Cross-Species Amplification of Microsatellite DNA Markers and Comparison on the Sequence of Microsatellite Regions Among Southeast Asian Catfish Species

Muhammad Fitri YUSOF[1,2]* Siti Shapor SIRAJ[1] and Masamichi NAKAJIMA[2]

[1] Institute of Oceanography and Marine Sciences, International Islamic University, Malaysia
[2] Graduate School of Agricultural Science, Tohoku University, Japan

Abstract

Thirty microsatellite markers developed for River catfish (*Hemibagrus nemurus*) were screened for cross amplification in six species of catfish. Out of 30 primers, only 3 (10%) to 6 (20%) produced successful amplifications in six catfish species. Six different loci could be amplified in *Pangasius pangasius* and *Clarias batrachus*, 5 loci in *Clarias gariepinus*, 4 loci in *Pseudomystus siamensis* and 3 loci in *Clarias macrocephalus* and *Pangasius nasutus*. The low rate of amplification in these 6 closely related species proved the primers are locus-specific. Sequence analysis of locus MnRm7-1 showed differences in the flanking region and repeat motif in certain species, suggesting the existence of indel mutations in catfish species in that particular region. The examined DNA sequence revealed that the repeat motif is conserved within Pangasiid catfishes and not conserved within Clariid catfishes.

（accepted January 15, 2015）

Introduction

Catfish are an important fishery resource both as a source of income and an alternative protein for most Southeast Asian nations[1–3]. Indeed, in the most recent accounting, catfish species made up for 67% of total freshwater aquaculture production in Malaysia[4]. Catfish have also been used as an animal model in reproductive, physiological and genetic studies. Species from three major families – namely, Bagriidae, Clariidae and Pangasiidae – are of particular commercial importance and widely cultured in Southeast Asia.

The study of genetics has been of major importance for the aid aquaculture sector and assist conservation programme. Microsatellite DNA markers are short tandem repeat motifs with high levels of polymorphism[5,6], and are distributed in both coding and non-coding regions[7]. These markers have been a subject of great interest due to their co-dominant characteristics, which make them more informative than dominant markers such as RAPD and AFLP. In aquaculture, microsatellites have become one of the most useful molecular markers in fish population studies and have supplemented conventional markers such as allozymes and mitochondrial DNA that show totally low overall levels of variation[8].

Microsatellite markers also show high allelic variation that makes them effective as markers in many genetic studies. Microsatellite markers are widely applied in studies on population genetic structures and genetic diversity of species. However, the development of microsatellite markers is very time- consuming and labor-intensive. As a potential alternative, some microsatellite markers have been shown to be conserved within related taxa[9,10]. Through cross-species amplification, microsatellite markers

*Corresponding author: Muhammad Fitri Yusof, 1-1 Tsutsumidori-Amamiyamachi, Aoba, Sendai, Miyagi 981-8555, Japan
e-mail: fitriyusof@yahoo.com

developed for source species can be amplified in target species[11,12]. Cross-species amplification in catfish species has been tested by many researchers, and has led to the identification of polymorphic microsatellite loci in target species[13–15].

In the present study, we examined cross-species amplification of primers developed for the river catfish *H. nemurus* in 6 catfish species collected in Malaysia. Our objective was to identify potential microsatellite markes that could be used in target species.

Materials and Method

1. Fish Samples

A total of 210 samples of 7 catfish species were collected from 7 locations in Peninsular Malaysia. Fresh samples were identified based on previous study[16], labelled, and immediately frozen at −20℃ in order to preserve their condition. Details of each sample are represented at Table 1.

2. DNA Extraction

The muscle tissues were taken from fresh samples for the DNA extraction process. The DNA extraction was performed using commercial Promega® Wizard Genomic DNA Purification Kit. DNA quantification was performed and DNA maintained at a temperature below −20℃ until further use. Thirty microsatellite primer pairs developed[17–20] were tested on 7 species in this study (Table 2).

3. PCR Conditions and Electrophoresis

Each primer selected was tested for cross-amplification in every species using Touchdown PCR or normal PCR. The PCR conditions are as follows: 95℃ for 3 min, followed by 5 cycles of 95℃ for 30s and annealing at different temperature (see Table 3) or following touchdown PCR for 45s, and 72℃ for 60s and extension of 72℃ for 15 minutes. The primers and its repeat sequence were represented at Table 2. Since several primers show problems during amplification, PCR touchdown (MJ Research, USA) was performed to screen for primers capable of amplifying the selected DNA region The PCR touchdown with 5 different annealing temperatures (60,58,56,54,52,48) consisting 5 cycles for each annealing temperatures respectively.

A 10 µl PCR reaction consisting of 1.5–2.5 mM $MgCl_2$, 0.2 mM dNTP, 0.5 µM of the forward and reverse primer, 1X of Buffer, 0.1 unit of Taq Polymerase and 0.1 µl genomic DNA as template. Initially, 3 random samples from each species were used during the optimization. The primers that showed positive amplifications were further evaluated using larger number of samples. PCR product was resolved by electrophoresis on 2.5% metaphor agarose gels, stained with GelRed™ 3X (GENTAUR) and viewed under UV and photographed using Alpha imager Gel Documentation System (Syngene).

