

## Indus and Nubra Valley hot springs affirm the geomicrobiological specialties of Trans-Himalayan hydrothermal systems

Nibendu Mondal<sup>1</sup>, Aditya Peketi<sup>2</sup>, Tarunendu Mapder<sup>3</sup>, Chayan Roy<sup>4</sup>, Aninda Mazumdar<sup>2</sup>,  
Ranadhir Chakraborty<sup>5</sup> and Wriddhiman Ghosh<sup>1\*</sup>

<sup>1</sup> Department of Microbiology, Bose Institute, P-1/12 CIT Scheme VIIM, Kolkata 700054, India.

<sup>2</sup> Division of Geological Oceanography, CSIR National Institute of Oceanography,  
Dona Paula, Goa 403004, India.

<sup>3</sup> Division of Clinical Pharmacology, Department of Medicine  
Indiana University School of Medicine, Indianapolis, IN 46202, USA

<sup>4</sup> College of Veterinary Medicine, Western University of Health Sciences,  
309 East Second Street, Pomona, CA 91766, USA

<sup>5</sup> Department of Biotechnology, University of North Bengal, Siliguri, West Bengal 734013, India

\* Correspondence: wriman@jcbose.ac.in / Wriman@rediffmail.com

**Abbreviations used in the paper:** TDS, total dissolved solids; ICPMS, inductively coupled plasma mass spectrometry; EDTA, ethylenediaminetetraacetic acid; PCR, polymerase chain reaction; OTUs, operational taxonomic units; RDP, Ribosomal Database Project; SILVA, it is the name (derived from Latin *silva*, meaning forest) of an online collection of aligned rRNA gene sequence databases.

### Abstract

Geomicrobiologies of the hot springs of Chumathang and Panamik (located in the Indus and Nubra valleys of eastern and northern Ladakh respectively) were revealed, and compared with the Lotus Pond spring of Puga Valley (eastern Ladakh), which is known for its mineralogical and ecological peculiarities. Physicochemically, the vent-waters of the explored springs, Chumathang\_01, Panamik\_01 and Panamik\_02, were distinct from Lotus Pond, with wide variations in boron, chloride, lithium, magnesium and potassium concentrations. Their microbiomes encompassed several unique constituents, but resembled Lotus Pond in being highly-diversified and bacterial-mesophiles-dominated. Higher diversities of thermophilic archaea were detected in Chumathang\_01, than Panamik\_01 or Panamik\_02. Statistical analysis of the geochemical and microbiological data highlighted the overall uniqueness of Lotus Pond, the constraint imposed by high-temperature on the diversity of most bacterial groups, and the potential role of *in-situ* geochemicals in helping mesophilic bacteria inhabit the high temperature environments. While the microbiome architecture of the 86°C Chumathang\_01 (having the highest bacterial species count) closely resembled that of the geochemically-similar, 78°C Panamik\_01, both the biomes were apparently shaped by temperature and pH. In contrast, the distinctive geochemistry of the 81°C Lotus Pond was apparently instrumental in sustaining a microbiome similar to that of the cooler (70°C) Panamik\_02.

**Key words:** Trans-Himalayan hot spring systems; hot spring geochemistry; hot spring microbiome; mesophilic bacteria in high-temperature habitats.

## 1. Introduction

Aspects of geochemistry and microbiology have been characterized so well for certain types of terrestrial and marine hydrothermal systems that they have been instrumental in establishing the hypothesis of high-temperature origin of life on a firm footing (Miller and Bada 1988; Russell and Hall 1997; Wächtershäuser G 2006; Martin *et al.* 2008; Ghosh and Dam 2009; Patel *et al.* 2015; Ranjan *et al.* 2018). Of all terrestrial hot springs, the best studied ones are those which (i) slowly vent sulfurous, acidic fluids (Jones *et al.* 2000; Johnson *et al.* 2001, 2003; Owen *et al.* 2008) or (ii) rapidly discharge circumneutral to alkaline pH, sulfidic fluids having high concentrations of silicon, chloride and carbonate/bicarbonate (Mathrani *et al.* 1993; Atkinson *et al.* 2000; Hetzer *et al.* 2007; Roeselers *et al.* 2007). Based on the large body of geomicrobiological information available from these two types of springs across the world, the connection between hydrothermal habitats and thermophilic microbial groups has become almost axiomatic (Schwartzman and Lineweaver 2004; Des Marais and Walter 2019). This said, geographically and geologically distinct hot spring systems, by virtue of affording different sets of ecosystem opportunities and constraints, do host distinctive microbiotas (Ghosh *et al.* 2015; Chiriatic *et al.* 2017; Ghilamical *et al.* 2017; Power *et al.* 2018; Colman *et al.* 2019). Physicochemical factors intrinsic to different hydrothermal systems potentially confer differential levels of habitability to distinct hot spring environments (Xie *et al.*, 2011; Alsop *et al.*, 2014; Cowan *et al.*, 2015; Najar *et al.* 2018; Roy *et al.* 2020b), thereby often allowing the colonization of high temperature sites by such microbial groups which have no known thermophilic members in their taxonomic ranks (Jimenez *et al.* 2012; Wemheuer *et al.* 2013; Chan *et al.* 2015; Ghosh *et al.* 2015; Menzel *et al.* 2015; Roy *et al.* 2016, 2020a, 2020b).

Recent explorations of geomicrobiology carried out for the sulfur-boron-rich hot springs of Puga Valley in eastern Ladakh have revealed peculiar, mesophiles-dominated microbiomes within this mineralogically special, Trans-Himalayan hydrothermal system (Roy *et al.* 2016, 2020a, 2020b). The unique geochemistry of the Puga hot springs - which includes the discharge of boiling or near-boiling water having circumneutral pH, accompanied by low levels of sulfide, silicon and total dissolved solids (TDS), but high concentrations of sodium, boron, elemental sulfur and sulfate - have been hypothesized as the key facilitator of the high *in-situ* diversity of microorganisms (Roy *et al.* 2020b). Furthermore, the characteristic geohydrology of the Puga hot spring area, located just south of the collision-boundary between the Asian and Indian continental plates involved in Himalayan orogeny (this tectonically-active terrain is called the Indus Tsangpo Suture Zone, abbreviated as ITSZ; Gansser 1964), has been identified as the key driver of microbial flux through the hydrothermal system (Roy *et al.* 2020a). In this scenario it is imperative, as well as timely, to reveal

the microbiomes of other Trans-Himalayan hot spring systems and consider the community architectures in the context of the systems' geology and chemistry. Increasing geomicrobiological knowledge on the Trans-Himalayan hot springs would enhance our overall understanding of the less-explored third category of hydrothermal systems which they represent, namely, the mineralogically diverse, neutral pH to slightly alkaline, hot springs that are typically located in areas of elevated groundwater level and/or copious melting snow or rain (Skirnisdottir *et al.* 2000; Huang *et al.* 2011). Moreover, the idiosyncratic pattern of microbiome composition discovered during the initial explorations of Trans-Himalayan hot springs suggests that these ecosystems may hold potentially paradigm-changing implications for our understanding of life's adaptation to heat, the nature of ancient metabolism, and the geobiological frameworks of early ecosystems on Earth as well as in potential extra-terrestrial locations.

In the present study centered on the Chumathang and Panamik hot spring systems located at 3950 m and 3200 m altitudes within the Indus and Nubra valleys of Eastern and Northern Ladakh respectively (figure 1), we explore the vent-waters' chemical and microbial compositions, and evaluate the geochemical dependencies of the microbiome architectures in the context of equivalent information available for the well studied Lotus Pond spring (Ghosh *et al.* 2012; Roy *et al.* 2016, 2020a) of the Puga geothermal area (altitude: 4436 m), which is also located in Eastern Ladakh, only ~20 km (aerial distance) away from Chumathang, but to the south of the Indus river across a watershed. Our comparative analyses reveal distinctive as well as unifying geomicrobiological features (physicochemical factors promoting and/or constraining the microbiota) for the hydrothermal systems located in the distinct Trans-Himalayan territories.

## **2. Materials and Methods**

### ***2.1 Geography and geology of the study area***

The Trans-Himalayan region of Ladakh lies in the rain shadow zone of the Great Himalayan Range, and features a pristine, cold arid territory having minimum winter temperature around -40°C and very low rainfall round the year (Shukla *et al.* 2017). It encompasses at least three such high energy geothermal area, namely Puga, Chumathang and Panamik (figure 1), which have documented potentials for power generation (Craig *et al.* 2013). All these geothermal systems apparently originate from the northward movement of the Indian continental plate beneath the Eurasian plate near the southern Tibet margin, and are controlled by the major tectonic fault boundaries such as ITSZ for Puga and Chumathang, and Shyok Suture Zone for Panamik (Tiwari *et al.* 2016). Whereas the Puga geothermal valley (GPS coordinates of the prototypical Lotus Pond spring is 33°13'46.3" N

and 78°21'19.7" E) is to the immediate south of ITSZ, Chumathang is to its north (GPS coordinates 33°21'37.03" N; 78°19'24.51" E). Panamik hot springs, on the other hand, are located in the Nubra valley (34°46'49.92" N and 77°32'32.49" E), south of the Karakoram fault system (Borneman *et al.* 2015).

## **2.2 Vent-water sampling**

At the time of sampling, i.e., 11:00 h of 12 August 2017, all the three venting points tested in the Chumathang geothermal area were found to discharge 86°C waters having pH 8.25, so only one of them, referred to as Chumathang\_01, was sampled for further geochemical and microbiological studies. In contrast, at the time of sampling, i.e., 11:00 h of 15 August 2017, all the four venting points tested in the Panamik geothermal area were found to discharge waters having pH 7.75, whereas two of the springs vented 78°C waters and the other two vented 70°C waters. At Panamik, therefore, one spring each was chosen from the two temperature categories for sampling and further investigation; these sites, located within a distance of 5 m from each other, were designated as Panamik\_01 (78°C) and Panamik\_02 (70°C).

For chemical analyses, vent-water samples were collected after filtration through 0.22 µm mixed cellulose ester membranes (Merck Life Science Private Limited, India) using separate sterile pipettes for individual sites. Batches of 100 mL sample meant for quantification of metallic elements were all acidified to pH ≤ 2 by adding 400-450 µL of 69% (w/v) HNO<sub>3</sub>. Batches of 40 mL sample meant for estimating sulfate concentration by the standard gravimetric precipitation method were first acidified to pH 2.0 by adding a few drops of 1.2 N HCl. 1 mL preheated BaCl<sub>2</sub> was then added and mixed vigorously to precipitate the dissolved sulfate as BaSO<sub>4</sub> (Roy *et al.* 2020a).

For culture-independent studies of microbiology, 300 mL batches of vent-water sample were individually passed through sterile 0.22 µm mixed cellulose ester membranes, following which the filters were inserted into separate cryovials containing 5 mL of 50 mM Tris:EDTA having pH 7.8 (Roy *et al.* 2016, 2020a, 2020b). The cryovials were sealed with Parafilm (Bemis Company Inc., USA), packed in polyethylene bags and put into heat-insulated boxes containing dry ice. The sample-containing boxes were immediately dispatched to the laboratory at Bose Institute, and total community DNA was extracted from the samples immediately upon arrival.

## **2.3 Chemical analyses**

Vent-water samples were tested for the concentrations of boron, calcium, lithium, magnesium and potassium with the help of inductively coupled plasma mass spectrometry (ICPMS) on an iCAP QICPMS (Thermo Fisher Scientific, USA), as per instructions provided by the manufacturer. ICPMS

standards (Sigma Aldrich, USA, and VHG Labs Inc., USA) were used to prepare the calibration curves for these analyses; after analyzing multiple concentrations of the standards in triplicates, <2%, <1%, <2.1%, <1% and <2.5% deviations from actual concentrations were observed for boron, calcium, lithium, magnesium and potassium respectively.

Sodium concentration in the vent-water samples was determined with the help of a 240 AA atomic absorption spectrometer (Agilent Technologies, USA). After analyzing multiple concentrations of the standard (Sigma Aldrich, USA) in triplicates, deviations from the actual concentrations of sodium used were found to be <3%. Concentration of silicon in the vent-water samples was estimated with the help of a CARY 100 UV-visible spectrophotometer (Varian Deutschland GmbH, Germany), as described elsewhere (Grasshoff *et al.* 1999). In order to quantify chloride ions, precipitation titration with 0.1 N silver nitrate was carried out, as described previously (Mazumdar *et al.* 2009), using a Titrino 799GPT auto titrator (Metrohm AG, Switzerland). Sulfate concentrations of the vent-water samples were determined in the laboratory, as described previously (Pyne *et al.* 2018; Rameez *et al.* 2020), via standard gravimetric analysis of the barium sulfate precipitates already generated during sampling at the study sites. Sulfate concentration was also measured directly in the vent-water samples by using an ion chromatograph (Basic IC plus 883, Metrohm AG, Switzerland) that was equipped with a Metrosep A Supp 5 anion exchange column and a suppressed conductivity detector (both from Metrohm AG, Switzerland), as described elsewhere (Fernandes *et al.* 2018).

#### **2.4 Assessment of microbial diversity**

For each study site (Chumathang\_01, Panamik\_01 or Panamik\_02), total community DNA (metagenome) was extracted, as described previously (Roy *et al.* 2016, 2020a), from the microbial biomass that was filtered from the corresponding vent-water sample *in-situ*. Previous studies of microbial ecology based on a number of Puga Valley hot springs, such as Lotus Pond, Shivlinga, PCPR\_1 and PCPR\_2 (Roy *et al.* 2016, 2020a, 2020b), had comprehensively revealed the *in-situ* bacterial diversities using oligonucleotide primers that exclusively amplify the V3 regions of the *Bacteria*-specific 16S rRNA genes present in the total community DNA. In contrast, a similar investigation of the Shivlinga hot spring-microbialite system of Puga Valley, which attempted to reveal the *in-situ* archaeal diversity using oligonucleotide primers targeting the V3 regions of *Archaea*-specific 16S rRNA genes, failed to obtain any PCR product (Roy *et al.* 2020b); presently, the same primer pair also failed to yield any PCR product from the vent-water community DNA of the Chumathang and Panamik hot springs. Explorations of various other habitats, however, have successfully detected taxonomically diverse archaea by using oligonucleotide primers that target the

V4-V5 regions of *Archaea*-specific 16S rRNA genes (Baker *et al.* 2003; Pal *et al.* 2020). Therefore, to reveal the bacterial or archaeal diversity present in the vent-water samples studied, V3 or V4-V5 regions of all the bacteria- or archaea-derived 16S rRNA genes present in the corresponding metagenomes were PCR-amplified and sequenced on an Ion S5 next-generation DNA sequencing machine (Thermo Fisher Scientific, USA), using the fusion primer protocol (Ghosh *et al.* 2015; Fernandes *et al.* 2018). While the forward primer 5' - CCTACGGGAGGCAGCAG - 3' and the reverse primer 5' - ATTACCGCGGCTGCTGG - 3' were used to amplify the V3 regions of the bacterial 16S rRNA genes, the V4-V5 regions of the archaeal counterparts were amplified using the forward and reverse primers 5' - GCYTAAAGSRICCGTAGC - 3' and 5' - TTTCAGYCTTGCGRCCGTAC - 3' respectively (Baker *et al.* 2003). The sequence datasets obtained from Ion S5 sequencing were submitted for public accession to Sequence Read Archive, National Center for Biotechnology Information, USA, under BioProject PRJNA296849, with separate run accession numbers for each dataset (table S1). Filtration of the reads of a given dataset for quality value 20 and sequence length threshold 100 bp, followed by their clustering (using modules of UPARSE; Edgar 2013) into operational taxonomic units (OTUs, or putative species-level entities unified at the 97% 16S rRNA gene sequence similarity level), were all carried out as described previously (Roy *et al.* 2020a, 2020b). Singletons were eliminated from the final OTU sets obtained, following which the online tool RDP Classifier, located at <http://rdp.cme.msu.edu/classifier/classifier.jsp>, was used to taxonomically identify the consensus sequence of each OTU present in a given set. Notably, searching against the taxonomic classifications curated semi-manually in the RDP and the SILVA databases classify 16S rRNA gene sequence data with equally high accuracy, so either of the two can be the database of choice for genus-level identification of sequences; in contrast, other databases available for this purpose have issues with size and/or presence of too many intermediate ranks/nodes in their taxonomic classifications (Balvočiūtė and Huson 2017). Furthermore, SILVA derives taxonomic rank information from Taxonomic Outline of the Archaea and Bacteria, published by John Wiley & Sons, Inc., in association with Bergey's Manual Trust (<https://doi.org/10.1002/9781118960608.bm00024>), and the List of Prokaryotic Names with Standing in Nomenclature (<https://www.bacterio.net/>), whereas RDP incorporates 16S rRNA gene sequences from International Nucleotide Sequence Database Collaboration (<https://www.insdc.org/>) and ascribes taxon names with reference to Prokaryotic Nomenclature Up-to-Date (<https://www.gbif.org/dataset/52a423d2-0486-4e77-bcee-6350d708d6ff>). In the present study, therefore, taxonomic identification of OTU sequences was carried out via RDP-based classification as it allowed the identification of the most contemporary taxa potentially represented in the datasets. Rarefaction curves, generated with the help of UPARSE

modules, indicated that the read-contents of all the datasets generated were adequate to discover almost the entire bacterial or archaeal diversities that were there in the samples (figure S1).

## **2.5 Mathematical analyses**

The *Clustergram* function of ‘MATLAB 2017b’ (Martinez *et al.* 2017) was used to perform hierarchical clustering, and generation of heat maps and two-way dendrograms, for the hot springs under consideration, based on their physicochemical or microbiological data. Within *Clustergram*, Euclidean distances matrices and average linkages were delineated to cluster the rows (study sites) and columns (physicochemical or microbiological parameters) of the individual datasets.

Canonical Correspondence Analysis (CCA, Ter Braak 1986) was carried out to reveal the influence of environmental (physicochemical) parameters on the overall bacterial diversity, as well as the diversity of major bacterial phyla, across the four Trans-Himalayan hydrothermal vents Chumathang\_01, Panamik\_01, Panamik\_02 and Lotus Pond. The CCA was derived in terms of the linear regression of the two data matrices on the basis of the row and column scores that actually determined the orthogonal contrasts of the two data matrices (De Leeuw and Mair 2009; notably, the two data matrices used in CCA were same as the ones used for hierarchical clustering). In the first data matrix, only those 12 environmental (physicochemical) parameters were considered for which data are available from all the four vents considered (table 1); in the second matrix, only those 10 (major) bacterial phyla were included which have five or more OTUs detected from at least one of the four vents (besides the data for the 10 major phyla, parameters of overall bacterial diversity were also included in the second matrix; see table 2). A two-dimensional solution was fitted for the entire geomicrobiological data encompassed in the two matrices, using the R package “anacor” (De Leeuw and Mair 2009). Notably, archaeal OTUs were detected in the present exploration of the Chumathang and Panamik hot springs, but those data were not included in the hierarchical clustering or CCA because of the lack of corresponding information from the comparator hot spring of Puga Valley.

## **3. Results**

### **3.1 Geochemical distinctions of the Trans-Himalayan hot springs studied**

The hot springs of Chumathang and Panamik were found to be distinguished considerably by their physicochemical characters (table 1). While all the vents surveyed in Chumathang discharged warmer waters, their pH was also slightly more alkaline. In terms of chemical constituents, Chumathang\_01, the only spring representing this geothermal area in the current study, discharged approximately 30 times more boron, 10 times more chloride, 5 times more lithium, 2-3 times more

sulfate, calcium, magnesium, potassium and sodium, than Panamik\_01 or Panamik\_02. Correspondingly, Chumathang\_01 had more than twice the concentration of TDS than the Panamik vents.

Although the Chumathang geothermal area is in close proximity of Puga Valley, its springs discharged water having slightly higher temperature and pH, and distinct chemical constitution as compared to those of Puga (table 1). Notably, springs of both Chumathang and Puga discharge boiling water, but since the valley of Puga is situated at a higher altitude (4436 m) boiling point of water is relatively lower in that area. In terms of chemical constitution, all the inorganic solutes tested, except calcium and sulfate, were found to have lower concentrations in the Chumathang vent-water. Correspondingly, Chumathang\_01 had approximately only half the concentration of TDS than Lotus Pond (table 1).

