Biodiversity and environmental function of new *Thermoplasmata*

Olga V Golyshina*, Stepan Toshchakov, Sergey Gavrilov, Rafael Bargiela, Ilya Kublanov and Peter Golyshin
Thermoplasmata Reysenbach 2002, class. nov.

Type order: Thermoplasmatales Reysenbach 2002.
The order *Thermoplasmales*
Class *Thermoplasmata*
Phylum *Euryarchaeota*

**The most acidophilic organisms**

- Acidic pH optima and range (lowest known, growth at pH below 2, some strains are able to growth at pH 0: *Picrophilus* spp., *Acidiplasma* spp. and *Cuniculiplasma* spp.)
- Unique morphology (cell wall-lacking organisms)
- A great diversity of uncultured *Thermoplasmales*
The new member *Cuniculiplasma divulgatum* (the family *Cuniculiplasmataceae*) with sequence identities:

*Thermogymnomonas acidicola* (86.3 %),

followed by *Thermoplasma acidophilum* (84.7 %) and *Thermoplasma volcanium* (84.3 %), *Picrophilus torridus* and *Picrophilus oshimae* (both 84.9 %),

with more distantly relatives being members of the Ferroplasmaceae; both species of *Acidiplasma* (77.0 %) and *Ferroplasma acidiphilum* (73.9 %).

Golyshina et al., 2016a
Places of isolation of strains of *Cuniculiplasma divulgatum*

Mynydd Parys/Parys Mountain, Anglesey, North Wales, UK

Cantareras mine site in Tharsis, Huelva, Spain
Uncultured archaea within the order *Thermoplasmales*: candidate clades

“G-plasma”, 100% identical with 16S rRNA of *Cuniculiplasma divulgatum*

Acid Mine Drainage System
Iron Mountain, USA

*Yelton et al.*, 2013
Physiological traits: *in silico* predictions in ‘G-plasma’ vs experimental data in *Cuniculiplasma* strains

- Iron oxidation
  - sulfocyanin

- Archaeal flagella and pili
  - the full operon encoding **FlaBCDEFGHIJ**

- S-layer prediction
  - oligosaccharyltransferase AgIB

- Methylotrophy
  - methenyl tetrahydrofolate cyclohydrolase and formyl-tetrahydrofolate synthetase

Electron micrographs of *Cuniculiplasma* *divulgatum* showing monolayer membranes and absence of the S-layer (**A**, **B**), pilus (**C**), arrow and pleomorphism of cells. Scale bars: 500 nm (**A**), 200 nm (**B**), 1 μm (**C,D**). Ultrathin sections (**A,B**) and Pt-C shadow castings (**C,D**). Figure shows cells of the strain PM4 (**B,C and D**) and SS (**A**). Arrowheads in **C and D** indicate the direction of shadow cast, arrows in **A and B** point to the cytoplasmic membrane.

*(Golyshina et al., 2016b)*

**Phylogenetic tree based on sulfocyanin/rusticyanin genes**
Overview of metabolic networks in acid mine drainage systems

Mendez-Garcia et al., 2015
<table>
<thead>
<tr>
<th>Characteristic</th>
<th><em>Acidiplasma</em></th>
<th><em>Ferroplasma</em></th>
<th><em>Thermoplama</em></th>
<th><em>Picrophilus</em></th>
<th><em>Thermogymnononas</em></th>
<th><em>Cuniculiplasma</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Cell morphology</strong></td>
<td>pleomorphic</td>
<td>pleomorphic</td>
<td>pleomorphic</td>
<td>Irregular coccoid</td>
<td>pleomorphic</td>
<td>pleomorphic</td>
</tr>
<tr>
<td><strong>Cell wall</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>S-layer</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Motility</strong></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Pili</strong></td>
<td>+</td>
<td>-</td>
<td>no information</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Fe (II/III) metabolism</strong></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>T, °C optimum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>pH range for growth</strong></td>
<td>0.5-4 (1-1.5)</td>
<td>0.3-3 (1-1.7)</td>
<td>0.5-4 (1-2)</td>
<td>0.3-3.5 (0.7)</td>
<td>1.8-4 (3)</td>
<td>0.5-4 (1-1.2)</td>
</tr>
<tr>
<td><strong>pH optimum</strong></td>
<td></td>
<td></td>
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<tr>
<td><strong>Anaerobic growth</strong></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>DNA G+C mol%</strong></td>
<td>34-37</td>
<td>35-37</td>
<td>38-46</td>
<td>36</td>
<td>56</td>
<td>37</td>
</tr>
<tr>
<td><strong>Respiratory quinones</strong></td>
<td>naphtoquinone derivatives</td>
<td>no information</td>
<td>menaquinones, thermoplasmaquinones, methionaoquinones</td>
<td>no information</td>
<td>menaquinones</td>
<td>menaquinones, thermoplasmaquinones</td>
</tr>
<tr>
<td><strong>Main sugar moiety in major lipid component</strong></td>
<td>b-galactose</td>
<td>b-glucose</td>
<td>b-gulose</td>
<td>b-glucose</td>
<td>no information</td>
<td>no information</td>
</tr>
</tbody>
</table>

Golyshina et al., 2016  *Bergey’s Manual*
Distribution of *Cuniculiplasmataceae* (Golyshina et al., 2016b)
Genomic islands (GIs) in *C. divulgatum* strains PM4 and S5

GI incorporate genes of toxin-antitoxin, restriction-modification systems and being metal-, efflux-, transport-, and oxidative stress response-related 

(Golyshina *et al.*, 2016b).
Coexistence of *C. divulgatum* PM4 with Mia14 (ARMAN-2-like) *in vitro* and *in situ*

General overview of Parys Mt community

**Distribution of Parys Mt. metagenomic contigs by coverage and GC content.** Clusters of contigs related to *C. divulgatum*—‘Ca. Mancarchaeum’ and uncultivated *Thermoplasmatales*—‘Ca. Micrarchaeota’ excluding ‘Ca. Mancarchaeum acidiphilum’ microbial consortia are shown with *dotted-line ovals* (Golyshina et al., 2017)
Conclusions

• Recently, the order *Thermoplasmatales* expanded by a number of cultured members from family *Cuniculiplasmataceae* with *Cuniculiplasma divulgatum* species.

• These archaea are globally ubiquitously distributed in low-pH settings.

• A number of physiological and genomic features suggest their environmental function linked to scavenger type of nutrition.

• Co-existence of *Cuniculiplasmataceae* with ARMAN-related archaea specify their common environmental strategy, metabolic interaction and evolution history.
Acknowledgements

The novel extremely acidophilic, cell-wall-deficient archaean *Cuniculiplasma divulgatum* gen. nov., sp. nov. represents a new family, *Cuniculiplasmataceae* fam. nov., of the order *Thermoplasmatales*

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‘ARMAN’ archaea depend on association with euryarchaeal host in culture and in situ

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