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TECHNICAL NOTE

Isolation and characterization of microsatellite loci from *Iguana delicatissima* (*Reptilia: Iguanidae*), new perspectives for investigation of hybridization events with *Iguana iguana*

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Abstract A (GA)*n* and (GT)*n* microsatellite-enriched library was constructed and 25 nuclear simple sequence repeat (SSR) loci were characterized in the Lesser Antillean Iguana (*Iguana delicatissima*). All SSR loci were found to be polymorphic after screening for diversity in different cultivars, and a cross-taxa amplification tests showed the potential transferability of most SSR markers in *Iguana iguana*. First to be published for *I. delicatissima*, this new SSR resource will be a powerful tool for intra-specific genetic studies and for investigation of hybridization events with *Iguana iguana*.

Keywords *Iguana delicatissima* · *Iguana iguana* · Microsatellite

Lesser Antillean Iguana (*Iguana delicatissima*) is endemic to the Lesser Antilles, inhabiting this archipelago from Martinique to Anguilla. In Guadeloupean Archipelago hybridization detected on a morphological basis between the common or green Iguana (*Iguana iguana*) and (*I. delicatissima*) have lead to the elimination of the endemic species from four islands (Basse-Terre, Grande-Terre, Terre-de-Haut and Terre-de-Bas des Saintes) (Breuil 2002). Because this situation will become worst, Iguana Specialist Group upgraded *I. delicatissima* from vulnerable to endangered (Breuil et al. 2010). In order to know what impacts could have this invasion, it is important to monitor genetic evolution in different populations of *I. delicatissima*. Several studies have shown that microsatellites are good tools to monitor genetic evolution of populations (Roy et al. 1994) but, to date, no microsatellite was described in the *Iguana* genus. In this study, we characterized 25 microsatellites in *I. delicatissima* and we tested them by cross-species amplifications on a population of *I. iguana.*

We extracted *I. delicatissima* genomic DNA from blood samples of an individual captured in Chancel, Martinique, using the Generation DNA purification Capture Plate Kit (QIAGEN). A microsatellite-enriched library was constructed using the protocol described by Billote et al. (1999). We restricted DNA with RsaI (Invitrogen). The fragments were ligated to two double-strand adapters (Rsa 21 and Rsa 25). Then we hybridized fragments to 50 μM of each biotin-labeled oligo [$(CA)_n$ and $(GT)_n$]. We bounded the hybrid complexes to streptavidin-coated magnetic beads (Streptavidin MagneSphere® Paramagnetic Particles, ref Z5481) and washed them. We cloned the enriched DNA obtained into pGEM-T (Promega, Ref A3600) and transformed them into XL1-Blue supercompetent cells (Stratagene, Ref 200236). We have let them incubate for one night at 37 °C. We have then randomly amplified 150 clones with Rsa 21 primer. We transferred PCR products on Hybond-N + nylon membranes (Amersham/GE Healthcare Biosciences, Pittsburgh,

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Table 1 Characteristics of the developed microsatellite loci for *I. delicatissima*

Sequence (5′–3′)					*I. delicatissima*			*I. iguana*		Usefulness
	Repeat Motif	Clone size (bp)	Forward	Reverse	No. of allele	Ho	HE		Allele size range	
IgdL1	(GT)10	190–194	CGCTCAGTGAGGAATGGAA	TCAGGTGATCAGGACTGCAA	2	0.04	0.04	3	188–204	H
IgdL2	(CA)9	207–212	GGCAGATTTGCAGGTTTCAC	TGTTCACTTGAGCAG^TGCTT	2	0.07	0	1	209	H
IgdL3	(GT)10	249–255	CAGAAGCATCAGCCAGCTCT	CCAGGGCATCTTTGA^CAG	1	0.15	0	3	249–259	–
IgdL4	(GT)10	168	GGCTGCTCACACCTTCTCAT	TTCTTGAACATCCCCTTTAGC	2	0	0	1	162	H
IgdL5	(GT)10	176–192	ATAGCTGAGGTGAGCCCTTG	TCAGAGCAAGGCAATGGTAA	1	0.08	0	2	192–194	H
IgdL6	(GT)10	250	TCGTTGTCGGTAACTCACCA	GAACAGGAGCGGCTGTACTC	1	0	0	1	254	H
IgdL7	(GT)10	186	CAGCACAACCAACATGGCTA	TGACACATCTGCAAGAATACCC	1	0	0	NS	–	–
IgdL8	(CA)9	226	GACTACATGCGATGTATCCCTTT	GGGTCTTAAATAAACTCCACTGGTT	1	0	0	1	220	H
IgdL9	(CA)9	190	TCAAACCGCCTCTGATTACC	TTTCTGCTTAAAGATGCCTATTG	1	0	0	NS	–	–
IgdL10	(GT)16	168	TGTATAGCCCAGGGTTGGTT	CTCATGAGGCTACTGGCACA	1	0	0	NS	–	–
IgdL11	(TG)19	270–280	GCTTCAGTGCATAGTTTCCTGTT	TCATATATGCACTTCCCTCTCC	2	0.5	0.41	NS	–	–
IgdL12	(TG)12	178–194	GAGCCCACCAATTAATGGAA	TCTCTGTTGCAATCCAGCAA	3	0.57	0.39	2	178–184	–
IgdL13	(TG)13	250	AGCTCTTCAGAGTGCTGAACC	TGGCCCTTGAGAAAGTAACTG	1	0	0	2	236–238	H
IgdL14	(CA)16	191–193	CCTACAGATCATATCTTGTGCATTC	TGGGAGAGATTCATCGGAAC	2	0.48	0.46	1	187	H
IgdL15	(CT)9	206	AATCCTTGCTGATCCACTGC	GCTGGCATGAGGATGAATG	1	0	0	1	204	H
IgdL16	(GT)13	182	GGTCTATTTCACAGGCAGCTAA	GTTCAGCGATGCAGGTGTTA	1	0	0	1	180	H
IgdL17	(GT)7	230	AACCATAATGTCCATCCACACA	TGGAAGTTCAGGTGAATCCAT	2	0.39	0.24	1	240	H
IgdL18	(GT)7	199	GGGAATAGCAGCAGCTTACAA	TTCTGCCAGGCT^TAGGAA	1	0	0	2	191–193	H
IgdL19	(GT)18	232–236	CCTGGGTACCACTCAAGCTC	GCTGCTGCAGAAGTCATAGC	2	0.51	0.48	2	238–240	H
IgdL20	(TC)13 + (CA)20	202–214	CCTGTGCTAGAACTTGCCATT	GATGAAAAGTGCCTTCCTAGACA	3	0.51	0.6	2	180–182	H
IgdL21	(AC)18	186–190	CCAGCTGTGTCCAAATGTCT	AAGTGACTGCCCAAGGTGAC	3	0.41	0.37	1	166	H
IgdL22	(TG)8	208–210	GGAATGAGCCAATTGAGGAA	GGAGGAAAGACTGAGCAACAA	2	0.05	0.05	1	206	H
IgdL23	(CT)4 + (GT)14	303	TTGGACAGAAGTTTTATGGCATT	GCCATGGGCAGTTAGTAAGG	1	0	0	2	313–317	H
IgdL24	(GT)14	188–200	CCTGTGGCAGCCAATTCTAT	GGGCAGGGAGGAATAGAGTAA	3	0.54*	0.14	1	172	H
IgdL25	(GT)6	186	GACTCTGGGATGGGAGTGAA	CCAATGAGTAGCCCACAGGT	1	0	0	2	196–198	H

