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Eleven novel polymorphic microsatellite loci in the ornate spiny lobster *Panulirus ornatus* (Decapoda: Palinuridae)

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Eleven polymorphic microsatellite loci were developed for the ornate spiny lobster (*Panulirus ornatus*). Polymorphism was assessed for 24 individuals from Zhanjiang sea area (the South China Sea). The number of alleles per locus in 24 individuals ranged from 4 to 9, the average observed heterozygosity per locus was from 0.375 to 1.000, and the average expected heterozygosity per locus was from 0.535 to 0.874, The polymorphic information content for 11 distinct loci varied between 0.493 and 0.861. Six loci followed Hardy–Weinberg expectations after Bonferroni correction for multiple comparisons. No significant linkage association was found among all these loci. The 11 polymorphic microsatellite loci might be helpful in planning strategies for conservation of the ornate spiny lobster.

P. ornatus are valuable marine fishery resource (Williams 2009) mainly distributed in the tropical eastern Indian Ocean, Southeast Asia, Australia and the West Pacific. Because of their high market value, lobsters are under severe fishing pressure and this level of exploitation is a serious threat to the sustainability of the stocks (Williams 2004). At the same time, marine environment pollution and climate change also contribute to white spot syndrome virus (WSSV) causing lobster population to decline (Musthaq *et al.* 2006). So far, the ornate spiny lobster is an important species under provincial protection and the artificial propagation technology has not yet been developed in China (Liang and He 2012).

Microsatellite DNA (simple sequence repeats, SSRs) marker is currently one of the best tool to study population genetics (Sekino *et al.* 2012). It has been widely used in studying genetic diversity and population genetic structure of marine animals. Fifteen polymorphic microsatellite loci of *P. ornatus* in Australia were recently published (Hoc *et al.* 2013). This study reports 11 polymorphic microsatellite loci isolated from *P. ornatus*, and it will help to evaluate ornate spiny lobster resources condition and develop its artificial propagation techniques in future.

A microsatellite-enriched genomic library of ornate spiny lobster was constructed using the method of fast isolation by AFLP sequences containing repeats (FIASCO) (Zane *et al.* 2002). Genomic DNA was extracted using the traditional phenol–chloroform method from lobster tentacles. Extracted DNA was digested with restriction enzyme *Mse*I (TaKaRa, Dalian, China) for 4 h, and DNA fragments between 300 and 1000 bp were isolated and purified using DNA purification kit (TaKaRa). The purified fragments were ligated to *Mse*I adapter (MA1: 5′-GACGATGAGTCCTGA G-3′, MA2: 5′-TACTCAGGACTCAT-3′) with T4 DNA ligase (TaKaRa) at 16°C for 16 h, and the ligation products were amplified by polymerase chain reaction using the adapter *Mse*I primers (*Mse*P) (5′-GATGAGTCCTGAGTAA-3′) with the following conditions: 94°C for 2 min, 30 cycles of 94°C for 30 s, 53°C for 30 s and 72°C for 1 min, followed by 72°C for 10 min. Amplified fragments containing SSRs sequences were captured by denatured and hybridized to $(CA)_{15}$ biotin-labelled probe, using streptavidin-coated-magnetic beads. The captured products were cloned into pMD18-T vector (TaKaRa) and transformed into *Escherichia coli* DH5α competent cells. The clones likely to contain SSRs were isolated and sequenced on an ABI-PRISM 3730 DNA Sequencer (Applera Corporation, Foster City, USA). Using the SSR-Hunter software search for microsatellites and suitable priming regions, 26 primer pairs were designed with Primer Premier 5.0 (http://www.premierbiosoft.com/primerdesign) based on the conserved flanking sequences. Eleven SSR sequences have been deposited in GenBank with accession numbers JX273368–JX273378.

Finally, a total of 11 pairs of polymorphic primers were tested with 24 wild individuals of *P. ornatus* from Zhanjiang

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Table 1. Characterization of 11 polymorphic microsatellite loci in the *Panulirus ornatus*.

