nature portfolio

Corresponding author(s): Brian P. Hedlund

Last updated by author(s): 2025-3-3

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 Editorial Policies 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	Commercial and open-source software: NCBI-BLAST suite, Kraken2, metaFLYE v2.8.2, metaSPAdes v3.12.1, CheckM v1.0.12, CheckM2 v1.0.1, barrnap v0.9, tRNAscan-SE v2.0.11, DESeq2 v1.40.2, R software, SignalP versions 5.0 and 6.0. DeepTMHMM, CD-HIT, MAFFT v7.3, TrimAl v1.4, IQ-TREE v2.2.6, FAStconCAT-G v3.4.2, ProtTest v3.4.2, RAxML v8.2.12, COUNT, ALEml_undated algorithm, QIIME2 v2023.2, L'Image, AlphaFold 3 webserver, Spectronaut v18, Prodigal v2.6.2, Hmmscan software 3.3, METABOLIC, GTDB Toolkit v2.3.2, Eggnog-mapper v2.1.9. Custom code is available on GitHub (https://github.com/alexximalayaunlv/Calditenuis_paper.git). The Python scripts in this repository were used to: (1) process ALE results and (2) select genomes with specific marker proteins for genealogical concordance analysis using Densitree.

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 raw sequences for nomenclatural type genomes are available in the Sequence Read Archive (SRA) under the accession numbers SRR30574052, SRR30574051, and SRR32545976. The sequence with temporary ID SUB15147207 is awaiting review by curators. MAGs are available under the accession numbers listed in Supplementary Table 18, with several still awaiting assignment. The 16S rRNA raw reads from lab cultures amended with BCAA, PAA, and BPAA are available under the temporary SRA accession SUB15150641, pending curator review. Metaproteomics data (MSV000093641) are accessible via the Center for Computational Mass Spectrometry's MassIVE repository through the ProteomeXchange Consortium. The description on the website should be modified to indicate that this is a metaproteome (and not specific to one organism), and we have informed the UC-Davis Metaproteomics Center to update it. Data availability will be updated for the next submission.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	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	Many thermophiles that are abundant in high-temperature geothermal systems have never been cultivated and are poorly understood, including deeply branching Thermoproteota. Here, we describe the genome-guided cultivation of one such organism, Calditenuis ramacidaminiphagus, and show that it has evolved a heterotrophic metabolism focused on branched-chain amino acids (BCAAs). Initially, fluorescence in situ hybridization and nanoscale secondary ion mass spectrometry (FISH-nanoSIMS) showed that Cal. ramacidaminiphagus assimilated amino acids rapidly in casamino acid-amended enrichment cultures. Genome and metaproteome analyses showed a high abundance and expression of BCAA transporter genes, suggesting a BCAA-focused metabolism. This inference was supported by the subsequent enrichment of Cal. ramacidaminiphagus in BCAA-fed cultures, reaching 2.66×106 cells/mL and 48.7% of the community, whereas it was outcompeted when polar amino acids (PAAs) were included. Metabolic reconstruction and metaproteomics suggest that BCAAs are channeled into the mevalonate pathway for lipid biosynthesis and fuel ATP production through oxidative Stickland reactions and the TCA cycle, both coupled with aerobic respiration. Ancestral state reconstructions and phylogenetic analyses of 62 Caldarchaeales genomes revealed multiple horizontal transfers of BCAA transporters to the ancestor of the genus Calditenuis. Our study highlights the crucial role of BCAAs in the early evolution and niche of this genus, and suggests a high degree of resource partitioning even within low-diversity thermophilic communities.
Research sample	Sediment samples that contained members of the Calditenuaceae (circumneutral pH, >60 Celsius) were used for metagenome sequencing, experiments, and collection for lab cultivation.
Sampling strategy	Sediment samples that contained members of the Calditenuaceae (circumneutral pH, >60 Celsius) were used for metagenome sequencing, experiments, and collection for lab cultivation.

Data collection	This study involved collection and analysis several types of data: (i) metagenomes and metagenome-assembled genomes (Nou, Lai, Seymour, Jiao, Dodsworth, Li, Lian, Mosier, Dimapilis, Palmer, Hedlund, Woyke, Li, Eloe-Fadrosh); (ii) Illumina 16S rRNA gene amplicons (Saldivar, Mosier, Dodsworth, Lai); (iii) cryo electron tomography (Muok, Briegel); (iv) identification of cells using fluorescence in situ hybridization (FISH) (Dodsworth, Mosier, Mayali, Johnston); (v) quantitative PCR (qPCR) (Lai); (vi) metaproteomes (Dodsworth, Mosier, Lai).
Timing and spatial scale	Precise sample dates are indicated in the manuscript.
Data exclusions	No data were excluded.
Reproducibility	Locations and details for all sample collection and experiments are described in as much detail possible to promote reproducibility. For nomenclatural types, all raw data are available to allow reanalysis of sequence data. In general, raw and processed data have been archived in public databases wherever possible.
Randomization	N/A
Blinding	N/A

Did the study involve field work? ☒ Yes ☐ No

Field work, collection and transport

Field conditions	N/A
Location	Most experiments were conducted at Site A of Great Boiling Spring in Gerlach, NV (40°39'41.3"N, 119°21'58.1"W). Sampling locations for metagenomes are indicated in Supplementary table 18.
Access & import/export	Most experiments were conducted at Great Boiling Spring in Gerlach, NV, with full permission from the landowners David and Sandy Jamieson. Samples for metagenomes were collected with permission from the operators of the Yunnan Tengchong Volcano and Spa Tourist Attraction, Gongxiaoshe Resort and Hotel, Jinze Resort and Hotel, and Yellowstone National Park under the National Park Service permit YELL-SCI-5544.
Disturbance	Samples were generally less than 5 g of sediment.

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 and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A