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Isolation and characterization of microsatellite loci in *Branchiostoma belcheri* Gray (Amphioxus)

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ABSTRACT. *Branchiostoma belcheri* Gray is a second-class, nationally protected protochordate in China. We developed 10 novel polymorphic sites in *B. belcheri*, which were examined using a population of 30 wild individuals from Xiamen, China. The polymorphism information content ranged between 0.141 and 0.681, and the number of alleles varied from 2 to 5. The expected and observed heterozygosities varied between 0.1528 and 0.6920, and between 0.1429 and 0.5000, respectively. These novel microsatellite markers will facilitate the genetic analysis and protection of wild *B. belcheri* individuals, and the possible re-stocking of the species in the long-term.

Key words: *Branchiostoma belcheri* Gray; FIASCO; Conservation; Microsatellite

INTRODUCTION

Branchiostoma belcheri Gray is one of the few remaining species of lancelet, and is a filter feeder that spends most of the time in the sediment. *B. belcheri* Gray is distributed in South Africa, the Indo-West Pacific, and Madagascar, and it also occurs in China, particularly along the coasts of Fujian, Guangdong, and Shandong Provinces. However, in recent decades its population has decreased sharply due to the effects of habitat destruction and overfishing (Fang, 1987). The conservation of this species is essential, in order to effectively protect it. It is not only globally endangered, but is also under second-class state protection in China (Dai et al., 2013).

Microsatellites are co-dominant, widespread, and important components of vertebrate genomes (Jarne and Lagoda, 1996), and are regarded as effective genetic markers in *B. Belcheri* Gray. Microsatellites have been extensively used in ecology and evolutionary biology (Selkoe and Toonen, 2006) in studies on population genetics and differentiation, linkage analysis, and evolution. To contribute to the conservation of *B. belcheri* Gray, we developed 10 novel microsatellites for the species.

MATERIAL AND METHODS

The microsatellite loci were developed using the fast isolation by amplified fragment length polymorphism of sequences containing repeats (FIASCO) protocol (Zane et al., 2002). We extracted DNA from the muscle tissue of *B. belcheri* Gray individuals using a Genomic DNA Extraction kit (Tiangen, Beijing, China). Genomic DNA was digested using the restriction enzyme *Mbo*I (Fermentas, Vilnius, Lithuania) and two biotinylated probes, $(GT)_{15}$ and $(CT)_{15}$, were used to construct genomic libraries (Chen et al., 2012). There were 152 positive clones, with fragment sizes ranging between 500 and 1000 bp, which were sent to Invitrogen (Guangzhou, China) for sequencing. Thirty-nine pairs of primers were designed by Primer Premier 6.0, based on the results of the sequencing.

We examined the 10 primer pairs using polymerase chain reactions (PCRs), from 30 wild *B. belcheri* Gray collected from Xiamen, China. The PCR final volume of 10 µL consisted of 50 ng template DNA, 1X *EasyTaq*® buffer for PAGE, 2.5 U *EasyTaq*® DNA Polymerase for PAGE (TransGen Biotech, Beijing, China), 0.4 mM dNTP, and 0.4 µM of each primer. The PCR conditions were as follows: an initial denaturation at 94°C for 5 min, 35 cycles at 94°C for 30 s, annealing temperature for 30 s, 72°C for 1 min, and a final extension at 72°C for 10 min. The amplification fragments were resolved by denaturing them on 6% polyacrylamide gels using Sequi-Gen™ Sequencing Cell (Bio-Rad, Hercules, CA, USA). The number of alleles per locus (N_A), the expected (H_E) and observed (H_O) heterozygosities, and the polymorphic information content (PIC) were calculated using PopGene32 (version 1.32) (Yeh et al., 2000) and CERVUS (version 3.0.3). Deviations from the Hardy-Weinberg equilibrium (HWE) and the genotypic linkage disequilibrium were also tested using PopGene32.

