Genetic variability in jack mackerel *Trachurus murphyi* Nichols: New SSRs loci and application

Variabilidad genetica en jurel *(Trachurus murphyi Nichols):* Nuevos loci SSRs y aplicación

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ABSTRACT

Nine novel microsatellite loci were developed for *Trachurus murphyi*. These loci were characterized in samples from three sites along the eastern South Pacific Ocean. One of the loci was monomorphic, whereas the rest of the loci had between six and 19 alleles per locus and showed the expected heterozygosity ranging from 0.653 to 0.933. These loci do not show genetic differentiation among the analyzed sites.

RESUMEN

Nueve nuevos loci microsatélites fueron desarrollados para *Trachurus murphyi*. Estos loci fueron caracterizados en muestras de tres localidades a lo largo del océano Pacífico suroriental. Uno de los loci fue monomórfico, mientras que para el resto de los loci el número de alelos por locus oscilo entre 6 a 16 y la heterocigosidad esperada entre 0.653 a 0.933. Estos loci no muestran diferenciación entre las diferentes localidades analizadas.

Jack mackerel (*Trachurus murphyi*) is a pelagic fish distributed along the eastern Pacific coastline and in the New Zealand Sea (Serra 1991). Due to its economic importance, *T. murphyi* has been the focus of several studies aiming to resolve its taxonomic status (Poulin *et al.* 2004) and phylogenetic relationships (Cárdenas *et al.* 2005) and to assess its population structure. For the latter, several approaches have been used, including morphometrics and meristic life history traits (e.g. Serra 1991), parasites (e.g. Oliva 1999), and genetics (González *et al.* 1996; Cárdenas *et al.* 2009). These approaches have revealed from one to three populations along the jack mackerel distribution. Genetic studies using allozymic markers (González *et al.* 1996), mtDNA, and heterologous microsatellites (Cárdenas *et al.* 2009) have only shown homogeneity among locations. Although species-specific microsatellite markers for jack mackerel have been described by Canales-Aguirre *et al.* (2010a), these authors only characterized the markers at one site.

This study describes nine new microsatellite loci isolated from *T. murphyi*. In addition, we tested these new loci to evaluate the genetic variability among sites in the eastern South Pacific Ocean.

In order to isolate the microsatellite loci, we obtained high-molecular-weight DNA from the muscle tissue of ten individuals of both sexes using a phenol/chloroform method (Sambrook *et al.* 1989). Microsatellite loci were isolated using an enrichment procedure involving magnetic beads (Jones *et al.* 2000). DNA was partially digested with a cocktail of seven blunt-end restriction enzymes (*Hae*III, *Stu*I, *Eco* RV, *Sca*I, *Bsr* BI, *Pvu*II, *Hiu* CII). Fragments between 300 and 700 bp were selected by gel extraction and ligated to a 20-bp oligonucleotide adaptor containing a *Hind* III restriction site at the 5' end. Microsatellite enrichment was achieved using streptavidin-coated magnetic beads and 5'-biotinylated $TAGA_{12}$ and $CATC_8$ oligonucleotide probes. The captured molecules were amplified by PCR using a primer complementary to the adaptor, digested with *Hind* III to remove the adaptor, and ligated into the *Hind* III site of the pUC19 vector. The plasmids were then electroporated into *Escherichia coli* DH5. Recombinant clones, identified by blue-white selection, were chosen arbitrarily for sequencing on an ABI 377 using the Big Dye Terminator Cycle Sequencing methodology (Applied Biosystems). Specific primers flanking the identified microsatellite sequences were designed using DesignerPCR v1.03 (Research Genetics)

(Table 1). The microsatellite loci were amplified in 10-µL reactions containing 1X PCR buffer, 2 mM $MgCl_2$, 0.2 mM forward primer (fluorescently-labeled), 0.2 mM reverse primer, 200 mM dNTPs, 0.03 U/µL Taq DNA polymerase (Invitrogen®), and 20 ng genomic DNA template. PCR was performed in a PTC-200 (MJ Research) thermal cycler with the following parameters: 94 °C for 3 min, followed by 35 cycles of 94 °C for 40 s, 57 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 5 min. All loci were successfully amplified under these conditions. The PCR products were analyzed on an ABI 3330 DNA sequencer. Alleles were scored using Peak Scanner™ software v1.0, with GS500 as the internal size standard.

