Chapter III

Results

DNA Extraction

The shrimp DNAs were prepared from the pleopods by using proteinase-K / phenol-chloroform extraction. The quality and quantity of DNA from individual shrimp were determined by electrophoresis on a 0.7% mini-gel and measuring absorbance at 260 and 260/280 nm.

The extracted DNA from one pleopod showed O.D. at 260 between 0.20-0.32 and O.D. 260/280 ratio between 1.8-2.0. Since $O.D._{260}$ of 1.0 corresponds to 50 μg double-stranded DNA per ml, thus, the recovery yield was about 250-400 μg/ml = 75-100 μg/one pleopod. The O.D. 260/280 ratio also indicated the purity of the extracted DNA. High MW. DNA (>23 kb) was observed after electrophoresed through a 0.7% agarose mini-gel (Fig 3.1).

Optimization of RAPD-PCR

Experiments were performed to optimize PCR program parameters for reproducible amplification of discrete *P.monodon* DNA.. Inconsistent amplification was minimized by including three quantities of template DNA

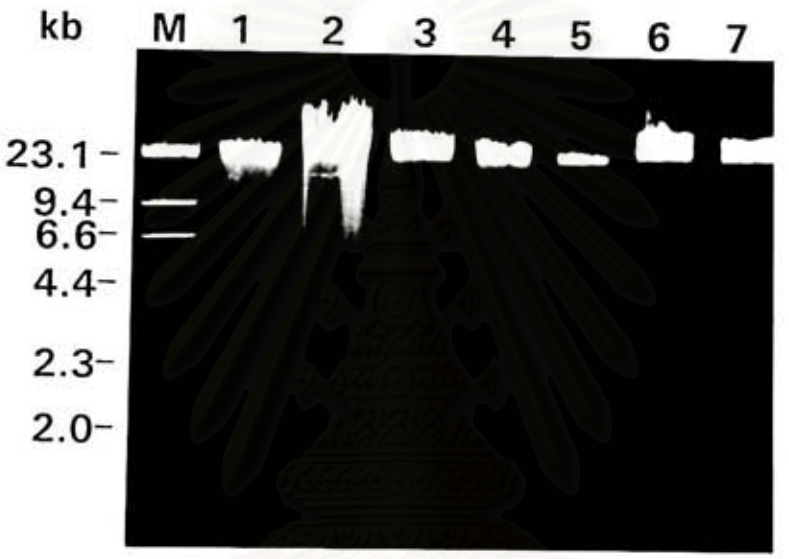


Figure 3.1. Ethidium bromide staining of 0.7% agarose gel showing DNA extracted from pleopods of *P.monodon*.

Lane M = λ DNAs / *Hin*d III size marker.

Lanes 1-7 = genomic DNAs of 7 individuals of *P.monodon*.

(25, 50 and 100 ng) in the program evaluation and selecting optimal program parameters based upon consistent amplification at all DNA quantities. The first assay was attempted with the following profiles: 35 cycles consisting of denaturation at 94°C for 5 sec, 15 sec, and 30 sec, annealing at 36°C for 45 sec and extension at 72°C for 90 sec. In this assay, denaturation for 5 sec yielded consistent amplifications at all three DNA concentrations (Fig 3.2 A), whereas denaturation for 15 and 30 sec yielded faint bands at higher DNA concentration.

The length of annealing step was attempted with the following profiles: 35 cycles consisting of denaturation at 94°C for 5 sec, annealing at 36°C for 30, 45 and 60 sec and extension at 72°C for 90 sec. The results of annealing for 45 and 60 sec gave bands of acceptable intensity (Fig 3.2 B) but annealing for 30 sec reduced the yield of PCR products. Annealing length of 45 sec was chosen.

The length of extension step was attempted with the following profiles: 35 cycles consisting of denaturation at 94°C for 5 sec, annealing at 36°C for 45 sec and extension at 72°C for 75, 90 and 105 sec. It was found that short extension for 75 sec resulted in faint bands, whereas too long extension for 105 sec resulted in smearing an agarose gel. Therefore, the optimized extension time was 90 sec (Fig 3.2 C).

The last assay was attempted by varying the number of cycles with the following profiles: 30, 35 and 40 cycles consisting of denaturation at 94°C for 5

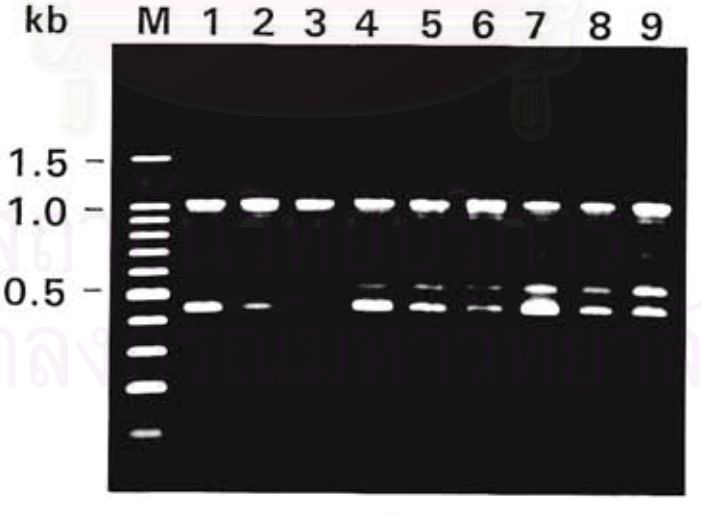
sec, annealing at 36°C for 45 sec and extension at 72°C for 90 sec. The 30 cycles of amplification produced lesser amounts of PCR products. Increasing the number of cycles from 35 to 40 cycles did not significantly increase the PCR products (Fig 3.2 D).

Therefore the optimal program parameters identified for the RAPD analysis of *P. monodon* were 35 cycles of 5 sec at 94°C, 45 sec at 36°C and 90 sec at 72°C.

Primer screening

A total of 300 ten-base primers were screened for their ability to prime PCR amplifications of the black tiger prawn DNA. Only 138 RAPD primers (46%) yielded amplification products while the rest of the primers did not amplified the DNA template or resulted in smear patterns (Table 3.1). Examples of primer screening are shown in Fig 3.3. The primers which yielded intense or consistent bands or both were selected for further analysis. In this study, seven positive primers 101, 174, 268, 428, 456, 457 and 459 were selected for the detection of genetic variation in wild populations of *P.monodon*.

A



B

Figure 3.2 Optimization of the PCR parameters for RAPD analysis of *P.monodon* using primer 174.

A . Variation of the length of denaturation step at 94 °C.

Lane M = 100 bp DNA ladder.

Lanes 1-3 = denaturation for 5 sec with 25, 50 and 100 ng template DNA, respectively.

Lanes 4-6 = denaturation for 15 sec with 25, 50 and 100 ng template DNA, respectively.

Lanes 7-9 = denaturation for 30 sec with 25, 50 and 100 ng template DNA, respectively.

B. Variation of the length of annealing step at 36 °C.

Lane M = 100 bp DNA ladder.

Lanes 1-3 = annealing for 30 sec with 25, 50 and 100 ng template DNA, respectively.

Lanes 4-6 = annealing for 45 sec with 25, 50 and 100 ng template DNA, respectively.

Lanes 7-9 = annealing for 60 sec with 25, 50 and 100 ng template DNA, respectively.

C. Variation of the length of extension step at 72°C.

Lane M = 100 bp DNA ladder.

Lanes 1-3 = extension for 75 sec with 25, 50 and 100 ng template DNA, respectively.

Lanes 4-6 = extension for 90 sec with 25, 50 and 100 ng template DNA, respectively.

Lanes 7-9 = extension for 105 sec with 25, 50 and 100 ng template DNA, respectively.

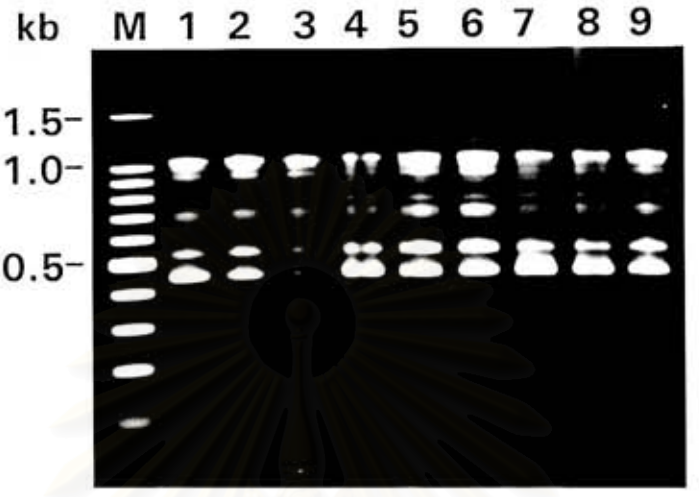
D) Variation of the number of cycles.

Lane M = 100 bp DNA ladder.

