Supplementary Information

Supplementary Materials and Methods

Alpha and beta diversity

Alpha diversity metrics (observed phylotype richness, Chao1 richness, Shannon index, equitability index, and Simpson index), sampling coverage, and principal coordinates analysis of samples based on weighted UniFrac distances (Lozupone & Knight, 2005) were calculated using QIIME (Caporaso et al, 2010) with re-sampling (bootstrapping and jackknifing: 1000 re-samples) at 800 reads to avoid sample size based artifacts (Lozupone et al, 2011).

Environmental distribution of putative thermophilic endospore phylotypes

The Short Read Archive (SRA) database (Kodama et al, 2012) was screened (May 2013) for metagenomic datasets containing 16S rRNA gene sequences obtained by PCR amplification and sequencing using the 454 platform. 226 amplicon metagenome files were downloaded and the sequences contained in them were extracted using fastq-dump (part of SRA tools freely available in SRA site) into environmental categories according to the NCBI taxonomic classification of their environmental origin (46 metagenomic categories). In addition, all datasets of the VAMPS database (http:////vamps.mbl.edu) spanning variable regions V4 to V6 were downloaded (May 2013). Finally, using makeblastdb (available in the NCBI BLAST stand-alone distributions) those files were formatted into databases containing a total of 36,178,644 sequences. Since these datasets contain short amplicon sequences of different regions of the 16S rRNA gene, the use of representative thermospore phylotype sequences as BLAST queries only yield results in the subset database sequences from the same region. Thus to simultaneously access all datasets for the

presence of thermospore phylotypes, we used proxy sequences of almost full length (>1400 nt) as queries. Proxy sequences were selected by BLAST searching [(Altschul et al, 1990) megablast default options] representative sequences of thermospore phylotypes against the NCBI nucleotide database (Wheeler et al, 2008) to identify, whenever possible, the closest, near full-length 16S rRNA sequences. Full-length sequences were only considered proxies of thermospore phylotypes if they shared more than 97% sequence identity across more than 80% of the query length. The BLAST hits for each proxy were quality filtered (longer than 300 nt with more than 97% identity across more than 80% of the amplicon length) and the results were normalized as relative abundance of the obtained sequences compared to the total number of sequences (longer than 300 nt) for each dataset. In addition, the L4-DeepSeq dataset (containing ~10 million 16S rRNA V6 reads from a deeply sequenced site in the English Channel) (Gibbons et al, 2013) was downloaded from the European Nucleotide Archive (ENA accession: PRJEB3249) and formatted to a database as described above. The full-length proxies of the thermospore phylotypes were then used as BLAST queries. Positive hits (>30 nt, ≥97% similarity, ≥80% coverage) were normalized against the 10,786,733 sequences longer than 30 nt in this dataset.

Supplementary Results and Discussion

Detecting thermophilic endospores as indicators for passive dispersal in the ocean

Direct identification of thermophilic endospores by DNA-based molecular methods is hampered by difficulties in (i) efficient DNA extraction from low abundant spores in marine sediments and (ii) distinguishing DNA from spores and vegetative cells in environmental nucleic acids extracts. Hence, an alternative means to identify thermophilic endospores is to record significant changes in community structure that are due to endospore germination and growth in incubations of pasteurized sediments at

high temperature. We initially evaluated different incubation conditions and times (56 h, 72 h, and 120 h) to increase the recovery of thermospore phylotypes from sediments of Svalbard stations J and A, and/or Aarhus Bay station M5. The temperature was set to 50°C because previous studies have shown that maximal thermophilic sulfate reduction rates and numbers of endospore-forming, sulfate-reducing *Desulfotomaculum* phylotypes were obtained at incubation temperatures of about 50°C (de Rezende et al, 2013; Hubert et al, 2009). Amendment of sediment incubations with a mixture of formate, lactate, acetate, succinate, propionate, butyrate, and ethanol and/or freeze-dried *Spirulina* cells considerably increased the number of detected thermospore phylotypes compared to incubations without supplemental organic compounds (Supplementary Figure S1A). To confirm that *Spirulina* cells were free of viable thermophiles, they were incubated under the same conditions as the sediment samples and no growth/enrichment was observed. While there were some differences in the identity of the phylotypes detected after different incubation times, more thermospore phylotypes were detected after longer incubation (Supplementary Figure S1B) and thus all subsequent incubations were performed for 120 h. While still not all thermophilic endospores may germinate and grow under these incubation conditions, the amendment of incubations with organic compounds has a normalizing effect on endospore recovery by providing similar germination conditions in all sediments and thus allows comparative analysis of spore phylotype richness between different locations. Surveys of 16S rRNA gene sequence diversity are commonly used for studies of microbial biogeography (Chu et al, 2010; Fierer et al, 2009; Galand et al, 2010; Horner-Devine et al, 2004; Martiny et al, 2011; Nemergut et al, 2011), although the phylogenetic resolution of the 16S rRNA gene is limited to species-level phylotypes or higher order taxa and some microbial biogeography patterns only become apparent at the strain-level (Cho & Tiedje, 2000; García-Martínez & Rodríguez-Valera, 2000; Miller et al, 2006; Papke et al, 2003; Silva et al, 2005; Whitaker et al, 2003). Despite this acknowledged caveat, we used 16S rRNA as phylogenetic marker for our study also because the high sequence conservation renders this gene particularly advantageous for selective

analysis of passive dispersal. Mutations in the 16S rRNA gene due to genetic drift are less likely to occur compared to mutations in other, more variable genetic markers.

