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Morphological characteristics and genetic diversity of the *Anadara antiquata* distribution in Central Vietnam

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Abstract*---Anadara antiquata*** belongs to the Mollusca phylum, class Bivalvia, distributed in the coastal areas of Vietnam, with high nutritional and economic value. This study focused on the morphological characteristics and genetic diversity of *A. antiquata* in six regions of central Vietnam (Da Nang, Quang Nam, Phu Yen, Nha Trang, Binh Thuan and Thanh Hoa). The analysis of morphology of *A. antiquata* had the following results: shell width (SW: 41.45 mm), shell length (SL: 22.54 mm), right shell height (HRV: 29.27 mm), height left sheath (Coach: 28.49 mm), ligament length (LL: 25.74 mm), right trochanteric cavity depth (UHR: 7.90 mm), left trochanteric space depth (UHL: 8, 33 mm), right case symmetry (SRV: 8.58 mm) and left case symmetry (SLV: 8.41 mm). The mean shell volume (SV) and shell cavity volume (DSWT) were 4.45 ml and 11.11 ml, respectively. The average dry peel weight was 11.17 g. Genetic diversity showed that the 8 random primers used to analyze DNA polymorphisms of 41 *A. antiquata* had 87 amplified polymorphic DNA bands. Among the 6 study areas, the *A. antiquata* in four regions in central Vietnam (Da Nang, Quang Nam, Nha Trang and Binh Thuan) had genetic similarity coefficients between individuals from 0.76 to 1.00. The genetic

distance between populations of *A. antiquata* was quite low, varying from 0.0172 to 0.0229.

***Keywords*---**Genetic diversity, morphology, population, shellfish.

Introduction

Currently, *A. antiquata* is a favorite food and a species of high economic value in Vietnam and some countries around the world. Shellfish is a very healthy type of seafood. The nutritional components in scallops, such as protein, lipids, total sugars, etc. have the effect of enhancing flexibility, providing energy for the body... In addition, to making real food, products, meat and shells are also used as medicine by traditional medicine. Traditional medicine calls *A. antiquata* meat as humiliation, sweet, salty, warm, nontoxic, has the effect of nourishing blood in the central region, nourishing the taste, laxative of the five organs, quenching thirst, aperitif, treating chronic dysentery, curing anemia, blood damage, poor digestion, and stomach pain. Their shells are mainly composed of calcium carbonate (over 97%), this is a medicinal herb with a sweet and salty taste, that is slightly cold, and has the effect of reducing accumulation, chemical phlegm, curing hematoma, bruises and paralysis, bloody stools, dysentery, orange teeth, etc. However, today, along with urbanization, the expansion of residential areas and over-exploitation, the resources of shellfish in the regions have decreased. Our country's lagoons and coastal areas have been seriously reduced.

Vietnam is located in the tropical monsoon region, with diverse topography, soil types and landscapes, creating favorable conditions for the development of these species. In our country, shellfish are mainly concentrated in some coastal areas, from Quang Ninh to Binh Thuan [18]. However, in our country, there have been no publications on the morphological and genetic characteristics of *A. antiquata*, especially for our central coastal region. For these reasons, a study on the morphological characteristics and genetic diversity of *A. antiquata* is necessary now for conservation work, as well as for teaching and research work at the university.

Study Materials and Methods

Study materials

The number, symbols and locations of *A. antiquata* samples in some study areas in central Vietnam are shown in Fig. 1 and Tables 1 and 2.

Table 1. Samples of *A. antiquata* shellfish used in the study

Sampling location	Number of samples	Sample symbols
Da Nang	30	ĐN1, ĐN2, ĐN3, ĐN4, ĐN5, ĐN6, ĐN7, ĐN8, ĐN9, ĐN10, ĐN11, ĐN12, ĐN13, ĐN14, ĐN15, ĐN16, ĐN17, ĐN18, ĐN19, ĐN20, ĐN21, ĐN22, ĐN23, ĐN24, ĐN25, ĐN26,

		ĐN27, ĐN28, ĐN29, ĐN30.
Quang Nam	13	QN1, QN2, QN3, QN4, QN5, QN6, QN7, QN8, QN9, QN10, QN11, QN12, QN13.
Phu Yen	30	PY1, PY2, PY3, PY4, PY5, PY6, PY7, PY8, PY9, PY10, PY11, PY12, PY13, PY14, PY15, PY16, PY17, PY18, PY19, PY20, PY21, PY22, PY23, PY24, PY25, PY26, PY27, PY28, PY29, PY30.
Nha Trang	30	NT1, NT2, NT3, NT4, NT5, NT6, NT7, NT8, NT9, NT10, NT11, NT12, NT13, NT14, NT15, NT16, NT17, NT18, NT19, NT20, NT21, NT22, NT23, NT24, NT25, NT26, NT27, NT28, NT29, NT30.
Binh Thuan	30	BT1, BT2, BT3, BT4, BT5, BT6, BT7, BT8, BT9, BT10, BT11, BT12, BT13, BT14, BT15, BT16, BT17, BT18, BT19, BT20, BT21, BT22, BT23, BT24, BT25, BT26, BT27, BT28, BT29, BT30.
Thanh Hoa	30	TH1, TH2, TH3, TH4, TH5, TH6, TH7, TH8, TH9, TH10, TH11, TH12, TH13, TH14, TH15, TH16, TH17, TH18, TH19, TH20, TH21, TH22, TH23, TH24, TH25, TH26, TH27, TH28, TH29, TH30.

Among the above samples, 41 samples from 4 study regions were qualified for genetic diversity analysis (Table 2).

