CHAPTER III

Results

3.1 DNA extraction

Genomic DNA was extracted from frozen pleopods of *P. monodon* using phenol-chloroform method modified from the procedure described in Pongsomboon (1996). The quality of isolated genomic DNA were determined electrophoretically in a 0.8 % agarose (w/v) gel while the amount of DNA was estimated spectrophotometrically. The recovery yield was about 50-100 μg/pleopod. The ratio of OD 260 / 280 was 1.8-2.0 reflecting reasonably good quality of DNA obtained (Fig. 3.1 ; Lanes 2, 4, 8 and 9). As can be seen from Figure 3.1, the extracted DNAs show high molecular weight DNA of approximately 23.1 kb (compared with a λ - *Hin*d III marker). Electrophoretic analysis also revealed slightly degraded DNA in all speciments (Fig. 3.1). Some extracted DNA samples contained RNA contamination as visualised by smeared bands at the bottom of gel (Fig 3.1; Lanes 1, 3 and 6). However, RNA contaminant did not interfere the success of amplification reactions.

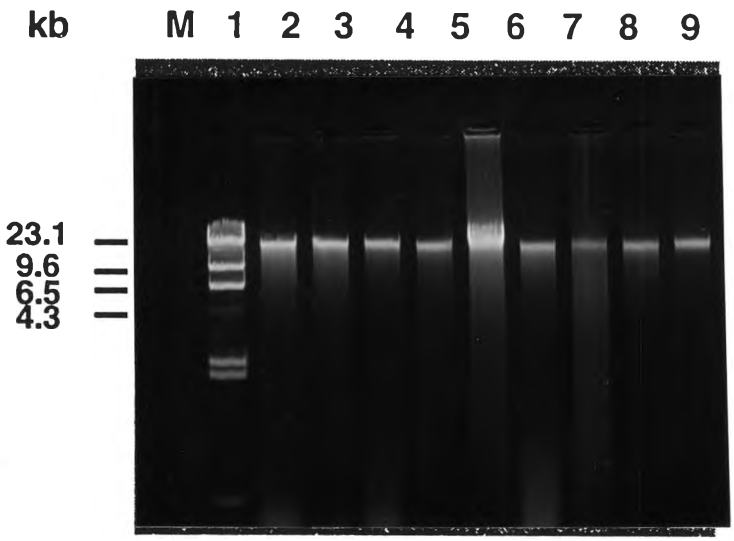


Figure 3.1 Ethidium bromide staining of a 0.8 % agarose gel showing DNA extracted from pleopods of *P. monodon*

Lane M = λ DNA / *Hin*d III

Lanes 1-9 = DNA extracted from frozen pleopod of *P. monodon*

3.2 Determination of genetic variation in wild population of *P. monodon* using RAPD analysis

When RAPD method is used to determine level of genetic variation in *P. monodon*, optimization of the PCR conditions is necessary for reproducible amplification of RAPD patterns. The amplification conditions used in this thesis was slightly modified from that of Pongsomboon (1996). Previously, the PCR profile of 35 cycles (denaturation at 94 °C for 5 sec, annealing at 36 °C for 45 sec and extention at 72 °C for 90 sec) was carried out as the original protocol but the RAPD products obtained were not consistently found with a Hybaid thermo-cycler. Presumably, the effeciency to switch temperature from one to the other step in a Perkin Elmer 2400 thermo-cycler used by Pongsomboon (1996) was greater than that of a Hybaid thermo-cycler used in the present study.

Accordingly, a slighly modified PCR profile suitable for this study was examined. Extending the denaturation and extension steps to be 10 sec and 15 sec longer for each cycle provided fairly successful amplication results. Further adjustment of various amount of $MgCl_2$ and primer concentrations did not yield any improved results. Therefore, all conditions except the PCR profile was carried out following the original publication.

On the basic of preliminary results by Pongsomboon (1996), ten primers are consistent and robust for RAPD-PCR. All ten primers were then chosen by this thesis (UBC101, UBC174, UBC228, UBC268, UBC273, UBC299, UBC428, UBC456, UBC457 and UBC459). Tassanakajon et al. (1998) published genetic variation of *P. monodon* using 6 of the total primers described there. Based on their analysis, primers UBC174 and UBC456 did not provide any useful information for population genetic studies while the primers UBC459

overestimated geographic population differentiation in *P. monodon*. The primer UBC101 was not included because it yielded the same result as did the primer UBC268 but the latter gave a better view on differentiation between Trat and Chumphon. Finally, only three primers were selected. Primers UBC273 and UBC299 were selected because results from these primers have not been reported.

Analysis of genetic diversity of *P. monodon* using primers UBC268, UBC273 and UBC299 produced 53 RAPD bands. The scorable bands were composed of 43 polymorphic bands (81.13 %) and 10 monomorphic bands (18.87%) ranging from 200 - 1600 bp in fragment sizes (Table 3.1).

Primers UBC299, UBC268 generated 18 scorable band whereas the primer UBC273 generated 17 scorable bands. The percentage of polymorphic bands generated by primers UBC268, UBC273 and UBC299 across all specimens were 77.78 %, 82.35 % and 83.33 %, respectively. The percentage of monomorphic bands generated by these respective primers for overall specimens were 22.22 %, 17.65% and 16.67%, respectively. Therefore the UBC299 was the most polymorphic primer in this study.

RAPD patterns generated by primers UBC268, UBC273 and UBC299 found in five geographic sample of *P. monodon* are shown in Fig. 3.2, 3.4 and 3.6, respectively. Using the primer UBC268, a band with 260 bp in length was found in almost all of the Trat specimens (the Gulf of Thailand) but disappeared for the Satun, Trang, Phangnga samples (the Andaman Sea). This DNA marker was also found in a few *P. monodon* individuals from Chumphon.

Table 3.1 Nucleotide sequences, number of scored bands and sizes ranges of fragments (bp) resulted from primers UBC268, UBC273 and UBC299 observed overall samples.

Primer	Sequence	Size-range (bp)	No. of overall amplified bands	No. of overall polymorphic bands(%)	No. of overall monomorphic bands(%)
UBC268	AGGCCGCTTA	260-1600	18	14 (77.78 %)	4 (22.22 %)
UBC273	AATGTCGCCA	400-1600	17	14 (82.35%)	3 (17.65 %)
UBC299	TGTCAGCGGT	200-1450	18	15 (83.33%)	3 (16.67 %)
Total primers	-	-	53	43 (81.13 %)	10 (18.87%

All RAPD patterns (genotypes) generated from each primer are shown in Fig 3.3, 3.5 and 3.7. A total of 88 genotypes from 3 primers were observed (Appendix B). Large numbers of unique genotypes were generally found in Trat for each primer.

