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	Methanonatronarchaeum thermophilum with the type strain AMET1T (DSM 26684T=NBRC 110805T=UNIQEM U982), and the salt lake methanogens - as a candidate genus and species 'Ca. Methanohalarchaeum thermophilum'. Together with uncultured SA1 group clones, these organisms are proposed to form a new class Methanonatronarchaeia within the phylum Euryarchaeota.

Methanonatronarchaeum thermophilum gen. nov., sp. nov, and '*Candidatus* Methanohalarchaeum thermophilum', extremely halo(natrono)philic methyl-reducing methanogens from hypersaline lakes comprise a new euryarchaeal class *Methanonatronarchaeia* classis nov.

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Running title: *Methanonatronarchaeum thermophilum* gen. nov., sp. nov, and '*Candidatus* Methanohalarchaeum thermophilum'

The genome of the type strain AMET1[T] and the metagenome of HMET1[T] have been deposited in the GenBank under the numbers MRZU00000000 and MSDW00000000, respectively. The 16S-rRNA gene sequences of the AMET strains are deposited under the numbers KY449317-KY4493127.

Methanogenic enrichments from hypersaline lakes at moderate thermophilic conditions resulted in cultivation of an unknown deep lineage of euryarchaeota related to the class *Halobacteria*. Both soda and salt lake isolates belong to methyl-reducing methanogens that utilize C_1 methylated compounds as electron acceptor and H_2 or formate as electron donor. They are extreme halophiles, growing optimally at 4 M total Na^+ and represent the first example of methanogens employing the "salt-in" osmoprotection mechanism. The salt lake subgroup is neutrophilic, whereas the soda lake isolates are obligate alkaliphiles, with an optimum around pH 9.5. Both grow optimally at 50^{o}C. The genetic diversity inside the two subgroups is very low, indicating that the soda and salt lake clusters consist of a single genetic species each. The phylogenetic distance between the two subgroups is in the range of distant genera, whereas the distance to other euryarchaea is below 83% identity of the 16S rRNA. These isolates and closely related environmental clones from hypersaline habitats (SA1 group) form a novel class-level clade in the phylum Euryarchaeota that is strongly supported by bootstrap analysis. On the basis of distinct phenotypic and genetic properties, the soda lake isolates are classified into a new genus and species *Methanonatronarchaeum thermophilum* with the type strain AMET1^T (DSM 26684^T=NBRC 110805^T=UNIQEM U982), and the salt lake methanogens - as a candidate genus and species 'Ca. Methanohalarchaeum thermophilum'. Together with uncultured SA1 group clones, these organisms are proposed to form a new class *Methanonatronarchaeia* within the phylum Euryarchaeota.

In hypersaline habitats, methylotrophic methanogenesis is usually considered to be the dominant pathway [1-2]. The organisms responsible for this process are members of the order *Methanosarcinales*. In neutral pH conditions, *Methanosarcinales* are represented by the high salt-tolerant genera *Methanohalophilus* and *Methanohalobium* that can grow at up to 4 M NaCl [2-4], and a single methylotrophic genus *Methanonatronum* has been identified that can grow in hypersaline soda brines [5-8]. All these methanogens, although able to tolerate salt-saturating conditions, belong to moderate halophiles that grow optimally at salinity around 2 M total Na^+ and utilize the bacterial type of osmoprotection based on organic compatible solutes [9-10].

Our recent exploration of methanogenic archaea in sediments of hypersaline inland lakes has shown that, at elevated temperatures, a previously unknown group of extremely halo(natrono)philic methanogens started to outcompete the salt-tolerant *Methanosarcinales* members when formate was supplied on the top of C_1 methylated compounds as methanogenic substrate. This suggested the methyl-reducing nature of the novel group [11]. In this hybrid methanogenic pathway, the C_1 methylated compounds are used as electron acceptors only, whereas external H_2 is required as the electron donor. This pathway, until recently, had been considered rare, having been characterized in only two species of methanogens, *Methanosphaera stadtmanae* (*Methanobacteriales*) and *Methanomicrococcus blatticola* (*Methanosarcinales*) [12-14]. However, virtually all recent discoveries of novel deep lineages of methanogens involve methyl-reducers, including the *Thermoplasmata* methanogens [15-16], the Candidate class "Methanofastidiosa" [17] and the Candidate phyla "Bathyarchaeota" [18] and "Verstraetearchaeota" [19]. These findings indicate that methyl-reduction has so far been overlooked as an important methanogenic pathway that might be able to compete with both classical methylotrophic and lithotrophic pathways. Here, we

describe the phenotypic and genetic properties of the novel group of extremophilic methyl-reducing methanogenic euryarchaea from hypersaline lakes which we propose as founding members of a new class *Methanonatronarchaeia*.

The source of the isolates was surface layer (5-15 cm) of anaerobic sediments from hypersaline salt and soda lakes from various geographical locations as shown in **Table 1**. Overall, eleven pure cultures of haloalkaliphilic and three highly enriched cultures of halophilic methyl-reducing methanogens were obtained at 4 M total Na^+ and 37-60^oC.

