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PERMANENT GENETIC RESOURCES

Isolation and characterization of 13 microsatellite loci for *Percichthys trucha* (Percichthyidae)

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Abstract

Thirteen polymorphic microsatellite loci are described for the South American freshwater fish *Percichthys trucha*. Number of alleles per locus ranged from two to 21 and observed heterozygosities ranged from 0.304 to 0.915 in a sample of 47 individuals from four different sampling locations.

Keywords: dinucleotide, microsatellite, *Percichthys trucha*, tetranucleotide

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Percichthys trucha is a member of the south temperate perch family Percichthyidae and is endemic to southern South America. It is distributed from ~33°S in the north (Tunuyán River, Maipo River), and east of the Andes to the southern tip of continental Patagonia (Santa Cruz River, 50°S).

Percichthys exhibits considerable morphological variation throughout its range, prompting some authors (Ringuelet *et al*. 1967; Arratia 1982; López-Arbarello 2004) to argue for the existence of up to five species. Recently, however, molecular evidence based on mtDNA control region and nuclear (gonadotropin growth releasing hormone 3 intron 2, GnRH3-2) sequence polymorphism (Ruzzante *et al*. 2006) indicates that there are only two species of *Percichthys*, the widely distributed *P. trucha*, and a Chilean species with a more restricted distribution (Maipo to Bio Bio), *P. melanops* (Arratia 1982; Dyer 2000). Phenotypic polymorphism within populations of *P. trucha* (Ruzzante *et al*. 1998, 2003) tends to parallel some of the variation previously used to distinguish species, suggesting that locally varying ecological conditions can produce morphological variants.

Here we report on the development of a suite of microsatellite loci for *P. trucha* intended for their use in studies on connectivity and population structure and on the nature of the phenotypic polymorphism exhibited by this species. Microsatellite loci were isolated using DNA of a single fish from Lake Argentino. To provide a large amount (> 5.0 μg) of high quality DNA, we extracted from blood tissue using a standard phenol:chloroform:isoamyl alcohol technique (Sambrook *et al*. 1989). A microsatellite-enriched library was constructed by following Glenn & Schable (2005). Genomic DNA was digested with *Rsa*1 (New England Biolabs) and ligated to Glenn & Schable's (2005) SuperSNX forward linker. Two oligonucleotide probe mixtures were created with biotinylated probes and used to enrich for microsatellite-containing fragments. Each mix contained five different oligonucleotide probes at 2 μM each: mix1) $(CATC)_4$, $(GACA)_4$, $(GATA)_4$, $(AG)_9$, $(AC)_9$ and mix2) $(AAAG)_7$, $(GGAT)_5$, $(GTAT)_5$, $(GATA)_7$, $(GACA)_7$. Hybridization and capture on streptavidin coated magnetic beads followed Glenn and Schable except stringency washes were done at 50 °C. The enriched libraries were amplified by polymerase chain reaction (PCR) using the SuperSNX-forward linker as the primer, ligated into vectors (QIAGEN PCR Cloning Kit), transformed into competent *Escherichia coli* (New England Biolabs) and plated on Invitrogen imMedia Amp Blue media. Positive clones (n = 456) were picked with a sterile toothpick to 0.8 mL LB broth and grown for 16 h at 37 °C with 250 rpm rotary shake. DNA inserts were PCR-amplified by directly placing 1 μL of the overnight cell culture in a 24 μL PCR cocktail containing 20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, 0.1% Triton X-100, 0.05 mM each dNTP, 0.08 μM M13 forward (–20) primer, 0.08 μM M13 reverse primer, 0.5 U *Taq* polymerase. One microlitre of each PCR product was visualized using standard gel electrophoresis in 0.8%

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Table 1 Characteristics and primer sequences for 13 microsatellite loci from *Percichthys trucha*

