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Diversity of novel archaeal viruses infecting methanogens discovered through coupling of stable isotope probing and metagenomics

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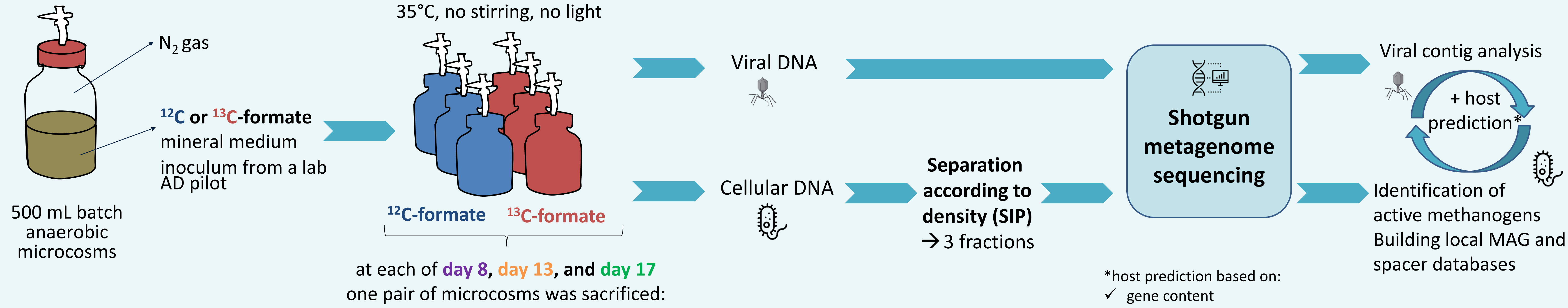
Context

Viruses of microbes are major players in various ecosystems, typically affecting the structuration and dynamics of microbial communities [1]. They nevertheless remain poorly explored in anaerobic digestion (AD) plants [2], where biowaste is valorised into methane-rich biogas. Here, we explored the diversity of viruses infecting methanogens involved in AD processes. We developed an original coupling of **metagenomics** and **stable isotope probing (SIP)**, to identify viruses infecting formate-assimilating methanogens during AD. Only a dozen of **viruses of methanogens** have been isolated so far, mostly head-tailed.

