720	730	740	750		
5CPY_ITS_R							
6CPY_ITS_R							
3ASL_ITS_R							
Pfiesteria	AGAACGTATC	TGCACTTCAC	TGTGTGGTGC	GGTATCCAGG	ACTTTTACTT		
Clustal							

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	760	0 77	0 78	0 79	0 800		
5CPY_ITS_R							
6CPY_ITS_R							
3ASL_ITS_R							
Pfiesteria	TGAGGAAATT	AGAGTGTTTC	AAGCAGGCAC	ACGCCTTGAA	TACGTTAGCA		
Clustal							

	•••• ••••				
	810	820	830	0 840	0 850
5CPY_ITS_R					
6CPY_ITS_R					
3ASL_ITS_R					
Pfiesteria	TGGAATAATA	AGATAGGACC	TTGGTTCTAT	TTTGTTGGTT	TCTAGAACTG
Clustal					

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	860	87	0 880	0 89	000 0			
5CPY_ITS_R								
6CPY_ITS_R								
3ASL_ITS_R								
Pfiesteria	AGGTAATGAT	TAATAGGGAT	AGTTGGGGGC	ATTCGTATTT	AACTGTCAGA			
Clustal								

	910	920	930	940	950
5CPY_ITS_R					
6CPY_ITS_R					
3ASL_ITS_R					
Pfiesteria	GGTGAAATTC	TTGGATTTGT	TAAAGACGGA	CTACTGCGAA	AGCATTTGCC
Clustal					

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	960	970	980	990	1000		
5CPY_ITS_R							
6CPY_ITS_R							
3ASL_ITS_R							
Pfiesteria	AAGGATGTTT	TCATTGATCA	AGAACGAAAG	TTAGGGGATC	GAAGACGATC		
Clustal							

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	10:	10 10	20 10	30 10	40 1050			
5CPY_ITS_R								
6CPY_ITS_R								
3ASL_ITS_R								
Pfiesteria	AGTATACCGT	CCTAGTCTTA	ACCATAAACC	ATGCCGACTA	GAGATTGGAG			
Clustal								

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	10	60 1	070 1	.080 10	90 1100			
5CPY_ITS_R								
6CPY_ITS_R								
3ASL_ITS_R								
Pfiesteria	GTCGTTATCT	TTACGACTC	C TTCAGCACC	T TATGAGAAAA	CAAAGTCTTT			
Clustal								

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	111	10 11	20 11	30 114	40 1150		
5CPY_ITS_R							
6CPY_ITS_R							
3ASL_ITS_R							
Pfiesteria	GGGTTCCGGG	GGGAGTATGG	TCGCAAGGCT	GAAACTTAAA	GGAATTGACG		
Clustal							

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	110	60 1	.170 11	180 11	90 1200		
5CPY_ITS_R							
6CPY_ITS_R							
3ASL_ITS_R							
Pfiesteria	GAAGGGCACC	ACCAGGAGI	G GAGCCTGCGG	G CTTAATTTGA	CTCAACACGG		
Clustal							

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	121	LO 12	20 12	30 12	40 1250		
5CPY_ITS_R							
6CPY_ITS_R							
3ASL_ITS_R							
Pfiesteria	GGAAACTTAC	CAGGTCCAGA	CATAGTAAGG	ATTGACAGAT	TGATAGCTCT		
Clustal							

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	12	60 12	.70 12	80 12	90 1300		
5CPY_ITS_R							
6CPY_ITS_R							
3ASL_ITS_R							
Pfiesteria	TTCTTGATTC	TATGGGTGGT	GGTGCATGGC	CGTTCTTAGT	TGGTGGAGTG		
Clustal							

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	13:	10 1	320	133	30 13	40	1350	
5CPY_ITS_R								
6CPY_ITS_R								
3ASL_ITS_R								
Pfiesteria	ATTTGTCTGG	TTAATTCCG	T TAACG	AACGA	GACCTTAACC	TGCTAAAT	AG	
Clustal								

....l...l....l....l....l....l136013701380139014005CPY_ITS_R------------------6CPY_ITS_R------------------3ASL_ITS_R------------------PfiesteriaTTACACTGTA ACTTCGGTTA CGTGGGCAAC TTCTTAGAGG GACTTTGTGTClustal

