GENETIC DIVERSITY OF *Hemibagrus guttatus* IN THAI NGUYEN PROVINCE BY RAPD MARKERS

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SUMMARY

Hemibagrus guttatus, which is a species of the *Hemibagrus* genus of Barridae, distributed in few large rivers in the mountains and only in the rapid and strong currents. Because the economic benefits from *H. guttatus* are very high, this is hunted in large numbers. This study evaluated genetic diversity of *H. guttatus* from 24 collected individuals in Thai Nguyen based on ten primers of RAPD marker. Results showed that the average genetic similarity value between the individuals studied was 69.6%, the genetic similarity was ranged from 0.57 to 0.89. The samples were divided into two groups based on the value of genetic similarity, in which the samples collected in Phu Luong district had a similarity value of 57% in one group, unlike the others with similarity value 65%. Following research creates a scientific basis and an important scientific proof to conservation and development of *H. guttatus* genetic resources in Thai Nguyen province.

Keywords: **Genetic diversity, *Hemibagrus guttatus*, RAPD, Thai Nguyen province**.

I. INTRODUCTION

Hemibagrus guttatus, which is a species of the *Hemibagrus* genus of Barridae. In Vietnam, it is only found in the big rivers of the Northern such as Hong River, Da River, Lo River, Ma River; *H. guttatus* is distributed in China (Yunnan) and Laos. Due to the economic benefits of this species, the locals exploit this species in the wild without preserving its feeding and living habitat. Therefore, up to now, the number of *H. guttatus* in the wild is rapidly declined. For that reason, *H. guttatus* has been listed in the Vietnam Red List, the threat level VU A1c, d B2a, b - will be endangered, while declining by at least 20% as estimated due to habitat decline.

Information on population structure is useful for the development of management strategies that will conserve the biodiversity associated with different species, sub-species, stocks and races (Turan et al., 2005). Thus, detailed knowledge of the population structure is needed for sound management and successful commercial fishing of this species. Genetic diversity and gene pools which help in adaptation and survival is considered to be a key component for conservation and management of populations (Andayani et al., 2001). Molecular markers along with the development of new statistical tools have indeed revolutionized the analytical power necessary to explore the genetic diversity, both in native populations and in captive lots. In recent years, a wide range of new molecular techniques has been explored and reported for fishes. Random amplified polymorphic DNA (RAPD) is one of such techniques which was first introduced by Williams et al. (1990). RAPD technique is the one of the most frequently used molecular methods for taxonomic and systematic analyses of various organisms and has provided important applications in fish. RAPD is a technique based on the PCR amplification of discrete regions of the genome with short oligonucleotide primers of arbitrary sequence (Welsh and McClelland, 1990; Williams et al., 1990). It utilizes single, arbitrary, decamer DNA oligonucleotide primers to amplify regions of the genome based on the polymerase chain reaction (Hadrys et al., 1992;

Williams et al., 1993). The characters assessed through RAPD are useful for genetic studies because they provide various types of data like taxonomic population or inheritance pattern of various organisms including fishes.

The study of genetic diversity of *H. guttatus* is very much limited, so in the present study, this technique was applied to analyze the genetic relationship among *H. guttatus* populations. The objectives of this study are focused on the genetic variation of *H. guttatus*. That creates a scientific basis and an important scientific proof to conservation and development of *H. guttatus* genetic resources in Thai Nguyen province.

II. RESEARCH METHODOLOGY

2.1. Materials

A total of 24 fish specimens were randomly collected from 4 areas: Dai Tu district, Phu Luong district, Song Cong district and Thai Nguyen city (Table 1). All the fish specimens were kept in the iceboxes and brought to the laboratory for further study. The muscle tissues were isolated from freshly caught fishes and preserved at -20°C for further use.

Table 1. List of *H. guttatus* samples used in this research

Samples	Collection area	Samples	Collection area
NL1	Thai Nguyen city	NL13	Song Cong city
NL2	Thai Nguyen city	NL14	Song Cong city
NL3	Thai Nguyen city	NL15	Song Cong city
NL4	Thai Nguyen city	NL16	Song Cong city
NL5	Thai Nguyen city	NL17	Song Cong city
NL6	Thai Nguyen city	NL18	Song Cong city
NL7	Thai Nguyen city	NL19	Dai Tu district
NL8	Thai Nguyen city	NL20	Dai Tu district
NL9	Thai Nguyen city	NL21	Dai Tu district
NL10	Thai Nguyen city	NL22	Dai Tu district
NL11	Thai Nguyen city	NL23	Phu Luong district
NL12	Song Cong city	NL24	Phu Luong district

Table 2. List of primers used during this study

Oligo name	Sequence 5'-3'
OPA-07	GGA ACG GGTG
OPA-09	GGG TAA CGCC
OPA-11	CAA TCG CCGT
OPA-20	GTT GCG ATCC
OPAC-14	GTC GGT TGTC
OPAH-01	TTC GCA ACCA
Rm-1	CTG GGC ACGA
Rm-2	TTC CGC CACC
Rm-4	CCG CTA CCGA
Rm-5	CCT TTC CCTC

In the present study, 10 commercially available RAPD primers (Table 2), which were purchased from IDT, USA, were used to initiate PCR amplifications. Primers were randomly selected on the basis of GC content and annealing temperature for RAPD-PCR amplifications.

