CHAPTER III

Results

3.1 DNA extraction

Genomic DNA was individually extracted from a frozen pleopod of each *P.monodon* using a phenol-chloroform method. The quality of extracted DNA was determined by electrophoresis through 0.8% agarose gels. High molecular weight extracted DNA was approximately 23 kb in size (Fig. 3.1). The amount of extracted DNA was estimated spectrophotometrically. The yield was about 50-100 µg per pleopod. The ratio of OD 260/280 was 1.8-2.0 indicating good quality of obtained DNA. Some DNA samples contained RNA contamination as visualized by smeared bands at the bottom of gel but contaminated RNA did not interfere the subsequent PCR reactions.

3.2 PCR amplification

Thirteen primers designed from mitochondrial and nuclear DNA genes were screened for the amplification success of *P. monodon* DNA. Five pairs of primers (an intergenic COI-COII, 16S rDNA, ND5, 12S rDNA and hemocyanin) were successfully used in this species (Fig. 3.2). Nevertheless, ND5 and 12S rDNA were not consistently amplified when the sample sizes were increased. The amplified hemocyanin gene segment showed low polymorphism when digested with restriction endonucleases initially screened in this study (Fig.3.3 - 3.9).

Conversely, an intergenic COI-COII and 16S rDNA were consistently amplified and provided high polymorphic results. As a result, these gene segments were chosen for population genetic studies of *P.monodon*. Sizes of an intergenic COI-COII and 16S rDNA were 1700 bp, 560 bp in length, respectively. The reaction conditions for amplification of these mitochondrial regions were further optimised. The selected amplification conditions were described in the Chapter II.

3.3 Restriction analysis of an intergenic COI-COII and the 16S rDNA genes

Each PCR-amplified product of 10 individuals of *P. monodon* was screened with 18 restriction enzymes including *Dra* I, *EcoR* I, *Alu* I, *Mbo* I , *Dde* I, *Bam* H I, *Hind* III, *Taq* I, *Acs* I, *Bfr* I, *Swa* I, *Hinf* I, *Bgl* II, *Rsa* I, *Nde* I, *Ssp* I, *Cla* I and *Hae* III. An intergene COI-COII restricted with *Alu* I, *Mbo* I, *Taq* I, *Hin* fI and *Dde* I and 16S rDNA restricted with *Mbo* I revealed polymorphism of restriction enzyme patterns in *P. monodon*.

Thirty-seven digestion patterns (single haplotypes) were observed from analysis of 154 shrimps with 6 restriction enzymes. Restriction patterns generated by each enzyme is shown in Table 3.1 and Figs. 3.10 - 3.15. Distributions of single haplotypes of each restriction enzyme in a particular geographic sample are shown in Table 3.2.

Three different haplotypes (A, B and C) were obtained from *Mbo* I digestion of an amplified 16S rDNA (Fig. 3.10). Haplotype A had 390 and 170 bp bands . Haplotype B had discrete bands of 280, 170 and 100 bp. Haplotype C had 380 and 170 bp band and was found in only one specimens singleton from Phangnga. The

interconnection between haplotype B and C could be explained by a loss of the restriction site which generated 280 bp and 100 bp fragments in haplotype B resulted in an appearance of a 380 bp fragment in the haplotype C. On the other hand, gaining of a restriction site within a 380 bp fragment in the haplotype A resulted in an occurrence of 280 bp and 100 bp fragments in haplotype B. Haplotypes A and B were found in all five locations. The frequencies of the haplotype A in all except the Trat samples were greater than those of the B haplotype.

Five different haplotypes (A, B, C, D and E) were observed from digestion of COI-COII with *Alu*I. Haplotype D was a unique pattern (found in specimens from only one geographic area) found in Chumphon whereas the haplotype E was a rare haplotype found in the Gulf of Thailand but disappeared in the Andaman Sea samples.

Five different haplotypes (A, B, C, D and E) were obtained from digestion of amplified COI-COII with *Mbo*I (Fig. 3.12). Haplotypes A and B were common haplotypes found all five geographic locations with different frequencies. Unique haplotypes C, D and E were found in Chumphon, Trat and Trang, respectively.

Digestion of the amplified COI-COII with *Taq* I generated five different haplotypes (A, B, C, D and E) (Fig. 3.13). Haplotype A and B were the common haplotypes in all geographic samples. The ratio of haplotype A/B found in the Trat sample (2.52) was much higher than that of the remaining samples (1.00 - 1.66). All five haplotypes were found in Chumphon implying that the gene pool of this

geographic sample may represent an interface between the Andaman and Trat *P. monodon*.

A total of eight different haplotypes were obtained from digestion of COI-COII with *Hinf* I (see Fig. 3.14 for examples of haplotypes A and B from Trat). Haplotypes A, B and C were distributed across all five locations. The highest frequency of haplotype C was found in Chumphon. The haplotype D was specifically found in the Gulf of Thailand. (Chumphon and Trat). The rare haplotype E was found in only one individual from each geographic sample whereas F, G and H were private haplotype (found in only one individual) for *P. monodon* from Satun, Trat and Trang , respectively.

The maximum number of eleven enzyme digestion profiles were generated from *Dde* I digested CO I - CO II. Haplotypes A and B were common for all samples. The A/B ratio in each of the Andaman and Chumphon (1.11 - 1.74) was greater than that in Trat (0.60). The haplotype C found in the Andaman *P. monodon* with frequencies of 0.13 - 0.20 was also observed in the Chumphon samples with a very low frequency but disappeared from Trat *P. monodon*. Haplotypes F and K were uniquely found in *P. monodon* from Satun. Other three private haplotypes (H from Phangnga, I from Trang and J from Chumphon) were observed. No unique haplotype was observed in *P. monodon* from Trat.

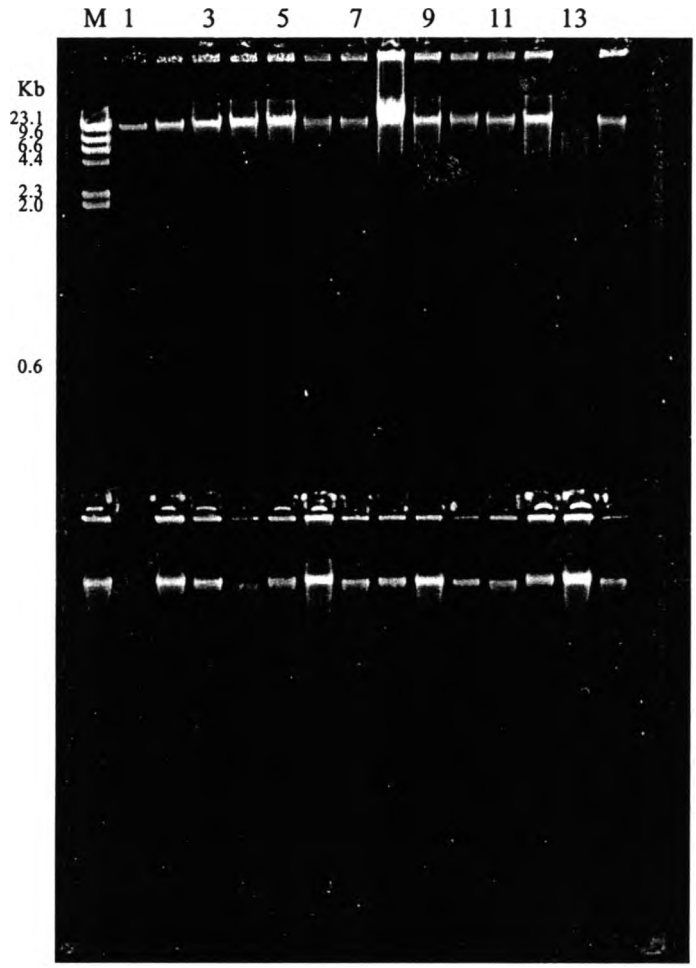

Figure 3.1 High molecular weight DNA extracted from frozen pleopods of *P. monodon.* The extracted DNA was electrophoresed through a 0.8% agarose gel and stained with ethidium bromide (0.5 μg/ml).