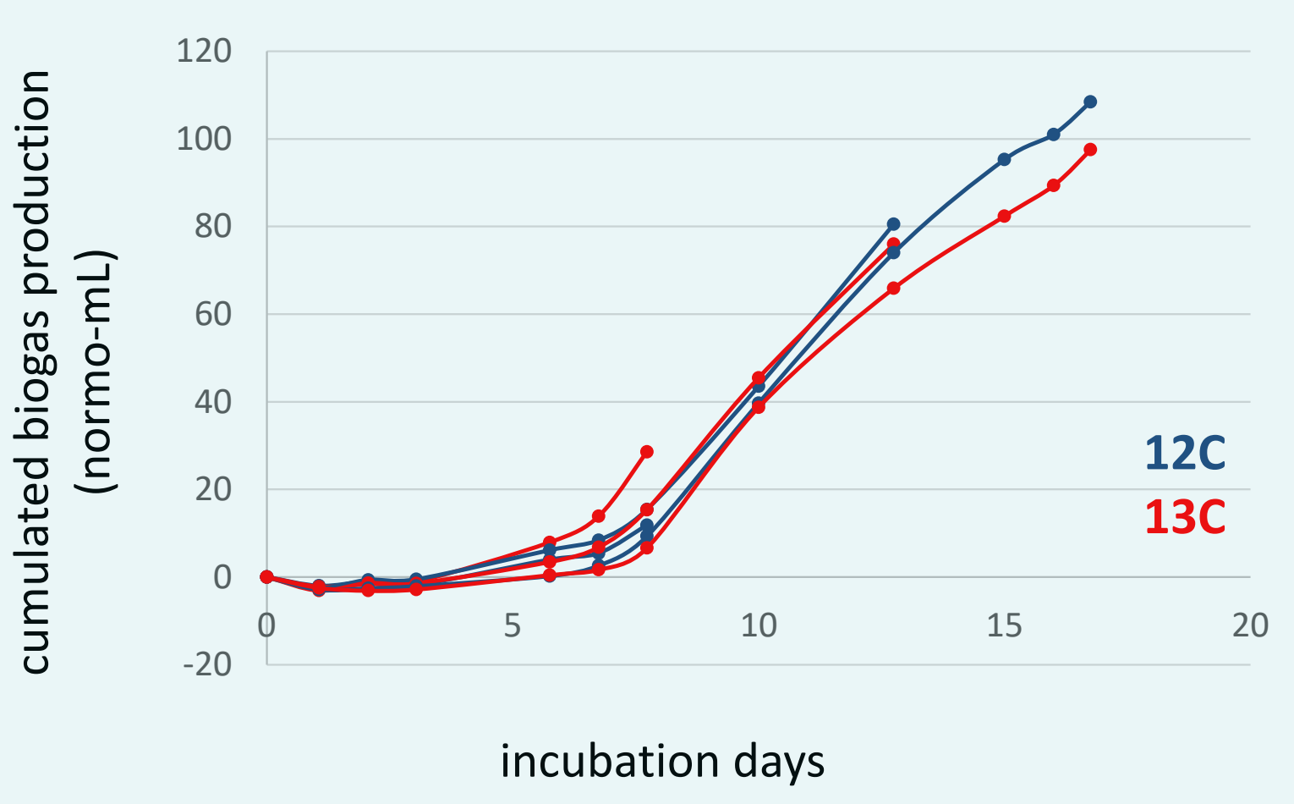
Experimental set-up

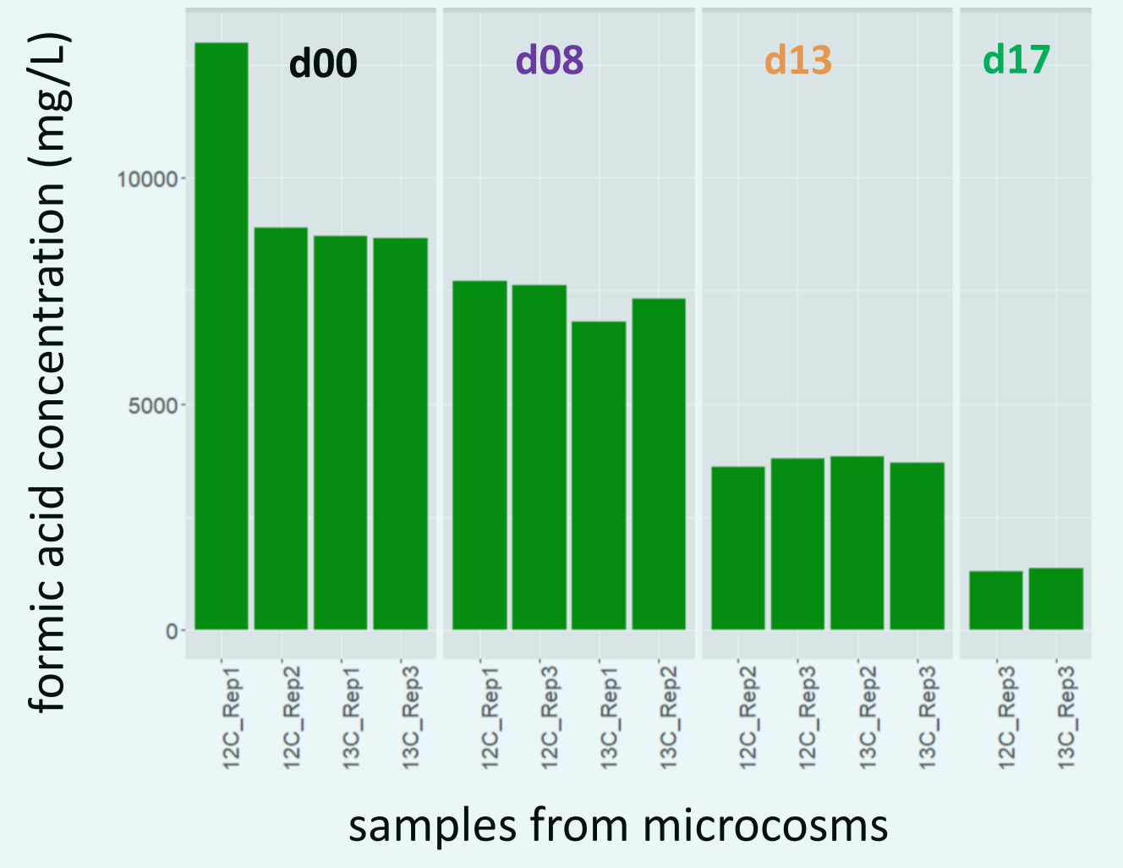


at each of day 8, day 13, and day 17
one pair of microcosms was sacrificed:
^{12}C-formate (control) + ^{13}C-formate

*host prediction based on:
- ✓ gene content
- ✓ alignment with public [3] and local spacer databases
- ✓ k-mer composition, using WIsH [4] with the local MAG database

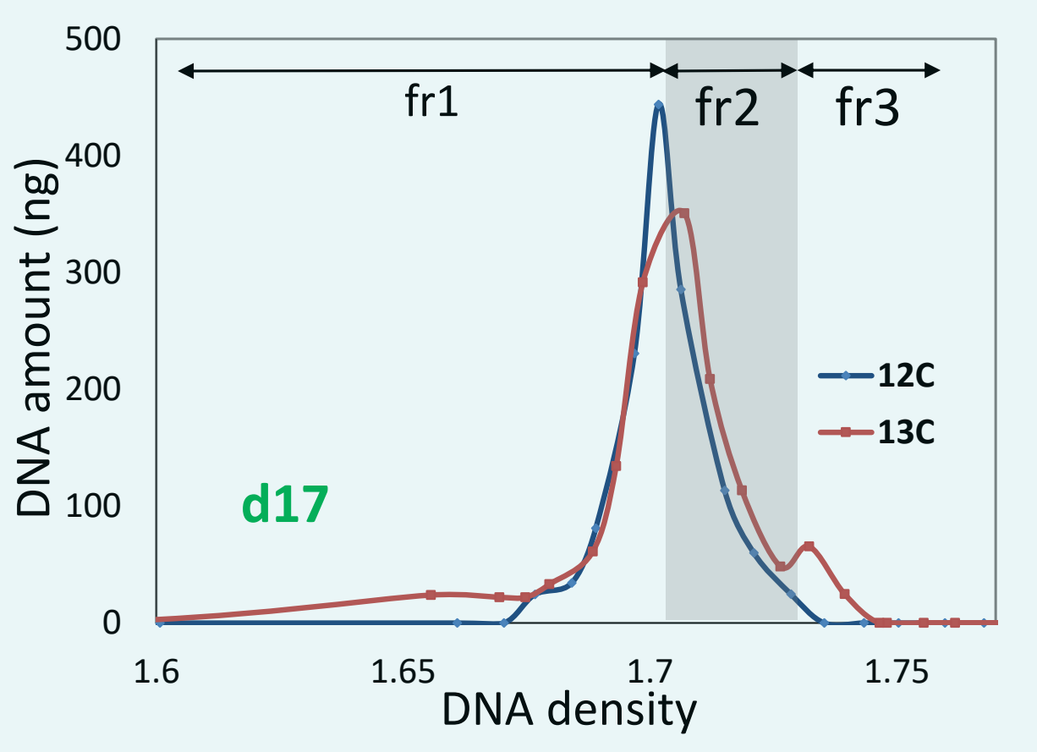
Biogas production and formate consumption