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	······································						
	141	.0 142	20 14:	30 . 14	40 1450		
5CPY_ITS_R							
6CPY_ITS_R							
3ASL_ITS_R							
Pfiesteria	GTCTAACGCA	AGGAAGTTTG	AGGCAATAAC	AGGTCTGTGA	TGCCCTTAGA		
Clustal							

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	14	60	14	70 1	480	149	90	1500
5CPY_ITS_R								
6CPY_ITS_R								
3ASL_ITS_R								
Pfiesteria	TGTTCTGGGC	TGCACG	CGCG	CTACACTGA	T GCAC	TCAACG	AGTTTA	CGAC
Clustal								

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	15	10 1	520 15	30 15	40 1550		
5CPY_ITS_R							
6CPY_ITS_R							
3ASL_ITS_R							
Pfiesteria	CTTGCCCGAT	AGGGTTGGG	T AATCTTTTA	AAATGCATCG	TGATGGGGAT		
Clustal .							

			1	···· ····		1
156	50 3	1570	158	30 15	590	1600
AGATTATTGC	AATTATTAA	AT CTTC	AACGAG	GAATTCCTAC	TAAGCAG	CGAG
	150 	1560	1560 1570	1560 1570 158	1560 1570 1580 15 	1560 1570 1580 1590 AGATTATTGC AATTATTAAT CTTCAACGAG GAATTCCTAG TAAGCAG

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	161	10 16	20 16	30 16	40 1650		
5CPY_ITS_R							
6CPY_ITS_R							
3ASL_ITS_R							
Pfiesteria	TCATCAGCTC	GTGCTGATTA	CGTCCCTGCC	CTTTGTACAC	ACCGCCCGTC		
Clustal							

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	166	50 16	70 16	80 169	90 1700	
5CPY_ITS_R						
6CPY_ITS_R						
3ASL_ITS_R						
Pfiesteria	GCTCCTACCG	ATTGAGTGAT	CCGGTGAATA	ATTCGGACCG	CAGCATTGTT	
Clustal						

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	171	LO 17	20 173	30 174	40 1750		
5CPY_ITS_R							
6CPY_ITS_R							
3ASL_ITS_R							
Pfiesteria	CAGCTTCTGA	ACGTTGCAGT	GGAAAGTTTA	GTGAACCTTA	TCACTTAGAG		
Clustal							

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	176	50 17	70 17	80 17	90 1800		
5CPY_ITS_R							
6CPY_ITS_R							
3ASL_ITS_R							
Pfiesteria	GAAGGAGAAG	TCGTAACAAG	GTTTCCGTAG	GTGAACCTGC	GGAAGGATCA		
Clustal							

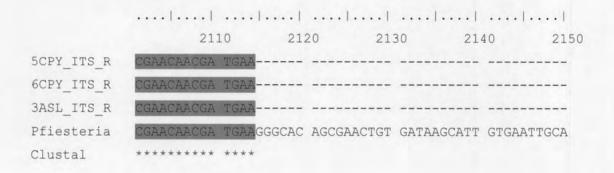
	····· · · · · · · · · · · · · · · · ·						
	181	.0 .	1820	1830	1840	1850	
5CPY_ITS_R							
6CPY_ITS_R							
3ASL_ITS_R							
Pfiesteria	TTCGCACGCA	TCCAAGCA	TC TCACCAC	CGT GAATT	CTGGG TGTG	AGGCTC	
Clustal							

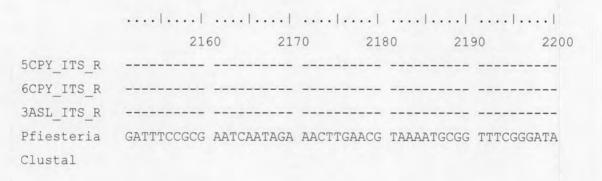
	····· · · · · · · · · · · · · · · · ·						
	18	60 18	70 18	80 18	90 1900		
5CPY_ITS_R							
6CPY_ITS_R							
3ASL_ITS_R							
Pfiesteria	TGTGTAGGGA	GGTATCGGTG	CTCGTCGCCG	AGATATCCCT	GTGCAGCAGG		
Clustal							

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	19:	10 1	920 1	930 19	40 1950
5CPY_ITS_R					
6CPY_ITS_R					
3ASL_ITS_R					
Pfiesteria	CTCGAGGGCG	GCTCAGTGC	A GGATGGGTA	G CATTTGCTTA	CCTCCTTTCT
Clustal					

