

Thermophilic photosynthetic bacterial communities exposed to different oxygen concentrations as candidates for photosynthetic communities on early earth

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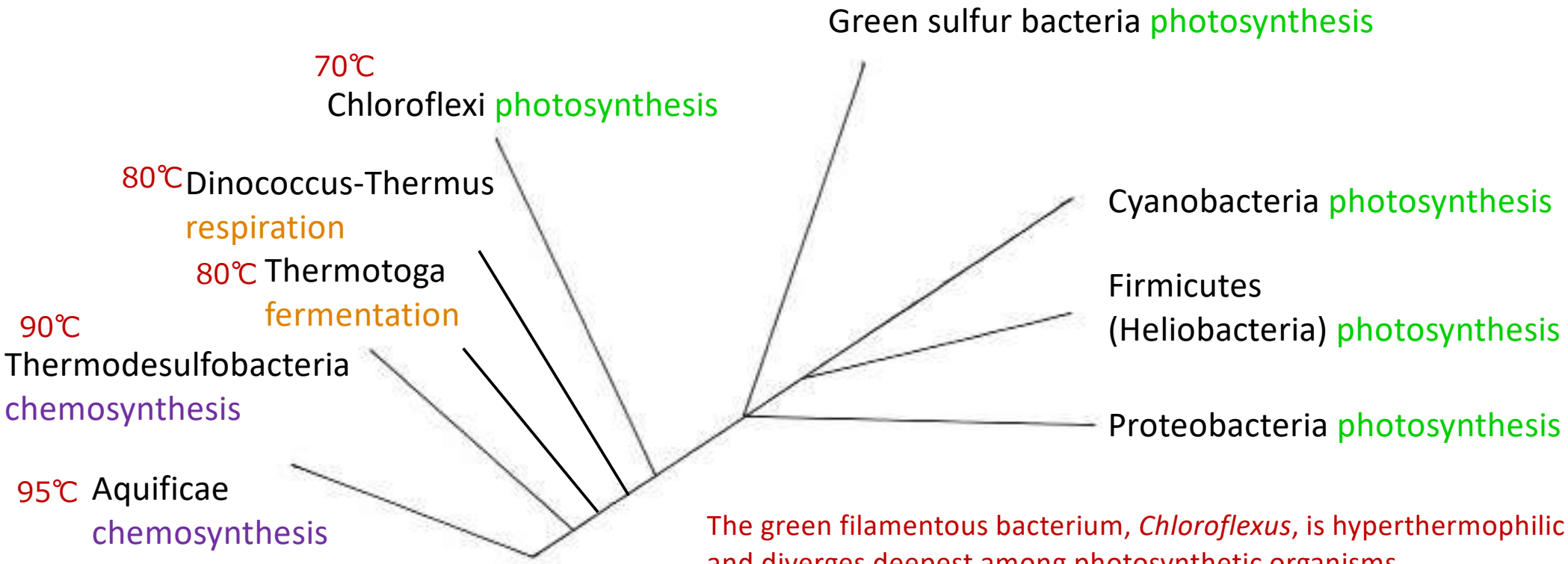
Background Hypothesis 1: Life was born at high temp. and early organisms were hyperthermophilic

1. The maximum temperature for biological growth is 122 ° C, and the organism branches deeply in Archaea.
2. The maximum temperature for bacterial growth is 95 ° C, and the organism branches deeply in Bacteria.

【 The phylogenetic tree of 16S rRNA of major phyla of hyperthermophilic and photosynthetic bacteria 】



