High Genetic Diversity and Gene Flow among Cultured and Wild Populations of Bighead Catfish (*Clarias macrocephalus*) in the Mekong Delta of Viet Nam Inferred from ISSR Markers

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ABSTRACT

Bighead catfish (*Clarias macrocephalus*) is an important aquaculture species in the Mekong Delta of Viet Nam, but its farming has been facing such difficulties as slow growth and low seed quality. To apply genetic improvement programs to enhance farming production, it is crucial to start with a base population with high genetic diversity. This study compared genetic diversity between cultured and wild bighead catfish populations using inter-simple sequence repeat (ISSR) markers. Samples were collected in the Mekong Delta from two cultured populations (in Can Tho and Long An provinces) and two wild populations (in Ca Mau and Hau Giang). A total of 112 individuals was screened using six highly polymorphic ISSR primers which generated 61 scorable bands (500-3,000 bp). The study found that levels of genetic diversity of bighead catfish populations were high, with the number of effective alleles from 1.42 to 1.50, Shannon Index from 0.381 to 0.433, and unbiased expected heterozygosity from 0.257 to 0.297; those values from cultured populations were lower than wild populations. Moreover, the value of Nei's unbiased genetic distance among populations was low, indicating high gene flow among bighead catfish populations distributed in the Mekong Delta, Viet Nam.

Keywords: Catfish, Dominant markers, Genetic variation, Hatchery fish, Intraspecific introgression

INTRODUCTION

Bighead catfish (*Clarias macrocephalus*) is a member of the family Clariidae, and has become important for freshwater aquaculture in Southeast Asian countries, especially Cambodia, Thailand and Viet Nam in recent decades (Na-Nakorn, 2004; Duong and Scribner, 2018). Due to its high quality and attractive yellow-colored flesh, it has received high interest from consumers. In Viet Nam, this species was domesticated successfully and has experienced approximately 30 generations in hatcheries around the Mekong Delta (Duong and Scribner, 2018). A question was raised regarding how the level of genetic diversity of bighead catfish varied between wild populations and cultured populations. The genetic diversity of cultured fish has a tendency to decrease over time because of imbalanced breeding sex ratios, genetic drift, and inbreeding (Tave, 1993; 1999). In addition, wild fish populations can be affected by over-exploitation or gene introgression, leading to a decrease of genetic diversity (Na-Nakorn *et al*., 2004; Frost *et al*., 2006; Ford and Myers, 2008).

There have been inconsistent findings regarding genetic variation between cultured and wild populations of fish species. Genetic diversity of cultured populations was lower than wild ones in some species such as black carp (*Mylopharyngodon piceus*) in Thailand (Zhou *et al*., 2020), swamp eel (*Monopterus albus*) in central China (Li *et al*., 2013), but the opposite was true in other species, such as striped catfish (*Pangasianodon hypophthalmus*) in Thailand (Na-Nakorn and Moeikum, 2009). In bighead catfish, Duong and Scribner (Duong

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and Scribner, 2018) used microsatellite markers to quantify levels of genetic diversity in samples from the Mekong Delta, and found that hatchery populations had lower genetic diversity than wild populations in conservation zones, but were similar to those in aquaculture-affected areas. Another study focusing on only wild populations with a wider sampling scale from Malaysia to Viet Nam revealed that the genetic diversity of bighead catfish populations from Cambodia and Viet Nam was higher than those in Malaysia (Nazia *et al*., 2021).

Evaluation of genetic diversity can be used for aquaculture purposes. Low genetic diversity of a captive population can be improved by crossing with introduced wild or genetically divergent individuals (Schönhuth *et al*., 2003; Wachirachaikarn *et al*., 2009). In this regard, genetic differentiation among stocks used for that purpose could be verified based on molecular genetic markers (Wachirachaikarn *et al*., 2009). In addition, for a long-term response of a selective breeding program, the genetic diversity of a base population can be maximized by using the combination of several populations with high genetic diversity (Hayes *et al*., 2006).

