9 **Uncultivated thermophiles: Current status and spotlight on**
10 **'Aigarchaeota'**

11
12 Brian P. Hedlund^{1,2*}, Senthil K. Murugapiran¹, Timothy W. Alba¹, Asaf Levy³, Jeremy
13 A. Dodsworth⁴, Gisele B. Goertz¹, Natalia Ivanova³, Tanja Woyke³

- 14
15 1. School of Life Sciences, University of Nevada Las Vegas, Las Vegas, NV 89154,
16 USA
17 2. Nevada Institute of Personalized Medicine, University of Nevada, Las Vegas,
18 Nevada, 89154, USA
19 3. DOE Joint Genome Institute, Walnut Creek, California 94598, USA
20 4. Department of Biology, California State University, San Bernardino, California,
21 92407, USA

22
23 ***Correspondence:**

24 Brian P. Hedlund, School of Life Sciences, University of Nevada, Las Vegas, 4505
25 Maryland Parkway, Las Vegas, NV 89154-4004, USA.

26 Tel.: +1 (702) 895-0809

27 E-mail: brian.hedlund@unlv.edu

HIGHLIGHTS

- Independent analyses show novel taxa and coding sequences are highly abundant in geothermal systems.
- Candidate taxa include 'Aigarchaeota', 'Korarchaeota', 'Calescamantes', and 'Fervidibacteria'.
- Genomic exploration provides a window into the biology of uncultivated microbes.
- 'Aigarchaeota' is a diverse, globally-distributed group of obligate thermophiles.

ABSTRACT

Meta-analysis of cultivation-independent sequence data shows that geothermal systems host an abundance of novel organisms, representing a vast unexplored phylogenetic and functional diversity among yet-uncultivated thermophiles. A number of thermophiles have recently been interrogated using metagenomic and/or single-cell genomic approaches, including members of taxonomic groups that inhabit both thermal and non-thermal environments, such as 'Acetothermia' (OP1) and 'Atribacteria' (OP9/JS1), as well as the exclusively thermophilic lineages 'Korarchaeota', 'Calescamantes' (EM19), 'Fervidibacteria' (OctSpA1-106), and 'Aigarchaeota' (HWCG-I). The 'Aigarchaeota', a sister lineage to the Thaumarchaeota, likely includes both hyperthermophiles and moderate thermophiles. They inhabit terrestrial, marine, and subsurface thermal environments and comprise at least nine genus-level lineages, several of which are globally distributed.

INTRODUCTION

Geothermal systems hosting thermophiles (optimum growth 45-80 °C) and hyperthermophiles (>80 °C) were some of the first environments to be explored by cultivation-independent approaches using 16S rRNAs and 16S rRNA gene amplicons [1, 2]. These studies and many others revealed a vast diversity that filled in and expanded the tree of life, with yet-uncultivated organisms spanning the phylum to species level. Cultivation-independent investigations of Obsidian Pool in Yellowstone National Park were particularly ground-breaking, revealing deeply branching *Archaea*, including 'Korarchaeota' [3, 4], and twelve novel lineages of *Bacteria*, provisionally named OP1-OP12 [5]. Many of these were subsequently shown to also inhabit non-thermal environments and have come into focus in a variety of habitats [e.g., 6, 7, 8]. Over the last ten years, a few thermophilic members of these groups and others have been isolated and described as axenic cultures [e.g., 9, 10, 11, 12, 13]; however, most of the diversity within them remains to be explored. Recent advancements in metagenomics and single-cell genomics have provided access to the genomes of yet-uncultivated thermophiles, enabling insight into their evolution, cell biology, and possible metabolic and ecological functions. Continued genomic exploration of these enigmatic organisms, along with experiments to test their functions *in situ* and cultivate them in the laboratory, will be an exciting frontier in biology for decades to come.

EXTENT OF YET-UNCULTIVATED THERMOPHILE DIVERSITY

Currently there are over 4.3 million 16S rRNA gene sequences in public databases (14). Despite exponential increases in the number of entries per year, the accumulation of new

operational taxonomic units at all levels is approaching an asymptote, with total bacterial species richness predicted to be $\sim 4 \times 10^5$ [●●15]. At present, it is difficult to estimate the taxonomic richness of thermophiles and hyperthermophiles, although well-established negative correlations between microbial diversity and temperature [●16, ●17] suggest it may be a small fraction of the total taxonomic richness. Nevertheless, with very few species of thermophiles and hyperthermophiles formally described (<1,000, estimated from $\sim 12,000$ total species on the *List of Prokaryotic Names with standing in Nomenclature* [18]), the vast majority remains to be carefully studied. More intriguingly, many deep branches of thermophiles also remain poorly represented, or unrepresented by axenic cultures. Estimates of the total number of phyla of *Bacteria* and *Archaea* commonly range from 50 to 100 [19, 20]; however, a conservative, unbiased approach recently estimated $\sim 1,600$ bacterial lineages equivalent to the existing bacterial phyla [●●15]. As with species, the number of phylum-level lineages containing some or many thermophiles is currently difficult to determine.

Cultivation-independent studies of geothermal systems provide a window to assess the abundance and distribution of yet-uncultivated thermophile diversity (Figure 1; Table S1; Table S2). The most comprehensive view currently available is gained by meta-analysis of 16S rRNA gene amplicon data. Parallel analysis of data from eight regions, comprising 372 samples from terrestrial geothermal systems [●16, ●17, 21-27] suggests a high abundance of uncultivated high-level taxa in geothermal systems globally, including phyla ($\bar{x}$ =16.8%), classes ($\bar{x}$ =34.0%), orders ($\bar{x}$ =42.1%), and families ($\bar{x}$ =46.9%). Most regions show a somewhat even distribution of uncultivated taxa among samples;

however, an exception is Tengchong, China, in which the Rehai Geothermal Field springs contain a very low apparent abundance of uncultivated organisms and the Ruidian (Diantan) springs contain a very high abundance of uncultivated organisms (24, 27). Hotspots of uncharacterized biodiversity like the Ruidian springs may be obscured in other regions by greater sampling effort or different sampling strategies.

