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PERMANENT GENETIC RESOURCES

Cross-species testing of 27 pre-existing microsatellites in *Podarcis gaigeae* and *Podarcis hispanica* (Squamata: Lacertidae)

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Abstract

We tested 27 microsatellite loci for cross-species amplification in the lacertids *Podarcis gaigeae* and *Podarcis hispanica*. We detected 11 and 15 polymorphic loci in the former and the latter species, respectively. In a larger sample of individuals from a single population of each species, the number of alleles ranged from five to 23 in 10 of the polymorphic loci in *P. gaigeae*, and between four and 13 in nine of polymorphic loci in *P. hispanica*. Two locus deviated from Hardy-Weinberg equilibrium in *P. hispanica*. Between 11 and 16 of the 27 loci also amplified successfully in three other *Podarcis species*.

Keywords: cross-species amplification, microsatellite, neutral molecular marker, *Podarcis*, population differentiation, Squamata

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The lacertid genus *Podarcis* is widely distributed across Europe and North Africa (Arnold & Ovenden 2002). Several species in the genus exhibit substantial population differentiation in morphology, including *Podarcis gaigeae* on the island of Skyros, Greece (A. Runemark and E. I. Svensson, unpublished data) and *Podarcis hispanica* in the Madrid area, Spain (M. Gabirot and J. Martín, unpublished data). This makes them suitable model systems for studying dispersal and gene flow between populations and investigating population level processes that promote divergence and eventually speciation. To study gene flow and population differentiation in *P. gaigeae* and *P. hispanica*, neutral molecular markers with high levels of variation such as microsatellites are required.

We tested 27 lacertid microsatellites isolated in *Podarcis muralis* (Nembrini & Oppliger 2003), *Lacerta vivipara* (Boudjemadi *et al*. 1999), *Podarcis bocagei* (Pinho *et al*. 2004) and *Podarcis erhardii* (Poulakakis *et al*. 2005a) for cross-species amplification in *P. gaigeae* and *P. hispanica*. We also tested primers on *Podarcis milensis* and *Podarcis taurica* which are closely related to *P. gaigeae* (Poulakakis *et al*. 2005b) as well as in *P. erhardii* for which five primers are already published (Poulakakis *et al*. 2005a).

Tail samples were collected and preserved in ethanol. DNA was extracted with an ammonium acetate extraction protocol (Sambrook *et al*. 1989) or with the DNeasy Tissue extraction kit (QIAGEN). Initially, the primers were tested in seven *P. gaigeae* individuals, four from a population on mainland Skyros and three individuals from a neighbouring islet, and in seven *P. hispanica* individuals from the Madrid area. The polymerase chain reaction (PCR) mix contained 4 pmol of each primer, 15 nM $MgCl_2$, 1.25 nM dNTP, 0.5 U Ampli*Taq* polymerase and 10 ng template in a 10-μL reaction. PCRs were carried out in a GeneAmp PCR system 9700 (Applied Biosystems) and the conditions were as follows: 94 °C for 2 min, then 35 cycles at 94 °C for 30 s, T_a for 30 s, 72 °C for 30 s followed by 72 °C for 10 min, where T_a is the locus specific annealing temperature (Table 1). The fluorescent-labelled PCR products were separated and alleles were detected in an ABI PRISM 3730 capillary sequencer (Applied Biosystems).

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Table 1 Source species, accession number, primer sequences, annealing temperatures and number of alleles for the 27 microsatellite loci in *Podarcis gaigeae* ($n = 7$), *P. hispanica* ($n = 6$), *P. taurica* ($n = 4$), *P. milensis* ($n = 10$) and *P. erhardii* ($n = 12$); numbers in brackets indicate the number of individuals successfully amplified, when deviating from the number initially tested

