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Short Communication

Novel tetranucleotide microsatellite markers for Chinese beard eel (*Cirrhimuraena chinensis* Kaup)

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ABSTRACT. In this study, we characterized 12 novel polymorphic microsatellite loci for Chinese beard eel (*Cirrhimuraena chinensis* Kaup) from a tetranucleotide microsatellite-enriched library. Loci screened on a sample of 37 individuals from Xiamen wild stocks revealed 8 to 24 alleles per locus, with a mean of 13.83 over all loci. Observed and expected heterozygosities ranged from 0.270 to 0.944 and 0.439 to 0.942, respectively. These efficient genetic markers thus provide useful tools in the study of the population genetics and phylogeography of Chinese beard eel.

Key words: Chinese beard eel; *Cirrhimuraena chinensis* Kaup; Microsatellite

INTRODUCTION

The Chinese beard eel (*Cirrhimuraena chinensis* Kaup) is one of the nearshore warm-water demersal fishes, which spreads widely from the eastern coast of Indo-Africa to Indonesia, China, and the Philippines. In China, it occurs along the coastal areas of the East China Sea, Taiwan Strait, and South China Sea (Zhu, 1984). It inhabits the sandy shallows of estuaries and burrows of low intertidal zones, and is generally considered to be a resident fish without long-distance swimming ability (Liu et al., 2005). In addition, it experiences a metamorphosis stage of leptocephalus in its life cycle (Zhang et al., 1982), as do most Anguilliformes fishes. These factors suggest that the population of *C. chinensis* may be sensitive to local environmental conditions and have different dispersal rates in the larval and adult stages. Thus, Chinese beard eel could be regarded as a favorable environmental monitoring candidate of local habitats. However, little genetic information is currently available and no studies on its population structure have been reported yet.

Microsatellite markers are extensively applied in genetic studies for their superiority of abundance, co-dominance, easy detection, and high polymorphism (Aggarwal et al., 2004). In order to avoid mistyping the real alleles, a tetranucleotide repeat motif strategy was used to make the stutter bands less common (Hoffman and Amos, 2005). Here, we describe the isolation and characterization of 12 polymorphic microsatellite markers for Chinese beard eel.

MATERIAL AND METHODS

Samples of Chinese beard eel were captured from Xiamen wild stocks. To construct a microsatellite-enriched library, genomic DNA was isolated from muscle tissue originally collected and stored in ethanol absolute, using a standard traditional phenol-chloroform procedure (Sambrook et al., 1989). The microsatellites were enriched according to the FIASCO protocol (Zane et al., 2002), with little modifications: approximately 250 ng genomic DNA was simultaneously digested with *Cvi*QI and ligated to a *Cvi*QI adaptor (5'-TAGTCAGGACTCAT-3'/5'-GACGATGAGTCCTGAC-3') overnight at 25°C. The digestion-ligation mixture was diluted (1:10), and directly amplified in a total volume of 20 µL with a pig-tailed *Cvi*QI-N primer (5'-GTTTGACGATGAGTCCTGACTACN-3'). Biotinylated $(GATA)_6$ and $(AAAT)_6$ were used as probes. The probe hybridization and capture of the probe-hybridized DNA by Streptavidin MagneSphere® paramagnetic particle (Promega) methods followed the protocol of Zane et al. (2002). After 3 nonstringency washes and 3 stringency washes, the recovered DNAs were amplified by 24 cycles of PCR using the above-mentioned primer. The PCR products were then purified and ligated into the PMD18-T vector (TaKaRa) and transformed into DH5α competent cells. A total of 214 positive clones selected randomly from the enriched library were amplified and sequenced. Microsatellite loci were sought with SSRHunter 1.3.0 (Li and Wan, 2005). Fifty-nine clones with microsatellite inserts and sufficient flanking sequences were chosen to design primers by the Primer 5 software (Premier Biosoft International). For all loci, the 5'-end of the forward primer was attached to an M13 tag (5'-TGTAAAACGACGGCCAGT-3') to facilitate incorporation of a 5'-fluorescent label (Schuelke, 2000).

Initially, new primer pairs were tested by amplifying 6 to 10 specimens to evaluate for polymorphic content. The PCR amplification was performed in a 16-µL reaction containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.1 µM forward primer, 0.4 µM reverse primer, 0.3 µM dye-labeled M13 primer (FAM/HEX/TAMRAD), 0.64 U *Taq* polymerase (TaKaRa), and 30 ng genomic DNA. The PCR profile consisted of an

initial denaturation step at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 45 s, primer-specific annealing temperature for 45 s, and extension at 72°C for 45 s, and then another 8 cycles of denaturation at 94°C for 45 s, annealing at 53°C for 45 s, and extension at 72°C for 45 s, and then terminating with a final extension at 72°C for 10 min. Of 59 primer pairs assessed, 12 primer pairs with highly polymorphic performance were selected for further screening with 37 individuals from Xiamen stocks. The PCR products were checked on a 1.5% agarose gel and then electrophoresed on an ABI 3730xl sequencer (Applied Biosystems, Foster City, CA, USA) using a ROX 500 size standard (Applied Biosystems). The GeneMarker software (SoftGenetics, State College, PA, USA) was used to confirm all the allele calls.

