academicJournals

Vol. 5(9), pp. 611-615, September 2013
DOI: 10.5897/IJBC2013.0562
ISSN 2141-243X © 2013 Academic Journals
http://www.academicjournals.org/IJBC

International Journal of Biodiversity and Conservation

Full Length Research Paper

Informative microsatellites for freshwater and marine shrimp species

Carla Guinart Marques, Camilla Alves Santos, Pedro Manoel Galetti Jr. and Patrícia Domingues de Freitas

Departamento de Genética e Evolução, Universidade Federal de São Carlos, Via Washington Luiz km 235, Caixa Postal 676, CEP 13565-905, São Carlos, SP, Brazil.

Accepted 2 July 2013

Marine and freshwater shrimp are overexploited worldwide due to their high commercial value being usually fished in a predatory manner. The concern about conserving the stocks for economical or ecological reasons has been threatened by the absence of molecular markers for the majority of these species. In this work, we described ten anonymous microsatellites isolated from an enriched-library with magnetic beads technology of *Penaeus* (*Litopenaeus) vannamei genome*, considered the most economically important marine shrimp in the world. In addition, we tested these loci and a panel of 21 Simple Sequence Repeat from Expressed Sequence Tag (EST-SSR) loci, also isolated from *P. vannamei*, in ten other marine and two freshwater shrimp species. The cross species amplification success rate ranged from 32 to 77% in *Macrobrachium jelskii* and *Penaeus (Litopenaeus) schmitti*, respectively. These microsatellites appear conservative in a wide spectrum of shrimp groups, facilitating genetic studies in those species in which no microsatellites have been yet described.

Key words: Conservative, Simple Sequence Repeat (SSR), shrimp genetic, penaeid, *Macrobrachium.*

INTRODUCTION

In the last two decades, many studies have described microsatellite loci for shrimp, mostly due to increasing need of access to the genetic information either for improvement of reared populations or in conservation of wild populations (Freitas et al., 2007; Artiles et al., 2011; Chiang et al., 2012; Santos et al., 2012; Mohanty et al., 2012). Despite this, several marine and freshwater shrimp species have no microsatellite thus far described and cross species amplification may be useful to assess genetic variation in such groups, reducing time and costs related to isolate specific markers for all existing species.

In this work, we described ten polymorphic microsatellites obtained from anonymous regions of *Penaeus (Litopenaeus) vannamei* genome, one of the most important marine shrimp of the world. These loci and a panel of other 21 EST-SRR loci, previously described for *P. vannamei* (Santos et al., 2012), were evaluated in 12 important species of marine and freshwater shrimp natives to Brazil and Mozambique.

MATERIALS AND METHODS

The sequences were isolated from genome arbitrary regions using the methodology based on construction of enriched-library with magnetic beads described by Hamilton et al. (1999) and sequenced on an ABI 377 automated sequencer (Applied Biosystems Inc.). Sequences were analyzed by CID tool (Freitas et al., 2008), a pipeline that allow the identification of microsatellite and flanking primer pair sequences based on the MISA (Thiel, 2001) and Primer 3 (Rozen and Skaletsky, 2000) computer programs. In addition, these loci and a panel of 21 EST-SSR (Expressed Sequence Tags-Simple Sequence Repeats) obtained from coding regions of *P. vannamei* (Santos et al., 2012) were tested in cross species

*Corresponding author. E-mail: guinartc@gmail.com. Tel:55-16-3351 8309. Fax: +55-16-3351 8377.

Table 1. Population analysis for the new microsatellite loci in *Penaeus* (*Litopenaeus) vannamei* and for the cross amplification results.