Potential physiology of thermospore phylotypes

Previous analyses have shown that anoxic high temperature incubation resuscitates a diverse community of dormant *Firmicutes* that collectively catalyze the interdependent series of organic carbon degradation transformations i.e. hydrolysis, fermentation, and mineralization through sulfate respiration (Hubert et al, 2010). The majority of thermospore phylotypes identified in this study belong to the class *Clostridia* (Supplementary Table S3, Supplementary Figure S3). *Clostridia* are anaerobic microorganisms that can ferment a wide range of organic compounds and produce a variety of metabolites (reviewed in Tracy et al, 2012). In addition, they produce extracellular enzymes to degrade large biological molecules into fermentable components. Thermospore phylotypes with ≥97% 16S rRNA sequence similarity to e.g. *Anaerosalibacter bizertensis*, *Brassicibacter mesophilus*, *Caloranaerobacter azorensis*, *Clostridium* spp., *Sporosalibacterium faouarense* (Supplementary Table S3) were thus likely involved in hydrolysis and fermentation (Fang et al, 2012; Rezgui et al, 2011; Wery et al, 2001; Wiegel et al, 1989) of complex substrates present in the sediments and the supplied *Spirulina* cells. In contrast, thermospore phylotypes related to known sulfate reducers of the genus *Desulfotomaculum* (Fardeau et al, 1995) and the iron reducer *Tepidimicrobium ferriphilum* (Slobodkin et al, 2006) probably used the amended organic compounds and products from primary fermenters as electron donors for thermophilic reduction of sulfate and iron, respectively, in the anoxic, high-temperature incubations. Thermospore phylotypes belonging to the class *Bacilli* were mostly related to facultative (e.g. *Anoxybacillus flavithermus*, *Bacillus azotoformans*, *B. licheniformis*, *B. themoamylovorans*, *B. thermolactis*, *Geobacillus thermoglucosidasius*, *Microaerobacter geothermalis, Virgibacillus proomii*) and obligate anaerobes (e.g. *Anaerobacillus alkalilustre, Bacillus infernus*, *Vulcanibacillus modesticaldus*)

(Supplementary Table S3). The metabolic capabilities of these *Bacilli*-related phylotypes are presumably as diverse as those of their next cultivated relatives, which are capable of hydrolysis, fermentation, and/or anaerobic respiration with nitrate, iron, manganese or arsenate as electron acceptors (Boone et al, 1995; Khelifi et al, 2010; L'Haridon et al, 2006; Voigt et al, 2006; Zavarzina et al, 2009).

Sequences belonging to thermophilic endospore-forming phylotypes are rare in available 16S rRNA sequence datasets

In order to gain insights into the general environmental distribution of thermospore phylotypes, we screened all available 16S rRNA amplicon datasets (that were deposited until May 2013 in the SRA database) for the presence of sequences with ≥97% similarity to near full-length proxy sequences of thermospore phylotypes. The use of proxy sequences was necessary because different amplicon sequencing studies targeted different regions of the 16S rRNA gene. We obtained suitable proxy full-length sequences (>1400 nt, ≥97% similarity, >80% coverage) for 78 of 146 thermospore phylotypes. Of over 36 million sequences analyzed in total only 0.005% were closely affiliated with thermospore phylotypes (Supplementary Table S4). Surprisingly, most of these hits were obtained with sequences from bioreactors and intestinal microbiomes. While these anoxic environments support presence of similar but not necessarily thermophilic bacteria, they are unlikely major sources of marine thermophilic endospores. In the datasets from marine environments (i.e., sediments, surface water, sponges, fish, hydrothermal vents, cold-seeps), sequences affiliated with thermospore phylotypes were only present at a very low relative abundance of 0.0003% (n=363/1,132,627). 93% of these hits (n=338/363) were derived from proxies of the cosmopolitan thermospore phylotypes TSP003, TSP005, TSP007, TSP010, TSP013, TSP016, TSP0017 or TSP021. We also analyzed the very deeply sequenced L4-DeepSeq dataset from the English Channel (Gibbons et al, 2013) and found that only 213 of 10,786,733 reads longer than 30 nt showed ≥97% similarity to 14 of our TSP proxy sequences. Under the premise that abundances of

inactive spores will be underestimated in nucleic acids-based diversity surveys, the low prevalence of sequences affiliated with thermospore phylotypes in marine environments suggests that thermophilic spores are members of the rare biosphere in the oceans (Hubert et al, 2009).

