Hydroperversive archaia near life limits at the polyextreme geothermal Dallol area

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Microbial life has adapted to various individual extreme conditions; yet, organisms simultaneously adapted to very low pH, high salt and high temperature are unknown. We combined environmental 16S/18S ribosomal RNA gene metabarcoding, cultural approaches, fluorescence-activated cell sorting, scanning electron microscopy and chemical analyses to study samples along such unique polyextreme gradients in the Dallol-Danakil area in Ethiopia. We identified two physicochemical barriers to life in the presence of surface liquid water defined by (1) high chaotropicity–low water activity in Mg2+/Ca2+-dominated brines and (2) hyperacidity–salt combinations (pH ~0/NaCl-dominated salt saturation). When detected, life was dominated by highly diverse ultrasmall archaea that were widely distributed across phyla with and without previously known halophilic members. We hypothesize that a high cytoplasmic K⁺ level was an original archaean adaptation to hyperthermophily, subsequently exapted during several transitions to extreme halophily. We detect active silica encrustment/fossilization of cells but also abiogenic biomorphs of varied chemistry. Our work helps circumscribing habitability and calls for cautious interpretations of morphological biosignatures on Earth and beyond.

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Results and discussion

To investigate the distribution and, eventually, type of microbial life along those polyextreme gradients, we analysed a large variety of brine and mineral samples collected mainly from two field expeditions (January 2016 and 2017; a few additional samples were collected in 2018) in four major zones (Fig. 1 and Extended Data Figs. 1–3). The first zone corresponded to the hypersaline (37–42%) hyperacidity (pH between ~0 and ~1; values down to pH ~1.6 were measured on highly concentrated and oxidized brines on site) and very low salt (≤−1.5 to 6.0).

Dallol is an uplifted (~40 m) dome structure located in the north of the Danakil Depression (~120 m below sea level). The Danakil Depression is a 200-km-long basin within the Afar Rift at the junction between the Nubian, Somalian and Arabian Plates. Lying only 30 km north of the hypersaline, hydrothermally influenced Lake Assale (Karum) and the Erta Ale volcanic range, Dallol does not display volcanic outcrops but intense degassing and hydrothermalism. These activities are observed on the salt dome and the adjacent Black Mountain and Yellow Lake (Gaët' Ale) areas (Fig. 1a,b). Gas and fluid isotopic measurements indicate that meteoritic waters, notably infiltrating from the high Ethiopian plateau (2,500 m), interact with an underlying geothermal reservoir (280–370°C). Further interaction of those fluids with the 1-km thick marine evaporites filling the Danakil Depression results in unique combinations of polyextreme conditions and salt chemistries, which have led some authors to consider Dallol as a Mars analogue.

Here, we use environmental 16S/18S ribosomal RNA gene metabarcoding, cultural approaches, fluorescence-activated cell sorting (FACS) and scanning electron microscopy (SEM) combined with chemical analyses to explore microbial occurrence, diversity and potential fossilization along Dallol–Danakil polyextreme gradients.

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were acidic (pH ~1.8), warm (~40 °C) and extremely hypersaline (≥50%). The Yellow Lake actively bubbles and emits toxic gases for animals, as illustrated by the presence of numerous dead birds. The gas phase includes light hydrocarbons. The fourth zone consisted of the hypersaline (36%), almost neutral (pH ~6.5), Lake Assale (Fig. 1b and Extended Data Fig. 2r), which we used as a milder, yet extreme Danakil system for comparison. In contrast to a continuous degassing activity, the hydrothermal manifestations were highly dynamic, particularly on the dome and the Black Mountain area. The area affected by hydrothermal activity in January 2017 was much more extensive than the previous year (Fig. 1 and Extended Data Fig. 1). Dallol chimneys and hyperacidic ponds can appear and desiccate in a matter of days or weeks, generating a variety of evaporitic crystalline structures observable in situ. Similarly, very active and occasionally explosive (salt bombs') hydrothermal activity that was characterized by hot (110 °C), slightly acidic (pH ~4.4) black hypersaline fluids was detected in the Black Mountain area in 2016 ('Little Dallol'; sample BL6–01; Extended Data Figs. 1b and 2l) but not in the following years. Active bischofite flows (116 °C) were also observed in the Black Mountain area in 2016 but not in 2017. To assess potential correlations between microbial life and local chemistry, we analysed the chemical composition of representative samples used in parallel for microbial diversity analyses (see Methods). Our results revealed three major types of solution chemistry depending on the dominant elements (Fig. 2f and Extended Data Fig. 4a). In agreement with recent observations, Dallol ponds were characterized by NaCl-supersaturated brines that were highly enriched in iron with different oxidation states, which explained the colour variation. Potassium and sulfur were also abundant (Supplementary Table 1). Many aromatic compounds were identified, particularly in Dallol and Yellow Lake fluids (Supplementary Table 2). High chaotropicity associated with MgCl-rich brines, high ionic strength and low water activity ($a_w$) is thought to be a limiting factor for life. We therefore determined these parameters in representative samples (Extended Data Fig. 5). Based on our experimental measures and theoretical calculations from dominant