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	196	50 19	70 198	80 19	90 2000		
5CPY_ITS_R							
6CPY_ITS_R							
3ASL_ITS_R							
Pfiesteria	GTGTTGGTCG	CCACCCATTT	CCCTACCATA	TTTACCGTCT	CTTCCATACT		
Clustal							

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	201	10 20	20 20	30 204	40 2050		
5CPY_ITS_R							
6CPY_ITS_R							
3ASL_ITS_R							
Pfiesteria	ATCCCTTTGC	TCGGGCTGTG	GGGTGCTGTG	TGTTCGCACA	CTCATCTCTC		
Clustal							





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	221	.0 22	20 22	30 224	40 2250	
5CPY_ITS_R						
6CPY_ITS_R						
3ASL_ITS_R						
Pfiesteria	TCCCTGAAAC	CATGCCTGCT	TCAGTGTCCC	AACCTCTCCT	TCCGGTCGAA	
Clustal						

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	226	50 22	270 22	80 22	90 2300		
5CPY_ITS_R							
6CPY_ITS_R							
3ASL_ITS_R							
Pfiesteria	CCTTTCGGTG	ACTGATGTAT	TTCGGTGCTC	CGAAGGTTCG	GTCTCGGTGT		
Clustal							

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	23	10 23	20 23	30 23	40 2350		
5CPY_ITS_R							
6CPY_ITS_R							
3ASL_ITS_R							
Pfiesteria	CGTTGTGTGT	TAAGGTGCTT	CGGCTCTCGT	CGTCGCCCTT	GACACATCTA		
Clustal							

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	23	50 2	370 2	380 2	390 240	C	
5CPY_ITS_R							
6CPY_ITS_R							
3ASL_ITS_R							
Pfiesteria	GGACATGGAG	ATATCCCTG	G CGGCTCTTT	G GCGACGAAT	C ATGCTCACCC		
Clustal							

	•••••		1		
	241	24	20 24	30 244	40 2450
5CPY_ITS_R					
6CPY_ITS_R					
3ASL_ITS_R					
Pfiesteria	ATGCATTTGC	TCGCCTCCCG	TCTTGGGGTT	TTGTCTCCCA	CAGTCTTCCT
Clustal					

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	24	60 24	70 24	80 24	90 2500		
5CPY_ITS_R							
6CPY_ITS_R							
3ASL_ITS_R							
Pfiesteria	TAGCACATTG	TCACACGACG	ATGGCTCTCA	AGACATGAAG	TCAGGTCAGC		
Clustal							

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	25	10	25:	20 25	30 25	640	2550
5CPY_ITS_R							
6CPY_ITS_R							
3ASL_ITS_R							
Pfiesteria	AAACCCGCTG	AATTTA	AGCA	TATAAGTAAG	CGGAGGAAAA	GAAACTA	AAT
Clustal							

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	25	60 25	70 25	80		
5CPY_ITS_R						
6CPY_ITS_R						
3ASL_ITS_R						
Pfiesteria	AGGATTCCCT	TAGTAATGGC	GAATGAACAG	GGAAATCAC		
Clustal						

APPENDIX5

The result from BioEdit program of ITS forward (ITS_F3 and ITS_F4) sequence of *N. scintillans* sample in outing group from Philippine (10MB).Asterisks symbols (*) expressed that all samples appear nitrogenous base (A, C, G, and T) identity. Purple label showed the area of nucleotide sequences from ITS_F3 primer and yellow label showed the area of nucleotide sequences from ITS_F4 primer, the results were compared with *Pfiesteria-like dinoflagellate* position.

