



Metagenomic analysis sheds light on the mixotrophic lifestyle of bacterial phylum *Zhuqueibacterota*

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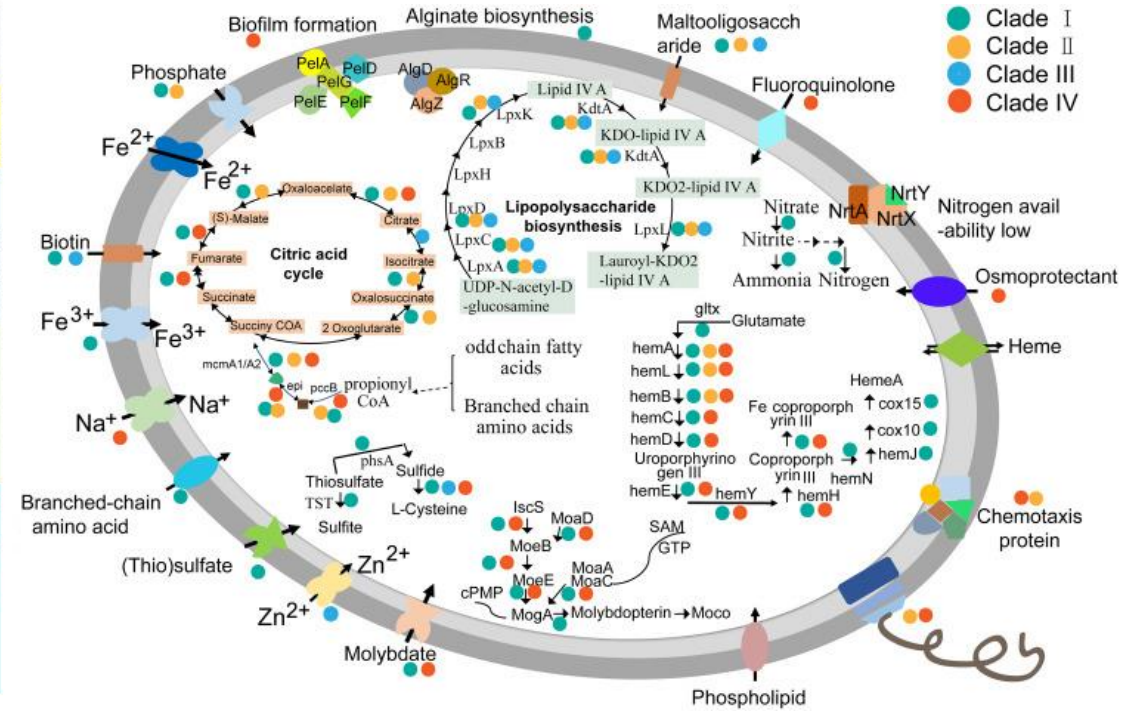
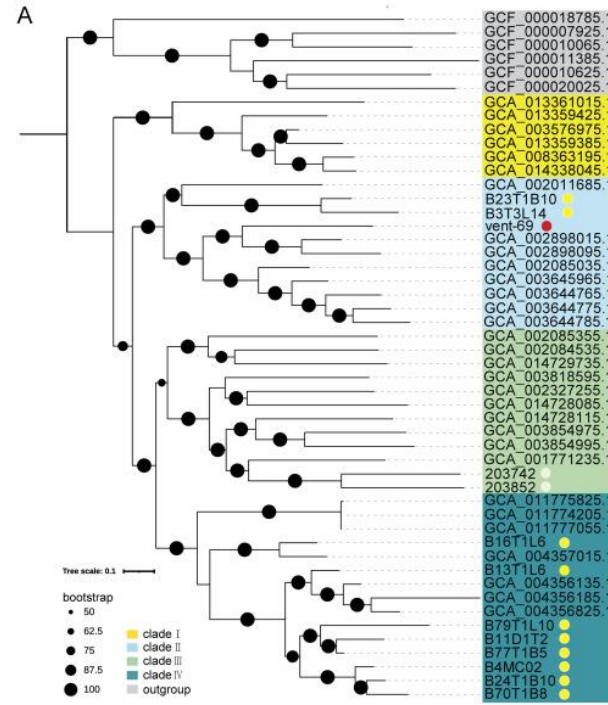
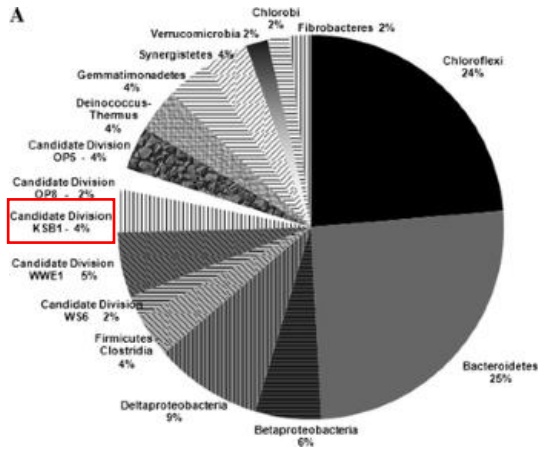
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Introduction