In this study, ISSR (inter-simple sequence repeat) markers were used to re-evaluate genetic diversity of bighead catfish between cultured and wild populations in the Mekong Delta. The findings of this study will provide valuable information for designing future genetic improvement programs of bighead catfish in the region.

MATERIALS AND METHODS

Sampling sites

Bighead catfish samples were collected from two main sources, including cultured populations from hatcheries in Can Tho (cultured CT) and Long An (cultured LA) and wild populations from conservation areas in Ca Mau (wild CM) and Hau Giang (wild HG). Sampling sites were based on the previous study by Duong and Scribner (2018). These four populations were hydrologically connected within the Mekong Delta of Viet Nam (Figure 1). The cultured populations from CT and LA hatcheries are located near the tributaries of the Vietnamese Mekong River, while

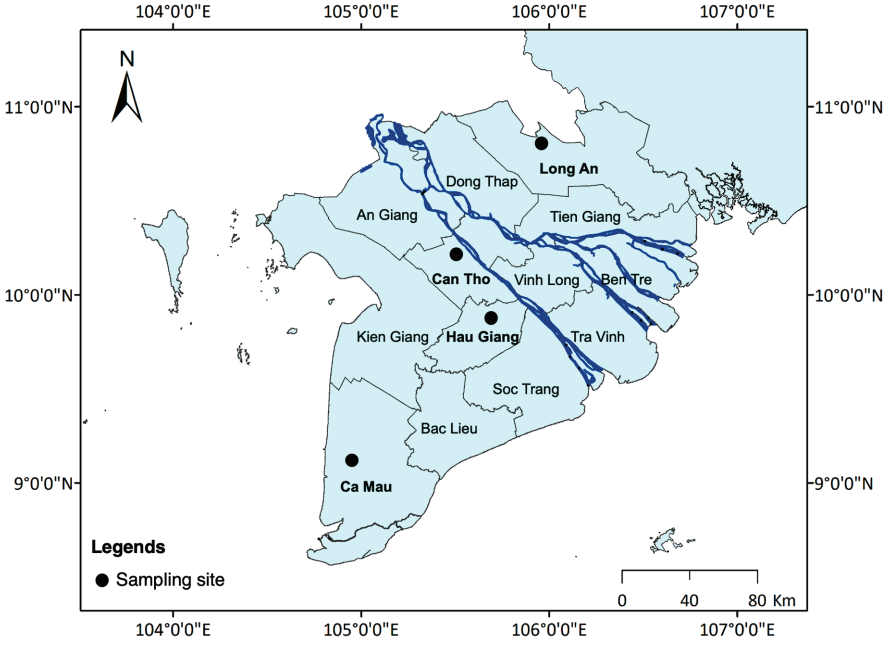

Figure 1. Sampling sites of bighead catfish in the Mekong Delta of Viet Nam.

the two wild populations are from wetlands of the conservation areas. Fin clips of 27-29 individuals from each population were preserved in 95 % ethanol for genetic analysis.

Extraction of DNA

About 25 mg of preserved fin clips of the collected samples was used to extract genomic DNA following the ammonium acetate method as modified by Saporito-Irwin *et al*. (1997). This method is based on the principle of using a salt solution (ammonium acetate) for protein precipitation. After protein elimination, DNA was precipitated by cool absolute ethanol. To remove other unwanted components, 70% ethanol was used to clean the precipitated DNA pellet. DNA was then air-dried and diluted with TE buffer (Tris-EDTA). The DNA solution was stored at -20 °C until analysis. The quality of DNA was checked by 1% agarose electrophoresis and viewed under an ultraviolet (UV) transilluminator.

