

## **Supplementary Materials and Methods**

### **Sample sources**

Four samples of *M. gongshanensis*, including one antler, two skulls, and one skin, were obtained from northern Myanmar between 2016 and 2018, with permission (1567/XTBG/2017) from the Forest Research Institute, Forest Department Ministry of Environmental Conservation and Forestry of Myanmar. All samples were stored in 95% ethanol (Supplementary Table S2). Total genomic DNA was extracted using a TIANamp Genomic DNA Kit (Tiangen, Beijing, China). Specimens and DNA were deposited in the Southeast Asia Biodiversity Research Institute, Chinese Academy of Sciences, Myanmar Lab (Yezin, Nay Pyi Taw, Myanmar). Based on previous studies (Li et al., 2017; Martins et al., 2017; Zhang et al., 2004), 14 primers were designed to amplify mitogenomes of three samples. Primer synthesis and sequencing were conducted by Beijing Tianyi Huiyuan Bioscience and Technology Incorporation (Beijing, China). In addition, the mitochondrial gene cyt *b* of the fourth sample was amplified (Supplementary Table S2).

### **Data analysis**

Sequences were edited using DNASTAR v.5.0 and aligned (mitogenomes and cyt *b* gene fragments in the same file) using default parameters in MAFFT v.7.245 (Rozewicki et al., 2019). Mitogenome sequences were assembled and annotated using software Geneious v 2019.1.1 (Kearse et al., 2012). Locations of genes were annotated by comparison with genes from *M. crinifrons* (NC\_004577). Identical haplotypes were collapsed using DNASP v.5.10 (Rozas et al., 2017) by removing

missing/gap sites and invariant sites from the dataset and the median-joining network was reconstructed using Network v.4.6.1.4 (Leigh & Bryant, 2015) based on *cyt b* gene fragments for *M. gongshanensis*, *M. crinifrons*, and *M. feae*.

Phylogenies were reconstructed using two different methods: maximum-likelihood (ML) and Bayesian inference (BI). To assess the phylogenetic relationships of *Muntiacus*, complete and partial mitochondrial sequences of *cyt b* were downloaded from GenBank, and mitogenome sequences of *Tragulus kanchil* (JN632709) and *Elaphodus cephalophorus* (DQ873526) were used as the outgroup according to Li et al. (2017). The best-fit model of nucleotide substitution for each marker was selected prior to analysis using the Akaike Information Criterion, implemented in the model selected by JModelTest 2 (Darriba et al., 2012). HKY+G was selected for mitochondrial DNA (mtDNA) sequences. Bayesian analyses were run in MrBayes v.3.2.2 (Ronquist et al., 2012) and consisted of two independent runs of 10 million generations, sampling trees every 100 generations under default priors. The average standard deviation of split frequencies between runs was always <0.001, indicating convergence. The first 25% of sampled trees were discarded as burn-in and the remaining topologies were combined to generate a 50% majority rule consensus tree. ML analysis was conducted using RAxML v.8.2.10 (Stamatakis, 2014) on the CIPRES Science Gateway (<http://www.phylo.org>). Rate heterogeneity and proportion of invariant sites were included as model parameters, with other options matching default settings. Support values for nodes and clades were estimated from 1 000 bootstrap replicates.

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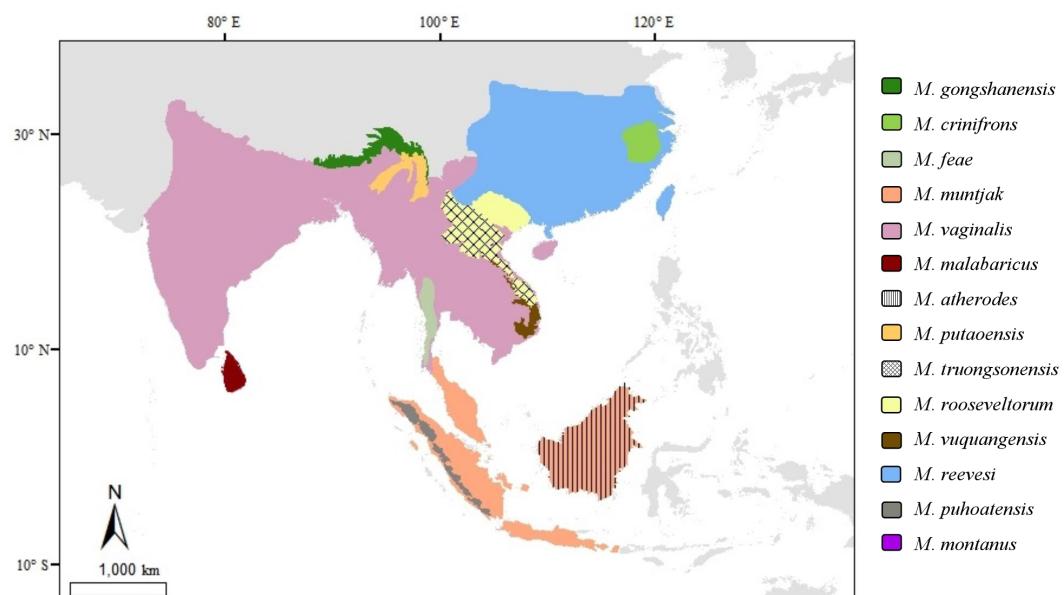
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## SUPPLEMENTARY FIGURE LEGENDS