Table 2. DNA samples used in genetic diversity studies

Population	Number of samples (n)	Sample symbols
Nha Trang	11	NT1, NT5, NT8, NT9, NT11, NT13, NT15, NT19, NT20, NT22, NT27.
Da Nang	12	ĐN3, ĐN10, ĐN11, ĐN13, ĐN14, ĐN15, ĐN18, ĐN19, ĐN22, ĐN24, ĐN28, ĐN29.
Quang Nam	9	QN1, QN2, QN3, QN4, QN5, QN6, QN7, QN8, QN9, QN10, QN11.
Binh Thuan	9	BT2, BT3, BT7, BT9, BT14, BT15, BT18, BT20, BT21

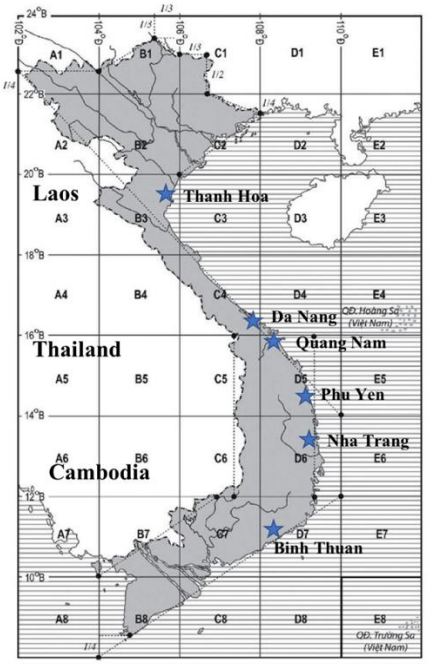

Figure 1. Sample collection sites *of A. antiquata. Note: the star is the sampling location*

Research Methods

Methods for studying morphological characteristics: The collected samples were washed with sandy soil and organic debris, and the morphological characteristics were analyzed. Nine morphological features were examined, including: width (SW), length (SL), right shell height (HRV), left shell height (HLV), ligament length (LL), depth right saliental space (UHR), left saliental space depth (UHL), right cortical symmetry (SRV), and left cortical symmetry (SLV) (Fig. 2) [11].

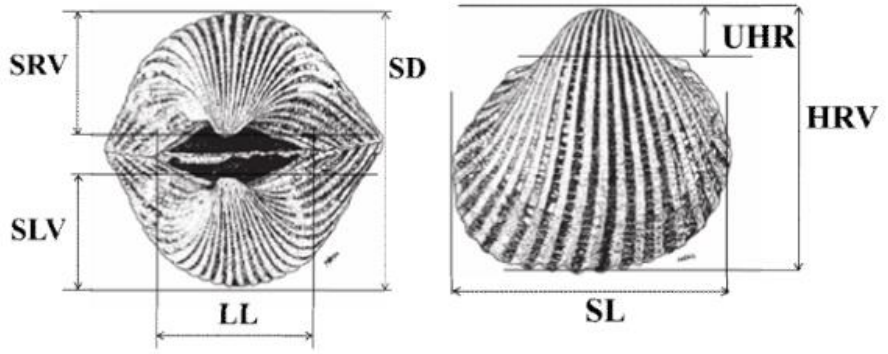

Figure 2. Indicators for measuring the morphological characteristics of scallops. (Source: Qonita Y et al 2015)

The dry weight of the shell (DSWT) was determined. The volume of the shell (SV) was calculated as the amount of water displaced. The shell cavity volume (SCV)

was calculated by measuring the volume of sand that filled the shell cavity. The shell shape is described by the height/length ratio (SH/SL). The shell density (SD) is the ratio of dry shell weight to shell volume (DSWT/SV) (Sambrook and Ruesel, 2001).

Genetic diversity analysis: Genomic DNA from muscle tissue was extracted using the modified phenol–chloroform protocol (Sambrook *et al.*, 1989). The quantity and quality of the extracted DNA were determined by measuring its absorbance value at 260 nm and estimating the ratio of absorbance values at 260 nm and 280 nm, respectively. The purified DNA was kept at -20°C until further analysis.
PCR-RAPD reaction, eight random primers: Primer use for PCR: OPA-03 (AGTCAGCCAC), OPA-04 (AATCGGGCTG), OPB-01 (GTTTCGCTCC), OPB-11 (CCACAGCAGT), OPF-04 (GGTGATCAGG), OPG-17 (ACGACCGACA), OPD-11 (AGCGCCATTG), and OPN-06 (GAGACGCACA). The amplifications were carried out in a 20 µl reaction mixture containing 10 µl GoTaq® Green Master Mix 2× (Promega, USA), 2 µl 10 pmol primers, 2 µl 25 ng of genomic DNA, and 6 µl nuclease-free water. The amplification reactions were performed on a thermocycler (MJ Research, USA) programmed at 94°C for 4 minutes, 92°C for 1 minute, 35°C for 1 minute, 72°C for 2 minutes, for 43 cycles, and finally 72°C for 10 minutes. The PCR products were analyzed by electrophoresis on 1.5% agarose gels, visualized by staining with ethidium bromide and photographed by a Gel Documentation.

Data analysis: The RAPD patterns of individuals were compared within and among earthworm populations. The RAPD bands were scored as 1 if present or 0 if absent. The sizes of the RAPD bands were estimated by using Quantity One software (ver. 4.1, Bio–Rad, USA). The genetic identity and genetic distance between earthworm populations were expressed using Nei's (1972) genetic distance (Nei, 1972).

The genetic parameters calculated were: the observed number of alleles (na), effective number of alleles (ne), number of polymorphic bands, Nei's (1973) gene diversity (h), Shannon's information index (I), total genotype diversity in populations (Ht), total genotype diversity within populations (Hs), and the mean coefficient of gene differentiation (Gst). The gene flow (Nm) was estimated for RAPD data using the POPGENE software (ver. 1.31) (Yeh *et al.*, 1999). RAPD data were analyzed using NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System) software. The dendrograms were generated using the UPGMA (Unweighted Pair-Group Method with Arithmeticmean) clustering method to estimate the relationships between two populations of earthworms. Building the phylogenetic tree: The construction of the phylogenetic tree and the cluster analysis according to the UPGMA algorithm of 163 *A. antiquata* samples studied was performed by NYSYS 2.1 (Exeter Software, USA) based on the genetic similarity coefficient (Jaccard, 1908).