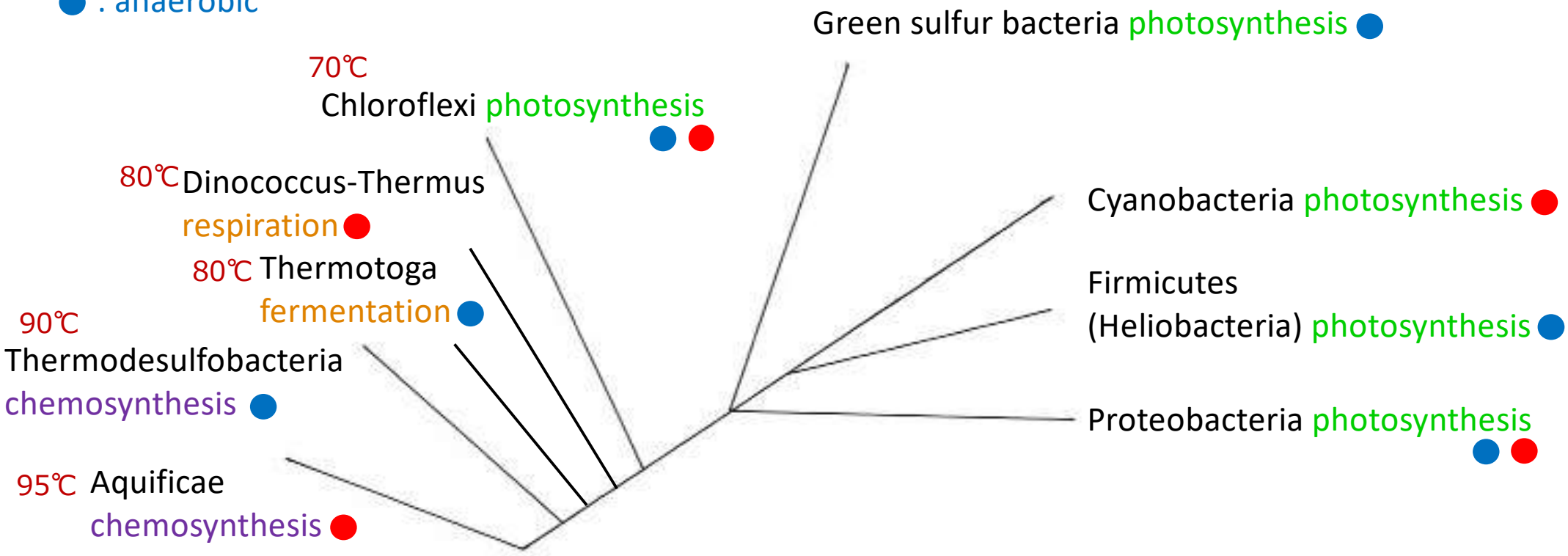
The green filamentous bacterium, *Chloroflexus*, is hyperthermophilic and diverges deepest among photosynthetic organisms.

Background Hypothesis 2: The green filamentous bacterium, *Chloroflexus*, resembles the oldest photosynthetic organisms

1. Deep divergence in 16S rRNA phylogenetic analysis
2. Hyperthermophilic
3. Photosynthetic reaction centers and photosynthetic pigments are relatively simple

● : aerobic

● : anaerobic



Background Hypothesis 3: Oxygen respiration is older than photosynthetic oxygen evolution

1. Cytochrome oxidase for oxygen respiration has existed before the increase in atmospheric oxygen due to oxygen-evolving photosynthesis.
2. It is highly possible that the common ancestor of life already had cytochrome c oxidase.

Evolution of cytochrome oxidase, an enzyme older than atmospheric oxygen
J. Castresana, M. Lubben, M. Saraste, D.G. Higgins (1994)
EMBO Journal, 13, 2516-2525

Subsequent studies supported these ideas.

The multiple evolutionary histories of dioxygen reductases: Implications for the origin and evolution of aerobic respiration
C. Brochier-Armanet, E. Talla, S. Gribaldo (2009), Mol. Biol. Evol. 26, 285-297

Availability of O_2 and H_2O_2 on pre-photosynthetic earth
J. Haqq-Misra, J.F. Kasting, S. Lee (2011), Astrobiology 11, 293-302

Evolution of oxygen-utilizing enzymes suggests early biosphere oxygenation
J. Jabloriska, D.S. Tawfik (2021), Nature Ecology & Evolution 5, 442–448

Upper questions of this research

1. What kinds of energy-transducing organisms were included in the microbial communities at the time when photosynthesis began?