Loci	Primer sequences (5' - 3')	Repeat motif	T_a (°C)	n_a	Size-range (bp)	H_O	H_E	GenBank
Specie	***Penaeus (Litopenaeus) vannamei***							
Lvan14	F: AGACAAAGGGAAGGAGAGAC R: GGCTTATTCCATGGTTGTT	(AGAC)5	53	3	119-135	0.48	0.55	EU419945
Lvan16	F: CACCCGAAAGATATGAGAGA R: TGGCTTCCCTTTGTTTATC	(AGAC)5(AG)3A(CAGG)5	49,5	17	112-210	0.31	0.89*	EU419946
Lvan17	F: GTAACATGCCCTCACTCACT R: GTCAAAAGCGCCTTAGTTTA	(TTCTT)5..(CT)10	50	11	220-329	0.75	0.79	EU419947
Lvan19	F: GAGCAAATCTATGCGACAAT R: GTAAAAAGCTTTGCGGGT	(TC)6..(CT)6..(TC)7	49,5	5	208-222	0.47	0.70	EU419948
Lvan21	F: AGCATTTGTTCATCTTCAGC R: GAATGGAAAGAAGGAGGAAG	(CT)23..(CT)6	50	3	319-379	0.91	0.50	EU419949
Lvan22	F: TGTGTTGCTTGTTTGCTACT R: TTCCCTTTTTCCTCCTCTAT	(ACAG)5G(CAGA)6	51	13	258-318	0.76	0.85	EU419950
Lvan23	F: GCACGTCAGGAAGATTTCTA R: CCATTTCTCTTTTCTTTCCC	(GA)12AT(G)11	53	9	190-286	0.77	0.88	EU419951
Lvan25	F: TGGTATTGTGGTTGCTGTTA R: AGAAGGTGAAACGTAATACTCG	(TC)6	47	4	125-135	0.27	0.38	EU419952
Lvan26	F: CTGTGGCAGAATTTTTCTCT R: TGTCTCCGTTTATGTCTCCT	(GA)8(CA)5(GACA)5..(AG)9	53	2	249-265	0.58	0.41	EU419953
Lvan33	F: TTTGTCTGTCTGTCCATCTG R: GTTCGTAAAATCCTCCTCAA	(TCAC)5	51	3	234-250	0.28	0.33	EU419954

amplification reactions for ten other penaeid species and two freshwater shrimp. The *P. vannamei* specimens were collected from Brazil captive stocks. The wild shrimp were collected along continental and coast regions of Brazil [*Penaeus* (*Litopenaeus) schmitti, Penaeus (Farfantepenaeus) brasiliensis, Penaeus (Farfantepenaeus) paulensis, Penaeus (Farfantepenaeus) subtilis, Penaeus (Farfantepenaeus) notialis, Xiphopenaeus kroyeri, Rimapenaeus constrictus, Macrobrachium amazonicum, Macrobrachium jelsk* and Mozambique [*Metapenaeus monocerus, Penaeus monodon, Penaeus indicus and Penaeus (Marsupenaeus) japonicas*]. Total DNA was extracted from nitrogen frozen pleopod tissue according to Sambrook et al. (1989). Amplification reactions were carried on Mastercycler Gradient Thermal Cycler (Eppendorf) using 10 µL reactions containing 5 ηg of DNA, 0.2 mM of dNTPs, 1 x PCR buffer (200 mM Tris-HCl, pH 8.4 and 500 mM KCl), 1.5 mM of $MgCl_2$, 1 U of Taq DNA Polymerase, (Invitrogen Life Technologies), 0.8 µM of reverse primer, 0.2 µM of forward primer with a M13 tail and 0.8 µM of M13 fluorescently labeled (Schuelke, 2000). The PCRs were performed through touchdown reactions, starting with annealing temperature at 54°C and dropping one degree every three cycles up to 49°C, followed by 15 min of final extension at 72°C. PCR-labeled products were analyzed on MegaBACE1000 (GE Healthcare) using the Genetic Profiler software (GE Healthcare). Expected and observed heterozygosity, linkage disequilibrium and deviation from Hardy-Weinberg equilibrium (HWE) were determined using the GenePop program, version 3.4 (Raymond and Rousset, 1995). Sequential Bonferroni correction (Rice, 1989) was implemented for data significance analysis. The Macro-Checker software, version 2.2.3 (Oosterhout et al., 2004) was used to determine the presence of null alleles, stutters or genotyping errors in the populations analyzed.