4. DNA Purification and Sequencing

The target PCR product from single individual of each species was excised from the gel and purified using GeneJET™ PCR Purification Kit-Fermentas, then used for sequencing analysis. DNA sequence obtained for each species was submitted to the online program WebSAT[21] (http://wsmartins.net/websat/) to identify tandem repeats in each sequence. Repeated sequence repeat was considered a microsatellite when the number of repeats was more than three. All sequences were deposited at DNA DATA Bank of Japan (DDBJ).

Table 1. Sample size of each species used in this study

Family	Species	Sample size	Collection Date	Location
Bagridae	*Hemibagrus nemurus*	30	March 2009	Tasik Banding, Perak
	Pseudomystus siamensis	30	April 2011	Jelebu, Negeri Sembilan
Pangasidae	*Pangasius pangasius*	30	March 2009	Kuala Kangsar, Perak
	Pangasius nasutus	30	June 2010	Perlok, Pahang
Clariidae	*Clarias macrocephalus*	30	Dis 2008	Maran, Pahang
	Clarias batrachus	30	Jan 2009	Yong Peng, Johor
	Clarias gariepinus	30	April 2009	UPM Hatchery, Selangor

Table 2. Information of microsatellite loci of Hemibagrus nemurus used in this study

No	Locus	Primer sequences	Expected size	Tandem repeat	Accession Number	Source
1	*MnBP5-1-115b*	F: TTTTGCTACTAGAGACTGAC R: TAGGCAAAACGTGTACTTG	179 bp	$(GT)_4$	AF544042	Hoh *et al*. 2013[19]
2	*MnBP5-2-05*	F: CAAGTGCAAAGACAGACAGA R: TCTCTAAGGCTATCCATCCA	186 bp	$(TGGA)_2N(AGAC)_3$	AY207448	Hoh *et al*. 2013[19]
3	*MnBP5-2-05d*	F: CATGGTGGATGGATAGATTT R: TGTTATCATACACCAGTCAC	168 bp	(TGGA)	AY207448	Hoh *et al*. 2013[19]
4	*MnBP5-2-06b*	F: CGTGTCCAGACATGGTTAAT R: GAGTGGGCGACTTTCAG	164 bp	$(CAT)_2CAC(CAT)_8$	AY671084	Hoh *et al*. 2013[19]
5	*MnBP5-2-06c*	F: CCCACTCTTCCTTCTTATCC R: TGGTCTGAGCGCTAGAG	132 bp	$(TCT)_3(CT)_7$	AY671084	Hoh *et al*. 2013[19]
6	*MnBP5-2-24b*	F: GTCATATTTGCTTTGGCAGT R: GTGGTTTTGAATGTTCTCTG	139 bp	$(AAG)_3(AG)_6$	AY207450	Hoh *et al*. 2013[19]
7	*MnBP5-2-27*	F: TTATAACAGGGGAGTGAAGG R: CAAGTGCAAAGACAGACAGA	270 bp	$(CT)_8$	AY207449	Hoh *et al*. 2013[19]
8	*MnBP5-2-2a*	F: GGTCGACAGCGAGAGAGAG R: TCCTGAACTGCTCAGATTTT	242 bp	$(GT)_{12}N_5(GA)_5$	AY205994	Hoh *et al*. 2013[19]
9	*MnBP5-2-2c*	F: GCCCAATACACTGAATGAAC R: TTCTCTGAGCGAAGAGAG	245 bp	$(CT)_8$	AY205994	Hoh *et al*. 2013[19]
10	*MnBP5-2-05c*	F: TGGATGGATAGCCTTAGAGA R: CCACCCAATCACTTATTTGT	215 bp	$(TGGA)_2N(AGAC)_3$	AY207448	Hoh *et al*. 2013[19]
11	*MnBP5-2-06a*	F: GGTTCGCCCGAGAGAGAGAG R: CGGGGTTAGAACACACATCC	168 bp	$(GA)_3$	AY804195	Hoh *et al*. 2013[19]
12	*MnBP5-2-13b*	F: CCGCTTTTTATTAGTCCTCA R: CACAGAAACAGGGTTTGAA	234 bp	$(CTCAT)_2$	AY804209	Hoh *et al*. 2013[19]
