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ORIGINAL PAPER

A phylogenomic reappraisal of family-level divisions within the class *Halobacteria*: proposal to divide the order *Halobacteriales* into the families *Halobacteriaceae*, *Haloarculaceae* fam. nov., and *Halococcaceae* fam. nov., and the order *Haloferacales* into the families, *Haloferacaceae* and *Halorubraceae* fam nov.

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Abstract The evolutionary interrelationships between the archaeal organisms which comprise the class *Halobacteria* have proven difficult to elucidate using traditional phylogenetic tools. The class currently contains three orders. However, little is known about the family level relationships within these orders. In this work, we have completed a comprehensive comparative analysis of 129 sequenced genomes from members of the class *Halobacteria* in order to identify shared molecular characteristics, in the forms of conserved signature insertions/deletions (CSIs) and conserved signature proteins (CSPs), which can provide reliable evidence, independent of phylogenetic trees, that the species from the groups in which they are found are specifically related to each other due to common ancestry. Here we present 20 CSIs and 31 CSPs which are unique characteristics of infra-order level groups of genera within the class *Halobacteria*. We also present 40 CSIs and 234 CSPs which are characteristic of *Haloarcula*, *Halococcus*, *Haloferax*, or *Halorubrum*. Importantly, the CSIs and CSPs identified here provide evidence that the order *Haloferacales* contains two main groups, one consisting of *Haloferax* and related genera supported by four CSIs and five CSPs and the other consisting of *Halorubrum* and related genera supported by four CSPs. We have also identified molecular characteristics that suggest that the polyphyletic order *Halobacteriales* contains at least two large monophyletic clusters of organisms in addition to the polyphyletic members of the order, one cluster consisting of *Haloarcula* and related genera supported by ten CSIs and nineteen CSPs and the other group consisting of the members of the genus *Halococcus* supported by nine CSIs and 23 CSPs. We have also produced a highly robust phylogenetic tree based on the concatenated sequences of 766 proteins which provide additional support for the relationships identified by the CSIs and CSPs. On the basis of the phylogenetic analyses and the identified conserved molecular characteristics presented here, we propose a division of the order *Haloferacales* into two families, an emended family *Haloferacaceae* and *Halorubraceae* fam. nov. and a division of the order *Halobacteriales* into three families, an emended family *Halobacteriaceae*, *Haloarculaceae* fam. nov., and *Halococcaceae* fam. nov.

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Introduction

The class *Halobacteria* consists of a large and phenotypically heterogeneous assemblage of halophilic archaea within the phylum Euryarchaeota (Grant et al. 2001; Oren 2012). The class *Halobacteria* currently contains over 175 species placed into fifty distinct genera (Parte 2013; NamesforLife 2015). Our understanding of interrelationships of the genera within the class *Halobacteria* was previously based on chemotaxonomic characteristics and analysis of the 16S rRNA gene (Grant et al. 2001; Oren 2006; Wright 2006; Oren 2012). However, chemotaxonomic properties have not proven useful for classification above the genus level (Oren 2006, 2012) and the presence of multiple highly divergent copies of the 16S rRNA gene in many members of the class *Halobacteria* has limited the inferences that can be drawn from its analysis (Mylvaganam and Dennis 1992; Vreeland et al. 2002; Boucher et al. 2004; Cui et al. 2009; Oren 2012). Analyses of other individual gene/protein sequences, such as *rpoB'*/*rpoC*, have also thus far proven of limited value in elucidating the evolutionary relationships of the members of the class *Halobacteria* due to concerns regarding recombination events and lateral gene transfers (Walsh et al. 2004; Enache et al. 2007; Minegishi et al. 2010b; Naor et al. 2012; Williams et al. 2012).

The use of a concatenated set of unlinked and conserved loci in phylogenetic reconstruction is able to mitigate the effects of any instances of recombination or lateral gene transfer and provide greater resolving power than trees based on single genes/proteins (Rokas et al. 2003; Ciccarelli et al. 2006; Wu et al. 2009). The advent of widely available genome sequencing technology has provided taxonomists with a wealth of data from which to elucidate the relationships between various prokaryotic groups (Gao and Gupta 2012a; Zhi et al. 2012; Oren and Garrity 2014). A genome-centric, polyphasic approach to taxonomy, in which categorisation is driven primarily by inferences drawn from the genome sequence data and secondarily by molecular, biochemical, and phenotypic traits is now the recommended approach for prokaryotic taxonomy (Klenk and Goker 2010; Oren and Garrity 2014; Rossello-Mora and Amann 2015; Sutcliffe 2015; Whitman 2015).

The increasing availability of genome sequencing technology has provided us with genome sequence data from 35 genera within the class *Halobacteria*, covering a majority of the diversity within the group (NCBI 2015). This genome sequence data has allowed for robust and in-depth phylogenetic reconstructions of the sequenced *Halobacteria* species based on multiple concatenated gene and protein sequences (Papke et al. 2011; Andam et al. 2012; Williams et al. 2012; Soucy et al. 2014; Gupta et al. 2015). The most comprehensive phylogenetic reconstructions to date have been based on the sequences of thirty-two concatenated housekeeping proteins from 98 *Halobacteria* genomes (Gupta et al. 2015) and fifty-five concatenated ribosomal proteins from 118 *Halobacteria* genomes (Soucy et al. 2014). This genome sequence data is also enabling the detection of conserved molecular characteristics shared by evolutionarily related groups of organisms. In particular, two classes of conserved molecular characteristics have recently been utilised in prokaryotic taxonomy (Bhandari and Gupta 2014; Gupta 2014; Naushad et al. 2014; Gupta et al. 2015): conserved signature insertions/deletions (CSIs), which are insertions or deletions (indels) that are present only in a related group of organisms, and conserved signature proteins (CSPs), which are whole proteins that are present only in a related group of organisms. Both classes of molecular characteristics represent synapomorphic characteristics and provide reliable evidence, independent of phylogenetic trees, that the species from the groups in which they are found are specifically related to each other due to common ancestry. Recently, the class *Halobacteria*, which previously contained a single order (*Halobacteriales*), was divided into three orders (*Halobacteriales*, *Haloferacales*, and *Natrialbales*) on the basis of CSIs and CSPs (Gupta et al. 2015). However, due to the size of the class *Halobacteria*, the previous analysis only focused on the higher level divisions within the class *Halobacteria*, placing a single family within each of the three orders despite the size, diversity, and, in the case of the order *Halobacteriales*, the polyphyly of the identified groups (Gupta et al. 2015).

In this work, we have employed the whole genome sequences of 129 genome sequenced members of the class *Halobacteria* to reconstruct a highly robust phylogenetic tree based on 766 shared proteins and to identify conserved molecular characteristics that can be used to determine the interrelationships of the halobacterial genera within the three orders. We present 20 CSIs and 31 CSPs which are unique characteristics of infra-order level groups of genera within the class *Halobacteria*. Additionally, we present 40 CSIs and 234 CSPs that are characteristic of *Haloarcula*, *Halococcus*, *Haloferax*, or *Halorubrum*. Importantly, the order *Haloferacales* has been found to contain two main groups, a group containing the genus *Haloferax* and related genera, which is supported by four CSIs and five CSPs and a group containing the genus *Halorubrum* and related genera, which is supported by four CSPs. We have also identified molecular characteristics that suggest that the polyphyletic order *Halobacteriales* contains at least two large monophyletic clusters of organisms in addition to the polyphyletic members of the order, one cluster containing the genus *Haloarcula* and related genera supported by ten CSIs and nineteen CSPs and the other containing the members of the genus *Halococcus* which is supported by nine CSIs and 23 CSPs. On the basis of the phylogenetic analyses and the identified conserved molecular characteristics presented here, we propose a division of the order *Haloferacales* into two families, an emended family *Haloferacaceae* and *Halorubraceae* fam nov. and a division of the order *Halobacteriales* into three families, an emended family *Halobacteriaceae*, *Haloarculaceae* fam. nov., and *Halococcaceae* fam. nov.

Methods

Phylogenetic analyses

A phylogenetic tree was produced based on the concatenated sequences of 766 proteins obtained from 129 genome sequenced members of the class *Halobacteria*. The protein families used in this phylogeny were identified using the UCLUST algorithm (Edgar 2010) to identify proteins families present in at least 80 % of the input genomes which shared at least 50 % sequence identity and 50 % sequence length. Input genomes that were not annotated had all of their open reading frames translated from nucleotide sequences to amino acid sequences using USEARCH 8 (Edgar 2010). Each identified protein family was individually aligned using Clustal Omega (Sievers et al. 2011) and trimmed using Gblocks 0.91b (Castresana 2000) with relaxed parameters (Talavera and Castresana 2007). The concatenated dataset of the trimmed sequence alignments contained 212,988 aligned amino acid residues. A maximum-likelihood tree based on this alignment was constructed using FastTree 2 (Price et al. 2010) employing the Whelan and Goldman model of protein sequence evolution (Whelan and Goldman 2001) and RAxML 8 (Stamatakis 2014) using the Le and Gascuel model of protein sequence evolution (Le and Gascuel 2008). SH-like statistical support values (Guindon et al. 2010) for each branch node in the final phylogenetic tree were calculated using RAxML 8 (Stamatakis 2014). The resultant phylogenetic tree was drawn and artificially rooted on the midpoint using MEGA 6 (Tamura et al. 2013). This process was completed using an internally developed software pipeline. A manuscript for this pipeline is currently under preparation and the pipeline will be available for public use on Gleans.net once released.

In parallel, a phylogenetic tree based on the 16S rRNA gene sequences of type strains covering all validly named genera within the class *Halobacteria* was also constructed. The 16S rRNA sequences were retrieved from Ribosomal Database Project (Cole et al. 2014) and aligned using the SINA aligner (Pruesse et al. 2012) to form a multiple sequence alignment that was 1604 nucleotides long with common gaps removed. A maximum-likelihood phylogenetic tree based on this multiple sequence alignment was created using in MEGA 6 (Tamura et al. 2013) employing the General Time-Reversible model of sequence evolution (Tavaré 1986) with branch support based on 1000 bootstrap replicates.

Identification of conserved signature indels

Conserved signature indels were identified as detailed by Gupta (2014). In summary, BLASTp (Altschul et al. 1997) searches were performed on each protein in the genome of *Halobacterium salinarum* R1 (Pfeiffer et al. 2008) against all available sequences in the GenBank non-redundant database. Multiple sequence alignments were then created using ClustalX (Jeanmougin et al. 1998) for proteins that returned high scoring matches from *Halobacteria* and other prokaryotes. The alignments were then visually inspected for the presence of insertions or deletions that were flanked on both sides by at least 5–6 conserved amino acid residues in the neighbouring

30–40 amino acids. Detailed BLASTp searches were then carried out on short sequence segments containing the indel and the flanking conserved regions (60–100 amino acids long) to determine the specificity of the indels. SIG_CREATE and SIG_STYLE (available on Gleans.net) were then used to create Signature files for CSIs that were specific to *Halobacteria* subgroups as described by Gupta (2014). Due to the large number of genome sequences available for *Halobacteria*, the sequence alignment files presented here contain sequence information for only a limited number of species (generally only the type species from different genera). However, unless otherwise indicated, all members of the specified groups displayed similar sequence characteristics.

Identification of conserved signature proteins

Identification of conserved signature proteins for different *Halobacteria* subgroups was carried out by completing BLASTp (Altschul et al. 1997) searches using all proteins in the genomes of *Halobacterium salinarum* R1, *Haloarcula marismortui* ATCC 43049, *Halococcus thailandensis* JCM 13552, *Haloferax volcanii* DS2 and *Halorubrum lacusprofundi* ATCC 49239 (Baliga et al. 2004; Pfeiffer et al. 2008; Hartman et al. 2010) as query sequences. BLASTp searches were performed against all available sequences in the GenBank non-redundant sequence database. The results of the BLAST searches were then manually inspected for proteins for which all significant hits were from well-defined groups within the class *Halobacteria* or proteins for which there was a large increase in E value from the last hit belonging to a particular group of organism within the class *Halobacteria* and the first hit from an organism for any other bacterial group and the E value for the latter hits were $>1 \times 10^{-4}$ (Gao and Gupta 2007; Gupta and Mok 2007; Naushad et al. 2014).

Results

Phylogenetic analysis of the class *Halobacteria* based on concatenated protein sequences

In this work, we have produced a phylogenetic tree containing 129 genome sequenced members of the class *Halobacteria* using a concatenated set of seven hundred and sixty-six proteins (Fig. 1). The phylogenetic reconstruction produced for this work is the most comprehensive phylogenetic analysis of the genome sequenced *Halobacteria* completed to date. The branching patterns in this concatenated protein based phylogenetic tree largely reflect those seen in prior publications (Soucy et al. 2014; Gupta et al. 2015). In this concatenated protein based phylogenetic tree, the orders *Natrialbales* and *Haloferacales* form monophyletic groups with strong statistical support. The order *Haloferacales* can be further divided into two smaller groups; one containing the genera *Haloferax* and other closely related *Halobacteria* and the other containing *Halorubrum* and its relatives, labelled HF1 and HF2, respectively, in Fig. 1. The order *Halobacteriales* is currently a polyphyletic assemblage of halobacterial groups which do not show strong affinity to either the order *Natrialbales* or *Haloferacales* (Gupta et al. 2015). In our concatenated protein tree, the order *Halobacteriales* spans at least three subgroups. Two of these subgroups consist of distinct pairings of genera (viz. *Halobacterium*/*Halarchaeum*, *Haladaptus*/*Halalkalicoccus*). In addition to these smaller subgroups, two large neighbouring clusters of *Halobacteriales*, one consisting of *Haloarcula*, *Halomicrobium*, *Halorhabdus*, *Halosimplex*, and *Natronomonas* (labelled HB1) and the other consisting of the members of the genus *Halococcus* (labelled HB2), are also observed in the phylogenetic tree.

