Isolation and characterization of microsatellite loci from the invasive delicate skink (*Lampropholis delicata*), with cross-amplification in other Australian *Eugongylus* group species

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Abstract The delicate skink (*Lampropholis delicata*), native to eastern Australia, is one of the most successful invasive reptiles in the Pacific region, with established populations in New Zealand, the Hawaiian Islands, and Lord Howe Island. Here we isolate and characterize 12 polymorphic microsatellite loci for *L. delicata*. Genetic variation was assessed in individuals from Sydney and also from the eastern Victoria and Tasmanian region. The number of alleles per locus ranged from 2 to 23, with high levels of heterozygosity. All microsatellite loci successfully amplified in individuals from the three invasive populations. Cross-amplification of these loci was assessed in 14 species from other *Eugongylus* group skink genera. These microsatellite markers will be used to investigate the invasion dynamics of *L. delicata*.

Keywords Hawaii · Invasive species · Lord Howe Island · New Zealand · Microsatellites · Plague skink · Rainbow skink

The delicate skink (*Lampropholis delicata*), known as the rainbow or plague skink in its introduced range, is one of the most successful invasive reptiles in the Pacific region (Lever 2003). It is native to eastern Australia (Wilson and Swan 2008), but is an invasive species in New Zealand, the Hawaiian Islands, and Lord Howe Island (Baker 1979; Lever 2003; Peace 2004; Hutchinson et al. 2005). *Lampropholis delicata* occurs in extremely high population densities across its introduced range and has the potential to have adverse impacts on the native herpetofauna of New Zealand and Lord Howe Island (Lever 2003; Peace 2004; Hutchinson et al. 2005). We developed 12 microsatellite markers to investigate the invasion dynamics of *L. delicata*.

Total genomic DNA was extracted from the liver of a *L. delicata*, collected from the grounds of the University of Sydney, Australia, using a Qiagen DNeasy Blood and Tissue Kit as per the manufacturer's instructions (Qiagen Pty Ltd, Melbourne, Australia). Microsatellite DNA library construction, enrichment and screening were completed as per the protocol contained in Jones et al. (2002). Genomic DNA was partially restricted with a cocktail of seven blunt-end cutting enzymes (*Rsa*I, *Hae*III, *Bsr* B1, *Pvu*II, *Stu*I, *Sca*I, *Eco* RV). Fragments in the size range of 300–750 bp were adapted and subjected to magnetic bead capture (CPG Inc., Lincoln Park, New Jersey, USA), using biotinylated capture molecules.

Libraries were prepared in parallel using Biotin-(AAC)₁₂, Biotin-(ATG)₁₂, Biotin-(CATC)₈, and Biotin-(TAGA)₈ as capture molecules in a protocol provided by the manufacturer. Captured molecules were amplified and restricted with *Hind*III to remove the adapters. The resulting fragments were ligated into the *Hind*III site of pUC19. Recombinant molecules were electroporated into *Escherichia coli* DH5 α (ElectoMaxTM, Invitrogen). One hundred and thirty-two recombinant clones, identified by blue-white

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Table 1 Characterization of 12 microsatellite loci in the delicate skink (*Lampropholis delicata*) from the Sydney region and eastern Victoria and Tasmania

