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Baraminological Analysis of a Set of Archaea Species Based on Genomic Data

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Abstract

Archaea have not yet been classified in detail by creationist taxonomy. Also, the Bible does not mention archaea or any other kinds of microbes specifically by name. However, clusters of orthologous genes have been determined for a set of 168 archaeal species. In this study an all-versus-all comparison of whole-gene content was performed on these 168 species, and eight groups, or tentative holobaramins, were determined based on their whole-gene content by using a new baraminology method that measures the Jaccard coefficient value. The member species of these holobaramins had a high mean Jaccard coefficient value compared to one another and a low value compared to other species from different archaeal baramins and bacterial taxa. This paper presents a holistic way of measuring species distance as compared to phylogenetic trees based on evolutionary methods. Open reading frames also were predicted for three ancient halophile archaea species (*H. hubeiense*, *H. salifodinae*, and *H. carlsbadense*) and compared to these 168 species. These three species may closely represent the archebaramin, or originally created ancestors, of one of the predicted archaeal holobaramins, which consist of extreme halophilic species. On average, baraminic boundaries could be set at the level of order or class for Archaea. Archaeal baramins can also be characterized by the ecological niche that they exist in, due to special sets of genes that are necessary to help these archaeal species to adapt to these sometimes extreme environmental conditions.

Introduction

Until now, very few creationist studies have been undertaken to analyze boundaries of different kinds of microbial holobaramins, such as archaea, bacteria, and protozoa. This is all the more difficult, as the Bible does not specifically mention microorganisms anywhere. According to some views, microbes were created after the Fall, due to the pathogenic characteristics of many bacteria. However, only about 5–10% of bacteria are pathogenic, and many viruses act as harmless passengers within their hosts. In fact, pathogens have not yet been discovered among archaea (Pace, 1997), yet some think they have the potential to become pathogenic (Cavicchioli et al., 2003). It has become increasingly

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evident that microbes aid in digestion and produce vitamins and minerals as part of a symbiotic relationship with their hosts. According to the biomatrix or organosubstrate theory (Francis, 2003; Linares, Ross, and Stanton, 2016), microbes were created as a link between multicellular organisms and the physical world to extract inorganic materials and to participate in the geochemical cycling of elements and compounds. Therefore, according to this view, microbes would have been created on Days 3, 5, and 6, along with their plant and animal hosts (Gillen, 2008).

So far, around 11,000 prokaryotic species have been discovered and given a Latin name (Kyrpides et al., 2014). Similar to bacteria, archaea form a separate domain of life; both are separate from eukaryotes. Both bacteria and archaea are prokaryotes, meaning that they lack a cell nucleus and membrane-bound organelles. Archaea share genetic properties with both eukaryotes and bacteria but also have unique cellular characteristics. They have isoprenoid ether lipids in their unique plasma membranes and are the sole group of organisms capable of methanogenesis (Gribaldo and Brochier-Armanet, 2006). According to secular taxonomy, the Archaea are divided into two phyla, the Crenarchaeota, which consist mainly of thermophiles and thermoacidophiles, and the Euryarchaeota, which are made up of halophiles, methanogens, thermophiles and thermoacidophiles (Woese, Kandler, and Wheelis, 1990). They also use a wide range of energy sources, such as ammonia, metals, and hydrogen. Archaea exist in extreme environmental niches, such as hydrothermal vents, or hypersaline environments, but also in non-extreme environments as well. Their cellular structures allow them to exist in such extreme conditions.

Due to their cellular structure, microbes such as bacteria and archaea are discontinuous (forming an apobaramin) from multicellular organisms and even protists. Elder (2015) describes both archaea and bacteria each as a specific cognitum, which is a grouping of creatures that seem to naturally go together by use of the senses. Because both bacteria and archaea are single-celled organisms, different biochemical and genetic characteristics are taken into account in order to classify them.

Genome decay, gene loss, and pseudogene accumulation have been observed in a number of bacterial genera (O'Micks, 2015), meaning that all species that are members of these genera share a common ancestral genome. In archaea, less than 10% of the genome resides within pseudogenes, compared to up to 50% in bacteria. The insertion-to-deletion ratio is also lower in archaea, as well as strand slippage due to mononucleotide repeats. Compared to bacteria, the number of inactivating mutations per gene as well as the proportion of truncated pseudogenes is greater in archaea (O'Micks, 2016). On the other hand, the average pseudogene-to-total-gene ratio is about the same (3.5–4.5%) between archaea and bacteria, as is their organization of genes into operons (Tenori-Salgado et al., 2011). Archaeal transcription factors (TFs) also make up a smaller proportion of the genome, and are also shorter, 43.5% of them between only 100 and 200 amino acids long.

Materials and Methods

A list of 412,531 archaea genes from 13,444 archaeal cluster of orthologous genes (arCOGs) belonging to 168 archaea species in 77 genera was downloaded from the NCBI COG database (ftp://ftp.ncbi.nih.gov/pub/wolf/COGs/arCOG/ar14.arCOG.csv). A cluster of orthologous genes, or a COG, is a specified gene with a copy in at least three lineages, which are paralogous or homologous to each other (thus an arCOG is an archaeal COG). Data for comparison with bacteria was taken from ftp://ftp.ncbi.nih.gov/pub/wolf/COGs/Prok1402/Prok1402.tar.gz. This data set was chosen because it involved refined algorithms for orthology identification and was combined with manual curation. The arCOG annotations were based on comparisons with pfam, Conserved Domain Database, tigrfams, and comparisons with COGS database (Makarova, Wolf, and Koonin, 2015).

An R script (JaccardClusters.R) was written that calculates the Jaccard coefficient value (JCV) for each of the 14,028 possible species pairs and depicts the JCV heat map for all species. It also creates a .noa and a .sif file for visualization in Cytoscape. The R script is available at https://github.com/jeanomicks/JCV. The JCV is calculated in the following way: $JCV = |A \cap B|/(|A|+|B|-|A \cap B|)$; that is, the intersection of common genes divided by the union of all genes for species A and B, where $0 \leq JCV \leq 1$. R version 3.1.3 was used. In the JCV heat map, lighter colors mean higher JCVs, closer to 1.0, whereas darker colors correspond to lower JCVs, closer to 0.0.

The genomes of the three ancient halophile species were downloaded from NCBI (*Halobacterium hubeiense*: NZ_LN831302.1; *Halococcus salifodinae*: AOME00000000.1; *Halosimplex carlsbadense*: AOIU00000000.1). CLC Genomics version 8.0 was used to find open reading frames (ORFs) in the genomes of these species. The ORFs found in these genomes were BLASTED (a sequence comparison algorithm) against the 168 archaeal proteomes using blastx without gap extensions (all six translated frames of a DNA sequence compared to a protein sequence) to find matching homologs for JCV calculation. An e-score cutoff of 1^{-10} was used.

Results

Principle of Investigation

Until now, baraminology studies have avoided using systematic data derived from DNA sequence comparisons,

because such comparisons are done on genes that are similar between species. Thus, if gene sequences were used in these studies, they would be biased toward similarity between species, which may not show up in the phenotype (Wood, 2002). Nevertheless, some baraminology studies have been carried out using molecular data. Wood (2013) compared alignments of a certain region of the mitochondrial DNA within species of the cat, dog, and horse kinds compared to outliers pertinent to these three kinds. Wood found that based on the number of transversions to transitions, species from these three kinds could be separated from their outliers.

