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TECHNICAL NOTE

Isolation and characterization of microsatellites in three overexploited penaeid shrimp species along the Brazilian coastline

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Abstract Most Brazilian commercial species of penaeid shrimp are currently overexploited. Thirteen polymorphic microsatellite loci herein isolated and characterized in *Farfantepenaeus brasiliensis*, *Rimapenaeus constrictus* and *Xiphopenaeus kroyeri* could be very useful for population studies on these penaeid species and proved to be potentially functional in cross-amplification with other species of shrimp. These microsatellites may be very helpful tools for research programs aimed at the sustainable management and conservation of these important fishery resources.

Keywords Conservation · Polymorphic microsatellites · *Farfantepenaeus brasiliensis* · *Rimapenaeus constrictus* · *Xiphopenaeus kroyeri*

The exploitation of species of penaeid coastal shrimp in tropical regions is a very long-standing activity that has resulted in their current overexploitation (Costa et al. 2004). The impact of shrimp fisheries in these regions has become comparable to the most intensively exploited temperate shelf ecosystems in the world, thereby causing significant loss in spawning biomass and biodiversity (Pauly et al. 2002).

The pink shrimp *Farfantepenaeus brasiliensis* (Latreille 1817) and the seabob shrimp *Xiphopenaeus kroyeri* (Heller 1862), showing a wide geographic distribution quite similar along the Brazilian coast, have predominantly been fished (Costa et al. 2004) and are currently considered overexploited (MMA 2004). *Rimapenaeus constrictus* (Stimpson 1874), although a non-commercial species due to its small body size (Castilho et al. 2007), can also undergone an extensive reduction in its population, being a typical by-catch species in commercial shrimp fisheries (Babler et al. 2000).

Microsatellites have been used to answer a wide variety of questions related to biological conservation (Taylor and Parkin 2007) and molecular analysis on Brazilian shrimp stocks should be very informative for their management and conservation (Gusmão et al. 2006). Thus far, microsatellites are unknown for *F. brasiliensis*, *R. constrictus* and *X. kroyeri* and 13 polymorphic loci are described in the present work.

A partially enriched genomic library of each target species was produced following Hamilton et al. (1999). DNA was isolated from muscle tissue through the method described by Aljanabi and Martinez (1997). After the digestion of this DNA with restriction enzymes, *Rsa*I and *BstU*I (GE Healthcare), fragments with 200–800 bp were isolated using agarose gel, with the Wizard SV Gel and Polymerase Chain Reaction (PCR) Clean-Up System kit (Promega), and linked to adaptors (Hamilton et al. 1999). These products were enriched with eight biotinylated tetranucleotides, which were hybridizated to digested DNA and captured with magnetic beads (Streptavidin Magnesphere Paramagnetic Particles, Promega). Recovered fragments were cloned using the pGEM-T Easy kit (Promega). The clones containing inserts were sequenced in an automatic sequencer (ABI 377 and MegaBace 1000). The sequences obtained were analyzed using CID software (Freitas et al. 2008) to extract the vector sequence, find the sequences containing microsatellites and design the primers anchored in the flanking regions.

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Table 1 Characteristics of 13 microsatellite loci developed for *Farfantepenaeus brasiliensis*, *Rimapaenaeus constrictus* and *Xiphopenaeus kroyeri*

Loci	Primer sequences	Motif	T_a (°C)	$MgCl_2$ (mM)	Allele size (bp)	# of alleles	Expected heterozygosity	Observed heterozygosity
Farfantepenaeus brasiliensis ($n = 27$, I)								
Fbra 01	F: ACGCACACAGAGCACATAC	$(CA)_{10}$	57.0	2.5	178–198	11	0.849	0.654
	R: CTTGTCTTGGAAACGTCCTA							
Fbra 02	F: ACTTTGTAATACGGAGTGCG	$(GTCT)_5...(CT)_6$	55.0	1.5	208–244	9	0.784	0.693
	R: TACATATTGGGAAACGAAGG							
Fbra 03	F: ACACACACGCACACACTTAT	$(AC)_7$	60.0	1.5	108–150	6	0.571	0.308*
	R: TAGCCTCTTTGTGGTTTGTT							
Fbra 04	F: CTTGTTTATGGTGGGATGAG	$(TGTC)_5...(TC)_{11}$	55.0	1.5	171–313	14	0.871	0.087*
	R: CACAGACAGAGATTTATGCG							
Fbra 05	F: TTCACATTACCCTTCTCTCG	$(TC)_6...(CT)_9$	60.0	1.5	261–377	15	0.894	0.5*
	R: AAGAGAGGGGAGACAGAGAG							
Rimapaenaeus constrictus ($n = 30$, II)								
Rcon 06	F: GTTGCTTATTGCTGAACC	$(GTT)_{12}$	57.8	1.5	194–363	11	0.653	0.143*
	R: GACAACGCCGACTATAAC							
Rcon 07	F: AATAGGATTCGGATACGC	$(GTTT)_5$	48.1	1.5	146–182	3	0.382	0.214
	R: AGCAAAAAGTCTGCGTTC							
Rcon 08	F: ACCCAAGCTATGGTCTTC	$(CT)_{13}(ATCT)_5$	51.3	1.5	166–218	7	0.568	0.241*
	R: ATGCCTGATAAGGGACATC							
Rcon 09	F: ATAGAATGTACACACGCC	$(AC)_6...(AC)_{10}$	49.5	1.5	240–276	7	0.458	0.533
	R: TATCAAACTCTGTCCTCTAG							
Xiphopenaeus kroyeri ($n = 41$,III)								
Xkro 10	F: GTGTTGAGACGAAACATGG	$(TC)_8$	55.6	1.5	260–262	2	0.366	0.195*
	R: CGACCTAGAGAATGTAGGCA							
Xkro 11	F: CGATAGCATCTACCTCTTCC	$(CTTCCT)_9$	55.6	1.5	164–200	7	0.643	0.560
	R: ACAGAGGGTATTTCACTTCATC							
Xkro 12	F: TCTCGGTTTCTCTTGTCACT	$(TCTG)_{6...}(TCTG)_7$ $(TC)_6CT(TC)_4$	55.6	1.5	184–248	13	0.865	0.536*
	R: AGCTGGGACTTCTCCTTAC							
Xkro 13	F: CACGCTCATATACACTCACA	$(CA)_6$	55.0	1.5	166–168	3	0.182	0.195
	R: GAACTGCAGGTGGTGAAGTC							

