DOI: http://dx.doi.org/10.15578/iaj.20.1.2025.75-86 *Indonesian Aquaculture Journal, 20 (1), 2025, 75-86*

Available online at: http://ejournal-balitbang.kkp.go.id/index.php/iaj

SPECIES IDENTIFICATION AND GENETIC DIVERSITY OF PORTUGUESE OYSTER (*Crassostrea angulata*): IMPLICATIONS FOR BREEDING IN VIETNAM

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(Received: April 19, 2025; Final revision: May 29, 2025; Accepted: June 11, 2025)

ABSTRACT

Cupped oyster has emerged as the predominant mollusk farmed domestically in Vietnam because of its exceptional adaptability to the local subtropical and tropical climates. However, considerable confusion remains regarding the identity of the cultivated species due to the taxonomic ambiguity between Portuguese cupped oyster (Crassostrea angulata) and Pacific cupped oyster (Crassostrea gigas). This study aims to clarify the species identity of the most commonly farmed cupped oysters in Vietnam and to evaluate genetic diversity and the suitability of oyster strains for a breeding program including three Vietnamese strains (Quang Ninh, Khanh Hoa, and Vung Tau) and one wild population from Taiwan. Based on mitochondrial DNA cytochrome c oxidase subunit I (COI) sequences, our results confirmed that all samples in Vietnam and Taiwan are C. angulata. Furthermore, this study performed genetic analyses using mtCOI sequences and five microsatellites. Populations in Vietnam and Taiwan maintain high levels of genetic diversity, with the average number of alleles per population varied between 7.80 to 16.0, and there was no statistical difference between observed and expected heterozygosity ($P > 0.05$), except in the hatchery population Vung Tau. The samples collected from this population suffered a great loss of heterozygosity and occupied the highest F_{is} value ($F_{is} = 0.3$), which is likely due to the small size of founding stock and long-term artificial breeding by local hatchery farmers, resulting in a strong genetic bottleneck and inbreeding depression. Pairwise F_{ST} calculated by microsatellites with a range of 0.043 to 0.093 revealed significant ($P < 0.05$) levels of genetic differentiation among oyster lines. Collectively, our findings clarify the taxonomic status of farmed oysters in Vietnam and highlight the importance of crossing among different strains in future breeding programs to maximize the genetic gain and avoid inbreeding, especially when using the oyster strain from Vung Tau.

KEYWORDS: genetic diversity; microsatellites; mtCOI marker; Portuguese cupped oysters; species identification

INTRODUCTION

The farming of bivalve mollusks, particularly oysters, clams, and scallops, is a rapidly expanding industry, accounting for 20% of global aquaculture and 53% of animal mariculture production (FAO, 2024). Among these mollusks, Portuguese cupped oyster (*Crassostrea angulata*) has become a key species for mariculture in subtropical and tropical regions due to its rapid growth, high survival rates, and economic value (Qin *et al*., 2023; Tan *et al*., 2021). Once nearly wiped out in Europe by an iridovirus outbreak in the late 1960s (Buestel *et al*., 2009), *C. angulata* has since become a dominant aquaculture species in Asia, particularly in Vietnam, Taiwan, and China (Ugalde *et al*., 2023; Yu, 2023). Despite being non-native, it now leads Vietnam's mollusk farming industry, with production reaching 50,000 metric tonnes in 2019, thanks to its adaptability to local environments (Le *et al*., 2023). However, the lack of wild *C. angulata* populations in Vietnam forces the industry to rely on hatchery-produced spat or imports from Taiwan and China, increasing the risk of inbreeding depression, reduced growth, smaller oyster size, and higher vulnerability to environmental stressors. To ensure long-term sustainability, establishing a selective breeding program for *C. angulata* is crucial.

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So far, a major challenge in launching a selective breeding program for *C. angulata* is the taxonomic uncertainty surrounding hatchery stocks. Historically, Vietnamese farmed oysters were classified as *Crassostrea gigas* due to imports from Taiwan, where *C. angulata* had been mislabeled for over a century (Hsiao *et al*., 2016). Recent DNA barcoding studies have confirmed that cupped oysters cultivated in northern Vietnam and Taiwan are indeed *C. angulata* (Hsiao *et al*., 2016; In *et al*., 2017). Furthermore, Vietnamese hatcheries source spat from China, a major producer of *C. gigas*, raising concerns that farmed oysters may include *C. angulata*, *C. gigas*, or their hybrids (FAO, 2024; Jiang *et al*., 2021; Jiang *et al*., 2025). This taxonomic ambiguity may compromise genetic integrity in breeding populations, underscoring the need for precise species identification.

