Supplementary Material

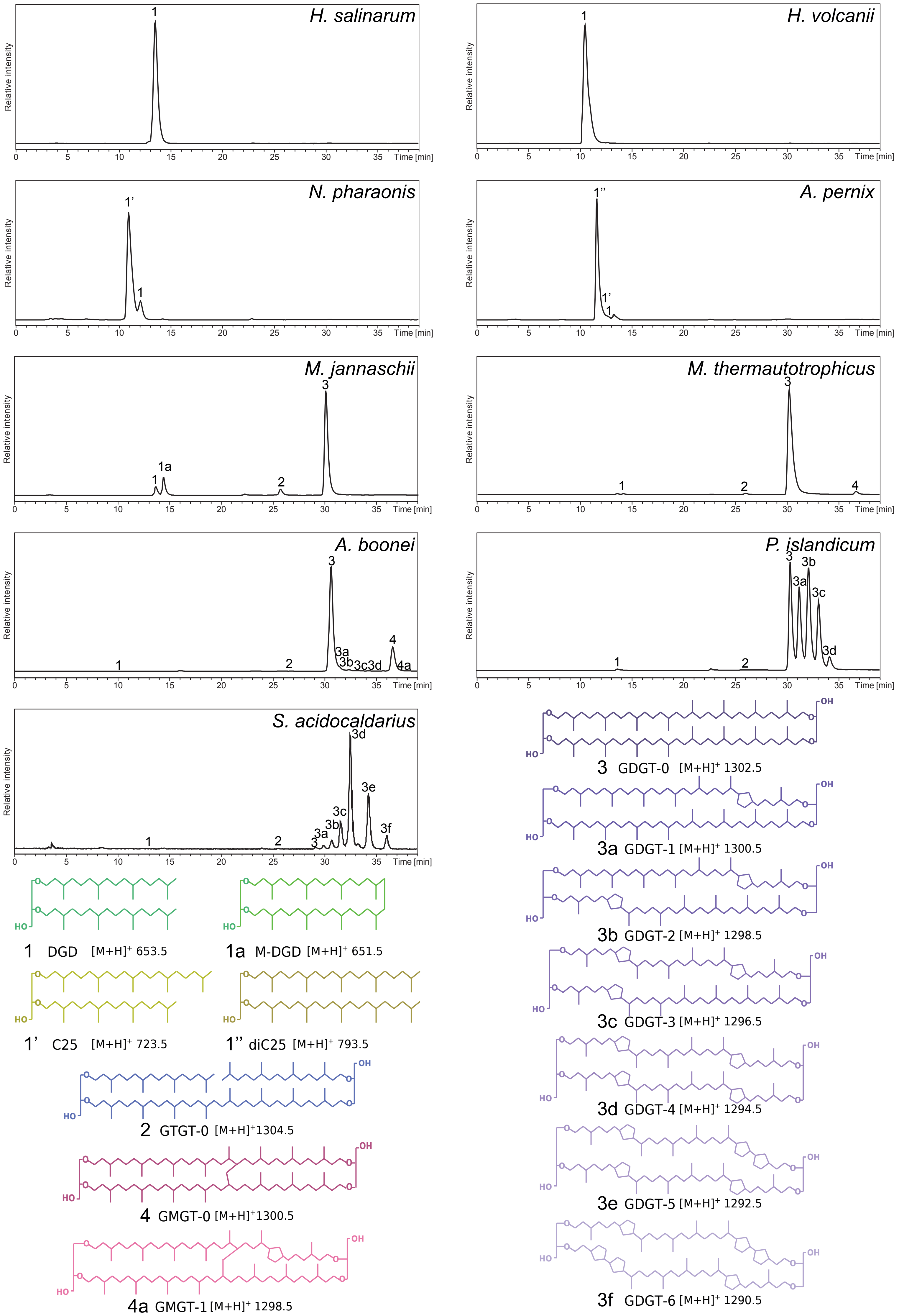
1.1 Supplementary Figures

Figure S1. HPLC chromatograms (top) and lipid core structures (bottom) identified in *Halobacterium salinarum*, *Haloferax volcanii*, *Natronomonas pharaonis*, *Aeropyrum pernix*, *Methanocaldococcus jannaschii*, *Methanothermobacter thermautotrophicus*, *Aciduliprofundum boonei*, *Pyrobaculum islandicum* and *Sulfolobus acidocaldarius*. Cells were grown under their respective optimal conditions as described in the literature. (1) Diphytanyl glycerol diether (DGD, green), (1a) Macrocyclic DGD (M-DGD, light green), (1') 2-O-phytanyl-3-O-sesterterpanyl-sn-glycerol and/or 2-O-sesterterpanyl-3-O-phytanyl-sn-glycerol diether (C25, light yellow), (1'') disesterterpanyl glycerol diether (diC25, dark yellow), (2) glycerol trialkyl glycerol tetraethers with no cyclopentane ring (GTGT-0, blue), (3 to 3f) glycerol dibiphytanyl glycerol tetraethers with 0 to 6 rings (GDGT-0 to -6, dark to light purple) and (4 and 4a) glycerol monoalkyl glycerol tetraethers with 0 (GMGT-0, dark red) and 1 ring (GMGT-1, light red). [M+H$^+$]: mass/charge ratio.

