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Short Communication

Characterization of single nucleotide polymorphism (SNP) markers for the Chinese black sleeper, *Bostrychus sinensis*

Hangjun Wang[1], Liyan Zhang[2], Xiaowan Chen[3] and Shaoxiong Ding[4]*

Department of Oceanography, the Chinese University of Xiamen, Xiamen 361005, P. R. China.

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We characterized 11 single nucleotide ploymorphism (SNP) markers for the Chinese black sleeper, *Bostrychus sinensis*. These markers were isolated from a genomic library and tested in ten geographically distant individuals of *B. sinensis*. Polymorphisms of these SNP loci were assessed using a wild population including 36 individuals. All the 11 loci exhibited bi-allelic polymorphism. The minor allele frequencies ranged from 0.042 to 0.486. The observed and expected heterozygosities ranged from 0.083 to 0.556 and from 0.081 to 0.507, respectively. These markers are supposed to be useful in population genetic studies of Chinese black sleeper.

Key words: *Bostrychus sinensis*, single nucleotide ploymorphism (SNP), PAMSA.

FULL TEXT

The Chinese black sleeper, *Bostrychus sinensis* Lacepede 1801, occurs from the northern Indian Ocean coast, reaching east to the Pacific, Melanesia and Polynesia, north to Japan and south to Australia (Kottelat et al., 1993; Masuda et al., 1984). It is a small warm-water fish that inhabits holes and caves of estuaries and inlets. Chinese black sleeper exhibits narrow habitat specificity, spawns in burrow and shows behavior of guarding eggs. These characteristics not only influence their expansion capability, but make them sensitive to the changes of the surrounding environment. Until now, studies on *B. sinensis* mostly focused on its tolerance to environmental factors and its biological characteristics (Hong et al., 2006; Chen et al., 2006; Hong et al., 2006; Chen et al., 2006), whereas no population-genetic studies have been conducted, even though this information is critically needed for conservation. As the most promising genetic marker, single nucleotide polymorphisms (SNPs) had been widely used in the study of population genetics (Morin et al., 2004). Here, we developed the first set of SNP markers for Chinese black sleeper which can be widely used in population genetics studies of this organism in the future.

*Corresponding author. E-mail: sxding@xmu.edu.cn.

Genomic DNA of *B. sinensis* individuals sampled from Xiamen, China was extracted and sheared. Fragments of approximately 1 to 2 kb were isolated from an agarose gel, following end-blunting, ligation with a vector and transformation into *Escherichia coli* cells, and then a genomic library was constructed. 45 random clone inserts were sequenced, and BLAST searches were performed to characterize the loci. Most loci were nuclear sequences with the exception of one mitochondrion gene which was not considered here, and discarded. Then, the 44 primer pairs were designed based on all the cloning nuclear sequence using Primer 3 (Rozen and Skaletsky, 2000). Polymerase chain reaction (PCR) were performed in 25 µl reaction mixtures containing 100 ng DNA, 0.2 mM each dNTP, 0.4 µM each primer, 1 U Taq polymerase and 1×PCR buffer. PCR conditions were applied to all the reactions including an initial denaturation at 95°C for 10 min, followed by 30 cycles of denaturation at 95°C for 45 s, annealing at optimized temperature for 45 s, extension at 72°C for 1 min and a final extension at 72°C for 10 min. A DNA pool, including 10 individuals from different populations, was used as the primary template. From the 44 loci, 26 were confirmed by high quality sequencing and were selected for second turn PCR in 10 individuals that had longer geographical distances than the previous ones. Finally, from 14,754 bp high quality DNA sequences, we identified 68 SNPs in 17

Table 1. Characteristics of 11 single nucleotide polymorphism markers in the Chinese black sleeper (*B. sinensis*).