The extremely haloalkaliphilic methyl-reducing AMET isolates were enriched and further purified by serial dilution using mineral base medium containing 4 M total Na^+ (2 M Na^+ as sodium carbonates + 2 M NaCl), 5 g l^{-1} KCl and and 1 g l^{-1} of K_2HPO_4 at pH 9.5 (4 mM NH_4Cl was added after sterilization). The extremely halophilic, neutrophilic methyl-reducing HMET cultures were enriched in 4 M NaCl/5 g l^{-1} KCl, buffered at pH 7 by K_2HPO_4-KH_2PO_4 (total 3 g l^{-1}) and supplemented with 0.5 g l^{-1} of NH_4Cl. After sterilization, both types of the mineral basic media were supplemented with two trace metal solutions, $MgCl_2$ and vitamins as described previously [8]. Further additions included CoM (50 μM), yeast extract (100 mg/l) and either 0.1 mM hydrotroillite (FeS x nH_2O) or heat-sterilized anaerobic sediment slurries (approx. 10 cm^3 l^{-1}) from either soda lakes or salt lakes. 50 mM each of MeOH and sodium formate were added as substrates and 0.5 mM sodium sulfide as a reductant. The media were dispensed into serum bottles (from 30 to 100 ml) at 75% volume capacity and made anoxic by sterile argon flushing-evacuation. Final reduction of the media was achieved by adding by syringe of a drop/10 ml of 10% dithionite solution in 1 M $NaHCO_3$. The colonial growth of strain AMET1^T was achieved in soft agar by mixing the 4 M complete sterile liquid alkaline medium and 4% agarose (0.8% final) at 50^oC and pouring 20 ml portions onto plates

116 containing 0.1 ml of serially diluted, fully grown liquid culture. All manipulations were
117 performed in an anaerobic glove box. The plates were incubated for one month in closed jars
118 under Ar atmosphere with the O_2-scavenging catalyzer (Oxoid) at 45^{o}C.
119
120 Phase contrast microphotographs were obtained using a Zeiss Axioplan Imaging 2
121 microscope (Göttingen, Germany). For the total cell electron microscopy, the cells were
122 centrifuged and resuspended in 3 M NaCl, fixed with paraformaldehyde (final concentration
123 3%, v/v) for 2 h at room temperature, then washed again with the same NaCl solutions. The
124 fixed cells were positively contrasted with 1% (w/v) uranyl acetate. For thin sectioning, the
125 cell pellets were fixed in 1% (w/v) OsO_4 containing 3.0 M NaCl for 1 week at 4^{o}C, washed
126 and resuspended in 3 M NaCl, stained overnight with 1% (w/v) uranyl acetate, dehydrated in
127 ethanol series, and embedded in Epon resin. Thin sections were poststained with 1% (w/v)
128 lead citrate. The core membrane lipids were obtained by acid hydrolysis (5% HCl in methanol
129 by reflux for 3 h) of the freeze-dried cells and subsequent analysis by HPLC-MS for GDGTs
130 and archaeol derivatives according to [20]. Intact polar lipids were obtained by Bligh Dyer
131 extraction of freeze-dried cells and subsequent HPLC-MS analysis as described in [21]. The
132 presence of intracellular organic compatible solutes in the lyophilized cells of strain AMET1^{T}
133 was analyzed by HPLC and ^{1}H-NMR after extraction with EtOH and the intracellular
134 potassium concentration was measured using ICP-MS. The cell protein was analyzed by the
135 Lowry method after removal of cell-bound FeS by several washing with acidic 4 M NaCl
136 solution.
137
138 In total, eleven pure and three highly enriched mixed methanogenic cultures were isolated
139 from anaerobic sediments of hypersaline soda and salt lakes, respectively, at 4 M total Na^{+}
140 and methyl-reducing conditions, i.e. with either methanol or trimethylamine (TMA) as the

electron acceptor and formate or H_2 as the electron donor (**Table 1)**. In addition to the extreme salinity, all but one AMET strain were enriched and isolated at elevated temperatures, between 48 and 60^{o}C. The soda lake AMET isolates were successfully purified from bacterial satellites using a combination of antibiotic treatment (strepromycin + vancomycin, or rifampicine 100 mg l^{-1} each) and filtration (0.45 μm). For the salt lake HMET cultures, although this procedure efficiently eliminated bacteria, a small fraction (approx. 5%) of other, non-methanogenic haloarcheal cells persisted in the serial dilutions. Furthermore, the growth rate and yield of the HMET cultures were extremely low compared to the AMET isolates, which made their purification problematic. All cultures exhibited obligate dependence on external CoM and FeS. Moreover, only three of the eleven AMET isolates grew in the minimal medium with these additions, whereas the rest were dependent on the presence of sterilized sediments either from soda (AMET) or salt (HMET) lakes. What exactly these organisms needed from the sediments, remains unclear, although a test with separated pore brines and the solid phase demonstrated that the latter was far more efficient as a growth factor. To our knowledge, similar observations have been reported in only one other case, for an unidentified methylotrophic methanogenic culture obtained from alkaline saline Mono Lake, but that culture most probably belonged to a classical methylotroph, because it grew in the presence of methanol as the only substrate [22].

