

## ABSTRACT

Magnetotactic bacteria (MTB) biomineralize intracellular magnetic nanocrystals called magnetosomes that are used to navigate in the geomagnetic field. MTB are considered important to study the origin and evolution of magnetotaxis and biomineralization. Evidences show that early life on Earth may occur in thermal environments. We analyzed the phylogenetic, morphological and genomic features of MTB from Tengchong hot springs of Yunnan Province. We found a considerable diversity of MTB affiliated to *Nitrospirae* phylum. We also obtained five draft genomes containing magnetosome gene clusters (MGCs) which regulate biomineralization. Based on the results above, we will use genomic data to reconstruct metabolic pathways of these organisms and precisely annotate the MGCs in order to get a glimpse of the mineralization mechanism of hot spring MTB. Results will shed new lights on the origin and evolution of magnetotaxis and biomineralization.

## INTRODUCTION

Over the forty years since first discovered in 1975, magnetotactic bacteria (MTB) have become a hot interdisciplinary research topic in the fields of biogeology and biomagnetism. MTB are important systems for investigating biomineralization mechanisms in prokaryotes as they synthesize intracellular magnetosomes that are membrane-bound nano-sized crystals of magnetite or greigite in nature. The biomineralization process is under strict genetic control, and genes involved are proposed as magnetosome gene clusters (MGCs). Recently, Lin (2017) used a Bayesian molecular clock-dating method to demonstrate an ancient origin of MTB in the Archean Eon, indicating that MTB are the earliest magnetic-sensing and biomineralizing organisms on Earth. There is evidence that early life on Earth may occur in thermal environments (hydrothermal vents and hot springs). Weiss (2016) has analyzed more than 6 million protein-encoding genes to find the protein families linked to the last universal common ancestor (LUCA) and proposed that LUCA appeared to have obtained energy from thermal environments. The study of MTB in hot springs may help us to better understand the early life of MTB and get a glimpse of the origin and early evolution of magnetotaxis and biomineralization.

## METHODS

We choose Tengchong for the research as the natural exposed hot springs here are distributed intensive, and it is one of the earliest area in China to carry out microbial research on terrestrial hot springs. Among the 18 sampling sites, we found MTB in 4 sites (SHD, DGB, HXQ and STY, Fig.1).