When analyzing microbial baramins, we have to take into account that they lack macromorphological characteristics, such as length of limbs or cranial capacity. Thus, we are forced to take genetic characteristics, such as gene content, into consideration. On the molecular and cellular level, genes code for proteins, which are responsible for different functions in the cell, such as structural proteins, enzymes, or transcription factors. Thus, a haploid single-celled organism's cellular phenotype is directly determined by its gene content.

Whereas the baraminic distance correlation (BDC) method measures the percentage of characters in which the two species differ in their character states (Wood, 2002), the JCV measures the percentage of common genes to all genes in both species. BDC measures distance and dissimilarity, whereas the JCV measures similarity. Since this method holistically takes the whole-gene content of a species into account, this should alleviate objections by previous workers as to the usefulness of genetic data in baraminology studies. As opposed to BDC, which captures the state of a given character, JCV captures binary information about whether a certain gene is absent or present in a given species. However, compared to BDC, the JCV does not suffer from information loss by losing characters between pairs of species. With the JCV, a higher number of orthologous genes between two single-species signifies continuity, whereas a smaller number of common genes corresponds to discontinuity. Species within a holobaramin would have high JCVs when compared to one another but low JCVs when compared to members of another holobaramin.

The JCV method can be used to detect discontinuity via additive evidence. If we start out with a small number of seed species that all belong to the same holobaramin, we can calculate the average JCV between all members of the holobaramin. Here we would expect this value to be relatively high. Afterwards, we could keep adding newer and newer members of the holobaramin and expect the average JCVs between all species pairs to remain relatively high. This would remain so until an outlier species is added, which statistically would have a significantly lower average JCV compared to the members of the existing holobaramin. This could be determined by using the Student's t-test. For example, the average JCV between seven nitrous archaea is 0.69, whereas the average JCV between these species drops to a value of 0.18 (p-value = 6.1x10-17) when the outlier species *Nanoarchaeum equitans* is added..

Archaeal Holobaramins

It is not clear from the Bible on which day microbes, such as archaea, were created. Neither do we know to what extent they exhibited genetic continuity when they were created. The latter is an important factor, as horizontal gene transfer (HGT) is widespread among archaea.

We can use data from NCBI's COG database delineating in which species which genes correspond to which orthologous group (archaeal COG, or arCOG). In archaea, Makarova, Wolf, and Koonin (2015) carried out a comparison listing which gene corresponds to which arCOG in 168 species. A list of these species and the number of proteins per species is given in Supplemental Table 1.

JCVs were calculated for all possible species pairs of the 168 archaea species that had information in the arCOG data set. These values were put into a matrix and then visualized in a heat map, which can be seen in Figure 1. Lighter colors correspond to higher JCVs close to 1, whereas darker colors correspond to lower JCVs, closer to 0. As we can see, a number of archaeal groups are visible that have high JCVs among their individual species members. In Figure 1 there are eight of these groups that have at least seven members. These groups of species that have high common gene content can be inferred to correspond to created archaeal holobaramins. As we can see, compared to all other species, these archaeal holobaramins have low JCVs, which are denoted with darker squares in Figure 1. Since this is the first gene-based baraminology study of its kind, and the first one to study Archaea, the holobaramins identified here should be considered tentative.

These archaeal holobaramins are listed in Table 1 along with their mean JCV±standard deviation and the number of member species within them. What is also interesting is that these archaeal holobaramins are comprised of species that generally belong to the same ecological niche (sulfur reducing, salt-rich environment, extreme heat, or methanogens). This could be due to the core genes belonging to most or all of these species enabling them to survive in extreme conditions, or code for enzymes that are capable of utilizing alternative energy sources.

Thermoacidophiles: *Sulfolobales*

The first predicted archaeal baramin is that of the genus *Sulfolobus*, along with two *Metallosphaera* species and one *Acidianus* species. *Sulfolobales* is an order of the Crenarcheota, which

live in extreme thermal and acidic environments and lack a cell envelope. According to Gao and Gupta (2007), 264 proteins were identified that are characteristic only of *Sulfolobales*, suggesting that these genes may be considered markers of this holobaramin.

Halophiles

Halophilic archaea are species that require 5–10 times the salinity of seawater. This holobaramin contains 27 species in 18 genera, meaning that these species are quite diverse. Characteristic of these species is a high GC content in their genomes, the presence of chloride pumps, and also the capability to use solar energy to synthesize ATP. Gao and Gupta (2007) found 127 proteins that are characteristic of almost all the species in this holobaramin.

Methanogens 1 and 2

Methanogens have been divided into two main groups according to the secular literature: Class I includes the orders *Methanobacteriales*, *Methanococcales*, and *Methanopyrales*; Class II consists of the orders *Methanomicrobiales* and *Methanosarcinales* (Bapteste, Brochier, and Boucher, 2005). These microbes are capable of producing methane from simple carbon compounds such as CO_2, formate, or acetate (Thauer et al., 2008). Anderson et al. (2009) state that methanogenic archaea can be divided into three groups, but this is only based on phylogenetic trees based on seven core proteins found in all methanogens. Phylogenetic methods for the most part give contradictory trees and thus do not present as holistic a picture of species relationships as the present method does. The present analysis shows two clusters of methanogenic archaea, covering the same groups as those outlined by Bapteste, Brochier, and Boucher. According to Gao and Gupta (2007), 31 proteins are exclusively characteristic of methanogenic archaea and have functions in the production of methane. Of these, 11 have been selectively lost from the methanogenic baranome in *Methanospharea stadtmanae*.

Nitrous archaea

Seven species from four genera (*Nitrosoarchaeum*, *Nitrosopumilus*, *Nitrososphaera*, and *Cenarcheaum*) grouped together based on their ability to oxidize ammonia to nitrite (Hallam et al., 2006; Bartossek et al., 2010). These species had an average JCV of 0.69, which is relatively high and had 844 genes in common. Each of these species also had two subunits of the nitrite reductase NirD in common (arCOG02852 and arCOG02854).

Thermophiles 1: *Thermoproteales*

The first group of thermophiles consists of twelve species from the genera *Pyrobaculum*, *Thermoproteus*, *Vulcanisaeta*, and *Caldivirga*. These are species that belong to the order *Thermoproteales*. These species share different combinations of introns at twelve specific loci (374, 548, 722, 781, 901, 907, 908, 919, 1093, 1205, 1213, and 1391) within the 16S rRNA gene, with *Pyrobaculum* and *Thermoproteus* sharing the most of them. What is surprising, however, is that these introns also occur at most of the same positions in *Desulfurococcales* (Jay and Inskeep, 2015). It might be suggested, therefore, that *Thermoproteales* and *Desulfococcales* should be classified as a single holobaramin. However, this is the only gene out of hundreds of genes that are common to both of these two groups (the median JCV between these two holobaramins is only 0.26). Species from the order *Thermoproteales* lack an intron at loci 802 that is present in *Desulfurococcales*, which would reflect differential intron loss if indeed the two groups in fact had a common ancestor—this as opposed to the evolutionary idea that this gene was newly gained. However, multiple copies of the 16S rRNA gene can be found in different groups of bacteria and archaea, such as *Aigarcheota* (Roux et al., 2011; Jay and Inskeep, 2015); therefore, it is possible that the intron-carrying variant was transferred between these two holobaramins via HGT, meaning no common ancestor.

