Can Tho University Journal of Science
website: sj.ctu.edu.vn

DOI: 10.22144/ctu.jen.2019.027

Genetic diversity of *Pangasius krempfi* in the Mekong River estuaries

Duong Thuy Yen[1*] and Nguyen Tien Vinh[2]

[1]*College of Aquaculture and Fisheries, Can Tho University, Vietnam*

[2]*Advanced Aquaculture Program Course 40, Can Tho University, Vietnam*

**Correspondence: Duong Thuy Yen (email: thuyyen@ctu.edu.vn)*

Article info.

Received 22Mar 2019
Revised 19 Jun 2019
Accepted 30 Jul 2019

Keywords

Genetic diversity, ISSR, Pangasius krempfi, population structure

ABSTRACT

Pangasius krempfi is an important catfish species for capture fisheries in the Mekong River basin. Overexploitation could lead to decreasing genetic diversity of this species. This study was aimed to quantify genetic diversity and structure of P. krempfi in the lower Mekong River using ISSR (Inter-simple sequence repeat) markers. Samples were collected from two estuaries of Tien (at Binh Dai, Ben Tre, BT) and Hau Rivers (at Cu Lao Dung, Soc Trang, ST). Twenty individuals per location (or group) were analyzed with six ISSR primers, generated a total of 32 bands with the size ranging from 500 – 2200 bp. The two fish groups had similarly moderate levels of genetic diversity. As the whole population, genetic parameters were (mean ± SE) 56.3 ± 3.1% of polymorphic loci, 1.365 ± 0.048 effective number of alleles, 0.215 ± 0.027 expected heterozygosity, and 0.310 ± 0.037 Shannon index. Genetic distance based on Nei's method between the two groups was 3.4%, accounting for 12% of total genetic variation. Principal coordinate and molecular genetic variance (AMOVA) analyses indicated that the two fish groups were genetically clustered at a low level, suggesting that they can be originated from different spawning groups of the same population.

Cited as: Yen, D.T. and Vinh, N.T., 2019. Genetic diversity of *Pangasius krempfi* in the Mekong River estuaries. Can Tho University Journal of Science. 11(2): 81-88.

1 INTRODUCTION

Pangasius krempfi Fang and Chaux, 1949, a member of Pangasiidae family, distributes along the Mekong River, from Luang Prabang province in northern Laos to coastal areas of Mekong estuaries in Vietnam (Poulsen and Valbo-Jørgensen, 2000; Poulsen and Hortle, 2004; Tran *et al.*, 2013). This is an anadromous species that migrates a long distance from the downstream in the Mekong Delta (Vietnam) to the upstream of the Mekong River (in Laos) for spawning (Baird, 1996; Hogan *et al.*, 2007). *P. krempfi* has high flesh quality and is a preferred food with high economic values. This species is an important fish for capture fisheries in the lower Mekong River basin (Baird, 1996; Poulsen and Hortle, 2004). Because of overexploitation, *P. krempfi* has been listed as vulnerable species (Baird, 2013) and thus needs to be set a high priority for conservation.

In a conservation program, knowledge of genetic diversity and genetic structure of a species is critical (Ellegren *et al.*, 2016). It is well documented that the decline in population sizes causes the loss of genetic diversity, reducing species adaptability to environmental changes in the future (Allendorf *et al.*, 2008; Pinsky and Palumbi, 2014). Up to now, no genetic diversity information of *P. krempfi* is available. In the Mekong Delta of Vietnam, the

species has been found in Hau and Tien Rivers, two branches of the Mekong River (herein fish in two locations are called "fish groups"). Previous studies proposed that *P. krempfi* larvae drift from spawning areas in Khone Fall to the downstream of the Mekong Delta (Baird, 1996; Hogan *et al.*, 2007). If larvae come from the same population and they enter randomly to Hau and Tien Rivers, they should have no genetic difference. Genetic data can be inferred to test this hypothesis.

Different DNA markers can be employed to investigate the genetic diversity of fish species (Liu and Cordes, 2004). Among which, the inter-simple sequence repeat (ISSR) is a dominant marker amplified by a polymerase chain reaction (PCR) with one primer that is complementary to a target microsatellite. Without prior knowledge on DNA of a target species, sequence segments between two neighboring microsatellites are amplified, yielding high polymorphic patterns (Bornet and Branchard, 2001). Thus, the ISSR technique is simple, inexpensive, and effective in population genetics studies.