The success of selective breeding programs relies on maintaining sufficient genetic diversity, which is essential for biological resilience, environmental adaptability, and sustained genetic improvement (Jiang *et al*., 2024; Vu *et al*., 2020a; Zhang *et al*., 2023). Since breeding populations rarely receive new genetic inputs, selecting a limited number of elite broodstock can lead to population bottlenecks and a decline in genetic diversity (Chen *et al*., 2017; Sonesson *et al*., 2023). Previous studies reported genetic diversity loss in selectively bred strains of oysters (Le *et al*., 2023), tilapia (Lal *et al*., 2021), and shrimp (Knibb *et al*., 2014). To mitigate inbreeding risks and genetic drift, base populations should be established using diverse strains (Mickett *et al*., 2003) or at least four distinct lineages from different ecological and geographical sources (Holtsmark *et al*., 2006).

As part of this genetic improvement initiative, our study examines the species identification and genetic diversity of oysters collected from hatcheries in three major oyster farming regions in Vietnam and a wild population in Taiwan. The objectives are (1) to accurately identify the species of farmed oysters and (2) to assess genetic diversity and population structure by molecular tools, providing a foundation for future selective breeding efforts in Vietnam's oyster industry.

MATERIALS AND METHODS

Sample collection and DNA extraction

Oyster samples were collected in 2022 from hatcheries located in three major oyster farming regions in Vietnam, namely Quang Ninh (21°N, 107.5°E), Khanh Hoa (12.4°N, 109.2°E), and Vung Tau (10.4°N, 107.1°E), and from one wild population in Penghu Island, Taiwan (23.6°N, 119.5°E). The abbreviations of each sampling site were denoted as follows: (1) Q as the stock cultured in Quang Ninh, North Vietnam; (2) K as the local oysters farmed in Khanh Hoa, Central Vietnam; (3) V as the stock cultured in Vung Tau, South Vietnam; and (4) T as the spat imported from Penghu Island, Taiwan.

Quang Ninh was the first region to engage in cupped oyster farming in Vietnam and has grown to be the largest area since the species was introduced from Taiwan in 2007. The province has a subtropical climate characterized by temperature fluctuations ranging from 15°C to 35°C, with an average around 23°C (Vu *et al*., 2020b). Its geographical proximity to China and Taiwan facilitates frequent importation of oyster spat from these regions to local hatcheries. Khanh Hoa, a tropical region with temperatures ranging from 22°C to 32°C and an average of about 26°C, began farming cupped oysters in 2010 through local hatcheries. However, detailed records regarding the introduction of *C. angulata* to this area are scarce. Cupped oyster farming in Vung Tau commenced in 2014, initiated by farmers from Quang Ninh experimenting. This region features a tropical climate with consistently warm water temperatures ranging from 25°C to 30°C, averaging about 27°C. After ten years of oyster farming, the number of hatcheries has significantly increased, leading to rapid oyster production growth despite the relatively small farming area of only 155 hectares. Penghu Island is an offshore island of Taiwan and is the native habitat of *C. angulata*. Thus, cupped oysters collected from this region served as wild Asian reference samples, which can be used for comparison with Vietnamese hatchery stocks.

The mantle samples of oysters were transferred on ice to Laboratory of Molecular Biology, RIA2 (Ho Chi Minh City, Vietnam) and stored in absolute ethanol at 4°C. After extraction using the salting-out method (Miller *et al*., 1988), the quality of genomic DNA was evaluated using agarose gel electrophoresis. Additionally, DNA purity and concentration were determined using OPTIZEN NanoQ Lite Spectrophotometer (K Lab Co. Ltd.).

Mitochondrial COI sequencing and analysis

Sixteen to twenty specimens from each population were used to amplify the fragment of mitochondrial cytochrome C oxidase subunit I (mtCOI) gene using universal primers LCO1490 and HCO2198 (Folmer *et al*., 1994). Each PCR reaction consisted of 2 μL DNA template (10 μg/ìL), 1 μL of each primer (50 μM), 1 μL dNTPs (10 mM), 10 μL 5X Felix Buffer, 3 μL $MgCl_2$ (25 mM), 1.25 unit of GoTaq® Felix DNA Polymerase (Promega), and nuclease-free water added up

to a total volume of 50 µL. PCR conditions included an initial denaturation for 3 min at 94°C; 34 cycles of 30s at 94°C, 30s at the annealing temperature of 55°C, and 45s at 72°C; and a final extension for 7 min at 72°C. The purification and sequencing of PCR products were conducted by the 1st BASE Manufacturer (Axil Scientific Pte Ltd, Singapore). The obtained mtCOI sequences were manually checked for base calling on chromatograms using FinchTV v.1.4 (Geospiza Inc.); modified and aligned by ClustalW algorithm in BioEdit v.7.2 (Hall, 1999). To reduce redundancy as high sequence similarity among, all mtCOI sequences were grouped into haplotypes by DnaSP v.6.12 (Rozas *et al*., 2017).