In a single case of strain AMET1^{T}, which can grow in presence of FeS alone, colonial growth was achieved. The colonies were disc-shaped, up to 1 mm in diameter and yellow-colored. The typical cell morphologies of the AMET and HMET type strains are shown on **Figs. 1** and **2**. The cells are irregular angular cocci of a characteristic small size (mean cell diameter is 0.4 μm). The cells of AMET strains were motile and possess multiple archaella, whereas no motility was observed for the cells in HMET cultures. Both groups have a thin, monolayer cell wall covered with a thick EPS layer. In addition, invaginations of cytoplasmic

membrane and large electron transparent inclusions (possibly, polyhydroxyalkanoates) were visible in the cells of HMET1. The cells lyzed immediately upon downshift in salinity below 2 M Na^+. The absence of blue autofluorescence indicated the absence of deazoflavine (F_{420}) normally present in classical methylotrophic methanogens.

The analysis of organic compatible solutes in cells of strain AMET1^T (grown at 4 M Na^+, pH 9.5) gave negative results. However, intracellular cation analysis demonstrated molar concentrations of K^+. These observations indicate that the novel methanogens employ the haloarchaeal type ("salt-in") osmoprotection mechanism which has not yet been demonstrated for any other halophilic methanogens.

The core membrane lipids of AMET1^T and HMET1^T are primarily composed of a mixture of GDGT-0 and archaeol (C20-C20 diphytanylglycerol diether) (**Supplementary Table S1**). AMET1 also contained small quantities of GDGT-1, which was not detected in HMET1^T. In addition to archaeol, minor amounts of two monophytanyl glycerol ethers (2-C20 MGE and 1-C20 MGE) in AMET1^T and only 1-C20 MGE in HMET1^T were detected. The complete absence of extended archaeols (C20-C25 and C25-C20 DGE) in membrane lipids differentiated the extremely halophilic methanogens from haloarchaea [23]. The intact polar lipid compositions of the two organisms were clearly different. In the alkaliphilic AMET1^T, the dominant polar lipids were phosphatidylglycerol (PG) and PG-PG with GDGT-0 as the core lipid. In the halophilic strain HMET1^T, the dominant polar lipids were identified as dihexose derivatives of both archaeol and GDGT-0 (**Supplementary table S1**).

Both AMET and HMET strains use the methyl-reducing pathway of methanogenesis, whereby the C_1 methylated compounds, such as methanol, methylamines or methylated sulfides are used only as electron acceptors, whereas H_2 serves as the external electron donor. For the AMET strains the best electron acceptor was methanol. Methylamines, including mono-, di- and trimethyamine and tetramethyammonium, can also be utilized in ammonia-

191 free media but were highly toxic at alkaline conditions and the growth was much less active.
192 The growth with dimethylsulfide demanded gradual adaptation starting from 2 mM, but after
193 several steps, the best adapted strain, AMET6-2, was able to grow in presence of up to 20 mM
194 DMS. On the other hand, although possible in principle, the utilization of methanethiol was
195 irregular and no adaptation was observed to this toxic methylated compound. The neutrophilic
196 HMET strains preferred trimethylamine as the acceptor over methanol and growth with the
197 other C_1 methyl compounds was not observed. The two groups also differed in their preferred
198 *e*-donor: while the AMET strains clearly preferred formate, the HMET strains used H_2 more
199 actively. Utilization of formate as the *e*-donor, as well as DMS as the acceptor, have not been
200 demonstrated previously for any cultured methyl-reducing methanogens.

201 A unique property of the novel methyl-reducing methanogens is their extreme
202 halophily. Both groups grew within the range of Na^+ concentrations that, among the cultured
203 archaea, are typical only for haloarchaea, i.e. from 3 to 5 M, with an optimum at
204 approximately 4 M. This preferred range of salt concentration is compatible with the evidence
205 indicating that these organisms employ the "salt-in" strategy for osmoprotection. The AMET
206 group from soda lakes belongs to obligate alkaliphiles growing within the pH range (at 4 M
207 Na^+ and 48^{o}C) from 8.2 to 10.2 (optimum at 9.5-9.8). In contrast to most of the extremely
208 natronophilic bacteria isolated from hypersaline soda lakes, the new archaea depend on molar
209 concentrations of NaCl and grow optimally in a medium containing 2 M NaCl and 2 M (Na)
210 carbonates. The HMET strains were typical neutraphiles with a pH range for growth from 6.5
211 to 8. Furthermore, both groups preferred elevated temperatures for growth despite being
212 isolated from moderate habitats. They grew optimally at 50^{o}C and some of the strains
213 tolerated up to 60^{o}C.

