

## SUPPORTING ONLINE INFORMATION

**Table S1.** Genes used to generate baits for capture enrichment.

<b>Gene</b>	<b>Length (bp)</b>	<b>NCBI accession</b>	<b>Species</b>
epsilon globin (HBE)	1706	AAA35404.1	<i>Cheracebus torquatus</i>
oxytocin receptor (promotor region) (OXTR)	1872	KF701386.1	<i>Plecturocebus cupreus</i>
Von Willebrand factor (vWF)	1238	JX856254	<i>Plecturocebus moloch</i>
interphotoreceptor retinoid binding (IRBP)	1275	JX856220.1	<i>Plecturocebus moloch</i>
growth hormone receptor (GHR)	904	JX856185.1	<i>Plecturocebus moloch</i>
taste receptor 2 member 38 (TAS2R38)	1002	JQ272231.1	<i>Plecturocebus moloch</i>
mitochondrial genome	16689	NC024630.1	<i>Cheracebus lugens</i>

**Table S2.** Genes used as reference sequences for alignment of raw reads during analysis.

<b>Gene</b>	<b>Length (bp)</b>	<b>Species</b>	<b>NCBI accession</b>
epsilon-globin	1706	<i>Callicebus toruatus</i>	AAA35404.1
oxytocin receptor (promotor region)	1872	<i>Callicebus cupreus</i>	KF701386.1
vWF (von Willebrand factor)	1238	<i>Callicebus moloch</i>	JX856254.1
IRBP (interphotoreceptor retinoid binding protein)	1275	<i>Callicebus moloch</i>	JX856220.1
GHR (growth hormone receptor)	904	<i>Callicebus moloch</i>	JX856185.1
TAS2R38 (Camopa bitter taste receptor)	1002	<i>Callicebus moloch</i>	JQ272231.1
mitochondrion whole genome	16689	<i>Callicebus lugens</i>	NC_024630.1
ABCA1 (ATP-binding cassette sub-family A)	804	<i>Callicebus lugens</i>	KU694299.1

**Table S3.** Substitution models chosen for the partitioned dataset.

<b>Subset</b>	<b>Best model</b>	<b>Subset Partitions</b>
Mitochondrial genome		
1	GTR+G	rrnL, rrnS, trnA, trnC, trnD, trnF, trnH, trnI, trnL1, trnL2, trnM, trnN, trnQ, trnR, trnS2, trnW, trnY
2	GTR+G	atp6, atp8, cob, cox3, nad1, nad2, nad3, nad4a, nad4b, nad4l, nad5, nad6, trnP, trnS1, trnV
3	GTR+G	cox1, cox2, trnE, trnG, trnK, trnT
<b>ADORA3</b> (adenosine A3 receptor)		
1	K80+G	gene
<b>APP</b> (Amyloid beta A4 protein)		
1	HKY	gene
<b>CREM</b> (CAMP Responsive Element Modulator)		
1	K80	gene
<b>DENND5A</b> (DENN Domain Containing 5A)		
1	K80	gene
<b>DMRT1</b> (Doublesex And Mab-3 Related Transcription Factor 1)		
1	HKY	gene
<b>ERC2</b> (ELKS/RAB6-Interacting/CAST Family Member 2)		
1	HKY	gene
<b>FAM123B</b> (APC Membrane Recruitment Protein 1)		
1	K80	gene
<b>FES</b> (FES Proto-Oncogene)		
1	HKY+G	gene
<b>FOX G1</b> (Forkhead box protein G1)		
1	HKY	gene
<b>MAPKAP1</b> (mitogen-activated protein kinase associated protein 1)		
1	HKY	gene