Figure S2. Thermococcales core lipid composition clustering. A hierarchical clustering on principal components was conducted with the FactoMineR(Lê et al., 2008) R package (version 1.38) using Euclidean distance and the average linkage method on the mean core lipid abundances without prior data scaling. The optimal number of clusters was automatically computed using the highest relative loss of inertia.

Figure S3. Thermococcales core lipid composition vs. lipid synthesis gene phylogeny. Unrooted ML phylogeny of Thermococcales was inferred from the aligned concatenated protein sequences of the five core genes of archaeal lipid synthesis, e.g. the CDR archaeaol synthase (TERMP_01811), the (S)-3-O-geranylgeranylglyceryl phosphate synthase (TERMP_01020), (S)-2,3-di-O-geranylgeranylglyceryl phosphate synthase (TERMP_02000), the Digeranylgeranylglyceryl phospholipid reductase (TERMP_00654) and the multifunctional geranyl diphosphate/farnesyl diphosphate/geranylgeranyl diphosphate synthase (TERMP_00147) (1,467 amino acid positions). The tree was inferred with PhyML (LG). The scale bar represents the average number of substitutions per site. Numbers at branches represent bootstrap values (1000 replicates, values > 50% are shown). Core lipid relative proportions from Table 1 are represented as bar charts using the same color code as that in Figure 1.

Figure S4. **Thermococcales core lipid composition vs. whole genome phylogeny.** Unrooted phylogeny of Thermococcales was inferred from whole genomes suing REALPHY. The scale bar represents the average number of substitutions per site. Core lipid relative proportions from Table 1 are represented as bar charts using the same color code as that in Figure 1.



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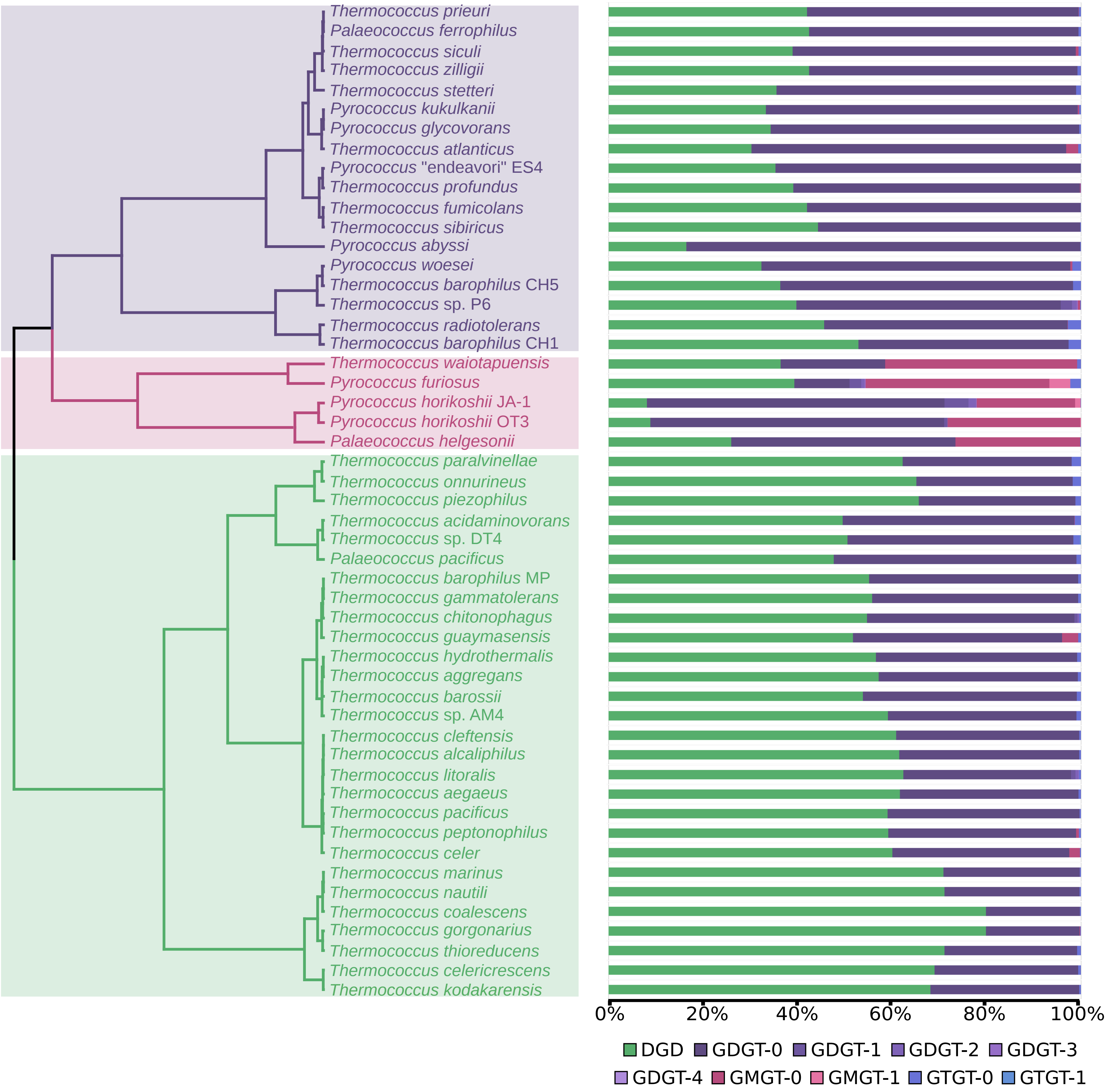

