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Taxonomy of halophilic Archaea: current status and future challenges

Aharon Oren

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Abstract Several groups of Archaea, all *Euryarchaeota*, develop in hypersaline environments (from >10 % salt up to saturation). The cultured diversity of halophilic Archaea includes the family *Halobacteriaceae* of aerobic or facultative anaerobic, generally red-pigmented species (47 genera and 165 species as of February 2014) and seven representatives of four genera of methanogens, most of which obtain energy from methylated amines under anaerobic conditions. Metagenomic studies have identified an additional deep lineage of Archaea in salt lakes and ponds with brines approaching NaCl saturation. Genomic information is now available for representatives of these 'Nanohaloarchaea', but no members of this lineage have yet been cultured. Multilocus sequence analysis is becoming increasingly popular in taxonomic studies of the *Halobacteriaceae*, and such studies have demonstrated that recombination of genetic traits occurs at an extremely high frequency at least in some genera. Metagenomic studies in an Antarctic lake showed that large identical regions of up to 35 kb in length can be shared by members of different genera living together in the same environment. Such observations have important implications not only for the taxonomy of the *Halobacteriaceae*, but also for species concepts and questions on taxonomy and classification for prokaryotic microorganisms in general.

Keywords *Halobacteriaceae* · Halophilic methanogens · Nanohaloarchaea · Taxonomy · Nomenclature · Species concepts

Abbreviations

ANI	Average nucleotide identity
MLSA	Multilocus sequence analysis
Hbt.	*Halobacterum*
Hfx.	*Haloferax*
Hht.	*Halohasta*
Hqr.	*Haloquadratum*
Hrr.	*Halorubrum*

Introduction

> "The halobacteria were once thought related to the pseudomonads, and possible ways in which they were derived from this group have been discussed (…). I think it a mistake to consider the red halophiles as very closely related to any other known bacterial genus. We may hope for further revelations of their taxonomic status by comparisons of specific ribosomal proteins, of RNA's, and of other macromolecules of known function."

These prophetic words were written by Donn Kushner in his classic review on "Life in high salt and solute concentrations: halophilic bacteria" (Kushner 1978). In the same year, Magrum et al. (1978) asked the question: "Are extreme halophiles actually "bacteria"?". This was the time when Carl Woese and coworkers first proposed the

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Three-letter abbreviations for genera of *Halobacteriaceae* were used as recommended (Oren and Ventosa 2013)

A. Oren (✉)
Department of Plant and Environmental Sciences, The Institute of Life Sciences, The Hebrew University of Jerusalem, Edmond J. Safra Campus, Givat Ram, 91904 Jerusalem, Israel
e-mail: aharon.oren@mail.huji.ac.il

existence of the third domain of life, the Archaea. *Halobacterium* and other red-pigmented extreme halophiles classified in the family *Halobacteriaceae* were quickly recognized to be part of the archaeal domain.

The *Halobacteriaceae* are the halophiles par excellence: many members of the family grow well in NaCl-saturated media and most species require molar concentrations of salt. However, they are by no means the only halophilic representatives of the Archaea. If is often forgotten that there are quite a few methanogenic Archaea that grow up to very high salt concentrations and play important roles in anaerobic hypersaline environments. Moreover, metagenomics studies have shown the presence of at least one additional deep lineage of Archaea (the 'Nanohaloarchaea') in salt lakes and saltern ponds with brines approaching NaCl saturation. No representatives of this lineage have yet been cultured. All these halophiles belong to the *Euryarchaeota* phylum.

This essay was written as background information for a taxonomy workshop to be held as part of the Extremophiles 2014 congress in Saint Petersburg, Russia, in September 2014. It aims at presenting the status of the taxonomy of the different groups of halophilic Archaea and discussing the current problems and possible future developments.

Taxonomy of the *Halobacteriaceae*: current status

As explained in an earlier review paper (Oren 2012), the *Halobacteriaceae* are an excellent group to show how the classification of microorganisms has changed over time following changes in taxonomic concepts and developments in methodology. At the time of writing (February 2014), the family encompassed 47 genera and 165 species with names with standing in the nomenclature of prokaryotes. A few genera have a large number of species (*Halorubrum*: 25; *Haloferax*: 12; *Haloarcula*: 10), but most contain a few species only.

The Subcommittee on the Taxonomy of *Halobacteriaceae* is undoubtedly one of the most active of all Subcommittees on Taxonomy of the International Committee on Systematics of Prokaryotes, as judged by the published minutes of its frequent meetings (for the most recent documents see Arahal et al. 2011; Oren and Ventosa 2008, 2010, 2013; Oren et al. 2007). The Subcommittee has recommended minimal standards for the description of new taxa belonging to the group (Oren et al. 1997), and from time to time updates these standards according to the current needs. The records of the Subcommittee give an excellent picture of the number of new taxa described: from 26 genera with 85 species in August 2007 (Oren et al. 2007) to 29 genera with 100 species in June 2010 (Oren and Ventosa 2010), 33 genera with 126 species in August 2011 (Arahal et al. 2011), and 40 genera with 144 species in June 2013 (Oren and Ventosa 2013). In June 2013, genome sequence information was available for 68 type strains and for 15 additional strains at least (Oren and Ventosa 2013).