RESULTS AND DISCUSSION

We successfully amplified 10 polymorphic loci (Table 1). The N_A ranged between 2 and 5, and the H_E and H_O varied between 0.1528 and 0.6920, and between 0.1429 and 0.5000, respectively. No linkage disequilibrium was detected after applying a Bonferroni's correction.

Eight loci were in HWE (P > 0.05), except for the loci WCY1 (P = 0.0000) and WCY2-22 (P = 0.0000). These highly significant HWE deviations were probably caused by the small sample size. In addition, the solitary nature of *B. belcheri* Gray, as well as possible invasions of individuals that had been cultured, might have affected wild populations.

In summary, the ten loci characterized in the present study are a set of microsatellite markers for *B. belcheri* Gray. They were successfully amplified and exhibited high levels of heterozygosity. Therefore, they will facilitate further genetic analysis of this species, and will be a valuable resource for its conservation and possible re-stocking in the long term.

Table 1. Statistic information of ten microsatellite loci in *B.belcheri* Gray (30 individuals).

GenBank accession No.	Locus ID	Primer sequences (5'-3')	Repeat motif	Ta (°C)	N_A	Allele size (bp)	PIC	H_O	H_E
KM029984	WCY1	F: GATCGTTTTCGGCTTGCTT R: TAAACCGATGAGTCTACCTCC	$(GT)_{21}$	44.5	5	290-315	0.681	0.1786	0.6920**
KM029985	WCY7	F: TACTGCCCACTCGATTCCAC R: ATGTATACTCCGCATTAACACT	$(AC)_{25}(CT)_{28}$	42.0	3	253-315	0.454	0.2500	0.3299
KM029986	WCY2-4	F: CATCTATAGCAGCAACACTTGGG R: CAAACATTAGCCATCTGAACAGG	$(TG)_{32}$	53.2	4	187-198	0.626	0.4762	0.5408
KM029987	WCY2-16	F: AGCCTTCTCAAGTTATTGTGTCC R: TAGACAAGCAAATGACAGGCAAC	$(AC)_{17}N(CA)_{20}$	44.5	2	225-235	0.141	0.1667	0.1528
KM029988	WCY2-22	F: GATCAAGCACACAATTGCAAG R: CTCCGGTGTACGATTTCCT	$(GT)_{25}$	48.0	4	280-300	0.489	0.1429	0.4917**
KM029989	WCY4-33	F: ACACATACACATTCACAGAG R: CTAATCGCATTTGCCATTG	$(AG)_{27}(GT)_{26}$	51.0	3	124-131	0.342	0.5000	0.4150
KM029990	WCY4-37	F: TGTAATTGTATGCCTGAGC R: TCCAATCAAGTGCTGACT	$(GA)_{17}(GT)_{36}$	53.2	3	100-108	0.368	0.3333	0.2778
KM029991	WCY4-38	F: ACCAAGGAGGTTAGAAAGG R: GTCTGTACTATTGTCTCAAGG	$(GA)_{24}(GT)_{71}$	46.8	3	270-300	0.351	0.2222	0.2524
KM029992	WCY4-50	F: GACACAGACACACAGACA R: ATGAACGGAAGAGTGAGAG	$(CA)_{31}N(CA)_{10}N(CT)_{21}$	51.0	4	172-182	0.498	0.2083	0.3620
KM029993	WCY4-58	F: GGAGGTATGGAGTCTATGG R: GGAACAACAGACGATAAACT	$(CT)_{32}N(CT)_{14}$	48.8	4	86-98	0.596	0.2857	0.5000

Ta = annealing temperature; N_A = number of polymorphic alleles per locus; PIC = polymorphism information content; H_O = observed heterozygosity; H_E = expected heterozygosity. **High significant deviations (P < 0.005) of locus from Hardy-Weinberg equilibrium after correction for multiple tests (k = 10).

Conflicts of interest

The authors declare no conflict of interest.

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