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**Fig. 1** Overview of sampling sites at the polyextreme geothermal field of Dallol and its surroundings in the Danakil Depression, Ethiopia. a, Location of the Dallol dome area in the Danakil Depression following the alignment of the Erta Ale volcanic range (Gada Ale, Alu-Dalafilla), Northern Ethiopia. b, Close view of the sampling zones in the Dallol area and Lake Assale or Karum (satellite image from Copernicus Sentinel 1; 19 January 2017). c–e, Geological maps showing the sampling sites at the Dallol dome summit (c), west salt canyons and Black Mountain, including the Black Lake (d) and Yellow Lake (Gaet’Ale) zone (e). Squares (solid samples) and circles (liquid samples) indicate the nature of the collected samples. The colour indicates the collection date (red, 2016; yellow, 2017; green, 2018). The size of circle is proportional to the collected brine volume for analyses. Specific sample names are indicated in the aerial view shown in Extended Data Fig. 1.
salts, only samples in the Yellow and Black Lake areas displayed life-limiting chaotropicity and $a_w$ values according to established limits$^{12,13,19,20}$. A principal component analysis (PCA) showed that the sampled environments were distributed in three major groups depending on solution chemistry, pH and temperature: Black and Yellow Lake samples, anticorrelating with $a_w$; Dallol dome samples, mostly correlating with $a_w$ but anticorrelating with pH; and Dallol canyon cave reservoir (Gt samples) and Lake Assale, correlating with $a_w$ and pH (Fig. 2g). These results are consistent with those obtained with analysis of variance and subsequent post-hoc analysis, which show significant differences between the three major chemical zones (coloured areas in Fig. 2f,g) among them for the variables tested (Supplementary Table 4).

To ascertain the occurrence and diversity of microbial life along these physiochemical gradients, we purified DNA from a broad selection of brine samples (0.2–30 µm size fraction) and solid samples (gypsum and halite-rich salt crusts, compacted sediment and soil-like samples; Extended Data Fig. 3). We carried out 16S/18S rRNA gene-based diversity studies by high-throughput short-amplicon sequencing (metabarcoding approach) but also sequenced almost-full length genes from clone libraries, providing local reference sequences for more accurate phylogenetic
Fig. 3 | Distribution and diversity of prokaryotes in samples from the Dallol dome and surrounding areas based on 16S rRNA gene metabarcoding data. a, Histograms showing the presence/absence and abundance of amplicon reads of archaea (upper) and bacteria (lower) obtained with universal prokaryotic primers. Samples yielding amplicons directly (negative PCR controls were negative) are shown on the right (direct). Samples for which amplicons were only obtained after nested PCR, all of which also yielded amplicons in ‘negative’ controls, are displayed on the left (nested PCR). Sequences identified in the ‘negative’ controls, considered as contaminants, are shaded in light grey in the corresponding Dallol samples. The phylogenetic affiliation of dominant archaeal and bacterial groups is colour coded. For details, see Supplementary Table 5. k reads, thousand reads. The names of the different samples are provided on the x axis. b, GC content of archaeal OTUs plotted on a graph showing the positive correlation of GC content (for the same 16S rRNA region) and growth temperature of diverse described archaeal species. c, Phylogenetic tree of archaeal 16S rRNA gene sequences showing the phylogenetic placement of archaeal OTUs identified in the different environmental samples (full tree provided in Supplementary Data 1). Sequences derived from metabarcoding studies are represented with blue branches (Illumina sequences); those derived from cloning and Sanger sequencing of environmental samples, cultures and FACS-sorted cells are labelled with a red dot. Reference sequences are in black. Concentric circles around the tree indicate the presence/absence of the corresponding OTUs in different groups of samples (groups shown in (a)).
analyses (see Methods). Despite intensive PCR efforts and extensive sampling in Dallol polyextreme ponds, including pools that were active in two consecutive years (Extended Data Fig. 1) to minimize ephemeral system-derived effects, we only amplified 16S/18S rRNA genes from Dallol canyon cave water, the dome-base geothermally influenced salt plain and Lake Assale, but never from the Dallol dome or Black/Yellow lakes (Fig. 3a).

To check whether this resulted from excessively low DNA amounts in those samples (although relatively large volumes were filtered), we carried out seminested PCR reactions using, as templates, potential amplicons produced during the first PCR-amplification reaction, including the first PCR negative controls. Almost all samples produced amplicons in seminested PCR reactions, including the first PCR blanks (Fig. 3a).

Metabarcoding analysis revealed that amplicons from direct PCR reactions (PS/PS3, Gt, Assale) were largely dominated by archaeal sequences (>85%) grouping in diverse and abundant operational taxonomic units (OTUs) (Extended Data Fig. 6). By contrast, amplicons derived from Dallol ponds, Black and Yellow lakes and also first PCR ‘negative’ controls were dominated by bacterial sequences. Most of them were related to well-known molecular biology kit and laboratory contaminants, whilst others were human-related bacteria probably introduced during intensive afa and touristor daily visits to the site. A few archaeal sequences might also result from aerosol cross-contamination, despite extensive laboratory precautions (see Methods). After the removal of contaminant sequences (grey bars in Fig. 3a, and Supplementary Table 5), only rare OTUs encompassing fewer reads (mosty archaeal) could be associated with Dallol dome or Yellow Lake brines, which we interpret as dispersal forms (dusty wind is frequent in the area). Slightly higher abundances of archaeal OTUs were identified in ‘soil’ samples, that is samples retrieved from salty consolidated mud or crusts where dust brought by the wind from the surrounding plateaux accumulates and starts constituting a proto-soil (with incipient microbial communities; for example, Extended Data Fig. 2i). Therefore, although we cannot exclude the presence of active life in these ‘soil’ samples, our results strongly suggest that active microbial life is absent from polyextreme Dallol ponds and the Black and Yellow lakes.