**Table S3** (continued).

<b>Subset</b>	<b>Best model</b>	<b>Subset Partitions</b>
1	HKY	gene
<b>NEGR1</b> (Neuronal Growth Regulator 1)		
1	F81	gene
<b>NPAS3</b> ( Neuronal PAS domain protein 3)		
1	HKY	gene
<b>RAG1</b> (Recombination Activating 1)		
1	K80+G	gene
<b>RAG2</b> (Recombination Activating 2)		
1	K80+G	gene
<b>RPGRIP1</b> (Retinitis Pigmentosa GTPase Regulator Interacting Protein 1)		
1	K80	gene
<b>SGMS1</b> (Sphingomyelin Synthase 1)		
1	F81	gene
<b>SIM1</b> (Single-minded homolog 1)		
1	F81	gene
<b>ZFX</b> (Zinc Finger Protein)		
1	HKY	gene

**Table S4.** Tests of alternative topologies suggested by previous studies (H1-H5; see main text, Fig. 1) and the new topology derived in this study from ancient DNA data (H6), made by submitting the sitewise log-likelihood values obtained from RAxML v.8 to CONSEL to calculate the P-values for each tree topology by using the AU test.

Hypothesis number	obs p-value	au p-value	np p-value	bp p-value	pp p-value	kh p-value	sh p-value	wsh p-value
<b>H1</b>	-430.9	1	1	1	1	1	1	1
<b>H2</b>	430.9	$1 \times 10^{-50}$	$4 \times 10^{-16}$	0	$7 \times 10^{-188}$	0	0	0
<b>H3</b>	722.6	0.003	$5 \times 10^{-8}$	0	$1 \times 10^{-314}$	0	0	0
<b>H4</b>	760.7	0.0002	0.0001	0	0	0	0	0
<b>H5</b>	543.9	$2 \times 10^{-53}$	$4 \times 10^{-19}$	0	$3 \times 10^{-228}$	0	0	0
<b>H6</b>	-523.9	1	1	1	1	1	1	1

**Table S5.** Fossil constraints and priors used in divergence date analysis.

<b>Root</b>	<b>Node</b>	<b>Fossil</b>	<b>Age</b>	<b>Prior distribution</b>
1	<b>Cebidae</b> <i>Cebus albifrons</i> – <i>Saimiri sciureus</i>	<i>Neosaimiri</i>	Mid Miocene (12.5 Ma)	logNormalPrior mean="1.287" stdev="0.8" offset="12.5"
2	<b>Pitheciidae</b> <i>Cacajao calvus</i> – <i>Chiropotes israelita</i> – <i>Pithecia pithecia</i>	<i>Proteropithecia</i>	Mid Miocene (15.7 Ma)	logNormalPrior mean="1.016" stdev="0.8" offset="15.7"

**Table S6.** Comparison of pre- and post-addition of target capture enrichment baits sequencing results, including number of mapped reads and average coverage of reads mapped to different genes.

Sample ID	Taxon	Total reads	Gene	Average coverage	Reads mapped	Sequencing method
AMNH 268010	<i>Xenothrix</i>	6,833,646	(NC_024630) <b>Whole mtgenome</b>	0.56	438	NextSeq
		after trim:	(JX856254)	3.66	253	
		<b>6,822,002</b>	(KT182969)	9.75	600	
AMNH 268010	<i>Xenothrix</i>	47,038,996	(NC_024630) <b>Whole mtgenome</b>	13.81	8,567	NextSeq <b>Capture</b>
		after trim:	(JX856254)	84.02	5,708	
		<b>39,632,332</b>	(KT182969)	48.51	3,236	

**Table S7.** BLAST results from the NCBI database for reads mapped to reference sequences.

Species mapped to: <i>Callicebus lugens</i>	Genbank ID NC_024630.1	
1st BLAST hit		
Description <i>Callicebus lugens</i> mt genome	Query cover 77%	Identity 85%
2nd BLAST hit		
Description <i>Callicebus cupreus</i> mt genome	Query cover 28%	Identity 78%
3rd BLAST hit		
Description <i>Callicebus donacophilus</i> mt genome	Query cover 36%	Identity 77%
Other hits: <i>Callicebus</i> 36-77%, <i>Alouatta</i> 19%, <i>Aotus</i> 13-18%, <i>Saguinus</i> 11%, <i>Callithrix</i> 14%, <i>Leontopithecus</i> 5%, <i>Cebus</i> 5%, and <i>Homo</i> below 13%		
Species mapped to: <i>Tarsius bancanus</i>	Genbank ID NC_002811.1	
1st BLAST hit		
Description <i>Tarsius bancanus</i> mt genome	Query cover 82%	Identity 84%
2nd BLAST hit		
Description <i>Canis adustus</i> mt genome	Query cover 9%	Identity 75%
3rd BLAST hit		
Description <i>Mustela</i>	Query cover 19%	Identity 74%
Other hits: <i>Tarsius</i> 7-82%, <i>Canis</i> 9-10%, <i>Mustela</i> 19%, <i>Cercocebus</i> 4%, <i>Pan</i> 12%		



