

Supplementary material for online publication only

## **Genetic structure of southernmost populations of black-and-gold howler monkeys (*Alouatta caraya*) and its conservation implications.**

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**Table A. Summary estimates of mitochondrial diversity.**

Genetic diversity estimates, results of neutrality tests and demographic parameters for black-and-gold howler monkeys sampled in ten populations from Northeastern Argentina and Southern Paraguay, based on 491-bp mtDNA Control Region fragment sequences. *H*: number of haplotypes (*n*: number of sequences). *PS*: number of polymorphic sites; *h*: haplotypic diversity  $\pm$  standard deviation (*SD*);  $\pi$ : nucleotidic diversity  $\pm$  standard deviation (*SD*); Tajima's (1989) *D*; Fu's (1997) *Fs*; Ramos-Onsins and Rozas's (2002) *R<sub>2</sub>*; *SSD*: Sum of Squared Deviations, *Rg*: Raggedness index; *Tau*: mode of the unimodal mismatch distribution; *TSE*: time since population expansion (in years before present).

<b>Statistic</b>	<b>Pop 1</b>	<b>Pop 2</b>	<b>Pop 3</b>	<b>Pop 4</b>	<b>Pop 5</b>	<b>Pop 6</b>	<b>Pop 7</b>	<b>Pop 8</b>	<b>Pop 9</b>	<b>Pop 10</b>
<i>H</i> ( <i>n</i> )	2 (2)	6 (10)	1 (11)	3 (8)	2 (8)	1 (8)	5 (6)	1(4)	3 (7)	2 (8)
<i>PS</i>	1	14	0	6	5	0	9	0	24	6
<i>h</i> $\pm$ <i>SD</i>	1 $\pm 0.035$	0.911 $\pm 0.035$	0 $\pm 0.123$	0.714 $\pm 0.123$	0.536 $\pm 0.123$	0 $\pm 0.122$	0.933 $\pm 0.122$	0 $\pm 0.209$	0.524 $\pm 0.123$	0.536 $\pm 0.123$
$\pi \pm SD$	0.001 $\pm 0.003$	0.012 $\pm 0.007$	0 $\pm 0.006$	0.005 $\pm 0.006$	0.005 $\pm 0.003$	0 $\pm 0.005$	0.008 $\pm 0.005$	0 $\pm 0.011$	0.019 $\pm 0.004$	0.006 $\pm 0.004$
<i>D</i>	-	1.199	-	0.806	1.764	-	-0.113	-	0.063	1.813
<i>Fs</i>	-	0.920	-	2.285	4.413	-	-0.807	-	5.712	5.011
<i>R<sup>2</sup></i>	0.500	0.223	-	0.226	0.268	-	0.203	-	0.176	0.268
<i>SSD</i>	0.000	0.039	0.000	0.111	<b>0.340</b>	0.000	0.028	0.000	<b>0.330</b>	0.000
<i>Rg</i>	0.000	0.060	0.000	0.204	0.790	0.000	0.093	0.000	0.685	0.789
<i>Tau</i>	0.000	9.797	0.000	7.072	6.211	0.000	5.328	0.000	0.000	0.000
<i>TSE</i>	-	12,756	-	9,208	8,087	-	6,937	-	-	-

**Table B. Results of modified STRUCTURE procedure.**

The most likely number of differentiated genetic groups ( $K = 4$ , column shaded in grey) found to better explain the variation observed in the genotypic dataset (ten microsatellite *loci*) of black-and-gold howler monkeys from Northeastern Argentina and Southern Paraguay. The Max-of-Median (*MaxMedK*) and Median-of-Median (*MedMedK*) indexes (Puechmaille, 2016), taken at the 0.80 threshold of membership proportion, were used as conservative estimators of  $K$ . The different sampling schemes tested were: 1) the full dataset ( $n = 138$ , ten sites), 2) subsampling Pop 2 and 3 to obtain more even sample sizes ( $n = 88$ , ten sites), 3) original dataset but removing the least sampled populations ( $n = 123$ , seven sites), and 4) the dataset obtained by a combination of the two latter strategies ( $n = 73$ , seven sites) (see main text for further details). For each sampling scheme, 20 replicate STRUCTURE runs were performed from  $K = 2$  to  $K = 10$ .

Sampling strategy	Index	<b><math>K=2</math></b>	<b><math>K=3</math></b>	<b><math>K=4</math></b>	<b><math>K=5</math></b>	<b><math>K=6</math></b>
1 – Full dataset	<i>MaxMedK_0.8</i>	2	3	4	4	3
1 – Full dataset	<i>MedMedK_0.8</i>	2	3	4	4	2
2 – Even sample sizes	<i>MaxMedK_0.8</i>	2	3	4	5	3
2 – Even sample sizes	<i>MedMedK_0.8</i>	2	3	4	3	2
3 – Remove samples $n \leq 6$	<i>MaxMedK_0.8</i>	2	3	4	5	4
3 – Remove samples $n \leq 6$	<i>MedMedK_0.8</i>	2	3	4	4	3
4 – Even sample sizes + remove samples $n \leq 6$	<i>MaxMedK_0.8</i>	2	3	4	4	4
4 – Even sample sizes + remove samples $n \leq 6$	<i>MedMedK_0.8</i>	2	3	4	2.5	4

**Table C. Genetic structure observed in populations of black-and-gold howler monkeys from Northeastern Argentina and Southern Paraguay.**

Pairwise  $F_{ST}$  values for ten microsatellite *loci* (below diagonal) and pairwise  $\Phi_{ST}$  values for 512-bp mtDNA Control Region fragment sequences (above diagonal). Significant values after Bonferroni correction are shown in bold ( $\alpha = 0.0005$ ).

	Pop 1	Pop 2	Pop 3	Pop 4	Pop 5	Pop 6	Pop 7	Pop 8	Pop 9	Pop 10
Pop 1		-0.013	0.710	0.585	0.582	0.985	0.509	0.961	0.505	0.601
Pop 2	0.039		0.352	0.164	0.141	0.468	0.263	0.345	0.506	0.287
Pop 3	<b>0.142</b>	<b>0.077</b>		<b>0.815</b>	<b>0.814</b>	<b>1.000</b>	<b>0.802</b>	<b>1.000</b>	<b>0.760</b>	<b>0.818</b>
Pop 4	0.050	0.015	<b>0.075</b>		0.062	<b>0.741</b>	0.438	0.628	0.577	0.442
Pop 5	<b>0.105</b>	<b>0.053</b>	<b>0.114</b>	0.039		<b>0.675</b>	0.433	0.686	0.599	<b>0.449</b>
Pop 6	<b>0.261</b>	<b>0.133</b>	<b>0.177</b>	<b>0.183</b>	<b>0.107</b>		<b>0.662</b>	<b>1.000</b>	<b>0.709</b>	<b>0.643</b>
Pop 7	<b>0.075</b>	<b>0.071</b>	<b>0.153</b>	0.006	<b>0.097</b>	<b>0.241</b>		0.544	0.579	0.309
Pop 8	<b>0.119</b>	<b>0.080</b>	<b>0.177</b>	<b>0.092</b>	<b>0.081</b>	<b>0.244</b>	0.029		0.562	0.543
Pop 9	<b>0.133</b>	<b>0.046</b>	<b>0.109</b>	<b>0.095</b>	0.044	<b>0.200</b>	0.028	0.027		<b>0.622</b>
Pop 10	<b>0.131</b>	<b>0.110</b>	<b>0.171</b>	0.063	<b>0.087</b>	<b>0.262</b>	-0.014	0.070	0.061	