Amplification of ISSR

The initial step of ISSR amplification is primer screening and optimization. Thirty ISSR primers from previous studies by Fernandes-Matioli *et al*. (2000), Pazza *et al*. (2007), Sharma *et al*. (2011), Saad *et al*. (2012), Raghuwanshi *et al*. (2013), Saxena *et al*. (2014) and Labastida *et al*. (2015) were selected for this step. Two samples from each population (n = 10) were randomly chosen to test with all library primers using the annealing temperatures reported in previous references. Six primers (Table 1) were then selected for this study after demonstrating bands with polymorphism, reproducibility and visibility. PCR reactions for ISSR amplification were performed in a 10-µL mixture comprising 5 µL Promega PCR Master Mix (containing Taq DNA polymerase supplied in a reaction buffer [pH 8.5], 400 µM dNTPs, and 3 mM $MgCl_2$), 0.4 µL primer (10 µM), 2 µL DNA, and 2.6 µL nuclease-free water. The cycling parameters included one cycle of initial denaturation at 95 °C for 5 min; 38 repeated cycles of denaturation at 95 °C for 30 s, annealing temperature (Table 1) for 40 s and extension at 72 °C for 1 min; and one cycle of final extension at 72 °C for 5 min.

Electrophoresis and visualization of ISSR bands

Electrophoresis was conducted to observe PCR products. The products were loaded into a 1.2% agarose gel with a 1kb-DNA ladder (ABM Canada) in TBE (Tris-borate-EDTA) buffer. The electrophoresis lasted for 80 min at 50 V (Consort EV243). The agarose gels were then submerged in ethidium bromide solution (0.5 $\mu g \cdot mL^{-1}$) for 10 to 15 min and bands were visualized under a UV transilluminator. Gel images were captured by a camera for later band scoring. The size of the bands was estimated based on the DNA ladder. Band scoring was carried out by two independent co-authors. A binary data matrix was created as the raw data for further analysis by scoring 1 or 0 for the presence or absence of a band, respectively.

Data analysis

Genetic variablity parameters, namely percentage of polymorphic loci (P), number of effective alleles (Ne), Shannon's information index (I), unbiased expected heterozygosity (uHe) and

Table 1. Primer sets of the genes used, their sequences, and amplification size.

No	Primer	Sequence (5'-3')	Annealing temperature (°C)	Reference
1	Chiu-SSR1	$[GGAC]_3A$	46	Pazza *et al*. (2007)
2	HB10	$[GA]_6CC$	46	Saad *et al*. (2012)
3	ISSR11	$[CAC]_3GC$	44	Sharma *et al*. (2011)
4	ISSR14	$[AGC]_4GT$	46	Raghuwanshi *et al*. (2013)
5	ISSR15	$[TCC]_5$	46	Saxena *et al*. (2014)
6	micro11	$[GGAC]_4$	46	Fernandes-Matioli *et al*. (2000)

Nei's unbiased genetic distance, were calculated to estimate levels of genetic diversity and genetic differentitation among all bighead catfish popualtions and between cultured and wild populations. All of the above parameters were calculated by GenAlEx 6.5 software (Peakall and Smouse, 2012). Differences in genetic diversity parameters among populations were tested using one-way analysis of variance (ANOVA), followed by Duncan's multiple range tests, which were performed using SPSS version 22.0 (IBM Corporation, USA). Furthermore, the evolutionary relationship among the bighead catfish populations was reconstructed by producing a dendrogram based on UPGMA (unweighted pair-group method with arithmetic average) algorithm using the Popgene software (Yeh *et al*., 1999) and visualized by MEGA 7 software (Kumar *et al*., 2016).