By contrast, PS/PS3, Gt and Assale samples harboured extremely diverse archaea (2,653 OTU conservatively determined at 95% identity; that is genus level) that virtually spanned the known archaeal diversity (Fig. 3, Extended Data Fig. 6 and Supplementary Table 5). Around half of that diversity belonged to Halobacteria, and an additional quarter to the Nanohaloarchaeota (Extended Data Fig. 7). The rest of archaea distributed in lineages typically present in hypersaline environments, for example, the Methanonatronororlocarchaeai2,23 and Candidate Division MSBL1, which is thought to encompass methanogens26 and/or sugar-fermentors27. However, they also included other archael groups not specifically associated with salty systems (although they can sometimes be detected in hypersaline settings, for example someThermoplasmataerWoesearchaeota). These excludedThermoplasmata and Archaeoglobi within Euryarchaeota, Woesearchaeota and other lineages (Aenigmarchaeota, Altarachaeota) usually grouped as DPANN24–30, and Thaumarchaeota and Crenarchaeota (Sulfolobales) within the TACK/Proteoarchaeota31 (Fig. 3a and Supplementary Table 5). In addition, because rRNA GC content correlates with growth temperature, around 27% and 6% of archaeal OTUs were inferred to correspond to thermophile and hyperthermophile organisms, respectively (see Methods; Fig. 3b). As previously observed12,28,29, common archaeal primers for near-full 16S rRNA genes (Fig. 3c, red dots) failed to amplify Nanohaloarchaeota and other divergent DPANN lineages. These probably encompass ectosymbionts or parasites28–30,32. Given their relative abundance and co-occurrence in these and other ecosystems, it is tempting to suggest that Nanohaloarchaeota are (ecto)symbionts of Halobacteria, and Woesearchaeota could potentially be associated with Thermoplasmata-like archaea. Although much less abundant, bacteria belonging to diverse phyla, including CPR (Candidate Phyla Radiation) lineages, were also present in these samples (710 OTUs; Extended Data Figs. 6 and 7 and Supplementary Table 5). In addition to typical extreme halophilic genera (for example, Salinitaer, Bacteroidetes), one Deltaproteobacteria group and two divergent bacterial clades were overrepresented in Dallol canyon Gt samples.

Eukaryotes, which were less abundant and diverse, were present in Lake Assale and occasionally in the salt plain and Gt. They were dominated by halophilic Dunaliella algae (Extended Data Fig. 8 and Supplementary Table 6).

Consistent with metabarcoding results, and despite the use of various culture media and growth conditions mimicking local environments (see Methods), cultural approaches did not yield enrichments for the Dallol dome, Black Lake and Yellow Lake samples. We obtained enrichments from the canyon cave (Gt/Gt) and salt plain (PS/PS3) samples in most culture media (except in benzoate/hexadecane and tested conditions (except at 70 °C in the dark). However, all attempts to isolate microorganisms at pH < 3 from these enrichments failed. The most acidophilic isolate obtained from serial dilutions (PS3–A1) only grew at 37 °C and optimal pH 5.5 (range pH 3–7). Its 16S rRNA gene was 98.3% identical to that of Halarchaeum rubiduriarum MH11–16–3 (NR_112764), an acidophilic halarchoan growing at pH 4.0–6.5 (ref. 23).

In agreement with metabarcoding and culture-derived observations, multiparametric fluorescence analysis showed no DNA fluorescence above background for Dallol and Yellow Lake samples (Extended Data Fig. 9). Because optical and SEM observations suggested that indigenous cells were unusually small, we applied FACS to samples from the different Dallol environments (Extended Data Fig. 3), followed by systematic SEM analysis of sorted events. Despite some samples showed no difference in fluorescence after incubation with DNA dyes, we sorted all events above background limit (as defined in Extended Data Fig. 9a). We only detected cells in Dallol cave water and salt plain samples, but not in Dallol dome ponds or Yellow Lake samples (Extended Data Fig. 9).