Species identification of obtained mtCOI haplotypes was performed by using BLAST search against NCBI BLAST Core Nucleotide Database (core_nt). Genetic diversity indices including haplotype diversity (H_d), percent nucleotide diversity (P_i), and average number of nucleotide differences (k), were calculated by DNASP v.6.12. The geographical distribution and evolutionary relationships of mtCOI haplotypes were represented in the geographic map and Median-joining (MJ) network constructed by POPART 1.7 (Leigh & Bryant, 2015).

Microsatellite genotyping and analysis

The genomic DNA of twenty samples from each sampling site was amplified in one multiplex PCR consisting of five microsatellite markers previously developed for *C. gigas* (Huvet *et al*., 2000a; Li *et al*., 2003; Sauvage *et al*., 2009) (Table 1). The total volume of PCRs was 10 µL containing 1 µL DNA template (10 µg/µL), 0.2-0.4 µM each primer, 5 µL QIAGEN Multiplex PCR Master Mix 2X, and 1 µL Q-solution (QIAGEN). The forward primers were labeled with different fluorescent dyes (FAM, SUN). The PCR amplification was performed on C1000 Touch™ Thermo Cyclers (Bio-Rad), comprising an initial denaturation for 15 min at 95°C; 28 cycles of 30s at 94°C, 90s at the annealing temperature of 55°C, and 60s at 72°C; and a final extension for 30 min at 60°C. Genotyping was performed on ABI 3500XL Genetic Analyzer (Applied Biosystems) with the internal standard GeneScan LIZ 500 (Applied Biosystems).

Table 1. Primer information of 6 microsatellite markers used in the study

ID	Repeat motif	Forward primer (5'-3')	Reverse primer (5'-3')	Labeled	Conc. (µM)	References
L10	$(AG)_n$	GGTCAATTCAAAGTCAATTTCCC	CATGTTTTCCCTTGACTGATCC	FAM	0.2	Huvet *et al*., 2000a
L16	$(AG)_n$	CGGACGAATAAGATATTTGGTC	TGGA TCTGCGCATCATCTCG	SUN	0.2	Huvet *et al*., 2000a
ucdCg-139	$(GA)_n$	CCCAAACACCTTTACATTCCA	GGCAATTGTCGTTTTCATTCA	FAM	0.2	Li *et al*., 2003
ucdCg-133	$(CT)_n$	GTTGGCGCGAAATATAGGAA	TGAACTTGTGATGTGCAGGA	FAM	0.2	Li *et al*., 2003
cgsili57	$(AAG)_n$	CAGTCCCTCTACGCTACATC	ACACTACCGCTTTCCTGATA	SUN	0.2	Sauvage *et al*., 2009

The allele sizes were manually identified using GeneMapper v.5 (Applied Biosystems). Micro-Checker v.2.2.3 (Van Oosterhout *et al*., 2004) was then used to determine scoring errors, allelic dropouts, and null alleles of microsatellite genotypes. The polymorphism information content (PIC) and null allele frequency (F_{null}) were estimated by Cervus v.3.0.7 (Kalinowski *et al*., 2007). Genetic diversity parameters of each population such as the number of alleles (N_a), number of effective alleles (N_e), number of private alleles (P_a), observed heterozygosity (H_o), and expected heterozygosity (H_e) were obtained using GenAlEx v.6.503 (Peakall & Smouse, 2012). Allelic richness (A_r) and population specific inbreeding coefficient (F_{is}) were calculated by FSTAT v.2.9.4 (Goudet, 1995) and Arlequin v.3.5 (Excoffier & Lischer, 2010), respectively. The differences in H_o and H_e in each population were addressed by the nonparametric Kruskal-Wallis test. Fisher exact test with Markow chain approximation was carried out to identify allelic deviation from HWE using Genepop v.4.7.5 (Raymond & Rousset, 1995).

The detection of population differentiation was carried out using AMOVA on Arlequin v.3.5. Possible population structure was further inferred using the admixture ancestry model with correlated allele frequencies between populations implemented in STRUCTURE v.2.3.4 (Falush *et al*., 2003). After 20 independent runs consisted of 5,000 burn-in iterations followed by 50,000 further iterations of Markov chain Monte Carlo (MCMC), the optimal number of genetic clusters (K) ranging from 1 to 10 was determined by

StructureHarvester software (Evanno *et al*., 2005). The Delta K graph of population structure analysis was obtained from CLUMPAK (https://clumpak.tau.ac.il/) (Kopelman *et al*., 2015). Furthermore, genetic distances between populations were calculated by GenAlEx v.6.5 (Peakall & Smouse, 2012) and were visualized on a principal coordinate analysis (PCoA) plot generated from *ggplot2* R package.

