Microsatellites from the White Shrimp Litopenaeus schmitti *(Crustacea, Decapoda)*

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ABSTRACT

Microsatellite sequences from *Litopenaeus schmitti*, a penaeid shrimp species used in Cuban aquaculture, were identified from a 300-700 bp insert DNA library. A total of 910 colonies were obtained and screened for repetitions; 114 resulted positive: 106 for the oligonucleotides $(TC)_{15}$, $(TG)_{15}$ and $(TAA)_{15}$ and 8 for the $(GTG)_{15}$ oligonucleotide. Dinucleotide repetitions prevailed over other motifs and GT was the most abundant, representing 50%. Optimal amplification conditions were determined for two loci *(Lsch*-1 and *Lsch*-2), which were used to analyze three shrimp populations (one wild and two hatchet). Six alleles were found for *Lsch*-1 $(GT)_n$ and 8 alleles for *Lsch*-2 (composite). The results suggest that the two loci are useful for studying *L. schmitti* populations.

Keywords: genetic polymorphism, *Litopenaeus schmitti,* microsatellites, shrimp

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RESUMEN

Microsatélites del camarón blanco *Litopenaeus schmitti* (Crustacea, Decapoda). A partir de una genoteca de ADN construida con insertos entre 300 pb y 700 pb, se identificaron secuencias microsatélites de *Litopenaeus schmitti,* especie cubana de camarón utilizada en la acuicultura. Las 910 colonias que se obtuvieron fueron analizadas en busca de repeticiones y resultaron positivos 114 clones de los cuales 106 lo fueron para los oligonucleótidos $(TC)_{15}$, $(TG)_{15}$ y $(TAA)_{15}$, y 8 para el oligonucleótido $(GTG)_{15}$. Las repeticiones de dinucleótidos prevalecieron sobre otras y el dinucleótido GT fue el más abundante, lo que constituyó 50% de las últimas. Se determinaron las condiciones óptimas para la amplificación de dos loci (*Lsch*-1 y *Lsch*-2), los que fueron utilizados en el análisis de tres poblaciones (una natural y dos de cultivo). El número de alelos encontrado para el locus *Lsch*-1 $(GT)_n$ fue de 6 mientras que el locus *Lsch*-2 (compuesto) presentó 8. Los resultados sugieren que los dos loci son útiles para el estudio de poblaciones del camarón blanco *L. schmitti*.

Palabras claves: camarón, *Litopenaeus schmitti,* microsatélites, polimorfismo genético

*I*ntroduction

Litopenaeus schmitti is one of the two economically relevant shrimp species in Cuba; particularly for its introduction in aquaculture more than fifteen years ago. Different human activities and natural events have deeply affected shrimp ecosystems endangering natural populations of this species. One way to protect this resource and indirectly sustain shrimp aquaculture industry is to evaluate genetic variability and natural population structure [1]. Previous works using allozymes have suggested a limited genetic variability among penaeids populations at limited geographic scales [2, 3]. However, a higher sensitivity and a discriminatory power have been described using other molecular markers such as mtDNA, RAPDs, and microsatellites, when studying the population genetics of different penaeid species [4–7]. Especially, a growing body of information on microsatellite sequences is available for some penaeids, and a number of primer sets for PCR amplifications has been designed [6, 8–10]. However, the usefulness of the primers for inter-species application has been questioned in some studies [11]. The aim of the present study is to characterize microsatellites loci isolated from *L. schmitti* and evaluate the usefulness of two of them in genetic population studies.

*M*aterials and Methods

Library construction and screening

Genomic DNA was extracted from a single ethanol preserved pleopod muscle of *L. schmitti* according to García-Machado *et al.* [12]. Partial genome library construction and screening was carried out following Estoup and Cornuet [13]. *Sau*3AI restriction fragments of sizes ranging from 300 bp to 700 bp were ligated to a previously *Bam*HI-digested and alkaline phosphatase-treated pUC18 vector. Ligations were used to transform Epicuriam Coli® XL1-Blue ultracompetent cells (Stratagene, USA).