In the present work, ISSR markers were used to quantify genetic diversity levels of *P. krempfi* in the Mekong Delta and test if two fish groups in Hau and Tien estuaries were genetically similar. Such information is important for better understanding the migratory pathway of the species in the lower Mekong River, contributing to the management and conservation of *P. krempfi*.

2 MATERIALS AND METHODS

2.1 Fish sampling

Fish samples were collected from fishermen at two estuaries at Binh Dai district, Ben Tre (BT) province and Cu Lao Dung district, Soc Trang (ST) province. These sampling sites are located at two branches, Tien branch and Hau branch, respectively, of the Mekong River. The fish was identified based on morphological keys provided from previous studies (Truong Thu Khoa and Tran Thi Thu Huong, 1993; Tran *et al.*, 2013; Duong *et al.*, 2016). Fin clips from 20 samples from each location were used for genetic analysis.

2.2 DNA extraction

DNA was extracted from fin clips using Promega genomic DNA purification kit. First, a piece of fin sample (20 mg) was placed in a 1.5 mL tube with 275 µL of digestion solution (containing 10 mg proteinase K) and then incubated overnight at 55°C. After incubation, 250µl of Wizard® SV Lysis Buffer was added and the entire lysate sample was transferred into a Wizard® SV Mini-column assembly put in a micro-centrifuge tube. The tube was centrifuged at 13,000 × g for three minutes to bind DNA into the Mini-column. Next, the DNA sample was washed four times with 650 µL of Wash Solution (containing 95% ethanol) and centrifuged at 13,000 × g for one minute. Finally, DNA was diluted in TE and stored at –20°C.

2.3 PCR amplification and visualization of ISSRs

Total 29 primers (Table 1) were screened by amplifying two random DNA samples from each sampling location. Primers were chosen based on three criteria including high polymorphisms, reproducibility and visibility on gels. Of the primers screened, six primers were selected for genetic diversity analysis (the first six rows in bold text, Table 1).

Primer amplifications (or PCR) 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), 400µM dNTPs, and 3mM $MgCl_2$), 0.4 µL primer (10 µM), 1.0 µL DNA, and 3.6 µL nuclease-free water. Thermal conditions for PCRs included one denaturing cycle at 94°C for four minutes, 40 cycles at [94°C for 45 seconds, annealing temperature (*Ta*) from 44°C to 51°C (according to primers) for 45 seconds, extension at 72 °C for two minutes], and one final extension cycle at 72°C for 10 minutes.

PCR products and a 1-kb ladder (Fermatas) were electrophoresed (80 minutes at 50 V) in 1.2% agarose gels. The gels were then soaked in ethidium bromide solution (0.5 µg/mL) before being visualized on a UV- transilluminator. The size of bands was estimated based on the ladder, and then each sample was scored as presence (1) or absence (0) for each band, forming a binary matrix data set for further analysis.

