Genetic diversity of the migratory Mekong endemic catfish species *Pangasius macronema* populating along the Hau and Tien Rivers

Mai Thy Le Nguyen, Yen Thuy Duong*

**ABSTRACT**

*Pangasius macronema* is an endemic and important species with high economic value for fisheries in the Mekong River basin. The rapid increase in fishing levels can have an impact on genetic diversity. This study evaluated the genetic diversity of three wild populations of *P. macronema* using intersimple sequence repeat (ISSR) markers. Six primers were used to analyze 72 samples from three locations (populations) along the Hau and Tien Rivers in An Giang (n=20), Soc Trang (n=29), and Dong Thap (n=23). The results showed that all populations of *P. macronema* had relatively high genetic diversity (effective number of alleles, Ne, from 1.381 to 1.454 and unbiased heterozygosity, uHe, from 0.243 to 0.286), with the Soc Trang population having the highest level. As a result, Nei’s unbiased genetic distances among the three populations were low, ranging from 0.015 to 0.035, with the smallest genetic distance between the An Giang and Dong Thap populations. The genetic differences among the three populations may be influenced by the species’ migration patterns and hydrological connectivity.

**Key words:** fish population, genetic diversity, ISSR markers, *Pangasius macronema*

**INTRODUCTION**

*Pangasius macronema* (Bleeker 1851) is a small migratory catfish belonging to the Pangasiidae family. This species is endemic to the Mekong River basin and can be found across the region, including Thailand, Cambodia, Laos, and Vietnam. The distribution of this fish generally occurs throughout the year, but it is more abundant during the migration period. Poulsen et al. (2004) reported that *P. macronema* migrates from the mainstream of the Mekong River into smaller tributaries and rivers from April to June. Migration can generate gene flow, inevitably affecting the genetic diversity of fish species. On the other hand, seasonal migration provides resources for fisheries in the area.

*P. macronema* also plays an important role in local fisheries with high yield and economic value, although it is a small fish. In the Mekong Delta, the main catch season for this species is from March to May. The catch production in Tien Giang Province – one branch of the Mekong River – is approximately 78.86 tons/year. Nevertheless, the diversity of wild populations in the Mekong Delta can currently be threatened by serious effects such as overexploitation, water pollution, and food shortages. Therefore, the size of wild fish populations tends to decrease rapidly, which can be associated with the decline in genetic diversity.

In recent years, *P. macronema* has been cultured in the Mekong Delta using wild-caught fingerlings. They can be raised in earthen ponds and cages along the Tien and Hau Rivers. The domestication process began because of an increase in seed demand for farming of this species. A good base broodstock population for domestication should be chosen based on genetic information. Based on the aforementioned factors, it is necessary to investigate the genetic diversity of *P. macronema*. Previous genetic studies of this species, however, have been limited. Among various molecular markers used to determine the genetic diversity of fish species, ISSR (intersimple sequence repeat) is a simple but high-efficiency marker. This study aimed to evaluate the genetic diversity of wild *P. macronema* populations along the Hau and Tien Rivers using ISSR markers. The results of this study provide preliminary genetic information for the management of wild populations and domestication program of this species.

**MATERIALS AND METHODS**

**Fish sampling**

*P. macronema* samples were collected from fishermen at three sites herein called three populations, including Thanh Binh district, Dong Thap province (DT), Chau Thanh district, An Giang province (AG), and

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Ke Sach district, Soc Trang province (ST). These sampling sites (Figure 1) are located at the two main river branches, Tien (DT) and Hau branch (AG and ST), of the Mekong River. The fish were identified based on morphological keys. This pangasiid species is characterized by a small body size (18-20 cm), large eyes, and long barbels reaching the pectoral-fin base. Samples from the three populations (n=23 for DT, n=20 for AG, and n=29 for ST) were chosen for genetic analysis. Fin clips of these samples were preserved in 95% ethanol until DNA analysis.

**Genetic diversity analysis methods**

**DNA extraction methods**

DNA was extracted from fin clips by using the ammonium acetate protocol. Approximately 25 mg of fin clip from each fish was first cut and then placed in an Eppendorf tube containing 650 μl of lysis buffer solution and 5 μl of Proteinase-K. After being incubated overnight at 55°C, all samples were added to 240 μl ammonium acetate and then incubated at 4°C for 30 minutes to precipitate the protein. Next, the tube was centrifuged at 10,000 rpm for 15 minutes two times, and 650 μl of the above solution was pipetted into a new tube. Then, 100% ethanol was added to the tube, incubated, and centrifuged to obtain DNA, which remained at the bottom of the tubes. Finally, the tube was air-dried, and 100 μl TE was added to obtain DNA.