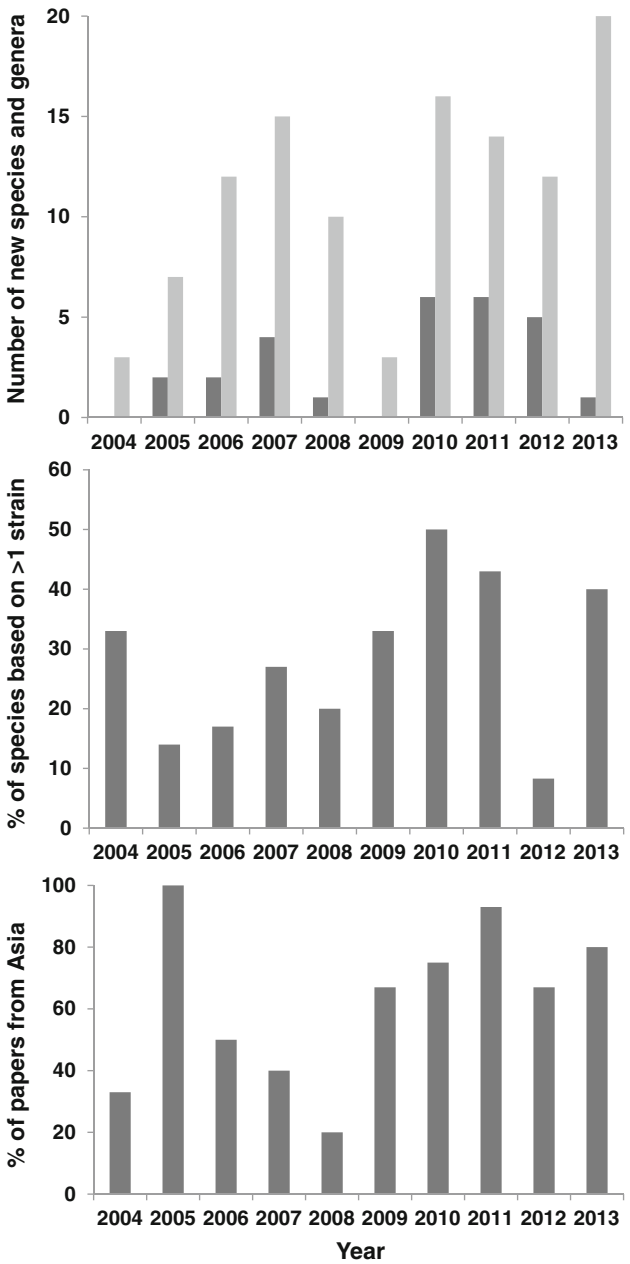

Fig. 1 The numbers of new taxa of *Halobacteriaceae* described in the past decade. The upper panel shows the numbers of names of new species (*grey bars*) and genera (*black bars*) effectively published each year and subsequently validated. Not included were new combinations and the species *Halobacterium piscisalsi*, later proposed as a later heterotypic synonym of *Hbt. salinarum*. The middle and the lower panels present the percentage of the new species descriptions based on more than one isolate and the percentages of new species described by Asian scientists, based on the address of the corresponding author of the publications

Figure 1 summarizes some of the statistics relating to the 112 new species and the 27 new genera of *Halobacteriaceae* described in the past 10 years. A surprisingly large number of these new species descriptions were based on more than one isolate: 33 out of the 112 species, i.e., 29 %. This is a good record compared with the value of 21.6 % calculated for the 194 new species described in the last three issues of the International Journal of Systematic and Evolutionary Microbiology published in 2013. The lower part of Fig. 1 shows that the great majority of papers presenting new taxa of *Halobacteriaceae* were submitted by authors from the Asian continent. China is the leading country here. These data can be compared with the overall percentage of new prokaryote taxa described by Asian authors in the past year: ~67 % in the years 2012–2013, as compared to only ~4 % in 2000–2003 (Oren and Garrity 2014).

When looking at the new species names of *Halobacteriaceae* added in the past 10 years, it may be noted that about 30 % of the specific epithets are 'geographical' names, indicating the location of the country, city, lake, etc. from which the type strain was isolated. There has been a call to avoid 'localimania' when giving names to new species (Trüper 2005). The frequency of such geographical epithets still used for new halophilic Archaea is similar to that for all prokaryotes in recent years.

The Subcommittee also alerted scientists active in the field of halophile taxonomy that many of the genus descriptions needed emendation as the properties of new species assigned to existing genera often did not agree with the earlier published genus protologues (Oren and Ventosa 2008). In response, emended genus descriptions were published to correct the problem (Oren et al. 2009). Overall, the phenotypic differences between many of the genera of *Halobacteriaceae* are rather small; although polar lipid analysis is also an important determinative feature for some groups (Oren 2014c; Oren et al. 2009), most strains can only be assigned to a genus on the basis of 16S rRNA sequence comparisons. This is an unfortunate situation, caused to a large extent by the limited phenotypic and metabolic diversity within the group. Although the range of substrates metabolized by different members of the family can be considerable (Andrei et al. 2012) and chemotaxonomic characters (notably polar lipid composition) may differ, the lack of clear phenotypic traits that can be used to assign a new isolate to one of the 47 currently recognized genera must be regretted. There are of course exceptions (e.g., *Haloquadratum*, *Halococcus*, genera that can be recognized based on their morphology), but these are very few. As long as 16S rRNA (possibly in combination with *rpoB′*, see below) will remain the primary trait on the basis of which a new species or genus is proposed, it will become ever more difficult to assign new isolates to existing taxa based on easy-to-determine phenotypic properties.

Thus far, the family *Halobacteriaceae* is the only family within the order *Halobacteriales*. It was noted that the family can be divided into two clusters based on the position of *pyrD* (coding for dihydroorotate dehydrogenase) in the genome. In one group, it is located immediate upstream the 16rRNA gene; in the second, *pyrD* is found in a different position. On the basis of this observation, Minegishi et al. (2012) suggested that "the time for proposal of a second family in the order *Halobacteriales* is not too far in the future". However, following discussions on Minegishi's proposal, the Subcommittee decided against the splitting of the *Halobacteriaceae* into two families as long as no phenotypic properties can be found to discriminate between the two clusters (Arahal et al. 2011).

Taxonomy of the halophilic methanogenic Archaea

Another group of halophilic Archaea, much less well known than the *Halobacteriaceae*, consists of the methanogens found in hypersaline sediments of salt lakes (Andrei et al. 2012; McGenity 2010). Most halophilic or highly salt-tolerant members belong to the *Methanosarcinaceae* (Oren 2014a); these are all methylotrophic types that obtain energy from methane production from methylated amines and other methyl group-containing compounds: methanol and dimethylsulfide. Compounds such as trimethylamine and dimethylsulfide are formed in hypersaline anaerobic environments as degradation products of glycine betaine and dimethylsulfoniopropionate, compounds that serve as osmotic solutes in many halophilic prokaryotes and in marine algae (McGenity 2010). In addition, a single halotolerant hydrogenotrophic methanogen has been described: *Methanocalculus halotolerans*, a representative of the small family *Methanocalculaceae* (Oren 2014b). No truly halophilic or highly halotolertant aceticlastic methanogens have yet been isolated. As methanogens are seldom mentioned in reviews on halophilic Archaea, the properties of those species able to grow at NaCl concentrations of 10 % or higher are summarized below.