Table 1: List of primers used for screening in the study

No.	Primer	Sequence	Annealing temperature	Reference
1	HB10	5' [GA]6CC 3'	45ºC	Saad *et al.*, 2012
2	ISSR11	5'[CAC]3GC 3'	46ºC	Sharma *et al.*, 2011
3	Chiu-SSR1	5'[GGAC]3A 3'	46ºC	Pazza *et al.*, 2007
4	Chiu-SSR2	5'[GGAC]3C 3'	48ºC	Pazza *et al.*, 2007
5	Micro11	5'[GGAC]4 3'	44ºC	Fernandes *et al.*, 2000
6	EL02B	5'[AG]8T 3'	51ºC	Labastida *et al.*, 2015
7	ISSR03	5'[GACA]4 3'	46ºC	Rout *et al.* 2009
8	ISSR06	5'[GA]8C 3'	46ºC	Labastida *et al.*, 2015
9	ISSR14	5'[GCT]6C 3'	46ºC	Tanhuanpaa *et al.*, 2008
10	ISSR15	5'[TCC]5 3'	46ºC	Raghuwanshi *et al.* 2013
11	EL02A	5'[AG]7C 3'	53ºC	Labastida *et al.*, 2015
12	EL04A	5'AT[GACA]4 3'	53ºC	Labastida *et al.*, 2015
13	EL06A	5'[GACA]4AT 3'	53ºC	Labastida *et al.*, 2015
14	EL03	5'[GTG]5GC 3'	56ºC	Labastida *et al.*, 2015
15	EL05	5'[GAG]5GC 3'	56ºC	Labastida *et al.*, 2015
16	EL06B	5'[GACA]4AC 3'	54ºC	Labastida *et al.*, 2015
17	EL06D	5'[GACA]4TC 3'	54ºC	Labastida *et al.*, 2015
18	17899A	5'[CA]6AG 3'	52ºC	Saad *et al.*, 2012
19	17898A	5'[CA]6AC 3'	48ºC	Saad *et al.*, 2012
20	17898B	5'[CA]6GT 3'	48ºC	Saad *et al.*, 2012
21	844A	5'[CT]8AC 3'	44ºC	Saad *et al.*, 2012
22	844B	5'[CT]8GC 3'	44ºC	Saad *et al.*, 2012
23	841	5'[AG]8T 3'	44ºC	Kumla *et al.*2012
24	ANSSR1	5'[AACC]4 3'	44ºC	Kumla *et al.*2012
25	ANSSR6	5'[GGAT]4 3'	44ºC	Kumla *et al.*2012
26	EL01	5'[AG]8T 3'	52ºC	Labastida *et al.*, 2015
27	HB08	5'[GA]6GG 3'	48ºC	Eshak *et al.*, 2010
28	HB09	5'[GT]6GG 3'	48ºC	Eshak *et al.*, 2010
29	HB11	5'[GT]6CC 3'	48ºC	Eshak *et al.*, 2010

Note: The first six primers in bold text were selected for genetic diversity analysis.

2.4 Data analysis

Genetic diversity parameters including the percentage of polymorphism, private alleles, effective number of alleles, expected heterozygosity, and the Shannon index were estimated for each fish group using GenAlEx 6.5 software (Peakall and Smouse, 2012). Estimates of genetic diversity parameters across loci were compared between the two fish groups using independent-sample T-test in SPSS (version 20.0). Genetic distance and genetic identity between the two fish groups were also evaluated to test whether they are genetically different. To better understand the genetic relationship between Bong Lau fish groups, a group of 10 samples of Tra Ban *Pangasius mekongensis* was used as an outgroup to generate a phylogenic tree. Tra Ban samples were also amplified with the same six ISSR primers as being done for Bong Lau. The phylogenetic tree based on UPGMA (Unweighted pair group method with arithmetic mean) approach was constructed by using programs POPGEN (Yeh *et al.*, 1999) and MEGA 7.0 (Kumar *et al.*, 2016).

3 RESULTS

The amplification of six ISSR primers on 40 fish samples generated a total of 32 allelic bands with the size range from 500 to 2200 bp, in which each primer yielded from 5 to 6 bands. Examples of bands amplified with two primers HB10 and Micro are illustrated in Fig. 2. Genetic diversity of the whole fish samples was moderate, with (Mean ± SE) 56.3 ± 3.1% of polymorphic loci, 1.365 ± 0.048 effective alleles, 0.215 ± 0.027 expected heterozygosity, and 0.310 ± 0.037 Shannon index. Estimates of genetic diversity of fish in BT was insignificantly higher than those of ST fish group (Table 2).