**Screening primers and PCR**

Two samples from each population were selected randomly for primer screening. A total of 15 primers from ISSR libraries were screened. Six primers that had high polymorphisms and were visible on gels were chosen (Table 1). PCR amplifications were conducted in a 10 μl mixture containing 5 μl Promega PCR Master Mix (including Taq DNA polymerase supplied in a proprietary reaction buffer (pH 8.5), 400 μM dNTPs, and 3 mM MgCl₂), 0.4 μl primer (10 μM),...
Table 1: Sequences and annealing temperatures of the six primers used in the study

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence (5' – 3')</th>
<th>Ta (°C)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro11</td>
<td>[GGAC]4</td>
<td>44</td>
<td>Fernandes Matioli et al. (2000)</td>
</tr>
<tr>
<td>Chiu-SSR1</td>
<td>[GGAC]3A</td>
<td>46</td>
<td>Paza et al. (2007)</td>
</tr>
<tr>
<td>Chiu-SSR2</td>
<td>[GGAC]3C</td>
<td>46</td>
<td>Paza et al. (2007)</td>
</tr>
<tr>
<td>HB10</td>
<td>[GTG]5GC</td>
<td>46</td>
<td>Paterson et al. (2009)</td>
</tr>
<tr>
<td>ISSR15</td>
<td>[TCC]5</td>
<td>46</td>
<td>Tiwari et al., (2009)</td>
</tr>
<tr>
<td>17898A</td>
<td>[CA]6AC</td>
<td>50</td>
<td>Paterson et al. (2009)</td>
</tr>
</tbody>
</table>

Ta: Annealing temperature

1.0 μL DNA, and 3.6 μL nuclease-free water. Thermal conditions for PCRs included one denaturing cycle at 95°C for 5 minutes, exceeded by 38 repeated cycles of 95°C for 30 seconds, annealing temperature (Ta) from 44°C to 50°C (Table 1) for 40 seconds, extension at 72°C for 1 minute; and one final extension has one cycle at 72°C for 5 minutes.

Electrophoresis and band scoring

PCR results after amplification were checked with 1.2% agarose electrophoresis. Each gel contained a 1 kb DNA ladder (ABM, Canada) and individuals representative of the three populations. The electrophoresis process was carried out at 50 V for 80 minutes. Then, the gels were dipped in ethidium bromide (5 μg/mL) solution for staining for 15 minutes. The bands were visualized by scanning in a UV transilluminator, and band sizes were estimated based on the DNA ladder.

ISSR bands of each gel were scored by using a binary data matrix, '1' for the presence of the band and '0' for the absence of the band. To minimize errors in band scoring, only clear and intensified bands were scored (low-intensity bands were not scored), and scored bands were compared between gels of the same primers. Furthermore, scoring was performed independently by two lab technicians, and the results were then discussed to finalize the data.

Data analysis

Genetic diversity parameters, including the effective number of alleles (Ne), percentage of polymorphism, private alleles, unbiased expected heterozygosity (uHe), and Shannon index (I), were estimated for each population by using GenALEx 6.5 software. Genetic differences among the three populations were evaluated based on the overall genetic difference (G_{ST}), Nei's genetic distance, and principal coordinates analysis (PCoA). In addition, a dendrogram was constructed based on the unweighted pair-group method with arithmetic average (UPGMA) method using Popgene version 1.3.1 and MEGA 6.0.

RESULTS

ISSR variability

There were 72 samples from three populations using six primers (Table 1) for ISSR analysis. A total of 67 bands were generated ranging from 390 bp (17898A) to 3000 bp (Chiu-SSR1, Chiu-SSR2, and Micro11) (Figure 2). The number of bands of each primer fluctuated from 7 (ISSR15) to 15 (Micro11). Band numbers from each population ranged from 65 bands (AG) to 66 bands (DT and ST), in which one private band was found in the ST population.