3.2.1 Analysis of genetic polymorphism of *P. monodon* using the primer UBC268

A total of thirty RAPD genotypes was generated from analysis of this primer with 89 individuals of *P. monodon* (Fig. 3.3a and Fig 3.3b). Eighteen RAPD fragment generated by the UBC268 were scored (1600, 1450, 880, 820, 800, 740, 720, 680, 620, 600, 540, 500, 480, 420, 400, 360, 300 and 260 bp) (see Appendix B). Four RAPD fragments (480, 400, 360 and 300 bp) are common for all genotypes while other 14 fragments contributed differences in similarity/ distance between different genotypes (Table 3.1).

The percentage of polymorphic RAPD bands within a geographic sample was between 40 % - 77.78 %. The highest percentage of polymorphic bands was observed in Trat (77.78%) followed by Trang (53.33%), Chumphon and Phangnga (50 %) and Sutun (40 %) (Table 3.2). Three common genotypes; A (19.5 %), B (14.9) and D (13.8 %), were found (Table 3.5). Generally, the remaining genotypes were possessed by less than 5% of investigated individuals. The most common genotype A was overlappingly found across all geographic samples while B and C were found in all the Andaman sample and Chumphon but did not observed in Trat. Relationships between these genotypes can be drawn. An absence of a 820 bp fragment in the genotype A resulted in the appearance of the genotype B and a further loss of a RAPD fragment of 500 bp in B yielded the genotype C (Appendix B). Thirteen unique (or private genotype, found in only one geographic sample) genotypes accounting for 86.4%

of *P. monodon* in Trat were found (Table 3.5) whereas lower numbers of these were observed in Satun(1), Trang(2) and Phangnga (2) and Chumphon (4).

Dissociation between *P. monodon* from Trat and the Andaman Sea are partially accomplished by the presence or absence of a 260 bp fragment. This RAPD marker was found in 22 of 24 investigated individuals from Trat and in 2 of 25 *P. monodon* individuals from Chumphon (accounting for 90.9% and 8%, respectively). All *P. monodon* from the Andaman Sea did not contain this RAPD marker.

Table 3.2 Total number of bands, percentage of polymorphic and monomorphic bands within a geographic sample observed when *P. monodon* from 5 conspecific samples were analysed by the primer UBC268

Sample	No. of bands	No. of polymorphic bands within a sample	No. of monomorphic bands within a sample
Satun (N=14)	15	6 (40.00%)	9 (60.00%)
Trang (N=10)	15	8 (53.33%)	7 (46.67%)
Phangnga (N=18)	16	8 (50.00%)	8 (50.00%)
Chumphon(N=25)	16	8 (50.00%)	8 (50.00%)
Trat (N=22)	18	14 (77.78%)	4 (22.22%)
	Average	54.22 %	45.78%

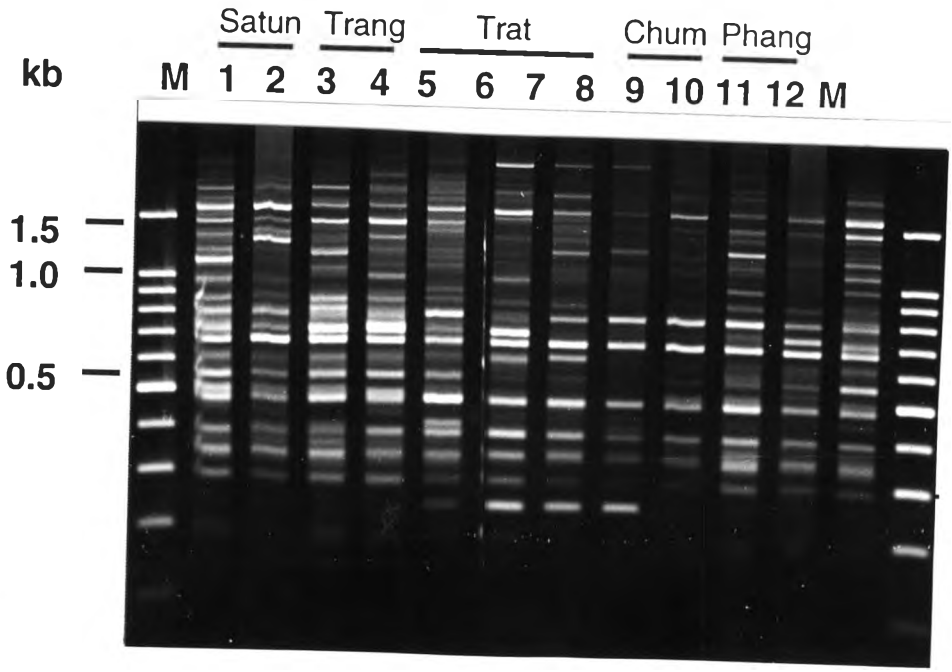


Figure 3.2 Examples of RAPD patterns using the primer UBC 268. An arrow indicates a 260 bp fragment found in Trat (90.9%) and Chumphon (8%) but absence in other samples

