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MICROSATELLITE LETTERS

Development of 27 novel cross-species microsatellite markers for the endangered *Hucho bleekeri* using next-generation sequencing technology

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Abstract In this study, we developed 27 polymorphic microsatellite loci in *Hucho bleekeri*, a glacial relict and freshwater resident salmonid fish in China. The number of alleles varied from 2 to 8 for each primer set. The observed and expected heterozygosity ranged from 0.250 to 0.906 and 0.508 to 0.845, respectively. The polymorphic information content ranged from 0.371 to 0.808. These microsatellite loci should be useful to study population genetics, paternity identification, speciation and adaptive evolution of this lineage.

Keywords *Hucho bleekeri* · Microsatellite · Endangered species

Hucho bleekeri, a glacial relict and freshwater resident salmonid fish, is endemic to the Yangtze River drainage in China. It became a critically endangered species mainly due to over-fishing and habitat destruction since 1960s (Hu et al. 2008). A series of measures have been taken to protect this species such as listing it into China Red Data Book to ban on fishing and establishing a national nature reserve (Wang 1998). And recently it has been performed successfully controlled breeding, which has great significance to the species preservation. To better address the conservation status of the endangered species, a management programme based on genetics is being devised to define the efficiency of these conservation measures (Zhu et al. 2005). Despite the increasing demand for preservation and management plans, no suitable high resolution molecular markers have been available for this species. In current study, we report a set of polymorphic microsatellite loci for *H. bleekeri* that can serve as effective genetic markers for conducting further preservation and management in this endangered fish.

We used the microsatellite makers isolated from closely related species *Brachymystax lenok*, that had been constructed DNA Library by Ion PGM^{TM} sequencer with Ion 318 chip kit v2 (Life tech LTD) in previous study (unpublished). 72 primer pairs were designed using Primer Premier 5.0 based on the flanking sequence of the repeat motifs. The polymorphisms of the loci were analyzed using a population of 24 wild individuals collected from Taibai River in Baoji City, Shaanxi Province, China. Total genomic DNA of *H. bleekeri* was extracted from tissue using the rapid salt-extraction method (Salah and Iciar 1997). The PCR amplification was carried out in a 25 μL volume that contained 1× PCR buffer, 50–100 ng genomic DNA, 0.25 μM of each primer, 200 μM dNTPs, 0.5 mM $MgCl_2$, and 0.25 U Taq DNA polymerase (TaKaRa). The PCR cycle comprised an initial denaturation at 94 °C for 5 min, followed by 35 cycles of at 94 °C for 30 s, at the appropriate temperature (Table 1) for 30 s and extension at 72 °C for 60 s, and then 72 °C for 10 min. The PCR products were separated on 12 % non-denaturing polyacrylamide gel and visualized by silver staining. A 20-bp DNA ladder molecular weight marker (TaKaRa) was used as the standard to determine the size of the alleles.

Among these primer sets synthesized, only 27 sets showed clearly polymorphism (Table 1 and Appendix).

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Table 1 Characterization of 27 polymorphic microsatellite loci in *H. bleekeri*