   Aerobic chemosynthesis, anaerobic chemosynthesis, fermentation, aerobic respiration, anaerobic respiration

2. What kinds of oxygen concentrations were the biological communities at the time when photosynthesis began?

   Anaerobic, semi-aerobic, aerobic

Objective

Objective：

1. To determine microbial compositions in various communities with *Chloroflexus* as a sole photosynthetic organism in Nakabusa hot springs, in relation with oxygen conditions.

2. To estimate the microbial compositions in early photosynthetic communities on Earth, in relation with oxygen conditions.

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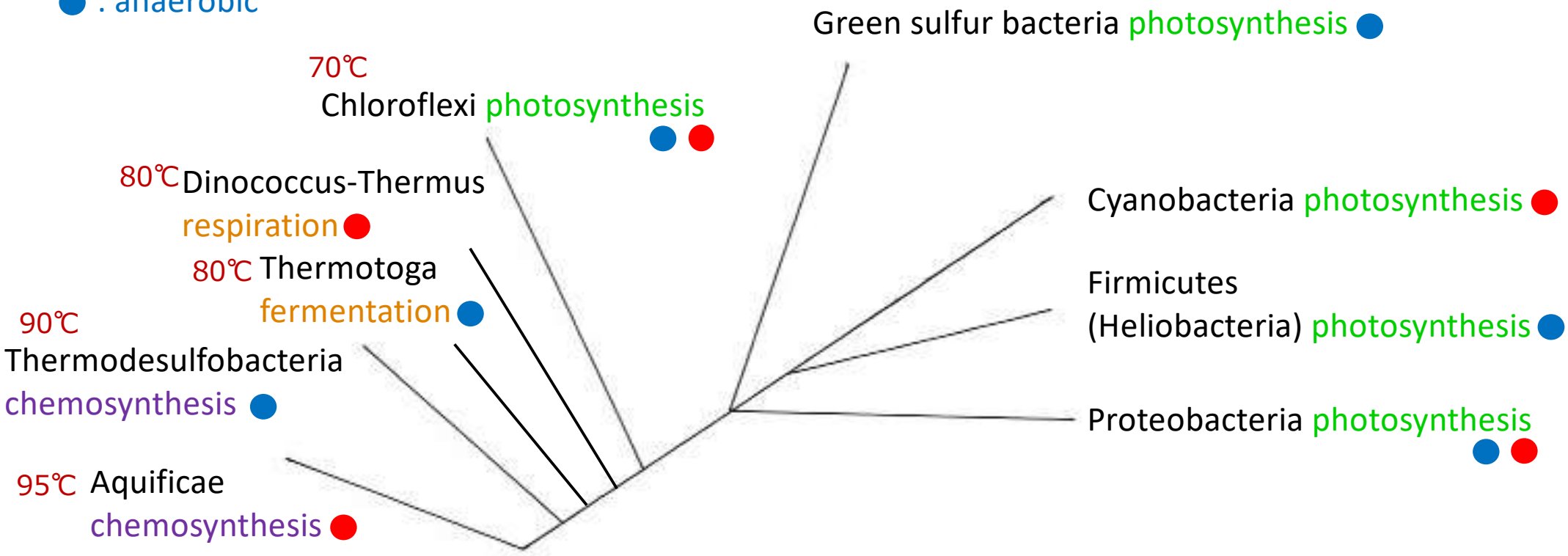


Nakabusa hot springs: Azumino City Nagano Pref. Alkaline hydrogen sulfide springs