Lanes M = a 100 bp DNA ladder

Lanes 1-2 = *P. monodon* individuals collected from Satun

Lanes 3-4 = *P. monodon* individuals collected from Trang

Lanes 5-8 = *P. monodon* individuals collected from Trat

Lanes 9-10 = *P. monodon* individuals collected from Chumphon

Lanes 11-12 = *P. monodon* individuals collected from Phangnga

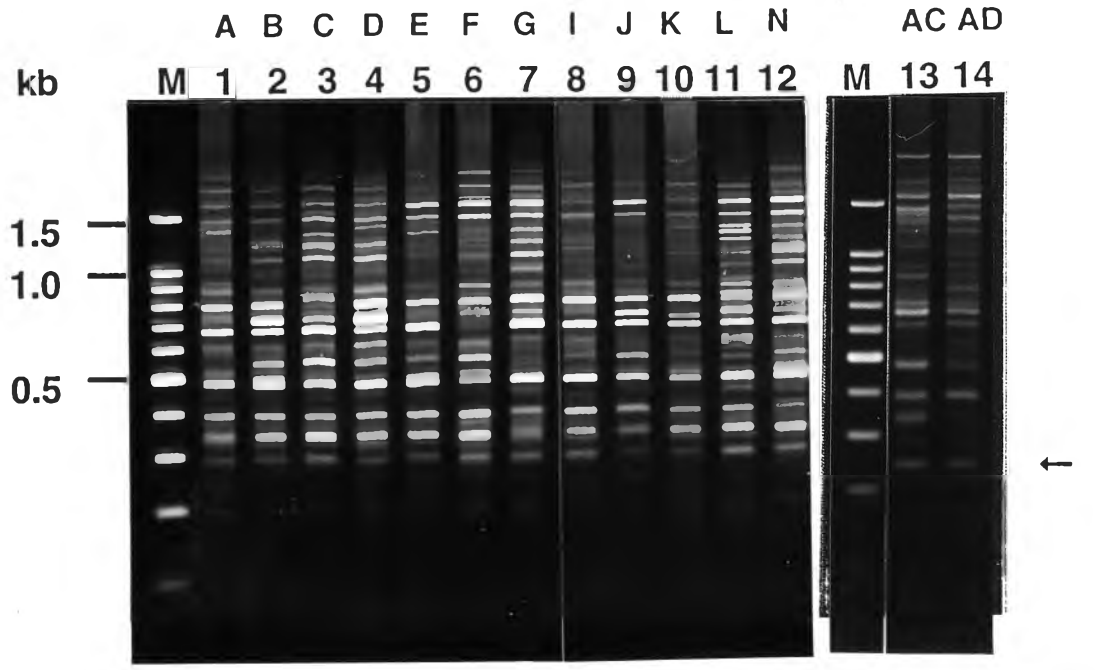
Figure 3.3 RAPD patterns generated from the primer UBC268. An arrow indicated a 260 bp RAPD fragment specifically found in 90.9% of Trat and 8 % of Chumphon *P. monodon*.

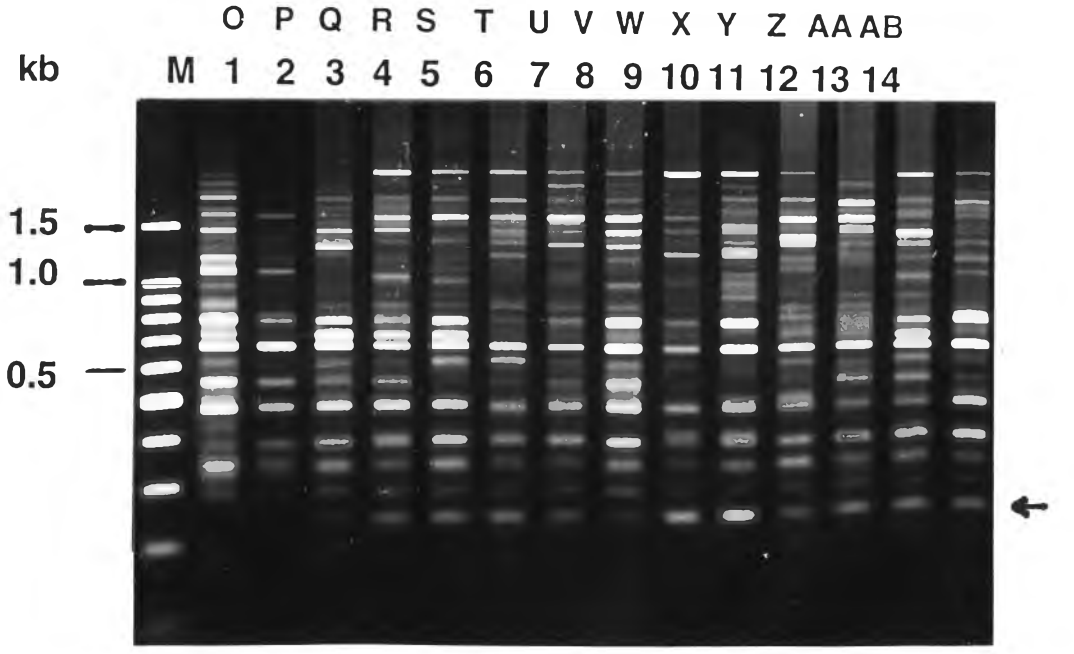
a) Lane M = a 100 bp DNA ladder

Lanes 1-14 = RAPD genotypes A – N, AC - AD

b) Lane M = a 100 bp DNA ladder

Lanes 1-14 = RAPD genotypes O – AB


A B C D E F G I J K L N AC AD
kb M 1 2 3 4 5 6 7 8 9 10 11 12 M 13 14
1.5
1.0
0.5


O P Q R S T U V W X Y Z AA AB
kb M 1 2 3 4 5 6 7 8 9 10 11 12 13 14
1.5
1.0
0.5

3.2.2 Analysis of genetic polymorphism of *P. monodon* using the primer UBC273

Thirty- two RAPD genotypes were generated when one hunderd *P. monodon* specimens were analysed with this primer (Fig. 3.5a and Fig. 3.5b). A total of seventeen RAPD generated fragments were scored (1600, 1400, 1100, 1050, 960, 900, 880, 860, 730, 700, 620, 600, 550, 500, 460, 440 and 400 bp) (see Appendix B). Three RAPD fragments (700, 440 and 400 bp) were monomorphic while other 14 fragments were polymorphic. The latter contributed differences in similarity/distance between different RAPD genotypes (Table 3.1).

The percentage of polymorphic RAPD bands within a geographic sample also varied tremendously for this primer and showed a different trend from that of the primer UBC268. Chumphon showed the highest percentage of polymorphic bands (71.43%) than did any others (66.67 % in Satun and Phangnga, 58.33 % in Trang and 41.67 % in Trat) (Table 3.3).

Only three genotypes were possessed by at least ten *P. monodon* individuals. The most common RAPD genotype D were found in one - quarter of the specimens. Apparently, this genotype was not observed in Trang and Trat. The second common genotype B was found in Satun, Phangnga and Chumphon accounting for 23.1 % of overall individuals. The genotype P was found in all geographic samples (19.2 %) with the exception of Trat (Table 3.5).

