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TECHNICAL NOTE

Isolation and characterization of microsatellite loci in the endangered lizard *Gallotia bravoana* and cross-species amplification in other Canarian *Gallotia*

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Abstract The *Gallotia bravoana* lizard, is an endangered species endemic to the Canary Island, entirely restricted to a very small area (~1 Ha) in La Gomera. Here we report the isolation and characterization of fifteen microsatellite loci following an enrichment protocol. Although five loci were monomorphic, an average of 2.1 alleles per locus and an average observed heterozygosity of 0.423 were found ($n = 33$) for the remaining loci. The loci were tested for their ability to cross amplify in all *Gallotia* species. These microsatellites will be used to manage a captive breeding programme for this endangered species.

Keywords Canarian *Gallotia* · Endangered lizard · *Gallotia bravoana* · Microsatellite

The genus *Gallotia* (Arnold 1973) is endemic to the Canary Islands. This genus comprises 7 species and 9 subspecies distributed around different islands. Particularly, the species *Gallotia bravoana* is restricted to a small area (Risco de La Mérica) situated on the western side of La Gomera island, and is clearly endangered, with censuses indicating a current population size of approximately 120 individuals. The extremely precarious state of this population and the need for continued action to prevent its extinction has led to the neccessity to isolate microsatellite markers for outlining a captive breeding plan.

Genomic DNA was extracted with the PureGene DNA Purification Kit (Gentra) using a fresh blood sample conserved in EDTA from a captive individual. The DNA was digested with *Sau*3AI and *Bam*HI (New England Biolabs) obtaining fragments between 200 and 1,100 base pairs. The digested DNA was adapter-ligated to the oligos S61 (5′-GGCCAGAGACCCCAAGCTTCG-3′) and S62 (5′ phosphorylated-GATCCGAAGCTTGGGGTCTCTGGCC-3′) (Refseth et al. 1997). The resulting DNA was hybridized with 3′ biotinylated oligonucleotides [$(AAAG)_6$, $(AAAT)_6$, $(AAGG)_6$ and $(GATA)_6$]. Microsatellite enrichment was completed using Streptavidine Magnetic Dynabeads (Dynal) according to Glenn and Schable (2005) with slight modifications. The enriched DNA was amplified by polymerase chain reaction (PCR) using the S61 primer and then cloned into the plasmid pGEM-T easy vector (Promega) using *Escherichia coli* strain JM109 (Promega). Screening of positive clones was performed by PCR amplification from white colonies using the S61 oligo and a mix of 1 μM of each repetitive probe. Forty-eight (25%) out of 192 screened clones contained potential microsatellite motives. Positive clones were PCR-amplified and sequenced with SP6 and T7 primers, using BigDye version 3.1 chemistry (Applied Biosystems) on an ABI PRISM 3130xl automatic sequencer (Applied Biosystems).

Of the 48 clones sequenced in both directions, 29 contained a microsatellite motif with adequate flanking regions, which were selected for primer design using Primer3 software (Rozen and Skaletsky 2000). Fifteen pairs of primers amplified a clear PCR-product and were selected for characterization. For these loci, the forward primer of each pair was labelled with a fluorescent dye (Table 1). PCR were carried out in 10 μl using 20 ng of genomic DNA, 1× buffer (Bioline; 16 mM $(NH_4)_2SO_4$, 67 mM Tris–HCl (pH 8.8) and 0.01% Tween 20), 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, and 0.4 U of Taq DNA polymerase (Bioline) with different primer concentration for loci (Table 1).

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Table 1 Primer sequences and descriptions of the fifteen microsatellite loci isolated from *Gallotia bravoana*

