# nature portfolio

Corresponding author(s):	Gabriel Wrobel
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### **Reporting Summary**

Nature Portfolio 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 Portfolio policies, see our <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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For	all st	atistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a	Cor	nfirmed
	×	The exact sample size $(n)$ for each experimental group/condition, given as a discrete number and unit of measurement
	x	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
x		The statistical test(s) used AND whether they are one- or two-sided Only common tests should be described solely by name; describe more complex techniques in the Methods section.
x		A description of all covariates tested
x		A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
x		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)
x		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 noted <i>Give P values as exact values whenever suitable.</i>
x		For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
×		For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
X		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

Plant material. The two archaeological Bolivian maize kernel samples were acquired prior to 1890 AD from the area south of modern La Paz, Bolivia, curated at the MSU Museum, Michigan State University, and based on AMS assay date from 401 to 582 cal. BP, with a median age of 1474 cal AD. In addition, data from 13 archaeological samples collected from previous research work were also used, including two from Chile dating from 750 to 1,100 BP, two from Brazil dating from 510 to 690 BP, three from Peru dating from 600 to 1,000 BP, two from Argentina, dating from 980 to 1040 BP and from 70 to 130 BP, three from Honduras dating from 1,740 to 2,300 BP, and one from Mexico dating to 5,310 BP 41-43.

aDNA extraction. Archaeological maize remains were processed in a clean laboratory environment at Michigan State University. Whole kernels were crushed in a mortar and pestle. Six hundred uL of fresh PTB (N-phenacylthiazolium bromide) buffer (0.4 mg/mL proteinase K, T2.5 mM PTB, 50 nM dithiothreitol) was added. The maize in the buffer was added to 1260 uL of lysis buffer. An additional 600 uL of PTB buffer was used to rinse the mortar and added to the lysis buffer. The individually ground maize kernels were rotated overnight at 37C. The remaining DNA extraction followed Swarts et al., 2017.

Library construction, amplification, and sequence. DNA extracts were converted to Illumina-compatible paired-end DNA libraries in a clean setting using SMARTer ThurPLEX DNA-seq from Takara Bio and amplified with 7 PCR cycles. The generated libraries were cleaned to remove remaining adaptors with AxyPrep Mag beads from Fisher Scientific and ran on a 2100 Bioanalyzer (Agilent Technologies) to check for quality. The libraries were indexed to enable pooling on the NovaSeq SP at University of Illinois.

No software was used.

The software packages used in this study are astq-dump sratoolkit.2.10.0-centos\_linux64, AdapterRemoval AdapterRemoval/2.3.1, BWA v0.7.17, Picard GATK/4.0.5.1-Python-3.6.4, MapDamage v.2.2.1, Qualimap v2.2.1, Trimmomatic Trimmomatic/0.38-Java-1.8, VCFools 0.1.15foss-2018a-Perl-5.26.1, VCF2PopTree, FigTree v1.4.4, plink v1.9, ggplot2 package, ADMIXTURE, pong v1.4.9, Treemix, OptM, XP-CLR.

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 and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The genome sequence data of ancient Bolivian Maize has been deposited in the NCBI database under BioProject accession PRJNA886637. All the data analysis code used in this study can be downloaded from https://datadryad.org/stash/share/bzhtYglrzpFqXP\_dmeTuaYXGxkpzJAPiL2aIXAQAQgg or can be found on Dryad (DOI: https://doi.org/10.5061/dryad.w6m905qtd).

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Policy	information	about studies	involving human	research partici	pants and Sex and	Gender in Research.
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Reporting on sex and gender	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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

### 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

## Ecological, evolutionary & environmental sciences study design

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

Study description This study was designed to identify the possible location from where Archaeological Bolivian maize may have come, and how maize diversity is associated with Inca cultural expansion and environmental variation in South America.

There all 6 sub-sample of archaeological Bolivian maize, and 13 ancient maize sample, 87-modern maize, 3-modern samples of Zea Research sample mays ssp. parviglumis, 34-modern samples of Zea mays ssp. Mexicana, and 1-Tripsacum sample. The reason we collected all these ancient and modern samples is to help identify the possible location of archaeological Bolivian maize and understand how it affects the diversity of modern maize because of Inca culture and environment.

Sampling strategy No sample-size calculation was performed. Sample size based on availability from the repository.

> The two archaeological Bolivian maize kernel samples were collected prior to 1890 AD from the area south of modern La Paz. Bolivia. curated at the MSU Museum, Michigan State University. 13 ancient maize sample, 87-modern maize, 3-modern samples of Zea mays ssp. parviglumis, 34-modern samples of Zea mays ssp. Mexicana, and 1-Tripsacum sample information were found in previous research which were listed by following and HapMap2 by downloading sequencing from Sequence Read Archive (SRA) (https://

- 1. Kistler, L. et al. Multiproxy evidence highlights a complex evolutionary legacy of maize in South America. Science 362, 1309-1313
- 2. Kistler, L. et al. Archaeological Central American maize genomes suggest ancient gene flow from South America. Proc Natl Acad Sci USA 117, 33124-33129 (2020).
- 3. Mangelsdorf, P.C., Macneish, R.S. & Galinat, W.C. Domestication of Corn. Science 143, 538-545 (1964).

Timing and spatial scale

Data collection

samples selected 11/28/2018. Original date or sample collection is ca. 1890 AD. 14-C median age cal 1470 AD. Samples derived from

	vicinity of La Paz, Bolivia.
Data exclusions	No data were excluded from the analyses.
Reproducibility	No repeated experiments were conducted/applied.
Randomization	Our study only collected six sub-samples from two archaeological Bolivian maize kernels, so they are not relevant to our research.
Blinding	N/A. This is not relevant to our research because our study did not use blinding.
Did the study involve fi	
Reporting for	or specific materials, systems and methods
system or method listed is re	n authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, elevant 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 & experim	
n/a Involved in the stud	·
Antibodies    X	es
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Clinical data	
Dual use research	of concern
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Specimen provenance	The two archaeological Bolivian maize kernel samples were collected prior to 1890 AD from the area south of modern La Paz, Bolivia, and then curated at the MSU Museum, Michigan State University. The six sub-samples were collected 11/28/2018 (original date is the 1890's).
Specimen deposition	Original research specimens destroyed during analysis. Remaining specimens are curated at the National Museum of Archaeology, La Paz, Bolivia.
Dating methods	A single AMS date was obtained on one kernel from a pouch woven of Camelid fibers associated with the Andean chullpa mortuary assemblage, yielding an age of 400 $\pm$ 26 BP (D-AMS-027148; maize kernel; corrected for fractionation, no $\delta$ 13C reported). For the date 400 $\pm$ 26 BP the two possible calibrated age ranges are 1440-1520 cal AD (p=0.823) and 1588-1621 cal AD (p=0.177), with a median age of cal AD 1474. (Calibrated at 2 $\sigma$ with Calib 8.2 [IntCal 20]; Stuiver and Reimer 1993)

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

Ethics oversight

No ethical approval or guidance was required.

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