CHAPTER III

RESULTS

3.1 DNA extraction

Genomic DNA was extracted from either the foot tissue or haemolymp of each abalone using a proteinase K-phenol-chloroform extraction method. The quality of extracted genomic DNA was electrophoretically determined in a 0.8% agarose (w/v) gel while the concentration of DNA was estimated spectrophotometrically and electrophoretically. As can be seen from Figure 3.1, the migration distance of the extracted DNA was as the same as a 23.1 kb band of a λ-*Hin*d III marker indicating that high molecular weight DNA was consistently obtained. The intensity of the extracted DNA were compare with series of undigested λ DNA (10, 25, 50, 75, 100, 250 ng) and DNA concentrations were determined by measuring the optical density at 260 nm (1 OD_{260} unit was equivalent to 50 μg DNA/ml). The ratio of OD_{260}/OD_{280} was 1.8-2.0 indicating a possible contamination of RNA in the extracted DNA samples. Some DNA samples contained RNA contamination as visualized by the smear at the bottom of gel. Approximately 25-50 μg DNA were usually obtained from each specimen.

3.2 Primer screening

DNA samples of *H. asinina*, *H. ovina* and *H. varia* were tested for the amplification success against one hundred and thirty primers (Table 3.1). Thirty-four RAPD primers yielded successful amplification and were further tested against a larger number of individuals of three abalone species. In this study, two primers (UBC101 and

OPB11) were used to study population genetics of three abalone species whereas intraspecific genetic diversity was examined for *H. asinina* by those two primers together with additional three primers (UBC195, UBC197 and UBC271).

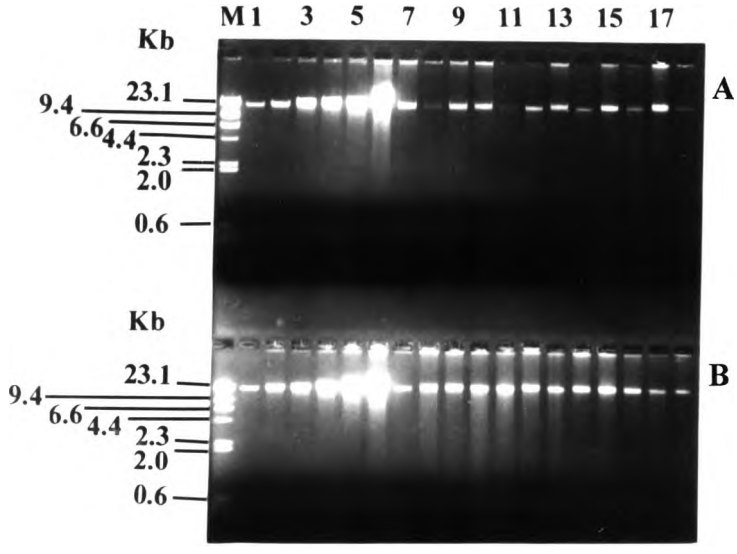

Figure 3.1 A 0.8% ethidium bromide stained-agarose gel showing the quality of total DNA extracted from the foot tissue of abalone.

lane M = λ-*Hin*d III

lanes 1-6 = undigested lamda DNA 10, 25, 50, 75, 100, 250 ng

lanes 7-18 (A and B) = Total DNA extracted from individuals of abalone (*H. asinina*)

Table 3.1 The amplification success of RAPD primers and their sequences initially screened by this study

Primer	Sequence	Amplification Strength
UBC101	GCG CCT GGA G	+++
UBC111	AGT AGA CGG G	-
UBC114	TGA CCG AGA C	-
UBC115	TTC CGC GGG C	-
UBC117	TTA GCG GTC T	-
UBC118	CCC GTT TTG T	-
UBC119	ATT GGG CGA T	++
UBC120	GAA TTT CCC C	-
UBC121	ATA CAG GGA G	-
UBC122	GTA GAC GAG C	-
UBC128	GCA TAT TCC G	-
UBC132	AGG GAT CTC C	-
UBC135	AAG CTG CGA G	-
UBC138	GCT TCC CCT T	-
UBC139	CCC AAT CTT C	-
UBC140	GTC GCA TTT C	-
UBC142	ATC TGT TCG G	-
UBC143	TCG CAG AAC G	-
UBC144	AGA GGG TTC T	-
UBC146	ATG TGT TGC G	-
UBC148	TGT CCA CCA G	-
UBC153	GAG TCA CGA G	-
UBC158	TAG CCG TGG C	-
UBC159	GAG CCC GTA G	-
UBC160	CGA TTC AGA G	++
UBC161	CGT TAT CTC G	-
UBC163	CCC CCC AGA T	-

Table 3.1 (continued)

Primer	Sequence	Amplification Strength
UBC164	CCA AGA TGC T	-
UBC166	ACT CCT ACA G	-
UBC167	CCA ATT CAC G	-
UBC168	CTA GAT GTG C	++
UBC169	ACG ACG TAG G	-
UBC170	ATC TCT CCT G	-
UBC171	TGA CCC CTC C	-
UBC174	AAC GGG CAG C	++
UBC175	TGG TGC TGA T	-
UBC187	AAC GGG GGA G	-
UBC189	TGC TAG CCT C	-
UBC191	CGA TGG CTT T	-
UBC193	TGC TGG CTT T	+++
UBC195	GAT CTC AGC G	+++
UBC197	TCC CCG TTC C	+++
UBC200	TCG CGA TAT G	++
UBC210	GCA CCG AGA G	++
UBC217	ACA GGT AGA C	-
UBC220	GTC GAT GTC G	++
UBC222	AAG CCT CCC C	-
UBC228	GCT GGG CCG A	-
UBC233	CTA TGC GCG C	-
UBC235	CTG AGG CAA A	-
UBC237	CGA CCA GAG C	-
UBC255	TTC CTC CGG A	-
UBC259	GGT ACG TAC T	-
UBC262	CGC CCC CAG T	-
UBC263	TTA GAG ACG G	-

Table 3.1 (continued)