In this work, we have also produced a 16S rRNA gene sequence based phylogenetic tree containing all named *Halobacteria* species (Fig. 2). The branching patterns observed in the 16S rRNA based phylogenetic tree are similar to those observed in the concatenated protein tree. As observed in the concatenated protein tree, the orders *Natrialbales* and *Haloferacales* are monophyletic and well-separated entities in the 16 rRNA gene based tree. Within the order *Natrialbales,* the members of the genus *Halopiger* form a monophyletic grouping that is not observed in the concatenated protein tree. We have further examined the significance of the monophyletic grouping of the *Halopiger* in the 16S rRNA tree by creating individual and concatenated phylogenetic trees for the five multi-locus sequence analysis proteins (viz. *atpB*, EF-2, *radA*, *rpoB'*, and *secY*) for the class *Halobacteria* proposed by Papke et al. (2011) (Supplemental Figs. 54–59). A monophyletic grouping of the members of the genus *Halopiger* was not observed in any of

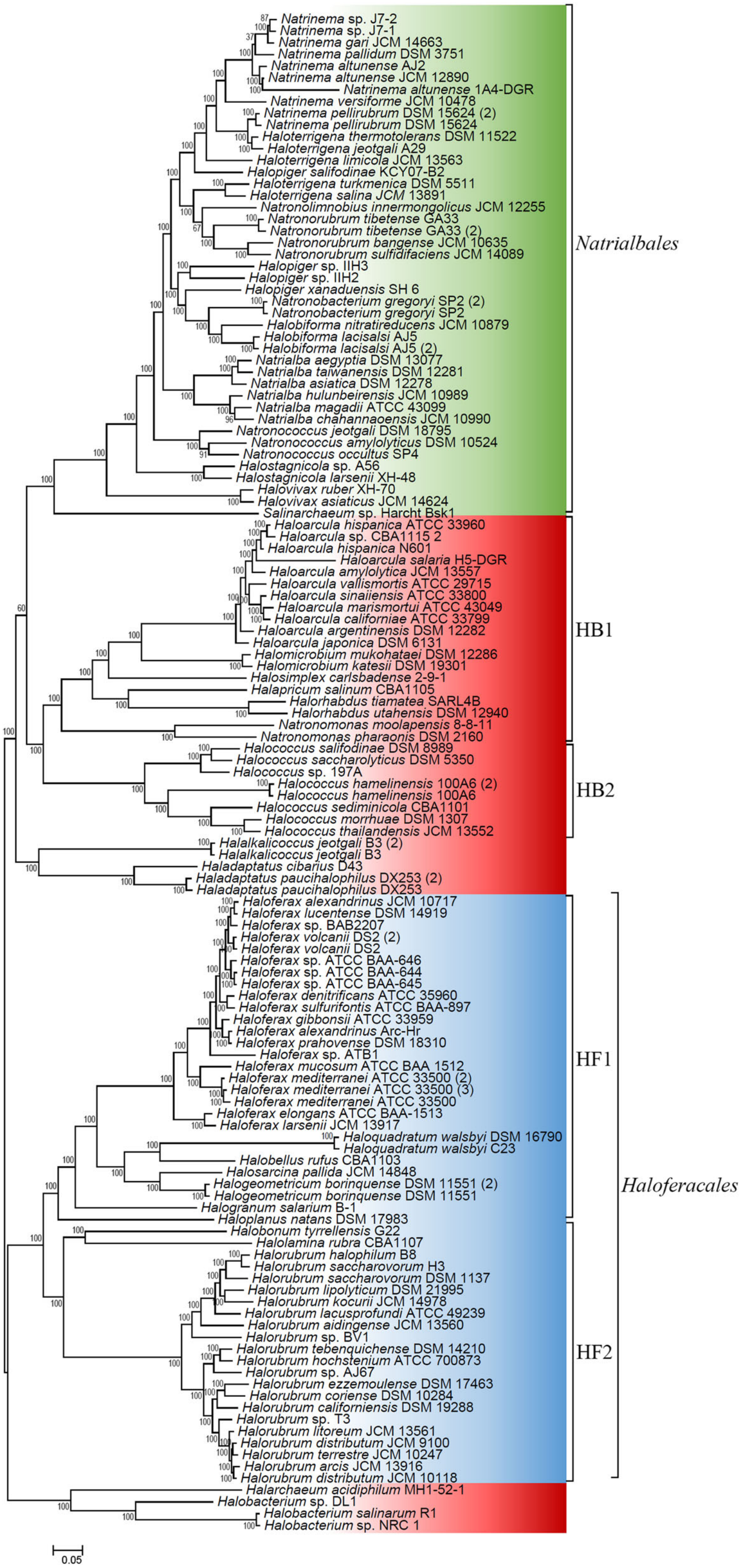
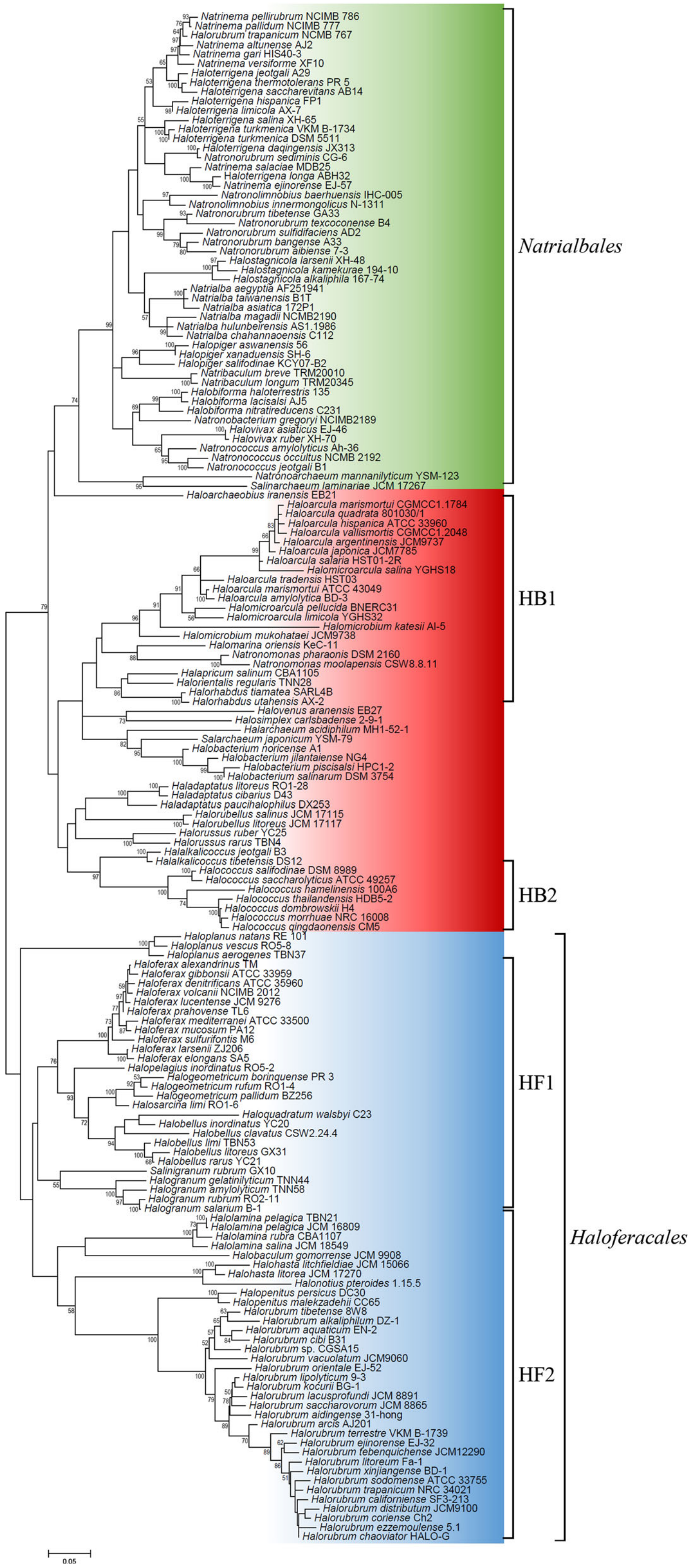


Fig. 1 A maximum likelihood phylogenetic tree based on the concatenated sequences of 766 proteins obtained from 129 genome sequenced members of the class *Halobacteria*. The members of orders *Natrialbales*, *Haloferacales*, and *Halobacteriales* are highlighted in *green*, *blue*, and *red*, respectively. Major clades are labelled. SH-like statistical support values are shown at branch nodes

Fig. 2 A maximum likelihood phylogenetic tree based on 144 16S rRNA gene sequences from members of the class *Halobacteria* covering all known species. The members of orders *Natrialbales*, *Haloferacales*, and *Halobacteriales* are highlighted in *green*, *blue*, and *red*, respectively. Major clades are labelled. Bootstrap support values above 50 % are shown at branch nodes



these phylogenetic trees and there was stochasticity in their branching patterns, indicating a high level of genetic heterogeneity within the members of this genus. In phylogenetic trees based on different protein sequences, *Natrinema altuense* 1AG-DGR consistently shows a longer branch in comparison to the other *Natrinema* spp., but its significance at present is unclear. Within the order *Haloferacales* in the 16S rRNA tree, the clades HF1 (*Haloferax* and related genera) and HF2 (*Halorubrum* and related genera) are identifiable and well separated with the sole exception of the members of the genus *Haloplanus* which branched within the *Haloferacales* adjacent to the clades HF1 and HF2. The order *Halobacteriales* weakly supported monophyletic branching in the 16S rRNA based phylogenetic tree. Within this weakly supported grouping of the *Halobacteriales*, there are nine distinct and well-supported subgroups. Importantly, the large clusters of *Halobacteriales* species observed in the concatenated protein tree (clades HB1 and HB2) are also identifiable in the 16S rRNA tree. However, the genus *Halococcus* (clade HB2) did not branch with clade HB1 in the 16S rRNA tree, instead *Halococcus* branched with the genus *Halalkalicoccus* alongside the genera *Haladaptus*, *Halorussus*, and *Halorubellus*. The genus *Halosimplex* did not branch within clade HB1 in the 16S rRNA tree. The single member of the genus *Halosimplex* formed a cluster with the lone member of the genus *Halovenus* which branched in the vicinity of clade HB1 and other *Halobacteriales*.

Molecular characteristics distinguishing the two main groups within the order *Haloferacales*

Molecular characteristics, such as CSIs and CSPs, that are uniquely found in a well-defined group of organisms are powerful tools for evolutionary studies (Rokas and Holland 2000; Gao and Gupta 2012a; Jones 2012; Gupta 2014). Recently, CSIs and CSPs have been used to revise the taxonomy of the class *Halobacteria* and a large number of other prokaryotic groups at varying taxonomic depths (Sawana et al. 2014; Gupta et al. 2015; Naushad et al. 2015). However, the previous analysis of the class *Halobacteria* focused only on the higher level divisions within the class. In this work we have identified four CSIs and five CSPs which are shared by clade HF1 (*Haloferax* and related genera). An example of one such CSI, consisting of a one amino acid insertion in the members of clade HF1 located in a conserved region of a hypothetical protein, is shown in Fig. 3. This insertion is uniquely found in all members of clade HF1 and absent in the members of clade HF2 and all other members of the class *Halobacteria*. The sequence alignments for three additional CSIs which are specific for clade HF1 are shown in Supplemental Figs. 1–3 and their properties are briefly summarised in Table 1. The second group within the order *Haloferacales*, clade HF2 (*Halorubrum* and related genera), is characterised by four identified CSPs. GenInfo Identifier numbers (GI Numbers) for the five CSPs specific for clade HF1 and the four CSPs specific for clade HF2 are provided in Table 3A, B, respectively.

