



Methodology

Development of microsatellite markers for the Neotropical endemic Brazilian Guanabara frog, *Euparkerella brasiliensis*, through 454 shotgun pyrosequencing

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ABSTRACT. The new-generation 454 GS-FLX Titanium pyrosequencing was used to isolate microsatellite markers for the Brazilian Guanabara frog, *Euparkerella brasiliensis*, an Atlantic forest endemic species. Three multiplex polymerase chain reaction sets were optimized for genotyping of 11 polymorphic (di- and tetranucleotide) microsatellite markers. Genetic diversity was assessed in 21 individuals from a population (Reserva Ecológica de Guapiaçu, REGUA) located

in the central region of the Rio de Janeiro State, in Brazil. The mean number of alleles per locus ranged from 3 to 12. Observed and expected heterozygosities ranged from 0.095 to 0.905 and from 0.094 to 0.904, respectively. After using the Bonferroni correction for multiple tests, there was no evidence of linkage disequilibrium between pairs of loci but deviations for Hardy-Weinberg equilibrium were found in 4 loci. We found no evidence for allele dropouts or stuttering, but we detected the presence of null alleles at loci Eb10 and Eb36. These markers will be useful for analyses of fine-scale population structure and determination of relative effects of habitat loss and fragmentation on population genetic variability within species.

Key words: Amphibians; Atlantic forest; *Euparkerella brasiliensis*; Microsatellites; 454 Sequencing