Marker name/accession number	Primer sequence (5'-3')	T_a(℃)	Allele and product size (bp)	Minor allele and frequency	H_O	H_E	*P*
Cbe1 HQ704695	ASF1: *ATTAATATCGGCC*CAGAGTTAGACATGGCCTGTAGG ASF2: *GCGGC*CAGAGTTAGACATGGCCTGGCGA R: GCTGCTGAAGATCAAGACCGA	60	T 214 C 222	C 0.4444	0.556	0.501	0.504
Cbg5 HQ704696	ASF1:*TTATATTAGTGTT*CACCATCTCATCCTTCTCAGTTCG *CCCGG*CACCATCTCATCCTTCTCAGCCCT R: CCGAAAAGCAGGCTACAGGAC	60	A 199 C 207	A 0.4583	0.417	0.504	0.293
Cbe7 HQ704697	ASF1: *TAATAATTGCCCG*TTCACAGATCTCTGAGCCAGAT ASF2: *CGACG*TTCACAGATCTCTGAGCCCTAC R: GACTGAGCGGCAGGCGTTGGTAA	60	C 141 T 149	G 0.4306	0.472	0.497	0.759
Cbe10 HQ704698	ASF1: *TATAATAAGGCGC*GAACATCCTACTAATCCTCTTATCT ASF2: *GCAGG*GAACATCCTACTAATCCTCTTCGCC R: GGCATTCGGCAGAACAGCAA	60	G 95 A 103	G 0.4861	0.472	0.507	0.679
Cbe16 HQ704699	ASF1: *AATATTTATCGCG*TTAGCGTCAAGAAGCTGAGGCG ASF2: *CGGCC*TTAGCGTCAAGAAGCTGATTCA R: TATGAGGAGGCTGCCACTACAAA	60	A 319 G 327	G 0.1944	0.389	0.317	0.075
Cbe17 HQ704700	ASF1: *TCTCTCTCCTTGG*TCTTCCAGCCATCTTTCCTTGGCAA ASF2: *TATAC*TCTTCCAGCCATCTTTCCTTGATAC R: CAGACAGCACAGTGTTGGCATA	60	C 207 A 215	C 0.3056	0.500	0.430	0.308
Cbg5 HQ704691	ASF1: *AATACCCCTACCC*ATCACCAGTCACAAAGTCAGTCTCG ASF2: *CGTTT*ATCACCAGTCACAAAGTCAGTAGCA R: CCCTTTGAGGCTACTTTTGTTGT	60	A 245 G 253	G 0.3750	0.528	0.475	0.450
Cbg1 HQ704690	ASF1: *TTCAGAATAGAAC*CTAATGCAATCACAGAGGTTTAAAC ASF2: *CGCAG*CTAATGCAATCACAGAGGTTTGGAA R: AAGTTCCTTCAGGGCATTTGTT	60	T 228 G 236	T 0.1364	0.091	0.239	0.002*
Cbg10 HQ704692	ASF1: *GTGCCGTGCCAGA*TAAAATGCTGAAAAAAATGGATGAT ASF2: *GGGGC*TAAAATGCTGAAAAAAATGGACAAC R: GCCATCACAACTGCGGTTACAA	60	G 233 A 241	A 0.0417	0.083	0.081	0.768
Cbg16 HQ704693	ASF1: *TATGGGTTCCTAT*AAGAATTGCGCAGTCCGCACCT ASF2: *TAGGG*AAGAATTGCGCAGTCCGCCACC R: TTGGCAAATGGTGGCAGAGCAG	60	C 245 T 253	T 0.3667	0.267	0.472	0.015*
Cbg18 HQ704694	ASF1: *TATATATCCGTTC*AACGTTTCCATGGTAACCGGCTC ASF2: *GGTGC*AACGTTTCCATGGTAACCGCGTG R: GGGGACTGTATGAAATGCTGTGCT	60	C 219 G 227	C 0.1250	0.194	0.222	0.476

ASF1 and ASF2, Allele-specific forward primers 1 and 2; R, reverse primers; T_a, annealing temperature; H_O, observed heterozygosity; H_E, expected heterozygosity; *P*, exact *P* value for Hardy-Weinberg equilibrium (HWE) test; underline bases denote internal mismatches; italic bases correspond to the tails. *Statistically significant after Bonferroni correction (adjusted P value = 0.05/11).

loci.

To make the SNP screening process quick and convenient, only one polymorphic site was selected in each locus to design PCR primers and perform (PCR Amplification of Multiple Specific Alleles) PAMSA procedure (Gaudet et al., 2007). Three unlabeled primers were designed for each (PCR Amplification of Multiple Specific Alleles) PAMSA procedure: one common reverse primer and two allele-specific primers having tails of different lengths to differentiate the two SNP alleles by the size of the amplification products on agarose gels. A standard 10 µl PCR reaction was mixed with 1×PCR buffer, 20 ng DNA, 0.2 mM each dNTP, 0.4 µM each primer, 1 U *Tag* polymerase and a standard cycling program was used: an initial denaturation at 95°C for 5 min, followed by 30 cycles of 30 s at 95°C, 30 s at 60°C, 30 s at 72°C, then a final extension of 5 min at 72°C. After PCR, 11 of the 17 loci that successfully amplified expected fragments were selected and applied to evaluate the polymorphism of a wild population, in which 36 individuals were randomly collected in 2010 from the coast of Hai fang, Vietnam. 10 previously sequenced samples were set as the controls in most genotyping batches.

We calculated the observed and expected heterozygosities, and tested for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) between pairs of loci using POPGENE32 software (version 1.32) (Yeh et al., 2000). All loci exhibited bi-allelic polymorphism. Minor allele frequencies ranged from 0.042 to 0.486. The observed and expected heterozygosities ranged from 0.083 to 0.556 and from 0.081 to 0.507, respectively. After Bonferroni corrections, two loci were found to depart significantly from HWE (Table 1). No significant linkage disequilibrium between loci pairs was detected. In summary, these markers developed here will be useful for population genetic analysis of Chinese black sleeper in the future.

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