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TECHNICAL NOTE

Microsatellite loci isolated from the lowland tapir (*Tapirus terrestris*), one of the largest Neotropical mammal

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Abstract We describe ten microsatellite loci from the lowland tapir (*Tapirus terrestris*), one of the last members of the Neotropical megafauna and considered a vulnerable species. A total of 74 alleles were detected, ranging from four to thirteen alleles per locus. The expected heterozygosity ranged from 0.34 to 0.88, with a mean of 0.635. All but one locus conformed to the Hardy–Weinberg expectations. These microsatellite loci can be used in population genetic studies of the genus *Tapirus*, in which all species are threatened globally.

Keywords Atlantic forest · Cerrado · Tapiridae · Population genetics · Microsatellites

The lowland tapir, *Tapirus terrestris* (Linnaeus 1978), one of the largest surviving members of the Neotropical megafauna (Hansen and Galetti 2009), is considered vulnerable by IUCN (www.redlist.org). *Tapirus terrestris* has a cis-Andean distribution (Emmons and Feer 1997), but its distribution has shrunk in the last years due to overhunting and habitat fragmentation (Schipper et al. 2008; Bodmer and Brooks 1997). Nowadays the largest populations of lowland tapirs occur in the Amazon basin and the Brazilian Pantanal. Lowland tapirs are rare or locally extinct in most of its former distribution in the Atlantic forest (Galetti et al. 2009). The birth of only one young after a long gestation period and the low population density highlights the threats to the species (Padilha and Dowler 1994). There is no study on population genetics of lowland tapirs and it is paramount to develop genetic markers to diagnosis the remaining populations. Here, we report the isolation and characterization of microsatellite loci in *T. terrestris*, in order to provide useful genetic markers for population studies and conservation of this species.

Microsatellite loci were isolated from a partially enriched genomic library for tetranucleotide loci following the method described by Hamilton et al. (1999). DNA was extracted from blood tissue from one individual from a natural population using the protocol of Lahiri and Nurnberger (1991). This DNA was digested with *Rsa*I and *Bstu*I (GE Healthcare). DNA fragments (400–1,000 bp) were purified from the gel (Wizard SV Gel and PCR Clean-Up System kit, Promega) and ligated to the SuperSNX linkers (Hamilton et al. 1999). The enrichment was performed with the hybridization of the DNA fragments to a mix of eight biotinylated oligonucleotides [$(AAAC)_6$, $(AAAG)_6$, $(AATC)_6$, $(AATG)_6$, $(ACCT)_6$, $(ACAG)_6$, $(ACTC)_6$, $(ACTG)_6$)]. The candidate fragments were captured on streptavidin-coated magnetic beads (Streptavidin Magnesphere Paramagnetic Particles, Promega). DNA enriched was recovered by PCR using the SuperSNX primer and cloned using the pGEM-T Easy kit (Promega). A total of 169 clones were sequenced on an automated sequencer MegaBACE 1000 (GE Healthcare) using the DYEnamic ET Dye Terminator kit (GE Heathcare). The extraction of

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the vector sequence, the search for the microsatellites, and the design of primers were performed using CID (Freitas et al. 2008).

Amplifications of 18 microsatellite loci were performed in 24 DNA samples: 10 blood samples from a natural population of Parque Nacional de Emas (Brazil) and 14 hair samples from five different Brazilian zoos. DNA extractions were performed following Lahiri and Nurnberger (1991). Ten loci yielded reliable polymorphic banding patterns (Table 1). PCRs were performed using a three primer labeling system (Schuelke 2000) in a final volume of 15 µl, containing 20 ng of DNA, 0.2 mM dNTPs, 1× PCR buffer, 8 pmol of reverse primer, 8 pmol of 6-FAM or NED M13(-21) primer, 2 pmol of the forward primer, 1.5 mM MgCl2 and 1 U of Taq DNA Polymerase (Invitrogen). PCR conditions for all loci were as follows: 95°C (5 min), 35 cycles at 94°C (30 s), 56–58°C (1 min), 72°C (45 s), followed by 15 cycles at 94°C (30 s), 53°C (45 s), 72°C (45 s) and a final extension 72°C (10 min). The microsatellite loci were analyzed on a MegaBACE 1000 automated sequencer and the alleles were scored with the Genetic Profiler (GE Heathcare).