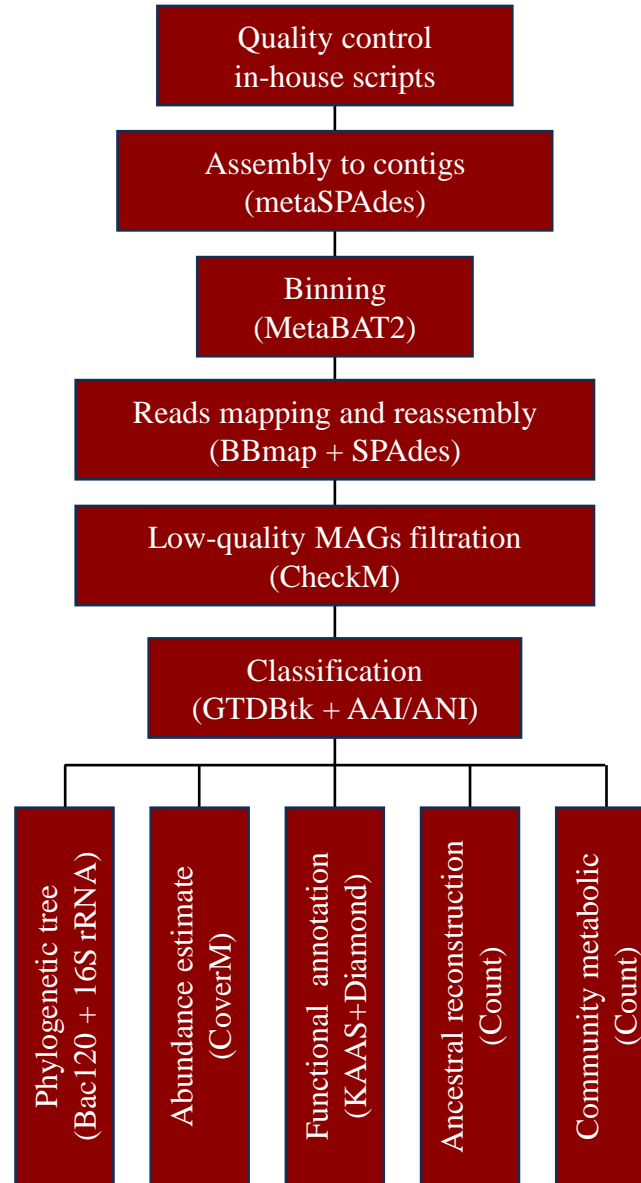
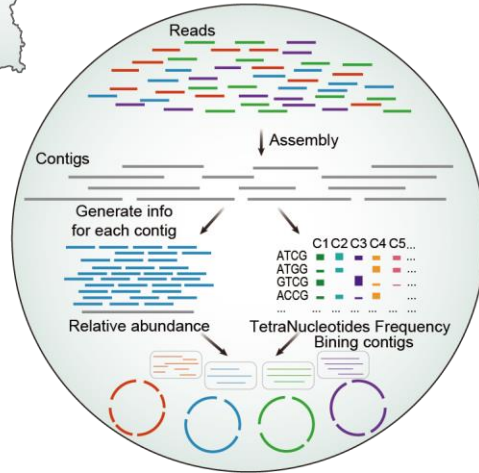
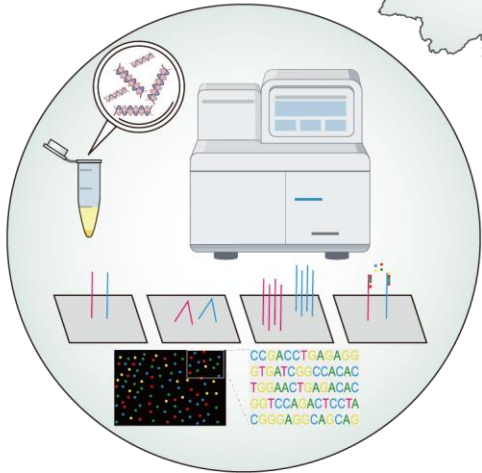
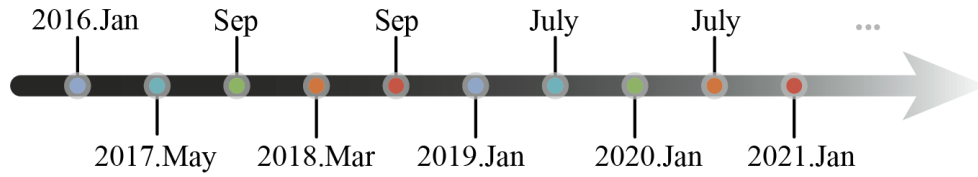
Clone type	Division	Salinity type
CE99	Candidate division KSB1	Brackish
CE45	Candidate division KSB1	Brackish