Examining metagenomes from geothermal systems in the Integrated Microbial Genomes-Microbiomes (IMG-M) database (28, 29) provides another view of the extent of yet-uncultivated thermophile biodiversity. Analysis of proteins extracted from assemblies for 62 datasets reveals a very broad range of apparent abundance of unexplored genomic diversity, with 10% to 92% of predicted coding sequences taxonomically unassigned at 60% amino acid identity ($\bar{x}$ =55.1%, n =62) (Table S2; calculated using UCHIME in IMG-M [29]). This level of amino acid identity is comparable with the family level since bacterial genus boundaries correspond to ~65% average amino acid identity (AAI)[30]; however, unassigned genes may also represent elements of the accessory genome residing within known taxa. Although these springs span a wide range of temperature (50-90 °C) and pH (2.1-9.3), no relationship between the percent of taxonomically unassigned sequences and either temperature (r^2 =0.07) or pH (r^2 =0.02) is evident (Table S2). Lastly, 16S rRNA genes extracted from these datasets represent a source of information, although read depth, which is necessary for abundance estimation, is only available for a subset of metagenomes. This approach also shows a wide variation in the abundance of yet-uncultivated microorganisms, with 0 to 82% of 16S rRNA gene sequences being taxonomically unassigned at the phylum level ($\bar{x}$ =32.1%, n =24) with a

higher abundance of unassigned sequences at the class ($\bar{x}$ =37.8%), order ($\bar{x}$ =40.1%), and family ($\bar{x}$ =44.1%) levels (Table S2; assigned using Greengenes). Despite many caveats deriving from differential DNA extraction efficiency, primer bias, uneven taxonomic/phylogenetic structure, and differences in phylogenetic resolution among different regions of the 16S rRNA gene [e.g., ●●15], these data suggest that uncultivated taxa are abundant in most geothermal systems.

[INSERT FIGURE 1 HERE]

PROGRESS ON THERMOPHILE METAGENOMICS AND SINGLE-CELL GENOMICS

A few studies have explicitly targeted yet-uncultivated, deep lineages of thermophiles by using metagenomics or single-cell genomics approaches in geothermal environments. Studies of a thermal spring in a Japanese gold mine (70 °C, pH 5.1) involved screening a fosmid library for 16S rRNA genes of interest, followed by both targeted and random fosmid clone sequencing, leading to a complete genomic assembly for '*Candidatus* Caldiarchaeum subterraneum' a member of a deeply branching archaeal group, Hot Water Crenarchaeotal Group I (HWCG I), or 'Aigarchaeota' [●●33], and a nearly complete genomic assembly for '*Candidatus* Acetothermum autotrophicum', belonging to candidate bacterial phylum OP1, 'Acetothermia' [●●34]. A shotgun metagenome study of iron oxide mats in One Hundred Springs Plain Spring (60-78 °C, pH 3.5) in Norris Geyser Basin, Yellowstone, yielded a near-complete consensus genome from a deeply branching archaeon, named NAG1, or 'Geoarchaeota' [●●35]. Another study purified 'Korarchaeota'

cells from an enrichment culture (85 °C, pH 6.5; Obsidian Pool) by SDS treatment followed by filtration (0.45 µm), enabling sequencing and assembly of a complete genome for '*Candidatus* Korarchaeum cryptofilum' [●●36]. In the U.S. Great Basin, one study combined single-cell genome sequencing of 15 single cells sorted and amplified using an optofluidic approach from Little Hot Creek, California (80 °C, pH ~7) and shotgun metagenomic sequencing of a cellulolytic enrichment from Great Boiling Spring, Nevada (77 °C, pH ~7) [●●37], yielding two fragmented but nearly complete genome assemblies for members of candidate bacterial phylum OP9 'Atribacteria', provisionally named '*Candidatus* Caldatribacterium californiense' and '*Ca.* Caldatribacterium saccharofermentans'. Samples from Great Basin springs were also part of the largest single-cell genomics study to date, the Genomic Exploration of Bacteria and Archaea-Microbial Dark Matter (GEBA-MDM) project, which used fluorescence activated cell sorting (FACS) followed by whole-genome amplification and sequencing [●●38]. More than 30 single cell genomes were obtained from natural sediments in Great Boiling Spring, including assemblies ranging from 50-95% estimated genome coverage for two lineages, EM19 ('*Calescamantes*'; '*Candidatus* Calescibacterium nevadense'; 10 single-amplified genomes (SAGs)), and OctSpA1-106 ('*Fervidibacteria*', '*Candidatus* Fervidibacter sacchari'; 5 SAGs), along with large assemblies for several unnamed 'Aigarchaeota' species (14 SAGs) and smaller datasets for additional deep lineages.

In aggregate, these studies and others [e.g., 39, 40] have significantly expanded the genomic coverage of thermophilic *Bacteria* and *Archaea*. The major metabolic features of these organisms seem to be diverse (Figure 2). Based on genome-enabled predictions,

the lineages described above potentially encode systems for autotrophy ('Acetothermum', 'Caldatribacterium'), aerobic respiration ('Acetothermum', 'Caldatribacterium', 'Calescibacterium', 'Fervidibacterium'), and various forms of anaerobic respiration ('Acetothermum', 'Caldatribacterium', 'Calescibacterium', 'Fervidibacter'). 'Caldatribacterium' and 'Korarchaeum' are predicted to be obligate fermenters. Most of these representative organisms are rod-shaped or filamentous, based on either direct observation [●●36, ●●37] or the presence of genes for MreB or crenactin, and all of the bacteria are probably diderm (i.e. Gram negative), based on genes encoding components for outer membrane synthesis and transport [41]. The Archaea are presumed to be monoderm. None of these lineages appear to be symbionts, based on genome size and predicted biosynthetic potential, distinguishing these organisms from a multitude of deep lineages of *Bacteria* and *Archaea* that have reduced genome sizes (<1.3 Mbp) and predicted symbiotic lifestyles [e.g., 20, ●●38, 42-49].

It is noteworthy that the provisional taxonomy of these organisms is uncertain on several grounds, including: the requirement in microbial systematics for well-described axenic cultures; current limitations on higher-order taxonomy, which does not cover classifications above class; and disagreements within the scientific community about these unofficial designations and their applications to various lineages [e.g., ●●50, 51-54]. Some members of the systematics community have recently encouraged discussion to emend *Candidatus* status to better take advantage of advancements in environmental genomics [55][56]; however, no recent changes have been ratified by the *International Committee on the Systematics of Bacteria and Archaea*.