The differences between B and D were from an absence of a 1100 bp band in the former and an available of an 880 bp fragment in the latter. An absence of a fragment of 1050 bp in A yielded the genotype P. To replace the genotype B with P, both gaining of a 1100 bp and losing of 1050 bp

are needed. These reflected the high genetic similarity between *P. monodon* carrying these genotypes.

Relatively low number of private haplotypes were generated by this primer. While four of which were found in Trat, three private genotypes were observed in Phangnga, Chumphon and Satun and only a single unique genotype was observed in Trang (Table 3.5).

Table 3.3 Total number of bands, percentage of polymorphic and monomorphic bands within a geographic sample observed when *P. monodon* from 5 conspecific samples were analysed by the primer UBC273

Sample	No. of bands	No. of polymorphic bands within a sample	No. of monomorphic bands within a sample
Satun (N=17)	15	10 (66.67%)	5 (33.33%)
Trang (N=8)	12	7 (58.33%)	5 (41.67%)
Phangnga (N=23)	15	10 (66.67%)	5 (33.33%)
Chumphon(N=36)	14	10 (71.43%)	4 (28.57%)
Trat (N=16)	12	5 (41.67%)	7 (58.33%)
	Average	60.95 %	39.05%

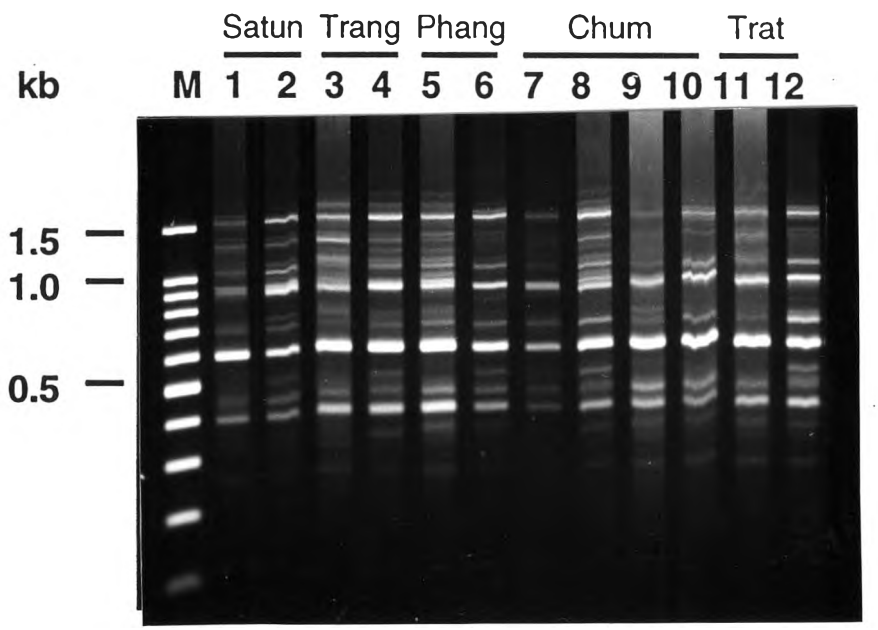


Figure 3.4 Examples of RAPD patterns using the primer UBC273

Lanes M = a 100 bp DNA ladder

Lanes 1-2 = *P. monodon* individuals collected from Satun

Lanes 3-4 = *P. monodon* individuals collected from Trang

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

Lanes 7-10 = *P. monodon* individuals collected from Chumphon

Lanes 11-12 = *P. monodon* individuals collected from Trat

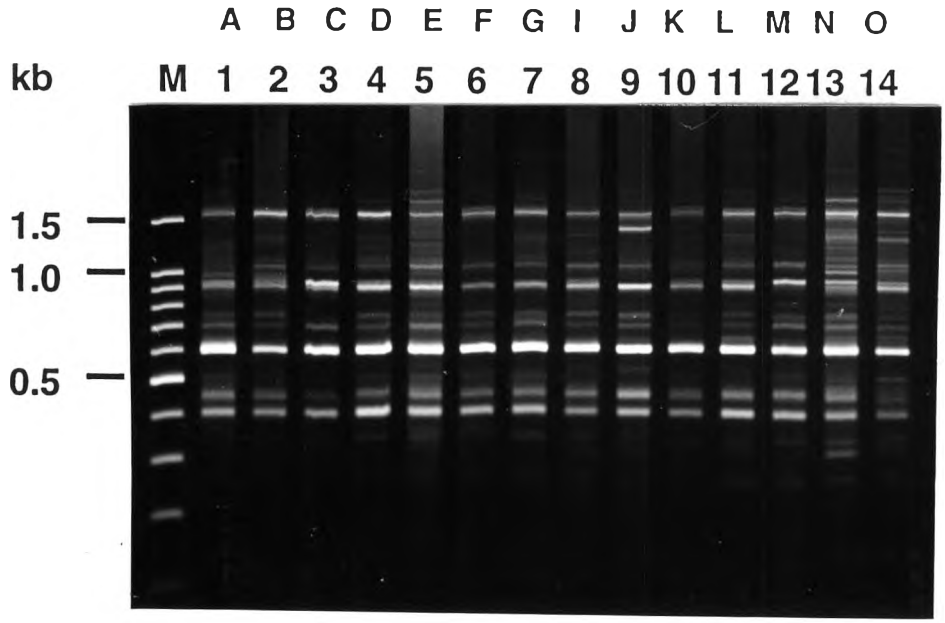
Figure 3.5 RAPD patterns generated from the primer UBC273