214 The Maximum Likelihood phylogenetic tree of 16S rRNA was constructed using
215 PhyML 3.0 with the Smart Model Selection [24], the SPR (Subtree Pruning and Regrafting)

type of tree improvement [25] and the aLRT (Approximate likelihood-ratio test) for branch support [26]. Only nearly complete sequences of 16S rRNA genes from the SILVA database [27] were included in the calculation. The results show that the AMET and HMET groups form two compact clades, with a maximum distance inside the groups of 1.5%. The distance between the two groups was about 10%, indicating that they represent two distinct genera. However, no close relatives of these organisms were identified among the cultivated members of Euryarchaeota, whereas among uncultured archaeal clones, the novel methanogens were clearly related to the SA1 group detected in various hypersaline habitats [28-30]. Further phylogenetic reconstruction [11] showed that the closest relatives of the AMET-HMET group in Euryarchaeota were haloarchaea of the class *Halobacteria* (**Fig. 3**) which, again, is compatible with the extreme halophily and the likely "salt-in" osmotic strategy of the novel methanogens.

Overall, on the basis of phylogenetic analysis and unique phenotypic properties, the novel moderately thermophilic and extremely halo(alkali)philic methyl-reducing methanogens from hypersaline lakes are proposed to form a new euryarchaeal class *Methanonatronarchaeia* including the alkaliphilic AMET isolates from soda lakes as a new genus and species *Methanonatronarchaeum thermophilum,* and a candidate genus and species '*Ca*. Methanohalarchaeum thermophilum' from salt lakes.

DESCRIPTION OF *METHANONATRONARCHAEUM* GEN. NOV.

Metha.no.na.tron.ar.chae'um. N.L. n. *methanum* [from French n. *méth*(*yle*) and chemical suffix *-ane*], methane; N.L. pref. *methano-*, pertaining to methane; N. Gr. n. *natron*, arbitrarily derived from the Arabic n. *natrun* or *natron*, soda; N. L. neut. n. *archaeum* [from Gr. adj. *archaios*, *-e*, *-on* ancient] archaeon; N. L. neut. n. *Methanonatronarchaeum* a soda-loving archaeon forming methane

Extremely halo(alkali)philic and moderately thermophilic methanogens that use the methyl-reducing pathway of methanogenesis. Utilize the "salt in" osmoprotection strategy. Found in hypersaline alkaline lakes. Member of the phylum Euryarchaeota.

DESCRIPTION OF *METHANONATRONARCHAEUM THERMOPHILUM* SP. NOV.

ther.mo.phi'lum Gr. adj. *thermos*, hot; N. L. adj. philum (from Gr. adj. philos -ê -on), friend, loving; N. L. adj. thermophilum, thermophilic).

The species description is based on eleven isolates. Cells are small irregular cocci, 0.4-0.5 μm in size, motile by 1-5 archaella. The cell wall is a thin monolayer covered with EPS. The cells lyze at salinity below 2 M Na^+. Accumulate potassium as compatible solute. The F_{420}-dependent cell autofluorescence is absent. The colonies are yellowish, lens-shaped, up to 1 mm. The core lipids are dominated by archaeol (C20-C20 DGE). Strictly anaerobic methanogens utilizing MeOH, methylamines and dimethylsulfide as electron acceptor and formate or H_2 as electron donor. Heterotrophic, can utilize yeast extract or acetate as C-source. Growth depends on external CoM, FeS/or sterilized anaerobic sediments from soda lakes. Obligately alkaliphilic with a pH range for growth from 8.2 to 10.2 (optimum at pH 9.5-9.7) and extremely halo(natrono)philic, growing optimally at 4 M total Na^+. Moderately thermophilic, with an optimum at 50°C and the upper limit for growth at 60°C. The G + C content of the genomic DNA in the type strain is 38 mol% (genome). The type strain, AMET1^T (DSM 26684=NBRC 110805=UNIQEM 982**),** was isolated from sediments of hypersaline soda lakes in Kulunda Steppe (Altai, Russia). The accession number of 16S rRNA gene sequence of the type strain in GenBank is KY449317.

DESCRIPTION OF '*CANDIDATUS* METHANOHALARCHAEUM THERMOPHILUM'

Metha.no.hal.ar.chae'um. N. L. n. *methanum* [from French n. *méth*(*yle*) and chemical suffix -*ane*], methane; N. L. pref. *methano*-, pertaining to methane; Gr. n. *hals halos*, sea salt, NaCl; N. L. neut. n. *archaeum* [from Gr. adj. *archaios*, *-e*, *-on* ancient] archaeon; N. L. neut. n. *Methanohalarchaeum* a salt-loving archaeon forming methane;
ther.mo.phi'lum Gr. adj. *thermos*, hot; N. L. adj. philum (from Gr. adj. philos -ê -on), friend, loving; N. L. adj. thermophilum, thermophilic).