RESULTS AND DISCUSSION

Confirmation of oyster species cultured in Vietnamese hatcheries

In this study, we sequenced partial mtCOI genes from 73 cupped oysters collected from three Vietnamese hatcheries (Q, K, and V) and one wild population (T) in Taiwan. After the alignment and modification of the 592-bp fragments of the mtCOI gene, no stop codons, deletions, or insertions were observed in any of the sequences. A total of 15 haplotypes were identified and were used to determine the oyster species by comparing them with sequences available in the NCBI BLAST Core Nucleotide Database (core_nt). Through BLAST search, all 73 sequences were confirmed to belong to *C. angulata*, exhibiting a high percent identity ranging from 99.66% to 100% (Table 2). This finding dismissed any previous confusion with *C. gigas*. It also aligns with earlier studies on species identity of cupped oysters cultivated in northern Vietnam and Taiwan (Hsiao *et al*., 2016; In *et al*., 2017) and further expands the understanding of phylogenetic information of oysters cultured in central and southern Vietnam.

So far, the taxonomic status of the *C. angulata* and *C. gigas* remains controversial. The two oysters are morphologically similar (In *et al*., 2017), hybridize under natural and artificial conditions, and produce viable and fertile progeny (Jiang *et al*., 2021; Tan *et al*., 2021). Some studies have suggested that they should be considered as a single species (Reece *et al*., 2008; Wang *et al*., 2010). Conversely, other authors concluded that *C. angulata* and *C. gigas* are closely related but genetically distinct species based on genetic divergence (Hsiao *et al*., 2016; In *et al*., 2017). Despite this unsettled taxonomic status, adaptive divergence of energy metabolism has been recognized in the two oysters, supposedly leading to their phenotypic differentiation: *C. gigas* adapts to cold environments, whereas *C. angulata* exhibits higher than adaptive plasticity to water temperature (Li *et al*., 2017; Wang *et al*., 2021). The accumulating physiological evidence highlights the importance of accurately classifying Vietnam's oyster stocks, as warm seawater temperatures in Vietnam's coast, ranging from near-tropical in the north (20-35°C) to tropical in the central and southern regions (23-32°C), may be favorable to *C. angulata* over *C. gigas*. Indeed, failed attempts to cultivate *C. gigas* in Vietnam using broodstock imported from Australia were reported (In *et al*., 2017). Therefore, we recommend conducting regular genetic audits to monitor genetic composition and preserve integrity, thereby preventing unintended hybridization between *C. angulata* and *C. gigas*.

Table 2. Species identification of 15 mtCOI haplotypes identified in 73 individuals. For each query sequence, the accession number with the highest percentage of identity in BLAST search was reported

Haplotype	No. of samples	Query length (bp)	Identity (%)	Accession number	Scientific taxonomy
Hap_1	28	592	100	LC383464.1	*Magallana angulata*
Hap_2	25	592	100	LC383458.1	*Magallana angulata*
Hap_3	1	592	99.83	LC383464.1	*Magallana angulata*
Hap_4	1	592	99.83	KC170323.1	*Magallana angulata*
Hap_5	3	592	100	LC383462.1	*Magallana angulata*
Hap_6	1	592	99.66	LC383464.1	*Magallana angulata*
Hap_7	5	592	99.83	LC383462.1	*Magallana angulata*
Hap_8	1	592	100	MH938693.1	*Magallana angulata*
Hap_9	1	592	100	LC383462.1	*Magallana angulata*
Hap_10	1	592	100	KC170323.1	*Magallana angulata*
Hap_11	1	592	99.83	LC383458.1	*Magallana angulata*
Hap_12	1	592	99.83	LC383461.1	*Magallana angulata*
Hap_13	2	592	99.83	LC383458.1	*Magallana angulata*
Hap_14	1	592	99.83	MH938693.1	*Magallana angulata*
Hap_15	1	592	99.93	LC383462.1	*Magallana angulata*

Note: *Magallana angulata* (formerly *Crassostrea angulata*) is the official taxonomy of Portuguese oyster according to World Register of Marine Species (LSID: 1039387). The taxonomic replacement was suggested by Salvi & Mariottini (2017)

Haplotypic diversity of mtCOI sequences suggests the origin of *C. angulata* stock

Among 15 mtCOI haplotypes identified from 73 oyster individuals across four populations (Q, K, V, T), there were 11 private and four shared haplotypes (Table 3). The two major haplotypes, Hap_1 and Hap_2, constituted 38.36% (28/73) and 34.25% (25/73) of all individuals, respectively, were present in all four populations. These two major haplotypes dominated in the center of the MJ network (Fig. 1) and 13 minor satellite haplotypes diverging from them. A previous study reported a similar complex star-like network of *C. angulata* featuring multiple connections and high-frequency internal haplotypes, as opposed to the simple star-like network with one most common and widespread ancestral haplotype observed in *C. gigas* (Hsiao *et al.*, 2016). Genetic differences among four studied populations were represented by the number of private haplotypes. While population V had only one private haplotype, populations Q, K, and T had 2,

Table 3. Genetic diversity and haplotype frequencies based on mtCOI sequences among four populations of *C. angulata*

