

Technical Note

Development and Characterization of 20 Microsatellite Markers for Chinese Black Sleeper, *Bostrychus sinensis*

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Abstract: Twenty microsatellite markers were isolated and characterized from the Chinese black sleeper, *Bostrychus sinensis*. Loci were screened in 30 individuals from Taiwan. For each locus, the number of alleles varied from 4 to 22 with mean expected and observed heterozygosity of 0.79 and 0.66, respectively. One locus significantly deviated from Hardy-Weinberg equilibrium after Bonferroni correction and no significant linkage disequilibrium was detected. This set of microsatellites will provide a suitable tool for population genetic studies of Chinese black sleeper.

Keywords: *Bostrychus sinensis*; microsatellites; population genetic

1. Introduction

The Chinese black sleeper, *Bostrychus sinensis* (Lacepede 1801), is one of the most widespread species of Indo-Pacific eleotrids, manifesting on the northern Indian Ocean coast, reaching east to the Pacific, Melanesia and Polynesia, north to Japan, and south to Australia [1,2]. In China, it is distributed in the coastal area of the East China Sea, Taiwan Strait and South China Sea. Chinese black sleepers are burrowing amphibians. They live in a limited territory, spawn in their burrow, and exhibit egg-guarding behavior. These factors suggest that populations will be sensitive to local environmental conditions and have a low rate of dispersal. Therefore, *Bostrychus sinensis* can be a useful biological indicator of the effects of long-term historical vicariant events and short-term human activities on intertidal habitats. In order to facilitate its population genetic studies, we developed and characterized 20 polymorphic microsatellite markers from Chinese black sleeper.

2. Results and Discussion

The number of observed alleles per locus ranged from 4 to 22. The observed and expected heterozygosity values ranged from 0.200 to 0.889 and 0.186 to 0.933, respectively. One locus (S98W83) significantly deviated from Hardy-Weinberg equilibrium after Bonferroi correction ($P < 0.0025$) and no significant genotypic linkage disequilibrium (LD) was found between all pairs of these 20 loci after Bonferroi correction ($P > 0.0025$). The levels of polymorphism uncovered at these loci suggest that they should be useful for population genetics as well as phylogeographic studies.

3. Experimental Section

3.1. Isolation of Microsatellite Markers

Microsatellites from *B.sinensis* were isolated using a modified enrichment technique described by Ding [3]. Genomic DNA was extracted from the muscle tissue using a standard traditional phenol-chloroform procedure [4] and digested with *Sau3AI* at 37 °C overnight. Fragments from 500–2000 bp were excised from agarose gels using a QIAquick Gel Extraction Kit (QIAGEN) and ligated to two oligo adapters (Oligo A 5'-GATCGTCGACGGTACCGAATTCT-3' and Oligo B 5'-GTCAAGAATTCGGTACCGTTCGAC-3') to facilitate amplification by PCR. The amplified genomic fragments were subsequently hybridized with the 5'- biotinylated oligo probes ATA(CA)₂₂C and fragments containing potential repeat motifs were captured with streptavidin-coated magnetic beads (Dynabeads[®] M-280, Invitrogen). To increase the amount of potential repeat motifs, a “recovery” PCR was performed using oligo B as the PCR primer. The PCR products were then purified and ligated into PMD19-T vector (TAKARA) and transformed into DH5 α competent cells. Cells were then plated onto LB agar, X-gal and ampicillin and incubated overnight at 37 °C.

Table 1. Details for 20 polymorphic microsatellite loci developed for *Bostrychus sinensis*.

Locus Genbank no.	Repeat motif	Primer sequence(5'-3')	T_a (°C)	Size range (bp)	N_a	H_o/H_e	P -value
BSD026 JN806116	(CA) ₆₁	F: CATAAAAGACCCATTGTAAGTCT R: CTGTAGCCCTCAGGAGCACATA	58	256–280	8	0.865/0.825	0.0505
BSD121 JN806117	(AC) ₁₉	F: CGCACTGTCATCATAGCACTC R: CCACCTGACAATGATTTAGTT	58	131–175	8	0.444/0.760	0.0060
BSD137 JN806118	(TG) ₂₃	F: CTGACCTGGACTTCCCCTGG R: CTGGGACAGGAGATGAGTTTT	58	202–330	22	0.879/0.933	1.0000
BSB006 JN806119	(TG) ₆ C(GT) ₁₃	F: TATTCTGTAATTACTGATATGTGCA R: TACACAAGACCAAAAAGGTTAGGAA	60	172–222	9	0.742/0.843	0.2209
BSW045 JN806120	(CT) ₆ ...(AC) ₇₀	F: ACTTTTTTCTCAATTTGGTTTCTAA R: TGTGCTCAGGGGTACCGGGA	52	128–220	14	0.833/0.867	0.9975
BSW068 JN806121	(AC) ₁₆	F: CTACAACAGCATCAGCCAACC R: ACTCCCAAACACTGTCCAAGAAC	58	113–133	7	0.684/0.766	0.3483
BSSD14 JN806122	(TG) ₄₃	F: ATTTAGCGAGGCTTTATGTT R: GGCTGGCTTCCATCTTTTCT	55	200–244	11	0.567/0.754	0.5198
BSSD21 JN806123	(TG) ₃₈	F: GATCCATTCTTAAAACACTCGTTAT R: CAGGAGCAGTATCCAGACAAAA	55	267–313	9	0.774/0.833	0.6818
BSSW83 JN806124	(TG) ₁₆	F: CCAGCAGCACCTGACACTCCAT R: TCCAGTGTTTGAAACTCCTGCC	58	144–156	8	0.452/0.808	0.0000 *
BSE020 JN806125	(GT) ₂₅	F: GATTTTCAGAGCAGCAGCGTTGGC R: CCACAAACGGAGCGTCCCAAATCT	66	239–339	10	0.769/0.824	0.9217
BSSW87 JN806126	(GT) ₃₅	F: CGCACAGTTGACGCTTCCTTTA R: GCCTCCCTGTCAGCCTTCACT	64	314–388	10	0.680/0.860	0.7399
BSSW89 JN806127	(GT) ₃₃	F: TTGTAGCATTCCTTCTGCCTGT R: CTCACTCCATCGGAATGTGTCTA	52	182–246	10	0.583/0.884	0.1589
BSD106 JN806128	(CA) ₂₀ T(AC) ₉	F: GAGATGAGCAACAGGTGAGTC R: CTGGCAGAAGAGGATTGATGG	56	338–388	10	0.667/0.842	0.5932
BSD045 JN806129	(GT) ₃₅	F: AAATGGATGTGTGAGAATGTGAGGCA R: TGTGAACTCGAATGTGGGAGGTACT	62	258–384	10	0.467/0.720	0.4404