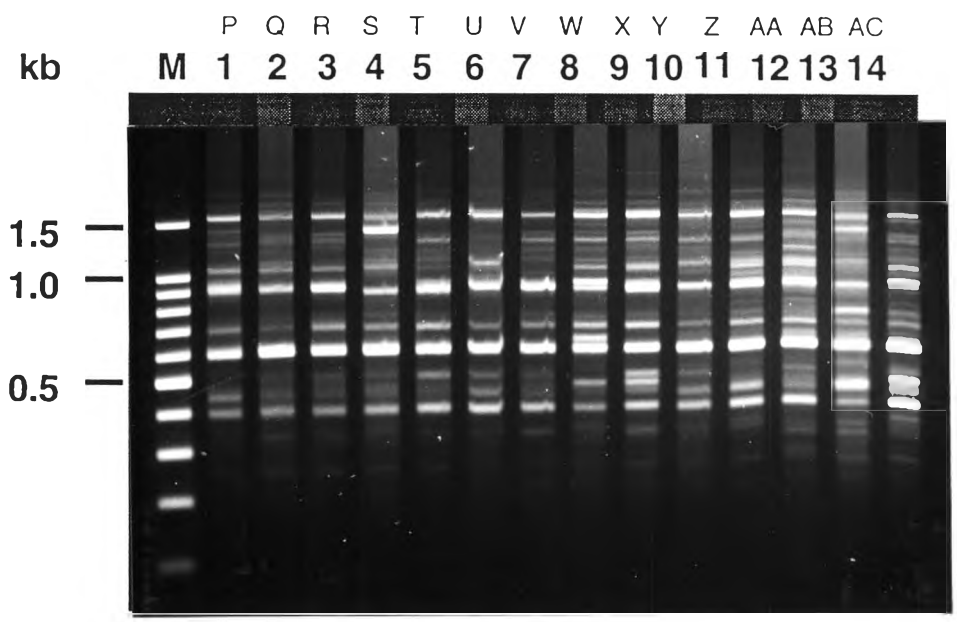
a) Lane M = a 100 bp DNA ladder

Lanes1-14 = RAPD genotypes A - O

b) Lane M = a 100 bp DNA ladder

Lanes 1-14 = RAPD genotypes P - AC


A B C D E F G I J K L M N O
kb
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14
1.5
1.0
0.5


P Q R S T U V W X Y Z AA AB AC
kb
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14
1.5
1.0
0.5

3.2.3 Analysis of genetic polymorphism of *P. monodon* using the primer UBC 299

Investigation of 136 *P. monodon* individuals with this primer resulted in 26 RAPD genotypes (Fig. 3.7a and 3.7b). A total of eighteen scorable RAPD fragments were generated by the UBC299 (1450, 1400, 1200, 1100, 1050, 950, 590, 570, 520, 500, 480, 450, 400, 340, 320, 300, 240 and 200 bp) (see Appendix B).

Three RAPD fragments (1200, 950 and 200 bp) are observed in all RAPD genotypes while other 15 fragments illustrated polymorphic results between genotypes. The percent polymorphic bands of this primer (83.33%) was comparable to that of the UBC273 (82.35%) but slightly greater than that (77.78%) of the UBC268 (Table 3.1).

The percentage of polymorphic RAPD bands within a geographic sample was between 42.85% - 64.28 %. The highest percentage of polymorphic bands was observed in Phangnga (64.28%) followed by Trat (57.14 %), Satun (53.33 %), Chumphon (50.00 %) and Trang (42.85 %) (Table 3.4).

Only four common genotypes were possessed by more than 10 % of investigated individuals. The common genotypes A and C carried by 24.3 % and 15.4 % of overall samples were not found in *P. monodon* from Trat whereas the genotype B (14.0 %) was not observed in the Trang sample. The haplotype K (11.0 %) was distributed in all geographic samples (Table 3.5).

Like primers UBC268 and UBC273, four common genotypes can be easily related by the presence or absence of a few RAPD bands (e.g. 1100 bp, 480 bp and 240 bp fragments). In terconnections between pairs of these common genotypes can be assessed with a single presence/ absence of a particular bands.

Ten RAPD singletons (a genotype carrying by a single individual) were found in Phangnga (7) and Trat (3). No unique genotype existed in Chumphon. While Satun showed one private genotype, large numbers were possessed by the Trat (4) and Trang (2) samples.

Table 3.4 Total number of bands, percentage of polymorphic and monomorphic bands whitin a geographic sanple observed when *P. monodon* from 5 conspecific samples were analysed by the primer UBC299

Sample	No. of bands	No. of polymorphic bands within a sample	No. of monomorphic bands within a sample
Satun (N=26)	15	8 (53.33%)	7 (46.67%)
Trang (N=18)	14	6 (42.85%)	7 (57.15%)
Phangnga (N=27)	14	9 (64.28%)	6 (42.85%)
Chumphon(N=40)	16	8 (50.00%)	8 (50.00%)
Trat (N=25)	14	8 (57.14%)	6 (42.86%)
	Average	53.52 %	46.48%