189

190 Metagenomic datasets provide a means to assess the distribution and abundance of yet-

191 uncultivated taxa for which reference genomes exist. We used a conservative approach of

192 >70% nucleotide identity and >100 bp long hits to recruit reads from 59 metagenomes

193 from terrestrial and marine geothermal systems [●28, 57-60] to seven reference genomes

194 [●●37, ●●36, 49, ●●38, ●●34]. This analysis roughly targets genus-level recruitment

195 [30] and suggests that members of the 'Aigarchaeota' ('*Ca. Caldiarchaeum*'),

196 'Korarchaeota' ('*Ca. Korarchaeum*'), 'Nanoarchaeota' ('*Ca. Nanobsidianus*'),

197 'Acetothermia' ('*Ca. Acetothermum*'), 'Atribacteria' ('*Ca. Caldatribacterium*'),

198 'Caescamantes' ('*Ca. Caescibacterium*'), and 'Fervidibacteria' ('*Ca. Fervidibacter*') are

199 present in a majority of geothermal systems (Figure 2; Table S3). "*Ca. Caldatribacterium*"

200 and "*Ca. Caescibacterium*" were particularly widespread, being detected in >80% of

201 metagenomes, including metagenomes from all terrestrial systems analyzed, as well as

202 marine hydrothermal systems. Close relatives of '*Candidatus Nanobsidiaunus stetteri*'

203 were particularly widespread in Yellowstone metagenomes, with >0.1% estimated

204 abundance in 29% of Yellowstone metagenomes, all of which are from acid sulfate

205 systems hosting the probable host, *Sulfolobales* [49]. Some springs hosted many of these

206 candidate taxa, including Great Boiling Spring and Little Hot Creek (Great Basin),

207 Octopus Spring, OSP Spring, and Beowulf Spring (Yellowstone), as well as Gongxiaoshe

208 and Gumingquan (Tengchong)(Figure 2). These candidate taxa were poorly represented

209 in marine hydrothermal samples; however, this is not surprising since the fragment

210 recruitment strategy was stringent. This high stringency also explains the low recruitment

of candidate phyla in springs in which they are known to be abundant and diverse (e.g., ●16).

[INSERT FIGURE 2 HERE]

'AIGARCHAEOTA': GENOMIC INSIGHTS, DIVERSITY, AND DISTRIBUTION

One deep lineage that has received considerable attention in the literature, but is still poorly studied is the HWCG I group, or 'Aigarchaeota'. The 'Aigarchaeota' was first highlighted as part of an investigation of a mildly acidic spring within a Japanese gold mine as a single fosmid clone and later an entire composite genome of '*Candidatus* Caldiarchaeum subterraneum' [●●33]. Analysis of predicted features led the authors to suggest that this organism may couple hydrogen or carbon monoxide oxidation to aerobic or anaerobic respiration using nitrate or nitrite as electron acceptors. However, the hydrogenases in "*Ca. Caldiarchaeum subterraneum*" belong to Group 3B and Group 4 NiFe hydrogenases [61], characterized members of which regulate redox homeostasis rather than hydrogenotrophy. '*Ca. Caldiarchaeum subterraneum*' may be autotrophic via the dicarboxylate/4-hydroxybutyrate pathway, but lacks a canonical 4-hydroxybutyryl-CoA dehydratase. Preliminary analysis of single-cell genomes of several 'Aigarchaeota' lineages from Great Boiling Spring has yielded additional insights, including potential capacity for heterotrophic utilization of proteins and sugars and, in some cases, anaerobic respiration using sulfite or nitrous oxide [●●38]. A more detailed interpretation and comparative analysis of these and other genomes from a diversity of 'Aigarchaeota'

lineages and thermal environments is necessary to reveal potential core traits and the extent of metabolic plasticity within the 'Aigarchaeota'.

Phylogenomic and comparative genomic studies have consistently revealed a deep relationship between Thaumarchaeota, 'Aigarchaeota', Crenarchaeota, and 'Korarchaeota' in the 'TACK superphylum' [●●38, 52, 62, 63], yet there is some uncertainty about whether 'Aigarchaeota' should be considered an independent phylum or a deep branch within the Thaumarchaeota [50, 51, 52]. This uncertainty is symptomatic of unrest among microbiologists seeking consensus on guidelines for higher-order taxonomy, particularly Archaea [51, 54]. The 16S rRNA gene identity between 'Aigarchaeota' and Thaumarchaeota (77-79%) is lower than the median sequence identity delimiting bacterial phyla (83.68% [●●15]) and close to the minimum distance between bacterial phyla (77.43% [●●15]), but has been reported to be similar to that delimiting euryarchaeal orders [●●50]. Regardless, it is clear that the cell biology of 'Aigarchaeota' shares many features with Thaumarchaeota [●●50, 64], although their ecology appears to be distinct. The evolution of metabolically specialized Thaumarchaeota from an apparently more flexible 'Aigarchaeota' ancestor [●●33, ●●38] may be one of many examples of streamlining in archaeal evolution [63].

The 'Aigarchaeota' are phylogenetically diverse and inhabit a wide array of thermal ecosystems, including terrestrial and marine geothermal systems, subsurface aquifers, and mine fracture waters (Figure 3; Figure 4; Table S4). Clustering and phylogenetic analysis of 'Aigarchaeota' 16S rRNA gene sequences from 16S rRNA gene surveys, single-cell

genomes, and metagenomes, shows at least nine genus-level groups (<95% 16S rRNA gene identity [●●15]), although some are not strongly supported and should be reevaluated as additional sequences become available (Figure 3; Table S5; Table S6). All groups are found in terrestrial geothermal systems, including deeply branching 'Aigarchaeota', suggesting a terrestrial origin (Figure 4). A few 'Aigarchaeota' 16S rRNA gene sequences have been recovered from moderate- or cold-temperature samples; however, the vast majority are from high-temperature environments and all have a 16S rRNA gene G + C content consistent with a thermophilic or hyperthermophilic lifestyle, with calculated optimal growth temperatures of 40.8-101.9 °C ($\bar{x}$ =78.4 °C, n =357)(Table S3)[65]. A general trend toward lower 16S rRNA gene G + C content in newer and longer branches suggests a high-temperature origin, with niche invasion to lower temperature systems. 'Aigarchaeota' are predominantly found in habitats with circumneutral pH. The U.S. Great Basin appears to be somewhat of a hotspot for 'Aigarchaeota' diversity and abundance, with several 'Aigarchaeota' lineages prominent in Great Boiling Spring [●16, 66], Mud Hot Springs [66], Grass Valley Spring [67], Little Hot Creek [68], and an aquifer at the Nevada National Security Test Site. However, sampling is currently highly biased and the full extent of 'Aigarchaeota' diversity is yet to be uncovered.

[INSERT FIGURE 3 AND FIGURE 4 HERE]

OUTLOOK

Both taxonomic and genomic diversity are poorly represented by thermophile laboratory cultures. Understanding thermophile diversity and ecology necessitates a polyphasic approach to biodiversity exploration, including microbial cultivation and characterization, cultivation-independent genomic exploration, and *in situ* activity measurements. Exploration of thermophile biodiversity will remain an exciting and important field for decades to come.