Population	Vung Tau	Khanh Hoa	Quang Ninh	Taiwan	Total
Sample size	18	19	20	16	73
Haplotype					
Hap_1	9	6	12	1	28
Hap_2	8	6	1	10	25
Hap_3	1				1
Hap_4		1			1
Hap_5		1	2		3
Hap_6		1			1
Hap_7		2	3		5
Hap_8		1			1
Hap_9		1			1
Hap_10			1		1
Hap_11			1		1
Hap_12				1	1
Hap_13				2	2
Hap_14				1	1
Hap_15				1	1
N_h	3	8	6	6	15
N_s	2	11	8	7	17
H_d	0.582 ± 0.061	0.819 ± 0.063	0.632 ± 0.113	0.617 ± 0.135	0.737 ± 0.036
P_i (%)	0.107	0.446	0.378	0.204	0.316
k	0.634	2.643	2.237	1.208	1.873

N_h: number of haplotypes, N_s: number of segregating sites, H_d: haplotype diversity (± standard deviations), P_i: percent nucleotide diversity (± standard deviations), k: average number of nucleotide differences.

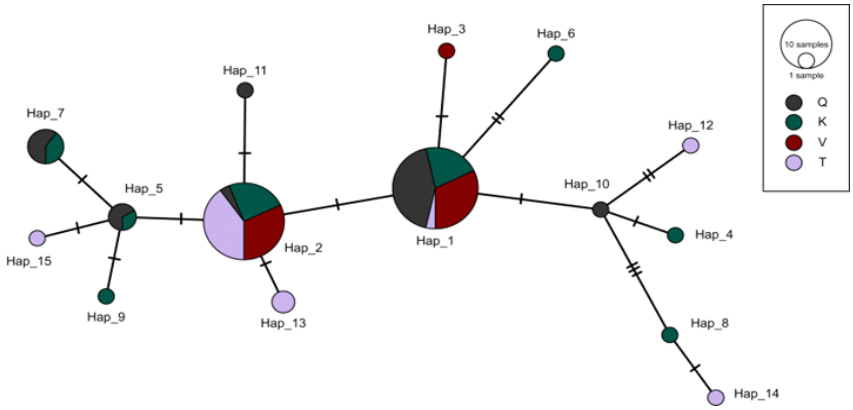

Figure 1. Median-joining (MJ) network for 15 mtCOI haplotypes identified in four populations of *C. angulata* (n = 73). Each circle represents a unique haplotype (Hap). Colors indicate population origin: Quang Ninh (Q, purple), Khanh Hoa (K, green), Vung Tau (V, red), and Taiwan (T, pink).

4, and 4 private haplotypes, respectively. Notably, only two samples shared the same haplotypes between population Q and T. This finding demonstrates that most oyster lines in Quang Ninh's hatcheries are not derived from Penghu Island in Taiwan, but probably from China instead. This speculation is given because Quang Ninh province is geographically close to China and the importation of oyster spat from Southern China occurs frequently to satisfy the increasing demand for production during peak season (Le *et al.*, 2023).

Regarding population genetic diversity, population K demonstrated the highest number of haplotypes, haplotype diversity, and percent of nucleotide diversity (N_h = 8, H_d = 0.819 ± 0.063, and P_i = 0.446), followed by population Q (N_h = 6, H_d = 0.632 ± 0.113, and P_i = 0.378) and population T (N_h = 6, H_d = 0.617 ± 0.135, and P_i = 0.204), whereas population V demonstrated the lowest level of genetic diversity (N_h = 3, H_d = 0.582 ± 0.061, and P_i = 0.107) (Table 3). Low haplotypic diversity (3-5 haplotypes) has been reported in hatchery lines in Northern Vietnam (In *et al.*, 2017). Similarly, only six haplotypes in *C. angulata* populations were previously found in 25 locations worldwide, including both Europe and Asia (Boudry *et al.*, 1998; Huvet *et al.*, 2000b). Therefore, it is reasonable to conclude that the limited number of haplotypes likely does not indicate inbreeding in the hatchery lines in Vietnam and in the wild population in Taiwan specifically but rather represents the overall haplotype diversity of the species. Nevertheless, why population K has an exceptionally high haplotypic diversity compared to other populations remains unclear.

Microsatellite diversity and inbreeding patterns in four *C. angulata* populations

The genotyping of 80 oysters from four populations using five microsatellite markers revealed no apparent evidence of large allele dropout and scoring error due to stuttering. The estimated null allele frequencies across loci ranged from 0.042 to 0.269, with the locus ucdCg-133 exceeding 0.2 (0.269) (Table 4). All five microsatellites were highly polymorphic (PIC > 0.5), with PIC values ranging from 0.593 to 0.939 and an average of 0.855, which allowed robust assessment of genetic diversity and differentiation among populations.