Locus	Repeat motifs	Primer sequence (5′–3′)	Tm (°C)	Size range (bp)	Na	PIC	Ho	He	*P*-HWE	Accession no.
BLT2	(AC)11	F: ATGACGCCACTATGAGACCG	60	230–310	4	0.681	0.656	0.742	0.018	KM051898
		R: CAGGAAGAAGAACCAGAACCA								
BLT4	(TG)11	F: TGAACAGACACTCACACAGGC	55	185–215	4	0.696	0.750	0.744	0.036	KM051900
		R: GTGTTTCAGCTGCTGCGTT								
BLT20	(GT)17	F: CCGTACTGCCTAGCAACACA	56	205–255	4	0.677	0.719	0.738	0.316	KM051916
		R: GGCTGTTTTCACAGAAAGGC								
BLT19	(CA)15	F: GTTCCTCTCTGTCCCCTTCC	55	133–185	5	0.718	0.818	0.792	0.009*	KM051915
		R: AAACACCATGGAACTCGACC								
BLT6	(GT)13	F: CCCAGGTCTGGTGTCCAGTA	55	105–123	4	0.696	0.708	0.760	0.024	KM051902
		R: GTACCGCGCTCAGCTCAT								
BLT27	(TC)11	F: GGGCAAGGTGTTATGGCTAA	58	135–153	4	0.691	0.719	0.751	0.501	KM051923
		R: ATGAAACAGGTCCATAGCGG								
BLT28	(GT)18	F: CCCTACCAAGCACCAATACC	56	155–200	5	0.746	0.563	0.794	0.003*	KM051924
		R: TGATGTCAGGTTGCTTATTCAGA								
BLT25	(TG)14	F: AATGAAACCAGCTCATTGCC	56	160–200	3	0.534	0.300	0.647	0.008*	KM051921
		R: CAAGTCCTTCCAAATGGTCC								
BLT30	(TG)14	F: CCGCTCACTTTGTTGACGTA	56	165–200	5	0.740	0.531	0.788	0.001*	KM051926
		R: CCCTGTCCAACCTCTCTCAG								
BLT16	(CA)17	F: ATCCAGTCAATAACCGCTGG	55	183–205	4	0.692	0.625	0.764	0.019	KM051912
		R: CCTCGAGAAACTCGGTTGTT								
BLT31	(AG)12	F: TGGATGGGTGTTACAAGCAA	56	90–100	3	0.505	0.833	0.594	0.142	KM051927
		R: CAGATCTTGAGACAAAGAGCCA								
Hbl1-101	(CA)13	F: TGTAATGTCACACACGCACG	55	190–240	4	0.673	0.625	0.742	0.220	KM385538
		R: CCAGACCAGAGGGACTTCAA								
Hbl2-2	(TTA)10	F: TCTAGTGCTGCATGTCTGCC	57	140–174	5	0.741	0.625	0.789	0.199	KM385539
		R: AGGCTCACATTGGCTCGTAT								
Hbl3-5	(TAA)11	F: TTGAAGTTGCCTTCTGGTCC	58	152–185	4	0.703	0.531	0.761	0.009*	KM385540
		R: GGCCACACATGCAAAACAT								
Hbl4-10	(ATC)12	F: GACAACAGCTACAGGGCACA	56	215–240	5	0.737	0.688	0.784	0.194	KM385541
		R: GACCTGGCTCTGGGTGATAG								
Hbl5-11	(TAT)11	F: TATGTGCCCAAAATGCTGTC	58	140–210	4	0.676	0.594	0.739	0.005*	KM385542
		R: AATGGGATGTATGGGACACG								
Hbl6-240	(AC)22	F: GGGGAATGCAGTTGAAATGT	55	140–180	6	0.808	0.531	0.845	0.001*	KM385543
		R: ATGGCATGTGTGGTGTGTTT								
Hbl7-290	(GT)14	F: ACTCTGATCGCTACCTGGGG	56	100–160	6	0.781	0.689	0.821	0.134	KM385544
		R: TCTGTTCGGACTGCTACATCA								
Hbl8-163	(GT)15	F: GACTGGTGAGTCACAGGCAA	60	83–105	8	0.800	0.750	0.833	0.001*	KM385545
		R: GCCTAGAGTGAGACCGATGC								
Hbl9-643	(TG)14	F: GGCACAGCATGTCCCTTTTA	55	100–125	4	0.702	0.818	0.766	0.031	KM385546
		R: CTGCAGCATGTTCTGGATGT								
Hbl10-16	(TAA)10	F: TTCCTCTCCTCCTCCTCCAT	56	155–185	5	0.749	0.719	0.796	0.063	KM385547
		R: GACTTCGGGGATGGCTCTAT								
Hbl11-21	(TCAT)10	F: CATTAATCCATCCAACCATGC	56	125–200	5	0.741	0.625	0.789	0.495	KM385548
		R: ACATCCCTGCCTTCGAAAC								
Hbl12-23	(AGTT)13	F: AATGCTTATTCACGCGAGGT	56	180–220	2	0.371	0.250	0.508	0.036	KM385549
		R: ACACACAGCTTGGGACACAG								
Hbl13-25	(TGAG)13	F: TTTCACTGTGACCTGCTGCT	56	88–135	5	0.748	0.688	0.796	0.074	KM385550
		R: CTCTCAACCAACACACACCG								