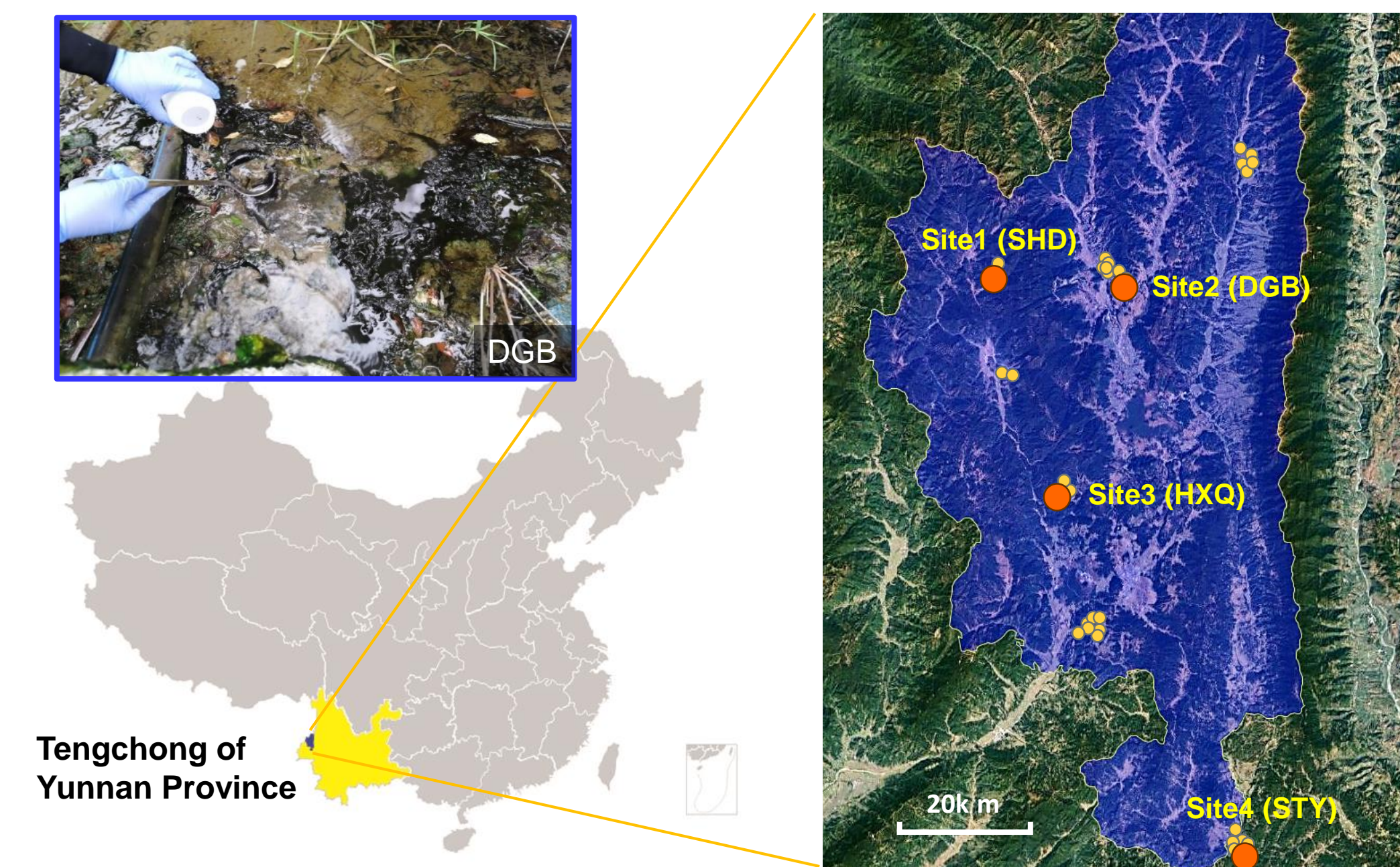


Figure 1. Sampling sites of Tengchong hot springs.

Over 400ml sediment samples with overlying water were collected and MTB cells were isolated in field through magnetic enrichment. Following field investigation and specimen preparation, we combine the approaches of electron microscopy with molecular ecology and metagenomics to analyze the phylogenetic, morphological and genomic features of MTB in Tengchong hot springs.