Primer	Sequence	Amplification Strength
UBC264	TCC ACC GAG C	++
UBC267	CCA TCT TGT G	++
UBC268	AGG CCG CTT A	-
UBC270	TGC GCG CGG G	-
UBC271	GCC ATC AAG A	+++
UBC272	AGC GGG CCA A	++
UBC273	AAT GTC GCC A	-
UBC277	AGG AAG GTG C	-
UBC281	GAG AGT GGA A	-
UBC282	GGG AAA GCA G	-
UBC286	CGG AGC CGG C	-
UBC293	TCG TGT TGC T	-
UBC295	CGC GTT CCT G	-
UBC297	GCG CATT TAG A	-
UBC298	CCG TAC GGA C	-
UBC299	TGT CAG CGG T	-
UBC428	GGC TGC GGT A	-
UBC456	GCG GAG GTC C	++
UBC457	CGA CGC CCT G	++
UBC459	GCG TCG AGG G	++
OPA1	CAG GCC CTT C	++
OPA2	TGC CGA GCT G	++
OPA3	AGT CAG CCA C	-
OPA4	AAT CGG GCT G	-
OPA5	AGG GGT CCT G	-
OPA6	GGT CCC TGA C	-
OPA7	GAA ACG GGT G	-
OPA8	GTG ACG TAG G	-

Table 3.1 (continued)

Primer	Sequence	Amplification Strength
OPA9	GGG TAA CGC C	-
OPA10	GTG ATC GCA G	++
OPA11	CAA TCG CCG T	-
OPA12	TCG GCG ATA G	-
OPA13	CAG CAC CCA C	-
OPA14	TCT GTG CTG G	-
OPA15	TTC CGA ACC C	++
OPA16	AGC CAG CGA A	-
OPA17	GAC CGC TTG T	-
OPA18	AGG TGA CCG T	-
OPA19	CAA ACG TCG G	++
OPA20	GTT GCG ATC C	++
OPB1	GTT TCG CTC C	-
OPB2	TGA TCC CTG G	-
OPB3	CAT CCC CCT G	-
OPB4	GGA CTG GAG T	-
OPB5	TGC GCC CTT C	-
OPB6	TGC TCT GCC C	-
OPB7	GGT GAC GCA G	-
OPB8	GTC CAC ACG G	-
OPB9	TGG GGG ACT C	-
OPB10	CTG CTG GGA C	-
OPB11	GTA GAC CCG T	+++
OPB12	CCT TGA CGG A	-
OPB13	TTC CCC CGC T	-
OPB14	TCC GCT CTG G	-
OPB15	GGA GGG TGT T	-
OPB16	TTT GCC CGG A	++

Table 3.1 (continued)

Primer	Sequence	Amplification Strength
OPB17	AGG GAA CGA G	++
OPB18	CCA CAG CAG T	-
OPB19	ACC CCC GAA G	-
OPB20	GGA CCC TTA C	-
OPM9		-
OPZ9	-	-
Microsatellites	$(CA)_8$	-
Microsatellites	$(CT)_8$	-
Microsatellites	$(CAC)_5$	+
Microsatellites	$(GTG)_5$	++
Microsatellites	$(GATA)_4$	+
Microsatellites	$(GACA)_4$	+
HRU33	CCC AAG GTC CCC AAG GTC AGG GAG GCG AAG GCT	-
HRU18	ACC CGG CGC TTA TTA GAG	-
PER I	GAC NGG NAC NGG	-
INS	ACA GGG GTG TGG GG	+++
M13	GAG GGT GGN GGN TCT	++
YNZ22	CTC TGG GTG TCG TGC	-
YN73	CCC GTG GGG CCG CCG	+++

+, ++ and +++ indicate that the strength of amplification success.
- indicates that the amplification was not successful.

The RAPD-PCR amplification was performed in a DNA thermal Cycle (Hybaid Model Omnigene-E for primer UBC271 and Sprint PCR for the other primer). The annealing temperature for UBC271 was carried out at 40°C. This high amplification temperature RAPD (HAT-RAPD) provided more reproducible results than the standard low amplification temperature RAPD (LAT-RAPD).

3.3 Genetic diversity of tropical abalone using RAPD

3.3.1 Between species diversity

High genetic diversity levels between *H. asinina, H. ovina and H. varia* was observed based on RAPD analysis. Two decanucleotide primers, UBC101 and OPB11, were used for genetic analysis of all three Thai abalone species. Seventy-two RAPD fragments ranged from 320 bp to 2300 bp in length were generated. The size of RAPD bands ranged from 320 bp to 1850 bp for primer UBC101 and 390 bp to 2300 bp for primer OPB11. The number of reproducible bands across all investigated samples were 37 and 35 bands for respective primers. One hundred percent of polymorphic bands (bands found in less than 95% of overall investigated individuals within a particular species) were found for both primers (Table 3.2A).

The percentage of polymorphic bands of *H. asinina*, *H. ovina* and *H. varia* using UBC101 and OPB11 primers were 84.91%, 94.74% and 91.23% respectively. *H. ovina* exhibited the greatest levels of polymorphic bands followed by *H. varia* and *H. asinin.* (Table 3.3). The total number of bands, percentage of polymorphic and monomorphic bands within each abalone species are shown in Table 3.3.

RAPD amplification patterns generated by primer UBC101 and primer OPB11 are shown by Figure 3.2 - 3.6. RAPD patterns of all specimens examined in this study are shown in the appendices B1 and B2.

3.3.2 Genetic diversity of H. asinina

High genetic diversity levels of *H. asinina* was also observed based on RAPD analysis using 5 decanucleotide primers. The number of reproducible bands across all investigated samples were 32, 21, 25, 23 and 12 bands for primers UBC101, OPB11, UBC195, UBC197 and UBC271, respectively. One hundred and thirteen RAPD fragments ranged from 250 bp to 2300 bp were generated. All primer provided high polymorphic levels in this abalone species. The percent polymorphic bands of UBC101, OPB11, UBC195, UBC197 and UBC271 were 81.25, 90.48, 84.00, 86.96 and 83.33, respectively. Size-range of RAPD bands in each primer are shown in Table 3.2B. RAPD amplification patterns generated by those primers are shown in Figures 3.2-3.10. RAPD patterns of all tested specimens in this study are shown in an appendix B.

Table 3.2 Sequence of RAPD primers, size-range, number of amplified bands, and the percentage of polymorphic and monomorphic bands resulted from RAPD analysis of three species of abalone; *H. asinina*, *H. ovina* and *H. varia* (A) and *H. asinina* (B)

A.

Primer	Sequence	Size-range (bp)	No. of RAPD bands	Polymorphic bands (%)	Monomorphic bands (%)
UBC101	GCG CCT GGA G	320-1850	37	100	0
OPB11	GTA GAC CCG T	390-2300	35	100	0
Total		320-2300	72	100	0

B.

