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Ten novel microsatellite loci characterized for a remarkably widespread fish: *Galaxias maculatus* (Galaxiidae)

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Abstract

Ten polymorphic microsatellite markers (five tetra-, one compound tetra-, one octa- and three dinucleotides) were isolated and characterized for *Galaxias maculatus*, a fish species widely distributed in the Southern Hemisphere. Markers were tested in 89 individual samples from a single location and the number of alleles ranged between 2 and 28. Observed and expected heterozygosities ranged from 0.103 to 0.910 and 0.098 to 0.935 respectively. No evidence was detected for either linkage disequilibrium (*P*-values > 0.05 for each locus pair) or deviations from HWE (*P*-values > 0.05 for every loci).

Keywords: compounds, dinucleotides, *Galaxias maculatus*, microsatellite markers, tetranucleotides

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Galaxias maculatus is arguably one of the most widely distributed freshwater fishes in the world. The species has a Gondwanan distribution and is found in landlocked and diadromous populations in Australia, New Zealand and Patagonian South America (McDowall 1972; Cussac *et al.* 2004). Waters *et al.* (2000) examined phylogeographical patterns throughout the species range using mtDNA control region sequence divergence and concluded that both marine dispersal and vicariance may have contributed to the wide geographical distribution. In South America, there are few studies examining genetic diversity within the Galaxiidae family. Zattara & Premoli (2004) compared allozyme diversity among lacustrine (landlocked) populations of *G. maculatus* and reported a significant correlation between gene diversity and lake size. More recently, Ruzzante *et al.* (2008) and Zemlak *et al.* (2008) examined phylogeographical patterns within Patagonia for *Galaxias platei*. Six microsatellite loci were described for the *Galaxias vulgaris* complex endemic to New Zealand, however, amplification in *G. maculatus* was reported to yield only ambiguous or faint products (Waters *et al.* 1999). In this study, we describe 10 new microsatellite loci developed specifically for *G. maculatus*. To our knowledge, no other microsatellite marker currently exists for this species.

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Genomic DNA was extracted from four individuals from the lake Gutierrez (Argentina) and one from the lake Huillinco (Chile) using standard phenol-chloroform-isoamyl alcohol technique (Sambrook *et al.* 1989). Subsequently, these DNAs were used to create microsatellite-enriched libraries following the protocol of Glenn & Schable (2005).

Three DNA samples were digested with *Rsa*I and the remaining two with *Hinc*II restriction enzymes. These were then ligated to superSNX linkers (Glenn & Schable 2005) and hybridized to four different biotinylated oligonucleotide probe mixtures of the following motifs: 5 μM

