

Supplementary Materials

Table S1. Gibbon samples used in the present study

Genus	Species	owner's ID/Barcode ID	Working ID ^b	Origin ^a	Current deposition ^a	
<i>Hylobates</i>	<i>agilis</i>	20050293D10	<u>T02</u>	Taipei Zoo	Taipei Zoo	
		20060466D10	T03	Taipei Zoo	Taipei Zoo	
		1138	1138	Bristol Zoo	German Primate Center	
	<i>lar</i>	20040083D10	<u>T05</u>	Taipei Zoo	Taipei Zoo	
		20040111D10	T08	Taipei Zoo	Taipei Zoo	
		20040112D10	<u>T09</u>	Taipei Zoo	Taipei Zoo	
		20040286D10	<u>T13</u>	Taipei Zoo	Taipei Zoo	
		23	23	Wuppertal Zoo	German Primate Center	
		103	103	Nuremberg Zoo	German Primate Center	
		501	<u>501</u>	Nuremberg Zoo	German Primate Center	
		2356	<u>J09</u>	Dusit Zoo	WRC, Kyoto University	
		1982	<u>J27</u>	Chiang Mai Zoo	WRC, Kyoto University	
		3400	<u>J29</u>	PRI, Kyoto University	WRC, Kyoto University	
		<i>muelleri</i>	1058	<u>1058</u>	Rostock Zoo	German Primate Center
		<i>klossii</i>	1230	<u>1230</u>	Twycross Zoo	German Primate Center
		<i>moloch</i>	2488	<u>J13</u>	Ragunan Zoo	WRC, Kyoto University
			1228	1228	Twycross Zoo	German Primate Center
<i>pileatus</i>	2360	<u>J16</u>	Dusit Zoo	WRC, Kyoto University		
	2361	<u>J17</u>	Dusit Zoo	WRC, Kyoto University		
<i>Symphalangus</i>	<i>syndactylus</i>	20060592D10	<u>T21</u>	Taipei Zoo	Taipei Zoo	
		980	<u>980</u>	Krefeld Zoo	German Primate Center	
		1973	<u>J33</u>	Padang, Sumatra, Indonesia	WRC, Kyoto University	
<i>Nomascus</i>	<i>leucogenys</i>	1006	<u>1006</u>	Duisburg Zoo	German Primate Center	
	<i>gabriellae</i>	Arnold	<u>Arnold</u>	Leipzig Zoo	MPI-EVA	
		Falco	<u>Falco</u>	Leipzig Zoo	MPI-EVA	
	<i>concolor</i>	1231	<u>Nc</u>	Twycross Zoo	German Primate Center	

^a Abbreviations: PRI, Primate Research Institute; WRC, Wildlife Research Center; MPI-EVA, Max-Planck Institute for Evolutionary Anthropology

^b The mtgenome sequences of the individuals underlined are retrieved for the reconstruction of mtgenome phylogeny, in which individuals T02, T05 and T09 are the sons of individuals T01, T05 and T10, respectively, as shown in Figure 2.

Table S2. List of primers tested in this study for the amplification of the non-recombining portion of Y chromosome

NCBI name	Gene name*	primer pair	Reference	Note
<i>DAZI</i>	DAZ	1990FY x 3760RYsiam	Makova and Li, 2002 [1]	Applied to all male individuals
		6996FY x 8754RY	Makova and Li, 2002 [1]	Failed to amplify in every species
		18219FY x 19599RY	Makova and Li, 2002 [1]	Applied to all male individuals
		18264F x 21252RY	Makova and Li, 2002 [1]	Amplification occurred in both sexes
		2384FY x 4809RY	Makova and Li, 2002 [1]	Failed to amplify in every species
		25321FY x 27521RY	Makova and Li, 2002 [1]	Failed to amplify in every species
<i>DDX3Y</i>	DBY	DBY1-F x DBY1-R	Hellborg and Ellegren, 2003 [2]	Failed to amplify in every species
		DBY5-F x DBY5-R	Hellborg and Ellegren, 2003 [2]	Applied to all male individuals
		DBY6-F x DBY6-R	Hellborg and Ellegren, 2003 [2]	Failed to amplify in every species
<i>EIF1AY</i>	EIF1AY	EI-1 x EI-2	Hong et al., 2007 [3]	Failed to amplify in every species
<i>RPS4Y1</i>	RPS4Y	RPS4Y4-F x RPS4Y4-R	Hellborg and Ellegren, 2003 [2]	Applied to all male individuals
		RPS4Y-F x RPS4Y-R	Samollow et al., 1996 [4]	Failed to amplify in every species
<i>KDM5D</i>	SMCY	SMCY7-F x SMCY7-R	Hellborg and Ellegren, 2003 [2]	Applied to all male individuals
		SMCY11-F x SMCY11-R	Hellborg and Ellegren, 2003 [2]	Failed to amplify in every species
<i>TSPY</i>	TSPY	TSPY-A x TSPY-B	Kim et al., 1996 [5]	Applied to all male individuals
<i>UTY</i>	UTY	UTY11-F x UTY11-R	Hellborg and Ellegren, 2003 [2]	Applied to all male individuals

*Gene name aliases used in the references and this study.