Molecular characteristics which provide evolutionary insights for the order *Halobacteriales*

In this work, we have also identified a number of CSPs and CSIs that provide novel insights into the interrelationships within the polyphyletic order *Halobacteriales*. Most importantly, we have identified three CSIs and four CSPs which are shared by clade HB1 as seen in the concatenated protein based phylogenetic tree (viz. the genera *Haloarcula*, *Halomicrobium*, *Halorhabdus*, *Halosimplex*, and *Natronomonas*) and seven CSIs and fifteen CSPs which are unique characteristics of all clade HB1 members except the genus *Natronomonas*, which forms the outermost branch of clade HB1. One example each of these two types of CSI are shown in Fig. 4. The first CSI, consisting of a one amino acid insertion in ATP-dependent DNA helicase (Fig. 4a), is uniquely found in the members of clade HB1 while the second CSI, consisting of a one amino acid deletion in a conserved region of the protein acetylglutamate kinase (Fig. 4b), is uniquely found in all members of clade HB1 except the genus *Natronomonas*. Neither of the CSIs shown in Fig. 4, or any of the other CSIs indicated to be specific for clade HB1, are found in the members of clade HB2 or other members of the class *Halobacteria*. The sequence alignments for eight additional CSIs which are specific for clade HB1 are shown in Supplemental Figs. 4–11 and their properties are briefly summarized in Table 2. In addition, we have also identified nineteen CSPs which are also specifically found in members of clade HB1. Identification

			86		129
Clade HF1	Haloferax volcanii	292655478	RVILGEDEEEELFPGVLVRR	V	PGHRFEIEADPEVARGRVFVFVE
	Haloferax denitrificans	491108947	-------D------------	-	-----------------------
	Haloferax sulfurifontis	494484389	-------D------------	-	-----------------------
	Haloferax mucosum	495596354	----A-G---------VA--	-	---Q--L----A-------A---
	Haloferax elongans	495599398	----A-G---------VA--	I	S-----VD---------------
	Haloferax larsenii	494804446	----A-GD--------VA--	I	S-----VD---------------
	Haloferax mediterranei	389846860	----A-E---------V---	L	------V----D-----------
	Haloferax gibbonsii	491118767	------G-------------	-	-----------------------
	Haloferax lucentense	448571277	--------------------	-	-----------------------
	Haloferax alexandrinus	448596112	--------------------	-	-----------------------
	Haloferax volcanii DS2	448291877	--------------------	-	-----------------------
	Haloferax prahovense	495368388	------G-------------	-	-----------A-----------
	Halogranum salarium	496767422	----NA----------T---	M	G-M-N-VV---D-----------
	Halogeometricum borinquense	313126648	----DAG---------S---	T	E-M-T----------------E-
	Haloquadratum walsbyi	110668661	--V-EQGSS-TV----T-S-	L	N-M-TT------T----------
	Halosarcina pallida	495665513	--V-D-----------R---	T	KSM-T----------------E-
Other *Halobacteria*	Halorubrum lacusprofundi	222480291	---VEDG-RD-V----I-G-		T-Q-N-VI----I-G--------
	Halorubrum terrestre	494585702	---VEDG-RD-V----I-G-		E-Q-N-VV------G--------
	Halorubrum distributum	490734406	---VEDG-RD-V----I-G-		E-Q-N-VV------G--------
	Halorubrum tebenquichense	493680221	---VEDG-RD-V----I-G-		D-Q-N-VV----I-G--------
	Halorubrum californiense	495717232	---VEDG-RD-V----I-G-		D-K-N-VV------G--------
	Halorubrum hochstenium	495858678	---VEDG-RD-V----I-G-		E-Q-N-VV----I-G--------
	Halorubrum lipolyticum	495281757	---VEDG-RD-V----I-G-		E-Q-N-VI----I-G--------
	Halorubrum saccharovorum	490146200	--MVEDG-RD-V----I-A-		E-Q-N-VI----I-G--------
	Halomicrobium mukohataei	257388393	-IM-DTG-Q--V----TITD		TSM-V-V-----T----------
	Halosimplex carlsbadense	493940218	--MVEDGD--P--Q--TA-S		TA--V-V---LS-VN--------
	Haladaptatus paucihalophilus	495255561	----EPG---------T--A		D-YAV-V--S-KI--------E-
	Halalkalicoccus jeotgali	300711238	--LVESG-R--V----E--S		E---VFV-G---I--------A-
	Halococcus thailandensis	495015612	--LVDDG--TT-----S--A		E---VTV-----T--------E-
	Halococcus morrhuae	490154608	--LVDDG--TT-----S--A		D---VTVA----T--------E-
	Halococcus saccharolyticus	492978668	--L-DQ---TT-----S-HE		T-Y-TIVD----T--------E-
	Halococcus salifodinae	491184249	--L-DQ---TT-----S--E		T-Y-TIVD---QS--------E-
	Halococcus hamelinensis	494968135	--FVDDGD-TT-----S-AA		GSY-IT-----G---------E-
	Halobacterium sp. DL1	497447202	--L-DPG---------T-QV		D-YAI-VT----E--------E-
	Halobacterium sp. NRC1	15790302	-LL-DPG-R-------T-QA		D-YAID-D----T--------A-
	Haloarcula vallismortis	490652929	----DDG--D------T--D		NQM-T-V-V-Y--TN------E-
	Haloarcula argentinensis	491678348	----DDG--D------T--D		NQM-T-V-V-YG-TN------E-
	Haloarcula hispanica	344211728	----DDG--D------T--D		NQM-TKV-V-YD-TN------E-
	Haloarcula japonica	490730463	----DDG--D------T--D		NQM-TAV-V-YD-TN------E-
	Natrinema pellirubrum	433590495	--M-DRND--------T-AT		EAYSIRV----S-VD------A-
	Natrinema versiforme	493476330	--M-DR-D--------T-AT		EAYSIRVD--LSLVD------A-
	Natrinema altunense	494169377	--M-DR-D-A------T-AT		DAYSVRV---LS-VD------A-
	Natronococcus occultus	435848614	----EGND--V-----T-ST		E-YSIRV------VD------A-
	Natronococcus amylolyticus	491714211	--M-EG-D--V-----T-ST		TAYAIQV--E-S-VD------A-
	Natronococcus jeotgali	495700714	--M-ER-D--V-Y---T-ST		A-YSIRV---L--VD------A-
	Haloterrigena turkmenica	284176159	--M-ER-D--------T-AT		E-YSIRV---LSAVD------A-
	Haloterrigena salina	496171027	--M-DR-D------A-T-AT		E-YSIRV---LSAVD------A-
	Haloterrigena limicola	495289203	--M-DR-D--------R-AT		EAYSVRVD--LSAVD------A-
	Natronomonas moolapensis	452207109	-IV-ER--TAS-----DAEN		H-Y-V--S---D---------A-
	Natronomonas pharaonis	76802724	---IEGG-TAS-----AAES		T-Y-V-VS--FD--D------A-
	Halopiger xanaduensis	336254372	--M-EA-D--V-----T-TT		EAYSIRV---L--VD----I-A-
	Natronorubrum bangense	492959254	--M-ED-D--V-----T-ST		AAYSIRV---LSLVD------A-
	Natronorubrum sulfidifaciens	495434352	--M-EG-D--V-----T-TT		E-YSIRV---LSLVD------A-
	Natronorubrum tibetense	493011678	--M-EG-D--V-----T-ST		EAYSIRVD--LSLVD------A-
	Natrialba hulunbeirensis	493705074	--M-SD-D--V-----T-TT		EAYSIRVD--LSLVD------A-
	Natrialba asiatica	493048335	----SG-D--V-Y---S-ST		DAYSIRV---LSLVD------A-
	Natronolimnobius innermongolic	494472057	--M-EG-D--T-----S-ST		EAYSIRV---LS-VD------A-
	Natronobacterium gregoryi	429193451	--M-E-NDA-V-----T-ST		DAYSIRV---L-AVD------A-
	Halobiforma lacisalsi	494234730	--M-ES-D--V-Y---T-ST		E-YSIRV---VSAVD--I---A-
	Halovivax ruber	433638560	--LIEQG-RSV-----SAAA		E--SVTVSVE-A-VD------A-
	Halovivax asiaticus	494970370	--LIEQG-RSV-----SAAA		E--SVTVSVE-A-VD------A-
	Halorhabdus tiamatea	495800449	--V-EPG--TTV----RA-N		ETQ-IVVD--F-AVD------E-
	Halorhabdus utahensis	257054087	--V-EPG-KTTV----RAAN		DAR-IVV---FDAVD------E-

◀**Fig. 3** A partial sequence alignment of a hypothetical protein showing a 1 amino acid insertion (*boxed*) that is characteristic of the members of clade HF1. Sequence information for a limited number of *Halobacteria* and other archaea are shown here, but unless otherwise indicated similar CSIs were detected in all members of the indicated group and not detected in any other species in the top 250 Blastp hits. The dashes (-) in the alignments indicate identity with the residue in the top sequence. GenInfo identification (GI) numbers for each sequence are indicated in the second column

numbers for these nineteen clade HB1-specific CSPs are provided in Table 3C. These CSIs and CSPs provide strong support that the genus *Halosimplex* shares an evolutionary lineage with the members of clade HB1 despite not branching within the clade in our 16S rRNA tree (Fig. 2).

We have also identified a number of CSIs and CSPs that are uniquely shared by groups within the order *Halobacteriales* that show inconsistent branching

Table 1 Conserved signature indels specific for infra-order level groups of genera within the class *Halobacteria*

Protein name	GI number	Figure	Indel size	Indel position	Specificity
Hypothetical protein	292655478	Fig. 3	1 aa ins	83–140	*Haloferacaceae* (HF1)
Thermosome	491114234	Supp. Fig. 1	1 aa ins	156–207	
Hypothetical Protein	495366528	Supp. Fig. 2	3 aa ins	17–69	
Ribonuclease BN	494808956	Supp. Fig. 3	1 aa del	179–234	
ATP-dependent DNA helicase	490727914	Fig. 4A	1 aa ins	152–187	*Haloarculaceae* (HB1)
DNA mismatch repair protein MutS	499543694	Supp. Fig. 4	1 aa del	333–389	
DEAD/DEAH box helicase	505220991	Supp. Fig. 5	1 aa ins	441–487	
Acetylglutamate kinase	257052575	Fig. 4B	1 aa del	61–99	*Haloarculaceae* (HB1) except *Natronomonas*
Ribonuclease R	517067816	Supp. Fig. 6	10 aa ins	182–246	
Metallo-beta-lactamase	55379160	Supp. Fig. 7	1 aa ins	145–183	
tRNA modifying enzyme	506242706	Supp. Fig. 8	1 aa ins	46–81	
Hypothetical protein	490727671	Supp. Fig. 9	2 aa ins	115–176	
Hypothetical protein	490731323	Supp. Fig. 10	1 aa del	7–78	
Carbamoyl phosphate synthase large subunit	490731427	Supp. Fig. 11	1 aa ins	814–872	
Deoxyribonuclease/rho motif-related TRAM	169236738	Supp. Fig. 12	1 aa ins	66–102	*Halobacterium* and *Halarchaeum*
Flap endonuclease 1	15790386	Supp. Fig. 13	1 aa ins	147–189	
Hypothetical protein VNG1087C	15790181	Supp. Fig. 14	1 aa ins	585–645	
Hypothetical protein VNG1809H	15790721	Supp. Fig. 15	2 aa ins	45–96	
Ribonuclease BN	495013437	Supp. Fig. 16	1 aa ins	185–214	*Halococcus* and *Halalkalicocccus*
Ferritin Dps family protein	495014878	Supp. Fig. 17	1 aa ins	22–72	

(A)

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                                                           152                                187
Clade HB1
Haloarcula japonica                     490727914          AMEARNAVVEELRDLQDEREEIE T ERSTCDHYYRNL
Haloarcula californiae                  494347427          ----------------------- - ------------
Haloarcula vallismortis                 490650916          ----------------------- - ------------
Haloarcula amylolytica                  495582601          ----------------------- - ------------
Haloarcula marismortui                  499543604          ----------------------- - ------------
Haloarcula hispanica                    503805053          ----------------------- - ------------
Haloarcula sinaiiensis                  491098379          ----------------------- - ------------
Haloarcula argentinensis                491680644          -------------E--------- - ------------
Halomicrobium katesii                   517068255          -L------MD--QAVEEDL--LQ - DATI--------
Halomicrobium mukohataei                506242027          -L------MD--QAVEEDL--LQ - DATI--------
Halosimplex carlsbadense                493938224          -AD--G--MD--DAVE--I-QLR E D-TV--------
Halorhabdus tiamatea                    495803037          -A---S--MD--ESVAE-V-TLK E -QA---------
Halorhabdus utahensis                   506269391          -A---S--MD--DAVAE-I-TLK E -QA---------
Natronomonas pharaonis                  499641645          -A---GT-LD--DE-EASA-ALR E --NV--R--K--
Natronomonas moolapensis                505223612          -AD--GT-LS--E--ETGA-A-R E -ETI-ER--N--
Other Halobacteria
Haladaptatus paucihalophilus            495251732          -A---S--MD--EQI---V-NLK   -KNV-EY--N--
Halococcus hamelinensis                 498297972          -A---S--MD--DAVE--L-DLR   -G-I-E---N--
Halococcus hamelinensis                 494964792          -A---S--MD--DAVE--L-DLR   -G-I-E---N--
Halococcus saccharolyticus              492986005          -T---S--MD--ESVES-VDDLR   GE-I-E---N--
Halalkalicoccus jeotgali                495694272          -A---S--MD--ESVDE-VSDL-   -EP--E---N--
Haloferax mediterranei                  490160096          -AD--S--TD--DTID--L-QLK   -GNY-----N--
Haloferax sulfurifontis                 494486341          -AD--S--TD--DA-D--L--LK   -GNY-E---N--
Haloferax denitrificans                 491109797          -AD--S--TD--DSID--L--LK   -GNY-E---N--
Haloferax gibbonsii                     491115547          -AD--S--TD--DSID--L--LK   -GNY-E---N--
Haloferax prahovense                    495366278          -AD--S--TD--DSID--L--LK   -GNY-E---N--
Haloferax elongans                      495597911          -AD--S--TD--DR-DE-LD-LQ   -GNY-----N--
Haloferax larsenii                      494808608          -AD--S--TD--DR-DE-LD-LQ   -GNY-----N--
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(B)

