

## Supplementary Information

### **The complex evolutionary history of big-eared horseshoe bats (*Rhinolophus macrotis* complex): insights from genetic, morphological and acoustic data**

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**Table S1** Sample information.

Colony	Sampling locality	Longitude	Latitude	Nmt	cytb haplotype	control region haplotype	Nnc	Chd1 haplotype
<b>small form (mt Clade 1)</b>				36			35	
<b>GD1</b>	Yangchun, Guangdong Province	111.94	22.44	1	H1	H1	1	H1, H20
<b>GD2</b>	Shaoguan, Guangdong Province	113.56	24.77	10	H2	H2-H9	9	H2, H20
<b>GX</b>	Nanning, Guangxi Province	108.70	22.82	5	H3	H10-H13	5	H2, H20
<b>HuN</b>	Lengshuijiang, Hunan Province	111.57	27.75	3	H2	H14	3	H2, H20
<b>JX1</b>	Jianggangshan, Jiangxi Province	114.20	26.60	1	H2	H15	4	H2, H3, H20
<b>YN1</b>	Jinning, Yunnan Province	102.33	24.50	4	H4-H6	H16-H18	4	H7, H8, H20
<b>YN2</b>	Yuanjiang, Yunnan Province	102.00	23.59	7	H7, H8	H19- H21	5	H2, H20
<b>YN3</b>	Baoshan, Yunnan Province	99.10	25.08	5	H9, H10	H22-H24	4	H4-H8, H20
<b>large form (mt Clade 2)</b>				22			19	
<b>CQ</b>	Wulong, Chongqing City	107.95	29.27	2	H11	H25	2	H12, H18, H20
<b>GD1</b>	Yangchun, Guangdong Province	111.94	22.44	6	H12, H13	H26, H27	6	H14-H18
<b>GZ</b>	Xishui, Guizhou Province	106.21	28.45	4	H14-H16	H28-H31	3	H10, H11, H18, H20
<b>SC</b>	Dazhou, Sichuan Province	107.74	31.20	6	H17, H18	H32, H33	6	H10, H11, H18-H20
<b>SXi</b>	Hanzhong, Shannxi Province	107.03	32.84	3	H18, H19	H32-H35	2	H9, H18, H20
<b>HeN</b>	Xichuan, Henan Province	111.55	32.87	1	H19	H36	0	—
<b>large form (mt Clade 3)</b>				21			17	
<b>CQ</b>	Wulong, Chongqing City	107.95	29.27	1	H20	H37	1	H18, H20
<b>HuN</b>	Lengshuijiang, Hunan Province	111.57	27.75	5	H21-H24	H38-H42	6	H15, H17, H18, H20
<b>JX1</b>	Jianggangshan, Jiangxi Province	114.20	26.60	6	H25, H26	H43-H46	4	H15, H17, H18
<b>JX2</b>	Ganzhou, Jiangxi Province	114.09	25.46	5	H27, H28	H47- H49	2	H18, H20
<b>SXi</b>	Hanzhong, Shannxi Province	107.03	32.84	4	H29	H50	4	H9, H18

Nmt, the number of mitochondrial DNA sequences. Nnc, the number of nuclear Chd1 gene sequences.

**Table S2** Diversity estimates for each form. Number of individuals (N), mean allele number (A), allelic richness ( $A_R$ ), expected heterozygosity ( $H_o$ ) and observed heterozygosity ( $H_e$ ).

<b>Taxon</b>	<b>Populations</b>	<b>N</b>	<b>A</b>	<b><math>A_R</math></b>	<b><math>H_E</math></b>	<b><math>H_O</math></b>
large form	GZ	4.0	5.38	1.86	0.86	0.84
	CQ	4.0	5.25	1.87	0.87	0.72
	SC	6.0	5.75	1.81	0.81	0.77
	SiX	7.0	5.75	1.78	0.78	0.71
	HeN	2.0	—	—	0.81	0.69
	JX1	5.0	6.00	1.85	0.85	0.83
	JX2	6.0	6.50	1.86	0.86	0.83
	GD2	6.0	4.38	1.71	0.71	0.67
	HuN	6.0	6.25	1.85	0.85	0.77
small form	HuN	3.0	3.75	1.80	0.80	0.75
	YN1	4.0	5.13	1.81	0.81	0.81
	YN2	5.0	5.25	1.80	0.80	0.73
	YN3	5.0	4.25	1.69	0.69	0.58
	GX	5.0	4.38	1.74	0.74	0.75
	GD1	9.0	4.38	1.69	0.69	0.71
	GD2	1.0	—	—	—	—

**Table S3** Resting frequency (RF) in echolocation calls and forearm length (FA) of the large form and small form in the *R. macrotis* complex populations from China.