Primer	Sequence	Size-range (bp)	No. of RAPD bands	Polymorphic bands (%)	Monomorphic bands (%)
UBC101	GCG CCT GGA G	320-1800	32	81.25	18.75
OPB11	GTA GAC CCG T	390-2300	21	90.48	9.52
UBC195	GAT CTC AGC G	520-1480	25	84.00	16.00
UBC197	TCC CCG TTC C	500-1480	23	86.96	13.04
UBC271	GCC ATC AAG A	250-1020	12	83.33	16.67
Total		250-2300	113	85.20	14.80

Table 3.3 Total number of bands, percentage of polymorphic and monomorphic bands within each abalone revealed by RAPD analysis

Primer No.	*H. asinina*			*H. ovina*			*H. varia*		
	No. of bands	No. of polymorphic bands	No. of monomorphic bands	No. of bands	No. of polymorphic bands	No. of monomorphic bands	No. of bands	No. of polymorphic bands	No. of monomorphic bands
UBC101	32	26	6	26	23	3	24	20	4
OPB11	21	19	2	31	31	0	33	32	1
UBC195	25	21	4						
UBC197	23	20	3						
UBC271	12	10	2						
Total	113	96 (84.96%) 45 (84.91%)*	17 (15.32%)	57	54 (94.74%)	3 (5.26%)	57	52 (91.23%)	5 (8.77%)

* When consider only UBC101 and OPB11

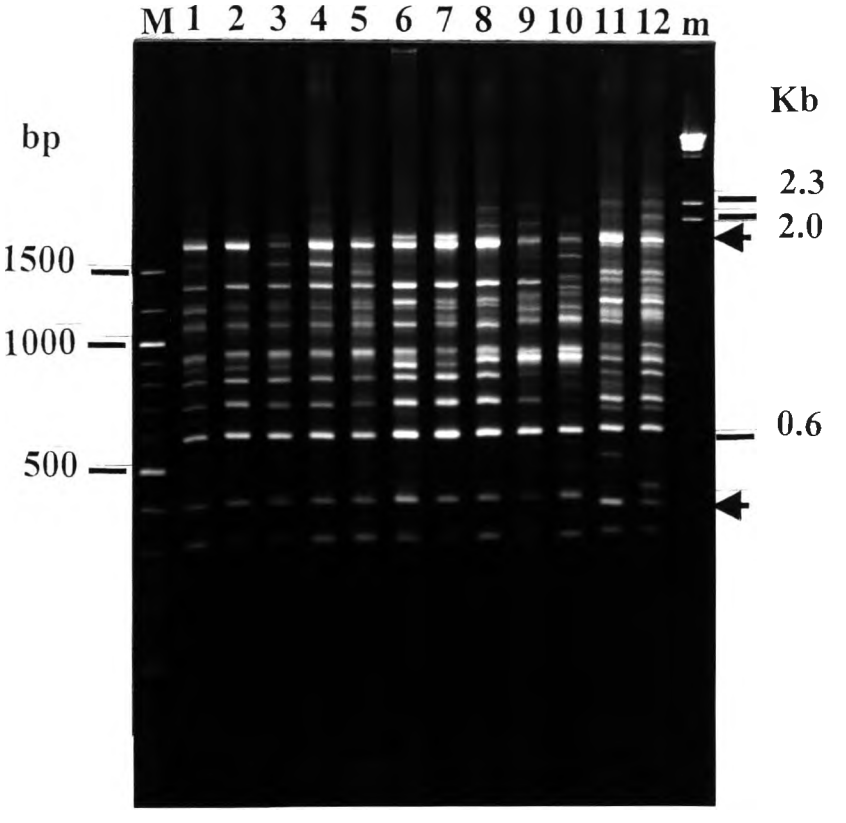

Figure 3.2 RAPD patterns resulted from analysis of *H. asinina* from Samet Island, Rayong (lanes 1-4), Cambodia (lanes 5-8), Talibong Island, Trang (lanes 9-10), and the Philippines (lanes 11-12) with the primer UBC101. Lanes M and m are a 100 bp ladder and λ-*Hin*d III, respectively.

Arrows indicated species-specific markers for *H. asinina* (1700 bp) and a marker specifically observed in *H. asinina* from the Philippines (380 bp).

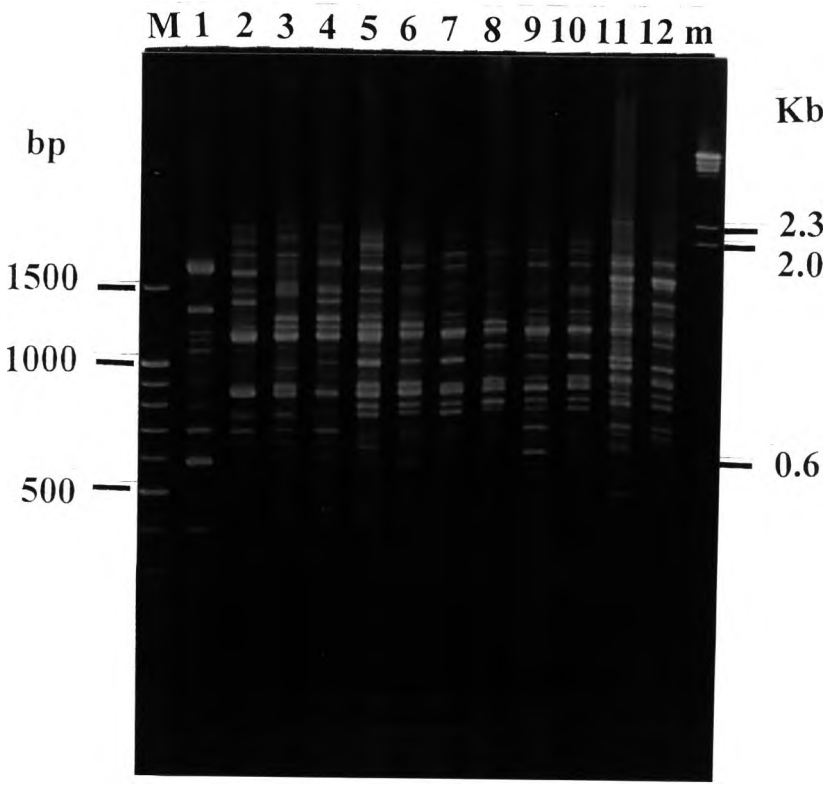

Figure 3.3 RAPD patterns resulted from analysis of *H. asinina* from Samet Island, Rayong (lanes 1), *H. ovina* from Khang Kao Island, Chon Buri (lanes 2-3), Samet Island, Rayong(lanes 4-5), Chuak Island, Trang (lanes 6-7), Similan Island, Phang-nga (lane 8), *H. varia* from Aeo Island, Phuket, (lanes 9-10), Similan Island, Phang-nga (lane 11-12) with the primer UBC101. Lanes M and m are a 100 bp ladder and λ-*Hin*d III, respectively.