Results and Discussion

Morphological characteristics of *A. antiquata*
Shell size: Research results show that the average length (SL), width (SW), right shell height (HRV), and left shell height (HLV) of *A. antiquata* scallops on average

are 41.45 mm (variable from 34.33-50.00 mm), 22.54 mm (variable from 18.50-29.00 mm), 29.27 mm (variable from 24.25-36.00 mm), and 28.49 mm (variable from 23.50-34.75 mm), respectively (Table 3). The research results of Siahainenia *et al.* (2018) show that shell length varies from 15.87 mm to 57.5 mm, width is from 15.50 mm to 48.60 mm and height is from 9.36 mm to 35.9 mm [16]. The *ligament length (LL), right mastoid space depth (UHR), left trochanteric space depth (UHL), right cortical symmetry (SRV) and left cortical symmetry (SLV) of A. antiquata were 25.74 mm (varying from 20.83 to 31.67 mm), 7.90 mm (variable from 6.33-10.25 mm), 8.33 mm (variable from 6.33-10.05 mm), 8.58 mm (variable from 6.41-11.50 mm), and 8.41 mm (variable from 6.08-11.25 mm), respectively.*

Table 3. *A. antiquata* feather shell size (mm)

Index	Location	Da Nang	Quang Nam	Phu Yen	Khanh Hoa	Binh Thuan	Thanh Hoa	Average
SL	$\overline{X}$ ±SD	44.46 ±5.86	45.00 ±3.43	40.16 ±2.42	40.90 ±5.72	40.80 ±2.10	37.43 ±4.10	41.45±3.93
	Min-Max	36-62	40-52	36-45	28-51	37-44	29-46	34.33±4.76 -50.00±6.72
	CV (%)	13.18	7.62	6.02	13.98	5.14	10.95	9.48±3.75
SW	$\overline{X}$ ±SD	22.50 ±3.69	23.92 ±2.32	24.03 ±1.56	24.46 ±4.44	19.58 ±1.13	20.80 ±3.46	22.54±2.76
	Min-Max	18-32	21-29	21-27	16-36	17.5-22	15-28	18.08±2.5 -29.00±4.73
	CV (%)	16.4	9.7	6.49	18.15	5.77	16.63	12.19±5.53
HRV	$\overline{X}$ ±SD	30.06 ±4.04	31.23 ±2.75	29.90 ±2.42	29.40 ±3.92	27.43 ±1.54	27.60 ±3.24	29.27±2.98
	Min-Max	25-40	27.5-37	26-36	21-37	23-31	23-35	24.25±2.36 -36.00±2.96
	CV (%)	13.43	8.80	8.09	13.30	5.61	11.74	10.16±3.15
HLV	$\overline{X}$ ±SD	29.86 ±4.00	30.50 ±2.72	29.10 ±2.01	28.90 ±3.95	26.26 ±1.43	26.36 ±3.40	28.49±2.92
	Min-Max	25-40	27-36.5	26-34	20-36	22-29	21-33	23.50±2.88 -34.75±3.71
	CV (%)	13.4	8.92	6.90	13.67	5.44	12.90	10.21±3.59
LL	$\overline{X}$ ±SD	25.85 ±4.06	27.30 ±2.4	26.33 ±2.41	24.71 ±3.1	25.36 ±1.49	24.90 ±1.9	25.74±2.56
	Min-Max	20-37	24-32	20-32	18-32	22-28	21-29	20.83±2.04 -31.67±3.14
	CV(%)	15.7	8.8	9.15	12.54	5.87	7.63	9.94±3.57
UHR	$\overline{X}$ ±SD	8.20 ±1.27	8.80 ±0.99	8.71 ±0.70	8.23 ±1.15	6.05 ±0.54	7.40 0±1.26	7.90±0.98
	Min-Max	6-12	8-11	7.5-11	6-10.5	5-7	5.5-10	6.33±1.17 -10.25±1.72
	CV(%)	15.48	11.25	8.03	13.97	8.92	17.02	11.11±6.16
UHL	$\overline{X}$ ±SD	8.21 ±1.14	8.96 ±1.10	8.71 ±0.55	8.23 ±1.08	6.00 ±0.54	7.36 ±1.46	8.33±0.98
	Min-Max	7-11.5	8-12	7.5-9.5	6-10	5-7	4.5-10	6.33±1.40 -10.05±1.74

	CV(%)	13.88	12.27	4.88	13.12	9.00	19.83	12.16±5.01
	$\bar{X}$ ±SD	8.83 ±1.88	9.27 ±1.26	9.20 ±0.92	8.71 ±1.43	7.38 ±0.66	8.11 ±1.74	8.58±1.331
SRV	Min-Max	6-13.5	8-12	8-11	5-11.5	6-9	5.5-12	6.41±1.28 -11.5±1.48
	CV(%)	21.30	13.60	10.00	16.41	8.94	21.45	15.28±5.41
	$\bar{X}$ ±SD	8.81 ±1.70	9.15 ±1.23	8.68 ±0.90	8.58 ±1.39	7.38 ±0.61	7.87 ±1.73	8.41±1.26
SLV	Min-Max	6-13	5.5-12	7-10	6-11.5	6.5-9	5.5-12	6.08±0.58 -11.25±1.47
	CV(%)	19.30	13.44	10.36	16.20	8.26	21.98	14.92±5.25

The lengths of the feather shells in different locations are different. The largest shell length was obtained from samples collected in Quang Nam Province (45.00 mm), and the smallest was obtained from samples collected from Thanh Hoa (37.43 mm). The widths of the feather shells in different locations are different. The largest shell width is in Nha Trang (24.46 mm), and the smallest is in Binh Thuan (19.58 mm). The heights of the right shell and the left shell of *A. antiquata* in different locations are different. The height of the right shell and the left shell of the largest A. antiquata collected in Quang Nam was on average 31.23 mm and 30.50 mm, respectively. The smallest samples were collected in Binh Thuan, with averages of 27.43 mm and 26.26 mm, respectively. The lengths of the ligaments are different in different locations. The longest ligament length was found in the samples collected from Quang Nam Province (27.30 mm), and the shortest was found in the samples collected from Nha Trang (24.71 mm).