The description is based on three highly enriched monomethanogenic cultures. Cells are small, irregular, non-motile cocci, 0.4-0.5 μm. The cell wall is a thin monolayer covered with EPS. The cells lyze at salinity below 2 M NaCl. The F_{420}-dependent cell autofluorescence is absent. The core lipids are dominated by archaeol (C20-C20 DGE). The colony formation was not observed. Strictly anaerobic methanogens utilizing MeOH and trimethylamine as electron acceptor and H_2 or formate as electron donor. Heterotrophic, utilize yeast extract as C-source. The growth depends on external CoM and sterilized anaerobic sediments from salt lakes. Extremely halophilic, grow optimally at 4-5 M NaCl. The pH optimum for growth is 7-

7.5. Moderately thermophilic with an optimum at 50^{o}C and the upper limit for growth at 60^{o}C. The G + C content of the genomic DNA in the type strain is 35.4 mol% (genome). The type strain, HMET1^{T}, was enriched from sediments of hypersaline lakes in Kulunda Steppe. The accession number of 16S rRNA sequence of the type strain in GenBank is KY449328.

DESCRIPTION OF *METHANONATRONARCHAEACEAE* FAM. NOV.

The description is the same as for the genus *Methanonatronarchaeum*.
Type genus: *Methanonatronarchaeum* gen. nov.

DESCRIPTION OF ORDER *METHANONATRONARCHAEALES* ORD. NOV.

The description is the same as for the genus *Methanonatronarchaeum*.
Type genus: *Methanonatronarchaeum* gen. nov.

DESCRIPTION OF *METHANONATRONARCHAEIA* CLASSIS NOV.

The class *Methanonatronarchaeia* is defined on the basis of comparative sequence analysis of the 16S rRNA obtained from 11 pure cultures of the genus *Methanonatronarchaeum*, 3 highly enriched cultures of 'Candidatus Methanohalarchaeum' and the cloned sequences from uncultured SA1 group found in various hypersaline habitats of terrestrial and marine origin.
Type order: *Methanonatronarchaeales* ord. nov.

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Conflicts of interest

The authors declared no conflicts of interest

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400 **Table 1**. Extremely halophilic and moderately thermophilic mixotrophic methanogens isolated from hypersaline
401 lakes at 4 M total Na^+ TMA - trimethylamine

Strain	Lake	Area	Brine parameters			Enrichment conditions		
			pH	Total salt g/l	Soluble carbonate alkalinity, M	Substrate	pH	T, °C
AMET1	Mix 5 soda lakes	Kulunda Steppe (Altai, Russia) 2013-2015	9.6-10.1	120-400	0.6-3.0	MeOH+formate	**9.6**	48
AMET3	Tanatar-1		10.1	350	2.8			48
AMET4	Picturesque Lake		9.8	250				48
AMET5	Mix 6 soda lakes		9.6-10.2	50-380	0.5-3.4	TMA +formate		48
AMET6-2	Tanatar-1		10.25	380	3.4	MeOH+formate		60
AMET7	Soda crystallizer		9.6	350	3.8			55
AMET8	Mix 6 soda lakes		9.6-10.2	50-380				30
AMET9	Soda crystallizer		10.1	340	3.9			43
AMET10	Stamp Lake		9.1	325	0.2			54
AMET2	Mix from 8 lakes	Wadi al Natrun (Egypt, 2000)	9.1-9.9	200-360	0.1-0.9	MeOH+formate		60
AMET-Sl	Searles Lake	California	9.8	350	0.2	MeOH+formate	**9.2**	48
HMET1 (mixed culture)	Mix from 4 salt lakers	Kulunda Steppe 2014	7.5-8.1	280-340	-	$TMA+H_2$	**7.0**	48
HMET-El (mixed culture)	Lake Elton	Southa Russia 2015	6.7	320	-	MeOH+formate		54
HMET-Eu (mixed culture)	Salt crystallizer	Crimea (Russia) 2015	7.2	220	-			55

Figure legends

Fig. 1 Cell morphology of *Methanonatronarchaeum thermophilum* strain AMET1^T grown with MeOH+formate at pH 9.5, 4 M total Na^+ and 48^{o}C. (**a)**, phase contrast microscopy; (**b** and **c**), electron microscopy of total cells and thin sections, respectively. **N**, nucleoide; **CM** - cytoplasmic membrane; **CW** - cell wall.

Fig. 2. Cell morphology of '*Ca*. Methanohalarchaeum thermophilum' strain HMET1 grown with TMA+H_2 at pH 7, 4 M NaCl and 50^{o}C. (**a)**, phase contrast microscopy; (**b** and **c**), electron microscopy of total cells and thin section, respectively. **N**, nucleoide; **ICPM** - intracytoplasmic membranes; **PHA** - possible polyhydroxyalkanoate storage granule; **CW** - cell wall.

Fig. 3. Phylogeny of novel halo(alkali)philic methanogens from hypersaline lakes based on the 16S rRNA gene sequence analysis. The bootstrap values above 70% are shown at the nods. Bar, 0.10 changes per position.