Thermophiles 2: *Desulfurococacceae*

Seven species from four genera (*Desulfurococcus*, *Staphylothermus*, *Thermosphaera*, and *Thermogladius*) make up a second group of thermophiles that belong to the family *Desulfurococacceae*. These species are anaerobic hyperthermophiles, which reduce organic sulfur to hydrogen sulfide (Kochetkova et al., 2016). Several archaeal potential holobaramins metabolize sulfur; however, this can be done seemingly in different manners between holobaramins, which is reflected in the difference in their gene content. Such species are *Hyperthermus butylicus*, and *Thermofilum pendens*, which also appear on the heat map, but it is not clear which holobaramin they belong to. These species differ in the way they process nutrients. This is reflected in their differing sets of transporters and other enzymes; for example, ABC transporters, glycosidases, sulfur reductases and other oxireductases (Anderson et al., 2009). The differences are so large that the JCV between *S. marinus* and *H. butylicus* and between *S. marinus* and *T. pendens* are only 0.31 and 0.24, respectively, while the mean JCV for this holobaramin is 0.69. These two JCVs fall outside of the range of intrabaraminic JCVs (see Tables 1 and 2).

Thermophiles 3: *Thermococcus* and *Pyrococcus*

The last large holobaramin is one that consists of nine *Thermococcus* and seven *Pyrococcus* species. These belong to the family *Thermococcaceae*, which is the sole family of the class Thermococci. These species differ in their temperature range, growth rate, and toxicity tolerance; they all transform sulfur into hydrogen sulfide. Both of these genera form monophyletic groups and form

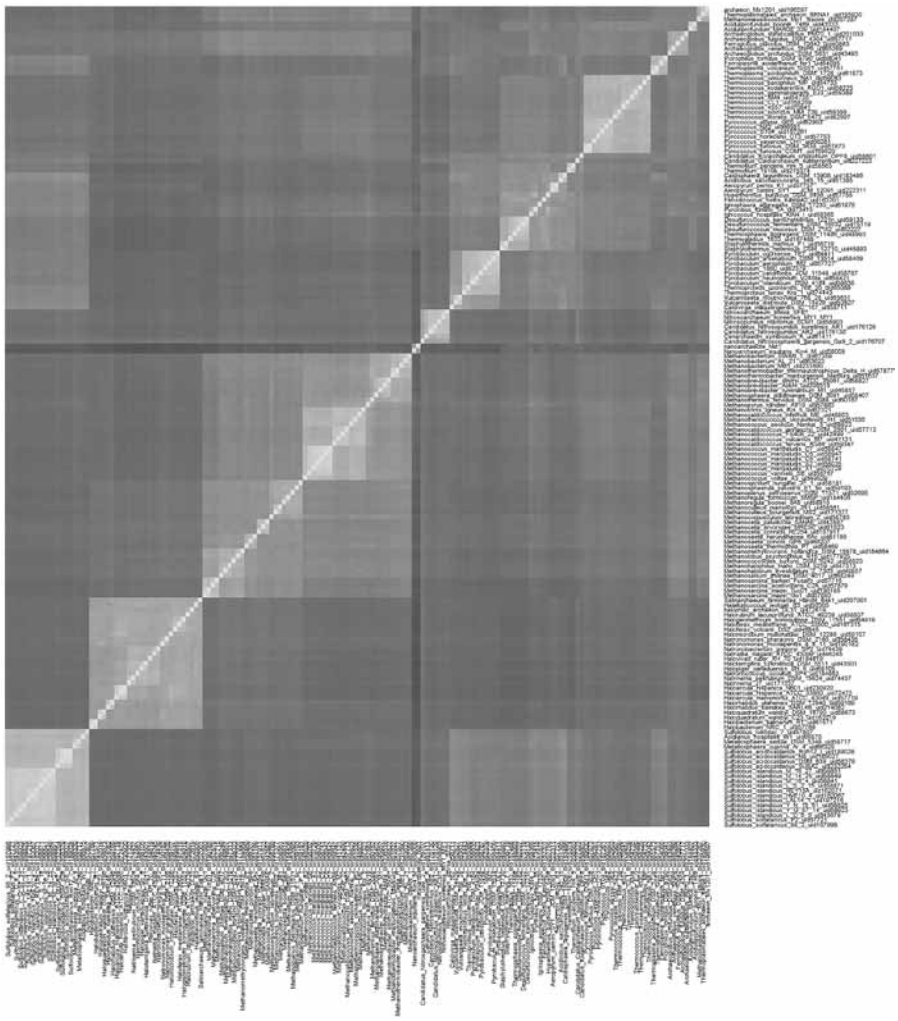

Figure 1. Heat map of Jaccard Coefficient Values for all pairs of 168 archaeal species with COG data from NCBI. Lighter colors correspond to higher JCVs, and darker ones correspond to lower values. Groups of archaeal species can be seen clustered together. Eight clusters of archaea with at least seven members were chosen for further analysis, as described in the text.

different clades based on differences in the DGGE fragment of their 16S rRNA gene (Teske et al., 2009). Interestingly, here the genus *Pyrococcus* intermingles between two different clades of *Thermococcus* species. According to Gao and Gupta (2007), 141 proteins are common to the four *Pyrococcus* species. However, according to Cohen et al. (2003), much HGT has occurred between these species and those of the genus *Thermococcus*, which warrants putting them in the same holobaramin..

Other groups

While the previous eight putative holobaramins have been delineated, there are other archaeal species in this analysis that merit further notice. For example, there are two nanoarchaeal species (*Nanoarchaeum equitans* and nanoarchaeote Nst1) that differ very much from all the other 168 species in this analysis. The JCV between them is 0.51. The median JCV between these two species and all other archaea in this study is 0.16, which is even lower than the median JCV between holobaramins in general (0.28). *N. equitans*, an obligate symbiote hyperthermophile, has the smallest cellular genome (490 Mbp) and is lacking one-third of all the genes in other archaeal species, yet about 15% of its genes are unique (Waters et al., 2003; Gribaldo and Brochier-Armanet, 2006) and has a volume 1/100th that of *E. coli*.

Interbaraminic and Intrabaraminic JCV Comparisons

Each of these eight archaeal holobaramins is made up of several genera, which would correspond to different monobaramins within these holobaramins. The mean and median JCV (±SD) was calculated for four taxonomical categories: (A) species belonging to the same genus, (B) species belonging to the same holobaramin, (C) species belonging to the different holobaramins, and (D) Archaea and Bacteria. Species from five bacterial genera were used as an outgroup in comparison D to see if we get the same kind of JCVs as between archaeal holobaramins. These bacterial species came from those genera that had the most species and had annotated COGs in the COG database.