Shell volume and shell cavity volume: The results showed that the average shell volume (SV) and shell cavity volume (SCV) of *A. antiquata* were on average 4.45 ml (variable from 2.76- 7.36 ml) and 11.11 ml (varying from 6.60-18.05 ml). The shell volumes in different locations are different. The largest volume of feather shells was in Quang Nam (6.03 ml), and the smallest was in Binh Thuan (2.78 ml) (Table 4).

Table 4. Shell volume and shell cavity volume (ml) of *A. Antiquata*

Sample	SV			SCV		
	$\bar{X}$ ±SD	Min-Max	CV (%)	$\bar{X}$ ±SD	Min-Max	CV (%)
Da Nang	5.44±1.95	3.4-11.6	35.84	12.21±4.56	6.9-24.5	37.34
Quang Nam	6.03±1.42	4.2-8.5	23.55	12.92±3.38	8.9-21.4	26.16
Phu Yen	4.54±0.72	3.2-6.1	15.86	11.89±2.19	8.4-16.9	18.42
Khanh Hoa	4.95±1.55	2.1-8.4	31.31	10.50±3.52	3.4-18.2	33.52
Binh Thuan	2.78±0.52	1.9-4.5	18.7	9.51 ±1.29	7-12.1	13.56
Thanh Hoa	2.99±0.89	1.8-5.1	29.76	9.65±2.86	5-15.2	29.63
Average	4.45±1.17	2.76±0.97 - 7.36±2.65	25.83±7.76	11.11±2.97	6.60±2.07 -18.05±4.42	26.43±9.04

The volumes of the shell cavity in different locations are different. The largest volume of the feather shell cavity was in Quang Nam (12.92 ml), and the smallest

was in Binh Thuan (9.51 ml). The volume of the shell cavity is arranged in ascending order as Binh Thuan<Thanh Hoa<Nha Trang<Phu Yen<Da Nang<Quang Nam. Dry shell weight and shell density: The average dry shell weight (DSWT) and shell density (DSWT/SV) of A. antiquata were 11.17 g (range 6.80-1917 g) and 2.51 g (variable from 6.80-19.17 g), respectively, and the shell density bias was from 1.89-3.00. The dry weights of shells of shellfish in different locations were different (Table 5).

Table 5. Dry shell weight (g) and shell density of A. Antiquata

Sample	DSWT			SD (DSWT/SV)		
	$\bar{X}$ ±SD	Min-Max	CV (%)	$\bar{X}$ ±SD	Min-Max	CV (%)
Da Nang	13.55±5.21	7.50-30.50	38.45	2.49±0.27	2.02-2.92	10.84
Quang Nam	14.90±3.46	11.00-21.80	23.22	2.49±0.26	2.10-2.93	10.44
Phu Yen	11.18±1.97	8.20-16.00	17.62	2.46±0.24	2.00-2.96	9.75
Khanh Hoa	12.64±3.98	5.20-22.30	31.48	2.56±0.31	1.62-3.28	12.10
Binh Thuan	7.18±1.20	5.10-10.30	16.71	2.60±0.26	1.97-2.89	10.00
Thanh Hoa	7.57±2.78	3.80-14.10	36.72	2.48±0.34	1.68-2.97	13.70
Average	11.17±3.10	6.80±2.60 -19.17±7.20	27.37±9.50	2.51±0.28	1.89±0.20 - 3.00±0.14	11.13±1.50

The largest dry weight of shells was collected from Quang Nam (14.90 g), and the smallest was collected from Binh Thuan (7.18 g). The dry weight of the peel is arranged in ascending order as Binh Thuan<Thanh Hoa<Phu Yen<Nha Trang<Da Nang< Quang Nam.

Research results on genetic diversity

Total DNA extraction results: The total DNA after being extracted must be clean enough and less broken to ensure the best efficiency for the next RAPD-PCR. Therefore, we need to pay attention and study to choose the appropriate extraction method for the object. After extraction, total DNA was electrophoresed on a 0.8% agarose gel to check for quality (Fig. 3).

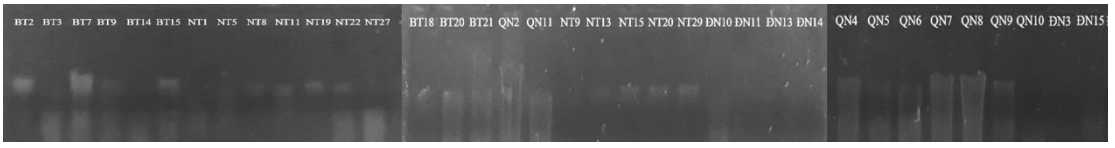

Figure 3. Total DNA electrophoresis of some samples of *A. antiquata*

The total DNA products of the extracted *A. antiquata* feathers were concentrated into clear bands, showing that the total extracted DNA was quite clean, less broken, and could be used for further studies. RAPD-PCR performance results: From the results of DNA extraction, 11 Nha Trang DNA samples, 12 samples from Da Nang, 9 samples from Quang Nam and 9 samples from Binh Thuan were selected for PCR-RAPD (Table 2). For effective RAPD analysis, we conducted exploratory reactions with 20 different random primers and selected 8 primers

(OPD11, OPN06, OPG17, OPA03, OPB01, OPA04, OPF04, OPB11) for RAPD analysis.