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                                                           61                                        99
Clade HB1
Halorhabdus utahensis                   257052575          GVVGRFTDEETMEVFEMAF   GHLNTQLVAGLQSQGVDAVG
Halorhabdus tiamatea                    529077976          -------------------   --------------------
Haloarcula japonica                     490727961          ---------T---------   -------------E------
Haloarcula vallismortis                 490652622          ---------T---------   -------------E------
Haloarcula amylolytica                  495582632          ---------T---------   -------------E------
Haloarcula argentinensis                491680684          ---------T---------   -------------E------
Haloarcula marismortui                  55379308           ---------T---------   -------------E------
Haloarcula californiae                  494347404          ---------T---------   -------------E------
Haloarcula hispanica                    344213322          ---------T---------   -------------E------
Haloarcula sinaiiensis                  448638524          ---------T---------   -------------E------
Halosimplex carlsbadense                493937912          --------A--------V-   --------------------
Halomicrobium katesii                   648498467          ---------A-------V-   -------------L------
Halomicrobium mukohataei                257386839          ---------A-------V-   -------------L------
Natronomonas moolapensis                452206098          --------A---D--K--L A -QV--D--T--RN-------
Natronomonas pharaonis                  76803258           --------A---D--K--M A -QV--D--V---N-------
Other Halobacteria
Natronobacterium gregoryi               429191590          --------A---DI-K-VM P -K---D--ES--NE------
Natronococcus occultus                  435845570          ------------D--K-VM P -K---D--ES--NE------
Natrinema pellirubrum                   433592856          ------------D--K-VM P -K---D--ES-HNE------
Haloterrigena thermotolerans            493698471          ------------D--K-VM P -K---D--ES-HNE------
Haloterrigena limicola                  495289096          ---------R--D--K-VM P -K---D--ES-HNE------
Halopiger xanaduensis                   336252785          ------------D--K-VM P -K---D--ES-HNE------
Halobiforma nitratireducens             493722902          --------A---D--K-VM P -K---D--ES--NE--N---
Natronolimnobius innermongolic          494469828          ------------D--K-VM P -K---D--ES--NA------
Natrialba aegyptia                      493715810          ------------D--K-VM P -K---D--ES--NE--N---
Natronorubrum sulfidifaciens            495436334          ---------R--D--K-VM P -K---D--ES--NE------
Halostagnicola larsenii                 573480006          ---------R--D--K-VM P -K---D--EA--NE------
Halarchaeum acidiphilum                 545902714          ---------A---A----M P -VV--D------N-------
Halovivax ruber                         433637417          ---------TA--A-T--M A -TV--D--CA--NE----I-
Halogeometricum borinquense             313124961          ---------R-----S-VM P -K---D--TT-REA----L-
Halococcus morrhuae                     490155296          ---------R-------VL P -K---D-T-Q-RAD----L-
Halosarcina pallida                     495661902          ---------R-----S-VM P -K---D--TT--GA--N-L-
Haloquadratum walsbyi                   385805021          -I-------R-----S-VL P -KI--D-TVS---A--N-L-
Haladaptatus paucihalophilus            495251741          --M------K-------VM P -K---E-T-N--NE--RSI-
Halogranum salarium                     496770389          --S------R-----T-VM P -K---E-T-T-RGA----L-
Haloferax mucosum                       495595510          --S------R-----S-VM P -K---D-T-LFREA----L-
Halalkalicoccus jeotgali                300709567          --T-----A----A-T--M A -QV--D-T-A-RNE--N-L-
Halobonum tyrrellensis                  557372244          --T-----A----T-S-VL P -K---D--VA-RNA----L-
Halorubrum californiense                495716159          --T-----A----A-S--M A -K---E-T-AFR-A------
```

◀**Fig. 4** Partial sequence alignments of **a** a ATP-dependent DNA helicase showing a one amino acid insertion deletion that is characteristic of the members of clade HB1, **b** acetylglutamate kinase showing a 1 amino acid deletion that is characteristic of the members of clade HB1 except the genus *Natronomonas*. Sequence information for a limited number of *Halobacteria* and other archaea are shown here, but the indicated CSIs were detected only in members of the indicated group and not detected in any other species in the top 250 Blastp hits. The dashes (-) in the alignments indicate identity with the residue in the top sequence. GenInfo identification (GI) numbers for each sequence are indicated in the second column

affinity in phylogenetic trees. An association between the members of the genera *Halococcus* and *Halalkalicoccus* is supported by two CSIs and one CSP identified in this work (Tables 1, 3D). Additionally, *Halococcus* and *Halalkalicoccus*, cluster together in the 16S rRNA sequence based phylogenetic tree alongside the genera *Haladaptus*, *Halorussus*, and *Halrubellus*. However, there is no association observed between *Halococcus* and *Halalkalicoccus* in the concatenated protein tree and no CSIs or CSPs are identified that are specific to a grouping of the

Table 2 Conserved signature indels specific for *Haloarcula*, *Halococcus*, *Haloferax*, or *Halorubrum*

Protein name	GI number	Figure	Indel size	Indel position	Specificity
Hypothetical protein	389847853	Fig. 5A	1 aa ins	31–76	*Haloferax*
Phospholipase D	495599015	Supp. Fig. 18	3 aa del	217–263	*Haloferax*
Endonuclease IV	495598530	Supp. Fig. 19	2 aa ins	228–268	*Haloferax*
Kynureninase	495370699	Supp. Fig. 20	1 aa ins	280–341	*Haloferax*
hypothetical protein C498_12368	490143326	Supp. Fig. 21	6 aa ins	3–49	*Haloferax*
Cobyrinic acid ac-diamide synthase	491119179	Supp. Fig. 22	1 aa ins	123–192	*Haloferax*
Adenosine deaminase	494808074	Supp. Fig. 23	1 aa ins	131–179	*Haloferax*
50S ribosomal protein L19	495276588	Fig. 5B	1 aa ins	101–133	*Halorubrum*
FAD dependent oxidoreductase	495273660	Supp. Fig. 24	1 aa ins	87–150	*Halorubrum*
Phosphate uptake regulator, PhoU	495273602	Supp. Fig. 25	1 aa ins	267–327	*Halorubrum*
Hypothetical protein	490152145	Supp. Fig. 26	3 aa ins	8–51	*Halorubrum*
HhH-GPD family protein	495643346	Supp. Fig. 27	2 aa ins	68–130	*Halorubrum*
PhnP-like protein	495642406	Supp. Fig. 28	1 aa ins	111–148	*Halorubrum*
Electron transfer flavoprotein, alpha subunit	493052510	Supp. Fig. 29	2 aa del	130–187	*Halorubrum*
DNA gyrase subunit B	495016103	Fig. 6A	2 aa ins	26–83	*Halococcus*/ *Halococcaceae* (HB2)
Chaperone protein DnaK	495016166	Supp. Fig. 30	1 aa del	50–122	*Halococcus*/ *Halococcaceae* (HB2)
Hypothetical protein	495013289	Supp. Fig. 31	1 aa ins	81–155	*Halococcus*/ *Halococcaceae* (HB2)
HAD-superfamily hydrolase	495015049	Supp. Fig. 32	1 aa del	118–167	*Halococcus*/ *Halococcaceae* (HB2)
Glycosyltransferase	494966438	Supp. Fig. 33	1 aa del	286–323	*Halococcus*/ *Halococcaceae* (HB2)

Table 2 continued

Protein name	GI number	Figure	Indel size	Indel position	Specificity
2-Succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylate synthase	495015818	Supp. Fig. 34	4 aa ins	298–347	*Halococcus/ Halococcaceae* (HB2)
Iron-regulated ABC transporter	495013404	Supp. Fig. 35	1 aa ins	1–49	*Halococcus/ Halococcaceae* (HB2)
Glycine dehydrogenase subunit 2	490157199	Supp. Fig. 36	1 aa del	374–407	*Halococcus/ Halococcaceae* (HB2)
GMP synthase	490155708	Supp. Fig. 37	1 aa del	20–58	*Halococcus/ Halococcaceae* (HB2)
Metal transporter family GufA protein	55379677	Fig. 6B	5 aa ins	114–175	*Haloarcula*
Cysteinyl-tRNA synthetase	503806672	Supp. Fig. 38	1 aa del	135–193	*Haloarcula*
FAD dependent oxidoreductase	490730545	Supp. Fig. 39	1 aa ins	217–276	*Haloarcula*
Hypothetical protein C437_19977	490653762	Supp. Fig. 40	1 aa del	8–49	*Haloarcula*
Hypothetical protein C443_17178	491681737	Supp. Fig. 41	13 aa ins	17–93	*Haloarcula*
Inosine-5′-monophosphate dehydrogenase	491100099	Supp. Fig. 42	1 aa ins	105–170	*Haloarcula*
Dihydroorotase	490650307	Supp. Fig. 43	2 aa ins	307–364	*Haloarcula*
Phosphoglyceromutase	55379940	Supp. Fig. 44	13 aa ins	55–139	*Haloarcula*
Pyruvate kinase	503806135	Supp. Fig. 45	7 aa ins	470–533	*Haloarcula*
Arginyl-tRNA synthetase	495586504	Supp. Fig. 46	1 aa del	399–434	*Haloarcula*
Aspartate kinase	344213312	Supp. Fig. 47	1 aa del	322–370	*Haloarcula*
DNA repair protein	494347396	Supp. Fig. 48	13 aa ins	31–93	*Haloarcula*
Signal recognition 54 kDa protein	344210686	Supp. Fig. 49	1 aa ins	286–346	*Haloarcula*
DNA-directed RNA polymerase subunit A’	344213107	Supp. Fig. 50	1 aa ins	900–946	*Haloarcula*
Archaeosine tRNA-ribosyltransferase	490731527	Supp. Fig. 51	11 aa ins	14–76	*Haloarcula*
Fructose-bisphosphate aldolase	490652215	Supp. Fig. 52	3 aa ins	137–177	*Haloarcula*
Geranylgeranyl hydrogenase	491096917	Supp. Fig. 53	1 aa del	38–74	*Haloarcula*

sequenced members of the genus *Halococcus* and associated genera identified in the 16S rRNA tree (viz. *Halalkalicoccus* and *Haladaptus*). Thus, clade HB2 is currently limited to the members of the genus *Halococcus* until the affinity between the genus *Halococcus* and the genera *Halalkalicoccus*, *Haladaptus*, *Halorussus*, and *Halrubellus* can be determined. Within the order *Halobacteriales*, we have also identified phylogenetic and molecular support for a supergeneric relationship between the genera *Halobacterium* and *Halarchaeum*. The genera *Halobacterium* and *Halarchaeum* cluster together in

Table 3 GI numbers for conserved signature proteins that are specific for family, infrafamilial, and genus level groups within the class *Halobacteria*

(A) CSPs specific for the family *Haloferacaceae* (Clade HF1)							
292656573	292656429	292656269	292654161	292654131			
(B) CSPs specific for the family *Halorubraceae* (Clade HF2)							
222478926	222478774	222478444	222480259				
(C) CSPs specific for the family *Haloarculaceae* (Clade HB1)							
55378836	55380168	55376411	55376265	55378970[a]	55378973[a]	55379019[a]	55379070[a]
55379099[a]	55377253[a]	55377399[a]	55377827[a]	55377943[a]	55377963[a]	55378635[a]	55378971[a]
55379068[a]	55379522[a]	55379574[a]	55379881[a]				
(D) CSPs specific for the family *Halococcaceae*/the genus *Halococcus* (Clade HB2)							
445806789	445806788	445806714	445806697	445806652	445806473	445806366	445806322
445806308	445806306	445806298	445806297	445806221	445806208	445806200	445806118
445806088	445806041	445806039	445803970	445803968	445803958	445803893	445803977[b]
(E) CSPs specific for the genus *Haloferax*							
292656225	292656227	292656259	292656310	292656334	292656343	292656364	292656365
292656387	292656409	292657038	292657046	292657072	292657095	292657102	292657119
292656438	292656445	292656480	292656555	292656581	292656595	292656602	292656612
292494027	292494096	292494305	292494306	292653578	292653664	292653693	292653698
292653726	292653732	292653742	292653892	292654020	292654041		
(F) CSPs specific for the genus *Halorubrum*							
222478461	222478465	222478541	222478552	222478554	222478568	222478624	222478629
222478793	222478794	222478856	222478860	222478897	222478981	222479032	222479239
222479297	222479341	222479366	222479380	222479450	222479677	222479703	222479781
222479801	222479807	222479982	222479998	222480013	222480054	222480074	222480091
222480093	222480122	222480128	222480130	222480147	222480153	222480184	222480245
222480288	222480289	222480337	222480338	222480432	222480552	222480553	222480562
222480596	222480599	222480725	222480772	222480798	222480818	222480822	222480944
222480952	222481000	222481094	222481118	222481133			
(G) CSPs specific for the genus *Haloarcula*							
55377010	55377039	55377054	55377105	55377127	55377136	55377137	55377183
55377272	55377329	55377394	55377415	55377429	55377435	55377658	55377720
55377784	55377807	55377844	55377958	55378029	55378062	55378081	55378112
55378114	55378115	55378117	55378116	55378125	55378140	55378150	55378173
55378219	55378261	55378405	55378416	55378418	55378521	55378547	55378567
55378609	55378814	55378870	55378914	55378958	55379039	55379157	55379176
55379236	55379253	55379331	55379343	55379376	55379433	55379435	55379471
55379491	55379609	55379622	55379650	55379681	55379682	55379688	55379870
55379899	55379968	55380000	55380001	55380040	55380130	55380142	55380160
55380174	55380182	55380183	55380200	55380201	55380209	55380327	55376585
55376586	55376628	55376662	55376675	55376676	55376767	55376774	55376859
55376501	55376303	55376321	55376358	55376359	55376362	55376370	55376372
55376373	55376379	55376383	55376261	55376269	55376199	55376208	55376215
55376145	55376153	55376169	55376170	55376171	55376174	55376124	55376140
55376141							

[a] CSP specific for *Haloarculaceae* (Clade HB1) except *Natronomonas*

[b] CSP specific for *Halococcus* and *Halalkalicocccus*

the concatenated protein and in the 16S rRNA gene based phylogenetic trees along with the genus *Salarchaeum*. A relationship between *Halobacterium* and *Halarchaeum* is further supported by four CSIs (Table 1) and one CSP (GI: 169236474) identified in this work which are uniquely shared by these two genera. We have also identified some CSIs that support a relationship *Halobacterium* and *Haladaptus*, the significance of which is currently unclear. The sequence alignments for the CSIs specific to either *Halococcus* and *Halalkalicoccus* or *Halobacterium* and *Halarchaeum* are shown in Supplemental Figs. 12–17 and their properties are briefly summarised in Table 2.

Molecular characteristics which characterise important genus level groups within the class *Halobacteria*

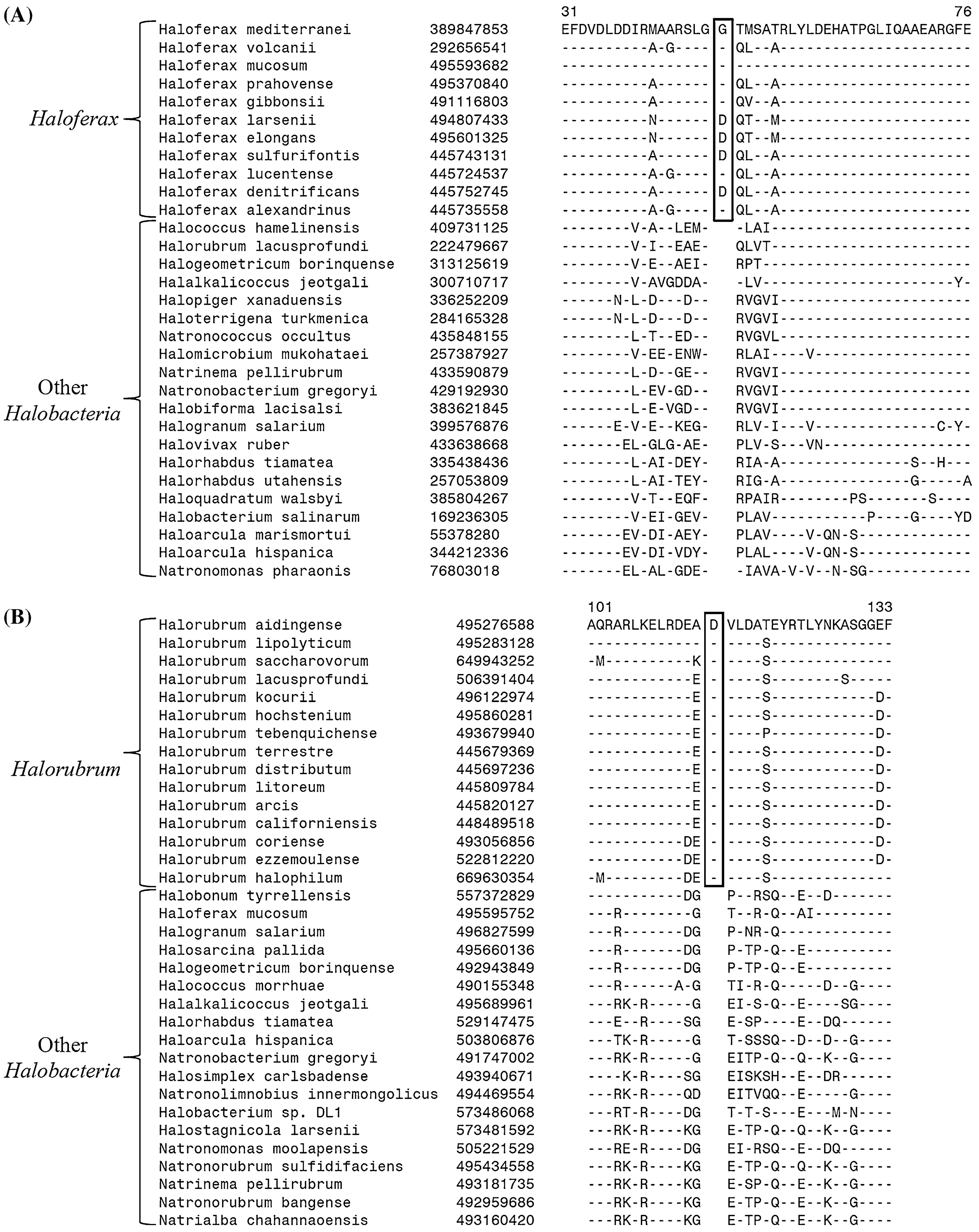
In addition to the CSIs and CSPs identified for groups of multiple genera within the class *Halobacteria*, we have also identified a number of CSIs and CSPs which are uniquely found within members of specific genera within the class. Within the order *Haloferacales*, we have identified seven CSIs and 38 CSPs which are restricted to the members of the genus *Haloferax*, and seven CSIs and 60 CSPs which are restricted to the members of the genus *Halorubrum*. Examples of CSIs which are uniquely found in members of the genera *Haloferax* and *Halorubrum*, respectively, are shown in Fig. 5. A one amino acid insertion that is specific for members of the genus *Haloferax* found in a hypothetical protein is shown in Fig. 5a whereas a one amino acid insertion that is specific for members of the genus *Halorubrum* which is present in the 50S ribosomal protein L19 is shown in Fig. 5b. In both cases, these inserts are unique characteristics of these genera and are not found in any other member of the class *Halobacteria*. The sequence alignments for the other identified *Haloferax* and *Halorubrum* CSIs are presented in Supplemental Figs. 18–29 and their properties are summarised in Table 2. The GI numbers for the seventeen CSPs specific for the genus *Haloferax* and the seven CSPs specific for the genus *Halorubrum* are provided in Table 3E, F, respectively.

We have also identified nine CSIs and twenty-three CSPs which are restricted to the large assemblage of sequenced species within the genus *Halococcus* and seventeen CSIs and 113 CSPs that are restricted to the genus *Haloarcula*, both of which are found within the order *Halobacteriales*. An example of a CSI which is specific for the genus *Halococcus* and another CSI which is specific for the genus *Haloarcula* are shown in Fig. 6. The first CSI, shown in Fig. 6a, consists of a two amino acid insertion in DNA gyrase subunit B that is uniquely found in the genus *Halococcus* whereas the second CSI, shown in Fig. 6b, consists of a five amino acid insertion in a *gufA* family protein that is uniquely found in the members of the genus *Haloarcula*. The sequence alignments for eight additional CSIs which are specific for the genus *Halococcus* and sixteen additional CSIs which are specific for the genus *Haloarcula* are shown in Supplemental Figs. 30–53 and their properties are briefly summarised in Table 2. The identification numbers for the 23 CSPs which are specific for the genus *Halococcus* and the 113 CSPs specific for the genus *Haloarcula* are provided in Table 3D, G, respectively.