Locus	Source species	EMBL Accession no.	Primer sequences (5′–3′)	T_a	*P. gaigeae*	*P. hispanica*	*P. taurica*	*P. milensis*	*P. erhardii*
A7F	*Podarcis*	AY147824	F: HEX-TGCTTATGGGTGATGACTGG	60	1	2	3	4	3
A7R	*muralis*		R: AGAATTGCAGAGGTGGAAGG						
B3F	*P. muralis*	AY147825	F: HEX-CTGTCCTCTCACAGTTCACTCC	57	0	0	1	1	1
B3R			R: AAAGAGCTAAGAAGCGAAGACC						
B4F	*P. muralis*	AY147826	F: HEX-AATCTGCAATTCTGGGATGC	57	5 (76)	5	1 (3)	5	11 (11)
B4R			R: AGAAGCAGGGGATGCTACAG						
B6F	*P. muralis*	AY147829	F: FAM-CTGCTGCTTCAATCACACTC	57	16 (76)	0	5	7	12
B6R			R: GCCTTGCCTCTCCAGAAC						
B7F	*P. muralis*	AY147823	F: FAM-GGGGAAAGCTACTGGCTACAC	60	1	1	0	0	0
B7R			R: AGTCCAGGTGAATTTGCAGAG						
C24F	*P. muralis*	AY147827	F: FAM-AGAGTGGCTGGGGGAAAC	60	1	1	1	1	1
C24R			R: GTAAGTAAACGGGCGGCTTG						
C8F	*P. muralis*	AY147828	F: HEX-GACAATCCAATGTACAGAGCAAG	57	1	6	1	2	2
C8R			R: AACACACATGCACAAACCAC						
C9	*P. muralis*	AY147822	F: FAM-CATTGCTGGTTCTGGAGAAAG	57	17 (76)	12 (19)	6 (3)	9	1
C9			R: CCTGATGAAGGGAAGTGGTG						
D1F	*P. muralis*	AY147830	F: HEX-GAGTGCCCAAGACAGTTGTAT	57	3	0	0	2	2
D1R			R: GAGGTCTTGAATCTCCAGGTG						
Lv-3-19	*Lacerta*	AF100289	F: NED-CTGTTGCTATTTTGTATGCTTAC	55	16 (76)	0	2	9	11
Lv-3-19	*vivipara*		R: CCTGTGACTGTCCTCAGAGG						
Lv-4-72	*L. vivipara*	AF100290	F: FAM-CCCTACTTGAGTTGCCGTC	53	23 (76)	4 (19)	6	8	7
Lv-4-72			R: CTTTGCAGGTAACAGAGTAG						
Lv-4-α	*L. vivipara*	AF100291	F: HEX-CTGCAGGGAACAGAATTAACC	60	6 (76)	7 (19)	2 (2)	4 (9)	3 (6)
Lv-4-α			R: CTGCCCAGAAAGCATTTCC						
Lv4x	*L. vivipara*	AF100292	F: FAM-CTGAAACATGGATTAGAGGC	54	1	7 (19)	1	1	0
Lv4x			R: GCACTCCTTGCGTGGC						
Pb10	*Podarcis*	AY545220	F: FAM-AGTGGAATCGGCTGCAATAC	56	15 (76)	7 (19)	1 (1)	15	15 (11)
Pb10	*bocagei*		R: ACCAGTCCCAGGAATTTAGG						
Pb11	*P. bocagei*	AY165221	F: HEX-TTTCTGGGAGGAGAAGACAC	56	0	13 (19)	1	1	1
Pb11			R: CTGGAAGAACACAGCAGGAG						
Pb20F	*P. bocagei*	AY545222	F: FAM-ACGCAAAGTCTCTCCACACC	57	0	0	0	0	0
Pb20R			R: CTTTGGCAGCTTCTTGCTTC						
Pb37F	*P. bocagei*	AY545223	F: HEX-GAGAGTATACCAACCGTG	54	1	6	3	4	3
PB37R			R: CTAATGCTGGAACTATCC						
Pb47	*P. bocagei*	AY165224	F: FAM-CTTGGTGGTTAACAATGTGGC	56	0	13 (19)	0	0	0
Pb47			R: GTGAGCTAATACAACTCTCCAC						
Pb50	*P. bocage*	AY165225	F: HEX-GGATGTTTCAGCATGCTTGG	54	0	9 (19)	3	3	4
Pb50			R: AGACCTCACTGGGCCATTAC						
Pb55F	*P. bocagei*	AY545226	F: FAM-CCCATCCTAACCCTTACCTTTG	55	0	1	0	0	1
Pb55R			R: GCAGCTCCATCACTGGCCCTG						
Pb66F	*P. bocagei*	AY545227	F: HEX-GGACAGCTAGTCCCATGGCTTAC	55	1	1	3	2	1 (3)
Pb66R			R: GGATTGCTGTCACCAGTCTCCCC						
Pb73	*P. bocagei*	AY545228	F: HEX-GCCCATGTCACTTCAGGTAGAAGC	58	8 (76)	13 (19)	1	7	4 (4)
Pb73			R: GAAAACTAGGAGTTAGGGAGAAGG						
POD-1AF*	*Podarcis*	AY924398	F: FAM-TGAGAAGCACATCTGCACAC	58	0	3	1	1	4
POD-1AR*	*erhardii*		R: TGAACGCATAATGGCTGAAGG						
POD-1B*	*P. erhardii*	AY924398	F: NED-CCTTCAGCCATTATGCGTTCATC	55	5 (76)	8	1	10	6
POD-1B*			R: AGGATGGGGATAACCCCAGT						
POD-2*	*P. erhardii*	AY924399	F: FAM-GGCAATGTTCCTGCATGACG	58	17 (76)	0	7	7	10
POD-2*			R: TGGGACAAAAAGGCAGAACG						
POD-3F*	*P. erhardii*	AY924400	F: HEX-TTATCAGACGTTGGGGAAAG	58	0	0	2	1	3
POD-3R*			R: GCACTTCAACCCGAGGTCTG						
POD-8F*	*P. erhardii*	AY924401	F: FAM-CCTCTAACTATCTGTTGCTGCTG	49	0	0	0	0	0
POD-8R*			R: CACAAAGGGTATCGAAGGAGG						

*Locus isolated in *Podarcis erhardii* (Poulakakis *et al*. 2005a).