Guaymas Basin sediments exhibit characteristics of a source environment for thermophilic endospores

The Guaymas Basin spreading center is the largest in the Gulf of California and harbors a unique hydrothermal vent system at a water depth of about 2000 m (Weber & Jørgensen, 2002). Unlike other deep-sea vent sites, the hydrothermal fluids in this basin are driven by deeply buried magmatic intrusions and rise up to the surface through a sediment cover that has a mean thickness of >100 m (Curray et al, 1982; Fisher & Becker, 1991). Hydrothermal fluid flow supplies oil compounds (Didyk & Simoneit, 1989), methane, and small organic compounds to the anaerobic microbial communities close to the sediment surface. These hydrothermal sediments are anoxic and temperatures at the hot spots increase rapidly with depth from 3°C to above 100°C within the uppermost 30-40 cm and thus provide ideal environments for a variety of anaerobic thermophiles (Martens, 1990; Meyer et al, 2013). Consequently, organisms related to sulfate-reducing *Desulfotomaculum* spp. (*Clostridiales*) (Dhillon et al, 2003; Kniemeyer et al, 2007) (which could contribute to the high thermophilic sulfate reduction rates measured in situ (Weber & Jørgensen, 2002)), members of the genus *Bacillus* (*Bacillales*) (Dick et al, 2006; Marteinsson et al, 1996), and other thermophilic, endospore-forming bacteria were previously detected in these sediments (Biddle et al, 2012; Lakhal et al, 2013; Phelps et al, 1998). The considerable flux of hydrothermal fluids emanating from hydrothermal mounds, chimneys and sediments (Campbell & Gieskes, 1984) could expel large amounts of thermophilic spores into the water column.

Supplementary Figure Legends

Figure S1. Maximizing detection of thermophile spores. Impact of different incubation conditions (A, amendment type; B, incubation time) on the number of thermospore phylotypes detected during germination experiments.

Figure S2. Beta-diversity analysis (PCoA of weighted UniFrac distances) of bacterial communities before and after incubation of pasteurized marine sediments at 50°C. Analysis was performed at 800 reads per library. Sphere sizes and shapes indicate 95% confidence intervals based on 1000 re-samplings. Red spheres indicate starting samples (T=0 h) and green spheres indicate after incubation (T=120 h).

Figure S3. Phylogeny and geographic distribution of all 146 *Firmicutes* thermospore phylotypes. Scale bar indicates 1% sequence divergence as inferred from RAxML. Colored bars indicate broad geographic regions where the thermospore phylotypes were present. Numbers indicate the number of sites at which a thermospore phylotype was detected.

Figure S4. Site occupancy of thermophilic endospore phylotypes. Graph shows the number of phylotypes versus the number of sites at which each phylotype was detected. The majority of the 146 thermospore phylotypes is present at 5 sites or less, while 21 phylotypes were present at 15 or more locations (arbitrarily designated as 'cosmopolitan phylotypes').

Figure S5. Geographic distribution of each cosmopolitan thermospore phylotype. Red circles show the locations where a phylotype was detected.

Figure S6. Network analysis of thermophile spore co-occurrence (**A**) and location (**B**). **A**, Networks of co-occurring thermospore phylotypes. Each node represents a thermospore phylotype. Presence of an edge between two nodes shows a strong correlation between these two phylotypes, which is indicative for co-occurrence. Circle size indicates site occupancy. **B**, Location networks. Each node represents a location, presence of an edge between two nodes corresponds to a high Bray Curtis similarity (≥0.6) between the endospore communities at these two locations. Circle size indicates thermospore phylotype richness.

Supplementary Tables

Table S1. Marine sediment sample description, sediment incubation conditions, thermospore phylotype richness, and thermophilic sulfate reduction rates.

Table S2. Read number, coverage, and alpha-diversity of bacterial 16S rRNA gene sequence libraries of pasteurized marine sediments before and after incubation at 50°C.

Table S3. Site occupancy, next relatives, presence/absence at sampling locations and representative 16S rRNA gene sequences of putative thermophilic *Firmicutes* endospore phylotypes

Table S4. Prevalence of proxy sequences of thermospore phylotypes in publically available 16S rRNA amplicon pyrosequencing datasets from various environments.

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354 transfer of Bacillus arseniciselenatis, Bacillus macyae, and Bacillus alkalidiazotrophicus to Anaerobacillus
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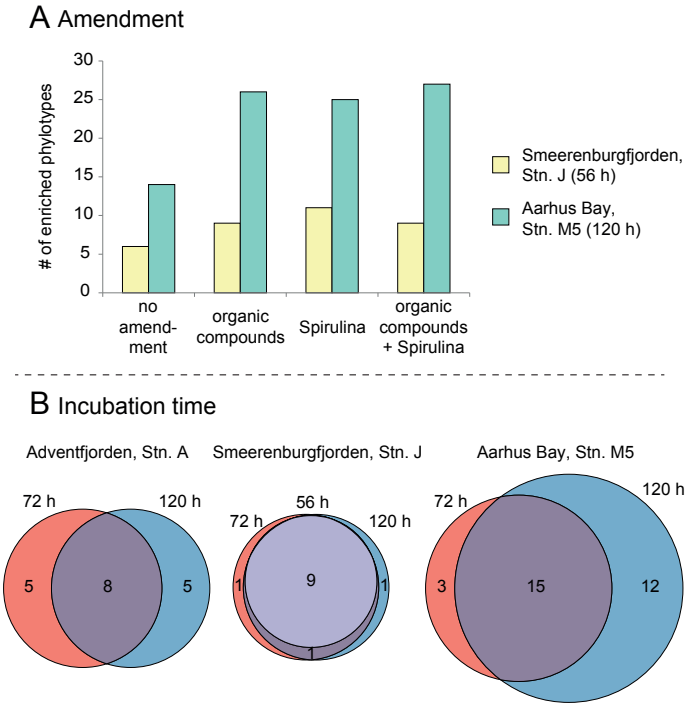