- Uncultured candidate division KSB1 was firstly detected in marine coastal with sulfur-rich black mud by 16S rRNA amplicon sequencing.
- KSB1 is widely distributed across different habitats such as marine coastal, microbial mat, cave sediment, aquifer and swine sludge, and might be enriched in an anoxic environment.
- Phylogenetic analysis divides KSB1 into four clades, each characterized by distinct gene profiles and niche adaptations.
- Metabolic analysis of KSB1 clades reveal an anaerobic heterotrophic lifestyle highlighting differences in their potential ecological roles.



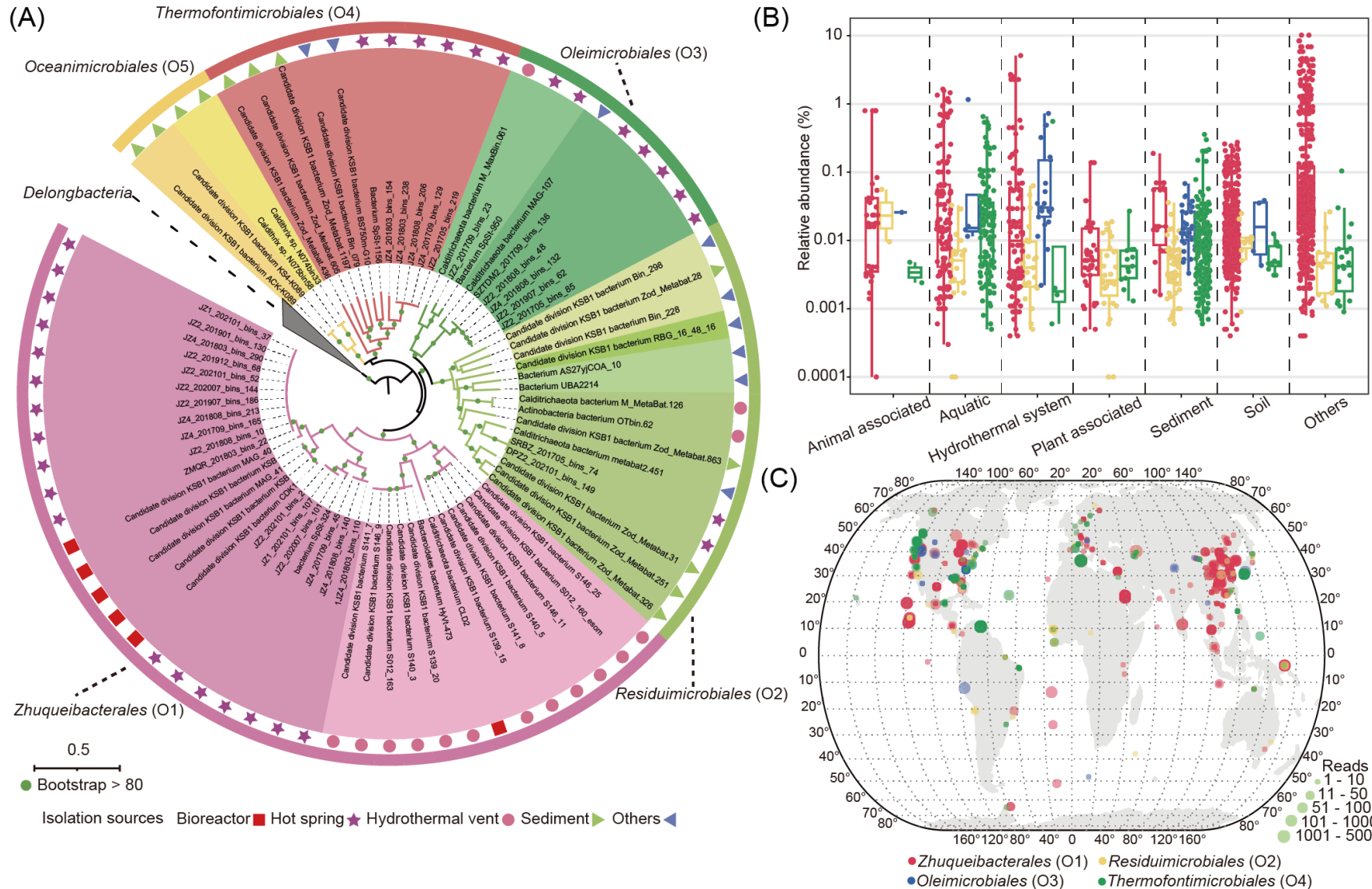
Methods



- ▣ Long-term collection of hot spring sediments from Tengchong.
 - A total of 17 samples were collected.
- ▣ Ultra-deep metagenomic sequencing ≥ 30 Gbp per sample, yielding 650 Gbp of paired-end reads (2×150 bp)
- ▣ Metagenome assembly and genome binning
 - 30 high-quality KSB1 MAGs obtained
- ▣ Public data collection
 - 45 high-quality KSB1 MAGs downloaded



Globally distributed bacterial phylum *Zhuqueibacterota*



- *Zhuqueibacterota*, is proposed based on hot spring metagenomes and public MAGs, classified within the FCB superphylum and comprising one class and five orders.
- *Zhuqueibacterota* exhibits a global distribution, with a strong presence in soil samples, while it thrives in biofilm, sludge, and hydrothermal environments.
- Distinct lineages exhibit distinct environmental preferences.