The two springs explored in Panamik differed not only in terms of their vent-water temperature but also the concentrations of TDS, silicon and sodium (standard deviation equal to 7%, 5% and 3.5% of the mean respectively). However, their concentrations of boron, chloride, calcium, lithium, potassium, magnesium and sulfate deviated <1% from the respective means. Furthermore, the Panamik springs were not only distinct from Chumathang\_01 but also Lotus Pond in terms of solute concentrations. All the chemical constituents tested, except calcium and sulfate, were found to have far lower concentrations in Panamik\_01 and Panamik\_02, as compared to Lotus Pond; TDS concentration of the Panamik vents was almost one third of the Lotus Pond TDS concentration (table 1).

Two-dimensional hierarchical clustering of Chumathang\_01, Panamik\_01, Panamik\_02 and Lotus Pond in terms of the physicochemical properties of their vent-waters highlighted the geochemical distinctiveness of Lotus Pond, besides a close relationship between Chumathang\_01 and Panamik\_01, and the intermediate position of Panamik\_02 (figure 2A). The heat map comparing the individual physicochemical parameters across the four vents (figure 2A) further illustrated the wide variations observed for the concentrations of boron, chloride, lithium, magnesium and potassium (standard deviation of the concentration data for each of these elements/ions was greater than the corresponding mean). Considerable variations were also highlighted for the concentrations of calcium, sodium, sulfate and TDS (for each of these parameters, standard deviation of the data was greater than 50% of the corresponding mean).



### 3.2 Archaea of the Chumathang and Panamik hot springs

Chumathang\_01, as well as Panamik\_01 and Panamik\_02, were found to encompass small but definite levels of archaeal diversity (table 2), even as no archaea has thus far been identified from the comparator springs of Puga Valley. Almost all the archaeal OTUs detected in the vent-waters of the Chumathang and Panamik hot springs were ascribed to either *Crenarchaeota* or unclassified (hitherto unknown) *Archaea* (table 2; figure S2A). Apart from these, two *Euryarchaeota* and one *Korarchaeota* OTUs were detected in Chumathang\_01, and only one *Thaumarchaeota*-affiliated OTU was there in Panamik\_02 (table 2). Thus, Chumathang\_01 encompassed no OTU from *Thaumarchaeota*, while Panamik\_01 had none affiliated to *Euryarchaeota*, *Korarchaeota* or *Thaumarchaeota*, and Panamik\_02 had no representative of *Euryarchaeota* or *Korarchaeota* (table 2).

Among the three vent-water samples investigated, Chumathang\_01 had the highest archaeal diversity (55 OTUs detected altogether). Out of the 26 *Crenarchaeota*-affiliated OTUs identified in Chumathang\_01 (table 2), only six could be classified under *Pyrodictium* (1), *Pyrobaculum* (4) and *Thermofilum* (1), while 20 were potential novel members of the phylum (table S3). Furthermore, in Chumathang\_01, both the *Euryarchaeota* OTUs detected belonged to *Archaeoglobus* while the sole *Korarchaeota* OTU identified belonged to *Candidatus Korarchaeum* (table S3). Apart from these, Chumathang\_01 harbored 26 more OTUs and all of them belonged to unclassified *Archaea* (table 2).

The vent-water of Panamik\_01 encompassed a total of 30 archaeal OTUs. Out of the 13 *Crenarchaeota*-affiliated OTUs present in this sample (table 2), only one could be identified as belonging to *Caldisphaera* (table S3) while the rest were potential novel members of the phylum. Apart from the *Crenarchaeota* OTUs, Panamik\_01 only had 17 more OTUs and all of them belonged to unclassified *Archaea* (table 2).

Of the three vent-water samples explored, Panamik\_02 had the lowest archaeal diversity (10 OTUs detected in all). Here, all the three *Crenarchaeota*-affiliated OTUs detected (table 2) were unclassified (table S3), so potentially belonged to novel taxa under this phylum. On the other hand, the sole *Thaumarchaeota*-affiliated OTU present in Panamik\_02 was classified under the genus *Nitrososphaera* (table S3). Panamik\_02 additionally had six OTUs belonging to unclassified *Archaea* (table 2).

### 3.3 Shared and unique components of the vent-water microbiomes

16S rRNA gene sequence-based diversity analysis showed that the vent-waters of Chumathang and Panamik hot springs harbor rich microbial diversities. The three springs investigated across the two

Trans-Himalayan territories encompassed 329-892 and 10-55 species-level entities or OTUs of bacteria and archaea respectively (table 2). They also contained 40-84 and 1-5 established genus-level entities over which the detected bacterial and archaeal OTUs could be classified respectively (tables S2 and S3); number of established phyla identified for the individual vent-water samples in the same way ranged between 9-13 and 1-3 for bacteria and archaea respectively (table 2). Bacterial diversity in the Chumathang and Panamik vent-water samples was typically higher than that of Lotus Pond in terms of species-level diversity (OTU count) but not the number of established genera or phyla detected after classifying the OTUs (table 2).

Despite having the highest temperature among the four Trans-Himalayan springs considered, species-level bacterial diversity (OTU count) of Chumathang\_01 was approximately double, more than double, and more than thrice that of Panamik\_01, Panamik\_02, and Lotus Pond, respectively (table 2). A large fraction of this high species-level diversity of Chumathang\_01 (511 out of the total 892 OTUs detected) was attributed to hitherto unknown, and therefore unclassified, *Bacteria*, in accordance with which the number of established phyla or genera over which the detected bacterial OTUs were classified was also quite low for Chumathang\_01 (table 2). Remarkably, only five phyla, *Actinobacteria*, *Chloroflexi*, *Deinococcus-Thermus*, *Firmicutes*, *Proteobacteria*, and the unclassified *Bacteria* accounted for 98% of all OTUs detected in Chumathang\_01 (figure S2B). Furthermore, in this context it is noteworthy that the vent-waters of the Chumathang and Panamik hot springs harbor only a few such established bacterial genera (namely, *Advenella*, *Bacillus*, *Chloroflexus*, *Meiothermus*, *Rubrobacter*, *Thermoflexus*, *Thermomicrobium* and *Thermus*) for which  $\geq 5$  OTUs were detected in at least one of the three sample sites (table S2). Out of these relatively predominant genera, *Advenella* and *Thermus* had the maximum number of OTUs across Chumathang\_01, Panamik\_01 and Panamik\_02.

Collective consideration of Chumathang\_01, Panamik\_01, Panamik\_02, and the comparator hot spring Lotus Pond of Puga, revealed the presence of a sum total of 169 established bacterial genera in the vent-waters of these four hot springs (table S2). Notwithstanding the unequal geographical distances separating the individual vents, out of the total 169 genera detected,

- 21 were found to be present in all four springs,
- 27, five, 23 and 46 were unique to Chumathang\_01, Panamik\_01, Panamik\_02 and Lotus Pond respectively,
- four were present exclusively in Chumathang\_01 and Lotus Pond, while three were there only in Panamik\_01 and 02 and nowhere else,

- 10 were there in Chumathang\_01, Lotus Pond and Panamik\_02, but not Panamik\_01,
- four were there in Chumathang\_01, Panamik\_01 and Panamik\_02 but not Lotus Pond,
- four were there in Panamik\_01, Panamik\_02 and Lotus Pond but not Chumathang\_01,
- two were there in Chumathang\_01 and Panamik\_01 but not Lotus Pond or Panamik\_02,
- seven were there in Chumathang\_01 and Panamik\_02 but not Lotus Pond or Panamik\_01,
- one was there in Panamik\_01 and Lotus Pond but not Chumathang\_01 or Panamik\_02,
- 12 were there in Panamik\_02 and Lotus Pond but not Chumathang\_01 or Panamik\_01.

Two-dimensional hierarchical clustering of the four Trans-Himalayan hot springs in terms of their phylum-level bacterial diversity highlighted the overall relatedness of the vent-water microbiomes of Lotus Pond and Panamik\_02 on one hand and Panamik\_01 and Chumathang\_01 on the other (figure 2B). Furthermore, the heat map comparing the OTU counts of the individual phyla present across the four springs (figure 2B) reflected the wide variations existing in the species richness of *Acidobacteria*, *Bacteroidetes*, *Chloroflexi*, *Firmicutes*, *Thermotogae* and unclassified Bacteria (table 2 shows that the standard deviation of the OTU count data for each of these groups was  $\geq 80\%$  of the corresponding mean). Among the other phyla for which  $\geq 5$  OTUs were detected in at least one of the four sample sites, standard deviations of the OTU count data for *Actinobacteria*, *Armatimonadetes*, *Cyanobacteria* and *Deinococcus-Thermus* were 50-80% of the corresponding means (see table 2) – these trends were also reflected in figure 2B.

### **3.4 Taxonomic relatives of mesophilic bacteria predominate the vent-water communities**

A comparison between the bacterial diversities of the two Eastern Ladakh hot springs, Chumathang\_01 and Lotus Pond, showed that a total of 138 genera are present in the two springs, out of which only 35 are common to both (table S2). Of the 35 genera found in both Chumathang\_01 and Lotus Pond, only 11 (*Anoxybacillus*, *Bacillus*, *Brevibacillus*, *Chloroflexus*, *Exiguobacterium*, *Fervidobacterium*, *Hydrogenobacter*, *Rubrobacter*, *Thermodesulfobivrio*, *Thermus* and *Truepera*) have members reported for laboratory growth at  $>45^{\circ}\text{C}$  (table S2). On the other hand, the vent-water of Chumathang\_01 harbored 40 such genera that were not present in Lotus Pond. Out of these, only 13 (*Anaerobranca*, *Caldalkalibacillus*, *Caldilinea*, *Clostridium sensu strict*, *Kallotenue*, *Meiothermus*, *Ornithinimicrobium*, *Piscibacillus*, *Rubellimicrobium*, *Salinicoccus*, *Thermoflexus*, *Thermoleophilum* and *Thermomicrobium*) have members reported for laboratory growth at  $>45^{\circ}\text{C}$  (table S2). The Lotus Pond vent-water, in turn, harbored 63 such genera that were absent in Chumathang\_01, of which again only 14 (*Actinomyces*, *Caldicellulosiruptor*, *Dictyoglomus*,

*Geobacillus*, *Halomonas*, *Marinobacter*, *Marinococcus*, *Photobacterium*, *Sporolituus*, *Sulfurihydrogenibium*, *Tepidimonas*, *Thermoactinomyces*, *Thermomonas* and *Thiofaba*) have members reported for laboratory growth at >45°C (table S2). From the above data it is evident that despite their close spatial proximity, the hot springs of Chumathang and Puga Valley harbor distinct microbiomes, which, however, are unified by the preponderance of bacterial mesophiles.

A comparison between the bacterial diversities of the two Panamik springs showed that despite being situated within 5 m of each other, their vent-waters harbor considerably distinct microbiomes. For instance, out of the total 92 genera detected in the two vents, only 32 were common to both (out of the 32, only seven - namely *Bacillus*, *Elioraea*, *Hydrogenobacter*, *Meiothermus*, *Thermoflexus*, *Thermomicrobium* and *Thermus* - have members reported for laboratory growth at >45°C; see table S2); eight were unique to Panamik\_01 (out of these, only one - *Ornithinimicrobium* – encompass members with laboratory growth at >45°C); and 52 were unique to Panamik\_02 (out of these, 14 - namely *Albidovulum*, *Anoxybacillus*, *Anaerobranca*, *Brevibacillus*, *Calditerricola*, *Chloroflexus*, *Fervidobacterium*, *Methylothermus*, *Rehaibacterium*, *Rhodothermus*, *Rubrobacter*, *Schleiferia*, *Thermoleophilum* and *Truepera* has members with laboratory growth at >45°C; see table S2).

### **3.5 Comparing the overall geomicrobiology of the Trans-Himalayan hot springs**

Canonical correspondence analysis of the entire geomicrobiological data yielded two principal inertias (Eigenvalues), 0.118 and 0.026, along the dimensions 1 and 2, represented as the X and Y axes in the CCA plot (figure 3A), respectively, with a total chi-square value of 658.451. These Eigenvalues implicated a bipolar spread of the environmental parameters, weighted essentially along the dimension 1 (X axis). Based on this ordination trend, pH, temperature, and concentrations of calcium and sulfate on one hand, and concentrations of TDS, boron, chloride, lithium, magnesium, potassium, silicon and sodium on the other, appeared to have opposite effects on the diversity of individual groups or the overall bacterial diversity of the vents represented by the parameters species (OTU) count, genus count and phylum count. In other words, if the first four parameters are limiting to the species-level diversity of a particular group, or to the overall microbial diversity of a particular vent, then the latter set of eight parameters tend not to be restrictive to them, and vice versa.

In the CCA plot (figure 3), pH, temperature, and concentrations of calcium and sulfate clustered in the positive ordinate of dimension 1 alongside the vents Chumathang\_01 and Panamik\_01, and the phyla *Chloroflexi*, *Deinococcus-Thermus*, *Firmicutes*, and the large number of unclassified (novel) bacterial taxa present especially in Chumathang\_01. On the contrary, TDS, and concentrations of boron, chloride, lithium, magnesium, potassium, silicon and sodium clustered in

the negative ordinate of dimension 1, alongside the vents Lotus Pond and Panamik\_02, and the phyla *Actinobacteria*, *Acidobacteria*, *Armatimonadetes*, *Bacteroidetes*, *Cyanobacteria*, *Proteobacteria* and *Thermotogae*. This bifurcation pattern indicated that in the context of the four Trans-Himalayan hydrothermal vents compared, pH, temperature, and calcium and sulfate concentrations do not favor the diversity of *Acidobacteria*, *Armatimonadetes*, *Bacteroidetes*, *Cyanobacteria*, *Proteobacteria* and *Thermotogae*, or for that matter the overall microbial diversity of Lotus Pond and Panamik\_02, but they support the overall diversity of Chumathang\_01 and Panamik\_01, plus the diversities of *Chloroflexi*, *Deinococcus-Thermus*, *Firmicutes* and the unclassified taxa. By contrast, TDS, boron, chloride, lithium, magnesium, potassium, silicon and sodium are indicated by CCA to be supportive of *Acidobacteria*, *Armatimonadetes*, *Bacteroidetes*, *Cyanobacteria*, *Proteobacteria* and *Thermotogae* (but not *Chloroflexi*, *Deinococcus-Thermus*, *Firmicutes* and the novel taxa) among the bacterial phyla, and Lotus Pond and Panamik\_02 (but not Chumathang\_01 and Panamik\_01) among the vents. Furthermore, out of the four parameters which were on the positive ordinate of dimension 1, calcium was inferred to have the maximum influence on diversity while temperature has the least. Likewise, out of the eight parameters which were on the negative ordinate of dimension 1, silicon has the least influence on diversity, whereas TDS, boron, chloride, lithium, magnesium, potassium and sodium have largely equivalent bearings on diversity. So far as the different bacterial groups are concerned, in the ordination diagrams, *Bacteroidetes*, *Thermotogae*, and the novel taxa are maximally differentiated along the dimension 1; there is not much discrimination along the dimension 2, except for *Chloroflexi* and *Thermotogae* (figure 3A and B). As for the individual springs, Chumathang\_01, Panamik\_01 and Panamik\_02 were located close to the origin, whereas only Lotus pond was separated widely along dimension 1; there was little discrimination along dimension 2 (figure 3A and C).

#### **4. Discussion**

Recent geomicrobiological investigations based on the sulfur-boron hot spring system of Puga Valley have shown that snow-melts infiltrating the shallow geothermal reservoir of this Trans-Himalayan region (seated ~450 m deep within the breccia) via local tectonic faults, introduce terrestrial microorganisms to the thermal waters which in turn are vented out of the copious hot springs scattered all over the valley (Roy *et al.* 2020a). This biogeodynamic process apparently gives rise to peculiar, mesophiles-dominated, high-diversity microbiomes across the vent-waters, outflow channels and apron areas of the hot springs of Puga (Roy *et al.* 2016, 2020a, 2020b). Furthermore, inorganic solutes such as ions of boron, lithium and sodium, present in the Puga hot spring waters (Roy *et al.* 2020a, 2020b), have been envisaged to potentially mitigate the biomacromolecule-

disordering effects of high heat and facilitate the *in-situ* survival of the phylogenetic relatives of mesophilic bacteria (Roy *et al.* 2020b).

The present study revealed highly diversified vent-water microbiomes in hot springs located in the Chumathang and Panamik regions of eastern and northern Ladakh respectively. Mirroring the bacterial-mesophiles-dominated microbiome architecture of the Puga hot springs located in eastern Ladakh (Roy *et al.* 2016, 2020a, 2020b), microbial diversities of the Chumathang and Panamik hot springs were also found to be remarkably dominated by such bacterial taxa whose members are incapable of laboratory growth at  $>45^{\circ}\text{C}$ . For instance, out of the 75, 40 and 84 genera detected in Chumathang\_01, Panamik\_01 and Panamik\_02 (table 1), 48, 31 and 53 have no constituent strain reported for laboratory growth at  $>45^{\circ}\text{C}$ , respectively (table S2). Notably, out of the 47 genera detected previously in the Lotus Pond vent-water 31 had no report of laboratory growth at  $>45^{\circ}\text{C}$  (Roy *et al.* 2020a). A mesophiles-dominated vent-water microbiome now appears to be a common feature of the hot spring systems situated in the Ladakh Trans-Himalayas, irrespective of their geographical distance or underlying geology. It is also not unlikely that introduction of microbes from surface or sub-surface sources into the hydrothermal reservoir via infiltrating snow-melts (Roy *et al.* 2020a) is the main driver of the high diversity of mesophilic bacteria encountered in all these hydrothermal vents. Notably, such a biogeodynamic phenomenon has been reported for hydrothermal systems located in other parts of the world as well (Power *et al.* 2018; Colman *et al.* 2019).

Notwithstanding their bacterial-mesophiles-dominated microbiomes, the Chumathang and Panamik vent-waters were found to harbor quite a few archaeal OTUs. Almost all the genera or candidate genera over which the archaeal OTUs of the Chumathang and Panamik hot springs were classified include members having laboratory growth at  $\geq 80^{\circ}\text{C}$  (table S3). Only *Nitrososphaera*, that was present exclusively in Panamik\_02, and which encompasses strains of terrestrial ammonia oxidizers (Kerou and Schleper 2016), grow at  $\leq 47^{\circ}\text{C}$  *in vitro* [notably, *Candidatus Korarchaeum* was detected in the vent-water of Chumathang\_01; although the upper limit of growth temperature is unknown for *Korarchaeota*, their thermophilic ecology is well known (Elkins *et al.* 2008; Reigstad *et al.* 2010)]. In the context of archaeal diversity, it is noteworthy that a previous study based on the Shivlinga hot spring-microbialite system of Puga Valley had not been able to detect archaea by using primers that target the V3 regions of archaeal 16S rRNA genes (Roy *et al.* 2020b). However, the present detection of small but definite archaeal diversities in the vent-waters of the Chumathang and Panamik hot springs using primers that target the V4-V5 regions of archaeal 16S rRNA genes indicates that future explorations utilizing similar strategies may reveal some archaeal diversity in

the vent-waters of the Puga hot springs as well. This said, overall microbiome depiction for the Puga hydrothermal system is likely to remain overwhelmingly bacteria-dominated because previous whole metagenome sequence-based microbiome analysis along the hydrothermal gradients of the Shivlinga spring had shown that archaea constitute less than 1% of all the *in-situ* microbial communities explored, including the white streamers which float on Shivlinga's vent-water and anchor to the sintered rim of the vent (Roy *et al.* 2020b).