ACKNOWLEDGEMENTS

This work was supported by NASA Exobiology grant EXO-NNX11AR78G; U.S. National Science Foundation grant OISE 0968421; U.S. Department of Energy (DOE) grant DE-EE-0000716; and the U.S. Department of Energy Joint Genome Institute (CSP-182), a DOE Office of Science User Facility, supported under Contract No. DE-AC02-05CH11231. B.P.H. acknowledges generous support from Greg Fullmer through the UNLV Foundation. T.W.A. was supported by the Richard and Beverly Hermesen Fellowship. G.B.G. was supported by the Robert E. McNair Foundation and NSF DBI - 1358896. We acknowledge Steve Quake, Paul Blainey, Jad Kanbar, and Iwijn De Vlamincx for collaboration with single-cell genomics and metagenomics and Hailiang Dong and Brandon Briggs for collaboration with metagenomics. William Inskeep and Jake Beam provided an 'Aigarchaeota' 16S rRNA gene sequence from Octopus Spring. Duane Moser and Scott Hamilton-Brehm provided 16S rRNA gene sequences from the Nevada National Security Site (NNSS). Scott Miller, Ken Jones, Eric Boyd, and Trinity Hamilton kindly provided raw 16S rRNA gene pyrotag reads. Fengping Wang, William Brazelton, and Matthew Schrenk kindly provided metagenomic datasets.

REFERENCES

● of special interest

●● of outstanding interest

1. Stahl DA, Lane DJ, Olsen GJ, Pace NR: **Analysis of hydrothermal vent-associated symbionts by ribosomal RNA sequences.** *Science* 1984, **224**:409-411.
2. Reysenbach AL, Wickham GS, Pace NR: **Phylogenetic analysis of the hyperthermophilic pink filament community in Octopus Spring, Yellowstone National Park.** *Appl Environ Microbiol* 1994, **60**:2113-2119.
3. Barns SM, Fundyga RE, Jeffries MW, Pace NR: **Remarkable archaeal diversity detected in a Yellowstone National Park hot spring environment.** *Proc Natl Acad Sci U S A* 1994, **91**:1609-1613.
4. Barns SM, Delwiche CF, Palmer JD, Pace NR: **Perspectives on archaeal diversity, thermophily and monophyly from environmental rRNA sequences.** *Proc Natl Acad Sci USA* 1996, **93**:9188–9193.
5. Hugenholtz P, Pitulle C, Hershberger KL, Pace NR: **Novel division level bacterial diversity in a Yellowstone hot spring.** *J Bacteriol* 1998, **180**:366-376.
6. Farag IF, Davis JP, Youssef NH, Elshahed MS: **Global patterns of abundance, diversity and community structure of the Aminicenantes (candidate phylum OP8).** *PLoS One* 2014, **9**:e92139.

7. Glöckner J, Kube M, Shrestha PM, Weber M, Glöckner FO, Reinhardt R, Liesack W: **Phylogenetic diversity and metagenomics of candidate division OP3.** *Environ Microbiol* 2010, **12**:1218-1229.
8. Lee KC, Herbold CW, Dunfield PF, Morgan XC, McDonald IR, Stott MB: **Phylogenetic delineation of the novel phylum *Armatimonadetes* (former candidate division OP10) and definition of two novel candidate divisions.** *Appl Environ Microbiol* 2013, **79**:2484-2487.
9. Dodsworth JA, Gevorkian J, Despujos F, Cole JK, Murugapiran SK, Ming H, Li WJ, Zhang G, Dohnalkova A, Hedlund BP. ***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.** *Int J Syst Evol Microbiol*. 2014, **64**:2119-2127.
10. Iino T, Mori K, Uchino Y, Nakagawa T, Harayama S, Suzuki K: ***Ignavibacterium album* gen. nov., sp. nov., a moderately thermophilic anaerobic bacterium isolated from microbial mats at a terrestrial hot spring and proposal of *Ignavibacteria* classis nov., for a novel lineage at the periphery of green sulfur bacteria.** *Int J Syst Evol Microbiol* 2010, **60**:1376-1382.
11. Lee KC, Dunfield PF, Morgan XC, Crowe MA, Houghton KM, Vyssotski M, Ryan JL, Lagutin K, McDonald IR, Stott MB: ***Chthonomonas calidirosea* gen. nov., sp. nov., an aerobic, pigmented, thermophilic microorganism of a novel bacterial class, *Chthonomonadetes* classis nov., of the newly described phylum *Armatimonadetes* originally designated candidate division OP10.** *Int. J. Syst. Evol. Microbiol* 2011, **61**:2482–2490.
12. Mori K, Yamaguchi K, Sakiyama Y, Urabe T, Suzuki K: ***Caldisericum exile* gen. nov., sp. nov., an anaerobic, thermophilic, filamentous bacterium of a novel**

bacterial phylum, *Caldiserica* phyl. nov., originally called the candidate phylum OP5, and description of *Caldiseriaceae* fam. nov., *Caldisericales* ord. nov. and *Caldisericia* classis nov. Int J Syst Evol Microbiol 2009, **59**:2894-2898.

13. Podosokorskaya OA, Kadnikov VV, Gavrilov SN, Mardanov AV, Merkel AY, Karnachuk OV, Ravin NV, Bonch-Osmolovskaya EA, Kublanov IV: **Characterization of *Melioribacter roseus* gen. nov., sp. nov., a novel facultatively anaerobic thermophilic cellulolytic bacterium from the class *Ignavibacteria*, and a proposal of a novel bacterial phylum *Ignavibacteriae*.** *Environ Microbiol* 2013, **15**:1759-1771.

14. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO: **The SILVA ribosomal RNA gene database project: improved data processing and web-based tools.** *Nucl Acids Res* 2013 **41**: D590-D596.

15. Yarza P, Yilmaz, P, Pruesse, E, Glöckner, FO, Ludwig, W, Schleifer, KH, Whitman, WB, Euzéby, J, Amann, R, Rosselló-Móra R: **Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences.** *Nat Rev Microbiol* 2014, **12**:635-645.

●● Although not focusing on thermophiles and focused only on *Bacteria*, this paper provides a great perspective on the yet-uncultivated microbial world. The authors use all taxa on the *List of Prokaryotic Names with Standing in Nomenclature* to calibrate 16S rRNA gene distance to the taxonomic structure and then use the calibration to expand the taxonomic structure to 16S rRNA genes from cultivation-independent studies, generating estimates of biodiversity and an up-to-date view on 16S rRNA gene exploration.