Lane M = λ - *Hind* III DNA marker

Lanes 1 - 29 = Total DNA of *P. monodon.*

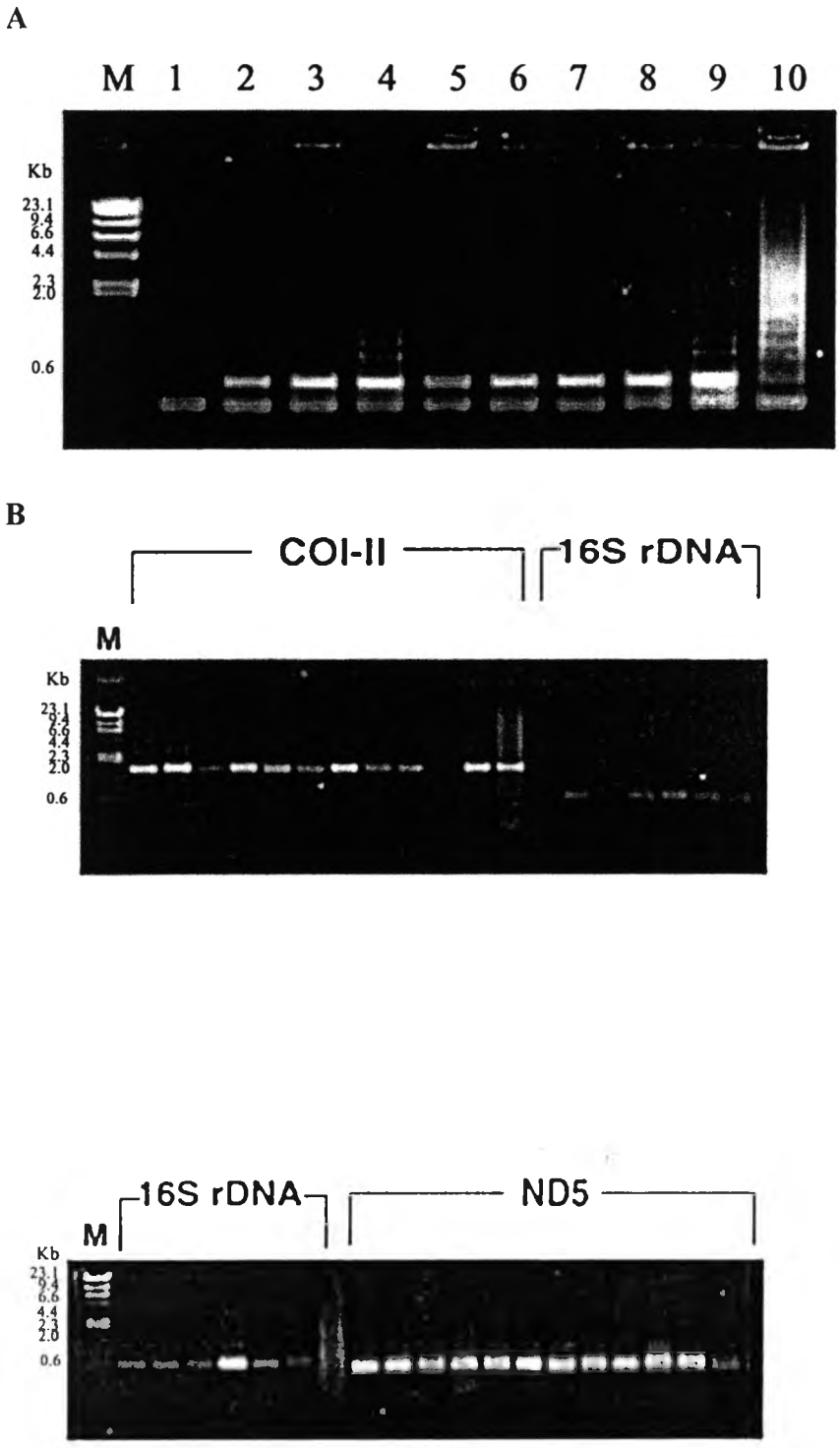

Figure 3.2 PCR products resulted from amplification of hemocyanin (A), 16S rDNA, ND5, and an intergenic COI-COII (B) genes. Only 16S rDNA and COI-COII gene segments were further used for population genetic studies of *P. monodon* in Thailand.

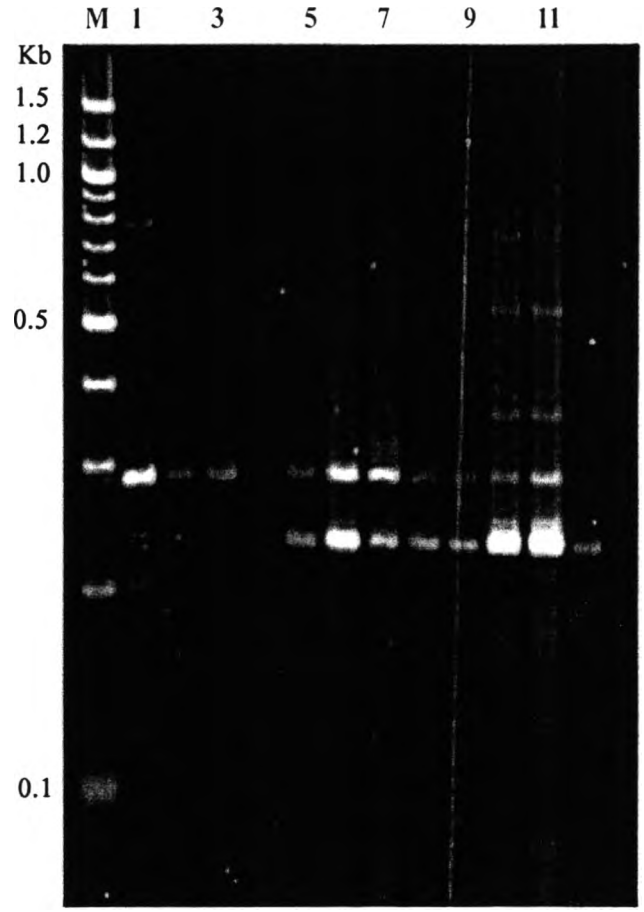

Figure 3.3 An example of restriction analysis of *P. monodon* haemocyanin gene segment with *Alu* I.

Lane M = A 100 bp DNA ladder (NEW ENGLAND BioLabs)

Lane 1 = An undigested hemocyanin (290 bp in length)

Lanes 2 - 4 = *P. monodon* from Chumphon

Lanes 5 - 6 = *P. monodon* from Phangnga

Lanes 7 - 9 = *P. monodon* from Satun

Lanes 10 - 12 = *P. monodon* from Trang

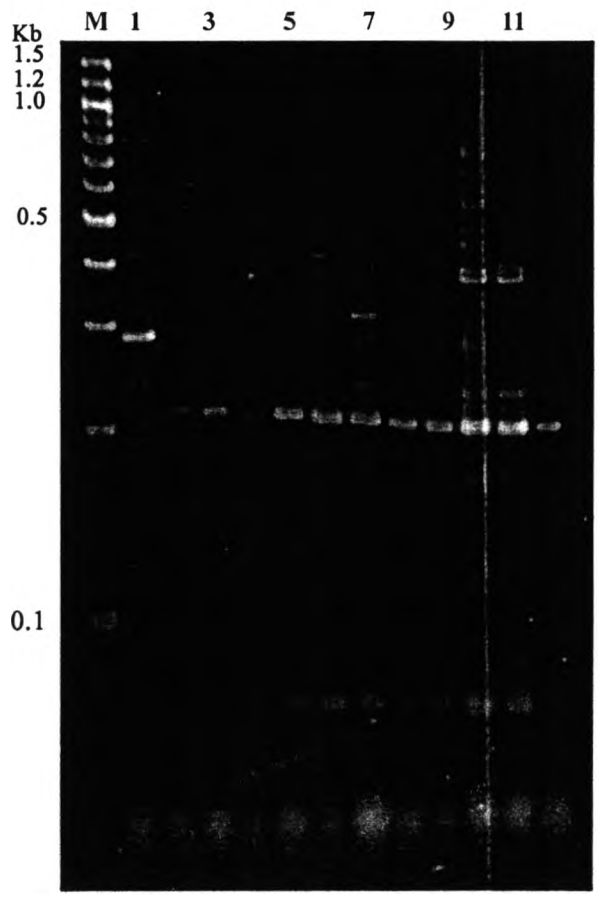

Figure 3.4 An example of restriction analysis of *P. monodon* hemocyanin gene segment with *Hae* III.

Lane M = A 100 bp DNA ladder (NEW ENGLAND BioLabs)

Lane 1 = An undigested hemocyanin (290 bp in length)

Lanes 2 - 4 = *P. monodon* from Chumphon

Lanes 5 - 6 = *P. monodon* from Phangnga

Lanes 7 - 9 = *P. monodon* from Satun

Lanes 10 - 12 = *P. monodon* from Trang

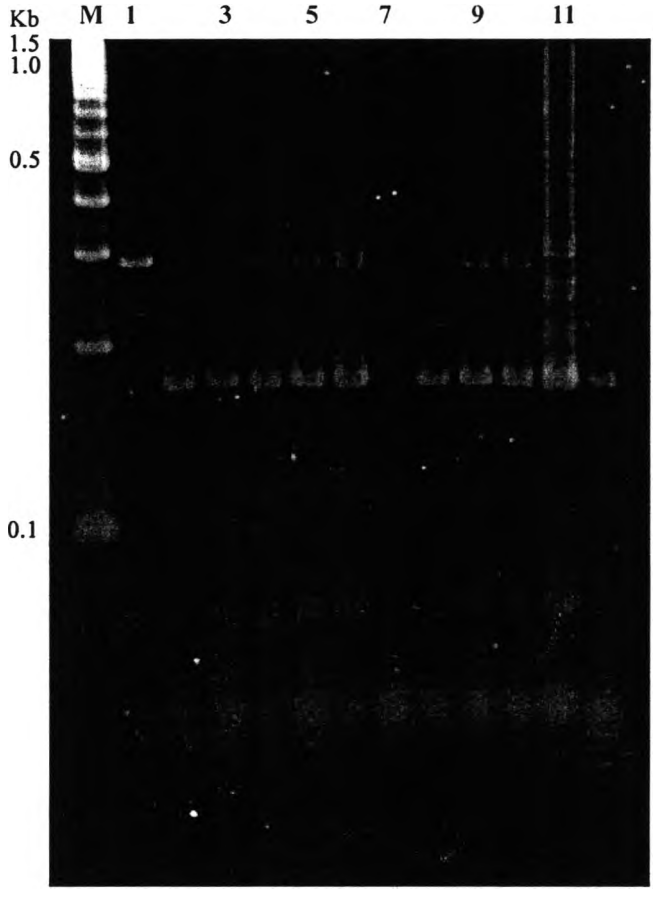

Figure 3.5 An example of restriction analysis of *P. monodon* hemocyanin gene segment with *Dde* I.

