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Isolation and characterization of polymorphic microsatellite markers in *Bagarius yarrelli* using RNA-Seq

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ABSTRACT. The yellow sisorid catfish (*Bagarius yarrelli*) is a vulnerable fish species. In this study, seven polymorphic microsatellite DNA markers for yellow sisorid catfish were described, using RNA-Seq methodology. In *B. yarrelli* (N = 44) from a Hekou wild population, allelic frequency, and observed and expected heterozygosities per locus varied from two to six, 0.0333 to 0.6793, and 0.0333 to 0.6004, respectively. One locus (Baya153) denoted notable separation from the Hardy-Weinberg equilibrium, after sequential Bonferroni correction ($P < 0.05$). The microsatellite markers described here will be useful for investigating population structure and genetic resource of *B. yarrelli* from different geographical locations.

Key words: Microsatellite markers; *Bagarius yarrelli*; Catfish; RNA-Seq

INTRODUCTION

The yellow sisorid catfish (*Bagarius yarrelli*) belongs to Osteichthyes, Siluriformes, Sisoridae (Cheng and Zheng, 1987). It inhabits China, where it is found mainly in the Yuanjiang, Nujing, and Lanchangjiang Rivers in Yunnan Province (Chu and Chen,1989), as well as in Vietnam, Burma, and India. In these regions, *B. yarrelli* has significant potential as an aquaculture production species, owing to its delicious flavor and high economic value. However, the present status of the wild resource of yellow sisorid catfish is vulnerable (Leng et al., 2011), because of overfishing and environmental pollution.

Published data around *B. yarrelli* are mostly concerning morphology (Tian et al., 2009a), physiology (Tian et al., 2009b), phylogeny (Guo et al., 2004; Sullivan et al., 2006; Yu and He, 2012), and random amplification polymorphic DNA (RAPD) method (Du et al., 2015). At present, there are no simple sequence repeat (SSR) data deposited in GenBank, The European Molecular Biology Laboratory (EMBL), or DNA Data Bank of Japan (DDBJ), which would allow studies of genetic diversity in *B. yarrelli* using microsatellite markers, an effective method of evaluating genetic resources. Recently, microsatellite markers from genomic and expressed sequence tags (ESTs) have been developed in a number of fish species, e.g., fat greenling (*Hexagrammos otakii*) (Chen et al., 2009) and eleotris fish (*Odontobutis potamophila*) (Zhang et al., 2014). To date, there is no microsatellite exploited for *B. yarrelli*. It is essential to develop useful SSR markers for genome mapping and parentage analysis, and for genetic resource management of *B. yarrelli*.

MATERIAL AND METHODS

Bagarius yarrelli specimens (N = 44) were collected from the farmers market of Hekou County, Honghe Hani, and Yi Autonomous Prefecture, Yunnan Province, China. Genomic DNA was isolated from fin tissues of each animal, using the phenol-chloroform method described by Du et al. (2012). Following extraction, DNA samples were diluted to 100 µg/µL and stored at -20ºC until further use.

RNA-Seq methodology was employed to develop microsatellite markers; a total of 151,911 sequences were generated. The MISA software program was used to identify SSRs (Microsatellite, http://pgrc.ipk-gatersleben.de/misa). In total, 14,812 microsatellite loci were confirmed; of these, 198 were selected for further characterization. Microsatellite primers were designed for 15 motifs containing five bases, using the PRIMER PREMIER 5.0 software program (Premier Biosoft International, Palo Alto, CA, USA) and were synthesized by Sangon Biotech Company (Shanghai, China). Polymerase chain reaction (PCR) assays were carried out using a thermal cycler (Eastwin-200, Beijing Dongsheng innovation Biological Technology Co., Ltd., Beijing, China). Reactions were in a 20-µL total volume, which contained 2X Taq PCR master mix (0.4 mM dNTPs, 4 mM $MgCl_2$, and 0.05 U/µL Taq DNA polymerase; Biomed Biological Technology Co., Ltd., Beijing, China) and 80 ng genomic DNA. Cycling parameters for PCR amplification were: initial denaturation at 94ºC for 4 min; 35 cycles of 45 s at 94ºC, 45 s at the annealing temperature for each specific primer (Table 1), and 45 s at 72ºC; and extension for 7 min at 72ºC (Miao et al., 2011).

RESULTS AND DISCUSSION

Amplified PCR products were analyzed on 8% non-denaturing polyacrylamide (w/v) gel and identified by ethidium bromide staining. Scopes of allele size were determined in the light of the pBR322/*Msp*I marker (Tian-Gen Biotech Co. Ltd., Beijing, China). The number of alleles,

and expected (H_E) and observed (H_O) heterozygosities were determined using the POPGENE version 1.32 program software (Yeh et al.,1999). Significant levels for all tests were converted by sequential Bonferroni method (Rice et al.,1989).

Characteristics of the seven *B. yarrelli* microsatellite loci analyzed in POPGENE are denoted in Table 1. Altogether, 22 alleles were confirmed in 44 individuals; H_O and H_E per locus ranged from 0.0333 to 0.6793, and 0.0333 to 0.06004, respectively. One of the seven loci presented in Table 1 displayed significant departure from Hardy-Weinberg equilibrium.

Table 1. Characterization of *Bagarius Yarrelli* microsatellite loci from 44 individuals.

Locus	Primer (5'-3')	Size (bp)	Repeat motif	Ta (ºC)	N_A	H_O	H_E	P value HWE	PIC
*Baya*153	F: TTAGTTCTCAGGGTCCGTCG R: CCCGCTCTCTTAGACTGTGC	123-160	$(TTTTA)_6$	57.8	4	0.4000	0.5621	0.0006*	0.5004
*Baya*155	F: TGTTTACGGTTACCGCTTCC R: ACACAGAGCCCACCTCAGAC	201-238	$(TTTTC)_5$	58.2	3	0.6429	0.4552	0.1178	0.3618
*Baya*157	F: ATTCGGTGTCTTTCCTCACG R: AAGTGCCCCTAAAACCCAAC	217-242	$(ATCTG)_5$	57.8	2	0.0333	0.0333	1.0000	0.0323
*Baya*166	F: TCCCTCCATCTTCTCTTCCA R: TGATTTGGGACACGATCTGA	217-404	$(CCTCT)_6$	55.7	5	0.6152	0.6004	0.6183	0.4810
*Baya*167	F: CGCACCTGAACACCCTTTAT R: TCTTCTCTTCTGTCTTATGCTATCC	90-110	$(GATAG)_5$	55.1	2	0.1304	0.1246	0.7787	0.1145
*Baya*160	F: TCATCGTACTGCTTTGCGTC R: CCCGCTTTTTACTTCACTGC	190-404	$(CGCTG)_5$	57.8	6	0.6793	0.4835	0.2276	0.3521
*Baya*161	F: AACGCAAATCCGATTGAAAC R: ACAGCTGCTCTCTCTCACCC	242-309	$(GAGGT)_5$	56.8	2	0.2500	0.3001	0.3000	0.2522

Ta, annealing temperature; N_A, observed number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; PIC, polymorphism information content. *Indicates significant deviation from Hardy-Weinberg equilibrium (HWE) after Bonferroni correction (adjusted P = 0.0006).

The novel polymorphism primers from EST-SSRs described here provide a foundation for future population genetic diversity studies and genetic resource management of *B. yarrelli.* In the future, we will use these primers for study the population genetics of *B. yarrelli* from other rivers of Yunnan Province.

Conflicts of interest

The authors declare no conflict of interest.

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