16. Cole JK, Peacock JP, Dodsworth JA, Williams AJ, Thompson DB, Dong H, Wu G, Hedlund BP: **Sediment microbial communities in Great Boiling Spring are controlled by temperature and distinct from water communities.** *ISME J* 2013, **7**:718-729.

● This study shows focuses on Great Boiling Spring, in the U.S. Great Basin, a hotspot for 'Aigarchaeota' diversity and abundance. This spring contains at least four genus-level groups of 'Aigarchaeota'. In the highest temperature sample, 'Aigarchaeota' comprise >50% of 16S rRNA gene pyrotags. Pyrotag-based estimates of 'Aigarchaeota' abundance in this spring are similar to metagenomic estimates (IMG #2053563014)

17. Sharp CE, Brady AL, Sharp GH, Grasby SE, Stott MB, Dunfield PF: **Humboldt's spa: microbial diversity is controlled by temperature in geothermal environments.** *ISME J* 2014, **8**:1166-1174.

● This study examines 16S rRNA gene pyrotag data from ~170 geothermal systems in Canada, New Zealand, United States, and China and shows a richness and diversity maximum at ~24 °C, with both measurements declining as temperature increases.

18. Parte, A.C. **LPSN-list of prokaryotic names with standing in nomenclature.** *Nucl. Acids Res* 2014, **42**:D613-D616.

19. Baker BJ, Dick GJ: **Omic Approaches in Microbial Ecology: Charting the Unknown.** *Microbe* 2013, **8**:353–360.

20. Kantor RS, Wrighton KC, Handley KM, Sharon I, Hug LA, Castelle CJ, Thomas BC, Banfield, JF: **Small genomes and sparse metabolisms of sediment-associated bacteria from four candidate phyla.** *mBio* 2013, **4**:e00708–00713.

21. Bowen De León K, Gerlach R, Peyton BM, Fields MW: **Archaeal and bacterial communities in three alkaline hot springs in Heart Lake Geyser Basin, Yellowstone National Park.** *Front Microbiol* 2013, **4**:330.

22. Hamilton TL, Koonce E, Howells A, Havig JR, Jewell T, de la Torre JR, Peters JW, Boyd ES: **Competition for ammonia influences the structure of**

chemotrophic communities in geothermal springs. *Appl Environ Microbiol* 2014, **80**:653-661.

23. Hedlund BP, Dodsworth JA, Cole JK, Panosyan HH: **An integrated study reveals diverse methanogens, Thaumarchaeota, and yet-uncultivated archaeal lineages in Armenian hot springs.** *Antonie Van Leeuwenhoek*. 2013, **104**:71-82.

24. Hou W, Wang S, Dong H, Jiang H, Briggs BR, Peacock JP, Huang Q, Huang L, Wu G, Zhi X, Li W, Dodsworth JA, Hedlund BP, Zhang C, Hartnett HE, Dijkstra P, Hungate BA: **A comprehensive census of microbial diversity in hot springs of Tengchong, Yunnan Province China using 16S rRNA gene pyrosequencing.** *PLoS One* 2013, **8**:e53350.

25. Huang Q, Jiang H, Briggs BR, Wang S, Hou W, Li G, Wu G, Solis R, Arcilla CA, Abrajano T, Dong H: **Archaeal and bacterial diversity in acidic to circumneutral hot springs in the Philippines.** *FEMS Microbiol Ecol* 2013, **85**:452-464.

26. Miller SR, Strong AL, Jones KL, Ungerer MC: **Bar-coded pyrosequencing reveals shared bacterial community properties along the temperature gradients of two alkaline hot springs in Yellowstone National Park.** *Appl Environ Microbiol* 2009, **75**:4565-4572.

27. Wang S, Hou W, Dong H, Jiang H, Huang L, Wu G, Zhang C, Song Z, Zhang Y, Ren H, Zhang J, Zhang L: **Control of temperature on microbial community structure in hot springs of the Tibetan Plateau.** *PLoS One* 2013, **8**:e62901.

28. Inskeep WP, Jay ZJ, Tringe SG, Herrgård MJ, Rusch DB; YNP Metagenome Project Steering Committee and Working Group Members: **The YNP Metagenome Project: Environmental Parameters Responsible for Microbial**

Distribution in the Yellowstone Geothermal Ecosystem. *Front Microbiol* 2013, 4:67.

- This paper summarizes the Yellowstone National Park Metagenome Project, which included twenty sites, roughly divided into phototrophic mat communities, *Aquificales*-dominate "streamer" communities, and *Archaea*-dominated communities. Although not focused on major, yet-uncultivated groups, the YNP Metagenome Project is the largest coordinated effort to obtain metagenomes from thermal environments.

29. Markowitz VM, Chen IM, Chu K, Szeto E, Palaniappan K, Pillay M, Ratner A, Huang J, Pagani I, Tringe S, Huntemann M, Billis K, Varghese N, Tennessen K, Mavromatis K, Pati A, Ivanova NN, Kyrpides NC: **IMG/M 4 version of the integrated metagenome comparative analysis system.** *Nucleic Acids Res* 2014, 42:D568-573.

30. Konstantinidis KT, Tiedje JM: **Prokaryotic taxonomy and phylogeny in the genomic era: advancements and challenges ahead.** *Curr Opin Microbiol* 2007, 10:504-509.

31. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R: **QIIME allows analysis of high-throughput community sequencing data.** *Nat Methods* 2010, 7:335-336.

32. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF: **Introducing mothur: open-source, platform-independent, community-supported software for describing and**

485 **comparing microbial communities.** *Appl Environ Microbiol* 2009, **75**:7537-
486 7541.

487

488 33. Nunoura T, Takaki Y, Kakuta J, Nishi S, Sugahara J, Kazama H, Chee GJ, Hattori
489 M, Kanai A, Atomi H, Takai K, Takami H: **Insights into the evolution of**
490 **Archaea and eukaryotic protein modifier systems revealed by the genome of**
491 **a novel archaeal group.** *Nucleic Acids Res* 2011, **39**:3204-3223.

492 ●● This study describes a complete genomic assembly for the founding member of the
493 'Aigarchaeota', "*Candidatus* Caldiarchaeum subterraneum", from a subterranean gold
494 mine in Japan. The paper describes possible metabolic features, including
495 hydrogenotrophy, autotrophy, aerobic respiration, and nitrate reduction, as well as some
496 eukaryotic features such as a possible ubiquitin protein modification system.