Locus	GenBank accession	Primer sequence (5′ → 3′)	T_a(°C)	Repeat motif in the coloned allele	Allele size range (bp)	Number of alleles	H_e	H_o	Test of HWE (P)	PIC
Por13	JX273368	F: CGGATGGAGGCGGCACAAAT R: CATCTCCAAACGGGGCGAAC	55	$(GT)_5N_2(GT)_{11}$	144–180	5	0.535	0.375	0.017	0.493
Por532	JX273369	F: GCATAGGAGGAAACTGTCA R: AAGAGGTACGCACGGGAGC	61	$(TG)_9$ $N_2(TG)_4$	294–326	5	0.729	0.542	0.009	0.683
Por731	JX273370	F: TTTCCAACTTTATGAGCCC R: ATCCACCCCGTTCGTCTGT	54	$(AC)_{12}$	72–116	8	0.820	1.000	0.000*	0.799
Por1121	JX273371	F: CTTGGTGACCCTATGCTCT R: CACATCAGCGGACATTTATC	58	$(ATT)_6$ $N(TG)_9$	164–210	4	0.720	1.000	0.000*	0.666
Por122	JX273372	F: TACCCTCCGCACATCTTCAC R: TGTTGTGGTTGACCTGCTT	58	$(AC)_{18}$	176–224	9	0.874	0.958	0.000*	0.861
Por123	JX273373	F: ACCCGTTCCTGGCGATTT R: AGGCGACCTTCGGTGTAA	60	$(AC)_{12}(CA)_8$	255–330	7	0.830	0.958	0.403	0.807
Por135	JX273374	F: ACCTGCGCTGCGTTATGT R: TCCGTCTTGCGTTCCTTC	60	$(TG)_{13}$	139–167	5	0.773	1.000	0.000*	0.736
Por1371	JX273375	F: ATCCGATTCCCCTCGCCTCT R: CCGCTTCCTCCAGCAGTCT	61	$(CT)_7$ N_2 $(CTCC)_2(CT)_3$	132–167	6	0.786	0.917	0.520	0.757
Por205	JX273376	F: TAGAATCCCTCAGTGTTGTAGC R: GGTAGGACCATTTCGTTGTT	61	$(AC)_6N(AC)_3$	251–312	7	0.790	0.792	0.258	0.758
Por213	JX273377	F: CCTTCAGATGAGGGCTAC R: GTGACTTCCTGGGACAAA	60	$(AC)_6$ N_2 $(CTT)_2$	191–235	6	0.810	0.917	0.000*	0.783
Por218	JX273378	F: CGGAAGGTGATGGTGAGTGT R: GTTGCAGTAGTTCTGATGTAAGG	60	$(TG)_7$	218–264	7	0.832	0.875	0.016	0.810

T_a, annealing temperature; H_o, observed heterozygosity; H_e, expected heterozygosity; PIC, polymorphic information content; HWE (P), a significant deviation from Hardy–Weinberg equilibrium ($P < 0.0045$).

sea area of China. PCRs were carried out in a Bio-Rad S1000 PCR with 15 μL reaction volumes that contained 1 μL of DNA (40–60 ng/μL), 1.5 μL 10× PCR buffer (TaKaRa, Dalian, China), 1.5 μL 20 mM dNTPs, 1 U *Taq* DNA polymerase (TaKaRa), 0.5 μL of each 25 μM primer, and distilled deionized water. Thermal cycling consisted of initial denaturation at 94°C for 3 min, 30 cycles of denaturation at 94°C for 42 s, annealing at 54–61°C for 50 s and 45 s at 72°C, followed by a final elongation step at 72°C for 5 min. PCR products were separated on 8% nondenaturing polyacrylamide gel electrophoresis and silver-stained, scanned and analysed using Gel-Pro analyzer software ver. 4.0 (Media Cybernetics, Rockville, USA).

The results of the microsatellite data were analysed by GenAlEx 6.41 (Peakall and Smouse 2005) (table 1). Hardy–Weinberg equilibrium (HWE) and *P* values were tested for using web-based Genepop 4.0 (Rousset 2008). Five loci showed significant deviations from HWE expectations after Bonferroni correction ($P < 0.0045$). No significant evidence of linkage disequilibrium was observed. Thus, the 11 polymorphic microsatellite loci showed high polymorphism, and they will be useful in population genetics, conservation and other relevant studies.

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