Lane M = A 100 bp DNA ladder (Promega)

Lane 1 = An undigested hemocyanin (290 bp in length)

Lanes 2 - 4 = *P. monodon* from Chumphon

Lanes 5 - 6 = *P. monodon* from Phangnga

Lanes 7 - 9 = *P. monodon* from Satun

Lanes 10 - 12 = *P. monodon* from Trang

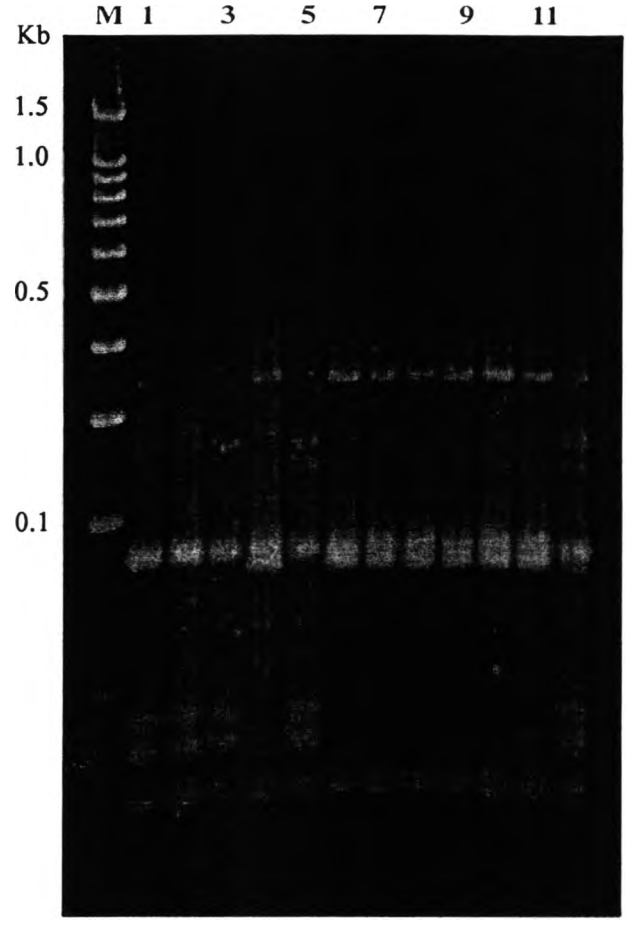

Figure 3.6 An example of restriction analysis of the NADH subnit 5 (ND5) gene segment of *P. monodon* with *Alu* I

Lane M = A 100 bp DNA ladder (Promega)

Lanes 1 - 3 = *P. monodon* from Chumphon

Lanes 4 - 6 = *P. monodon* from Phangnga

Lanes 7 - 9 = *P. monodon* from Satun

Lanes 10 - 12 = *P. monodon* from Trat

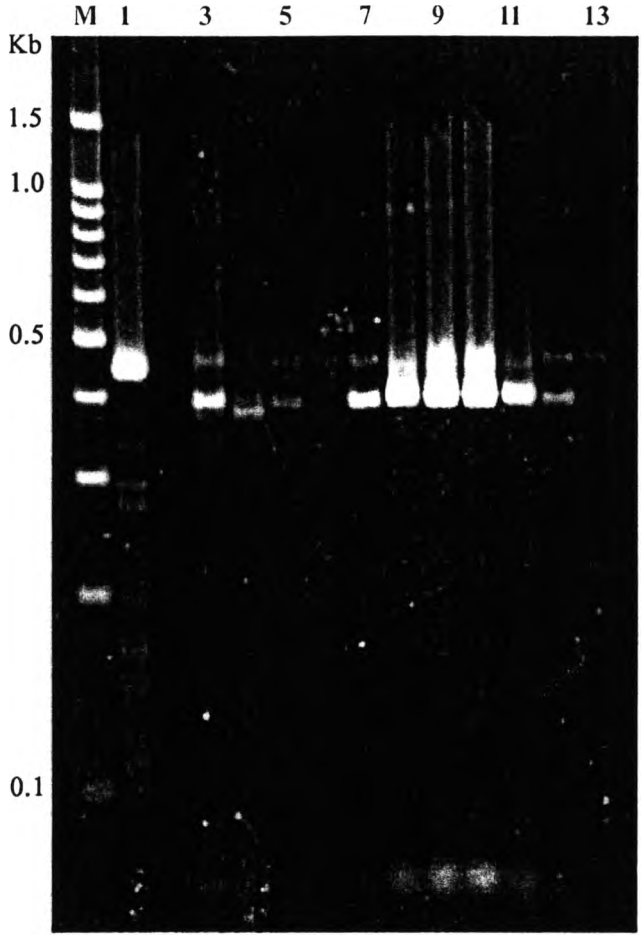

Figure 3.7 An example of restriction analysis of the NADH subnit 5 (ND5) gene segment of *P. monodon* with *Dde* I

Lane M = A 100 bp DNA ladder (Promega)

Lane 1 = An undigested ND5 (480 bp in length)

Lanes 3 - 4 = *P. monodon* from Chumphon

Lanes 5 - 7 = *P. monodon* from Phangnga

Lanes 8 - 10 = *P. monodon* from Satun

Lanes 11 - 13 = *P. monodon* from Trang

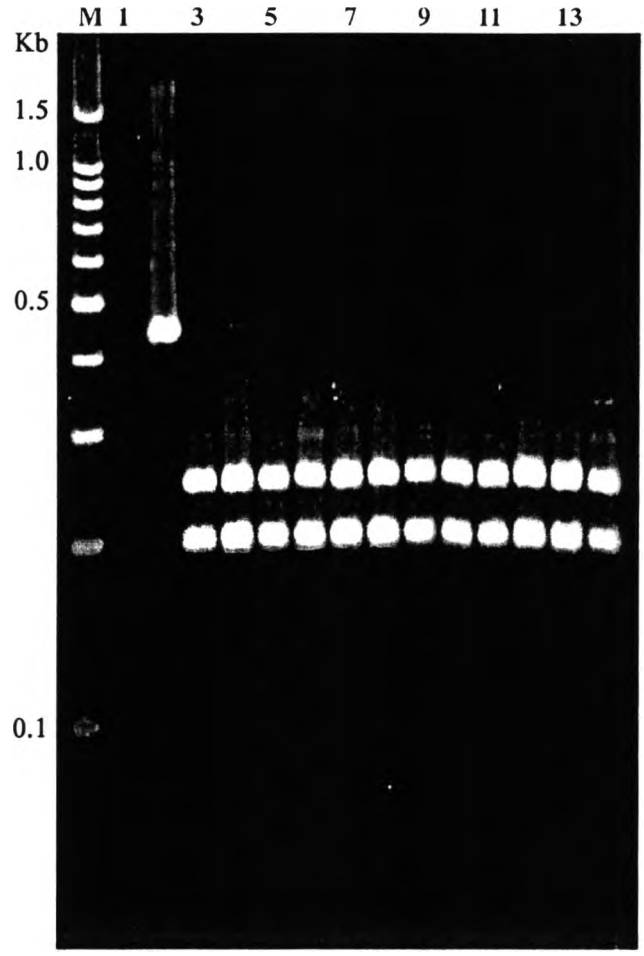

Figure 3.8 An example of restriction analysis of the NADH subnit 5 (ND5) gene segment of *P. monodon* with *Mbo* I

Lane M = A 100 bp DNA ladder (Promega)

Lane 2 = An undigested ND5 (480 bp in length)

Lanes 3 - 5 = *P. monodon* from Chumphon

Lanes 6 - 8 = *P. monodon* from Phangnga

Lanes 9 - 11 = *P. monodon* from Satun

Lanes 12 - 14 = *P. monodon* from Trang

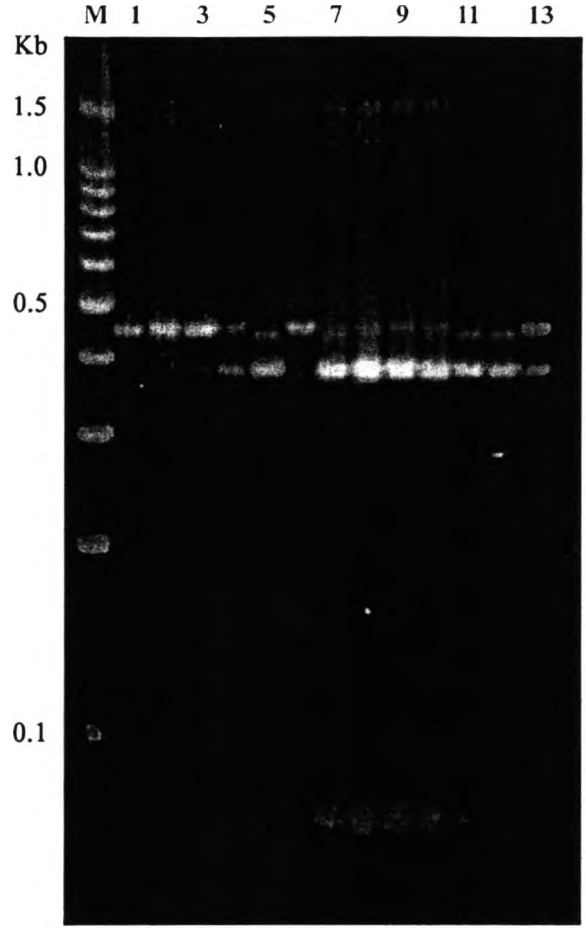

Figure 3.9 An example of restriction analysis of the NADH subnit 5 (ND5) gene segment of *P. monodon* with *Rsa* I

Lane M = A 100 bp DNA ladder (Promega)

Lane 1 = An undigested ND5 (480 bp in length)