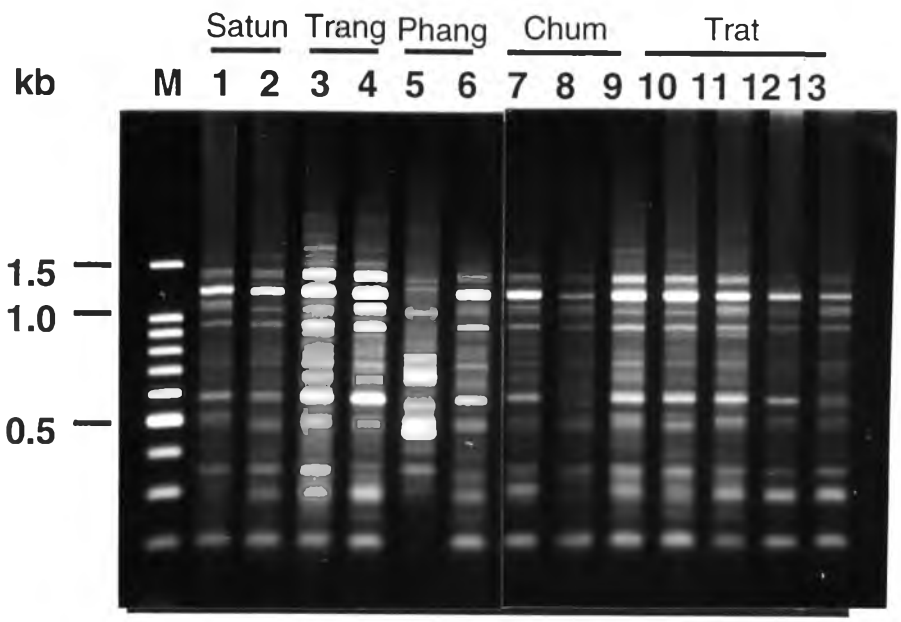


Figure 3.6 Examples of RAPD patterns using the primer UBC299

Lanes M = a 100 bp DNA ladder

Lanes 1-2 = *P. monodon* individuals collected from Satun

Lanes 3-4 = *P. monodon* individuals collected from Trang

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

Lanes 7-9 = *P. monodon* individuals collected from Chumphon

Lanes 10-13 = *P. monodon* individuals collected from Trat

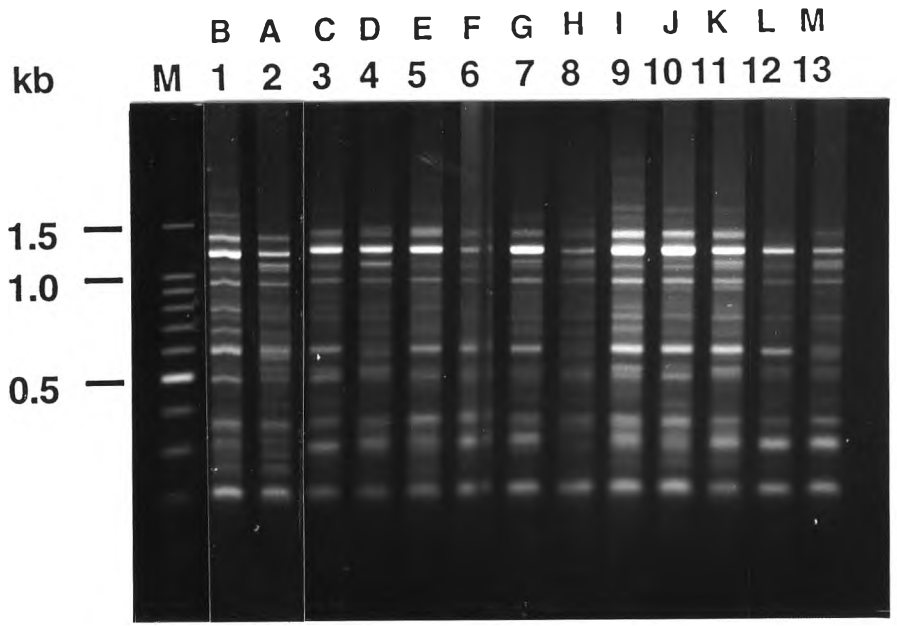
Figure 3.7 RAPD patterns generated from the primer UBC299

a) Lane M = a 100 bp DNA ladder

Lanes 1-14 = patterns of RAPD genotype A – M

b) Lane M = a 100 bp DNA ladder

Lanes 1-14 = RAPD genotypse N – Z



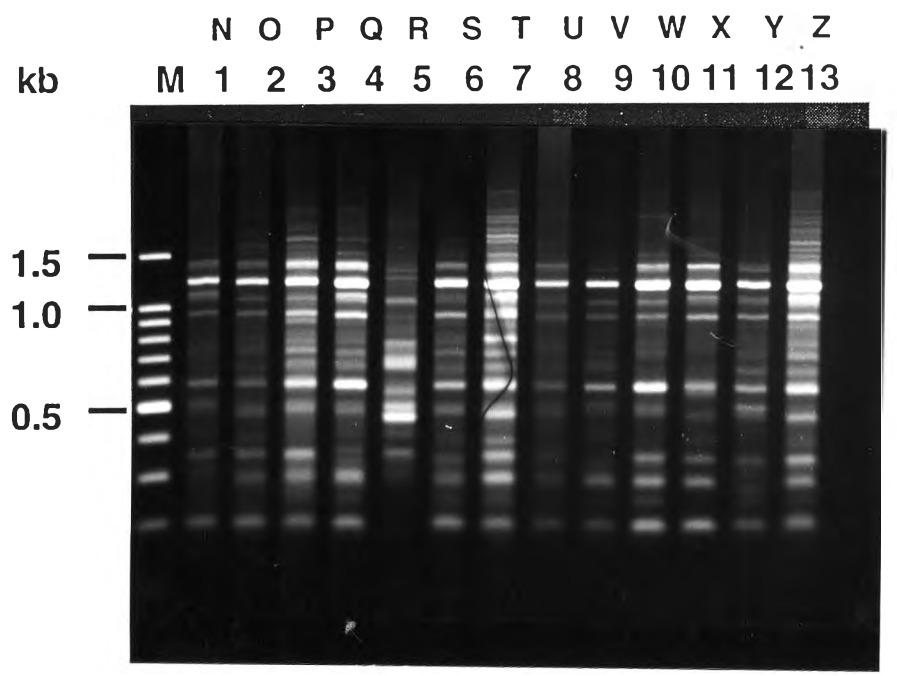


Table 3.5 Geographic distribution of RAPD genotypes in 5 geographic samples of wild *P. monodon* in Thailand

A. Primer UBC268

	Genotype																													
Population	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD
Satun	2	5	1	3	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trang	3	1	1	1	0	0	2	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phangnga	6	2	1	4	0	1	1	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chumphon	4	5	1	4	4	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Trat	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	1	2	2	1	2	1	1	1	1	2	1	1

B. Primer UBC273

	Genotype																														
Population	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF
Satun	0	5	1	1	2	1	0	0	1	0	1	0	0	0	0	1	0	1	0	0	0	0	1	1	0	0	0	1	0	0	0	0
Trang	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	2	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Phangnga	2	4	2	5	0	1	0	0	0	1	0	1	0	2	0	2	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0
Chumphon	2	3	3	7	3	0	1	2	0	1	2	0	1	0	1	5	1	2	0	1	1	0	0	0	0	0	0	0	0	0	0	0
Trat	0	0	0	0	3	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	6	2	1

C. Primer UBC299

	Genotype																									
Population	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
Satun	5	3	8	0	0	0	1	0	0	1	1	3	1	0	2	0	0	0	0	0	0	1	0	0	0	0
Trang	7	0	3	0	0	1	1	0	1	0	2	0	0	1	0	0	1	0	0	0	0	1	0	0	0	0
Phangnga	4	3	6	1	1	0	0	1	1	0	4	0	0	0	1	1	0	1	1	0	1	0	0	0	1	0
Chumphon	17	6	4	2	0	0	1	0	1	0	4	0	1	0	3	0	1	0	0	0	0	0	0	0	0	0
Trat	0	7	0	1	0	0	0	0	0	1	5	0	0	0	0	0	0	0	0	1	0	0	7	1	0	2

3.3 Genetic similaries, distances and differentiation in Thai *P. monodon*

Estimation of similarity indices within and between 5 geographic samples of wild *P. monodon* calculated when 89, 100 and 136 individuals of *P. monodon* were analyzed with primers UBC268, UBC273 and UBC299 was shown by Table 3.6 and 3.7.

