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MICROSATELLITE LETTERS

Novel and cross-amplified microsatellite loci for the critically endangered São Paulo marsh antwren *Formicivora paludicola* (Aves: Thamnophilidae)

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Abstract The São Paulo marsh antwren (*Formicivora paludicola*) is a critically endangered bird endemic to marshes in the metropolitan region of São Paulo city, Brazil. The total population is estimated to be around 300 individuals, distributed among 15 small (<50 ha) fragments, suggesting that loss of genetic variability may affect the long-term viability of this species. To develop genetic tools for gaining information on effective population sizes, inbreeding and gene flow between populations, we describe nine polymorphic microsatellite loci isolated from a *F. paludicola* library using next-generation sequencing. We report on levels of variation in these novel microsatellites and eight additional heterologous loci in these birds. Expected (H_E) and observed (H_O) heterozygosities averaged 0.72 and 0.70, respectively, and the number of alleles per locus ranged from 3 to 10. These loci will permit evaluation of whether artificial translocations are necessary for long-term viability of this rare bird.

Keywords Passeriformes · Microsatellites · Next-generation sequencing · Conservation genetics

The São Paulo marsh antwren, *Formicivora paludicola* (Aves: Thamnophilidae) is a small insectivorous bird endemic to São Paulo State, Brazil. The total population is estimated to be 250–300 individuals which are distributed among 15 isolated marshland fragments (all smaller than 50 ha), located in the metropolitan region of the city of São Paulo. This species has been classified as "Critically Endangered" in São Paulo State Red List (Buzzetti et al. 2013). The small population sizes and isolation of marsh areas suggest that loss of genetic variability may be occurring. The goal of this work was to isolate microsatellite markers for *F. paludicola*, and characterize these and a set of heterologous microsatellite loci previously isolated from the Chestnut-backed Antbird, *Myrmeciza exsul*

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Table 1 Data of novel (Fpa loci) and cross-amplified (MyEx and Mex loci) polymorphic microsatellites in *F. paludicola*

Locus	Motif	Primer sequence (5′–3′)	N	T_A (°C)	N_A	Allele Range (bp)	H_O	H_E	P
Fpa11	$(AAGGAG)_7$	F: CAACCAGCTCTGGTGTTTGC R: GATGAGCCATTCACTGAGCC	26	60.0	10	395–467	0.81	0.84	0.308
Fpa13	$(TTTGG)_{16}$	F: ACCAGGGAAGGATTCAAGGC R: AGTGTCCAGGGAAATGTGGG	26	55.0	3	368–393	0.35	0.36	0.544
Fpa14	$(ATCT)_{15}$	F: ATGGTGGCCTGTAAACTGGG R: CACAGACTGCTGTTTCCAAGG	26	60.0	6	575–599	0.73	0.70	0.450
Fpa15	$(TTCC)_{15}$	F: CTAACAGCTCCAGCTCACAGG R: TGTTCCTCACTGAAGTGCCC	26	60.0	4	216–264	0.77	0.73	0.586
Fpa17	$(ATCT)_{14}$	F: ACATGCTGCCTGTCCTTGG R: GAAGCAGGTGTAAGGTGCCC	26	60.0	6	452–472	0.89	0.82	0.807
Fpa18	$(ATCT)_{17}$	F: AACTGCTAAGACTGACTTGCTGG R: GGACTGTGGTGATAGAGCTGG	26	60.0	6	364–388	0.69	0.77	0.297
Fpa21	$(ATCT)_{17}$	F: TAAGGGATGGCAGCTTCTGG R: GTGGGCTTCATGGAATCTGG	26	60.0	10	417–489	0.58	0.83	0.001*
Fpa23	$(ACTCCC)_{11}$	F: ACTCAGGAACATGTCGTCGG R: ATGGAGGAGACATGATGGGG	26	60.0	4	237–255	0.73	0.73	0.112
Fpa24	$(AATAG)_{18}$	F: CTCAGGAAAGCAGGTCATGG R: TGGAATGCAACAGAACGG	26	60.0	7	429–479	0.65	0.76	0.207
Fpa25	$(AAAG)_{18}$	F: GGAAGGGGATGATTGTTTTCC R: TCCTCAATATGCCAAATGCG	26	60.0	5	252–288	0.69	0.67	0.457
MyEx19	$(ATC)_{16}$	Barnett et al. (2007)	26	58.0	5	292–313	0.65	0.62	0.300
MyEx41	$(GGAT)_{16}$	Barnett et al. (2007)	26	45.0	5	247–275	0.65	0.67	0.413
MyEx46	$(AT)_5(GT)_8$	Barnett et al. (2007)	26	51.0	10	298–346	0.85	0.87	0.378
Mex024	$(TAGA)_6TCGA(TAGA)_8$	Feldheim et al. (2010)	24	60.0	11	472–696	0.58	0.86	0.001*
Mex034	$(TAGA)_{13}$	Feldheim et al. (2010)	26	60.0	6	251–275	0.81	0.79	0.559
Mex089	$(TTTC)_{17}$	Feldheim et al. (2010)	25	60.0	17	542–638	0.64	0.91	0.000*
Mex120	$(AG)_{22}$	Feldheim et al. (2010)	26	60.0	6	223–243	0.89	0.80	0.852
Mex140	$(TCCA)_{11}$	Feldheim et al. (2010)	26	60.0	9	196–240	0.58	0.82	0.003
Mex162	$(TAGA)_{13}$	Feldheim et al. (2010)	25	60.0	5	232–252	0.60	0.66	0.029
Mex176	$(TAGA)_{12}$	Feldheim et al. (2010)	26	55.0	5	206–234	0.58	0.54	0.587