Locus	GenBank accession no.	Primer sequence (5'–3')	F 5' label	Repeat motif in cloned allele	Population/region	Size range	n	N _A	H _O	H _E
Lamp1	FJ854556	F: TGGAGGTCAGAGTGGATTG R: TTGGGAAATGAATGTGCC	VIC	(AAT) ₃ (AAC) ₁ (AAT) ₁ (AAC) ₇ (AAT) ₃	Sydney VIC/TAS	223–263 226–235	19 24	7 3	0.632 0.250	0.684 0.288
Lamp2	FJ854557	F: GTGCTCATAGCCTCTTTCATC R: AAGCCAGAAATCCTCAGTGG	PET	(CA) ₁₀ (AC) ₂	Sydney VIC/TAS	280–312 282–316	19 24	12 11	0.789 0.667	0.868 0.793
Lamp3	FJ854558	F: TAGGAGAGTTGGAGAGGAAAGTG R: TAAAGCAATCGGTGAAGGAC	PET	(AAC) ₆	Sydney VIC/TAS	268–302 265–302	18 25	10 8	0.722 0.720	0.823 0.758
Lamp4	FJ854559	F: ATAGCAATGGCAATGATGGTA R: GCAGTTTCCTACACAGGTAAGG	VIC	(AAC) ₁₁	Sydney VIC/TAS	262–367 274–289	19 18	9 5	0.842 0.167	0.762 0.414
Lamp5	FJ854560	F: CTTCAAGTTCACAAATCTCTGTG R: GCTTGGCAGTTGCTATTATC	NED	(TTG) ₇ (TCC) ₃ TCTT(TCC) ₆ (TTA) ₈	Sydney VIC/TAS	166–244 175–265	17 24	16 23	0.824 0.875	0.920 0.959
Lamp6	FJ854561	F: CACTCTTGGAACTTCACACAC R: GCATCTGGCTATTTAGGAGGTT	PET	(TAA) ₄ (CAA) ₇	Sydney VIC/TAS	235–261 241–252	19 25	8 3	0.789 0.080	0.798 0.114
Lamp7	FJ854562	F: GCAGAAAGTGATGCCATACTATC R: TCCTCGTCAGTGGTCAATC	VIC	(CAT) ₇	Sydney VIC/TAS	263–323 266–323	19 25	16 10	0.895 0.720	0.907 0.832
Lamp8	FJ854563	F: AGAGGAGCTTTGGTGTGTATC R: CCTGGGATTACTGGTACATAAG	6-FAM	(ATC) ₉	Sydney VIC/TAS	141–172 147–157	19 24	8 4	0.737 0.333	0.722 0.345
Lamp9	FJ854564	F: AGAAGGTGTGTGTGAGTCG R: AGGTTTGAGACCCAGGTTTC	VIC	(CAT) ₂ (CGT) ₂ (CAT) ₁ (CGT) ₁ (CAT) ₁₂	Sydney VIC/TAS	143–209 170–200	19 25	15 6	0.789 0.640	0.903 0.694
Lamp10	FJ854565	F: GAGCCAGATTATCCACACG R: TTAGCCTTAGCATCTCTCCC	NED	(ATG) ₇	Sydney VIC/TAS	158–209 161–164	19 25	13 2	0.737 0.240	0.898 0.216
Lamp12	FJ854566	F: AACTTCTGTGAAATGTCATTC R: AGCGTTGAGTACGATACCATAG	6-FAM	(GATG) ₅ (GATA) ₉	Sydney VIC/TAS	214–266 233–331	17 22	11 17	0.765 0.818	0.869 0.839
Lamp13	FJ854567	F: CTATCCGTGTGCATCACTCAG R: CTTAACCACCTTGTGCGTTGTG	6-FAM	(CATC) ₇ (TATC) ₂	Sydney VIC/TAS	149–189 157–215	19 25	10 10	0.579 0.440	0.837 0.570

n Number of individuals tested, N_A number of alleles, H_O observed heterozygosity, H_E expected heterozygosity

selection, were selected at random for sequencing, and enrichment levels were expressed as the fraction of sequences that contained a microsatellite. Sequences were obtained on an ABI 377 automated sequencer (Applied Biosystems Inc.), using ABI Prism Taq dye terminator cycle sequencing methodology.

Primer pairs were designed from 78 of the 99 microsatellite containing sequences using DESIGNERPCR version 1.03 (Research Genetics Inc). Seven samples from across the native range of *L. delicata* were selected to screen the markers for polymorphism. Twenty-four of the best loci were amplified in 10 µl volumes containing the following components: 1× Biolase Buffer (from 10× stock supplied by the manufacturer), 2 mM MgCl₂, 0.2 mM each dNTP, 0.6 µM each primer, 0.025 U/µl Biolase Taq polymerase (BioExpress, UK), and 0.2 ng/µl of template DNA. Samples were amplified in a Perkin-Elmer-Cetus thermal cycler by an initial 3 min denaturation at 94°C, followed by 35 cycles of denaturation (94°C, 40 s), annealing (55°C, 40 s), and extension (72°C, 30 s), with final extension time of 4 min at 72°C. The PCR products were run on agarose gels to test for amplification and polymorphism. Seventeen microsatellite loci were polymorphic in the seven *L. delicata* samples.