Physicochemically, the Chumathang and Panamik hot springs were found to be considerably diverged among themselves, as well as from the springs of Puga Valley. This is not only consistent with the location of the three spring systems in distinct geographical and geological territories but also indicative of the disconnected nature of their underlying geothermal reservoirs. In particular, the concentrations of boron, lithium and sodium, were considerably lower in Chumathang\_01, Panamik\_01 and Panamik\_02 as compared to Lotus Pond. Sulfate, on the other hand, was more abundant in Chumathang\_01 and comparable in concentration across the other three vents. In the context of vent-water chemistry, it is noteworthy that the biomacromolecule-stabilizing properties of boron, lithium, sodium and sulfate have been previously envisaged as the key mitigators of microbial cell-system disruption by the thermal water, and therefore as the key drivers of the high diversity of mesophilic bacteria, in the Puga hot spring ecosystem (Roy *et al.* 2020b). With the current revelation of the predominance of mesophilic bacteria in the Chumathang and Panamik hot springs amidst much lower concentrations of boron, lithium and sodium (as compared to the Puga hot springs), it seems plausible that even low concentrations of these inorganic solutes are sufficient to impart biomacromolecule-stabilizing effects on the expectedly low density of microbial cells present in the thermal waters. This said, chances remain that the Trans-Himalayan hot spring systems are endowed with some hitherto-unknown biogeochemical factors which are supportive of high microbial diversity amidst high heat. In relation to the high habitability apparent for the Trans-Himalayan hot spring systems in general, it is further noteworthy that the ordination trends exhibited by the physicochemical and microbiological parameters in CCA (figure 3A) were consistent not only with the reported temperature constraints on the diversity of most bacterial phyla, but also with the potential role of *in-situ* geochemicals in supporting high species-level diversity of most bacterial phyla (Roy *et al.* 2020a, 2020b). For instance, in CCA, most bacterial phyla (excepting *Chloroflexi*, *Deinococcus-Thermus*, *Firmicutes* and the unclassified taxa) ordinated opposite to temperature, but in association with TDS and most of the native inorganic solutes present in the vent-waters of the Trans-Himalayan hydrothermal systems compared.

Hierarchical clustering of the four Trans-Himalayan hot springs in terms of the physicochemical properties of their vent-waters revealed the geochemical uniqueness of Lotus Pond (figure 2A). In contrast, clustering based on bacterial diversity pointed out the relatively greater closeness between the vent-water microbiomes of Lotus Pond (81°C) and Panamik\_02 (70°C) on one hand, and Chumathang\_01 (86°C) and Panamik\_01 (78°C) on the other. CCA, in turn, reflected the overall geomicrobiological uniqueness of Lotus Pond, besides clustering Panamik\_02 with Lotus Pond in the negative ordinate (opposite to temperature), and Chumathang\_01 and Panamik\_01 in the positive ordinate (alongside temperature), of dimension 1. A holistic consideration of the dendrogram data (figure 2) and the CCA results (figure 3) suggests that the distinctive vent-water chemistry of Lotus Pond could be instrumental in sustaining such a microbiome *in-situ* which is similar in architecture to the microbiome of a cooler vent-water environment (the 70°C Panamik\_01). This is not only consistent with the hypothesis of native-solutes-enabled survival of mesophiles in Lotus Pond (Roy *et al.* 2020a), but also the general perception that environmental factors other than temperature and pH potentially determine hot spring microbiome architecture in geographically and geologically distinct hydrothermal systems (Xie *et al.*, 2011; Alsop *et al.*, 2014; Cowan *et al.*, 2015). On the other hand, microbial diversity of the 78°C vent-water of Panamik\_01 closely resembled its counterpart in the geochemically similar, 86°C vent-water of Chumathang\_01, and both the microbiomes are apparently shaped by their high *in-situ* temperatures. This inference is also consistent with the detection of higher diversity of thermophilic archaea in the vent-waters of Chumathang\_01 and Panamik\_01, as compared to Panamik\_02 (tables 2 and S3). Furthermore, in this context, it is noteworthy that in CCA (figure 3) Chumathang\_01 and Panamik\_01 clustered in the positive ordinate of dimension 1 alongside not only temperature but also pH, with the locus for Chumathang\_01 being closer to the loci of these two environmental parameters, as compared to the distance of Panamik\_01 from the temperature and pH loci. In the light of previous global comparisons showing that neutral-pH to moderately-alkaline spring-waters harbor higher microbial diversities than their acidic counterparts irrespective of *in-situ* temperature (Roy *et al.* 2020a), it seems likely that the exceptionally high microbial diversity of Chumathang\_01 amidst high heat (water boils at 86°C in Chumathang, located at an altitude of 3950 m) could be supported - among other geomicrobial factors - by the vent-water's pH 8.25.

The geomicrobiological specialties revealed here for distinct Trans-Himalayan hot springs highlight the significance of these less-explored hydrothermal ecosystems in understanding how the habitability of high temperature environments is governed by *in-situ* physicochemical parameters. The geochemical dependencies identified for the microbiome architecture of the distinct spring



systems of Ladakh hold critical implications for life's challenges and opportunities in the thermodynamic extremes, whether on Earth or in potential extra-terrestrial locations. More explorations of biogeochemistry, followed by environment-inspired studies of culture-based microbiology, are required across Trans-Himalayan hot spring systems to comprehensively decipher the *in-situ* biochemical and biophysical factors which sustain their rich microbial diversities amidst high heat.

### **Acknowledgements**

This research was financed by Bose Institute as well as Science and Engineering Research Board (SERB), Government of India (SERB grant numbers were SR/FT/LS-204/2009 and EMR/2016/002703). Sri Pankaj Kumar Ghosh (Chinsurah, West Bengal, India) provided additional travel grants for field expedition. NM received fellowships from SERB and Bose Institute. On field assistance provided by Sri Asgar Ali is gratefully acknowledged.

### **Author statement**

WG conceived the program, carried out the fieldwork, designed the experiments, interpreted the results, and wrote the paper. NM and CR carried out the studies of microbiology. AP and AM carried out the studies of geochemistry. TM carried out the mathematical analyses. RC made critical intellectual contributions to the work and the manuscript. All authors read and vetted the paper.

The authors have no conflict of interest to declare.

### **Supplementary material**

The online version of this article contains supplementary materials.

### **References**

- Alsop E B, Boyd E S and Raymond J 2014 Merging metagenomics and geochemistry reveals environmental controls on biological diversity and evolution; *BMC Ecol.* **14** 16.
- Atkinson T, Cairns S, Cowan D A, Danson M J, Hough D W, Johnson D B, Norris P R, Raven N, Robinson C, Robson R and Sharp R J 2000 A microbiological survey of Montserrat Island hydrothermal biotopes; *Extremophiles* **4** 305-313.
- Baker G C, Smith J J and Cowan D A 2003 Review and re-analysis of domain-specific 16S primers; *J. Microbiol. Methods* **55** 541-555.
- Balvočiūtė M, Huson D H 2017 SILVA, RDP, Greengenes, NCBI and OTT - how do these taxonomies compare? *BMC Genomics* **18** 114.

- Borneman N L, Hodges K V, Van Soest M C, Bohon W, Wartho J A, Cronk S S and Ahmad T 2015 Age and structure of the Shyok suture in the Ladakh region of northwestern India: Implications for slip on the Karakoram fault system; *Tectonics* **34** 2011-2033.
- Chan C S, Chan K G, Tay Y L, Chua Y H and Goh K M 2015 Diversity of thermophiles in a Malaysian hot spring determined using 16S rRNA and shotgun metagenome sequencing; *Front. Microbiol.* **6** 177.
- Chiriac C M, Szekeres E, Rudi K, Baricz A, Hegedus A, Dragoş N and Coman C 2017 Differences in temperature and water chemistry shape distinct diversity patterns in thermophilic microbial communities; *Appl. Environ. Microbiol.* **83** e01363-17.
- Colman D R, Lindsay M R and Boyd E S 2019 Mixing of meteoric and geothermal fluids support hyperdiverse chemosynthetic hydrothermal communities; *Nat. Commun.* **10** 681.
- Cowan D A, Ramond J B, Makhalanyaane T P and De Maayer P 2015 Metagenomics of extreme environments. *Curr. Opin. Microbiol.* **25** 97-102.
- Craig J, Absar A, Bhat G, Cadel G, Hafiz M, Hakhoo N, Kashkari R, Moore J, Ricchiuto T E, Thurow J and Thusu B 2013 Hot springs and the geothermal energy potential of Jammu Kashmir State, NW Himalaya, India; *Earth Sci. Rev.* **126** 156-177.
- De Leeuw J and Mair P 2009 Simple and canonical correspondence analysis using the R package anacor; *J. Stat. Softw.* **31** 1-8.
- Des Marais D J and Walter M R 2019 Terrestrial hot spring systems: introduction. *Astrobiology* **19** 1419-1432.
- Edgar R C 2013 UPARSE: highly accurate OTU sequences from microbial amplicon reads; *Nat. Methods* **10** 996-998.
- Elkins J G, Podar M, Graham D E, Makarova K S, Wolf Y, Randau L, Hedlund B P, Brochier-Armanet C, Kunin V, Anderson I and Lapidus A 2008 A korarchaeal genome reveals insights into the evolution of the Archaea; *Proc. Natl. Acad. Sci. USA* **105** 8102-8107.
- Fernandes S, Mazumdar A, Bhattacharya S, Peketi A, Mapder T, Roy R, Carvalho M A, Roy C, Mahalakshmi P, Silva R, Rao P S, Banik S and Ghosh W 2018 Enhanced carbon-sulfur cycling in the sediments of Arabian Sea oxygen minimum zone center; *Sci. Rep.* **8** 8665.
- Gansser A 1964 *Geology of the Himalayas*. London: Interscience Publishers.
- Ghilamical A M, Budambula N L, Anami S E, Mehari T and Boga H I 2017 Evaluation of prokaryotic diversity of five hot springs in Eritrea; *BMC Microbiol.* **17**, 203.
- Ghosh W and Dam B 2009 Biochemistry and molecular biology of lithotrophic sulfur oxidation by taxonomically and ecologically diverse bacteria and archaea; *FEMS Microbiol. Rev.* **33** 999-1043.
- Ghosh W, Mallick S, Haldar P K, Pal B, Maikap S C and Gupta S K 2012 Molecular and cellular fossils of a Mat-like microbial community in geothermal boratic sinters; *Geomicrobiol. J.* **29** 879-885.

- Ghosh W, Roy C, Roy R, Nilawe P, Mukherjee A, Haldar P K, Chauhan N K, Bhattacharya S, Agarwal A, George A, Pyne P, Mandal S, Rameez M J and Bala G 2015 Resilience and receptivity worked in tandem to sustain a geothermal mat community amidst erratic environmental conditions; *Sci. Rep.* **5** 12179.
- Grasshoff K, Kremling K and Ehrhardt M 1999 *Methods of seawater analysis*; Wiley-VCH, Weinheim.
- Hetzer A, Morgan H W, McDonald I R and Daughney C J 2007 Microbial life in Champagne Pool, a geothermal spring in Waiotapu, New Zealand. *Extremophiles* **11** 605-614.
- Huang Q, Dong C Z, Dong R M, Jiang H, Wang S, Wang G, Fang B, Ding X, Niu L, Li X and Zhang C 2011 Archaeal and bacterial diversity in hot springs on the Tibetan Plateau, China; *Extremophiles* **15** 549-563.
- Jiménez D J, Andreote F D, Chaves D, Montaña J S, Osorio-Forero C, Junca H, Zambrano M M and Baena S 2012 Structural and functional insights from the metagenome of an acidic hot spring microbial planktonic community in the Colombian Andes; *PloS One* **7** e52069.
- Johnson D B, Body D A, Bridge T A M, Bruhn D F and Roberto F F 2001 Biodiversity of acidophilic moderate thermophiles isolated from two sites in Yellowstone National Park and their roles in the dissimilatory oxido-reduction of iron; In: Reysenbach A L, Voytek M, Mancinelli R (eds) *Thermophiles Biodiversity, Ecology, and Evolution*, Springer, Boston, pp 23-39.
- Johnson D B, Okibe N and Roberto F F 2003 Novel thermo-acidophilic bacteria isolated from geothermal sites in Yellowstone National Park: physiological and phylogenetic characteristics; *Arch. Microbiol.* **180** 60-68.
- Jones B, Renaut R W and Rosen M R 2000 Stromatolites forming in acidic hot-spring waters, North Island, New Zealand; *Palaios* **15** 450-475.
- Kerou M and Schleper C 2015 Nitrososphaera. *Bergey's manual of systematics of archaea and bacteria*; pp.1-10.
- Martin W, Baross J, Kelley D and Russell M J 2008 Hydrothermal vents and the origin of life; *Nat. Rev. Microbiol.* **6** 805-814.
- Martinez W L, Martinez A R and Solka J 2017 *Exploratory data analysis with MATLAB*; Abingdon: CRC Press.
- Mathrani I M, Nielsen P, Sonne-Hansen J, Kristjansson J K and Ahring B K 1993 Influence of pH and temperature on enumeration of cellulose- and hemicellulose-degrading thermophilic anaerobes in neutral and alkaline Icelandic hot springs; *Appl. Environ. Microbiol.* **59** 1963-1965.
- Mazumdar A, Dewangan P, João H M, Peketi A, Khosla V R, Kocherla M, Badesab F K, Joshi R K, Ramamurty P B, Karisiddaiah S M, Patil D J, Dayal A M, Ramprasad T, Hawkesworth C J and Avanzinelli R 2009 Evidence of paleo-cold seep activity from the Bay of Bengal, offshore India; *Geochem. Geophys. Geosy.* **10** 1-15.

- Menzel P, Gudbergsdóttir S R, Rike A G, Lin L, Zhang Q, Contursi P, Moracci M, Kristjansson J K, Bolduc B, Gavrillov S, Ravin N, Mardanov A, Bonch-Osmolovskaya E, Young M, Krogh A and Peng X 2015 Comparative metagenomics of eight geographically remote terrestrial hot springs; *Microb. Ecol.* **70** 411-424.
- Miller S L and Bada J L 1988 Submarine hot springs and the origin of life; *Nature* **334** 609.
- Najar I N, Sherpa M T, Das S, Das S and Thakur N 2018 Microbial ecology of two hot springs of Sikkim: Predominate population and geochemistry. *Sci. Total Environ.* **637** 730-745.
- Owen R B, Renaut R W and Jones B 2008 Geothermal diatoms: a comparative study of floras in hot spring systems of Iceland, New Zealand, and Kenya; *Hydrobiologia* **610** 175-192.
- Pal S, Biswas R, Misra A, Sar A, Banerjee S, Mukherjee P and Dam B 2020 Poorly known microbial taxa dominate the microbiome of hypersaline Sambhar Lake salterns in India; *Extremophiles* **24** 875-885.
- Patel B H, Percivalle C, Ritson D J, Duffy C D and Sutherland J D 2015 Common origins of RNA, protein and lipid precursors in a cyanosulfidic protometabolism; *Nat. chem.* **7** 301.
- Power J F, Carere C R, Lee C K, Wakerley G L, Evans D W, Button M, White D, Climo M D, Hinze A M, Morgan X C, McDonald I R, Cary S C and Stott M B 2018 Microbial biogeography of 925 geothermal springs in New Zealand; *Nat. Commun.* **9** 2876.
- Pyne P, Alam M, Rameez M J, Mandal S, Sar A, Mondal N, Debnath U, Mathew B, Misra A K, Mandal A K and Ghosh W 2018 Homologs from sulfur oxidation (Sox) and methanol dehydrogenation (Xox) enzyme systems collaborate to give rise to a novel pathway of chemolithotrophic tetrathionate oxidation; *Mol. Microbiol.* **109** 169-191.
- Rameez M J, Pyne P, Mandal S, Chatterjee S, Alam M, Bhattacharya S, Mondal N, Sarkar J and Ghosh W 2020 Two pathways for thiosulfate oxidation in the alphaproteobacterial chemolithotroph *Paracoccus thiocyanatus* SST; *Microbiol. Res.* **230** 126345.
- Ranjan S, Todd Z R, Sutherland J D and Sasselov D D 2018 Sulfidic anion concentrations on early earth for surficial origins-of-life chemistry; *Astrobiology* **18** 1023-1040.
- Reigstad L J, Jorgensen S L and Schleper C 2010 Diversity and abundance of Korarchaeota in terrestrial hot springs of Iceland and Kamchatka. *ISME J.* **4** 346-356.
- Roeselers G, Norris T B, Castenholz R W, Rysgaard S, Glud R N, Kuhl M and Muyzer G 2007 Diversity of phototrophic bacteria in microbial mats from Arctic hot springs (Greenland); *Environ. Microbiol.* **9** 26-38.
- Roy C, Alam M, Mandal S, Haldar P K, Bhattacharya S, Mukherjee T, Roy R, Rameez M J, Misra A K, Chakraborty R, Nanda A K, Mukhopadhyay S K and Ghosh W 2016 Global Association between Thermophilicity and Vancomycin Susceptibility in Bacteria; *Front. Microbiol.* **7** 412.
- Roy C, Mondal N, Peketi A, Fernandes S, Mapder T, Volvoikar S P, Haldar P K, Nandi N, Bhattacharya T, Mazumdar A and Chakraborty R 2020a Geomicrobial dynamics of Trans-Himalayan sulfur-borax spring system reveals mesophilic bacteria's resilience to high heat; *J. Earth Syst. Sci.* **129** 157.

Roy C, Rameez M J, Haldar P K, Peketi A, Mondal N, Bakshi U, Mapder T, Pyne P, Fernandes S, Bhattacharya S, Roy R, Mandal S, O'Neill W K, Mazumdar A, Mukhopadhyay S K, Mukherjee A, Chakraborty R, Hallsworth J E and Ghosh W 2020b Microbiome and ecology of a hot spring-microbialite system on the Trans-Himalayan Plateau; *Sci. Rep.* **10** 5917.

Russell M J and Hall A J 1997 The emergence of life from iron monosulphide bubbles at a submarine hydrothermal redox and pH front; *J. Geol. Soc. London.* **154** 377-402.

Schwartzman D W and Lineweaver C H 2004 The hyperthermophilic origin of life revisited; *Biochem. Soc. Trans.* **32** 168-171.

Shukla A D, Ray D, and Bhattacharya S 2017 Sulfate Mineral from Hot Springs in Cold Desert (Ladakh, India): Analogue to Martian Sulfate Deposit?; *LPSC.* **1964** 1939.

Skirnisdottir S, Hreggvidsson G O, Hjorleifsdottir S, Marteinson V T, Petursdottir S K, Holst O and Kristjansson J K 2000 Influence of sulfide and temperature on species composition and community structure of hot spring microbial mats; *Appl. Environ. Microbiol.* **66** 2835-2841.

Ter Braak C J F 1986 Canonical Correspondence Analysis: A New Eigenvector Technique for Multivariate Direct Gradients; *Ecology* **67** 1167-1179.

Tiwari S K, Rai S K, Bartarya S K, Gupta A K and Negi M 2016 Stable isotopes ( $\delta^{13}\text{C}_{\text{DIC}}$ ,  $\delta\text{D}$ ,  $\delta^{18}\text{O}$ ) and geochemical characteristics of geothermal springs of Ladakh and Himachal (India): Evidence for  $\text{CO}_2$  discharge in northwest Himalaya; *Geothermics* **64** 314-330.

Wächtershäuser G 2006 From volcanic origins of chemoautotrophic life to Bacteria, Archaea and Eukarya. *Phil. Trans. R. Soc. B.* **361** 1787-1806.

Wemheuer B, Taube R, Akyol P, Wemheuer F and Daniel R 2013 Microbial diversity and biochemical potential encoded by thermal spring metagenomes derived from the Kamchatka Peninsula; *Archaea* **2013** 136714.

Xie W, Wang F, Guo L, Chen Z, Sievert S M, Meng J, Huang G, Li Y, Yan Q, Wu S, Wang X, Chen S, He G, Xiao X and Xu A 2011 Comparative metagenomics of microbial communities inhabiting deep-sea hydrothermal vent chimneys with contrasting chemistries. *ISME J.* **5** 414-426.

## Figure legends

**Figure 1.** Geographical location of the Trans-Himalayan hot springs explored or considered in this study. (A) Map of northern India showing the location of the Puga, Chumathang and Panamik geothermal areas; their distances (by road) from Leh, the capital of the union territory of Ladakh, are also marked in the map. (B) Satellite view of the Puga, Chumathang and Panamik hot spring areas.

**Figure 2.** Two-way clustering (dendrograms) of the four Trans-Himalayan hydrothermal vents and (A) their bacterial diversity data or (B) physicochemical properties represented as a heat map. In the heat maps, each cell represents either (A) OTU count for a phylum present a vent-water sample or species (OTU) count of a vent-water sample, or (B) a physicochemical parameter tested. Cells have been colored based on the log ratios of the values recorded for the parameters considered: individual values for any parameter tested across the four vents were normalized by first dividing them with the median value of the data series, following which  $\text{Log}_{10}$  of the ratios were determined. Log ratios of 0 are indicated in white, increasing positive log ratios are represented in increasing intensities of red, and numerically increasing negative log ratios are shown in increasing intensities of blue.

