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TECHNICAL NOTE

Characterization of 22 microsatellite marker loci in the Madagascar rousette (*Rousettus madagascariensis*)

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Abstract Twenty-two nuclear microsatellite loci were isolated from a genomic DNA library derived from Madagascar's *Rousettus madagascariensis*. Marker characteristics were determined from a single population (37 individuals) from Fort Dauphin (southeastern Madagascar). Sixteen of the 22 loci were within Hardy–Weinberg expectations. These loci are highly informative with polymorphic information content values ranging between 0.757 and 0.916. These loci will provide valuable information for the study of population genetics and gene flow within this species of bats. Due to the dramatic reduction and alteration of their habitat, data generated utilizing this marker suite will potentially provide additional information for the effective long-term management of this near-threatened bat species.

Keywords Genetic markers · *Rousettus madagascariensis* · Pteropodidae · Madagascar · Microsatellites

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Rousettus madagascariensis is the smallest of the three endemic frugivorous bats of Madagascar (Bergmans 1990). The ability of this genus to echolocate and hover (Kulzer 1956; Novock 1958) enables it to forage within forests where it is likely to be an important nocturnal pollinator. This is among the few endemic species of bats covering a large distribution in lowland areas of the island, however, few roost sites are known. Roost sites were located in both shallow and deep caves (Juste et al. 1999) with infrequent human disturbance. Colony sizes ranged from 300 to several thousand. According to the IUCN Red List (IUCN 1999), this species is listed as one of lower risk, near threatened (LR/nt). Twenty-two nuclear microsatellite loci were isolated from genomic DNA derived from *R. madagascariensis* to estimate population genetic parameters for a population in southeastern Madagascar (Fort-Dauphin).

Genomic DNA was isolated from tissue from *R. madagascariensis* sampled in Kianjavato Classified Forest, Madagascar. Procedures for construction of the genomic DNA library, identification of plasmids containing (GT)n inserts, plasmid preparation, and sequencing were carried out as generally described by Hillis et al. (1996). Isolated genomic DNA was digested using Sau3A restriction enzyme. The digested DNA was sized using Clontec© chromaspin columns to remove fragments under 400 bp. Sized DNA was ligated to primers forming blunt-ended DNA pieces. Ligated DNA was enriched using PCR based on Moraga-Amador et al. (2001), a modification of Kandpal et al. (1994). Enriched DNA was denatured and a biotinilated probe annealed to the DNA. The biotinilated DNA was captured using Vector Laboratory (Burlingame, CA) Vectrex Avidin D© and non-annealed DNA was washed away. After releasing captured DNA from the Vectrex Avidin D©, a second round of PCR enrichment was performed. An Invitrogen (Carlsbad, CA) TOPO A©

Table 1 Primer sequences with fluorescent dye labels, optimized annealing temperature, locus characteristics, and GenBank accession numbers of 22 *Rousettus madagascariensis*—specific microsatellite loci