Repeat motifs, primer sequences (of unpublished loci) and references in the literature (of cross-amplified loci), number of screened individuals (N), PCR annealing temperatures (T_A), number of alleles (N_A), allele range in base pairs (bp), observed (H_O) and expected (H_E) heterozygosities, and probability of heterozygote deficits (P)

* Significant deviation after Bonferroni correction ($P < 0.0025$)

(Thamnophilidae) (Barnett et al. 2007; Feldheim et al. 2010) to support conservation genetic analyses.

Genomic DNA extracted from four individuals of *F. paludicola* was used to construct a paired-end shotgun library with Illumina Nextera reagents according to the manufacturer's protocol, except that custom primers were used and cleaned-up using Speedbeads instead of the supplied reagents. Sequencing was conducted on the Illumina MiSeq platform with version 2 250 base paired-end reads. A total of 3,108,418 reads were analyzed in the program PAL_FINDER_v0.02.03 (Castoe et al. 2012) to identify 49,093 reads containing 58,694 microsatellites (from dinucleotide to hexanucleotide) and 8,619 primer pairs were designed. Twenty loci were chosen for amplification and polymorphism analyses. Another 26 heterologous microsatellite loci, developed for *M. exsul* (Barnett et al. 2007; Feldheim et al. 2010), were also tested.

Variation at these loci was analyzed by PCR for up to 26 individuals of *F. paludicola* from a single population located in Mogi das Cruzes (23°32′S, 46°07′W). PCRs were performed in Eppendorf MasterCycler Gradient thermal cyclers in a 10 μL volume containing 150 ng of DNA, 0.2 mM of each dNTP, 1X PCR buffer (200 mM Tris–HCl, pH 8.4, and 500 mM KCl), 0.2 μM of each

primer, 3 mM $MgCl_2$, and 1 U of *Taq* DNA-Polymerase. Amplification conditions were 94 °C (5 min), 30 cycles of 94 °C (30 s), 30 s at the annealing temperature specified in Table 1, and 30 s at 72 °C, followed by a final extension of 72 °C (10 min). Amplified products were scored on an automated sequencer (ABI 3500). We calculated observed (H_O) and expected (H_E) heterozygosities, probability of heterozygosity deficit (P), and linkage disequilibrium using GENEPOP 4.0 (Raymond and Rousset 1995).

All 20 microsatellite loci isolated from *F. paludicola* amplified, and 10 were polymorphic. Among the 26 loci isolated from *M. exsul*, 24 could be amplified in *F. paludicola*, and 10 were polymorphic (Table 1). Heterozygosity deficits were detected in loci *Fpa*21, Mex024, and Mex089, suggesting the presence of null alleles. No significant linkage disequilibrium was detected among pairs of loci. The number of alleles among polymorphic loci in HW equilibrium ($N = 17$) ranged from 3 to 10, and observed (H_O) and expected (H_E) heterozygosities ranged from 0.35 to 0.89, and from 0.36 to 0.87, respectively.

Genetic analyses using the microsatellite loci described above will permit assessment of genetic variability within and between the currently small and recently isolated populations of this bird, and indicate whether reestablishing gene flow among them using translocations is a desirable management activity.

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