RESULTS AND DISCUSSION

The microsatellite loci obtained by enriched library from genome of *P. vannamei* showed no match with any other known microsatellite sequence available at GenBank from NCBI, distinguishing a new set of these molecular markers for this species. The number of alleles ranged from two to 17, averaging 7.2 per locus. The observed and expected heterozygosity ranged from 0.27 to 0.91 and 0.33 to 0.89, respectively for *P. vannamei* population evaluated. One locus (Lvan16) showed significant HWE deviation following sequential Bonferroni correction, exhibiting a significant heterozygote deficit ($P<0.005$). Micro-Checker software indicated a possible presence of null alleles on this marker, although Wahlund effect and nonrandom sampling should not be discarded. No linkage disequilibrium was detected (Table 1).

The cross species amplification success rate ranged from 32 to 77% for *M. jelskii* and*. P. schmitti,* respectively, using either anonymous SSR and EST-SSR loci. Positive amplification patterns were obtained for at least one of the species analyzed for all tested loci (Table 2). Polymorphisms in some of these loci were tested in native populations of *P. schmitti, P. brasiliensis* and *P. paulensis* Lvan 61 locus showed significant HWE deviation for *P. schmitti* population (Table 1). Cross species amplification rate differences between SSR and EST-SSR were not detected.

The *P. schmitti* showed the highest heterologous amplification rate (77%) followed by *P. paulensis* (51%) and *P. brasiliensis* (41%). Both *Macrobrachium* species showed the lowest amplification rates (32 – 35%), likely due to their lower phylogenetic relationship with *P. vannamei* (Table 2). The new set of anonymous SSR described here proved to be useful to assess genetic variation in *P. vannamei* and, together with the EST-SSR loci also evaluated, show to be conservative within marine and freshwater shrimp, and can be

Table 1. Contd.

Specie	*Penaeus (Litopenaeus) schmitti*							
Lvan 202	F: TAGTGTTCACAGATTCCG R: GAAGTATCAAACAGAACAGC	(CT)17	Touchdown	7	261-291	0,5	0,73	HS412016
Lvan 204	F: AGAACTGAACTTTGACCTTG R: CATACAATTCCAAGACCG	(TTC)5	Touchdown	9	154-206	1	0,86	HS412020
Lvan 183	F: GTGAAGCCTCTCATTACTC R: CATGACTACCAAGATTTCTC	(GA)7	Touchdown	7	281-308	0,5	0,81	HS412014
Lvan 205	F: TACAACCGCAAGTAGATG R: ATAGAAGAGTATAGGTAGGCG	(CA)6	Touchdown	4	185-200	0,3	0,28	HS412021
Lvan 61	F: ATCACACTAAGCAGGATATG R: GTCACGTCTAACAAGCAG	(TG)10	Touchdown	7	149-261	0,4	0,67*	HS412004
Lvan16	F: CACCCGAAAGATATGAGAGA R: TGGCTTCCCTTTGTTTATC	(AGAC)5(AG)3A(CAGG)5	Touchdown	7	156-244	0,75	0,67	EU419946
Lvan19	F: GAGCAAATCTATGCGACAAT R: GTAAAAAGCTTTGCGGGT	(TC)6..(CT)6..(TC)7	Touchdown	4	161-247	0,28	0,32	EU419948
Specie	***Penaeus (Farfantepenaeus) paulensis***							
Lvan 99	F: CAACACTAAAGGAACACACAC R: CGTTTCTTGTTTTCTCTGTG	(AAG)5	Touchdown	4	110-121	0,28	0,3	HS412007
Lvan 202	F: TAGTGTTCACAGATTCCG R: GAAGTATCAAACAGAACAGC	(CT)17	Touchdown	5	249-290	0,3	0,69*	HS412016
Lvan 205	F: TACAACCGCAAGTAGATG R: ATAGAAGAGTATAGGTAGGCG	(CA)6	Touchdown	5	142-199	0,1	0,19	HS412021
Lvan 16	F: CACCCGAAAGATATGAGAGA R: TGGCTTCCCTTTGTTTATC	(AGAC)5(AG)3A(CAGG)5	Touchdown	5	154-240	0,93	0,71	EU419946
Lvan 19	F: GAGCAAATCTATGCGACAAT R: GTAAAAAGCTTTGCGGGT	(TC)6..(CT)6..(TC)7	Touchdown	11	197-229	0,31	0,81*	EU419948
Specie	***Penaeus (Farfantepenaeus) brasiliensis***							
Lvan 16	F: CACCCGAAAGATATGAGAGA R: TGGCTTCCCTTTGTTTATC	(AGAC)5(AG)3A(CAGG)5	Touchdown	10	166-232	0,53	0,84*	EU419946
Lvan 19	F: GAGCAAATCTATGCGACAAT R: GTAAAAAGCTTTGCGGGT	(TC)6..(CT)6..(TC)7	Touchdown	8	162-208	0,41	0,82*	EU419948