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11_MB_ITS3 11 MB ITS4	10 20 30 40 50
Pfiesteria Clustal	TCAACCTGGT GATCCTGCCA GTAGTCATAT GCTTGTCTCA AAGATTAAGC
	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$
11_MB_ITS3 11_MB_ITS4	
Pfiesteria Clustal	CATGCATGTC TCAGCATAAA TTCCTGCACG GTAAGGCTGC GAATGGCTCA
11_MB_ITS3	110 120 130 140 150
11_MB_ITS4 Pfiesteria Clustal	TTAAAACAGT TATAGTTTAT TTGATGGTCA TTCCTACATG GATAACTGTG
11_MB_ITS3 11_MB_ITS4 Pfiesteria Clustal	l l <t< td=""></t<>
	GTAATTCTAG AGCTAATACA TGCACCCAAA CCCGACTCCG TGGAAGGGTT
	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
11_MB_ITS3 11_MB_ITS4	
Pfiesteria Clustal	GTGTTTATTA GTTACAGAAC CAACCCAGGC TTGCCTGGTC TTGTGGTGAT
	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
11_MB_ITS3 11 MB ITS4	
Pfiesteria Clustal	TCATAATAAC CGAACGAATC GCATGCCTTC GGCGGCGATG GATCATTTAA
Continued	

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 310
 320
 330
 340
 350

 11 MB ITS3 ------ -------11 MB ITS4 GTTTCTGACC TATCAGCTTT CGACGGTAGG ATATAGGCCT ACCGTGGCGA Pfiesteria Clustal 11 MB ITS3 11 MB ITS4 Pfiesteria CGACGGGTAA CGGAGAATTA GGGTTCGATT CCGGAGAGGG AGCCTGAGAA Clustal Co 410 420 430 440 450 11 MB ITS3 11 MB ITS4 Pfiesteria ACGGCTACCA CATCTAAGGA AGGCAGCAGG CGCGCAAATT ACCCAATCCT Clustal Co 11 MB ITS3 --- ----- ------- ----- ------11 MB ITS4 _____ _____ Pfiesteria GACACAGGGA GGTAGTGACA AGAAATAACA ATACAGGGCA TCCATGTCTT Clustal Co 11 MB ITS3 -----11 MB ITS4 Pfiesteria GTAATTGGAA TGAGTAGAAT TTAAATCCCT TTATGAGTAT CAATTGGAGG Clustal 11 MB ITS3 11 MB ITS4 Pfiesteria GCAAGTCTGG TGCCAGCAGC CGCGGTAATT CCAGCTCCAA TAGCGTATAT Clustal 610 620 630 640 650 11 MB ITS3 11 MB ITS4 Pfiesteria TAAAGTTGTT GCGGTTAAAA AGCTCGTAGT TGGATTTCTG CCGAGGACGA Clustal ---- ------ -------- ---11 MB ITS --- -11 MB ITS Pfiesteria CCGGTCCGCC CACTGGGTGT GCATCTGGCT CGGCCTGGGC ATCTTCTTGG Clustal Co

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 <t 11 MB ITS3 11 MB ITS4 AGAACGTATC TGCACTTCAC TGTGTGGTGC GGTATCCAGG ACTTTTACTT Pfiesteria Clustal 11 MB ITS3 11 MB ITS4 Pfiesteria TGAGGAAATT AGAGTGTTTC AAGCAGGCAC ACGCCTTGAA TACGTTAGCA Clustal

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 810
 820
 830
 840
 850

 11 MB ITS3 11 MB ITS4 ---- --------TGGAATAATA AGATAGGACC TTGGTTCTAT TTTGTTGGTT TCTAGAACTG Pfiesteria Clustal Co

 11 MB ITS3 ----- ------11 MB ITS4 Pfiesteria AGGTAATGAT TAATAGGGAT AGTTGGGGGGC ATTCGTATTT AACTGTCAGA Clustal 11 MB ITS3 11 MB ITS4 ----- ----- ------Pfiesteria GGTGAAATTC TTGGATTTGT TAAAGACGGA CTACTGCGAA AGCATTTGCC Clustal 11 MB ITS3 11 MB ITS4 Pfiesteria AAGGATGTTT TCATTGATCA AGAACGAAAG TTAGGGGATC GAAGACGATC Clustal ···· [····] ····] ····] ····] ····] ····] ····] ····] 1010 1020 1030 1040 1050 11 MB ITS3 11 MB ITS4 Pfiesteria AGTATACCGT CCTAGTCTTA ACCATAAACC ATGCCGACTA GAGATTGGAG Clustal 1060 1070 1080 1090 1100 11 MB ITS3 ---- ------ --11 MB ITS4 ----- ------ ------ -----GTCGTTATCT TTACGACTCC TTCAGCACCT TATGAGAAAT CAAAGTCTTT Pfiesteria Clustal