RAPD results with primer OPD11: The results showed that with the primer OPD11, 25 individuals were amplified, with the number of amplification bands being 14. The individuals with the most amplification bands had 4 bands (individuals DN29 and QN9), and some had at least 1 amplification band (individuals DN10, DN11, DN13, QN6, BT7, BT20) (Fig. 4).

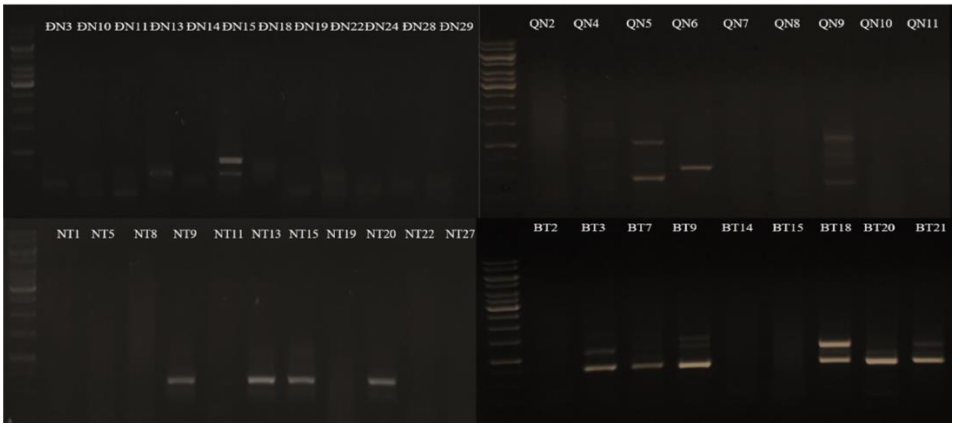

Figure 4. PCR-RAPD electrophoresis image with primer OPD11. M: Marker DNA 1 kb ladder

PCR-RAPD electrophoresis analysis showed that the 450 bp DNA band (presented in 11/41 individuals) was the main product of this primer. The 1850 bp DNA band appeared in individual BT3. The 1650 bp DNA band appeared in the BT9 individual and the 1280 bp DNA band appeared in the QN4 individual. These are the 3 bands that were amplified in the fewest individuals when PCR-RAPD was performed with primer OPD11. RAPD results with primer OPN06: The results showed that with primer OPN06, 34 individuals were amplified with amplification bands of 11. The individuals with the most amplification bands had 3 bands (individuals NT11, NT22, QN5, QN6, BT2, BT3, BT21), and some had at least 1 gain band (individuals NT5, NT9, DN3, DN11, DN18, DN24, BT20) (Fig. 5).

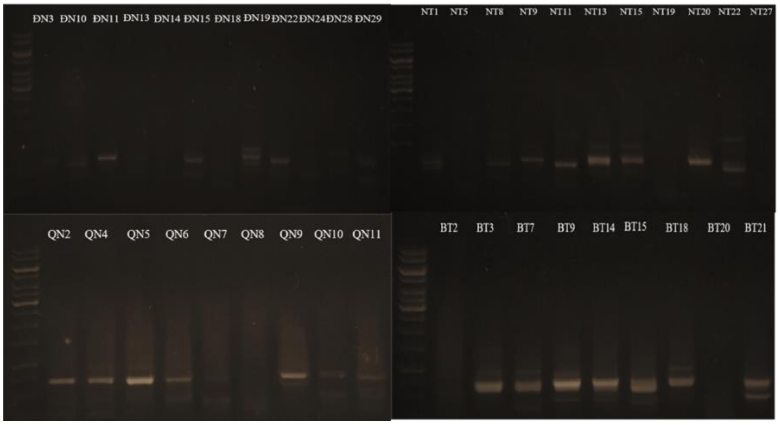

Figure 5. PCR-RAPD electrophoresis image with primer OPN06. M: Marker DNA 1 kb ladder

Analysis of PCR-RAPD electrophoresis images showed that the 500 bp DNA bands (appearing in 19/41 individuals) were the main product of this primer. The 1200 bp DNA band appeared in individual NT22. This is the band that was amplified in the fewest individuals when PCR-RAPD was performed with the primer OPN06.

RAPD results with primer OPG17: The results showed that with primer OPG17, 28 individuals were amplified with a number of amplification bands of 13. The individuals with the most amplification bands are 5 bands (individuals DN13, DN22, DN29) and some of them have at least 1 band of amplification (individuals DN11, DN14, DN19, BT2, BT3, BT7, BT20, B21). The analysis of PCR-RADP electrophoresis images revealed that the DNA bands were 1250 bp in size. The 1000 bp and 350 bp sequences (present in 9/41 individuals) were the major products of this primer. The 600 bp DNA band appeared in DN22 BT9, and the 300 bp DNA band appeared in DN2. These are the two bands that were amplified in the fewest individuals when PCR-RADP was performed with primer OPNG17 (Fig. 6).

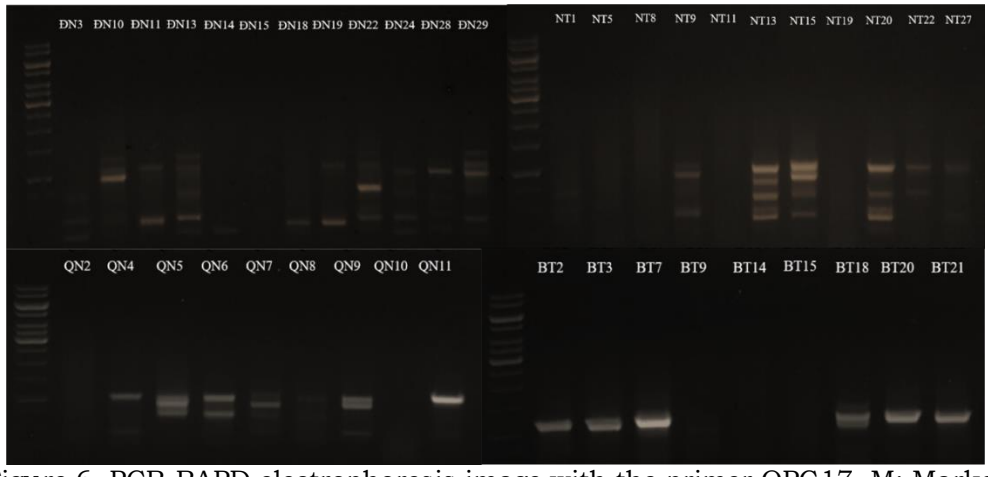

Figure 6. PCR-RAPD electrophoresis image with the primer OPG17. M: Marker DNA 1 kb ladder