Lanes2 - 4 = *P. monodon* from Chumphon

Lanes 5 - 7 = *P. monodon* from Phangnga

Lanes 8 - 10 = *P. monodon* from Satun

Lanes 11 - 13 = *P. monodon* from Trang

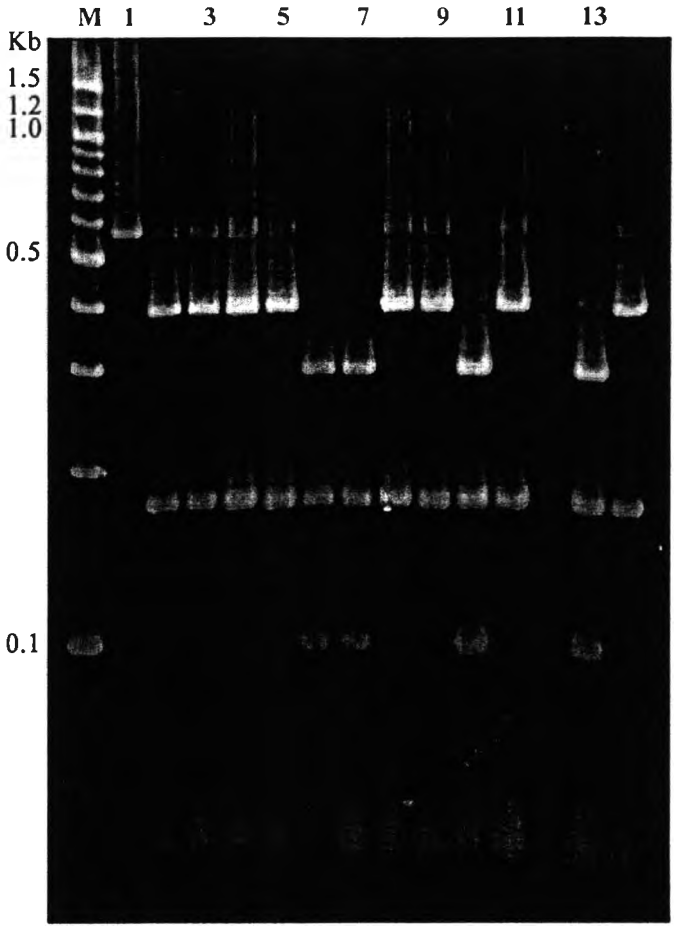

Figure 3.10 An example of *P. monodon* restriction fragment length polymorphism illustrating patterns A (lanes 2 - 5, 8, 9, 11 and 14) and B (lanes 6, 7, 10 and 13) generated from digestion of an amplified 16S rDNA with *Mbo* I. Three haplotypes were observed in this enzyme digestion.

Lane M = A 100 bp DNA ladder (NEW ENGLAND BioLabs)

Lane 1 = An undigested 16SrDNA (560 bp in length)

Lanes 2 - 6 = *P. monodon* from Chumphon

Lanes 7 - 10 = *P. monodon* from Phangnga

Lanes 11,13 and 14 = *P. monodon* from Satun

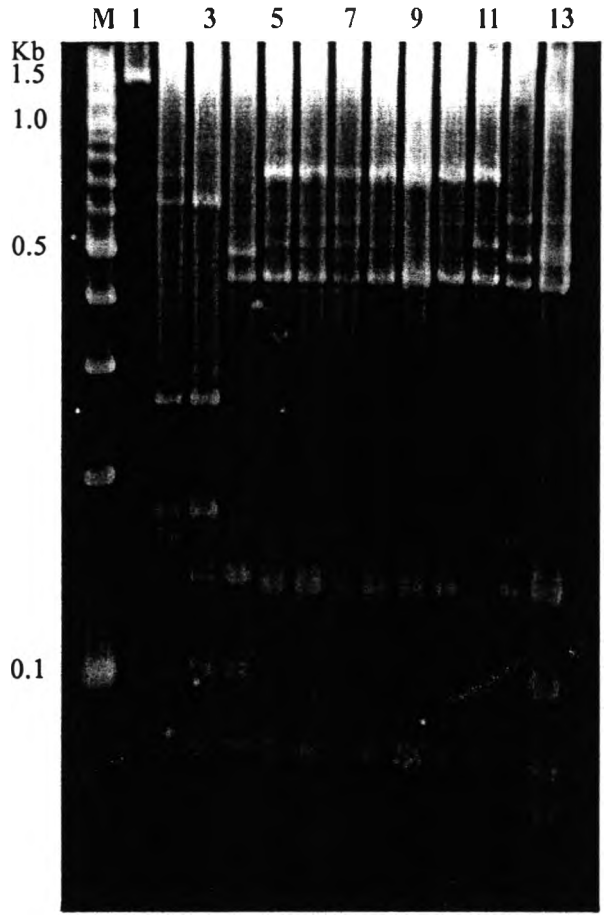

Figure 3.11 An example of *P. monodon* restriction fragment length polymorphism illustrating patterns A (lanes 5-11), B (lanes 4 and 13), C (lane 12) and D (lanes 2 and 3) generated from digestion of an intergenic COI-COII with *Alu* I. Five haplotypes were observed in this enzyme digestion.

Lane M = A 100 bp DNA ladder (NEW ENGLAND BioLabs)

Lane 1 = An undigested COI-II (1700 bp in length)

Lanes 2 - 5 = *P. monodon* from Chumphon

Lanes 6 - 9 = *P. monodon* from Phangnga

Lanes 10 - 13 = *P. monodon* from Satun

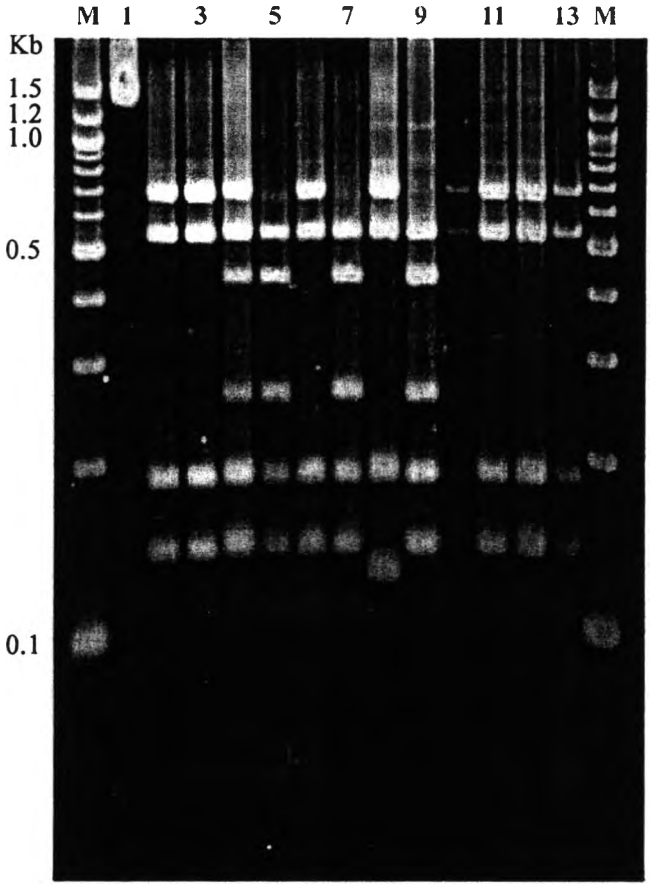

Figure 3.12 An example of *P. monodon* restriction fragment length polymorphismillustrating patterns A (lanes 2, 3, 6, and 10-13), B (lanes 5, 7 and 9), and D (lane 8) generated from digestion of an intergenic COI-COII with *Mbo* I. Incomplete digestion was observed in the *P. monodon* individuals in lane 4. This shrimp possess a restriction pattern B after subsequent confirmation. Five haplotypes were observed in this enzyme digestion.

Lanes M = A 100 bp DNA ladder

Lane 1 = An undigested COI-II (1700 bp in length)

Lanes 2 - 13 = *P. monodon* from Trat

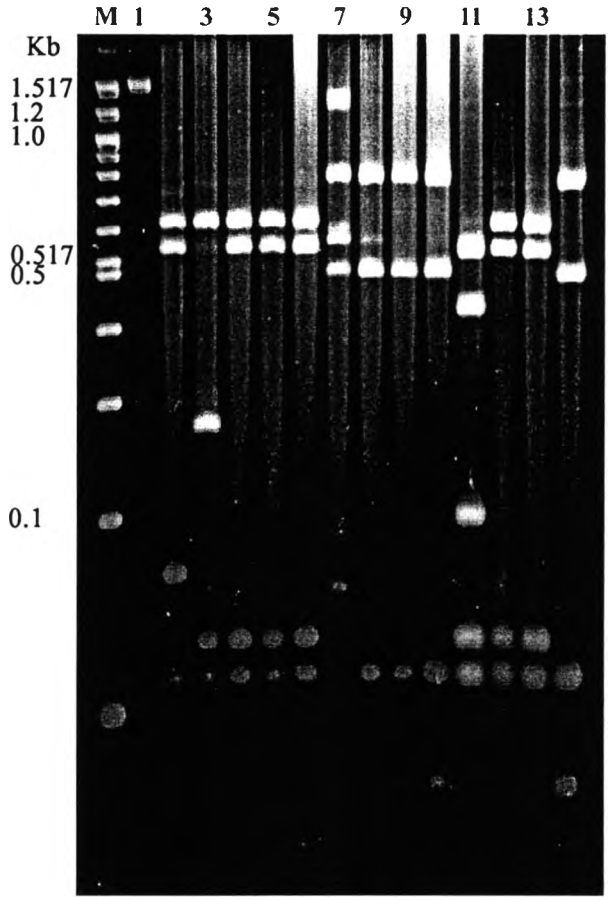

Figure 3.13 An example of *P. monodon* restriction fragment length polymorphism illustrating patterns A (lanes 8 - 10, and 14), B (lanes 4 - 6, 12 and 13), C (lane 3), D (lane 2), E (lane 11) generated from digestion of an intergenic COI-COII with *Taq* I. The *P. monodon* individual in lane 7 exhibit a pattern A after reanalysed. Five haplotypes were observed in this enzyme digestion.