源泉数　30カ所以上
（すべて自然湧出）
温度　60℃～95℃
湧出量　1500 L/min 以上

pH 8.0～9.5
硫化水素イオン　200～400 μmol/L
硫酸イオン　150～300 μmol/L
炭酸水素イオン　1.5～2.5 mmol/L

Photosynthetic and chemosynthetic microbial communities in Nakabusa hot springs

Kassen spring: Source 86 ℃, almost horizontal flow

86℃

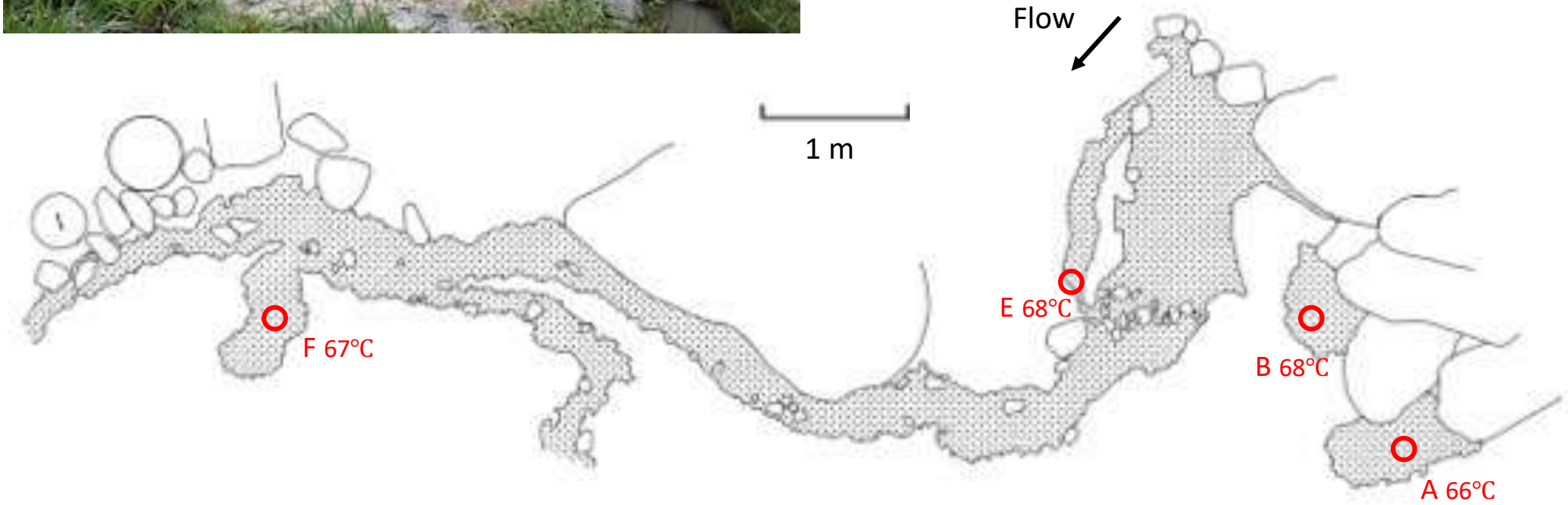
66℃

Kojiki spring: Source 80 ℃, almost vertical flow

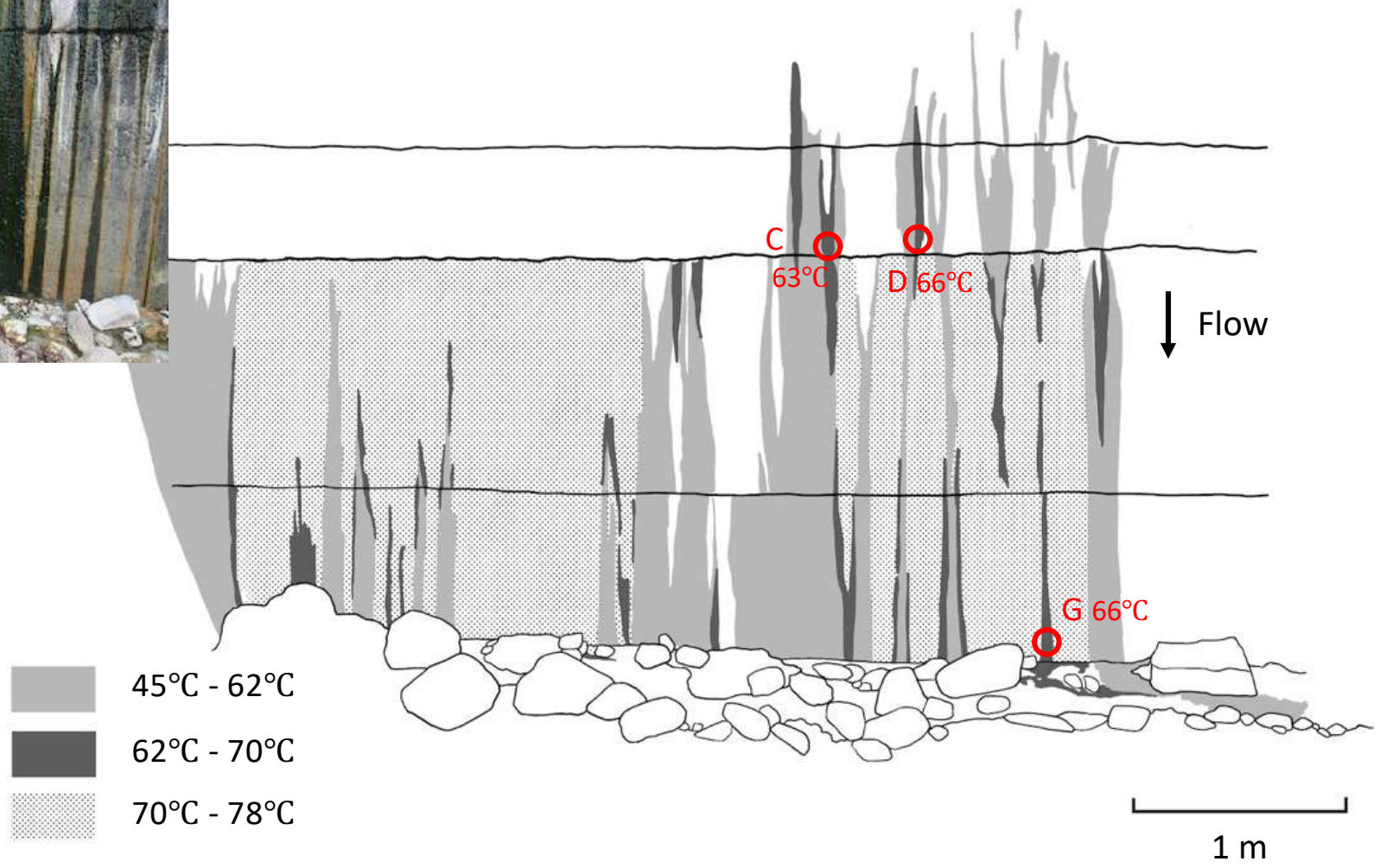
78℃

63℃

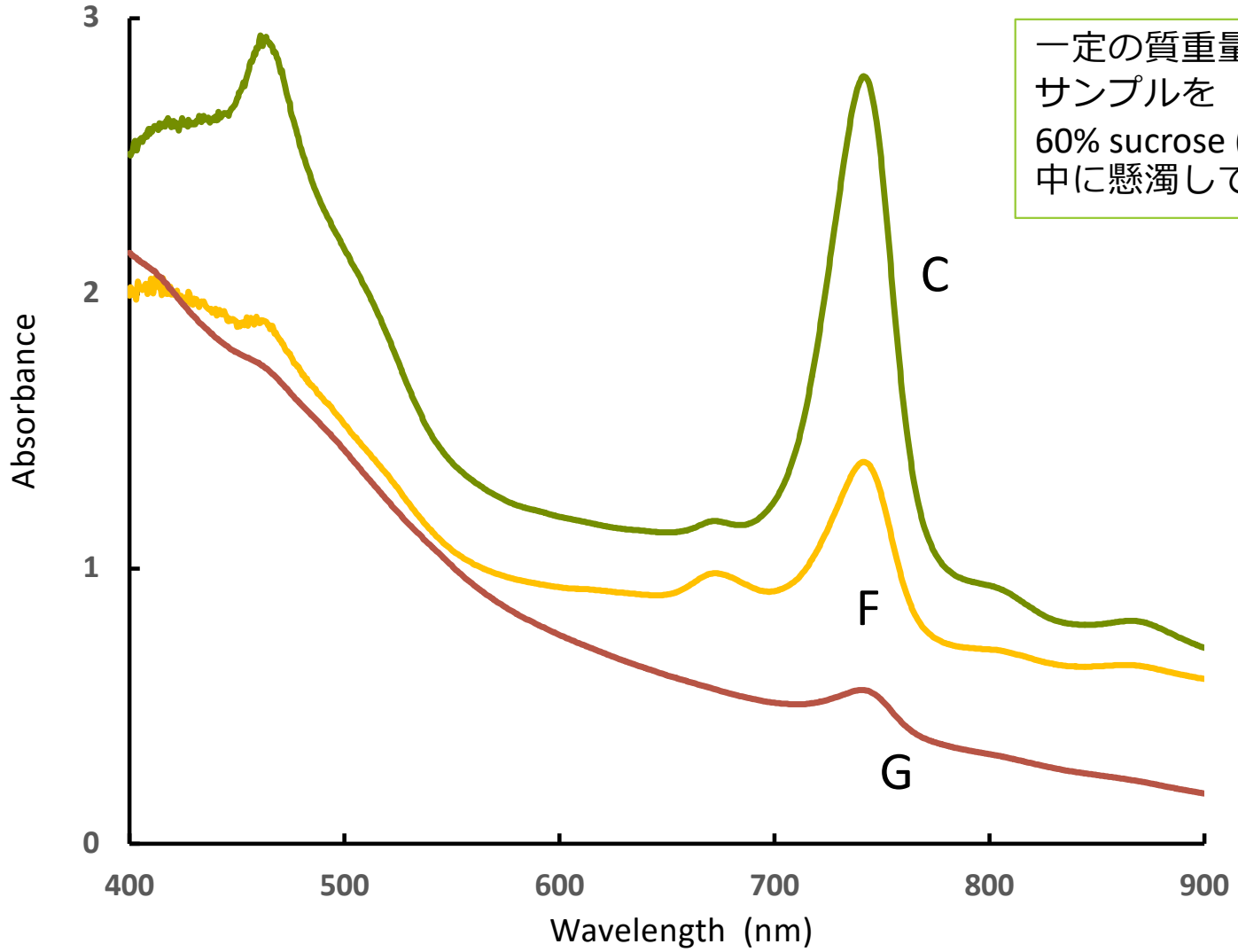
Site 1: Nakabusa hot springs/Kassen Spring: Flow on the sandy ground near the river



Site 2: Nakabusa hot springs/Kojiki spring: Flow of the wall of the Sabo dam



Absorption spectra of photosynthetic pigments in suspensions of homogenized communities