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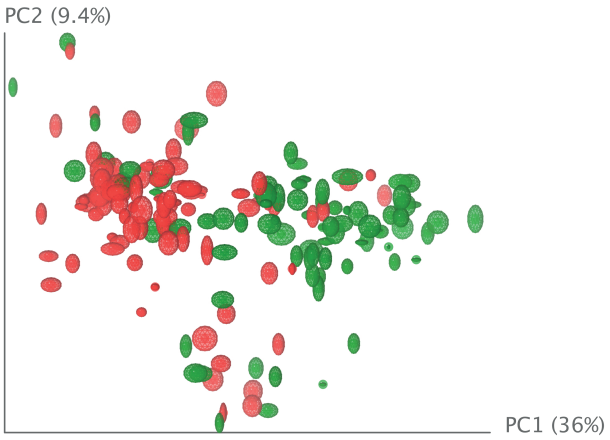

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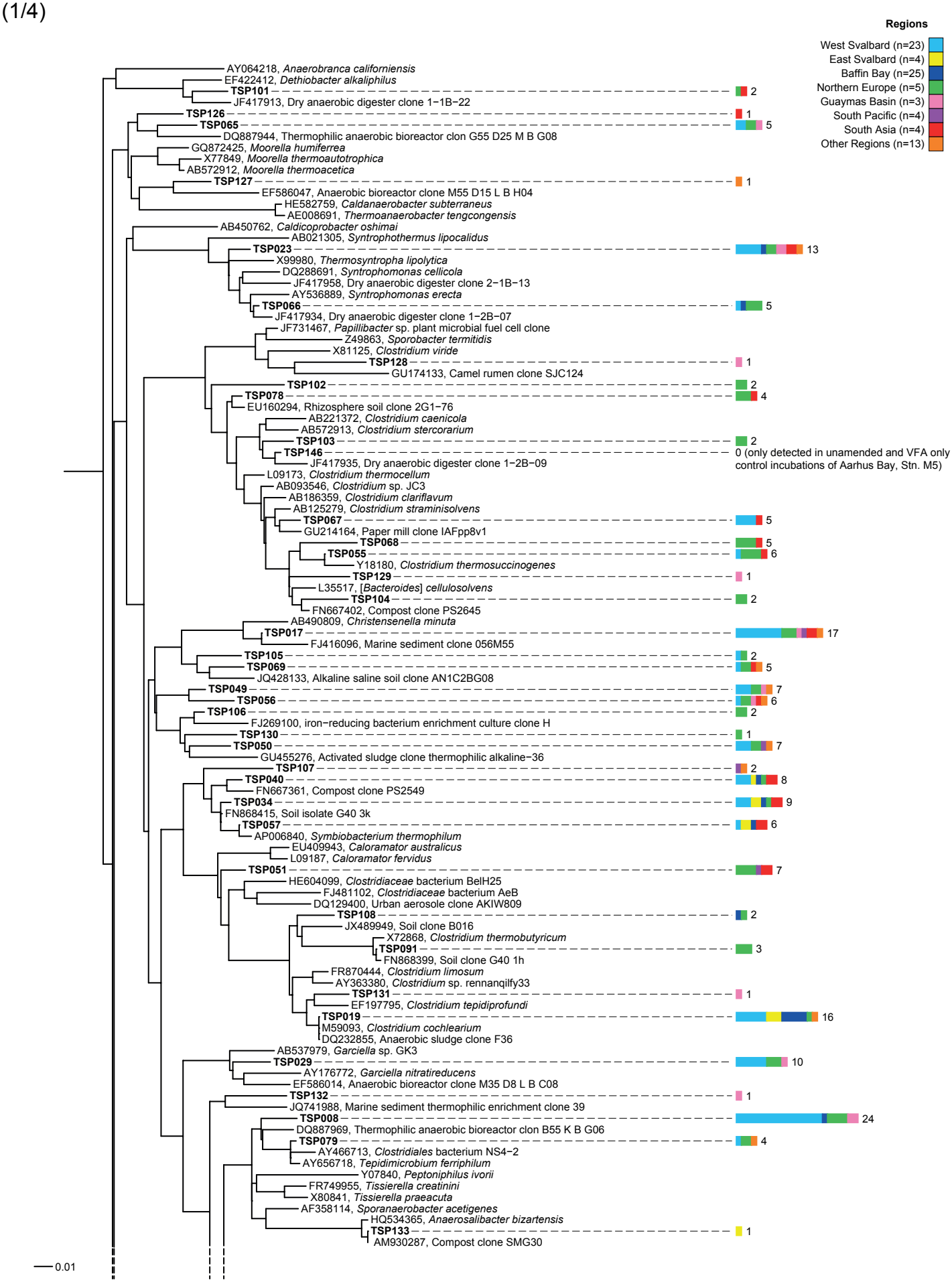
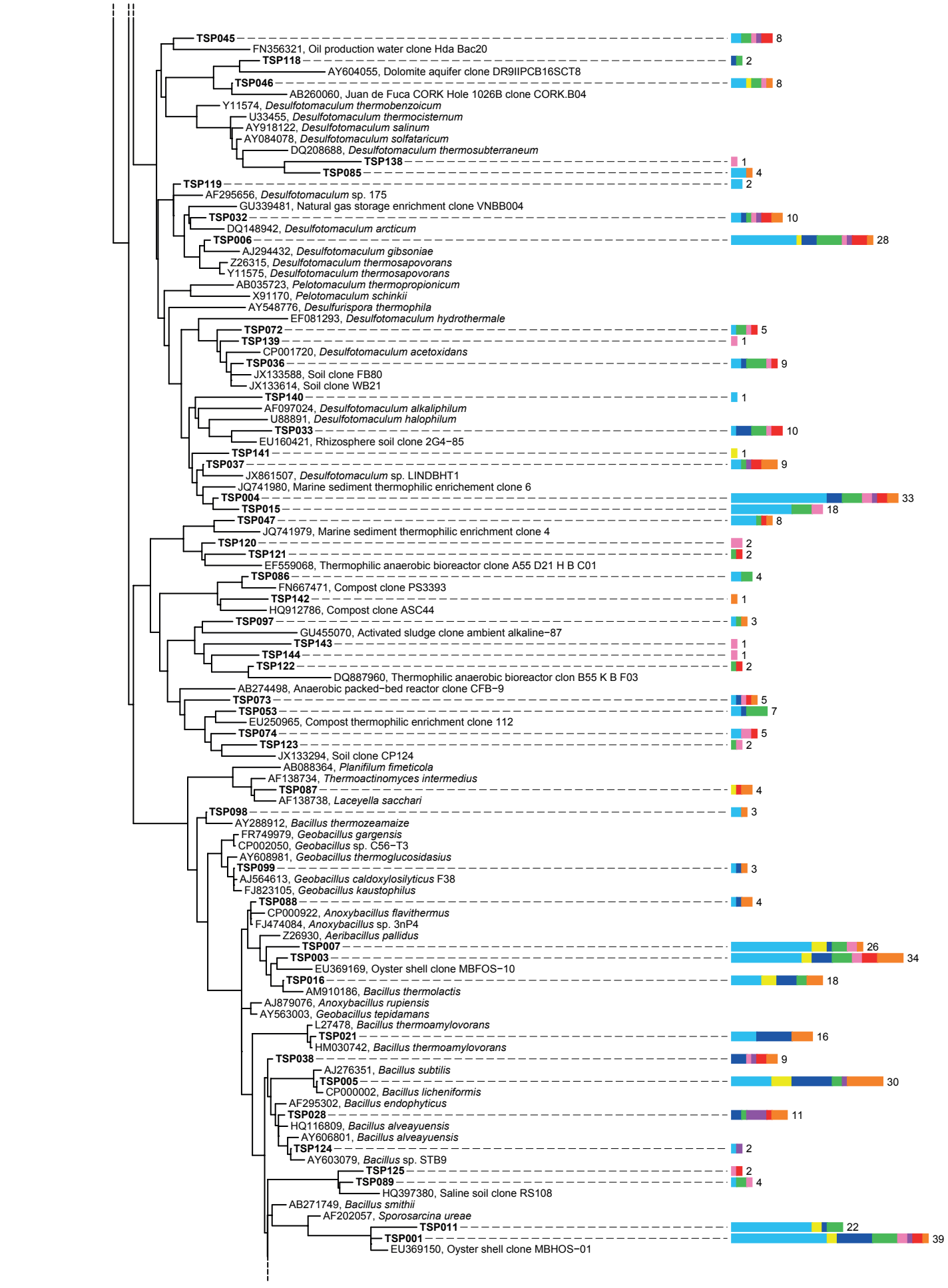
Supplementary Figure S3
(1/4)