Locus (accession no.)	Primer sequence (5′–3′)	Label	Repeat array	T[a]	Primer concentration (μM)	Clon size (Size range)	N_A	H_O	H_E
Gbr03	TGCACAATGGACAGGAAAAG	VIC	$(AAAG)_7AG(AAAG)_4$	58	0.15	400	2	0.518	0.475
GU355904	GTCTGCTCTGAATAAAACTTAGTTGA					(357–381)			
Gbr04	CAAGAAGCAATGCTGTCCAA	6-FAM	$(GAAA)_4(GGAA)_4(GAAA)_5$	55	0.15	458	2	0.347	0.464
GU355905	CTGGTTTGTTTGCTTTCCTTG					(374–380)			
Gbr08	GCCATAGATGCACATGGAGA	NED	$(GA)_9(GAAA)_{12}$	55	0.05	333	2	0.423	0.382
GU355906	AGCTCCTTCCCTGTGAGATG					(135–151)			
Gbr09	CGTTAAGACTGCGTTCTTGG	NED	$(AAGG)_4(AAAGCAGG)_3$	58	0.3	420	2	0.182	0.169
GU355907	CATCTGGCTGCTTGCACTAA					(380–398)			
Gbr10	AACCTGGACGTAGGGCAAC	VIC	$(GAAA)_{11}$	62	0.05	568	2	0.633	0.463
GU355908	GGGCATATTCTGAAGAGTGAAA					(211–222)			
Gbr11	TCACTTTGGGCAAGTCACTG	PET	$(GATA)_{12}$	60	1	406	2	0.259	0.372
GU355909	CACCTGCTGAATTTCCGTCT					(374–380)			
Gbr12	TCCCCAAAAAGCTCAAAAAG	6-FAM	$(GAAA)_{10}$	55	0.1	169	2	0.679	0.508
GU355910	TGAAACCTAGGGTTGGCATA					(142–162)			
Gbr15	CACCAGGGATATTGGATTGG	PET	$(GAAA)_{12}$	55	0.1	509	2	0.414	0.492
GU355911	CTCTCAGTGTGGAGGCACAG					(171–205)			
Gbr16	AGAGCAATGACAATGCCAGA	VIC	$(GGAA)_5$	55	0.05	536	1		
GU355912	AACCTGGCAAGGCTGTGTAA					(298)			
Gbr17	CAGAAAAGAGTCAGAAAGAGAAAGC	6-FAM	$(GAAA)_9$	60	0.2	361	2	0.187*	0.476
GU355913	GATGTGGAGCACCTTCTTCTG					(317–487)			
Gbr19	GCCAAGAAGATTTCCCAATG	6-FAM	$(GAAA)_6(GGAA)_2(GAAA)_4$	62	0.1	502	3	0.586	0.565
GU355914	TCCAAAAACAGCTGGAGACC					(190–200)			
Gbr20	CAAGAATCATAAGGACATGGAAA	VIC	$(GAAA)_{12}$	55	0.05	478	1		
GU355915	CAGCAGGCAAGAAATAGTGC					(188)			
Gbr22	GATTGTTAGCCGCTTTGAGG	6-FAM	$(GGAA)_{11}$	55	0.25	588	1		
GU355916	TCCTTTGTTGCCTGTAACTCC					(455)			
Gbr24	AAACTCAGGCAGGTGGAGTG	NED	$(GT)_8(GA)_8AA(GA)_7$	55	0.1	535	1		
GU355917	AAGAATTGCAGGGCTTTTCA					(203)			
Gbr28	TGTAACTCAATGGGGCTTGC	6-FAM	$(GAAA)_{10}(GGAA)_4$	55	0.1	313	1		
GU355918	CACAGGCCATCAGTACAACG					(234)			

N_A Number of alleles, H_O observed heterozygosity, H_E expected heterozygosity

* Significant HWE deviation ($P < 0.05$, after sequential Bonferroni correction)