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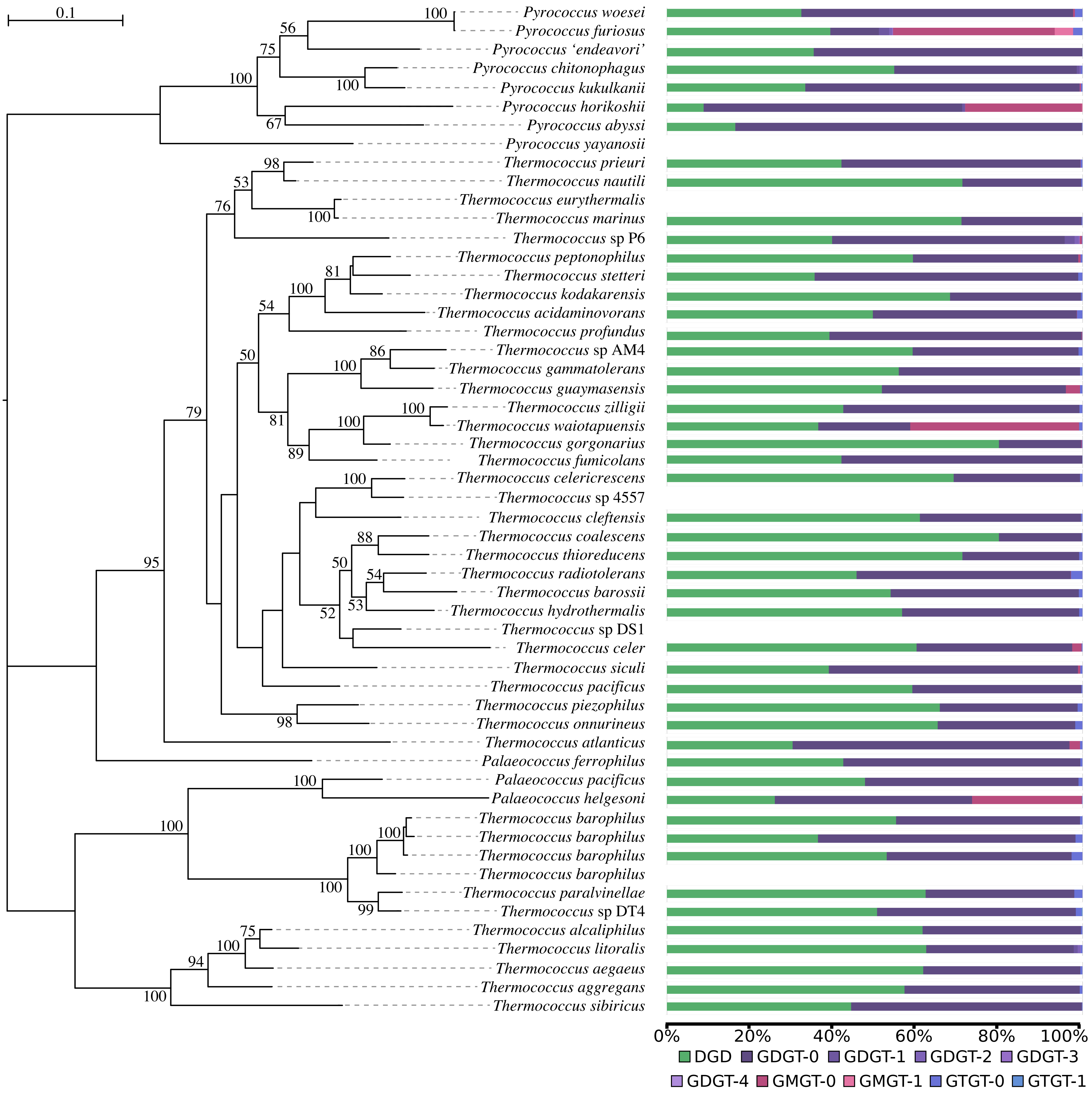

