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PRIMER NOTE

Microsatellite primers for three Western Atlantic *Farfantepenaeus* prawn species

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Abstract

Microsatellite loci were identified for three closely related penaeid species, *Farfantepenaeus subtilis*, *F. paulensis* and *F.* sp., from genomic libraries enriched for CA repeats. Seven out of nine highly polymorphic loci detected were amplified across all three species. Between four and 64 alleles were recorded per locus (average = 36). The average observed heterozygosity ranged from 0.094 to 0.897 (mean = 0.613), while the expected heterozygosity ranged from 0.091 to 0.985 (mean = 0.822).

Keywords: Brazil, enriched library, microsatellite, Penaeidae, *Penaeus*, shrimp

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The interest in the molecular genetics of penaeid prawns is high because of their value in commercial fisheries and aquaculture. Whilst allozyme studies have previously detected low levels of polymorphism in penaeids, higher levels of genetic variability have been found through DNA markers such as random amplified DNA polymorphisms and microsatellites (Benzie 2000). Three species of *Penaeus* (*Farfantepenaeus*), *F. brasiliensis*, *F. subtilis* and *F. paulensis*, are commercially fished along the Brazilian coast, between 4° N and 34° S (Perez-Farfante 1969). Recently evidence has been presented for a new Brazilian *Farfantepenaeus* species, based on data from allozymes and partial sequences of cytochrome oxidase I and on 16S rDNA partial sequences (Gusmão *et al*. 2000; Maggioni *et al*. 2001). These studies have provided evidence for a close evolutionary relationship among *F. paulensis*, *F. subtilis* and *F.* sp. We have exploited this close evolutionary relationship to develop a suite of microsatellite loci that amplify for all three species.

Specimens were collected along the Brazilian coast at five different locations. *F. paulensis* was obtained at Rio Grande (32° S); *F. subtilis*, at São Luís (2° S) and Fortaleza (4° S); *F.* sp., at Recife (8° S) and Vitória (20°) (see Maggioni *et al*. 2001). Tail muscle was sampled and preserved in 95% ethanol. DNA extractions followed the phenol–chloroform–isoamyl alcohol protocol of Sambrook *et al*. (1989). Genomic libraries enriched for $(CA)_n$ repeats were constructed from DNA of 20 individuals per species, following Kandpal *et al*. (1994), but probing and detection follow the digoxygenin protocol described by Morgan *et al*. (1999). The resulting libraries constituted of approximately 150–300 clones per species. $(CA)_n$ inserts were detected on 19–100 clones per species. Ten clones were sequenced for *F. subtilis* and *F.* sp., and 30 for *F. paulensis*. Plasmids were cycle-sequenced through Perkin-Elmer kits (#4303149 or #4307175) in 10 µL reactions (primers T7-TAATACGACTCACTATAGGG- or SP6-GATTTAGGTGACACTATAG-), which were read on an ABI 377 automated sequencer. Six, eight and four primer pairs were designed, respectively, for *F. subtilis*, *F. paulensis* and *F.* sp., using PRIMER 3 (Rozen & Skaletsky 1998). Polymerase chain reaction (PCR) amplification was conducted on a Hybaid 'PCR Express' or on an MWG-Biotech 'Primus 96 Plus' thermocycler. Ten µL of PCRs contained 1× Qiagen buffer (Tris-HCl, KCl, 1.5 mM $MgCl_2$); 1× Qiagen 'Q solution'; 200 µM each dNTP; 0.5 µM each primer (fluorescent labelling as presented in Table 1), 1 U Qiagen *Taq* DNA polymerase, 10–50 ng template DNA and chemical water (Sigma). PCR cycling conditions included an initial denaturing step of 2 min at 95 °C; followed by five cycles of 30 s at 95 °C, 1 min at annealing temperature, 45 s at 72 °C; followed by 25 cycles of 30 s at 94 °C; 1 min at annealing temperature; 45 s at 72 °C; followed by a final elongation and dATP-tailing step of 30 min at 72 °C. For the locus Fpa046 amplification was performed using Qiagen 'HotStar' *Taq* DNA polymerase requiring an initial denaturing step of 15 min at 95 °C. Genotypes were

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Table 1 Primer pairs for the identified microsatellite loci (fluorescent phosphoramidite label indicated)

Locus	GenBank Accession no.	Primers (5′–3′)	Repeat motif	T_a (°C)	Clone size	Size range
Fpa034	AY049195	ATCAACACTACATTATGCAT$^{6\text{-FAM}}$ CAGTTTCAACAATTTTATTC	$(CA)_3(CAAA)_3(CA)_7(CAAA)_4$ $(CA)_{10}CAAA((CA)_6TA)_2(CA)_2$	51	182	165–325
Fpa046	AY049196	AGCCATGTCTTTATTCATTATTGC ACGTGCACTGTGTGTGAGTG^{NED}	$(CA)_{23}$	57	121	124–337
Fpa104	AY049197	ACGTGCATGAGTATGTGTA$^{6\text{-FAM}}$ GAATATTATCGCTGTACCAA	$(CA)_{36}CG(CA)_7$	55	160	99–128
Fpa307	AY049198	CCCAATGTGAAAATATCTCAAACC^{HEX} TGTAATGGCAACTCGCACAC	$(GT)_7(GA)_2(GT)_2CAGA$ $((GT)_2GA)_4(GT)_{30}GC(GT)_{16}$	55	216	195–456
Fpa413	AY049199	TCTTTATATTCAGGGTGAGGGGAG CAGCCCAGTTCCTTTTCAAG^{NED}	$(GT)_{11}$	54	211	207–213
Fpa413a	—	AGTAGTGGTTCTTTGAGCTGGAAC CAGCCCAGTTCCTTTTCAAG^{NED}	—	54	—	160–197
Fpa421	AY049200	TTAGCTCCTTTGGTGGTGATG^{HEX} CTGGTTACCTGCCTGAGTGAG	$(CA)_{11}CG(CA)_7$	60	104	80–155
Fsu121	AY049201	AGCAGACTCTCAAGGTGTGTACC^{HEX} TGAGTGTAAGTATTTTCCGTTTGG	$(CG)_4(CA)_{45}(TA(CA)_4CG$ $(CA)_3)_2$	55	220	121–315
Fsu141	AY049202	GTACTCCCTCTACTCTCCAC GTGAGGGTTTTGTAAACTG$^{6\text{-FAM}}$	$(CA)_5AGCACG(CA)_{34}$	55	140	133–319
Fsp120	AY049203	GATCAGTGACACAAGTCTATG ATGCGTATGTAGTCATAAGG^{NED}	$(GT)_{20}ATTT(GT)_{18}ATTT(GT)_{13}$	40	196	121–286