Figure 1(ABC). Phylogenetic placement and global distribution of *Zhuqueibacterota*



The metabolic potential of *Zhuqueibacterota*

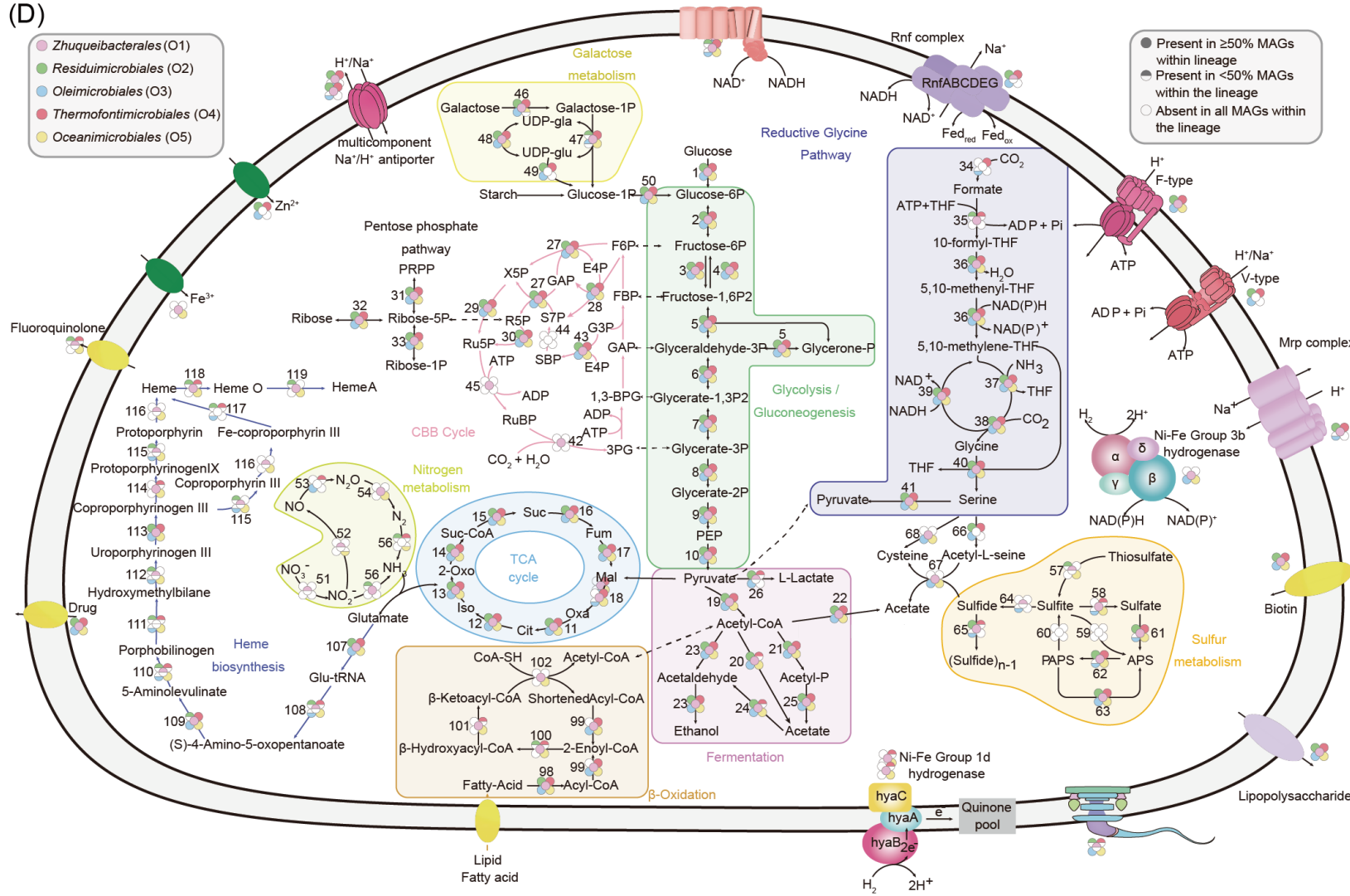


Figure 1D. Metabolic model of *Zhuqueibacterota*

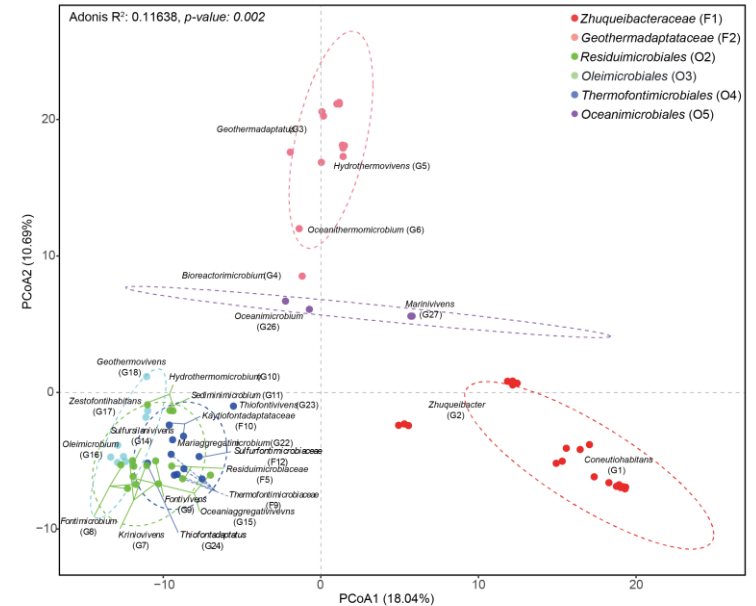


Figure S9. PCoA plot

- *Zhuqueibacterota* possesses diverse metabolic potential, with different lineages exhibiting significant functional differences.
- *Zhuqueibacterales* (O1) and *Oceanimicrobiales* (O5) are more likely facultative anaerobes