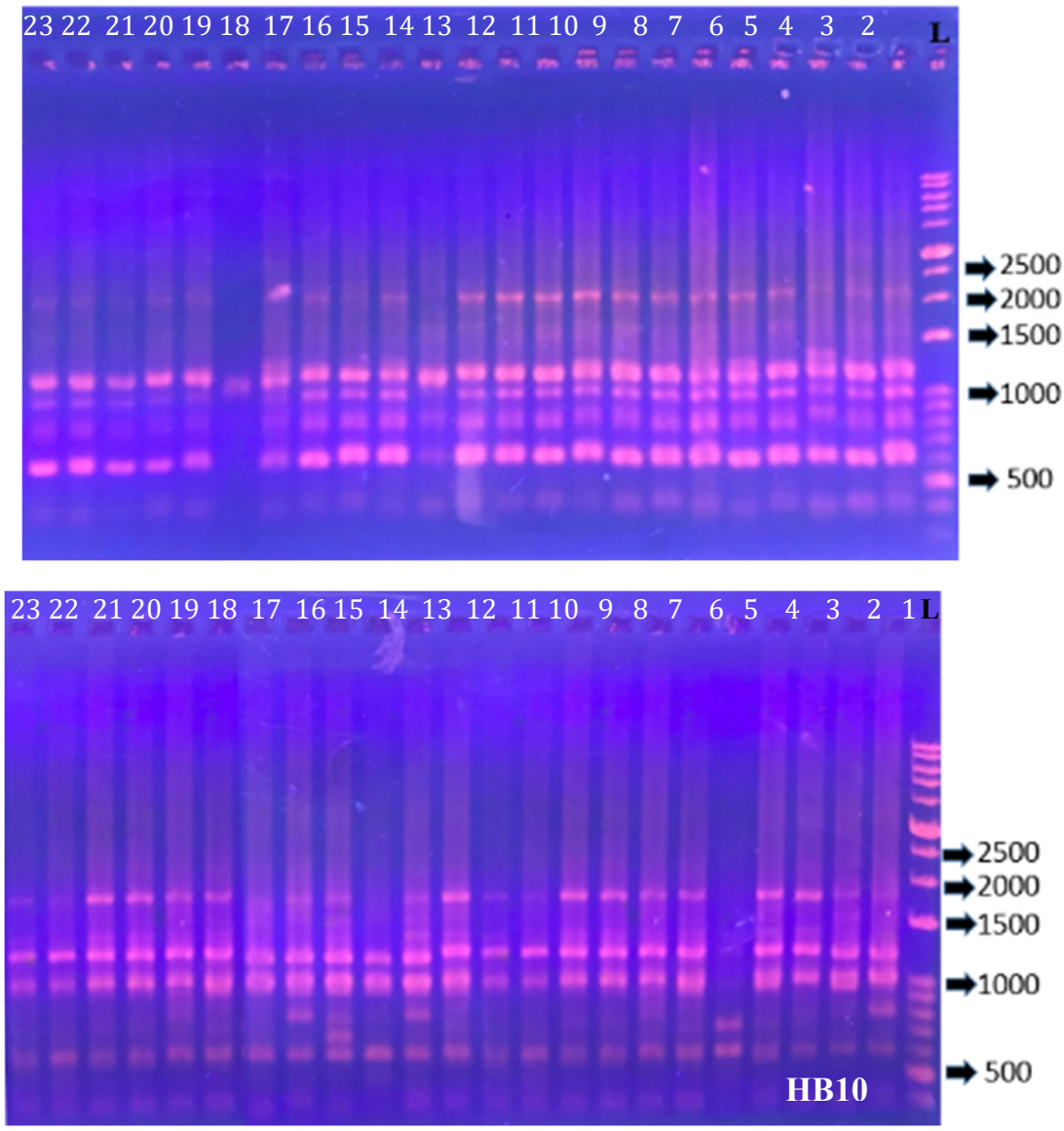

Fig. 2: Examples of ISSR bands from primers Micro11 and HB10 on P. krempfi samples

Note L: ladder; 1 – 12 Ben Tre samples; 13 – 23 Soc Trang samples

Table 2: Genetic diversity indices (SE) of P. krempfi groups produced by ISSR makers

Fish groups	N	Np	%P	Ne	He	I
Ben Tre	20	2	59.4	1.376 (0.069)	0.222 (0.038)	0.320 (0.037)
Soc Trang	20	0	53.1	1.352 (0.069)	0.208 (0.038)	0.299 (0.053)
Total	40	2	56.3 (3.1)	1.365 (0.048)	0.215 0.027)	0.310 (0.037)

Note: N: sample size, Np: private alleles, %P: Percentage of polymorphic loci, Ne: Number of effective alleles, He: Expected heterozygosity, I: Shannon index

Genetic difference between the two fish groups was revealed by Nei's genetic distance, principal coordinates analysis (PCoA), and phylogenetic relationship. Genetic distance between BT and ST groups was 3.4%, or their genetic identity was 96.6%. Analysis of molecular variance (AMOVA) showed that a majority of genetic variation (88%) was from within fish groups, while 12% of genetic variation resulted from between fish groups (Table 3). The PCoA plot (Fig. 3) indicated that the two fish groups were clustered (only a few individuals were mixed

between two fish groups) in the coordinate 1, contributing to 19.1% genetic variation. However, in the presence of Tra Ban data as an outgroup, the two fish groups were more genetically similar and both were distinct from Tra Ban (Fig. 4). The intra-specific genetic distance of Bong Lau was approximately 48-folder less than genetic distance between the two species (estimated based on branch lengths of the phylogenetic tree, Fig. 4).

