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Note

Development and characterisation of polymorphic microsatellite markers in the pearl oyster *Pteria penguin* (Roding, 1798)

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ABSTRACT

We developed and characterised 12 polymorphic microsatellite loci for *Pteria penguin* (Roding, 1798), an important pearl oyster species for pearl production in China. The number of observed alleles per locus ranged from 5 to 26 within 46 individuals. The observed and expected heterozygosities per locus ranged from 0.2 to 0.925 and 0.707 to 0.945, respectively. The polymorphism information content values per locus ranged from 0.656 to 0.929. Ten loci were in accordance with Hardy-Weinberg equilibrium and no significant linkage disequilibrium was detected between loci. These new microsatellite markers will be useful for population and conservation genetic studies of *P. penguin.*

Keywords: Conservation, Genetics, Microsatellite locus, Pearl oyster, *Pteria penguin*

The pearl oyster *Pteria penguin* (Roding, 1798) (Family: Pteriidae), is mainly distributed along the coastal areas of South China, south of Kyushu and Ryukyu Islands in Japan and Philippines. *P. penguin* is extensively cultured in South China and has been used to produce large round pearls and blister pearls (Mao *et al.,* 2003). Currently, the germplasm of *P. penguin* has degenerated significantly due to overfishing and environmental pollution. Reduced growth in hatchery produced seeds leading to decrease in yield of the pearl oyster, has been haunting this aquaculture industry. Therefore, reasonable stock management as well as genetic improvement programmes are required for the sustainable development of the pearl oyster aquaculture industry. Microsatellite markers have been considered as useful tools to identify population genetic variation due to their codominance, abundance and unambiguous scoring of alleles (Tautz, 1989). In this study, we attempted to develop and characterise polymorphic microsatellite markers aimed at conservation of genetic diversity of *P. penguin*.

P. penguin (46 nos.) were collected from Beihai, Guangxi Province, China were dissected and the adductor muscles were immediately preserved in 95% ethyl alcohol (EtOH) and stored in -20°C freezer until DNA extraction. Total genomic DNA was extracted using the CTAB (cetyl trimethylammonium bromide) method (Doyle, 1987). A dinucleotide-enriched genomic library was constructed following the method described by Ma and Chen (2009). The sample DNA was digested by *Mse*I restriction enzyme (New England Biolabs, USA). The DNA fragments were ligated to adapters, OligoA (5'-TACTCAGGACTCAT-3') and OligoB (5'-GACGATGAGTCCTGAG-3'). The ligated products were pre-amplified in a 25-µl reaction system using a specific primer (5'-GAT GAG TCC TGA GTA A-3') to validate successful ligation and to increase DNA concentration. The DNA fragments were eluted from the magnetic beads and then further amplified by the adapter specific primer. The final amplification products were ligated into the pMD18-T vectors (Takara, Japan) and transformed into *Escherichia coli* DH5α. The positive clones were randomly sequenced on ABI 3730xl DNA analysers at Life Technologies (Guangzhou, China). A total of 198 positive clones were sequenced and 125 microsatellite repeats were obtained. Subsequently, 53 microsatellite loci were selected to design primer pairs by PRIMER PREMIER 5 software (Premier Biosoft International, USA).

The designed primers were used to evaluate the polymorphisms of each locus within 12 individuals of *P. penguin*. Polymerase chain reaction (PCR) was done in a reaction volume of 15 µl, comprising 1× buffer, 0.2 mm dNTP, 0.2 µm primer pairs, 1.5 mm $MgCl_2$,

1 U Taq polymerase (Takara, Japan) and about 50 ng genomic DNA. The PCR amplifications were carried out under the following conditions: initial denaturatiuon at 94°C for 5 min, followed by 35cycles of denaturation at 94°C for 45 sec, annealing at the optimised annealing temperature (Table 1) for 30 sec, extension at 72°C for 30 sec and a final extension at 72°C for 10 min. The PCR products were separated on 8% denaturing polyacrylamide gel and visualised by silver staining. We assessed the polymorphism of the twelve loci with 46 individuals of the wild populations. The PCR products were detected on a 3130xl capillary DNA analyser (Applied Biosystems) using GS-500LIZ (Applied Biosystems) as the size standard. GENEPOP 4.0 software was used to calculate the observed (*Ho*) and expected heterozygosities (*He*), Hardy-Weinberg equilibrium and linkage disequilibrium (Rousset, 2008). Bonferroni's correction was used to correct all the results obtained in the multiple tests (Rice, 1989). Polymorphism information content (*PIC*) was assessed by Cervus 3.0 (Kalinowski *et al.* 2007).