The average of similarity within a samples from primers UBC268, UBC273 and UBC299 were 0.8341, 0.8771 and 0.9290, respectively (Table 3.6). The average similaries within each geographic across all primers was 0.8871 - 0.9125, the highest values were observed in Chumphon (0.9125), followed by Satun (0.9029), Phangnga (0.9007), Trat (0.0881) and Trang (0.8871), respectively (Table 3.6). Chumphon showed the highest similarity index within samples whereas Trang showed the lowest. The results suggested that *P. monodon* from Chumphon was genetically less diverse than that of others .

The similarity indices between 5 geographic samples of wild *P. monodon* in Thailand ranged from 0.8000 - 0.9276, 0.8429 - 0.8790 and 0.8834-0.9427 when analysed with primers UBC268, UBC273 and UBC299, respectively (Table 3.7). The lowest average similaries between samples were 0.8491 (Trang – Trat) whereas the highest of such a parameter was 0.9072 (Phangnga- Chumphon).

It should be noted that similarity indices described above do not eliminate effects of within population similarities and this must be taken into account for population genetic studies. Nevertheless, such an effect can be obviated if each of all the primers used give unique or nearly unique genotypes across individuals. On the basic of similarity among pairs of geographic samples with a correction of within similarity effects, the most

genetically closed samples were Satun – Phangnga and Chumphon - Phangnga (1.000) whereas the most diverse samples were between Satun - Trat (0.9608).

All possible pairwise comparisons of similaries between pairs of samples within the Andaman *P. monodon* was between 0.9952 - 1.0000 indicating their closed relationships (Table 3.8). Surprisingly, Chumphon located on the opposite coast of the Andaman Sea showed closer genetic relationships to those samples (0.9967 - 1.0000) than did the proximal geographic sample like Trat (0.9651). The similarity indices between Chumphon – Phangnga (1.0000) and Chumphon - Trang (0.9990) were among the highest values for all possible pairwise comparisons. The similarity indices indicated less similarity between *P. monodon* of Trat and that from other samples. The Chumphon *P. monodon* was genetically more similar to three *P. monodon* samples from the Andaman Sea rather than the Trat *P. monodon*.

Genetic distances were then converted from similarity values ($D_{ij} = 1 - S'_{ij}$, equation 2.4). The average genetic distance between pairs of geographic samples overall primers were 0.0000 – 0.0392. Large genetic distance were observed when comparing the Trat *P. monodon* with each of the remaining samples including Chumphon (0.0349 – 0.0392).

Disregrading the Trat samples, genetic distance levels for all possible comparisons were much lower (0.0000- 0.0048). The results indicated that *P. monodon* from Trat was genetically separated from that of the 4 geographic samples whereas *P. monodon* from Chumphon did not difference genetically from that of the Andaman Sea.

Table 3.6 Estimated similarity (S) within each of the 5 geographic samples of wild *P. monodon*

Sample	Primers			Average similarity within a sample across all primers
	UBC268	UBC273	UBC299	
Satun	0.9312	0.8505	0.9271	0.9029
Trang	0.8398	0.8772	0.9445	0.8871
Phangnga	0.9231	0.8696	0.9094	0.9007
Chumphon	0.9157	0.8809	0.9411	0.9125
Trat	0.8341	0.9072	0.9230	0.8881
Average similarity for each primer across all samples	0.8341	0.8771	0.9290	

Genetic distances between pairs of samples resulted from each RAPD primer and their average were subjected to phylogenetic reconstruction using unweighted pair group method with arithmetic averages (UPGMA). Generally, phenograms generated from a single primer and from the average distance across all primers showed similar topology. The UPGMA phenograms from primers UBC268, UBC273 and overall primers were identical and allocated all investigated samples to 2 separated groups ; A (Trat) and B (Chumphon, Phangnga, Satun and Trang). It was surprised that Chumphon well clustered with either Trang (UBC 299) or Phangnga (UBC268 and UBC 273). These indicated its closer relationships to the Andaman Sea samples rather than Trat. Large genetic distance was observed between these two phylogenetically groups.

Table 3.7 Estimated similarity (S_{ij}) between each of 5 geographic samples of wild *P. monodon*

	Primers			
Sample	UBC268	UBC273	UBC299	Average
Satun/Trang	0.8830	0.8570	0.9306	0.8902
Satun/Phangnga	0.9276	0.8608	0.9181	0.9021
Satun/Chumphon	0.9217	0.8620	0.9295	0.9044
Satun/Trat	0.8329	0.8429	0.8932	0.8563
Trang/Phangnga	0.8781	0.8683	0.9253	0.8905
Trang/Chumphon	0.8747	0.8790	0.9427	0.8988
Trang/Trat	0.8000	0.8501	0.8973	0.8491
Phangnga/Chumphon	0.9218	0.8769	0.9230	0.9072
Phangnga/Trat	0.8336	0.8556	0.8834	0.8575
Chumphon/Trat	0.8341	0.8551	0.9070	0.8654

Table 3.8 The average genetic distances (below diagonal) and genetic similaritieses with a correction of within sample similarity effects (S'_{ij}, above diagonal) of all primers between 5 geoghaphic samples of wild *P. monodon*

-	Satun	Trang	Phangnga	Chumphon	Trat
Satun	-	0.9952	1.0000	0.9967	0.9608
Trang	0.0048	-	0.9966	0.9990	00.9615
Phangnga	0.0000	0.0034	-	1.0000	0.9631
Chumphon	0.0033	0.0010	0.0000	-	0.9651
Trat	0.0392	0.0385	0.0369	0.0349	-

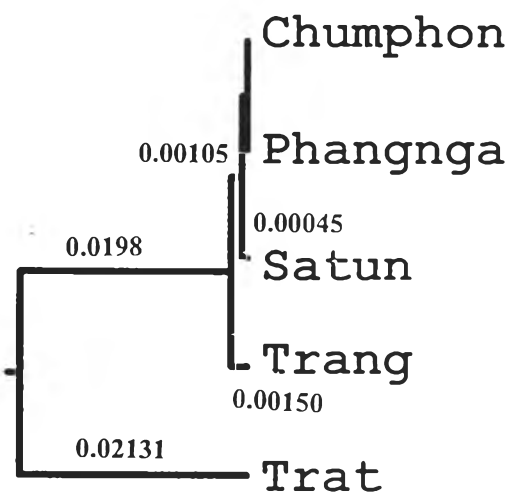


Figure 3.8 UPGMA dendrogram showing relationships among the 5 geographic samples of wild *P. monodon* based on genetic distance of the primer UBC268