The number of alleles, observed and expected heterozygosities (H_O and H_E, respectively), deviation from Hardy-Weinberg equilibrium, and linkage disequilibrium were estimated by Genepop (Rousset, 2008). Sequential Bonferroni's corrections were conducted for multiple comparison tests.

RESULTS

The loci exhibited high levels of polymorphism, with H_O and H_E varying from 0.270 to 0.944 and 0.439 to 0.942, respectively. The number of alleles per locus ranged from 8 to 24, with a mean of 13.83 per locus. After Bonferroni's correction, 3 loci (CCG24, CCG39, and CCI52) departed significantly from Hardy-Weinberg equilibrium in the screened samples, and linkage disequilibrium between a pair of loci (G30 and G71) was detected. Details of the microsatellite markers, including the name of each locus and its GenBank No., repeat motif, primer sequences, primer melting temperature, base-pair size range of alleles/locus, number of alleles/locus, H_O and H_E, and P value, are shown in Table 1.

Table 1. Details for 12 polymorphic microsatellite loci developed for *Cirrhimuraena chinensis* Kaup.

Locus GenBank No.	Repeat motif	Primer sequences (5'→3')	Ta (°C)	Size range (bp)	N_A	H_O	H_E	P value
CCA10 JX644946	$(TAAA)_8$	F: *TTCAAAATAATTGGGTTATAC R: CTCATCATCTTTGTTAATGTC	51	105-197	13	0.595	0.712	0.1531
CCB20 JX644947	$(ATTT)_{11}(TG)_7$	F: *CAATGCAGAGTTAGGGGC R: GATAAGGTCCGCACAGCT	56	141-283	17	0.686	0.798	0.0867
CCG24 JX644948	$(TATC)_{19}TATG(TATC)_{18}$	F: *AATTAAATAATTGCGCCAG R: CCACAAATACCCTCATCCA	51	147-265	9	0.270	0.439	0.0000#
CCG30 JX644949	$(TATT)_{11}$	F: *TAAGGGTCCATGTGCTTCAC R: GAGCTGCTTCCATCAATTTC	56	109-181	12	0.784	0.892	0.3248
CCG39 JX644950	$(TAAA)_4TG(TAAA)_6$	F: *GTCGTCAGACTATGGACAGCAC R: TAATACGCCGTCGTAATTAACA	50	117-197	12	0.629	0.916	0.0035#
CCG71 JX644951	$(GATA)_{22}$	F: *CGATACTTTCATTTAGGTT R: GTCTGCTTTGTAGTGTTCA	55	179-283	24	0.919	0.928	0.9059
CCH22 JX644952	$(ATTT)_8$	F: *AAGGGTTTGGGGTTACATT R: GGTTGATTTGAAACAGGCA	51	132-204	8	0.500	0.672	0.0055
CCI34 JX644953	$(TTTA)_9$	F: *GCTGTTGGGCTTCCTCTT R: TCCTGGCAGTCATTTGTT	55	97-137	10	0.622	0.631	0.5450
CCI52 JX644954	$(TCTA)_6TTTA(TCTA)_{30}$	F: *CTGTCATTTGGTTTTAGC R: TTTGTGTGTATGCGTGTA	55	147-313	17	0.306	0.912	0.0000#
CCI55 JX644955	$(TAAA)_{10}$	F: *CAAAAATAGTAACCCTCC R: CAAATGAGATGTTGGATG	56	99-167	13	0.757	0.839	0.7991
CCJ20 JX644956	$(CAAT)_{15}CAAC(CAAT)_4$	F: *CCAAAACATACAGAACCGTG R: GAAATGCCTGCTCCATTACA	55	98-164	13	0.806	0.778	0.0098
CCJ29 JX644957	$(ATCA)_{10}ATCG(ATCA)_{11}$ $(ATCT)_{13}$	F: *CAAATAACAAATCAAGAAAG R: AGAGACAGACAGACACAAAT	55	199-295	19	0.944	0.942	0.6095

Ta = annealing temperature; N_A = number of alleles; H_O and H_E = observed and expected heterozygosities, respectively; P value = probability of deviation from Hardy-Weinberg equilibrium. *M13 tag (5'-GTAAAACGACGGCCAG-3') label. #Significant deviations from Hardy-Weinberg equilibrium after sequential Bonferroni's corrections.

DISCUSSION

As far as we know, this set of markers represents the first microsatellites developed for *C. chinensis* and thus contributes available molecular resources to the genetic study of this species. The preliminary results of high variability over these loci indicate that they could be useful in the evaluation of the genetic variation and genetic population structure of Chinese beard eel.

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