RESULTS

Results of ISSR amplification

Six ISSR primers were used to explore the genetic diversity of 112 individuals of bighead catfish belonging to four different populations. There was a total of 61 bands detected with the sizes from 500 bp (HB10 and micro11) to 3,000 bp (ISSR11) (Figure 2). The number of bands produced using

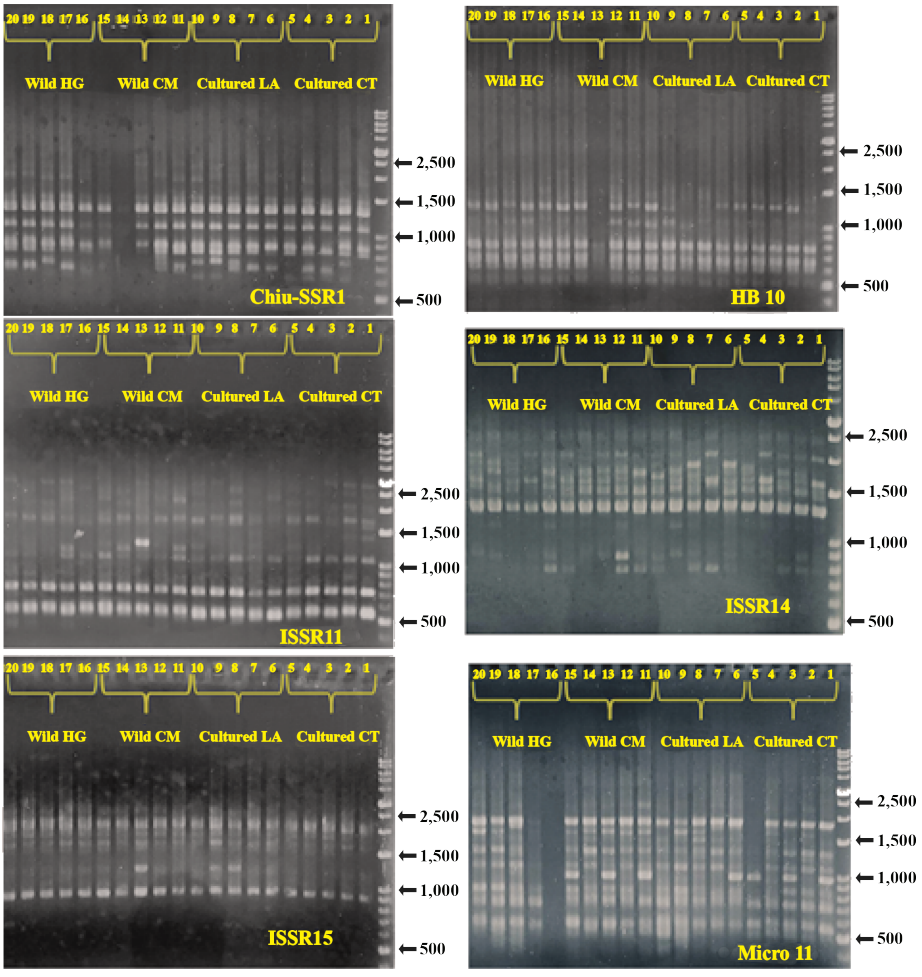

Figure 2. Gel electrophoresis images of ISSR amplifications using six primers for four bighead catfish populations.

each primer ranged from six (ISSR15) to 12 (ISSR11, ISSR14 and micro11), while band numbers from each population ranged from 58 (cultured CT) to 60 (cultured LA). No private bands were found among these bighead catfish populations.

Genetic diversity of bighead catfish populations

Levels of genetic diversity of bighead catfish populations were quantified by the genetic parameters presented in Table 2. The percentage of polymorphic loci of bighead catfish from the studied populations ranged from 75.4 % to 82.0 %. Values of the number of effective alleles, Shannon Index and unbiased expected heterozygosity varied from 1.42-1.50, 0.381-0.433 and 0.257-0.297, respectively.

When the genetic diversity of bighead catfish populations was compared, the wild HG population had the highest values of genetic diversity parameters (Ne = 1.50±0.05, I = 0.433±0.033 and uHe = 0.297±0.024), while the cultured LA had the lowest (Ne = 1.42±0.05, I = 0.381±0.034 and uHe = 0.257±0.024). Between cultured and wild populations, the genetic parameters of wild populations tended to be higher than those of the cultured ones. However, none of these parameters was significantly different ($p>0.05$), as all of the means had overlapping confidence intervals.