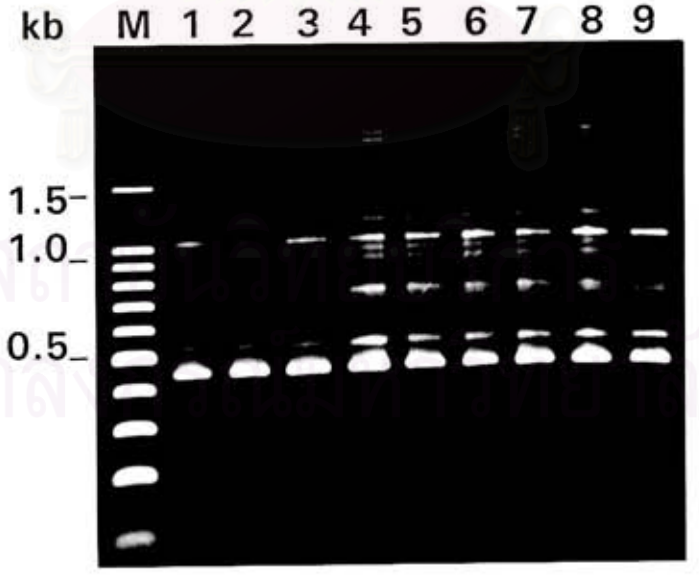
Lanes 1-3 = amplification for 30 cycles with 25, 50 and 100 ng template DNA, respectively.

Lanes 4-6 = amplification for 35 cycles with 25, 50 and 100 ng template DNA, respectively.

Lanes 7-9 = amplification for 40 cycles with 25, 50 and 100 ng template DNA, respectively.



C



D

Figure 3.3. RAPD patterns obtained from amplification of *P.monodon* DNA with various primers.

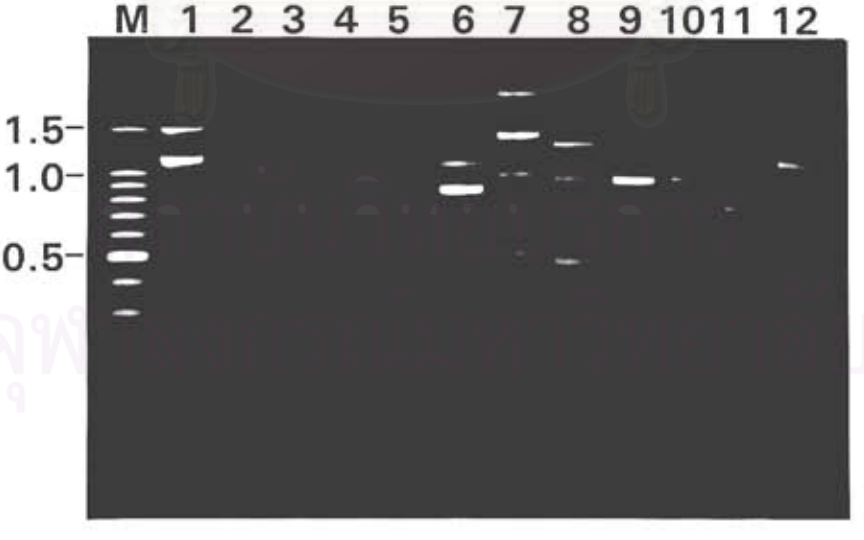
A. Lane M = 100 bp DNA ladder.

Lanes 1-12 = amplifications with primers 421- 432, respectively.

B. Lane M = 100 bp DNA ladder.

Lanes 1-12 = amplifications with primers 450- 460 and 174, respectively.

A



B

Table 3.1 The sequences of random primers included in the screening and amplification strength values for *P.monodon*.

Primer	Sequence	Strength*	Primer	Sequence	Strength*
101	GCGCCTGGAG	+++	126	CTTTCGTGCT	-
102	GGTGGGGACT	-	127	ATCTGGGAGC	-
103	GTGACGCCGC	+	128	GCATATTCCG	-
104	GGGCAATGAT	-	129	GCGGTATAGT	-
105	CTCGGGTGGG	-	130	GGTTATCCTC	-
106	CGTCTGCCCG	-	131	GAAACAGCGT	+
107	CTGTCCCTTT	-	132	AGGGATCTCC	++
108	GTATTGCCCT	-	133	GGAAACCTCT	++
109	TGTACGTGAC	-	134	AAGCTGCGAG	-
110	TAGCCCGCTT	-	135	AAGCTGCGAG	++
111	AGTAGACGGG	-	136	TACGTCTTGG	-
112	GCTTGTGAAC	-	137	GGTCTCTCCC	+
113	ATCCCAAGAG	-	138	GCTTCCCCTT	+
114	TGACCGAGAC	-	139	CCCAATCTTC	-
115	TTCCGCGGGC	-	140	GTCGCATTTC	-
116	TACGATGACG	-	141	ATCCTGTTCG	-
117	TTAGCGGTCT	-	142	ATCTGTTCGG	-
118	CCCGTTTTGT	-	143	TCGCAGAACG	-
119	ATTGGGCGAT	-	144	AGAGGGTTCT	-
120	GAATTTCCCC	-	145	TGTCGGTTGC	-
121	ATACAGGGAG	-	146	ATGTGTTGCG	-
122	GTAGACGAGC	+	147	GTGCGTCCTC	-
123	GTCTTTCAGG	-	148	TGTCCACCAG	+
124	ACTCGAAGTC	-	149	AGCAGCGTGG	-
125	GCGGTGAGAGG	-	150	GAAGGCTCTG	-

Table 3.1 Continued

Primer	Sequence	Strength*	Primer	Sequence	Strength*
151	GCTGTAGTGT	+++	176	CAAGGGAGGT	-
152	CGCACCGCAC	+++	177	TCAGGCAGTC	+++
153	GAGTCACGAG	++	178	CCGTCATTGG	-
154	TCCATGCCGT	-	179	TCACTGTACG	-
155	CTGGCGGCTG	++	180	GGCCACGCT	+++
156	GCCTGGTTGC	++	181	ATGACGACGG	++
157	CGTGGGCAGG	-	182	GTTCTCGTGT	-
158	TAGCCGTGGC	-	183	CGTGATTGCT	-
159	GAGCCCGTAG	-	184	CAAACGGCAC	+++
160	CGATTCAGAG	-	185	GTGTCTTCAG	+
161	CGTTATCTCG	-	186	GTGCGTCGCT	++
162	AACCTACCGC	-	187	AACGGGGGAG	-
163	CCCCCCAGAT	-	188	GCTGGACATC	++
164	CCAAGATGCT	+	189	TGCTAGCCTC	-
165	GAAGGCACGT	++	190	AGAATCCGCC	+++
166	ACTCCTACAG	-	191	CGATGGCTTT	-
167	CCAATTCACG	-	192	GCAAGTCACT	++
168	CTAGATGTGC	-	193	TGCTGGCTTT	-
169	ACGACGTAGG	-	194	AGGACGTGCC	-
170	ATCTCTCCTG	-	195	GATCTCAGCG	-
171	TGACCCCTCC	+++	196	CTCCTCCCCC	++
172	ACCGTCGTAG	-	197	TCCCCGTTCC	-
173	CAGGCGGCGT	+++	198	GCAGGACTGC	++
174	AACGGGCAGC	+++	199	GCTCCCCCAC	-
175	TGGTGCTGAT	++	200	TCGGGATATG	-

Table 3.1 Continued

Primer	Sequence	Strength*	Primer	Sequence	Strength*
201	CTGGGGATTT	-	226	GGGCCTCTAT	+++
202	GAGCACTTAC	-	227	CTAGAGGTCC	+++
203	CACGGCGAGT	+	228	GCTGGGCCGA	++
204	TTCGGGCCGT	+	229	CCACCCAGAG	++
205	CGGTTTGGAA	++	230	CGTCGCCCAT	+++
206	GAGGACGTCC	+	231	AGGGAGTTCC	+++
207	CATATCAGGG	-	232	CGGTGACATC	+++
208	ACGGCCGACC	-	233	CTATGCGCGC	-
209	TGCACTGGAG	++	234	TCCACGGACG	+++
210	GCACCGAGAG	+	235	CTGAGGCAAA	++
211	GAAGCGCGAT	+++	236	ATCGTACGTG	+
212	GCTGCGTGAC	+++	237	CGACCAGAGC	+++
213	CAGCGAACTA	+++	238	CTGTCCAGCA	+++
214	CATGTGCTTG	+	239	CTGAAGCGGA	+++
215	TCACACTTGC	++	240	ATGTTCCAGG	++
216	CATAGACTCC	++	241	GCCCGACGCG	+++
217	ACAGGTAGAC	++	242	CACTCTTTGC	-
218	CTCAGCCCAG	++	243	GGGTGAACCG	+++
219	GTGACCTCAG	+++	244	CAGCCAACCG	+++
220	GTCGATGTCG	+++	245	CGCGTGCCAG	+++
221	CCCGTCAATA	+++	246	TATGGTCCGG	+
222	AAGCCTCCCC	+++	247	TACCGACGGA	-
223	GATCCATTGC	++	248	GAGTAAGCGG	+++
224	TCTCCGGTAT	-	249	GCATCTACCG	+++
225	CGACTCACAG	+++	250	CGACAGTCCC	+++

