

PERMANENT GENETIC RESOURCES NOTE

Polymorphic microsatellite markers of freshwater prawn *Palaemon paucidens* (De Haan, 1844) (Crustacea: Palaemonidae)

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Abstract

We report 11 polymorphic microsatellite loci developed from the freshwater prawn *Palaemon paucidens*. Their genetic characteristics were assessed in 48 individuals selected from six Korean populations. The number of alleles ranged from two to 21, and the observed and expected heterozygosities were between 0.13 and 0.83, and 0.46 and 0.95, respectively. We examined the cross-specific amplification of each locus in three species of palaemonid prawn and one species of atyid prawn.

Keywords: cross-specific amplification, freshwater prawn, microsatellites, *Palaemon paucidens*

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Palaemon paucidens (De Haan, 1844) is the most abundant species among freshwater prawns (Kim 1977; Chow & Fujio 1985), distributed widely over the East Asian region, including in reservoirs and river systems of China, Japan, Russia and Korea (Kim 1976, 1977). Although several of the 19 species belonging to the genus *Palaemon* have developed adaptations for various aquatic habitats, *P. paucidens* is an obligatory freshwater species. Given this fact, evolutionary studies of *P. paucidens* may provide clues for adaptive radiation of *Palaemon* in the East Asian region. To date, a handful of studies have been performed on this species in order to assess genetic variation and population divergence variation using allozyme markers (Seinen & Yoshihisa 1985; Seinen *et al.* 1988). In this study, we develop and characterize 11 polymorphic microsatellite loci from *P. paucidens*, for population genetic studies.

Total DNA was extracted from the legs of each individual specimen using the DNeasy Tissue Kit (QIAGEN). Partial genomic library was constructed according to the protocol of Estoup & Turgeon (1996). Accordingly, 300 ng of DNA were digested with 1.5 µL of *Sau3A1* (10 µ/µL) and loaded in a 2% agarose gel. Fragments ranging between 200 bp and 800 bp were recovered from gel and cloned into a pUC118 plasmid vector digested by *Bam*HI (Promega). More than 1200 recombinant clones were obtained and screened for tandem repeats using a mixture

of six digoxigenin (DIG)-labelled oligonucleotides: (CT)₁₀ (TG)₁₀ (CAC)₅CA, CT(CCT)₅, CT(ATCT)₆ (TGTA)₆TG, and detected with the DIG detection kit (Roche). Strongly positive clones were selected and incubated in liquid Luria-Bertani (LB) medium, and the plasmids from these clones were recovered using the QIAprep Spin Miniprep kit (QIAGEN). Inserted fragments were sequenced using the BigDye Terminator Ready Reaction kit (Applied Biosystems) and M13 universal primers. Sequences were determined using the ABI PRISM 3730 Genetic Analyser system (Applied Biosystems), and were finally confirmed visually using Sequence Navigator 1.1 (PerkinElmer). Polymerase chain reaction (PCR) primers used to amplify the microsatellite regions were designed from each clone sequence using the Primer 3 program (Rozen & Skaletsky 2000). One of the two primers for each locus was labelled with a fluorescent dye including HEX, NED and FAM.

Twenty-one primer pairs were designed from 64 clone sequences. Each microsatellite locus was tested by PCR amplification of sequences acquired from 30 individuals. These specimens were collected from eight locations across Korea, the genomic DNAs of which were extracted according to standard protocol. Microsatellites were amplified under the following conditions: the reaction mixture (total volume 25 µL) comprised of 0.5 µL of template DNA, 0.5 µL of each primer (10 µM), 1.0 µL of dNTP solution (10 µM), 5 µL of 5× PCR buffer, 0.3 µL of *Taq* DNA polymerase (5 µ/µL) (Promega), and 17.2 µL of deionized water. PCR analysis was performed using the GeneAmp 9700 system (Applied

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Table 1 Characterization of 11 microsatellite loci in *Palaemon paucidens* (Crustacea: Decapoda)