The genera of *Methanosarcinaceae* relevant to hypersaline environments are *Methanohalobium*, *Methanohalophilus*, and *Methanosalsum*. *Halomethanococcus doii*, isolated from salterns in San Francisco Bay and reportedly growing between 10 and 22 % NaCl with an optimum at 17.5 % (Yu and Kawamura 1987) probably also belongs to this group, but the culture was lost, and the genus and species are not available for further study. The most halophilic or all methanogens described is *Methanohalobium evestigatum* isolated from a saline lagoon of Lake Sivash, Ukraine. It grows between 15 and 30 % salt with an

optimum at 25 % (Zhilina 2001; Zhilina and Zavarzin 1987). The species were reported to grow not only on methylated amines but also on H_2/CO_2, formate, and acetate.

The genus *Methanohalophilus* (Boone 2001) contains the following halophilic or highly halotolerant species:

- *Methanohalophilus mahii* from Great Salt Lake, Utah. It grows within the range 3–20 % NaCl with an optimum at 6–15 % (Paterek and Smith 1988). Analysis of its genome sequence (Spring et al. 2010) showed significant differences in energy metabolism when compared to non-halophilic members of the *Methanosarcinaceae*.
- *Methanohalophilus halophilus* from Shark Bay, W. Australia, growing optimally at 7–9 % NaCl and tolerating up to 15 % (Wilharm et al. 1991; Zhilina 1983).
- *Methanohalophilus portucalensis* from a Portugese saltern. It grows optimally at 3–12 % NaCl and tolerates up to 25 % (Boone et al. 1993).

Another halotolerant *Methanohalophilus* strain is *Methanohalophilus euhalobius* from an oil deposit in Russia reported to grow up to 13.5 % NaCl (optimum: 6 %) (Davidova et al. 1997). However, the name has no standing in the nomenclature. Not all members of the genus *Mehanohalophilus* qualify as a true halophiles or halotolerant organisms: *Methanohalophilus oregonensis* grows up to 8 % salt only (Liu et al. 1990).

The genus *Methanosalsum* (Boone and Baker 2001) currently contains a single species, *Methanosalsum zhilinae*. This alkaliphilic methylotrophic methanogen was isolated from the Wadi an Natrun soda lakes, Egypt. It grows optimally at pH 9.2 and 4 % salt (Mathrani et al. 1988).

The second family of methanogens that contain halophilic or highly halotolerant representatives is the *Methanocalculaceae* (order *Methanomicrobiales*) (Oren 2014b; Zhilina et al. 2013). This family consists of methanogens that obtain energy from the reduction of CO_2 with H_2 as the electron donor. Two highly salt-tolerant species have been described:

- *Methanocalculus halotolerans* from an oil field in Alsace, France. It grows over a wide range of NaCl concentration (0–12.5 %) with an optimum at 5 % (Ollivier et al. 1998).
- *Methanocalculus natronophilus* from a soda lake in the Altai region, Russia (Zhilina et al. 2013). This alkaliphilic isolate grows best at 0.5–1.6 M total carbonates and 0.9–3.3 M Na^+ (optimum: 1.4–1.9 M).

Very little work has been done on the halophilic methanogens in the past decade. It may be assumed that the true diversity of methanogens in hypersaline environments is much greater than that represented by the brief list of strains in culture. However, these methanogens are slow growers, and because of their great sensitivity to molecular oxygen they are difficult to handle in the laboratory. Therefore, they never were a popular group object of research.

Novel gene- and genome-based approaches to the taxonomy of the *Halobacteriaceae*

New species of prokaryotes are constantly being described, in the past 8 years at a rate of ~630 per year on average. Still a clear well-defined concept how to delineate species is lacking (Oren and Garrity 2014). Current practice is based on a polyphasic approach that includes comparison of phenotypic, chemotaxonomic and genotypic properties. For the *Halobacteriaceae* (as well as for many other groups of prokaryotes), the primary classification of species and genera is based on 16S rRNA sequence comparisons. DNA–DNA reassociation assays are still used for the delineation of species, as also recommended by the above-mentioned Subcommittee (Oren et al. 1997). This rRNA-based approach has led to the creation of large numbers of species and genera of halophilic Archaea, often with very few readily recognizable phenotypic differences. Comparison of the protologues of many genera, including the many emended versions published in recent years (Oren et al. 2009), shows that most of these descriptions are insufficient to indicate to what genus most new isolates must be assigned: the protologues are very much alike, and their practical use is limited.

Approaches based on 16S rRNA gene sequences to determine the phylogenetic position of isolates of *Halobacteriaceae* are not always straightforward. Strains of some genera such as *Haloarcula* and *Halomicrobium* contain divergent copies of 16S rRNA with differences of up to 5 % and higher—a level normally associated with differences at the genus level (Cui et al. 2009). Occurrence of multiple highly different copies of the 16S rRNA gene was first reported for *Haloarcula marismortui* (Dennis et al. 1998; Mevarech et al. 1989). Both rRNA operons of *Haloarcula marismortui* can be expressed within a single cell (Amann et al. 2000). Differential expression of these operons under different conditions of temperature and salinity may confer adaptive advantages to organisms exposed to fluctuating environmental conditions (López-López et al. 2007). Presence of multiple divergent 16S rRNA sequences was also reported for *Halosimplex carlsbadense* (Vreeland et al. 2002) and for *Natronoarchaeum* and *Halobaculum* spp.

Multilocus sequence analysis (MLSA) is a highly valuable tool for the evaluation of the taxonomic position of members of the *Halobacteriaceae*, to complement 16S rRNA sequence data. The importance of MLSA was repeatedly stressed at meetings of the ICSP Subcommittee on the taxonomy of *Halobacteriaceae*, and ad hoc committees to recommend a suite of appropriate genes were appointed (Arahal et al. 2011; Oren and Ventosa 2013; Oren et al. 2007). Out of a large number of candidate genes, the genes selected for MLSA of the *Halobacteriaceae* are *atpB* (ATPase subunit), EF-2 (elongation factor), *radA* (DNA repair), *rpoB′* (RNA polymerase subunit) and *secY* (protein export through the membrane) (Oren 2012; Oren and Ventosa 2010, 2013; Papke et al. 2011).