To assess these loci, we genotyped samples of *T. murphyi* from three sites in the eastern South Pacific Ocean (Fig. 1). These sites were: 1) Lobos de Afuera Islands, Perú (LA), and 2) Talcahuano (THNO) and 3) Chiloé (CH), Chile. To detect scoring errors due to large allele drop-out, stuttering, and null alleles, we used MICRO-CHECKER v.2.2.3 software (Van Oosterhout *et al.* 2004). The total number of alleles (Na) and the observed (Ho) and expected heterozygosities (He) were calculated for all microsatellite loci using ARLEQUIN 3.1 (Excoffier *et al.* 2005). The Hardy–Weinberg equilibrium was tested with ARLEQUIN, and linkage disequilibrium was tested using GENEPOP 3.1 (Raymond & Rousset 1995). Genetic differentiation was estimated by pairwise Fst and analyses of molecular variance (AMOVA) with ARLEQUIN. The significance of the F-statistics was achieved using 10,000 permutations.

Only the TmurD101 locus was monomorphic (Table 1). The data base did not contain scoring errors such as drop-out alleles and stutter bands. Null alleles may be present at the TmurC110 locus, as suggested by the general excess of homozygotes (Table 2). The *T. murphyi* samples showed high genetic variability of the eight microsatellite loci. The number of alleles per locus ranged from six to 19 (TmurC109a and TmurC12-TmurD5, respectively). He ranged from 0.653 to 0.933 (TmurC109a and TmurD5, respectively) and Ho ranged from 0.143 to 1.000 (Table 2). Significant deviations from the Hardy-Weinberg equilibrium were found for locus TmurC110 at all sites and for locus TmurC12 at Chiloé. No loci were found to be in linkage disequilibrium, suggesting that the eight loci are independent.

Table 1: Characteristics of the microsatellite loci isolated for *T. murphyi*. For each marker, we report the locus name, primer sequence, repeat unit, allele size range (bp), and Genbank accession number. Tmur: ***T****rachurus* ***mur****phyi*. Mono = monomorphic locus

Tabla 1: Características de los loci microsatelites aislados para *T. murphyi*. Reportamos para cada marcador el nombre del locus, la secuencia del primer, el rango del tamaño alelico (pb) y el número de acceso a Genbank. Tmur significa ***T****rachurus* ***mur****phyi*. Mono = locus monomórfico.

Locus	Primer sequence (5'-3')	Repeat unit	Size range	Genbank Acc.
TmurC12	F: TCTGAACAACTGCTGGAAGAC	$CATC_{(26)}$	128-284	GU562609
	R: CTACGCATAGTCCGATCCAC			
TmurC102	F: CAGCAGAGCAGAGTGACTG	$CATC_{(9)}$	219-477	GU562612
	R: CACGGATAGGTGGTAAAGC			
TmurC104	F: AGCAGAACCGTCCGATAGTC	$TGGA_{(10)}$	157-277	GU562613
	R: AGCATCCTGGCAGAAGAATC			
Tmurc109a	F: AGCCATCATTGGACTGGC	$GATG_{(10)}$	262-350	GU562614
	R: AGGCAAGCAAGGTCAAGG			
TmurC110	F: GTTCCCTCTGACTGTAACCTG	$GATG_{(14)}$	174-286	GU562615
	R: GGTGGTGATGTGATGTGG			
TmurD5	F: GCAGGAGTTACACATTTACAGC	$TAGA_{(23)}$	262-458	GU562610
	R: AACCAGGTCGCCATAGAG			
TmurD8	F: TAATGCCTTGAGGGTATGTTC	$CTAT_{(18)}$	237-369	GU562611
	R: GATGGATGGGTTCTGATAGTG			
TmurD101	F: TAAACCGAGCCGAGTAGAGC	$GACA_{(15)}GATA_{(11)}$	Mono	GU562616
	R: ATTTCCCTTTGGAGCATCCT			
TmurD112	F: CAGAGGACAACTAACGGAGTC	$CTAT_{(8)}$	213-297	GU562617
	R: GACCTAATGCTGGTTTACAGTG			

Table 2: Parameters of the microsatellite loci for jack mackerel (*Trachurus murphyi*). Sample size (N), number of alleles (Na), observed (Ho) and expected (He) heterozygosity, and the *p*-value from the exact test for Hardy–Weinberg equilibrium (H&W). Values in bold show $p \leq 0.05$ after Bonferroni correction.

Tabla 2: Parámetros de los loci microsatélites para el jurel (*Trachurus murphyi*). Tamaño de la muestra (N), número de alelos (Na), heterocigosidad observada (Ho) y esperada (He), y valor-p asociado al test exacto para el equilibrio de Hardy & Weinberg (H&W). Valores en negrita muestran un valor-$p \leq 0.05$ después de la corrección de Bonferroni.