Lane M = A 100 bp DNA ladder (NEW ENGLAND BioLabs)

Lane 1 = An undigested COI-II (1,700 bp in length)

Lanes 2 - 14 = *P. monodon* from Chumphon

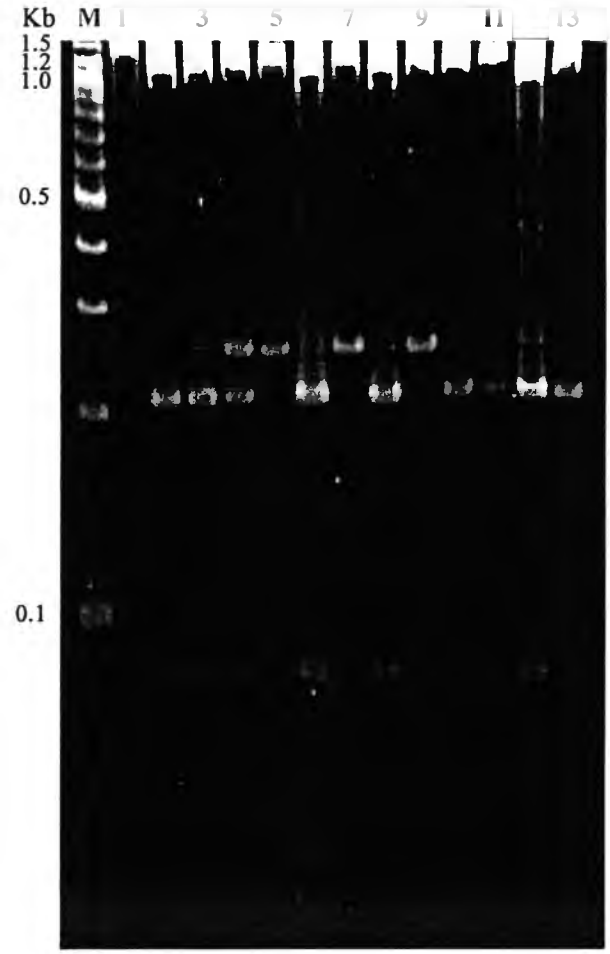

Figure 3.14 An example of *P. monodon* restriction fragment length polymorphism illustrating patterns A (lanes 2, 3, 6, 8, and 10 - 13), B (lanes 5, 7 and 9) and G (lane 4) generated from digestion of an intergenic COI-COII with *Hin*f I. Eight haplotypes were observed in this enzyme digestion.

Lane M = A 100 bp DNA ladder (NEW ENGLAND BioLabs)

Lane 1 = An undigested COI-II (1,700 bp in length)

Lanes 2 - 13 = *P. monodon* from Trat

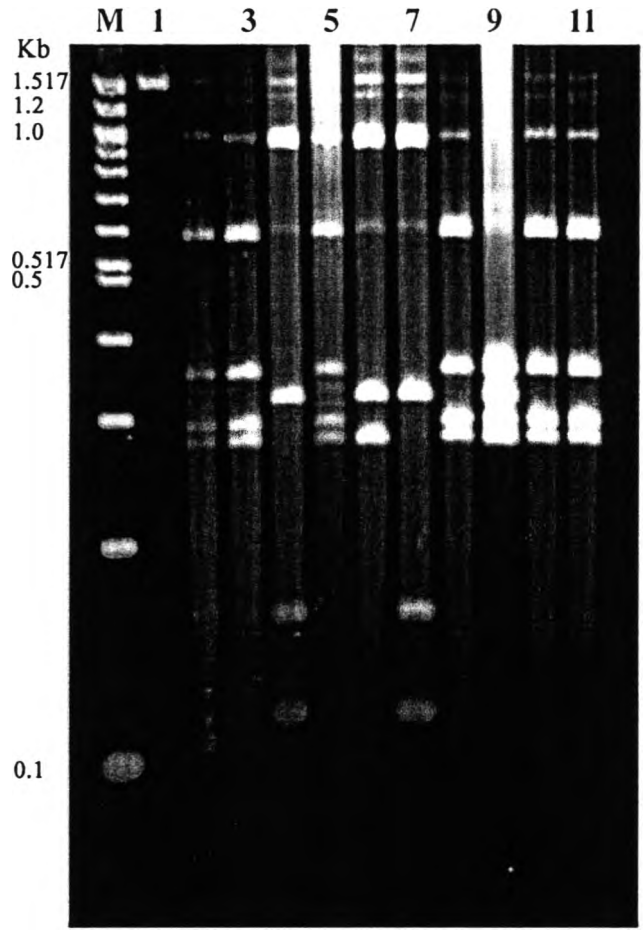

Figure 3.15 An example of *P. monodon* restriction fragment length polymorphism illustrating patterns A (lane 6), B (lanes 2, 3, 5, 8, 10 and 11), C (lane 9) and G (lanes 4 and 7) generated from digestion of an intergenic COI-COII with *Dde* I. Eleven haplotypes were observed in this enzyme digestion.

Lane M = A 100 bp DNA ladder (NEW ENGLAND BioLabs)

Lane 1 = An undigested COI-II (1,700 bp in length)

Lanes 2 - 4 = *P. monodon* from Chumphon

Lanes 5 - 6 = *P. monodon* from Satun

Lanes 7 - 11 = *P. monodon* from Trang

Table 3.1 Restriction fragment patterns observed from digestion of the 16S rDNA and an intergenic COI-COII of *P. monodon* with restriction endonucleases used in this study

Restriction pattern (single haplotype)		
16S rDNA/*Mbo* I (bp)		
A	**B**	**C**
390	-	-
-	-	380
-	280	-
170	170	170
-	100	-

Restriction pattern (single haplotype)									
COI-COII/*Alu* I (bp)					**COICOII/*Mbo*I (bp)**				
A	**B**	**C**	**D**	**E**	**A**	**B**	**C**	**D**	**E**
725	-	-	-	725	700	-	700	700	700
-	-	-	625	-	565	565	-	565	565
-	510	510	-	-	-	440	-	-	-
455	455	455	-	455	-	-	325	-	325
-	-	-	280	-	-	-	300	-	-
-	-	-	175	-	-	260	-	-	-
-	-	165	-	165	-	-	250	-	-
135	-	-	-	-	185	185	-	185	-
135	135	135	135	135	140	140	-	-	-
-	105	105	105	-	-	-	-	135	-
75	75	75	75	75					
-	65	65	65	-					

Table 3.1 Restriction fragment patterns (cont.)

Restriction pattern (single haplotype)												
COI-COII/*Taq* I (bp)					**COI-COII/*Hin* fI (bp)**							
A	**B**	**C**	**D**	**E**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
800	-	-	-	-	1200	1200	1200	1200	1200	1200	1200	1200
-	625	625	625	-	-	250	250	-	-	-	250	-
-	575	-	575	575	210	-	-	210	-	210	210	-
500	-	-	-	-	-	-	-	-	160	-	-	160
-	-	-	-	425	-	-	-	-	100	-	-	-
-	-	290	-	-	90	90	90	90	90	90	90	90
-	-	290	-	-	65	65	-	-	65	65	65	65
-	-	-	-	200	-	-	-	60	-	60	-	-
-	-	-	175	-								
-	125	125	-	125								
115	115	115	115	115								
75	-	-	-	-								
-	50	-	-	-								

Table 3.1 Restriction fragment patterns (cont.)

Restriction pattern (single haplotype)										
COI-COII/*Dde* I (bp)										
A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**
980	-	-	-	980	-	980	-	-	980	-
-	-	-	900	-	900	-	-	900	-	900
-	600	-	-	-	-	-	600	-	-	-
-	360	360	-	-	-	-	-	-	-	-
325	-	325	325	325	325	325	-	325	325	325
-	300	300	-	-	-	-	300	300	-	-
295	295	295	-	295	295	-	295	295	-	-
-	-	-	-	-	-	-	225	-	-	-
-	-	-	175	-	-	175	-	-	175	175
-	-	-	-	-	-	-	140	-	-	-
-	-	-	125	-	-	125	-	-	125	125
-	-	-	-	110	110	-	-	-	110	110
50	50	50	50	50	50	50	50	-	50	50

Table 3.2 Frequency distributions of restriction enzyme patterns across five geographic samples of *P. monodon* in Thailand

Restriction pattern	**Geographic location**				
	Satun (*N* = 30)	**Phangnga** (*N* = 31)	**Trang** (*N* = 30)	**Chumphon** (*N* = 39)	**Trat** (*N* = 24)
16S rDNA - *Mbo* I					
A	0.70(21)	0.52(16)	0.67(20)	0.64(25)	0.42(10)
B	0.30(9)	0.45(14)	0.33(10)	0.36(14)	0.58(14)
C	-	0.03(1)	-	-	-
COI - COII - *Alu I*					
A	0.40(12)	0.58(18)	0.50(15)	0.38(15)	0.58(14)
B	0.40(12)	0.26(8)	0.33(10)	0.18(7)	0.21(5)
C	0.20(6)	0.16(5)	0.17(5)	0.28(11)	0.17(4)
D	-	-	-	0.10(4)	-
E	-	-	-	0.05(2)	0.04(1)
COI - COII - *Mbo* I					
A	0.40(12)	0.58(18)	0.50(15)	0.44(17)	0.54(13)
B	0.6(18)	0.42(13)	0.47(14)	0.49(19)	0.42(10)
C	-	-	-	0.08(3)	-
D	-	-	-	-	0.04(1)
E	-	-	0.03(1)	-	-
COI - COII - *Taq* I					
A	0.40(12)	0.58(18)	0.50(15)	0.44(17)	0.63(15)
B	0.40(12)	0.35(11)	0.40(12)	0.28(11)	0.25(6)
C	0.07(2)	0.03(1)	-	0.15(6)	0.13(3)
D	0.13(4)	0.03(1)	0.10(3)	0.05(2)	-
E	-	-	-	0.08(3)	-

Table 3.2 Frequency distributions of restriction enzyme patterns across five geographic samples of *P. monodon* in Thailand (cont.)