Supplementary Figure S3
(2/4)

Supplementary Figure S3
(3/4)



Supplementary Figure S3
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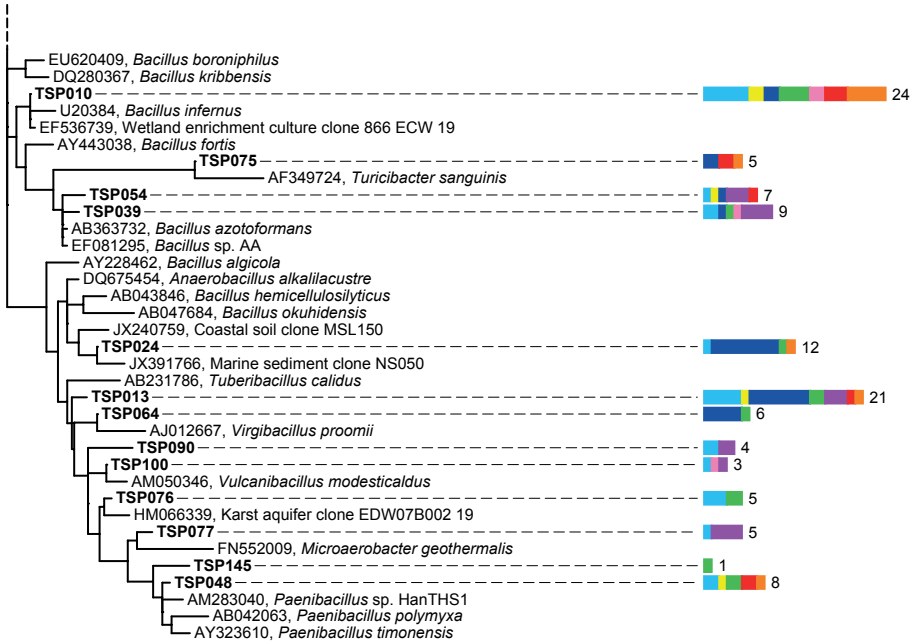

