

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 [Authors & Referees](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- | n/a | Confirmed |
|-------------------------------------|--|
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of all covariates tested |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
<i>Give P values as exact values whenever suitable.</i> |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

Data were collected by authors in Mexico (Universities of Zacatecas, Autónoma de San Luis Potosí, Nacional Autónoma de México, Instituto Nacional de Antropología e Historia INAH), United Kingdom (Universities of Oxford and Cambridge), Denmark (University of Copenhagen), U.S.A. (Kansas State University), and Brazil (University of São Paulo), using the softwares detailed below and institutional computers/databases, following the appropriate guidelines.

Data analysis

Genetic analysis involved Fastq-tools v0.8 for grepping poly A and T tails, SGA v0.10.15 for removing low complexity reads and duplicates, Bowtie2 v2.3.2 for database indexing and aligning reads against database, Samtools v1.10 for merging and sorting alignment files, ngsLCA - <https://github.com/miwipe/ngsLCA> for performing LCA on the alignment files, R v3.6.0, R studio v1.1.463 for parsing taxonomic profiles and figure production, MapDamage2.0 v2.0.8 for deamination estimates and read length distributions, Seqtk v1.2-r102 for generating fastq files from read IDs.

Chronometric analysis involved OxCal v.4.3.

Phytolith and pollen analysis involved Zen lite, C2 v.1.7.7. and TILIA/TILIAGRAPH v.2.0.41.

OLS analysis involved the the Analyst v. 4.57 and R luminescence package v. 0.8.6.

In-field finds were plotted using ArcMap v.10.3 and ArcScene v.10.3 (ESRI).

Photogrammetry involved Adobe Photoshop CC19 v. 20.0.2 and Blender v.2.77.

Figures were made using Adobe Photoshop CC19 v. 20.0.2.

Further details on the methods and relevant code are included in the manuscript and the Supplementary Information.

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 [data availability statement](#). 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

The data that support the findings of this study are available from the main text or Supplementary Information files. All sequence alignments are available from the European Nucleotide Archive under accession no. PRJEB37914.

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 [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Ecological, evolutionary & environmental sciences study design

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

Study description	This is an archaeological study centred on the site of Chiquihuite Cave, and its cultural, palaeoenvironmental and geological findings.
Research sample	Lithic artefacts (N=1930), radiocarbon and OSL samples (N=46), eDNA sediment samples (N=31), pollen and phytolith samples (N=9), chemical residue samples (N=3), micromorphology samples (N=1), petrography samples (N=12).
Sampling strategy	No statistical methods were used. Our sampling strategy was dependent on the available material within the excavation (number of lithic samples found, available locations for OSL and eDNA sampling, number of organic materials suitable for radiocarbon dating, etc).
Data collection	In the field, all archaeological finds were recorded at a station installed near the dig. Everything resembling an artefact was collected, avoiding on-spot subjectivity. Charcoals for AMS were immediately given separate codes. Every find bag received a number (1 to 2168). Each item got inventory numbers. Each find can be identified by the bag number plus inventory number, e.g., 206-13456. The final processing was done at the University of Zacatecas. If not laser-measured, the finds received 3D UTM coordinates based on provenience and depth, calculated manually, avoiding point overlap. This was carried out by the excavation director, C. Ardelean, and his students.
Timing and spatial scale	Data was collected between 2011 and 2018 in Mexico (Universities of Zacatecas, Autónoma de San Luís Potosí, Nacional Autónoma de México, United Kingdom (Universities of Oxford and Cambridge), Denmark (University of Copenhagen), U.S.A. (Kansas State University), and Brazil (University of São Paulo).
Data exclusions	No data were excluded. All results are duly reported.
Reproducibility	Multiple replicates, of subsamples from the same sample and multiple extracts, as well as replicated genomic libraries were performed and showed a remarkable similarity to samples from the same layers. Details of replication and results are described in the Supplementary Information. Sensitivity testing for Bayesian age modeling was carried out and described in the Supplementary Information.
Randomization	Our study was not experimental, therefore randomization does not apply.
Blinding	Our study was not experimental, therefore randomization does not apply.
Did the study involve field work?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No