13	*MnBP5-2-22a*	F: TGTCTGAGCCAGAGAGAGA R: GTCTCTGATGGTGTTTGCTT	365 bp	$(GA)_9$	AY205998	Hoh *et al*. 2013[19]
14	*MnBP8- 4-26b*	F: GCAACTTGCACAGTATTT R: ATGCGAAATTTGCACAGA	174 bp	$(T)_8(TAA)_4$	AY860214	Hoh *et al*. 2013[19]
15	*MnBP-8-4-34a*	F: GCCTACTGTTGTTGTTGT R: GTGGCCAGAAAAGTGTAGAA	232 bp	$(GTT)_5(ATT)_{11}$	AY806222	Hoh *et al*. 2013[19]
16	*MnBP8-4-26a*	F: TTTCTGTTGTTGTTGTTG R: GCACAAAATACTGTGCAA	138 bp	$(GTT)_7(TTA)_3$	AY860214	Hoh *et al*. 2013[19]
17	*MnBP4-2-08*	F: GCCCAGGACACACACACA R: TATGGCTCCCTCACACA	183 bp	$(AC)_5(TG)_7$	AY804197	Hoh *et al*. 2013[19]
18	*MnRmB1-2*	F: TTAGCTGACAGGATGCACTG R: GTTCCGTATGATGATGATGATG	160-230 bp	$(CAT)_6$	-	Chan *et al*. 2005b[18]
19	*MnRmC8-1*	F: TGTGCGCGATGTGTGTGT R: GAAACTGCTGGTTTTGTCAGC	280 bp	$(GT)_8$	-	Chan *et al*. 2005b[18]
20	*MnRm D11-1*	F: GATCCCGAAAGAAATTCCA R: GTTAGCGGATAGATAGATAG	180-240 bp	$(CT)_{29}$	-	Chan *et al*. 2005b[18]
21	*MnRm7-1*	F: TTTTTCTCTCGCTGTCTCTC R: GCAGAGTTTGGGTGACATAC	174 bp	$(CT)_5$	-	Chan *et al*. 2005b[18]
22	*MnSC4-3b*	F: GCCAAGGAGCTATGAACTGG R: GACGCAACTATGTCCACCAC	208 bp	$(GAT)_6(TGG)_6$	-	Keong *et al*. 2008[20]
23	*MnSC3-15b*	F: TCCCTTTGTTGGAGTTAGGG R: GGAGGAAAAACCACAGAGTC	166 bp	$(GAC)_4$	-	Chan *et al*. 2005b[18]
24	*MnLR2-1-15b*	F: TACGGAATCCGAGGTCACTC R: AAGCGGGCGGCTCTCTCT	206 bp	$(CT)_{17}(GAATG)_2$ $(GA)_5(AG)_6$	-	Chan *et al*. 2005b[18]
25	*MnLR2-1-17b*	F: GCAGTTTCCTTCTCTTCACT R: GGGGGGCGCGCAACTCTCTC	132 bp	$(TG)_{11}(AG)_{12}$	-	Chan *et al*. 2005b[18]
26	*MnLR2-1-21a*	F: GGAAAGGGCGAGGCTCTC R: GTCGAGGGTGAAGAGGGAAG	199 bp	$(CT)_6$	-	Chan *et al*. 2005b[18]
27	*MnLR2-1-21c*	F: GGAAAGGGGAGGCTCTCTCT R: AGCTCAATAAGGTGCCATGC	177 bp	$(CT)_6$	-	Chan *et al*. 2005b[18]
28	*MnB10-2-17*	F: CGGATACGTGTTGCTTTC R: GCTCCTGTGCGCGGCTCT	248 bp	$(CTTTC)_2(GA)_{26}$	-	Chan *et al*. 2005b[18]
29	*MnLR2-2-11*	F: GGAAGGCGCGAGGCTC R: GGGAGAAGGGCCTCTC	204 bp	$(CT)_9(T)_9$ $(A)_8T(GA)_6$	-	Chan *et al*. 2005b[18]
30	*MnLR2-1-52a*	F: TCCCCTTTTTATTGCCATTC R: GGAACGAGGAGGGCTCTCTCT	189 bp	$(GA)_{22}(T)_8$	-	Chan *et al*. 2005b[18]

Result

1. Cross-species Amplification

Thairty heterologous microsatellite loci were tested on 6 species of freshwater catfish in the cross-species amplification experiment. Only 3 (10%) to 6 (20%) primers produced successful amplifications in the 6 catfish species. The amplifications were considered successful when they produce amplicons in size ranges similar to those in *H. nemurus*. In the species in which the amplification was succeeded the amplicons were observed in all individuals. The numbers of alleles and annealing temperatures (Ta) of successfully amplified primers were listed in Table 3.

