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Isolation and characterization of polymorphic microsatellite loci in the ridgetail white prawn *Exopalaemon carinicauda*

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ABSTRACT. The ridgetail white prawn *Exopalaemon carinicauda* is one of the major economic shrimp species cultured in China. In this study, 30 microsatellite loci were isolated and characterized for the ridgetail white prawn *E. carinicauda* using a microsatellite-enriched library. Polymorphisms were tested in 30 individuals from a single wild population. The number of alleles at each locus ranged from 2 to 14. The observed and expected heterozygosities varied from 0.1000 to 0.8000 and from 0.2299 to 0.9228, respectively. The PIC value ranged from 0.2002 to 0.8939. These new loci will be useful in the study of population genetic structure and genetic diversity in this species.

Key words: *Exopalaemon carinicauda*; Enriched library; Microsatellites

INTRODUCTION

The ridgetail white prawn *Exopalaemon carinicauda* (Crustacea: Decapoda: Palaemonidae) is an endemic shrimp species in China and is widely distributed in the coastal waters of China and west coast of the Korean Peninsula, especially in the Yellow and Bohai Seas. Due to the multiple merits of good reproductive performance, fast growth, and good environmental adaptability, the culture area of the ridgetail white prawn in China has expanded in recent years (Wang and Cao, 2010). Although the ridgetail white prawn is one of the major commercial shrimp species cultured in China and contributes one-third of the gross revenues from the polyculture ponds in eastern China (Xu et al., 2010), its seeding is dependent on wild resources.

So far, studies have focused on culture (Shi and Ye, 2007), biology (Li, 1994), and ecology (Wang and Cao, 2010), but there are few reports about population genetics. The conservation and culture of the prawns require information about the genetic diversity and structure of *E. carinicauda*. To provide some available information on developing conservation and culture, efficient molecular markers are needed to study the genetic diversity and structure of the species. Microsatellite markers are regarded as a useful tool for such studies, because they show a high degree of polymorphism, co-dominance and neutrality. In this study, we developed 30 polymorphic microsatellite loci in the ridgetail white prawn.

MATERIAL AND METHODS

Samples of the ridgetail white prawn were collected from the coastal waters of the Bohai Sea, Laizhou Bay, Shandong Province, China. The samples were stored at -80°C in the laboratory.

The isolation of microsatellites from the prawn was performed using the enrichment protocols reported by Song (2010), with minor modifications. Genomic DNA was extracted from the muscle of a single individual using phenol-chloroform procedures described by Liu et al. (2000). Approximately 6 μg genomic DNA was digested with the restriction endonuclease *Hae*III. DNA fragments from 400 to 1200 bp were separated on a 1.5% agarose gel, extracted using a Gel Extraction kit (Tiangen, China), and ligated to *Hae*III adaptors generated by annealing together oligo A (5'-CTCTTGCTTGAATTCGGACTA-3') and oligo B (5'-pTAGTCCGAATTCAAGCAAGAGCACA-3') using T_4 DNA ligase (TaKaRa, Dalian, China). The ligated DNA fragments were amplified by polymerase chain reaction (PCR) using oligo A as the primer. The PCR products were cleaned using a DNA purification kit. The PCR products were then hybridized with the biotinylated probes $(AC)_{15}$ and $(AG)_{15}$, and the hybridization complexes were captured using streptavidin magnetic beads (Promega). Captured fragments were amplified again using oligo A as primer, ligated to pMD18-T vector (TaKaRa), and transformed into competent *Escherichia coli* DH5α cells. The clones were identified on LB plus ampicillin plates. Approximately 460 clones were randomly picked and screened by colony PCR and 314 clones were positive. A total of 227 positive colonies were selected from the 314 clones and sequenced on an ABI 3730 DNA sequencer (Applied Biosystems), which showed that 186 clones contained microsatellites.

After we discarded duplicates using the DNAMAN version 6.0 software and discarded those with short unique regions flanking the microsatellite array, 132 primer pairs were designed by the Primer 5.0 software (http://www.premierbiosoft.com/). Primers were selected

and PCR conditions were optimized using genomic DNA obtained from 5 individuals. The polymorphisms of the microsatellite loci were examined in 30 individuals of *E. carinicauda*. The PCR amplifications were done in a 20-μL reaction volume containing 90 ng DNA template, 1.0 μM forward and reverse primers, 1X PCR buffer, 2.4 mM $MgCl_2$, 0.32 mM dNTPs, and 1.0 U Taq DNA polymerase. PCR profiles involved an initial denaturation at 94°C for 5 min, followed by 28 cycles of 94°C for 45 s, annealing at 48° to 62°C (Table 1) for 45 s and extension at 72°C for 45 s, and a final extension at 72°C for 5 min.