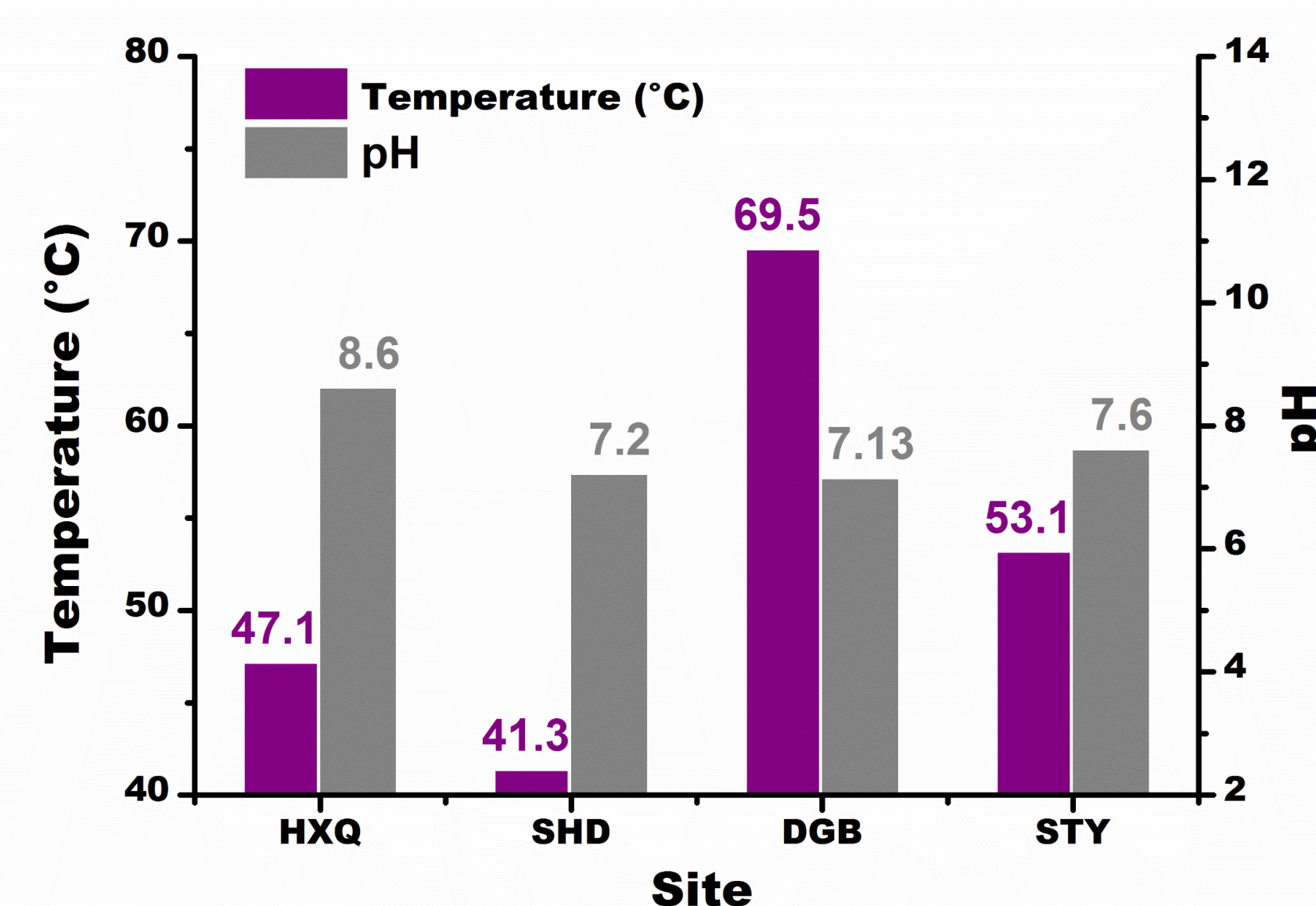


Figure 2. Temperature and pH of four sampling sites.

Temperature, pH and salinity of each sampling sites were measured in situ with three replicates using portable pH and salinity meters and high temperature electrodes. Results show that MTB with abundance of  $10^2$ - $10^4$  cells/cm<sup>3</sup> live in hot springs of 41.3-69.5 °C and neutral and alkaline conditions (Fig.2).

## RESULTS

The magnetotactic bacteria found in Tengchong hot springs are varied in morphology, not only the vibrioid-helicoid bacteria similar to those found in the United States, but also cocci and small vibrios (Fig.3A-C). All the MTB cells synthesize bullet-shaped magnetosomes (Fig.3D-E). Energy-dispersive X-ray spectrum (EDXS) analyses show the main components of magnetosomes are Fe and O (Fig.3G). Further, selected area electron diffraction (SAED) patterns show the magnetite (Fe<sub>3</sub>O<sub>4</sub>) nature of these minerals (Fig.3F).

We obtained 14 MTB related OTUs through DNA extraction and 16s rDNA sequencing, all of which belonged to new species in *Nitrospirae* phylum (Fig.4). They are located in distinct branches on the phylogenetic tree from *Nitrospirae* MTB in normal temperature habitats. From the perspective of phylogenetic relationship, *Nitrospirae* MTB in Tengchong hot springs are closely related to the ones found in American hot springs, but also cluster into two adjacent branches on the phylogenetic tree. We also obtained five draft genomes containing MGCs which will be used for the subsequent studies on biomineralization mechanisms.