Species mapped to: <i>Leontopithecus rosalia</i>	Genbank ID NC_021952.1	
1st BLAST hit		
Description <i>Leontopithecus</i> whole mt genome	Query cover 69%	Identity 89%
2nd BLAST hit		
Description <i>Homo</i> secernin (SCRN1)	Query cover 1%	Identity 95%
3rd BLAST hit		
Description <i>Pan troglodytes</i> BAC CH251-499L3	Query cover 1%	Identity 94%
Other hits: <i>Leontopithecus rosalia</i> 69%, <i>Homo</i> and <i>Pan</i> below 1%		

Species mapped to: <i>Callicebus donacophilus</i>	Genbank ID NC_019801.1	
1st BLAST hit		
Description <i>Callicebus donacophilus</i> mt genome	Query cover 69%	Identity 86%
2nd BLAST hit		
Description <i>Callicebus moloch</i> 16s	Query cover 5%	Identity 86%
3rd BLAST hit		
Description <i>Homo</i> nmt	Query cover 9%	Identity 77%
Other hits: <i>Callicebus</i> 2-65%, <i>Homo</i> below 9%		

Species mapped to: <i>Saguinus oedipus</i>	Genbank ID NC_021960.1	
1st BLAST hit		
Description <i>Saguinus</i> mt genome	Query cover 76%	Identity 85%
2nd BLAST hit		
Description <i>Callithrix</i> mt genome	Query cover 9%	Identity 79%
3rd BLAST hit		
Description <i>Leontopithecus</i>	Query cover 3%	Identity 81%
Other hits: <i>Saguinus</i> 1-76%, <i>Callithrix</i> 9%, <i>Leontopithecus</i> 3%, <i>Alouatta</i> 3%, <i>Aotus</i> 7%, <i>Lagothrix</i> 13%, <i>Cebus</i> 55%, <i>Ateles</i> 1%		

Species mapped to: <i>Macaca fuscata</i>	Genbank ID NC_025513.1	
1st BLAST hit		
Description <i>Macaca fuscata</i> mt genome	Query cover 90%	Identity 85%
2nd BLAST hit		
Description <i>Macaca mulatta</i> mt genome	Query cover 85%	Identity 82%
3rd BLAST hit		
Description <i>Macaca arctoides</i> mt genome	Query cover 79%	Identity 80%
Other hits: All <i>Macaca</i> 6-90%		

Species mapped to: *Alouatta seniculus* Genbank ID  
gi|927371820|ref|NC\_027825.1

1st BLAST hit

Description	Query cover	Identity
<i>Alouatta seniculus</i> mt genome	68%	89%

2nd BLAST hit

Description	Query cover	Identity
<i>Ateles belzebuth</i> mt genome	14%	79%

3rd BLAST hit

Description	Query cover	Identity
<i>Lagothrix</i> mt genome	13%	79%

Other hits:

*Alouatta* 11-68%, *Ateles* 8-14%, *Pan* and *Homo* below 12%



**Fig. S1.** *Xenothrix* specimens from Somerville Cave, Clavendon, Jackson's Bay, Jamaica, which were selected for aDNA extraction in this study. **(A)** femur, AMNH 268003; **(B)** proximal ulna, AMNH 268010.