Table 3: Analysis of molecular variance (AMOVA)

Source	df	Sum of square	Mean of square	Estimated variation	% of total variation
Between fish groups	1	12.1	12.1	0.45	12%
Within fish groups	38	121.9	3.2	3.21	88%
Total	39	134.0		3.66	100%

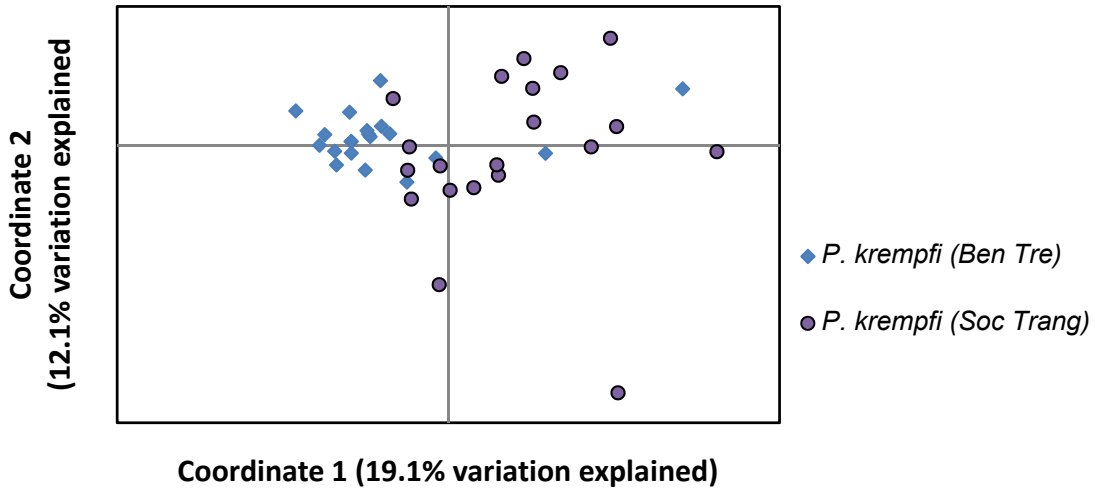

Fig. 3: A plot of principal coordinates analysis (PCoA) of two *P. krempfi* groups

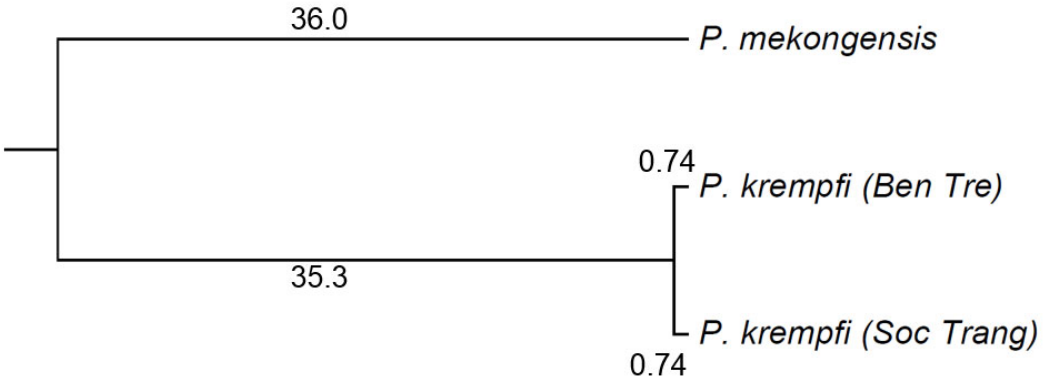

Fig. 4: UPGMA-based phylogenetic tree of *P. krempfi* groups and *P. mekongensis* (numbers present branch lengths estimated based on Nei's genetic distance)