All the colonies were screened for repetitions using the DIG-labeled oligonucleotides $(TC)_{15}$, $(TG)_{15}$, $(TAA)_{15}$ and $(GTG)_{15}$. Labeling was achieved using the Tailing Kit (Boehringer Mannheim, Germany). The $(TC)_{15}$, $(TG)_{15}$ and $(TAA)_{15}$ oligonucleotides were pooled in the same hybridization mix, and the hybridization temperature was set to 48 ºC, whereas hybrid-

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ization temperature for $(GTG)_{15}$ oligonucleotide was 65 ºC. Complete or partial sequences from 30 positive clones were obtained using an automatic sequencer ALF express (Amersham Pharmacia-Biotech, Sweden), and using as sequencing primers the universals M13-40 forward and M13 reverse primers.

DNA was isolated as described above from samples of three populations. Different amplification conditions were assayed with unique pair of primers designed for each locus, changing $MgCl_2$, DNA, and primer concentrations and the annealing temperature (Table). PCR products were run on 6% sequencing gels and scored against a known sequence. The corresponding bands were developed using the Silver Stain Sequencing Kit (Promega, USA).

The deviations from Hardy-Weinberg equilibrium were estimated using the program Byosis [14]. The observed and expected heterozigosity, the number of alleles per locus and the linkage disequilibrium and the genetic diversity were calculated according to Nei [15] using the program Genepop 3.1 version [16] and Genetix [17].

Results and Discussion

Of the 910 colonies obtained, 114 were positives: 106 for the oligonucleotides $(TC)_{15}$, $(TG)_{15}$ and $(TAA)_{15}$ and 8 for $(GTG)_{15}$. Ten of the 30 clones sequenced had 16 repeated motifs. Dinucleotide repetitions were found in 8 arrays, prevailing over penta- (3), tri- (3), and tetranucleotide motifs (2). Among the dinucleotide repeated motifs, GT (50%) was the most abundant followed by TA (25%) and CT (12.5%), respectively. A similar prevalence of dinucleotide repeats was also reported for *L. setiferus* [6] and *P. monodon* [7]. As for *L. schmitti,* dinucleotide GT was the most abundant in *L. setiferus* [6] but as frequent as CT in *P. monodon* [7]. GT motive is also more frequent in fish [18, 19] as well as in animals than in plants [20].

Optimal amplification conditions were determined for two loci: *Lsch*-1 and *Lsch*-2 (EMBL accession numbers AJ277641 and AJ277642). Reaction mix consisted in 10 ng of template DNA, 20 pmol of forward and reverse primers, 0.1 mM of each dNTP, 1.5 mM of $MgCl_2$ and 0.5 units of DNA Polymerase (Amersham Pharmacia-Biotesh, Sweden). PCR reaction was developed in 12 cycles of 1 min at 94 ºC, 30 s at 2 ºC below annealing temperature, 30 s at 72 ºC, 22 cycles of 30 s at 94 ºC, 30 s at annealing temperature, 30 s at 72 ºC, preceded by an initial denaturation step (5 min at 94 ºC) and followed by a final elongation step (10 min at 72 ºC) (Table).

Allele frequencies for the two loci are shown in the Figure. The size of the *Lsch*-1 alleles seems to vary according to the repetition of two bases. Two variants, differing by four nucleotides, were far more frequent in locus *Lsch*-2. However, other alleles appear apparently resulting from the loss or gain of 1, 2 or 3 bases. They could be related to the presence of 4 or 5 T instead of 3 in some places, since such sequences are observed once each in the sequenced clone. For this reason the variation of the *Lsch*-2 locus was not well defined as a change of the repetition number.