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Table 2 Basic population statistics for 10 polymorphic microsatellite loci in a *Podarcis gaigeae* population (76 individuals genotyped) and for nine loci in a *Podarcis hispanica* population (19 individuals genotyped)

Locus	*A*	H_O	H_E	HWP	Allele size range
P. gaigeae					
B4	5	0.428	0.506	0.514	134–138
B6	16	0.693	0.899	0.014	151–192
C9	17	0.727	0.798	0.235	128–175
Lv-3-19	16	0.796	0.912	0.195	125–169
Lv-4-72	23	0.89	0.919	0.578	102–180
Lv-4-α	6	0.417	0.452	0.973	105–124
Pb10	15	0.855	0.9	0.119	209–233
Pb73	8	0.646	0.793	0.585	125–148
POD-1B	5	0.356	0.452	0.513	144–155
POD-2	17	0.885	0.827	0.862	100–128
P. hispanica					
C9	12	0.706	0.912	0.004	135–175
Lv-4-72	4	0.769	0.75	0.501	110–218
Lv-4-α	7	0.611	0.802	0.024	100–135
Lv4x	7	0.533	0.771	0.019	105–160
Pb10	7	0.529	0.827	0.005	155–200
Pb11	13	0.778	0.879	0.103	142–180
Pb47	13	0.875	0.921	0.33	142–232
Pb50	9	0.688	0.74	0.272	100–216
Pb73	13	0.933	0.924	0.414	128–168

Shown are number of alleles (*A*), observed heterozygosity (H_O), expected heterozygosity (H_E), Hardy–Weinberg *P* value (HWP) and allele size range.

Of the 27 primer pairs tested in the seven test individuals of *P. gaigeae*, 11 were found to be potentially useful since they had polymorphic products, whereas 15 primer pairs seemed to be useful in *P. hispanica*. One locus (D1) that amplified in *P. gaigeae* was later discarded due to the product being very long (*c.* 640 bp), and six were not further tested for *P. hispanica* (Table 1). The remaining loci were either monomorphic, did not amplify or had nonspecific products (Table 1).

The 10 polymorphic and easily scored loci in *P. gaigeae* were further tested in 76 individuals from the mainland of Skyros, and the nine selected polymorphic loci in *P. hispanica* were tested in 19 individuals from the Madrid area (Table 2). At these loci, the number of alleles ranged between five and 23 in *P. gaigeae* and between four and 13 in *P. hispanica*. The expected heterozygosity ranged between 0.45 and 0.92, and between 0.74 and 0.92, for *P. gaigeae* and *P. hispanica*, respectively. No loci for *P. gaigeae*, but the loci Pb10 and C9 for *P. hispanica*, departed from Hardy-Weinberg equilibrium after Bonferroni correction (one loci departed for *P. gaigeae* and four for *P. hispanica* before correction) in tests conducted in FSTAT 2.9.3.2 (Table 2; Goudet 2001). This may indicate the presence of null alleles at Pb10 in *P. hispanica*.

Tests of linkage equilibrium between all pairs of loci were performed in Arlequin version 2.00 (Schneider *et al*. 2000). Two pairs of loci showed a significant deviation after Bonferroni correction for *P. gaigeae* (between Lv-319 and Pb10, and between Lv-472 and Pb10; adjusted nominal level $P = 0.0011$), whereas no loci deviated significantly after Bonferroni correction for *P. hispanica* (adjusted nominal level $P = 0.0014$).

All 27 loci were also tested for amplification in three other *Podarcis* species. The majority of primers successfully amplified polymorphic loci in *P. milensis* (16 loci) and *P. taurica* (11 loci; Table 1). Four of the five microsatellites that were isolated in *P. erhardii* (Poulakakis *et al*. 2005a) were also polymorphic in our sample of *P. erhardii*, as were 12 of the other 23 primers (Table 1). Thus, the identified loci seem to be a valuable resource for future research in those species, for example, for analyses of parentage and population differentiation. Our results also indicate that primers developed for particular *Podarcis* species are potentially applicable to other members of the genus.

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