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SUPPORTING INFORMATION

HOLOCENE RANGE COLLAPSE OF GIANT MUNTJACS AND PSEUDO-ENDEMISM IN THE ANNAMITE LARGE MAMMAL FAUNA

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APPENDIX S1 Ancient biomolecule analysis methods.

ZooMS was carried out on four unaccessioned antler fragments probably representing sambar deer (*Rusa unicolor*) from Hemudu available from ZMNH, to assess the potential for preservation of ancient biomolecules from the early-mid Holocene of the Yangtze region of China. Collagen peptide isolation methods followed Buckley *et al*. (2009), with an extended initial bone demineralization step of 42 hours at 4˚C. Peptide solutions were spotted onto Brucker anchorchip PACII 96HCCA plates and run on a MALDI-TOF autoflex III mass spectrometer, with calibrated spectra of monoisotopic peptides retrieved over a mass range of m/z 560-5340. Triplicate results for each sample were normalized and averaged to create one average spectrum per sample. Peaks were picked by mMass 5.2.0 (Strohalm *et al*., 2010), with a sound to noise ratio of 7. Spectra were calibrated against a collagen sequence of *Bos taurus*. Published relative peptide mass values (Buckley *et al*., 2009; Buckley & Kansa, 2011) were utilized to identify samples.

Ancient DNA extraction techniques (Brace *et al*., 2012) were then applied to attempt to analyze mitochondrial DNA from the four probable sambar antler samples described above, plus a further unaccessioned probable sambar fragment from Hemudu, a small bone sample of *M. gigas* from Hemudu (ZMNH M10009.25), two unaccessioned bone samples of *M. gigas* from Tianluoshan, and further samples from Hemudu of Sumatran rhinoceros (ZMNH M10013) and Javan rhinoceros (ZMNH M10014). All extractions were carried out in a dedicated ancient DNA laboratory at Royal Holloway University of London, followed by targeted amplification of small fragments of the cytochrome *b* region of mitochondrial DNA using PCR. Protocols to prevent contamination, PCR reactions, amplicon purification and sequencing were performed as described in Brace *et al*. (2012), with a PCR annealing temperature of 52˚C. Details of primer pairs used are given in Table S1.

REFERENCES

Brace, S., Barnes, I., Powell, A., Pearson, R., Woolaver, L., Thomas, M.G. & Turvey, S.T. (2012) Population history of the Hispaniolan hutia *Plagiodontia aedium* (Rodentia: Capromyidae): testing the model of ancient differentiation on a geotectonically complex Caribbean island. *Molecular Ecology*, **21**, 2239-2253.

Buckley, M., Collins, M.J., Thomas-Oates, J. & Wilson, J. (2009) Species identification by analysis of bone collagen using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry. *Rapid Communications in Mass Spectrometry*, **23**, 3843-3854.

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Strohalm, M., Kavan, D., Novák, P., Volný, M. & Havlíček, V. (2011) mMass 3: a cross-platform software environment for precise analysis of mass spectrometric data. *Analytical Chemistry*, **82**, 4648-4651.

Table S1. Details of primer pairs used in mtDNA (cytochrome *b*) analyses.

Taxon	Forward sequence 5' to 3'	Reverse sequence 5' to 3'	Product length (base pairs)
Cervid	GCCTATACTACGGGTCATA	AGAAGGTTGGTAATGACTG	148
Cervid	CCACAGCATTCGTAGGGTATG	GAATCGAGTTAAGGTTGCTTTGTC	141
Cervid	CCTCTCAGCAATCCCATACAT	CTGTTGGGTTATTGGATCCTGTCTC	162
Cervid	ATTGCAGCACTTGCCATAGTAC	TCCTAACACGTCTGGTGAGAATA	189
Rhinoceros	GCCTACGCAATCCTACGATC	CGGAATATTATGCTTCGTTGTTT	125