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PRIMER NOTE

Isolation and characterization of nine microsatellite loci in *Podarcis bocagei* (Squamata: Lacertidae)

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Abstract

Nine dinucleotide microsatellite loci were developed through an enrichment protocol for Bocage's wall lizard, *Podarcis bocagei* Seoane 1884, a lacertid endemic to the Iberian Peninsula. Nineteen primer pairs were designed and tested. From these, nine loci yielded satisfactory results and were screened on 15–19 individuals. These loci revealed a high level of polymorphism (8–15 alleles) and heterozygosity (0.611–0.947) and will certainly be useful in the study of population structure and evolutionary history of this species.

Keywords: Lacertidae, microsatellites, *Podarcis*, polymorphism, wall lizards

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Podarcis is a lacertid genus comprising at least 15 species, which is widely distributed across Europe and North Africa, including some Mediterranean and Atlantic islands. In the Iberian Peninsula, these lizards have been extensively studied using morphological (Harris & Sá-Sousa 2001), mitochondrial DNA (Harris & Sá-Sousa 2002) and protein electrophoretic (Pinho *et al*. 2003) data. Although these studies were useful in differentiating evolutionary entities, pointing out the need for a taxonomical revision, the applied markers gave little or no information on differentiation within each of the described forms. Neutral molecular markers with higher variability levels are therefore required to study in more detail the evolutionary history of each species, especially in determining population structure, gene flow, glacial refugia and potentially important conservation areas.

To this end we have developed a set of microsatellite markers in *Podarcis bocagei*, an endemic species from northwest Iberia, with the expectation that in the future these markers could be applied in other species of this genus.

Genomic DNA, extracted from tail tissue of a single individual, was digested with *Mbo*I. Fragments with the desired size (300–750 bp) were isolated and submitted to an enrichment protocol for dinucleotide microsatellite loci (GT and AC motifs) following Armour *et al*. (1994). A partial genomic library containing sequences enriched for these particular repeats was constructed by ligation of the selected fragments to pUC19 vector followed by transformation in *Escherichia coli* competent cells. Colonies were transferred to Hybond-N+ nylon membranes (Amersham) by colony-lift and hybridized with labelled probes containing the GT/CA and AG/TC tandem arrays. Labelling of probes and detection of positive clones were performed using ECL direct nucleic acid labelling and detection system (Amersham-Pharmacia Biotech) based on enhanced chemiluminescence. Positive clones were polymerase chain reaction (PCR)-amplified using universal M13 primers and re-hybridized in order to exclude false positives. A total of 29 positive clones were sequenced using the ABI Prism BigDye Terminator Cycle sequencing protocol in an ABI Prism 310 automated sequencer (Applied Biosystems). Primers were designed by eye in the flanking regions of 19 microsatellite loci.

DNA was extracted from individuals of *P. bocagei* belonging to the same population, following standard procedures (Sambrook *et al*. 1989). PCR was carried out in 10 μL volumes, containing 1 μL of reaction buffer [166 mM $(NH_4)_2SO_4$, 670 mM Tris-HCl, 0.1% Tween-20, pH = 8.8; Ecogen], 1–1.5 mM $MgCl_2$, 5 μM each dNTP, 0.2 μM each primer, 0.5 U of *Ecotaq* DNA polymerase (Ecogen) and approximately 25 ng of genomic DNA. For the microsatellite *Pb47*, better results were accomplished adding 5% dimethyl sulfoxide in PCR reactions. PCR products were visualized in 2% agarose gels stained with ethidium bromide. Thirteen loci were

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Table 1 Characterization of nine microsatellite loci isolated from *Podarcis bocagei*

Locus	Accession no.	Repeat motif	Primer sequences (5′–3′)	T_a (°C)	Allele size (bp)	n	No. of alleles	H_O	H_E	H–W (P-value)
Pb10	AY545220	$(GT)_nGC(GT)_nGC(GT)_n(AG)_n$	F: AGTGGAATCGGCTGCAATAC R: ACCAGTCCCAGGAATTTAGG	56	178–204	18	15	0.889	0.864	0.123
Pb11	AY545221	$(TG)_n$	F: TTTCTGGGAGGAGAAGACAC R: CTGGAAGAACACAGCAGGAG	56	152–180	18	9	0.611	0.711	0.217
Pb20	AY545222	$(AC)_n$	F: ACGCAAAGTCTCTCCACA CC R: CTTTGGCAGCTTCTTGCTTC	57	124–155	18	10	0.889	0.843	0.914
Pb37	AY545223	$(CA)_n$	F: GAGAGTATACCAACCGTG R: CTAATGCTGGAACTATCC	54	129–158	18	11	0.778	0.858	0.153
Pb47	AY545224	$(GT)_n$	F: CTTGGTGGTTAACAATGTTGGC R: GTGAGCTAATACAACTCTCCAC	56	203–238	19	15	0.947	0.895	0.917
Pb50	AY545225	$(CA)_n$	F: GGATGTTTCAGCATGCTTGG R: AGACCTCACTGGGCCATTAC	54	113–135	18	12	0.833	0.877	0.063
Pb55	AY545226	$(TG)_n$	F: CCCATCCTAACCCTTACCTTTG R: GCAGCTCCATCACTGGCCCTG	55	228–242	15	8	0.667	0.822	0.127
Pb66	AY545227	$(TG)_n$	F: GGACAGCTAGTCCCATGGCTTAC R: GGATTGCTGTCACCAGTCTCCCC	55	138–171	19	12	0.947	0.889	0.968
Pb73	AY545228	$(CA)_nCT(CA)_n$	F: GCCCATGTCACTTCAGGTAGAAGC R: GAAAACTAGGAGTTAGGGAGAAGG	58	146–178	18	11	0.889	0.892	0.017

T_a, annealing temperature; n, number of individuals analysed; H_O, observed heterozygosity; H_E, expected heterozygosity; H–W (P-value), Hardy–Weinberg probability.

successfully amplified within the expected size range and were run on 6% denaturing polyacrylamide gels and visualized by silver staining. Four of these did not show interpretable patterns and were excluded from further analyses. The results obtained for the remaining nine loci are shown in Table 1. Two microsatellite loci are compound (*Pb10* and *Pb73*) and the other seven are simple dinucleotides.

In six out of the nine microsatellites typed (*Pb10*, *Pb20*, *Pb37*, *Pb47*, *Pb55*, *Pb66*), both even and odd-sized alleles were detected, suggesting the occurrence of insertions or deletions in the flanking regions.

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 3.3, Raymond & Rousset 1995). The observed allelic richness ranged from eight (*Pb55*) to 15 alleles (*Pb10* and *Pb47*), with a mean of 11.4, and observed heterozygosities ranged from 0.611 (*Pb11*) to 0.947 (*Pb47* and *Pb66*), averaging 0.827 across loci. Of the nine markers, one (*Pb73*) showed a deviation to Hardy–Weinberg's expectations ($P < 0.05$). It is common that the presence of null alleles is invoked to explain such deviations in microsatellite markers. However, in this marker, heterozygote deficiency, tested using GENEPOP option 1.1, was not significant ($P < 0.05$). An alternative hypothesis can be an insufficient sampling, especially if one considers the high number of alleles at this locus. Linkage disequilibrium between pairs of loci was not detected ($P < 0.05$).

The high levels of polymorphism observed at these markers contrast with the very low levels of variation described in mitochondrial DNA and morphology (Harris & Sá-Sousa 2001, 2002). Therefore, this set of loci will certainly be useful in uncovering the yet to be studied evolutionary history of *Podarcis bocagei*.

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