Taxon and colony	mRF(kHz)			FA(mm)		
	Nf/Nm	female	male	Nf/Nm	female	male
<b>large form (<i>mt</i> Clade 2)</b>						
<b>CQ</b>	0/2	—	50.05	0/2	—	44.84
<b>GD1</b>	2/4	52.78	51.66	2/4	48.21	46.47
<b>GZ</b>	0/4	—	49.33	0/4	—	45.63
<b>SC</b>	2/4	49.80	49.86	2/4	45.22	47.51
<b>SXi</b>	3/0	52.82	—	3/0	46.71	—
<b>HeN</b>	0/2	—	54.44	0/2	—	44.95
<b>large form (<i>mt</i> Clade 3)</b>						
<b>CQ</b>	0/1	—	49.32	0/1	—	46.94
<b>HuN</b>	10/12	46.52	46.16	10/12	48.45	47.25
<b>JX1</b>	9/2	49.39	46.80	9/2	45.17	48.00
<b>JX2</b>	4/2	47.64	46.50	4/2	49.60	49.62
<b>SXi</b>	4/0	52.26	—	4/0	—	46.93
<b>Small form (<i>mt</i> Clade 1)</b>						
<b>GD1</b>	0/0	—	—	—	—	39.57
<b>GD2</b>	8/3	69.01	67.79	12/3	40.12	38.50
<b>GX</b>	1/2	67.76	66.29	2/3	39.75	39.67
<b>HuN</b>	2/1	67.12	66.06	2/1	39.95	39.69
<b>JX1</b>	2/0	64.70	—	2/0	39.75	—
<b>YN1</b>	3/9	57.83	57.41	6/14	43.42	41.93
<b>YN2</b>	0/0	—	—	3/7	40	39.14
<b>YN3</b>	0/0	—	—	11/1	43.05	45

**Fig. S1** Phylogenetic tree based on the analysis of the mitochondrial *Cytb* of the *R. macrotis* complex. Numbers above the branches are the bootstrap support obtained in maximum likelihood and posterior probabilities from Bayesian analyses.

**Fig. S2** Phylogenetic tree based on the analysis of the mitochondrial control region of the *R. macrotis* complex. Numbers above the branches are the bootstrap support obtained in maximum likelihood and posterior probabilities from Bayesian analyses.

**Fig. S3** Output from Geneland showing assignments to clusters for  $K = 2$ . Black dots represent localities analysed in this study. In five independent runs, three runs resulted in the clusters of (a) and (b), while two runs resulted in the clusters of (c) and (d).

**Fig. S4** Phylogenetic tree based on the analysis of the mitochondrial *Cytb* of all the *R. macrotis* complex and *Rhinolophus sp1.* (EU434937). Numbers above the branches are the bootstrap support obtained in maximum likelihood analyse.