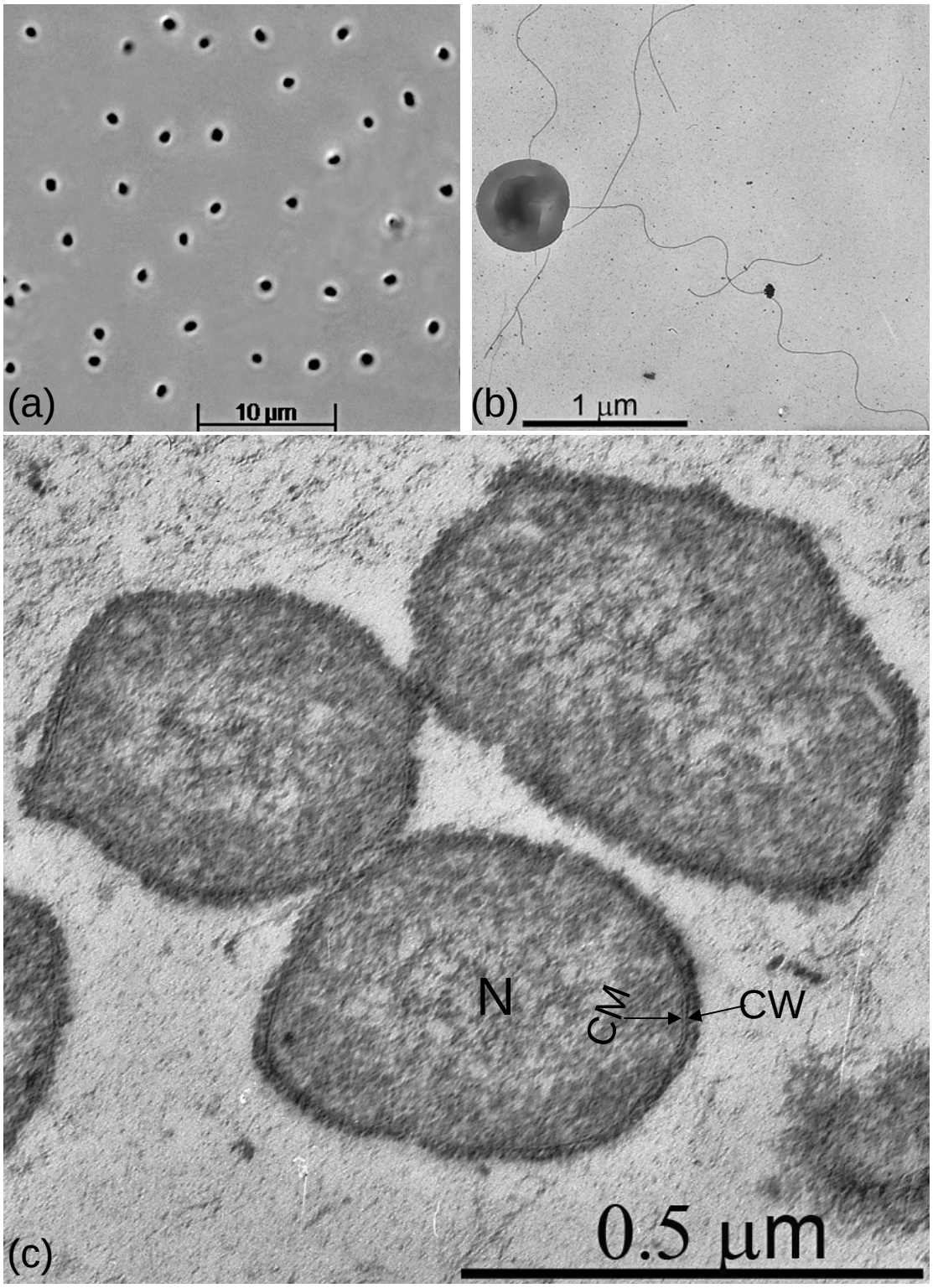


Fig.1