screened through multiplex electrophoresis on an ABI 377 automated sequencer running GENESCAN 3.1 software (Perkin-Elmer). Four *F. paulensis*, homozygous for locus Fpa104, were PCR amplified and cycle-sequenced to confirm the flanking regions (GeneBank accession no. AY049204). Basic statistics and Fisher's (1935) exact tests for conformation to Hardy–Weinberg equilibrium were carried out using GDA software (Lewis & Zaykin 2001).

The microsatellite loci described here were amplifiable across the three *Farfantepenaeus* species, except for loci Fpa421 and Fpa413 (Table 2). Amplification of locus Fpa413 produced a strong nonspecific product (named Fpa413a in Table 1), which is monomorphic for *F. paulensis* and *F.* sp., but variable for *F. subtilis* where it behaves as a codominant microsatellite marker. One null allele was detected in locus Fpa413 when comparing the alleles amplified by the three primer sets. Null alleles may be present in other loci where Hardy–Weinberg expectations were not met. However, other reasons for departure from Hardy–Weinberg equilibrium include selection, assortative mating or population structure among the studied samples. The Wahlund effect was possible for *F.* sp. and *F. subtilis* because the sample set for these species was drawn from geographically separate localities. This effect was observed at least once, in *F.* sp. for locus Fpa104: p(all samples) = 0.034 (Table 2); p(Vitória) = 1.000, p(Recife) = 0.132.

The microsatellite loci described were more variable in size than usually expected for this kind of marker (Goldstein & Schlötterer 1999). However, the size range usually brackets clone size and, along with the interspecific variation, some of the variation may be attributed to complex repeat patterns. As an example of this complexity, nested repeat motifs can be observed on loci Fpa034, Fsu121 and Fpa307 (Table 1). Large sizes in microsatellite loci appear to be common for penaeid prawns (Ball *et al*. 1998; Benzie 2000).

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Table 2 Allele frequencies for the described loci. n, sample size; Na, number of alleles; H_E, estimated heterozygosity (Lewis & Zaykin 2001); H_O, observed heterozygosity; p(H–W), probability of conformance to Hardy–Weinberg equilibrium

Locus	Species	n	Na*	H_E	H_O	p(H–W)
Fpa034	*Farfantepenaeus* sp.	20	22	0.967	0.550	0.000
	F. paulensis	24	19	0.943	0.625	0.000
	F. subtilis	20	17	0.814	0.750	0.134
			47			
Fpa046	*F.* sp.	20	20	0.959	0.300	0.000
	F. paulensis	21	13	0.880	0.286	0.000
	F. subtilis	18	22	0.971	0.556	0.000
			45			
Fpa104	*F.* sp.	20	6	0.523	0.450	0.034
	F. paulensis	22	6	0.541	0.455	0.182
	F. subtilis	19	10	0.844	0.632	0.018
			19			
Fpa307	*F.* sp.	20	24	0.951	0.600	0.000
	F. paulensis	20	20	0.958	1.000	0.047
	F. subtilis	20	27	0.973	0.850	0.013
			64			
Fpa413	*F.* sp.	—	—	—	—	—
	F. paulensis	21	4	0.475	0.333	0.032
	F. subtilis	—	—	—	—	—
			4			
Fpa413a	*F.* sp.	20	1	0.000	0.000	1.000
	F. paulensis	24	1	0.000	0.000	1.000
	F. subtilis	20	4	0.273	0.300	1.000
			4			
Fpa421	*F.* sp.	—	—	—	—	—
	F. paulensis	20	16	0.923	1.000	0.002
	F. subtilis	11	12	0.909	0.364	0.000
			27			
Fsu121	*F.* sp.	19	24	0.973	1.000	1.000
	F. paulensis	19	20	0.950	1.000	0.005
	F. subtilis	20	25	0.976	0.700	0.000
			52			
Fsu141	*F.* sp.	19	23	0.960	0.632	0.000
	F. paulensis	21	17	0.941	1.000	0.086
	F. subtilis	20	26	0.973	0.950	0.461
			53			
Fsp120	*F.* sp.	20	25	0.971	0.950	0.485
	F. paulensis	19	17	0.936	0.737	0.000
	F. subtilis	20	21	0.874	0.800	0.170
			45			

*In bold = total number of different alleles across the three species.

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