2.2. Methods

2.2.1. Isolation of genomic DNA from fish tissue

For the isolation of total genomic DNA, a modified protocol was followed using DNA isolation Kit (Norgen, Canada). UV-VIS spectrophotometer was used to check quality as well as quantity of isolated DNA. Optical densities of the DNA samples were measured at 260 nm and 280 nm and the concentration of extracted DNA was adjusted to 50 ng/µl for PCR amplification.

2.2.2. PCR amplification

The reaction mixture (25 µl) for PCR was composed of H_2O deion (9.5 µl), 2x PCR Master mix Solution (12.5 µl), 10 pmol/µl primer (1.0 µl), template ADN (2 µl). A negative control, without template DNA was also included in each round of reactions. After preheating for 5 minutes at 94°C, PCR was run for 35 cycles. It consisted of a 94°C denaturation step (1 min), 37°C annealing step (1 min) and 72°C elongation step (2 min) in a thermal cycler (Applied Biosystems, USA). At the end of the run, a final extension period was appended (72°C, 10 min) and then samples were stored at 4°C until the PCR products were analyzed.

2.2.3. Agarose gel electrophoresis

The amplified DNA fragments were separated on 1.2% agarose gel and stained with Redsafe solution (Norgen, Canada). A low range DNA marker of 1Kb from Norgen, Canada was run with each gel. The amplified pattern was visualized on an UV transilluminator and photographed by gel documentation system (BIORAD, USA).

2.2.4. Statistical analysis

The DNA fragments were scored for the presence and absence of fragments on the gel photographs and DNA fragments were compared among the *H. guttatus* individuals. RAPD banding patterns were recorded on spreadsheets, which were used to determine gene diversity, gene flow, number of polymorphic loci and genetic distance through a construct by an un-weighted pair group method of the arithmetic mean of UPGMA (Nei, 1978).

Genetic relationships among 24 individuals were constructed by UPGMA method using NTSYS v.2.1.

III. RESULTS AND DISCUSSION

3.1. Qualitative estimation of DNA

The quality of DNA extracted from different *H. guttatus* samples were analyzed by staining with Redsafe solution and the bands were seen under UV light. The single sharp bands in all the 24 lanes clearly indicated the presence of DNA in all samples. Spectrophotometric analysis of the DNA samples showed the 260 nm/280 nm ratio of the samples obtained in range from 1.8 to 2.0 which indicates the presence of pure DNA.

3.2. RAPD polymorphic analysis

The RAPD profile of bands obtained in the 24 varieties of *H. guttatus* with 10 random primers is shown in Table 3. These 10 random primers generated a total of 56 bands in all the

24 individuals out of which 66.70% bands were polymorphic. Bands size ranged from 0.3 – 2.0 Kb. Among these 10 primers, there was one primer which has polymorphism 100% (Rm-2), 6 primers had polymorphism over 50% (OPA-07, OPA-09, OPA-20, OPAH-01, Rm-1, Rm-4) and 3 primers had polymorphism less than 50% (OPA-11, OPAC-14, Rm-5).

Table 3. Pattern of polymorphism of 10 random primers

Primer	Total No. of DNA fragments	Total No. of polymorphic DNA fragments	The rate of polymorphism (%)
OPA-07	6	4	67.7
OPA-20	5	3	60.0
OPA-09	6	4	67.7
OPA-11	5	2	40.0
OPAC-14	6	2	33.3
Rm-5	5	3	36.0
OPAH-01	6	5	83.0
Rm-1	6	4	67.0
Rm-2	6	6	100
Rm-4	5	4	80.0
Total	56	37	66.1

The genetic similarity reflects the genetic relationship of the *H. guttatus* individuals. The greater is the similarity between two samples, the higher is their genetic similarity, and if there is the genetic similarity between two samples, the genetic relationship between them is closer. Results showed that the correlation coefficient between lobed tick samples ranged from 0.393 (NL13 and NL24) to 0.893 (NL19 and NL20) respectively from 39.3% to 89.3%. and the average genetic similarity value between the individuals studied was 69.6%.