The Table summarizes the main characteristics and some variability estimates of the two-microsatellite markers from the studied populations. The quantity of alleles was different at each population. Yaguanabo, one of the hatchery populations, presented only 2 *Lsch*-1 alleles and 5 *Lsch*-2 alleles (Figure). This result could be expected as genetic variation is not taken into account during stock foundation and maintenance across

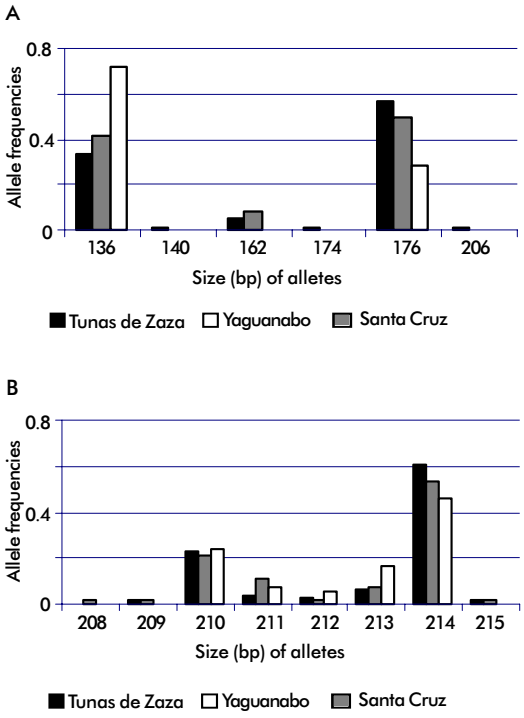

Figure. Allele sizes (bp) and frequencies at each *Litopenaeus schmitti* populations. A) *Lsch*-1; B) *Lsch*-2.

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Table. Primer, repeat motif, amplification conditions and results of amplification of two microsatellite loci in three populations of *L. schmitti*.

Locus	Repeat motif	Type of repeat	Size range (bp)	Annealing temperature (°C)	Relative diversity (%)	(N = 37) Tunas de Zaza[a]	(N = 30) Santa Cruz[b]	(N = 29) Yaguanabo[b]
						Observed / expected heterozigosity		
Lsch-1	$(GT)_{25}$	Perfect	136–206	60	7.7	0.59 / 0.57	0.48 / 0.58	0.43 / 0.41
Lsch-2	$(CTTT)_9$ (CTTTT) (CTTTTT) $(CATT)_5$ $(CATG)_2$ $(CATT)_2$	Imperfect-composite	208–215	50	1.1	0.43 / 0.58	0.71 / 0.66	0.56 / 0.70
Number of alleles per locus						6.5	5.5	3.5

Primer sequences were: *Lsch*-1, 5'-GGCTTCTATTGTGTGTCTTCTC-3' and 5'-GAGGTGGTTACATATTTGCACGC-3';
Lsch-2, 5'-TAAGGCGATTTGGTTCACGT-3' and 5'-TTAGTCATTATGAACGAATG-3'.
[a]Samples from a wild population; [b]samples from hatchery populations.

generations, which result in most cases in a bottleneck effect and inbreeding [21].

Santa Cruz population is the first generation from wild brood stock reared at the hatchery center. The allele number of this population is similar to Tunas de Zaza's wild population. This type of domesticate group of animals has been reported with a higher heterozigosity of some isozyme loci and a particular behavior of number of rostral tooth [22].

Relative diversity was higher for locus *Lsch*-1 (Table) indicating stronger resolution of populations. This can be related with a higher variation of dinucleotide repeat; according with the results of Charkraborty *et al.* [23] that found a high mutation rate in a survey of microsatellite variability in natural populations.

The difference between the observed and expected heterozigosity was higher for *Lsch*-2 than *Lsch*-1, but no significant departure from the Hardy-Weinberg equilibrium for both loci in all three populations and no linkage disequilibrium were found.

Taking into account that both loci resulted polymorphic and that the genetic variability correlates with population histories, it could be suggested that *Lsch*-1 and *Lsch*-2 are useful markers for the study of *L. schmitti* populations.

Other potentially useful microsatellite loci from the present library are under analysis for making a better characterization of *L. schmitti* wild and cultured populations.

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