**Figure 3.** Canonical correspondence analysis of the bacterial diversity data and the physicochemical properties determined/available for the four Trans-Himalayan hydrothermal vents. (A) Ordination diagram depicting the alignment patterns of the different diversity parameters [OTU count of various phyla present or species(OTU)/genus/phylum count of the vent-water samples] with the underlying gradients of the physicochemical (environmental) parameters, across the vent-water samples. The ordinations (grey arrows) depicted for the individual physicochemical parameters signify their relative influences on the bacterial diversity parameters (red dots) across the different hydrothermal vents (green dots). Panels (B) and (C) represent the relative significance of the two canonical environmental variables (dimension 1 in black solid line and dimension 2 in red dashed line) on the bacterial diversity parameters and the hydrothermal vents respectively.

Figure 1

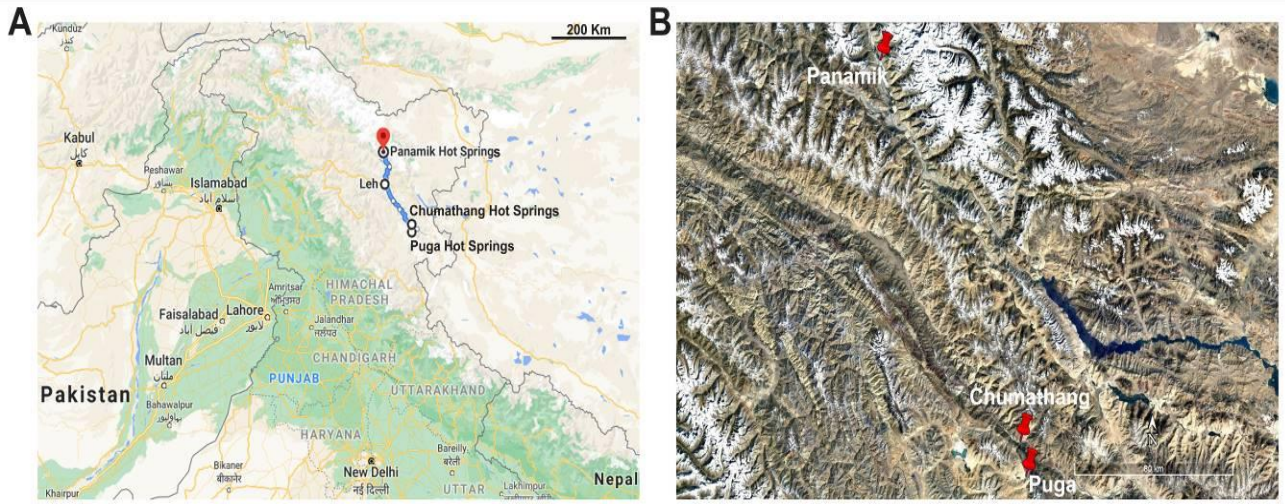


Figure 2

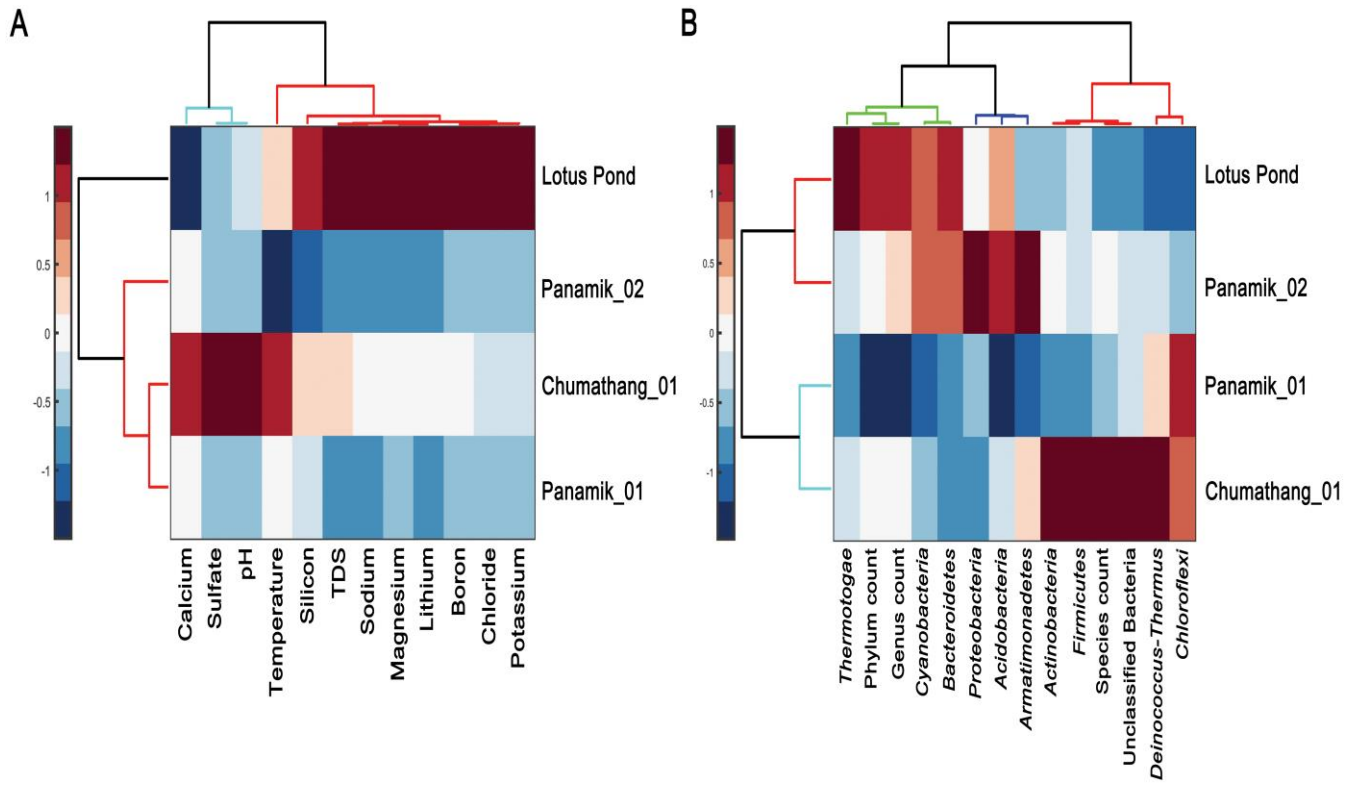
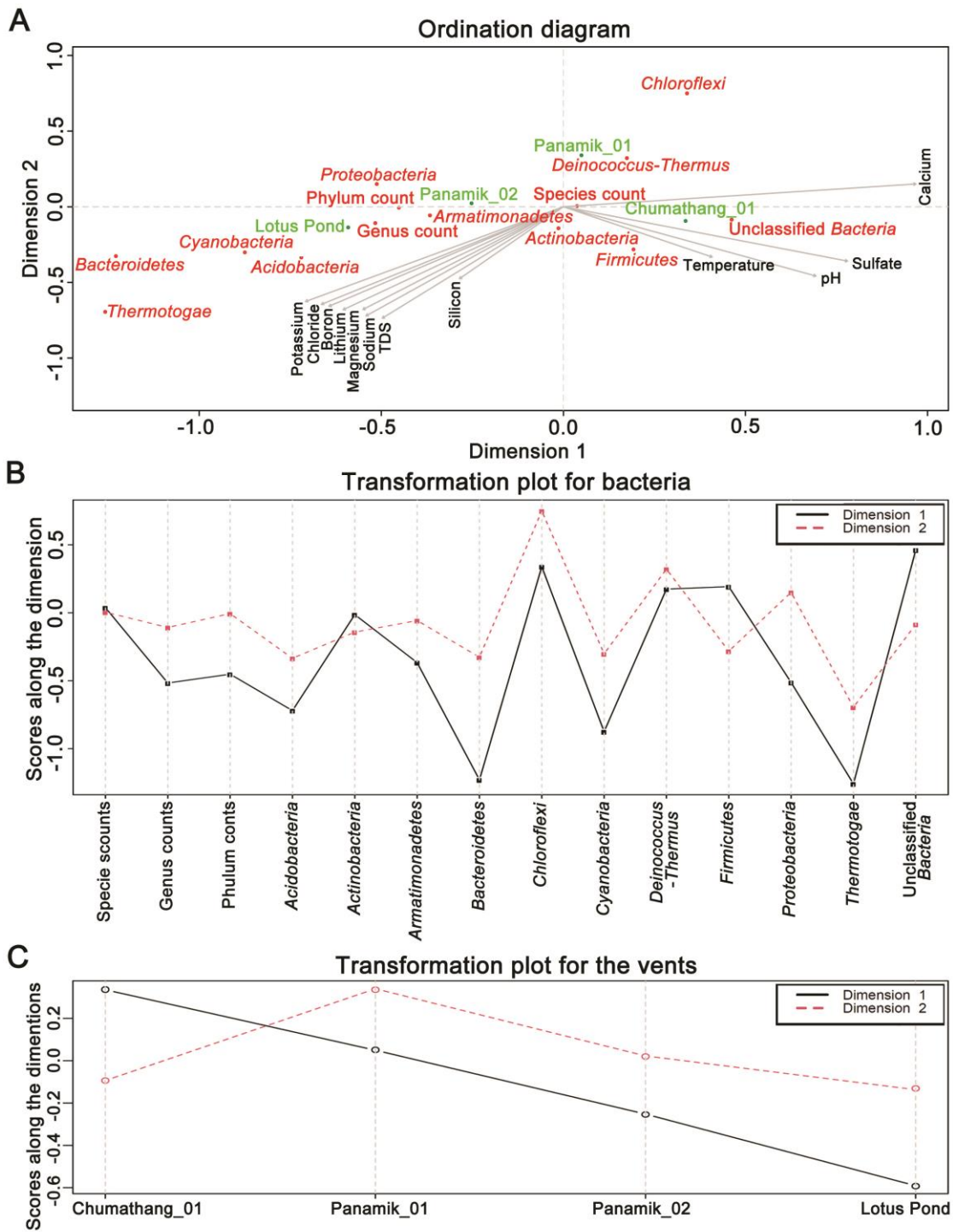




Figure 3



**Table 1.** Physicochemical characteristics<sup>1</sup> of the vent-water samples investigated for the hot springs of the Chumathang and Panamik geothermal areas of eastern and northern Ladakh respectively (NT = not tested; corresponding data for the vent-water of the Lotus Pond hot spring<sup>2</sup> located in the Puga geothermal area of eastern Ladakh have been included for comparative purposes).

Physicochemical parameter	Chumathang_01	Panamik_01	Panamik_02	Lotus Pond
Temperature (in °C)	86	78	70	81
pH	8.25	7.75	7.75	7.8
Total dissolved solids (mg L <sup>-1</sup> )	1150	530	540	1950
Boron concentration (ppm)	32.4	1.064	0.996	126.7
Silicon concentration (ppm)	59.373	55.329	48.279	66.7
Lithium concentration (ppm)	1.635	0.32	0.313	4.7
Sodium concentration (ppm)	300	140	145	553.5
Chloride concentration (ppm)	88.4	7.9	8.2	370.6
Calcium concentration (ppm)	18	11	10	2.1
Potassium concentration (ppm)	18.07	6.1	7.48	75
Magnesium concentration (ppm)	0.995	0.226	0.119	2.5
Rubidium concentration (ppm)	0.4	NT	NT	1.2
Cesium concentration (ppm)	2.5	NT	NT	7.5
Sulfate concentration (ppm)	241.7	99.85	100.9	92.5

- 1 Values presented for all the parameters are averages of the data obtained from three sampling replicates, with standard deviations <5% of their respective means.
- 2 Physicochemical properties of Lotus Pond vent-water have been studied by Roy *et al.* (2020a) at multiple hours of a day; all the data presented here, except total dissolved solid concentration, correspond to the values recorded by Roy *et al.* (2020a) at 14:30 h of 20 October 2014; the total dissolved solid concentration given here for Lotus Pond vent-water was determined at 11:00 h of 11 August 2017.

**Table 2.** Taxonomic classification of all the bacterial and archaeal OTUs that were detected in the vent-water samples of the Chumathang and Panamik hot springs explored (corresponding data for the vent-water of the Lotus Pond hot spring<sup>1</sup> located in Puga Valley have been included for comparative purposes).

	<b>Chumathang_01</b>	<b>Panamik_01</b>	<b>Panamik_02</b>	<b>Lotus Pond</b>
<b>Total bacterial OTUs detected</b>	892	329	496	279
<b>No. of established genera over which the bacterial OTUs were classified</b>	75	40	84	100
<b>No. of established phyla over which the bacterial OTUs were classified</b>	13	9	13	16
<i>Acidobacteria</i>	3	0	7	5
<i>Actinobacteria</i>	69	17	37	26
<i>Aquificae</i>	3	2	2	3
<i>Armatimonadetes</i>	3	1	5	2
<i>Bacteroidetes</i>	0	1	15	18
Candidatus Saccharibacteria	0	0	1	1
<i>Chloroflexi</i>	31	36	8	2
<i>Cyanobacteria</i>	4	1	12	12
<i>Deinococcus-Thermus</i>	77	52	35	15
<i>Dictyoglomi</i>	0	0	0	1
<i>Firmicutes</i>	101	14	29	27
<i>Nitrospirae</i>	1	0	1	1
<i>Proteobacteria</i>	86	94	204	120
<i>Spirochaetes</i>	0	0	0	1
Synergistetes	1	0	0	0
<i>Thermodesulfobacteria</i>	1	0	0	2
<i>Thermotogae</i>	1	0	1	6
Unclassified <i>Bacteria</i>	511	111	139	9
<b>Total archaeal OTUs detected</b>	55	30	10	NT <sup>2</sup>
<b>No. of established genera over which the archaeal OTUs were classified</b>	5	1	1	NA <sup>2</sup>
<b>No. of established phyla over which the archaeal OTUs were classified</b>	3	1	2	NA
<i>Crenarchaeota</i>	26	13	3	NA
<i>Euryarchaeota</i>	2	0	0	NA
<i>Korarchaeota</i>	1	0	0	NA
<i>Thaumarchaeota</i>	0	0	1	NA
Unclassified <i>Archaea</i>	26	17	6	NA

<sup>1</sup> Bacterial diversity of the Lotus Pond vent-water has been studied by Roy *et al.* (2016, 2020a) at multiple sampling hours of a day; the data presented here shows the diversity recorded by Roy *et al.* (2020a) at 14:30 h of 20 October 2014.

<sup>2</sup> NT = not tested. NA = not applicable. Roy *et al.* (2016, 2020a) did not explore the archaeal diversity of Lotus Pond; however, another study exploring the microbiome of the hot spring-microbialite system called Shivlinga (Roy *et al.* 2020b), also located in the Puga valley and within 50 m of Lotus Pond, failed to detect any OTU in the vent-water by using the universal archaeal 16S rRNA gene sequence primers 344F (5' - AATTGGANTCAACGCCGG - 3') and 522R (5' - TCGRCGGCCATGCACCWC - 3').

## Supplementary Materials

### Indus and Nubra Valley hot springs affirm the geomicrobiological specialties of Trans-Himalayan hydrothermal systems

Nibendu Mondal<sup>1</sup>, Aditya Peketi<sup>2</sup>, Tarunendu Mapder<sup>3</sup>, Chayan Roy<sup>4</sup>, Aninda Mazumdar<sup>2</sup>, Ranadhir Chakraborty<sup>5</sup> and Wriddhiman Ghosh<sup>1\*</sup>

#### Author Address:

<sup>1</sup> Department of Microbiology, Bose Institute, P-1/12 CIT Scheme VIIM, Kolkata 700054, India.

<sup>2</sup> Division of Geological Oceanography, CSIR National Institute of Oceanography, Dona Paula, Goa 403004, India.

<sup>3</sup> Division of Clinical Pharmacology, Department of Medicine, Indiana University School of Medicine, Indianapolis, IN 46202, USA

<sup>4</sup> College of Veterinary Medicine, Western University of Health Sciences, 309 East Second Street, Pomona, CA 91766, USA

<sup>5</sup> Department of Biotechnology, University of North Bengal, Siliguri, West Bengal 734013, India

\* **Correspondence:** wriman@jbose.ac.in / Wriman@rediffmail.com

---

## Contents

### Supplementary Tables

**Table S1.** Summary statistics of the OTU analyses carried out for the vent-water communities of Chumathang\_01, Panamik\_01 and Panamik\_02.

**Table S2.** Genera over which the bacterial OTUs of Chumathang\_01, Panamik\_01, Panamik\_02 and Lotus Pond were classified.

**Table S3.** Genera over which the archaeal OTUs of Chumathang\_01, Panamik\_01 and Panamik\_02 were classified.

### Supplementary Figures

**Figure S1.** Rarefaction curves showing the proportionality between OTU-level diversity revealed and the number of 16S rRNA gene sequence (V3 region) reads analyzed for (A-C) bacteria or (D-F) archaea in the different vent-water samples.

**Figure S2.** Microbial community architecture of the vent-waters of the Panamik\_01, Panamik\_02 and Chumathang\_01 hot springs.

### Supplementary Tables

**Table S1.** Summary statistics of the OTU analyses carried out for the vent-water communities of Chumathang\_01, Panamik\_01 and Panamik\_02.

Vent-water sample	Type of 16S rRNA gene analyzed	BioSample accession number	Run accession number	Total number of sequencing reads generated	Number of reads used for OTU clustering after quality filtering	Number of OTUs obtained (minus singletons)	Number of singletons obtained	Shannon index (base e)	Simpson index
Chumathang_01	Bacterial	SAMN16132276	SRR12665107	1506571	1428010	892	215393	1.10	0.556
Panamik_01	Bacterial	SAMN16132277	SRR12665106	1326973	1233603	329	187518	1.10	0.407
Panamik_02	Bacterial	SAMN16132384	SRR12665227	1620870	1520319	496	184796	0.284	0.890
Chumathang_01	Archaeal	SAMN16132383	SRR12665228	565096	515467	55	401081	0.869	0.476
Panamik_01	Archaeal	SAMN16132382	SRR12640680	913153	835732	30	571136	0.690	0.503
Panamik_02	Archaeal	SAMN16132278	SRR12640556	127103	115652	10	91789	0.692	0.501

**Table S2.** Established genera over which the bacterial OTUs of Chumathang\_01, Panamik\_01, Panamik\_02 and Lotus Pond were classified<sup>1</sup> (the highest temperatures reported for laboratory growths of the members of the different genera have also been shown together with the relevant references).

