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*Short Communication*

# Isolation and characterization of 10 microsatellite loci for Pallas' long-tongued bat *Glossophaga soricina* (Phyllostomidae)

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**ABSTRACT.** *Glossophaga soricina* is a widespread Neotropical nectarivorous bat. We characterized 10 microsatellite loci isolated from a shotgun genomic library. We analyzed tissues from wing membrane of 67 individuals collected from two populations of Central Brazil (Brasília and Alto Paraíso). The number of alleles per locus ranged from 2 to 20, and the observed and expected heterozygosities ranged from 0.015 to 0.666 and from 0.016 to 0.915, respectively. The high combined probability of genetic identity ($4.369 \times 10^{-8}$) and probability of paternity exclusion (0.996) showed that these microsatellite loci would be useful for population genetic structure and parentage studies in natural populations of *G. soricina*.

**Key words:** Cerrado; Chiroptera; Conservation; Genetic diversity; Neotropical bat; Shotgun genomic library

 

## INTRODUCTION

*Glossophaga soricina* (Chiroptera: Phyllostomidae) is one of the most common and widespread bats in the Neotropics (Simmons, 2005). It feeds mainly on floral nectar, where it is an important pollinator of many Neotropical plants (Nogueira et al., 2007). Despite the current knowledge about species life history, there is still much to learn about population genetic structure, and also kinship and mating system within social groups. Microsatellite markers may provide useful tools for genetic studies on populations of animals, especially those where information is difficult to obtain by direct observations, such as bats (Bryja et al., 2009). Hence, we report the development and characterization of a set of microsatellite loci for *G. soricina*, to serve as a tool to study population genetic structure, patterns of gene flow and mating system of this species.

## MATERIAL AND METHODS

Microsatellites were developed from a shotgun genomic library. DNA from blood of one individual of *G. soricina* was extracted following the protocol described by Sambrook and Russell (2001). The DNA was sheared using a sonicator (120 W for 1 h and 45 min) to obtain fragments from 500 bp to 1 kb. Fragments were recovered, cloned into pMOSBlue vector using the Blunt-ended PCR Cloning kit® (GE HealthCare, Sweden), and transformed into pMOSBlue® competent cells (GE HealthCare). Recombinant clones were selected on Luria-Bertani (LB) plates, containing ampicillin and X-Gal. For plasmid DNA extraction, recombinant clones were grown overnight in LB-ampicillin liquid medium, following the protocol described by Sambrook and Russell (2001). Finally, the DNA inserts were sequenced on a 3100 automated DNA sequencer (Applied Biosystems, CA, USA), using the U19 primer and the DYEnamicET Terminator kit (GE Healthcare), according to manufacturer instructions. Sequences were analyzed for their nucleotide content and filtered by their quality and length (phred value ≥20; length ≥150). The selected reads were screened for microsatellites using the WEBSAT software (Martins et al., 2009), and primers were designed using the Primer3 software (Rozen and Skaletsky, 2000).

Wing membranes from 67 individuals of *G. soricina* were sampled for characterization of loci. DNA was extracted using the DNeasy Tissue Kit (QIAGEN). Microsatellite amplifications were performed in a 15-µL final volume, containing 12.5 ng template DNA, 0.13 µM of each primer, 1 U Taq DNA polymerase (Phoneutria, Brazil), 0.21 mM each dNTP, 0.21 mg BSA and 1X reaction buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$) with the following conditions: 94°C for 5 min (1 cycle), followed by 30 cycles of 94°C for 1 min, annealing temperature (see Table 1) for 1 min and 72°C for 1 min, and 72°C for 7 min (1 cycle). To detect the polymorphisms, we used 6% denaturing polyacrylamide gels stained with silver nitrate (Creste et al., 2001), and the alleles were sized by comparison to a 10-bp DNA ladder standard (Invitrogen, USA).

The number of alleles per locus, the observed and expected heterozygosities under Hardy-Weinberg equilibrium (HWE) (Nei, 1978), and inbreeding coefficient (Weir and Cockerham, 1984) were estimated using FSTAT 2.9.3.2 (Goudet, 2002). The probability of genetic identity (Paetkau et al., 1995) and paternity exclusion probability (Weir, 1996), for each polymorphic locus and overall loci, were estimated using Identity 1.0 (Wagner and Sefc, 1999).