Fig. 5 Partial sequence alignments of **a** a hypothetical protein ▶ showing a one amino acid insertion (*boxed*) identified in all sequenced members of the genus *Haloferax*, **b** 50S ribosomal protein L19 showing a one amino acid insertion (*boxed*) identified in all sequenced members of the genus *Halorubrum*. Sequence information for a limited number of *Halobacteria* and other archaea are shown here, but unless otherwise indicated similar CSIs were detected in all members of the indicated group and not detected in any other species in the top 250 Blastp hits. The characteristics of all identified CSIs specific for the genera *Haloferax* and *Halorubrum* are summarized in Table 2

Discussion

The analyses presented here, in the form of a highly robust phylogenetic tree based on 266 proteins obtained from 129 genome sequenced members of the class *Halobacteria* (Fig. 1), the 20 identified CSIs (Table 1), and the 31 identified CSPs (Table 3) provide a reliable basis for understanding the infraorder level relationships of genera within the class *Halobacteria*. These analyses have identified a number of family level clusters within the orders *Haloferacales* and *Halobacteriales* which are supported by a large number of CSIs and CSPs (Fig. 7). The result of phylogenomic studies and identified molecular characteristics provide strong evidence that the order *Haloferacales* contains two main groups, one consisting of *Haloferax* and related genera and the other



(A)

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                                                         31                                            76
            Haloferax mediterranei           389847853   EFDVDLDDIRMAARSLG G TMSATRLYLDEHATPGLIQAAEARGFE
            Haloferax volcanii               292656541   ----------A-G---- - QL--A----------------------
            Haloferax mucosum                495593682   ----------------- - ---------------------------
            Haloferax prahovense             495370840   ----------A------ - QL--A----------------------
            Haloferax gibbonsii              491116803   ----------A------ - QV--A----------------------
Haloferax   Haloferax larsenii               494807433   ----------N------ D QT--M----------------------
            Haloferax elongans               495601325   ----------N------ D QT--M----------------------
            Haloferax sulfurifontis          445743131   ----------A------ D QL--A----------------------
            Haloferax lucentense             445724537   ----------A-G---- - QL--A----------------------
            Haloferax denitrificans          445752745   ----------A------ D QL--A----------------------
            Haloferax alexandrinus           445735558   ----------A-G---- - QL--A----------------------
            Halococcus hamelinensis          409731125   --------V-A--LEM-   -LAI-----------------------
            Halorubrum lacusprofundi         222479667   --------V-I--EAE-   QLVT-----------------------
            Halogeometricum borinquense      313125619   --------V-E--AEI-   RPT------------------------
            Halalkalicoccus jeotgali         300710717   --------V-AVGDDA-   -LV----------------------Y-
            Halopiger xanaduensis            336252209   ------N-L-D---D--   RVGVI----------------------
            Haloterrigena turkmenica         284165328   ------N-L-D---D--   RVGVI----------------------
            Natronococcus occultus           435848155   --------L-T--ED--   RVGVL----------------------
            Halomicrobium mukohataei         257387927   --------V-EE-ENW-   RLAI----V------------------
            Natrinema pellirubrum            433590879   --------L-D--GE--   RVGVI----------------------
Other       Natronobacterium gregoryi        429192930   --------L-EV-GD--   RVGVI----------------------
Halobacteria Halobiforma lacisalsi           383621845   --------L-E-VGD--   RVGVI----------------------
            Halogranum salarium              399576876   ------E-V-E--KEG-   RLV-I---V--------------C-Y-
            Halovivax ruber                  433638668   -------EL-GLG-AE-   PLV-S---VN-----------------
            Halorhabdus tiamatea             335438436   --------L-AI-DEY-   RIA-A---------------S--H---
            Halorhabdus utahensis            257053809   --------L-AI-TEY-   RIG-A---------------G-----A
            Haloquadratum walsbyi            385804267   --------V-T--EQF-   RPAIR--------PS-------S----
            Halobacterium salinarum          169236305   --------V-EI-GEV-   PLAV-----------P----G----YD
            Haloarcula marismortui           55378280    -------EV-DI-AEY-   PLAV----V-QN-S-------------
            Haloarcula hispanica             344212336   -------EV-DI-VDY-   PLAL----V-QN-S-------------
            Natronomonas pharaonis           76803018    -------EL-AL-GDE-   -IAVA-V-V--N-SG------------
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(B)

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                                                        101                          133
            Halorubrum aidingense               495276588   AQRARLKELRDEA D VLDATEYRTLYNKASGGEF
            Halorubrum lipolyticum              495283128   ------------- - ----S--------------
            Halorubrum saccharovorum            649943252   -M----------K - ----S--------------
            Halorubrum lacusprofundi            506391404   ------------E - ----S--------S-----
            Halorubrum kocurii                  496122974   ------------E - ----S------------D-
            Halorubrum hochstenium              495860281   ------------E - ----S------------D-
            Halorubrum tebenquichense           493679940   ------------E - ----P------------D-
Halorubrum  Halorubrum terrestre                445679369   ------------E - ----S------------D-
            Halorubrum distributum              445697236   ------------E - ----S------------D-
            Halorubrum litoreum                 445809784   ------------E - ----S------------D-
            Halorubrum arcis                    445820127   ------------E - ----S------------D-
            Halorubrum californiensis           448489518   ------------E - ----S------------D-
            Halorubrum coriense                 493056856   -----------DE - ----S------------D-
            Halorubrum ezzemoulense             522812220   -----------DE - ----S------------D-
            Halorubrum halophilum               669630354   -M---------DE - ----S--------------
            Halobonum tyrrellensis              557372829   -----------DG   P--RSQ--E--D-------
            Haloferax mucosum                   495595752   ---R--------G   T--R-Q--AI---------
            Halogranum salarium                 496827599   ---R-------DG   P-NR-Q-------------
            Halosarcina pallida                 495660136   ---R-------DG   P-TP-Q--E----------
            Halogeometricum borinquense         492943849   ---R-------DG   P-TP-Q--E----------
            Halococcus morrhuae                 490155348   ---R------A-G   TI-R-Q-----D--G----
            Halalkalicoccus jeotgali            495689961   ---RK-R-----G   EI-S-Q--E----SG----
            Halorhabdus tiamatea                529147475   ---E--R----SG   E-SP----E--DQ------
            Haloarcula hispanica                503806876   ---TK-R-----G   T-SSSQ--D--D--G----
Other       Natronobacterium gregoryi           491747002   ---RK-R-----G   EITP-Q--Q--K--G----
Halobacteria Halosimplex carlsbadense           493940671   ----K-R----SG   EISKSH--E--DR------
            Natronolimnobius innermongolicus    494469554   ---RK-R----QD   EITVQQ--E-----G----
            Halobacterium sp. DL1               573486068   ---RT-R----DG   T-T-S---E---M-N----
            Halostagnicola larsenii             573481592   ---RK-R----KG   E-TP-Q--Q--K--G----
            Natronomonas moolapensis            505221529   ---RE-R----DG   EI-RSQ--E--DQ------
            Natronorubrum sulfidifaciens        495434558   ---RK-R----KG   E-TP-Q--Q--K--G----
            Natrinema pellirubrum               493181735   ---RK-R----KG   E-SP-Q--E--K--G----
            Natronorubrum bangense              492959686   ---RK-R----KG   E-TP-Q--E--K--G----
            Natrialba chahannaoensis            493160420   ---RK-R----KG   E-TP-Q--E--K--G----
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(A)

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                                                                       45                                  83
               Halococcus thailandensis         495016103    DNAIDEALAGYCDSISVTVHD GE HPSVSVTDDGRGIPVD
               Halococcus hamelinensis          657898863    ---------------T--I-- -- ------A---------
Halococcus     Halococcus hamelinensis          494964320    ---------------T--I-- -- ------A---------
(Clade HB2)    Halococcus morrhuae              490156877    ----------H---------- D- ----------------
               Halococcus saccharolyticus       492982518    ----------H----E--I-- D- N---------------
               Halococcus salifodinae           491184798    ----------H----E--I-- D- D---------------
               Halococcus sediminicola          684138127    ----------H----T--I-- -- ----------------
               Halobacterium salinarum          501246227    --S----------R-D--L--    DG--------------
               Natrialba taiwanensis            493879799    --S----------E-T--I-E    DG--------------
               Natronobacterium gregoryi        491746858    --S-------H--D-T--I-E    DR--------------
               Halorhabdus utahensis            502460418    ----------H--H-E--I--    DG------------I-
               Halorhabdus tiamatea             495799274    ----------H--T-A--M--    DG----V-------I-
               Halalkalicoccus jeotgali         495692047    -------------T-G--I-E    DG----A---------
               Natronococcus amylolyticus       491714379    --S-------H--D-T--INA    DD--T-S---------
               Halorubrum kocurii               496125396    --S----------D-T-----    DN----S---------
               Haloarcula californiae           494342561    -------------N-D--I--    DG----S---------
               Halosimplex carlsbadense         493939945    --S---------------I--    DG----S---------
               Halosarcina pallida              495659081    --S----------E-----N-    DG----S-N-------
               Natronomonas pharaonis           499642726    -------------T-E-VIN-    DG--T-R---------
               Halostagnicola larsenii          573481605    --S-------H--D-T-SI--    DG--T-----------
               Natronorubrum tibetense          493008696    --S-------H--D-T--I-E    DN----A---------
               Natronorubrum sulfidifaciens     495434624    --S-------H--D-T-SI--    DG----A---------
               Halarchaeum acidiphilum          519065728    --S----------E-T--L--    DG----E---------
               Halarchaeum acidiphilum          545903639    --S----------E-T--L--    DG----E---------
               Haloterrigena turkmenica         502708250    --S-------H--D-T-SIN-    DG----A---------
Other          Haloterrigena limicola           495287608    --S----------D-T--I-E    DN----A---------
Halobacteria   Halogranum salarium              496767231    --S----------N-D--I-E    DD----E---------
               Haladaptatus paucihalophilus     495255637    --S------------D--I-E    DN---IA---------
               Natronolimnobius innermongolic   494469520    --S----------D-T--I-E    DD----A---------
               Halopiger xanaduensis            503644380    --S----------D-T-SI-E    DG----A---------
               Halobiforma lacisalsi            494239720    --S-------H--D-T--I-E    DR----A---------
               Halomicrobium mukohataei         506243902    --S-------H--E-T--I-E    DD----S---------
               Halomicrobium katesii            517069442    --S-------H--E-T--I-E    DD----S---------
               Halogeometricum borinquense      492946030    --S----------E-G-S--E    DG------N-------
               Haloferax mediterranei           490158128    --S----------A-E-AL-E    DG------N-------
               Haloquadratum walsbyi            544615984    ----------H--Q-D--L-E    DG------N-------
               Halovivax asiaticus              494970680    ----------H-ED-T--I-E    DG----V---------
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(B)

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                                                                117                                                   171
               Haloarcula marismortui           55379677    HSFPEGVAVGVSFAELGLEAATP ESAVG IVGVSVPLLAVFMTVAISIHNIPEGTA
               Haloarcula sinaiiensis           491103161   ----------------------- ----- ---------------------------
               Haloarcula japonica              490731674   -------------------S--- ----- -I-------------------------
               Haloarcula argentinensis         491680145   -------------------S--- D---- -I-------------------------
Haloarcula     Haloarcula amylolytica           495586597   -------------------S--- ----- -L-------------------V-----
               Haloarcula hispanica             344210671   -------------------S--- ----- -L-------------------V-----
               Haloarcula vallismortis          490653351   -------------------S--- A---S -I-------------------------
               Haloarcula californiae           448655660   ----------------------- ----- ---------------------------
               Haloarcula salaria               743691969   -------------------S--- ----- -L-------------------V-----
               Natronomonas moolapensis         452208417   -------------------GGL-       -L-------------------V-----
               Halopiger xanaduensis            336251720   -------------------GGLS       -L---I--------I------------
               Natrialba aegyptia               493717623   ------------------GGGVS       -L---I--------I------V-----
               Natronococcus jeotgali           495694848   ------------------DGGAS       -L-F-I--------I------------
               Natronolimnobius innermongolic   494470948   --------I-----D----GG-E       LL-FT-----I----------V-----
               Haloterrigena thermotolerans     493697943   --------I-----D----GGAQ       FL-FT--V--I---I------V-----
               Haloterrigena salina             496168000   -------------------GGVS       -L-F-I--------I------V-----
               Haloterrigena limicola           495286294   ------I-------D----GGAE       LL-FT---------I------------
               Halorubrum californiense         495719720   ------I-------D----GG-A       LF-FT----------------------
               Natronorubrum sulfidifaciens     495438555   ------I-------D----DGVA       FL-FT-----I----------------
               Natronorubrum bangense           492959967   ------I------SD----DGVA       FL-FT---------I------------
Other          Halobiforma lacisalsi            494234608   ------I-------D----GG-Q       -L-FT--V--I---I------V-----
Halobacteria   Natrinema versiforme             493475745   --------------D----GG-E       LL-FT--V--I----------V-----
               Natrinema pallidum               493195418   --------I-----D----GG-E       LL-FTI-V-------------------
               Natrinema gari                   495730226   --------I-----D----GG-E       LL-FTI-I--L---I------------
               Halobacterium sp. DL1            497447325   --------------DM--DGGF-       -L-FT---------I------V---L-
               Haloferax mediterranei           389846465   --------------D----GGFQ       LL-FA--------------------L-
               Halosarcina pallida              495665861   -----------A--D---DGGLR       VL--A-------------V--V---V-
               Halovivax ruber                  433638179   -----------------FDGGLS       VL-F-I---------------------
               Halovivax asiaticus              494971373   -----------------FDGGLA       VL-F-I---------------------
```

