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# Screening and characterization of new microsatellite markers in *Fenneropenaeus penicillatus*

**J.B. Shangguan[1,2], Z.B. Li[1,2], Q.H. Li[1,2], G. Dai[1,2] and Y.F. Ning[1,2]**

[1]Fisheries College, Jimei University, Xiamen, China
[2]Fujian Provincial Key Laboratory of Marine Fishery Resources and Eco-Environment, Xiamen, China

Corresponding author: Z.B. Li
E-mail: lizhongbao@jmu.edu.cn

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**ABSTRACT.** *Fenneropenaeus penicillatus*, with high protein and low fat, is a commercially important aquatic product in China. Microsatellite loci were developed according to the protocol of fast isolation by amplified fragment length polymorphism of sequences containing repeats. Eight new polymorphic microsatellite markers for *F. penicillatus* were identified, and 32 wild individuals were used to evaluate the degree of polymorphism of these markers. The polymorphism information content ranged from 0.2703 to 0.7598, and the number of alleles per locus varied from 3 to 6. The observed and expected heterozygosities were 0.1613-0.5556 and 0.2347-0.7387, respectively. No significant deviations from Hardy-Weinberg equilibrium ($P > 0.00625$) were detected. These polymorphic microsatellite loci will be useful to study the genetic diversity and population structure of *F. penicillatus*.

**Key words:** *Fenneropenaeus penicillatus*; Polymorphic microsatellite loci; Magnetic bead enrichment

 

## INTRODUCTION

*Fenneropenaeus penicillatus*, generally called red tail shrimp by the Food and Agriculture Organization, is widely distributed in the India and Indonesia coastal waters, especially in the East China Sea and South China Sea. It became a major breeding object in Guangdong, Taiwan, and the south of Fujian of China for its delicious taste, rich nutrition, and rapid growth (Zhang et al., 2010). However, in recent years, due to the destruction of habitats, spawning, and other natural environments, as well as the overfishing of wild resources and continuous outbreak of popular shrimp diseases, the aquaculture and resource capacity of *F. penicillatus* both sharply declined. In 2005, *F. penicillatus* was included in the Red List by the Chinese government as an endangered species (Wang and Xie, 2009). Protecting and rejuvenating *F. penicillatus* resources are imperative. Currently, microsatellite molecular markers are widely and expertly applied to the research of many species for genetic conservation. Polymorphic microsatellite loci of *F. penicillatus* were developed (Cao et al., 2012). Nevertheless, information about the genetic background of *F. penicillatus* remains limited (Voloch et al., 2005). Therefore, we developed eight novel polymorphic microsatellite loci that could contribute to protect the natural resources and promote the development of aquaculture for *F. penicillatus*.

## MATERIAL AND METHODS

The microsatellite makers were developed using a modified fast isolation by amplified fragment length polymorphism of sequences containing repeat protocol (Zane et al., 2002). Genomic DNA was extracted from the muscle of a single wild *F. penicillatus* that was captured in Beihai, China, by using a modified cetyltrimethylammonium bromide extraction. High-quality genomic DNA (20 µL 100 ng/µL) was digested with 1 µL FastDigest Tru1I (Fermentas, Canada) restriction enzyme in a 25-µL volume for 10 min at 65°C. The digested fragments, ranging from 400 to 1200 bp, were ligated to *Mse*I adapter1 (5'-ACGATGAGTCCTGAG-3')/*Mse*I adapter2 (5'-TACTCAGGACTCAT-3') by T4 DNA ligase at 22°C for 10 h. The digestion-ligation fragments were denatured at 95°C for 10 min and were immediately hybridized to the biotinylated oligonucleotide probes (CT)15 and (GT)15 at 61°C for 1 h. Afterwards, the fragments containing microsatellite repeats were captured and gathered by streptavidin-coated magnetic sphere particles (Promega, USA), and the noncaptured and loose DNA fragments were washed away. The recovered DNA fragments were amplified using *Mse*I adapter1. After purification by GenClean Cycle Pure Kit (Omega Bio-Tek) to remove the extra adapters and dNTPs, the purified products (4 µL) were ligated to 1 µL pMD19-T (TaKaRa, Japan) at 16°C for 3 h and then were transformed into *Escherichia coli* DH5α (TIANGEN, China) for further selection on ampicillin plates. The positive clones were amplified by M13 general primers, and the polymerase chain reaction (PCR) products were separated on 1% agarose gels. One hundred fragments that ranged from 500 to 1000 bp were selected to sequence by the Majorbio Company (Shanghai, China).