These JCVs were visualized in the boxplot depicted in Figure 2. This was done in order to better characterize distance relationships within holobaramins and between holobaramins. What we can see in Figure 2 is that the range of JCVs is about the same for genera and within holobaramins (categories A and B). However, there is a large drop in the median JCV when comparing species from the same holobaramin (category B) to different holobaramins (category C) (0.64 to 0.26). This is a clear signal of discontinuity between archaeal holobaramins as characterized

Main baraminic property	Number of species	Number of genera	Species to genus ratio	Mean JCV±stdev	Number of core genes
Thermoacidophiles	20	3	6.67	0.74±0.08	1071
Halophiles	27	18	1.5	0.57±0.06	799
Methanogens	50	25	2	0.45±0.12	420
Methanogens 1	26	10	2.6	0.56±0.12	670
Methanogens 2	24	15	1.6	0.49±0.09	33
Nitrous archaea	7	4	1.75	0.69±0.1	844
Thermophiles 1	12	4	3	0.63±0.13	821
Thermophiles 2	7	4	1.75	0.69±0.09	782
Thermophiles 3	16	2	8	0.68±0.05	865

Table 1. List of archaeal holobaramins and the number of member species and genera predicted by JCV analysis.

by their common gene content. The median JCV (0.18) between archaeal species and bacterial species (category D) is comparable to that of category C, though slightly lower. This illustrates additive evidence when adding species to a given holobaramin in that the JCVs between members of different holobaramins should be statistically significantly lower than JCVs between members of an individual holobaramin.

Genomic Comparisons of Ancient Halophile Species to Other Species

Jaakkola et al. (2016a) have sequenced the whole genome sequences of three extreme halophilic archaeal species, *Halobacterium hubeiense* (Jaakkola et al., 2016b), *Halococcus salifodinae*, and *Halosimplex carlsbadense*. Characteristics of these species are listed in Table 3. These three species were discovered in evaporate basins and are capable of existing in extremely high ion concentrations and anoxic conditions. These are also allegedly the oldest known organisms to live on Earth. Therefore, it would be extremely interesting to measure their common gene content with that of other archaeal species. These species could more closely represent the archebaramin of the halophilic archaeal holobaramins.

The whole genome sequence for these three species was available either as contigs or a full genome. If contigs, they were first assembled into whole-genome sequences. ORFs of at least 300 bp were determined using CLC Genomics software version 8.0. The six-frame translation products (frames 1, 2, and 3 in the forward and reverse direction of the DNA) of these ORFs were matched (blastx) against protein sequences for the examined 168 archaeal species. JCVs were then calculated between each of the three ancient halophiles and all of the 168 archaeal species. These JCVs are depicted in Figure 3 for all three species compared to the 168 archaeal species that have data from the COG database. The JCVs for all three species comparisons are available in Supplemental Table 2.

What we can see in Figure 3 for all three archaic species is that their JCV distribution resembles a hockey stick graph in that a smaller group of these archaeal species have a larger than average JCV compared to the rest of the species. This smaller group of species is made up of extreme halophiles from the second discovered holobaramin (all

	Within genus	Within baramin	Between baramins	Between archaea and bacteria
Mean	0.75±0.08	0.64±0.13	0.28±0.06	0.18±0.06
Median	0.74	0.64	0.26	0.18
Range	0.53–0.98	0.37–0.98	0.11–0.56	0.01–0.37

Table 2. Statistical characteristics of JCVs according to three taxonomic categories with three separate methanogen holobaramins.

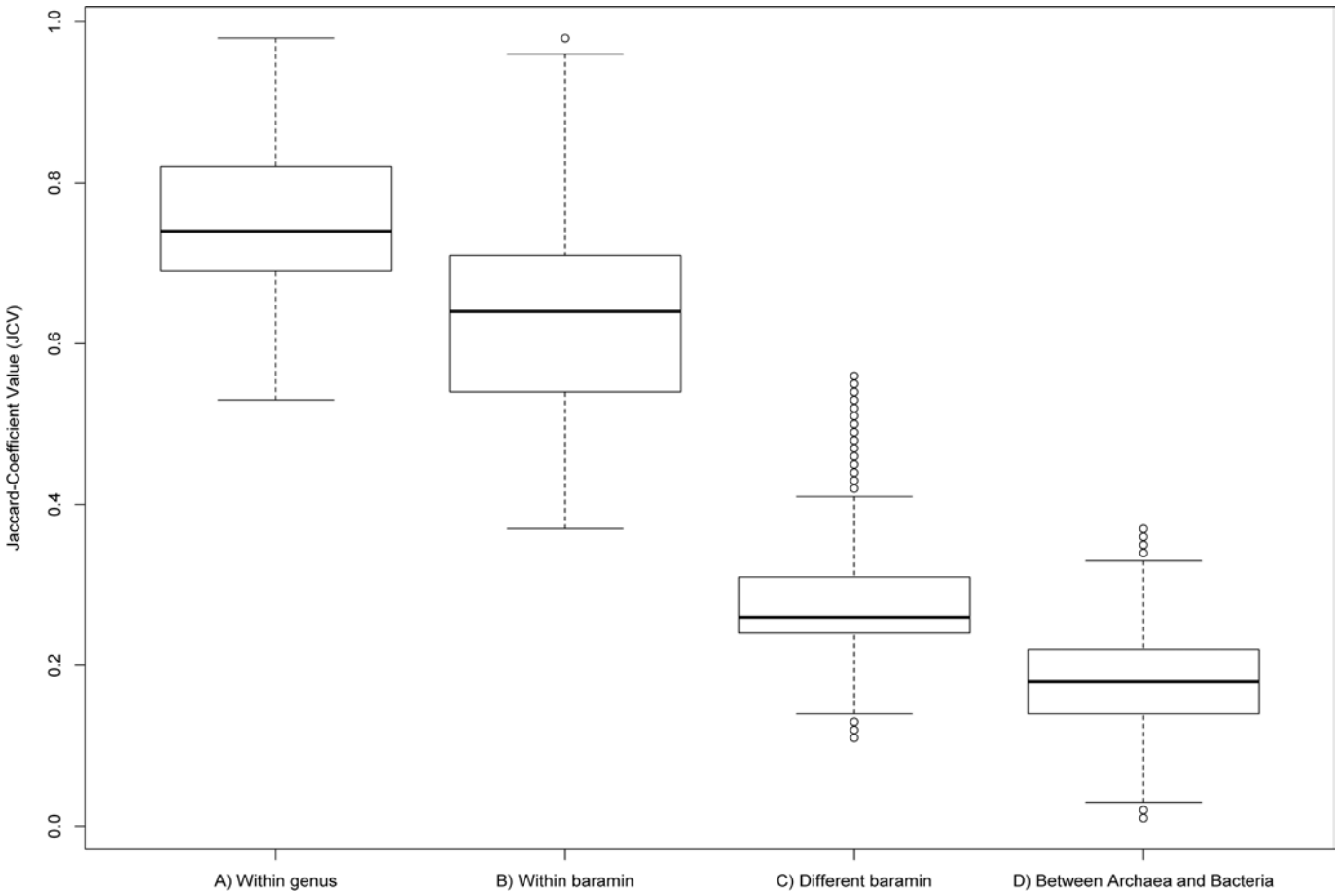

Figure 2. Boxplot diagram of JCVs for four different taxonomic categories: (A) within archaeal genera, (B) within the eight archaeal holobaramins studied in detail, (C) between different archaeal holobaramins, and (D) between archaea and five bacterial genera with the most species with data from the COG database. The four categories depict the range, the 50% percentile, and the median for the JCVs.