4 DISCUSSION

The results of the present study show that genetic diversity of *P. krempfi* was moderate, and the species had a low level of genetic difference between fish in two branches of the Mekong River. Estimates of genetic diversity parameters of *P. krempfi* were not significantly different between the two fish groups, and these parameters of the whole population (effective alleles Ne: 1.365 ± 0.048, expected heterozygosity He: 0.215 ± 0.027, and Shannon index I: 0.310 ± 0.037) were comparable to those of other species based on similar dominant markers such as ISSR and RADP (Random amplified polymorphic DNA). On climbing perch (*Anabas testudineus*), mean estimates of Ne, He, and I of four wild and cultured populations were 1.364, 0.223, and

0.347, respectively (Pham Thi Trang Nhung and Duong Thuy Yen, 2014). On kissing gourami (*Helostoma temminckii*) in the Mekong Delta, levels of genetic diversity varied among populations, expected heterozygosity ranging from 0.180 to 0.245, and Shannon index from 0.269 to 0.386 (Duong *et al.*, 2018). Another study using ISSR found that lionfish (*Pterois species*) populations in Guanahacabibes (Cuba) had heterozygosity value of 0.253 ± 0.019 (Labastida *et al.*, 2015), relatively higher than that of *P. krempfi*. However, some other species were reported to have lower genetic diversity compared to *P. krempfi*. The yellow catfish (*Mystus nemurus*) populations in Thailand had He (based on seven ISSR markers) from 0.134 to 0.171, and I range of 0.202 to 0.247 (Kumla *et al.*, 2012). In Japanese flounder (*Paralichthys olivaceus*), genetic diversity of three hatchery populations based on 12 ISSR markers was low with He from 0.092 to 0.108, and I from 0.117 to 0.143 (Liu *et al.*, 2006).

Principal coordinates analysis (variation showed by coordinate 2, Fig. 3) and between-group genetic variation (12% of total variance, Table 3) indicate that two groups of *P. krempfi* had clustering structure at a low level. Their genetic distance (3.4%) was lower than that of inter-populations (based on ISSR markers) in other species. For example, genetic distances among populations of yellow catfish in Japan were high, from 14.9% to 61.9% and correlated with geographic distance, indicating genetic isolation by distance of these populations (Kumla *et al.*, 2012). Genetic distance among four kissing gourami populations in the Mekong Delta was from 2.3% to 10.2 (Duong *et al.*, 2018), comparable to or higher than that of *P. krempfi*.

Low genetic distance and weak genetic structure of *P. krempfi* groups indicated that they originate from the same population. This result can be explained by migration behavior of the species and water connectivity in Mekong estuaries. *P. krempfi* is anadromous, the adult fish migrates upstream of the Mekong River (in Laos) for spawning (Baird, 1996; Hogan *et al.*, 2007). When young fish individuals drift downstream to southern Vietnam, they can enter two branches (Tien and Hau Rivers) of the Mekong River. Thus, fish samples collected from different downstream locations can be originated from the same population. In addition, in estuary areas, *P. krempfi* can migrate along the coastal line including two sampling sites in Hau and Tien estuaries.

However, the two fish groups exhibited a small level of genetic structure (Table 3 and Fig. 3), probably because young fish in two locations could be produced by different broodstock groups spawning at different times of the same population. The spawning season of *P. krempfi* can be from May to early November when this species has been observed to be in maturation conditions at Khone Falls in Laos (Baird, 1996). Such a long spawning season is more likely attributed by several spawning groups returning to the spawning ground at different times. Several studies in other migratory species such as pink salmon *Oncorhynchus gorbuscha* (Smoker *et al.*, 1998) or lake sturgeon *Acipenser fulvescens* (Forsythe *et al.*, 2012) found that difference in spawning time had a genetic basis. Consequently, spawning groups can be genetically different. Coulson *et al.* (2006) found that early and late spawning adults of rainbow smelt *Osmerus mordax* along the east coast of Canada had genetic differentiation with the magnitude of difference comparable with that of spatially separated populations. Similarly, *Prochilodus costatus*, a freshwater migratory fish, also displays genetic structure among adult groups within a spawning season (Braga-Silva and Galetti, 2016). Based on the result of the present study, a hypothesis is proposed that spawning adults of *P. krempfi* can consist of several groups differing in spawning time and/or genetics. This hypothesis is similar to a prediction from previous studies (Rainboth, 1996; Sokheng *et al.*, 1999). These authors predicted that there may be at least two spawning populations of *P. krempfi*, one population in the upper Khone Falls migrates for spawning from May to September; the other population in the lower Khone Falls spawn between May and August. Further ecological and genetic studies in upstream sites (in Laos) can test this hypothesis.