Site	Parameter	Locus							
		TmurC12	TmurC102	TmurC104	TmurC109a	TmurC110	TmurD5	TmurD8	TmurD112
Lobos de Afuera Island	N	15	15	14	14	11	14	13	14
	Na	17	15	10	10	10	15	15	13
	Ho	0.867	0.867	0.786	0.857	0.364	1.000	1.000	1.000
	He	0.907	0.911	0.714	0.804	0.839	0.921	0.923	0.890
	H&W	0.128	0.084	0.936	0.784	**0.000**	1.000	1.000	1.000
Talcahuano	N	15	14	14	13	14	15	15	15
	Na	19	15	12	9	9	19	17	13
	Ho	0.933	0.857	0.857	0.846	0.286	1.000	0.933	0.800
	He	0.931	0.908	0.842	0.834	0.867	0.933	0.927	0.902
	H&W	0.519	0.479	0.499	0.511	**0.000**	1.000	0.825	0.187
Chiloé	N	15	15	15	15	14	15	15	15
	Na	18	16	11	6	11	17	16	17
	Ho	0.733	0.867	0.733	0.800	0.143	1.000	1.000	0.867
	He	0.931	0.916	0.818	0.653	0.857	0.920	0.931	0.922
	H&W	**0.000**	0.007	0.125	0.675	**0.000**	0.776	1.000	0.060
	Fst by locus	0.007	-0.006	-0.006	0.023	0.024	0.001	-0.006	-0.009

Figure 1: Map indicating the *T. murphyi* sampling sites in the eastern South Pacific Ocean.

Figura 1: Mapa indicando las localidades de muestreo de *T. murphyi* desde el Océano Pacífico sureste.

The AMOVA of the eight microsatellite loci analyzed revealed a non-significant Fst value among the *T. murphyi* samples (Fst = 0.003; P = 0.591). Pairwise Fst tests for genetic differentiation provided non-significant p-values among all sample pairs: LA-THNO = 0.004, LA-CH = 0.001, THNO-CH = 0.007, indicating a lack of genetic discontinuities in the analyzed *T. murphyi* distribution.

The development of new microsatellite loci in *T. murphyi* to evaluate patterns of genetic variation is necessary for adequate resource conservation and to address other ecological questions. The observed genetic variability is similar to that found by Cárdenas *et al.* (2009), using heterologous microsatellite loci isolated from *Trachurus trachurus* (Kasapidis & Magoulas 2008). Heterologous markers are useful when no species-specific markers are available (e.g. Canales-Aguirre *et al.* 2010b). Notwithstanding the usefulness of specific microsatellite loci for *T. murphyi*, those at the population level (as described in Canales-Aguirre *et al.* 2010a) could provide more information on the genetic diversity of the species.

Although the small sample sizes, 15 individuals, used herein are one possible reason for not observing statistically significant differences among the samples studied, our results agree with previous studies (e.g. González *et al.* 1996; Cárdenas *et al.* 2009). The characteristics of jack mackerel, such as its large population size (e.g. in 2008, spawning biomass was estimated at 1,934,723 tons by the daily egg production method according to Ruiz *et al.* (2009), seasonal migrations between spawning and feeding grounds, and reproductive strategies that facilitate larval dispersal across large geographic distances as well as the lack of obvious physical barriers in the marine environment promote genetic connectivity and the presence of a single reproductive group in this species. This genetic homogeneity is usual in marine fishes (e.g. Canales-Aguirre *et al.* 2010c) and especially in migratory pelagic species of the Humboldt Current System (e.g. Galleguillos *et al.* 1997; Ferrada *et al.* 2002). Ward (2000) reports that mixing rates of 1% result in genetic homogeneity and a failure to identify stock structure, even if it exists. Notwithstanding a lack of genetic structure may imply the presence of one genetic stock, nevertheless, more than one harvest stocks may exist. For instance, these results are not consistent with morphometric and meristic characteristics and life histories (e.g. Serra 1991), which show more than one population. Incongruence between life history traits and genetics evidence could be to outcome of phenotypic plasticity, more than local adaptation, which implies a genetic component for these differences.

In summary, the new microsatellite loci presented here do not show differences between the three sites along the *T. murphyi* distribution. On the other hand, these results must be applied with caution due to the small sample sizes used herein. Finally, these loci could be useful for elucidating ecological issues such as genetic structure when dealing with a large sample and the identification of recent reductions in the effective population size, the recent geographic expansion of *T. murphyi* to New Zealand, and contemporaneous migration along its distribution.

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