Table 1 continued

Locus	Repeat motifs	Primer sequence (5′–3′)	Tm (°C)	Size range (bp)	Na	PIC	Ho	He	*P*-HWE	Accession no.
Hbl14-29	(TGTA)10	F: GAGGTGCACGTGTTCAAAGA	55	178–190	4	0.697	0.594	0.756	0.047	KM385551
		R: TGGTTAAGACCAAGACCAACG								
Hbl15-31	(GTTA)10	F: GAGCTGGCTTGGTTGGTTAG	57	120–200	5	0.727	0.906	0.778	0.010	KM385552
		R: GCACCAGTCTTCTTTTTCGG								
Hbl16-128	(GT)13	F: AGCCTGTCAGCACTGATGATT	52	100–120	4	0.696	0.594	0.756	0.093	KM385553
		R: TGGTCTGGCCTATGAACACA								

Tm annealing temperature, *Size range (bp)* allele range, *Na* observed number of alleles, *PIC* polymorphic information content, *Ho* observed heterozygosity, *He* expected heterozygosity, *P-HWE* probability of Hardy-Weinberg equilibrium

* $P < 0.01$, significant departures from Hardy-Weinberg equilibrium

The polymorphic parameters, including the number of alleles (Na), observed heterozygosity (H_O), expected heterozygosity (H_E), and Hardy–Weinberg equilibrium (HWE) test, for each locus were performed using Popgen32 software. And the polymorphic information content (PIC) was calculated using PIC_CALC (Cheng et al. 2013). The number of alleles per locus varied from 2 to 8. H_O and H_E were 0.250–0.906 and 0.508–0.845, respectively (Table 1). The polymorphic information content (PIC) was 0.371–0.808 (Table 1). Eight loci (BLT19, BLT28, BLT25, BLT30, Hbl3-5, Hbl5-11, Hbl6-240, Hbl8-163) deviated significantly from the Hardy–Weinberg equilibrium ($P < 0.01$; Table 1). This is the first time to develop polymorphic microsatellite markers for *H. bleekeri* and the markers are expected to be useful for further studies of genetic diversity and paternity identification.

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Appendix: Sequences of *H. bleekeri*