Phylum	Genus	Chumathang_01	Panamik_01	Panamik_02	Lotus Pond <sup>2</sup>	Upper limit of temperature (°C) for laboratory growth	Reference
<i>Acidobacteria</i>	<i>Geothrix</i>	0	0	0	+	35	Coates <i>et al.</i> 1999
	Gp16	3	0	1	-	-	-
	Gp2	0	0	2	+	-	-
	Gp3	0	0	1	+	-	-
	Gp6	0	0	1	-	-	-
<i>Actinobacteria</i>	<i>Actinomyces</i>	0	0	0	+	55	An <i>et al.</i> 2006
	<i>Bifidobacterium</i>	2	0	0	-	37	Killer <i>et al.</i> 2013
	<i>Brachybacterium</i>	0	1	1	+	30	Collin <i>et al.</i> 1988
	<i>Brevibacterium</i>	1	1	1	+	42	Kim <i>et al.</i> 2013
	<i>Collinsella</i>	1	0	0	-	37	Kageyama <i>et al.</i> 1999
	<i>Corynebacterium</i>	2	3	3	+	42	Bernard <i>et al.</i> 2016
	<i>Dietzia</i>	0	0	1	+	30	Kim <i>et al.</i> 2011
	<i>Euzebya</i>	1	0	0	-	28	Kurahashi <i>et al.</i> 2010
	<i>Friedmanniella</i>	1	0	0	-	30	Kim <i>et al.</i> 2016
	<i>Gaiella</i>	2	0	1	-	37	Albuquerque <i>et al.</i> 2011
	<i>Ilumatobacter</i>	2	0	0	-	31	Matsumoto <i>et al.</i> 2009
	<i>Marmoricola</i>	0	0	0	+	40	Lee <i>et al.</i> 201
	<i>Microbacterium</i>	0	0	1	+	30	Young <i>et al.</i> 2010
	<i>Mycobacterium</i>	1	1	1	+	43	Kusunoki and Ezaki 1992
	<i>Nesterenkononia</i>	1	0	0	-	42	Luo <i>et al.</i> 2009
	<i>Nitriliruptor</i>	1	0	2	-	32	Sorokin <i>et al.</i> 2009
	<i>Nocardia</i>	0	1	0	-	37	Jurado <i>et al.</i> 2008
	<i>Nocardioides</i>	1	0	0	+	37	Prauser 1976
	<i>Ornithinimicrobium</i>	1	1	0	-	50	Groth <i>et al.</i> 2001
	<i>Patulibacter</i>	0	0	0	+	39	Almeida <i>et al.</i> 2013
	<i>Pedomicrobium</i>	0	0	0	+	40	Gebers 1981
	<i>Propionibacterium</i>	1	2	1	+	45	Koussémon <i>et al.</i> 2001
	<i>Pseudonocardia</i>	2	0	0	-	38	Duangma <i>et al.</i> 2009
	<i>Rothia</i>	1	0	0	-	37	Fan <i>et al.</i> 2002
<i>Rubrobacter</i>	7	0	1	+	60	Carreto <i>et al.</i> 1996	

	<i>Solirubrobacter</i>	1	0	0	-	30	Singleton <i>et al.</i> 2003
	<i>Thermoleophilum</i>	1	0	1	-	60	Zarilla and Perry 1984
	<i>Turicella</i>	0	0	0	+	37	Funke <i>et al.</i> 1994
<i>Aquificae</i>	<i>Hydrogenobacter</i>	3	2	2	+	85	Takai <i>et al.</i> 2001
<i>Armatimonadetes</i>	Armatimonadetes_gp4	0	0	1	-	-	-
	Armatimonadetes_gp5	0	0	0	+	-	-
	Armatimonadetes_gp6	1	0	1	-	-	-
	Armatimonadetes_gp7	2	1	3	+	-	-
<i>Bacteroidetes</i>	<i>Algoriphagus</i>	0	0	1	-	37	Li <i>et al.</i> 2001
	<i>Chryseobacterium</i>	0	0	0	+	37	Yang <i>et al.</i> 2015
	<i>Cloacibacterium</i>	0	0	0	+	40	Chun <i>et al.</i> 2017
	<i>Cruoricaptor</i>	0	0	0	+	37	Yassin <i>et al.</i> 2012
	<i>Elizabethkingia</i>	0	1	1	-	37	Kampfer <i>et al.</i> 2011
	<i>Flavobacterium</i>	0	0	2	-	25	Lee <i>et al.</i> 2012
	<i>Pedobacter</i>	0	0	0	+	35	Zhang <i>et al.</i> 2015
	<i>Porphyromonas</i>	0	0	1	-	37	Saha and Collins 1998
	<i>Rhodothermus</i>	0	0	1	-	80	Sako <i>et al.</i> 1996
	<i>Schleiferia</i>	0	0	1	-	50	Albuquerque <i>et al.</i> 2011
<i>Sulfurihydrogenibium</i>	0	0	0	+	80	O'Neill <i>et al.</i> 2008	
<i>Candidatus Saccharibacteria</i>	Saccharibacteria_genera_incertae_sedis	0	0	1	+	-	-
<i>Chlorobi</i>	<i>Dehalogenimonas</i>	0	0	0	+	42	Bowman <i>et al.</i> 2013
<i>Chloroflexi</i>	<i>Caldilinea</i>	1	0	0	-	65	Sekiguchi <i>et al.</i> 2003
	<i>Chloroflexus</i>	6	0	1	+	59	Gaisin <i>et al.</i> 2017
	<i>Kallotenue</i>	3	0	0	-	65	Cole <i>et al.</i> 2013
	<i>Thermoflexus</i>	2	32	4	-	75	Dodsworth <i>et al.</i> 2014
	<i>Thermomicrobium</i>	9	3	2	-	70	King <i>et al.</i> 2014
<i>Cyanobacteria</i>	GpI	0	0	0	+	-	-
	GpIV	0	0	1	+	-	-
	GPXIII	0	0	0	+	-	-
<i>Deinococcus-Thermus</i>	<i>Deinococcus</i>	3	0	1	+	45	Hussain <i>et al.</i> 2016
	<i>Meiothermus</i>	8	1	2	-	60	Zhang <i>et al.</i> 2010
	<i>Thermus</i>	48	47	29	+	79	Brock and Freeze 1969
	<i>Truepera</i>	4	0	1	+	50	Albuquerque <i>et al.</i> 2005
<i>Firmicutes</i>	<i>Anaerobranca</i>	1	0	1	-	66	Engle <i>et al.</i> 1995
	<i>Anoxybacillus</i>	2	0	1	+	70	Coleri <i>et al.</i> 2014
	<i>Bacillus</i>	5	5	6	+	70	Yang <i>et al.</i> 2013a

	<i>Brevibacillus</i>	2	0	1	+	65	Inan <i>et al.</i> 2012
	<i>Caldalkalibacillus</i>	1	0	0	-	65	Xue <i>et al.</i> 2006
	<i>Caldicellulosiruptor</i>	0	0	0	+	90	Yang <i>et al.</i> 2010
	<i>Calditerricola</i>	0	0	1	-	83	Moriya <i>et al.</i> 2011
	<i>Clostridium sensu stricto</i>	1	0	0	-	70	Shiratori <i>et al.</i> 2009
	<i>Clostridium XIVa</i>	0	0	0	+	-	-
	<i>Dictyoglomus</i>	0	0	0	+	80	Saiki <i>et al.</i> 1985
	<i>Exiguobacterium</i>	2	0	0	+	49	Crapart <i>et al.</i> 2007
	<i>Finegoldia</i>	0	0	0	+	37	Murdoch and Shah 1999
	<i>Gemella</i>	0	0	0	+	37	Kilpper-Bälz and Schleifer 1988
	<i>Gemmiger</i>	1	0	0	-	45	Gossling and Moore 1975
	<i>Geobacillus</i>	0	0	0	+	80	Coorevits <i>et al.</i> 2012
	<i>Lysinibacillus</i>	0	0	0	+	45	Ahmed <i>et al.</i> 2007
	<i>Marinococcus</i>	0	0	0	+	50	Balderrama-Subieta <i>et al.</i> 2013
	<i>Oxalophagus</i>	0	0	1	-	35	Dehning and Schink 1989
	<i>Paenibacillus</i>	0	0	0	+	37	Shida <i>et al.</i> 1997
	<i>Peptoniphilus</i>	0	0	0	+	43	Patel <i>et al.</i> 2016
	<i>Piscibacillus</i>	1	0	0	-	55	Amoozegar <i>et al.</i> 2009
	<i>Planococcus</i>	1	0	0	+	42	Suresh <i>et al.</i> 2007
	<i>Planomicrobium</i>	1	0	0	-	38	Luo <i>et al.</i> 2014
	<i>Romboutsia</i>	1	0	0	-	40	Wang <i>et al.</i> 2015
	<i>Salinicoccus</i>	1	0	0	-	49	Zhang <i>et al.</i> 2002
	Sporolactobacillaceae_in certae_sedis	0	0	1	+	-	-
	<i>Sporolituus</i>	0	0	0	+	60	Ogg and Patel 2009
	<i>Staphylococcus</i>	2	1	1	+	40	Fuente <i>et al.</i> 1985
	<i>Streptococcus</i>	1	1	1	+	45	Sherman and Stark 1931
	<i>Thermoactinomyces</i>	0	0	0	+	60	Yao <i>et al.</i> 2014
<i>Nitrospirae</i>	<i>Nitrospira</i>	0	0	1	-	30	Watson <i>et al.</i> 1986
	<i>Thermodesulfovibrio</i>	1	0	0	+	70	Sekiguchi <i>et al.</i> 2008
<i>Proteobacteria</i>	<i>Achromobacter</i>	1	0	1	+	42	Vandamme <i>et al.</i> 2016
	<i>Acidovorax</i>	0	0	0	+	37	Heylen <i>et al.</i> 2008
	<i>Acinetobacter</i>	3	1	3	+	37	Smet <i>et al.</i> 2014
	<i>Advenella</i>	28	42	77	+	35	Wang <i>et al.</i> 2016
	<i>Aeromonas</i>	0	0	1	+	41	Huys <i>et al.</i> 2002
	<i>Albidovulum</i>	0	0	1	-	58	Yin <i>et al.</i> 2012



<i>Alcanivorax</i>	0	1	0	+	43	Kwon <i>et al.</i> 2015
<i>Amaricoccus</i>	0	1	0	-	37	Maszenan <i>et al.</i> 1997
<i>Aquabacterium</i>	0	0	1	-	40	Chen <i>et al.</i> 2012a
<i>Aquicella</i>	0	0	1	-	43	Santos <i>et al.</i> 2003
<i>Azospira</i>	1	0	1	-	40	Reinhold-Hurek <i>et al.</i> 2000
<i>Azospirillum</i>	1	1	1	-	37	Mehnaz <i>et al.</i> 2007
<i>Beijerinckia</i>	1	1	1	+	30	Oggerin <i>et al.</i> 2009
<i>Bradyrhizobium</i>	1	1	1	+	37	Araújo <i>et al.</i> 2017
<i>Brevundimonas</i>	1	0	1	+	42	Choi <i>et al.</i> 2010
<i>Burkholderia</i>	1	1	1	+	37	Aizawa <i>et al.</i> 2011
<i>Caulobacter</i>	0	1	1	-	32	Jin <i>et al.</i> 2013
<i>Craurococcus</i>	2	0	0	-	32	Saitoh <i>et al.</i> 1998
<i>Comamonas</i>	0	0	0	+	44	Chang <i>et al.</i> 2002
<i>Delftia</i>	0	0	1	+	40	Chen <i>et al.</i> 2012b
<i>Desulfovibrio</i>	0	0	0	+	37	Maarel <i>et al.</i> 1996
<i>Elioraea</i>	0	1	1	-	50	Albuquerque <i>et al.</i> 2008
<i>Enhydrobacter</i>	0	1	1	+	41	Staley <i>et al.</i> 1987
<i>Ensifer</i>	1	0	0	-	37	Casida Jr 1982
<i>Escherichia_Shigella</i>	1	2	1	+	37	Liu <i>et al.</i> 2015
<i>Ferrovibrio</i>	1	0	0	-	45	Sorokina <i>et al.</i> 2012
<i>Filomicrobium</i>	0	0	1	-	45	Wu <i>et al.</i> 2009
<i>Geminicoccus</i>	0	0	1	-	45	Foesel <i>et al.</i> 2007
<i>Gemmobacter</i>	0	1	0	-	37	Liu <i>et al.</i> 2014
<i>Halomonas</i>	0	0	0	+	50	Guan <i>et al.</i> 2010
<i>Hydrogenophaga</i>	0	0	1	-	37	Yoon <i>et al.</i> 2008
<i>Hyphomicrobium</i>	2	2	1	+	30	McDonald <i>et al.</i> 2001
<i>Hyphomonas</i>	0	0	1	-	42	Moore <i>et al.</i> 1984
<i>Klebsiella</i>	0	0	0	+	37	Bergey <i>et al.</i> 1923
<i>Limnobacter</i>	1	0	0	-	42	Lu <i>et al.</i> 2011
<i>Litoreibacter</i>	0	0	1	+	37	Romanenko <i>et al.</i> 2011
<i>Marinobacter</i>	0	0	0	+	50	Wang <i>et al.</i> 2009
<i>Methylobacillus</i>	1	0	0	-	30	Kaparullina <i>et al.</i> 2007
<i>Methylobacterium</i>	1	1	1	+	37	Doronina <i>et al.</i> 2002
<i>Methylocystis</i>	1	0	0	-	40	Bowman <i>et al.</i> 1993
<i>Methylophaga</i>	1	1	0	-	40	Janiver <i>et al.</i> 1985
<i>Methylothermus</i>	0	0	2	-	65	Hirayama <i>et al.</i> 2011
<i>Methyloversatilis</i>	0	0	1	+	37	Kalyuzhnaya <i>et al.</i> 2006

	<i>Neisseria</i>	0	0	1	–	37	Hansen <i>et al.</i> 2017
	<i>Nevskia</i>	0	0	0	+	25	Leandro <i>et al.</i> 2012
	<i>Paracoccus</i>	1	1	1	+	45	Sun <i>et al.</i> 2015
	<i>Pelomonas</i>	0	0	1	+	37	Gomila <i>et al.</i> 2007
	<i>Photobacterium</i>	0	0	0	+	50	Wang <i>et al.</i> 2017
	<i>Proteus</i>	0	0	1	–	30	Hyun <i>et al.</i> 2016
	<i>Providencia</i>	0	0	1	–	41	Somvanshi <i>et al.</i> 2006
	<i>Pseudomonas</i>	2	2	2	+	42	Yang <i>et al.</i> 2013b
	<i>Ralstonia</i>	0	0	0	+	41	Yabuuchi <i>et al.</i> 1995
	<i>Rehaibacterium</i>	0	0	1	–	55	Yu <i>et al.</i> 2013
	<i>Reyranella</i>	0	0	1	–	35	Pagnier <i>et al.</i> 2011
	<i>Rheinheimera</i>	0	0	0	+	35	Ryu <i>et al.</i> 2008
	<i>Rhodobacter</i>	0	1	0	–	40	Arunasri <i>et al.</i> 2008
	<i>Rhodoplanes</i>	0	0	0	+	43	Okamura <i>et al.</i> 2009
	<i>Rubellimicrobium</i>	1	0	0	–	52	Denner <i>et al.</i> 2006
	<i>Serratia</i>	0	0	0	+	35	Geiger <i>et al.</i> 2010
	<i>Sneathiella</i>	0	1	0	–	37	Jordan <i>et al.</i> 2007
	<i>Sphingobium</i>	0	0	0	+	37	Kumari <i>et al.</i> 2009
	<i>Sphingomonas</i>	0	1	1	+	45	Gao <i>et al.</i> 2016
	<i>Stenotrophomonas</i>	1	1	1	+	42	Lee <i>et al.</i> 2011
	<i>Sulfitobacter</i>	0	1	1	+	40	Kumari <i>et al.</i> 2016
	<i>Sulfurimonas</i>	0	0	0	+	40	Inagaki <i>et al.</i> 2003
	<i>Tepidimonas</i>	0	0	0	+	60	Chen <i>et al.</i> 2013
	<i>Thermomonas</i>	0	0	0	+	50	Busse <i>et al.</i> 2002
	<i>Thiofaba</i>	0	0	0	+	51	Mori and Suzuki 2008
	<i>Thiothrix</i>	0	0	0	+	37	Howarth <i>et al.</i> 1999
	<i>Variovorax</i>	1	0	1	+	37	Miwa <i>et al.</i> 2008
	<i>Vibrio</i>	1	0	0	–	37	Macian <i>et al.</i> 2001
<i>Spirochaetes</i>	<i>Treponema</i>	0	0	0	+	30	Evans <i>et al.</i> 2009
<i>Synergistetes</i>	<i>Aminivibrio</i>	1	0	0	–	42	Honda <i>et al.</i> 2013
<i>Thermotogae</i>	<i>Fervidobacterium</i>	1	0	1	+	90	Cai <i>et al.</i> 2007

<sup>1</sup> Numbers of OTUs classified under each genus across the vent-water samples have been shown for Chumathang\_01, Panamik\_01 and Panamik\_02. OTU counts under the individual genera identified in Lotus Pond are not available in the corresponding literature (Roy *et al.* 2020a), so only the presence or absence of the genera has been indicated as ‘+’ or ‘–’.

<sup>2</sup> Bacterial diversity of the Lotus Pond vent-water has been studied by Roy *et al.* (2016, 2020a) at multiple sampling hours of a day; the data presented here shows the diversity recorded by Roy *et al.* (2020a) at 14:30 h of 20 October 2014.

**Table S3.** Established genera over which the archaeal OTUs of Chumathang\_01, Panamik\_01 and Panamik\_02 were classified<sup>1</sup> (the highest temperatures reported for laboratory growths of the members of the different genera have also been shown together with the relevant references).

Phylum	Genus	Chumathang_01	Panamik_01	Panamik_02	Upper limit of temperature (°C) for laboratory growth	References
<i>Crenarchaeota</i>	<i>Pyrodictium</i>	1	0	0	110	Pley <i>et al.</i> 1991
	<i>Pyrobaculum</i>	4	0	0	104	Huber <i>et al.</i> 1987; Völkl <i>et al.</i> 1993
	<i>Thermofilum</i>	1	0	0	100	Zillig <i>et al.</i> 1983
	<i>Caldisphaera</i>	0	1	0	80	Itoh <i>et al.</i> 2003
<i>Euryarchaeota</i>	<i>Archaeoglobus</i>	2	0	0	90	Burggraf <i>et al.</i> 1990
<i>Korarchaeota</i>	<i>Candidatus Korarchaeum</i>	1	0	0	Not known	-
<i>Thaumarchaeota</i>	<i>Nitrososphaera</i>	0	0	1	47	Stieglmeier <i>et al.</i> 2014

<sup>1</sup> Numbers of OTUs classified under each genus across the vent-water samples have been shown.

## References used in Tables S2 and S3

- Ahmed, I., Yokota, A., Yamazoe, A. and Fujiwara, T., 2007. Proposal of *Lysinibacillus boronitolerans* gen. nov. sp. nov., and transfer of *Bacillus fusiformis* to *Lysinibacillus fusiformis* comb. nov. and *Bacillus sphaericus* to *Lysinibacillus sphaericus* comb. nov. *International Journal of Systematic and Evolutionary Microbiology*, 57(5), pp.1117-1125.
- Aizawa, T., Vijarnsorn, P., Nakajima, M. and Sunairi, M., 2011. *Burkholderia bannensis* sp. nov., an acid-neutralizing bacterium isolated from torpedo grass (*Panicum repens*) growing in highly acidic swamps. *International journal of systematic and evolutionary microbiology*, 61(7), pp.1645-1650.
- Albuquerque, L., França, L., Rainey, F.A., Schumann, P., Nobre, M.F. and da Costa, M.S., 2011. *Gaiella occulta* gen. nov., sp. nov., a novel representative of a deep branching phylogenetic lineage within the class Actinobacteria and proposal of *Gaiellaceae* fam. nov. and *Gaiellales* ord. nov. *Systematic and applied microbiology*, 34(8), pp.595-599.
- Albuquerque, L., Rainey, F.A., Nobre, M.F. and da Costa, M.S., 2008. *Elioraea tepidiphila* gen. nov., sp. nov., a slightly thermophilic member of the Alphaproteobacteria. *International journal of systematic and evolutionary microbiology*, 58(4), pp.773-778.
- Albuquerque, L., Simoes, C., Nobre, M.F., Pino, N.M., Battista, J.R., Silva, M.T., Rainey, F.A. and de Costa, M.S., 2005. *Truepera radiovictrix* gen. nov., sp. nov., a new radiation resistant species and the proposal of *Trueperaceae* fam. nov. *FEMS microbiology letters*, 247(2), pp.161-169.
- Almeida, B., Vaz-Moreira, I., Schumann, P., Nunes, O.C., Carvalho, G. and Crespo, M.T.B., 2013. *Patulibacter medicamentivorans* sp. nov., isolated from activated sludge of a wastewater treatment plant. *International journal of systematic and evolutionary microbiology*, 63(7), pp.2588-2593.
- Amoozegar, M.A., Sánchez-Porro, C., Rohban, R., Hajighasemi, M. and Ventosa, A., 2009. *Piscibacillus halophilus* sp. nov., a moderately halophilic bacterium from a hypersaline Iranian lake. *International journal of systematic and evolutionary microbiology*, 59(12), pp.3095-3099.
- An, D., Cai, S. and Dong, X., 2006. *Actinomyces ruminicola* sp. nov., isolated from cattle rumen. *International journal of systematic and evolutionary microbiology*, 56(9), pp.2043-2048.
- Araújo, J., Flores-Félix, J.D., Igual, J.M., Peix, A., González-Andrés, F., Díaz-Alcántara, C.A. and Velázquez, E., 2017. *Bradyrhizobium cajani* sp. nov. isolated from nodules of *Cajanus cajan*. *International Journal of Systematic and Evolutionary Microbiology*, 67(7), pp.2236-2241.
- Arunasri, K., Ramana, V.V., Spröer, C., Sasikala, C. and Ramana, C.V., 2008. *Rhodobacter megalophilus* sp. nov., a phototroph from the Indian Himalayas possessing a wide temperature range for growth. *International journal of systematic and evolutionary microbiology*, 58(8), pp.1792-1796.
- Balderrama-Subieta, A., Guzmán, D., Minegishi, H., Echigo, A., Shimane, Y., Hatada, Y. and Quillaguamán, J., 2013. *Marinococcustarijensis* sp. nov., a moderately halophilic bacterium isolated from a salt mine. *International journal of systematic and evolutionary microbiology*, 63(9), pp.3319-3323.
- Bernard, K.A., Pacheco, A.L., Loomer, C., Burdz, T., Wiebe, D., Huynh, C., Kaplen, B., Olson, A.B., Cnockaert, M., Eguchi, H. and Kuwahara, T., 2016. *Corynebacterium lowii* sp. nov. and *Corynebacterium oculi* sp. nov., derived from human clinical disease and an emended description of *Corynebacterium mastitidis*. *International journal of systematic and evolutionary microbiology*, 66(8), pp.2803-2812.