Fig. S1

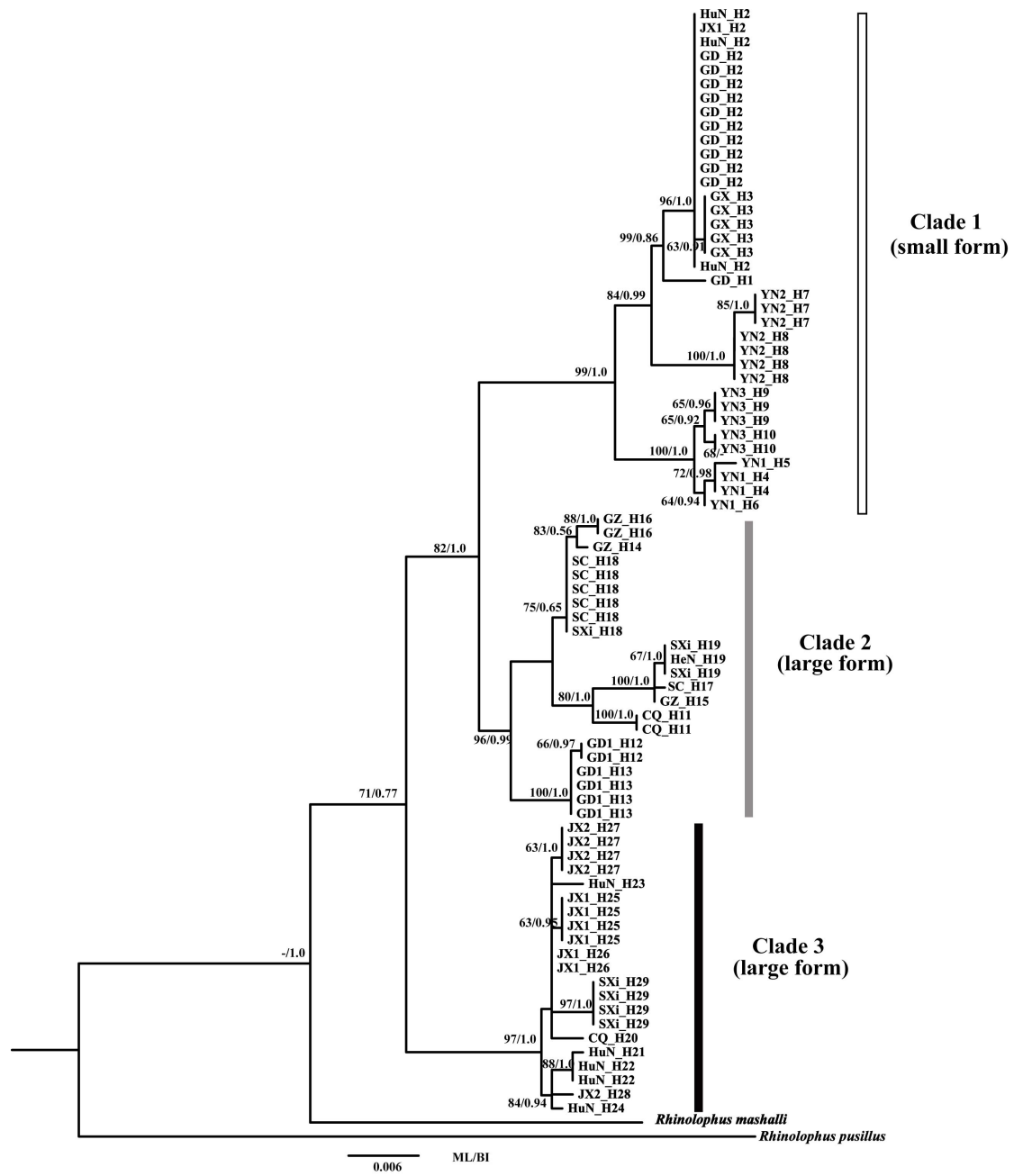
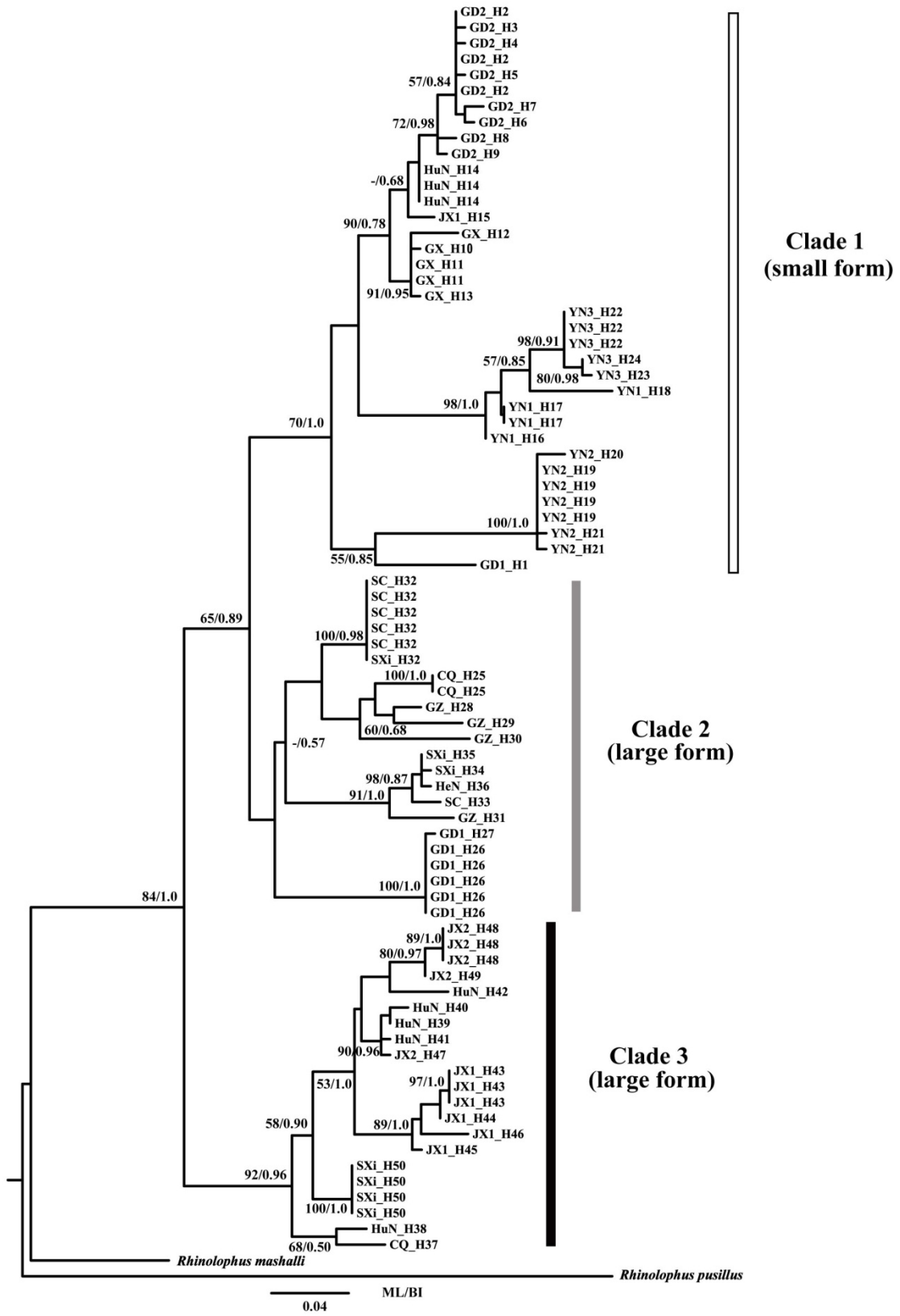


Fig. S2



**Fig. S3**