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 11 MB ITS3 _____ ____ 11 MB ITS4 Pfiesteria GGGTTCCGGG GGGAGTATGG TCGCAAGGCT GAAACTTAAA GGAATTGACG Clustal 1160 1170 1180 1190 1200 11 MB ITS3 11 MB ITS4 Pfiesteria GAAGGGCACC ACCAGGAGTG GAGCCTGCGG CTTAATTTGA CTCAACACGG Clustal

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 11 MB ITS3 11 MB ITS4 _____ Pfiesteria GGAAACTTAC CAGGTCCAGA CATAGTAAGG ATTGACAGAT TGATAGCTCT Clustal 1260 1270 1280 1290 1300 11 MB ITS3 11 MB ITS4 Pfiesteria TTCTTGATTC TATGGGTGGT GGTGCATGGC CGTTCTTAGT TGGTGGAGTG Clustal

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 11 MB ITS3 11 MB ITS4 Pfiesteria ATTTGTCTGG TTAATTCCGT TAACGAACGA GACCTTAACC TGCTAAATAG Clustal ···· [····] ···· [····] ····] ····] ····] ····] ····] 1360 1370 1380 1390 1400 11 MB ITS3 _____ 11 MB ITS4 Pfiesteria TTACACTGTA ACTTCGGTTA CGTGGGCAAC TTCTTAGAGG GACTTTGTGT Clustal ···· [····] ···· [····] ····] ····] ····] ····] ····] 1410 1420 1430 1440 1450 11 MB ITS3 11 MB ITS4 Pfiesteria GTCTAACGCA AGGAAGTTTG AGGCAATAAC AGGTCTGTGA TGCCCTTAGA Clustal ·····|·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ···| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ··| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ··| ···| ···| ···| ··| ···| ···| ···| 11_MB_ITS3 11 MB ITS4 Pfiesteria TGTTCTGGGC TGCACGCGCG CTACACTGAT GCACTCAACG AGTTTACGAC Clustal

···· [····] ···· [····] ···· [····] ···· [····] ···· [····] 1510 1520 1530 1540 1550 11_MB_ITS3 11 MB ITS4 Pfiesteria CTTGCCCGAT AGGGTTGGGT AATCTTTTTA AAATGCATCG TGATGGGGGAT Clustal 1560 1570 1580 1590 1600 _____ 11 MB ITS3 _____ 11 MB ITS4 Pfiesteria AGATTATTGC AATTATTAAT CTTCAACGAG GAATTCCTAG TAAGCACGAG Clustall....l....l....l....l....l....l....l 1610 1620 1630 1640 1650 11 MB ITS3 11 MB ITS4 _____ ____ Pfiesteria TCATCAGCTC GTGCTGATTA CGTCCCTGCC CTTTGTACAC ACCGCCCGTC Clustal ···· [···· [····] ····] ···· [····] ···· [····] ····] ····] ····] 1660 1670 1680 1690 1700 11 MB ITS3 11 MB ITS4 _____ Pfiesteria GCTCCTACCG ATTGAGTGAT CCGGTGAATA ATTCGGACCG CAGCATTGTT Clustal 1710 1720 1730 1740 1750 11 MB ITS3 11 MB ITS4 Pfiesteria CAGCTTCTGA ACGTTGCAGT GGAAAGTTTA GTGAACCTTA TCACTTAGAG Clustal ····· | ····· | ····· | ····· | ····· | ····· | ····· | ····· | ····· | ····· | ····· | 11 MB ITS3 11 MB ITS4 _____ Pfiesteria GAAGGAGAAG TCGTAACAAG GTTTCCGTAG GTGAACCTGC GGAAGGATCA Clustal ···· [···· [····] ···· [····] ····] ····] ····] ····] ····] 1810 1820 1830 1840 1850 TTCACACAT TCCAG---CT TCATGTTGT GCATGTTGCG T-TGTGGCCA 11 MB ITS3 11 MB ITS4 -----CCA TTCAG---CT TCATGTTTGT GCATGTTGCG T-TGTGGCCA Pfiesteria TTCGCACGCA TCCAAGCATC TCACCACCGT GAATTCTGGG TGTGAGGCTC *** ** ** ** * ** *** Clustal * ** 1860 1870 1880 1890 1900 IGA-IGA CGTCTCATAC GICTT----- ------ -----11 MB ITS3 TGTGGAATGA CGTCTCATAC GTCTT----- -----11 MB ITS4 Pfiesteria TGTGTAGGGA GGTATCGGTG CTCGTCGCCG AGATATCCCT GTGCAGCAGG **** ** ** ** ** ** Clustal

