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Short communication

Isolation and characterization of 15 novel microsatellite loci from an endangered bream *Megalobrama pellegrini* (Tchang, 1930)

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Introduction

Megalobrama pellegrini is a cyprinid fish species endemic to the upper Yangtze River basin in China (Chen, 1998). In recent decades due to dam construction, over fishing, and water pollution, the wild population of *M. pellegrini* was found only in the Longxi-He River, a tributary of the upper Yangtze River in Sichuan basin, after three consecutive years of field investigations (Li et al., 2007). Even worse, its population size has continued to decline in recent years (e.g. Gao et al., 2009). Biological genetic diversity is an important parameter to evaluate the present situation of biological resources, and the Simple Sequence Repeats (SSRs) have long been used for population genetic studies and in this regard are of great applicational value in the conservation and management of biological resources. However, most of the existing microsatellite markers were moderate polymorphic (e.g. Wang et al., 2012), and only a few genetic studies have been conducted for this endangered bream due to a lack of appropriate molecular markers (Liu and Wang, 1997; Wang et al., 2012, 2014). Therefore, more polymorphic microsatellite markers are needed for *M. pellegrini*. Previous studies have shown that the 454-sequencing is a powerful technique for the isolation of polymorphic microsatellite markers (e.g. Zeng et al., 2013; Zhao et al., 2014). Here we isolated and characterized 15 novel polymorphic microsatellite loci (6 di- and 9 tri-nucleotide) in the genome of *M. pellegrini* based on the remaining 454-sequencing dataset after Wang et al. (2012). These microsatellite markers should be useful in a wide range of population and evolutionary studies of this and other closely related species.

Materials and methods

Thirty-three *M. pellegrini* individuals were collected from the Longxi-He River, a tributary of the upper Yangtze River, and used for subsequent analysis. Genomic DNA was extracted from fins using the DNeasy Tissue Kit (QIAGEN; Shanghai, China). MSATCOMMANDER version 0.8.2 (Faircloth, 2008) was used for the microsatellite-containing sequence identification with default parameters. Minimum repeats of 12 and 8 respective for the di- and tetra-nucleotides (perfect type) were selected for primer design in the present study. The primers were designed by the software Primer Premier version 6.0 (www.PremierBiosoft.com).

Polymerase chain reaction (PCR) amplifications were conducted in 12.5 μl volumes and each reaction system contained 1.25 μl of 10 × buffer, 1.0 μl of 2.5 mM of each dNTP, 0.75 μl of 50 mM $MgCl_2$, 0.5 U Taq DNA polymerase (rTaq, TaKaRa; Dalian, China), 3.0 pmoles each of forward and reverse primer, 1–2 μl of genomic DNA (30 ng μl^{-1}) and distilled water up to 12.5 μl. Amplification was performed with the conditions of an initial denaturation at 94°C for 5 min, followed by 36 cycles of 35 s at 94°C, 35 s at Tm, 30 s at 72°C and a final elongation step at 72°C for 7 min; the Tm was optimized according to different pairs of primers (Table 1). PCR products were separated on a 6% denaturing polyacrylamide gel (PAGE gel) using the pBR322 DNA/MspI molecular weight marker (TIANGEN; Beijing, China) as a standard for the assessment of products size. Furthermore, the PCR reactions were performed at least twice per polymorphic locus to confirm their reproducibility.

The number of alleles (N_A), observed and expected heterozygosities (H_O and H_E, respectively) and the polymorphic information content (PIC) of each polymorphic locus were calculated using CERVUS 3.0 software (Kalinowski et al., 2007). Deviation from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium at each locus was calculated using POPGENE version 1.31 (Yeh and Boyle, 1997). The significance level of Hardy–Weinberg equilibrium (HWE) departures and linkage disequilibrium were corrected using Bonferroni correction (Rice, 1989). The presence of null alleles and scoring errors were investigated using MICRO-CHECKER version 2.2.3 (Van Oosterhout et al., 2004) at a 95% confidence interval.