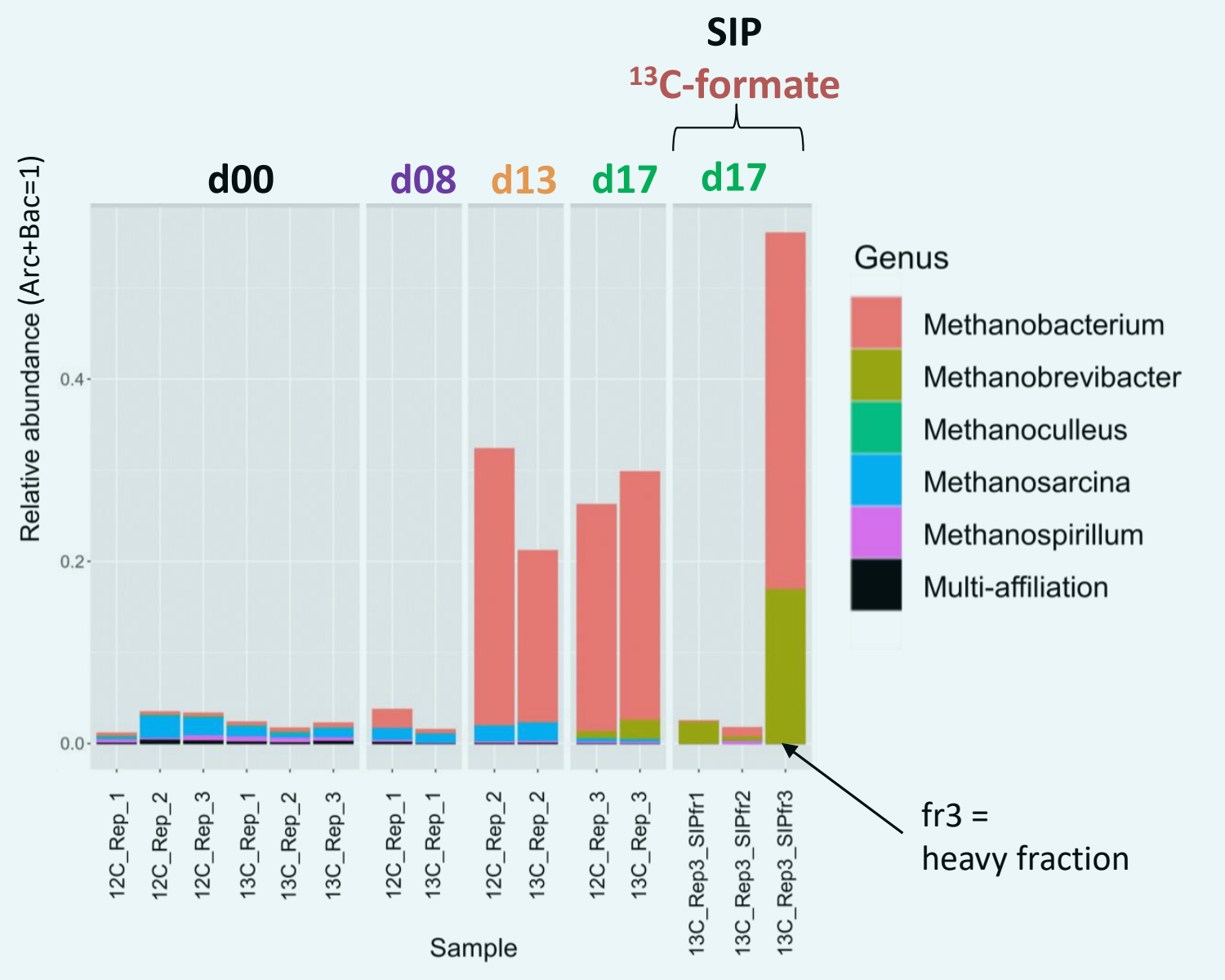




→ As expected, biogas was produced and formate was consumed, with a good reproducibility among replicates