The results showed that the samples of *H. guttatus* in Thai Nguyen have a moderate genetic diversity compared to some other authors' studies using the same primers. According to Yoon et al. (2001), the polymorphic ratio of five RAPD primers including OPA09 (as used in this study) in Korean catfish *(Silurus asotus*) ranged from 56.4% to 59.6%. Abdul Muneer et al. (2009) have investigated the polymorphic random DNA amplification (RAPD) to investigate the patterns and distribution of genetic diversity in the natural population of tilapia in the Western Ghat-India. The study used 32 samples and 10 random primers to evaluate genetic diversity, resulted in a total of 124 bands, including 75 polymorphic bands (60.48%). The heterozygosity of 0.72 indicates a high level of genetic diversity in the studied population.

Table 4. Nei's (1972) genetic similarity among 24 samples of H. guttatus estimated from RAPD profiles

	NL1	NL2	NL3	NL4	NL5	NL6	NL7	NL8	NL9	NL10	NL11	NL12	NL13	NL14	NL15	NL16	NL17	NL18	NL19	NL20	NL21	NL22	NL23	NL24
NL1	1.00																							
NL2	0.71	1.00																						
NL3	0.71	0.68	1.00																					
NL4	0.79	0.79	0.64	1.00																				
NL5	0.73	0.84	0.70	0.80	1.00																			
NL6	0.61	0.68	0.64	0.64	0.66	1.00																		
NL7	0.71	0.68	0.75	0.68	0.66	0.75	1.00																	
NL8	0.70	0.66	0.63	0.63	0.68	0.80	0.73	1.00																
NL9	0.71	0.61	0.71	0.64	0.63	0.79	0.79	0.77	1.00															
NL10	0.63	0.63	0.66	0.55	0.61	0.66	0.66	0.75	0.73	1.00														
NL11	0.75	0.68	0.71	0.68	0.66	0.64	0.68	0.73	0.68	0.70	1.00													
NL12	0.75	0.82	0.71	0.75	0.77	0.68	0.71	0.63	0.64	0.66	0.71	1.00												
NL13	0.79	0.79	0.71	0.79	0.84	0.64	0.68	0.63	0.61	0.55	0.75	0.86	1.00											
NL14	0.66	0.59	0.55	0.66	0.57	0.63	0.52	0.68	0.59	0.61	0.70	0.73	0.70	1.00										
NL15	0.71	0.61	0.64	0.68	0.66	0.64	0.64	0.73	0.68	0.66	0.64	0.61	0.61	0.63	1.00									
NL16	0.71	0.64	0.68	0.75	0.70	0.68	0.68	0.66	0.71	0.70	0.71	0.71	0.64	0.66	0.64	1.00								
NL17	0.64	0.79	0.71	0.57	0.77	0.82	0.71	0.73	0.68	0.70	0.68	0.75	0.71	0.59	0.61	0.61	1.00							
NL18	0.70	0.70	0.77	0.66	0.75	0.73	0.84	0.75	0.77	0.75	0.77	0.77	0.77	0.64	0.63	0.77	0.77	1.00						
NL19	0.71	0.68	0.61	0.71	0.73	0.54	0.61	0.59	0.61	0.52	0.75	0.71	0.79	0.70	0.61	0.61	0.64	0.63	1.00					
NL20	0.64	0.64	0.61	0.68	0.73	0.57	0.57	0.55	0.61	0.52	0.71	0.75	0.75	0.70	0.57	0.68	0.61	0.63	0.89	1.00				
NL21	0.68	0.64	0.68	0.68	0.63	0.64	0.57	0.59	0.64	0.63	0.75	0.71	0.75	0.77	0.57	0.61	0.68	0.63	0.79	0.75	1.00			
NL22	0.71	0.61	0.64	0.64	0.70	0.64	0.68	0.66	0.75	0.59	0.75	0.68	0.75	0.55	0.68	0.64	0.61	0.77	0.68	0.68	0.64	1.00		
NL23	0.59	0.55	0.63	0.48	0.57	0.59	0.59	0.57	0.63	0.57	0.59	0.52	0.48	0.54	0.59	0.55	0.73	0.54	0.59	0.55	0.59	0.63	1.00	
NL24	0.54	0.50	0.57	0.46	0.48	0.61	0.64	0.59	0.68	0.59	0.61	0.50	0.39	0.55	0.57	0.61	0.61	0.59	0.54	0.54	0.54	0.61	0.84	1.00

Based on the genetic similarity between samples, NTSYS software automatically arranges samples with similarity coefficients into one group and results are shown in a systematic tree graph. Jaccard numbers and UPGMA subtypes indicate the degree of genetic variation between the 24 specimens. Different levels are expressed by the difference coefficient between the samples.