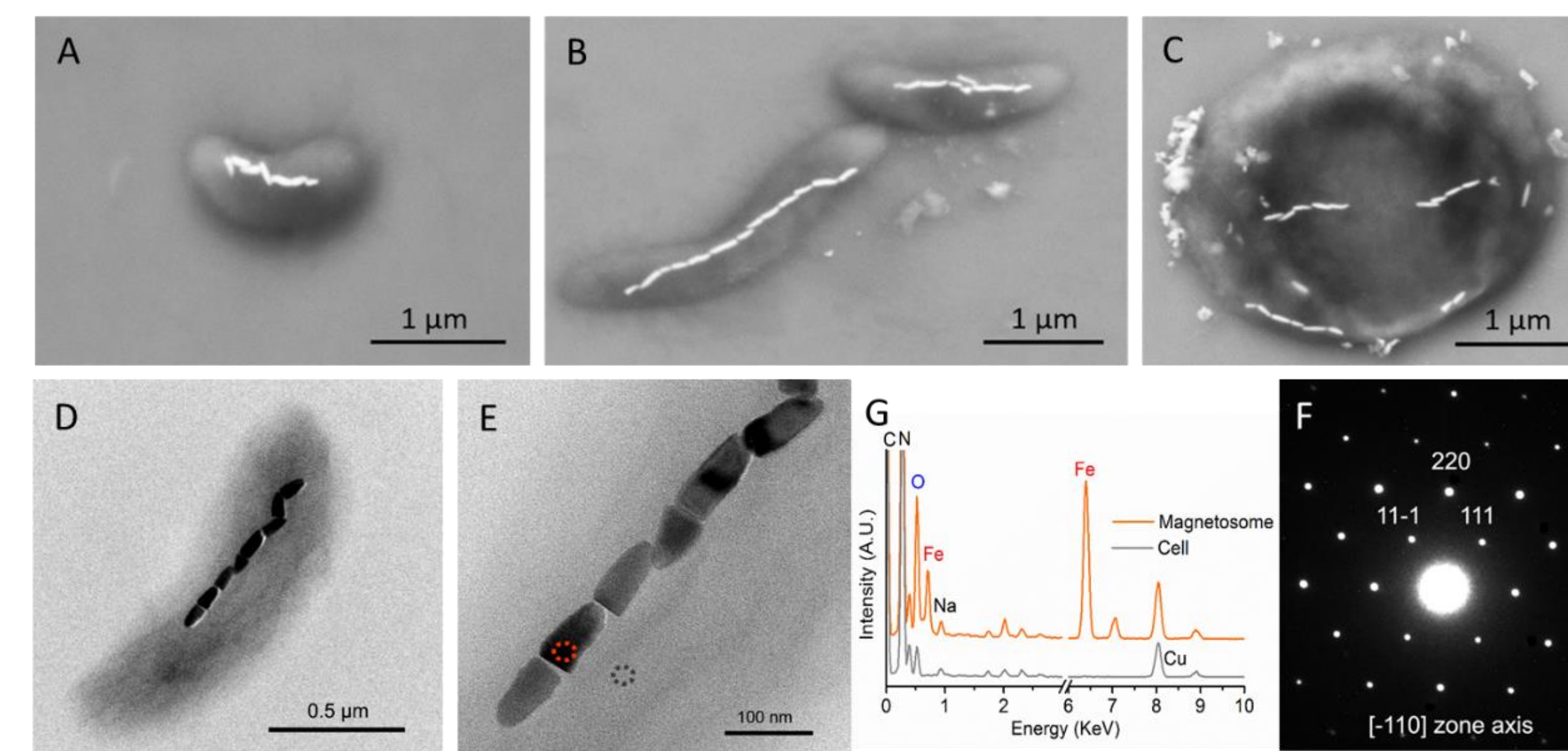


Figure 3. Scanning and Transmission electron micrographs of MTB in Tengchong hot springs.