◀**Fig. 6** Partial sequence alignments of **a** a *gufA* family protein showing a five amino acid insertion (*boxed*) identified in all sequenced members of the genus *Haloarcula*, **b** DNA gyrase subunit B showing a two amino acid insertion (*boxed*) identified in all sequenced members of the genus *Halococcus*. Sequence information for a limited number of *Halobacteria* and other archaea are shown here, but unless otherwise indicated similar CSIs were detected in all members of the indicated group and not detected in any other species in the top 250 Blastp hits. The characteristics of all identified CSIs specific for the genera *Haloarcula* and *Halococcus* are summarized in Table 2

consisting of *Halorubrum* and related genera, each of which is supported by multiple lines of evidence.

The order *Halobacteriales*, as currently described (Gupta et al. 2015), is a polyphyletic assemblage of members of the class *Halobacteria* that do not fall into the monophyletic orders *Haloferacales* and *Natrialbales*. The present analysis has identified one large and consistently observed group of multiple genera within the order *Halobacteriales*, referred to as clade HB1, and a second clade consisting of the large assemblage of species which make up the genus *Halococcus*, referred to as clade HB2, which are both supported by a large number of identified CSIs and CSPs (Fig. 7). Prior research on the interrelationships of the species within the genus *Halococcus* has noted that the members of the genus are more genetically diverse than other genera within the class *Halobacteria* and that the genus *Halococcus* contains two phylotypes which may eventually be separated into two genera

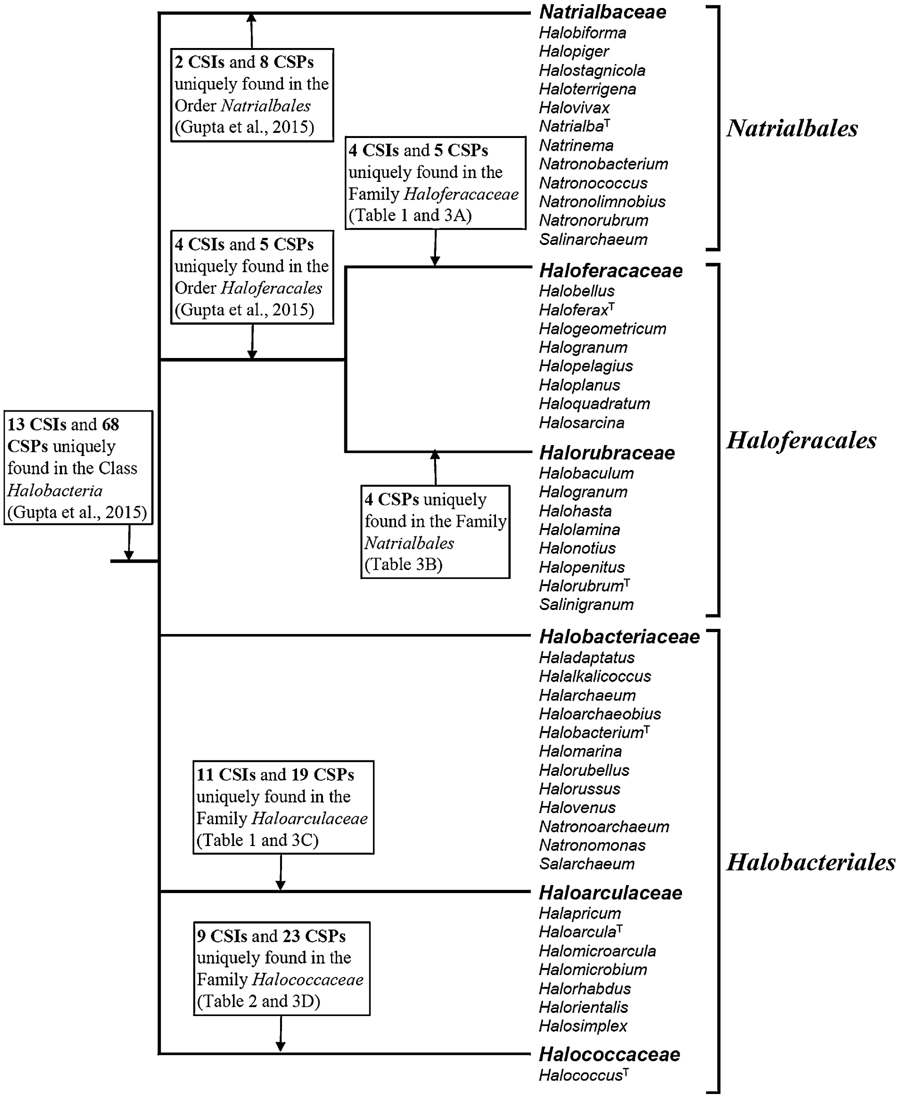

Fig. 7 A summary diagram depicting the distribution of identified CSIs and CSPs within the class *Halobacteria* and the proposed families described in this study. The genera within each family are listed under the corresponding family name. The superscript letter T beside a genus indicates that it is the type genus of the family

(Montero et al. 1993; Goh et al. 2006). It should be mentioned that in the rRNA tree, the genera *Halalkalicoccus*, *Haladaptus*, *Halorussus* and *Halrubellus* also group together with the *Halococcus* spp. (Clade HB2). An affinity of *Halococcus* and *Halalkalicoccus* is also supported by a number of identified CSIs and CSPs. Although we are presently limiting the clade HB2 to the genus *Halococcus*, with some additional research it is likely that this group will be expanded to include the genera *Halalkalicoccus*, *Haladaptus*, *Halorussus* and *Halrubellus*, which show some affinity to this clade. Further work is also required to clearly delimit the groups within the genus *Halococcus*.

The phylogenetic trees and molecular characteristics presented here also provide strong evidence for the separation of the genera *Halobacterium, Halarchaeum*, and *Salarchaeum* from the other members of the order *Halobacteriales* as a novel family level taxa. However, the Bacteriological Code (Lapage et al. 1992) states that a family consisting of the genera *Halobacterium, Halarchaeum*, and *Salarchaeum* must retain the name *Halobacteriaceae* precluding any reclassification of this group. Thus, an important task for the future will be to identify reliable, genome-derived characteristics, utilising novel techniques and the increasing wealth of genomic data, which can be used to classify the other members of the order *Halobacteriales* as members of distinct clades/families or clearly establish their close affinity to the grouping of the genera *Halobacterium, Halarchaeum*, and *Salarchaeum*.

The analyses presented here have also lead to the identification of 31 CSIs and 211 CSPs which are characteristic of *Haloferax*, *Halorubrum*, and *Haloarcula* (Table 2, 3E–G). Based upon these molecular characteristics, it is now possible to differentiate these groups of species from all other *Halobacteria* on the basis of the presence or absence of unique molecular features. Earlier work by our group on other groups of prokaryotes has shown that these CSIs and CSPs have strong predictive value and will likely be found in other members of these groups as more members are sequenced (Gao and Gupta 2012b; Gupta and Lali 2013; Bhandari and Gupta 2014; Howard-Azzeh et al. 2014). Additionally, the conserved nature of these CSIs and CSPs make them promising targets for the development of diagnostic assays that can be used to identify novel members of these genera from isolates or environmental samples (Ahmod et al. 2011; Wong et al. 2014). Further analyses of these genus specific molecular characteristics should also lead to the discovery of novel functions in these organisms mediated by CSIs and CSPs which may provide important insights into the physiology, evolution, and novel adaptations of these groups of *Halobacteria*.

Overall, the results presented here provide strong support for the presence of two family-level groups within the order *Haloferacales* and for two novel family level clusters within the polyphyletic order *Halobacteriales*. Based on the phylogenetic analyses, the identified CSIs and the identified CSPs presented in this work, we propose a division of the order *Haloferacales* into two families, an emended family *Haloferacaceae* (clade HF1) and *Halorubraceae* fam nov. (clade HF2) and a division of the order *Halobacteriales* into three families, *Haloarculaceae* fam. nov. (clade HB1), *Halococcaceae* fam. nov. (clade HB2), and an emended family *Halobacteriaceae,* containing all members of the order *Halobacteriales* that do not fall into clade HB1 or HB2. Descriptions of the new and emended families are provided below.