Table 1 Characterization of 10 microsatellite loci for *Galaxias maculatus*

Locus	Accession No	Primer sequence (5′–3′)	Repeat	No. alleles/ No. individuals*	Allele range (bp)	T_a (°C)	H_E	H_O	*P*-value
Gmac_1	FJ571610	F: CATCATCGTTTTGTCATTAGCC R: TCAGGCCAAAGATGTGTTTT	$(GTGA)_{12}$	10/86	148–192	62	0.641	0.674	0.54
Gmac_2	FJ571608	F: CACTTACGAACACCCATGC R: CACCGTATCTAGAGTTGATACTCAAAG	$(AC)_{15}N_{12}(CA)_6$	10/76	200–318	55	0.755	0.697	0.06
Gmac_3	FJ571611	F: TCAGTATGCCTGTCTTTCAACC R: GACAGACCACAATAATAAATGTCAGG	$(TGTC)_{22}$	11/82	198–238	60	0.77	0.756	0.20
Gmac_4	FJ571612	F: GACCCAGACACAAAGAACAGC R: TTTGTCCCCTCTTTTCCGTA	$(GAAA)_{14}$	8/81	210–242	64	0.740	0.703	0.28
Gmac_5	FJ571613	F: CTTCTTACCTGGCTGGCTCA R: TGGCCCCAATTAATTATCCA	$(CACTCACA)_9$	7/84	188–236	60	0.389	0.440	0.59
Gmac_6	FJ571614	F: GAACGTGGGATGGGTTTATG R: GAGGACGAGGACTCTGACCT	$(TG)_5cc(TG)_{28}c(GT)_4$	7/73	210–228	59	0.389	0.410	0.37
Gmac_7	FJ571607	F: CAAAAGGCAGACCAATCAGG R: TTGTTGAGATAGGCCGAGGT	$(AGAC)_4N_{26}$ $(AGAC)_4$ $(AGGT)_4$	2/73	146–154	60	0.387	0.301	0.06
Gmac_8	FJ571609	F: CAGGAAGGAAAGTTGGACGA R: AACATGAATCAAGCGGGAAG	$(GAGT)_{10}$	2/87	190–194	62	0.0984	0.103	1
Gmac_9	FJ571616	F: CTCAATCACCCGCTCCTC R: ATCCCGATTCCTTCTGAGGT	$(AG)_4cagac(AG)_{11}$	5/81	150–166	63	0.531	0.580	0.22
Gmac_10	FJ571615	F: TTGGAGAAAGTGAGCAATGG R: CTTTCAGCCCTCCACCTCAT	$(ACAG)_5a(ACAG)32$	28/67	184–272	59	0.935	0.910	0.15

P-value corresponds to Hardy–Weinberg Exact Test.
*The number of successfully genotyped individuals is specified for each locus.

each of $(GACA)_4$, $(CACG)_4$ (mixture 1); 5 μM each of $(GACA)_4$, $(CATC)_4$ (mixture 2); 2 μM each of $(AAAC)_6$, $(ACTG)_6$, $(GACA)_6$, $(GATG)_6$, $(ACAG)_6$ (mixture 3); and 2 μM each of $(AATC)_6$, $(ACTC)_6$, $(ACCT)_6$, $(GTAT)_6$, $(AAAG)_6$, (mixture 4). Enriched fragments were captured using streptavidin-coated magnetic beads (Dynal, Invitrogen), ligated into vectors (Qiagen PCR Cloning Kit), transformed into New England Biolabs 5-alpha competent *Escherichia coli* and plated on Invitrogen imMedia™ Amp Blue media. Two-hundred and eighty-eight positive clones were polymerase chain reaction (PCR) amplified using M13 primers under standard PCR conditions. PCR products were screened in 1% agarose gels and 150 suitably sized inserts (>500 bp) were sent for sequencing to Macrogen USA. A total of 138 sequences were aligned and edited using Sequencher 4.5 and searched for microsatellite repeats using the open source Simple Sequence Repeat Identification Tool (http://www.gramene.org/db/markers/ssrtool). Primer pairs were designed using Primer 3 software (Rozen & Skaletsky 2000) for 46 candidate loci, 10 of which proved useful when tested with 89 samples from a single location in Nahuel Huapi Lake, Argentina (Table 1).

DNA was extracted from 89 tissue samples following Elphinstone *et al*. (2003). PCR mixture contained 20–100 ng DNA, 20 mM Tris–HCl, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 0.1 % Triton X-100, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 U Tsg DNA polymerase (BioBasic D0081) and 0.1–0.2 μM of each primer. The cycling conditions used in Eppendorf thermocyclers were 95 °C for 5 min, followed by 30 cycles of 95 °C for 45 s, primer-specific annealing temperature for 60 s, 72 °C for 60 s, and a final extension at 72 °C for 10 min.

Genotypes were examined with MICRO-CHECKER (van Oosterhout *et al*. 2004) and no evidence of null alleles or large allele drop out was detected. Observed and expected heterozygosities were calculated using GENEPOP 4.0 (Raymond & Rousset 1995). No evidence of genotypic linkage disequilibrium between any paired loci (all *P*-values > 0.05) or deviations from Hardy–Weinberg (Table 1) was detected.

The present microsatellite markers are being used to assess population structure and connectivity among natural populations in South America.

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