Genetic variance of bighead catfish populations

The overall genetic differentiation (GST) of bighead catfish populations was 0.038, and the estimated number of migrants per generation (Nm) among populations was 12.79. The range of Nei's unbiased genetic distance of bighead catfish populations was 0.007-0.019 (Table 3). The highest value of genetic differentiation was found between the cultured CT and the cultured LA, and the lowest one was between wild CM and wild HG. Overall genetic distance between cultured populations and wild populations was 0.004. These values illustrated that genetic differentiation among populations of bighead catfish was low.

Table 2. Genetic diversity (mean±SE) of bighead catfish populations.

Population	n	P (%)	Ne	I	uHe
Cultured CT	27	78.7	1.43±0.04	0.394±0.032	0.265±0.024
Cultured LA	28	75.4	1.42±0.05	0.381±0.034	0.257±0.024
Wild CM	28	78.7	1.49±0.05	0.424±0.033	0.290±0.024
Wild HG	29	82.0	1.50±0.05	0.433±0.033	0.297±0.024
Overall					
Cultured	55	82.0	1.45±0.04	0.410±0.031	0.273±0.023
Wild	57	86.9	1.51±0.05	0.440±0.032	0.298±0.023

Note: SE = standard error; n = sample size; P = Percentage of polymorphic loci; Ne = Number of effective alleles; I = Shannon's information index; uHe = Unbiased expected heterozygosity. uHe was calculated by the equation of uHe = (2n/(2n-1)) * (2pq), in which p and q are allele frequencies.

Table 3. Nei's unbiased genetic distance (below diagonal) and genetic identity (above diagonal) of bighead catfish populations.

Population	Cultured CT	Cultured LA	Wild CM	Wild HG
Cultured CT		0.981	0.991	0.991
Cultured LA	0.019		0.988	0.982
Wild CM	0.009	0.012		0.993
Wild HG	0.009	0.018	0.007	

From Nei's unbiased genetic distance, an UPGMA dendrogram was constructed to show the evolutionary relationship among populations of bighead catfish (Figure 3). The dendrogram divided bighead catfish populations into two main clusters, with the cultured LA alone in one cluster and the other three populations (cultured CT, wild CM, and wild HG) in the other. The wild CM and wild HG with the smallest genetic distance (0.007) were placed in the same smaller cluster in the dendrogram.

Analysis of molecular variance (AMOVA) using ISSR data demonstrated that genetic variance within populations accounted for 97.76 % of the total, while the remaining portion (2.24 %) was among populations of bighead catfish. Principal coordinates analysis (PCoA) also revealed no divisions among populations of bighead catfish (Figure 4). Just 6.79 % and 6.14 % of genetic variation was explained by coordinates one and two, respectively.

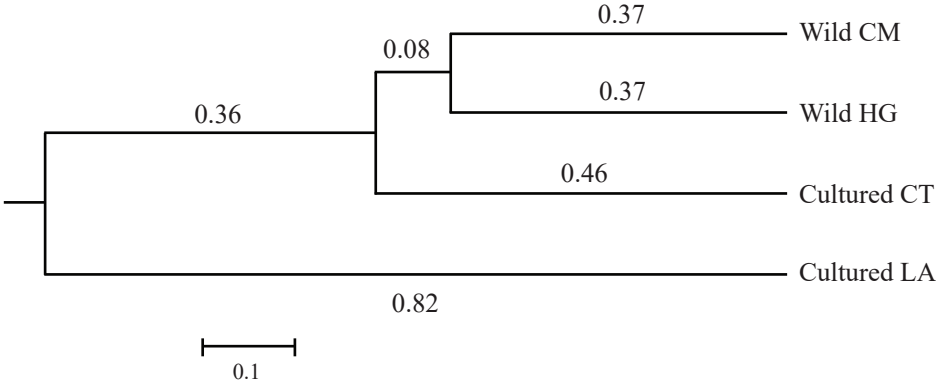

Figure 3. UPGMA dendrogram from Nei's unbiased genetic distance of bighead catfish populations.