The polymorphic loci were then characterized in 19 individuals from the Sydney region. Since there is substantial phylogeographic structure present within *L. delicata* (5–8% mitochondrial sequence divergence among the major genetic lineages; our unpublished data), we also assessed the level of genetic variation in these loci in 25 individuals from eastern Victoria and Tasmania (a region that represents a single phylogeographic lineage within *L. delicata*; our unpublished data). PCR was performed with fluorescently labelled forward primers (see Table 1) on a PTC-225 DNA Engine Tetrad™ cycler (MJ Research), and multiplexed PCR products were run on an Applied Biosystems ABI3730 DNA analyser with LIZ-500 size standard. The output was analysed with GENEMAPPER 3.7 (Applied Biosciences). Five loci were excluded from further analysis due to difficulties with amplification, repeat structure or scoring. Observed and expected heterozygosity, deviation from Hardy–Weinberg equilibrium (HWE), and likelihood of linkage disequilibrium (LD) were calculated in GenAEx 6.2 (Peakall and Smouse 2006) and GENEPOP 4.0 (Rousset 2008). The loci exhibited high levels of polymorphism with the number of alleles ranging from 7 to 16 in the Sydney population and 2–23 in the Victoria-Tasmania region (Table 1). The observed heterozygosity was high (Sydney: 0.579–0.895, Victoria-Tasmania: 0.080–0.875), and the level of genetic variation was generally greater in the Sydney population than in the Victoria-Tasmania region (Table 1). There was no significant deviation from HWE in any of the loci following Bonferroni adjustment for multiple tests, except for

Table 2 Cross-amplification of the 12 microsatellite loci in 14 Australian *Eugongylus* group skink species

Species	Lamp loci											
	1	2	3	4	5	6	7	8	9	10	12	13
<i>Acritoscincus duperreyi</i>	○	●	○	○	○	●	○	○	○	◇	◇	○
<i>Anepischtos maccayi</i>	●	●	●	●	○	○	●	●	●	●	○	○
<i>Carlia rubrigularis</i>	○	○	◇	○	○	○	●	●	●	○	○	●
<i>Cryptoblepharus carnabyi</i>	○	○	◇	○	○	○	○	○	●	●	○	○
<i>Erotoscincus graciloides</i>	○	○	●	○	○	○	○	●	○	●	○	○
<i>Harrisoniascincus zia</i>	○	○	○	○	○	○	○	○	●	●	○	○
<i>Lampropholis guichenoti</i>	○	○	○	○	○	○	○	●	●	●	○	○
<i>Lygisaurus foliorum</i>	○	○	◇	○	○	○	●	●	○	●	●	○
<i>Menetia greyii</i>	○	○	○	●	○	○	●	●	●	●	○	○
<i>Morethia boulengeri</i>	○	○	○	●	○	○	○	○	○	◇	○	○
<i>Niveoscincus coventryi</i>	○	○	●	●	○	○	○	●	●	●	○	○
<i>Niveoscincus metallicus</i>	●	○	○	●	○	●	●	●	●	●	○	○
<i>Proablepharus kinghorni</i>	○	●	○	○	○	○	○	○	●	●	○	○
<i>Pseudemoia pagenstecheri</i>	○	○	●	○	○	○	○	●	●	○	◇	○

A single individual per species was tested

● Indicates amplification of a product of comparable size to that amplified from *L. delicata*. ○ Indicates no amplification of any products. ◇ Indicates amplification of a product of a substantially different length from that amplified from *L. delicata*

Lamp4 in the Victoria-Tasmania region due to difficulties in amplifying this locus in all samples (Table 1). No significant LD was detected between any of the locus pairs.

All 12 microsatellite loci were successfully amplified in each of the three invasive populations (New Zealand, Hawaiian Islands, Lord Howe Island; two individuals screened per population). These primers will be used to compare the level of genetic variation between source and introduced populations, and investigate the invasion dynamics of the *L. delicata*. Cross-species amplification of the loci was assessed in *Lampropholis guichenoti* and 13 species from other Australian genera within the *Eugongylus* Group lineage of skinks (Table 2). Amplification success was high, with 1–8 loci successfully amplifying in each species (Table 2), indicating the potential for these loci to be useful in other skink species within the *Eugongylus* lineage.

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