A comprehensive study of *rpoB′* sequences and their use in the phylogenetic characterization of the *Halobacteriaceae* showed *rpoB′* -based trees to be coherent with 16S rRNA-based trees in most cases. A few exceptions were found: based on the *rpoB′* sequence comparisons, *Natronolimnobius innermongolicus* is related to the *Haloterrigena—Natrinemea* cluster, while *Natronolimnobius baerhuensis* clusters with *Halostagnicola larsenii*. *Natronorubrum tibetense* was segregated from three other *Natronorubrum* species in the RpoB′ protein tree, but all four species formed a cluster in the gene tree (Minegishi et al. 2010).

Full use has not yet been made of the above-recommended genes for MLSA characterization of new isolates toward their description as new species or genera. However, in an increasing number of publications, *rpoB′* sequences are analyzed in addition to 16S rRNA gene sequences. Sequences of *rpoB′* were first included in descriptions of new species of *Halobacteriaceae* in 2011 (3 out of 14 new species descriptions = 21 %), and the percentage of species descriptions that included the *rpoB′* sequence increased to 58 % (7 out of 12) and 30 % (6 out of 20) in 2012 and in 2013, respectively. The genes encoding 16S rRNA gene, *atpB*, *bop*, EF-2, and *radA* were used as markers in a study of the diversity of *Halorubrum* spp. in salterns in Spain and in Algeria to assess the frequency of homologous recombination in natural populations of *Halobacteriaceae* (Papke et al. 2007; see below for more information).

Now complete genomes are increasingly becoming available and nearly half of all type strains of the species of *Halobacteriaceae* have been sequenced (Oren and Ventosa 2013), whole-genome comparisons for taxonomic purposes are becoming feasible as well as a taxonomic tool. The average nucleotide identity (ANI) value, the result of comparison of all shared orthologous protein-coding genes between two genomes and defined as the mean identity of all BLASTN matches that show >30 % overall sequence identity over an alignable region of at least 70 % of their length (Konstantinidis and Tiedje 2005), is currently the most widely accepted parameter for such comparisons (Oren and Garrity 2014). ANI values to compare different genomes of selected *Halorubrum* spp. and *Haloferax* spp. were quoted by DeMaere et al. (2013) and by Naor et al. (2012), respectively (see below).

The *Halobacteriaceae* as model systems to study horizontal gene transfer and speciation in prokaryotes

The *Halobacteriaceae* are an interesting group to study processes of gene transfer, speciation, and evolution. Numbers of halophilic Archaea in hypersaline brines such as found in saltern crystallizer brines, in the northern part of Great Salt Lake, Utah, in the Dead Sea during rare events of microbial blooms, and in many other high-salinity environments are in the order of 10^7–10^8 per milliliter, i.e., two orders of magnitude higher than in typical ocean and fresh lake waters. Such high densities implicate that there may be plenty of opportunities for exchange of genetic material. The recent finding that many members of the family can form biofilms on solid surfaces (Di Meglio et al. 2014; Fröls et al. 2012) indicates that there may be more possibilities for close contact between cells in natural environments.

A mechanism for direct exchange of genetic material between cells of halophilic Archaea (intraspecific as well as interspecific) was documented thus far only in the genus *Haloferax*. DNA can be transferred between *Hfx. volcanii* cells through cytoplasmic bridges up to 2 μm long and ~0.1 μm in diameter. During this mating process, which is most efficient on a solid support, no mixing of the cytoplasms of the parental types occurs, and each parental type can serve as a donor or as a recipient. Cell fusion is even possible under conditions that lead to the destabilization of the structure of the cytoplasmic bridges (Rosenshine et al. 1989). Free DNA in solution is not taken up to be used as genetic material in *Hfx. volcanii*, but it is digested to serve as a source of phosphorus, nitrogen and carbon (Chimileski et al. 2014). Recombinant hybrids can be formed by mating of cells of *Hfx. volcanii* and *Hfx. mediterranei*. These two species have 98.6 % 16S rRNA identity and have an ANI value of 86.6 % in shared protein-coding genes. Very large DNA fragments were found to be transferred: 310–530 kb, equivalent to 10–17 % of the total chromosome length. Thus, only low barriers exist between species of the genus *Haloferax* (Naor et al. 2012).

For other genera of *Halobacteriaceae*, we know little how genes are transferred horizontally, but much evidence has accumulated on the transfer of genes within and between communities of halophilic Archaea both from culture-dependent studies (MLSA of multiple isolates

belonging to the same genus) and from culture-independent, metagenomic analysis. The square flat *Haloquadratum walsbyi* has been the object of such culture-independent studies as it is difficult to grow and only few cultured strains exist. With a G+C content of 47.9 mol % of the DNA, *Haloquadratum* with *Hqr. walsbyi* as the only described species is atypical, as most members of the family have a G+C value of 60–70 mol %. The genome sequence of *Hqr. walsbyi* strain HBSQ001 (not the type strain) was published (Bolhuis et al. 2006). At the level of 16S rRNA, very similar *Haloquadratum* phylotypes were recovered from saltern crystallizer ponds at three geographically distant sites in Australia. Sequences 99.5 % identical to the type strain were present at all three sites, and 98 % of the *Haloquadratum* sequences recovered differed less than 2 % from that of the nomenclatural type (Oh et al. 2010). However, metagenomic analysis of the *Hqr. walsbyi* population in a single crystallizer pond of a saltern in Spain showed presence of a large pool of accessory genes, including many transposition and phage-related genes. Thus, a large 'pan-genome' can be present even in this highly specialized organism at a single geographic location (Legault et al. 2006). Information on how genes are transferred in *Haloquadratum* is still lacking.

