

## Isolation and characterization of microsatellite loci for the jack mackerel (*Trachurus murphyi* Nichols, 1920)

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Received: 4 February 2009 / Accepted: 15 April 2009  
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**Abstract** Eight microsatellite loci were developed for the jack mackerel (*Trachurus murphyi*), a fish of significant commercial importance in the Southeast Pacific. Genetic variation at these loci was examined in 15 samples from the locality of Talcahuano (Chile). All eight were highly polymorphic, with a number of alleles per locus ranging from 4 to 22 and an observed heterozygosity from 0.429 to 1. These markers will be useful to address issues of population genetics, ecology, conservation and fisheries management related to that species.

**Keywords** Population genetics · Fishery management · Jurel · *Trachurus murphyi* · Southeast Pacific

Due to its economical importance, the jack mackerel (*Trachurus murphyi*; Nichols 1920) represents one of the most significant resources in the Southeastern Pacific. *T. murphyi* is transzonal. Its distribution ranges from the coasts of Ecuador to the end of Chile in the Southeastern Pacific, and along the New Zealand coasts in the Southwestern Pacific (Serra 1991). Previous genetic studies using allozymes (González et al. 1996) and RFLP (Sepúlveda et al. 1996) have not showed any differentiation across its geographical distribution. In the present study,

we report the isolation and characterization of eighth polymorphic loci for *T. murphyi*. These loci were developed as a study tool useful for the estimation of genetic diversity and population genetic structure in this species, with the goal of providing baseline information for management plans aiming to protect that resource.

High molecular weight DNA was extracted from muscle tissue of ten individuals from 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. 2002). Briefly, the 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, *Hii* 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 CA<sub>15</sub>, ATG<sub>12</sub> and CATC<sub>8</sub> 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 Designer PCR version 1.03 (Research Genetics) (Table 1). The microsatellite loci were amplified in 10 µl reactions containing 1 × PCR buffer, 2 mM MgCl<sub>2</sub>, 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 of genomic DNA template. PCR was performed in a PTC-200 (MJ Research)

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**Table 1** Characteristics of the microsatellite loci isolated for the jack mackerel (*Trachurus murphyi*)

Locus	Primer sequence (5′–3′)	Repeat unit	Size range	$N_a$	$H_o$	$H_e$	$F$	H & W	Genbank
TmurA101	F: ACTCCAGAGATAAAGGCAACAC R: TGGGTAATAGTCAGTTCACAGC	CA <sub>(45)</sub>	158–198	14	0.733	0.891	<b>0.177</b>	0.152	FJ666131
TmurA104	F: ACCTGAGGGAGCAGAAAC R: TGCCATCTAACATTACTGTAC	TG <sub>(23)</sub>	159–203	17	1.000	0.929	–0.077	1.000	FJ668657
TmurA115	F: GTCACTGGAGCAATCAATAGAC R: TGCAATGTTACATGACTCAGAG	TG <sub>(28)</sub>	157–227	22	0.857	0.946	<b>0.094</b>	0.050	FJ668658
TmurB2	F: AATCTTCACGTCACATAAACAC R: TCGACACTGTTGAGTCATC	TG <sub>(5)</sub> AG <sub>(2)</sub> TG <sub>(5)</sub>	139–151	5	0.429	0.702	<b>0.389</b>	0.083	FJ668659
TmurB6	F: CAACGGCTCCAGTCAGAG R: GAGGCTCAATGAAGGGTAGC	CA <sub>(13)</sub>	163–208	9	0.786	0.852	0.078	0.354	FJ668660
TmurB104	F: TGAAGCACAAGTTTCCAAATC R: AAAGGTCAGAGAGAGAACAACG	ATC <sub>(14)</sub>	147–168	7	0.467	0.724	<b>0.356</b>	0.044	FJ668661
TmurB116	F: CTCTCGTCTTCATTGAGGTCAC R: AAGTCGTCTGACTCATCTGTGC	ATC <sub>(7)</sub>	127–142	4	0.533	0.429	–0.244	1.000	FJ668662
TmurC4	F: TGAATGCTCTCCAGATGAATAC R: GCAAACACTTTCTCCTCAGATT	CATC <sub>(9)</sub>	225–281	9	0.867	0.811	–0.068	0.987	FJ688270

The values reported for each marker are the allele size range (in bp), the number of alleles ( $N_a$ ), the observed ( $H_o$ ) and the expected ( $H_e$ ) heterozygosity, the fixation index ( $F$ ), the  $P$  value from the exact test for Hardy–Weinberg equilibrium (H & W) and the Genbank accession number. Values in bold show homozygote excess

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 successfully amplified under the same conditions. The PCR products were analysed on an ABI 3330 DNA sequencer. Alleles were scored using Peak Scanner v1.0 (Applied Biosystems), with GS500 (Applied Biosystems) as the internal size standard.

We characterized the polymorphism of each marker in a sample of 15 individuals from the locality of Talcahuano, Chile. We first checked for the potential presence of null alleles using MicroChecker v2.2.3 (Van Oosterhout et al. 2004). The number of alleles ( $N_a$ ), the expected ( $H_e$ ) and observed ( $H_o$ ) levels of heterozygosity were obtained using GENALEX v6 (Peakall and Smouse 2006). Deviations from Hardy–Weinberg equilibrium and gametic disequilibrium between markers were tested using Arlequin v3.01 (Schneider et al. 2000).

Null alleles may be present at four loci, TmurA101, TmurA115, TmurB2 and TmurB104, as suggested by the general excess of homozygotes (Table 1). The number of alleles per locus ranged from 4 to 22, corresponding to markers TmurB116 and TmurA115, respectively, and observed heterozygosity values from 0.429 to 1, for TmurB2 and TmurA104, respectively. Only TmurA115 and TmurB104 showed significant deviation from Hardy–Weinberg equilibrium ( $P \leq 0.05$ ). No pair of loci was found to be in linkage disequilibrium.

These eight microsatellite loci are the first developed for *T. murphyi*. They will be useful in studies related to population genetics, ecology, conservation and fisheries management in that species.

**Acknowledgments** We thank Claudio Díaz, Andrea Barrera, Mariem Dib, Maritza Seguel for technical support, and Nicolas Gouin for helpful comments and reviewing the English. This work forms part of the FIP 2007-27 project.

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