 

## RESULTS AND DISCUSSION

We sequenced 2112 clones, and 474 showed microsatellite sequences. Primers were designed for 15 sequences and 10 amplified successfully (Table 1). All loci were polymorphic with numbers of alleles ranging from 2 to 20 (Table 1). Observed heterozygosity ranged from 0.015 to 0.666, whereas expected heterozygosity ranged from 0.016 to 0.915 (Table 1).

**Table 1.** Characterization of the 10 microsatellite loci developed for *Glossophaga soricina*, based on 67 individuals.

| Locus | Repeat motif | Primer sequence (5'-3') | Ta | $N_A$ | $H_O$ | $H_E$ | *F* | *I* | Q |
|---|---|---|---|---|---|---|---|---|---|
| GS02 | $(AG)_8$ | (F) TGGACCAACATGACAATATG<br>(R) CACATATAGGAATCAACCAATG | 56 | 6 | 0.224 | 0.537 | 0.582 | 0.266 | 0.303 |
| GS03 | $(TG)_{20}$ | (F) GGATCTGGGTCCAAAATAAA<br>(R) ACAGACCCAAGTACCCTACAG | 56 | 20 | 0.666 | 0.915 | 0.272 | 0.016 | 0.810 |
| GS04 | $(TG)_7$ | (F) CCGACTGAGGTCAAGTTGTA<br>(R) CTGATGCAGGAGTATGGAAA | 56 | 6 | 0.358 | 0.735 | 0.512 | 0.117 | 0.493 |
| GS05 | $(TC)_{24}$ | (F) TGCTTTACTTCTCCCAACAA<br>(R) CCAATCACATGGTGAGAGC | 56 | 5 | 0.606 | 0.663 | $0.086^{NS}$ | 0.161 | 0.423 |
| GS08 | AC(8) | (F) TAGACCCCATAAACCTGGAC<br>(R) AGAAAATTAGCTCAGAGGAACC | 66 | 2 | 0.016 | 0.016 | $0.000^{NS}$ | 0.967 | 0.008 |
| GS10 | CAAA(6) | (F) AATTCGAGCTCCCCTACC<br>(R) TCCTCCCAACAGTTTTATGA | 56 | 3 | 0.015 | 0.515 | 0.970 | 0.363 | 0.197 |
| GS12 | AC(19) | (F) AACGGGAAGGACATATCAAT<br>(R) AGGCATTGAGCAGTTTCTAGT | 60 | 13 | 0.415 | 0.897 | 0.537 | 0.023 | 0.771 |
| GS13 | TG(7) | (F) CCGACAGAGGTCAAGTTGTA<br>(R) CTGATGCAGGAGTATGGAAA | 64 | 4 | 0.363 | 0.309 | $-0.176^{NS}$ | 0.511 | 0.148 |
| GS14 | AGGA(5) | (F) ATAAATGGTGGTGGAAGGAC<br>(R) GACTGGGAGACATGGGTAAC | 64 | 3 | 0.338 | 0.316 | $-0.072^{NS}$ | 0.515 | 0.137 |
| GS15 | AG(8) | (F) GCACACCAAAGTGACAGACT<br>(R) CACATATAGGAATCAACCAA | 60 | 4 | 0.184 | 0.586 | 0.685 | 0.243 | 0.316 |
| All | | | | | | | 0.419 | 4.369798e-008 | 0.996 |

Ta = annealing temperature (°C); $N_A$ = number of alleles; $H_O$ = observed heterozygosity; $H_E$ = expected heterozygosity; *F* = fixation index; *I* = probability of genetic identity; Q = paternity exclusion probability; NS = not significant (P > 0.005).

Other studies using non-enriched libraries also observed low heterozygosity for bat species (e.g., Rossiter et al., 1999; Mayer et al., 2000). The deviation from HWE observed in some loci (Table 1) and linkage disequilibrium between loci GS04 and GS13 were most likely due to a Wahlund effect, because of the sampling of individuals from different social groups. However, the high combined paternity exclusion and low genetic identity probabilities for overall loci (Table 1) demonstrated that the developed microsatellite loci are suitable for population genetic structure and detailed parentage studies in natural populations.

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