

# Omnitrophota: a diverse and ubiquitous candidate phylum of putative syntrophs, predators, and free-living nanobacteria

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## Overview

Candidate bacterial phylum *Omnitrophota* has never been isolated and is poorly understood. Here, we explored the systematics, distribution, cell size, and predicted physiology of *Omnitrophota* based on 316 genomes. Phylogenetic concordance using several marker sets identified seven classes accommodating 144 species. Calibration of the genome taxonomy to 16S rRNA gene phylogenies revealed *Omnitrophota* to be prevalent in the Earth Microbiome Dataset, particularly rhizosphere (96%), bulk soils (73%) and freshwater (92%) and saline (79%) sediments. Phylogenetic placement of single-amplified genomes with cell size measurements and differential size filtration experiments showed ultra-small (<400 nm) cells to be common across the phylum. Energy metabolism is conserved; classes share genes encoding either respiratory or fermentative pathways. Conserved fermentative pathways include the Wood-Ljungdahl pathway, suggesting syntrophic acetogenesis. Parasitism-related genes are common: a conserved tight adherence (Tad) complex and ATP/ADP translocase are encoded by several classes. However, neither genome size nor biosynthetic pathways are reduced. These results suggest that candidate phylum *Omnitrophota* is diverse and ubiquitous, including putative syntrophs, predators, and free-living nanobacteria. Study of this lineage will provide insight into the evolution of parasitism and predation in bacteria, which likely evolved prior to eukaryotic multicellularity.

## Introduction

*Omnitrophota* comprise a yet-uncultivated candidate phylum of bacteria about which **very little is understood**. First observed as a single 16S rRNA gene sequenced from **Obsidian Pool in Yellowstone National Park**<sup>[1]</sup>, *Omnitrophota* have been reported in 16S rRNA gene amplicon and environmental metagenomic surveys **around the world**.



Obsidian Pool hot spring, Yellowstone National Park. *img src: hotmicrobiol.soup.space*

Although amplicon surveys provide information on the occurrence and diversity of *Omnitrophota*, they provide little insight on physiology or ecology.

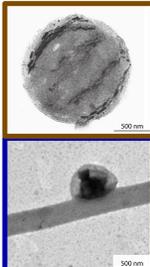
Only two *Omnitrophota* species have been microscopically observed:

- (i) *Candidatus* *Omnitrophus* magneticus SKK-01<sup>[2]</sup>, a large, ovoid, magnetic bacterium containing many sulfur inclusions.
- (ii) *Candidatus* *Vampirococcus* archaeovorans LiM<sup>[3]</sup>, a very small (~250 nm) parasitic coccus, which has been reported to attach to the outside of other prokaryotes.

These characteristics suggest that LiM is a **novel parasitic member of the *Bdellovibrio*** and like organisms (BALOs)<sup>[3]</sup>.

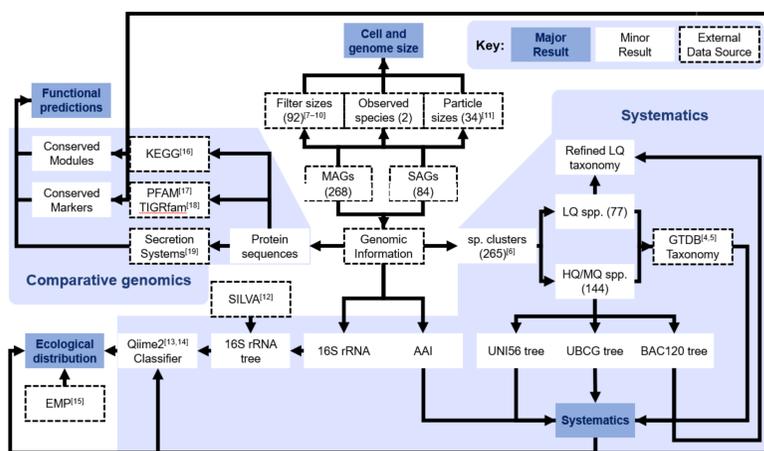
However, while these observations have provided rare and valuable descriptions of individual *Omnitrophota* species, the nature of the phylum as a whole remains unclear, as there has been no systematic attempt to extrapolate these data points to the rest of the phylum.