Genetic diversity parameters of the three P. macronema populations

The genetic diversity parameters of P. macronema are presented in Table 2. The percentage of polymorphic loci varied from 80.6% to 95.03%. The number of effective alleles (Ne) fluctuated from 1.381 to 1.454; the Shannon index (I) ranged from 0.369 to 0.435, and the unbiased expected heterozygosity (uHe) ranged from 0.243 to 0.286. The ST population had the highest genetic diversity values (Ne=1.454±0.038, I=0.435±0.024, and uHe=0.286±0.019), and the lowest was the AG population (Ne=1.381±0.040, I=0.369±0.029, and uHe=0.243±0.021).

Genetic differences among the three Pangasius macronema populations

The overall genetic difference among populations (G_{ST}) was 0.053, and the number of migrants per generation (Nm) was 8.90. The values of Nei's unbiased genetic distance and genetic identity among the three P. macronema populations ranged from 0.015 to 0.035 and from 0.966 to 0.985, respectively (Table 3). The genetic distance between the ST and AG populations
Figure 2: ISSSR bands of *P. macronema* samples from three populations (Dong Thap, An Giang, and Soc Trang) using six primers (Micro11, 17898A, Chiu-SSR1, Chiu-SSR2, HB10, and ISSR15)

Table 2: Genetic diversity parameters of three *P. macronema* populations from Dong Thap, An Giang, and Soc Trang

<table>
<thead>
<tr>
<th>Population</th>
<th>Number of samples</th>
<th>P (%)</th>
<th>Ne</th>
<th>I</th>
<th>uHe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dong Thap</td>
<td>23</td>
<td>86.57</td>
<td>1.423 ± 0.039</td>
<td>0.404 ± 0.028</td>
<td>0.268 ± 0.021</td>
</tr>
<tr>
<td>An Giang</td>
<td>20</td>
<td>80.60</td>
<td>1.381 ± 0.040</td>
<td>0.369 ± 0.029</td>
<td>0.243 ± 0.021</td>
</tr>
<tr>
<td>Soc Trang</td>
<td>29</td>
<td>94.03</td>
<td>1.454 ± 0.038</td>
<td>0.435 ± 0.024</td>
<td>0.286 ± 0.019</td>
</tr>
</tbody>
</table>

Note: P: polymorphism, Ne: number of effective alleles, I: Shannon’s information index, uHe: unbiased expected heterozygosity

was the largest, and the AG - DT pair was the lowest. In addition, the genetic relationship among populations of this fish was shown by the UPGMA dendrogram using Nei’s unbiased genetic distance (Figure 3). The AG and DT populations are more genetically similar than the ST population.

The molecular analysis of variance (AMOVA) showed that the genetic variation within populations (97%) was larger than that among populations (3%). Principal coordinates analysis (PCoA) indicated the genetic difference among the three populations in a two-axis (coordinate) plot, with 8.54% and 6.51% of the total genetic variation explained by coordinates 1 and 2,

Figure 3: UPGMA dendrogram constructed using Nei’s unbiased genetic distances among three populations of *P. macronema* from Dong Thap, An Giang, and Soc Trang
Table 3: Nei’s unbiased genetic distance (below diagonal) and identity (above diagonal) among P. macronema populations

<table>
<thead>
<tr>
<th>Populations</th>
<th>Dong Thap</th>
<th>An Giang</th>
<th>Soc Trang</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dong Thap</td>
<td>*</td>
<td>0.985</td>
<td>0.983</td>
</tr>
<tr>
<td>An Giang</td>
<td>0.015</td>
<td>*</td>
<td>0.966</td>
</tr>
<tr>
<td>Soc Trang</td>
<td>0.017</td>
<td>0.035</td>
<td>*</td>
</tr>
</tbody>
</table>

respectively (Figure 4). The AG and the majority of individuals from DT populations were clustered together in the same region, whereas most individuals from the ST population were distributed further apart from the plot. The ST population, followed by the DT population, was more widely scattered on the coordinate plot than the AG population, indicating that these two (ST and DT) populations had larger levels of genetic diversity.