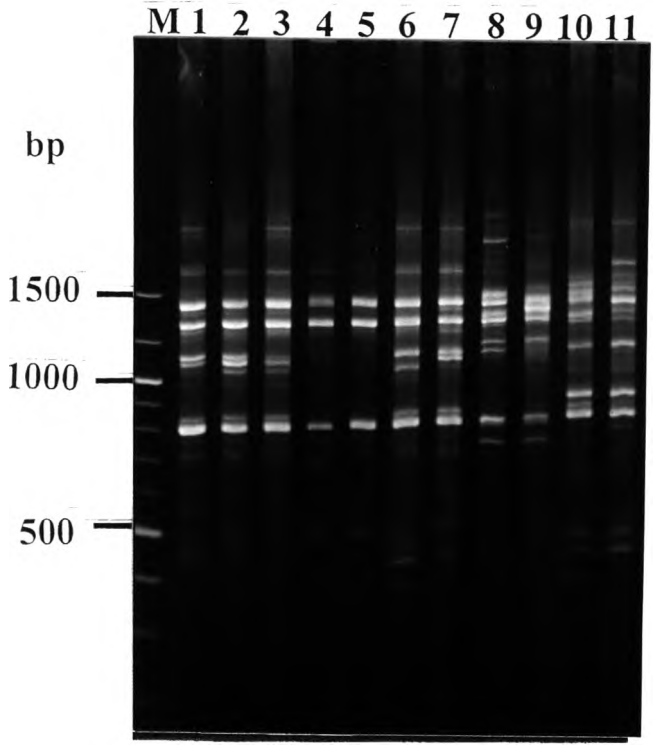

Figure 3.4 RAPD patterns resulted from analysis of *H. asinina* from Samet Island, Rayong (lanes 1-3), Cambodia (lanes 4-7), Talibong Island, Trang (lanes 8-9), and the Philippines (lanes 10-11) with the primer OPB11. Lane M is a 100 bp ladder.

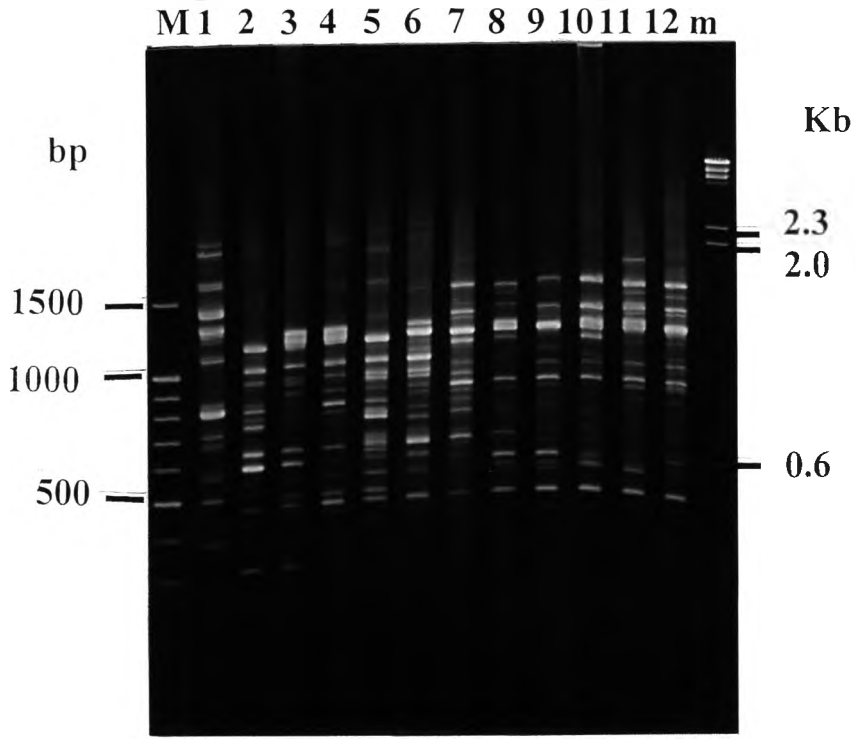

Figure 3.5 RAPD patterns resulted from analysis of *H. asinina* from Samet Island, Rayong (lanes 1), *H. ovina* from Khang Kao Island, Chon Buri (lanes 2-4), Samet Island, Rayong(lanes 5-6), Similan Island, Phang-nga (lane 7-9), Chuak Island, Trang (lanes 10-12) with the primer OPB11. Lanes M and m are a 100 bp ladder and λ-*Hin*d III, respectively.

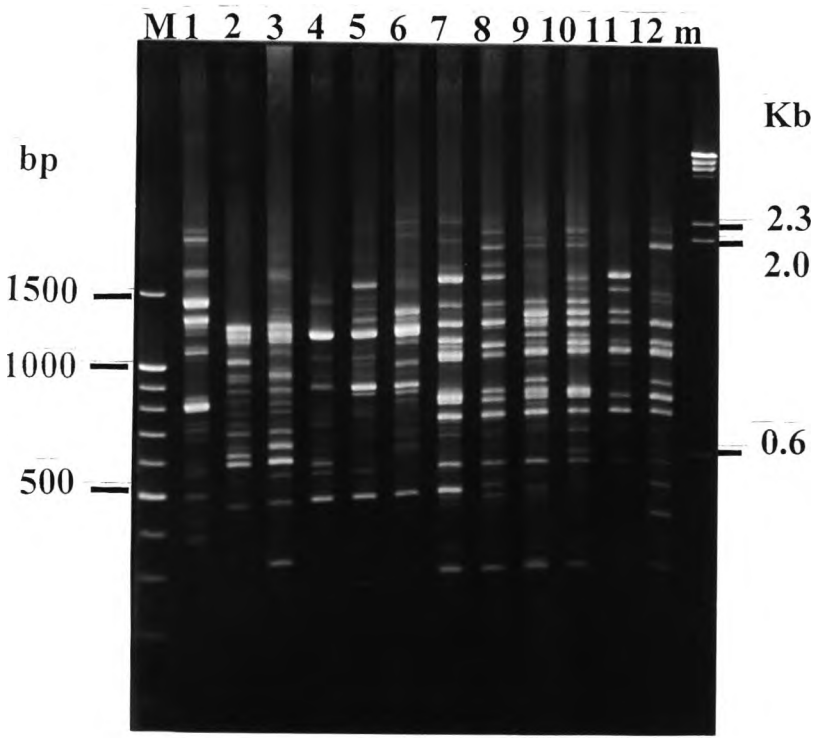

Figure 3.6 RAPD patterns resulted from analysis of *H. asinina* from Samet Island, Rayong (lanes 1), *H. ovina* from Samet Island, Rayong(lanes 2-3), Similan Island, Phang-nga (lane 4), Chuak Island, Trang (lanes 5-6), *H. varia* from Aeo Island, Phuket, (lanes 7-10), Similan Island, Phang-nga (lane 11-12) with the primer OPB11. Lanes M and m are a 100 bp ladder and λ-*Hin*d III, respectively.