Population-level genetic diversity parameters derived from genotypic data are presented in Table 5. The average number of alleles (N_a) per population varied between 7.80 to 16.0, while the average number of effective alleles (N_e) and allelic richness (A_r) ranged from 4.71 to 11.2, and from 7.54 to 14.9, respectively. Surprisingly, while population V exhibited the highest N_a (16.0 ± 3.31), N_e (11.2 ± 2.48), A_r (14.9 ± 6.73), and number of private alleles (P_a = 17), it showed a significantly lower observed heterozygosity (H_o) compared to expected heterozygosity (H_e) (P = 0.028 < 0.05) and a high inbreeding coefficient (F_{is} = 0.3 > 0.2). Additionally, allele frequencies for three microsatellite loci in population V

Table 4. Genetic diversity parameters per locus across four populations of *C. angulata* using 5 microsatellites

Locus	k	N	H_o	H_e	PIC	F_{null}
L10	30	80	0.863	0.948	0.939	0.042
L16	24	77	0.727	0.944	0.935	0.125
ucdCg133	25	74	0.527	0.919	0.907	0.269
ucdCg139	17	80	0.788	0.913	0.900	0.069
cgsili57	6	80	0.500	0.631	0.593	0.096
Mean	20.4	78.2	0.681	0.871	0.855	0.120
Std.	9.30	2.68	0.160	0.135	0.147	0.089

k: number of alleles; N: number of genotyped individuals; H_o: observed heterozygosity; H_e: expected heterozygosity; PIC: polymorphism information content; F_{null}: null allele frequency; Std.: standard deviation.

Table 5. Genetic diversity parameters of four populations of *C. angulata* based on five microsatellite loci

Pop.	*N*	N_a	N_e	A_r	P_a	H_o	H_e	F_{is}	*dHWE*
Q	20	11.0 ± 1.92	6.64 ± 1.15	10.5 ± 3.91	3	0.67 ± 0.12	0.84 ± 0.05	0.208	3
K	20	8.60 ± 1.60	6.12 ± 1.23	8.35 ± 3.40	5	0.75 ± 0.08	0.80 ± 0.08	0.041	1
V	20	16.0 ± 3.31	11.2 ± 2.48	14.9 ± 6.73	17	0.63 ± 0.06[a]	0.89 ± 0.06[a]	0.300	3
T	20	7.80 ± 1.02	4.71 ± 0.69	7.54 ± 2.18	3	0.67 ± 0.10	0.78 ± 0.06	0.148	1
Total	80	10.9 ± 1.23	7.16 ± 0.90	10.3 ± 3.29	-	0.68 ± 0.04	0.80 ± 0.03	0.179	-

Pop.: Population; V: Vung Tau, K: Khanh Hoa, Q: Quang Ninh, T: Taiwan, *N*: number of samples; N_a: number of alleles (± standard deviations); N_e: number of effective alleles (± standard deviations); A_r: allelic richness (± standard deviations); P_a: number of private alleles; H_o: observed heterozygosity (± standard deviations); H_e: unbiased expected. [a] significant difference at *P*-value < 0.05.

significantly deviated from HWE expectations (P < 0.05). The disparities between expected and observed heterozygosity were not observed in three other populations (Q, K, and T). However, population Q also experienced relatively high level of inbreeding (F_{is} = 0.208 > 0.2) and heterozygote deficits related to Hardy-Weinberg disequilibrium (3 loci deviated from HWE).

Aquacultural practices have been shown to reduce genetic variation in hatchery-produced stocks and lines, leading to undesirable traits and decreased fitness. In this study, five microsatellite loci revealed relatively high allelic variation across all populations (Q, K, V, and T), averaging about 11 alleles per locus (Table 5). This aligns with previous research on the genetic diversity of *C. angulata* hatchery lines in Northern Vietnam (In *et al*., 2017). The observed and expected heterozygosity were not significantly different (P < 0.05) between Vietnamese hatchery stocks and wild populations in Taiwan, except for population V. The high level of diversity suggests that these oyster lines are suitable for breeding programs, despite some evidence of inbreeding indicated by positive F_{is} values.

On the other hand, population V showed significant heterozygote deficiencies, as evidenced by significantly lower observed heterozygosity compared to expected values (P < 0.05), high F_{is} values (F_{is} > 0.2) and a substantial number of loci deviated from Hardy-Weinberg expectation (Table 5). Such heterozygote deficiencies commonly occur in population genetic studies of marine mollusks using microsatellites (De Meeûs, 2018). The loss of heterozygosity was reportedly correlated with null alleles in many marine or freshwater bivalves (Yu *et al*., 2015). Additionally, the excess of homozygosity may stem from founder effects and genetic bottlenecks due to the limited stocks initially introduced into hatchery (Hillen *et al*., 2017). Inbreeding and genetic drift can also occur in closed stocks, influenced by significant disparities in reproductive success among highly fecund species (Varney & Wilbur, 2020). Other factors during the domestication of captive populations, such as Wahlund effects and non-random mating, may contribute to the observed deficits of heterozygotes in hatchery lines (Chen *et al*., 2017). Given these considerations, future breeding programs involving the oyster strain from population V should be carefully managed and regularly monitored using genetic markers for pedigree control to prevent unintended genetic bottlenecks.