Here, we leverage the information contained within 316 *Omnitrophota* genomes—268 Metagenome-Assembled Genomes (MAGs) and 84 Single cell-Amplified Genomes (SAGs)—to explore the evolution, environmental distribution, cell size, and genomic potential of *Omnitrophota*.

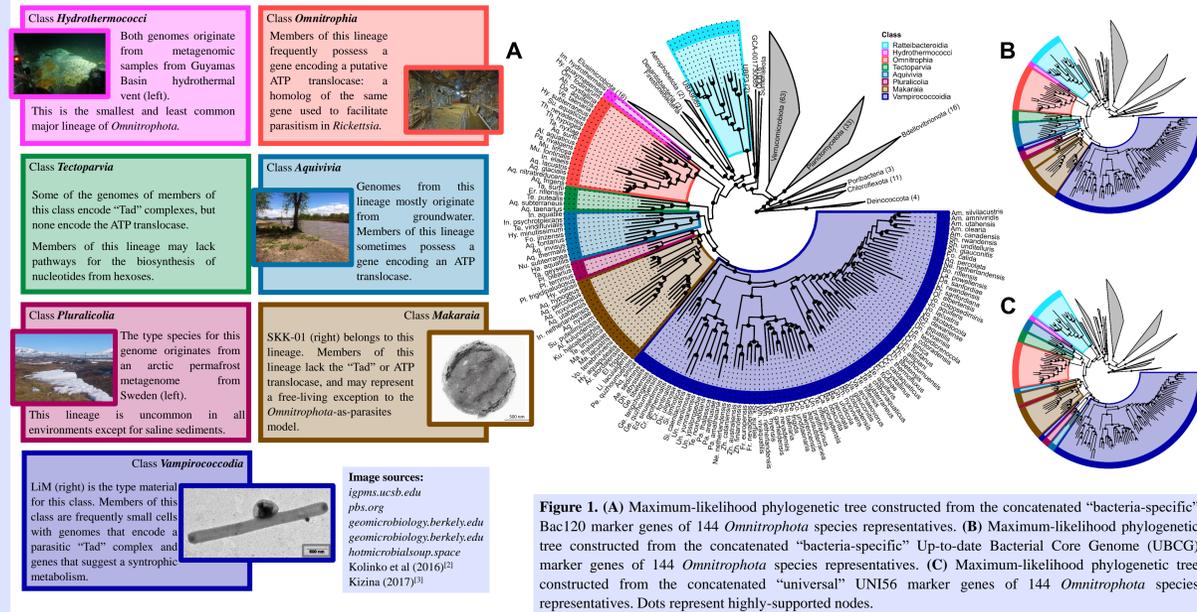


SKK-01 (top) and LiM (bottom), modified from: Kizina et al. (2017) and Kolinko et al. (2016).