Locus	GenBank Accession no.	Primer sequence (5′–3′)	Repeat	No. of alleles (No. of samples)	Allele range	T_a	H_E	H_o
Ptru-2*	EU085432	F: ACGCCCCCTACAGGTGTCTC R: ACGGACCGCAGGTCAGTTTA	$(CTGT)_8$	7 (45)	122–138	55	0.609	0.600
Ptru-3	EU085433	F: AGCCCCCAGGTAGAGAGCAC R: CACGCATGCTTTCTGTCTGACT	$(GT)_{28}$	15 (45)	137–187	60	0.847	0.778
Ptru-11†	EU085434	F: CAAACCTCCACCCTCCTTCA R: TTAGCCTGCTGCCACAACAA	$(CATC)_7$	7 (47)	243–267	50	0.755	0.617
Ptru-13	EU085435	F: CATCCTGATTCACGCACCAA R: CAGCACACAGCATGAGGCTAA	$(AC)_{37}$	18 (46)	118–184	55	0.832	0.826
Ptru-16	EU085436	F: AGAACTCGTCTCGGACTGC R: CAAATTTAAGAGAAAGAAAAGTCTG	$(ACAG)_6(CAGG)_5$	4 (47)	125–161	55	0.536	0.532
Ptru-17	EU085437	F: CGGCCATTAGGTGGTTGTTT R: GAAAGTTTGGAGAGGGAAGGACA	$(TATG)_6$	4 (46)	112–128	60	0.424	0.413
Ptru-21	EU085438	F: CGTGGAGGAGGAGAGAGCTG R: ATAGAGAAGCCATGCCAGGA	$(CA)_{22}$	10 (47)	126–148	60	0.821	0.787
Ptru-24	EU085439	F: GTCCCTTTGGCAGTGAAAT R: CCTGCCAGCCAAAAATTCAT	$(GT)_{25}$	13 (47)	100–136	60	0.823	0.808
Ptru-28	EU085440	F: CGCTGTGGCATGTCTGTAGC R: GGTCTCCCTACTCCCGACCT	$(GATA)_4GAAA(GACA)_5$	5 (46)	133–149	60	0.402	0.304
Ptru-30	EU085441	F: TTTTACATTTCTGCTCTTACATCTGA R: TGTGTGAGAGGACTTGATGG	$(AGAT)_{18}$	21(47)	129–337	50	0.897	0.915
Ptru-33	EU085442	F: AGATACACTGGATGTAGGTTGGA R: CTGGGGCAGAGTGCAATGTT	$(ACAG)_5(ATAG)_3$	2 (46)	141–145	55	0.340	0.391
Ptru-37	EU085443	F: GGCTACATCCTGCGTTTTGC R: TCCTTTAGAAGCCATCTCAACCAG	$(GTCT)_6t(TGTC)_4$	3 (47)	113–125	55	0.514	0.489
Ptru-38	EU085444	F: TTTGTGAGCAATGACTAACGAAAGA R: TGCTCTAATTTCATTGTTCCTTT	$(TCTA)_4g(ATCT)_5$	6 (47)	146–166	50	0.729	0.702

Repeat, repetitive sequence of the original clone; (No. of samples), number of individuals successfully genotyped; T_a, annealing temperature of the PCR; H_E, expected heterozygosity; H_O, observed heterozygosity.*Ptru2 has a 1-bp shift, see text. †Ptru11 amplifies as a trinucleotide, see text.

agarose gels, stained with GelGreen and imaged on a Darkreader transilluminator. Images were captured with a Canon Powershot A640 camera. One hundred forty-three PCR products of appropriate size (> 500 bp) and quality (single intense band) were sequenced using M13-forward and M13-reverse primers with BigDye version 3 on ABI 3730xl DNA analysers in the Genome Quebec Innovation Centre at McGill University, Canada. Sequences were edited and aligned using SEQUENCHER 4.5 (http://www.genecodes.com/) and examined for microsatellite repeats using PERFECT MICROSATELLITE REPEAT FINDER (http://sgdp.iop.kcl.ac.uk/nikammar/repeatfinder.html). Primers were designed for 27 loci using PRIMER 3 (Rozen & Skaletsky 2000).

Samples from four populations in Argentina — Lake San Martin (N = 17), Rio Chalia (N = 8), Rio Chico (N = 16) and Lake Viedma (N = 6) — were used for primer testing. DNA was extracted from blood or fin tissue using a silica-binding method modified from Elphinstone *et al*. (2003). Individuals were genotyped in 5.0 μL PCR volumes containing 20–100 ηg DNA, 20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, 0.1% Triton X-100, 0.05 mM each dNTP, 0.1–0.2 μM each primer, 0.25 U DNA polymerase. Forward primers were 5′-labelled with either IR700 or IR800 dye. Typical PCR conditions were 94 °C for 3 min, 25 cycles of 94 °C for 30 s, locus-specific T_a (Table 1) for 30 s, 72 °C for 30 s, followed by a 72 °C, 3 min finishing step. PCR products were imaged on LICOR 4200 DNA sequencers. Images were scored by eye using a size standard constructed from pUC18-derived PCR fragments. Microsatellite data were appraised using EXCEL MICROSATELLITE TOOLKIT (http://animalgenomics.ucd.i.e./sdepark/ms-toolkit/), and MICROCHECKER (van Oosterhout *et al*. 2004). Heterozygosity data, Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were calculated using GENETIX 4.02 (Belkhir *et al*. 2004).