Table 3.1 Continued

Primer	Sequence	Strength*	Primer	Sequence	Strength*
251	CTTGACGGGG	++	276	AGGATCAAGC	+++
252	CTGGTGATGT	+++	277	AGGAAGGTGC	++
253	CCGTGCAGTA	+++	278	GGTTCCAGCT	+++
254	CGCCCCCATT	+++	279	AGACATTAGA	-
255	TTCCTCCGGA	-	280	CTGGGAGTGG	+++
256	TGCAGTCGAA	+++	281	GAGAGTGGAA	+++
257	CGTCACCGTT	+++	282	GGGAAAGCAG	++
258	CAGGATACCA	++	283	CGGCCACCGT	+++
259	GGTACGTACT	-	284	CAGGCGCACA	++
260	TCTCAGCTAC	+	285	GGGCGCCTAG	+++
261	CTGGCGTGAC	+++	286	CGGAGCCGGC	-
262	CGCCCCCAGT	+++	287	CGAACGGCGG	+++
263	TTAGAGACGG	-	288	CCTCCTTGAC	+
264	TCCACCGAGC	+++	289	ATCAAGCTGC	+++
265	CAGCTGTTCA	+++	290	CCGCGAGCAC	+++
266	CCACTCACCG	+++	291	AGCTGAAGAG	++
267	CCATCTTGTG	-	292	AAACAGCCCG	+++
268	AGGCCGCTTA	+++	293	TCGTGTTGCT	-
269	CCAGTTCGCC	+++	294	TGATTGGCCA	+++
270	TGCGCGCGGG	++	295	CGCGTTCCTG	+++
271	GCCATCAAGA	+++	296	CCGCTGGGAG	+++
272	AGCGGGCCAA	++	297	GCGCATTAGA	+
273	AATGTCGCCA	+++	298	CCGTACGGAC	++
274	GTTCCCGAGT	-	299	TGTCAGCGGT	++
275	CCGGGCAAGC	++	300	GGCTAGGGCG	+++

Table 3.1 Continued

Primer	Sequence	Strength*	Primer	Sequence	Strength*
401	TAGAACAGTC	-	426	CTCCCGGTG	-
402	CCCGCCGTTG	-	427	GTAATCGACG	-
403	GGAAGGCTGT	+++	428	GGCTGCGGTA	+++
404	TCTCTACGAC	-	429	AAACCTGGAC	-
405	CTCTCGTGCG	++	430	AGTCGGCACC	+
406	GCCACCTCCT	+++	431	CTGCGGGTCA	+
407	TGGTCCTGGC	+++	432	AGCGTCGACT	-
408	CCGTCTCTTT	-	433	TCACGTGCCT	+++
409	TAGGCGGCGG	+++	434	TCGCTAGTCC	-
410	CGTCACAGAG	+++	435	CTAGTAGGGG	-
411	GAGGCCCGTT	+	436	GAGGGGGCCA	-
412	TGCGCCGGTG	++	437	AGTCCGCTGC	+++
413	GAGGCGGCGA	+	438	AGACGGCCGG	+++
414	AAGGCACCAG	+	439	GCCCCTTGAC	+++
415	GTTCCAGCAG	-	440	CTGTCGAACC	++
416	GTGTTTCCGG	+++	441	CTGCGTTCTT	-
417	GACAGGCCAA	++	442	CTACTCGGTT	-
418	GAGGAAGCTT	+	443	TGATTGCTCG	-
419	TACGTGTTTG	-	444	GCAGCCCCAT	+++
420	GCAGGGTTCG	++	445	TAGCAGCTTG	+++
421	ACGGCCCACC	+++	446	GCCAGCGTTC	++
422	CACCTGCGGG	-	447	CAGGCTCTAG	++
423	GGGTCTCGAA	++	448	GTTGTGCCTG	+
424	ACGGAGGTTC	-	449	GAGGTTCAAC	+
425	CGTCGGGCCT	+++	450	CGGAGAGCCC	+++

Table 3.1 Continued

Primer	Sequence	Strength*	Primer	Sequence	Strength*
451	CTAATCTCGC	-	476	TTGAGGCCCT	+
452	CTAATCACGG	-	477	TGTTGTGCCC	-
453	AGTACAAGGG	-	478	CAGGCTGGTC	++
454	GCTTACGGCA	-	479	CTCATACGCG	-
455	AGCAAGCCGG	+++	480	GGAGGGGGGA	++
456	GCGGAGGTCC	+++	481	GTAAGGGCGC	-
457	CGACGCCCTG	+++	482	CTATAGGCCG	-
458	CTCACATGCC	+++	483	GCACTAAGAC	-
459	GCGTCGAGGG	++	484	CTGGCAAGGA	-
460	ACTGACCGGC	++	485	AGAATAGGGC	-
461	CCCGTATGTC	-	486	CCAGCATCAG	++
462	CATAGCGGCA	++	487	GTGGCTAGGT	++
463	AGGCGGAAGC	-	488	TTCGCTTCTC	-
464	CACAAGCCTG	+++	489	CGCACGCACA	-
465	GGTCAGGGCT	-	490	AGTCGACCTT	+++
466	TTCTTAGCGG	-	491	TCCTGTCCAG	-
467	AGCACGGGCA	++	492	GTGACTGCTC	+
468	ACGGAAGCGC	+++	493	CCGAATCACT	-
469	CTCCAGCAAA	-	494	TGATGCTGTC	-
470	AGGAGCTGGG	++	495	CTTTCCTTCC	-
471	CCGACCGGAA	-	496	CCTTTCAAGG	-
472	AGGCGTGCAA	++	497	GCATAGTGCG	-
473	ATCCCCAAGA	-	498	GACAGTCCTG	-
474	AGGCGGGAAC	+++	499	GGCCGATGAT	++
475	CCAGCGTATT	-	500	TTGCGTCATG	+

* Primer amplification strength was scored as -, +, ++, +++, where - indicates no amplification, + indicates weak amplification, ++ indicates moderately amplification, and +++ indicates strong amplification.

Detection of genetic variation in wild populations of *P.monodon*

Four geographically separated wild populations of *P. monodon* were collected from the Andaman Sea and the Gulf of Thailand. Samples from the Andaman Sea were from Satun-Trang and Medan (Indonesia) provinces. Samples from the Gulf of Thailand were from Chonburi (Angsila district) and Trad provinces.

The RAPD analysis of 2 geographically separated populations using the 7 selected primers produced a total of 80 scorable bands ranging in size from 200 to 2,200 bp. Eighteen of these bands (22.5%) were monomorphic (present in at least approximately 95% of all individuals) and 62 bands (77.5%) were polymorphic (present in some individuals, absent in others) (Table 3.2). The percentages of polymorphic bands varied between primers. The percentages of polymorphic bands generated by primers 101, 174, 268, 428, 456, 457 and 459 were 84.6, 70.0, 88.9, 100.0, 66.7, 69.2 and 72.7% respectively. Each primer generated between 9-15 scorable bands. The complexity of the banding patterns varied between the primers. Primer 456 gave the highest number of amplified bands (15 bands) but yielded least percentage of polymorphic bands (66.7%). Primers 428 yielded the highest percentage of polymorphic bands which was 100 %.

Individuals of the shrimps from Satun-Trang (17), Trad (28), Angsila

(15) and Medan (15) produced 71, 69, 68 and 73 scorable bands respectively (Table 3.3). The percentages of polymorphic bands from individual shrimps of the Satun-Trang, Trad, Angsila and Medan were 57.7, 52.2, 45.6 and 53.4%, respectively. The results suggested that the shrimps collected from Satun-Trang were the highest polymorphic, while the shrimps collected from Angsila were the least polymorphic among the 4 geographic samples.

The RAPD patterns of all samples tested are shown in Appendix A. Examples of RAPD amplification patterns and the bands scored generated by the selected primers are shown in Fig 3.4-3.10. Only reproducible bands were scored for their presence or absence. Difference in staining intensity of RAPD fragments between profiles was not scored as real variation. Primer 428 appeared to identify a more variable region among the samples of Thai *P.monodon* from the Andaman Sea and the Gulf of Thailand, whereas the remaining primers showed less variable RAPD patterns. When comparing RAPD patterns among wild populations of *P.monodon* from Indonesia (Medan) and Thailand, all of the seven primers gave different RAPD patterns.

RAPD analysis by primer 101

When genomic DNAs of the 4 geographically separated *P.monodon* were amplified by primer 101, bands at about 900 and 520 were found in all individuals of the 4 geographic samples (Table 3.4A). A band at about 1550 bp, were found in 66.7 and 100% of the Angsila and Medan samples, respectively,

Table 3.2 Nucleotide sequences of seven selected primers, number of amplified bands and range of sizes of amplified fragments (bp) shown in the RAPD analysis in *P.monodon*.

Primer No.	Sequences	Size-rang	No. of amplified bands	No. of polymorphic bands	No. of monomorphic* bands
101	GCGCCTGGAG	1800-420	13	11(84.6%)	2(15.4%)
174	AACGGGCAGG	1500-450	10	7(70.0%)	3(30.0%)
268	AGGCCGCTTA	1300-400	9	8(88.9%)	1(11.1%)
428	GGCTGCGGTA	1800-200	9	9(100.0%)	0(0.0%)
456	GCGGAGGTCC	2200-260	15	10(66.7%)	5(33.3%)
457	CGACGCCCTG	2000-350	13	9(69.2%)	4(30.8%)
459	GCGTCGAGGG	1550-430	11	8(72.7%)	3(27.3%)
		Total	80	62(77.5%)	18(22.5%)

*These bands are present in at least approximately 95% of total investigated individuals.