- Bowman, J.P., SLY, L.I., NICHOLS, P.D. and Hayward, A.C., 1993. Revised taxonomy of the methanotrophs: description of *Methylobacter* gen. nov., emendation of *Methylococcus*, validation of *Methylosinus* and *Methylocystis* species, and a proposal that the family *Methylococcaceae* includes only the group I methanotrophs. *International Journal of Systematic and Evolutionary Microbiology*, 43(4), pp.735-753.
- Bowman, K.S., Nobre, M.F., da Costa, M.S., Rainey, F.A. and Moe, W.M., 2013. *Dehalogenimonas alkenigignens* sp. nov., a chlorinated-alkane-dehalogenating bacterium isolated from groundwater. *International Journal of Systematic and Evolutionary Microbiology*, 63(4), pp.1492-1498.
- Brock, T.D. and Freeze, H., 1969. *Thermus aquaticus* gen. n. and sp. n., a nonsporulating extreme thermophile. *Journal of bacteriology*, 98(1), pp.289-297.
- Burggraf, S., Jannasch, H.W., Nicolaus, B. and Stetter, K.O., 1990. *Archaeoglobus profundus* sp. nov., represents a new species within the sulfate-reducing archaeobacteria. *Systematic and applied microbiology*, 13(1), pp.24-28.
- Busse, H.J., Kämpfer, P., Moore, E.R.B., Nuutinen, J., Tsitko, I.V., Denner, E.B.M., Vauterin, L., Valens, M., Rosselló-Mora, R. and Salkinoja-Salonen, M.S., 2002. *Thermomonas haemolytica* gen. nov., sp. nov., a gamma-proteobacterium from kaolin slurry. *International journal of systematic and evolutionary microbiology*, 52(2), pp.473-483.
- Cai, J., Wang, Y., Liu, D., Zeng, Y., Xue, Y., Ma, Y. and Feng, Y., 2007. *Fervidobacterium changbaicum* sp. nov., a novel thermophilic anaerobic bacterium isolated from a hot spring of the Changbai Mountains, China. *International journal of systematic and evolutionary microbiology*, 57(10), pp.2333-2336.
- Carreto, L., Moore, E., Nobre, M.F., Wait, R., Riley, P.W., Sharp, R.J. and DA COSTA, M.S., 1996. *Rubrobacter xylanophilus* sp. nov., a new thermophilic species isolated from a thermally polluted effluent. *International Journal of Systematic and Evolutionary Microbiology*, 46(2), pp.460-465.
- Casida Jr, L.E., 1982. *Ensifer adhaerens* gen. nov., sp. nov.: a bacterial predator of bacteria in soil. *International Journal of Systematic and Evolutionary Microbiology*, 32(3), pp.339-345.
- Chang, Y.H., Han, J.I., Chun, J., Lee, K.C., Rhee, M.S., Kim, Y.B. and Bae, K.S., 2002. *Comamonas koreensis* sp. nov., a non-motile species from wetland in Woopo, Korea. *International journal of systematic and evolutionary microbiology*, 52(2), pp.377-381.
- Chen, W.M., Cho, N.T., Yang, S.H., Arun, A.B., Young, C.C. and Sheu, S.Y., 2012a. *Aquabacterium limnoticum* sp. nov., isolated from a freshwater spring. *International journal of systematic and evolutionary microbiology*, 62(3), pp.698-704.
- Chen, W.M., Huang, H.W., Chang, J.S., Han, Y.L., Guo, T.R. and Sheu, S.Y., 2013. *Tepidimonas fonticaldi* sp. nov., a slightly thermophilic betaproteobacterium isolated from a hot spring. *International journal of systematic and evolutionary microbiology*, 63(5), pp.1810-1816.
- Chen, W.M., Lin, Y.S., Sheu, D.S. and Sheu, S.Y., 2012b. *Delftia litopenaei* sp. nov., a poly- $\beta$ -hydroxybutyrate-accumulating bacterium isolated from a freshwater shrimp culture pond. *International journal of systematic and evolutionary microbiology*, 62(10), pp.2315-2321.
- Choi, J.H., Kim, M.S., Roh, S.W. and Bae, J.W., 2010. *Brevundimonas basaltis* sp. nov., isolated from black sand. *International journal of systematic and evolutionary microbiology*, 60(7), pp.1488-1492.
- Chun, B.H., Lee, Y., Jin, H.M. and Jeon, C.O., 2017. *Cloacibacterium caeni* sp. nov., isolated from activated sludge. *International journal of systematic and evolutionary microbiology*, 67(6), pp.1688-1692.

- Cihan, A.C., Cokmus, C., Koc, M. and Ozcan, B., 2014. *Anoxybacillus calidus* sp. nov., a thermophilic bacterium isolated from soil near a thermal power plant. *International journal of systematic and evolutionary microbiology*, 64(1), pp.211-219.
- Coates, J.D., Ellis, D.J., Gaw, C.V. and Lovley, D.R., 1999. *Geothrix fermentans* gen. nov., sp. nov., a novel Fe (III)-reducing bacterium from a hydrocarbon-contaminated aquifer. *International journal of systematic and evolutionary microbiology*, 49(4), pp.1615-1622.
- Cole, J.K., Gieler, B.A., Heisler, D.L., Palisoc, M.M., Williams, A.J., Dohnalkova, A.C., Ming, H., Yu, T.T., Dodsworth, J.A., Li, W.J. and Hedlund, B.P., 2013. *Kallotenua papyrolyticum* gen. nov., sp. nov., a cellulolytic and filamentous thermophile that represents a novel lineage (Kallotenuales ord. nov., Kallotenuaceae fam. nov.) within the class Chloroflexia. *International journal of systematic and evolutionary microbiology*, 63(12), pp.4675-4682.
- Collin MD., Brown J. and Jones D. 1988. *Brachybacterium faecium* gen. nov., sp. nov., a coryneform bacterium from poultry deep litter. *International Journal of Systematic and Evolutionary Microbiology*, 38(1), pp.45-48.
- Crapart, S., Fardeau, M.L., Cayol, J.L., Thomas, P., Sery, C., Ollivier, B. and Combet-Blanc, Y., 2007. *Exiguobacterium profundum* sp. nov., a moderately thermophilic, lactic acid-producing bacterium isolated from a deep-sea hydrothermal vent. *International journal of systematic and evolutionary microbiology*, 57(2), pp.287-292.
- De la Fuente, R., Suarez, G. and Schleifer, K.H., 1985. *Staphylococcus aureus* subsp. *anaerobius* subsp. nov., the causal agent of abscess disease of sheep. *International Journal of Systematic and Evolutionary Microbiology*, 35(1), pp.99-102.
- Dedysh, S.N., Kulichevskaya, I.S., Huber, K.J. and Overmann, J., 2017. Defining the taxonomic status of described subdivision 3 Acidobacteria: proposal of Bryobacteraceae fam. nov. *International journal of systematic and evolutionary microbiology*, 67(2), pp.498-501.
- Dehning, I. and Schink, B., 1989. Two new species of anaerobic oxalate-fermenting bacteria, *Oxalobacter vibrioformis* sp. nov. and *Clostridium oxalicum* sp. nov., from sediment samples. *Archives of microbiology*, 153(1), pp.79-84.
- Denner, E.B., Kolari, M., Hoornstra, D., Tsitko, I., Kämpfer, P., Busse, H.J. and Salkinoja-Salonen, M., 2006. *Rubellimicrobium thermophilum* gen. nov., sp. nov., a red-pigmented, moderately thermophilic bacterium isolated from coloured slime deposits in paper machines. *International journal of systematic and evolutionary microbiology*, 56(6), pp.1355-1362.
- Dodsworth, J.A., Gevorkian, J., Despujos, F., Cole, J.K., Murugapiran, S.K., Ming, H., Li, W.J., Zhang, G., Dohnalkova, A. and Hedlund, B.P., 2014. *Thermoflexus hugenholtzii* gen. nov., sp. nov., a thermophilic, microaerophilic, filamentous bacterium representing a novel class in the Chloroflexi, Thermoflexia classis nov., and description of Thermoflexaceae fam. nov. and Thermoflexales ord. nov. *International journal of systematic and evolutionary microbiology*, 64(6), pp.2119-2127.
- Doronina, N.V., Trotsenko, Y.A., Kuznetsov, B.B., Tourova, T.P. and Salkinoja-Salonen, M.S., 2002. *Methylobacterium suomiense* sp. nov. and *Methylobacterium lusitanum* sp. nov., aerobic, pink-pigmented, facultatively methylotrophic bacteria. *International Journal of Systematic and Evolutionary Microbiology*, 52(3), pp.773-776.

- Duangmal, K., Thamchaipenet, A., Matsumoto, A. and Takahashi, Y., 2009. *Pseudonocardia acaciae* sp. nov., isolated from roots of *Acacia auriculiformis* A. Cunn. ex Benth. *International journal of systematic and evolutionary microbiology*, 59(6), pp.1487-1491.
- Engle, M., Li, Y., Woese, C. and Wiegel, J., 1995. Isolation and Characterization of a Novel Alkalitolerant Thermophile, *Anaerobranca horikoshii* gen. nov., sp. nov. *International Journal of Systematic and Evolutionary Microbiology*, 45(3), pp.454-461.
- Evans, N.J., Brown, J.M., Demirkan, I., Murray, R.D., Birtles, R.J., Hart, C.A. and Carter, S.D., 2009. *Treponema pedis* sp. nov., a spirochaete isolated from bovine digital dermatitis lesions. *International journal of systematic and evolutionary microbiology*, 59(5), pp.987-991.
- Fan, Y., Jin, Z., Tong, J., Li, W., Pasciak, M., Gamian, A., Liu, Z. and Huang, Y., 2002. *Rothia amarae* sp. nov., from sludge of a foul water sewer. *International journal of systematic and evolutionary microbiology*, 52(6), pp.2257-2260.
- Foesel, B.U., Gößner, A.S., Drake, H.L. and Schramm, A., 2007. *Geminicoccus roseus* gen. nov., sp. nov., an aerobic phototrophic Alphaproteobacterium isolated from a marine aquaculture biofilter. *Systematic and applied microbiology*, 30(8), pp.581-586.
- Funke, G., Stubbs, S., Altwegg, M., Carlotti, A. and Collins, M.D., 1994. *Turicella otitidis* gen. nov., sp. nov., a coryneform bacterium isolated from patients with otitis media. *International Journal of Systematic and Evolutionary Microbiology*, 44(2), pp.270-273.
- Gaisin, V.A., Kalashnikov, A.M., Grouzdev, D.S., Sukhacheva, M.V., Kuznetsov, B.B. and Gorlenko, V.M., 2017. *Chloroflexus islandicus* sp. nov., a thermophilic filamentous anoxygenic phototrophic bacterium from a geyser. *International Journal of Systematic and Evolutionary Microbiology*, 67(5), pp.1381-1386.
- Gao, J.L., Sun, P., Wang, X.M., Cheng, S., Lv, F., Qiu, T.L., Yuan, M. and Sun, J.G., 2016. *Sphingomonas zeicaulis* sp. nov., an endophytic bacterium isolated from maize root. *International journal of systematic and evolutionary microbiology*, 66(9), pp.3755-3760.
- GEBERS, R., 1981. Enrichment, isolation, and emended description of *Pedomicrobium ferrugineum* Aristovskaya and *Pedomicrobium manganicum* Aristovskaya. *International Journal of Systematic and Evolutionary Microbiology*, 31(3), pp.302-316.
- Geiger, A., Fardeau, M.L., Falsen, E., Ollivier, B. and Cuny, G., 2010. *Serratia glossinae* sp. nov., isolated from the midgut of the tsetse fly *Glossina palpalis gambiensis*. *International journal of systematic and evolutionary microbiology*, 60(6), pp.1261-1265.
- Gomila, M., Bowien, B., Falsen, E., Moore, E.R. and Lalucat, J., 2007. Description of *Pelomonas aquatica* sp. nov. and *Pelomonas puraquae* sp. nov., isolated from industrial and haemodialysis water. *International journal of systematic and evolutionary microbiology*, 57(11), pp.2629-2635.
- Gossling, J. and Moore, W.E.C., 1975. *Gemmiger formicilis*, n. gen., n. sp., an anaerobic budding bacterium from intestines. *International Journal of Systematic and Evolutionary Microbiology*, 25(2), pp.202-207.
- Groth, I., Schumann, P., Weiss, N., Schuetze, B., Augsten, K. and Stackebrandt, E., 2001. *Ornithinimicrobium humiphilum* gen. nov., sp. nov., a novel soil actinomycete with L-ornithine in the peptidoglycan. *International journal of systematic and evolutionary microbiology*, 51(1), pp.81-87.

- Guan, T.W., Xiao, J., Zhao, K., Luo, X.X., Zhang, X.P. and Zhang, L.L., 2010. *Halomonas xinjiangensis* sp. nov., a halotolerant bacterium isolated from a salt lake. *International journal of systematic and evolutionary microbiology*, 60(2), pp.349-352.
- Hansen, C.M., Himschoot, E.A., Hare, R.F., Meixell, B.W., Van Hemert, C. and Hueffer, K., 2017. *Neisseria arctica* sp. nov., isolated from nonviable eggs of greater white-fronted geese (*Anser albifrons*) in Arctic Alaska. *International journal of systematic and evolutionary microbiology*, 67(5), p.1115.
- Heylen, K., Lebbe, L. and De Vos, P., 2008. *Acidovorax caeni* sp. nov., a denitrifying species with genetically diverse isolates from activated sludge. *International Journal of Systematic and Evolutionary Microbiology*, 58(1), pp.73-77.
- Hirayama, H., Suzuki, Y., Abe, M., Miyazaki, M., Makita, H., Inagaki, F., Uematsu, K. and Takai, K., 2011. *Methylothermus subterraneus* sp. nov., a moderately thermophilic methanotroph isolated from a terrestrial subsurface hot aquifer. *International journal of systematic and evolutionary microbiology*, 61(11), pp.2646-2653.
- Holt, J.G., 1984. *Bergey's Manual of Determinative Bacteriology*, Vol. 1. *Williams and Wilkins, Baltimore*. 964p.
- Honda, T., Fujita, T. and Tonouchi, A., 2013. *Aminivibrio pyruvatiphilus* gen. nov., sp. nov., an anaerobic, amino-acid-degrading bacterium from soil of a Japanese rice field. *International journal of systematic and evolutionary microbiology*, 63(10), pp.3679-3686.
- Howarth, R., Unz, R.F., Seviour, E.M., Seviour, R.J., Blackall, L.L., Pickup, R.W., Jones, J.G., Yaguchi, J. and Head, I.M., 1999. Phylogenetic relationships of filamentous sulfur bacteria (*Thiothrix* spp. and Eikelboom type 021N bacteria) isolated from wastewatertreatment plants and description of *Thiothrix eikelboomii* sp. nov., *Thiothrix unzii* sp. nov., *Thiothrix fructosivorans* sp. nov. and *Thiothrix defluvii* sp. nov. *International Journal of Systematic and Evolutionary Microbiology*, 49(4), pp.1817-1827.
- Huber, R., Kristjansson, J.K. and Stetter, K.O., 1987. *Pyrobaculum* gen. nov., a new genus of neutrophilic, rod-shaped archaeobacteria from continental solfataras growing optimally at 100 C. *Archives of microbiology*, 149(2), pp.95-101.
- Hussain, F., Khan, I.U., Habib, N., Xian, W.D., Hozzein, W.N., Zhang, Z.D., Zhi, X.Y. and Li, W.J., 2016. *Deinococcus saudiensis* sp. nov., isolated from desert. *International journal of systematic and evolutionary microbiology*, 66(12), pp.5106-5111.
- Huys, G., Kämpfer, P., Albert, M.J., Kühn, I., Denys, R. and Swings, J., 2002. *Aeromonas hydrophila* subsp. *dhakensis* subsp. nov., isolated from children with diarrhoea in Bangladesh, and extended description of *Aeromonas hydrophila* subsp. *hydrophila* (Chester 1901) Stanier 1943 (approved lists 1980). *International journal of systematic and evolutionary microbiology*, 52(3), pp.705-712.
- Hyun, D.W., Jung, M.J., Kim, M.S., Shin, N.R., Kim, P.S., Whon, T.W. and Bae, J.W., 2016. *Proteus cibarius* sp. nov., a swarming bacterium from Jeotgal, a traditional Korean fermented seafood, and emended description of the genus *Proteus*. *International journal of systematic and evolutionary microbiology*, 66(6), pp.2158-2164.
- Inagaki, F., Takai, K., Kobayashi, H., Neilson, K.H. and Horikoshi, K., 2003. *Sulfurimonas autotrophica* gen. nov., sp. nov., a novel sulfur-oxidizing  $\epsilon$ -proteobacterium isolated from hydrothermal sediments in the Mid-Okinawa Trough. *International Journal of Systematic and Evolutionary Microbiology*, 53(6), pp.1801-1805.



- Inan, K., Canakci, S., Belduz, A.O. and Sahin, F., 2012. *Brevibacillus aydinogluensis* sp. nov., a moderately thermophilic bacterium isolated from Karakoc hot spring. *International journal of systematic and evolutionary microbiology*, 62(4), pp.849-855.
- Itoh, T., Suzuki, K., Sanchez, P.C. and Nakase, T., 2003. *Caldisphaera lagunensis* gen. nov., sp. nov., a novel thermoacidophilic crenarchaeote isolated from a hot spring at Mt Maquiling, Philippines. *Journal of Medical Microbiology*, 53(4), pp.1149-1154.
- Janvier, M., Frehel, C., Grimont, F. and Gasser, F., 1985. *Methylophaga marina* gen. nov., sp. nov. and *Methylophaga thalassica* sp. nov., marine methylotrophs. *International Journal of Systematic and Evolutionary Microbiology*, 35(2), pp.131-139.
- Jin, L., Lee, H.G., Kim, H.S., Ahn, C.Y. and Oh, H.M., 2013. *Caulobacter daechungensis* sp. nov., a stalked bacterium isolated from a eutrophic reservoir. *International journal of systematic and evolutionary microbiology*, 63(7), pp.2559-2564.
- Jordan, E.M., Thompson, F.L., Zhang, X.H., Li, Y., Vancanneyt, M., Kroppenstedt, R.M., Priest, F.G. and Austin, B., 2007. *Sneathiella chinensis* gen. nov., sp. nov., a novel marine alphaproteobacterium isolated from coastal sediment in Qingdao, China. *International journal of systematic and evolutionary microbiology*, 57(1), pp.114-121.
- Jurado, V., Boiron, P., Kroppenstedt, R.M., Laurent, F., Couble, A., Laiz, L., Klenk, H.P., González, J.M., Saiz-Jimenez, C., Mounié, D. and Bergeron, E., 2008. *Nocardia altamirensis* sp. nov., isolated from Altamira cave, Cantabria, Spain. *International journal of systematic and evolutionary microbiology*, 58(9), pp.2210-2214.
- Kageyama, A., Benno, Y. and Nakase, T., 1999. Phylogenetic and phenotypic evidence for the transfer of *Eubacterium aerofaciens* to the genus *Collinsella* as *Collinsella aerofaciens* gen. nov., comb. nov. *International Journal of Systematic and Evolutionary Microbiology*, 49(2), pp.557-565.
- Kalyuzhnaya, M.G., De Marco, P., Bowerman, S., Pacheco, C.C., Lara, J.C., Lidstrom, M.E. and Chistoserdova, L., 2006. *Methyloversatilis universalis* gen. nov., sp. nov., a novel taxon within the Betaproteobacteria represented by three methylotrophic isolates. *International Journal of Systematic and Evolutionary Microbiology*, 56(11), pp.2517-2522.
- Kämpfer, P., Matthews, H., Glaeser, S.P., Martin, K., Lodders, N. and Faye, I., 2011. *Elizabethkingia anophelis* sp. nov., isolated from the midgut of the mosquito *Anopheles gambiae*. *International journal of systematic and evolutionary microbiology*, 61(11), pp.2670-2675.
- Kaparullina, E.N., Trotsenko, Y.A. and Doronina, N.V., 2017. *Methylobacillus methanolivorans* sp. nov., a novel non-pigmented obligately methylotrophic bacterium. *International journal of systematic and evolutionary microbiology*, 67(2), pp.425-431.
- Kielak, A.M., Barreto, C.C., Kowalchuk, G.A., van Veen, J.A. and Kuramae, E.E., 2016. The ecology of Acidobacteria: moving beyond genes and genomes. *Frontiers in microbiology*, 7, p.744.
- Killer, J., Sedláček, I., Rada, V., Havlik, J. and Kopečný, J., 2013. Reclassification of *Bifidobacterium stercoris* Kim et al. 2010 as a later heterotypic synonym of *Bifidobacterium adolescentis*. *International journal of systematic and evolutionary microbiology*, 63(11), pp.4350-4353.
- Kilpper-Bälz, R. and Schleifer, K.H., 1988. Transfer of *Streptococcus morbillorum* to the Genus *Gemella* as *Gemella morbillorum* comb. nov. *International Journal of Systematic and Evolutionary Microbiology*, 38(4), pp.442-443.