Culture-dependent studies of the populations of *Halorubrum* spp. in saltern ponds in Spain and in Algeria using MLSA led to the recognition that recombination of genetic traits occurs at an extremely high frequency in this genus. Sequencing of the *atpB*, EF-2, *radA* and *secY* genes for a large number of *Halorubrum* isolates showed highly mosaic alleic profiles and the occurrence of promiscuous exchange of genetic information, comparable to that of a sexual population. It was concluded that the *Halorubrum* population is near linkage equilibrium and that random mating and recombination occur both within each pond and even between ponds of different salinities. Clusters could be defined by concatenation of multiple marker sequences, but barriers to exchange between them are leaky (Papke et al. 2004, 2007). The mechanisms of horizontal gene transfer between *Halorubrum* cells are still unknown, and no direct mating such as found in *Haloferax* was ever demonstrated in the genus. Very little is known about transduction and natural competence in the halophilic Archaea, and no evidence for natural transformation was ever reported. The paper entitled "Searching for species in haloarchaea" (Papke et al. 2007) concluded that no non-arbitrary way to circumscribe "species" is likely to emerge for the genus *Halorubrum*, and the same may well apply to other genera of halophilic Archaea and even of prokaryotes in general. These studies show that the *Halobacteriaceae* family is an excellent group to experimentally test different models of speciation and evolution, and the outcome of such studies has a direct impact on prokaryote taxonomy and nomenclature. Horizontal gene transfer is both a homogenizing and a diversifying force, and the result is a balance between recombinations as a cohesive force holding populations together as entities recognizable as taxonomic units, and barriers to that transfer for promoting diversification (Papke and Gogarten 2012; Papke et al. 2007).

Further interesting information on the large extent of horizontal gene transfer among different members of the *Halobacteriaceae* came recently from studies of the halophilic communities of Deep Lake, Antarctica. This hypersaline (210–280 g/l total dissolved salts) lake reaches temperatures above zero only in the top few meters in the summer months. The higest recorded temperature is +11.5 °C. In winter, the water temperature reaches −20 °C, but because of the high salinity, the lake remains ice-free. Different strains of halophilic Archaea were isolated from the lake, including two new species. One is *Halorubrum lacusprofundi*, which grows optimally at 31–37 °C, but still can grow at low rates at 4 °C or even lower (Franzmann et al. 1988). One of its adaptations to life in the cold is the presence of unsaturated diether lipids (Gibson et al. 2005). The second species retrieved from the lake are *Halohasta litchfieldiae*, an isolate that is not psychrotolerant: its temperature range for growth is 25–45 °C with an optimum at 30 °C (Mou et al. 2012). Both *Hrr. lacusprofundi* and *Hht. litchfieldiae* can form aggregates and biofilms (Fröls et al. 2012). It was estimated that in situ no more than ~6 generations can develop per year (DeMaere et al. 2013).

Metagenomic analysis of the prokaryote plankton of Deep Lake showed that four types of Archaea, belonging to distinct genera, account for ~72 % of the community. The genomes of these four organisms could be reconstructed from the metagenomic data: *Halohasta* sp. (1 replicon; 3.33 Mb), *Hrr. lacusprofundi* (3 replicons; 3.69 Mb), DL1 (*Halobacterium* sp.; 2 replicons, 3.16 Mb), and DL31, belonging to a yet undescribed genus (3 replicons, 3.64 Mb). Large identical regions of up to 35 kb in length were found to be shared between all four taxa: 30 regions of >5 kb were common to 7 of the 9 replicons, and 13 regions of >10 kb were shared between 6 replicons. Thus, we have here an extreme example of gene exchange between four phylogenetically disparate members of the *Halobacteriaceae* that have overall only ~73 % ANI values on the average. These high-identity regions did not match any metagenomic data from other hypersaline environments. Thus, in Deep Lake, a large extent of interspecies gene exchange occurs, but different coexisting genera still maintain their identities. Deep Lake, Antarctica is thus a lake ecosystem that sustains a high level of intergeneric gene exchange while selecting for ecotypes that maintain sympatric speciation (DeMaere et al. 2013).

The uncultured halophilic archaeal diversity

The 47 genera and 165 species of *Halobacteriaceae* and the 4 genera and 7 species of methanogens discussed above represent only part of the true diversity of halophilic Archaea in nature. Culture-independent approaches such as analyses of 16S rRNA gene libraries and metagenomic data show additional types of Archaea to be present in many hypersaline environments. One example was presented in the previous section: the recognition of organism DL31 in Deep Lake, Antarctica, representing a novel type of *Halobacteriaceae* at the genus level (DeMaere et al. 2013).

The first evidence for the existence of a new lineage of aerobic halophilic members of the *Euryarchaeota* with very small cells, only remotely related to the *Halobacteriaceae* and now known as the 'Nanohaloarchaea', came from 16S rRNA gene libraries of the biota of East African alkaline salterns (Grant et al. 1999). Sequences affiliated with the MSP8 and the MSP41 clades from these African salt ponds were later found also in other hypersaline environments, including e.g., the above-discussed Australian saltern ponds (Oh et al. 2010). Metagenomic information based on DNA isolated from Lake Tyrrell, NW Victoria, Australia has now enabled the reconstruction of two complete genomes of members of the group ('*Candidatus* Nanosalina'; 43.5 mol % G+C, and '*Candatus* Nanosalinarum'; 56 mol % G+C). Both draft genomes are small ($\sim$1.2 Mb) and compact (Narasingarao et al. 2012). Similar organisms were recovered from saltern ponds near Alicante, Spain by cell sorting by flow cytometry followed by single-cell sequencing; one of these genomes has 42.1 mol % G+C, the lowest value reported for any halophilic archaeon (Ghai et al. 2011). Fluorescence in situ hybridization with rRNA-targeted probes shows that the members of this lineage are very small cells, only $\sim$0.6 µm in diameter. One rhodopsin-like gene is present in each genome, suggesting a potentially facultative photoheterotrophic lifestyle of these aerobic chemoheterotrophs. Nanohaloarchaea were estimated to represent at least 10–25 % of the total archaeal community in Lake Tyrrell surface water and in the Chula Vista, California salterns (Narasingarao et al. 2012).

At the time of writing (March 2014), no reports existed of the cultivation of a representative of the Nanohaloarchaea. There have been cases in the past where many years passed from the time the existence of a certain type of archaeon was recognized until its cultivation and characterization in culture. A well-known example is *Haloquadratum*: its distinctive morphology was described in 1980 (Walsby 1980), but the isolation of *Hqr. walsbyi* was first reported only in 2004 (Bolhuis et al. 2004; Burns et al. 2004a), leading to the taxonomic description of the new genus and species (Burns et al. 2007). The small cell size of the Nanohaloarchaea and the available metagenomic information may well help designing selective cultivation approaches. Hopefully, pure cultures of these intriguing organisms will soon become available so that formal description and naming of the new taxa will become possible.