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1910 .1920 1930 1940 1950 11 MB ITS3 _____ 11 MB ITS4 CTCGAGGGCG GCTCAGTGCA GGATGGGTAG CATTTGCTTA CCTCCTTTCT Pfiesteria Clustal 11 MB ITS3 11 MB ITS4 Pfiesteria GTGTTGGTCG CCACCCATTT CCCTACCATA TTTACCGTCT CTTCCATACT Clustal 2010 2020 2030 2040 2050 11 MB ITS3 11 MB ITS4 ATCCCTTTGC TCGGGCTGTG GGGTGCTGTG TGTTCGCACA CTCATCTCTC Pfiesteria Clustall....l....l....l....l....l....l....l 2060 2070 2080 2090 2100 11 MB ITS3 11 MB ITS4 Pfiesteria ATCTCTGGCA TTTCATTTTC TTACAACTTT CAGCGACGAA TGTCTCGGCT Clustal Co 2110 2120 2130 2140 2150 11 MB ITS3 11 MB ITS4 Pfiesteria CGAACAACGA TGAAGGGCAC AGCGAACTGT GATAAGCATT GTGAATTGCA Clustal Co ···· [····] ···· [····] ···· [····] ···· [····] ···· [····] 2160 2170 2180 2190 2200 11 MB ITS3 11 MB ITS4 ----- ------ ------ ------GATTTCCGCG AATCAATAGA AACTTGAACG TAAAATGCGG TTTCGGGATA Pfiesteria Clustal 2210 2220 2230 2240 2250 11 MB ITS3 ----- ---------11 MB ITS4 Pfiesteria TCCCTGAAAC CATGCCTGCT TCAGTGTCCC AACCTCTCCT TCCGGTCGAA Clustal ···· [····] ····] ····] ····] ····] ····] ····] ····] 2260 2270 2280 2290 2300 11_MB_ITS3 11 MB ITS4 Pfiesteria CCTTTCGGTG ACTGATGTAT TTCGGTGCTC CGAAGGTTCG GTCTCGGTGT Clustal

 11_MB_ITS3 11_MB_ITS4 Pfiesteria CGTTGTGTGT TAAGGTGCTT CGGCTCTCGT CGTCGCCCTT GACACATCTA Clustal

 11 MB ITS3 11 MB ITS4 Pfiesteria GGACATGGAG ATATCCCTGG CGGCTCTTTG GCGACGAATC ATGCTCACCC Clustal ···· [···· [····] ····] ···· [····] ····] ····] ····] ····] 2410 2420 2430 2440 2450 11 MB ITS3 11 MB ITS4 ATGCATTTGC TCGCCTCCCG TCTTGGGGGTT TTGTCTCCCA CAGTCTTCCT Pfiesteria Clustal ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ·····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ····| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ···| ··| ···| ···| ···| ···| ··| ···| ···| ···| ··| ···| ···| ···| ···| ···| ···| ···| ···| ··| ···| ···| ··| ···| ···| ···| ···| ···| ··| ···| ···| ··| ··| ···| ··| ···| ···| ···| ···| ···| ··| ···| ···| ···| ··| ···| ··| ···| ···| ··| ···| ···| ··| ··| ··| ··| ··| ··| ···| ··| ··| ···| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| ··| 11 MB ITS3 ---- ----11 MB ITS4 ----- -------Pfiesteria TAGCACATTG TCACACGACG ATGGCTCTCA AGACATGAAG TCAGGTCAGC Clustal ···· [····] ···· [····] ···· [····] ···· [····] ···· [····] 2510 2520 2530 2540 2550 11 MB_ITS3 11 MB ITS4 Pfiesteria AAACCCGCTG AATTTAAGCA TATAAGTAAG CGGAGGAAAA GAAACTAAAT Clustal Co ···· [····] ···· [···· [···· [····] ···· [···· 2560 2570 2580 11 MB ITS3 ----- -------- ------ ------11 MB ITS4 Pfiesteria AGGATTCCCT TAGTAATGGC GAATGAACAG GGAAATCAC Clustal