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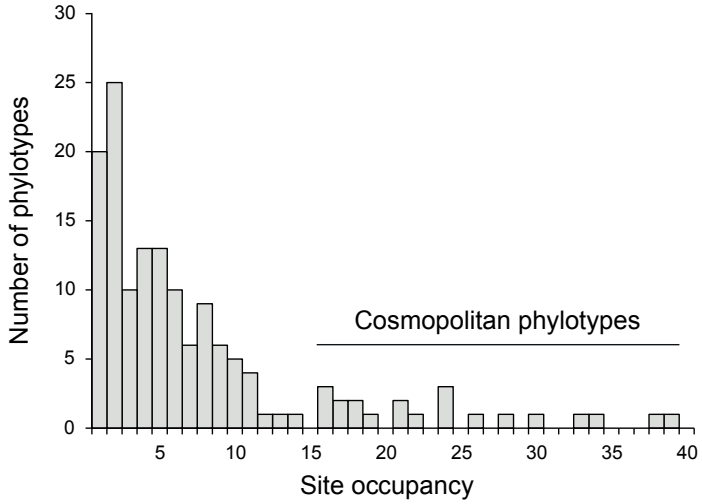
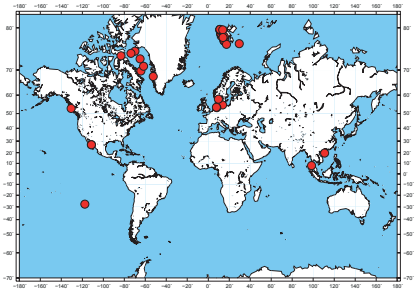


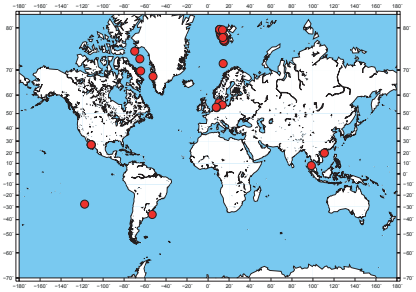
Supplementary Figure S4. Site occupancy of thermophilic endospore phylotypes. Graph shows the number of phylotypes versus the number of sites at which each phylotype was detected. The majority of the 146 thermospore phylotypes is present at 5 sites or less, while 21 phylotypes were present at 15 or more locations (arbitrarily designated as 'cosmopolitan phylotypes').

Supplementary Figure S5
(1/2)

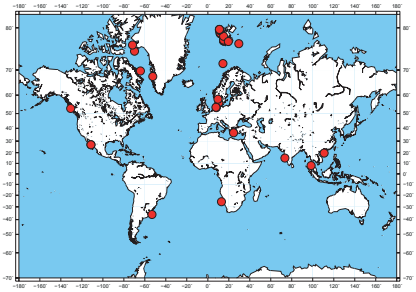
TSP001
Unclassified *Bacillales*



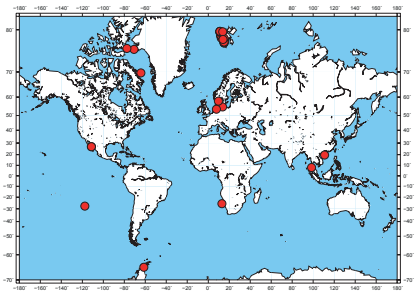
TSP002
Brassicibacter-Sporosalibacterium



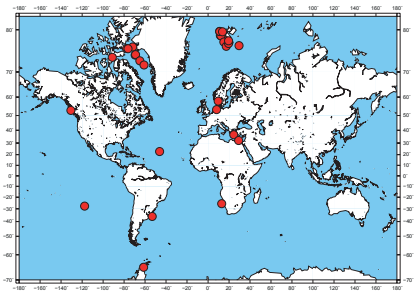
TSP003
Aeribacillus-Geobacillus



TSP004
Desulfotomaculum



TSP005
Bacillus



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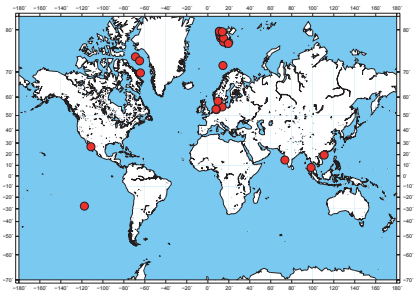
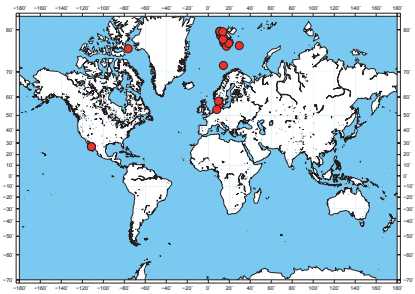
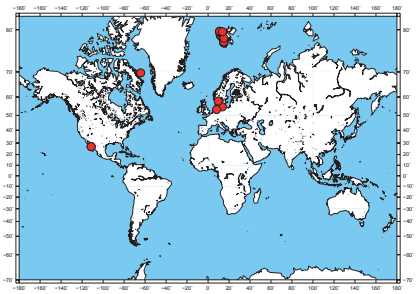
Desulfotomaculum