Another yet-uncultured lineage of Archaea, thus far recognized only on the basis of 16S rRNA sequences retrived from hypersaline environments (saltern ponds in Inner Mongolia and South Africa), is related to the Nanoarchaeota, a lineage thus far represented only by the hyperthermophilic *Nanoarchaeum equitans* which grow in symbiotic association with *Ignicoccus* (Crenarchaeota) (Casanueva et al. 2008).

Final comments

The halophilic Archaea are a diverse group of microorganisms. While no organisms growing at salt concentrations above 10 % were yet identified among the *Crenarchaeota*, there are at least three groups of *Euryarchaeota* that consist entirely or in part of halophiles: the order *Halobacteriales* with the single family *Halobacteriaceae*, the yet-uncultured Nanohaloarchaea, and two families of methanogens. The *Halobacteriaceae* have become popular objects of taxonomic research. The fact that, with some skill and much patience, nearly all types recognized in culture-independent studies can be grown in culture (Burns et al. 2004b) is very helpful in such studies.

Many species of *Halobacteriaceae* have been described and named. But for the prokaryotes in general, there is no clear generally accepted species concept, the same is true for these halophiles. A number of studies discussed above show that horizontal gene transfer can be extremely rapid for some members of the family, so that it becomes nearly impossible to define species boundaries if indeed genes are being transferred and rearranged at a speed comparable to sexual reproduction in eukaryotes (Papke et al. 2007). On the other hand, the fact that many members of the *Halobacteriaceae* can be considered polyploid may be a stabilizing factor as multiple copies exist for each gene within each cell. Thus, exponentially growing cells of *Halobacterium salinarum* contain $\sim$25 copies of the genome, and cells in the stationary phase have $\sim$15 copies; exponential and stationary cells of *Hfx. volcanii* have $\sim$15 and $\sim$10 genome copies, respectively. Multiple genome copies are also present in *Hfx. mediterranei*. Having many copies of the chromosome may result in low apparent mutation rates, high radiation and desiccation resistance, and survival over geological times (Soppa 2013).

From an evolutionary point of view, the *Halobacteriaceae* are an interesting group. Based on the analysis by

Nelson-Sathi et al. (2012), the origin of the halobacteria may have resulted from an influx of genes from the bacterial domain into an anaerobic chemolithoautotrophic methanogen, which reshaped its metabolism to that of a facultative aerobic heterotroph. Acquisition of 1,089 bacterial genes by lateral gene transfer may have sufficed for the formation of a heterotrophic, oxygen-respiring, and bacteriorhodopsin-phototrophic common ancestor of the *Halobacteriaceae*. If indeed so many genes in this group were derived from different branches of the Bacteria, including the *Proteobacteria*, then there may be some truth in the old assumption that the halobacteria may be related to the pseudomonads (see the quotation from Donn Kushner (1978) at the beginning of this article). More than three decades have passed since Kushner wrote: "We may hope for further revelations of their taxonomic status by comparisons of specific ribosomal proteins, of RNA's, and of other macromolecules of known function". Today, we are not limited by the amount of sequence information, but basic questions in the field of taxonomy of the halophilic Archaea still remain unsolved.

References

Amann G, Stetter KO, Llobet-Brossa E, Amann R, Antón J (2000) Direct proof for the presence and expression of two 5% different 16S rRNA genes in individual cells of *Haloarcula marismortui*. Extremophiles 4:373–376

Andrei A-Ş, Banciu HL, Oren A (2012) Metabolic diversity in Archaea living in saline ecosystems. FEMS Microbiol Lett 330:1–9

Arahal DR, Oren A, Ventosa A (2011) International Committee on Systematics of Prokaryotes. Subcommittee on the taxonomy of *Halobacteriaceae* and Subcommittee on the taxonomy of *Halomonadaceae*. Minutes of the joint open meeting, 6 September 2011, Sapporo, Japan. Int J Syst Evol Microbiol 61:2792–2795

Bolhuis H, te Poele EM, Rodríguez-Valera F (2004) Isolation and cultivation of Walsby's square archaeon. Environ Microbiol 6:1287–1291

Bolhuis H, Palm P, Wende A, Falb M, Rampp M, Rodriguez-Valera F, Pfeiffer F, Oesterhelt D (2006) The genome of the square archaeon *Haloquadratum walsbyi*: life at the limits of water activity. BMC Genom 7:169

Boone DR (2001) Genus IV. *Methanohalophilus* Paterek and Smith 1988, 122^{VP}. In: Boone DR, Castenholz RW, Garrity GM (eds) Bergey's manual of systematic bacteriology, vol 1, 2nd edn. The *Archaea* and the deeply branching and phototrophic *Bacteria*. Springer, New York, pp 281–283

Boone DR, Baker CC (2001) Genus VI. *Methanosalsum* gen. nov. In: Boone DR, Castenholz RW, Garrity GM (eds) Bergey's manual of systematic bacteriology, vol 1, 2nd edn. The *Archaea* and the deeply branching and phototrophic *Bacteria*. Springer, New York, pp 287–289

Boone DR, Mathrani IM, Liu Y, Menaia JAGF, Mah RA, Boone JE (1993) Isolation and characterization of *Methanohalophilus portucalensis* sp. nov. and DNA reassociation study of the genus *Methanohalophilus*. Int J Syst Bacteriol 43:430–437

Burns DG, Camakaris HM, Janssen PH, Dyall-Smith ML (2004a) Cultivation of Walsby's square haloarchaeon. FEMS Microbiol Lett 238:469–473

Burns DG, Camakaris HM, Janssen PH, Dyall-Smith ML (2004b) Combined use of cultivation-dependent and cultivation-independent methods indicates that members of most haloarchaeal groups in an Australian crystallizer pond are cultivable. Appl Environ Microbiol 70:5258–5265

Burns DG, Janssen PH, Itoh T, Kamekura M, Li Z, Jensen G, Rodríguez-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 57:387–392

Casanueva A, Galada N, Baker GC, Grant WD, Heaphy S, Jones B, Yanhe M, Ventosa A, Blamey J, Cowan DA (2008) Nanoarchaeal 16S rRNA gene sequences are widely dispersed in hyperthermophilic and mesophilic halophilic environments. Extremophiles 12:651–656

