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Discovery and characterization of a first set of polymorphic microsatellite markers in red crab (*Charybdis feriatus*)

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Introduction

The red crab (*Charybdis feriatus*) is a large size marine portunid species, broadly distributed in the Indo–Pacific sea areas. In mainland China, it is naturally found in the coastal areas of Zhejiang, Fujian, Guangdong, Guangxi and Hainan provinces. The juvenile crab lives in the sandy shore, while the adult inhabits muddy offshore areas (Baylon and Suzuki 2007). Because of its taste, fast growth rate and colour, *C. feriatus* has been considered as one of the most high-potential species for aquaculture. To date, very few information about the population genetic diversity and differentiation of *C. feriatus* are available, except that a wild population sampled from coast of Shanwei city of China showed moderate genetic variation as revealed by cytochrome *c* oxidase subunit I (*COI*) gene (Huang 2009). Microsatellites are an ideal molecular marker system for investigating population genetic diversity and variation, and have been widely isolated in other portunid species, such as *Callinectes sapidus* (Steven *et al.* 2005), *Scylla paramamosain* (Ma *et al.* 2010) and *Portunus trituberculatus* (Cui *et al.* 2012). Lack of microsatellite markers has limited the population genetic studies in *C. feriatus*. The purpose of this study was to isolate and characterize polymorphic microsatellite markers so as to facilitate the studies on genetic background and genetic structure in *C. feriatus* and other related portunid species.

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Materials and methods

5′-Anchored PCR and isolation of microsatellites

First, we designed three degenerate primers with the sequences KKVRVRV$(CT)_6$, KKRVRVR$(GT)_6$ and KKBDDBD$(CAC)_4$, where K = G/T, V = A/C/G, R = A/G, B = G/T/C and D = G/A/T. The repeat region of the primer matched with microsatellites in genomic DNA and the seven nucleotides at 5′ part of the primer works as an 'anchor'. The 5′ anchor PCR was carried out as described by Cui *et al.* (2011). The PCR products were separated on 1.0% agarose gel. The fragments with the length between 250 and 700 bp were recovered and ligated into pMD19-T vector (TaKaRa, Lalian, China), and then transformed into *Escherichia coli* DH 5α competent cells. One hundred and twenty-three positive clones were sequenced using ABI Prism 3730 DNA sequencer (ABI, USA) in order to obtain target sequences. Additionally, four gene sequences of *C. feriatus* were downloaded from GenBank database (http://www.ncbi.nlm.nih.gov/). All sequences derived from sequencing and from internet were screened for microsatellites using the software SSRHunter 1.3 (Li and Wan 2005). Finally, 47 pairs of primers were designed successfully based on the flanking sequences of microsatellites using Primer Premier 5.0 software (http://www.premierbiosoft.com/).

Microsatellite genotyping and data analysis

We collected 31 individuals from a wild population of *C. feriatus* from Zhoushan islands of China. Genomic DNA was isolated from the muscle tissues using a traditional proteinase K and phenol–chloroform extraction protocol as

Keywords. microsatellite markers; 5′-anchored PCR; function gene; genetic variation; *Charybdis feriatus*.

Table 1. Characterization of eight polymorphic microsatellite markers in the red crab (*Charybdis feriatus*).

Locus	Repeat sequence	Primer sequences (5' - 3')	T_a (°C)	N_a/N_e	H_O	H_E	PIC	P	GenBank acc. no.
Cfe-1	$(ACC)_9AA(CAA)_{11}$	ATGCTTGCTATTATTTTCTACTG GAGCAAATTAAGATTGACTGA	51	5.0/1.2	0.19	0.18	0.18	1.00	KF016938
Cfe-2	$(TG)_2G(TG)A(TG)$	GAGTGGTAATGGGAGCAAATG TCCACATGCTCGTAAAACAAA	54	5.0/3.2	0.79	0.70	0.64	0.24	KF016939
Cfe-3	$(GACA)_6$	GGGATGTAAGACAATGTGAAC TTATACACCAATCTATATTAATTTC	50	2.0/1.2	0.17	0.16	0.14	0.66	KF016940
Cfe-4	$(AC)_{13}$	ACCAGCCGTAATGCAGAACAC AAAACCCTGAGAAAGGATTGCA	59	6.0/3.7	0.74	0.74	0.69	0.27	KF016941
Cfe-5	$(TCC)_7$	CTTCCCCTTGGATGACGCTC GACTTAAACTCCCTTTGCTACCTG	60	2.0/1.6	0.32	0.39	0.31	0.33	KF016942
Cfe-MIH-1	$(CA)_{23}$	TGCGAGACTCACTCAACACT TTATGTTGTACACCGCTCCA	55	7.0/4.8	1.00	0.81	0.77	0.48	AF092945
Cfe-MIH-2	$(GT)_4N_6(GT)_8GC(GT)_2$	CTTAGTTATTCCGTCCCGTTTA TTAGCCCGCCCTATCTTCTC	55	2.0/1.1	0.13	0.12	0.11	0.74	AF092945
Cfe-MIH-3	$(GT)_{14}$	GTAGCGAAGGTACGAGAAGC GAAGATCGGAAACAATGAGC	56	3.0/1.7	0.50	0.41	0.36	0.38	AF092945
Mean	–	–	–	4.0/2.3	0.48	0.44	0.40		–

T_a, annealing temperature; N_a, observed number of alleles; N_e, effective number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; PIC, polymorphism information content.

described by Ma *et al.* (2009). Polymerase chain reaction (PCR) was performed on a Peltier Thermal Cycler (PTC-200, MJ Research, USA) in 12.5 μL total volume that included 0.4 μM each primer, 0.2 mM each dNTP, 1× PCR buffer, 1.5 mM $MgCl_2$, 0.3 unit *Taq* DNA polymerase, and approximately 50 ng template DNA under the following conditions: one cycle of denaturation at 94°C for 4 min; 30 cycles of 30 s at 94°C, 50 s at a primer-specific annealing temperature (table 1), and 50 s at 72°C. As a final step, products were extended for 7 min at 72°C. The PCR products were separated on 6% denaturing polyacrylamide gel, and visualized by silver-staining. The size of allele was estimated according to the pBR322/*Msp*I marker (TianGen Biotech, Beijing, China). Genetic diversity indices were calculated using the software PopGene ver. 1.31 (Yeh *et al.* 1999).

Results and discussion

Of the 47 pairs of primers, eight were found to be polymorphic in the wild population (table 1), while others were monomorphic, smears or yielded no products. The number of alleles (N_a), observed (H_O) and expected heterozygosity (H_E) and polymorphism information content (PIC) per locus ranged from 2 to 7, 0.13 to 1.00, 0.12 to 0.81 and 0.11 to 0.77, respectively. Compared with the other crab species, microsatellites variation level in *C. feriatus* is lower than that reported in *Scylla paramamosain* and *Portunus trituberculatus* (Ma *et al.* 2010; Cui *et al.* 2012). All loci were in Hardy–Weinberg equilibrium ($P > 0.05$) and no evidence for stuttering and allelic dropout were found in all loci. Further, no significant linkage disequilibrum between pairs of loci were found.

In conclusion, this study isolated eight polymorphic microsatellite markers using the 5′-anchored PCR assay and the bioinformatic mining of GenBank database that will be helpful for studies on population genetic diversity and differentiation in the red crab (*Charybdis feriatus*) and other related crab species.

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