Results

Of the 46 pairs of primers designed (22 di- and 24 tri-nucleotide types), 35 primer pairs amplified successfully, but only 15 loci demonstrated polymorphism (the other 20 showed monomorphism in the Longxi-He River population with 33 individuals). Characteristics of these 15 loci are presented in Table 1. Mean N_A was 2.87, with a range of 2–6 alleles per locus. Mean H_O and H_E were 0.461 and 0.531, ranging from 0.091 to 0.879 and from 0.416 to 0.728, respectively. The

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Table 1
Characteristics of the 15 microsatellite loci in *Megalobrama pellegrini*

Locus	Repeat motif	Primer sequence (5′-3′)		Tm (°C)	Population (N = 33)				GenBank Accession No.
		Forward primer	Reverse primer		N_A	H_O	H_E	PIC	P_{HWE}	
MP01	(AC)17	CGATCGAGTCAGAGACGCA	CACACACACTGCTGTCCATC	58	6	0.818	0.702	0.639	0.014	KP749779
MP02	(AC)14	GCTGTAGACGTTGAACTTCAGC	TCCGCTCCAGTTCTGTTCTC	52	2	0.546	0.507	0.375	0.844	KP749780
MP03	(TG)16	TAGCAGCGACTGGAAGTG	AGCAGCAATCGACTAACCAG	52	2	0.636	0.500	0.371	0.030	KP749781
MP04	(GT)12	TCCTAATGCAAACCTCCTGTG	GCACCTGTGATGCTGATTGG	52	4	0.879	0.728	0.665	0.844	KP749782
MP05	(CA)15	TATGATCCCTGGGCTGTACG	GCAGATGGCAGACTATGTTGG	50	2	0.515	0.508	0.375	0.813	KP749783
MP06	(TC)12	AAGAAACGTACGGCACTGTG	TTGTACGAGGGTGGAGTGCG	46	2	0.455	0.461	0.351	0.011	KP749784
MP07	(AAT)10	GGTTCCACAAGTAGTCGTGC	GGTTGTTAAATGCCTCAGGGG	56	2	0.242	0.470	0.356	0.185	KP749785
MP08	(GAA)8	GTGGCGCTAGAGGGATTGA	TGCTGCTGTTGCTTGTAGGC	52	2	0.333	0.461	0.351	0.887	KP749786
MP09	(TAA)12	ACTTCCACAACATCAAGGAGC	CACCAAGGCTGCATTTAGTTTG	58	2	0.485	0.470	0.356	0.869	KP749787
MP10	(GTT)16	TCGCTCTTTTGCCCCTCA	TGCCAGAACGTTAGCCAAAG	52	5	0.727	0.652	0.575	0.592	KP749788
MP11	(GAA)8	CAACTTTGGACAGCTGGTGG	CTGCTGCTGTTGCTTGTAGGC	52	3	0.242	0.552	0.448	0.000*	KP749789
MP12	(GAT)11	TTGAGCTCTTCTGCCCACAC	GCCTTGTCTGTGAAACCAGG	52	2	0.091	0.416	0.326	0.000*	KP749790
MP13	(TTA)11	TACTCTGTGCATCACTTTGGTC	ATGAGCTCTCCCAGGGATAC	54	2	0.272	0.478	0.360	0.026	KP749791
MP14	(TAA)9	TCTCTCCACCACACACTGTC	GTGAACTGATTTAGGCCATTGC	54	3	0.303	0.446	0.366	0.163	KP749792
MP15	(CAA)11	CACAAGACCCACGAGTCAAC	CATGGCTGTTTAGGGCTTCC	48	4	0.364	0.610	0.520	0.164	KP749793

Number of alleles (N_A); observed heterozygosity (H_O); expected heterozygosity (H_E); Polymorphic information content (PIC); Probability of Hardy–Weinberg equilibrium (P_{HWE}).
*Significance of deviation from Hardy–Weinberg equilibrium at $P < 0.001$ for loci in HWE after Bonferroni correction.

polymorphic information content (PIC) for allelic diversity ranged from 0.326 to 0.665 (mean 0.429), with 4 loci demonstrating moderately high polymorphism (MP01, MP04, MP10 and MP15; PIC > 0.5). There was no evidence for linkage disequilibrium at any locus. However, deviation from Hardy–Weinberg equilibrium was found in two loci (MP11 and MP12; $P < 0.001$) after Bonferroni correction and five loci (MP07, MP11, MP12, MP13 and MP15) showed signs of null alleles.

Discussion

The genetic diversity of *M. pellegrini* based upon the 15 polymorphic microsatellite loci in the present study was similar to that in Wang et al. (2012) showing a moderate genetic diversity (mean $N_A = 2.87$, $H_O = 0.461$, $H_E = 0.531$ vs mean $N_A = 3.08$, $H_O = 0.47$, $H_E = 0.51$). Of the 15 polymorphic loci, four were assessed to contain moderately high (PIC > 0.50) polymorphism (Table 1). Two loci (MP11 and MP12) significantly deviated from HWE, which was most likely due to the presence of null alleles as that occurred in 2 of these loci. In conclusion, several novel polymorphic microsatellite loci were isolated and characterized here, which can potentially be used to investigate the genetic diversity and population structure in *M. pellegrini* and, thus, contribute to the development of conservation management programs.

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