Consistent with this, after DNA purification of FACS-sorted particles, 16S rRNA gene amplicons could only be obtained from different cave
and salt plain samples but not from Dallol dome or Yellow Lake samples. Cell counts estimated from FACS for the cave and salt plain samples were low (average $3.1 \times 10^4$ cells ml$^{-1}$ and $5.3 \times 10^4$ cells ml$^{-1}$ for the cave and PS samples, respectively). Sorted cells were usually small to ultrasmall (down to 0.25–0.3 µm diameter; Fig. 4). In PS samples, some of these small cells formed colonies (Extended Data Fig. 9 and Fig. 4c), which were sometimes surrounded by an exopolymeric matrix cover (Fig. 4h). The presence of cytoplasmic
bridges and/or potential cell fusions (Extended Data Fig. 9 and Fig. 4c) suggest that they might be archaeal colonies.  
FACS-sorted fluorescent particles in Dallol pond samples appeared to correspond exclusively to salt crystals or cell-sized amorphous minerals morphologically resembling cells, that is, biominerals and/or silica (for example, Fig. 4d compared with Fig. 4c). This prompted us to carry out a more systematic search for abiotic biominerals in our samples. SEM observations coupled with chemical mapping by energy-dispersive X-ray spectrometry (EDXRS) showed a variety of coccolike biomineral structures of diverse elemental compositions. Many of them were Si biominerals (Dallol ponds, Yellow Lake and Assale Lake), but we also detected Fe−Al silicates (Gt), S or S-rich biominerals (Dallol ponds), and Ca or Mg chlorides (Yellow Lake, BLPS samples) (Fig. 4, Extended Data Fig. 10 and Supplementary Figs. 1 and 2). We also observed Si-encrusted rod-shaped cells in Lake Assale samples (Fig. 4f). Therefore, silicified precipitates represent ultrasmall cell-like biominerals in samples with no detectable life but they contribute to cell encrustation and potential fossilization when life is present.  
Our work has three major implications. First, by studying the microbial distribution along gradients of polye xtreme conditions in the geothermal area of Dallol and its surroundings in the Danakil Depression, we identify two major physicochemical barriers that prevent life from thriving in the presence of liquid water on Earth and, potentially, elsewhere, despite the presence of liquid water at the surface of a planet being a widely accepted criterion for habitability. In line with previous studies, one barrier is imposed by high chaotropicty and low aKþ which are associated with high Mg2+-brines in the Black Lake and Yellow Lake areas. The second barrier seems to be imposed by the hyperacidic−hyperhaline combinations found in the Dallol dome ponds (pH ~1−0; salt >35%), regardless of temperature. This suggests that molecular adaptations to simultaneous very low pH and high salt extremes are incompatible beyond those limits. In principle, more acidic proteins, intracellular K+ accumulation (‘salt-in’ strategy) or internal positive membrane potential generated by cations or H+/cation antiporers serve both acidophilic and halophilic adaptations. However, membrane stability/function problems and/or high external Cl− concentrations that induce H+ and cation (K+/N+) import, potentially disrupting membrane bioenergetics, might be deleterious under these conditions. We cannot exclude other explanations linked to the presence of several stressors, such as high metal content or an increased susceptibility to the presence of local chaotropics in the Dallol hyperacidic ponds even if measured chaotropicty values are relatively low (~31 to +19 kJ kg−1) compared to the established limit for life (87.3 kJ kg−1) (Extended Data Fig. 6). Future studies should help to identify the molecular barriers limiting the adaptation of life to this combination of extremes. Second, although extreme environments are usually low-diversity systems, we identify exceptionally diverse and abundant archaea spanning known major taxa in hypersaline, mildly acidic systems near life-limiting conditions. A wide archaela (and to a lesser extent, bacterial) diversity seems to be present in Lake Assale samples (Fig. 4l). Therefore, silica−silicates (Yellow Lake, BLPS samples) (Fig. 4, Extended Data Fig. 10 and Supplementary Figs. 1 and 2) are likely to correspond exclusively to salt crystals or cell-sized biominerals, that is, biominerals and/or silica. This suggests that intracellular K+ accumulation is an ancestral archaela trait linked to thermophilic adaptation that has been independently exapted in different taxa for adaptation to hypersaline habitats. Finally, the extensive occurrence of abiotic, mostly Si-rich, biominerals mimicking the simple shape and size of ultrasmall cells in the hydrothermally influenced Dallol settings reinforces the equivocal nature of morphological ‘microfossils’ and calls for the combination of several biosignatures before claiming the presence of life on the Earth and beyond.
DNA purification and 16S/18S rRNA gene metabarcoding. DNA from filters, cell-trap concentrates and grinded solid samples was purified using the Power Soil DNA Isolation Kit (MoBio) under an ultraviolet-irradiated Erlab Captair50 Bio DNA/RNA PCR Workstation. Before DNA purification, filters were cut into small pieces with a sterile scalpel and the ethanol remaining in cryotubes was removed. DNA/RNA PCR Workstation. Before DNA purification, filters were cut into small pieces with a sterile scalpel and the ethanol remaining in cryotubes was filtered through 0.2 µm pore-diameter filters and processed in the same way. Ethanol was removed from the filters using vacuum technology (Erichsen). Nucleic acids were suspended in 25 µl of PCR Buffer (Promega), 0.1 µM of each primer, 1–5 µl of purified DNA and 1 µl of the hot-start ‘Taq’ Platinum polymerase (Invitrogen). Dig’EaQ (Promega) was also used when amplifications were not detected, but did not yield better results. Amplification reactions were performed for 35 cycles (94°C for 15 s, 50–55°C for 30 s and 72°C for 10 min). Amplicons were visualized after gel electrophoresis and staining with ultrasensitive GelRed nucleic acid gel (Biotium) on an ultraviolet-light transilluminator. When direct PCR reactions failed to yield amplicons after several assays, PCR conditions and using increasing amounts of input potential DNA, we carried out seminested reactions. For seminested reactions, we used those same primers but for seminested we used up to 5 µl of DNA 1 µl of PCR products, from a first amplification reaction performed with universal prokaryotic primers U340F (5'-CCTACGGGGBRCCAGG-3') and U806R, including the negative controls from the first PCR reaction. Eukaryotic 18S rRNA gene fragments that included the V4 hypervariable region were amplified using primers EK-565F (5'-GAGTTTAA AAAACGCTCAGGT-3') and 18S-EUK-1134E-R-UmOmet (5'-TT AAATTTT GAGTTTAA AAAACGCTCAGGT-3'). Primers were tagged with different molecular identifiers (MID) to allow multiplexing and subsequent sequence sorting. Amplicons from at least five independent PCR products were pooled together and then purified using the QiAquick PCR purification kit (Qiagen). Whenever seminested PCR reactions yielded amplicons, seminested reactions using first PCR negative controls as the input of second PCR amplification were repeated. When enrichments were obtained, we attempted isolation by serial dilutions.