- Kim, J., Roh, S.W., Choi, J.H., Jung, M.J., Nam, Y.D., Kim, M.S., Park, E.J., Shin, K.S. and Bae, J.W., 2011. *Dietzia alimentaria* sp. nov., isolated from a traditional Korean food. *International journal of systematic and evolutionary microbiology*, 61(9), pp.2254-2258.
- Kim, J., Srinivasan, S., You, T., Bang, J.J., Park, S. and Lee, S.S., 2013. *Brevibacterium ammoniilyticum* sp. nov., an ammonia-degrading bacterium isolated from sludge of a wastewater treatment plant. *International journal of systematic and evolutionary microbiology*, 63(3), pp.1111-1118.
- Kim, S.J., Hamada, M., Ahn, J.H., Weon, H.Y., Suzuki, K.I. and Kwon, S.W., 2016. *Friedmanniella aerolata* sp. nov., isolated from air. *International Journal of Systematic and Evolutionary Microbiology*, 66(5), pp.1970-1975.
- King, C.E. and King, G.M., 2014. *Thermomicrobium carboxidum* sp. nov., and *Thermorudis peleae* gen. nov., sp. nov., carbon monoxide-oxidizing bacteria isolated from geothermally heated biofilms. *International journal of systematic and evolutionary microbiology*, 64(8), pp.2586-2592.
- Koussémon, M., Combet-Blanc, Y., Patel, B.K., Cayol, J.L., Thomas, P., Garcia, J.L. and Ollivier, B., 2001. *Propionibacterium microaerophilum* sp. nov., a microaerophilic bacterium isolated from olive mill wastewater. *International journal of systematic and evolutionary microbiology*, 51(4), pp.1373-1382.
- Kumari, H., Gupta, S.K., Jindal, S., Katoch, P. and Lal, R., 2009. *Sphingobium lactosutens* sp. nov., isolated from a hexachlorocyclohexane dump site and *Sphingobium abikonense* sp. nov., isolated from oil-contaminated soil. *International journal of systematic and evolutionary microbiology*, 59(9), pp.2291-2296.
- Kumari, P., Bhattacharjee, S., Poddar, A. and Das, S.K., 2016. *Sulfitobacter faviae* sp. nov., isolated from the coral *Faviaveroni*. *International journal of systematic and evolutionary microbiology*, 66(10), pp.3786-3792.
- Kurahashi, M., Fukunaga, Y., Sakiyama, Y., Harayama, S. and Yokota, A., 2010. *Euzebya tangerina* gen. nov., sp. nov., a deeply branching marine actinobacterium isolated from the sea cucumber *Holothuria edulis*, and proposal of *Euzebyaceae* fam. nov., *Euzebyales* ord. nov. and *Nitriliruptoridae* subclassis nov. *International journal of systematic and evolutionary microbiology*, 60(10), pp.2314-2319.
- Kusunoki, S. and Ezaki, T., 1992. Proposal of *Mycobacterium peregrinum* sp. nov., nom. rev., and Elevation of *Mycobacterium chelonae* subsp. *abscessus* (Kubica et al.) to Species Status: *Mycobacterium abscessus* comb. nov. *International Journal of Systematic and Evolutionary Microbiology*, 42(2), pp.240-245.
- Kwon, K.K., Oh, J.H., Yang, S.H., Seo, H.S. and Lee, J.H., 2015. *Alcanivorax gelatiniphagus* sp. nov., a marine bacterium isolated from tidal flat sediments enriched with crude oil. *International journal of systematic and evolutionary microbiology*, 65(7), pp.2204-2208.
- Leandro, T., França, L., Nobre, M.F., Schumann, P., Rosselló-Móra, R. and da Costa, M.S., 2012. *Nevskia aquatilis* sp. nov. and *Nevskia persephonica* sp. nov., isolated from a mineral water aquifer and the emended description of the genus *Nevskia*. *Systematic and applied microbiology*, 35(5), pp.297-301.
- Lee, H.Y., Liu, Q., Kang, M.S., Kim, S.K., Lee, S.Y. and Im, W.T., 2016. *Marmoricola ginsengisoli* sp. nov. and *Marmoricola pocheonensis* sp. nov. isolated from a ginseng-cultivating field. *International journal of systematic and evolutionary microbiology*, 66(5), pp.1996-2001.
- Lee, M., Woo, S.G., Chae, M., Shin, M.C., Jung, H.M. and Ten, L.N., 2011. *Stenotrophomonas daejeonensis* sp. nov., isolated from sewage. *International journal of systematic and evolutionary microbiology*, 61(3), pp.598-604.

- Lee, S., Weon, H.Y., Han, K. and Ahn, T.Y., 2012. *Flavobacterium dankookense* sp. nov., isolated from a freshwater reservoir, and emended descriptions of *Flavobacterium cheonanense*, *F. chungnamense*, *F. koreense* and *F. aquatile*. *International journal of systematic and evolutionary microbiology*, 62(10), pp.2378-2382.
- Li, Y., Yan, S., Yang, Q., Qi, Z., Zhang, X.H. and Fu, Y.B., 2011. *Algoriphagus faecimaris* sp. nov., isolated from coastal sediment. *International journal of systematic and evolutionary microbiology*, 61(12), pp.2856-2860.
- Liu, J.J., Zhang, X.Q., Chi, F.T., Pan, J., Sun, C. and Wu, M., 2014. *Gemmobacter megaterium* sp. nov., isolated from coastal planktonic seaweeds. *International journal of systematic and evolutionary microbiology*, 64(1), pp.66-71.
- Liu, S., Jin, D., Lan, R., Wang, Y., Meng, Q., Dai, H., Lu, S., Hu, S. and Xu, J., 2015. *Escherichia marmotae* sp. nov., isolated from faeces of *Marmota himalayana*. *International journal of systematic and evolutionary microbiology*, 65(7), pp.2130-2134.
- Lu, H., Sato, Y., Fujimura, R., Nishizawa, T., Kamijo, T. and Ohta, H., 2011. *Limnobacter litoralis* sp. nov., a thiosulfate-oxidizing, heterotrophic bacterium isolated from a volcanic deposit, and emended description of the genus *Limnobacter*. *International journal of systematic and evolutionary microbiology*, 61(2), pp.404-407.
- Luo, H.Y., Wang, Y.R., Miao, L.H., Yang, P.L., Shi, P.J., Fang, C.X., Yao, B. and Fan, Y.L., 2009. *Nesterenkonia alba* sp. nov., an alkaliphilic actinobacterium isolated from the black liquor treatment system of a cotton pulp mill. *International journal of systematic and evolutionary microbiology*, 59(4), pp.863-868.
- Luo, X., Zhang, J., Li, D., Xin, Y., Xin, D. and Fan, L., 2014. *Planomicrobium soli* sp. nov., isolated from soil. *International journal of systematic and evolutionary microbiology*, 64(8), pp.2700-2705.
- Macián, M.C., Ludwig, W., Schleifer, K.H., Pujalte, M.J. and Garay, E., 2001. *Vibrio agarivorans* sp. nov., a novel agarolytic marine bacterium. *International journal of systematic and evolutionary microbiology*, 51(6), pp.2031-2036.
- Maszenan, A.M., Seviour, R.J., Patel, B.K.C., Rees, G.N. and McDougall, B.M., 1997. *Amaricoccus* gen. nov., a gram-negative coccus occurring in regular packages or tetrads, isolated from activated sludge biomass, and descriptions of *Amaricoccus veronensis* sp. nov., *Amaricoccus tamworthensis* sp. nov., *Amaricoccus macauensis* sp. nov., and *Amaricoccus kaplicensis* sp. nov. *International Journal of Systematic and Evolutionary Microbiology*, 47(3), pp.727-734.
- Matsumoto, A., Kasai, H., Matsuo, Y., Ōmura, S., Shizuri, Y. and Takahashi, Y., 2009. *Ilumatobacter fluminis* gen. nov., sp. nov., a novel actinobacterium isolated from the sediment of an estuary. *The Journal of general and applied microbiology*, 55(3), pp.201-205.
- McDonald, I.R., Doronina, N.V., Trotsenko, Y.A., McAnulla, C. and Murrell, J.C., 2001. *Hyphomicrobium chloromethanicum* sp. nov. and *Methylobacterium chloromethanicum* sp. nov., chloromethane-utilizing bacteria isolated from a polluted environment. *International journal of systematic and evolutionary microbiology*, 51(1), pp.119-122.
- Mehnaz, S., Weselowski, B. and Lazarovits, G., 2007. *Azospirillum canadense* sp. nov., a nitrogen-fixing bacterium isolated from corn rhizosphere. *International Journal of Systematic and Evolutionary Microbiology*, 57(3), pp.620-624.
- Miwa, H., Ahmed, I., Yoon, J., Yokota, A. and Fujiwara, T., 2008. *Variovorax boronicumulans* sp. nov., a boron-accumulating bacterium isolated from soil. *International journal of systematic and evolutionary microbiology*, 58(1), pp.286-289.

- Moore, R.L., Weiner, R.M. and Gebers, R., 1984. Genus *Hyphomonas* Pongratz 1957 nom. rev. emend., *Hyphomonas polymorpha* Pongratz 1957 nom. rev. emend., and *Hyphomonas neptunium* (Leifson 1964) comb. nov. emend. (*Hyphomicrobium neptunium*). *International Journal of Systematic and Evolutionary Microbiology*, 34(1), pp.71-73.
- Mori, K. and Suzuki, K.I., 2008. *Thiofaba tepidiphila* gen. nov., sp. nov., a novel obligately chemolithoautotrophic, sulfur-oxidizing bacterium of the Gammaproteobacteria isolated from a hot spring. *International journal of systematic and evolutionary microbiology*, 58(8), pp.1885-1891.
- Moriya, T., Hikota, T., Yumoto, I., Ito, T., Terui, Y., Yamagishi, A. and Oshima, T., 2011. *Calditerricola satsumensis* gen. nov., sp. nov. and *Calditerricola yamamurae* sp. nov., extreme thermophiles isolated from a high-temperature compost. *International journal of systematic and evolutionary microbiology*, 61(3), pp.631-636.
- Murdoch, D.A. and Shah, H.N., 1999. Reclassification of *Peptostreptococcus magnus* (Prevot 1933) Holdeman and Moore 1972 as *Finegoldia magna* comb. nov. and *Peptostreptococcus micros* (Prevot 1933) Smith 1957 as *Micromonas micros* comb. nov. *Anaerobe*, 5(5), pp.555-559.
- Ogg, C.D. and Patel, B.K., 2009. *Sporolithuus thermophilus* gen. nov., sp. nov., a citrate-fermenting thermophilic anaerobic bacterium from geothermal waters of the Great Artesian Basin of Australia. *International journal of systematic and evolutionary microbiology*, 59(11), pp.2848-2853.
- Oggerin, M., Arahal, D.R., Rubio, V. and Marín, I., 2009. Identification of *Beijerinckia fluminensis* strains CIP 106281T and UQM 1685T as *Rhizobium radiobacter* strains, and proposal of *Beijerinckia doebereineriae* sp. nov. to accommodate *Beijerinckia fluminensis* LMG 2819. *International journal of systematic and evolutionary microbiology*, 59(9), pp.2323-2328.
- Okamura, K., Kanbe, T. and Hiraishi, A., 2009. *Rhodoplanes serenus* sp. nov., a purple non-sulfur bacterium isolated from pond water. *International journal of systematic and evolutionary microbiology*, 59(3), pp.531-535.
- O'Neill, A.H., Liu, Y., Ferrera, I., Beveridge, T.J. and Reysenbach, A.L., 2008. *Sulfurihydrogenibium rodmanii* sp. nov., a sulfur-oxidizing chemolithoautotroph from the Uzon Caldera, Kamchatka Peninsula, Russia, and emended description of the genus *Sulfurihydrogenibium*. *International journal of systematic and evolutionary microbiology*, 58(5), pp.1147-1152.
- Pagnier, I., Raoult, D. and La Scola, B., 2011. Isolation and characterization of *Reyranella massiliensis* gen. nov., sp. nov. from freshwater samples by using an amoeba co-culture procedure. *International journal of systematic and evolutionary microbiology*, 61(9), pp.2151-2154.
- Patel, N.B., Tito, R.Y., Obregon-Tito, A.J., O'Neal, L., Trujillo-Villaroel, O., Marin-Reyes, L., Troncoso-Corzo, L., Guija-Poma, E., Lewis Jr, C.M. and Lawson, P.A., 2016. *Peptoniphilus catoniae* sp. nov., isolated from a human faecal sample from a traditional Peruvian coastal community. *International journal of systematic and evolutionary microbiology*, 66(Pt 5), p.2019.
- Pley, U., Schipka, J., Gambacorta, A., Jannasch, H.W., Fricke, H., Rachel, R. and Stetter, K.O., 1991. *Pyrodictium abyssi* sp. nov. represents a novel heterotrophic marine archaeal hyperthermophile growing at 110 °C. *Systematic and applied microbiology*, 14(3), pp.245-253.
- Prauser, H., 1976. *Nocardioides*, a new genus of the order Actinomycetales. *International Journal of Systematic and Evolutionary Microbiology*, 26(1), pp.58-65.
- Reinhold-Hurek, B. and Hurek, T., 2000. Reassessment of the taxonomic structure of the diazotrophic genus *Azoarcus* sensu lato and description of three new genera and new species, *Azovibrio restrictus* gen. nov., sp.

- nov., *Azospira oryzae* gen. nov., sp. nov. and *Azonexus fungiphilus* gen. nov., sp. nov. *International journal of systematic and evolutionary microbiology*, 50(2), pp.649-659.
- Romanenko, L.A., Tanaka, N., Frolova, G.M., Svetashev, V.I. and Mikhailov, V.V., 2011. *Litoreibacter albidus* gen. nov., sp. nov. and *Litoreibacter janthinus* sp. nov., members of the class Alphaproteobacteria isolated from the seashore. *International journal of systematic and evolutionary microbiology*, 61(1), pp.148-154.
- Ryu, S.H., Chung, B.S., Park, M., Lee, S.S., Lee, S.S. and Jeon, C.O., 2008. *Rheinheimera soli* sp. nov., a gammaproteobacterium isolated from soil in Korea. *International journal of systematic and evolutionary microbiology*, 58(10), pp.2271-2274.
- SAIKI, T., KOBAYASHI, Y., KAWAGOE, K. and BEPPU, T., 1985. *Dictyoglomus thermophilum* gen. nov., sp. nov., a chemoorganotrophic, anaerobic, thermophilic bacterium. *International Journal of Systematic and Evolutionary Microbiology*, 35(3), pp.253-259.
- Saitoh, S., Suzuki, T. and Nishimura, Y., 1998. Proposal of *Craurococcus roseus* gen. nov., sp. nov. and *Paracraurococcus ruber* gen. nov., sp. nov., novel aerobic bacteriochlorophyll a-containing bacteria from soil. *International Journal of Systematic and Evolutionary Microbiology*, 48(3), pp.1043-1047.
- Sako, Y., Takai, K., Ishida, Y., Uchida, A. and Katayama, Y., 1996. *Rhodothermus obamensis* sp. nov., a modern lineage of extremely thermophilic marine bacteria. *International Journal of Systematic and Evolutionary Microbiology*, 46(4), pp.1099-1104.
- Santos, P., Pinhal, I., Rainey, F.A., Empadinhas, N., Costa, J., Fields, B., Benson, R., Veríssimo, A. and da Costa, M.S., 2003. Gamma-Proteobacteria *Aquicella lusitana* gen. nov., sp. nov., and *Aquicella siphonis* sp. nov. Infect Protozoa and Require Activated Charcoal for Growth in Laboratory Media. *Applied and environmental microbiology*, 69(11), pp.6533-6540.
- Sekiguchi, Y., Muramatsu, M., Imachi, H., Narihiro, T., Ohashi, A., Harada, H., Hanada, S. and Kamagata, Y., 2008. *Thermodesulfovibrio aggregans* sp. nov. and *Thermodesulfovibrio thiophilus* sp. nov., anaerobic, thermophilic, sulfate-reducing bacteria isolated from thermophilic methanogenic sludge, and emended description of the genus *Thermodesulfovibrio*. *International journal of systematic and evolutionary microbiology*, 58(11), pp.2541-2548.
- Sekiguchi, Y., Yamada, T., Hanada, S., Ohashi, A., Harada, H. and Kamagata, Y., 2003. *Anaerolinea thermophila* gen. nov., sp. nov. and *Caldilinea aerophila* gen. nov., sp. nov., novel filamentous thermophiles that represent a previously uncultured lineage of the domain Bacteria at the subphylum level. *International journal of systematic and evolutionary microbiology*, 53(6), pp.1843-1851.
- Shah, H.N. and Collins, M.D., 1988. Proposal for reclassification of *Bacteroides asaccharolyticus*, *Bacteroides gingivalis*, and *Bacteroides endodontalis* in a new genus, *Porphyromonas*. *International journal of systematic and evolutionary microbiology*, 38(1), pp.128-131.
- Sherman, J.M. and Stark, P., 1931. Streptococci which grow at high temperatures. *Journal of bacteriology*, 22(4), p.275.
- Shida, O., Takagi, H., Kadowaki, K., Nakamura, L.K. and Komagata, K., 1997. Transfer of *Bacillus alginolyticus*, *Bacillus chondroitinus*, *Bacillus curdlanolyticus*, *Bacillus glucanolyticus*, *Bacillus kobensis*, and *Bacillus thiaminolyticus* to the genus *Paenibacillus* and emended description of the genus *Paenibacillus*. *International Journal of Systematic and Evolutionary Microbiology*, 47(2), pp.289-298.
- Shiratori, H., Sasaya, K., Ohiwa, H., Ikeno, H., Ayame, S., Kataoka, N., Miya, A., Beppu, T. and Ueda, K., 2009. *Clostridium clariflavum* sp. nov. and *Clostridium caenicola* sp. nov., moderately thermophilic, cellulose-