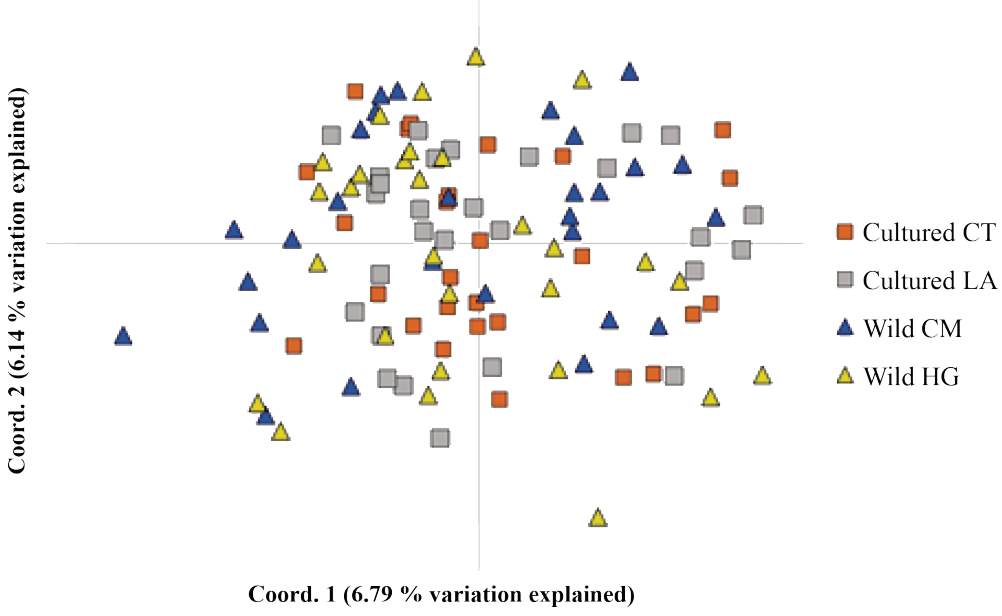

Figure 4. Principal coordinates analysis (PCoA) of bighead catfish populations.

DISCUSSION

Genetic diverstiy of bighead catfish populations

The present study revealed slightly higher genetic diversity of wild bighead catfish populations than cultured populations in the Mekong Delta. This results is consistent with the study by Duong and Scribner (2018), although the two studies employed different markers (dominant as ISSR and co-dominant as microsatellite). Lower genetic diversity in cultured populations could be due to genetic drift in small-size populations. However, opposite results were observed in a bighead catfish study in Thailand (Na-Nakorn *et al*., 2004), where reduction of genetic diversity of wild populations and high genetic variation of hatchery populations were revealed by isozyme analysis. Low genetic diversity of wild populations in Thailand was explained by population isolation, small population size, and/or historical population bottleneck. In other fish species, a trend similar to the present study has been recognized in genetic diversity between cultured and wild populations. Results from a study of swamp eel in central China showed higher genetic variation of wild populations than cultured populations using ISSR markers (Li *et al*., 2013). Another study using ISSR markers assessed the genetic structure of tambaqui (*Colossoma macropomum*), a native fish species in the Brazilian Amazon, and reported that all genetic variation indices of farmed populations were lower than in wild populations (Oliveira *et al*., 2019).

