



HAL
open science

Evolutionary placement of Methanonatronarchaeia

Monique Aouad, Guillaume Borrel, Céline Brochier-Armanet, Simonetta Gribaldo

► **To cite this version:**

Monique Aouad, Guillaume Borrel, Céline Brochier-Armanet, Simonetta Gribaldo. Evolutionary placement of Methanonatronarchaeia. *Nature Microbiology*, 2019, 10.1038/s41564-019-0359-z . pasteur-02059654

HAL Id: pasteur-02059654

<https://pasteur.hal.science/pasteur-02059654>

Submitted on 6 Mar 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - ShareAlike 4.0 International License

1 **Subject ontology**

2 [URI /631/326/26/2526]

3 [URI /631/326/171]

4
5
6 **Evolutionary placement of *Methanonatronarchaeia***

8
9 Monique Aouad^{1#}, Guillaume Borrel^{2#}, Céline Brochier-Armanet¹, and Simonetta Gribaldo²

10 ¹Univ Lyon, Université Lyon 1, CNRS, UMR5558, Laboratoire de Biométrie et Biologie
11 Évolutive, 43 bd du 11 novembre 1918, F-69622 Villeurbanne, France.

12 ²Institut Pasteur, Unit Evolutionary Biology of the Microbial Cell, Department of Microbiology,
13 15 rue du Dr Roux, 75015 Paris, France.

14 # these authors contributed equally to this work

15 Correspondence:

16 celine.brochier-armanet@univ-lyon1.fr

17 simonetta.gribaldo@pasteur.fr

18
19 ***Methanonatronarchaeia*, a newly discovered archaeal lineage of extremely halophilic**
20 **methanogens, were proposed to represent an evolutionary intermediate between**
21 **archaeal methanogens and the extremely halophilic *Halobacteria*. Here, we show that**
22 **the sistership between *Methanonatronarchaeia* and *Halobacteria* results from a tree**
23 **reconstruction artefact and that the divergence of *Methanonatronarchaeia* is in fact**
24 **much deeper. This sheds a new light on the adaptation to extreme halophilic lifestyle**
25 **in archaea and on the evolution of methanogenesis.**

26
27 Sorokin and colleagues recently reported the identification of *Methanonatronarchaeia*, a
28 fascinating archaeal lineage of extremely halophilic, moderately thermophilic, methyl-
29 reducing methanogens^{1,2}. Similar to most recently discovered methanogens,
30 *Methanonatronarchaeia* perform methanogenesis based on H₂ and methyl compounds, a
31 metabolism not previously reported from hypersaline environments. Together with
32 *Halobacteria* and *Nanohaloarchaea*³, *Methanonatronarchaeia* represent the third discovered
33 lineage of extreme halophilic archaea and the most halophilic methanogens ever found. They
34 have likely adapted to this lifestyle by employing a salt-in osmoprotection strategy¹, unlike
35 previously known halophilic methanogens and similarly to the two other extreme halophilic
36 archaeal lineages⁴. Moreover, *Methanonatronarchaeia* rely on cytochromes for
37 methanogenesis¹, a characteristic previously thought to be restricted to the
38 *Methanosarcinales*⁵. A maximum Likelihood (ML) phylogenetic analysis of a supermatrix
39 gathering ribosomal proteins indicated *Methanonatronarchaeia* as the closest relatives to
40 *Halobacteria* (Fig. 1A, red branches)¹. They were therefore proposed to be evolutionary
41 intermediates on the path from methanogens to extreme halophiles¹. However, multiple
42 substitutions occurring at the same site in sequences can mask the original phylogenetic
43 signal and provoke tree reconstruction artefacts⁶, a phenomenon particularly evident in
44 lineages that adapted to extreme salinity⁷.

45

46 To test the phylogenetic position of *Methanonatronarchaeia*, we reanalyzed the original
47 supermatrix of ribosomal proteins used by Sorokin et al.¹, through the progressive removal of
48 the fastest evolving sites, a method that is frequently used to reduce artefacts linked to
49 multiple substitutions⁷. This analysis, both by ML and Bayesian approaches including non-
50 homogeneous evolutionary models, shows that the clustering of *Halobacteria* and
51 *Methanonatronarchaeia* (Fig. 1B-C, red line) was recovered only when the fastest evolving-
52 sites are included in the analysis, while the progressive removal of these sites shifted the
53 position of *Methanonatronarchaeia* away from *Halobacteria* and to a deeper branching
54 position at the base of the superclass 'Methanotecta'⁸ (Fig. 1B-C, green line). This placement
55 is also consistently and robustly recovered when *Methanonatronarchaeia* were included in
56 two recently published supermatrices comprising a larger number of markers⁶ (over 250
57 conserved protein families) or a larger taxonomic sampling of the Methanotecta⁹ (including
58 ANME1, Syntrophoarchaeales, Methanoliparia, and a third *Methanonatronarchaeia*
59 member). In contrast with the dataset of Sorokin et al.¹, the new placement of
60 *Methanonatronarchaeia* was robust to the removal of the fastest-evolving sites for both these
61 supermatrices (Fig. 1D-G).