In *P. siamensis*, 4 primers (*MnBP5-2-24b*, *MnRm7-1*, *MnSC4-3b* and *MnRmC8-1*) were amplified and 3 of them showed polymorphism, with *MnSC4-3b* being the monomorphic. Six loci were amplified in *Pangasius pangasius* (*MnBP5-2-24b*, *MnRmB1-2*, *MnRmC8-1*, *MnRm7-1*, *MnSC4-3b* and *MnLR2-1-21c*) and 3 showed polymorphism (*MnRmB1-2*, *MnRm7-1*, and *MnLR2-1-21C*). In *P. nasutus*, only *MnBP5-2-24b* and *MnRm7-1* produced polymorphic bands while *MnBP5-2-24b* and *MnRmC8-1* were monomorphic. For Clariid catfish, 6 loci were amplified in *C. batrachus* (*MnRmC8-1*, *MnRm7-1*, *MnSC4-3B*, *MnLR2-1-15b*, *MnLR2-1-17b* and *MnLR2-2-11*), 3 in *C. macrocephalus* (*MnRmC8-1*, *MnRm7-1*, and *MnSC4-3b*) and 5 in *C. gariepinus* (*MnBP5-2-24b*, *MnRm7-1*, *MnSC4-3b*, *MnLR2-1-15b* and *MnLR2-2-11*). Only the primer for locus *MnRm7-1* was amplified in all 7 species. Because of metaphor agarose gels high resolution performance, a single band was assumed as monomorphic.

2. DNA Sequence of MnRm7-1 in 6 Catfish Species

Locus *MnRm7-1* showed amplification in all species thus selected to get the sequence information of *H. nemurus* and other 6 catfish species. The variations in sequence were observed and cross-amplified microsatellite motifs were highlighted in different colours in Fig. 1.

In *P. siamensis*, the forward flanking regions (10 bases) exhibit high similarity to the source sequences. On the other hand, the sequences from the other 5 species were different from that of *H. nemurus*. Most of them seem to have undergone substitutions. In the source species, the tandem repeat was CT with 5 repeats. The same repeats were also observed in *P. siamensis* (4 repeats), *P. nasutus* (5 repeats) and *C. macrocephalus* (5 repeats). For *P. pangasius*, the tandem repeat was TC, and the number of repeats was 8. A compound simple sequence repeat for locus *MnRm7-1* was observed in *C. batrachus* and *C. gariepinus*.

Discussion

Primer pairs developed for the river catfish, *Hemibagrus nemurus* could be successfully amplified in the other 6 catfish species selected for this study. Out of the 30 primers selected, 9 showed promising results. In previous studies, various groups have attempted cross-species amplification in freshwater fishes[22]. These attempts have generally involved obtaining amplicons from species closely related to the source species. To a certain extent, the loci that can be amplified in distant taxa, such as between loci in

Table 3. Number of alleles and annealing temperature (Ta) of amplified microsatellite loci in six catfishes

Species	*H. nemurus*	*P. siamensis*	*P. pangasius*	*P. nasutus*	*C. batrachus*	*C. macrocephalus*	*C. gariepinus*
Locus	Number of alleles and Ta (℃)						
MnBP5-2-24b	48	(2)Tc	(1)Tc	(2)48	-	-	(1)Tc
MnRmB1-2	55	-	(2)48	-	-	-	-
MnRmC8-1	54	(2)Tc	(1)48	(1)58	(1)48	(1)Tc	-
MnRm7-1	48	(2)60	(3)58	(2)60	(2)Tc	(2)58	(3)Tc
MnSC4-3b	50	(1)Tc	(1)Tc	-	(1)Tc	(2)Tc	(2)Tc
MnLR2-1-15b	48	-	-	-	(1)Tc	-	(1)Tc
MnLR2-1-17b	48	-	-	-	(1)Tc	-	-
MnLR2-1-21c	54	-	(3)Tc	-	-	-	-
MnLR2-2-11	54	-	-	-	(4)Tc	-	(2)48

Tc is Touchdown procedure.
Number of alleles is written in bracket.