Restriction pattern	**Geographic location**				
	Satun (*N* = 30)	**Phangnga** (*N* = 31)	**Trang** (*N* = 30)	**Chumphon** (*N* = 39)	**Trat** (*N* = 24)
COI - COII - *Hin* fI					
A	0.40(12)	0.58(18)	0.43(13)	0.38(15)	0.25(6)
B	0.50(15)	0.29(9)	0.47(14)	0.28(11)	0.33(8)
C	0.03(1)	0.10(3)	0.03(1)	0.26(10)	0.04(1)
D	-	-	-	0.05(2)	0.29(7)
E	0.03(1)	0.03(1)	0.03(1)	0.03(1)	0.04(1)
F	0.03(1)	-	-	-	-
G	-	-	-	-	0.04(1)
H	-	-	0.03(1)	-	-
COI - COII - *Dde* I					
A	0.47(14)	0.39(12)	0.40(12)	0.51(20)	0.38(9)
B	0.27(8)	0.35(11)	0.30(9)	0.38(15)	0.63(15)
C	0.13(4)	0.16(5)	0.2(6)	0.05(2)	-
D	0.03(1)	-	0.03(1)	-	-
E	-	0.03(1)	-	0.03(1)	-
F	0.07(2)	-	-	-	-
G	-	0.03(1)	0.03(1)	-	-
H	-	0.03(1)	-	-	-
I	-	-	0.03(1)	-	-
J	-	-	-	0.03(1)	-
K	0.03(1)	-	-	-	-

3.5 Analysis of geographic population structure of *P. monodon* base on mtDNA-RFLP approach

Thirty-seven composite haplotypes were generated from digestion of 16S rDNA with *Mbo* I and an intergenic COI-COII with *Alu* I, *Mbo* I, *Taq* I, *Hinf* I and *Dde* I of 154 *P. monodon* individuals. The distribution frequencies of these composite haplotypes among geographically different locales are shown in Table 3.3.

Of these, twenty-two composite haplotypes were possessed by single individuals and nine haplotypes were found in 2-4 individuals. Six composite haplotypes (I, ABBBBA, II, ACBBBA, III, AAAAAC, IV, ABBCBA, VII, BAAAAB and XXVII, BAAADB) were carried by at least 5% of overall investigated individuals. The composite haplotype I (ABBBBA) and VII (BAAAAB) were found in 16.23% and 28.57% of all individuals, respectively. These haplotypes did not fix in a particular sample but they overlapping distributed in all geographic samples with different frequencies. The ratio between composite haplotypes I and VII in the Andaman (1.0, 0.75 and 0.45 in Satun, Trang and Phangnga) and Chumphon (0.45) was greater than that of the Trat sample (0.17).

Five composite haplotypes (I, IV, VII, XXVII and XXXIV) were shared in both Chumphon and Trat. A similar number of shared composite haplotypes (5-6 haplotypes) were also observed between Chumphon and each of the Andaman samples. Pie charts illustrating composite haplotype frequencies of each geographic samples of Thai *P. monodon* are shown by Figure 3.16.

Using a band sharing method, genetic distances between pairs of composite haplotypes were calculated (Appendix D). The distances ranged from 0.00153 to 0.09349.

3.6 Phylogeny and distributions of mitochondrial DNA haplotypes

Genetic distances between all possible comparisons of paired composite haplotypes were subjected to phylogenetic reconstruction using a UPGMA method. As can be seen in Figure 3.17, two major phylogenetic clusters of mtDNA haplotypes (clusters I and II) are observed. The cluster I contained thirteen composite haplotypes: VII (BAAAAB), V (BAAAAC), XXVI (BEAAAB), XXXV (BAAAEB), XV (BAAAAH), XXXVII (BADADB), XXVII (BAAADB), XVIII (BAEAAB), III (AAAAAC), XIV (AAAAAB), XIX (AAAABC), XXV (AEAAAC), XXXVI (AEBAGB), whereas the cluster II was composed of twenty-four composite haplotypes: II (ACBBBA), XVI (ACBBBE), XII (ACBBCA), I (ABBBBA), XXXIV (ABBBCA), XXII I(ABBBAA), XIII (CBBBBA), XXII (ABABBA), XI (ABBDBA), XXIV (ACBDBA), XV (ABBCBA), XXXII (ABBCCA), XXX (ACBCBE), VIII (ACBBBF), X (ACBBEF), XX (ACBBCI), XXXI (ADBBCA), XXI (ACBDHD), VI (ACBDED), IX (ACBDBK), XVII (ABBDEG), XXIX (ACBDEJ), XXVIII (ADCECA) and XXXIII (ADCEBA).

The highest frequency of the cluster I haplotype was observed in Trat (0.625) followed by that of Phangnga (0.581), Trang (0.500), Chumphon (0.436) and Satun (0.400), respectively. The distribution frequency of cluster II was in the opposite direction. A ratio of clusters I/II in samples from Trat (A/B = 1.67) was greater than

Table 3.3 Geographic distributions of 37 composite haplotypes of five *P. monodon* samples based on restriction analysis of 16S rDNA with *Mbo* I and an intergenic COI-COII with *Alu* I, *Mbo* I, *Taq* I, *Hin* f I and *Dde* I

Composite haplotype		Geographic distribution frequency					Overall frequency ($N = 154$)
		Satun ($N = 30$)	Phangnga ($N = 31$)	Trang ($N = 30$)	Chumphon ($N = 39$)	Trat ($N = 24$)	
I.	ABBBBA	0.2667(8)	0.1613(5)	0.2000(6)	0.1282(5)	0.0417 (1)	0.1623(25)
II.	ACBBBA	0.0333(1)	0.0323(1)	0.1000(3)	0	0.1664 (4)	0.0584(9)
III.	AAAAAC	0.1000(3)	0.0968(3)	0.0667(2)	0.0256(1)	0	0.0584(9)
IV.	ABBCBA	0.0667(2)	0.0323(1)	0	0.0769(3)	0.1250 (3)	0.0584(9)
V.	BAAAAC	0.0333(1)	0.0645(2)	0.0333(1)	0	0	0.0260(4)
VI.	ACBDED	0.0333(1)	0	0	0	0	0.0065(1)
VII.	BAAAAB	0.2667(8)	0.3548(11)	0.2667(8)	0.2821(11)	0.2500 (6)	0.2857(44)
VIII.	ACBBBF	0.0333(1)	0	0	0	0	0.0065(1)
IX.	ACBDBK	0.0333(1)	0	0	0	0	0.0065(1)
X.	ACBBEF	0.0333(1)	0	0	0	0	0.0065(1)
XI.	ABBDBA	0.0667(2)	0	0.0333(1)	0	0	0.0195(3)
XII.	ACBBCA	0.0333(1)	0.0968(3)	0	0.0513(2)	0	0.0390(6)
XIII.	CBBBBA	0	0.0323(1)	0	0	0	0.0065(1)
XIV.	AAAAAB	0	0.0323(1)	0	0.0256(1)	0	0.0130(2)
XV.	BAAAAH	0	0.0323(1)	0	0	0	0.0065(1)
XVI.	ACBBBE	0	0.0323(1)	0	0	0	0.0065(1)
XVII.	ABBDEG	0	0.0323(1)	0.0333(1)	0	0	0.0130(2)
XVIII.	BAEAAB	0	0	0.0333(1)	0	0	0.0065(1)
XIX.	AAAABC	0	0	0.1000(3)	0	0	0.0195(3)

