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## **Reporting Summary**

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see <u>Authors & Referees</u> and the <u>Editorial Policy Checklist</u>.

### Statistics

For	Il statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods sectio	n.
n/a	Confirmed	
$\ge$	The exact sample size ( <i>n</i> ) for each experimental group/condition, given as a discrete number and unit of measurement	
$\ge$	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeat	tedly
$\boxtimes$	<ul> <li>The statistical test(s) used AND whether they are one- or two-sided</li> <li>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</li> </ul>	
$\boxtimes$	A description of all covariates tested	
$\boxtimes$	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons	
$\boxtimes$	<ul> <li>A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression</li> <li>AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)</li> </ul>	n coefficient)
$\boxtimes$	For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value <i>Give P values as exact values whenever suitable.</i>	e noted
$\boxtimes$	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings	
$\boxtimes$	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes	
$\ge$	Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated	
	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.	

#### Software and code

Policy information about <u>availability of computer code</u>				
Data collection	no code was use for data collection			
Data analysis	Oxcal computer program (v4.3)(Ramsey & Lee, 2013) bcl2Fastq v2.17.1 SeqPrep v1.1(John JS, 2011) BWA 0.7. (Li & Durbin, 2010) SAMtools v1.9 (Li, 2011) Picard v1.141 (http://broadinstitute.github.io/picard) GATK v3.4.0 (McKenna et al., 2010) Geneious® v7.0.336 (Kearse et al., 2012) BLASTn (Altschul, Gish, Miller, Myers, & Lipman, 1990; Johnson et al., 2008) Mitobim v1.9.0 (Hahn, Bachmann, & Chevreux, 2013) BEAST2 v2.4.0 (Bouckaert et al., 2014) Tracer v.1.6 (Rambaut, Suchard, Xie, & Drummond, 2014) TreeAnnotator v.1.7.5 (Rambaut & Drummond, 2013) FigTree v.1.4.3 (Rambaut, 2007)			
	References Altschul, S. F., Gish, W. R., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. Journal of Molecular Biology, 215(3), 403–410. Retrieved from http://linkinghub.elsevier.com/retrieve/pii/S0022283605803602 Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C. H., Xie, D., Drummond, A. J. (2014). BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. PLoS Computational Biology, 10(4). doi:10.1371/journal.pcbi.1003537			
	Hahn, C., Bachmann, L., & Chevreux, B. (2013). Reconstructing mitochondrial genomes directly from genomic next-generation			

sequencing reads - A baiting and iterative mapping approach. Nucleic Acids Research, 41(13). doi:10.1093/nar/gkt371 John JS. (2011). SeqPrep 1.1. Https://Github.Com/Jstjohn/ SeqPrep. Johnson, M., Zaretskaya, I., Raytselis, Y., Merezhuk, Y., McGinnis, S., & Madden, T. L. (2008). NCBI BLAST: a better web interface. Nucleic Acids Research, 36(Web Server issue). doi:10.1093/nar/gkn201

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., ... Drummond, A. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics, 28(12), 1647–1649. doi:10.1093/bioinformatics/bts199

Li, H. (2011). A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. Bioinformatics, 27(21), 2987–2993. doi:10.1093/bioinformatics/btr509 Li, H., & Durbin, R. (2010). Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics, 26(5), 589–595. doi:10.1093/bioinformatics/btp698 McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., ... DePristo, M. A. (2010). The Genome Analysis Toolkit: A

McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., ... DePristo, M. A. (2010). The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. Genome Research, 20, 1297–1303. doi:10.1101/gr.107524.110.20

Rambaut, A. (2007). FigTree, a graphical viewer of phylogenetic trees. See Http://Tree.Bio.Ed.Ac.Uk/Software/Figtree. Rambaut, A., & Drummond, A. (2013). TreeAnnotator v1.7.0. University of Edinburgh, Institute of Evolutionary Biology. Rambaut, A., Suchard, M., Xie, D., & Drummond, A. (2014). 'Tracer' 1.6. Available at: http://beast.bio.ed.ac.uk/Tracer. Ramsey, C. B., & Lee, S. (2013). Recent and Planned Developments of the Program OxCal. Radiocarbon, 55(2), 720–730. doi:10.1017/ s0033822200057878

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

#### Data

Policy information about availability of data

All manuscripts must include a <u>data availability statement</u>. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

all raw genomic data will be publicly available on ENA (study number: PRJEB35255)

Figures:

Figure 1. (a, b) Dorsal and ventral view of the c. 46 ka old bird carcass recovered from the Siberian permafrost. (c) Map indicating its sampling location (red dot). Figure 2. Bayesian phylogeny for the concatenated ND2 and cytb (2034 bp) for 31 horned lark from Ghorbani et al.20 and the ancient horned lark highlighted in grey. Nodes with <0.5 support should be considered collapsed.

### Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Life sciences 📃 Behavioural & social sciences 📈 Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>

### Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	This study uses ancient DNA techniques to identify the age, sex and species identity of a near-intact Pleistocene bird carcass				
Research sample	The feather and tissue sample used was taken from near-complete bird carcass found in Siberia				
Sampling strategy	The sample was obtained opportunistically from a permafrost tunnel				
Data collection	Boris Berezhnov and Spartak Khabrov found the bird remains				
Timing and spatial scale	only one sample alone dating back some 46 ka BP was obtained				
Data exclusions	NA				
Reproducibility	NA				
Randomization	NA				
Blinding	NA				
Did the study involve field work? Yes XNo					

### Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems	Methods
n/a Involved in the study	n/a Involved in the study
Antibodies	ChIP-seq
Eukaryotic cell lines	Flow cytometry
Palaeontology	MRI-based neuroimaging
Animals and other organisms	
Human research participants	
Clinical data	

### Palaeontology

Specimen provenance	Nort-estearn Slberia, Tirekhtyakh River, Belaya Gora
Specimen deposition	Sakha Academy of Sciences in Yakutsk (accession ID: 2018-Tir-Shorelark-01)
Dating methods	radiocarbon dating was performed at the Oxford Radiocarbon Accelerator Unit (ORAU) using the Oxcal computer program (v4.3)
<u></u>	

X Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

### Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	NA
Wild animals	Near-intact bird carcass found in permafrost
Field-collected samples	NA
Ethics oversight	NA

Note that full information on the approval of the study protocol must also be provided in the manuscript.