Table 3.3 Total number of bands, percentage of polymorphic and monomorphic bands found in the 4 geographic samples of *P.monodon*.

	Satun-Trang			Trad			Angsila			Medan		
Primer No.	No. of bands	No.of polymor-phic bands	No.of monomor-phic bands	No. of bands	No. of polymor-phic bands	No. of monomor-phic bands	No. of bands	No. of polymor-phic bands	No. of monomor-phic bands	No. of bands	No. of polymor-phic bands	No. of monomor-phic bands
101	11	5	6	11	4	7	12	6	6	10	4	6
174	10	5	5	10	6	4	10	5	5	10	6	4
268	9	7	2	9	6	3	9	6	3	8	5	3
428	8	6	2	7	5	2	5	2	3	6	2	4
456	13	7	6	12	4	8	12	3	9	15	9	6
457	12	7	5	12	8	4	12	5	7	13	7	6
459	8	4	4	8	3	5	8	4	4	11	6	5
Total	71	41 (57.7%)	30 (42.3%)	69	36 (52.2%)	33 (47.8%)	68	31 (45.6%)	37 (54.4%)	73	39 (53.4%)	34 (46.6%)

whereas in the Satun-Trang and Trad samples, this band was rare. Bands at about 690 and 790 bp were absent in the Medan sample but showed approximately 64-100% in Thai *P.monodon*. By contrast, bands at about 1800 and 810 bp were found in 93.3 and 100% of the Medan sample, respectively but approximately 0-7% were found in Thai *P.monodon*. The bands at 1800 and 810 bp make it possible to distinguish between Thai and Indonesian *P. monodon* (Fig 3.4).

RAPD analysis by primer 174

By using primer 174, bands at about 1050 and 700 were found in all individuals of the 4 geographic samples (Table 3.4B). When comparing RAPD patterns among the samples of Thai *P.monodon*, they were quite similar. The RAPD patterns compared between Thai and Medan (Indonesia) *P.monodon* were observed to contain a few differences. A band at about 520 bp was found in 100% and 53.3% of Thai *P.monodon* and Medan respectively, and bands at about 1450 and 1350 bp were found more frequently (>33%) in Thai *P.monodon* than in Medan (6.7%) (Fig 3.5).

RAPD analysis by primer 268

When using primer 268 for the amplification, a common band at about 690 bp was shown in all individuals of the 4 samples (Table 3.4C). This primer showed some variable RAPD patterns between Thai and Indonesian *P.monodon*. A band with size about 890 bp was found in approximately 88.2%

of Satun-Trang, whereas this band was found in approximately 3.6 and 40% of Trad and Angsila respectively. In Medan, this band was absent. A band about 400 bp was found in 100% of Thai *P.monodon* but only 42.9% was found in Medan (Fig 3.6).

RAPD analysis by primer 428

For amplification by primer 428, some RAPD fragments were monomorphic in particular samples. Several distinctive scorable bands were detected between Thai and Indonesian *P.monodon.* Bands at about 590 and 200 bp were found in approximately 73-89 % and 100% of Thai *P.monodon* respectively but were not found in Medan (Table 3.4D). In contrast, a band at about 1800 bp was absent in all Thai *P.monodon* but was found in 100% of Medan. The results suggested that these bands were possible to distinguish between Thai *P.monodon*; Satun-Trang, Trad, and Angsila and Indonesian *P.monodon*; Medan. A band with size about 950 bp was present in 100% of Satun-Trang and Medan, but absent in samples from Trad and Angsila. This band can be used as a region-specific marker (Fig 3.7).

RAPD analysis by primer 456

Bands at size range about 2000, 480 and 290 were monomorphic in the 4 geographic samples (Table 3.4E). When compared between Thai and Indonesian *P.monodon*, slightly different patterns were found but no specific fragment for either population was observed. A band at 1,300 bp was a

monomorphic band in *P.monodon* from the Gulf of Thailand but found only in 30.8% of Indonesian *P.monodon*. Bands at 2,100 and 1,400 bp were absent in Thai *P.monodon* but were present in 53.8 and 76.9% of Medan, respectively. Bands at 1,250 and 700 bp were found in 100 and 76.9% of Medan but were found approximately in 0-18% of Thai *P.monodon* (Fig 3.8).

RAPD analysis by primer 457

Bands at size range about 1250, 550 and 420 were monomorphic in all 4 geographic samples (Table 3.4F). A band at 950 bp was found at 70.6, 78.6 and 57.1% in Satun-Trang, Trad and Medan, respectively but was found only in 40.0% of Angsila. A band at about 900 bp was found in 52.9% and 32.1% of Satun-Trang and Trad but was found in 86.7% of Angsila. Moreover, it was found only in 7.1% of Medan. Bands at about 730 and 600 bp were found approximately in 18-46% of Satun-Trang, Trad and Medan but were found in 100% of Angsila. A band at 500 bp was found in 88.2, 80 and 100% of Satun-Trang, Angsila and Medan, respectively, but was found only in 28.6% of Trad. Although the majority of bands appeared in all 4 geographic samples, there was a specific band for Medan. A band at 2,200 bp was found in 100% of Medan but absent in all Thai *P.monodon* samples (Fig 3.9).

RAPD analysis by primer 459

Bands at 480 and 430 bp were found in all individuals of the 4 different geographic samples. A band at 900 bp found in 96.4% of Trad was monomorphic in the remaining geographic samples (Table 3.4G). A band at 620 bp was rare in all 4 samples. A band specific for Thai *P.monodon* was not found. In contrast, a band at 730 bp was found in 100% of Medan but absent in Thai *P.monodon*. Moreover, it was shown that the frequency of several bands differed between Thai and Indonesian *P.monodon*, such as the bands at 1,550, 1,450 and 790 bp. Bands at 1,550 and 1,450 were absent in Thai *P.monodon* but were found approximately in 66-73% of Medan. A band at 790 bp was found approximately in 60-100% of Thai *P.monodon* but was found only in 20% of Medan (Fig 3.10).

Table 3.4 The number of each amplified band found in each of the 4 geographic sample of *P. monodon*

A. Primer 101

Size-range	Satun-Trang (17 individuals)	Trad (28 individuals)	Angsila (15 individuals)	Medan (15 individuals)
1800	0(0.0.0%)	0(0.0%)	0(0.0%)	14(93.3%)
1550	2(11.7%)	0(0.0%)	10(66.7%)	15(100.0%)
1350	2(11.7%)	7(25.0%)	2(13.3%)	15(100.0%)
1050	13(76.5%)	7(25.0%)	9(60.0%)	15(100.0%)
900	17(100.0%)	28(100.0%)	15(100.0%)	15(100.0%)
810	0(0.0%)	1(3.6%)	1(6.7%)	15(100.0%)
790	17(100.0%)	27(96.4%)	14(93.3%)	0(0.0%)
710	14(82.3%)	27(96.4%)	13(86.4)	14(93.3%)
690	11(64.7%)	20(71.4%)	15(100.0%)	0(0.0%)
590	17(100.0%)	27(96.4%)	15(100.0%)	12(80.0%)
520	17(100.0%)	28(100.0%)	15(100.0%)	15(100.0%)
470	17(100.0%)	28(100.0%)	15(100.0%)	5(33.3%)
420	17(100.0%)	28(100.0%)	15(100.0%)	0(0.0%)

B. Primer 174

Size-range	Satun-Trang (17 individuals)	Trad (28 individuals)	Angsila (15 individuals)	Medan (15 individuals)
1500	8(47.1%)	7(25.0%)	7(46.7%)	5(33.3%)
1450	16(94.1%)	17(60.7%)	5(33.3%)	1(6.7%)
1350	7(41.2%)	9(32.1%)	11(73.3%)	1(6.7%)
1200	17(100.0%)	21(75.0%)	15(100.0%)	15(100.0%)
1050	17(100.0%)	28(100.0%)	15(100.0%)	15(100.0%)
720	10(58.8%)	19(67.9%)	10(66.7%)	15(100.0%)
700	17(100.0%)	28(100.0%)	15(100.0%)	15(100.0%)
600	8(47.1%)	6(21.4%)	4(26.7%)	1(6.7%)
520	17(100.0%)	28(100.0%)	15(100.0%)	8(53.3%)
420	17(100.0%)	28(100.0%)	15(100.0%)	14(93.3%)

C. Primer 268

Size-range	Satun-Trang (17 individuals)	Trad (28 individuals)	Angsila (15 individuals)	Medan (14 individuals)
1250	3(17.6%)	13(46.4%)	4(26.3%)	14(100.0%)
1150	13(76.5%)	9(32.2%)	15(100.0%)	4(28.6%)
980	7(41.2%)	5(17.9%)	3(20.0%)	14(100.0%)
890	15(88.2%)	1(3.6%)	6(40.0%)	0(0.0%)
790	14(82.3%)	28(100.0%)	14(93.3%)	9(64.3%)
710	4(23.5%)	9(32.1%)	8(53.3%)	9(64.3%)
690	17(100.0%)	28(100.0%)	15(100.0%)	14(100.0%)
490	14(82.3%)	26(92.9%)	11(73.3%)	4(28.6%)
400	17(100.0%)	28(100.0%)	15(100.0%)	6(42.9%)