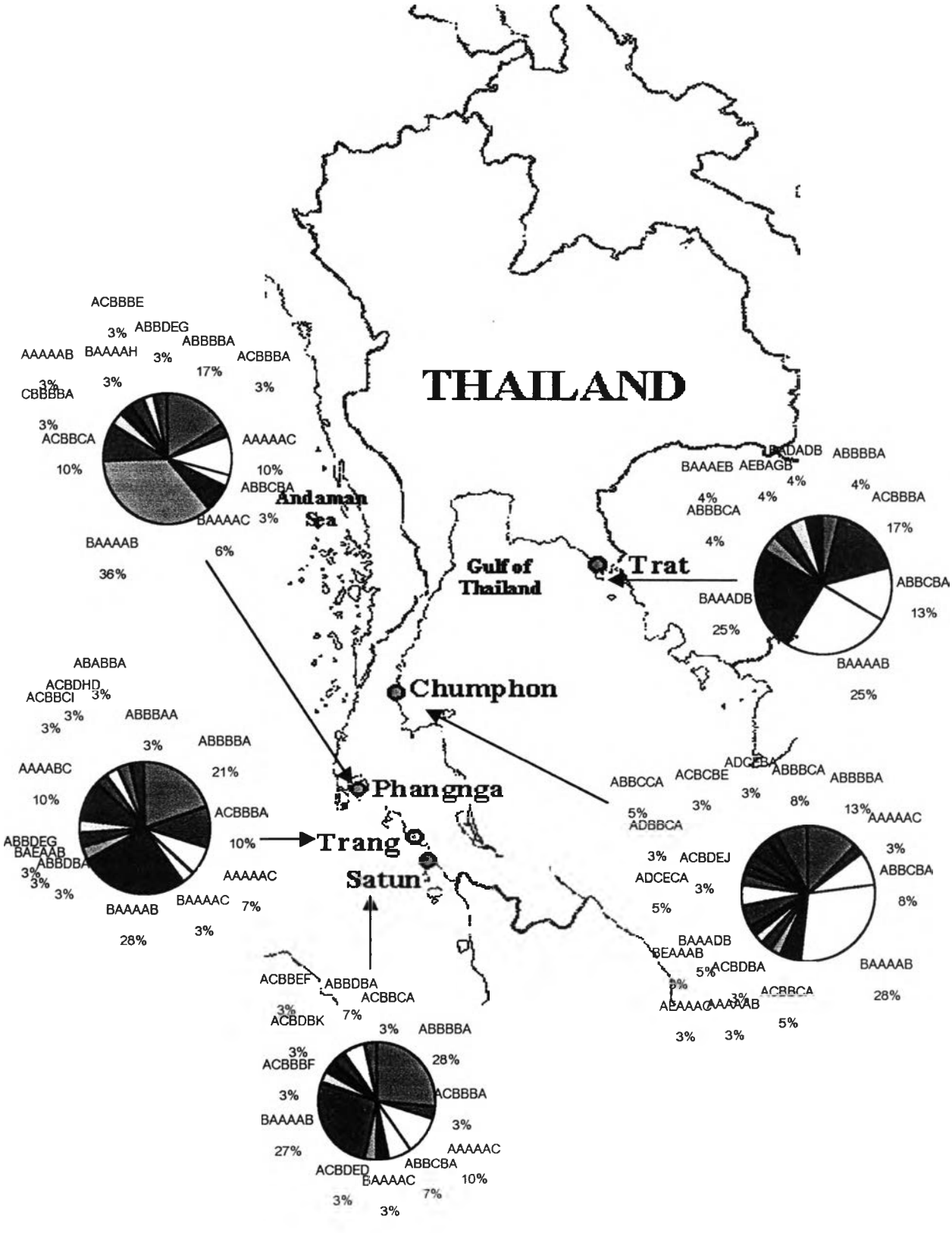

Figure 3.16 Pie charts showing distributions of mtDNA composite haplotypes within each geographic sample of *P.monodon* in Thailand.

Table 3.3 (cont.)

Composite haplotype	Geographic distribution frequency					Overall frequency (*N*)
	Satun (*N* = 30)	Phangnga (*N* = 31)	Trang (*N* = 30)	Chumphon (*N* = 39)	Trat (*N* = 24)	
XX. ACBBCI	0	0	0.0333 (1)	0	0	0.0065(1)
XXI. ACBDHD	0	0	0.0333 (1)	0	0	0.0065(1)
XXII. ABABBA	0	0	0.0333 (1)	0	0	0.0065(1)
XXIII. ABBBAA	0	0	0.0333 (1)	0	0	0.0065(1)
XXIV. ACBDBA	0	0	0	0.0256(1)	0	0.0065(1)
XXV. AEAAAC	0	0	0	0.0256(1)	0	0.0065(1)
XXVI. BEAAAB	0	0	0	0.0256(1)	0	0.0065(1)
XXVII. BAAADB	0	0	0	0.0513(2)	0.2500(6)	0.0519(8)
XXVIII. ADCECA	0	0	0	0.0513(2)	0	0.0130(2)
XXIX. ACBDEJ	0	0	0	0.0256(1)	0	0.0065(1)
XXX. ACBCBE	0	0	0	0.0256(1)	0	0.0065(1)
XXXI. ADBBCA	0	0	0	0.0256(1)	0	0.0065(1)
XXXII. ABBCCA	0	0	0	0.0513(2)	0	0.0130(2)
XXXIII. ADCEBA	0	0	0	0.0256(1)	0	0.0065(1)
XXXIV. ABBBCA	0	0	0	0.0769(3)	0.0417(1)	0.0260(4)
XXXV. BAAAEB	0	0	0	0	0.0417(1)	0.0065(1)
XXXVI. AEBAGB	0	0	0	0	0.0417(1)	0.0065(1)
XXXVII. BADADB	0	0	0	0	0.0417(1)	0.0065(1)
Total (*N*)	30	31	30	39	24	154

that of the Andaman sea (0.67 in Satun, 1.39 in Phangnga and 1.00 in Trang and Chumphon 0.77) (Figure 3.18). The phylogeny of composite haplotypes allocated 4 unique haplotypes into the cluster I (XIV, XV, XVIII and XIX) and 10 unique haplotypes into the cluster II (VI, VIII, IX, X, XIII, XVI, XX, XXI, XXII, XXIII) for those found in the Andaman Sea *P. monodon* and 5 unique haplotypes in the cluster I (XXV, XXVI, XXXV, XXXVI, XXXVII) and 7 unique haplotypes in the cluster II (XXIV, XXVIII, XXIX, XXX, XXXI, XXXII, XXXIII) for those found in the Gulf of Thailand *P. monodon*. Two composites haplotype XXVIII (ADCECA) and XXXIII (ADCEBA) were not further dissociated from the cluster II because they were found in only 2 and 1 individuals of *P. monodon* from Chumphon.

Considering restriction patterns obtained, only those generated from restriction of an intergenic COI-II with *Alu* I and *Taq* I represented the phylogenetic mtDNA linages in *P. monodon* accurately. The restriction patterns B, C and D of *Alu* I digested COI - COII were specifically allocated in the cluster II while patterns A and E were allocated in the cluster I. In addition, the single haplotype A from an intergenic COI-II digested with *Taq* I represent the cluster I haplotypes while the remaining haplotypes were allocated in the cluster II.

On the basis of this knowledge, the frequency of evolutionary clusters I/II in a given sample can be simply examined by digestion of an intergenic COI-II with either *Alu* I or *Taq* I rather than the use of a large number of restriction enzymes.

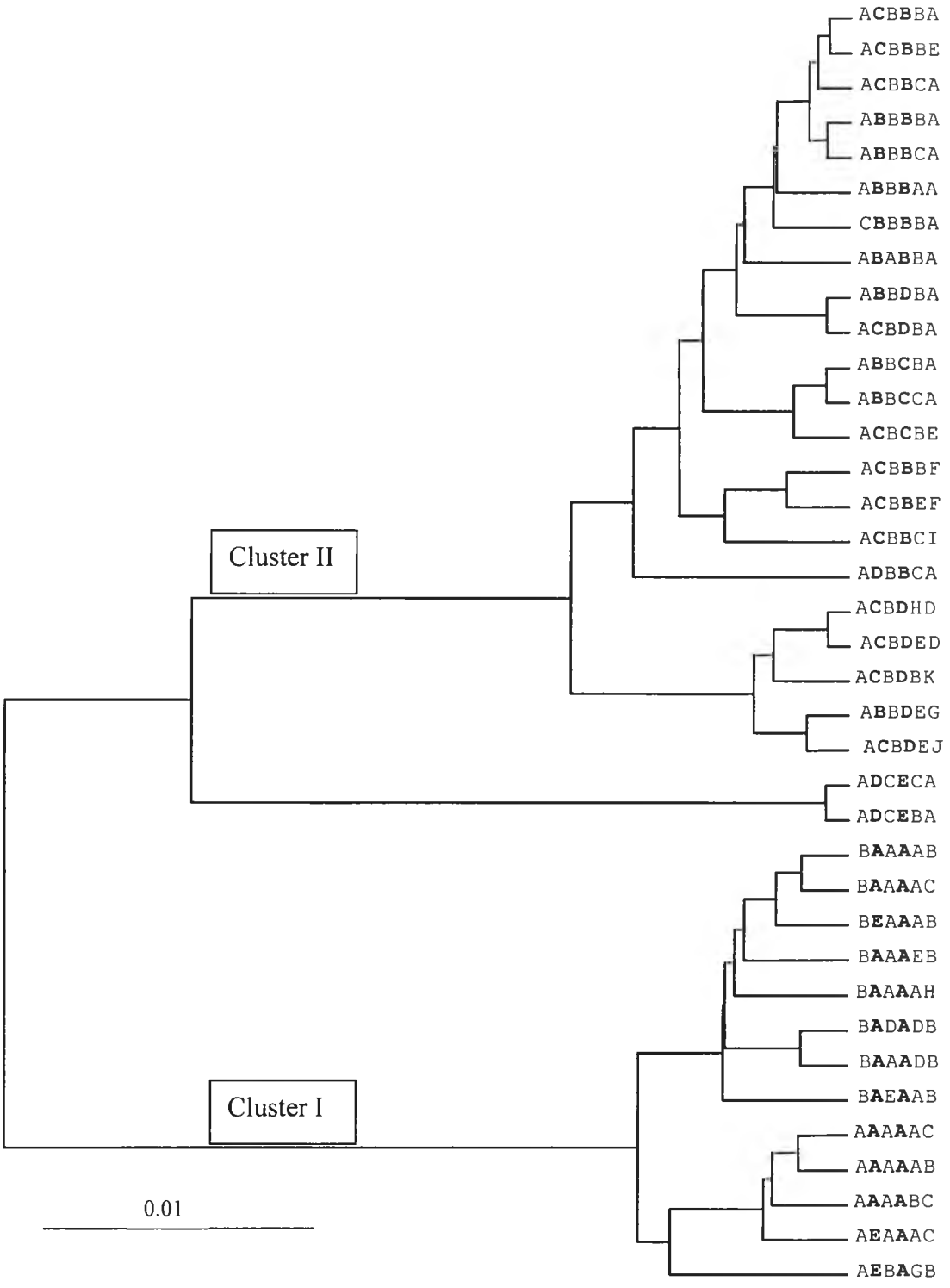

Figure 3.17 A UPGMA dendrogram showing the relationship among thirty-seven composite haplotypes found in Thai *P. monodon* based on sequence divergence between pairs of composite haplotype (arranged from 16S rDNA-*Mbo* I, an intergenic COI-CO II digested with *Alu* I, *Mbo*I, *Taq* I, *Hin* fI and *Dd* I). Bold faces refer to single haplotypes of *Alu* I- an *Mbo*I-digested COI-COII that can represent the frequencies of phylogenetic cluster I and II accurately.