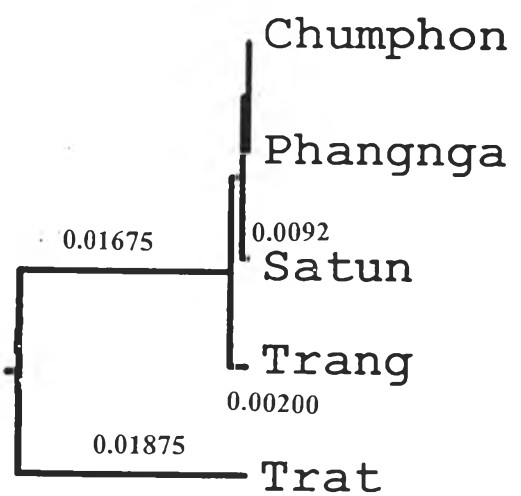


Figure 3.9 UPGMA dendrogram showing relationships among the 5 geographic samples of wild *P. monodon* based on genetic distance of the primer UBC273

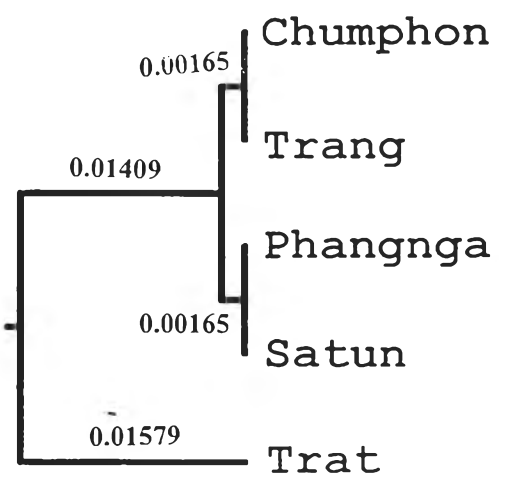


Figure 3.10 UPGMA dendrogram showing relationships among the 5 geographic samples of wild *P. monodon* based on genetic distance of the primer UBC299

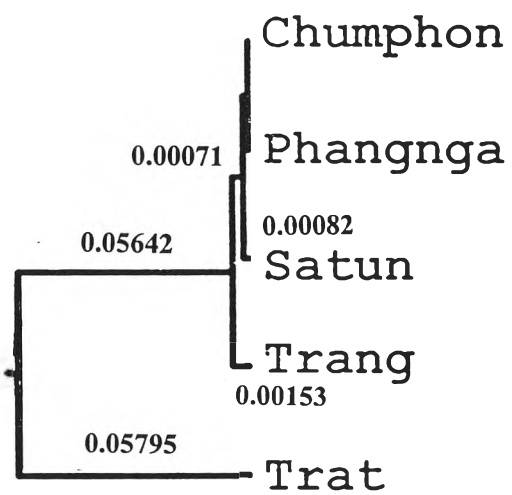


Figure 3.11 UPGMA dendrogram showing relationships among the 5 geographic samples of wild *P. monodon* based on genetic distance average over – all primers

Analysis of geographic heterogeneity was carried out whether distribution of RAPD genotype frequencies between pairs of samples/regions were significant (Table 3.9). Considering overall samples, all primer used in this study indicated highly significant genetic differences of *P. monodon* in Thailand ($P < 0.0001$). Therefore, population subdivision did exist in Thai *P. monodon*. Determination of geographic heterogeneity between the Andaman Sea and the Gulf of Thailand was then further carried out and revealed highly significant in genotype distributions for all primers ($P < 0.0001$).

Regarding all possible pairwise comparisons, no genetic differences within the Andaman Sea *P. monodon* were observed ($P > 0.05$). Not only did all primers indicated highly significant differences in genotype distribution between *P. monodon* originating from Trat and each of the Andaman Sea samples ($P < 0.0001$ for UBC268 and UBC299 and $P = 0.0022$ for UBC273). but also those when Trat and Chumphon were compared ($P = 0.0001$ for UBC268 and $P < 0.0001$ for UBC273 and UBC299). Chumphon did not showed any difference when separately compared with either Phangnga, Satun or Trang ($P > 0.05$) or when compared with the combined Andaman samples ($P > 0.05$). Based on all analyses, Trat was considered to be different *P. monodon* stock (called stock A) while the Andaman sample was regarded as another (B). The anomalous Chumphon *P. monodon* was then further dissociate from Trat and regarded as the other stocks (C).

Table 3.9 Analysis of geographic heterogeneity in genotype frequency distributions generated from RAPD patterns of wild *P.monodon* using a Monte Carlo simulation

	Primer		
Samples	UBC268	UBC273	UBC299
Satun/Trang	P=0.3895^{ns}	P=0.2286^{ns}	P=0.1905^{ns}
Satun/Phangnga	P=0.5308^{ns}	P=0.4629^{ns}	P=0.4151^{ns}
Satun/Chumphon	P=0.7783^{ns}	P=0.3812^{ns}	P=0.0715^{ns}
Satun/Trat	P=0.0011	P<0.0001	P<0.0001
Trang/Phangnga	P=0.8660^{ns}	P=0.0419^{ns}	P=0.4552^{ns}
Trang/Chumphon	P=0.2902^{ns}	P=0.3880^{ns}	P=0.3188^{ns}
Trang/Trat	P=0.0535^{ns}	P=0.0008	P<0.0001
Phangnga/Chumphon	P=0.6534^{ns}	P=0.3906^{ns}	P=0.2027^{ns}
Phangnga/Trat	P=0.0012	P<0.0001	P=0.0004
Chumphon/Trat	P=0.0001	P<0.0001	P<0.0001
Andaman/Chumphon	P=0.2654^{ns}	P=0.8511^{ns}	P=0.7859^{ns}
Andaman/Trat	P<0.0001	P=0.0022	P<0.0001
Andaman/Gulf of Thailand	P<0.0001	P<0.0001	P<0.0001
Over all samples	P<0.0001	P<0.0001	P<0.0001

ns = not significant at the probability level adjused to $P < 0.003$ based on the sequential Bonferoni method.