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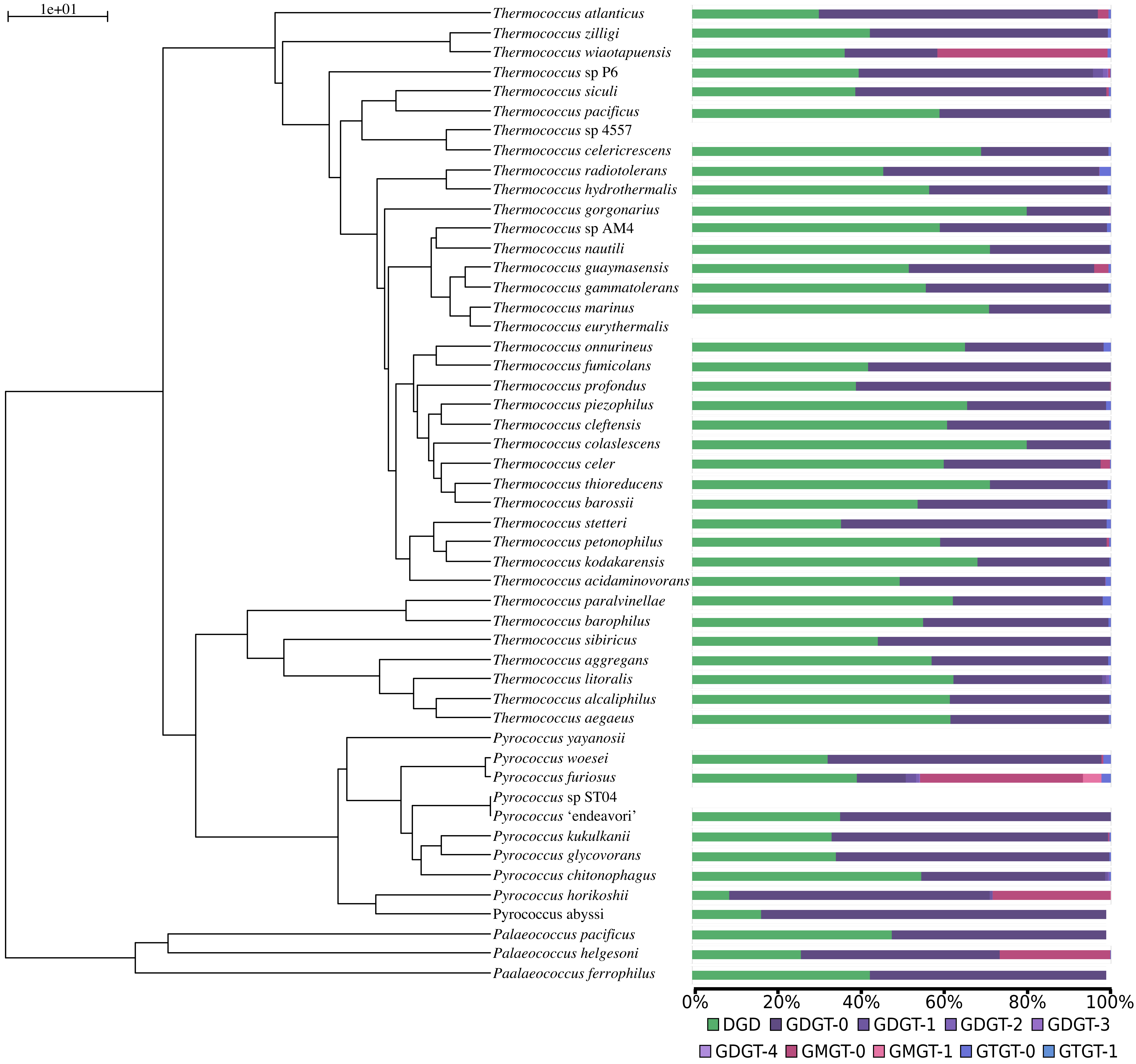

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1.2 Supplementary Tables

Table S1. Original site characteristics and cultivation conditions of the 51 *Thermococcales* strains analyzed. (XLSX format)

Table S2. Literature survey of the lipid compositions of 440 archaeal species. (XLSX format)

Table S3. Core lipid composition of the 9 additional strains. For each strain, values are the average of 3 biological replicates, except for *M. thermautotrophicus* which core lipid composition has been assessed only once (relative % ± standard deviation). (XLSX format)

2 Supplementary References

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