In this study, 28 out of the 53 selected loci were successfully amplified in the wild population of *P. penguin* and 12 loci were found to have polymorphism (Table 1). The numbers of alleles ranged from 5 to 26, with a mean value of 12.1. The *Ho* and *He* ranged from 0.2 to 0.925 and from 0.707 to 0.945, with a mean value of 0.741 and 0.856, respectively. The *PIC* values ranged from 0.656 to 0.929. Results of the study indicates that the genetic diversity of *P. penguin* in the wild population is high and this result is consistent with the findings obtained by using AFLP analysis (Peng *et al.,* 2012). Loci Ptpe_134 and Ptpe_128 significantly deviated from Hardy-Weinberg equilibrium after Bonferroni's correction (p<0.0042). There was no evidence of linkage disequilibrium between any pairs of loci.

Table 1. Characteristics of the 12 selected microsatellite loci in *P. Penguin*

Locus	Repeat motif	Ta (°C)	Primer sequence (5'-3')	Size range (bp)	N_a	H_O	H_E	*P*	*PIC*	Accession No.
Ptpe_134	$(CA)_{34}$	58	F: CAGACTTTGGTTACATTTGCG R: GGTCGTCTTGAGGTTTCGTT	184-192	5	0.2	0.707	<0.001	0.656	KJ606766
Ptpe_137	$(TG)_7TA(TG)_{10}$	60	F: CCATTCTTTACTTCAGAGGCAA R: CATTTACGCAAATTTCCACG	278-294	8	0.738	0.796	0.611	0.756	KJ606767
Ptpe_425	$(TG)_5TA(TG)_{24}$	60	F: GCTACCACAACATGACAACAGA R: TGCGTCAGACAGGTAACTTTG	245-271	12	0.867	0.918	0.437	0.900	KJ606768
Ptpe_429	$(GT)_{11}A(TG)_{23}$	60	F: GTCGTCTTGAGGTTTCGTTTC R: AGAAGGCTAAAGCTGCACCA	216-228	6	0.795	0.815	0.522	0.780	KJ606769
Ptpe_535	$(TG)_{11}(TGTA)_8G(GTAG)_8$	60	F: CCAGTGACTGTGGATGAATATCA R: GCAAGGTTTCGGGATCATAA	300-366	17	0.923	0.945	0.350	0.929	KJ606770
Ptpe_128	$(TACA)_{20}N(ACAT)_{17}$	55	F: ATGCATGTTACTTGTATTTGTTGA R: CCTCTAAATATGCCACTGGAATAG	240-370	22	0.308	0.939	<0.001	0.923	KJ606771
Ptpe_010	$(TG)_{13}N(TGTA)_{18}$	60	F: AGTCTGGGTTTTCACTCGTAAT R: CAGTTTGCCGTACAAGGTTT	261-311	26	0.911	0.944	0.805	0.929	KJ606772
Ptpe_016	$(CA)_{35}$	60	F: ATACCGAGTCAAGTGTCAAGTTC R: TGTTCAGGTTAGCATCAATGTC	215-281	15	0.925	0.938	0.133	0.921	KJ606773
Ptpe_025	$(TG)_6N(TG)_5$	60	F: GGACTATTACAGTGTCGGTAACAA R: GGACGATTTACACTCATAAGCAC	212-234	5	0.778	0.796	0.207	0.752	KJ606774
Ptpe_062	$(TG)_{17}$	60	F: AAACACGGGCTAAGTGAACA R: ACGTAGAAGTCGATCGGTATAAG	213-227	8	0.854	0.858	0.529	0.828	KJ606775
Ptpe_071	$(TG)_{11}N(TG)_6$	66	F: CTGGCAGCCTGAAAGACAGT R: CCAGATAACTTGAATACGATGACA	231-261	15	0.818	0.809	0.922	0.775	KJ606776
Ptpe_229	$(AC)_8$	58	F: CTGCCAAGGGAGAAGTCTGT R: GCGTATTTCCTTTGCTTAGTTG	283-297	6	0.778	0.805	0.154	0.768	KJ606777

*T*a: optimised annealing temperature, N_a: Number of alleles per locus, H_O: Observed heterozygosity, H_E: Expected heterozygosity, *P:* Probabilities of deviation from Hardy-Weinberg equilibrium, *PIC:* Polymorphism information content

The present study developed 12 polymorphic microsatellite markers in *P. penguin* using the magnetic beads enrichment procedure. These polymorphic loci (except Ptpe_134 and Ptpe_128) can be used in study of the population structure and conservation of *P. penguin*.

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