nature research

Corresponding author(s): Louys and Roberts

Last updated by author(s): Jul 2, 2020

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
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ 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.*
☒	☐ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ 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)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ 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
☐	☒ 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	N/A
Data analysis	PAST ver. 2.17c

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

All raw data reported in this paper is available in the main text and Supporting Information

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	Stable carbon and oxygen isotope analysis was applied to modern mammals found in Southeast Asia. These were compared with published records of fossil mammals in order to examine large-scale changes in vegetation in the region and its relationships to extinction and conservation risk.
Research sample	Mammals with known distribution in Southeast Asia were targeted. Historical mammal specimens were selected for stable carbon and oxygen isotope analysis of tooth enamel from the collections of the Zoologische Staatssammlung München, Germany, the Muséum National d'Histoire Naturelle, Paris, France, the American Museum of Natural History, New York, United States of America, and the Lee Kong Chian Natural History Museum, Singapore. Specimens with full adult dentition in occlusion and with clear provenance and collection information were preferentially selected. In collaboration with the curatorial teams of each Institution, specimens were only sampled where duplicate specimens existed for the same taxa.
Sampling strategy	We sampled mammalian species from Southeast Asia from the Museum Zoologische Staatssammlung München (ZSM), Germany, the Muséum National d'Histoire Naturelle (MNHN), Paris, France, the American Museum of Natural History (AMNH), New York, United States of America, and the Lee Kong Chian Natural History Museum (LKCNHM), Singapore. We compared this sample to all of the existing published isotopic data for Southeast Asia dating to the Pleistocene. Specimens with full adult dentition in occlusion and with clear provenance and collection information were preferentially selected. Under the direction of the curatorial teams of each institution, all specimens made available to us were sampled. Sampling was done under the CITES registration of Griffith University, Australia (no. AU 062) and the Department of Archaeology, Max Planck Institute for the Science of Human History (no. DE 215-07). Specimens were identified based on existing labels within the museum collections with taxonomy updated according to latest available systematic information. The resulting database (n = 269) represents by far the largest isotopic study of modern mammalian fauna in the tropics beyond Africa. The fossil data extracted from published sources cover 31 sites across Indochina and Sundaland, and represent the largest compilation of stable isotope data from anywhere in Asia. Based on similar published studies in Africa (published in Nature journals, Science, and PNAS) our modern and fossil databases provide sample sizes sufficient for our analyses. Sampled teeth were cleaned using portable air-abrasion to remove any adhering external material. Enamel powder for bulk analysis was obtained using gentle abrasion with a diamond-tipped drill along the full length of the buccal surface in order to ensure a representative measurement for the entire period of enamel formation. All enamel powder was pretreated to remove organic or secondary carbonate contaminates following established protocols. This consisted of a series of washes in 1.5% sodium hypochlorite for 60 minutes, followed by three rinses in purified H2O and centrifuging, before 0.1M acetic acid was added for 10 minutes, followed by another three rinses in purified H2O. When comparing the novel data presented here with that from the existing literature it is worth noting that different pretreatment protocols have been applied in each case, though for tooth enamel pre-treatment induced variation is limited (<0.5‰ for δ13C and δ18O), and these differences have a negligible effect on the scale of the questions examined here
Data collection	Specimen sampling at the American Museum of Natural History and the Lee Kong Chian Natural History Museum was undertaken by Julien Louys. Sampling at the Zoologische Staatssammlung München and Muséum National d'Histoire Naturelle, Paris was undertaken by Patrick Roberts. All enamel powder was pretreated to remove organic or secondary carbonate contaminates. This consisted of a series of washes in 1.5% sodium hypochlorite for 60 minutes, followed by three rinses in purified H2O and centrifuging, before 0.1M acetic acid was added for 10 minutes, followed by another three rinses in purified H2O. Following reaction with 100% phosphoric acid, gases evolved from the samples were analyzed to stable carbon and oxygen isotopic composition using a Thermo Gas Bench 2 connected to a Thermo Delta V Advantage Mass Spectrometer at the Department of Archaeology, Max Planck Institute for the Science of Human History. Carbon and oxygen isotope values were compared against international standards (NBS 19, MERCK) registered by the International Atomic Energy Agency. Replicate analysis of OES standards suggests that machine measurement error is c. ± 0.1‰ for δ13C and ± 0.2‰ for δ18O. Overall measurement precision was studied through the measurement of repeat extracts from a bovid tooth enamel standard (n=20, ± 0.2‰ for δ13C and ± 0.3‰). Analysis was performed by Patrick Roberts. Compilation of published isotope data was undertaken by Julien Louys.
Timing and spatial scale	The analyses performed were not time dependent
Data exclusions	No data were excluded from the analyses
Reproducibility	Replicate analysis of MERCK standards suggests that machine measurement error is c. ± 0.1‰ for δ13C and ± 0.2‰ for δ18O showing good machine replicability. Each sample was run only once to preserve material for future research, as is standard for this type of analysis. However, replicate analysis of the in-house bovid standard (n=20) provides an idea into overall reproducibility (± 0.2‰ for δ13C and ± 0.3‰ for δ18O). The small standard deviation for this standard show that our pretreatment and sampling protocols yield replicable results. Each δ13C and δ18O measurement on the Thermo Gas Bench 2 connected to a Thermo Delta V Advantage Mass Spectrometer at MPI-SHH is the product of 10 measurements of 10 pulses of gas produced from each sample during reaction with the phosphoric acid.
Randomization	Randomization is not relevant as sampling was undertaken on specimens of known species to establish a baseline.
Blinding	Blinding is not relevant as the experiments performed are based on all available material. In the case of stable carbon and oxygen isotope analysis all measurement data and calibration have been reported.

Did the study involve field work? ☐ Yes ☒ No

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	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other organisms

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

Laboratory animals	No laboratory animals were used in this study.
Wild animals	No wild animals were used in this study.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	Sampling was done under the CITES registration of Griffith University, Australia (no. AU 062) and the Department of Archaeology, Max Planck Institute for the Science of Human History (no. DE 215-07)

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