**Cultures.** Parallel culture attempts were carried out in two different laboratories (Osray and Madrid). We used several culture media derived from a classical halophilic base mineral growth medium containing NaCl (234 g l⁻¹), KCi (6 g l⁻¹), NH₄Cl (0.5 g l⁻¹), KHP0₄ (0.5 g l⁻¹), (NH₄)2SO₄ (1 g l⁻¹), MgSO₄·7H₂O (30.5 g l⁻¹), MnCl₂·4H₂O (19.5 g l⁻¹), CaCl₂·6H₂O (1.1 g l⁻¹) and Na₂CO₃ (0.2 g l⁻¹). The pH was adjusted to 4 and 2 with HCl and NaOH. The autoclaved medium was amended with filter-sterilized cyanocobalamin (1 µM final concentration) and 5 ml of an autoclaved CaCl₂·6H₂O 1 M stock solution. Our medium MDH2 contained yeast extract (1 g l⁻¹) and glucose (0.5 g l⁻¹). The MDSH1 medium had both two-thirds of each base medium salt concentration plus FeCl₃ (0.1 g l⁻¹) and 10 ml of Allen’s trace solution. It was supplemented with three energy sources (prepared in 10 ml distilled water at pH 2 and sterilized by filtration): yeast extract (1 g l⁻¹) and glucose (0.5 g l⁻¹) (MDSH1–org. medium); Na₂SO₄ (5 g l⁻¹) (MDSH1–thio. medium) and Fe₂O₃·7H₂O (30 g l⁻¹) (MDSH1–Fe. medium). Medium MDH2 mimicked more closely some Dallol salts as it also contained FeCl₃ (0.1 g l⁻¹), MnCl₂·4H₂O (0.7 g l⁻¹), CuSO₄ (0.02 g l⁻¹), ZnSO₄·7H₂O (0.05 g l⁻¹) and LaCl₃ (0.05 g l⁻¹). To Allen’s trace solution we added the same energy sources used for MDH1, yielding media MDH2–org., MDH2–thio. and MDH2–Fe. For enrichment cultures, we added 0.1 ml liquid samples to 5 ml medium at pH 2 and 4 and incubated at 37 °C, 50 °C and 70°C in 10 ml sterile glass tubes depending on the original sample temperatures. Three additional variants of the base salt medium, which was supplemented with FeCl₃ and trace minerals, contained 0.2 g l⁻¹ yeast extract (SALT–YE), 0.5 g l⁻¹ thiosalicylic acid (SALT–THIO) and 0.6 g l⁻¹ benzoate and 5 mM hexadecane (SALT–BH). The pH of these media was adjusted with 34% HCI to pH 1.5 for Dallol and Black Lake samples, and to pH 3.5 for Yellow Lake, PS3 and PSBL samples. We added 1 ml of sample to 4 ml of medium and incubated it at 45 °C in light conditions and at 37 °C and 70°C in dark conditions. We also tried cultures in anaerobic conditions but they were not successful. The enrichment cultures were monitored by optical microscopy and, for some samples, SEM. In the rare cases where enrichments were obtained, we attempted isolation by serial dilutions.

**Flow cytometry and FACS.** The presence of cell/particle populations above background levels in Dallol samples was assessed with a flow-cytometer cell-sorter FACSaria11 (Becton Dickinson). Seven Dallol samples were analyzed with low-background signal in forward scatter (FSC) red (695 ± 20 nm) and green (530 ± 15 nm) fluorescence (Extended Data Fig. 9a) using sterile SALT-YE medium as blank. DRAQ5 and SYTO13 (ThermoFisher) were retained and used at 5 µM final concentration to stain samples in the dark at room temperature for 1 h. Cell-trap concentrated, read samples were diluted at 20% with 0.1 µM-filtered and autoclaved MilliQ water. The FACSaria11 was set at purity sort mode triggering on the forward scatter (FSC). Fluorescent target cells/particles were gated based on the FSC and red or green fluorescence (Extended Data Fig. 9b) and flow-sorted at a rate of 1–1,000 particles s⁻¹. Sorting was conducted using the FACSData software (Becton Dickinson) and figures were produced using FCSExpress 6 software.
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Author contributions

P.L.-G. and D.M. designed and supervised the research. P.L.-G. organized the scientific expeditions. J.B., P.L.-G., D.M., L.J. and J.M.L.-G. collected samples and took measurements in situ. J.B., P.L.-G. and P.B. carried out molecular biology analyses. J.B., A.I.L.-A. and D.M. performed culture, chemistry analyses and water–salt related measurements. A.I.L.-A. and J.B. performed statistical analyses. J.B., G.R. and D.M. analysed metabarcoding data. K.B. performed SEM and EDX analyses. J.M.L.-G. mapped geothermal activity and georeferenced all samples. L.J. and J.B. performed FACS-derived analyses. P.L.-G. and J.B. wrote the manuscript. All authors read and commented on the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41559-019-1005-0. Supplementary information is available for this paper at https://doi.org/10.1038/s41559-019-1005-0.