Table S3. Primer sequences, sources of primers, and PCR conditions used in this study

Locus	Primer name	Primer sequence (5'-3')	PCR condition	Estimated size (bp)	PCR Master Mix*
DAZ	1990FY (DAZ-1F)	F: TGGGGAAACATAAGGGAGAAAAAAGTA	92°C 2 min, (92°C 10 sec, 57°C 15 sec, 68°C 4 min) × 9 cycles, (92°C 10 sec, 57°C 15 sec, 68°C 4 min) × 21 cycles with 20 sec extension/cycle, 68°C 7 min	1350	A
	3760RYsiam (DAZ-1R)	R: GGCTTGGCACGGAAAGACG			
DAZ	18219FY (DAZ-2F)	F: GCTTAATCCAAACAATCAGTGAGACA	92°C 2 min, (92°C 10 sec, 57°C 15 sec, 68°C 4 min) × 9 cycles, (92°C 10 sec, 57°C 15 sec, 68°C 4 min) × 21 cycles with 20 sec extension/ cycle, 68°C 7 min	1600	A
	19599RY (DAZ-2R)	R: GCCTTAAC TT TAAGAGAATATTGGGGA			
DBY	DBY5-F	F: CTTTGAGAAATATGATGATATA	95°C 10 min, touchdown with an annealing temperature of 55°-45°C over 20 cycles, (95°C 1 min, 45°C 1 min, 72°C 1 min 30 sec) × 30 cycles, 72°C 10 min	750	B
	DBY5-R	R: CGAGTAAGTTCAATGTTC			
RPS4Y	RPS4Y4-F	F: GAACAGATTCTCTTCCGTCG	95°C 10 min, touchdown with an annealing temperature of 63°-53°C over 20 cycles, (95°C 1 min, 53°C 1 min, 72°C 1 min 30 sec) × 30 cycles, 72°C 10 min	600	B
	RPS4Y4-R	R: GCTGCAACACGCTTTAAGTG			
SMCY	SMCY7-F	F: TGGAGGTGCCCRAARTGTA	95°C 10 min, touchdown with an annealing temperature of 63°-53°C over 20 cycles, (95°C 1 min, 53°C 1 min, 72°C 1 min 30 sec) × 30 cycles, 72°C 10 min	500	B
	SMCY7-R	R: AACTCTGCAA STR TACTCCT			
TSPY	TSPY-A	F: AGCCAGGAAGGCCTTTTCTCG	94°C 4 min, (94°C 1 min, 60°C 1 min, 72°C 1 min 30 sec) × 30 cycles, 72°C 7 min	780	B
	TSPY-B	R: CCATGTAGCTCAGCATGTCTTCAT			
UTY	UTY11-F	F: CATCAATTTTGTAYMAATCCAAAA	95°C 10 min, touchdown with an annealing temperature of 63°-53°C over 20 cycles, (95°C 1 min, 53°C 1 min, 72°C 1 min 30 sec) × 30 cycles, 72°C 10 min	900	B
	UTY11-R	R: TGGTAGAGAAAAGTCCAAGA			

*The final concentration of each reagent in a PCR Master Mix of 50 ul reaction volume. A, a PCR Master Mix consists of 100 ng of the purified WGA products, 0.3 μM of each forward and reverse primer, 500 μM of each dNTP, and 3.5 units of the Expand Long Range Enzyme mix (Roche) with 1X Expand Long Range Buffer containing MgCl₂. B, a PCR Master Mix consists of 100 ng of the purified WGA products, 2.2 mM MgCl₂, 0.2 μM of each forward and reverse primer, 200 μM of each dNTP, and 2 units of AmpliTaq Gold DNA polymerase (Applied Biosystems, Roche) with 1X AmpliTaq Gold PCR buffer II.

Table S4. GenBank IDs for outgroup sequences and newly obtained sequences of gibbons in this study

Amplicons	<i>Homo sapiens</i>	<i>Pan troglodytes</i>	<i>Macaca mulatta</i>	Gibbons
DAZ-1	AF483577	AC147143 (62937-64614)	AC217138 (113167-114796)	JN871346-JN871371
DAZ-2	AF483578	AC147143 (51140-52443)	AC217138 (98953-100288)	JN871320-JN871345
DBY	NG_012831 (13912-14646)	NC_006492 (18025162-18025912)	AC213321 (74334-75043)	JN871424-JN871449
RPS4Y	AF041427 (19681-20250)	NC_006492 (23753790-23754359)	AC217136 (84539-85106)	JN871398-JN871423
SMCY	AF273841 (45121-45592)	AF440161	AC215644 (72205-71739)	JN871476-JN871501
TSPY	M98524 (1158-1895)	Kim and Takenaka 1996*	AF425276	JN871372-JN871397
UTY	AF265575	NC_006492 (18457945-18458832)	AC212004 (1240-2129)	JN871450-JN871475

The numbers in parenthesis next to the GenBank accession numbers indicate the regions used as outgroup sequences in the phylogenetic analyses. * The TSPY sequence of *Pan troglodytes* is shown in Kim and Takenaka (1996) [6].

References

1. Makova KD, Li W-H: **Strong male-driven evolution of DNA sequences in humans and apes.** *Nature* 2002, **416**:624-626.
2. Hellborg L, Ellegren H: **Y chromosome conserved anchored tagged sequences (YCATS) for the analysis of mammalian male-specific DNA.** *Mol Ecol* 2003, **12**:283-291.
3. Hong KW, Huh JW, Kim DS, Ha HS, Kim HS: **Molecular relationship of hylobates based on Alu elements of the Y chromosome.** *Korean J Genet* 2007, **29**:379-387.
4. Samollow PB, Cherry LM, Witte SM, Rogers J: **Interspecific variation at the Y-linked RPS4Y locus in hominoids: implications for phylogeny.** *Am J Phys Anthropol* 1996, **101**:333-343.
5. Kim HS, Hirai H, Takenaka O: **Molecular features of the TSPY gene of gibbons and Old World monkeys.** *Chromosome Res* 1996, **4**:500-506.
6. Kim HS, Takenaka O: **A comparison of TSPY genes from Y-chromosomal DNA of the great apes and humans: sequence, evolution, and phylogeny.** *Am J Phys Anthropol* 1996, **100**:301-309.