DISCUSSION

Levels of genetic diversity of P. macronema populations

The study found that all populations of P. macronema had high levels of genetic diversity (with Ne from 1.381 to 1.454, I from 0.369 to 0.435, and uHe from 0.243 to 0.286) in comparison with other fish species based on ISSR markers. A study on Pangasius krempfi showed lower values of genetic diversity parameters, with Ne from 1.352 to 1.376, I from 0.299 to 0.320, and uHe from 0.208 to 0.222 \(^22\). In yellow catfish (Mystus nemurus) \(^23\), the results of Ne ranged from 1.225 to 1.284, lower compared to the present study. On the other hand, P. macronema has a higher and equivalent level of genetic diversity than some scale fish species. Kissing gourami (Helostoma temminckii) had less genetic diversity than P. macronema (Ne from 1.295 to 1.387, I from 0.269 to 0.386) \(^24\). In addition, the genetic parameters based on eight ISSR primers of three Channa punctatus populations in the Mekong Delta were comparable to P. macronema, with Ne ranging from 1.422 to 1.458, I from 0.364 to 0.395, and uHe from 0.250 to 0.271 \(^25\). Although the AG population of P. macronema had lower genetic parameters than the other two populations, the magnitudes of the differences were small, ranging from 3% to 5% for Ne and from 9% to 15% for I or uHe.

The genetic diversity of a population depends on internal factors of the population, such as population size and random genetic drift, and external factors of the gene exchange process due to the migration of individuals among populations \(^4\). The level of genetic diversity has a positive correlation with population size (or the number of individuals) \(^26\). Compared to other Pangasius species, P. macronema can be found in abundance, especially during the flooding season (from July to November) in tributaries of the Tien and Hau Rivers \(^6,27\). Based on the catch data, 78.86 tons/year in Tien Giang Province, it can be predicted that P. macronema has a large population size in the Mekong Delta, resulting in the high level of genetic diversity of this fish. In small populations, random genetic variation is powerful, causing some genes to be completely lost \(^28\). In kissing gourami (Helostoma temminckii), a low level of genetic diversity was observed in a closed population resulting from a small population size \(^24\). Moreover, the ability to exchange genes during migration is also a factor that can increase the genetic diversity of the population \(^4\). Hypostomus ancistroides in Brazil was detected to have low genetic diversity, which might be related to the sedentary habits of the species, reducing gene flow between populations of different locations \(^29\).

Genetic differentiation among P. macronema populations

The genetic differentiation of the P. macronema populations was low, as indicated by the low values of G\(_{ST}\) (0.053) and Nei’s unbiased genetic distances and the high value of the number of migrants per generation (Nm=8.9). These parameters are indicative of
The findings of the present study have implications for the domestication and wild population management of *P. macronema*. In the Mekong Delta, domestication of *P. macronema* has been in progress with preliminary results of artificial reproduction (unpublished information). Because of their high genetic diversity, the three *P. macronema* populations, of which the ST population is relatively better, could be good resources for domestication. Furthermore, combining these groups would boost genetic variety in a base population, allowing for more effective domestication programs. In terms of wild fish management, low genetic differentiation among the three populations implies that they can be considered a "panmictic population", in which free genetic exchange occurs among individuals from different locations. In this case, it is suggested that they can be managed as one population.

**CONCLUSIONS & RECOMMENDATIONS**

The results from the ISSR markers showed that the three wild *P. macronema* populations had high levels of genetic diversity, of which the ST population had the highest level. The genetic differentiation among populations was low, as indicated by the low values of *G*_{ST} and Nei’s unbiased genetic distances and the high gene flow value.

It is recommended that the three populations be managed as the entire population of the Lower Mekong River system. These populations can be utilized to generate a base population for domestication efforts (either individually or better in combination).

**LIST OF ABBREVIATIONS**

AMOVA: analysis of molecular variance

*G*_{ST}: overall genetic difference among populations

I: Shannon index

ISSR: intersimple sequence repeat

Ne: number of effective alleles

Nm: number of migrants per generation

P: polymorphism

PCoA: principal coordinates analysis

PCR: polymerase chain reaction

uHe: unbiased expected heterozygosity

UPGMA: unweighted pair-group method with arithmetic average

**CONFLICT OF INTEREST**

The authors declare no conflicts of interest.

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AUTHORS’ CONTRIBUTIONS
Le Nguyen Mai Thọ contributed to the investigation, data analysis and writing.
Duong Thuy Yen contributed to conceptualization, funding, writing and editing.

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Thời gian phản biện: 50 ngày
Lập chỉ mục (Indexed): Google Scholar, Scilit

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