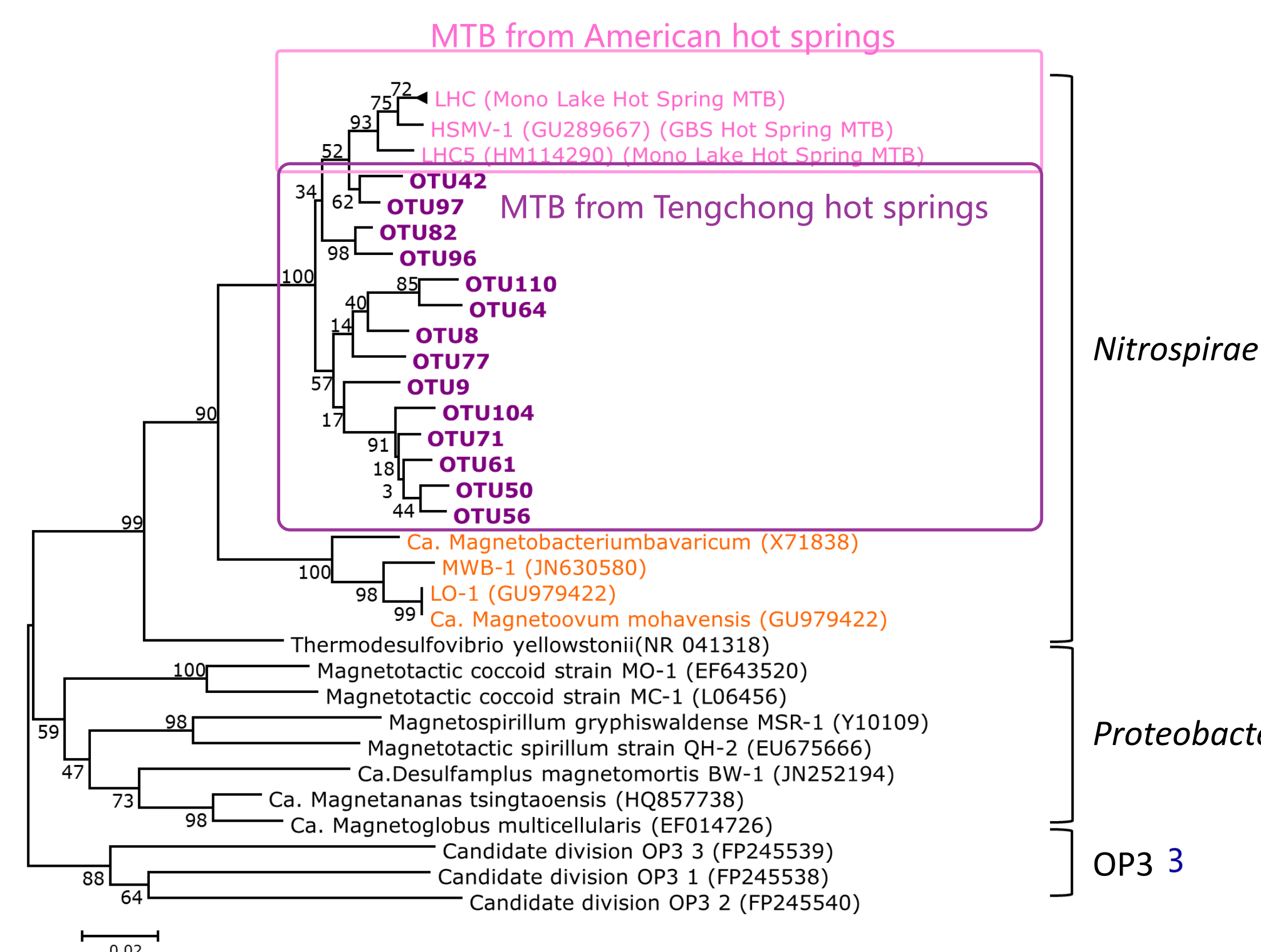


Figure 4. Phylogenetic tree based on 16S rDNA for hot spring MTB from Tengchong and United States.

Table 1. The quality of metagenomic assembled genomes of *Nitrospirae* MTB in Tengchong hot springs.

Bins	Taxonomy	Integrity (%)	Contamination (%)	MGCs gene numbers		
				<i>mam</i>	<i>mad</i>	<i>man</i>
TCH1	<i>Nitrospirae</i>	94.49	0.97	8	7	2
TCH2	<i>Nitrospirae</i>	91.82	2.73	8	8	5
TCH3	<i>Nitrospirae</i>	87.62	2.73	10	3	4
TCH4	<i>Nitrospirae</i>	64.60	4.55	1	5	3
TCH5	<i>Nitrospirae</i>	51.69	5.73	8	4	7

## CONCLUSION

- Magnetotactic bacteria of DGB hot spring were found living at 69.5 °C, which was higher than the highest temperature reported in the literature (GBS hot spring, 63 °C).
- There is a considerable diversity of MTB in Tengchong hot springs (14 OTUs). Both magnetotactic cocci and magnetotactic vibrios were found in the hot spring environments for the first time.
- Based on 16s rRNA genes, all the MTB in Tengchong hot spring affiliated to the *Nitrospirae* phylum. They formed a different evolutionary branch from the *Nitrospirae* MTB in the normal temperature habitats on the phylogenetic tree. MTB here have special evolutionary positions, and may have special characteristics in metabolism and biomineralization.
- Genomic analysis initially obtained five draft genomes of MTB, which contain varied genes of magnetosome gene clusters (MGCs).
- The next work will be focused on the reconstruction of metabolic pathways of these organisms and precise annotation of the MGCs. We are aimed to reveal the mineralization mechanism of MTB in hot springs. Results will shed new lights on the origin and evolution of magnetotaxis and biomineralization.

## REFERENCES

- Lin, W. *et al.* (2017) *Proc Natl Acad Sci U S A*.
- Lin, W. *et al.* (2018) *ISME J*.
- Lefèvre, C.T. *et al.* (2010) *Appl Environ Microbiol*.
- Weiss, M.C. *et al.* (2016) *Nat Microbiol*.
- Djokic, T. *et al.* (2017) *Nat Commun*.