Locus	Repeat motif	Primer sequence (5'–3')	N_A	Size range (bp)	T_a (°C)	H_E	H_O	GenBank no.
PAU0081	(GA) ₃₃	F: FAM-CTAGAGTGCGATGCTAATAA R: CTAAACTCGGCCATGTAACC	5	293–336	51	0.94	0.83	EU781708
PAU0133	(CCT) ₆	F: HEX-CACCCGAGATGGAGTACTT R: TCAAAAGGGGAGGAGGGGAA	2	189–192	55	0.46	0.37	EU781709
PAU0316*	(CT) ₃₁	F: HEX-CCAATAACCATTGATGTTGGG R: CAGTTGAGTCGCTAAGACATTGA	21	238–284	53	0.93	0.80	EU781711
PAU0329*	(CT) ₄₂	F: NED-TTGGACAGAGGGTTGGCTAC R: GGGCAGCCCTTTGAAGTCTAGT	19	204–252	54	0.95	0.77	EU781712
PAU0431*	(GA) ₂₅	F: FAM-CGAAGTGGCGGGTCTCAAACCC R: CGACCCCTACCTCTTCTTC	16	210–244	59	0.93	0.53	EU781713
PAU0593	(GA) ₂₂	F: FAM-CACCGAATGTTGCTTCAATTT R: ACAAACCAACATCATTCCTC	17	212–246	49	0.95	0.80	EU781714
PAU0625*	(GT) ₈ (GA) ₁₁	F: HEX-TGTCTGCCACAATGCTAGA R: TCCGAAGCGTCTAATTTTCG	7	242–276	52	0.49	0.13	EU781715
PAU0818	(GA) ₁₁	F: FAM-GACGGAGCTCCTCCAGAAT R: ACAATCTCCTCTTTTGGCATGT	11	192–220	52	0.85	0.80	EU781716
PAU0930*	(GA) ₂₇	F: HEX-ATCCCCCTTCCATCAATTC R: AGCAGTAGCTAAACCCACCC	20	179–221	53	0.95	0.70	EU781717
PAU0975*	(CT) ₂ CC(CT) ₂₃	F: NED-AAGGCATTCTCTCCCTCTCTC R: CGGGTCGTCGTTTGTAAAGAT	13	164–204	54	0.89	0.57	EU781718
PAU1164	(GA) ₄ CA(GA) ₂₈	F: NED-CAATGGCGTTAGGAGCATTT R: CAATGGCCAAATGATTTAAGTATG	14	188–236	51	0.61	0.53	EU781719

F, forward primer; R, reverse primer; N_A , number of alleles; T_a , optimal annealing temperature; H_E , expected heterozygosity; H_O , observed heterozygosity; *indicates loci which had significant heterozygote deficits ($P < 0.05$).

Table 2 Results of cross-species amplification

Species locus	PAU0133	PAU0431	PAU0593
<i>Palaemon (Palaemon) miyadai</i> (Kubo, 1938)	–	–	–
<i>Exopalaemon modestus</i> (Heller, 1862)	–	–	–
<i>Macrobrachium nipponense</i> (De Haan, 1849)	–	–	+
<i>Caridina denticulata</i> (De Hann, 1844)	+	+	–

+, successfully amplified; –, not amplified

Biosystems) and included the following steps; initial denaturation at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at a set temperature depending on the locus (Table 1) for 30 s, and elongation at 72 °C for 50 s. PCR products were sized and analysed by GeneScan 3.7 and GeneMapper 4.0 (Applied Biosystems). As a result, we could amplify 11 microsatellites successfully. Of 21 sets, one locus showed zero heterozygosity, and the others were not amplified.

Characterization of the microsatellite loci are shown in Table 1. Range of the allele number among 11 microsatellite markers was two to 21, while the loci PAU0316, PAU0329

and PAU0930, which are included in dinucleotide repeats, are more polymorphic than other loci. The range of expected heterozygosity was between 0.46 and 0.95 at the 11 polymorphic loci. Microsatellite data were analysed with GenePop 3.4 (Raymond & Rousset 1995) to test for Hardy–Weinberg equilibrium and linkage disequilibrium. This test indicated that loci PAU316, PAU329, PAU431, PAU625, PAU930 and PAU975 showed a significant deviation from Hardy–Weinberg equilibrium ($P < 0.05$), caused by a considerable heterozygote deficiency. For the possibility of null alleles, we tested with Micro-Checker (2.2.3) (van Oosterhout *et al.* 2004). Analysis indicated that all loci were no evidence for scoring error due to stuttering and large allele dropout. Null alleles may be present at several loci, as is suggested by the general excess of homozygotes for most allele size classes (Table 1). In part, excess homozygosity may be due to that specimens used in the genotyping were from several separated locations (i.e. Wahlund effect). As a result of linkage disequilibrium analysis, there was no significant nonrandom association among all the loci. These loci were tested in other prawn species inhabiting Korea; *Palaemon (Palaemon) miyadai* (Kubo 1938), *Exopalaemon modestus* (Heller, 1862), *Macrobrachium nipponense* (De Haan, 1849) and *Caridina denticulata* (De Hann, 1844). This showed that PAU0133, PAU0431 and PAU0593 markers could be amplified in two species (Table 2).

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