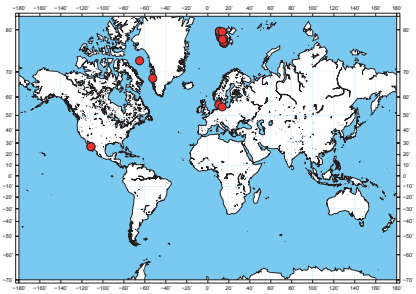
TSP007
Aeribacillus-Geobacillus



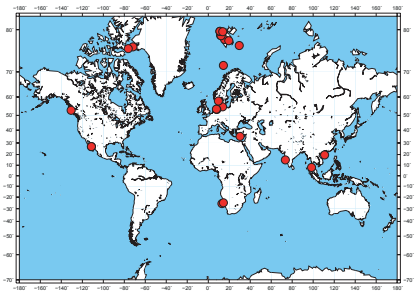
TSP008
Unclassified *Clostridiales*



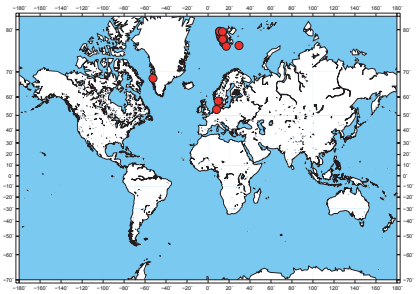
TSP009
Brassicibacter-Sporosalibacterium



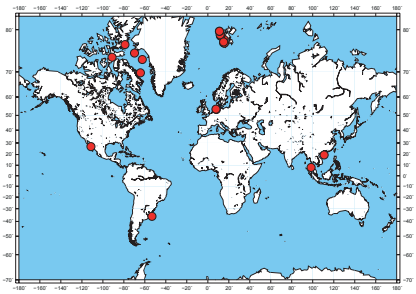
TSP010
Bacillus



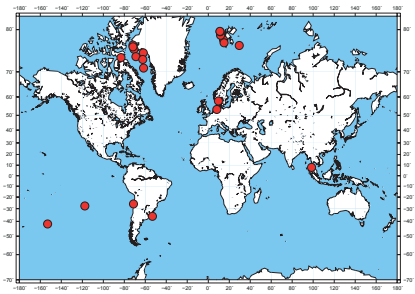
TSP011
Unclassified *Bacillales*



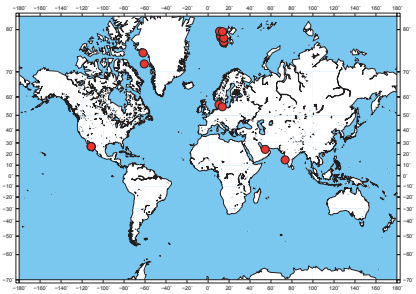
TSP012
Brassicibacter-Sporosalibacterium