D. Primer 428

Size-range	Satun-Trang (17 individuals)	Trad (28 individuals)	Angsila (15 individuals)	Medan (15 individuals)
1800	0(0.0%)	0(0.0%)	0(0.0%)	15(100.0%)
1400	6(35.3%)	2(7.1%)	0(0.0%)	15(100.0%)
1250	15(88.2%)	27(96.4%)	15(100.0%)	15(100.0%)
1150	9(52.9%)	20(71.4%)	12(80.0%)	6(40.0%)
950	17(100.0%)	0(0.0%)	0(0.0%)	15(100.0%)
900	15(82.3%)	17(60.7%)	15(100.0%)	5(33.3%)
880	1(5.9%)	10(35.7%)	0(0.0%)	0(0.0%)
590	15(88.2%)	25(89.3%)	11(73.3%)	0(0.0%)
200	17(100.0%)	28(100.0%)	15(100.0%)	0(0.0%)

E.Primer456

Size-range	Satun-Trang (17 individuals)	Trad (28 individuals)	Angsila (15 individuals)	Medan (13 individuals)
2100	0(0.0%)	0(0.0%)	0(0.0%)	7(53.8%)
2000	17(100.0%)	28(100.0%)	15(100.0%)	13(100.0%)
1400	0(0.0%)	0(0.0%)	0(0.0%)	10(76.9%)
1300	16(94.1%)	28(100.0%)	15(100.0%)	4(30.8%)
1250	3(17.6%)	5(17.9%)	0(0.0%)	13(100.0%)
1200	14(82.3%)	25(89.3%)	15(100.0%)	13(100.0%)
1100	11(64.7%)	17(60.8%)	14(93.3%)	2(15.4%)
920	17(100.0%)	28(100.0%)	15(100.0%)	8(61.5%)
750	16(94.1%)	24(85.7%)	14(93.3%)	9(69.2%)
700	3(17.6%)	0(0.0%)	1(6.7%)	10(76.9%)
650	16(94.1%)	27(96.4%)	15(100.0%)	13(100.0%)
600	17(100.0%)	28(100.0%)	15(100.0%)	12(92.3%)
520	17(100.0%)	28(100.0%)	15(100.0%)	11(84.6%)
480	17(100.0%)	28(100.0%)	15(100.0%)	13(100.0%)
290	17(100.0%)	28(100.0%)	15(100.0%)	13(100.0%)

F. Primer 457

Size-range	Satun-Trang (17 individuals)	Trad (28 individuals)	Angsila (15 individuals)	Medan (14 individuals)
2200	0(0.0%)	0(0.0%)	0(0.0%)	14(100.0%)
1250	17(100.0%)	28(100.0%)	15(100.0%)	14(100.0%)
950	12(70.6%)	22(78.6%)	6(40.0%)	8(57.1%)
900	9(52.9%)	9(32.1%)	13(86.7%)	1(7.1%)
850	13(76.5%)	25(89.3%)	10(66.7%)	8(57.1%)
800	17(100.0%)	27(96.4%)	14(93.3%)	14(100.0%)
730	5(29.4%)	13(46.4%)	15(100.0%)	3(21.4%)
650	17(100.0%)	26(92.9%)	15(100.0%)	5(35.7%)
600	4(23.5%)	5(17.9%)	15(100.0%)	5(35.7%)
550	17(100.0%)	28(100.0%)	15(100.0%)	14(100.0%)
500	15(88.2%)	8(28.6%)	12(80.0%)	14(100.0%)
420	17(100.0%)	28(100.0%)	15(100.0%)	14(100.0%)
350	11(64.7%)	20(71.4%)	15(100.0%)	5(35.7%)

G. Primer 459

Size-range	Satun-Trang (17 individuals)	Trad (28 individuals)	Angsila (15 individuals)	Medan (15 individuals)
1550	0(0.0%)	0(0.0%)	0(0.0%)	11(73.3%)
1450	0(0.0%)	0(0.0%)	0(0.0%)	10(66.7%)
900	17(100.0%)	27(96.4%)	15(100.0%)	15(100.0%)
800	17(100.0%)	25(89.3%)	15(100.0%)	15(100.0%)
790	11(64.7%)	28(100.0%)	11(73.3%)	3(20.0%)
730	0(0.0%)	0(0.0%)	0(0.0%)	15(100.0%)
700	11(64.7%)	28(100.0%)	11(73.3%)	7(47.7%)
690	15(88.2%)	26(92.9%)	14(93.3%)	9(60.0%)
620	1(5.9%)	5(17.9%)	2(13.3%)	1(6.7%)
480	17(100.0%)	28(100.0%)	15(100.0%)	15(100.0%)
430	17(100.0%)	28(100.0%)	15(100.0%)	15(100.0%)

Figure 3.4 RAPD patterns using primer 101.

Lane M = 100 bp DNA ladder.

Lanes 1-4 = individuals collected from Medan.

Lanes 5-8 = individuals collected from Satun-Trang.

Lanes 9-12 = individuals collected from Trad.

Lanes 13-15 = individuals collected from Angsila.

— = indicate bands that were scored.

Figure 3.5 RAPD patterns using primer 174.

Lane M = 100 bp DNA ladder.

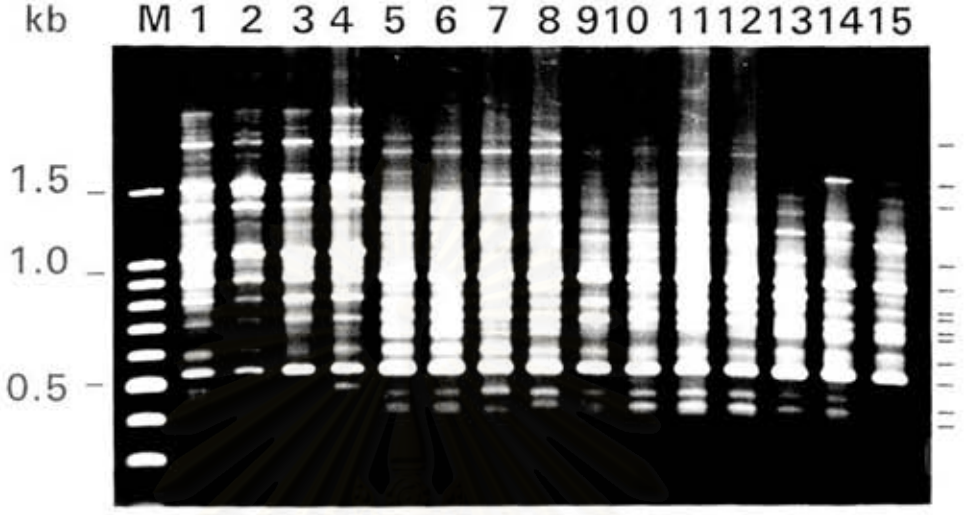
Lanes 1-4 = individuals collected from Medan.

Lanes 5-8 = individuals collected from Satun-Trang.

Lanes 9-12 = individuals collected from Trad.

Lanes 13-15 = individuals collected from Angsila.

— = indicate bands that were scored.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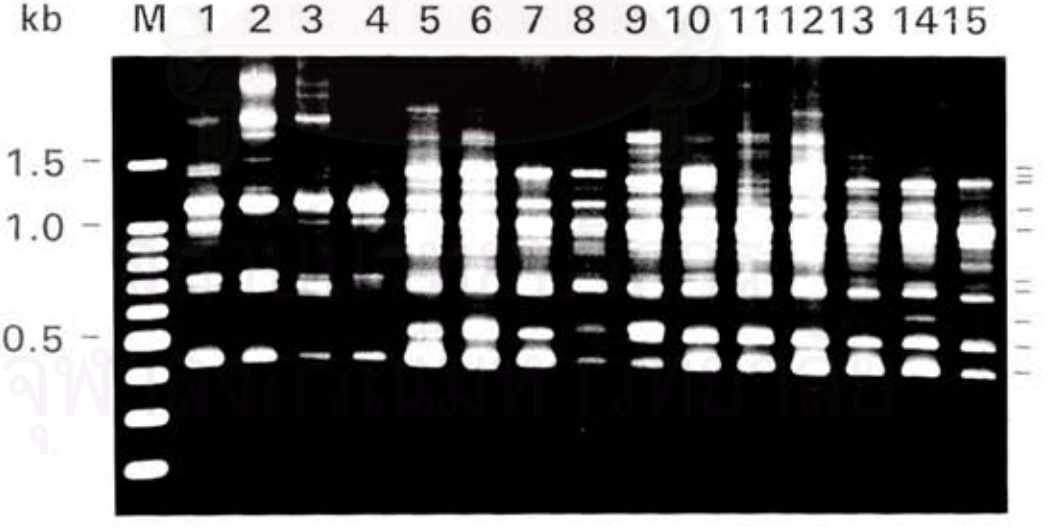
1.0
0.5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1.5
1.0
0.5

Figure 3.6 RAPD patterns using primer 268.