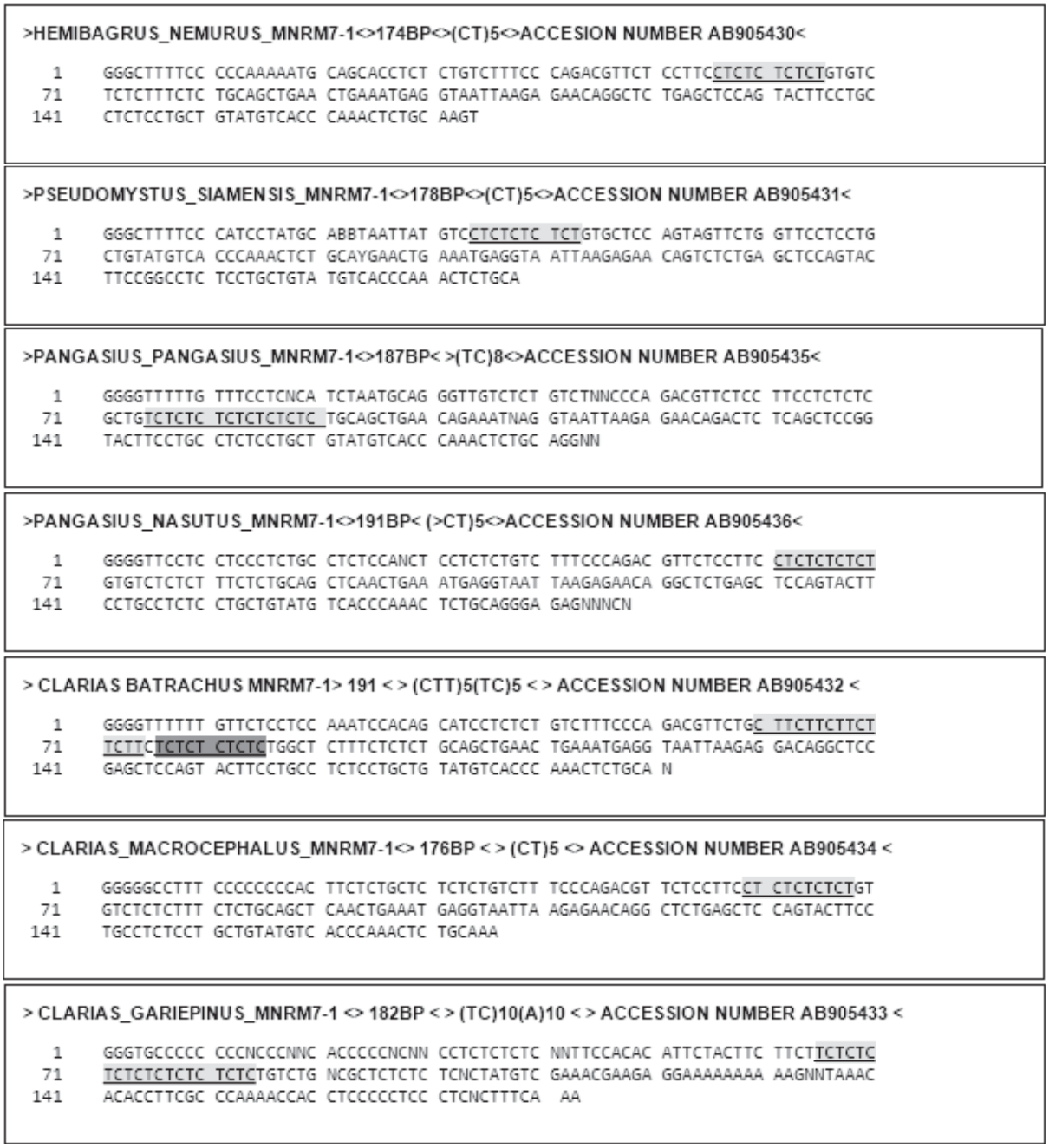

Fig. 1. Repeat motifs in microsatellite sequences of locus *MnRm7-1* in seven selected catfish species.

fishes, can also be amplified in arthropods.

The rate of success in cross-species amplification studies is also affected by the method adopted. The most commonly used method is to use the primers developed for various source species and to attempt to cross-amplify them in one or two target species[23, 24]. Another method is to diversify the target species and test the primer developed from a single source species[14,15]. In this study, the second method was adopted because the number of target species was 6 and the primers developed for the source species had already been tested for cross-species amplification in other species[25].

The rate of successful cross-species amplification is normally predicted based on their relatedness in phylogeny[26, 27]. Indeed, one theory has suggested that the more evolutionarily distant a taxon, the lower the chance of successful amplification[25]. A species in the same genus is generally assumed to have a greater chance of success. However, our present result did not follow the phylogenetic relationship theory. The data obtained herein suggested that the

rate of successful cross-species amplification is locus specific, and independent of the genetic distance between source species and target species. In this study, *MnRmB1-2* and *MnLR2-1-21C* showed no amplification in *Pseudamystus siamensis* belonging to the family as source species but were amplified in *P. pangasius* from the Family Pangasiidae. The same phenomenon was observed for the loci *MnLR2-1-15b*, *MnLR2-1-17b* and *MnLR2-2-11* which were amplified in *C. batrachus* but not in *P. siamensis*. These loci showed their specificity only when amplified in certain species that are relatively distant from the source species. *MnSC4-3b* showed amplifications in 5 of the 6 other catfish species in this study, with the exception being *P. nasutus*, suggesting that the locus is conserved in certain catfish and cyprinid species. However, *MnLR2-1-17b* was not amplified in 5 of the 6 catfish species, with the exception being *C. batrachus*, suggesting that this locus is species specific. The results in this study suggested that the loci developed for *H. nemurus* are locus specific and conserved among various species without regard to genetic relationship with source species. The length of the primers and the degree of conservation in that particular locus appear to have a minimal effect on the probability of amplification[28].

The results of this study also disclose that the optimum annealing temperature (Ta) of the 6 species of catfish tested differs from that of in *H. nemurus* for each primer with except in the case of *MnBP5-2-24b* (Table 3), with the Ta in *P. nasutus* being similar to the Ta in *H. nemurus*. Some successfully amplified primers in this study showed lower annealing temperature than reported in source species while the other showed amplification when using touchdown PCR protocol. *MnRm7-1* showed higher annealing temperature in some sample species than in source species. A general trend that cross-species amplification has lower annealing temperature than original amplification[20, 28].