We tested 27 loci and report the results of 13 useful microsatellites. Of the remaining 14 loci, six amplified well but were monomorphic among 20 individuals representing four widely separated source populations, and eight were irresolvable or failed to produce a PCR product. No evidence of null alleles was detected at any locus. The number of alleles ranged from two to 21, expected heterozygosities

ranged from 0.402 to 0.897 and observed heterozygosities ranged from 0.304 to 0.915. HWE and LD were calculated for San Martin ($N = 17$) and Rio Chico ($n = 16$) populations; other populations were omitted from this calculation due to small sample sizes. No loci deviated from HWE and no loci were in LD following Bonferroni correction ($k = 156$, $P > 0.0003$). Locus Ptru2 was noted to have a 1-bp shift which may make accurate scoring of a larger data set difficult. Alleles at this locus included 122 bp, 123 bp, 127 bp, 130 bp, 134 bp, 135 bp, and 138 bp. Locus Ptru11 was expected (based on the clone sequence) to be a tetranucleotide repeat with alleles ~114 bp. All individuals ($n = 47$) had a single band at this size, but the same primers additionally amplified a polymorphic trinucleotide locus of 243–267 bp (Table 1). These loci are being used to examine population genetics from *P. trucha* from throughout the species' range.

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References

Arratia G (1982) A review of freshwater percoids from South America. *Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft*, **540**, 1–52.

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (2004) GENETIX 4.02. *Logiciel sous Windows pour la Genetique des Populations*. Laboratoire Genome, Populations, Interactions, CNRS UPR 9060, Universite de Montpellier II, Montpellier, France.

Dyer B (2000) Systematic review and biogeography of the freshwater fishes of Chile. *Estudios Oceanológicos*, Chile, **19**, 77–98.

Elphinstone MS, Hinten GN, Anderson MJ, Nock CJ (2003) An inexpensive and high-throughput procedure to extract and purify total genomic DNA for population studies. *Molecular Ecology Notes*, **3**, 317–320.

Glenn TC, Schable NA (2005) Isolating microsatellite DNA loci. *Methods in Enzymology*, **395**, 202–222.

López-Arbarello A (2004) Taxonomy of the genus *Percichthys* (Perciformes: Percichthyidae). *Ichthyological Exploration of Freshwaters*, **15**, 331–350.

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535–538.

Ringuelet RA, Aramburu RH, Alonso de Aramburu A (1967) *Los Peces Argentinos de Agua Dulce*. Gobernación: Comisión de Investigación Científica, Provincia de Buenos Aires, Argentina.

Rozen S, Skaletsky H (2000) PRIMER 3 on the WWW for general users and for biologist programmers. In: *Bioinformatics Methods and Protocols: Methods in Molecular Biology* (eds Krawetz S, Misener S), pp. 365–386. Humana Press, Totowa, New Jersey.

Ruzzante DE, Walde SJ, Cussac VE, Macchi PJ, Alonso MF (1998) Trophic polymorphism, habitat and diet segregation in *Percichthys trucha* (Pisces: Percichthyidae) in the Andes. *Biological Journal of the Linnean Society*, **65**, 191–214.

Ruzzante DE, Walde SJ, Cussac VE, Macchi PJ, Alonso MF, Battini M (2003) Resource polymorphism in a Patagonian fish *Percichthys trucha* (Percichthyidae): phenotypic evidence for interlake pattern variation. *Biological Journal of the Linnean Society*, **78**, 497–515.

Ruzzante DE, Walde SJ, Cussac VE *et al*. (2006) Phylogeography of the Percichthyidae in Patagonia: roles of orogeny, glaciation, and volcanism. *Molecular Ecology*, **15**, 2949–2968.

Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: A Laboratory Manual Cold Spring*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.