Lane M = 100 bp DNA ladder.

Lanes1-4 = individuals collected from Medan.

Lanes 5-8 = individuals collected from Satun-Trang.

Lanes 9-12 = individuals collected from Trad.

Lanes 13-15 = individuals collected from Angsila.

— = indicate bands that were scored.

Figure 3.7 RAPD patterns using primer 428.

Lane M = 100 bp DNA ladder.

Lanes 1-4 = individuals collected from Medan.

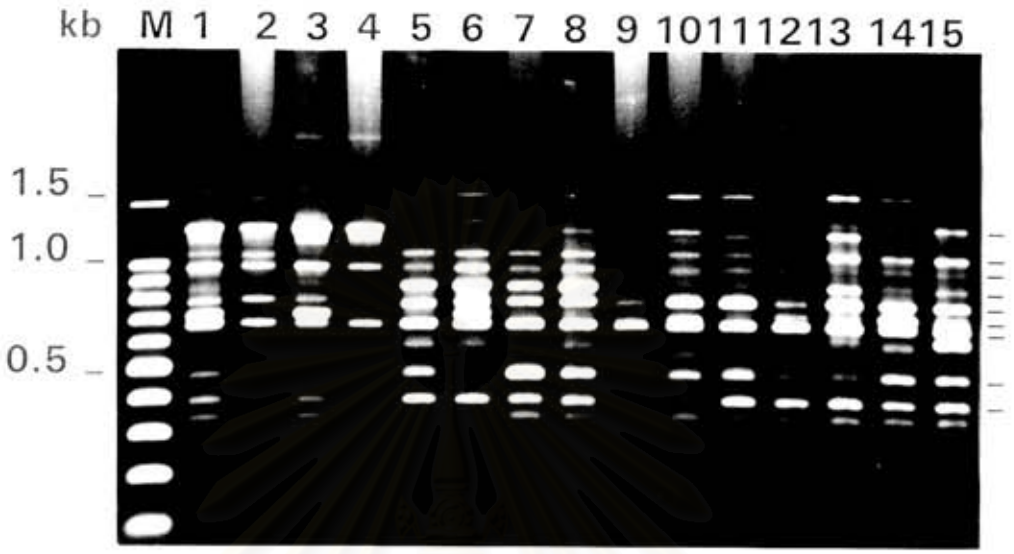
Lanes 5-8 = individuals collected from Satun-Trang.

Lanes 9-12 = individuals collected from Trad.

Lanes 13-15 = individuals collected from Angsila.

— = indicate bands that were scored.

← = indicate a band that was found only in the Andaman Sea.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1.5
1.0
0.5


kb M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
1.5
1.0
0.5

Figure 3.8 RAPD patterns using primer 456.

Lane M = 100 bp DNA ladder.

Lanes 1-5 = individuals collected from Medan.

Lanes 6-9 = individuals collected from Satun-Trang.

Lanes 10-12 = individuals collected from Trad.

Lanes 13-15 = individuals collected from Angsila.

— = indicate bands that were scored.

Figure 3.9. RAPD patterns using primer 457.

Lane M = 100 bp DNA ladder.

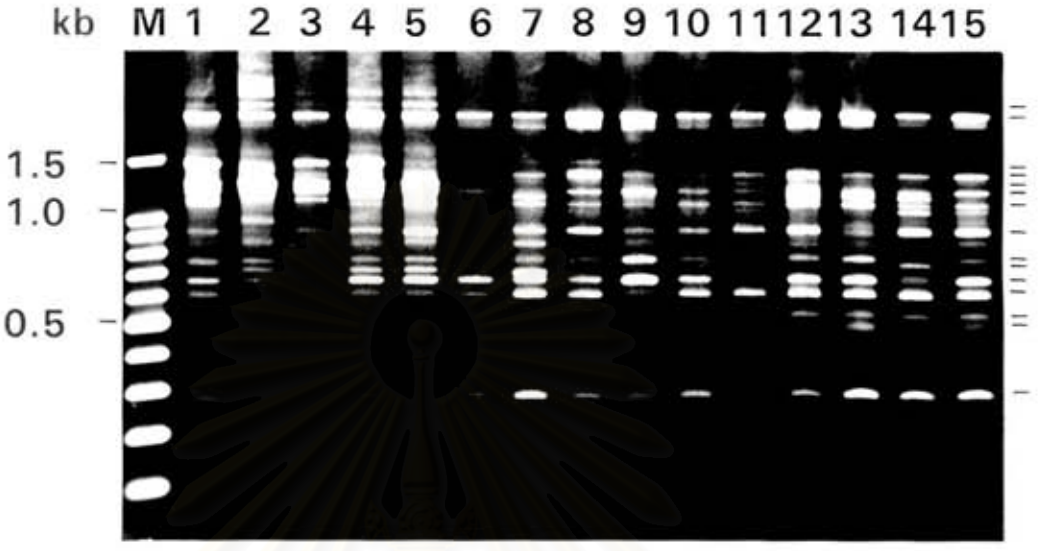
Lanes 1-4 = individuals collected from Medan.

Lanes 5-8 = individuals collected from Satun-trang.

Lanes 9-12 = individuals collected from Trad.

Lanes 13-15 = individuals collected from Angsila.

— = indicate bands that were scored.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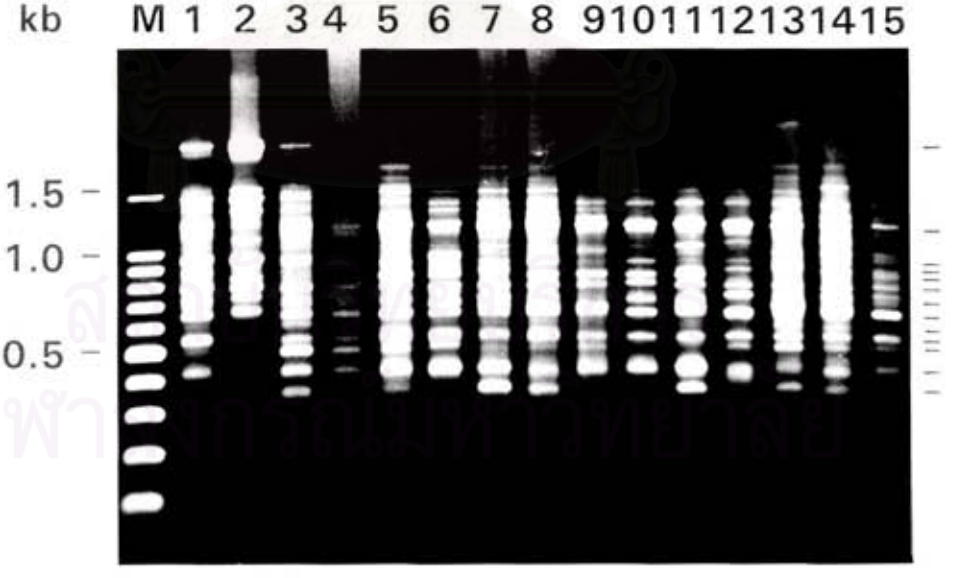
1.0
0.5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1.5
1.0
0.5

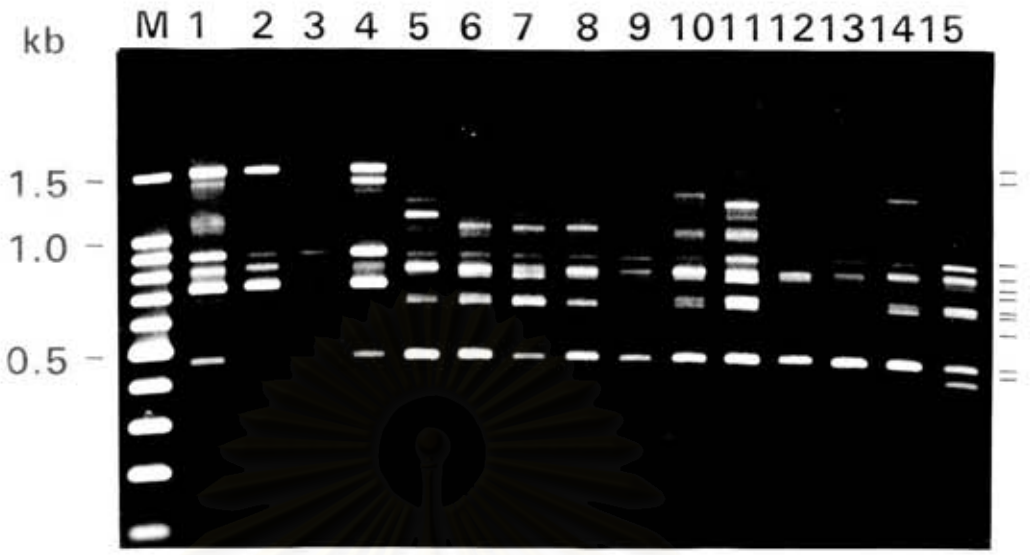


Figure 3.10 RAPD patterns using primer 459.