Annealing temperature (T_a), number of specimens (n) and sample locality (I = Baía Formosa, RN; II = Ubatuba, SP; III = Itajái, SC)

* $P < 0.05$ Hardy–Weinberg equilibrium; GenBank accession number (EU559711–EU559722)

Table 2 Cross-species amplification in different penaeid shrimp

Primer	*F. brasiliensis*	*F. paulensis*	*L. schmitti*	*L. vannamei*	*R. constrictus*	*X. kroyeri*
Fbra 01	+	+	+	−	+	+
Fbra 02	+	+	+	+	+	+
Fbra 03	+	+	+	+	+	+
Fbra 04	+	+	+	+	+	+
Fbra 05	+	+	−	−	+	−
Rcon 06	−	−	−	−	+	+
Rcon 07	+	−	−	+	+	+
Rcon 08	−	−	−	−	+	−
Rcon 09	−	+	−	−	+	−
Xkro 10	+	−	−	−	+	+
Xkro 11	−	−	−	−	−	+
Xkro 12	+	−	−	−	−	+
Xkro 13	−	−	+	−	+	+

The PCR optimum annealing temperature of each set of primers was achieved in a gradient Mastercycler thermocycler (Eppendorf). PCR conditioning was initial denaturation at 95°C for 5 min and 30 cycles of 94°C (30 s), 48–60°C (Table 1) (45 s) and 72°C (45 s), followed by 8 cycles of 94°C (30 s), 53°C (45 s), 72°C (45 s), with a final extension at 72°C for 10 min. An M13-tail was end-added in the forward primer and an additional fluorescently-labeled M13 primer was used in the PCR (Schuelke 2000), as described elsewhere (Sanches and Galetti 2006). Each PCR reaction used 2 µl of 1.25 mM of dNTPs, 1.5 µl of 10× amplification buffer (200 mM Tris–HCl, pH 8.4 and 500 mM KCl; Invitrogen and/or Fermentas), 0.45–0.6 µl of 50 mM of $MgCl_2$, 1 µl of 2 pmol/µl of the primer containing the complement tail to primer M13, 1 µl of 8 pmol/µl of the primer without the tail, 1 µl of 8 pmol/µl of fluorescently-labeled M13 primer (NED, 6-FAM or HEX), 0.1 µl of 5 U/µl of *Taq* DNA Polymerase and 1 µl of 5 ng/µl of DNA, completing a total volume of 15 µl. In *X. kroyeri*, some reactions used 7.5 µl of GoTaq Colorless Master Mix (Promega) containing 1.5 mM of $MgCl_2$ and 200 µM of each dNTP.

The PCR products were genotyped in a MegaBace 1000 automatic sequencer (Amersham Biosciences). The samples were analyzed using the GENETIC PROFILER software. Tests for deviation from the Hardy-Weinberg equilibrium and linkage disequilibrium were performed using the GENEPOP 3.4 program (Raymond and Rousset 1995) and corrected for multiple comparisons using the sequential Bonferroni procedure (Rice 1989). Significance values were estimated through the Markov chain method with 10,000 repetitions.

The results for each species are listed in Table 1. Linkage disequilibrium was not observed between loci. HWE deviations were probably related to null alleles, sample errors, preferential mating and inbreeding effects. The number of alleles per locus ranged from two (Xkro 10) to 15 (Fbra 05) and the expected heterozygosity ranged from 0.182 (Xkro 13) to 0.894 (Fbra 05). Cross-species amplification was successful in several penaeid species using the same PCR conditions (Table 2).

The microsatellites described here could be very useful for population studies on the target species and could constitute a set of molecular tools that are very helpful to research programs aimed at the sustainable management and conservation of these commercial shrimp species. Brazil has about 13% of its coastline protected in conservation units and the importance of marine reserves in maintaining and enhancing the yield of adjacent fisheries is well known (Kelly et al. 2000). The knowledge on an intra-specific diversity level for a set of sympatric species could be used to orient the establishment of biological and genetically representative conservation units. In addition, these microsatellites proved to be informative for population studies on other penaeids, mostly native in the Brazilian coast.

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