62
63 Our analyses indicate that the placement of *Methanonatronarchaeia* as the methanogenic
64 closest relatives of *Halobacteria* proposed in Sorokin et al.¹ is likely the consequence of a
65 tree reconstruction artefact induced by a multiple substitution-bias which is particularly strong
66 in their ribosomal protein dataset, but not in the other two datasets. The alternative position
67 of the *Methanonatronarchaeia* disclosed here provides a new perspective on the evolution of
68 this fascinating lineage. For example, it indicates that their adaptation to extreme halophily
69 would have occurred independently from the *Halobacteria*. Moreover, following the recent
70 proposal for the placement of *Nanohaloarchaea* as sister to the *Methanocellales*⁶, the salt-in
71 strategy used for thriving in hypersaline environments would have emerged three times
72 independently in the *Archaea*, a remarkable example of convergent evolution for adaptation
73 to similar environments. Finally, the new placement of *Methanonatronarchaeia* is highly
74 relevant for the evolution and diversity of methanogenesis, as their characteristics may
75 reflect those of the methanogenic ancestor of the whole 'Methanotecta' superclass. For
76 example, the fact that *Methanonatronarchaeia* rely on cytochromes for methanogenesis³
77 raises the question of whether this feature may be ancestral to all Class II methanogens and
78 was retained only in *Methanosarcinales* while *Methanomicrobiales*, *Methanocellales* and
79 *Methanoflorentaceae* shifted secondarily to methanogenesis without cytochromes, or if
80 instead it emerged twice independently.

81 The current pace in the acquisition of genomic data and the discovery of new lineages^{8,10} will
82 certainly allow to tackle these fundamental questions in the evolution and ecology of
83 methanogens and of *Archaea* in general.

84 85 **References**

- 86 1) Sorokin et al. (2017) *Nat Microbiol* 2:17081.
- 87 2) Sorokin et al. (2018) *Int J Syst Evol Microbiol* 68(7):2199-2208.
- 88 3) Narasingarao et al. (2012) *ISME J* 6, 81–93
- 89 4) Oren (2008) *Saline Syst* 4, 2.
- 90 5) Thauer et al. (2008) *Nat Rev Microbiol* 6(8):579-91.
- 91 6) Delsuc (2005) *Nat Rev Genet* 6(5):361-75.
- 92 7) Aouad et al. (2018) *Mol Phylogenet Evol* 127:46-54
- 93 8) Adam et al. (2017) *ISME J* (11):2407-2425.
- 94 9) Borrel et al. *Nat Microbiol* (accepted).
- 95 10) Spang et al. (2017) *Science* 357(6351).

96
97 Correspondence and request for materials should be addressed to Simonetta Gribaldo
98 (simonetta.gribaldo@pasteur.fr) or Céline Brochier-Armanet (celine.brochier-armanet@univ-lyon1.fr).

101 **Acknowledgements**

102 S.G., G.B., and C.B.A acknowledge funding from the French National Agency for Research,
103 Grant ArchEvol (ANR-16-CE02-0005-01). M.A. is funded by a doctoral fellowship from the
104 Région Rhône-Alpes-ARC 1 Santé. We thank the PRABI (Pôle Rhône-Alpes de
105 Bioinformatique) and the computational and storage services (TARS cluster) provided by the
106 IT department at Institut Pasteur, Paris.

107
108 **Author contributions**

109 S.G. and C.B.A. supervised the study. M.A. and G.B. assembled the datasets and performed
110 all analyses. All authors analysed the data and wrote the manuscript.

111
112 **Competing interests**

113 The authors declare no competing interest.

114
115 **Legend of Fig. 1**

116 **(A)** Schematic phylogeny of the *Archaea*, with a focus on the ‘Methanotecta’ superclass⁸.
117 Dotted lines indicate two alternative branchings of *Methanonatronarchaeia*: as the sister-
118 lineage of *Halobacteria* (red) or at the base of ‘Methanotecta’ (green).

119 **(B-G)**: Impact on the placement of *Methanonatronarchaeia* of the progressive removal of the
120 fastest-evolving sites from the three analysed supermatrices (see Supplementary Information
121 (SI) for details). **(B-C)**: the supermatrix of ribosomal proteins (8,072 amino acid positions)
122 derived from Sorokin et al.¹, **(D-E)**: the supermatrix, derived from Adam et al.⁸ (40 conserved
123 protein families, 9,228 amino acid positions), and **(F-G)**: the supermatrix derived from Aouad
124 et al.⁶ (258 conserved protein families, 62,398 amino acid positions).

125 The x-axis indicates the percentage of amino acid positions of the supermatrices that were
126 kept for phylogenetic analyses during the progressive removal of the fastest evolving sites.
127 The y-axis corresponds to bootstrap values associated to the ML trees inferred using the
128 LG+G4 evolutionary model **(B, D, and F)** or the PMSF+LG+G4 evolutionary model **(G)**, or to
129 posterior probabilities associated to the Bayesian trees inferred with the CAT+GTR+G4
130 evolutionary model **(C, and E)**. The green and red lines shown on these graphs correspond
131 to the bootstrap values and posterior probabilities supporting the two alternative placements
132 of *Methanonatronarchaeia* as illustrated in Figure 1A. In all trees, the clustering of
133 *Methanonatronarchaeia* with ‘Methanotecta’ was strongly supported, excluding the branching
134 of *Methanonatronarchaeia* elsewhere in the archaeal phylogeny. For two supermatrices on
135 panel C (86, 82, indicated by an asterisk), *Methanonatronarchaeia* branched in-between
136 *Archaeoglobales* and ‘Ca. Methanophagales’ (ANME-1). All trees and corresponding
137 supermatrices are provided in Supplementary Information (SI).