497

498 34. Takami H, Noguchi H, Takaki Y, Uchiyama I, Toyoda A, Nishi S, Chee GJ, Arai
499 W, Nunoura T, Itoh T, Hattori M, Takai K: **A deeply branching thermophilic**
500 **bacterium with an ancient acetyl-CoA pathway dominates a subsurface**
501 **ecosystem.** *PLoS One* 2012, **7**:e30559.

502 ●● This paper describes a complete genomic assembly for the founding member of
503 the 'Acetothermia' (OP1), "*Candidatus* Acetothermum autotrophicum", from a
504 subterranean gold mine in Japan. This organism is likely to be an acetogen capable of
505 autotrophy via the Wood-Ljungdahl pathway. The paper also speculates on the deep
506 evolutionary origin of H₂-dependent acetogenesis.

507

508 35. Kozubal MA, Romine M, Jennings Rd, Jay ZJ, Tringe SG, Rusch DB, Beam JP,
509 McCue LA, Inskeep WP: **Geoarchaeota: a new candidate phylum in the**
510 **Archaea from high-temperature acidic iron mats in Yellowstone National**
511 **Park.** *ISME J.* 2013, **7**:622-634.

512 ● The 'Geoarchaeota' (NAG1) is described here as a candidate phylum of Archaea,
513 represented by a composite metagenome assembled from One Hundred Springs Plain
514 Spring in Yellowstone; however, this group is monophyletic with the Thermoprotei
515 so the candidate phylum status is in question.

36. Elkins JG, Kunin V, Anderson I, Barry K, Goltsman E, Lapidus A, Hedlund BP, Hugenholtz P, Kyrpides N, Graham D, Keller M, Wanner G, Richardson P, Stetter KO: **A korarchaeal genome reveals insights into the evolution of archaea.** *Proc Natl Acad Sci U S A* 2008, **105**: 8102-8107.

●● This study describes a complete genomic assembly for a member of the 'Korarchaeota', "*Candidatus* Korarchaeum thermofilum", from an enrichment from Obsidian Pool, Yellowstone National Park.

37. Dodsworth JA, Blainey PC, Murugapiran SK, Swingley WD, Ross CA, Tringe SG, Chain PSG, Raymond J, Quake SR, and Hedlund BP: **Single-cell and metagenomic analyses indicate a fermentative, saccharolytic lifestyle for members of the OP9 lineage.** *Nature Comm* 2013, **4**:1854.

●● This study describes a synergistic analysis of single-cell genomics data and metagenomics data, leading to the assembly of nearly complete genomes for two different species of 'Atribacteria' (OP9) inhabiting different springs in the U.S. Great Basin. Genomic data and *in situ* enrichment on lignocellulose indicate a strictly fermentative, saccharolytic lifestyle.

38. Rinke C, Schwientek P, Sczyrba A, Ivanova NN, Anderson IJ, Cheng JF, Darling A, Malfatti S, Swan BK, Gies EA, Dodsworth JA, Hedlund BP, Tsiamis G, Sievert SM, Liu WT, Eisen JA, Hallam SJ, Kyrpides NC, Stepanauskas R, Rubin EM, Hugenholtz P, Woyke T, **Insights into the phylogeny and coding potential of microbial dark matter.** 2013, *Nature* **499**:431-437.

●● This study describes single-cell genomic analysis of 201 microbial cells from eight different biodiversity hotspots, including Great Boiling Spring, Nevada, leading to a significant expansion of genomic coverage for thermophilic candidate phyla, including 'Aigarchaeota' (HWCG I), 'Fervidibacteria' (OctSp-106), 'Calescamantes' (EM19), and EM3.

39. Beam JP, Jay ZJ, Kozubal MA, Inskeep WP. **Niche specialization of novel Thaumarchaeota to oxic and hypoxic acidic geothermal springs of Yellowstone National Park.** *ISME J* 2014, **8**:938-951.
40. Garcia Costas AM, Liu Z, Tomsho LP, Schuster SC, Ward DM, Bryant DA: **Complete genome of *Candidatus Chloracidobacterium thermophilum*, a chlorophyll-based photoheterotroph belonging to the phylum *Acidobacteria*.** *Environ Microbiol* 2012, **14**:177-190.
41. Sutcliffe IC: **Cell envelope architecture in the Chloroflexi: a shifting frontline in a phylogenetic turf war.** *Environ Microbiol* 2011, **13**:279-282.
42. Junglas B, Briegel A, Burghardt T, Walther P, Wirth R, Huber H, Rachel R: ***Ignicoccus hospitalis* and *Nanoarchaeum equitans*: ultrastructure, cell-cell interaction, and 3D reconstruction from serial sections of freeze-substituted cells and by electron cryotomography.** *Arch Microbiol* 2008, **190**:395-408.
43. Baker BJ, Comolli LR, Dick GJ, Hauser LJ, Hyatt D, Dill BD, Land ML, Verberkmoes NC, Hettich RL, Banfield JF: **Enigmatic, ultrasmall, uncultivated Archaea.** *Proc Natl Acad Sci U S A* 2010, **107**:8806-8811.
44. Castelle CJ, Wrighton KC, Thomas BC, Hug LA, Brown CT, Wilkins MJ, Frischkorn KR, Tringe SG, Singh A, Markillie LM, Taylor RC, Williams KH, Banfield JF: **Genomic expansion of domain Archaea highlights roles for organisms from new phyla in anaerobic carbon cycling.** *Curr Biol* 2015, doi: 10.1016/j.cub.2015.01.014.
45. Ghai R, Pašić, Fernández AB, Martin-Cuadrado AB, Mizuno CM, McMahon KD, Papke RT, Stepanauskas R, Rodriguez-Brito B, Rohwer F, Sánchez-Porro C, Ventosa A, Rodríguez-Valera F: **New abundant microbial groups in aquatic hypersaline environments.** *Sci Rep* 2011, **1**:135.