RAPD results with primer OPA03: The results showed that with the OPA03 primer, 8 amplification bands were amplified from 30 individuals. The individual with the most amplification bands had 3 bands (BT3) and amplified at least 1 band (individuals NT2, NT27, DN10, DN18, DN19, DN22, DN24, DN29, QN8, BT7, BT15, BT20) (Fig. 7).

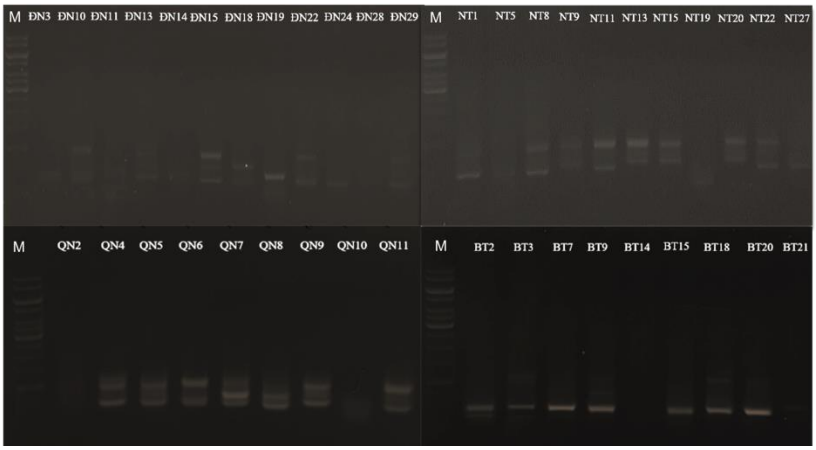

Figure 7. PCR-RAPD electrophoresis image with primer OPA03. M: Marker DNA 1 kb ladder

PCR-RAPD electrophoresis analysis showed that the 1000 bp DNA bands (present in 13/41 individuals) were the major products of this primer. The 500 bp DNA band appeared in the BT2 individual, and the 300 bp DNA band appeared in DN19. These are the two bands that were amplified in the fewest individuals when PCR-RAPD was performed with primer OPA03. RAPD results with primer OPB01: The results showed that with the primer OPB01, 26 individuals were amplified with 10 amplification bands. The individuals with the most amplification bands had 3 bands (individuals NT20, DN19, DN20, QN5, QN6, BT2, BT3, BT21) and some with at least 1 gain band (individuals NT5, BT7, BT20) (Fig. 8).

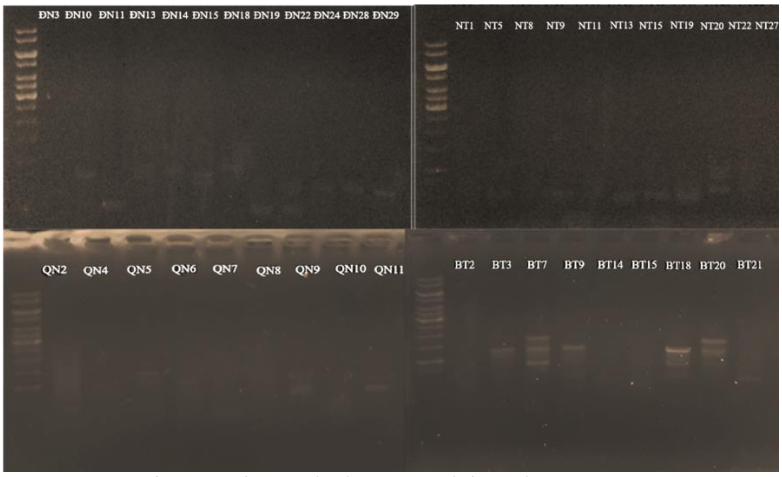

Figure 8. PCR-RAPD electrophoresis image with primer OPB01 M: Marker DNA 1 kb ladder

Analysis of PCR-RAPD electrophoresis images showed that the 500 bp DNA bands (present in 9/41 individuals) were the main product of this primer. The 2000 bp DNA band appeared in individual BT7. The 1800 bp DNA band appeared in individual BT20. The 600 bp DNA band appeared in individual BT21. The DNA band with a size of 400 bp appeared in individual DN29. The DNA band with a size of 350 bp appeared in individual DN11, and these are the 4 bands that were amplified in the fewest individuals.

RAPD results with primer OPA04: The results showed that with primer OPA04, 23 individuals were amplified, and the number of amplification bands was 11. The individuals with the most amplification bands had 3 bands (individual DN18), and the numbers with at least 1 amplification band were NT9, DN3, DN13, DN15, DN19, DN24, DN28, QN7, BT2, BT3, BT7, BT18, BT20, and BT21 (Fig. 9). The analysis of PCR-RAPD electrophoresis images showed that the 1000 bp DNA bands (present in 5/41 individuals) were the major product of this primer. The 800 bp DNA band appeared in QN5, and the 650 bp DNA band appeared in DN28; these are the two bands that were amplified in the fewest individuals.