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Extended Data Fig. 1 | Aerial view of the main sampling sites in the Dallol area. **a**, Dallol dome summit showing the acidic green-yellow-brown coloured hydrothermal ponds and active degassing areas during our 2017 sampling trip; the orange-shaded area shows the active hydrothermal zone in January 2016. **b**, Dallol West salt canyons and Black Mountain area. **c**, Black Lake. **d**, Yellow Lake and surroundings. Names of samples and sampling sites are indicated. The size of circles is proportional to the water volume collected or filtered for subsequent analyses. Aerial photographs were taken from a drone by O. Grunewald, except **b**, which is a Google Earth aerial image (09/03/2016) obtained by the Sentinel satellite (ESA Copernicus program) provided by Image © 2019 CNES/Airbus.
Extended Data Fig. 2 | see figure caption on next page.
Extended Data Fig. 2 | Views of different sampling sites in the Dallol dome and surroundings in the Danakil Depression. a, DAL4 sampling site ponds; b, DAL5 pond and active degassing area; c, active hydrothermal springs in DAL9 ponds; d, in situ cell-trap filtration at the 7DA7 sampling area; e, 7DA9 sampling site; f, 7DA10 ponds showing increasingly darker and brownish colours along the oxidation gradient; g, water samples from the different 7DA10 ponds; h, DAL8 mineral precipitates; i, ‘proto-soil’-like salt crust (7YL-S1) near the Yellow Lake; j, Yellow Lake showing active degassing; k, YL3, salt-mud volcano in the Yellow Lake area; l, ‘Little Dallol’ hydrothermal very active area in 2016 on the way to the Black Mountain (in the distance; inlet, chimney emitting hydrocarbon-rich fluids at 110 °C); m, Black Lake; n, PSBL2 (Black Lake area ponds); o, wet salt plain, influenced by hydrothermal activity, corresponding to PS3 sample area; p, the cave in the salt canyons where Gt, 7Gt and 8Gt samples were collected; q, salt canyons; r, Assale (Karum) lake. Sample names starting by 7 indicate collection in 2017. Pictures from all other samples/sampling sites were taken during the 2016 expedition.
Extended Data Fig. 3 | see figure caption on next page.
Extended Data Fig. 3 | List and description of samples from the Dallol area analysed in this study and type of analyses performed. DO, dissolved oxygen; ORP, oxido-reduction potential; SEM-/EDXS, scanning electron microscopy/energy-dispersive x-ray spectrometry; FACS, fluorescence-activated cell sorting analysis; n.a., not applicable; n.d. not determined. Refractometry-derived salinity refers to the percentage (w/v) of local salt composition (see Supplementary Tables 1 and 3 for elementary and ionic analyses) measured in situ. Salinity was also directly measured by weighting the total solids (dry weight experimentally measured in triplicates; SD, standard deviation).
Extended Data Fig. 4 | Principal Component Analyses (PCA) of Dallol area sampling sites as a function of physicochemical parameters. PCA of 29 samples according to their chemical composition; only relatively abundant elements (see Supplementary Table 1) are included in the analysis. A summary of this analysis is shown in Fig. 2f. b. PCA including the same variables as Fig. 2f but additionally including dissolved oxygen (DO). Measured parameters on site can be found in Extended Data Fig. 3. Coloured zones in PCA analyses correspond to the three major chemical zones identified in this study.
Extended Data Fig. 5 | Chaotropicity, ionic strength and water activity for a selection of samples of the Dallol area. Chaotropicity was measured experimentally (see Methods) and also calculated, together with ionic strength values were from dominant Na, K, Mg, Ca, Fe chemistry data; water activity values were measured using a probe (see Methods). Known limits for life for each parameter are listed at the top of the table. Samples beyond that threshold for one or more of those parameters are shaded in grey.

| Sample Type | Chaotropicity (kJ/kg) | Calculated chaotropicity (kJ/kg) | Ionic strength (mol/L) | Water activity (a_w) |
|-------------|-----------------------|----------------------------------|------------------------|---------------------|
| Cave water  |                       |                                  |                        |                     |
| 7Gt         | -18.3                 | -23.80                           | 4.751                  | 0.729               |
| 8Gt         | -57.5                 | -56.65                           | 6.873                  | 0.731               |
| Lake Assale |                        |                                  |                        |                     |
| 8Ass        |                       | 7.10                             | 7.274                  | 0.718               |
| Geothermally influenced Salt Plain |                |                                  |                        |                     |
| PS3         |                       | 24.09                            | 7.138                  | n.d                 |
| Dallol dome hydrothermal pools | |                                  |                        |                     |
| DAL 4.00    | -21.7                 | -17.87                           | 6.104                  | 0.719               |
| DAL 4.0     | n.d.                  | -18.71                           | 7.307                  | n.d                 |
| DAL 4A      | n.d.                  | -9.61                            | 6.346                  | n.d                 |
| DAL 4D      | n.d.                  | 2.14                             | 7.104                  | n.d                 |
| DAL 6A      | n.d.                  | -23.97                           | 7.203                  | n.d                 |
| DAL 9A      | n.d.                  | -7.77                            | 7.529                  | n.d                 |
| DAL 9C      | n.d.                  | -16.15                           | 8.349                  | n.d                 |
| 7DAL4-W1    | 19.3                  | 40.44                            | 6.314                  | 0.667               |
| 7DAL4-W2    | 8.3                   | 14.28                            | 5.383                  | 0.698               |
| 7DAL7       | 8.8                   | 19.64                            | 5.989                  | 0.694               |
| 7DAL-N1     | 9.2                   | 20.84                            | 6.472                  | 0.694               |
| 7DAL-N2     | 11.5                  | 11.01                            | 5.940                  | 0.698               |
| 7DAL9       | -8.2                  | 2.95                             | 5.176                  | 0.708               |
| 7DAL10      | 2.1                   | -7.46                            | 5.037                  | 0.714               |
| 7DAL10-1    | n.d.                  | n.d                              | n.d                    | 0.580               |
| 7DAL12      | -31.2                 | -20.57                           | 5.793                  | n.d                 |
| 7DAL13-W1   | -24.8                 | -20.13                           | 4.785                  | 0.723               |
| 7DAL14      | -11.7                 | 7.54                             | 5.307                  | 0.748               |
| Black Lake area pools | |                                  |                        |                     |
| PSBL1       | 108.3                 | n.d                              | n.d                    | 0.334               |
| PSBL2       | 93.5                  | n.d                              | n.d                    | 0.345               |
| PSBL3       | 63.4                  | n.d                              | n.d                    | 0.722               |
| PSBL4       | 61.8                  | n.d                              | n.d                    | 0.711               |
| Black Lake  |                        |                                  |                        |                     |
| BL          | 288.3                 | 354.19                           | 19.155                 | 0.319               |
| 7BL-W1      | 198.5                 | 259.41                           | 14.206                 | 0.322               |
| 7BL-W2      | 201.3                 | 268.89                           | 14.721                 | n.d                 |
| Yellow Lake |                        |                                  |                        |                     |
| YL1         | n.d.                  | 492.06                           | 19.141                 | n.d                 |
| YL2         | n.d.                  | 574.04                           | 22.085                 | n.d                 |
| YL3         | 231.8                 | n.d                              | n.d                    | 0.319               |
| 7YL-W1      | 320.8                 | 495.01                           | 18.446                 | 0.261               |
| 7YL-W2      | 308.2                 | 328.92                           | 13.796                 | 0.467               |
| 7YL-W3      | n.d.                  | 466.64                           | 17.609                 | n.d                 |