PCR products were resolved on 8% denaturing polyacrylamide gels and visualized by silver staining. We calculated the number of alleles per locus. The observed and the expected heterozygosities, and test for Hardy-Weinberg equilibrium (HWE) were determined with POPGENE32 (Yeh et al., 1999). Linkage disequilibrium was tested using GENEPOP 4.0 (Rousset, 2008). Polymorphism information content (PIC) was calculated using the method described by Botstein et al. (1980).

RESULTS AND DISCUSSION

Of a total of 132 microsatellite loci, 48 were polymorphic in *E. carinicauda.* Thirteen markers were judged to be multilocus markers because they amplified more than two alleles in most individuals. Five markers could not be fully amplified in 30 individuals of *E. carinicauda*. The number of alleles at the remaining 30 SSR markers ranged from 2 to 14 with an average of 8.2 alleles. The observed and the expected heterozygosities ranged from 0.1000 to 0.8000 and from 0.2299 to 0.9228, with an average of 0.4438 and 0.8220, respectively. The PIC value ranged from 0.2002 to 0.8939 (Table 1). Botstein et al. (1980) regarded any locus with a PIC ≥ 0.5 as highly polymorphic. In our study, only 1 of the 30 loci analyzed did not meet this criterion. Sixteen of the 30 loci deviated significantly from HWE after a Bonferroni's correction ($P < 0.0017$). These deviations might have been caused by the presence of null alleles (Pemberton et al., 1995) and sampling effect. Significant linkage disequilibrium was not detected between any pairs of loci ($P < 0.01$). The results suggested that these polymorphic loci should be useful in surveying the population genetic structure and genetic diversity of *E. carinicauda*.

Table 1. Levels of variability at 30 polymorphic microsatellite loci in the ridgetail white prawn *Exopalaemon carinicauda*.

Locus	Accession No.	Repeat motif	Primer sequence (5'-3')	Ta (°C)	N_A	Size range (bp)	H_O	H_E	P	PIC
EC65	JQ319400	$(CT)_{28}CC(CT)_6$	CCTGAGTAACGGTCCATA TACACCTCGCTCCTAAAA	58	5	507-532	0.2759	0.7338	0.0000*	0.6692
EC1125	JN408494	$(TC)_{33}$	TCAGCCTGAGGGTTTTGT GTAGTCGCCGCAGAAGTT	54	6	502-543	0.4333	0.7401	0.0018	0.6830
EC909	JQ319401	$(TC)_{11}C(CT)_{14}$	GAACCCTTTACCTACACGACTG GATTGTGTTTGAGTGGAGCCCT	48	6	416-439	0.5862	0.7949	0.0336	0.7485
EC7	JQ319402	$(GA)_{29}$	AATATGCAGTGGCAAGCT TTCCCATCATCTTCCTCC	56	7	503-559	0.4000	0.8114	0.0000*	0.7647
EC54	JQ319403	$(GA)_{36}$	GGCTGTCCCTTGGAACTA ACGAAATCCGAATAACCC	56	11	328-367	0.7000	0.8898	0.1137	0.8621
EC915	JN408481	$(AG)_{34}$	AAAGTGCGTTACAGGAAG GTCTGGAAACACCGAATG	51	9	358-427	0.5600	0.8465	0.0000*	0.8086
EC13	JQ319404	$(GA)_5A(AG)_{30}$	GAACTCAAGAAGAATAAGGATG TTGGTCGGCTAAGGATAC	51	7	210-263	0.4074	0.8379	0.0001	0.7980

Continued on next page

Table 1. Continued.