Mixotrophic lifestyle of *Zhuqueibacterota*

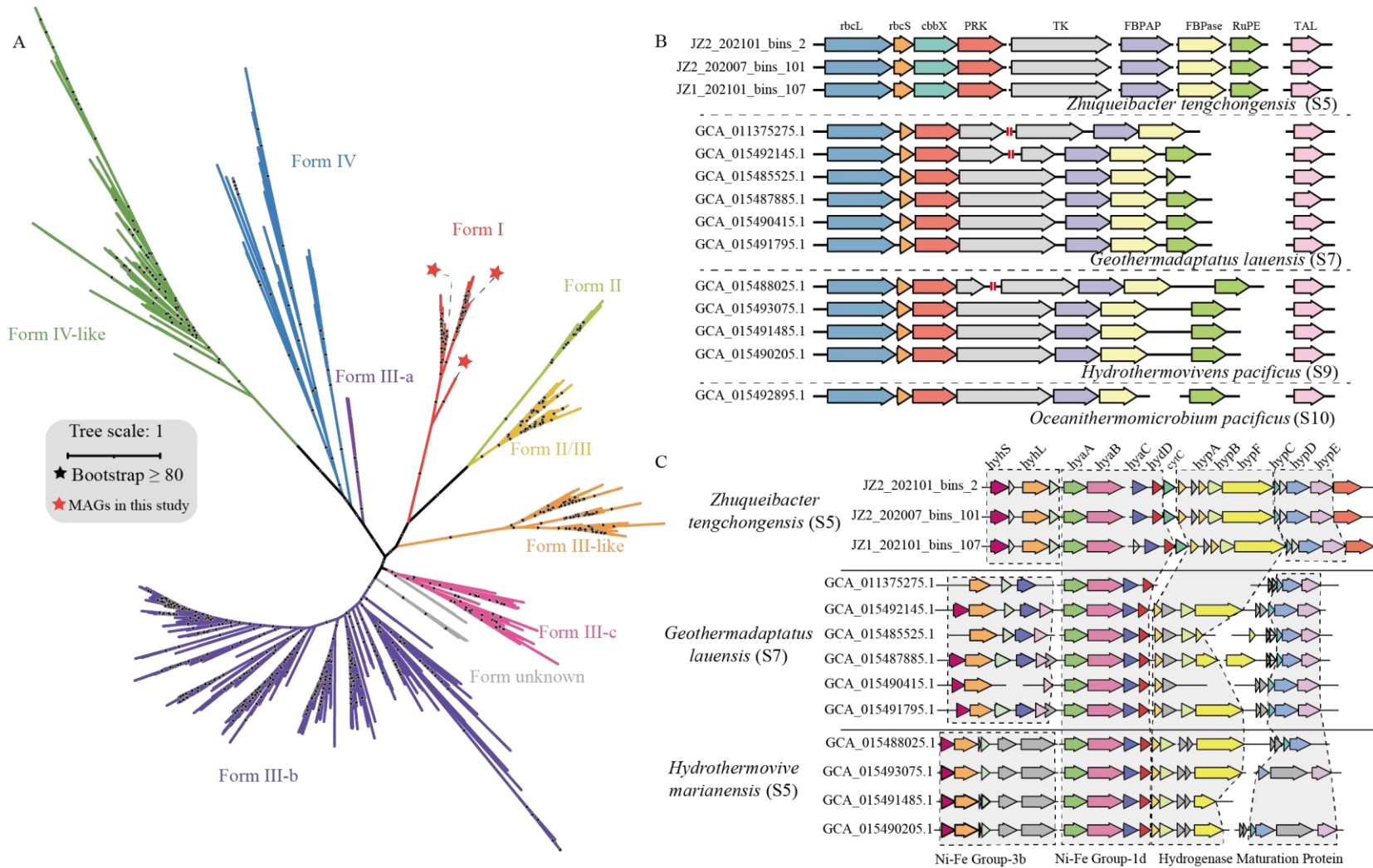
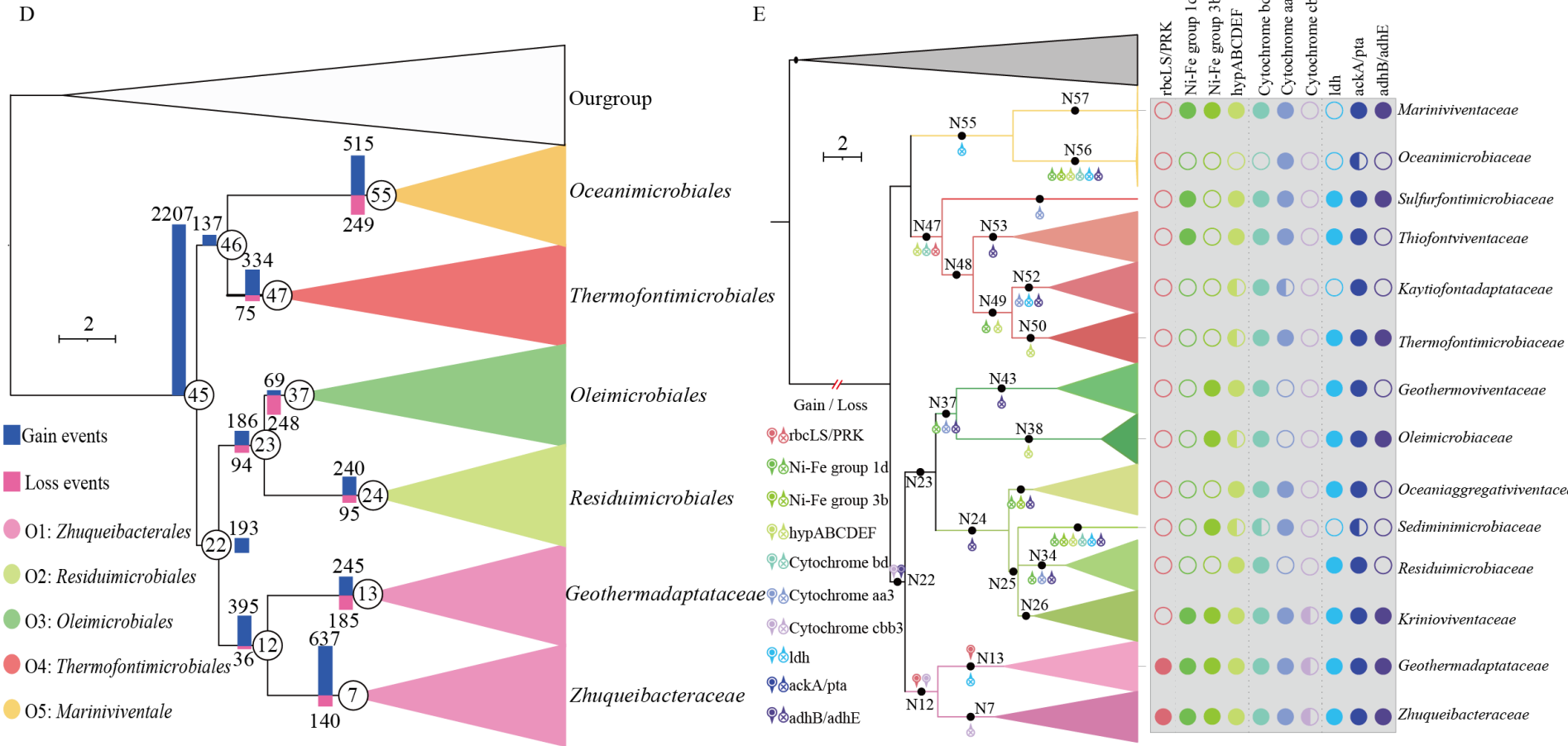