Locus	Primer sequence (5′ to 3′)	Temp (°C)	Repeat motif	PIC	Size range	GenBank accession no.
66HDZ20	F: ^{HEX}TCC CCC TCT CTC CGA CTC R: CCT GCG TTT TCT GTT CTG G	62	$(GT)_{20}$	0.843	194–216	EU883996
66HDZ80	F: ^{HEX}GGC TAT CAA GGG AAG GGT G R: GGG AAG ACA GCA ATA ACA ACC	58	$(GT)_{14}$	0.866	172–216	EU883997
66HDZ82	F: ^{FAM}TCT CTC AAT GCC CGT CCT C R: CAC TTT CAA ATG CCC TGC TC	60	$(CA)_{26}$	0.898	237–273	EU883998
66HDZ91	F: ^{FAM}GAC TTT GCT TCT TTC AGA TAC CA R: GAG GA GAC AGA GTT TTC CCT TTA	62	$(CA)_{21}$	0.901	125–173	EU883999
66HDZ95	F: ^{HEX}CAG GAT TTA TTA CCC CGC C R: GCT GTG ACC ACT CTG CCC	60	$(CA)_{20}$	0.873	245–275	EU884000
66HDZ105	F: ^{HEX}GAA AGA CCA GAA TCC TCA AAA TG R: TGA TAA CAA GTG GAA AAA AAT AAA GTA G	58	$(CA)_{13}$	0.863	190–220	EU884001
66HDZ106	F: ^{HEX}TTT GTT TGT TTT ATG TTT TTT TGC R: TGT TCC TTT TTA GCA TCT CCA G	56	$(CA)_{21}$	0.757	171–189	EU884002
66HDZ110	F: ^{HEX}CTT TGG TTT TCT GTG TTT CTG C R: CAG GGA ATA GGG TGT CTT TGA	54	$(GT)_{24}$	0.877	177–291	EU884003
66HDZ117	F: ^{FAM}TTT GTC TTT TCA TCT CTC TAT CCC R: TTT TTG TGC CAG AAT ACT TTG AC	60	$(CA)_{18}$	0.808	165–197	EU884004
66HDZ139	F: ^{FAM}CCA TAG CCA TCT CTC CTT CC R: TGT TGG GTA TTT CTG GTT TGG	56	$(CA)_{26}$	0.849	105–135	EU884005
66HDZ304	F: ^{FAM}CAC TAT GAT GAA GGG GAG GG R: GGT CAG TCA GGG GAG GAA C	62	$(GT)_{22}$	0.851	157–179	EU884006
66HDZ311	F: ^{FAM}GCC AAA TCT CTC ATC TTC AGC R: CAA TAG GAA AAA CAG CAA GGA G	60	$(CA)_{28}$	0.886	174–216	EU884007
66HDZ327	F: ^{HEX}ACG TCT GTC CCC ACT ATT GC R: GAA CCC GAG AGA GCA GGC	50	$(GT)_3$ $(GT)_{19}$	0.88	149–173	EU884008
66HDZ334	F: ^{FAM}ATA ATG AAT CAC GAC AGA ACG AG R: CTA CAA CAG GCT TTG GGC A	60	$(CA)_{17}$	0.884	173–197	EU884009
66HDZ337	F: ^{FAM}TGA AGC AGA ACC AAG ATA GGG R: GTG GGA GAG ACG GCG TG	54	$(CA)_{27}$	0.916	169–203	EU884010
66HDZ339	F: ^{HEX}AGG AAA GAC TCC AAA AGA CAA TG R: GGA AAA AGC AAA TCT CGT ATG TT	58	$(CA)_{24}$	0.860	153–179	EU884011
66HDZ340	F: ^{HEX}CCA CCC TGT TTG GTC CC R: TCC CCC TCC TCT GTT TCC	60	$(CA)_{14}$	0.851	136–152	EU884012
66HDZ341	F: ^{FAM}CAA GCA TAC AAA GTG GGG A R: ATG TCA TCT GTC TCT GAA ATG G	58	$(CA)_9$ CG $(CA)_{13}$	0.811	231–257	EU884013
66HDZ343	F: ^{HEX}CCA CCC ATC TTG GCT TCT R: CGA TAG GTA ATC ATA GGA ACG AA	58	$(CA)_{22}$	0.880	114–140	EU884014
66HDZ361	F: ^{HEX}GGT TTT GTC CCC CAG CC R: CAG AAG CCC CAG CAC AGT C	60	$(CA)_{15}$	0.856	181–205	EU884015
66HDZ407	F: ^{FAM}ACC CAG GAC GGC ATC AC R: CTC TTC GGC TTC CAC TTA GG	56	$(GT)_2$ $(GA)_3$ $(GT)_{15}$	0.765	142–162	EU884016
66HDZ413	F: ^{FAM}ATG CCC TTA GCA CTG GAC A R: TAA ATG TTT GTG TGT GGA GGT G	54	$(CA)_{21}$	0.845	202–226	EU884017

plasmid ligation was performed following this PCR. Following transformation, cells were plated onto LB agar plates including ampicillin and X-gal. Plates were picked for positive white colonies that were placed on Pall (East Hill, NY) Biodyne B nylon membranes. A Southern blot of the colonies was done using DIG-labeled oligonucleotide. Plasmid preps of the positive colonies from the Southern blot were sequenced and primers were designed from the two regions flanking the microsatellite repeat motif. Of 4,430 clones screened, 1,080 were positive for a microsatellite insert and the first 22 designed and tested polymorphic are reported here.