Ta, locus-specific annealing temperature; na, number of alleles; Ho, observed heterozygosity; He, expected heterozygosity and significant HWE deviation; * following Sequential Bonferroni correction. *Significant values considering sequential Bonferroni correction.

Table 2. SSR and EST-SSR loci cross amplification in 12 species. Positive (+) and negative amplification (-).

Loci	*Penaeus (Litopenaeus) schmitti*	*Penaeus (Farfantepenaeus) brasiliensis*	*Penaeus (Farfantepenaeus) Paulensis*	*Xiphopenaeus kroyeri*	*Rimapenaeus constrictus*	*Metapenaeus monoceros*	*Metapenaeus stebbing*	*Penaeus (Marsupenaeus) japonicus*	*Fenneropenaeus indicus*	*Penaeus monodon*	*Macrobrachium jelskii*	*Macrobrachium amazonicum*	Total (%)
SSRs randon													
Lvan14	-	+	-	-	+	+	+	+	+	+	-	+	66
Lvan16	+	+	+	-	-	+	-	-	+	+	-	-	50
Lvan17	+	-	-	-	+	+	+	+	+	+	+	+	66
Lvan19	+	+	+	+	-	+	+	+	+	+	+	+	91
Lvan21	-	-	-	-	-	-	-	-	-	-	-	-	0
Lvan22	-	-	-	-	-	+	+	+	+	-	-	-	33
Lvan23	+	-	-	-	-	-	-	+	-	-	-	-	16
Lvan25	+	+	+	+	+	+	+	+	+	+	+	+	100
Lvan26	-	+	-	-	+	+	-	+	-	-	+	+	50
Lvan33	+	+	-	-	+	+	+	+	+	+	-	-	66

Table 2. Contd.

SSR-ESTs													
Lvan 59	+	+	-	-	+	-	-	+	+	+	+	-	58
Lvan 61	+	+	-	+	-	-	-	-	+	-	-	-	33
Lvan 50	+	+	+	+	+	+	+	-	+	+	-	-	75
Lvan 51	+	+	+	-	-	+	+	-	-	-	+	-	50
Lvan 94	+	+	+	+	+	-	-	-	-	+	+	-	58
Lvan 99	+	+	+	-	-	-	-	-	-	-	-	-	25
Lvan 104	+	-	-	-	+	+	-	+	+	-	-	-	41
Lvan 117	+	-	-	-	-	+	-	+	-	+	-	-	33
Lvan 102	+	+	-	+	-	+	-	-	+	-	-	-	41
Lvan 93	+	-	+	+	-	+	+	+	+	-	-	+	66
Lvan 96	+	-	-	-	-	+	+	+	-	+	-	-	41
Lvan 98	+	+	-	-	-	+	+	-	+	-	-	+	50
Lvan 116	-	-	+	-	+	+	+	+	-	-	-	+	50
Lvan 134	-	-	-	+	-	-	+	-	-	-	+	+	33
Lvan 154	+	-	-	-	-	-	-	-	-	-	+	-	16
Lvan 183	+	-	+	+	+	-	-	-	-	-	-	-	33
Lvan 202	-	-	+	+	+	-	-	-	-	+	-	-	33
Lvan 204	+	-	+	+	+	-	+	-	-	+	-	+	58
Lvan 205	+	+	-	+	-	-	-	+	+	+	+	-	58
Lvan 195	+	-	+	-	+	+	+	+	+	+	-	-	66
Lvan 232	+	+	-	+	-	-	-	-	-	-	-	+	33
Total	77	51	41	41	38	58	48	51	51	48	32	35	