Locus designation, repeat motif, temperature of annealing, forward and reverse primer sequences for the 25 developed microsatellite loci, allele range and number of allele for both *Iguana* species, heterozygosity value are indicated for *I. delicatissima*

H hybridization

* Significant value ($p < 0.001$)

PA) and hybridized them at 56 °C with [γ32P] dATP 5′ end-labeled $(CA)_{15}$ and $(GT)_{15}$ probes. We sequenced 80 clones and designed pairs of primers using Primer 3 (Rozen and Skaletsky 2000) for 40 of them which contained pure, compound and interrupted microsatellites.

To test for good amplification and usefulness of each primer of microsatellite loci, we performed PCR amplifications with 29 individuals from one population from Chancel as well as on 5 individuals of the closely related species *I. iguana* from one population from Gosier, Guadeloupe (Grande-Terre). We extracted DNA from samples of blood with the same kit as previously. We performed all PCR reactions using the QIAGEN® Multiplex PCR Kit, in a 10 µL final volume containing 1 µL of genomic DNA (ranging from 25 to 60 ng), 10 µM of each primer, 5 µL of QIAGEN's multiplex master mix (containing HotStar*Taq* DNA polymerase, $MgCl_2$, dNTPs and PCR buffers), 1 µL of QIAGEN's Q-solution, and 2 µL of RNase-free water. This study only investigated primers which have good amplification for an annealing temperature of 56 °C. We used the same PCR profile for all microsatellites with 35 cycles at 95 °C for 40 s, an annealing temperature at 56 °C for 90 s, and 90 s of extension at 72 °C. Before the first cycle, we performed a prolonged denaturation step of 15 min at 95 °C and the last cycle was followed by one final step of 30 min at 60 °C.

We have then diluted by 20, the PCR products with a corresponding volume of ultrapure Milli-Q water. We mixed 1.2 µL of these diluted products with a solution containing 20 µL of GENESCAN 500 ROX (Applied Biosystems) and 1 mL of deionized Formamide and we analyzed these products on an ABI 3130XL Genetic Analyser (Applied Biosystem) following the manufacturer's protocols. Then, we scored alleles using the GENEMAPPER version 3.7 program from Applied Biosystems. Finally, 25 microsatellites were amplified and developed (Table 1).

The 29 individuals from Chancel have an average total number of 1.64 (±0.15) alleles per locus for these 25 microsatellites. Number of alleles ranged from 1 to 3 (Table 1) with observed heterozygosities for polymorphic loci ranging from 0.04 (IgdL1) to 0.5724 (IgdL12) (Table 1). In total, 13 loci are monomorphic in *I. delicatissima* and but have different alleles in *I. iguana*. One locus (IgdL24) departed from Hardy–Weinberg equilibrium ($p < 0.001$) revealing a deficit in heterozygosity, probably due to the presence of null allele. After a sequential Bonferroni correction, no linkage disequilibrium was found using GENEPOP in these microsatellites.

Cross-species amplification tests on *I. iguana* showed amplification for 21 on 25 microsatellites showing an average total number of 1.57 (±0.14) alleles per locus for these 25 microsatellites. Number of alleles ranged from 1 to 3 (Table 1). Among the studied microsatellite loci, 19 seem to have no allele in common between *I. iguana* and *I. delicatissima* and therefore could be diagnostic and serve as a powerful tool to study hybridization.

To date, our microsatellites were the first to be published in *I. delicatissima* and *I. iguana.* These data could enable to survey the evolution of populations of *I. delicatissima,* to distinguish the two species and potentially to investigate hybridization phenomena.

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