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TECHNICAL NOTE

Characterization of 10 polymorphic microsatellite loci for White-breasted mesites (*Mesitornis variegata*)

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Abstract We characterized 10 specific microsatellite loci for White-breasted mesites (*Mesitornis variegata*), an endemic bird species from western Madagascar. Nine loci were in Hardy–Weinberg equilibrium, and we detected 4–10 alleles per locus (mean = 6.1). These primers will be used to study the mating system and social organization of White-breasted mesites and may have applications for the conservation of the few remaining populations of this vulnerable and still poorly studied species.

Keywords Mesitornithidae · Population genetics · Primers

White breasted-mesites (*Mesitornis variegata*) are endemic birds from the dry deciduous forests of western Madagascar. They are monomorphic, ground-dwelling, medium-sized birds found in pairs or small groups (Hawkins and Seddon 2003). There is only little known on the general biology of this species, and its population genetic structure has not been studied. Here we describe the isolation and characterization of 10 microsatellite loci that were developed to study the social organization and mating system of *M. variegata*. White-breasted mesites are classified as vulnerable in the IUCN red list of threatened species (BirdLife International 2012). Available specific microsatellite markers can be an important tool for species conservation and could be used to assess the viability of the few remaining populations of this species by determining their genetic variability and degree of isolation (Hedrick 2001).

Feather, blood and tissue samples were collected in Kirindy Forest (Kappeler and Fichtel 2012), from birds and embryo remains of predated eggs. Sample collection and export were conducted according to local authority permits.

Microsatellite sequences were isolated by ecogenics GmbH (Switzerland). Size selected fragments from genomic DNA were enriched for simple sequence repeat (SSR) content by using magnetic streptavidin beads and biotin-labeled CT and GT repeat oligonucleotides. The SSR enriched library was analyzed on a Roche 454 platform using the GS FLX titanium reagents. The total 18,013 reads had an average length of 201 base pairs. Of these, 1,021 contained a microsatellite insert with a tetra- or a tri-nucleotide of at least 6 repeat units or a dinucleotide of at least 10 repeat units. Suitable primer design was possible in 340 reads, of which 36 were tested for polymorphism. We extracted DNA from 75 individuals using DNeasy Blood and Tissue Kit (Qiagen). Reactions of 10 µl containing 1× buffer, 200 µM of dNTPs, 0.04 µM M13 tailed locus specific forward primer, 0.16 µM locus specific reverse primer, 0.16 µM universal M13 primer 5′-end labeled with FAM (Metabion), 0.5 units of Hotstar Taq (Qiagen) were used to amplify each locus via the nested PCR procedure described by Schuelke (2000). The PCR profile was 95 °C for 15 min, 30 cycles of 30 s at 95 °C, 45 s at 56 °C (annealing temperature), 45 s at 72 °C, followed by 8 cycles of 30 s at 95 °C, 45 s at 53 °C and 45 s at 72 °C; and a final elongation phase of 30 min at 72 °C. PCR

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Table 1 Characterization of 10 microsatellite loci for *M. variegata* from 75 individuals

Locus		Primer sequences 5′–3′	Repeat type	Size range (bp)	Number of alleles	T_a (°C)	Number of individuals	Ho	He
Mesvar_01929	F	ACGAGATAAAACGCGGATGC	$(GT)_{15}$	68–82	6	56	75	0.680	0.621
	R	GGGGTTGCAAATGGGGAC							
Mesvar_05395	F	AGCAAAGAGGATGTTCTGCC	$(AC)_{19}$	181–188	5	56	74	0.689	0.705
	R	CTCAGTCTATTGCATGCTTGTG							
Mesvar_06758	F	GGACGCTAGGGCAGAGATG	$(CA)_{17}$	113–129	7	56	75	0.853	0.825
	R	CTCGCCAACTACGTGGAGG							
*Mesvar_07236	F	TGTCGTAGGGAGAGCTGAAC	$(TG)_{17}$	81–90	4	56	73	0.288	0.552
	R	GCACTTCGCTAATGCACAG							
Mesvar_07348	F	TGGTCCCCATTCCGCCTC	$(TG)_{16}$	109–169	10	56	75	0.813	0.830
	R	AGACCTCGGCGTAAAGGAAG							
Mesvar_08218	F	GAGGTGCGCCAATACCAAAG	$(GT)_{16}$	193–210	7	56	75	0.627	0.694
	R	CCTGCCCTAAGAACGACAAG							
Mesvar_09677	F	GCTGGCCCCATTGATTTACG	$(AC)_{17}$	75–95	7	56	75	0.813	0.772
	R	TGCTCATTAGCGTGGTTTCAG							
Mesvar_12782	F	ACACTTTCAGATGACAGGCTC	$(TG)_{12}$	177–182	4	56	75	0.733	0.736
	R	GCAGCTTAATGCTCCACCTG							
Mesvar_14701	F	AGGCCAGGTAATCTGAAGGG	$(AC)_{13}$	153–158	5	56	75	0.680	0.686
	R	AGGTGATCTGGTAGGGTTGC							
Mesvar_17549	F	GCAGAATGGTTATCCTATCTTTTACG	$(GT)_{12}$	118–132	6	56	75	0.573	0.601
	R	CGTGAAGTCAGCGGGAATAC							

T_a, annealing temperature; Ho, observed heterozygosity; He, expected heterozygosity; * deviations from Hardy–Weinberg equilibrium $P < 0.05$

products were sized on a 3130XL Genetic Analyzer (Applied Biosystems/Hitachi) and GENEMAPPER V4 (Applied Biosystems) was used to assign genotypes. Observed (Ho) and expected (He) heterozygosity and Hardy–Weinberg equilibrium were calculated using GENEPOP V4.1.4 (Rousset 2008). Description of 10 polymorphic microsatellite loci is provided in Table 1. We detected 4–10 alleles per locus (mean = 6.1), Ho ranged from 0.288 to 0.853 and nine loci were in Hardy–Weinberg equilibrium. Overall, the described microsatellite markers should be an adequate tool for the study of the social system and conservation of *M. variegata*.

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References

BirdLife International (2012) *Mesitornis variegatus*. IUCN 2012, IUCN Red List of Threatened Species, Version 2012.2. http://iucnredlist.org. Accessed 15 Nov 2012

Hawkins AFA, Seddon N (2003) Mesitornithidae, mesites. In: Goodman SM, Benstead JP (eds) The natural history of Madagascar. The University of Chicago Press, Chicago, pp 1095–1098

Hedrick PW (2001) Conservation genetics: where are we now? Trends Ecol Evol 16:629–636. doi:10.1016/S0169-5347(01)02282-0

Kappeler PM, Fichtel C (2012) A 15-year perspective on the social organization and life history of sifaka in Kirindy Forest. In: Kappeler PM, Watts DP (eds) Long-term field studies of primates. Springer, Berlin, pp 101–121

Rousset F (2008) Genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Mol Ecol Resour 8:103–106. doi:10.1111/j.1471-8286.2007.01931.x

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. Nat Biotechnol 18:233–234. doi:10.1038/72708