Lane M = 100 bp DNA ladder.

Lanes 1-4 = individuals collected from Medan.

Lanes 5-8 = individuals collected from Satun-Trang.

Lanes 9-12 = individuals collected from Trad.

Lanes 13-15= individuals collected from Angsila.

— = indicate bands that were scored.

Data analysis

The similarities in the RAPD patterns of all 75 individuals *P.monodon* were used to calculated the similarity index within and between populations as described by Lynch (1991). Within population, similarity (S) was calculated as the average of the index of similarity between individuals (S_{xy}) across all possible comparisons between individuals within a population. The index of similarity between individuals was calculated using the formula: $S_{xy}= 2n_{xy}/n_x+n_y$, where n_{xy} is the number of fragments shared by individuals x and y and n_x and n_y are the number of fragments scored for each individual. Between population, similarity (S_{ij}) is calculated using: $S_{ij} = 1+S'_{ij}-0.5\ (S_i+S_j)$, where S_i and S_j are the values of S for populations i and j, respectively and S'_{ij} is the average similarity between randomly paired individuals from populations i and j. Table 3.5 and 3.6 show the similarity within (S) and between (S_{ij}) populations for the 4 geographic samples based on RAPD patterns with 7 primers. The average similarity within populations (S) across all the primers ranged from 0.8626 to 0.9047. Samples from Angsila showed the highest similarity index (0.9047) where that of Satun-Trang, Trad and Medan was 0.8655, 0.8646 and 0.8626, respectively. The calculation suggested that Angsila was the least polymorphic. The average of similarity between populations (S_{ij}) across all the primers ranged from 0.7548 to 0.9578. The comparison of Satun-Trang with Angsila gave highest similarity index (S_{ij} = 0.9578), while comparison of Trad

and Medan showed the least similarity index (S_{ij} = 0.7548). The average of similarity between populations across all the primers of Thai *P.monodon*; the comparisons of Satun-Trang with Trad, Satun-Trang with Angsila and Trad with Angsila were 0.9478, 0.9578 and 0.9463, respectively. The results indicated that the 3 geographic samples of Thai *P.monodon* were similar. When compared between Thai and Indonesian *P.monodon*, the index of similarity between populations indicated significant difference between the two populations. The comparisons of Satun-Trang, Trad and Angsila with Medan showed similarity index of 0.7882, 0.7548 and 0.7563, respectively. The results indicated that Satun-Trang was genetically more similar to Medan than Trad and Angsila. S'_{ij} was converted to measure the genetic distance (D_{ij}) using the equation: $D_{ij} = -\ln[S'_{ij}/\sqrt{(S_iS_j)}]$. The results of the calculations are shown in Table 3.7. The average of genetic distance across all the primers yielded the values ranging from 0.0487 to 0.334. The comparisons of the Satun-Trang with Trad, Angsila and Medan; Trad with Angsila and Medan; and Angsila with Medan were 0.0622, 0.0487, 0.2812, 0.0624, 0.3340 and 0.3225, respectively. The comparison of Satun-Trang with Angsila showed the least genetic distance (D_{ij} = 0.0487), while comparison of Trad with Medan showed the highest (D_{ij} = 0.334). Distance values were used to construct dendrograms using the unweighted pair-group method of analysis (UPGMA). The resultant phenograms based on UPGMA are shown in Fig 3.11. The dendrogram across all the primers presented clusters that the 4 geographic samples of *P.monodon*

clearly divided into 2 distinct groups. Group 1 contained the 3 georaphic samples of Satun-Trang, Trad and Angsila. Group 2 contained the 1 geographic sample of Medan. For group 1, Satun-Trang and Angsila were more closely linked than Trad. To assess the significance of this grouping, a compatibility analysis was carried out by constructing separate dendrograms for each of the 7 primers. All primers clearly divided the isolates into 2 distinct groups which were Satun-Trang, Trad and Angsila as 1 group and the other was Medan. Samples of Thai *P.monodon*, Satun-Trang and Angsila were more closely linked than Trad for dendrograms of primers 101, 174, 268, 456 and 459. For dendrogram of primer 428, Trad and Angsila were more closely linked than Satun-Trang, while dendrogram of primers 457 Trad and Satun-Trang were more closely linked than Angsila.

Although the samples of Satun-Trang are separated from Trad and Angsila by geographic distribution, the genetic distances showed slight difference among the 3 geographic samples of Thai *P.monodon*. When compared RAPD patterns for each primer, the results showed that only primer 428 gave a specific marker for the samples of Satun-Trang (Fig 3.7). Thus, the samples of Thai *P.monodon* appeared to evolve from a single ancestral gene pool.

Table 3.5 Estimated similarity (S) for each primer within the 4 geographic samples of *P. monodon*

Primer	Satun-Trang	Trad	Angsila	Medan
101	0.9149	0.9099	0.9146	0.9605
174	0.8619	0.8216	0.8412	0.8651
268	0.8044	0.8239	0.8283	0.7640
428	0.8208	0.8086	0.9151	0.9420
456	0.9149	0.9284	0.9937	0.8016
457	0.8512	0.8279	0.9269	0.8648
459	0.8904	0.9316	0.9120	0.8403
Mean	0.8655	0.8646	0.9047	0.8626
SD	0.0437	0.0557	0.0556	0.0705

Table 3.6 Estimated similarlity (S_{ij}) for each primer between the 4 geographic samples of *P. monodon*

Primer	S-T/T*	S-T/A*	S-T/M*	T/A*	T/M*	A/M*
101	0.9614	0.9704	0.6518	0.9303	0.6448	0.7011
174	0.9442	0.9813	0.9144	0.9552	0.8762	0.8902
268	0.9512	0.9743	0.6886	0.8787	0.7186	0.7000
428	0.8801	0.8621	0.6344	0.9863	0.5174	0.5151
456	0.9820	0.9868	0.8712	0.9717	0.8696	0.8369
457	0.9641	0.9318	0.9133	0.9219	0.8831	0.8117
459	0.9516	0.9978	0.8435	0.9799	0.7739	0.8389
Mean	0.9478	0.9578	0.7882	0.9463	0.7548	0.7563
SD	0.0323	0.047	0.125	0.0385	0.1382	0.1283

*S-T = Satun-Trang, T = Trad, A = Angsila and M = Medan

Table 3.7 Estimated of genetic distance for each primer between the 4 geographic samples of *P. monodon*

A. All primers

	S-T*	T*	A*	M*
S-T	-			
T	0.0622	-		
A	0.0487	0.0624	-	
M	0.2812	0.3340	0.3225	-

B. Primer 101

	S-T	T	A	M
S-T	-			
T	0.0432	-		
A	0.0330	0.0795	-	
M	0.4638	0.4774	0.3837	-

C . Primer 174

	S-T	T	A	M
S-T	-			
T	0.0684	-		
A	0.0222	0.0553	-	
M	0.1044	0.1585	0.1378	-

D. Primer 268

	S-T	T	A	M
S-T	-			
T	0.0618	-		
A	0.032	0.1588	-	
M	0.5056	0.4370	0.4722	-

E. Primer 428

	S-T	T	A	M
S-T	-			
T	0.1592	-		
A	0.1716	0.0142	-	
M	0.5334	0.7987	0.7386	-

F. Primer 456

	S-T	T	A	M
S-T	-			
T	0.0197	-		
A	0.0131	0.0294	-	
M	0.1605	0.1607	0.1948	-

G. Primer 457

	S-T	T	A	M
S-T	-			
T	0.0437	-		
A	0.0790	0.0916	-	
M	0.1066	0.1484	0.2354	-

H. Primer 459

	S-T	T	A	M
S-T	-			
T	0.0544	-		
A	0.0023	0.0220	-	
M	0.1991	0.2934	0.2023	-

*S-T = Satun-Trang, T = Trad, A = Angsila and M = Medan

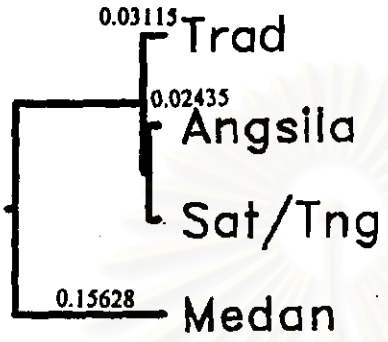
Figure 3.11 Phenograms showing the relationships among the 4 geographic samples of *P.monodon*, generated according to UPGMA method of cluster analysis based on distance matrix in Table 3.7.