一定の質重量の
サンプルを
60% sucrose (w/w)
中に懸濁して測定

Olive
Green

O_2 33µmol/L

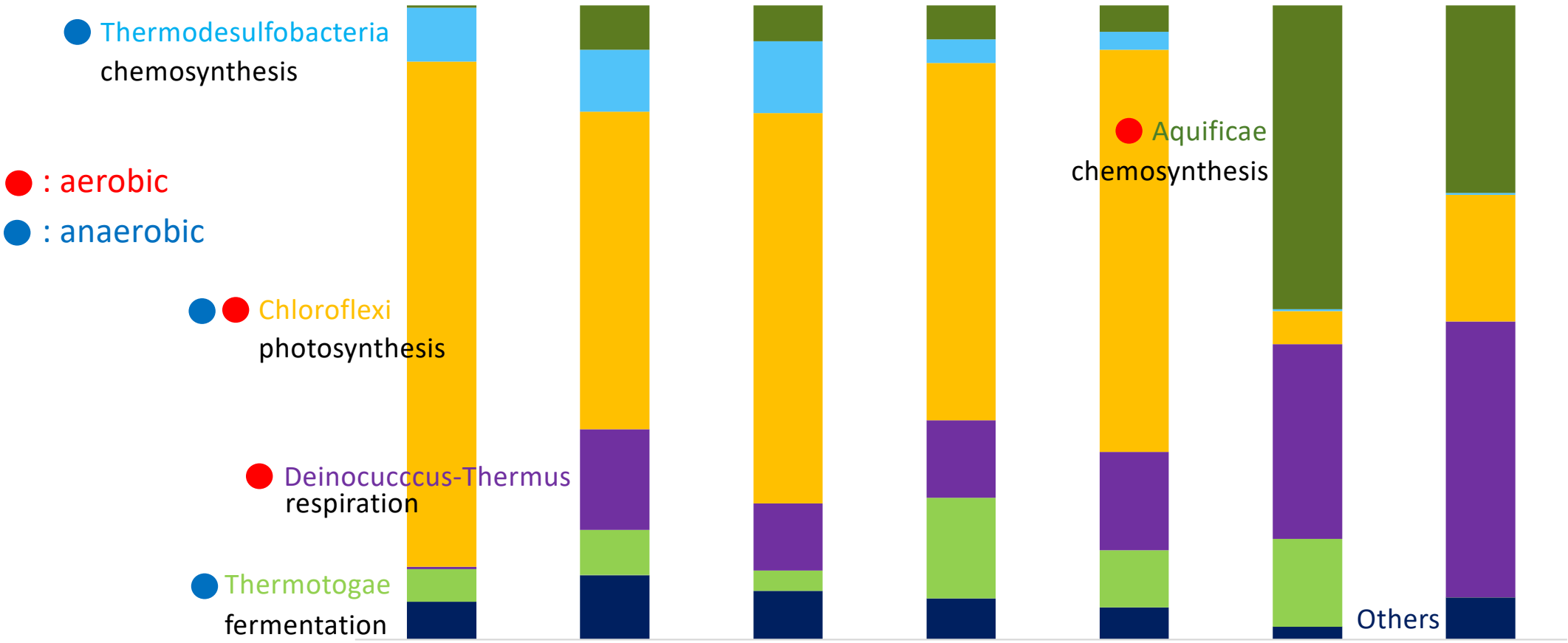
Orange

O_2 84µmol/L

Pale
Brown

O_2 134µmol/L

Differences in phylum-level composition of microbial communities due to differences in ambient oxygen concentrations



O_2 µmol/L	33	42	84	116	130	74	134

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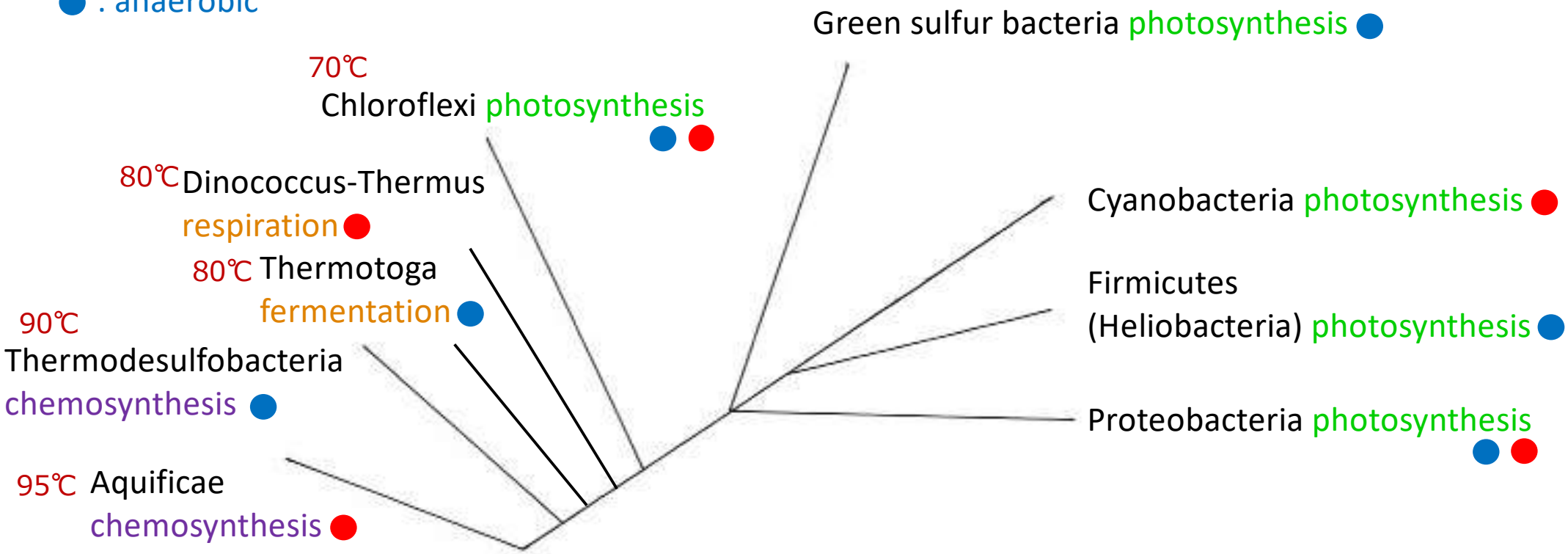