Emended description of the family *Halobacteriaceae* Gibbons 1974 (approved lists 1980)

The family *Halobacteriaceae* contains the type genus *Halobacterium* (Oren et al. 2009) and the genera *Haladaptatus* (Cui et al. 2010d)*, Halalkalicoccus* (Xue et al. 2005), *Halarchaeum* (Minegishi et al. 2010a), *Haloarchaeobius* (Yuan et al. 2015), *Halomarina* (Inoue et al. 2011), *Halorubellus* (Cui et al. 2012), *Halorussus* (Cui et al. 2010c), *Halovenus* (Makhdoumi-Kakhki et al. 2012), *Natronoarchaeum* (Shimane et al. 2010), *Natronomonas* (Burns et al. 2010b) and *Salarchaeum* (Shimane et al. 2011). The description of the family is the same as that of the order *Halobacteriales* given by Gupta et al. (2015) with the following modifications: The members of this order also lack the CSIs and CSPs that are specific for the families *Haloarculaceae* and *Halococcaceae*.

Description of *Haloarculaceae* fam. nov.

Haloarculaceae (Ha.lo.ar.cu.la.ce’ae. N.L. fem. n. *Haloarcula* type genus of the family; -*aceae* ending to denote a family; N.L. fem. pl. n. *Haloarculaceae* the family whose nomenclatural type is the genus *Haloarcula*).

The family *Haloarculaceae* contains the type genus *Haloarcula* (Oren et al. 2009) and the genera *Halapricum* (Song et al. 2014), *Halomicroarcula* (Echigo et al. 2013), *Halomicrobium* (Oren et al. 2002), *Halorhabdus* (Antunes et al. 2008), *Halorientalis* (Amoozegar et al. 2014), and *Halosimplex* (Han and Cui 2014). The description of the family is the same as that of the order *Halobacteriales* given by Gupta et al. (2015) with the following modifications: the members of the family *Haloarculaceae* can be distinguished from the *Halobacteriaceae* and all other archaea by the ten CSIs listed in Table 1 and by the twenty CSPs listed in Table 3C.

Description of *Halococcaceae* fam. nov.

Halococcaceae (Ha.lo.coc.ca.ce'ae.N.L. masc. n. *Halococcus* type genus of the family; -*aceae* ending to denote a family; N.L. masc. pl. n. *Halococcaceae* the family whose nomenclatural type is the genus *Halococcus*).

The family *Halococcaceae* contains only the type genus *Halococcus* (Oren et al. 2009). The description of the family is the same as that of the order *Halobacteriales* given by Gupta et al. (2015) with the following modifications: the members of the family *Halococcaceae* can be distinguished from the *Halobacteriaceae* and all other archaea by the nine CSIs listed in Table 2 and by 23 CSPs CSPs listed in Table 3D.

Emended description of the family *Haloferacaceae* Gupta 2015

The family *Haloferacaceae* contains the type genus *Haloferax* (Oren et al. 2009) and the genera *Halobellus* (Cui et al. 2011b), *Halogeometricum* (Cui et al. 2010e), *Halogranum* (Cui et al. 2011c), *Halopelagius* (Zhang et al. 2013), *Haloplanus* (Qiu et al. 2013), *Haloquadratum* (Burns et al. 2007), and *Halosarcina* (Cui et al. 2010a). The description of the family is the same as that of the order *Haloferacales* given by Gupta et al. (2015) with the following modifications: the members of the family *Haloferacaceae* can be distinguished from the *Halorubraceae* and all other archaea by their branching in phylogenetic trees and by the four CSIs listed in Table 1 and by the five CSPs listed in Table 2.

Description of *Halorubraceae* fam. nov.

Halorubraceae (Ha.lo.ru.bra.ce'ae. N.L. neut. n. *Halorubrum* type genus of the family; -*aceae* ending to denote a family; N.L. neut. pl. n. *Halorubraceae* the family whose nomenclatural type is the genus *Halorubrum*).

The family *Halorubraceae* contains the type genus *Halorubrum* (Oren et al. 2009) and the genera *Halobaculum* (Oren et al. 1995), *Halogranum* (Cui et al. 2010b), *Halohasta* (Mou et al. 2012), *Halolamina* (Cui et al. 2011a), *Halonotius* (Burns et al. 2010a), *Halopenitus* (Amoozegar et al. 2012), and *Salinigranum* (Cui and Zhang 2014). The description of the family is the same as that of the order *Haloferacales* given by Gupta et al. (2015) with the following modifications: the members of the family *Halorubraceae* can be distinguished from the *Haloferacaceae* and all other archaea by their branching in phylogenetic trees and by the four CSPs listed in Table 2.

References

Ahmod NZ, Gupta RS, Shah HN (2011) Identification of a *Bacillus anthracis* specific indel in the *yeaC* gene and development of a rapid pyrosequencing assay for distinguishing *B. anthracis* from the *B. cereus* group. J Microbiol Methods 87(3):278–285

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25(17):3389–3402

Amoozegar MA, Makhdoumi-Kakhki A, Shahzadeh Fazeli SA, Azarbaijani R, Ventosa A (2012) *Halopenitus persicus* gen. nov., sp. nov., an archaeon from an inland salt lake. Int J Syst Evol Microbiol 62(Pt 8):1932–1936

Amoozegar MA, Makhdoumi-Kakhki A, Mehrshad M, Fazeli SA, Sproer C, Ventosa A (2014) *Halorientalis persicus* sp. nov., an extremely halophilic archaeon isolated from a salt lake and emended description of the genus *Halorientalis*. Int J Syst Evol Microbiol 64(Pt 3):940–944

Andam CP, Harlow TJ, Papke RT, Gogarten JP (2012) Ancient origin of the divergent forms of leucyl-tRNA synthetases in the *Halobacteriales*. BMC Evol Biol 12:85

Antunes A, Taborda M, Huber R, Moissl C, Nobre MF, da Costa MS (2008) *Halorhabdus tiamatea* sp. nov., a non-pigmented, extremely halophilic archaeon from a deep-sea, hypersaline anoxic basin of the Red Sea, and emended description of the genus *Halorhabdus*. Int J Syst Evol Microbiol 58(Pt 1):215–220

Baliga NS, Bonneau R, Facciotti MT, Pan M, Glusman G, Deutsch EW, Shannon P, Chiu Y, Weng RS, Gan RR et al (2004) Genome sequence of Haloarcula marismortui: a halophilic archaeon from the Dead Sea. Genome Res 14(11):2221–2234

Bhandari V, Gupta RS (2014) Molecular signatures for the phylum (class) *Thermotogae* and a proposal for its division into three orders (*Thermotogales*, *Kosmotogales* ord. nov. and *Petrotogales* ord. nov.) containing four families (*Thermotogaceae*, *Fervidobacteriaceae* fam. nov., *Kosmotogaceae* fam. nov. and *Petrotogaceae* fam. nov.) and a new genus *Pseudothermotoga* gen. nov. with five new combinations. Anton Leeuw Int J G 105(1):143–168

Boucher Y, Douady CJ, Sharma AK, Kamekura M, Doolittle WF (2004) Intragenomic heterogeneity and intergenomic recombination among haloarchaeal rRNA genes. J Bacteriol 186(12):3980–3990

Burns DG, Janssen PH, Itoh T, Kamekura M, Li Z, Jensen G, Rodriguez-Valera F, Bolhuis H, Dyall-Smith ML (2007) *Haloquadratum walsbyi* gen. nov., sp. nov., the square haloarchaeon of Walsby, isolated from saltern crystallizers in Australia and Spain. Int J Syst Evol Microbiol 57(Pt 2):387–392

Burns DG, Janssen PH, Itoh T, Kamekura M, Echigo A, Dyall-Smith ML (2010a) *Halonotius pteroides* gen. nov., sp. nov., an extremely halophilic archaeon recovered from a saltern crystallizer. Int J Syst Evol Microbiol 60(Pt 5):1196–1199

Burns DG, Janssen PH, Itoh T, Minegishi H, Usami R, Kamekura M, Dyall-Smith ML (2010b) *Natronomonas moolapensis* sp. nov., non-alkaliphilic isolates recovered from a solar saltern crystallizer pond, and emended description of the genus *Natronomonas*. Int J Syst Evol Microbiol 60(Pt 5):1173–1176

Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol Biol Evol 17(4):540–552

Ciccarelli FD, Doerks T, Von Mering C, Creevey CJ, Snel B, Bork P (2006) Toward automatic reconstruction of a highly resolved tree of life. Science 311(5765):1283–1287

Cole J, Wang Q, Fish J, Chai B, McGarrell D, Sun Y, Brown C, Porras-Alfaro A, Kuske C, Tiedje J (2014) Ribosomal database project: data and tools for high throughput rRNA analysis. Nucleic Acids Res 42(1):D633

Cui HL, Zhang WJ (2014) *Salinigranum rubrum* gen. nov., sp. nov., a member of the family *Halobacteriaceae* isolated from a marine solar saltern. Int J Syst Evol Microbiol 64(Pt 6):2029–2033

Cui HL, Zhou PJ, Oren A, Liu SJ (2009) Intraspecific polymorphism of 16S rRNA genes in two halophilic archaeal genera, *Haloarcula* and *Halomicrobium*. Extremophiles 13(1):31–37

Cui HL, Gao X, Li XY, Xu XW, Zhou YG, Liu HC, Zhou PJ (2010a) *Halosarcina limi* sp. nov., a halophilic archaeon from a marine solar saltern, and emended description of the genus *Halosarcina*. Int J Syst Evol Microbiol 60(Pt 10):2462–3466

Cui HL, Gao X, Sun FF, Dong Y, Xu XW, Zhou YG, Liu HC, Oren A, Zhou PJ (2010b) *Halogranum rubrum* gen. nov., sp. nov., a halophilic archaeon isolated from a marine solar saltern. Int J Syst Evol Microbiol 60(Pt 6):1366–1371

Cui HL, Gao X, Yang X, Xu XW (2010c) *Halorussus rarus* gen. nov., sp. nov., a new member of the family *Halobacteriaceae* isolated from a marine solar saltern. Extremophiles 14(6):493–499

Cui HL, Sun FF, Gao X, Dong Y, Xu XW, Zhou YG, Liu HC, Oren A, Zhou PJ (2010d) *Haladaptatus litoreus* sp. nov., an extremely halophilic archaeon from a marine solar saltern, and emended description of the genus *Haladaptatus*. Int J Syst Evol Microbiol 60(Pt 5):1085–1089

Cui HL, Yang X, Gao X, Li XY, Xu XW, Zhou YG, Liu HC, Zhou PJ (2010e) *Halogeometricum rufum* sp. nov., a halophilic archaeon from a marine solar saltern, and emended description of the genus *Halogeometricum*. Int J Syst Evol Microbiol 60(Pt 11):2613–2617

Cui HL, Gao X, Yang X, Xu XW (2011a) *Halolamina pelagica* gen. nov., sp. nov., a new member of the family *Halobacteriaceae*. Int J Syst Evol Microbiol 61(Pt 7):1617–1621

Cui HL, Yang X, Gao X, Xu XW (2011b) *Halobellus clavatus* gen. nov., sp. nov. and *Halorientalis regularis* gen. nov., sp. nov., two new members of the family *Halobacteriaceae*. Int J Syst Evol Microbiol 61(Pt 11):2682–2689

Cui HL, Yang X, Gao X, Xu XW (2011c) *Halogranum gelatinilyticum* sp. nov. and *Halogranum amylolyticum* sp. nov., isolated from a marine solar saltern, and emended description of the genus *Halogranum*. Int J Syst Evol Microbiol 61(Pt 4):911–915

Cui HL, Mou YZ, Yang X, Zhou YG, Liu HC, Zhou PJ (2012) *Halorubellus salinus* gen. nov., sp. nov. and *Halorubellus litoreus* sp. nov., novel halophilic archaea isolated from a marine solar saltern. Syst Appl Microbiol 35(1):30–34

Echigo A, Minegishi H, Shimane Y, Kamekura M, Itoh T, Usami R (2013) *Halomicroarcula pellucida* gen. nov., sp. nov., a non-pigmented, transparent-colony-forming, halophilic archaeon isolated from solar salt. Int J Syst Evol Microbiol 63(Pt 10):3556–3562

Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26(19):2460–2461

Enache M, Itoh T, Fukushima T, Usami R, Dumitru L, Kamekura M (2007) Phylogenetic relationships within the family *Halobacteriaceae* inferred from *rpoB* ‘ gene and protein sequences. Int J Syst Evol Microbiol 57:2289–2295

Gao B, Gupta R (2007) Phylogenomic analysis of proteins that are distinctive of Archaea and its main subgroups and the origin of methanogenesis. BMC Genomics 8(1):86

Gao B, Gupta RS (2012a) Microbial systematics in the post-genomics era. Anton Leeuw Int J G 101(1):45–54

Gao B, Gupta RS (2012b) Phylogenetic framework and molecular signatures for the main clades of the phylum Actinobacteria. Microbiol Mol Biol Rev 76(1):66–112

Goh F, Leuko S, Allen MA, Bowman JP, Kamekura M, Neilan BA, Burns BP (2006) *Halococcus hamelinensis* sp. nov., a novel halophilic archaeon isolated from stromatolites in Shark Bay, Australia. Int J Syst Evol Microbiol 56(6):1323–1329

Grant WD, Kamekura M, McGenity TJ, Ventosa A (2001) Class III. *Halobacteria* class. nov. In: Boone, Castenholz RW, Garrity GM, Bergey DH (eds) Bergey’s manual of systematic bacteriology, vol 1, 2nd edn. Springer, New York