Ninety-five fragments were successfully sequenced, and 70 microsatellite sequences meeting the requirements were hunted by SSRhunter1.3. Thirty-eight pairs of microsatellite amplification primers were designed by Primer Premier5.0.32. Genomic DNA samples that were extracted from 32 wild individuals from Beihai, China, were used to test the polymorphism of these 38 microsatellite markers. The PCR amplification was in a volume of 10 µL and was performed on a gradient thermal cycler (Bio-Rad). The PCR protocol was designed as

 

follows: denaturation for 5 min at 94°C; 30 cycles of 45 s at 94°C, 45 s at annealing temperature (Table 1), and 1 min at 72°C; a final extension at 72°C for 10 min; and storage at 4°C. The amplified products were separated on polyacrylamide gels in a Sequi-Gen Sequencing Cell (Bio-Rad) and were visualized by silver staining. Finally, the important genetic information and index of the 8 polymorphic microsatellite loci were calculated by POPGENE 32 (version 1.32) (Yeh et al., 2000) and CERVUS 3.0 (version 3.0) softwares.

**Table 1.** Basic genetic information of eight microsatellite primers in *Fenneropenaeus penicillatus* (sample size = 32 individuals).

| GenBank accession No. | Locus ID | Primer sequences (5'-3') | Ta (°C) | Repeat motif | Allele size (bp) | $N_A$ | PIC | $H_O$ | $H_E$ | P-HWE |
|---|---|---|---|---|---|---|---|---|---|---|
| KF055840 | FP1-13 | F: AGGGCTCTCTCCTCAGGCAAG<br>R: TCACTGGGATTCTCAGCGACG | 60 | $(CA)_{11}$ | 110-120 | 3 | 0.4057 | 0.3125 | 0.5059 | 0.0290 |
| KF055841 | FP2-13 | F: CCTCCCTACCAACCACAGTC<br>R: TATCATAACGCAGAGCAAAA | 60 | $(CATCC)_3$ | 170-190 | 4 | 0.3643 | 0.3226 | 0.3522 | 0.7230 |
| KF055842 | FP-14 | F: CTCTCACTCCGAGATGGG<br>R: GCTGGTTGGTGTTGCTTT | 43 | $(CAC)_3CAT[(CAC)_{36}(CTT)_2]_3(CAC)$ | 260-340 | 4 | 0.5644 | 0.3704 | 0.5178 | 0.3612 |
| KF055843 | FP-15 | F: TGAGTGACTGCTTGGTAA<br>R: GAGAGGATGAGAGGGTAT | 55 | $T_{15}$ | 220-245 | 4 | 0.2812 | 0.1613 | 0.2555 | 0.0194 |
| KF055844 | FP-25 | F: CCACCAACTCTCACACC<br>R: CATAGCACTCGGGCATC | 55 | $(TTC)_3N(TC)_8$ | 190-210 | 4 | 0.4268 | 0.3448 | 0.3609 | 0.7327 |
| KF055845 | FP-40 | F: TTCCCTCGCCCCCCTTTC<br>R: CGCCCCTCTGGCTTGCTTT | 60 | $(AG)_{14}$ | 185-240 | 6 | 0.7598 | 0.5556 | 0.7387 | 0.0460 |
| KF055846 | FP-43 | F: TAAGGAAGGGGAGGGAAG<br>R: ACCTGCCTGCCTATCTGT | 56 | $(GA)_4N(AAAG)_3$ | 125-135 | 5 | 0.2703 | 0.2581 | 0.2347 | 0.9966 |
| KF055847 | FP-67 | F: AGAGAGAGTGAAAGACAAGGAATA<br>R: TCAAGATGAACAATGTGATAATAA | 55 | $(AAAG)_3$ | 128-150 | 4 | 0.3080 | 0.2812 | 0.3262 | 0.0075 |

Ta = annealing temperature; $N_A$ = number of polymorphic alleles per locus; PIC = polymorphism information content; $H_O$ = observed heterozygosity; $H_E$ = expected heterozygosity; P-HWE = P values for the Hardy-Weinberg expectation test (adjusted P = 0.00625).

## RESULTS AND DISCUSSION

Four monomorphic microsatellite loci and eight polymorphic microsatellite loci were screened, and the characterization of the eight loci is presented in Table 1. The number of alleles per locus ranged from 3 to 6, and the polymorphism information content varied from 0.2703 to 0.7598 by CERVUS3.0. The observed and expected heterozygosities were 0.1613-0.5556 and 0.2347-0.7387 by POPGENE 32, respectively. No loci significantly deviated from Hardy-Weinberg equilibrium, and there was no genotypic linkage disequilibrium in the tested population after Bonferroni's correction (adjusted P = 0.00625).

The eight novel polymorphic microsatellite loci presented here could provide useful and important information for further population studies and cultivation in *F. penicillatus*.

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