## Methods

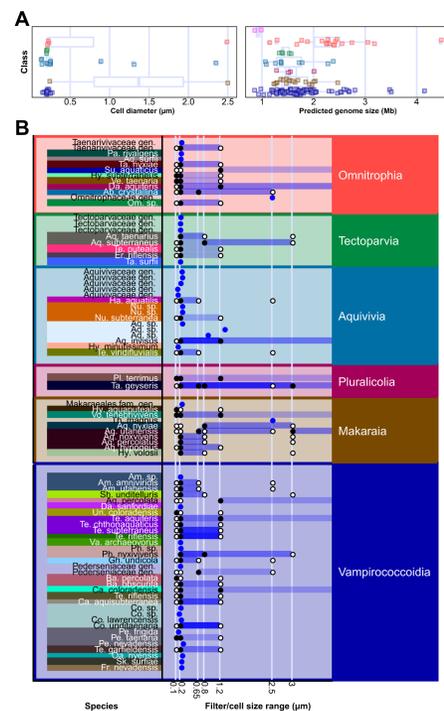


## Systematics: Meet the Classes



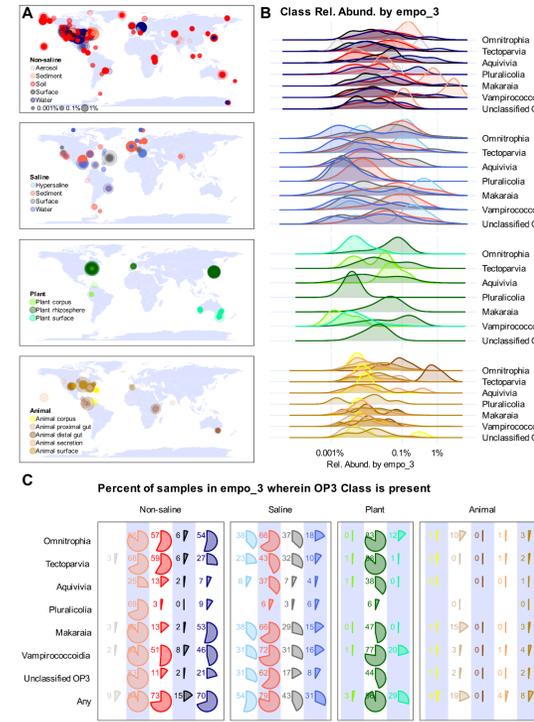
**Figure 1.** (A) Maximum-likelihood phylogenetic tree constructed from the concatenated "bacteria-specific" Bac120 marker genes of 144 *Omnitrophota* species representatives. (B) Maximum-likelihood phylogenetic tree constructed from the concatenated "bacteria-specific" Up-to-date Bacterial Core Genome (UBCG) marker genes of 144 *Omnitrophota* species representatives. (C) Maximum-likelihood phylogenetic tree constructed from the concatenated "universal" UNIS56 marker genes of 144 *Omnitrophota* species representatives. Dots represent highly-supported nodes.

## Cell and genome size



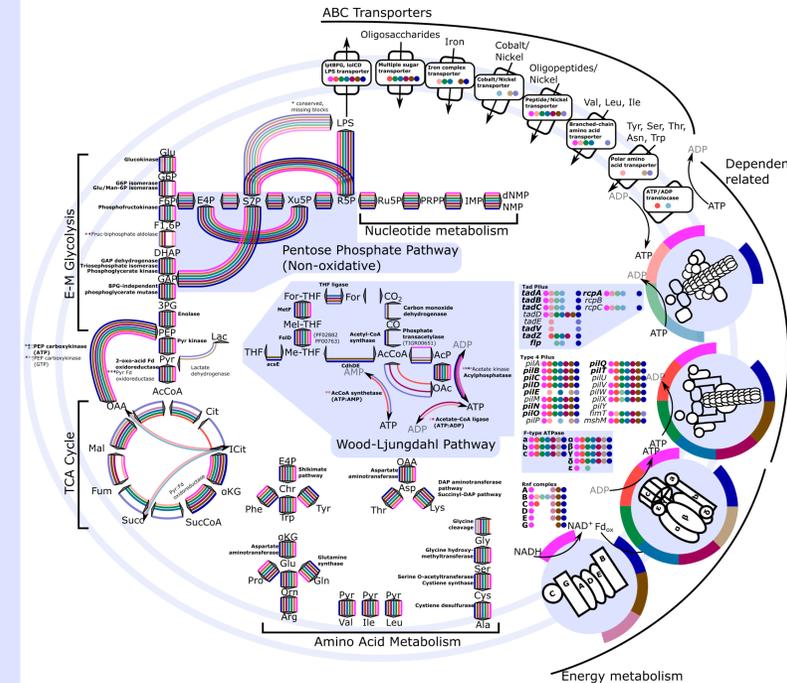
**Figure 3.** (A) Particle size of *Omnitrophota* cells associated with SAGs (left) and estimated genome size of *Omnitrophota* species representatives (right). (B) Predicted cell diameters (blue) including particle sizes<sup>[11]</sup> and serial-filtered *Omnitrophota* MAGs from Rifle, CO<sup>[7-9]</sup> and Crystal Geyser, UT<sup>[10]</sup>. Blue dots represent particle sizes associated with SAG assemblies. MAG size data are shown as black circles; filled circles indicate a filter from which a MAG was recovered, while unfilled indicated filters connected in serial. Lines connecting these dots represent the possible range of each cell size given the observed data.