5 CONCLUSION AND RECOMMENDATION

P. krempfi in the downstream of the Mekong River has moderate genetic diversity and low genetic structure inferred from ISSR markers. The results suggest that the two fish groups can be originated from different spawning groups of the same population in the upstream.

The species should be concerned under proper management strategies. In addition, more ecological and genetic information of this species in the upstream sites should be investigated for conservation purposes of this species.

ACKNOWLEDGMENTS

This research is a part of the project "Reproductive biology and genetic diversity of *Pangasius krempfi* and *Pangasisus mekongensis*" (Project number B2017-TCT-22ĐT), funded by the Ministry of Education and Training. The authors thanks MSc. Nguyen Thi Ngoc Tran for lab work assistance.

REFERENCES

Allendorf, F. W., England, P. R., Luikart, G., Ritchie, P. A., and Ryman, N., 2008. Genetic effects of harvest on wild animal populations. Trends in Ecology and Evolution, 23(6): 327–337.

Baird, I., 1996. Khone falls fishers. Catch and Culture: Mekong Fisheries Network Newsletter, 2(2): 2-3.

Baird, I., 2013. *Pangasius krempfi*. The IUCN Red List of Threatened Species: e.T181328A7668262.

Bornet, B., and Branchard, M., 2001. Nonanchored inter simple sequence repeat (ISSR) markers: reproducible and specific tools for genome fingerprinting. Plant Molecular Biology Reporter, 19 (3): 209–215.

Braga-Silva, A., and Galetti, P. M., 2016. Evidence of isolation by time in freshwater migratory fish *Prochilodus costatus* (Characiformes, Prochilodontidae). Hydrobiologia, 765(1): 159–167.

Coulson, M. W., Bradbury, I. R., and Bentzen, P., 2006. Temporal genetic differentiation: Continuous v. discontinuous spawning runs in anadromous rainbow smelt *Osmerus mordax* (Mitchill). Journal of Fish Biology, 69(SUPPL. C): 209–216.

Tran D.D., Koichi, S., Phương, N. T., *et. al.,* 2013. Fishes of the Mekong Delta, Vietnam. Can Tho University Publishing House.

Duong T. Y., Nguyen Phuong Thao, Tieu Van Ut, and Tran Dac Dinh, 2018. Genetic diversity of kissing gourami (*Helostoma temminkii*) in the Mekong Delta. Can Tho University Journal of Science, 54(7B): 86–93 (in Vietnamese).

Duong, T. Y., Nguyen, K., Bui, S. N., Nguyen, V. T., Nguyen, B. L., & Tran, D. D., 2016. DNA barcode and morphology of *P. krempfi, P. mekongensis,* and *P. elongatus*. Journal of Biotechnology 14(1): 29-37 (in Vietnamese)

Ellegren, H., Galtier, N., and Ellegren, N., 2016. Determinants of genetic diversity. Nature Reviews Genetics, 17(7): 422–433.

Eshak, M. G., Ebeed, N. M., Booles, H. F., and Fahmy, K., 2010. Genetic assessment of salinity tolerance in red tilapia. New York Science Journal, 3(12): 132–141.

Fernandes-Matioli, F.M.C., Matioli, S.R., Almeida-Toledo, L. F., 2000. Species diversity and geographic distribution of Gymnotus (Pisces: Gymnotiformes) by nuclear (GGAC)n microsatellite analysis. Genetics and Molecular Biology, 23(4): 803–807.

Forsythe, P. S., Crossman, J. A., Bello, N. M., Baker, E. A., Scribner, K. T., and Tonn, W., 2012. Individual-based analyses reveal high repeatability in timing and location of reproduction in lake sturgeon (*Acipenser fulvescens*). Canadian Journal Of Fisheries and Aquatic Science, 69(1): 60–72.