* Data from Hallsworth et al (2007) and Stevenson et al (2015 and 2017)
Extended Data Fig. 6 | Sequence data and diversity measurements. *Contaminant sequences included sequences identified in negative controls and/or high similarity to human-associated bacteria; s.e., standard error. Eventual mitochondrial and chloroplast 16S rRNA gene sequences were also removed at this step.
Extended Data Fig. 7 | Phylogenetic tree of bacterial 16S rRNA gene sequences showing the phylogenetic placement of OTUs identified in the different Dallol area samples. Sequences derived from metabarcoding studies are represented by blue lines (Illumina sequences); those derived from cloning and Sanger sequencing of environmental samples, cultures and FACS-sorted cells are labelled with a red dot. Reference sequences are in black. Concentric circles around the tree indicate the presence/absence of the corresponding OTUs in different groups of samples (groups shown in Fig. 3a). Only sequences not deemed contaminant (see Supplementary Table 5) were included in the tree. The full tree is provided as Supplementary Data 1.
Extended Data Fig. 8 | Eukaryotic presence, diversity and relative abundance in Dallol area samples. Histogram showing the phylogenetic affiliation and abundance of 18S rRNA gene amplicon reads of eukaryotes (upper panel) obtained with universal eukaryotic primers and the associated OTU diversity (lower panel). Only a few samples yielded amplicons; negative PCR controls were always negative. Sequences corresponding to macroscopic plants and fungi (probably derived from pollen or spores) were considered contaminant (light grey). The phylogenetic affiliation of dominant eukaryotic groups is colour-coded.
Extended Data Fig. 9 | Multiparametric fluorescence analyses and fluorescence-activated cell sorting (FACS) analyses of representative Dallol area samples. **a**, effect of DNA fluorescent dyes on background fluorescence emission; natural (sterile medium-only) and DNA dye-induced fluorescence in the sterile hypersaline SALT-YE medium used to dilute/sort Dallol samples. Fluorescence is plotted against the size of the analysed particles (forward scatter); events concentration is colour-coded, red being high concentration and blue, low concentration. DRAQ5 and SYTO13 introduced less background and were chosen for FACS of natural samples. The approximate background threshold (ca. $10^2$) is indicated by a broken grey line. **b**, multiparametric fluorescence analyses of different Dallol samples before (left panels) and after (right panels) adding fluorescent DNA dyes. Events (particles) above background (red squares) were FACS-sorted and filtered on 0.1µm pore-size filters prior to SEM observations. **c**, SEM photographs showing examples of sorted particles. Cells are observed in samples PS, Gt and 7Gt; halite crystals in 7DA7 and amorphous mineral particles in 7DA9 and 7YL. Arrows indicate ultrasmall cells. The scale bar is 1µm.
Extended Data Fig. 10 | Mineral phases observed by SEM-EDX in precipitates of typical abiotic morphology and ‘biomorphs’. Biomorphs correspond to rounded-shaped crystalline morphs resembling cell structures (cocci, rods) and compatible with cellular sizes. Observed dominant phases are highlighted in bold.

| Site                        | Samples           | Mineral phases                   |
|-----------------------------|-------------------|----------------------------------|
| Cave water                  | Gt2016, 7Gt, 8Gt_1| Si, Ca sulfate, Fe-K sulfate, Al-Mg Fe oxides, Fe and Ca oxides |
|                             |                   | Fe-Al silicates                  |
| Lake Assale (Karum)         | 8Ass_2, 8Ass_3,   | NaCl, Na-K-Mg chloride           |
|                             | 8Ass_4, 8Ass_6,   | Si biomorphs                     |
|                             | 8Ass_7, 8Ass_8    | (and encrustment)                |
| Dallol dome (ponds)         | Dal4.0, 7DA7_07,  | NaCl, Na-K-Mg chloride, Fe-K     |
|                             | DAL4D, 7DA9-P1,   | oxides, Ti oxides                |
|                             | 7CA9_P1_3,        |                                  |
|                             | 7DA7_04, 7DA7_05, |                                  |
|                             | 7DA7_06, 7DA9_P1_2|                                  |
|                             | 7DA9_P1_5,        |                                  |
|                             | 7DA9_P3_10,       |                                  |
|                             | 7DA9_P3_12        |                                  |
| Yellow lake                 | YL1-03_4, 7YL_4,  | Fe chloride, Mg chloride          |
|                             | YL1-03_5, 7YL_6   | Si, CaCl₂, Ca phosphate          |
| Black lake area (ponds)     | BLPS_05_5         | Mg-Fe-K chloride                 |
|                             |                   | Mg chloride                      |
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  - 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 not collected by software. We produced primary data through environmental sampling, DNA purification and 16S/18S rRNA gene amplicon sequencing. |
|-----------------|----------------------------------------------------------------------------------------------------------------------------------|
| Data analysis   | Software used for sequence analysis: To clean chimeric sequences, VSEARCH (v2.3.4, Rognes et al., 2016) OTU clustering, CDHIT-EST (v4.6, Li et al., 2006) To align gene markers, Codon Code Aligner (v8.0.1, CodonCode Corporation, www.codoncode.com) Database SILVA v.128 (Glöckner et al., 2017) To align sequences retrieved from SILVA, MAFFT (v7.388, Katoh and Standley, 2013, accurate linsi option) TRIMAL (v1.4rev15; Capella-Gutierrez et al., 2009, automated 1 option) IQ-TREE using using the GTR model of sequence evolution with a gamma law and taking into account invariable sites (GTR+G+I) |