However, in bighead catfish, the cultured populations still maintained a relatively high level of genetic diversity, comparable to other catfishes belonging to the order Siluriformes. Slender walking catfish (*Clarias nieuhofii*) collected from three wild populations in Thailand exhibited values of Ne = 1.383-1.586 and I = 0.344-0.505 (Pechsiri and Vanichanon, 2016). Smaller values of genetic diversity, with Ne = 1.225-1.284 and I = 0.202-0.259, were also found in 102 individuals of yellow catfish (*Mystus nemurus*) distributed in Northeast Thailand (Kumla *et al*., 2012). In the Mekong Delta of Viet Nam, genetic diversity parameters of *Pangasius krempfi* were reported to be lower than the study species, with the average values of Ne = 1.365±0.048 and I = 0.310±0.037 (Duong and Nguyen, 2019). Similar to bighead catfish in Viet Nam, two cultured and wild populations in Northeast Thailand were found to have higher heterozygosity (based on four microsatellites) than the average of other freshwater fish species (Muiocha *et al*., 2017). Duong and Scribner (2018) explained the relatively high genetic diversity in cultured populations of bighead catfish as a result of large numbers of breeders used for seed production and annual exchanges of mature males. This is a good sign for using cultured bighead catfish populations for genetic improvement programs.

Genetic differentiation of bighead catfish populations

Values of the parameters GST and Nm indicated a high level of apparent gene flow among bighead populations, resulting in low pairwise Nei's unbiased genetic distance. Low genetic differences among wild populations of bighead catfish can be the consequence of the highly connected water system in the Mekong Delta (Duong and Scribner, 2018). In addition, small genetic differences between wild and cultured populations may result from two-way gene flow. On one hand, cultured bighead catfish can escape easily to the wild in the Mekong Delta during the flooding season, resulting in intraspecific introgression of escaped fish into wild populations. On the other hand, wild fish are sometimes supplemented to hatchery populations (Duong *et al*., 2017). In contrast to this study, Muiocha *et al*. (2017) reported a moderate genetic distance between one cultured strain (from Kasetsart University) and a wild strain collected in Northeast Thailand. Values of genetic distance of bighead catfish in the present study were lower than those of other fish species evaluated by the same ISSR markers conducted in one specific region. In endangered snakehead (*Channa lucius*) populations in the Mekong Delta, the range of genetic distance among four populations was higher, varying from 0.022 to 0.057 (Sawasawa and Duong, 2020). Likewise, roughskin sculpin (*Trachidermus fasciatus*) exhibited high genetic distance among populations, ranging from 0.032 to 0.151 (Bi *et al*., 2011). Comparing to other Siluriformes species, the genetic differentiation

of bighead catfish in Viet Nam was considerably lower than those of slender walking catfish (0.1381-0.2213) (Pechsiri and Vanichanon, 2016) and yellow catfish (0.149-0.619) (Kumla *et al*., 2012).

In addition, the major portion of genetic variance of bighead catfish was within populations (97.76 %). This level was also higher when compared to other fish species. Within-population variation was only 69.59 % in swamp eel from central China (Li *et al*., 2013) and 94.7 % in black sharkminnow (*Labeo chrysophekadion*) migrating along the Mekong River.

The findings from this study have implications for genetic improvement programs of bighead catfish. Crossbreeding between wild and cultured populations has been applied to look for heterosis (Tave, 1993; Dunham, 2011). However, for bighead catfish in the Mekong Delta, with so little genetic differentiation among populations, heterosis is unlikely upon crossing. Indeed, experiments on crossbreeding between these wild and cultured bighead populations did not result in heterosis for growth or survival of crossbreeds (Duong *et al*., 2022). Alternatively, selective breeding is another approach in genetic improvement (Tave, 1993; Dunham, 2011). For a selection program, a good base stock can be created by sampling across the delta because all bighead catfish populations have similar genetic diversity.

CONCLUSION

Bighead catfish populations analyzed using six ISSR primers had relatively high levels of genetic diversity and low genetic differentiation among four populations in the Mekong Delta of Viet Nam. There was less genetic variation in cultured populations than in wild populations.

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