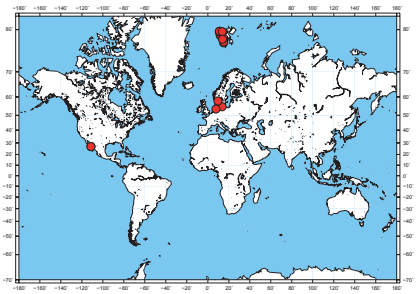
TSP013
Bacillus



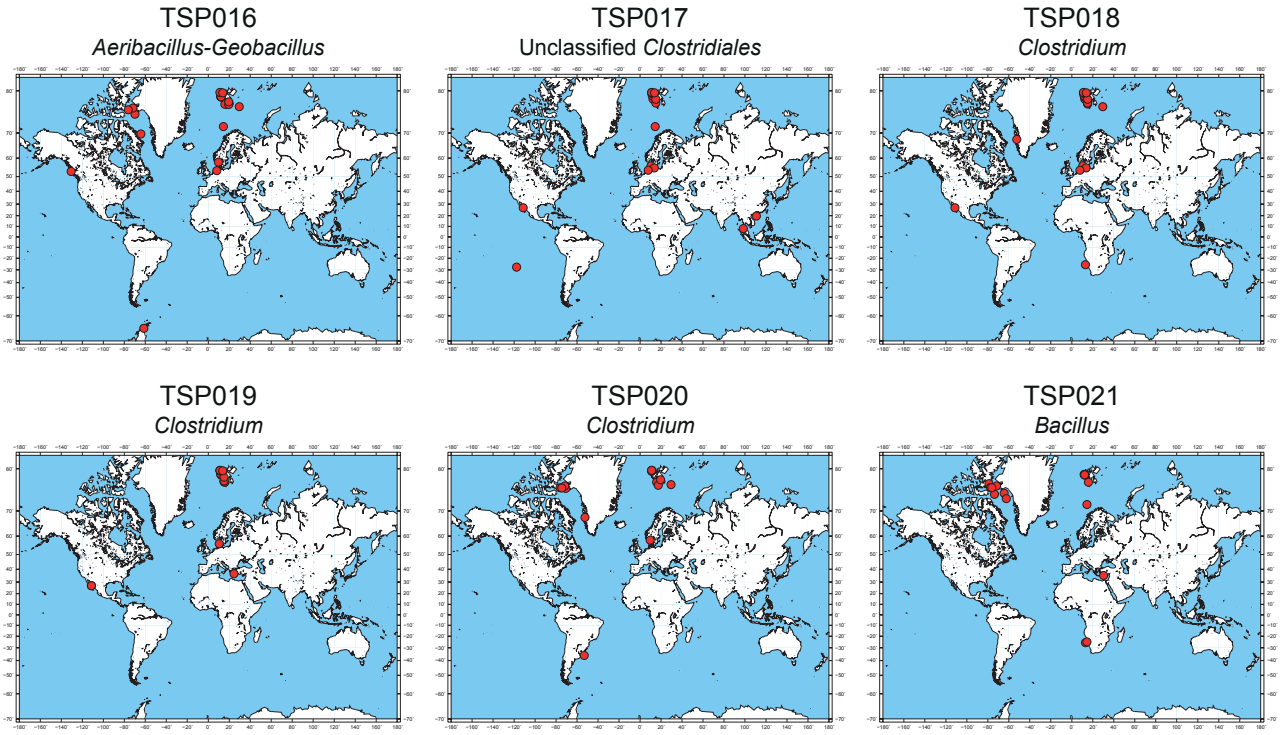
TSP014
Desulfonispora



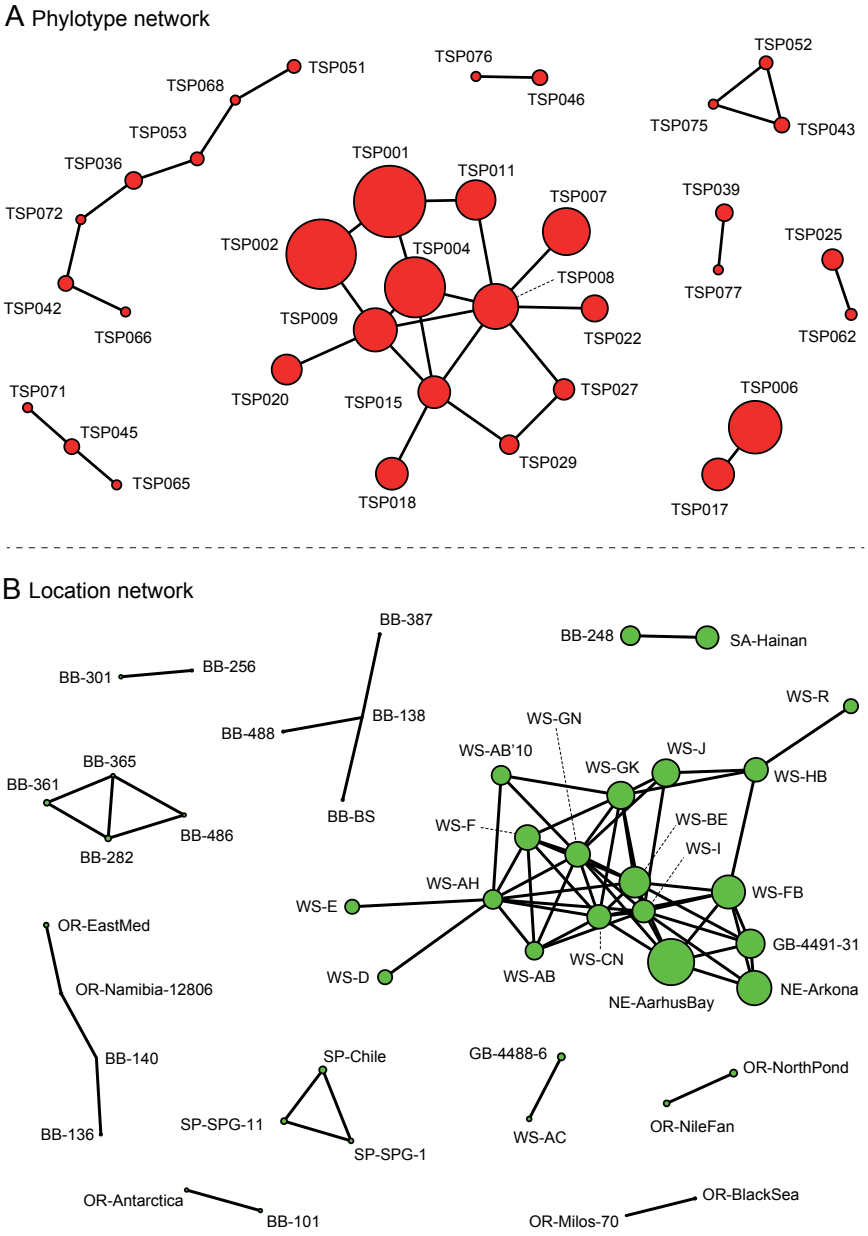
TSP015
Desulfotomaculum



Supplementary Figure S5
(2/2)



Supplementary Figure S5. Geographic distribution of each cosmopolitan thermospore phylotype. Red circles show the locations where a phylotype was detected.



Supplementary Figure S6. Network analysis of thermophile spore co-occurrence (**A**) and location (**B**). **A**, Networks of co-occurring thermospore phylotypes. Each node represents a thermospore phylotype. Presence of an edge between two nodes shows a strong correlation between these two phylotypes, which is indicative for co-occurrence. Circle size indicates site occupancy. **B**, Location networks. Each node represents a location, presence of an edge between two nodes corresponds to a high Bray Curtis similarity (≥0.6) between the endospore communities at these two locations. Circle size indicates thermospore phylotype richness.