Table 1. Cont.

Locus Genbank no.	Repeat motif	Primer sequence(5'-3')	T_a (°C)	Size range (bp)	N_a	H_o/H_e	P -value
BSW115 JN806130	(GT) ₃₇ C(TG) ₁₇	F: TGTGATGTGTGTTTTGGGTGGTTA R: TGTGTCCTCTGAAGTACCTGAAGC	64	437–541	7	0.708/0.764	0.8613
BSW053 JN806131	(AC) ₅ ...(CA) ₇ ...(AC) ₅₀	F: TGCCCCCAGATACCGACATTA R: CGAGAGGTGAGCCAGGTTTCAGGACT	66	308–394	17	0.889/0.912	0.9997
BSD125 JN806132	(CA) ₆ CGCACG(CA) ₃₅	F: CGCTTCAGTTCTGTGGAGGTA R: CTGTCTGCCAAAGTTCCTGTTA	56	137–185	9	0.714/0.827	0.9570
BSC001 JN806133	(GT) ₄₈	F: CTTGTTATGTCAAACCGTAGCCTTA R: CCCTATCGTCCCCTGTAGACCG	56	341–401	15	0.615/0.909	0.4762
BSE008 JN806134	(TG) ₁₉	F: GCTGCTCATAAACAATCACTTC R: GTTGTCTGTAATCAGTGGCTCTA	58	138–154	6	0.656/0.734	0.7305
BSC002 JN806135	(ACT) ₁₈	F: ATCAGCATCACAATGACCTGGGAG R: CTTGGTGGAACCTACAGACTTTTACA	55	247–271	4	0.200/0.186	0.9926

T_a Annealing temperature (°C), N_a Number of alleles, H_o/H_e Observed heterozygosity and Expected heterozygosity, P -value, P -values for exact tests for Hardy-Weinberg equilibrium(HWE), * Show significant deviation from HWE after Bonferroni correction ($P < 0.0025$)

The positive clones were identified by PCR with vector-specific primers. After being identified, 196 positive clones were randomly selected for sequencing using ABI3730XL sequencer (Applied Biosystems). Chromatograms were assembled and edited using SEQUENCHER 4.9 (Gene Codes Corporation), and 70 primers were designed for each unique amplicon containing a target microsatellite repeat. Initially, eight samples from different localities were used to test amplification of loci and evaluate polymorphic content. The PCR amplification was performed in 15 μ L volume containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂ 0.2 mM each dNTPs, 0.4 μ M each primer, 1 U *Taq* polymerase (TAKARA) and 30 ng genomic DNA. After denaturation for 5 min at 94 °C, followed by amplification for 30 cycles (94 °C for 30 s, annealing temperature for each pair of primers (Table 1) for 30 s, 72 °C for 30 s) and a final step at 72 °C for 5 min. PCR products were mixed with the GS500LIZ size standard (Applied Biosystems) and formamide and run on an ABI3130xl DNA Analyzer. Fragment analysis and genotyping were performed using Genemapper version 4.0 (Applied Biosystems). Out of the 70 primer pairs tested, 20 pairs were successfully amplified by PCR and further characterized using additional samples at Chiku Lagoon (23°55'05" N-120°02'57" E) from Taiwan ($n = 30$).

3.2. Data Analysis

The number of alleles, observed and expected heterozygosities, P value of Hardy-Weinberg and linkage disequilibria were estimated by using POPGENE [5].

4. Conclusions

In the present study, we describe 20 polymorphic microsatellite loci shown as the first set of microsatellite markers designed specifically for *Bostrychus sinensis*. These loci would be useful in providing an effective tool for investigating genetic variation and population structure in *B. sinensis*. Microsatellites are an excellent choice of genetic marker for genome mapping due to their hyper-variability and abundance throughout most vertebrate genomes. In our results, there are some strong heterozygosities for microsatellite loci, like BSD137/BSW045/BSW053/BSC001, which are suitable for identifying genetic mapping in *B. sinensis*. These markers will prove helpful in the management of fisheries and in the design of conservation strategies.

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