Chimileski S, Dolas K, Naor A, Gophna U, Papke RT (2014) Extracellular DNA metabolism in *Haloferax volcanii*. Frontiers Microbiol 5:57

Cui H-L, Zhou P-J, Oren A, Liu S-J (2009) Intraspecific polymorphism of 16S rRNA genes in two halophilic archaeal genera, *Haloarcula* and *Halomicrobium*. Extremophiles 13:31–37

Davidova IA, Harmsen HJM, Stams AJM, Belyaev SS, Zehnder AJB (1997) Taxonomic description of *Methanococcoides euhalobius* and its transfer to the *Methanohalophilus* genus. Antonie van Leeuwenhoek 71:313–318

DeMaere MZ, Williams TJ, Allen MA, Brown MV, Gibson JAE, Rich J, Lauro FM, Dyall-Smith M, Davenport KW, Woyke T, Kyrpides NC, Tringe SG, Cavicchioli R (2013) High level of intergenera gene exchange shapes the evolution of haloarchaea in an isolated Antarctic lake. Proc Natl Acad Sci 110:16939–16944

Dennis PP, Ziesche S, Mylvaganam S (1998) Transcription analysis of two disparate rRNA operons in the halophilic archaeon *Haloarcula marismortui*. J Bacteriol 180:4804–4813

Di Meglio L, Busalamen J, Pastore JI, Ballarin D, Nercessian D (2014) Hyperhalophilic archaeal biofilms: growth kinetics, structure, and antagonistic interaction in continuous culture. Biofouling 30:237–245

Franzmann PD, Stackebrandt E, Sanderson K, Volkman JK, Cameron DE, Stevenson PL, McMeekin TA, Burton HR (1988) *Halobacterium lacusprofundi* sp. nov., a halophilic bacterium isolated from Deep Lake, Antarctica. Syst Appl Microbiol 11:20–27

Fröls S, Dyall-Smith M, Pfeifer F (2012) Biofilm formation by haloarchaea. Environ Microbiol 14:3159–3174

Ghai R, Fernández AB, Martin-Cuadrado A-B, Megumi Mizuno C, McMahon KD, Papke RT, Stepanauskas R, Rodriguez-Brito B, Rohwer F, Sánchez-Porro C, Ventosa A, Rodríguez-Valera F (2011) New abundant microbial groups in aquatic hypersaline environments. Sci Rep 1:135

Gibson JAE, Miller MR, Davies NW, Neill P, Nichols DS, Volkmann JK (2005) Unsaturated diether lipids in the psychrotrophic archaeon *Halorubrum lacusprofundi*. Syst Evol Microbiol 28:19–26

Grant S, Grant WD, Jones BE, Kato C, Li L (1999) Noval archaeal phylotypes from an East African alkaline saltern. Extremophiles 3:139–145

Konstantinidis KT, Tiedje JM (2005) Genomic insights that advance the species definition for prokaryotes. Proc Natl Acad Sci 102:2567–2572

Kushner DJ (1978) Life in high salt and solute concentrations: halophilic bacteria. In: Kushner DJ (ed) Microbial life in extreme environments. Academic Press, London, pp 317–368

Legault BA, Lopez-Lopez A, Alba-Casado JC, Doolittle WF, Bolhuis H, Rodríguez-Valera F, Papke RT (2006) Environmental genomics of "*Haloquadratum walsbyi*" in a saltern crystallizer indicates a large pool of accessory genes in an otherwise coherent species. BMC Genom 7:171

Liu Y, Boone DR, Choy C (1990) *Methanohalophilus oregonense* sp. nov., a methylotrophic methanogen from an alkaline, saline aquifer. Int J Syst Bacteriol 40:111–116

López-López A, Benlloch S, Bonfá M, Rodríguez-Valera F, Mira A (2007) Intragenomic 16S DNA divergence in *Haloarcula marismortui* is an adaptation to different temperatures. J Mol Evol 65:687–969

Magrum LJ, Luehrsen KR, Woese CR (1978) Are extreme halophiles actually "bacteria"? J Mol Evol 11:1–8

Mathrani IM, Boone DR, Mah RA, Fox GE, Lau PP (1988) *Methanohalophilus zhilinae* sp. nov., an alkaliphilic, halophilic, methylotrophic methanogen. Int J Syst Bacteriol 38:139–142

McGenity TJ (2010) Methanogens and methanogenesis in hypersaline environments. In: Timmis KN (ed) Handbook of hydrocarbon and lipid microbiology. Springer-Verlag, Berlin, pp 665–680

Mevarech M, Hirsch-Twizer S, Goldman S, Yakobson E, Eisenberg H, Dennis PP (1989) Isolation and characterization of the rRNA gene clusters of *Halobacterium marismortui*. J Bacteriol 171:3479–3485

Minegishi H, Kamekura M, Itoh T, Echigo A, Usami R, Hashimoto T (2010) 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

Minegishi H, Kamekura M, Kitajima-Ihara T, Nakasone K, Echigo A, Shimane Y, Usami R, Itoh T, Ihara K (2012) Gene orders in the upstream of 16S rRNA genes divide genera of the family *Halobacteriaceae* into two groups. Int J Syst Evol Microbiol 62:188–195

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

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 22:1444–1448

Narasingarao P, Podell S, Ugalde JA, Brochier-Armanet C, Emerson JB, Brocks JJ, Heidelberg KB, Banfield JF, Allen EE (2012) De novo assembly reveals abundant novel major lineage of Archaea in hypersaline microbial communities. ISME J 6:81–93

Nelson-Sathi S, Dagan T, Landan G, Janssen A, Steel M, McInterney JO, Deppenmeier U, Martin WF (2012) Acquisition of 1,000 eubacterial genes physiologically transformed a methanogen at the origin of Haloarchaea. Proc Natl Acad Sci 109:20537–20542

Oh D, Porter K, Russ B, Burns D, Dyall-Smith M (2010) Diversity of *Haloquadratum* and other haloarchaea in three, geographically distant, Australian saltern crystallizer ponds. Extremophiles 14:161–169

Ollivier B, Fardeau M-L, Cayol J-L, Magot M, Patel BKC, Prensier G, Garcia J-L (1998) *Methanocalculus halotolerans* gen. nov., sp. nov., isolated from an oil-producing well. Int J Syst Bacteriol 48:821–828

