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Characterisation of nine European wall lizard (*Podarcis muralis*) microsatellite loci of utility across sub-species

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Abstract Human-assisted dispersal of the European wall lizard (*Podarcis muralis*) has created conservation concerns due to extensive introgression between normally allopatric and genetically distinct lineages. We characterised nine microsatellite loci that amplified in four mtDNA clades belonging to two main evolutionary lineages (broadly belonging to *P. m. brogniardii* and *P. m. nigriventris*) known to hybridise in England and Germany. All loci were autosomal and displayed 5–28 alleles. These loci will prove useful for population genetic and parentage studies in hybrid zones.

Keywords Hybridisation · Introgression · Paternity · *Podarcis muralis*

The European wall lizard (*Podarcis muralis*) consists of multiple lineages distributed across Europe. Human introductions of this species have created conservation concerns due to extensive hybridisation between genetically distinct and naturally allopatric lineages (Schulte et al. 2012). Since hybridisation is asymmetrical with respect to sex (Schulte et al. 2012), its study requires nuclear markers with cross-lineage amplification. We isolated microsatellite markers in *P. muralis* and characterised them in four mtDNA clades; two from France ('French' lineage, broadly corresponding to *P. m. brogniardii*) and two from Italy ('Italian' lineage, broadly corresponding to *P. m. nigriventris*) (Michaelides et al. 2013).

Genomic DNA was extracted from tail-tips using the ammonium acetate precipitation method (Nicholls et al. 2000). We created a microsatellite-enriched library using an Italian female from Ventnor, England (ID:497; Tuscan mtDNA clade) (following Mills et al. 2013). Twenty-two 454 microsatellite sequences were selected that were a consensus of multiple sequences. Their uniqueness was confirmed using BLASTN v.2.2.4 (Altschul et al. 1997). Primer sets were designed using Primer3v.0.4.0 and initially tested in 24 French animals caught from an introduced population at Cheyne Weare, Dorset, UK.

Fifteen markers were discarded that were monomorphic, amplified non-specific products or failed to amplify in multiple individuals. Nine markers amplified reproducibly across all 24 individuals, and were used to genotype adults from the French and Italian lineages being used in an experiment investigating behavioural causes of hybridisation. These consisted of 90 Italian individuals (40 males and 50 females from populations WS; DL; SH; VB; VT; PO) and 70 French individuals (40 males and 30 females from populations CWS; CWN; WE; BU; population details in Michaelides et al. 2013).

The nine loci were amplified in three multiplexes containing three primer sets each (Table 1). 1 µl of primer mix at 0.21 µM was added to 1 µl of QIAGEN Multiplex PCR Master Mix, and then added to ~10–20 ng of air-dried

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Table 1 Primer sequences and genetic diversity estimates of nine microsatellite markers in *P. muralis*

Locus	EMBL accession number	Primer sequences (5′–3′); F: forward; R: reverse	Repeat motif	MS	Sub–spp	N	Expected allele size based on Pmn497 (bp)	Observed allele size range (bp)	a	H_O/H_E
Pmur028	LM993853	F: [6–FAM] TTGCTTCTGAATACGCCTAGC	TATC	2	PmI	89	287	260–328	13	0.67/0.78
		R: AGTGTATTGCGACTGTCAATGG			PmF	70		260–314	7	0.70/0.63
Pmur038	LM993854	F: [HEX] CAATGTGCAGTGTTGGGTTG	TATC	2	PmI	86	210	211–548	18	0.70/0.92*
		R: ATGTGAGCGACTCCTGGATG			PmF	70		234–422	18	0.81/0.91*
Pmur103	LM993855	F: [6–FAM] CCAGGTCTTGTGATCGAGTG	GATA	3	PmI	87	350	329–480	18	0.60/0.90*
		R: CCCGACCCAAACTAGTGC			PmF	70		325–430	13	0.76/0.84
Pmur109	LM993856	F: [HEX] AGGAGCCCAGCAGCTGAA	GTA	3	PmI	89	309	297–328	11	0.72/0.87
		R: TTTACATAGACCTGCGGGTATGG			PmF	70		306–337	10	0.60/0.72
Pmur150	LM993857	F: [6–FAM] GTCAGCTTTGCAGCACCTTAG	CA	1	PmI	90	193	176–204	11	0.58/0.88
		R: GCGATTAGAGAAGGCGTTTG			PmF	69		176–204	10	0.68/0.72
Pmur164	LM993858	F: [6–FAM] ATCGATGAATGAAGGGCAGT	GATA	2	PmI	90	216	185–222	10	0.63/0.84
		R: CCAGGCATTGTCAAACTATCTG			PmF	70		189–209	5	0.04/0.14
Pmur168	LM993859	F: [HEX] GGTCCGGCTTCAAAGAATAAG	TTTC	1	PmI	90	244	225–257	10	0.73/0.82
		R: CAGAGGACTCGCTCAAGGAC			PmF	70		225–257	10	0.69/0.85*
Pmur275	LM993860	F: [6–FAM] CTTAAAATTAATGCTGCTATTGTATC	TATC	1	PmI	90	245	219–551	28	0.76/0.92*
		R: ATAGGTAGAAAATTTATAAACCCTTGG			PmF	70		219–497	23	0.84/0.91*
Pmur356	LM993861	F: [6–FAM] GATCTTCAGATGAAGGGTAGTTAGAT	GTTA	3	PmI	90	159	137–171	7	0.53/0.73
		R: ATGAAGACAAACAGGCTTGG			PmF	70		150–167	6	0.46/0.58

All primer sets were amplified with an annealing temperature of 57 °C. MS: multiplex set; N: number of individuals genotyped; Pmn497: the female individual (Italian lineage and Tuscan mtDNA clade, taxonomically *Podarcis muralis nigriventris*) sequenced and used to design the primer sets; a: number of alleles observed; PmI: Italian lineage animals; PmF: French lineage animals; H_O/H_E: observed/expected heterozygosity

* Deviation from Hardy–Weinberg equilibrium ($p < 0.001$)

DNA. PCRs were amplified in an MJ Research model PTC DNA Engine Tetrad thermal cycler set at 95 °C for the first 15 min and then in 35 repeated cycles of 94 °C at 30 s, 57 °C for 90 s, and 72 °C for 60 s. Samples were diluted 1:358 times before being separated on an ABI3730 48-well capillary DNA Analyser (Applied Biosystems, California, USA) using a 500-bp ROX size-standard marker. Alleles were categorised using GENEMAPPER. Expected and observed heterozygosities were calculated with CERVUS v.3.0.3, and deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium were assessed using GENEPOP.

Heterozygotes were observed in both sexes at all loci, indicating all loci were autosomal. Deviation from HWE was observed in both lineages for *Pmur038* and *Pmur275,* in the French for *Pmur168*, and in the Italian for *Pmur103* ($p < 0.001$ in each). Deviation from HWE may be due to allelic dropout, since *Pmur038, Pmur103* and *Pmur275* amplify large products. Other causes of deviation could be population sub-structure due to the multiple native origins of these populations, including founder effects, or null alleles. No locus pair displayed linkage disequilibrium in either lineage.

These markers will be useful for detecting the direction and extent of population-level hybridisation in secondary contact zones, particularly where introductions threaten native populations, and for paternity analysis in experiments aimed at elucidating the factors predicting hybridisation. The microsatellites may also prove valuable in closely related *Podarcis* species, several of which are of conservation concern [seven out of 21 *Podarcis* species are considered endangered, critically endangered, or vulnerable (IUCN 1996)].

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