Isolation and characterization of seven microsatellite loci in *Chioglossa lusitanica* (Urodela: Salamandridae)

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**Abstract**

Seven polymorphic microsatellite markers were isolated and characterized for golden-striped salamander, *Chioglossa lusitanica* (Bocage 1864), a salamandrid endemic to the northwestern part of the Iberian Peninsula, from genomic libraries enriched for (GATA)$_{8}$ (GACA)$_{8}$ (ATG)$_{9}$ and (CA)$_{16}$. These loci were screened in 37–51 individuals from two populations. The number of alleles per locus ranged from five to 19. Heterozygosity ranged from 0.241 to 1.0. The high level of polymorphism revealed by these loci will be extremely useful for the study of population structure and evolutionary history of this species.

**Keywords**: *Chioglossa lusitanica*, golden-striped salamander, microsatellites, polymorphism, Salamandridae

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The golden-striped salamander (*Chioglossa lusitanica*) is an endemic species restricted to the northwestern corner of the Iberian Peninsula. Recent studies based on allozyme and mitochondrial DNA loci indicated that genetic diversity was partitioned over two groups of populations, located north and south of the Mondego River valley, with the northern part of the distribution resulting from a postglacial range expansion from the south to the north (Alexandrino et al., 2000, 2002). The pattern of genetic variation observed across this river valley highlighted the existence of a contact zone between two lineages with limited introgression. Additionally, those studies suggested that other major rivers located in the northern part of the range appear to be at least partial barriers to dispersal because they are linked to the successive loss of genetic diversity along the route of postglacial recolonization. Although these studies were useful in describing the pattern of genetic variation across the species range, highly polymorphic markers are required to investigate in more detail the evolutionary history of this species, and in particular the spatial organization and interpatch dynamics of the species within the contact zone.

Three *C. lusitanica* genomic DNA libraries, enriched for di- (CA), tri- (ATG) and tetrancleotide motifs (GATA and GACA) were produced using a magnetic bead capture method, adapted from Carleton et al. (2002) and the Kocher Laboratory protocol (http://tilapia.unh.edu/WWWPages/carleton/protocol%20/pagesMicrosat%20Library). Genomic DNA, extracted from tail tissue of a single individual, was digested with *Sau*3AI and fragments in a size range of 500–1000 bp were isolated from 2% agarose gel using the Nucleopsin kit (BD Biosciences, Clonetech). Oligonucleotide adaptors (RBgl24, 5′-AGCACTCTCCAGCCTCTCACCGCA-3′, and RBgl12, 5′-GATCTGCGGTGA-3′) were ligated to the genomic DNA fragments using the enzyme T4 DNA ligase (Promega). The resulting DNA was hybridized to 5′—biotinylated probes, which consisted of a 3′-ATAAGATAT tail and microsatellite motifs (CA)$_{16}$ (GATA)$_{8}$ (GACA)$_{8}$ and (ATG)$_{9}$ at 68 °C, 64 °C and 65 °C, respectively. Hybridized fragments were captured with Streptavidin Magnesphere Paramagnetic Particles (1 mg, Promega). The enriched DNA was amplified by polymerase chain reaction (PCR), digested with *Sau*3AI to remove the adaptors, ligated into the plasmid PUC19 (MBI) and cloned in *Escherichia coli* strain pBluescript II KS. Positive clones were amplified by polymerase chain reaction using universal M13 primers.

Seventy-four, 49 and 114 inserts from tetra-, tri- and dinucleotide libraries, respectively, were sequenced using the ABI Prism BigDye Terminator Cycle sequencing protocol in an ABI prism 3100 capillary sequencer. Primers were designed for six of the 10 identified tetrancleotide loci,
four of the five identified trinucleotide loci and 14 of the 45 identified dinucleotide loci using oligo 6.8. Polymerase chain reaction (PCR) amplifications of microsatellite loci were done in 10 µL volumes in a GeneAmp 9700 thermal cycler (Perkin Elmer). Each reaction contained 1 µL 10× PeqLab Buffer B, 0.7–0.55 µL 25 mM MgCl₂, 0.2 µL 10 mM dNTP mix, 0.07 µL PeqLab Taq-Polymerase (Peq Laboratory) and approximately 25 ng of genomic DNA. The PCR conditions were as follows: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation for 30 s at 94 °C, annealing for 20 s at 50 °C to 62 °C, extension for 15 s at 72 °C, and a final extension at 72 °C for 1 min. PCR products were visualized in 2% agarose gels stained with ethidium bromide. Twenty-one loci (four tetra-, three tri- and 13 dinucleotide) were successfully amplified within the expected size range and fragments were separated on an automated sequencer (ABI 3100, Perkin Elmer) using an internal size standard (Rox — genescan 350, Applied Biosystems), and analysed using GENESCAN software (Applied Biosystems). However, one tri- and 12 dinucleotides did not show interpretable patterns and were excluded from further analysis. Here we report the results obtained for the remaining seven loci (Table 1). We characterized 37–51 individuals from two C. lusitanica populations located in the Center of Portugal (Linharg de Pala and Lousã Fiscal). Allele size ranges, number of alleles, observed and expected heterozygosity and P-values for Hardy–Weinberg equilibrium test are reported in Table 2. Observed and expected heterozygosities were calculated using the GENETIX software (version 4.04, Belkhir et al. 1996–2002). Tests for Hardy–Weinberg and linkage disequilibria were performed using GENEPop (version 1.2, Raymond & Rousset 1995). The number of alleles per locus ranged from five to 19 (two to 16 in L. Pala and three to 14 in L. Fiscal, with a mean of 7.57 in L. Pala and 7.71 in L. Fiscal). The observed heterozygosity ranged from 0.241 to 1.0 (µ = 0.725) in L. Pala and from 0.529 to 0.880 (µ = 0.742) in L. Fiscal. Locus Cl5 showed a significant deviation from
Hardy–Weinberg expectations ($P < 0.05$) in the population of L. Fiscal. Linkage disequilibrium was not detected, with exception between the loci Cl6 and Cl17 in L. Pala ($P < 0.05$).

The high levels of polymorphism observed at these markers supports their utility for genetic studies in C. lusitanica, especially directed toward a finer-scaled understanding of gene flow and dispersal dynamics within and between groups of populations.

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References