Hardy–Weinberg and linkage disequilibrium tests were performed using GENEPOP 3.4 (10,000 batches, Raymond and Rousset 1995). The critical values for multiple comparisons were adjusted using the Benjamin and Yekutieli correction (Benjamin and Yekutieli 2001). Null allele frequencies were estimate for each locus using Micro-Checker (van Oosterhout et al. 2004). These tests were performed only with the natural population.

A total of 74 alleles were detected with the analysis of all individuals, ranging from four (Tter3) to thirteen (Tter14) alleles per locus (mean of 7.4 alleles/locus). The expected heterozygosity of the natural population ranged from 0.34 (Tter9) to 0.88 (Tter13), with a mean of 0.635 (Table 1). All but one (Tter13) conformed to the Hardy–Weinberg expectations with a heterozigosity deficit due to the presence of null alleles at a frequency of 0.26 according to the Micro-Checker analysis. No pairwise test of the loci for genotypic disequilibrium was significant.

Tapirus terrestris is one of the four surviving tapir species in the world and all of them are threatened (www.redlist.org). In this way, these set of microsatellite loci will be an important tool for studies on population genetic of the tapir species, providing data on population size and structure, genetic diversity, dispersal patterns of these vulnerable animals, contributing to the conservation of the species.

Table 1 Characterization of ten microsatellite loci isolated from lowland tapir (*Tapirus terrestris*)

Locus	Primer sequence (5′–3′)	Motif	n_a	Size range (bp)	H_o	H_e	*P*-value
Tter3	ATCTCAGAGGTTCCACACTG GCTGGAAGGTAAGATCTGTG	(CATT)6	4	156–168	0.50	0.67	0.022
Tter4	CGTTAGCATGATCTCTAGACC CCAGATGAGAAGCAGGATAG	$(TG)_{20}$	11	230–264	0.86	0.85	0.552
Tter5	TGCCCTGATTTAGAGAAAAC AGGAGAAGTTAGAAGGGGAA	$(GT)_{10}$	7	199–209	0.40	0.64	0.365
Tter7	CCTGTGCAGCATTTGATAAC GGTTGACCAGTTTAATGCAG	(AATG)5	6	142–174	0.30	0.44	0.220
Tter9	GGACACTCAAGTGGGTCAAG AGTGTATGCTTGTCGCGC	$(CAGG)_7$	5	168–192	0.37	0.34	1.000
Tter11	GCTCTCTGGCTTTTACACACT GGAAAGCTGAAAAGGAGGA	$(TCTG)_7$ $(TC)_8$tttctcca $(TG)_6$	7	156–192	0.55	0.68	0.272
Tter13	CCATGCAATTAAGAGAAAGC CAGCTAAGGACAGGAAAATG	$(CA)_{20}$	9	252–282	0.40	0.88	0.001*
Tter 14	GATCCTCCTGTTTGCAGAT AGCCAAATGTTTTAGACTGAG	$(CA)_{22}$	13	174–208	0.60	0.84	0.146
Tter17	TGCCACATTTGTTCAGTCTC GGCTGAAATATTGTATCTGCA	$(TC)_{29}$	5	274–300	0.30	0.57	0.099
Tter18	AGAGTGTCAGATGTCCTGCC TGCTTTGTGTTTGAGTGTGC	$(CA)_7$	7	98–120	0.30	0.44	0.133

Number of alleles (n_a) based on a sample of 24 individuals, observed (H_o), expected (H_e) heterozygosities and *P*-value of the Hardy–Weinberg equilibrium test based on the natural population. GenBank accession numbers: GQ141515 to GQ141524

* Significant *P*-value < 0.01707, following Benjamin and Yekutiele correction

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