useful to genetic studies in those species in which no microsatellites have been yet described.

ACKNOWLEDGEMENTS

The authors thank the Brazilian agencies Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Associação Brasileira de Criadores de Camarão (ABCC).

REFERENCES

Artiles A, Rodríguez I, Pérez A, Pérez L, Espinosa G (2011). Low genetic variability in the fifth introduction of *Litopenaeus vannamei* in Cuba, as estimated with microsatellite markers. Biotecnologia Aplicada 28: 147-150.

Chiang TY, Tzeng TD, Lin HD, Cho CJ, Lin FJ (2012). Isolation and characterization of polymorphic microsatellite loci from *Metapenaeopsis barbata* using PCR-based Isolation of Microsatellite Arrays (PIMA). Int. J. Mol. Sci. 13: 2763-2768.

Freitas PD, Calgaro MR, Galetti Jr PM (2007). Genetic diversity within and between broodstocks of the white shrimp *Litopenaeus vannamei* (Boone, 1931) (Decapoda, Penaeidae) and its implication for the gene pool conservation. Braz. J. Biol. 67: 939-943.

Freitas PD, Martins DS, Galetti Jr PM (2008). CID (Clipping vectors, Identifying SSR, Design of primers): a rapid and efficient bioinformatic tool for the detection of simple sequence repeats (SSR) from genomic libraries. Mol. Ecol. Res. 8: 107-108.

Hamilton MB, Pincus EL, Di Fiore A, Flesher RC (1999). Universal linker and ligation procedures for construction of genomic DNA libraries enriched for microsatellites. Biotechniques 27: 500-507.

Mohanty P, Sahoo L, Parida K, Das P (2012). Development of polymorphic EST-SSR markers in *Macrobrachium rosenbergii* by data mining. Conserv. Genet. Res. 5 (1): 133-136.

Oosterhout CV, Hutchinson WF, Wills DPM, Shipley P (2004). MICRO-CHECKER: *Software* for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4: 535-538.

Raymond M, Rousset F (1995). Population genetics software for exact tests and ecumenecism. J. Hered. 86: 248-249.
Rice WR (1989) Analyzing tables of statistical tests. Evolution 43: 223–225.
Rozen S, Skaletsky HJ (2000) PRIMER 3 on the WWW for general users and for biologist programmers. In: Bioinformatics Methods and Protocols: Methods in Molecular Biology, (ed. by S. Misener & S.A. Krawetz), Humana Press, Totowa, NJ, USA. pp. 365–386.
Sambrook J, Fritsch EF, Maniatis T (1989). Molecular cloning: A laboratory manual. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.
Santos CA, Rossini BC, Marques CG, Galetti Jr PM, Freitas PD (2012). Characterization and genomic annotation of polymorphic EST-SSR loci in *Litopenaeus vannamei* shrimp. Aquac. Res. 43: 1567-1570.
Schuelke M (2000). An economic method for the fluorescent labeling of PCR fragments. Nat. Am. 18: 233-234.
Thiel T (2001). MISA; Microsatellite Identification Tool, Version 1.0. Available at http://pgrc.ipk-gatersleben.de/misa/. (Accessed on 21 June 2013).