27 species). The mean and median JCV, as well as their value range, can be seen in Table 3 for all three archaic species. Their intrabaraminic and interbaraminic JCVs also overlap with those calculated in Table 2. The JCVs that correspond to halophiles differ very significantly from the rest of the 168 species (p-values: *H. hubeiense*: 2.4e-99; *H. salifodinae*: 1.9e-126; *H. carlsbadense*: 9e-115) and is another illustration of additive evidence of adding species to an existing holobaramin until a statistically significant difference in gene content is encountered.

Discussion

Evolutionary explanations of the origin of Archaea are fraught with difficulties. Archaea were assigned to a third domain of life beside Bacteria and Eukarya based on protein trees based on universal small subunit ribosomal RNA (SSU rRNA) (Woese, Kandler, and Wheelis, 1990). However, Gribaldo and Brochier-Armanet (2006) bemoan that it is possible that there will be a demoralizing lack of resolution in the evolutionary history of Archaea based on molecular data similar to bacterial and eukaryotic phylogenies. What is interesting is that Archaea seemingly use eukaryotic proteins but in a bacteria-like context.

Two views of the origin of Archaea exist, and they are contradictory. According to the first view, Archaea are derived from Bacteria. However, this does not explain how and why the bacterial replication apparatus, an irreducibly complex information-integration system, was replaced by an unrelated archaeal one, or how the glycerol backbone of bacterial lipids in their plasma membrane was changed. This would

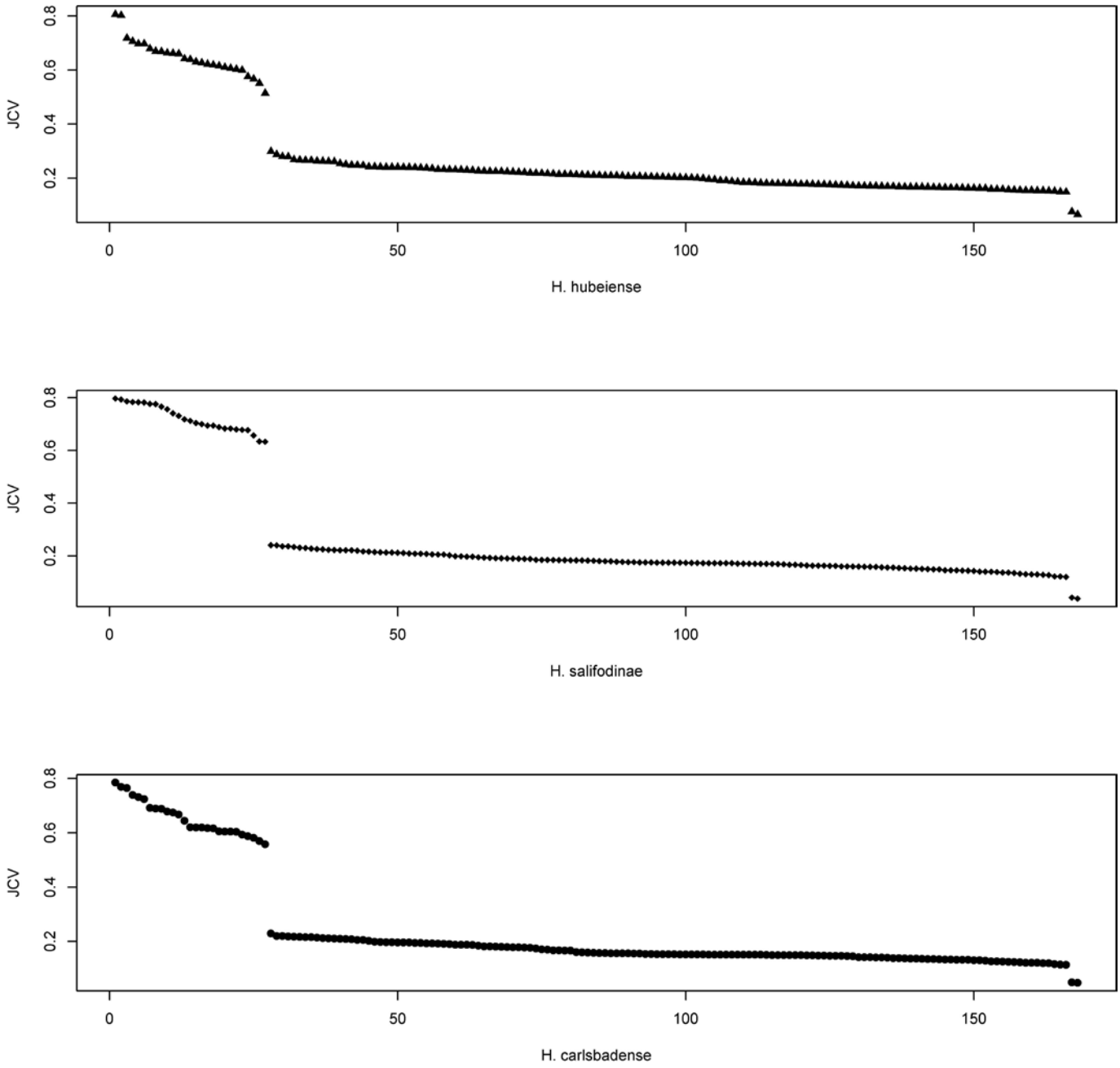

Figure 3. JCV distribution for three ancient halophile species, *Halobacterium hubeiense*, *Halococcus salifodinae*, *Halosimplex carlsbadense*, compared to the 168 archaeal species with data from the COG database. For all three species, distinctly high JCVs were calculated between each ancient halophile species as well as the 27 halophiles from the COG database. P-values for differences in JCVs between the halophiles and all other archaea were statistically significant: *H. hubeiense*: 2.4e-99; *H. salifodinae:* 1.9e-126; *H. carlsbadense:* 9e-115.

require unobservable, highly accelerated molecular evolution leading from Bacteria to Archaea (Gupta, 1998). In this view, methanogenic Archaea must have suddenly acquired the complete set of enzymes for methanogenesis (Gribaldo and Brochier-Armanet, 2006). According to the second view, Archaea are more ancient than both Bacteria and Eukarya (their name, ἀρχαῖα meaning "ancient things"), of which Eukarya retained ancestral traits resembling those of Archaea

	Halobacterium hubeiense	*Halococcus salifodinae*	*Halosimplex carlsbadense*
GenBank id	NZ_LN831302.1	AOME00000000.1	AOIU00000000.1
Estimated evolutionary age	123 Mya	225–280 Mya	250 Mya
Genome size	2.51 Mbp	4.27 Mbp	4.77 Mbp
No. of ORFs	4,074	7,968	7,540
Mean intrabaramin JCV	0.65	0.72	0.65
Median intrabaramin JCV	0.64	0.71	0.62
Range	0.51–0.81	0.63–0.8	0.56–0.78
Mean interbaramin JCV	0.2	0.18	0.16
Median interbaramin JCV	0.2	0.17	0.15
Range	0.07–0.3	0.05–0.24	0.05–0.23

Table 3. Biological characteristics of the three ancient halophile species whose whole genome sequence was studied in Jaakkola et al. (2016a).