Supplementary Figure S1 Currently accepted distribution of 14 muntjac species (IUCN). Map shows locations of *Muntiacus* species (adapted from: [www.maps.iucnredlist.org](http://www.maps.iucnredlist.org)).

**Supplementary Table S1** Comparison of number of accepted *Muntiacus* species among four recent publications

Species name	Ma et al. (1986)	Groves & Grubb (2011)	IUCN (2018)	This study	Red List Category
<i>M. muntjak</i> (Zimmermann, 1780)	<i>M. muntjak</i>	<i>M. muntjak</i>	<i>M. muntjak</i>	<i>M. muntjak</i>	LC
<i>M. vaginalis</i> (Boddaert, 1785)		<i>M. vaginalis</i>	<i>M. vaginalis</i>	<i>M. vaginalis</i>	LC
<i>M. aureus</i> (Hamilton Smith, 1826)		<i>M. aureus</i>			
<i>M. nigripes</i> (G. M. Allen, 1930)		<i>M. nigripes</i>			
<i>M. malabaricus</i> (Lydekker, 1915)		<i>M. malabaricus</i>		<i>M. malabaricus</i>	
<i>M. crinifrons</i> (Sclater, 1885)	<i>M. crinifrons</i>	<i>M. crinifrons</i>	<i>M. crinifrons</i>	<i>M. crinifrons</i>	VU
<i>M. gongshanensis</i> (Ma et al., 1990)		<i>M. gongshanensis</i>	<i>M. gongshanensis</i>	<i>M. gongshanensis</i>	DD
<i>M. feae</i> (Thomas & Doria, 1889)	<i>M. feae</i>	<i>M. feae</i>	<i>M. feae</i>	<i>M. feae</i>	DD
<i>M. vuquangensis</i> (Tuoc et al., 1994)		<i>M. vuquangensis</i>	<i>M. vuquangensis</i>	<i>M. vuquangensis</i>	CR

<i>M. rooseveltorum</i> (Osgood, 1932)	<i>M. rooseveltorum</i>	<i>M. rooseveltorum</i>	<i>M. rooseveltorum</i>	<i>M. rooseveltorum</i>	DD
<i>M. putaoensis</i> (Amato et al., 1999)		<i>M. putaoensis</i>	<i>M. putaoensis</i>	<i>M. putaoensis</i>	DD
<i>M. truongsonensis</i> Giao et al., 1998)		<i>M. truongsonensis</i>	<i>M. truongsonensis</i>	<i>M. truongsonensis</i>	DD
<i>M. reevesi</i> (Ogilby, 1839)	<i>M. reevesi</i>	<i>M. reevesi</i>	<i>M. reevesi</i>	<i>M. reevesi</i>	LC
<i>M. atherodes</i> (Groves & Grubb, 1982)		<i>M. atherodes</i>	<i>M. atherodes</i>	<i>M. atherodes</i>	NT
<i>M. montanus</i> (Robinson & Kloss, 1918)		<i>M. montanus</i>	<i>M. montanus</i>		DD
<i>M. puhoatensis</i> (Binh Chau, 1997)		<i>M. puhoatensis</i>	<i>M. puhoatensis</i>		DD
Total	5	16	13	12	

Number of recognized species ranges from 5 to 16. Red List category: LC, least concern; VU, vulnerable; DD, data deficient; CR, critically endangered; NT, near threatened.

