Development of eight polymorphic microsatellite markers in the Black and Rufous sengi, **Rhynchocyon petersi **

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Microsatellite Letters:

From 01 May 2013 all papers on microsatellite data should be submitted as microsatellite letters and should conform to the following format. Microsatellite letters might be no longer than 800 words, can include a maximum of 5 references, abstract must include rationale for conservation angle and not be longer than 150 words, method should refer to standard methodology and detail only deviations or specific details, ***sequences should be submitted as Electronic Supplementary Material.*** *Microsatellite Letters should focus exclusively on species of conservation value and this must be explained in the introduction of the paper (if possible, IUCN status should be provided). They should provide a minimum of* ***eight useful microsatellite DNA loci,*** *and should not be based exclusively on the cross-species application of previously published primers. If derived from EST markers, they must be determined to be novel. Cross-species testing of new primers is, however, very much encouraged as part of the primer note where new primers are reported.*

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Microsatellite Letter

Isolation and characterization of eight polymorphic microsatellite DNA markers from an Afrotherian, the Black and rufous sengi, *Rhynchocyon petersi*.

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Abstract

The Black and Rufous sengi *Ryhnchocyon petersi,* is endemic to a limited range in East Africa inhabiting Eastern Arc Mountains and coastal forests of Tanzania and Kenya. We report the development of eight polymorphic microsatellites using next generation sequencing technology. Eighteen individuals from Zaraninge forest (Saadani National Park, Tanzania) were genotyped. The number of alleles per locus ranged from 2 to 6, while the observed and expected heterozygosities varied from 0.17 to 0.82 and from 0.25 to 0.81, respectively. No locus deviated from Hardy–Weinberg equilibrium. These microsatellite markers will be useful tools for further studies of population genetic structure of *R. petersi* in its fragmented habitat.

Keywords: *Rhynchocyon petersi,* vulnerable Afrotheria, Microsatellites, 454 sequencing

Rhynchocyon petersi Bocage, 1880, the Black and rufous sengi (elephant-shrew) is one of the four known giant sengis from the subfamily Rhynchocyoninae which belong to the supercohort Afrotheria grouping together aardvark, tenrecs, golden-moles, elephants, hyraxes and sea-cows. This species is endemic to East Africa, distributed in the Eastern Arc Mountains and costal forests of Tanzania and Kenya. These habitats show a high level of biodiversity and endemism but are threatened by fragmentation and degradation due agricultural expansion, timber for various uses and charcoal production (Rathbun and Butynski, 2008). *Rhynchocyon petersi* is categorized as vulnerable due to the loss of habitats (IUCN, 2014).. Little is known about this species because no detailed field studies have ever been conducted (Rathbun and Butynski, 2008). Our aim was to develop microsatellite loci for future studies of the genetic structure and dispersal of this sengi species.

DNA was extracted using Nucleopsin kit (Machery Nagel) from a piece of tissue cut from ears. The library construction and microsatellite optimisation were outsourced to Genoscreen (Lille, France). The high-throughput method used for isolating microsatellite markers is based on coupling multiplex microsatellite enrichment and next-generation sequencing on a Roche 454 GS-FLX Titanium platform (Malausa, 2011). DNAs from 11 samples of *R. petersi* from 3 different forest patches from Saadani National park (Zaraninge (UTM E 457957/N 9324703, Gendagenda (UTM E 460395/N 9383565), Kamsisi (UTM E 455100/ N 9348677) were pooled for this step. In total 8310 sequenced reads allow us to identify 1833 microsatellite containing sequences and validate 172 primer pairs framing a microsatellite motif. 70 primer pairs were tested with 8 samples. PCR amplification was performed in a final volume of 10 µL containing 6 pmol dNTP, 37.5 pmol $MgCL_2$, 10 pmol of forward and reverse primers, 1 µL DNA (diluted 1/10) and 0.5 U of FastStart Taq DNA polymerase (Roche). The PCR conditions consisted in an initial denaturation of 10 min at 95°C, 40 cycles of 30 s at 95°C, 30 s at 55°C, 60 s at 72°C, and a final extension of 10 min at 72°C. 2 µL of PCR products were visualised on a 2 % agarose gel. Twenty-seven primer pairs were validated from this step and we selected 24 primer pairs to study their polymorphism in 7 samples (3 from Zaraninge, 2 from Gendagenda and 2 from Kwamsisi). The PCR mix and conditions were similar to the previous step but with the forward primer labelled with a fluorescent dye (Table 1). 1 µL of the PCR amplicons were diluted and run on an ABI-3730xl DNA Analyzer using

the GenScan-500LIZ (Applied Biosystems) as a size standard. Alleles were visualized and scored using GeneMapper Version 3.7 (Applied Biosystems). Nine microsatellite motifs showed polymorphism but for one locus, Rhpe20, all 7 individuals showed the same genotype, the same 2 alleles present in all individuals. Sequences obtained from the Roche 454 sequencer for the 9 loci are listed in Supplementary Material. The 9 polymorphic markers were pooled in two multiplex PCR (Table 1) and tested for effectiveness on 15 additional samples of *R. petersi* from Zaraninge forest. The PCRs were performed in 10 µL reaction volume containing the QIAGEN Multiplex PCR Master Mix (1x), 0.2 µM of each primer and 1 µL of genomic DNA. The PCR conditions consisted in an initial denaturation of 15 min at 95°C, 35 cycles of 30 s at 94°C, 90 s at 57°C, 90 s at 72°C, and a final extension of 10 min at 72°C. Microsatellite fragment analysis was performed as above. The number of alleles, and observed and expected heterozygosities were estimated by HW-QuickCheck software (Kalinowski, 2006). Tests for linkage disequilibrium were performed using GENEPOP 4.0 (Rousset 2008).

For the 18 samples from Zaraninge forest, allelic diversity ranged from 2 to 6 alleles per locus (Table 1). For the locus Rhpe20, all individuals show the same genotype. Given this result, we would not recommend the use of this locus. The 8 remaining loci have an average allelic diversity of 3.4 alleles. Observed and expected heterozygosity averaged across all loci were both 0.52. Between pairs of loci, no significant linkage disequilibrium was detected after Bonferroni correction ($P < 0.002$). No evidence of deviation from Hardy–Weinberg equilibrium was detected. The new microsatellite markers developed should be useful for examining the impact of the habitat fragmentation on the population structure and genetic diversity in this sengi species.

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