## Ecological distribution



**Figure 2.** (A) World map showing coordinates of Earth Microbiome Project (EMP)<sup>[14]</sup> samples wherein *Omnitrophota* sequence variants were observed in emp environmental ontology (emp) level 2 categories Non-saline, Saline, Plant-associated, and Animal-associated. Bubbles are sized proportional to relative abundance of the entire phylum within the sample and are colored to indicate emp level 3. (B) Log<sub>10</sub>-scale distribution of relative abundances of each class of *Omnitrophota* within empo 3 categories. (C) Multi-pie chart displaying percent of samples belonging to each emp level 3 category wherein at least one sequence variant from each class of *Omnitrophota* was observed.

## Metabolic and functional predictions



**Figure 4.** Schematic summarizing and comparing the conservation of KEGG<sup>[16]</sup> pathways within each class of *Omnitrophota*. Lines represent genes or modules as appropriate. Reactions are represented by multiple-line segments. Components of a complex are represented by colored circles. Segments of a circle surrounding a complex indicate the completeness of that complex. The colors of each of these shapes correspond to each class. Shapes are opaque if the gene or gene set catalyzing a given reaction is predicted to be present in the representative genomes of ≥50% of species, transparent if >1 and <50% of species, and deleted if one or no species.

## Conclusions

*Omnitrophota* is a diverse phylum of at least seven classes, accommodating ≥144 species. **The systematic framework** described here represents a major step forward in understanding this lineage.

Comparative genomics suggests **predation, parasitism, and syntrophy**—"eating together"—are common across the phylum, suggesting a conserved propensity toward **dependency or symbiosis**. However, the mechanism differs between classes.

**Small cell sizes** comparable to obligately parasitic bacteria are common across the phylum.

However, biosynthetic pathways and genome size are **not substantially reduced**.

A survey of *Omnitrophota* in the Earth Microbiome Project dataset indicated that *Omnitrophota* of every class are nearly **absent from host-associated biomes but ubiquitous in the environment**, especially soils and sediments, albeit at **low abundance**.

**No highly correlated partners** could be identified from amplicon data; *Omnitrophota* may be parasites or symbionts that interact with either **micro-eukaryotes** or **multiple species** of Bacteria and/or Archaea.

*Omnitrophota* possess genes indicative of a parasitic or syntrophic lifestyle. Study of this lineage will provide insight into the **evolution of parasitism and predation in bacteria**, which likely evolved prior to **eukaryotic multicellularity**.

## References and Acknowledgements

**References.** [1] Hugenoltz, P. et al. *Journal of Bacteriology* **180**, 366–376 (1998). [2] Kolinko, S. et al. *Environmental Microbiology* **18**, 21–37 (2016). [3] Kizina, J. Insights into the biology of Candidate Division OP3 LiM populations. (2017). [4] Parks, D. H. et al. *Nat Biotechnol* **36**, 996–1004 (2018). [5] Chaumeil, P.-A. et al. *Bioinformatics* **36**, 1925–1927 (2019). [6] Jain, C. et al. *Nat Commun* **9**, 1–8 (2018). [7] Anantharaman, K. et al. *Nature Communications* **7**, 13219 (2016). [8] Hug, L. A. et al. *Environ Microbiol* **18**, 159–173 (2016). [9] Hug, L. A. et al. *ISME J* **9**, 1846–1856 (2015). [10] Probst, A. J. et al. *Nature Microbiology* **3**, 328–336 (2018). [11] Beam, J. P. et al. *Front. Microbiol.* **11**, (2020). [12] Probst, A. et al. *Nucleic Acids Res* **35**, 7188–7196 (2007). [13] Bolten, E. et al. *Nat Biotechnol* **37**, 852–857 (2019). [14] Bokulich, N. A. et al. *Microbiome* **6**, 1–17 (2018). [15] Thompson, L. R. et al. *Nature* **551**, 457–463 (2017). [16] Aramaki, T. et al. *Bioinformatics* **btz859**, (2019). [17] Bateman, A. et al. *Nucleic Acids Research* **32**, D138–D141 (2004). [18] Haft, D. H. et al. *Nucleic Acids Res* **31**, 371–373 (2003). [19] Abby, S. S. et al. *Sci Rep* **6**, 1–14 (2016).

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