Figure 2(ABC). Key gene identification

- The Form I *rbcL* gene, responsible for CO₂ fixation, is widespread in *Zhuqueibacterales* (O1).
- A CBB cycle-related gene cluster suggests carbon fixation ability within *Zhuqueibacterales* (O1).
- The coexistence of membrane-bound hydrogenase, soluble hydrogenase, and hydrogenase maturation protein suggests the potential for H₂ oxidation, supplying energy and reducing equivalents.
- The *Zhuqueibacterales* (O1) may be a chemoautotrophic hydrogen-oxidizing bacteria.



The evolutionary history of *Zhuqueibacterota*



Zhuqueibacterota may have undergone genome expansion, with frequent HGT occurring within *Zhuqueibacterales* (O1), particularly among MAGs derived from hot spring environments.

The ancestors of were likely facultative anaerobic bacteria, later acquiring the ability for carbon fixation via the Calvin-Benson-Bassham cycle.

Figure 2(DE). The evolutionary history of *Zhuqueibacterota*

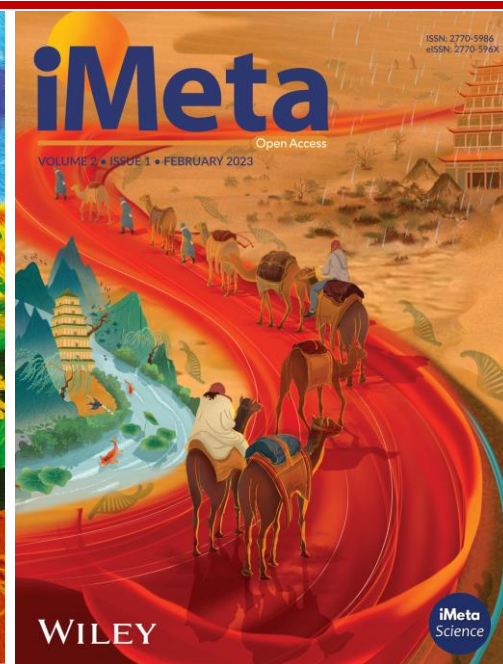


Summary

- ❑ In this study, we proposed *Zhuqueibacterota* based on hot spring metagenomes and public MAGs, classified within the FCB superphylum and comprising one class and five orders.
- ❑ *Zhuqueibacterota* is globally distributed, with most members being facultative anaerobes; one order (*Zhuqueibacterales*) utilizes hydrogen as an electron donor for carbon fixation via the CBB cycle.
- ❑ Phylogenetic and metabolic analyses highlight critical role of *Zhuqueibacterota* in the carbon cycle and frequent horizontal gene transfer events throughout its evolution.




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