Field work, collection and transport

Field conditions	Most information in this section has already been included in detail in the paper and in Methods. The field conditions were extreme. The excavation crew (only a few of the co-authors) spent 80 days in the field, in two separate seasons, living continuously inside the cave for up to 7 weeks in a row. The temperature inside the cave is stable at 12°C in winter months. Light enters the cave only for two hours a day. The use of artificial light is mandatory and permanent. Due to extremely difficult conditions of work and the very remote location of the cave, which demands a 9 hrs climb from the nearest village, the crew must live at the cave for the entire duration of the season and also must involve the local community in the logistics. A new sampling trip has been specifically carried out for the current resubmitted manuscript in order to obtain new samples and rerun the eDNA genetic studies.
Location	Chiquihuite Cave, Conception del Oro, Zacatecas, Mexico. Coordinates: 24.61666, -101.13333 (WGS84)

Access and import/export	<p>All information is stated in the paper. The fieldwork, extraction of samples, as well as the export of samples (for dating, palaeobotanics studies and ancient eDNA analysis) were done with special and separate permits issued by the Mexico's Archaeology Council in Mexico City (see list below). The first author and several other co-authors work for Mexican institutions and have been following Mexican archaeological legislation since the inception of this research project.</p> <ol style="list-style-type: none"> Permits for the third and fourths field seasons (that include Chiquihuite's seasons of 2016-2017 and 2018-2019): a) Oficio 401.B(4)19.2015/36/2938 of December 21, 2015; b) Oficio 401.1S.3-2018/2224 of December 19, 2018. Exportation permit for OSL tubes: 401-3-9554 of October 17, 2016. Exportation permit for radiocarbon and eDNA samples: 401.3S.16-2017/1769&1770 of August 28, 2017. Exportation permits for eDNA samples: 401.3S.16-2019/054 of January 15, 2019 & 676/2019-A-02, of January 29, 2019.
Disturbance	<p>The disturbance has been minimal. The excavation spot followed a series of criteria meant to minimize impact on the cave structure and environment, as well as to the fauna, temperature and sound. We excavated in an area far from the entrance, inside the cave, near the northern wall where there is no risk of collapse from the roof, damage to the walls, danger for the crew or significant change in the cave's topography. We used a low-emission eco-friendly Honda generator, powered by gasoline, with very low noise and almost zero gas emissions. We did not obstruct the entrance to the cave, so the bats and birds could access it freely. No fireplaces were lit inside the cave. Cooking, fire, use of stove, biological needs, all was done outside in the forest in specifically designated areas considering safety protocols and environmental protection.</p>

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

n/a	Involvement in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input type="checkbox"/>	<input checked="" type="checkbox"/> Palaeontology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Human research participants
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data

Methods

n/a	Involvement in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Palaeontology

Specimen provenance	<p>All specimens were excavated in excavation X-12, seasons 2016-2017, under the authorization issued by the Archaeology Council of the National Institute of Anthropology and History (INAH) in Mexico (see list of permits above). The study also involves ancient DNA obtained as environmental DNA directly from sediments in four stratigraphic layers (detailed in the paper). Same with the 9 sediment samples that served as basis for the pollen and phytolith studies (also detailed in the paper and SI).</p>
Specimen deposition	<p>Only fauna material can be referred to as specimens. The fauna specimens are currently in the possession of the Project crew at the University of Zacatecas, Mexico, in Zacatecas City. They will become freely available once the project has been terminated and the materials have been given to the national authority, the National Institute of Anthropology and History, according to the Mexican laws. Another part of the inventory is stored at the National Labs in Mexico City (INAH's Zooarchaeology laboratory). However, the specimens can be studied by other academics upon request. In the near future they will all be deposited at the INAH's national zooarchaeology lab and palaeontological repository, under the curatorship of Dr Joaquin Arroyo (co-author of this paper).</p>
Dating methods	<p>A few specimens have been dated by radiocarbon (AMS) and optically stimulated luminescence methods. Details of these are provided in the Supplementary Information.</p>
<p><input checked="" type="checkbox"/> Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.</p>	