(Gribaldo and Brochier-Armanet, 2006). This view fits better with evolutionary conceptions of the early stages of life on Earth, since Earth's putative early atmosphere reflected the metabolism of Archaea such as methanogens. There is no sound explanation as to why one group of organisms would retain ancestral genes yet another group would undergo major genetic derivation.

In a common ancestry scenario, the ancestral single cell could have been a combination of all three cell types, but this would entail early complexity followed by simplification across the board. Otherwise, the ancestral cell could have been extremely simplistic with few specific features of the three cell types, which would not be biologically feasible either, in order to evolve and replace underlying genetic structures as we observe in the three basic cell types. Alternatively, according to Woese (1998), early life existed not as a single cell but rather as a set of diverse cell types, but this still leaves the sudden origin of diversity unexplained.

It is precisely because of these difficulties in evolutionary models of Archaea that the creation model is more consistent with the data. Treating Archaea, Bacteria, and Eukarya as separately created, discontinuous holobaramins and calculating species similarities based on whole-gene content makes much more sense in light of the data than the idea that they all have a common evolutionary origin.

In this paper the tentative boundaries of eight putative archaeal baramins were on the level of order and even class. For the most part, especially in eukaryotic organisms, the boundary line is usually on the level of genus, family, or order. This might be reflective of the ever-changing status of microorganismal taxonomy. For example, the taxon Nanoarcheota was proposed as a new kingdom by Huber et al. (2002), but by now is reclassified as a member of the phylum Euryarchaeota. We will have to wait until further archaeal species are classified and described in more detail.

With more species to examine, current clusters of archaeal species might become more finely tuned. What we can see clearly is that archaea tend to form groups based on the specific ecological niche that they inhabit. In the case of specific archaeal groups that inhabit niches under extreme conditions (excessive heat, anaerobic atmosphere), this means that different types of basic genetic apparatuses would be necessary to process different basic metabolites.

Studying the three ancient halophiles, we can see that these three species are very similar to other halophilic archaea. The average JCV within the extreme halophile baramin is 0.57, yet the average JCV between these three species and the 27 members of the halophile baramin are all at least 0.65. Even if they do not represent members of the halophilic archebaramin, these species show that not too many genes have changed during their existence here on Earth. These three archaic archaeal halophile species can be viewed as microbial living fossils.

This study is the first attempt to use genomic data to determine continuity and discontinuities among putative microbial holobaramins. Therefore, further analyses should be done to corroborate

the conclusions of this paper. The JCV method could be used to complement existing baraminology studies—for example, those for which holobaraminic status has been proposed based on the BDC. If the results agree, then this novel technique has been corroborated, but if not, then either the JCV method should be refined, or problems should be found with the BDC. The JCV method could also be applied on bacteria or eukaryotes to see if we get meaningful results.

With this analysis we can be hopeful that with more data and more species examined, we will be able to study archaeal species in more depth in order to help classify them into possible holobaramins. This would help microbial baraminology, an area of creation science that has not yet been developed much recently.

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Supplemental Table 1.

Species	Number of Proteins
Acidianus hospitalis W1	2329
Acidilobus saccharovorans 345-15	1499
Aciduliprofundum boonei T469	1544
Aciduliprofundum sp. MAR08-339	1525
Aeropyrum camini SY1 = JCM 12091	1645
Aeropyrum pernix K1	1700
Archaeoglobus fulgidus DSM 4304	2420
Archaeoglobus profundus DSM 5631	1823
Archaeoglobus sulfaticallidus PM70-1	2216
Archaeoglobus veneficus SNP6	2090
Caldisphaera lagunensis DSM 15908	1478
Caldivirga maquilingensis IC-167	1963
Candidatus Caldiarchaeum subterraneum	1730
Candidatus Korarchaeum cryptofilum OPF8	1603
Candidatus Methanomassiliicoccus intestinalis Issoire-Mx1	1820
Candidatus Methanomethylophilus alvus Mx1201	1651
Candidatus Nitrosoarchaeum koreensis MY1	1945
Candidatus Nitrosoarchaeum limnia SFB1	2038
Candidatus Nitrosopumilus koreensis AR1	1890
Candidatus Nitrosopumilus sp. AR2	1974
Candidatus Nitrososphaera gargensis Ga9.2	3565
Cenarchaeum symbiosum A	2014
Desulfurococcus fermentans DSM 16532	1421
Desulfurococcus kamchatkensis 1221n	1471
Desulfurococcus mucosus DSM 2162	1345

Species	Number of Proteins
Ferroglobus placidus DSM 10642	2480
Ferroplasma acidarmanus fer1	1951
Fervidicoccus fontis Kam940	1385
Halalkalicoccus jeotgali B3	3873
Haloarcula hispanica ATCC 33960	3859
Haloarcula hispanica N601	3918
Haloarcula marismortui ATCC 43049	4243
Halobacterium salinarum R1	2749
Halobacterium sp. NRC-1	2622
Haloferax mediterranei ATCC 33500	3863
Haloferax volcanii DS2	4015
Halogeometricum borinquense DSM 11551	3898
Halomicrobium mukohataei DSM 12286	3349
Halopiger xanaduensis SH-6	4221
Haloquadratum walsbyi	1
Haloquadratum walsbyi C23	2652
Haloquadratum walsbyi DSM 16790	2643
Halorhabdus tiamatea SARL4B	3023
Halorhabdus utahensis DSM 12940	2998
Halorubrum lacusprofundi ATCC 49239	3560
Haloterrigena turkmenica DSM 5511	5113
Halovivax ruber XH-70	3099
Hyperthermus butylicus DSM 5456	1603
Ignicoccus hospitalis KIN4/I	1434
Ignisphaera aggregans DSM 17230	1930
Metallosphaera cuprina Ar-4	2029
Metallosphaera sedula DSM 5348	2256

Supplemental Table 1 (continued).