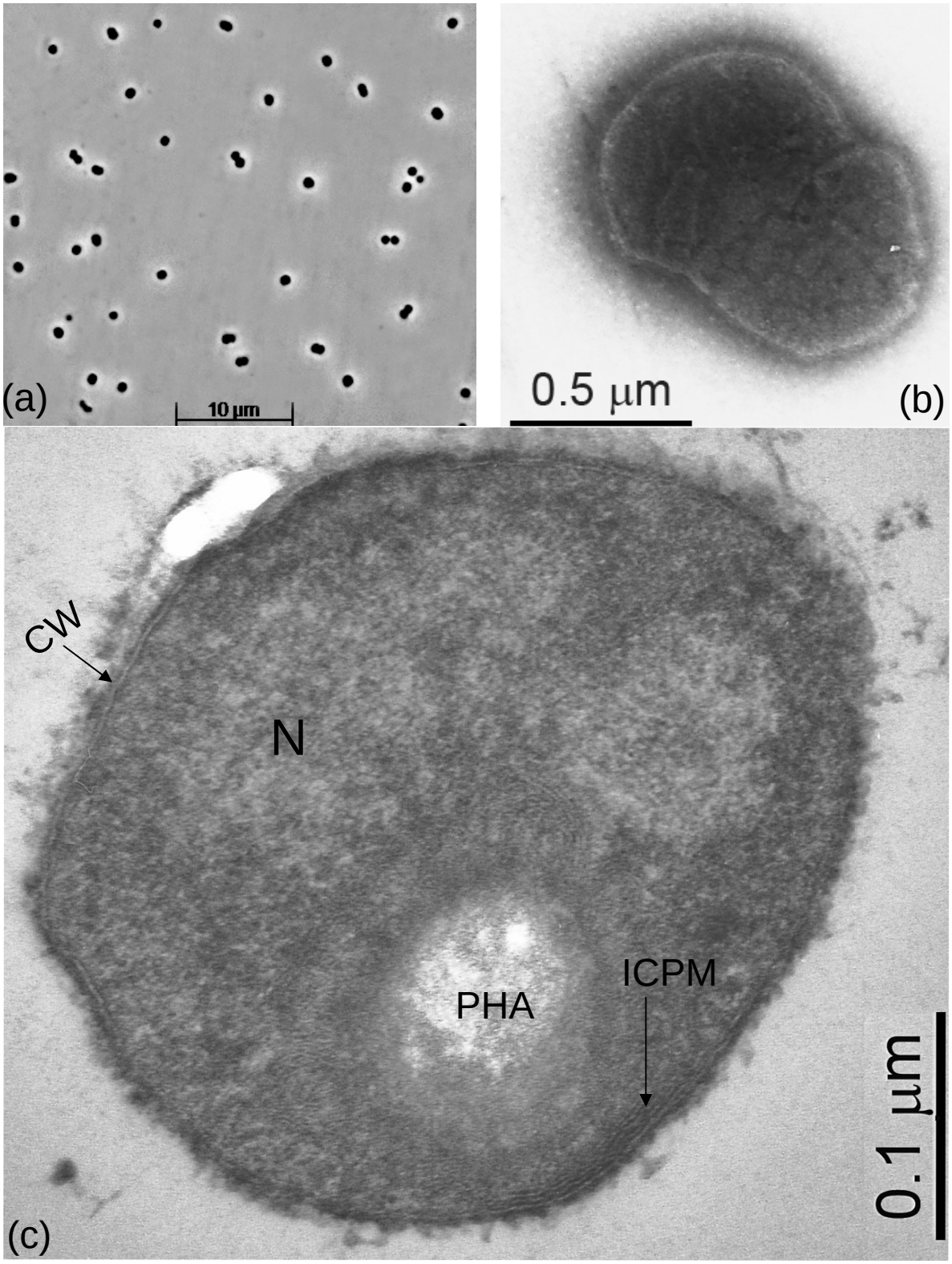
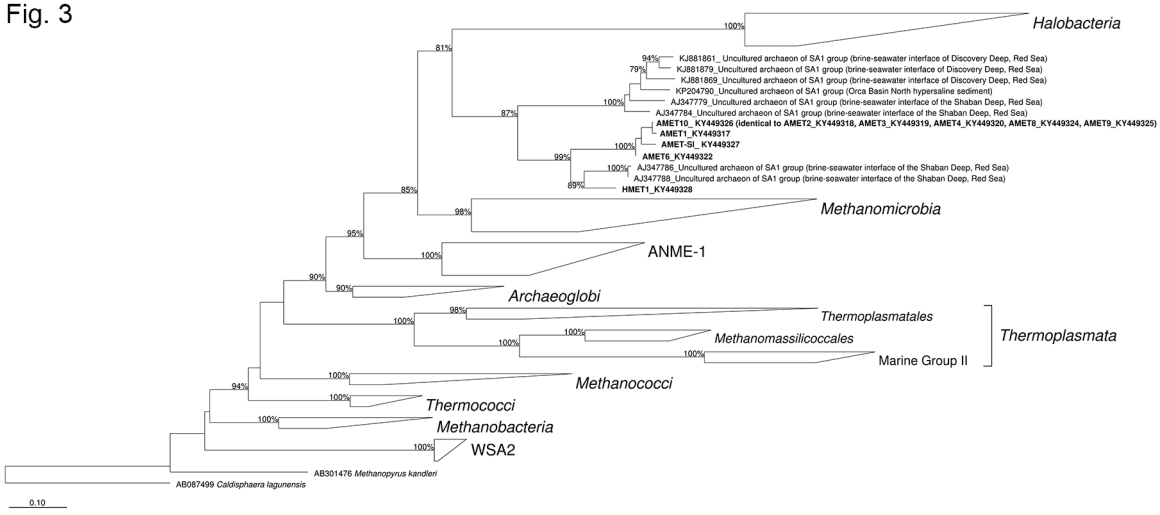