46. He X, McLean JS, Edlund A, Yooseph S, Hall AP, Liu SY, Dorrestein PC, Esquenazi E, Hunter RC, Cheng G, Nelson KE, Lux R, Shi W: **Cultivation of a human-associated TM7 phylotype reveals a reduced genome and epibiotic parasitic lifestyle.** *Proc Natl Acad Sci U S A* 2015, **112**:244-249.
47. Huber H, Hohn MJ, Rachel R, Fuchs T, Wimmer VC, Stetter KO: **A new phylum of Archaea represented by a nanosized hyperthermophilic symbiont.** *Nature* 2002, **417**:63-67.
48. Narasingarao P, Podell S, Ugalde JA, Brochier-Armanet C, Emerson JB, Brocks JJ, Heidelberg KB, Banfield JF, Allen EE: **De novo metagenomic assembly reveals abundant novel major lineage of Archaea in hypersaline microbial communities.** *ISME J* 2012, **6**:81-93.
49. Podar M, Makarova KS, Graham DE, Wolf YI, Koonin EV, Reysenbach AL. **Insights into archaeal evolution and symbiosis from the genomes of a nanoarchaeon and its inferred crenarchaeal host from Obsidian Pool, Yellowstone National Park.** *Biol Direct* 2013, **8**:9.
50. Brochier-Armanet C, Forterre P, Gribaldo S: **Phylogeny and evolution of the Archaea: one hundred genomes later.** *Curr Opin Microbiol* 2011, **14**:274-281.
- This review describes the landscape of archaeal genomics, including a summary of genes predicted to encode various components of cell division, DNA maintenance, protein ubiquitinylation, and transcription systems, including systems in 'Aigarchaeota', 'Korarchaeota', and 'Nanoarchaeota'. The paper also presents a cogent discussion of archaeal phylogeny and taxonomy from a phylogenetic and genomic perspective.
51. Gribaldo S, Brochier-Armanet C: **Time for order in microbial systematics.** *Trends Microbiol* 2012, **20**:209-210.

52. Guy L, Ettema TJ: **The archaeal 'TACK' superphylum and the origin of eukaryotes.** *Trends Microbiol* 2011, **19**: 580-587.
53. Guy L, Spang A, Saw JH, Ettema TJ: **'Geoarchaeote NAG1' is a deeply rooting lineage of the archaeal order Thermoproteales rather than a new phylum.** *ISME J* 2014, **8**:1353-1357.
54. Sutcliffe, I.C., Trujillo, M.E., Whitman, W.B., Goodfellow, M: **A call to action for the international committee on systematics of prokaryotes.** *Trends Microbiol* 2013, **21**:51-52.
55. Konstantinidis KT, Rosselló-Móra R: **Classifying the uncultivated microbial majority: A place for metagenomic data in the Candidatus proposal.** *Syst Appl Microbiol* 2015, doi: 10.1016/j.syapm.2015.01.001.
56. Hedlund BP, Dodsworth JA, Staley JT: **The changing landscape of microbial biodiversity exploration and its implications for systematics.** *Syst Appl Microbiol* 2015, in review.
57. Anderson RE, Sogin ML, Baross JA: **Evolutionary strategies of viruses, bacteria and archaea in hydrothermal vent ecosystems revealed through metagenomics.** *PLoS One.* 2014, **9**:e109696.
58. Inskeep WP, Rusch DB, Jay ZJ, Herrgard MJ, Kozubal MA, Richardson TH, Macur RE, Hamamura N, Jennings Rd, Fouke BW, Reysenbach AL, Roberto F, Young M, Schwartz A, Boyd ES, Badger JH, Mathur EJ, Ortmann AC, Bateson M, Geesey G, Frazier M: **Metagenomes from high-temperature chemotrophic systems reveal geochemical controls on microbial community structure and function.** *PLoS One* 2010, **5**:e9773.

59. Nakai R, Abe T, Takeyama H, Naganuma T: **Metagenomic analysis of 0.2- μ m-passable microorganisms in deep-sea hydrothermal fluid.** *Mar Biotechnol (NY)* 2011, **13**:900-908.
60. Xie W, Wang F, Guo L, Chen Z, Sievert SM, Meng J, Huang G, Li Y, Yan Q, Wu S, Wang X, Chen S, He G, Xiao X, Xu A: **Comparative metagenomics of microbial communities inhabiting deep-sea hydrothermal vent chimneys with contrasting chemistries.** *ISME J* 2011, **5**:414-426.
61. Vignais PM: **Hydrogenases and H(+)-reduction in primary energy conservation.** *Results Probl Cell Differ* 2008, **45**:223-252.
62. Petitjean C, Deschamps P, López-García P, Moreira D, Brochier-Armanet C: **Extending the conserved phylogenetic core of Archaea disentangles the evolution of the third domain of Life.** *Mol Biol Evol* 2015, doi: 10.1093/molbev/msv015.
63. Wolf YI, Makarova KS, Yutin N, Koonin EV: **Updated clusters of orthologous genes for Archaea: a complex ancestor of the Archaea and the byways of horizontal gene transfer.** *Biol Direct* 2012, **14**:46.
64. Raymann K, Forterre P, Brochier-Armanet C, Gribaldo S: **Global phylogenomic analysis disentangles the complex evolutionary history of DNA replication in archaea.** *Genome Biol Evol* 2014, **6**:192-212.
65. Kimura H, Sugihara M, Kato K, Hanada S: **Selective phylogenetic analysis targeted at 16S rRNA genes of thermophiles and hyperthermophiles in deep-subsurface geothermal environments.** *Appl Environ Microbiol* 2006, **72**:21-27.

- 668 66. Costa KC, Navarro JB, Shock EL, Zhang CL, Soukup D, Hedlund BP:
669 **Microbiology and geochemistry of great boiling and mud hot springs in the**
670 **United States Great Basin.** *Extremophiles* 2009, **13**:447–459.
- 671
- 672 67. Dodsworth JA and Hedlund BP: **Microbiology and geochemistry of Smith**
673 **Creek and Grass Valley hot springs: Emerging evidence for wide distribution**
674 **of novel thermophilic lineages in the US Great Basin.** *J Earth Sci* 2010,
675 **21**:315-318.
- 676
- 677 68. Vick TJ, Dodsworth JA, Costa KC, Shock EL, Hedlund BP: **Microbiology and**
678 **geochemistry of Little Hot Creek, a hot spring environment in the Long**
679 **Valley Caldera.** *Geobiology* 2010, **8**:140-154.
- 680
- 681 69. Blank CE, Cady SL, Pace NR. **Microbial composition of near-boiling silica-**
682 **depositing thermal springs throughout Yellowstone National Park:** *Appl*
683 *Environ Microbiol* 2002, **68**:5123-5135.
- 684
- 685 70. Stamatakis A: **RAxML version 8: a tool for phylogenetic analysis and post-**
686 **analysis of large phylogenies.** *Bioinformatics* 2014, **30**:1312-1313.
- 687

Figure 1. Summary of unclassified reads in 16S rRNA gene amplicon datasets. Raw 16S rRNA gene pyrotags or Illumina tags from [●16,●17,21-27] were processed using Qiime [31] and mothur [32], respectively, using default parameters (97% OTU clustering) and identified using the Greengenes taxonomy, accounting for unclassified classes, orders, and families. Phyla were not plotted due to uncertainty about archaeal phylum-level taxonomy. *n* represents the number of rRNA gene tag datasets per each site. Violin plots depict the range and data model (colored area), 25th and 75th percentile (box), mean (dark bar), and median (light bar). Individual data points are statistical outliers. Data are detailed in Table S1. Newly published data from the Great Basin were submitted to the NCBI under SRA# XXXXXX.