Accession no.	Locus	Sequence
KM051898	BLT2	ATGACGCCACTATGAGACCGGTCAAACATACACATGTACACACACACACACACACACACAGAGAGA GACAGACAGACAGATATTAATCAATCAGCCGATAGATCAAAGACAAGACTCACTTCTCCCTGACTGTC TTACAGTAGAGTGGTTCTGGTTCTTCTTCCTG
KM051900	BLT4	TGAACAGACACTCACACAGGCTGATAAGAAGTACAGAAGATGAGAAGGTGTCCACACTTCATAGACT GAAAGGTCAGGAGTAAACAAAACAGTGATTTCTTGTGTGTGTGTGTGTGTGTGTGTGTACTCACAGGCT GGGGCTGCTCTCCAACGCAGCAGCTGAAACAC
KM051916	BLT20	CCGTACTGCCTAGCAACACAGCAACTAGTAGAAAAAGTGTAAACATATATGTGATGTATTACGTGGTC ACTGTGGAATAACAGCTTGAAGTTTAGGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTATGTGTG CGTGTGTACTTAAAGAGGTGATACCTGCAGTAACCTGTAACCAAATAGTTAACCACTTAACCTCTGCC TTTCTGTGAAAACAGCC
KM051915	BLT19	GTTCCTCTCTGTCCCCTTCCCTTTGCATCTCTCTTCACACACACACACACACACACACACACACACACGC ACGCACGCACGCACACGAGACACACTCGTCGTCTCTTCTTCAGCTTGCGCTGACTTGAATGTACACT CTTGTTCAAATTAGGTCGAGTTCCATGGTGTTT
KM051902	BLT6	CCCAGGTCTGGTGTCCAGTATGTCTGTCGTGTGTGTGTGTGTGTGTGTGTGTGTGCGTGTGTACATAC CTGTGTCTGCTAGGTCTCTCAGTGATGAGCTGAGCGCGGTAC
KM051923	BLT27	GGGCAAGGTGTTATGGCTAAACTAAGCATAGTTTACAAATGTATCTCTCTCTCTCTCTCTCTCTCTACA ACACGTGTCATATTGTGTTGGTCCGCTATGGACCTGTTTCAT
KM051924	BLT28	CCCTACCAAGCACCAATACCTGATATAAAAGATATCAATGTTTGCTAAGCGAATTATGCTAATTGTAAT GCTTATATTCTATGTGTGTGTGCGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGACGAGCATG ATTTTGTGTGCTATACTCTGTACTCTGAATAAGCAACCTGACATCA
KM051921	BLT25	AATGAAACCAGCTCATTGCCACACTTTGTCGGTTAAAACACCTGTGTGTGTGTGTGTGTGTGTGTGTG TGTAAACACTACACATTTTACACAGTGAACATATCAAGCAGCAACATACCAAACAATAAAACAGTTAA TTACAAAATACCTCCCAGAGTAACCTAGGACCATTTGGAAGGACTTG
KM051926	BLT30	CCGCTCACTTTGTTGACGTAGATGTGTACGTTATTTGTTTCAAAATTATATCACAGGTCAACTGTATTGC TGCTTAAGTGAGCAGGTGAAGGCTGAGTTTGTGTGTCTGTGTGTGTGTGTGTGTGTGTGTGCTT GTCTGGGTGAAGGTCTGATCTGCTCTGAGAGAGGTTGGACAGGG
KM051912	BLT16	ATCCAGTCAATAACCGCTGGCCAGGGGAATAAAGAGATGGAGTTAGGGAAAGTGTAGAAAGGAAGG GAGTAGCACACATGCGCACACACACACACACACACACACACACACACACACACTCTGACGTACACACA ATGCTATTGATTGCAGACTATCACAAAAAGGGTTGCCGGGGCAACAACCGAGTTTCTCGAGG
KM051927	BLT31	TGGATGGGTGTTACAAGCAATGTGGCAGAAGCTCCACATAGACCCAATCTAAAGAGAGAGAGAGAG AGAGAGAGAGTCCTTAGTTCTGGCTCTTTGTCTCAAGATCTG
KM385538	Hbl1-101	TGTAATGTCACACACGCACGCACGCACGCACACGCATGCACACACACACACACACACACACACACG AGTGTTGTCATTATCCATTACTTATTAGCTTACCTAACTTCGTTGCTAAAGTATAAAAGAATGAGATACC AAACTCGCTCGCAGGATTGGTTAACTCTTGAAGTCCCTCTGGTCTGG
KM385539	Hbl2-2	TCTAGTGCTGCATGTCTGCCTGGAGCAGGGTTTCCGGACATTTGACCTGCAATTTTATTTATCTGCTTA GTTATTATTATTATTATTATTATTATTATTATTATTTCAAAAGCCGGCTAAGCCAGCTAACAGAAACCCTAGCC TGCATACAGTGGCCCCATACGAGCCAATGTGAGCCT
KM385540	Hbl3-5	TTGAAGTTGCCTTCTGGTCCCAGCAATGGCATTGATAAAAGGTAACGTATTGTTTGTCCTTTTCTCTCA TTTATTAATAATAATAATAATAATAATAATAATAATAAAAATTTATGTCATGTAGCAGATGCTTTTATCCCA AAGAGACTAAATGTTTTGCATGTGTGGCC
KM385541	Hbl4-10	GACAACAGCTACAGGGCACAATAACGTTCTTTATTGATAAGGTTACAAACTCTTGACAAAGGCTTCAT CATATGCTTCATGCTTGGATTGATTGCTAATGAGCTAATGGTGAGTACTGACAATCAAGTTCAGGAATT CTCCTGAATTGAAATCTGCTCGAGCCTCAATTGTCAACTAAATGTATCATCATCATCATCATCATCA TCATCATCATCAATAAGCTTCTATCACCCAGAGCCAGGTC
KM385542	Hbl5-11	TATGTGCCCAAAATGCTGTCCAATTTCAGCATGCTCCTAATGATGAAATATCTGTGGATTAGAATTATAT ATTATTATTATTATTATTATTATTATTATTATATATAACAGTGCTTGCATAATAAGTAATGGTAGCCTTAG TATCGTGTCCCATACATCCCATT
KM385543	Hbl6-240	GGGGAATGCAGTTGAAATGTGTACACACACACACACACACACACACACACACACACACACACACAC TAGCAGGAGGAGCAGAGGGCGATGCAGTACACTTGTGAATACACAAACACAGGATTAGGCTTTAGGC TACATGTACAGACAGGCATATTCACAAAACACACCACACATGCCAT
KM385544	Hbl7-290	ACTCTGATCGCTACCTGGGGTGAGGCAGCAGTCAAAGTGTGTGTGTGTGTGTGTGTGTGTGTGCT GCTTGCTACCCGGCGGCGGTGATGTAGCAGTCCGAACAGA
KM385545	Hbl8-163	GACTGGTGAGTCACAGGCAAGTCACCATGTTGCCATGGGAGTGTGTGTGTGTGTGTGTGTGTGTG TGTGAGTATGCATGCATCGGTCTCACTCTAGGC
KM385546	Hbl9-643	GGCACAGCATGTCCCTTTTAGATGTGACATTGACACTCTTCGGTGTGTGTGTTTGTGTGTGTGTGTGT GTGTGTGTGTGTGTACTTGGTTAGTGTCTCTTTCCTCTCTTCACATCCAGAACATGCTGCAG
KM385547	Hbl10-16	TTCCTCTCCTCCTCCTCCATGTCCAGCTTGGCTTCCAGCTCCTTCCTCTCCTCCTCGATCTTCGCCTGC ATCTCCACCATCTTGTCAGGAGAAACCTTCTTCTTCTTCCCTTTAATAATAATAATAATAATAATAATAAT AATAGTGATTATCATTTATTTGTCTTATAGAGCCATCCCCGAAGTC