A. For all primers.

B. For primer 101.

C. For primer174.

D. For primer 268.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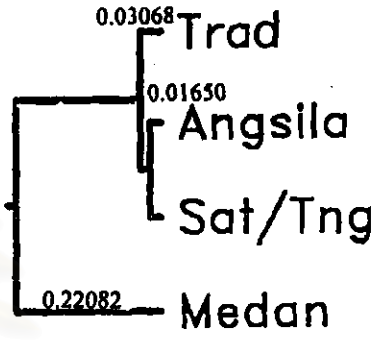
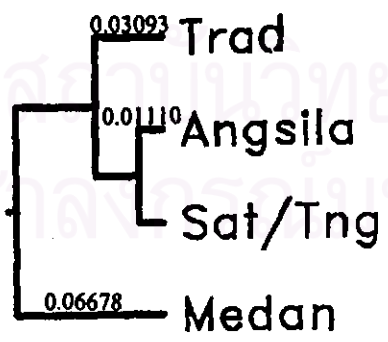
Trad
0.02435
Angsila
Sat/Tng
0.15628
Medan

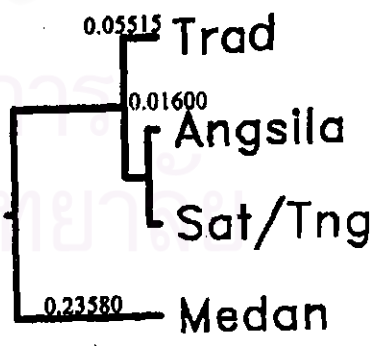
A


0.03068
Trad
0.01650
Angsila
Sat/Tng
0.22082
Medan

B


0.03093
Trad
0.01110
Angsila
Sat/Tng
0.06678
Medan

C


0.05515
Trad
0.01600
Angsila
Sat/Tng
0.23580
Medan

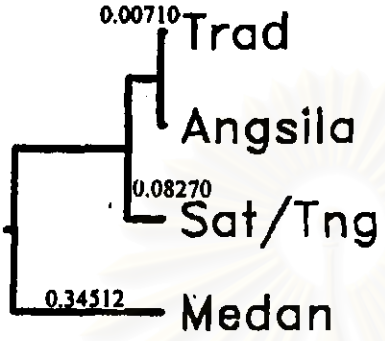
D

E . For primer 428

F . For primer 456

G . For primer 457

H . For primer 459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Angsila
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Sat/Tng
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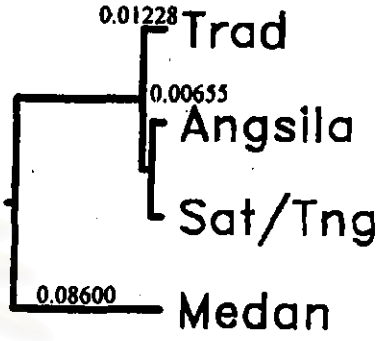
Medan

E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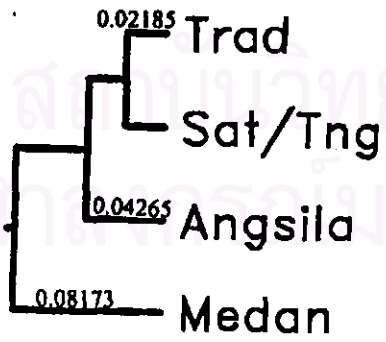
0.00655
Angsila
Sat/Tng
0.08600
Medan

F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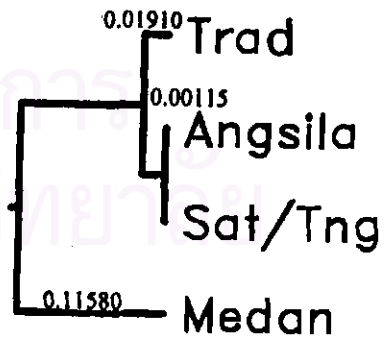
Trad
Sat/Tng
0.04265
Angsila
0.08173
Medan

G


0.01910
Trad
0.00115
Angsila
Sat/Tng
0.11580
Medan

H

Geographic heterogeneity in genotype frequency distributions

A RAPD pattern is referred to as a genotype which is generated from randomly amplified polymorphic DNA. In this study using 7 RAPD primers, 214 genotypes were observed from the 4 geographic samples; 29 individuals from Satun-Trang, 28 individuals from Trad, 15 individuals from Angsila and 15 individuals from Medan (for details of each genotype see Appendix B). One hundred and sixty of these were population-specific genotypes and 10 were region-specific genotypes for the sample from the Gulf of Thailand (Table 3.8). Ninety-seven of these genotypes were only represented by single individual. Primers 101, 174, 268, 428, 456, 457 and 459 yielded 24, 34, 35, 30, 22, 45 and 24 genotypes, respectively. Population-specific genotypes of primers 101, 174, 268, 428, 456, 457 and 459 were 18, 20, 27, 27, 15, 35 and 18, respectively. Only primers 174, 268, 428 and 457 gave region-specific genotypes which were 4, 2, 2 and 2, respectively. Genotypes from the 4 geographic samples of *P.monodon* which were generated by each primer, are shown in Table 3.8.

A chi-square (χ^2) analysis, a Monte Carlo simulation was used to analyze heterogeneity of the frequencies of 213 genotypes among the 4 geographic samples of *P.monodon* (Table 3.9). For overall samples, significant differences were observed for every primers ($P<0.0001$). Therefore, it could be concluded that the heterogeneity among samples existed. Highly significant differences were also observed between Thai and Indonesian *P.monodon*.

Heterogeneity for all Thai samples was not significantly different when primer 174 and 456 were employed (P = 0.1582 and P = 0.5600, respectively). When comparing between Thai *P.monodon* from the Gulf of Thailand and the Andaman Sea, there was no significant difference when using primers 174 and 456 (P = 0.0599 and P = 0.6663, respectively). Significant differences were observed for primers 101, 268, 428, 457 and 459 (P = 0.0049, <0.0001, <0.0001, 0.0014 and 0.0156, respectively). Therefore, the chi-square analysis showed the existent of region heterogeneity among Thai *P.monodon*.

Table 3.8 Geographic heterogeneity in genotype frequency distributions generated from randomly amplified polymorphic DNA patterns of *P. monodon*

A. primer 101

Population	genotype																								
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
Satun-Trang	3	0	4	3	1	4	5	0	0	2	0	2	1	0	1	1	1	0	0	0	0	0	0	1
Trad	13	0	2	5	0	0	0	3	0	1	3	0	0	0	0	0	0	1	0	0	0	0	0	0
Angsila	0	0	3	0	6	0	0	0	0	0	0	0	1	2	0	0	0	0	1	1	1	0	0	0
Medan	0	10	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0

B. primer 174

Population	genotype																																
	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	AG	AH
Satun-Trang	6	5	0	0	0	1	0	1	3	0	0	1	2	1	1	0	0	1	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0
Trad	3	3	0	0	3	1	0	2	0	1	1	1	1	1	1	1	1	1	2	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0
Angsila	0	1	0	0	1	1	4	0	0	2	2	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Medan	0	0	5	5	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1

C. primer 268

Population	genotype																																		
	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	AG	AH	AI
Satun-Trang	7	0	5	4	2	0	0	0	0	0	0	2	2	0	0	0	0	1	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
Trad	0	7	0	0	1	0	1	4	3	3	3	0	0	2	0	0	0	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Angsila	1	0	1	2	2	0	3	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0
Medan	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1

D. primer 428

Population	genotype																														
	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-Trang	0	0	1	6	0	0	5	4	0	2	2	2	0	0	0	1	1	1	1	1	0	0	0	0	1	1	0	0	0	0
Trad	7	0	4	0	5	0	0	0	3	0	0	0	2	1	0	0	0	0	0	0	1	1	1	1	0	0	1	1	0	0
Angsila	9	0	2	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Medan	0	9	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

E. primer 456

Population	genotype																						
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V
Satun-Trang	13	5	3	2	0	1	0	1	1	0	1	1	0	0	0	0	0	0	0	0	1	0
Trad	13	3	4	2	0	1	0	1	1	2	0	0	1	0	0	0	0	0	0	0	0	0
Angsila	14	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Medan	0	0	0	0	4	0	2	0	0	0	0	0	0	1	1	1	1	1	1	1	0	0

F. primer 457

Population	genotype																																														
	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	AG	AH	AI	AJ	AK	AL	AM	AN	AO	AP	AQ	AR	AS
Satun-Trang	4	0	3	0	0	0	0	2	0	0	2	2	2	2	2	0	0	0	0	0	1	1	1	1	1	0	0	1	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Trad	1	0	1	3	3	0	0	1	2	2	0	0	0	0	0	2	2	0	0	0	1	0	1	1	1	1	0	0	0	0	0	1	1	1	0	1	1	1	0	0	0	0	0	0	0	0
Angsila	0	7	0	0	0	3	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
Medan	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	2	2	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1

G. primer 459

Population	genotype																								
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
Satun-Trang	11	1	3	3	3	3	0	2	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0
Trad	17	6	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0
Angsila	8	1	2	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Medan	0	0	0	0	0	0	3	0	0	2	2	2	2	0	0	0	0	0	0	0	1	1	1	1

Table 3.9 Analysis of geographic heterogeneity in genotype frequency distributions generated from randomly amplified polymorphic DNA of *P.monodon* using a Monte Carlo simulation

	Primer						
	101	174	268	428	456	457	459
Satun-Trang v Gulf of Thailand	P=0.0049	P=0.0599	P<0.0001	P<0.0001	P=0.6663	P=0.0014	P=0.0156
Andaman v Medan	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001
Gulf of Thailand v Medan	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001
All Thai samples	P<0.0001	P=0.1582	P<0.0001	P<0.0001	P=0 5600	P<0.0001	P=0.0006
Over all samples	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001