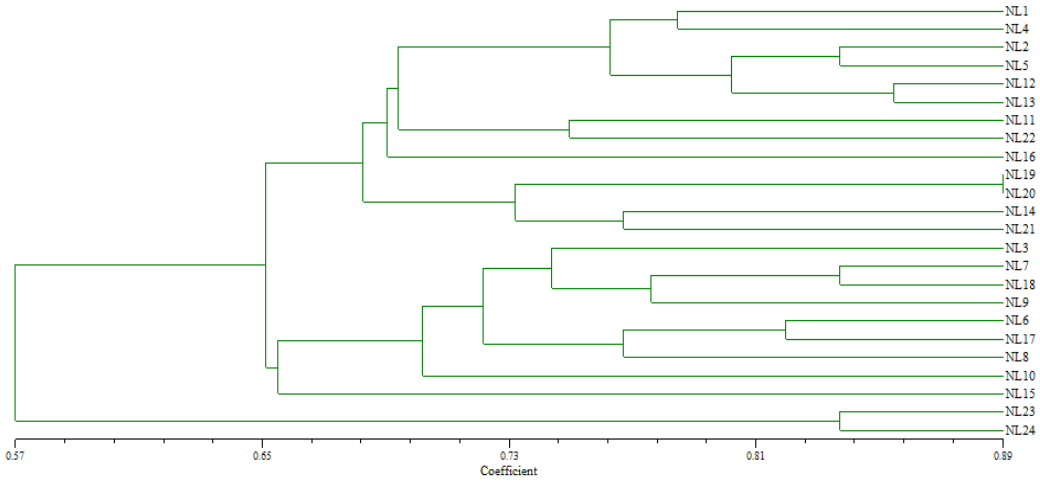

Figure 1. UPGMA dendrogram on the basis of Nei's (1972) genetic distance values at nodes represent proportion of similar replicates in H. guttatus species

Figure 1 shows that 24 specimens of *H. guttatus* were divided into two groups with a genetic similarity 0.57. Group 1 consisted of 22 samples divided into 5 subgroups with a genetic similarity 0.65. Of which, group 1 is divided into two sub-groups: Sub-group 1 has a similarity 0.682, consisting of 9 samples: NL1, NL2, NL4, NL5, NL11, NL12, NL13, NL16, NL22; And sub-branch NL14, NL19, NL20, NL21.Sub-group 2: has a similarity 0.698, consisting of 9 samples: NL3, NL6, NL7, NL8, NL9, NL10, NL15, NL17, NL18. Group 2 contains only samples NL23 and NL24 with high genetic similarity 0.835.

In conclusion, group 1 consisted of individuals which were collected in Thai Nguyen City, Song Cong City, and Dai Tu District. In particular, the individuals collected in two cities and district are scattered in both sub-groups 1 and 2, so there is a possibility of cross-genetic transfer between individuals of the two regions. Group 2 includes only individuals from Phu Luong District. Results of this analysis showed that the genetic similarity was ranged from 0.57 to 0.89.

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ĐA DẠNG DI TRUYỀN LOÀI CÁ LĂNG CHẤM (*Hemibagrus guttatus)* TẠI THÁI NGUYÊN BẰNG CHỈ THỊ RAPD

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TÓM TẮT

Cá lăng chấm (*Hemibagrus guttatus*) thuộc chi Cá lăng (*Hemibagrus*), họ Cá lăng (*Bagridae*), phân bố ở các con sông lớn thuộc các tỉnh phía Bắc như ở sông Hồng, sông Đà, sông Lô, sông Mã. Cá lăng chấm có giá trị kinh tế rất cao do đó chúng bị săn bắt với số lượng lớn nên đến nay số cá thể còn lại trong tự nhiên không nhiều. Đánh giá mức độ đa dạng di truyền của 24 mẫu Cá lăng chấm thu được tại Thái Nguyên dựa trên mười chỉ thị RAPD; kết quả cho thấy mức độ tương đồng di truyền trung bình giữa các mẫu nghiên cứu 69,6%, dao động trong khoảng 0,57 đến 0,898. Các mẫu nghiên cứu chia thành hai nhóm chính dựa vào hệ số tương đồng di truyền, trong đó các mẫu thu được tại huyện Phú Lương có hệ số tương đồng 57% thuộc một nhóm, khác với các mẫu còn lại có hệ số tương đồng 65%. Kết quả nghiên cứu là cơ sở khoa học phục vụ cho việc bảo tồn và phát triển nguồn gen Cá lăng chấm tại Thái Nguyên.

Từ khóa: **Cá lăng chấm, đa dạng di truyền, RAPD, Thái Nguyên.**

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