KM385548	Hbl11-21	CATTAATCCATCCAACCATGCTATCATTCATTCATTCATTCATTCATTCATTCATTCATTGGCCTAC ACAATAAAAATGCATCACTATGATTCTGACCATTGGTGCGGTTCTGAAGGCGAGGGTTTCGAAGGCA GGGATGT
KM385549	Hbl12-23	AATGCTTATTCACGCGAGGTATGCTACATAATTACAGCAGTGCCACATGACAAGCATAATAACATCAGC CATTATGAACTGTGGATCCAAATAAACAAGTTAGTTAGTTAGTTAGTTAGTTAGTTAGTTAGTTAGTTA GTTAGTTAGTTTGTTAATTGTGTGCTCTCTGACTCGTAGTTTAATTAATTATTAATATATTATCATGTCATT CCCTACATGCCTCATCTGTGTCCCAAGCTGTGTGT
KM385550	Hbl13-25	TTTCACTGTGACCTGCTGCTGGTGACTATCAGGCAGTAAGGCAAGCTGTTGCTGAGTGAGTGAGTGA GTGAGTGAGTGAGTGAGTGAGTGAGTGAGTGAGTGAGTGGTGGGGAGCGCCAGCGGTGTGTGTTGG TTGAGAG
KM385551	Hbl14-29	GAGGTGCACGTGTTCAAAGATGGAGCACTGTGTATGTATGTATGTATGTATGTATGTATGTATGTATGTA TCAGTGCGTCTCACTCTGCCGCCTCAAAGAGCAGACAGAGTTTATTGATAGGTCTCTGTAGGACGTTG GTCTTGGTCTTAACCA
KM385552	Hbl15-31	GAGCTGGCTTGGTTGGTTAGTTGGTTAGTTGGTTGGTTAGTTAGTTAGTTAGTTAGTTAGTTAGTTAGT TAGTTAGCTAGCTGTGGCATGCTAGTTTGCTAATCCCCCACATGCCTTCAGGTAAAGCCAGCTACAGA AACTGAAAGATGCCGAAAAAGAAGACTGGTGC
KM385553	Hbl16-128	AGCGAGACAGAGCGATAGAGAAATAGAAAGAGAGAGAGGGGCAATCAGAGATATAAAATATAGACA AGCCTGTCAGCACTGATGATTGATGAGAGGGTGTGTGTGTGTGTGTGTGTGTGTGCTACAAAGAC AGTCTGTGTGTTAGCAGCACAGACAGACCAGTCTGTGTTCATAGGCCAGACCA

The orange parts are primers
The highlighted parts are repetitive sequences

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