Cross-species amplification is a method of utilising primer pairs designed for a specific species (source species) in other species (target species) which normally closely related[27, 29]. However, the data of all the PCR products generated in the target species have only been analyzed based on their banding patterns[29]. There have been few analyses of the successful cross-species amplification data at the sequence level[30,31]. Thus, heterozygosity cannot be compared between species.

Previous study proposed that the PCR product generated from cross-species amplification to be analyzed at the sequence level to identify the homology between source and target species[31]. In this study, the primer designated for the flanking region of locus *MnRm7-1* showed amplification in 6 species. Heterozygosity could be observed in the DNA sequences among the species.

Amplification products of target species were sequenced directly to confirm their identity whether the flanking sequences and repeat motifs were conserved in length. Each species showed sequences in flanking regions with slightly differed among the species, which means the possibility that heterologous or orthologous loci were amplified. Microsatellite primer pairs that can amplify loci in non-specific species are called orthologous or heterologous primers[32]. The amplification of non-homologous loci has been observed in reptilia[33]. In fish, however, cross-species amplification varies, showing a tendency to amplify homologous loci in species such as flatfish[34] and non-orthologous loci in catfish[31]. Heterologous microsatellites have been suggested for use in genetic polymorphism tests and genetic variability evaluations[35, 36]. The present analysis also disclosed that the repeat motifs are conserved in Pangasiid catfishes but not in Clariid catfishes. A notable feature is that the repeat structure of this locus was slightly different among these species: $(CT)_5$ in the source species, *P. Siamensis*, *P. nasutus* and *C. macrocephalus*, $(TC)_8$ in *P. Pangasius*, $(TC)_{10}N(A)_{10}$ in *C. gariepinus* and are $(CTT)_5$ and $(TC)_6$ in *C. batrachus*. The variation among species occurs with respect to the pattern of tandem repeats and the number of repeats in microsatellite DNA. The WebSat program uses the color orange to highlight overlapping pairs of SSRs (have something in common the base T). Thus, the last base (T) of the first SSR $(CTT)_5$ is also the first base (T) of the second SSR (TC). This contradicts the idea that the rate of base substitution in aquatic organisms is lower than that in terrestrial organisms[9]. The amplification products in Clariid catfish provided evidence of indels in the flanking sequences and repeat motifs.

Amplification of non-orthologous loci will lead to obvious complications in phylogenetic inference, population genetics and evolutionary studies[31]. Therefore, we recommend that sequence analysis of cross-amplification products be performed prior to their application, as previously suggested[31].

Cross-species amplification is a method for identifying potential DNA microsatellite markers in source species to be amplified in target species. The polymorphic marker in cross-species amplification can be used in the genetic study of target species. This obviates the need for developing new DNA microsatellite markers for target species which requires high skill, consumed more labour, time and money. However, the possibility of obtaining polymorphic loci is uncertain. In this study, the results showed uncertainty in the success of cross-species amplification, with few loci exhibit polymorphism. In the future, further studies of cross-species amplification in catfish and also another species will need to be performed with more closely related species to obtain the DNA sequences of successfully amplified loci. Since the idea of close relationship having high success rate in cross-amplification is not applicable to this study, it is suggested that the markers that showed good performance in previous cross-amplification attempts should be used in the next study. Greater numbers of samples (100 specimens for species) and larger populations should be examined using more primer pairs in the screening process to increase the likelihood of obtaining polymorphic markers. Indeed, there are shortcomings in previous researches on the cross-amplification of non-homolog loci. However, cross-species amplification is not useful for the comparison of genetic diversity and differentiation among different species. It is necessary to identify the homology of DNA sequence in microsatellite DNA regions of species and/or local strains to compare.

Acknowledgements

This was part of Master thesis of the first author. This study was partly supported by JSPS Grants-in-Aid for Scientific Research, grant no.25252035.

References

1）Khan, M. S., K. J. Ang, M. A. Ambak and C. R. Saad, 1993. Optimum dietary protein requirement of a Malaysian freshwater catfish, *Mystus nemurus*. Aquacult., 112: 227-235.

2）Senanan, W., A. R. Kapuscinski, U. Na-Nakorn and L. M. Miller, 2004. Genetic impacts of hybrid catfish farming (*Clarias macrocephalus* x *C. gariepinus*) on native catfish populations in central Thailand. Aquacult. 235 (1-4): 167-184.

3）Sang, N. V., M. Thomassen, G. Klemetsdal and H. M. Gjøen, 2009. Prediction of fillet weight, fillet yield, and fillet fat for live river catfish (*Pangasianodon hypopthalmus*). Aquacult. 288: 166-171.

4）Department of Fisheries Malaysia (DOF). 2009. Perangkaan Perikanan Tahunan.