Genetic differentiation and population structure occurs in hatchery populations in Vietnam and a wild population in Taiwan.

The analysis of molecular variance (AMOVA) of microsatellites revealed a small but significant genetic differentiation among all populations with an overall F_{ST} of 0.062 (P < 0.001) (Table 6). Specifically, the percentage of genetic variances among and within populations were 6.24% and 16.87%, and further pairwise F_{ST} analysis demonstrated statistical significance from 0.043-0.093 (P < 0.05) for all population comparisons (Table 7). These values fall within or slightly above those reported in previous oyster species, such as *C. gigas* (F_{ST} = 0.0189 – 0.0609; Zhang *et al*., 2018), *C. virginica* (F_{ST} = 0.008 – 0.009; Thongda *et al*., 2018), and *Saccostrea glomerata*

Table 6. Analysis of molecular variance (AMOVA) among four populations of *C. angulata* based on five microsatellite loci

Source of variation	df	SS	Var	% Var	F_{ST}	P-value
Among populations	3	23.49	0.138	6.24	0.062	< 0.001
Among individuals within populations	76	180.86	0.373	16.87		
Within individuals	80	133.50	1.702	76.89		
Total	159	337.85	2.214			

df: degree of freedom; SS: sum of square; Var: Variance component; % Var: Percent variance component; F_{ST}: fixation index.

Table 7. Pairwise genetic differentiation (F_{ST}) among four populations of *C. angulata* based on five microsatellite loci

Population	Q	K	V	T
Q	-	-	-	-
K	0.053*	-	-	-
V	0.043*	0.063*	-	-
T	0.068*	0.093*	0.073*	-

Q: Quang Ninh, K: Khanh Hoa, V: Vung Tau, and T: Taiwan population.
* significant difference at P < 0.05.

(0.0033 – 0.0972; Thompson *et al.*, 2017). The slightly elevated differentiation observed here may partly reflect differences in population origins, as suggested by mtCOI analysis of populations Q and T. Additionally, the influence of local artificial selection is an alternative explanation. In Vietnam, spontaneous selection by farmers for enhanced growth performance has occurred since at least 2007, which is equivalent to approximately 18 generations (Le *et al.*, 2023). This ongoing selection, particularly in tropical hatchery environments such as Vung Tau and Khanh Hoa, likely contributed to the higher FST values relative to oyster species from subtropical regions.

In addition, clustering analysis using STRUCTURE algorithms assigned 80 oyster samples across 4 populations into 3 distinct genetic clusters (Figure 2A; Table 8). The boundaries of these genetic clusters aligned with the geographical distribution of two Vietnamese hatchery lines (populations K and V) and wild population in Taiwan (T), whereas population Q demonstrated a certain degree of genetic admixture with three other populations. Moreover, the genetic composition of population V, K, and T was clearly separated with a low degree of genetic admixture within individuals, whereas population Q consisted of admixed individuals from the other three populations. This finding was further evident in the principal coordinate analysis (PCoA) that illustrated the genetic relationships of individuals among populations (Figure 2B). Principal coordinate axis 1 (12.68%) and axis 2 (9.21%) could distinguish some individuals originating from population V, K, and T, but other individuals, especially those in population Q, were not easily separated from four populations. Therefore, we inferred that while Vietnamese hatchery lines originated from a subset of the original stocks in China and Taiwan, the three lines may have diverged from their ancestries over time as the result of artificial breeding and selection to local environments.