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Sanger sequences have been deposited in GenBank (NCBI) with accession numbers MK894601-MK894820 and Illumina sequences in GenBank Short Read Archive
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Study description
This is an interdisciplinary study of the occurrence and diversity of microorganisms in samples collected along polyextreme gradients (low pH, high salt, high temperature) involving culture attempts, amplification and sequencing of 16S/18S rRNA genes followed by molecular phylogenetic analyses, chemical analyses, fluorescence-activated cell sorting and electron microscopy observations combined with energy dispersive X-ray spectrometry.

Research sample
A total of 69 different samples were analyzed for different purposes. The detailed list of samples, their description and the type of analyses carried out are specifically provided in Supplementary Table 1.

Sampling strategy
Samples were chosen by their distribution along defined gradients of extreme physico-chemical parameters.

Data collection
Solid and water samples for microbial diversity analyses and culturing assays were collected under the most possible aseptic conditions to prevent contamination (gloves, sterile forceps and containers). Samples for culture assays were kept at room temperature. Salts and mineral fragments for DNA-based analyses were conditioned in Falcon tubes and fixed with absolute ethanol.

Timing and spatial scale
Samples were collected during two field trips carried out in January 2016 and January 2017 (when air temperature rarely exceeded 40-45°C); a few additional samples were collected in January 2018 (Fig. 1; Supplementary Fig. 1 and Supplementary Table 1). The exact collection date for each sample is provided in Supplementary Table 1.

Data exclusions
Data were not excluded from our study.

Reproducibility
This is a descriptive study involving only ordination methods and principal component analyses. Different samples collected from each specific area and bearing similar physico-chemical characteristics acts as replicate.

Randomization
Not applicable

Blinding
Not applicable

Did the study involve field work?  ☑ Yes  ☐ No

Field work, collection and transport

Field conditions
Physicochemical parameters (Supplementary Table 1) were measured in situ with a YSI Professional Series Plus multiparameter probe (pH, temperature, dissolved oxygen, redox potential) up to 70°C and a Hanna HI991001 pH probe (working pH range -2.00/16.00) at higher temperatures. Salinity was measured on site with a refractometer on 1:10 dilutions in MilliQ water. Water samples for chemical analyses were collected in 50 ml glass bottles after prefiltration through 0.22 μm pore-diameter filters; bottles were filled to the top and sealed with rubber stoppers to prevent the (further) oxidation of reduced fluids.

Location
All sampling points and mapping data were georeferenced using a Trimble® handheld GPS (Juno SB series) equipped with ESRI software ArcPad® 10. Cartography of hydrogeothermal activity areas was generated using ESRI GIS ArcMap™ mapping software ArcGis® 10.1 over georeferenced Phantom-4 drone images taken by O. Grunewald during field campaigns, compared with and updating previous local geological cartography. Samples were collected in three major areas at the Dallol dome and its vicinity (Fig.1b): i) the top of the Dallol dome, comprising various hydrothermal pools with diverse degrees of oxidation (Fig.1c); ii) the Black Mountain area (Fig.1d), including the Black Lake and surrounding bischofite flows and the South-Western salt canyons harboring water reservoirs often influenced by the geothermal activity and iii) the Yellow Lake (Gaet’Ale) area (Fig.1e). We also collected water samples from the hypersaline Lake Assale (Karum), located a few kilometers to the South in the Danakil Depression (Fig1b).

Access and import/export
Our field expeditions were locally organized by Luigi Cantamesnna (Géodécouvertes) in collaboration with the University of Mekelle and the Afar and Ethiopian authorities. All our samples transited by the Ministry of Mines, Ethiopia, where they were inspected and sealed prior to their shipment to the airport for a Custom’s declaration of export.
Reporting for specific materials, systems and methods

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Methodology

Sample preparation: Water samples were concentrated using CellTraps and directly used for fluorescent activated cell-sorting.

Instrument: FACSriaTMIII (Becton Dickinson)

Software: Sorting was conducted using the FACSDivaTM software (Becton Dickinson); figures were done with the FCSExpress 6 software (De Novo Software).

Cell population abundance: Cell population abundance varied and in some cases it was 0 - in these points only abiotic biomorphs could be detected.

Gating strategy: The FACSriaTMIII was set at purity sort mode triggering on the forward scatter (FSC). Fluorescent target cells/particles were gated based on the FSC and red or green fluorescence (Supplementary Fig. 6b) and flow-sorted at a rate of 1-1,000 particles per second.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.