Species	Number of Proteins
Methanobacterium sp. AL-21	2493
Methanobacterium sp. MB1	2021
Methanobacterium sp. SWAN-1	2397
Methanobrevibacter ruminantium M1	2217
Methanobrevibacter smithii ATCC 35061	1793
Methanobrevibacter sp. AbM4	1671
Methanocaldococcus fervens AG86	1581
Methanocaldococcus infernus ME	2
Methanocaldococcus jannaschii DSM 2661	1771
Methanocaldococcus sp. FS406-22	1816
Methanocaldococcus vulcanius M7	1742
Methanocella arvoryzae MRE50	3089
Methanocella conradii HZ254	2455
Methanocella paludicola SANAE	3004
Methanococcoides burtonii DSM 6242	2273
Methanococcus aeolicus Nankai-3	1490
Methanococcus maripaludis C5	1822
Methanococcus maripaludis C6	1826
Methanococcus maripaludis C7	1788
Methanococcus maripaludis S2	1722
Methanococcus maripaludis X1	1848
Methanococcus vannielii SB	1678
Methanococcus voltae A3	1717
Methanocorpusculum labreanum Z	1741
Methanoculleus bourgensis MS2	2618
Methanoculleus marisnigri JR1	2490
Methanohalobium evestigatum Z-7303	2254
Methanohalophilus mahii DSM 5219	1987
Methanolobus psychrophilus R15	3167
Methanomethylovorans hollandica DSM 15978	2556
Methanoplanus petrolearius DSM 11571	2785
Methanopyrus kandleri AV19	1687
Methanoregula boonei 6A8	2452
Methanoregula formicica SMSP	2816
Methanosaeta concilii GP6	2850
Methanosaeta harundinacea 6Ac	2371
Methanosaeta thermophila PT	1696
Methanosalsum zhilinae DSM 4017	1976

Species	Number of Proteins
Methanosarcina acetivorans C2A	4540
Methanosarcina barkeri str. Fusaro	3625
Methanosarcina mazei Go1	3368
Methanosarcina mazei Tuc01	3252
Methanosphaera stadtmanae DSM 3091	1535
Methanosphaerula palustris E1-9c	2655
Methanospirillum hungatei JF-1	3131
Methanothermobacter marburgensis str. Marburg	1757
Methanothermobacter thermautotrophicus str. Delta H	1873
Methanothermococcus okinawensis IH1	1595
Methanothermus fervidus DSM 2088	1283
Methanotorris igneus Kol 5	1772
Nanoarchaeum equitans Kin4-M	540
Natrialba magadii ATCC 43099	4212
Natrinema pellirubrum DSM 15624	4199
Natrinema sp. J7-2	4302
Natronobacterium gregoryi SP2	3656
Natronococcus occultus SP4	4154
Natronomonas moolapensis 8.8.11	2749
Natronomonas pharaonis DSM 2160	2820
Nitrosopumilus maritimus SCM1	1796
Picrophilus torridus DSM 9790	1537
Pyrobaculum aerophilum str. IM2	2602
Pyrobaculum arsenaticum DSM 13514	2299
Pyrobaculum calidifontis JCM 11548	2149
Pyrobaculum islandicum DSM 4184	1978
Pyrobaculum neutrophilum V24Sta	1966
Pyrobaculum oguniense TE7	2835
Pyrobaculum sp. 1860	2824
Pyrococcus abyssi GE5	1783
Pyrococcus furiosus COM1	2064
Pyrococcus furiosus DSM 3638	2122
Pyrococcus horikoshii OT3	1950
Pyrococcus sp. NA2	1979
Pyrococcus sp. ST04	1748
Pyrococcus yayanosii CH1	1865

Supplemental Table 1 (continued).

Species	Number of Proteins
Pyrolobus fumarii 1A	1967
Salinarchaeum sp. Harcht-Bsk1	3013
Staphylothermus hellenicus DSM 12710	1599
Staphylothermus marinus F1	1573
Sulfolobus acidocaldarius DSM 639	2224
Sulfolobus acidocaldarius N8	2275
Sulfolobus acidocaldarius Ron12/I	2317
Sulfolobus acidocaldarius SUSAZ	2146
Sulfolobus islandicus HVE10/4	2720
Sulfolobus islandicus L.D.8.5	2948
Sulfolobus islandicus L.S.2.15	2737
Sulfolobus islandicus LAL14/1	2601
Sulfolobus islandicus M.14.25	2608
Sulfolobus islandicus M.16.27	2657
Sulfolobus islandicus M.16.4	2735
Sulfolobus islandicus REY15A	2644
Sulfolobus islandicus Y.G.57.14	2902
Sulfolobus islandicus Y.N.15.51	2900
Sulfolobus solfataricus 98/2	2679
Sulfolobus solfataricus P2	2978
Sulfolobus tokodaii str. 7	2826
Thermococcus barophilus MP	2265

Species	Number of Proteins
Thermococcus gammatolerans EJ3	2156
Thermococcus kodakarensis KOD1	2306
Thermococcus litoralis DSM 5473	2516
Thermococcus onnurineus NA1	1975
Thermococcus sibiricus MM 739	2035
Thermococcus sp. 4557	2133
Thermococcus sp. AM4	2222
Thermococcus sp. CL1	2017
Thermofilum pendens Hrk 5	1878
Thermofilum sp. 1910b	1896
Thermogladius cellulolyticus 1633	1413
Thermoplasma acidophilum DSM 1728	1484
Thermoplasma volcanium GSS1	1501
Thermoplasmatales archaeon BRNA1	1523
Thermoproteus tenax Kra 1	2049
Thermoproteus uzoniensis 768-20	2186
Thermosphaera aggregans DSM 11486	1387
Vulcanisaeta distributa DSM 14429	2493
Vulcanisaeta moutnovskia 768-28	2320
halophilic archaeon DL31	3476
methanocaldococcus infernus ME	1439
nanoarchaeote Nst1	647

Supplemental Table 2.

Species	JCV
Halobacterium salinarum R1	0.805982
Halobacterium sp. NRC-1	0.801938
Halorubrum lacusprofundi ATCC 49239	0.717917
Halogeometricum borinquense DSM 11551	0.706177
Halomicrobium mukohataei DSM 12286	0.697199
Haloferax volcanii DS2	0.697074
Haloarcula hispanica ATCC 33960	0.678588
Haloferax mediterranei ATCC 33500	0.669529
Haloarcula hispanica N601	0.667998
Natronomonas pharaonis DSM 2160	0.662895

Species	JCV
Natronomonas moolapensis 8.8.11	0.661668
Haloarcula marismortui ATCC 43049	0.659813
Salinarchaeum sp. Harcht-Bsk1	0.641794
Halopiger xanaduensis SH-6	0.639166
Natrinema pellirubrum DSM 15624	0.630244
halophilic archaeon DL31	0.626956
Halovivax ruber XH-70	0.62168
Halalkalicoccus jeotgali B3	0.618537
Natronobacterium gregoryi SP2	0.615274
Natrinema sp. J7-2	0.61016

Supplemental Table 2 (continued).

Species	JCV
Halorhabdus utahensis DSM 12940	0.606383
Natronococcus occultus SP4	0.602737
Halorhabdus tiamatea SARL4B	0.599638
Haloquadratum walsbyi DSM 16790	0.575917
Natrialba magadii ATCC 43099	0.567238
Haloquadratum walsbyi C23	0.551104
Haloterrigena turkmenica DSM 5511	0.514257
Methanosalsum zhilinae DSM 4017	0.29912
Methanohalophilus mahii DSM 5219	0.28793
Methanohalobium evestigatum Z-7303	0.280453
Archaeoglobus veneficus SNP6	0.279635
Methanocella conradii HZ254	0.268999
Methanoculleus marisnigri JR1	0.267426
Ferroglobus placidus DSM 10642	0.266229
Methanomethylovorans hollandica DSM 15978	0.265848
Archaeoglobus fulgidus DSM 4304	0.263587
Archaeoglobus sulfaticallidus PM70-1	0.263135
Methanococcoides burtonii DSM 6242	0.262196
Methanocella paludicola SANAE	0.261176
Methanoculleus bourgensis MS2	0.254358
Methanosarcina mazei Go1	0.25097
Methanolobus psychrophilus R15	0.248545
Methanocella arvoryzae MRE50	0.248017
Methanosarcina barkeri str. Fusaro	0.24725
Methanosaeta harundinacea 6Ac	0.242733
Methanosaeta thermophila PT	0.242659
Methanosphaerula palustris E1-9c	0.241725
Methanoplanus petrolearius DSM 11571	0.240747
Methanoregula boonei 6A8	0.24055
Methanosarcina mazei Tuc01	0.24054
Archaeoglobus profundus DSM 5631	0.240346
Methanoregula formicica SMSP	0.239977
Pyrococcus sp. ST04	0.239583
Methanosarcina acetivorans C2A	0.238499
Pyrococcus furiosus COM1	0.237579
Pyrococcus abyssi GE5	0.23574
Pyrococcus furiosus DSM 3638	0.232478
Pyrococcus sp. NA2	0.232234