Locus	Accession No.	Repeat motif	Primer sequence (5'-3')	Ta (°C)	N_A	Size range (bp)	H_O	H_E	P	PIC
EC505	JQ319405	$(GA)_{31}..(AG)_{23}$	CGAAGCAAACGAGAAGAG ACAAGAATTGACGAGGGA	56	8	260-312	0.3478	0.8425	0.0000*	0.8011
EC41	JQ319406	$(TC)_{27}$	ATGACTCTTGGACGATTT GCATTTATGGCTTTGGTAT	54	10	294-336	0.5000	0.8513	0.0185	0.8159
EC35	JQ319407	$(GA)_{24}$	CCTCAGTATGCCGTGTAG CGAGCGGATGTAATAAAC	54	7	330-387	0.5185	0.8302	0.0076	0.7897
EC72	JQ319408	$(GA)_{26}$	GTCGGGAGAAGACAGGGAG TGAAGAGGCGAGGTTGGT	58	7	392-437	0.5185	0.8204	0.0053	0.7776
EC21	JQ319409	$(GA)_{29}$	ACGTGCTTCTGGCAATCC CTCGCCTTCATCCTCCTT	58	10	258-312	0.5000	0.8821	0.0000*	0.8487
EC1204	JN408482	$(CT)_{27}$	GAGTAGATAAAGAACACTGTGA CTATTGAACAGGTTGACATAAG	51	11	351-402	0.6552	0.9026	0.0154	0.8763
EC1217	JN408483	$(CT)_{27}..(ACAT)_6..A_{18}$	CTATTGAACAGGTTGACATAAG TCCTCCAACAAAACCCTCAAAC	62	8	352-390	0.8000	0.8633	0.1022	0.8305
EC1218	JQ319410	$(TC)_{20}$	AAAGGAGTTATTCTCACCAAGG ATATTGCTCTAATGCACCCAGG	60	9	126-179	0.6000	0.8506	0.0075	0.8142
EC38	JQ319411	$(GA)_{19}GG(GA)_{12}$	GCGAATAGATTGGAAGAC CAAATAAGAATTATGGGTGT	51	10	392-437	0.7083	0.8528	0.3060	0.8168
EC58	JQ319412	$(CT)_{32}$	CACCGCTTCTGGTATCTT GTTGATAATAATGCCGAGA	53	6	331-418	0.1667	0.7934	0.0000*	0.7408
EC1222	JQ319413	$(AG)_{22}$	CTCTTCCACTCCCCAACATCTC AAACACCCATACTACCTCCCCG	58	12	104-123	0.5556	0.8560	0.0040	0.8242
EC620	JN408484	$(TC)_9G(CT)_{29}$	CGATTCATCCGATACCAC TTTCTGTCAATGCGTCCT	62	8	189-225	0.5217	0.8599	0.0268	0.8212
EC56	JQ319414	$(CT)_{24}$	CACTTCCACGCTGGTAAA GACTGCGACTGGGTAATG	62	2	154-167	0.2593	0.2299	0.4773	0.2002
EC4	JQ319415	$(CT)_{31}$	CACCGCTTCTGGTATCTT GTTGATAATAATGCCGAGA	53	14	342-419	0.3636	0.9228	0.0000*	0.8939
EC93	JQ319416	$(GA)_{14}$	AGGGGTGGGGTAAAAGCAAATA CCGATGTTCTGGCTGTTCTCTA	53	9	259-296	0.2105	0.8734	0.0008	0.8341
EC1202	JQ319417	$(CT)_{30}$	TACTCAAATGGCAACACTCT AGATAGAGGTAAAGTGAGCA	56	8	320-413	0.3478	0.8222	0.0000*	0.7792
EC506	JN408487	$(GA)_{23}$	TTTAGTTGGCGTCTTGGT AGAACGAACAATGGAGGA	56	10	316-348	0.5200	0.8735	0.0000*	0.8411
EC1	JQ319418	$(GA)_{28}..(AG)_{26}$	TGTATTGGAGGAGAAGCAGAAG AGACAAGTCGGTGATGGTTTAG	62	7	441-452	0.4545	0.8312	0.0165	0.7649
EC92	JQ319419	$(CA)_6(AG)_{32}$	ATTCACAACCTAACCAGGAAAT TTCTCAAAACGATTGTAAACCA	56	9	79-126	0.3684	0.8578	0.0000*	0.8167
EC503	JQ319420	$(TC)_{33}$	TACTTTCGCTCGCTAACA TCCCGTTTCAAATTCTTGT	54	7	224-263	0.2500	0.7903	0.0000*	0.7347
EC619	JQ319421	$(CT)_{26}$	TGGTCATATTAAGAAAAGGTTG ATGTCTGCGTTCCAATCC	51	7	184-222	0.2500	0.8659	0.0000*	0.8074
EC10	JQ319422	$(AG)_{23}AA(AG)_5$	ACTTGAGACTGTTAGTGGAAAA TATTGCGTGCTGGTTCTC	53	10	444-521	0.1000	0.8872	0.0000*	0.8516
EC1029	JN408491	$(GA)_{23}G_9$	GAGATGGGTGAAGCAGAG TACGTCGTAATGTGATGTATGT	58	6	109-123	0.4348	0.8048	0.0000*	0.7549

Ta = annealing temperature; N_A = number of alleles; H_O = observed heterozygosity; H_E = expected heterozygosity; P = probability of being in Hardy-Weinberg equilibrium; PIC = polymorphism information content; *significant departure from expected Hardy-Weinberg equilibrium conditions after correction for multiple tests.

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