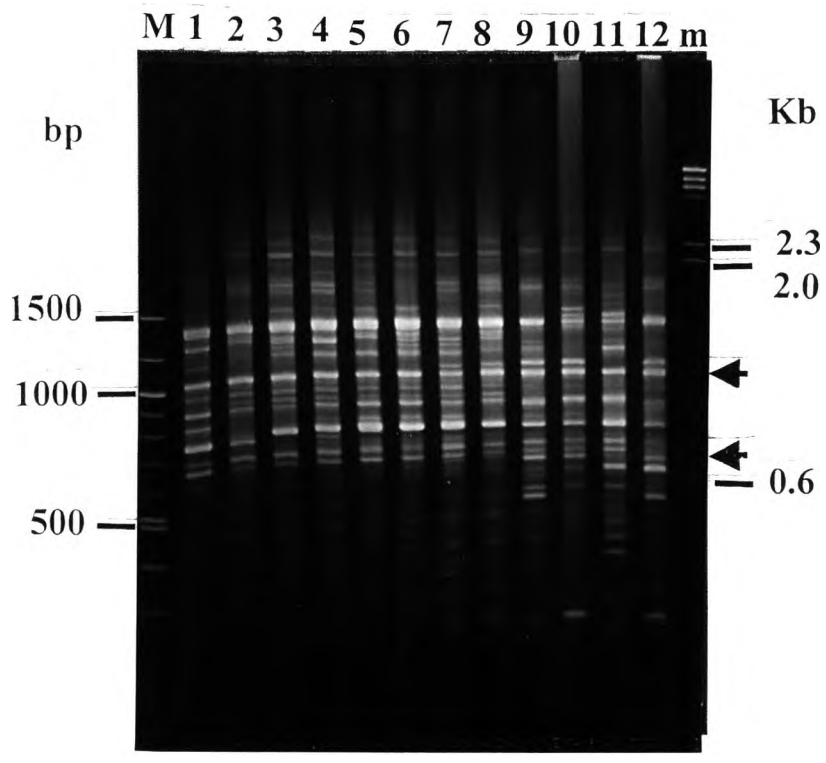

Figure 3.7 RAPD patterns resulted from analysis of *H. asinina* from Samet Island, Rayong (lanes 1-4), Cambodia (lanes 5-8), and The Philippines (lanes 9-12) with the primer UBC195. Lanes M and m are a 100 bp ladder and λ-*Hin*d III, respectively. Arrows indicate species-specific markers for *H. asinina* (1030 bp and 650 bp) found in this study.

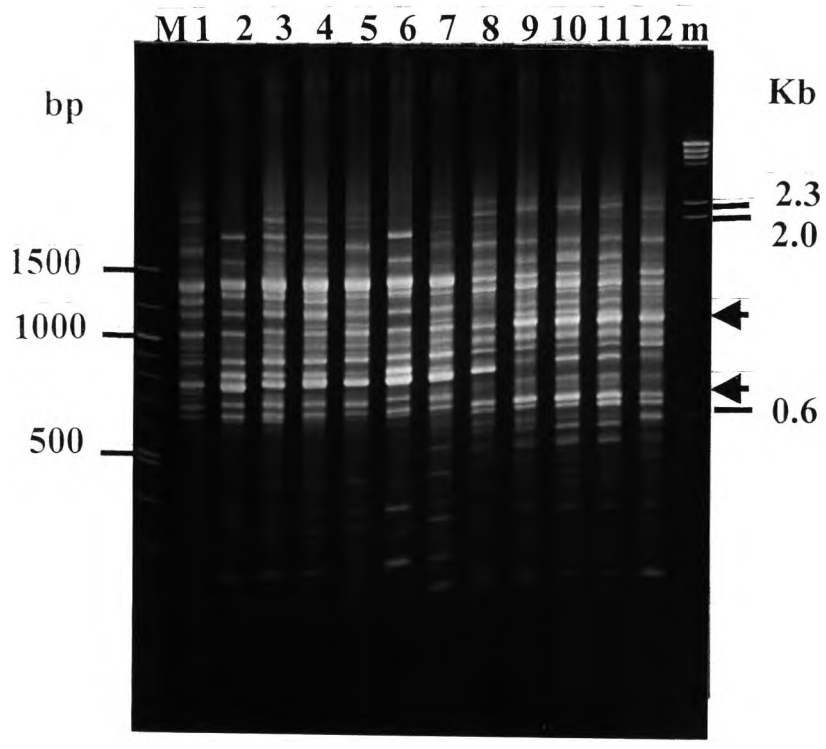

Figure 3.8 RAPD patterns resulted from analysis of *H. asinina* from Samet Island, Rayong (lanes 1), Cambodia (lanes 2-7), and Talibong Island, Trang (lanes 8-12) with the primer UBC195. Lanes M and m are a 100 bp ladder and λ-*Hin*d III, respectively. Arrows indicate species-specific markers for *H. asinina* (1030 bp and 650 bp) found in this study