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol 59(3):307–321

Gupta RS (2014) Identification of conserved indels that are useful for classification and evolutionary studies methods in microbiology, vol 41. Academic Press, Oxford

Gupta RS, Lali R (2013) Molecular signatures for the phylum Aquificae and its different clades: proposal for division of the phylum Aquificae into the emended order *Aquificales*, containing the families *Aquificaceae* and *Hydrogenothermaceae*, and a new order *Desulfurobacteriales* ord. nov., containing the family *Desulfurobacteriaceae*. Anton Leeuw Int J G 104(3):349–368

Gupta RS, Mok A (2007) Phylogenomics and signature proteins for the *Alphaproteobacteria* and its main groups. BMC Microbiol 7(1):106

Gupta RS, Naushad S, Baker S (2015) Phylogenomic analyses and molecular signatures for the class Halobacteria and its two major clades: a proposal for division of the class Halobacteria into an emended order Halobacteriales and two new orders, Haloferacales ord. nov. and Natrialbales ord. nov., containing the novel families Haloferacaceae fam. nov. and Natrialbaceae fam. nov. Int J Syst Evol Microbiol 65(Pt 3):1050–1069

Han D, Cui HL (2014) *Halosimplex pelagicum* sp. nov. and *Halosimplex rubrum* sp. nov., isolated from salted brown alga Laminaria, and emended description of the genus *Halosimplex*. Int J Syst Evol Microbiol 64(Pt 1):169–173

Hartman AL, Norais C, Badger JH, Delmas S, Haldenby S, Madupu R, Robinson J, Khouri H, Ren Q, Lowe TM et al (2010) The complete genome sequence of Haloferax volcanii DS2, a model archaeon. PLoS One 5(3):e9605

Howard-Azzeh M, Shamseer L, Schellhorn HE, Gupta RS (2014) Phylogenetic analysis and molecular signatures defining a monophyletic clade of heterocystous cyanobacteria and identifying its closest relatives. Photosynth Res 122(2):171–185

Inoue K, Itoh T, Ohkuma M, Kogure K (2011) *Halomarina oriensis* gen. nov., sp. nov., a halophilic archaeon isolated from a seawater aquarium. Int J Syst Evol Microbiol 61(Pt 4):942–946

Jeanmougin F, Thompson JD, Gouy M, Higgins DG, Gibson TJ (1998) Multiple sequence alignment with Clustal X. Trends Biochem Sci 23(10):403

Jones AL (2012) The future of taxonomy. In: Gadd GM, Sariaslani S (eds) Advances in applied microbiology, vol 80, 1st edn. Academic Press Inc, San Diego, pp 23–35

Klenk HP, Goker M (2010) En route to a genome-based classification of Archaea and Bacteria? Syst Appl Microbiol 33(4):175–182

Lapage SP, Sneath PHA, Lessel EF, Skerman VBD, Seeliger HPR, Clark WA (1992) International code of nomenclature of bacteria: bacteriological code, 1990 revision. ASM Press International Union of Microbiological Societies, Washington, DC

Le SQ, Gascuel O (2008) An improved general amino acid replacement matrix. Mol Biol Evol 25(7):1307–1320

Makhdoumi-Kakhki A, Amoozegar MA, Ventosa A (2012) *Halovenus aranensis* gen. nov., sp. nov., an extremely halophilic archaeon from Aran-Bidgol salt lake. Int J Syst Evol Microbiol 62(Pt 6):1331–1336

Minegishi H, Echigo A, Nagaoka S, Kamekura M, Usami R (2010a) *Halarchaeum acidiphilum* gen. nov., sp. nov., a moderately acidophilic haloarchaeon isolated from commercial solar salt. Int J Syst Evol Microbiol 60(Pt 11):2513–2516

Minegishi H, Kamekura M, Itoh T, Echigo A, Usami R, Hashimoto T (2010b) Further refinement of the phylogeny of the *Halobacteriaceae* based on the full-length RNA polymerase subunit B ‘ (*rpoB* ‘) gene. Int J Syst Evol Microbiol 60:2398–2408

Montero CG, Klenk H-P, Nieto JJ, Ventosa A (1993) DNA-rRNA hybridization studies on *Halococcus saccharolyticus* and other halobacteria. FEMS Microbiol Lett 111(1):69–72

Mou YZ, Qiu XX, Zhao ML, Cui HL, Oh D, Dyall-Smith ML (2012) *Halohasta litorea* gen. nov. sp. nov., and *Halohasta litchfieldiae* sp. nov., isolated from the Daliang aquaculture farm, China and from Deep Lake, Antarctica, respectively. Extremophiles 16(6):895–901

Mylvaganam S, Dennis PP (1992) Sequence heterogeneity between the two genes encoding 16S rRNA from the halophilic archaebacterium *Haloarcula marismortui*. Genetics 130(3):399–410

NamesforLife (2015) NamesforLife. http://www.namesforlife.com/

Naor A, Lapierre P, Mevarech M, Papke RT, Gophna U (2012) Low species barriers in halophilic archaea and the formation of recombinant hybrids. Curr Biol 22(15):1444–1448

Naushad HS, Lee B, Gupta RS (2014) Conserved signature indels and signature proteins as novel tools for understanding microbial phylogeny and systematics: identification of molecular signatures that are specific for the phytopathogenic genera *Dickeya*, *Pectobacterium* and *Brenneria*. Int J Syst Evol Microbiol 64(2):366–383

Naushad S, Adeolu M, Wong S, Sohail M, Schellhorn HE, Gupta RS (2015) A phylogenomic and molecular marker based taxonomic framework for the order *Xanthomonadales*: proposal to transfer the families *Algiphilaceae* and *Solimonadaceae* to the order *Nevskiales* ord. nov. and to create a new family within the order *Xanthomonadales*, the family *Rhodanobacteraceae* fam. nov., containing the genus *Rhodanobacter* and its closest relatives. Antonie Van Leeuwenhoek 107(2):467–485

NCBI (2015) NCBI genome database. http://www.ncbi.nlm.nih.gov/genome/

Oren A (2006) The order *Halobacteriales*. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E (eds) The prokaryotes. Springer, New York, pp 113–164

Oren A (2012) Taxonomy of the family *Halobacteriaceae*: a paradigm for changing concepts in prokaryote systematics. Int J Syst Evol Microbiol 62(Pt 2):263–271

Oren A, Garrity GM (2014) Then and now: a systematic review of the systematics of prokaryotes in the last 80 years. Antonie Van Leeuwenhoek 106(1):43–56

Oren A, Gurevich P, Gemmell RT, Teske A (1995) *Halobaculum gomorrense* gen. nov., sp. nov., a novel extremely halophilic archaeon from the Dead Sea. Int J Syst Bacteriol 45(4):747–754

Oren A, Elevi R, Watanabe S, Ihara K, Corcelli A (2002) *Halomicrobium mukohataei* gen. nov., comb. nov., and emended description of *Halomicrobium mukohataei*. Int J Syst Evol Microbiol 52(Pt 5):1831–1835

Oren A, Arahal DR, Ventosa A (2009) Emended descriptions of genera of the family *Halobacteriaceae*. Int J Syst Evol Microbiol 59(Pt 3):637–642

Papke RT, White E, Reddy P, Weigel G, Kamekura M, Minegishi H, Usami R, Ventosa A (2011) A multilocus sequence analysis approach to the phylogeny and taxonomy of the *Halobacteriales*. Int J Syst Evol Microbiol 61(Pt 12):2984–2995

Parte AC (2013) LPSN-list of prokaryotic names with standing in nomenclature. Nucleic Acids Res 42:D613–D616

Pfeiffer F, Schuster SC, Broicher A, Falb M, Palm P, Rodewald K, Ruepp A, Soppa J, Tittor J, Oesterhelt D (2008) Evolution in the laboratory: the genome of Halobacterium salinarum strain R1 compared to that of strain NRC-1. Genomics 91(4):335–346

Price MN, Dehal PS, Arkin AP (2010) FastTree 2–approximately maximum-likelihood trees for large alignments. PLoS One 5(3):e9490

Pruesse E, Peplies J, Glockner FO (2012) SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. Bioinformatics 28(14):1823–1829

Qiu XX, Zhao ML, Han D, Zhang WJ, Cui HL (2013) *Haloplanus salinus* sp. nov., an extremely halophilic archaeon from a Chinese marine solar saltern. Arch Microbiol 195(12):799–803

Rokas A, Holland PWH (2000) Rare genomic changes as a tool for phylogenetics. Trends Ecol Evol 15(11):454–459

Rokas A, Williams BL, King N, Carroll SB (2003) Genome-scale approaches to resolving incongruence in molecular phylogenies. Nature 425(6960):798–804

Rossello-Mora R, Amann R (2015) Past and future species definitions for Bacteria and Archaea. Syst Appl Microbiol 38(4):209–216

Sawana A, Adeolu M, Gupta RS (2014) Molecular signatures and phylogenomic analysis of the genus Burkholderia: proposal for division of this genus into the emended genus Burkholderia containing pathogenic organisms and a new genus Paraburkholderia gen. nov. harboring environmental species. Front Genet 5:429

Shimane Y, Hatada Y, Minegishi H, Mizuki T, Echigo A, Miyazaki M, Ohta Y, Usami R, Grant WD, Horikoshi K (2010) *Natronoarchaeum mannanilyticum* gen. nov., sp. nov., an aerobic, extremely halophilic archaeon isolated from commercial salt. Int J Syst Evol Microbiol 60(Pt 11):2529–2534

Shimane Y, Hatada Y, Minegishi H, Echigo A, Nagaoka S, Miyazaki M, Ohta Y, Maruyama T, Usami R, Grant WD et al (2011) *Salarchaeum japonicum* gen. nov., sp. nov., an aerobic, extremely halophilic member of the Archaea isolated from commercial salt. Int J Syst Evol Microbiol 61(Pt 9):2266–2270

Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J et al (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol 7(1):539

Song HS, Cha IT, Yim KJ, Lee HW, Hyun DW, Lee SJ, Rhee SK, Kim KN, Kim D, Choi JS et al (2014) *Halapricum salinum* gen. nov., sp. nov., an extremely halophilic archaeon isolated from non-purified solar salt. Antonie Van Leeuwenhoek 105(5):979–986

Soucy SM, Fullmer MS, Papke RT, Gogarten JP (2014) Inteins as indicators of gene flow in the halobacteria. Front Microbiol, 5

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9):1312–1313

Sutcliffe IC (2015) Challenging the anthropocentric emphasis on phenotypic testing in prokaryotic species descriptions: rip it up and start again. Front Genet 6:218

Talavera G, Castresana J (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst Biol 56(4):564–577

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30(12):2725–2729

Tavaré S (1986) Some probabilistic and statistical problems in the analysis of DNA sequences. In: Miura RM (ed) Lectures on mathematics in the life sciences, 17th edn. American Mathematical Society, Providence, RI, pp 57–86

Vreeland RH, Straight S, Krammes J, Dougherty K, Rosenzweig WD, Kamekura M (2002) *Halosimplex carlsbadense* gen. nov., sp. nov., a unique halophilic archaeon, with three 16S rRNA genes, that grows only in defined medium with glycerol and acetate or pyruvate. Extremophiles 6(6):445–452

Walsh DA, Bapteste E, Kamekura M, Doolittle WF (2004) Evolution of the RNA polymerase B' subunit gene (*rpoB*') in *Halobacteriales*: a complementary molecular marker to the SSU rRNA gene. Mol Biol Evol 21(12):2340–2351

Whelan S, Goldman N (2001) A general empirical model of protein evolution derived from multiple protein families using a maximum-likelihood approach. Mol Biol Evol 18(5):691–699

Whitman WB (2015) Genome sequences as the type material for taxonomic descriptions of prokaryotes. Syst Appl Microbiol 38(4):217–222

Williams D, Gogarten JP, Papke RT (2012) Quantifying homologous replacement of loci between haloarchaeal species. Genome Biol Evol 4(12):1223–1244

Wong SY, Paschos A, Gupta RS, Schellhorn HE (2014) Insertion/deletion-based approach for the detection of *Escherichia coli* O_{157}:H_7 in freshwater environments. Environ Sci Technol 48(19):11462–11470

Wright AD (2006) Phylogenetic relationships within the order *Halobacteriales* inferred from 16S rRNA gene sequences. Int J Syst Evol Microbiol 56(Pt 6):1223–1227

Wu D, Hugenholtz P, Mavromatis K, Pukall R, Dalin E, Ivanova NN, Kunin V, Goodwin L, Wu M, Tindall BJ (2009) A phylogeny-driven genomic encyclopaedia of Bacteria and Archaea. Nature 462(7276):1056–1060

Xue Y, Fan H, Ventosa A, Grant WD, Jones BE, Cowan DA, Ma Y (2005) *Halalkalicoccus tibetensis* gen. nov., sp. nov., representing a novel genus of haloalkaliphilic archaea. Int J Syst Evol Microbiol 55(Pt 6):2501–2505

Yuan PP, Zhang WJ, Han D, Cui HL (2015) *Haloarchaeobius salinus* sp. nov., isolated from an inland salt lake, and emended description of the genus *Haloarchaeobius*. Int J Syst Evol Microbiol 65(Pt 3):910–914

Zhang X, Zhang WY, Shen AH, Huo YY, Zhu XF, Wu M (2013) *Halopelagius longus* sp. nov., a member of the family *Halobacteriaceae* isolated from a salt mine, and emended description of the genus *Halopelagius*. Int J Syst Evol Microbiol 63(Pt 10):3585–3590

Zhi X-Y, Zhao W, Li W-J, Zhao G-P (2012) Prokaryotic systematics in the genomics era. Anton Leeuw Int J G 101(1):21–34