Fig.2

Fig. 3


Halobacteria
KJ881861_ Uncultured archaeon of SA1 group (brine-seawater interface of Discovery Deep, Red Sea)
KJ881879_Uncultured archaeon of SA1 group (brine-seawater interface of Discovery Deep, Red Sea)
KJ881869_Uncultured archaeon of SA1 group (brine-seawater interface of Discovery Deep, Red Sea)
KP204790_Uncultured archaeon of SA1 group (Orca Basin North hypersaline sediment)
AJ347779_Uncultured archaeon of SA1 group (brine-seawater interface of the Shaban Deep, Red Sea)
AJ347784_Uncultured archaeon of SA1 group (brine-seawater interface of the Shaban Deep, Red Sea)
AMET10_ KY449326 (identical to AMET2_KY449318, AMET3_KY449319, AMET4_KY449320, AMET8_KY449324, AMET9_KY449325)
AMET1_KY449317
AMET-SI_ KY449327
AMET6_KY449322
AJ347786_Uncultured archaeon of SA1 group (brine-seawater interface of the Shaban Deep, Red Sea)
AJ347788_Uncultured archaeon of SA1 group (brine-seawater interface of the Shaban Deep, Red Sea)
HMET1_KY449328
Methanomicrobia
ANME-1
Archaeoglobi
Thermoplasmatales
Methanomassiliococcales
Marine Group II
Thermoplasmata
Methanococci
Thermococci
Methanobacteria
WSA2
AB301476 Methanopyrus kandleri
AB087499 Caldisphaera lagunensis
0.10

Methanonatronarchaeum thermophilum gen. nov., sp. nov, and '*Candidatus* Methanohalarchaeum thermophilum', extremely halo(natrono)philic methyl-reducing methanogens from hypersaline lakes comprise a new euryarchaeal class *Methanonatronarchaeia* classis nov.

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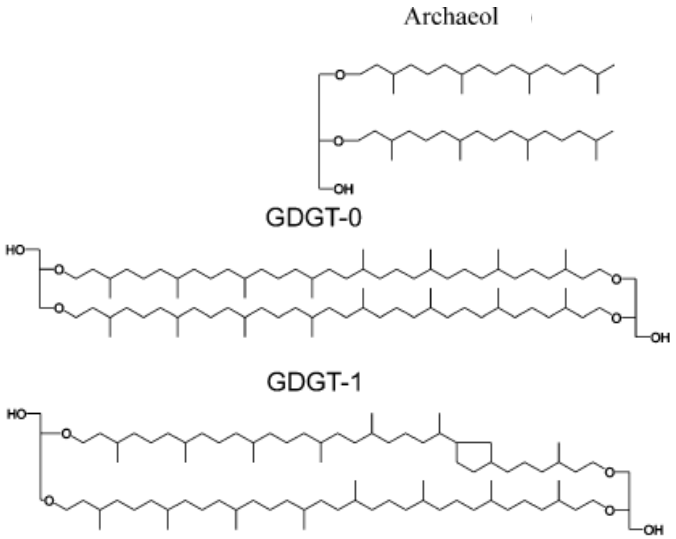
Supplementary Table S1.
Membrane lipid composition of extremely halophilic methyl-reducing methanogens

A: core lipids

Strain	Di- and monophytanyl glycerol ethers (%)			Glycerol dibiphytanyl glycerol tetraethers	
	Archaeol	2-C20 MGE	1-C20 MGE	GDGT-0/archaeol	GDGT-1
AMET1	96.0	2.4	1.6	1:1	+
HMET1	93.4	-	6.6	3:1	-



B: Intact polar lipids composed of a core lipid with attached polar head group(s)

Strain	Archaeol							GDGT-0	
	PG	PGP	PGP-Me	PS	HPH	DH	Cardiolipin Ar-P-G-P-Ar*	PG PG	DH
AMET1	++		+	+				++	
HMET1	+	+			+	+++	+		+++

PG=Phosphatidylglycerol
PGP=Phosphatidylglycerolphosphate
PGP-Me=Phosphatidylglycerophosphate methyl ester
PS=Phosphatidylserine
HPH=hexosephosphatidylhexose
DH=dihexose
Ar-P-G-P-Ar = archaeol-phosphatidyl-glycerol-phosphatidyl-archaeol
(*small part of the cardiolipin with extended archaeol (C25/C20) was also present)

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Moscow, Russia 12 September 2014

Confirmation of the availability of a strain for the purpose of valid publication of a new name according to the Bacteriological Code

The following information is confidential and serves only to allow the Microbiology journal to confirm that a strain has been deposited and will be available from the UNIQEM (Unique and Extremophilic Microorganisms Collection of Winogradsky Institute of Microbiology RAS).

Methanonatronaerchaeium thermophilum" strain **AMET1(T)** has been deposited in the UNIQEM under the number **U982**.

The strains are available in the open section of the UNIQEM and restrictions have not been placed on access to information concerning the presence of the strain in the UNIQEM. It will be included in published and online catalogues after publication of this number by the authors.

The strain has been checked for viability and is stored using 20% glycerol stock culture in liquid nitrogen.

Prof. Dr. Valery F. Galchenko
Director of UNIQEM