Table 2 Cross-amplification in other Canarian *Gallotia*

	Gallotia atlantica			*Gallotia caesaris*		*Gallotia galloti*				*Gallotia intermedia*	*Gallotia simonyi*	*Gallotia stehlini*
	atlantica $n = 5$	*laurae* $n = 5$	*mahoratae* $n = 10$	*caesaris* $n = 5$	*gomerae* $n = 5$	*eisentrauti* $n = 5$	*galloti* $n = 5$	*insulanagae* $n = 5$	*palmae* $n = 5$	$n = 4$	$n = 4$	$n = 5$
Gbr03	o (2; 374–378)	o (3; 370–378)	o (2; 370–374)	o (2; 354–358)	o (2; 350–354)	o (2; 354–358)	o (4; 332–362)	o (3; 336–358)	o (1; 362)	o (2; 358–362)	n	o (4; 354–372)
Gbr04	o (6; 344–392)	o (4; 344–366)	o (6; 344–386)	o (6; 354–376)	o (7; 364–384)	o (5; 340–376)	o (5; 356–372)	o (2; 368–372)	o (5; 364–422)	o (2; 374–384)	n	o (2; 360–364)
Gbr08	o (5; 138–170)	o (5; 134–182)	o (7; 136–164)	o (3; 106–118)	o (5; 112–152)	o (7; 104–118)	o (7; 94–124)	o (3; 110–114)	o (5; 98–120)	o (4; 132–140)	o (4; 132–148)	o (5; 94–106)
Gbr09	o (8; 398–474)	o (7; 378–474)	o (10; 374–464)	o (2; 338–344)	o (1; 344)	o (6; 326–356)	o (5; 326–356)	o (4; 334–360)	o (5; 338–392)	o (3; 352–380)	n	o (1; 330)
Gbr10	o (5; 205–239)	o (3; 221–235)	o (5; 205–237)	n	n	o (3; 192–204)	o (2; 190–198)	o (2; 213–224)	o (2; 208–214)	o (3; 200–226)	o (2; 201–215)	n
Gbr11	o (3; 280–312)	o (3; 300–340)	o (3; 280–288)	o (2; 266–270)	o (2; 270–292)	o (2; 252–262)	o (3; 262–296)	o (1; 262)	o (1; 262)	o (3; 248–268)	n	n
Gbr12	o (7; 148–202)	o (6; 152–194)	o (11; 164–234)	o (6; 122–174)	o (7; 118–156)	o (8; 116–160)	o (5; 138–156)	o (2; 122–142)	o (3; 130–152)	o (2; 146–150)	o (3; 134–146)	n
Gbr15	o (1; 168)	o (2; 156–168)	o (2; 156–168)	o (3; 274–292)	o (8; 208–250)	o (6; 176–210)	o (5; 184–206)	o (2; 190–206)	o (5; 190–208)	o (4; 180–200)	o (2; 198–206)	o (5; 174–206)
Gbr16	o (1; 294)	o (1; 294)	o (3; 270–294)	n	o (2; 294–298)	o (4; 290–302)	o (3; 290–298)	o (1; 298)	o (2; 290–302)	o (1; 300)	o (2; 298–302)	o (3; 292–300)
Gbr17	o (5; 321–357)	o (6; 321–347)	o (6; 321–361)	o (4; 309–341)	o (4; 309–325)	o (7; 297–325)	o (5; 309–325)	o (2; 305–317)	o (4; 305–329)	o (1; 309)	o (5; 293–325)	o (5; 305–355)
Gbr19	o (1; 172)	o (1; 170)	o (2; 156–170)	o (1; 178)	n	o (7; 178–200)	o (6; 174–204)	o (2; 196–200)	o (5; 182–204)	o (2; 168–180)	o (2; 172–194)	o (2; 156–172)
Gbr20	n	n	n	o (4; 170–190)	o (4; 162–182)	o (6; 158–196)	o (6; 154–198)	n	o (5; 170–196)	o (2; 162–196)	o (2; 174–190)	o (2; 162–166)
Gbr22	o (5; 462–492)	o (5; 458–496)	o (4; 458–526)	o (4; 426–444)	o (4; 428–458)	o (7; 440–520)	o (5; 436–464)	o (2; 454–462)	o (4; 452–468)	o (1; 460)	n	o (5; 474–508)
Gbr24	o (2; 180–192)	o (1; 188)	o (10; 176–240)	o (5; 200–222)	o (7; 190–236)	o (3; 200–210)	o (5; 198–210)	o (2; 198–202)	o (3; 198–222)	o (1; 208)	o (3; 198–202)	o (6; 186–216)
Gbr28	o (3; 210–228)	o (6; 220–262)	o (6; 206–242)	o (2; 180–184)	o (4; 180–198)	o (6; 180–218)	o (6; 180–214)	o (1; 188)	o (3; 180–202)	o (1; 210)	o (3; 202–216)	o (7; 168–214)

n Indicates no amplification of any product; o indicates amplification of a product of comparable size to that amplified from *Gallotia bravoana*. Values in parenthesis indicate the number of alleles detected and size ranges, respectively

Samples were amplified as follows: 94°C for 3 min, followed by 30 cycles at 94°C for 30 s, 55–62°C for 30 s and 72°C for 30 s with a final extension at 72°C for 10 min. Fluorescently labelled fragments were run on an ABI PRISM 3130xl genetic analyzer (Applied Biosystems) with G5 matrix and GeneScan-500 (LIZ) as size standard.

A total of 33 individuals from La Mérica population were used for obtaining polymorphism data (Table 1). Five loci were monomorphic (Gbr16, Gbr20, Gbr22, Gbr24 and Gbr28), but when these loci were used in other *Gallotia* species, different size alleles were obtained (see below). The remaining loci presented more than one allele and were selected for Hardy–Weinberg equilibrium (HWE) and Linkage Disequilibrium (LD) analyses. All calculations were performed using the program ARLEQUIN version 3.11 (Excoffier et al. 2005). Significant deviation from HWE due to heterozygote deficiency was only detected for Gbr17, possibly due to the presence of null alleles. LD was observed for Gbr04-Gbr08, Gbr08-Gbr10, Gbr08-Gbr12, Gbr11-Gbr17 and Gbr12-Gbr15 ($P < 0.05$, after sequential Bonferroni correction (Rice 1989)).

Cross-species priming was assessed by typing at least four individuals of each *Gallotia* species and subspecies. Five loci (Gbr08, Gbr15, Gbr17, Gbr24 and Gbr28) were successfully amplified in all species (1–10 alleles). The remaining loci gave variable results (Table 2).

Despite the low variability uncovered at these loci in *Gallotia bravoana*, these markers will provide a useful tool for future research concerning management of this endangered species and for outlining a captive breeding plan. Furthermore, these loci will be useful for high-resolution genetic studies of any *Gallotia* species.

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