5）Xu, T. J., D. Q. Sun, G. Shi and R. X. Wang, 2010. Development and characterization of polymorphic microsatellite markers in the gray mullet (*Mugil cephalus*). Genet. Mol. Res., 9(3): 1791-1795.

6）Ma, C. Y., H. Y. Ma and L. B. Ma, 2011. Development and testing of 13 polymorphic microsatellite markers in *Larimichthys polyactis* (Scianidae) using 5` anchored PCR. Genet. Mol. Res. 10(3): 1455-1460.

7）Zhang, L., D. Yuan, S. Yu, Z. Li, Y. Cao, Z. Miao, H. Qian and K. Tang, 2004. Preference of simple sequence repeats in coding and non-coding regions of *Arabidopsis thaliana*. Bioinformatics, 20: 1081-1086.

8）Bentzen, P., A. Harris and J. M. Wright, 1991. Cloning of hypervariable minisatellite and simple sequence microsatellite repeats for DNA fingerprinting of important aquacultural species of salmonids and tilapia. In: DNA Fingerprinting: Approaches and Applications, Burtke, T., G. A. Dolf, A. J. Jeffreys, and R. Wolf (Eds.). Basel: Birkhauster Verlag., pp: 243-262.

9）Rico, C., I. Rico and G. Hewitt, 1996, 470 million years of conservation of microsatellite loci among fish species. Proceedings of Royal Society: Biol. Sci., 263: 459-557.

10）Scribner, K. T., J. R. Gust and R. L. Field, 1996. Isolation and characterisation of novel salmon microsatellite loci: cross species amplification and population genetics application. Can. J. Fisheries. Aquatic. Sci., 53: 833-841.

11）Zheng, W., N. E. Stacey, J. Coffin and C. Strobeck, 1995. Isolation and characterization of microsatellite loci in goldfish *Carassius auratus*. Mol. Ecol., 4: 791-792.
12）Dubut, V., M. Sinama, J.-F. Martin, E. Meglécz, J. Fernandez, R. Chappaz, A. Gilles and C. Costedoat, 2010. Cross-species amplification of 41 microsatellites in European cyprinids: A tool for evolutionary, population genetics and hybridization studies. BMC Res. Notes., 3: 135.
13）Hogan, Z. S. and B. P. May, 2002. Twenty-seven new microsatellites for the migratory Asian catfish family Pangasiidae. Mol. Ecol. Notes., 2: 38-41.
14）Yue, G. H., B. Kovacs and L. Orban, 2003. Microsatellites from *Clarias batrachus* and their polymorphism in seven additional catfish species. Mol. Ecol. Notes., 3: 465-468.
15）Na-Nakorn, U., K. Phairoj, S. Sukmanomon, S. Poompuang and W. Kamonrat, 2006. Polymorphic microsatellite primers developed from DNA of the endangered Mekong giant catfish , *Pangasianodon gigas* (Chevey) and cross-species amplification in three species of *Pangasius*. Mol. Ecol. Notes., 6: 1174-1176.
16）Mohsin, A. K. M. and M. A. Ambak, 1983. Freshwater Fishes of Peninsular Malaysia, first ed. Universiti Pertanian Malaysia Publisher. Serdang, Selangor, Malaysia, pp. 119-241.
17）Chan, S. C., S. S. Siraj, S. G. Tan and K. Yusoff, 2005. Rapid and non-radioactive detection method of microsatellites in *Mystus nemurus*: A refined technique. Pert. J. of Trop. Agri. Sci., 28(1): 73-77.
18）Chan, S. C., Tan, S. G., Siraj, S. S. and K. Yusoff. 2005. Newly Developed Microsatellite Markers of *Mystus nemurus* Tested for Cross-Species Amplification in Two Distantly Related Aquacultured Catfish Species. Asian-Aust. J. Anim. Sci., 18 (11): 1513-1518.
19）Hoh. B. P., Siraj, S. S., Tan, S. G. and K. Yusoff. 2013. Segregation and genetic linkage analyses of river catfish, Mystus nemurus, based on microsatellite markers. Genetics and Molecular Research 12(3): 2578-2593.
20）Keong, R. B. P., Siraj, S. S. and Daud, S. K. 2008. Cross species amplification of ikan kelah, Tor tambroides by using Mystus nemurus microsatellite markers. Pertanika Journal of Tropical Agricultural Science 31(2):179-189.
21）Martin, W. S., D. C. Lucas, K. F. Neves and D. J. Bertioli, 2009. WebSat- a web software for microsatellite marker development. Bioinformation 2(6): 282-283.
22）Ghiasi, N., Z. A. Rashid, S. Hoohsmand, K. Yusoff, S. G. Tan and S. Bhassu, 2009. The use of locus specific microsatellite markers for detecting genetic variation in hatchery bred *Probarbus jullienii.* Biotech., 8 (1): 166-170.
23）Munenara, H., S. Awata, R. Katoh, M. Kohida and T. Sunobe, 2001. Primer sequences and cross-species amplification for parentage discrimination of Tanganyikan Cichlid fishes. Bull. Fisheries Sci. Hokkaido Univ. 52(3): 131-133.
24）Holmen, J., L. A. Vøllestad, K. S. Jakobsen and C. R. Primmer, 2009. Cross-species amplification of 36 cyprinid microsatellite loci in *Phoxinus phoxinus* (L.) and *Scardinius eryphtalmus* (L.). BMC Res. Notes., 2: 248.
25）Volckaert, F., B. Hellemans and L. Pouyaud, 1998. Preliminary data on genetic variation in the genus Clarias and Pangasius on the basis of DNA microsatellite loci. In: Marc, L. and P. Antoine (Eds.) The biological diversity and aquaculture of Clariid and Pangasiid catfishes in South-East Asia: proceedings of the mid-term workshop of the “Catfish Asia project”. Jakarta (IDN); Can Tho: IRD; Can Tho University,1998. pp. 57-60.
26）Ma, H. Y., J. Z. Bi, C. W. Shao, Y. Chen, G. D. Miao and S. L. Chen, 2009. Development of 40 microsatellite markers in spotted halibut (*Verasper variegatus*) and the cross-species amplification in barfin flounder (*Verasper moseri*). Anim. Genet., 40(4): 576-578.
27）Liu, J., L. M. Gao, D. Z. Li, D. Q. Zhang and M. Möller, 2011. Cross-species amplification and development of new microsatellite loci for *Taxus wallichiana* (Taxaceae). Am. J. Bot.,: e70-e73.
28）Housley D. J., Z. A. Zalewski, S. E. Beckett and P. J. Venta, 2006. Design factors that influence PCR amplification success of cross-species primers among 1147 mammalian primer pairs. BMC Genomics., 7: 253.
29）Zane, L., L. Bargelloni and T. Patarnello, 2002. Strategies for microsatellite isolation: a review. Mol. Ecol., 11: 1-16.
30）Berrebi, P., B. Laserre., F. Barbisan and L. Zane, 2006. Isolation of microsatellite loci anc cross-species amplifications in three gobiid fish of the genus *Pomatoschistus*. Mol. Ecol. Notes., 6: 724-727.
31）Yue, G. H., B. Kovacs and L. Orban, 2010. A new problem with cross-species amplification of microsatellites:

generation of non-homologous products. Zool. Res. 31(2): 131-140.
32）Moore, S. S., L. L. Sargenat, T. J. King, J. S. Mattilla, M. Georges and D. J. S. Metzel, 1991. The conservation of dinucleotide microsatellite among mammalian genomes allows the use of heterologous PCR primer pairs in closely related species. Genomics 10: 654-660.
33）Hille, A., Janssen, I. A. W., Menken, S. B. J., Schlegel, M. and Thorpe, R. S. 2002. Heterologous amplification of microsatellite markers from Colubroid snakes in European Natricines (Serpentes: Natricinae). J. of Hered., 93(1): 63-66.
34）Kim, S. G., K. Morishima and K. Arai, 2009. Cross-species amplification of microsatellite markers for the brown sole in the family Pleuronectidae. Fish. Sci., 75(5): 1103-1107.
35）Canales-Aguire, C. B., S. Ferrada, C. E. Hernandez and G. Ricardo, 2010. Usefulness of heterologous microsatellites obtained from *Genypterus blacodes* (Schneider 1801) in species Genypterus off the Southeast Pacific. Gayana., 74(1): 74-77.
36）Olivatti, A. M., T. A. Boni, N. J. Silva-Júnior, L. V. Resende, F. O. Gouveia and M. P. C. Telles, 2011. Heterologous amplification and characterization of microsatellite markers in the Neotropical fish *Leporinus friderici.* Genet. Mol. Res., 10(3): 1403-1408.

River Catfish で開発されたマイクロサテライト DNA マーカーの他魚種への応用と増幅領域の配列比較

ムハマド　フィトリ　ユソフ（東北大院農）・
シティ　シャポール　シラジ（国際イスラム大学マレーシア）・
中嶋正道（東北大院農）

マレーシアをはじめとする東南アジアにおいて重要な産業対象種である *Hemibagrus nemurus* で開発された30マイクロサテライト DNA マーカーの他の淡水ナマズ 6 種への利用の可能性を検討した。30マーカーを調べた結果、3 から 6 マーカーにおいて 6 種のいずれかにおいて増幅が確認され、そのうちのいくつかにおいては多型が観察された。*MnRm7-1* は全ての種で増幅が観察され、全ての種で多形が観察された。*MnRm7-1* において増幅断片のシーケンスを行いリピート配列とその隣接領域の配列を種間で比較した。リピート配列は Pangasiid 内では保存され *Hemibagrus nemurus* と同じモチーフであったが、Clariid 内は種間で異なっており、*Hemibagrus nemurus* とも異なったモチーフであった。また、隣接領域の配列は種間で異なっており、それぞれの種で異なった変異が蓄積されていた。