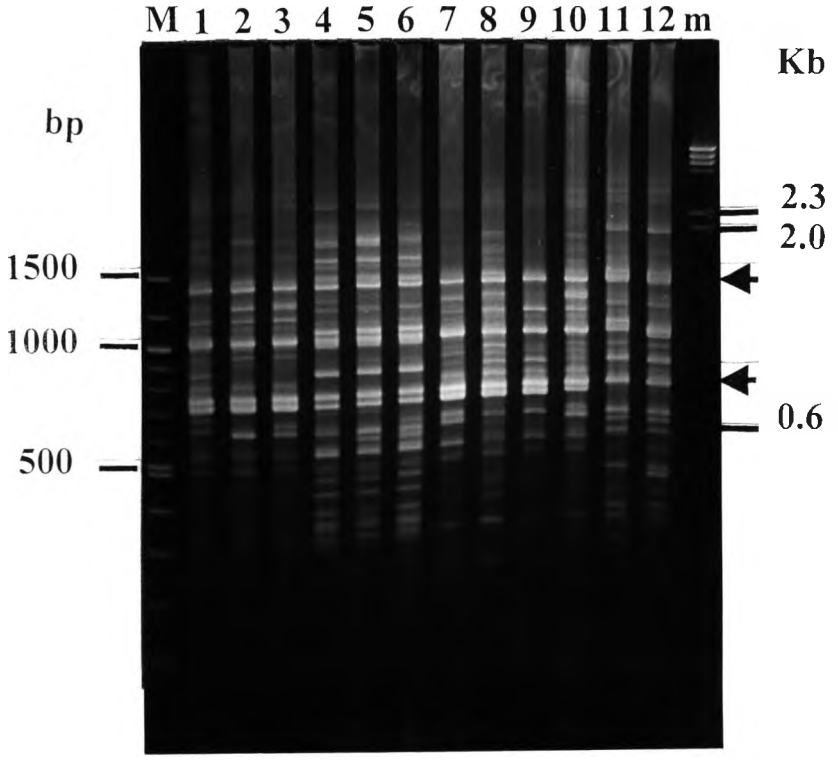

Figure 3.9 RAPD patterns resulted from analysis of *H. asinina* from Samet Island, Rayong (lanes 1-3), Talibong Island, Trang (lanes 4-6), Cambodia (lanes 7-10), and The Philippines (lanes 11-12) with the primer UBC197. Lanes M and m are a 100 bp ladder and λ-*Hin*d III, respectively.

Arrows indicate species-specific fragments (1450 bp and 750 bp) for *H. asinina* found in this study

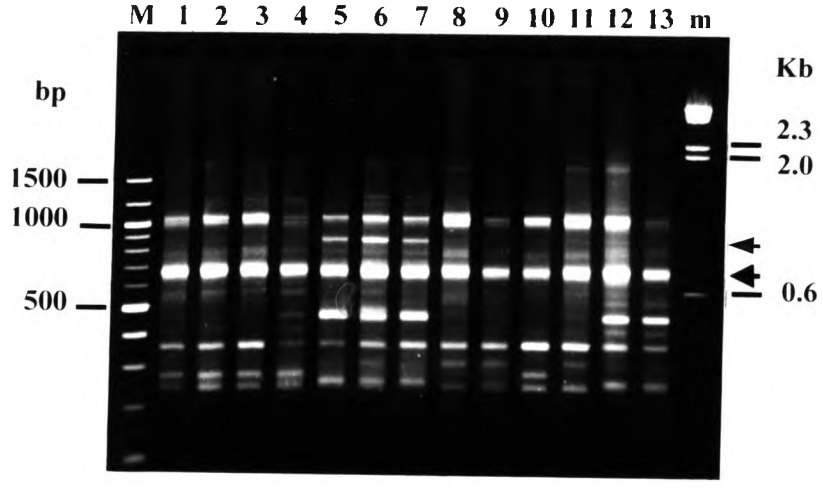

Figure 3.10 RAPD patterns resulted from analysis of *H. asinina* from Samet Island, Rayong (lanes 1-4), Talibong Island, Trang (lanes 5-7), Cambodia (lanes 8-11), and The Philippines (lanes 12-13) with the primer UBC271. Lanes M and m are a 100 bp ladder and λ- *Hin*d III, respectively.

Arrows indicated a species-specific marker (680 bp) for *H. asinina* and a population-specific marker for *H. asinina* originating from Talibong Islans (880 bp), respectively.

3.4 Genetic relationships of tropical abalone

3.4.1 Similarity index and Genetic distances

The similarity indices between and within species of 12 groups of samples (Table 2.1) analyzed by 5 primers (UBC101, OPB11, UBC195, UBC197 and UBC271) are illustrated by Table 3.4.

The average similarity index across overall samples resulted from primer UBC101, OPB11, UBC195, UBC197 and UBC271 were 0.7715, 0.6830, 0.8002, 0.8444 and 0.8396, respectively (Table 3.4). The mean similarity index within each geographic sample overall primers ranged from 0.5259 (HVPG) - 0.8496 (HALB). Basically, *H. asinina* exhibited the highest level of the similarity index within a species (0.8297) and ranged from 0.7927 (HASM) - 0.8496 (HALB) whereas *H. varia* showed the lowest similarity level (0.5681) within species. These results suggested that *H. asinina* has genetically closer related within species than that of *H. ovina* and *H. varia.* The average similarity index within samples of *H. asinina, H. ovina* and *H. varia* were between 0.7927-0.8496, 0.6010-0.7032 and 0.5259-0.6102, respectively.

Using primers OPB11 and UBC101, the similarity index between samples (S_{ij}) of each primer were calculated. Genetic distances (D_{ij}) were calculated from the similarity index between samples by the equation $D_{ij} = 1 - S_{ij}$. The average genetic distance between geographic samples within species across primers of *H. asinina, H. ovina* and *H. varia* ranged from 0.1578 - 0.4208, 0.3259 - 0.4827 and 0.4295, respectively (Table 3.5). Average genetic differences within 3 species were 0.2995, 0.4328 and 0.4295 for *H. asinina, H. ovina* and *H. varia,* respectively. The results from each primer are shown in an Appendix D. Notably, *H. ovina* showed the highest genetic diversity within species compared to *H. varia* and *H. asinina.*

Table 3.4 Estimated similarity indices (S) within geographic samples of abalone in Thailand using 5 selected RAPD primers. Detail information and abbreviations of sample sites are shown in Appendix A.

Sample	Primer					Average similarity within a sample across all primers
	UBC101	OPB11	UBC195	UBC197	UBC271	
HASH	0.8812	0.8112	0.8652	0.8666	0.8011	**0.8451±0.0362**
HASM	0.8571	0.7831	0.7066	0.8192	0.7974	**0.7927±0.0556**
HACH	0.8686	0.7973	0.8048	0.8941	0.8432	**0.8416±0.0412**
HACB	0.8640	0.8411	0.7749	0.8667	0.8317	**0.8357±0.0371**
HALB	0.8146	0.8133	0.8674	0.8385	0.9141	**0.8496±0.0423**
HAPH	0.8392	0.8143	0.7825	0.7811	0.8503	**0.8135±0.0317**
HOSC	0.7027	0.6431	-	-	-	**0.6729±0.0421**
HOSM	0.7092	0.6971	-	-	-	**0.7032±0.0086**
HOPG	0.7371	0.4648	-	-	-	**0.6010±0.1925**
HOTR	0.7053	0.5382	-	-	-	**0.6218±0.1182**
HVPK	0.7313	0.4891	-	-	-	**0.6102±0.1713**
HVPG	0.5482	0.5036	-	-	-	**0.5259±0.0315**
Average similarity of each primer across all samples	**0.7715 ± 0.0997**	**0.6830 ± 0.1474**	**0.8002 ± 0.0608**	**0.8444 ± 0.0403**	**0.8396 ± 0.0424**	