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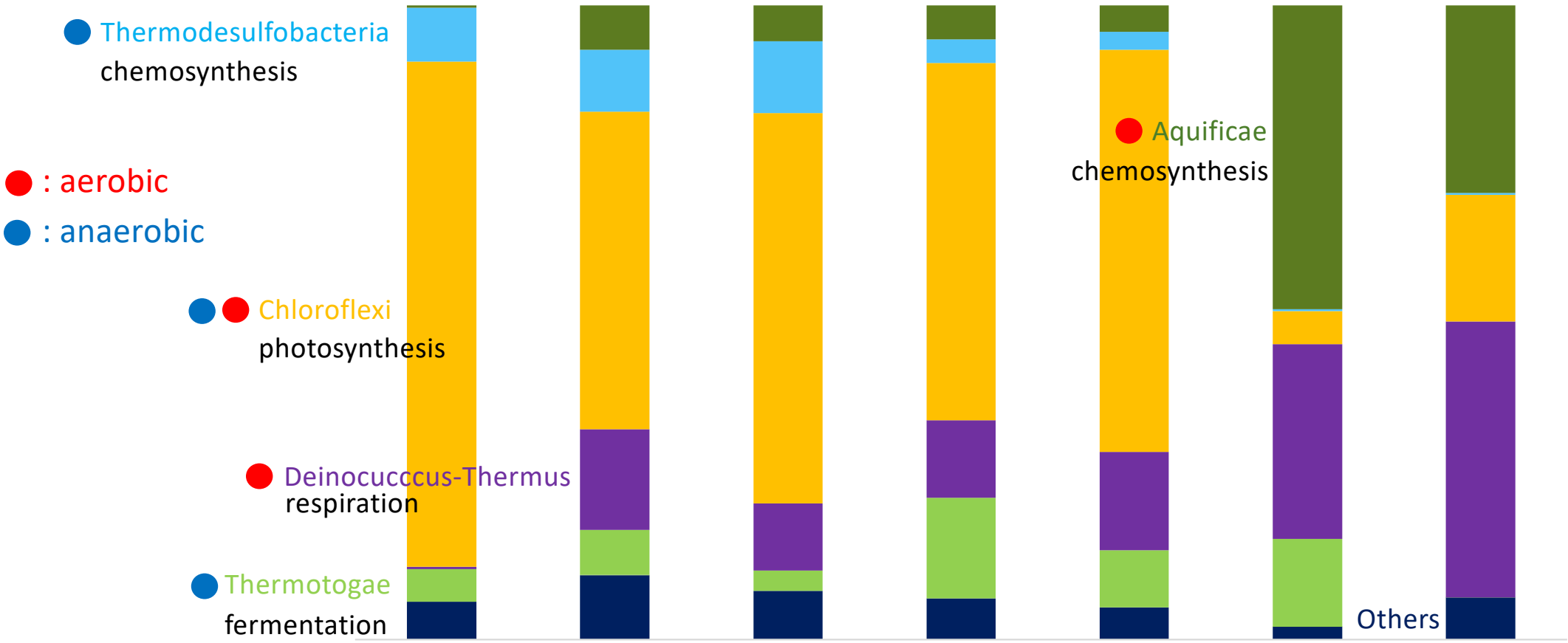

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Conclusions

1. In the microbial communities of Nakabusa hot springs with *Chloroflexus*, the most organisms are in the five hyperthermophilic phyla, and their relative ratios changed according to the oxygen concentration.

2. *Chloroflexus* was present in all oxygen-conditions, but more abundant in anaerobic and semi-aerobic conditions.

3. Archean photosynthetic communities before the emergence of oxygen-evolving photosynthesis may have been both anaerobic and semi-aerobic.