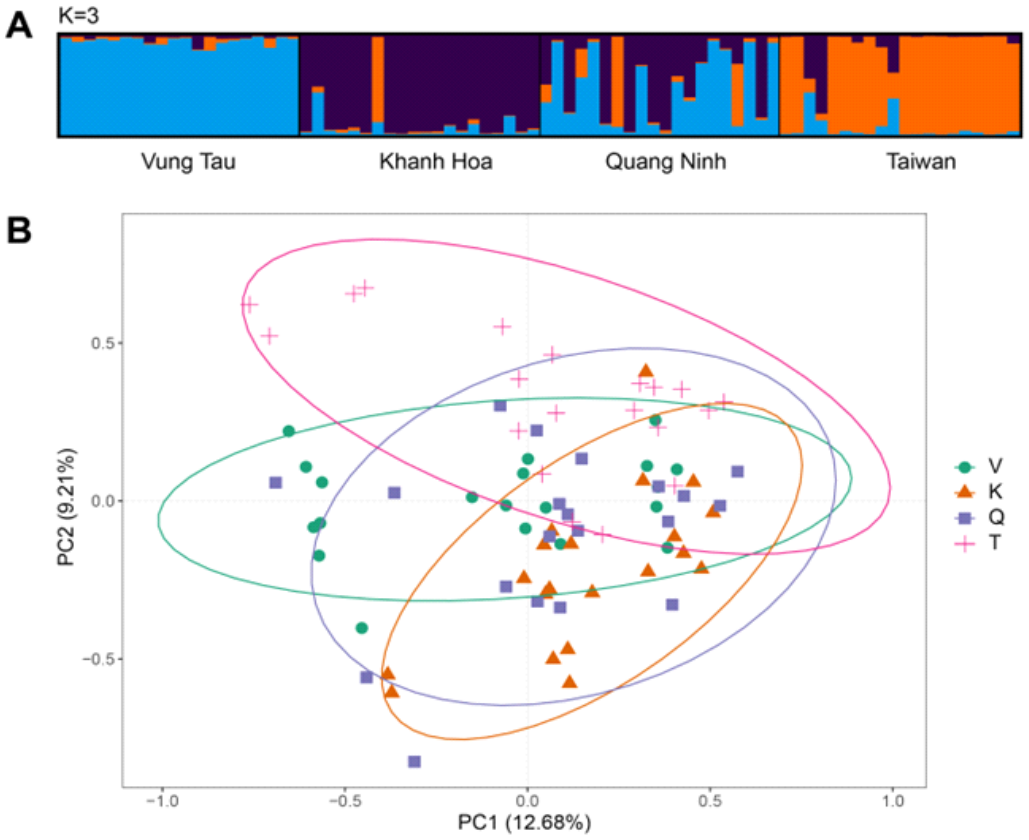

Figure 2. Genetic differentiation and population structure identified in four populations of *C. angulata* (n = 80) using five microsatellites. (A) Clustering analysis using admixture ancestry model in STRUCTURE identified three genetic clusters (K = 3). Each vertical bin represents the genetic proportion in one individual and color represents a unique cluster. (B) Principal Coordinate Analysis (PCoA) based on the genetic distance matrix derived from microsatellite genotypes. Colors and symbols represent studied populations: Quang Ninh (Q, purple square), Khanh Hoa (K, orange triangle), Vung Tau (V, green circles), and Taiwan (T, pink cross). The confidence ellipses were plotted around each population.

Table 8. Estimation of number of ancestral populations (K). The optimal number of K (bold) was determined through the maximum likelihood L(K) and the highest ΔK value

K	L(K)	Std.	L'(K)	Abs[L"(K)]	ΔK
1	-2029.455	0.4763	NA	NA	NA
2	-1940.26	13.0308	89.195	15.7	1.204839
3	-1835.365	2.5763	104.895	180.06	69.89193
4	-1910.53	57.391	-75.165	35.705	0.622136
5	-1949.99	66.1091	-39.46	7.665	0.115945
6	-1997.115	128.1111	-47.125	17.61	0.137459
7	-2026.63	77.5568	-29.515	34.335	0.442708
8	-2021.81	125.6133	4.82	409.665	3.261319
9	-2426.655	1494.5887	-404.845	193.9	0.129735
10	-2637.6	2365.5651	-210.945	NA	NA

CONCLUSION

This study confirms all oyster samples from Vietnamese hatcheries and a wild population in Taiwan belong to *C. angulata*, eliminating confusion with *C. gigas*. Further genetic analysis identified 15 mtCOI haplotypes, with two dominant haplotypes present across all populations. This result, along with microsatellite data, demonstrated high levels of genetic diversity and the presence of genetic differentiation in studied populations Nevertheless, signs of inbreeding were detected in population Vung Tau. Moreover, although the hatchery stocks in Vietnam originates from wild populations in China or Taiwan, they have genetically diverged probably due to artificial selection by local farmers and adaptation to local environments, resulted in low but significant genetic differentiation. Yet, gene flow persists among some hatchery populations, particularly in population Quang Ninh. Collectively, this study underscored the importance of implementing genetic monitoring and refining breeding strategies such as crossbreeding among different strains not only to preserve genetic diversity but also to prevent inbreeding depression and loss of adaptive potential in future breeding and conservation programs of oyster in Vietnam.

ACKNOWLEDGMENTS

We sincerely acknowledge the financial support from the Ho Chi Minh City Department of Science and Technology for funding the research project entitled “Selective breeding for growth improvement of milk oyster (*Crassostrea angulata*) and farming trials of Pacific oyster (*Crassostrea gigas*) in Ho Chi Minh City and southern provinces of Vietnam”, under project number 62/2020/HĐ-QPTKHCN.. We also express our gratitude to the Directorates and other colleagues at RIA2 for their valuable assistance in laboratory work and data analysis. Special thanks to the local oyster farmers and hatcheries in Vietnam and Taiwan for providing essential samples and sharing their insights. Finally, we appreciate the constructive feedback from anonymous reviewers, which contributed to the improvement of this article.

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