Table 3.5 The average genetic distance (D_{ij}, below diagonal) and similarity indices (S_{ij}, above diagonal) between and within species of 3 species of tropical abalone

	HASH	HASM	HACH	HACB	HALB	HAPH	HOSC	HOSM	HOTR	HOPG	HVPK	HVPG
HASH	-	**0.8068**	**0.8346**	**0.8423**	**0.6304**	**0.6268**	0.2528	0.2359	0.2556	0.2703	0.2957	0.2436
HASM	**0.1933**	-	**0.7985**	**0.8235**	**0.6122**	**0.6282**	0.2682	0.2493	0.2626	0.2872	0.2917	0.2464
HACH	**0.1655**	**0.2015**	-	**0.8302**	**0.6172**	**0.6093**	0.2706	0.2535	0.2660	0.2865	0.2825	0.2253
HACB	**0.1578**	**0.1765**	**0.1698**	-	**0.6182**	**0.6508**	0.2726	0.2553	0.2620	0.2907	0.3081	0.2451
HALB	**0.3697**	**0.3878**	**0.3828**	**0.3818**	-	**0.5793**	0.2969	0.2830	0.3384	0.3491	0.3508	0.3011
HAPH	**0.3732**	**0.3718**	**0.3907**	**0.3492**	**0.4208**	-	0.3038	0.5873	0.3489	0.3737	0.3182	0.2945
HOSC	0.7473	0.7319	0.7295	0.7275	0.7032	0.6962	-	**0.6741**	**0.5173**	**0.5201**	0.4388	0.3981
HOSM	0.7641	0.7508	0.7466	0.7447	0.7170	0.4127	**0.3259**	-	**0.5518**	**0.5464**	0.4209	0.4101
HOTR	0.7445	0.7374	0.7340	0.7381	0.6616	0.6512	**0.4827**	**0.4483**	-	**0.5933**	0.3761	0.3684
HOPG	0.7298	0.7128	0.7135	0.7093	0.6509	0.6264	**0.4799**	**0.4536**	**0.4067**	-	0.3803	0.3558
HVPK	0.7043	0.7084	0.7175	0.6919	0.6493	0.6818	0.5612	0.5791	0.6240	0.6197	-	**0.5706**
HVPG	0.7565	0.7536	0.7747	0.7550	0.6990	0.7055	0.6020	0.5899	0.6316	0.6443	**0.4295**	-

The similarity index between populations of *H. asinina* were calculated by the same procedure but concerning the similarity index within geographic sample. The genetic distances (D_{aij}) between pairs of geographic samples were corrected for effects of similarity within samples. The average genetic distance between geographic samples within species across all primers of *H. asinina* ranged from 0.0156-0.2381 (Table 3.6). The average genetic distance of *H. asinina* in the Gulf of Thailand (HASH, HASM, HACH and HACB) was 0.0243 indicating that they are mostly resembling compared to the Andaman (HALB) and The Philippines (HAPH).

Table 3.6 The average genetic distance (D_{aij}, below diagonal) and similarity indices (S_{aij}, above diagonal) within species of *H. asinina*

	HASH	**HASM**	**HACH**	**HACB**	**HALB**	**HAPH**
HASH	-	0.9730	0.9737	0.9844	0.7619	0.7781
HASM	0.0270	-	0.9683	0.9781	0.7686	0.7871
HACH	0.0263	0.0317	-	0.9766	0.7904	0.7764
HACB	0.0156	0.0219	0.0234	-	0.7691	0.7933
HALB	0.2381	0.2314	0.2096	0.2309	-	0.8203
HAPH	0.2219	0.2129	0.2236	0.2067	0.1797	-

3.4.2 Phylogeography

A neighbor-joining tree constructed from the average genetic distance between paired geographic samples indicated phylogenetically clear separation between investigated abalone species (using two primers, Figure 3.11) and geographic samples (using five primers, Figure 3.12). The neighbor-joining trees from each RAPD primer are shown in the Appendix E and F, respectively.

Genetic relationships from six geographic samples of *H. asinina* illustrated by Figure 3.12, demonstrated clearly differences between the Andaman (HALB) and The Philippines (HAPH) from the Gulf of Thailand populations (HASH, HASM, HACH and HACB). Likewise, phylogeographic differentiation from different coastal areas were observed in *H. ovina*. The phylogenetic relationships clearly divided in *H. ovina* to 2 different groups, HOSC and HOSM from the Gulf of Thailand and HOPG and HOTR from the Andaman Sea. For *H. varia*, due to small sample size from Similan Island, Phang-nga (HVPG), such a conclusion could not be made because population size were too small to be concluded accurately.