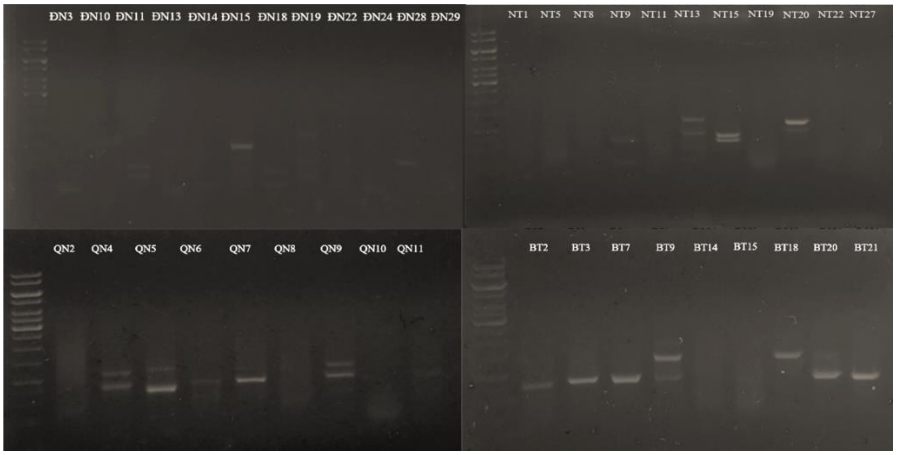

Figure 9. PCR-RAPD electrophoresis image with primer OPA04. M: Marker DNA 1 kb ladder

RAPD results with primer OPF04: The results showed that with the primer OPF04, 25 individuals were amplified, and the number of amplification bands was 9.

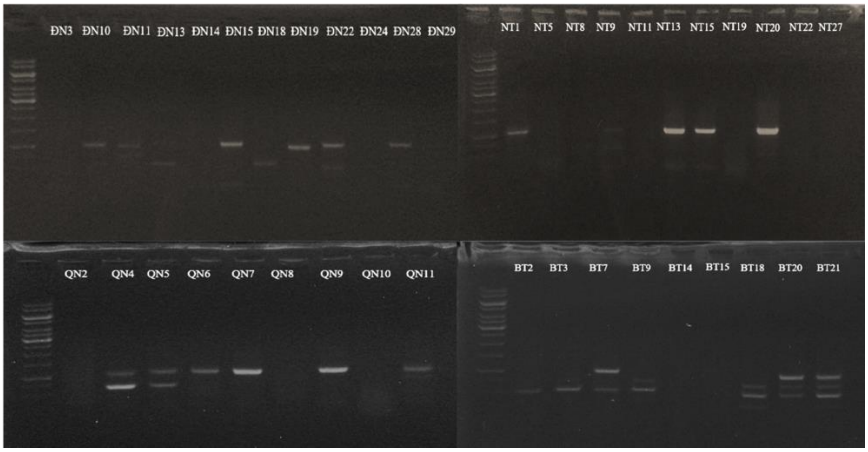

Figure 10. PCR-RAPD electrophoresis image with primer OPF04.M: Marker DNA 1 kb ladder

The individuals with the most amplification bands had 3 bands (individuals BT18, BT20, BT21) and some had at least 1 gain band (individuals NT1, NT15, NT20, DN10, DN11, DN15, DN18, DN19, DN28, QN6, QN7, QN9, BT2, BT3) (Fig. 10). Analysis of PCR-RAPD electrophoresis images showed that the 1000 bp DNA bands (present in 8/41 individuals) were the major products of this primer. The 700 bp DNA band appeared in individual BT9, this is the band that was amplified in the fewest individuals when PCR-RAPD was performed with the primer OPF04.

RAPD results with primer OPB11: The results showed that with the primer OPB11, 32 individuals were amplified, and the number of amplification bands was 11. The individuals with the most amplification bands had 4 bands (NT13 individuals) (Fig. 11). The PCR-RAPD electrophoresis analysis showed that the 500 bp DNA bands (presented in 14/41 individuals) were the major products of this primer. The 2000 bp DNA band appeared in the BT9 individual; this is the band that was amplified in the fewest individuals when PCR-RAPD was performed with the primer OPB11.

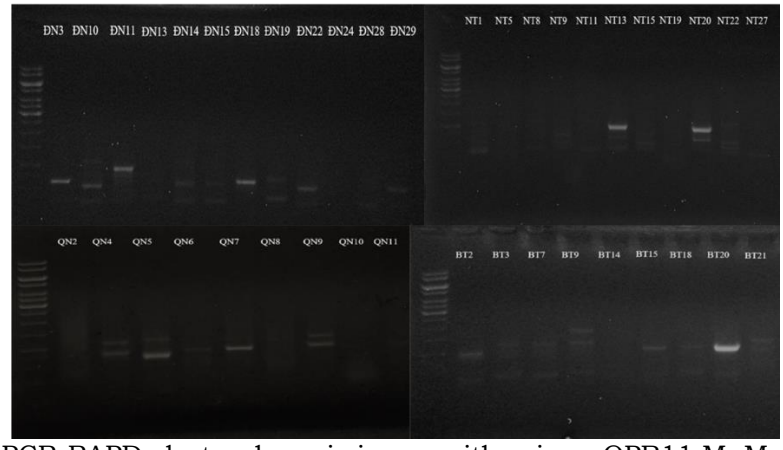

Figure 11. PCR-RAPD electrophoresis image with primer OPB11.M: Marker DNA 1 kb ladder

Analysis of the genetic relationship of *A. antiquata:* the PCR-RAPD results with 8 random primers after statistics were processed using NTSYS 2.1 software to build the pedigree diagrams (Fig. 12).