Hogan, Z., Baird, I. G., Radtke, R., and Vander Zanden, M. J., 2007. Long distance migration and marine habitation in the tropical Asian catfish, *Pangasius krempfi*. Journal of Fish Biology, 71(3): 818–832.

Kumar, S., Stecher, G., and Tamura, K., 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular Biology and Evolution, 33(7): 1870–1874.

Kumla, S., Doolgindachbaporn, S., Sudmoon, R., *et al.*, 2012. Genetic variation, population structure and identification of yellow catfish, *Mystus nemurus* (CandV) in Thailand using RAPD, ISSR and SCAR marker. Molecular Biology Reports, 39(5): 5201–5210.

Labastida, E., Cobián, D., Hénaut, Y., *et al.,* 2015. ISSR markers for Pterois species The use of ISSR markers for species determination and a genetic study of the invasive lionfish in Guanahacabibes, Cuba. J. Aquat. Res, 43(5): 1011–1018.

Liu, Y.-G., Chen, S.L., Li, J., 2006. Genetic diversity in three Japanese flounder (*Paralichthys olivaceus*) populations revealed by ISSR markers. Aquaculture, 255(1): 565–572.

Liu, Z. J., and Cordes, J. F., 2004. DNA marker technologies and their applications in aquaculture genetics. Aquaculture, 238(1-4): 1–37.

Pazza, R., Kavalco, K. F., Prioli, S. M., *et al.,* 2007. Chromosome polymorphism in *Astyanax fasciatus* (Teleostei, Characidae), Part 3: Analysis of the RAPD and ISSR molecular markers. Biochemical Systematics and Ecology, 35(12):843–851.

Peakall, R., and Smouse, P. E., 2012. GenALEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics, 28(19): 2537–2539.

Pham Thi Trang Nhung and Duong Thuy Yen, 2014. Genetic diversity of climbing perch (*Anabas testudineus*, Bloch 1792) populations based on RAPD and ISSR markers. Can Tho University Journal of Science, 1 (Special issue in Aquaculture): 86–93 (in Vietnamese).

Pinsky, M. L., and Palumbi, S. R., 2014. Meta-analysis reveals lower genetic diversity in overfished populations. Molecular Ecology, 23(1): 29–39.

Poulsen, A. F., and Valbo-Jørgensen, J., 2000. Fish Migrations and spawning habits in the Mekong mainstream: A survey using local knowledge. AMFC Technical Report, (0005), 1–149.

Poulsen, A., and Hortle, K., 2004. Distribution and ecology of some important riverine fish species of the Mekong River Basin. MRC Technical Paper No. 10.

Rainboth, W.J., 1996. Fishes of the Cambodian Mekong, FAO species identification field guide for fishery purposes. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.

Saad, Y.M., Rashed, M.A., Atta, A.H., *et al.,* 2012. Genetic diversity among some tilapia species based on ISSR markers. Life Science Journal, 9(4): 4841–4846.

Sharma, S. K., Kumaria, S., Tandon, P., *et al.,* 2011. Single primer amplification reaction (SPAR) reveals inter- and intra-specific natural genetic variation in five species of *Cymbidium* (Orchidaceae). Gene, 483(1–2): 54–62.

Smoker, W. W., Gharrett, A. J., and Stekoll, M. S., 1998. Genetic variation of return date in a population of

pink salmon: a consequence of fluctuating environment and dispersive selection? Alaska Fishery Research Bulletin, 5(1): 46–54.

Sokheng, C., Chhea, C.K., Viravong, S., et al., 1999. Fish migrations and spawning habits in the Mekong mainstream: a survey using local knowledge (basin-wide). Assessment of Mekong fisheries: Fish Migrations and Spawning and the Impact of Water Management Project (AMFC). AMFP Report 2/99. Vientiane, Lao P.D.R.

Truong Thu Khoa and Tran Thi Thu Huong, 1993. The freshwater fish species in the Mekong Delta. Faculty of Fisheries. Can Tho University (in Vietnamese).

Yeh, F., Yang, R., and Boyle, T., 1999. Popgene version 1.3.1. Mircosoft Window-based Freeware for Population Genetic Ananlysis. University of Alberta and Centre for International Forestry Research. Edmonton, Alto, 1–29.