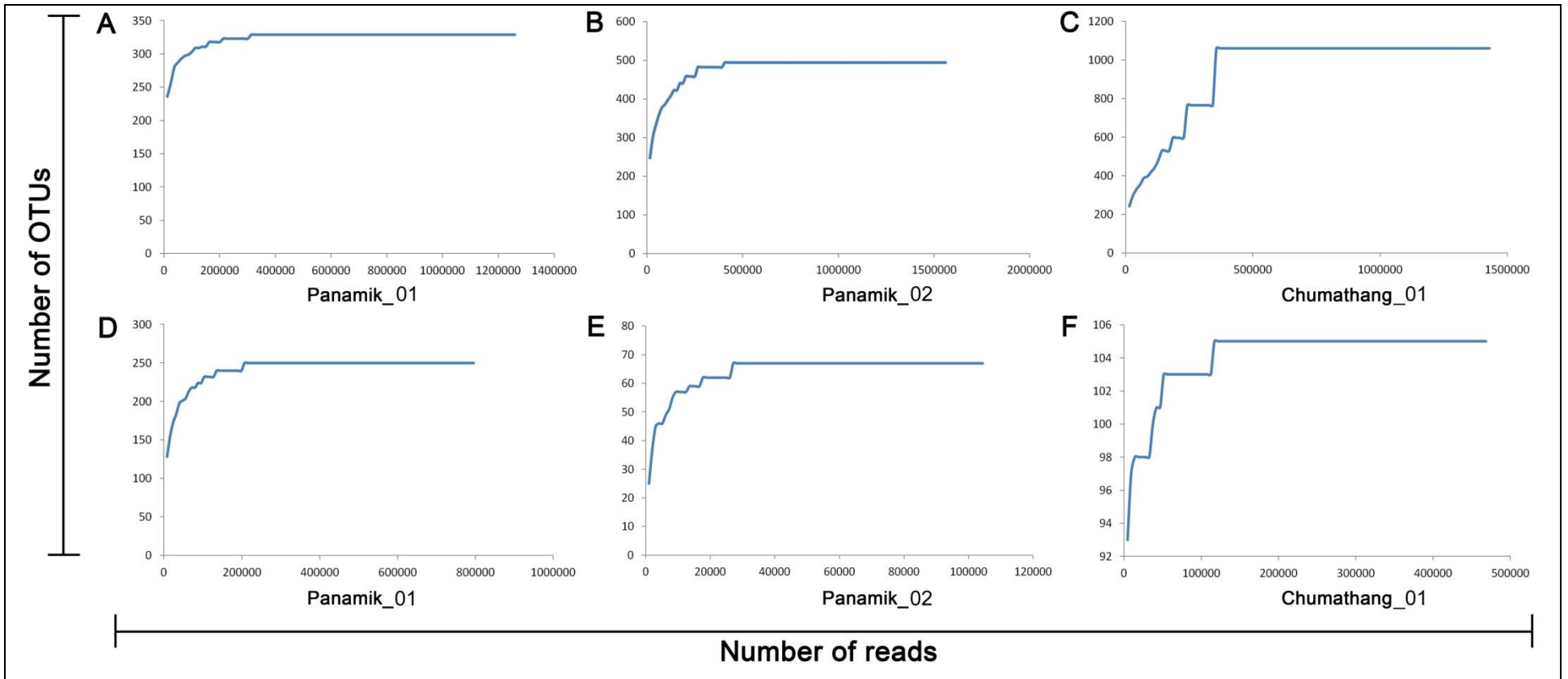
- /cellobiose-digesting bacteria isolated from methanogenic sludge. *International journal of systematic and evolutionary microbiology*, 59(7), pp.1764-1770.
- Singleton, D.R., Furlong, M.A., Peacock, A.D., White, D.C., Coleman, D.C. and Whitman, W.B., 2003. *Solirubrobacter pauli* gen. nov., sp. nov., a mesophilic bacterium within the Rubrobacteridae related to common soil clones. *International journal of systematic and evolutionary microbiology*, 53(2), pp.485-490.
- Smet, A., Cools, P., Krizova, L., Maixnerova, M., Sedo, O., Haesebrouck, F., Kempf, M., Nemeč, A. and Vanechoutte, M., 2014. *Acinetobacter gandensis* sp. nov. isolated from horse and cattle. *International journal of systematic and evolutionary microbiology*, 64(12), pp.4007-4015.
- Somvanshi, V.S., Lang, E., Sträubler, B., Spröer, C., Schumann, P., Ganguly, S., Saxena, A.K. and Stackebrandt, E., 2006. *Providencia vermicola* sp. nov., isolated from infective juveniles of the entomopathogenic nematode *Steinernema thermophilum*. *International Journal of Systematic and Evolutionary Microbiology*, 56(3), pp.629-633.
- Sorokin, D.Y., van Pelt, S., Tourova, T.P. and Evtushenko, L.I., 2009. *Nitriliruptor alkaliphilus* gen. nov., sp. nov., a deep-lineage haloalkaliphilic actinobacterium from soda lakes capable of growth on aliphatic nitriles, and proposal of Nitriliruptoraceae fam. nov. and Nitriliruptorales ord. nov. *International journal of systematic and evolutionary microbiology*, 59(2), pp.248-253.
- Sorokina, A.Y., Chernousova, E.Y. and Dubinina, G.A., 2012. *Ferrovibrio denitrificans* gen. nov., sp. nov., a novel neutrophilic facultative anaerobic Fe (II)-oxidizing bacterium. *FEMS microbiology letters*, 335(1), pp.19-25.
- Staley, J.T., Irgens, R.L. and Brenner, D.J., 1987. *Enhydrobacter aerosaccus* gen. nov., sp. nov., a gas-vacuolated, facultatively anaerobic, heterotrophic rod. *International Journal of Systematic and Evolutionary Microbiology*, 37(3), pp.289-291.
- Stieglmeier, M., Klingl, A., Alves, R.J., Rittmann, S.K.M., Melcher, M., Leisch, N. and Schleper, C., 2014. *Nitrososphaera viennensis* gen. nov., sp. nov., an aerobic and mesophilic, ammonia-oxidizing archaeon from soil and a member of the archaeal phylum Thaumarchaeota. *International Journal of Systematic and Evolutionary Microbiology*, 64(Pt 8), p.2738.
- Sun, X., Luo, P. and Li, M., 2015. *Paracoccus angustae* sp. nov., isolated from soil. *International Journal of Systematic and Evolutionary Microbiology*, 65(10), pp.3469-3475.
- Suresh, K., Mayilraj, S., Bhattacharya, A. and Chakrabarti, T., 2007. *Planococcus columbae* sp. nov., isolated from pigeon faeces. *International journal of systematic and evolutionary microbiology*, 57(6), pp.1266-1271.
- Takai, K., Komatsu, T. and Horikoshi, K., 2001. *Hydrogenobacter subterraneus* sp. nov., an extremely thermophilic, heterotrophic bacterium unable to grow on hydrogen gas, from deep subsurface geothermal water. *International journal of systematic and evolutionary microbiology*, 51(4), pp.1425-1435.
- Tamaki, H., Tanaka, Y., Matsuzawa, H., Muramatsu, M., Meng, X.Y., Hanada, S., Mori, K. and Kamagata, Y., 2011. *Armatimonas rosea* gen. nov., sp. nov., of a novel bacterial phylum, Armatimonadetes phyl. nov., formally called the candidate phylum OP10. *International journal of systematic and evolutionary microbiology*, 61(6), pp.1442-1447.
- Van der Maarel, M.J., van Bergeijk, S., van Werkhoven, A.F., Laverman, A.M., Meijer, W.G., Stam, W.T. and Hansen, T.A., 1996. Cleavage of dimethylsulfoniopropionate and reduction of acrylate by *Desulfovibrio acrylicus* sp. nov. *Archives of microbiology*, 166(2), pp.109-115.

- Vandamme, P.A., Peeters, C., Inganäs, E., Cnockaert, M., Houf, K., Spilker, T., Moore, E.R. and LiPuma, J.J., 2016. Taxonomic dissection of *Achromobacter denitrificans* Coenye et al. 2003 and proposal of *Achromobacter agilis* sp. nov., nom. rev., *Achromobacter pestifer* sp. nov., nom. rev., *Achromobacter kerstersii* sp. nov. and *Achromobacter deleyi* sp. nov. *International Journal of Systematic and Evolutionary Microbiology*, 66(9), pp.3708-3717.
- Völkl, P., Huber, R., Drobner, E., Rachel, R., Burggraf, S., Trincone, A. and Stetter, K.O., 1993. *Pyrobaculum aerophilum* sp. nov., a novel nitrate-reducing hyperthermophilic archaeum. *Applied and Environmental Microbiology*, 59(9), pp.2918-2926.
- Wang, C.Y., Ng, C.C., Tzeng, W.S. and Shyu, Y.T., 2009. *Marinobacter szutsaonensis* sp. nov., isolated from a solar saltern. *International journal of systematic and evolutionary microbiology*, 59(10), pp.2605-2609.
- Wang, H., Zhou, S., Wang, Y., Kong, D., Guo, X., Zhu, J., Dong, W. and Ruan, Z., 2016. *Advenella alkanexedens* sp. nov., an alkane-degrading bacterium isolated from biogas slurry samples. *International journal of systematic and evolutionary microbiology*, 66(2), pp.906-911.
- Wang, X., Wang, Y., Yang, X., Sun, H., Li, B. and Zhang, X.H., 2017. *Photobacterium alginatilyticum* sp. nov., a marine bacterium isolated from bottom seawater. *International journal of systematic and evolutionary microbiology*, 67(6), pp.1912-1917.
- Wang, Y., Song, J., Zhai, Y., Zhang, C., Gerritsen, J., Wang, H., Chen, X., Li, Y., Zhao, B., Zhao, B. and Ruan, Z., 2015. *Romboutsia sedimentorum* sp. nov., isolated from an alkaline-saline lake sediment and emended description of the genus *Romboutsia*. *International journal of systematic and evolutionary microbiology*, 65(4), pp.1193-1198.
- Watson, S.W., Bock, E., Valois, F.W., Waterbury, J.B. and Schlosser, U., 1986. *Nitrospira marina* gen. nov. sp. nov.: a chemolithotrophic nitrite-oxidizing bacterium. *Archives of Microbiology*, 144(1), pp.1-7.
- Wu, X.L., Yu, S.L., Gu, J., Zhao, G.F. and Chi, C.Q., 2009. *Filomicrobium insigne* sp. nov., isolated from an oil-polluted saline soil. *International journal of systematic and evolutionary microbiology*, 59(2), pp.300-305.
- Xue, Y., Zhang, X., Zhou, C., Zhao, Y., Cowan, D.A., Heaphy, S., Grant, W.D., Jones, B.E., Ventosa, A. and Ma, Y., 2006. *Caldalkalibacillus thermarum* gen. nov., sp. nov., a novel alkalithermophilic bacterium from a hot spring in China. *International journal of systematic and evolutionary microbiology*, 56(6), pp.1217-1221.
- Yabuuchi, E., Kosako, Y., Yano, I., Hotta, H. and Nishiuchi, Y., 1995. Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia* gen. nov.: proposal of *Ralstonia pickettii* (Ralston, Palleroni and Doudoroff 1973) comb. nov., *Ralstonia solanacearum* (Smith 1896) comb. nov. and *Ralstonia eutropha* (Davis 1969) comb. nov. *Microbiology and immunology*, 39(11), pp.897-904.
- Yang, F., Liu, H.M., Zhang, R., Chen, D.B., Wang, X., Li, S.P. and Hong, Q., 2015. *Chryseobacterium shandongense* sp. nov., isolated from soil. *International journal of systematic and evolutionary microbiology*, 65(6), pp.1860-1865.
- Yang, G., Chen, M., Yu, Z., Lu, Q. and Zhou, S., 2013a. *Bacillus composti* sp. nov. and *Bacillus thermophilus* sp. nov., two thermophilic, Fe (III)-reducing bacteria isolated from compost. *International journal of systematic and evolutionary microbiology*, 63(8), pp.3030-3036.
- Yang, G., Han, L., Wen, J. and Zhou, S., 2013b. *Pseudomonas guangdongensis* sp. nov., isolated from an electroactive biofilm, and emended description of the genus *Pseudomonas* Migula 1894. *International journal of systematic and evolutionary microbiology*, 63(12), pp.4599-4605.

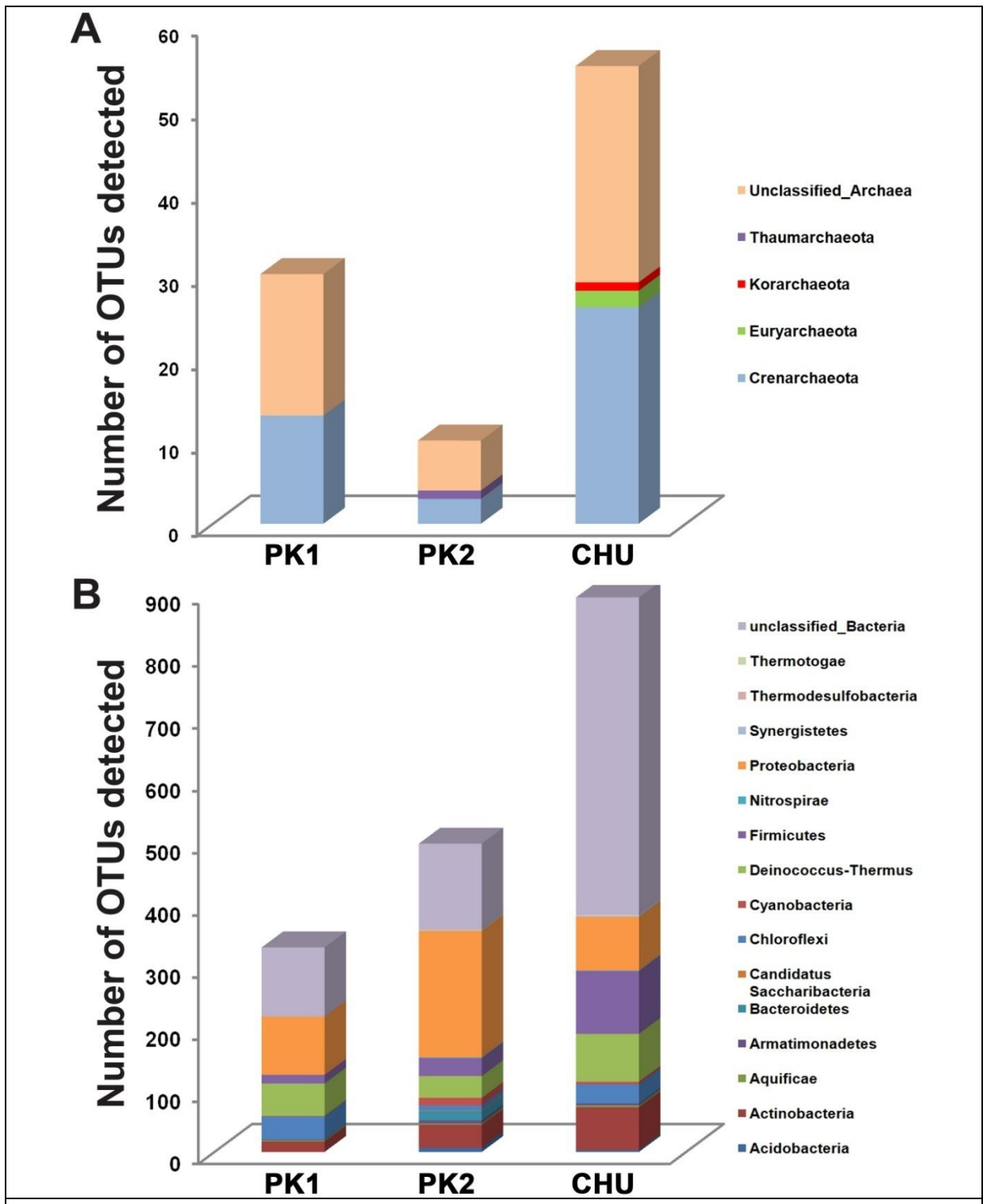
- Yang, S.J., Kataeva, I., Wiegel, J., Yin, Y., Dam, P., Xu, Y., Westpheling, J. and Adams, M.W., 2010. Classification of 'Anaerocellum thermophilum' strain DSM 6725 as *Caldicellulosiruptor bescii* sp. nov. *International journal of systematic and evolutionary microbiology*, 60(9), pp.2011-2015.
- Yao, S., Liu, Y., Zhang, M., Zhang, X., Li, H., Zhao, T., Xin, C., Xu, L., Zhang, B. and Cheng, C., 2014. *Thermoactinomyces daqus* sp. nov., a thermophilic bacterium isolated from high-temperature Daqu. *International journal of systematic and evolutionary microbiology*, 64(1), pp.206-210.
- Yassin, A.F., Inglis, T.J.J., Hupfer, H., Siering, C., Schumann, P., Busse, H.J. and Aravena-Roman, M., 2012. *Cruoricaptor ignavus* gen. nov., sp. nov., a novel bacterium of the family Flavobacteriaceae isolated from blood culture of a man with bacteraemia. *Systematic and applied microbiology*, 35(7), pp.421-426.
- Yin, D., Xiao, J., Ao, J., Ai, C. and Chen, X., 2012. *Albidovulum xiamenense* sp. nov., a moderately thermophilic bacterium from a terrestrial hot spring. *International journal of systematic and evolutionary microbiology*, 62(7), pp.1609-1612.
- Yoon, J.H., Kang, S.J., Ryu, S.H., Jeon, C.O. and Oh, T.K., 2008. *Hydrogenophaga bisanensis* sp. nov., isolated from wastewater of a textile dye works. *International journal of systematic and evolutionary microbiology*, 58(2), pp.393-397.
- Young, C.C., Busse, H.J., Langer, S., Chu, J.N., Schumann, P., Arun, A.B., Shen, F.T., Rekha, P.D. and Kämpfer, P., 2010. *Microbacterium agarici* sp. nov., *Microbacterium humi* sp. nov. and *Microbacterium pseudoresistens* sp. nov., isolated from the base of the mushroom *Agaricus blazei*. *International journal of systematic and evolutionary microbiology*, 60(4), pp.854-860.
- Yu, T.T., Yao, J.C., Yin, Y.R., Dong, L., Liu, R.F., Ming, H., Zhou, E.M. and Li, W.J., 2013. *Rehaibacterium terrae* gen. nov., sp. nov. isolated from a geothermally heated soil sample. *International journal of systematic and evolutionary microbiology*, 63(11), pp.4058-4063.
- Zarilla, K.A. and Perry, J.J., 1984. *Thermoleophilum album* gen. nov. and sp. nov., a bacterium obligate for thermophily and n-alkane substrates. *Archives of microbiology*, 137(4), pp.286-290.
- Zhang, H., Zhang, J., Song, M., Cheng, M.G., Wu, Y.D., Guo, S.H., Li, Q., Hong, Q. and Huang, X., 2015. *Pedobacter nanyangensis* sp. nov., isolated from herbicide-contaminated soil. *International journal of systematic and evolutionary microbiology*, 65(10), pp.3517-3521.
- Zhang, W., Xue, Y., Ma, Y., Zhou, P., Ventosa, A. and Grant, W.D., 2002. *Salinicoccus alkaliphilus* sp. nov., a novel alkaliphile and moderate halophile from Baer Soda Lake in Inner Mongolia Autonomous Region, China. *International journal of systematic and evolutionary microbiology*, 52(3), pp.789-793.
- Zhang, X.Q., Zhang, W.J., Wei, B.P., Xu, X.W., Zhu, X.F. and Wu, M., 2010. *Meiothermus cateniformans* sp. nov., a slightly thermophilic species from north-eastern China. *International journal of systematic and evolutionary microbiology*, 60(4), pp.840-844.
- Zillig, W., Gierl, A., Schreiber, G., Wunderl, S., Janekovic, D., Stetter, K.O. and Klenk, H.P., 1983. The archaeobacterium *Thermofilum pendens* represents, a novel genus of the thermophilic, anaerobic sulfur respiring Thermoproteales. *Systematic and applied microbiology*, 4(1), pp.79-87.



## Supplementary Figures



**Figure S1.** Rarefaction curves showing the proportionality between OTU-level diversity revealed and the number of 16S rRNA gene sequence (V3 region) reads analyzed for (A-C) bacteria or (D-F) archaea in the different vent-water samples. For each sample, number of reads used in OTU-clustering is plotted along the X-axis, while number of OTUs (excluding singletons) created is plotted along the Y-axis.



**Figure S2.** Microbial community architecture of the vent-waters of the Panamik\_01 (PK1), Panamik\_02 (PK2) and Chumathang\_01 (CHU) hot springs. Panels (A) and (B) show the phylum-level classification of the archaeal and bacterial OTUs identified respectively.