Species	JCV
Methanocorpusculum labreanum Z	0.231562
Thermococcus barophilus MP	0.231113
Methanospirillum hungatei JF-1	0.230499
Methanosaeta concilii GP6	0.229698
Methanothermobacter marburgensis str. Marburg	0.228814
Thermococcus kodakarensis KOD1	0.226953
Thermococcus litoralis DSM 5473	0.226053
Thermococcus onnurineus NA1	0.225493
Methanobacterium sp. MB1	0.22506
Methanothermobacter thermautotrophicus str. Delta H	0.224931
Thermococcus gammatolerans EJ3	0.223997
Thermococcus sibiricus MM 739	0.223054
Thermococcus sp. 4557	0.22181
Thermococcus sp. CL1	0.221164
Pyrococcus yayanosii CH1	0.21956
Pyrococcus horikoshii OT3	0.218608
Thermococcus sp. AM4	0.21809
Methanobacterium sp. SWAN-1	0.216914
Methanobacterium sp. AL-21	0.215924
Aciduliprofundum sp. MAR08-339	0.214506
Methanotorris igneus Kol 5	0.21444
Methanocaldococcus fervens AG86	0.214375
Methanococcus maripaludis S2	0.21306
Methanococcus maripaludis C5	0.212729
Methanococcus maripaludis C7	0.211571
Aciduliprofundum boonei T469	0.210907
methanocaldococcus infernus ME	0.210429
Methanothermus fervidus DSM 2088	0.209474
Methanocaldococcus sp. FS406-22	0.20917
Methanocaldococcus vulcanius M7	0.209148
Methanococcus maripaludis X1	0.208453
Methanobrevibacter sp. AbM4	0.206629
Methanococcus aeolicus Nankai-3	0.206612
Methanococcus maripaludis C6	0.206601
Methanobrevibacter smithii ATCC 35061	0.205713
Candidatus Methanomassiliicoccus intestinalis Issoire-Mx1	0.205558

Supplemental Table 2 (continued).

Species	JCV
Methanococcus vannielii SB	0.204824
Methanocaldococcus jannaschii DSM 2661	0.204632
Methanothermococcus okinawensis IH1	0.203844
Candidatus Caldiarchaeum subterraneum	0.203291
Aeropyrum pernix K1	0.202427
Thermoplasma volcanium GSS1	0.202154
Thermoplasma acidophilum DSM 1728	0.201165
Methanobrevibacter ruminantium M1	0.200214
Aeropyrum camini SY1 = JCM 12091	0.199203
Picrophilus torridus DSM 9790	0.195476
Methanopyrus kandleri AV19	0.19493
Methanosphaera stadtmanae DSM 3091	0.191252
Ferroplasma acidarmanus fer1	0.191074
Methanococcus voltae A3	0.188363
Sulfolobus acidocaldarius SUSAZ	0.186037
Pyrobaculum calidifontis JCM 11548	0.184536
Vulcanisaeta distributa DSM 14429	0.1843
Metallosphaera sedula DSM 5348	0.183755
Sulfolobus acidocaldarius N8	0.182216
Sulfolobus acidocaldarius DSM 639	0.18097
Sulfolobus islandicus M.14.25	0.180849
Sulfolobus islandicus HVE10/4	0.18055
Sulfolobus acidocaldarius Ron12/I	0.180506
Sulfolobus islandicus LAL14/1	0.179206
Sulfolobus islandicus M.16.27	0.178934
Sulfolobus islandicus M.16.4	0.178181
Candidatus Methanomethylophilus alvus Mx1201	0.17802
Pyrobaculum arsenaticum DSM 13514	0.177958
Thermoproteus uzoniensis 768-20	0.176821
Sulfolobus solfataricus 98/2	0.176699
Sulfolobus islandicus REY15A	0.175787
Sulfolobus islandicus L.S.2.15	0.174969
Metallosphaera cuprina Ar-4	0.174417
Nitrosopumilus maritimus SCM1	0.17367
Caldivirga maquilingensis IC-167	0.172348
Thermoproteus tenax Kra 1	0.171506
Thermoplasmatales archaeon BRNA1	0.171491

Species	JCV
Pyrobaculum aerophilum str. IM2	0.171199
Candidatus Korarchaeum cryptofilum OPF8	0.170973
Sulfolobus solfataricus P2	0.169996
Sulfolobus islandicus Y.N.15.51	0.169672
Vulcanisaeta moutnovskia 768-28	0.169581
Sulfolobus islandicus L.D.8.5	0.168642
Sulfolobus islandicus Y.G.57.14	0.167831
Pyrobaculum sp. 1860	0.167609
Pyrobaculum oguniense TE7	0.167601
Pyrobaculum islandicum DSM 4184	0.167343
Candidatus Nitrosoarchaeum limnia SFB1	0.166667
Sulfolobus tokodaii str. 7	0.166217
Candidatus Nitrososphaera gargensis Ga9.2	0.165636
Pyrobaculum neutrophilum V24Sta	0.165042
Candidatus Nitrosoarchaeum koreensis MY1	0.164613
Pyrolobus fumarii 1A	0.164511
Acidianus hospitalis W1	0.164219
Candidatus Nitrosopumilus sp. AR2	0.163523
Cenarchaeum symbiosum A	0.163027
Caldisphaera lagunensis DSM 15908	0.162498
Candidatus Nitrosopumilus koreensis AR1	0.16228
Hyperthermus butylicus DSM 5456	0.159038
Acidilobus saccharovorans 345-15	0.158885
Thermofilum pendens Hrk 5	0.158879
Thermofilum sp. 1910b	0.157409
Staphylothermus marinus F1	0.156657
Desulfurococcus fermentans DSM 16532	0.155684
Staphylothermus hellenicus DSM 12710	0.154927
Ignicoccus hospitalis KIN4/I	0.154739
Ignisphaera aggregans DSM 17230	0.153766
Desulfurococcus mucosus DSM 2162	0.153701
Desulfurococcus kamchatkensis 1221n	0.152839
Thermogladius cellulolyticus 1633	0.152361
Thermosphaera aggregans DSM 11486	0.149747
Fervidicoccus fontis Kam940	0.149091
Nanoarchaeum equitans Kin4-M	0.076277
nanoarchaeote Nst1	0.065929