Figure 2. Phenotypic characteristics and fragment recruitment-based prevalence of yet-uncultivated thermophiles. (A) Genomic anchoring. Characteristics were either directly observed [e.g., ●●37, ●●36, 49, 69] or inferred from genomic data. Predictions of cell shape (MreB or crenactin) or membrane structure (e.g., BamA/YaeT, OmpH, TolC, TonB, secretin [41]) were predicted using BLAST ($< 1e^{-10}$). (B) Genomic anchoring. Reads were recruited from 68 metagenomic datasets from geothermal sites [●28, 57-60], using a >100 bp, 70% nucleic acid identity cutoff using seven thermophile genomes [●●37, ●●36, 49, ●●38, ●●34]. The figure summarizes data from the ten metagenomic datasets with the highest apparent abundance of the target groups; data for the additional metagenomes are shown in Table S3.

Figure 3. Phylogenetic diversity of ‘Aigarchaeota’ lineages. 16S rRNA gene sequences with BLASTN identity >80% to “*Ca. Caldiarchaeum subterraneum*” were mined from GenBank, along with sequences from single-cell genomes and metagenomes in IMG-M [29]. Potential ‘Aigarchaeota’ 16S rRNA gene sequences >1,200 nucleotides ($n=82$; Table S4) were aligned against the SILVA reference alignment in mothur, hard filtered using Lane mask, soft filtered (positions with >50% gaps), and processed in RAxML with 1,000 pseudoreplicates [14, 32, 70]. The resulting phylogenetic tree was delineated into nine genus-level groups (G1-G9) according to >95% nucleotide identity [●●15]. Taxon jackknifing was performed by randomly removing 30% of the sequences (10X) and repeating the bootstrapping analysis (Table S6), further supporting the monophyly of all groups. A similar phylogenetic analysis with the Lane mask but without a soft filter yielded similar results.

Figure 4. Distribution of ‘Aigarchaeota’ based on 16S rRNA gene sequences. (A) Geographic distribution of ‘Aigarchaeota’ genus-level groups, including short sequences (<1,200 bp) in GenBank that could be confidently mapped to groups (Table S4). (B) Environments in which ‘Aigarchaeota’ groups are found. Colors correspond to genus-level groups in Figure 3; open dots are not confidently placed in a genus-level group.

Yellowstone (n=71)
31.2-88.4 °C; pH 2.1-9.6

Western Canada (n=77)
31.3-85.9 °C; pH 6.4-8.6

Armenia (n=2)
44-53 °C; pH 7.2-7.5

Tibet (n=15)
37-97 °C; pH 6-9.5

Great Basin (n=112)
31-95 °C; pH 6.6-9.5

Tengchong (n=43)
55-93 °C; pH 2.5-9.4

Philippines (n=6)
60-92 °C; pH 3.7-6.6

New Zealand (n=46)
31.4-94.7 °C; pH 2.1-8.9

Percent unclassified
at class level

P: Phylum
C: Class
O: Order
F: Family

A

Characteristics

Aerobic respiration	-----	●	○	○	●	○	●	●
Anaerobic respiration	-----	●	○	○	●	○	●	●
Fermentation only	-----	○	●	●	○	●	○	○
Autotrophic	-----	●	○	○	●	○	○	○
Monoderm	-----	●	●	●	○	○	○	○
Diderm	-----	○	○	○	●	●	●	●
Rod or filament	-----	●	●	○	●	●	●	●
Symbiotic	-----	○	○	●	○	○	○	○

Archaea				Bacteria		
'Caldiarchaeum'	'Korarchaeum'	'Nanobsidianus'	'Acetothermus'	'Caldatribacterium'	'Calescibacterium'	'Fervidibacterium'

Characteristics

Direct observation or genomic prediction
● Direct observation
● Genomic prediction/presumed
○ Not observed/not predicted

B

Locations with highest recruitment

1. Great Boiling Spring, Great Basin (82°C, pH 6.4)	●	●	○	●	●	●	●
2. Octopus Spring, Yellowstone (82°C, pH 8.0)	○	○	●	○	●	●	●
3. Cistern Spring, Yellowstone (80°C, pH 4.4)	○	○	●	○	○	○	●
4. Gongxioshe, Tengchong (74°C, pH 7.3)	●	○	○	●	○	○	●
5. OSP Spring, Yellowstone (72°C, pH 3.4)	○	○	●	○	○	○	●
6. Beowulf Spring, Yellowstone (65°C, pH 2.9)	○	○	●	○	○	○	●
7. Little Hot Creek, Great Basin (80°C, pH 6.9)	○	○	○	○	○	○	●
8. Nymph Lake, Yellowstone (88°C, pH 4.0)	○	○	○	○	○	○	●
9. Gumingquan, Tengchong (82°C, pH 9.3)	○	○	○	○	○	○	●
10. Bechler Spring, Yellowstone (80°C, pH 7.8)	○	○	○	○	○	○	●

Genus abundance							Sum
-----------------	--	--	--	--	--	--	-----

Percent reads recruited per Mb query genome
○ <0.001%
○ 0.001%
○ 0.01%
○ 0.1%
○ 1%
○ >5%

Yellowstone

Bechler ●
 Bison Pool ● ● ○
 Boulder Spring ● ●
 Fairy Falls ● ● ●
 Heart Lake ● ● ●
 Mitch's Last Stand ● ●
 Obsidian Pool ● ● ● ●
 Octopus Spring ●
 Perpetual Spouter ●
 Queen's Laundry ● ●
 Washburn ●

Great Basin

Grass Valley Spring ● ●
 Great Boiling Spring ● ● ● ●
 Little Hot Creek ● ● ● ● ●
 Mud Hot Springs ● ● ● ● ●
 Nevada National Security Site ● ● ● ● ●

Japan

Gold Mine ●
 Nakabusa Hot Spring ● ●
 Toyoha Mine ●
 Nagasaki ●
 Yunohama ●

Tengchong

Gongxiaoshe ● ●
 Jinze ● ● ●
 Tengchong ● ● ● ●