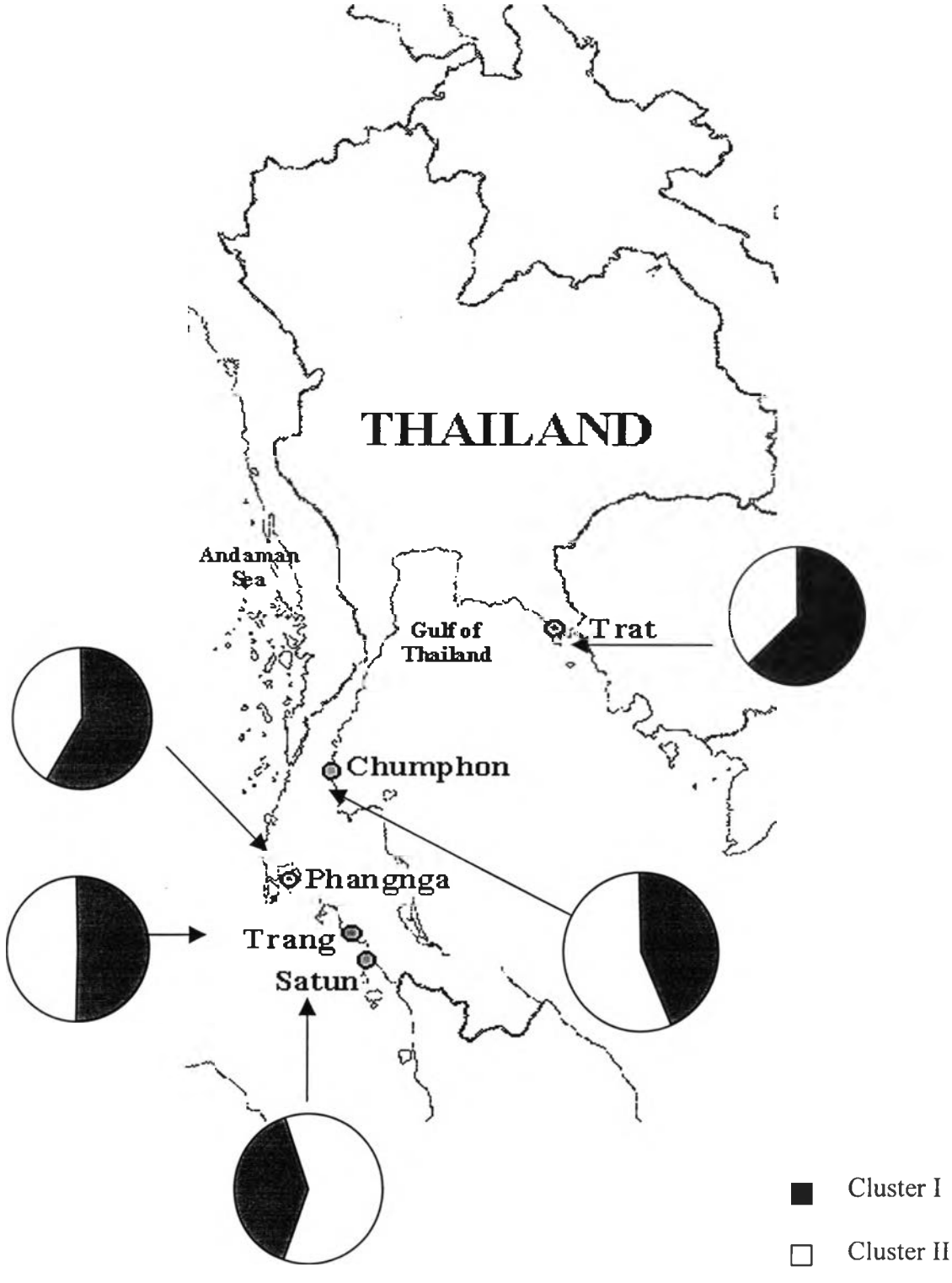

Figure 3.18 Pie charts showing distributions of major mtDNA phylogenetic cluster I and II among five geographic samples of *P. monodon* in Thailand.

3.7 Haplotype and nucleotide diversity within and between geographic samples and nucleotide divergence of *P. monodon* in Thailand

Haplotype and nucleotide diversity within geographic samples of *P. monodon* in Thai waters are shown Table 3.4. The average haplotype diversity ranged form 0.8404 (Trat) - 0.8871 (Chumphon) whereas the average percentage of nucleotide diversity within geographic samples was 3.1601 (Satun) – 3.8302 (Chumphon). These indicated high levels of genetic diversity of *P. monodon* in Thailand at both haplotype and nucleotide levels.

Considering the nucleotide diversity of each *P. monodon* sample, the value of this parameter of *P. monodon* originating from the Gulf of Thailand was slightly higher than that of *P. monodon* from the Andaman Sea (Satun, Trang and Phangnga). High genetic diversity and the distribution pattern of composite haplotypes in the Chumphon samples implied that the gene pool of *P. monodon* in this location may have mixed by *P. monodon* originating from a number of geographic samples.

Nucleotide diversity between pairs of geographic samples (3.3648%) was slightly higher than that within geographic samples (3.3283%) indicating degrees of population differentiation of *P. monodon* in Thailand. As can be seen Table 3.5, the highest nucleotide diversity between population was 3.7069% (Chumphon – Trat). The Andaman samples did not show any differentiation within the region as indicated by the negative values of nucleotide divergence. The greatest nucleotide divergence between samples was 0.2714% (Satun – Trat). Surprisingly, Chumphon and Trat

Table 3.4 Haplotype and nucleotide diversity within five geographic samples of Thai *P. monodon* examined by restriction analysis of 16S rDNA and an intergenic COI-COII

Geographic location	Haplotype diversity ± SE	Nucleotide diversity (x100)
Satun	0.8452 ± 0.02858	3.1601
Phangnga	0.8313 ± 0.03497	3.1631
Trang	0.8701 ± 0.02529	3.1697
Chumphon	0.8871 ± 0.02404	3.8302
Trat	0.8404 ± 0.02621	3.3186
Average	0.8548 ± 0.00011	3.3283 ± 0.0000017

showed reasonable levels of nucleotide divergence (0.1325%). The remaining populations had lower level of divergence (-0.0501 - 0.0909).

3.8 Geographic heterogeneity of *P. monodon* in Thailand

Geographic heterogeneity between *P. monodon* from different samples was tested using a Monte Carlo simulation. Significant differences in composite haplotype distribution frequencies for overall samples were observed ($P < 0.0005$), and between the Andaman and Gulf of Thailand samples ($P < 0.0001$) indicating that interspecific genetic differentiation does exist in Thai *P. monodon* (Table 3.6). Nevertheless, geographic heterogeneity was not observed within the Andaman Sea (between Satun, Trang and Phangnga) and the Gulf of Thailand (between Chumphon and Trat) ($P >$

0.0083). The Chumphon *P. monodon* exhibited genetic heterogeneity with Trang (P = 0.0080) but not Satun and Phangnga *P. monodon* (P . 0.0083). The pooled Andaman samples showed significant genetic heterogeneity with both Trat (P = 0.0002) and Chumphon (P = 0.0011).

On the basis of geographic heterogeneity test, five investigated samples of *P. monodon* in this study can be allocated to 2 different group (populations) composing of the Andaman (Satun, Trang and Phangnga) and the Gulf of Thailand (Trat and Chumphon) populations. (Table 3.6)

Table 3.5 Percent nucleotide diversity (above diagonal) and percent nucleotide divergence (below diagonal) between pairs of five geographic samples of *P. monodon* in Thailand

	Satun	Phangnga	Trang	Chumphon	Trat
Satun	-	3.2263	3.1089	3.4678	3.5107
Phangnga	0.0647	-	3.1088	3.5274	3.1907
Trang	-0.0560	-0.0576	-	3.4656	3.3350
Chumphon	-0.0273	0.0307	-0.0344	-	3.7069
Trat	0.2714	-0.0501	0.0909	0.1325	-

Average percent nucleotide diversity between samples = 3.3648%

Average percent nucleotide divergence between samples = 0.0365%

Table 3.6 Geographic heterogeneity analysis in distribution frequency of composite haplotype among 5 geographic samples of *P. monodon* in Thailand using a Monte Carlo simulation

	Satun	Phangnga	Trang	Chumphon	Trat
Satun	-				
Phangnga	P = 0.7612 ^{ns}	-			
Trang	P= 0.5111 ^{ns}	P = 0.3756 ^{ns}	-		
Chumphon	P= 0.1552 ^{ns}	P= 0.2465 ^{ns}	P= 0.0080 *	-	
Trat	P= 0.0014 *	P= 0.0017 *	P= 0.0021 *	P= 0.0497 ^{ns}	-
Andaman – Chumphon		P= 0.0011 *			
Andaman – Trat		P= 0.0002 *			
Andaman – Gulf of Thailand		P< 0.0001 *			
Overall sample		P= 0.0005 *			

^{ns} = not significant

* = P < 0.0083 following a sequential Bonferroni method (Rice, 1989)