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

Oren A (2014a) Family *Methanosarcinaceae*. In: Rosenberg E, DeLong EF, Thompson F, Lory S, Stackebrandt E (eds) The prokaryotes. A handbook on the biology of bacteria: ecophysiology and biochemistry, 4th edn. Springer, New York

Oren A (2014b) Family *Methanocalculaceae*. In: Rosenberg E, DeLong EF, Thompson F, Lory S, Stackebrandt E (eds) The prokaryotes. A handbook on the biology of bacteria: ecophysiology and biochemistry, 4th edn. Springer, New York

Oren A (2014c) Family *Halobacteriaceae*. In: Rosenberg E, DeLong EF, Thompson F, Lory S, Stackebrandt E (eds) The prokaryotes. A handbook on the biology of bacteria: ecophysiology and biochemistry, 4th edn. Springer, New York

Oren A, Garrity GM (2014) Then and now: a systematic review of the systematics of prokaryotes in the last 80 years. Antonie van Leeuwenhoek. doi:10.1007/s10482-013-0084-1

Oren A, Ventosa A (2008) International Committee on Systematics of Prokaryotes. Subcommittee on the taxonomy of *Halobacteriaceae*. Minutes of the meetings, 7 August 2008, Istanbul, Turkey. Int J Syst Evol Microbiol 58:2465–2467

Oren A, Ventosa A (2010) International Committee on Systematics of Prokaryotes. Subcommittee on the taxonomy of *Halobacteriaceae* and Subcommittee on the taxonomy of *Halomonadaceae*. Minutes of the meeting 30 June 2010, Beijing, China. Int J Syst Evol Microbiol 60:2257–2259

Oren A, Ventosa A (2013) International Committee on Systematics of Prokaryotes. Subcommittee on the taxonomy of *Halobacteriaceae* and Subcommittee on the taxonomy of *Halomonadaceae*. Minutes of the joint open meeting, 24 June 2013, Storrs, Connecticut, USA. Int J Syst Evol Microbiol 63:3540–3544

Oren A, Ventosa A, Grant WD (1997) Proposal of minimal standards for the description of new taxa in the order *Halobacteriales*. Int J Syst Bacteriol 47:233–238

Oren A, Vreeland RH, Ventosa A (2007) International Committee on Systematics of Prokaryotes. Subcommittee on the taxonomy of *Halobacteriaceae* and Subcommittee on the taxonomy of *Halomonadaceae*. Minutes of the joint open meeting, 3 September 2007, Colchester, UK. Int J Syst Evol Microbiol 57:2975–2978

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

Papke RT, Gogarten JP (2012) How bacterial lineages emerge. Science 336:45–46

Papke RT, Koenig JE, Rodríguez-Valera F, Doolittle WF (2004) Frequent recombination in a saltern population of *Halorubrum*. Science 306:1928–1929

Papke RT, Zhaxybayeva O, Feil EJ, Sommerfeld K, Muise D, Doolittle WF (2007) Searching for species in haloarchaea. Proc Natl Acad Sci 104:14092–14097

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 61:2984–2995

Paterek JR, Smith PH (1988) *Methanohalophilus mahii* gen. nov., sp. nov., a methylotrophic halophilic methanogen. Int J Syst Bacteriol 38:122–123

Rosenshine I, Tchelet R, Mevarech M (1989) The mechanism of DNA transfer in the mating system of an archaebacterium. Science 245:1387–1389

Soppa J (2013) Evolutionary advantages of polyploidy in halophilic archaea. Biochem Soc Trans 41:339–343

Spring S, Scheuner C, Lapidus A, Lucas S, Glavina Del Rio T, Tice H, Copeland A, Cheng JF, Chen F, Nolan M, Saunders E, Pitluck S, Liolios K, Ivanova N, Mavromatis K, Lykidis A, Pati A, Chen A, Palaniappan K, Land M, Hauser L, Chang YJ, Jeffries CD, Goodwin L, Detter JC, Brettin T, Rohde M, Göker M, Woyke T, Bristow J, Eisen JA, Markowitz V, Hugenholtz P, Kyrpides NC, Klenk HP (2010) The genome sequence of *Methanohalophilus mahii* SLP^T reveals differences in the energy metabolism among members of the *Methanosarcinaceae* inhabiting freshwater and saline environments. Archaea 2010:690737

Trüper HG (2005) Is 'localimania' becoming a fashion for prokaryote taxonomists? Int J Syst Evol Microbiol 45:1753

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 6:445–452

Walsby AE (1980) A square bacterium. Nature 283:69–71

Wilharm T, Zhilina TN, Hummel P (1991) DNA–DNA hybridization of methylotrophic halophilic methanogenic bacteria and transfer of *Methanococcus halophilus*^{VP} to the genus *Methanohalophilus as Methanohalophilus halophilus* comb. nov. Int J Syst Bacteriol 41:558–562

Yu IK, Kawamura F (1987) *Halomethanococcus doii* gen. nov., sp. nov.: an obligately halophilic methanogenic bacterium from solar salt ponds. J Gen Appl Microbiol 33:303–310

Zhilina TN (1983) A new obligate halophilic methane-producing bacterium. Mikrobiologiya 52:375–382

Zhilina TN (2001) Genus III. *Methanohalobium* Zhilina and Zavarzin 1988, 136^{VP} (Effective publication: Zhilina and Zavarzin 1987a, 467). In: Boone DR, Castenholz RW, Garrity GM (eds) Bergey's manual of systematic bacteriology, vol 1, 2nd edn. The *Archaea* and the deeply branching and phototrophic *Bacteria*. Springer, New York, pp 279–281

Zhilina TN, Zavarzin GA (1987) *Methanohalobium evestigatus* n. gen., n. sp. The extremely halophilic methanogenic *Archaebacterium*. Dokl Akad Nauk SSSR 293:464–468 (in Russian)

Zhilina TN, Zavarzina DG, Kevbrin VV, Kolganova TV (2013) *Methanocalculus natronophilus* sp. nov., a new alkaliphilic hydrogenotrophic methanogenic archaeon from a soda lake, and proposal of the new family *Methanocalculaceae*. Microbiology (Russ) 82:686–694