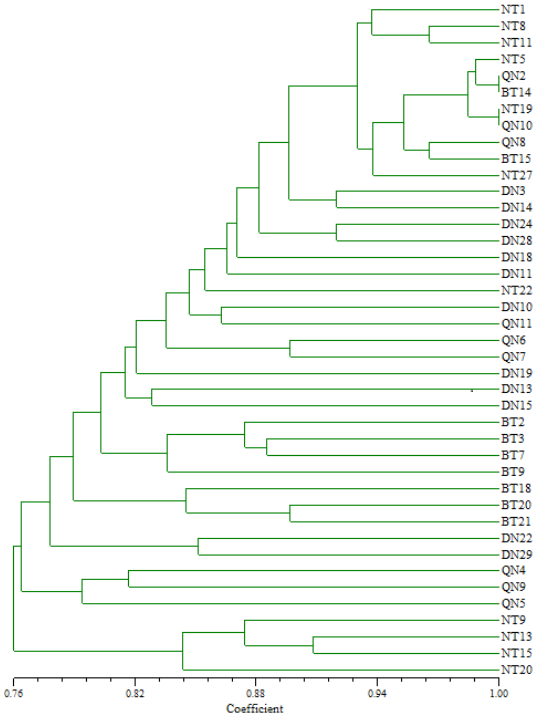

Figure 12. DNA pedigree of *A. antiquata* individuals

The pedigree chart shows that the coefficient of genetic similarity between the studied *A. antiquata* is quite high, ranging from 0.76 - 1.00 (76-100%). Genetic relationships of *A. antiquata* populations: The genetic distance and genetic similarity coefficient between four populations of *A. antiquata* in Nha Trang, Da Nang, Quang Nam, and Binh Thuan were calculated according to the Nei index (1972) (Table 6).

Table 6. Genetic distance (below the diagonal) and genetic similarity coefficient (above the diagonal) among four populations of *A. Antiquata*

Population	Nha Trang	Da Nang	Quang Nam	Binh Thuan
Nha Trang	****	0.9830	0.9830	0.9773
Da Nang	0.0172	****	0.9813	0.9794
Quang Nam	0.0172	0.0189	****	0.9810
Binh Thuan	0.0229	0.0208	0.0191	****

The results show that the genetic distance between populations of *A. antiquata* was low, varying from 0.0172 to 0.0229. The population pair Binh Thuan - Nha Trang had the largest genetic distance (0.0229). The population pair Nha Trang - Da Nang and the population pair Nha Trang - Quang Nam had the smallest genetic distance (0.0172). The coefficient of genetic similarity between the

populations of *A. antiquata* was high, varying from 0.9773 to 0.9830. The Da Nang -Nha Trang population pair and the Quang Nam - Nha Trang population pair had the highest genetic similarity coefficient (0.9830), and the Binh Thuan - Nha Trang population pair had the lowest genetic similarity coefficient. From the results obtained, we constructed a pedigree showing the genetic relationship between four populations of *A. antiquata* (Fig. 13).

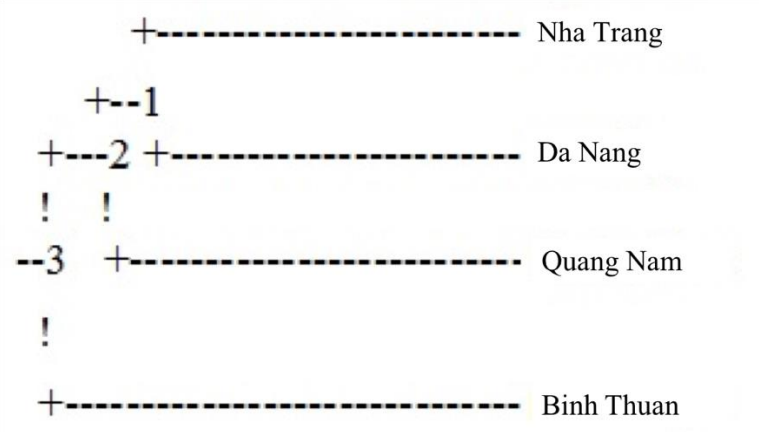

Figure 13. Pedigree chart obtained by the UPGMA method based on the genetic distance of Nei (1972) of four populations of *A. antiquata*

The pedigree shows two groups: three populations in Nha Trang, Da Nang and Quang Nam form a group, and the population of Binh Thuan forms a group.

Conclusion

A. antiquata in six regions of central Vietnam (Da Nang, Quang Nam, Phu Yen, Nha Trang, Binh Thuan and Thanh Hoa) had shell width (SW), shell length (SL), and shell height, right (HRV), left cortical height (HLV), ligamentum length (LL), right trochanteric space depth (UHR), left trochanteric space depth (UHL), right cortical symmetry (SRV), and symmetry of the left shell (SLV) of 41.45 mm, 22.54 mm, 29.27 mm, 28.49 mm, 25.74 mm, 7.90 mm, 8.33 mm, 8.58 mm, and 8.41 mm, respectively. The mean shell volume (SV) and shell cavity volume (DSWT) were 4.45 ml and 11.11 ml, respectively. The average dry peel weight was 11.17 g.

Eight random primers were used to analyze the DNA polymorphisms of 41 individuals of *A. antiquata*. 87 polymorphic DNA bands were amplified. Primer OPD11 had the most amplified DNA bands (14 bands). The OPG17 primer had 13 bands, and the least amplified band was OPA03 (8 bands). The primer OPB11 had the highest number of amplified individuals (32/41 individuals), and the primer OPA04 had the lowest number of amplified individuals (23/41 individuals). The *A. antiquata* in four study areas (Da Nang, Quang Nam, Nha Trang and Binh Thuan) has a genetic similarity coefficient between 0.76 and 1.00 individuals. The genetic distance between the populations of *A. antiquata* scallops was low, varying from 0.0172 to 0.0229. The pair of Binh Thuan - Nha Trang populations had the largest genetic distance (0.0229). The pair of Nha Trang - Da Nang populations and the pair of Nha Trang - Quang Nam populations had the smallest genetic

distance (0.0172). The coefficient of genetic similarity between populations of *A. antiquata* is high, varying from 0.9773 to 0.9830. The Da Nang - Nha Trang population pair and the Quang Nam - Nha Trang population pair had the highest genetic similarity coefficient (0.9830), and the Binh Thuan - Nha Trang population pair had the lowest genetic similarity coefficient.

Acknowledgments
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