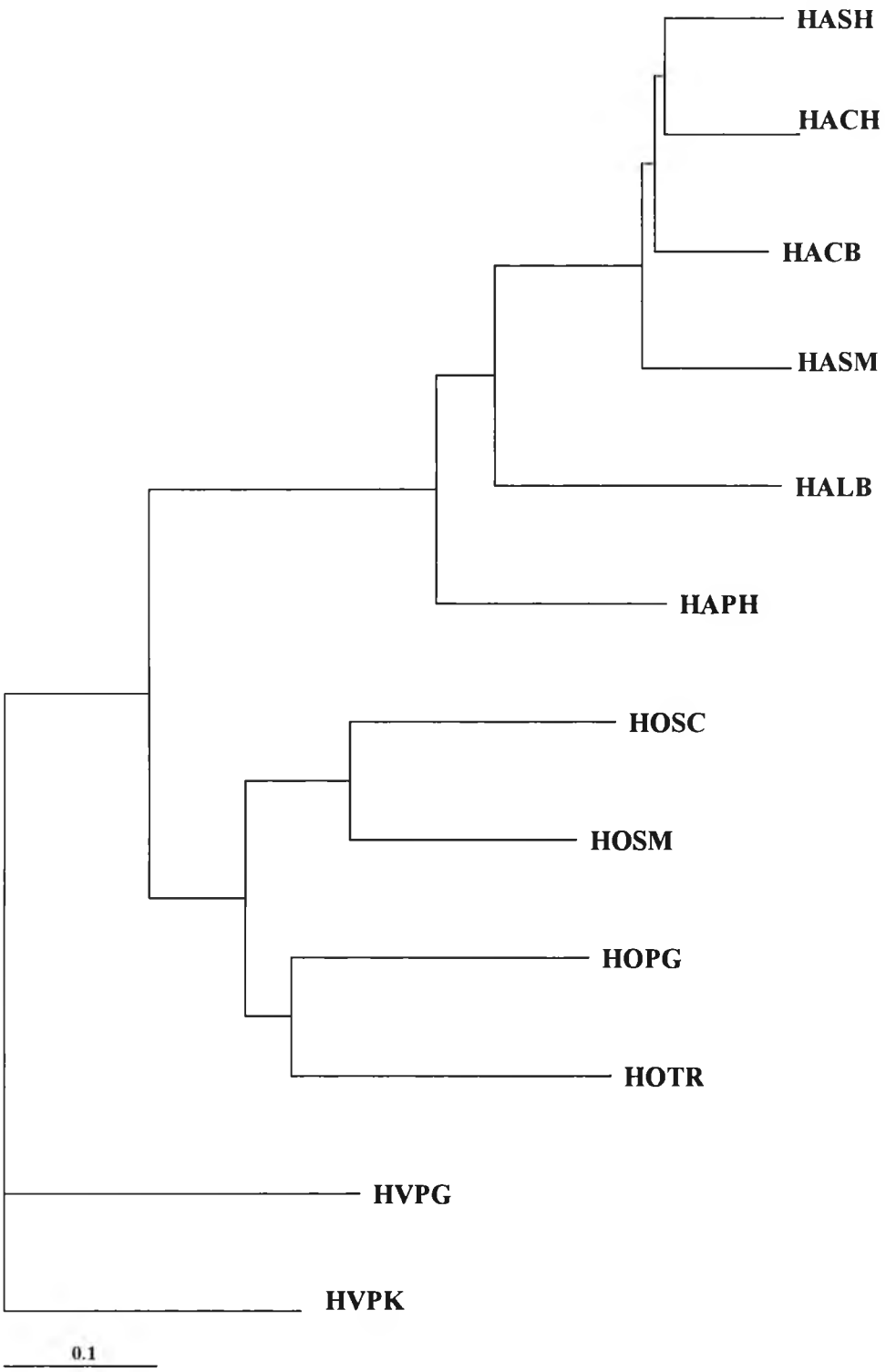

Figure 3.11 A neighbor-joining tree illustrating genetic relationships of Thai abalone in Thailand based on genetic distances resulted from RAPD analysis using two primers (UBC101 and OPB11). Detailed information and abbreviations of sample sites are shown in the Appendix A.

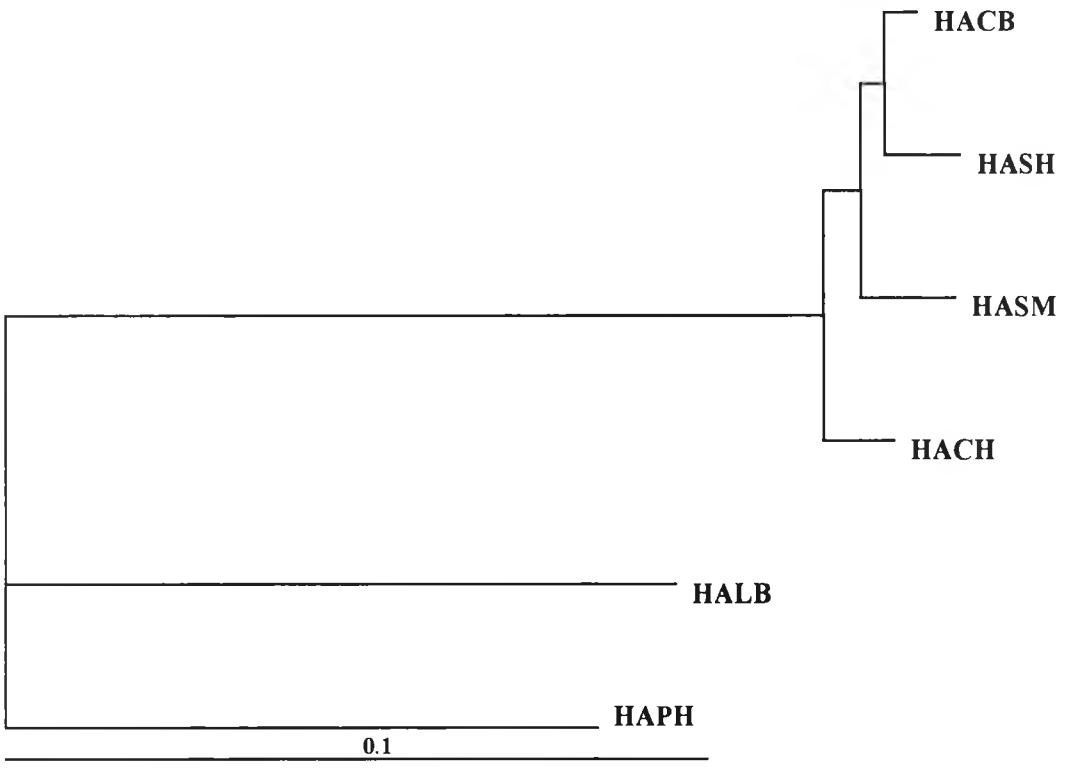

Figure 3.12 A neighbor-joining tree illustrating genetic relationships of *H. asinina* based on genetic distances resulted from RAPD analysis using five primers (UBC101, OPB11, UBC195, UBC197 and UBC271). Detailed information and abbreviations of sample sites are shown in the Appendix A.

3.5 Specific genetic markers of Tropical abalone found in Thailand

Four primers (UBC101, UBC195, UBC197 and UBC271) generated RAPD fragment exhibiting at least 95% of overall investigated specimens. The primer UBC101 yielded a species-specific marker in *H. asinina* (1700 bp) and the the Philippines samples (380 bp). The primer UBC195 showed species-specific nature in *H. asinina* (1030 and 650 bp) while the primer UBC197 provided RAPD markers in *H. asinina* (1450 and 750 bp). Additional species-specific RAPD markers in *H. asinina* (680 bp) and *H. asinina* from Talibong Island (880bp) were identified by the primer UBC271. No species-specific RAPD fragments in *H. ovina* and *H. varia* were found (Table 3.7).

Table 3.7 Specific RAPD markers of tropical abalone in Thailand revealed by RAPD analysis

Species markers	Primer	RAPD marker
Haliotis asinina	**UBC101**	**1700**
	UBC195	**1030, 650**
	UBC197	**1450, 750**
	UBC271	**680**
Population markers		
H. asinina **Talibong Island, Trang**	**UBC271**	**880**
H. asinina **The Philippines**	**UBC101**	**380**