Fig. S2a. Coverage of the reference (whole mitochondrial genome) by mapped reads. Reads mapped using CLC genomics work bench v.8 (CLC Bio-Qiagen, Aarhus, Denmark)

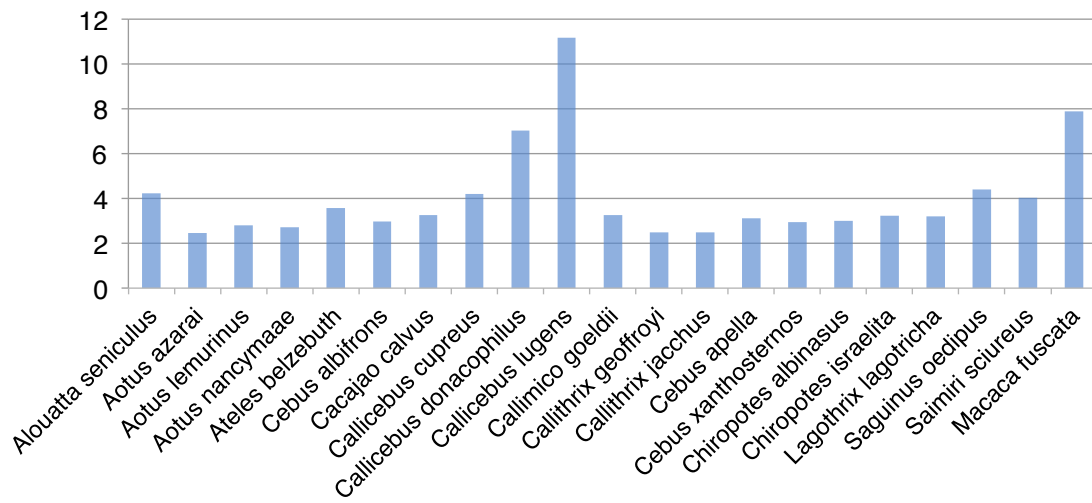
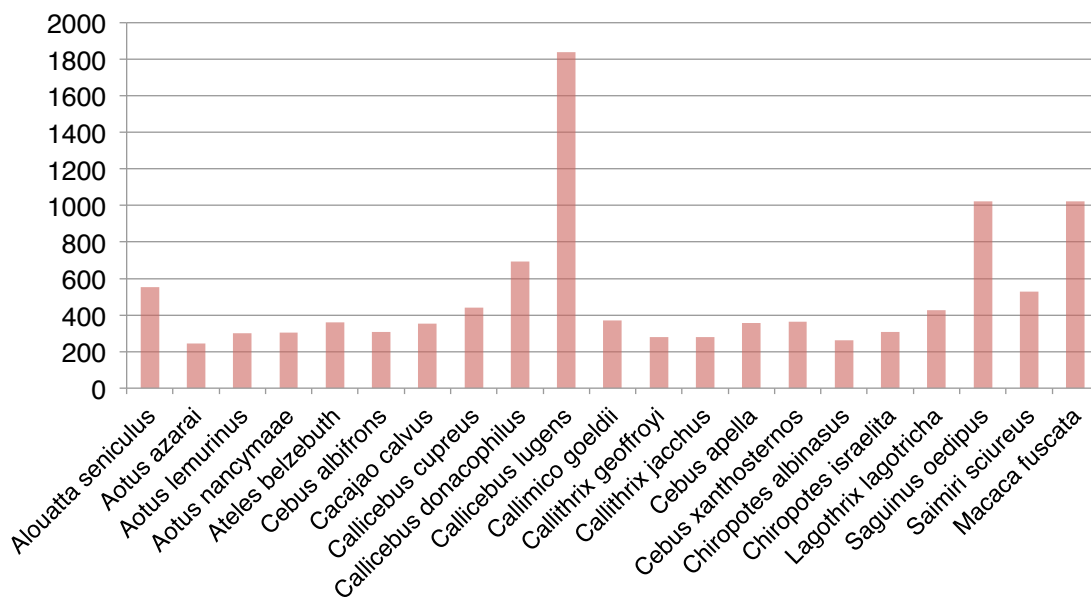
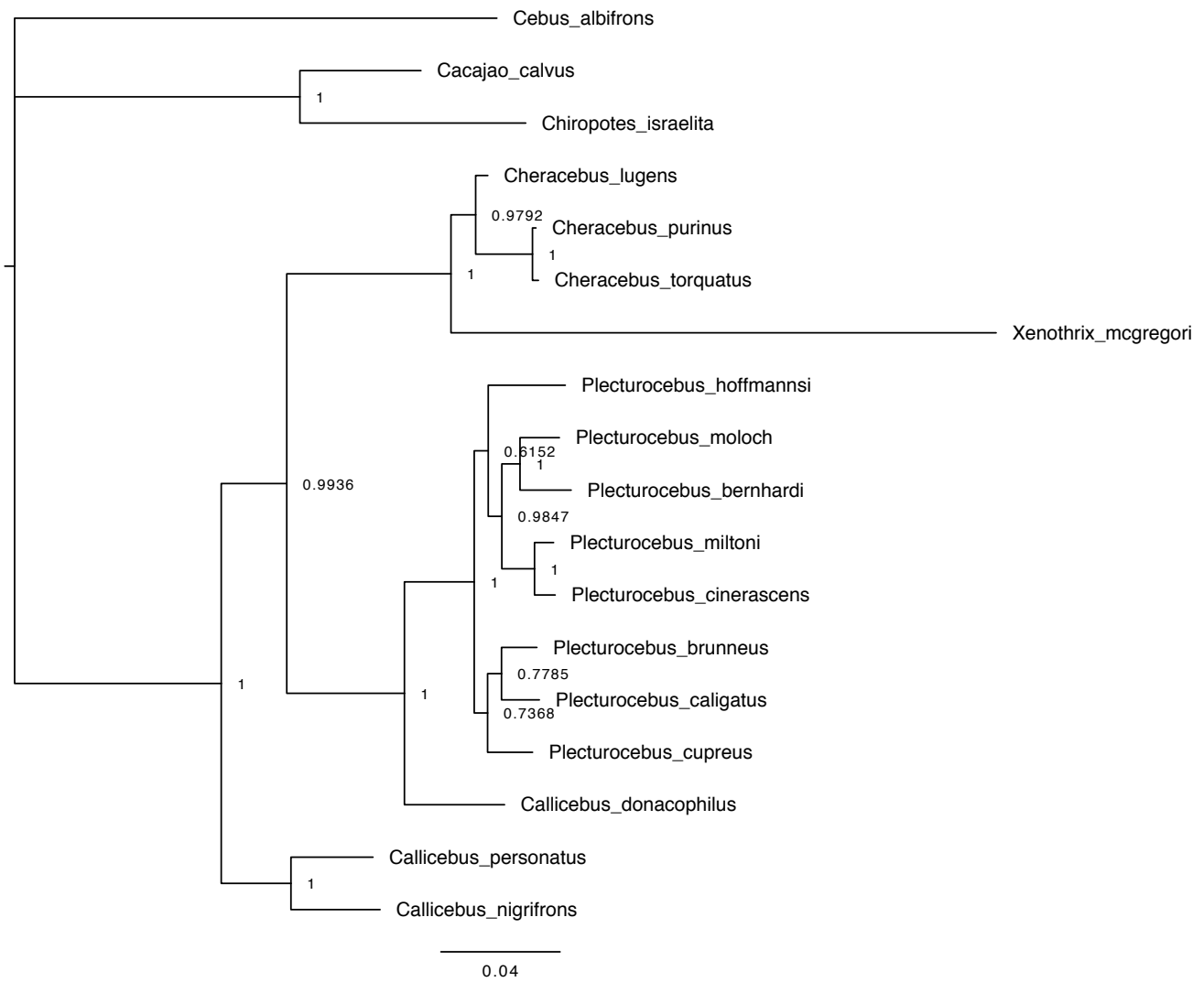


Fig. S2b. Total number of reads mapped to the reference (whole mitochondrial genome). Reads mapped using CLC genomics work bench v.8 (CLC Bio-Qiagen, Aarhus, Denmark)





**Fig. S3.** Bayesian tree constructed using MrBayes. Node values represent posterior probabilities. Scale indicates nucleotide substitutions per site.