**Supplementary Table S2** Detailed information on mtDNA sequences used in this study

Species name	GenBank accession No.		Source material	Latitude & longitude	Haplotypes	References
	Mitogenome	cyt b				
<i>M. gongshanensis</i>	MW596243		Ziradam, Putao	27° 34' 43.67" N / 97° 5' 53.89" E	10 M	This study
<i>M. gongshanensis</i>	MW596244		Ziradam, Putao	27° 30' 43.67" N / 97° 24' 53.89" E	9 M	This study
<i>M. gongshanensis</i>		MW596246	Putao	27° 20' 31.20 "N / 97° 24' 3.60" E	11 I/M	This study
<i>M. gongshanensis</i>	MW596245		Hkakabo Razi	28° 19' 59.43" N / 97° 37' 19.12" E	7 M/C	This study
<i>M. gongshanensis</i>	MK882935		Gongshan, Yunnan China.		7 M/C	Zhang et al., 2019
<i>M. gongshanensis</i>	DQ445732				13 C	Chen et al., 2008
<i>M. gongshanensis</i>	DQ445733				4 C	Chen et al., 2008
<i>M. gongshanensis</i>	DQ445734	Tibet, China,			3 C	Chen et al., 2008
<i>M. gongshanensis</i>	DQ445735				12 C	Chen et al., 2008
<i>M. gongshanensis</i>	EF523661				5 I	James et al., unpublished
<i>M. gongshanensis</i>	EF523662				6 I	James et al., unpublished
<i>M. gongshanensis</i>	EF523663	Namdapha, China			8 I	James et al., unpublished
<i>M. gongshanensis</i>	EF523664				11 I/M	James et al., unpublished

<i>M. crinifrons</i>	NC_004577		2	Li et al., unpublished
<i>M. feae</i>	MG857663		1	
<i>M. feae</i>	MG857664	Khao Kheow Open Zoo, Chon Buri, Thailand.	1	Srisodsuk et al., 2018
<i>M. feae</i>	NC_041100		1	
<i>M. muntjak</i>	KY052143			Martins et al., 2017
<i>M. muntjak</i>	KY052150			Martins et al., 2017
<i>M. muntjak</i>	KY052144			Martins et al., 2017
<i>M. muntjak</i>	KY052155			Martins et al., 2017
<i>M. muntjak</i>	KY052156			Martins et al., 2017
<i>M. muntjak</i>	KY117560			Martins et al., 2017
<i>M. muntjak</i>	KY052141			Martins et al., 2017
<i>M. vaginali</i>	KY052084			Martins et al., 2017
<i>M. vaginali</i>	KY052088			Martins et al., 2017
<i>M. vaginali</i>	KY052104			Martins et al., 2017
<i>M. vaginali</i>	KY052105			Martins et al., 2017
<i>M. vaginali</i>	KY052114			Martins et al., 2017
<i>M. vaginali</i>	MH547032			Singh et al., 2018
<i>M. vaginali</i>	MK050505			Singh et al., 2018
<i>M. vaginali</i>	MK050506			Singh et al., 2018
<i>M. vaginali</i>	MK050507			Singh et al., 2018
<i>M. malabaricus</i>	KY052116			Martins et al., 2017

<i>M. malabaricus</i>	KY052117	Martins et al., 2017
<i>M. atherodes</i>	KY117559	Mohd et al., 2017
<i>M. putaoensis</i>	NC036430	Li et al., 2017
<i>M. putaoensis</i>	MF737181	Li et al., 2017
<i>M. rooseveltorum</i>	KJ425279	Le et al., 2014
<i>M. truongsonensis</i>	KJ425276	Le et al., 2014
<i>M. vuquangensis</i>	NC_016920	Alexandre et al., 2012
<i>M. vuquangensis</i>	AF042720	Giao et al., 1998
<i>M. vuquangensis</i>	KJ425275	Le et al., 2014
<i>M. vuquangensis</i>	KJ425283	Le et al., 2014
<i>M. vuquangensis</i>	KJ425284	Le et al., 2014
<i>M. vuquangensis</i>	KJ425285	Le et al., 2014
<i>M. reevesi</i>	KY117561	Mohdet al., unpublished
<i>M. reevesi</i>	NC_004069	
<i>M. reevesi</i>	NC_008491	Wang et al., unpublished
<i>M. reevesi</i>	AF042719	Giao et al., 1998
<i>Tragulus kanchil</i>	NC_020753	Hassanin et al., 2011
<i>Elaphodus cephalophorus</i>	DQ873526	Alexandre et al., 2012