Tissue samples collected from the patagium of each bat were stored in an ambient temperature storage buffer (Longmire et al. 1992) from 37 *R. madagascariensis* individuals representing one roost site. Genomic DNA was isolated using standard protocols (Sambrook et al. 1989). PCR amplification was carried out in a 25 μl reaction volume using an MBS thermocycler (Thermo Electron Corporation, Milford, MA) with approximately 50 ng of genomic DNA template. Final amplification conditions consisted of 12.5 pmol unlabelled reverse primer, 12.5 pmol fluorescently labeled forward primer, 1.5 mM $MgCl_2$, 200 μM each dNTP, and 0.5 units of Taq DNA polymerase (Promega; Madison, WI). The thermal profile for PCR amplification was 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, a primer-specific annealing temperature for 30 s (Table 1), 72°C for 30 s, ending with a single extension of 72°C for 10 min. Allele sizes were determined by separation of the PCR products via POP 4 capillary buffer electrophoresed in an ABI 3100 DNA Analyzer (Applied Biosystems, Inc; Foster City, CA). Fragment length genotypes were assigned by GeneScan (Applied Biosystems, Inc.) using GeneScan-500 [Tamra] size standard. Loci characterizations are presented in Table 1.

The data set was analyzed for errors using MICRO-CHECKER (Van Oosterhaut et al. 2004) and MSA (Dieringer and Schlötterer 2003). Null alleles and polymorphic information content (PIC) were estimated using CERVUS v.2.0 (Marshall et al. 1998; Slate et al. 2000). Marker independence was tested following a Bonferroni correction for multiple tests in FSTAT using the linkage disequilibrium option (Goudet 1995, 2001) before population genetic parameters were estimated using Genepop 4.0 (Raymond and Rousset 1995) and FSTAT (Table 2).

Six of the loci showed departure from HWE owing to a deficit of heterozygotes. This deficit was diagnosed in MICROCHECKER and CERVUS as high frequencies (freq > 0.10) of null alleles in this population. The number of alleles ranged from 7 to 19 and informativeness, interpreted from polymorphic information content (PIC), ranged from 0.757 to 0.916 (66HDZ106 and 66HDZ337 respectively for both parameters). This marker suite should be useful in population genetic studies comparing *Rousettus madagascariensis* across the island of Madagascar.

Table 2 Number (k) of alleles detected, allelic richness (A.R.), observed (Ho) and expected (He) heterozygosities, *P*-values and standard errors (SE) for Hardy–Weinberg Exact Tests (HWE), and among 22 microsatellite loci screened across a population of 37 individuals of *R. madagascariensis* from southeastern Madagascar

	k	A.R.	Ho	He	HWE[a]	SE
66HDZ20	10	9.946	0.568	0.871	0.0003*	0.3514
66HDZ80	12	11.941	0.838	0.890	0.371	0.0594
66HDZ82	16	15.935	0.892	0.917	0.6647	0.0282
66HDZ91	18	17.723	0.784	0.921	0.0525	0.1505
66HDZ95	12	11.939	0.703	0.896	0.009*	0.2180
66HDZ105	13	12.941	0.784	0.887	0.1327	0.1179
66HDZ106	7	6.998	0.541	0.793	0.0008*	0.3217
66HDZ110	16	14.937	0.892	0.898	0.5789	0.0063
66HDZ117	11	10.836	0.811	0.840	0.7725	0.0357
66HDZ139	11	10.972	0.611	0.875	0.0001*	0.3047
66HDZ304	13	12.916	0.778	0.873	0.088	0.1103
66HDZ311	17	16.621	0.865	0.907	0.2967	0.0471
66HDZ327	13	13.000	0.943	0.902	0.3505	−0.0457
66HDZ334	13	12.944	0.946	0.905	0.6746	−0.0456
66HDZ337	19	18.777	0.595	0.934	0.0000*	0.3664
66HDZ339	12	11.833	0.811	0.886	0.4712	0.0855
66HDZ340	12	11.784	0.703	0.877	0.1759	0.2014
66HDZ341	12	11.89	0.676	0.837	0.0143*	0.1946
66HDZ343	13	12.836	0.757	0.903	0.2072	0.1635
66HDZ361	13	12.838	0.703	0.881	0.0114	0.2044
66HDZ407	11	10.836	0.838	0.799	0.52	−0.0499
66HDZ413	11	10.89	0.784	0.872	0.8063	0.1023

* Null allele frequency detected >0.10

[a] Probability of satisfying Hardy Weinberg Expectations following χ^2 test. Monte Carlo Markov Chain parameters: 100 batches and 5,000 iterations per batch

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