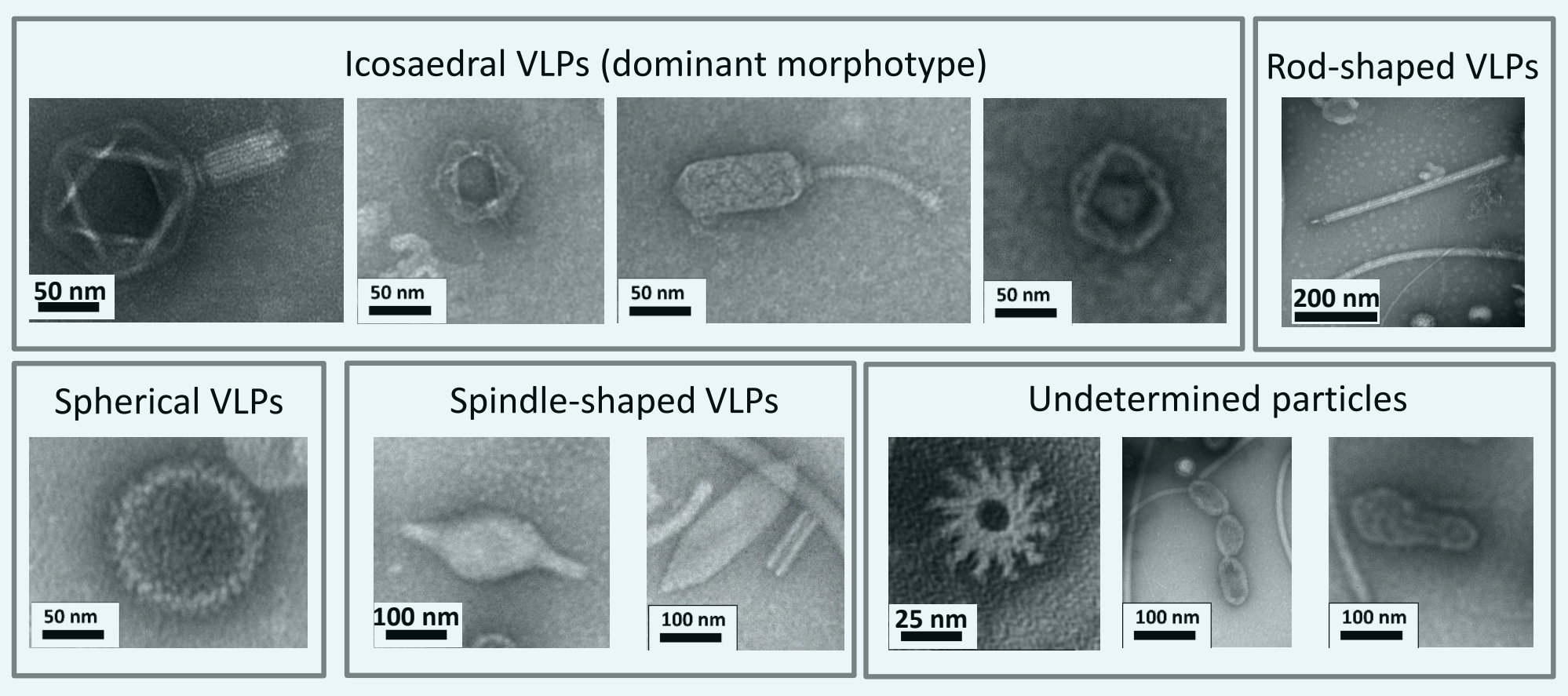
SIP and microbial community composition (16S metabarcoding)





→ Methanogenic archaea of the genera *Methanobacterium*, and to a lesser extent *Methanobrevibacter*, assimilated ^{13}C-formate and reached high proportions at d17, including in the heavy fraction (fr3)

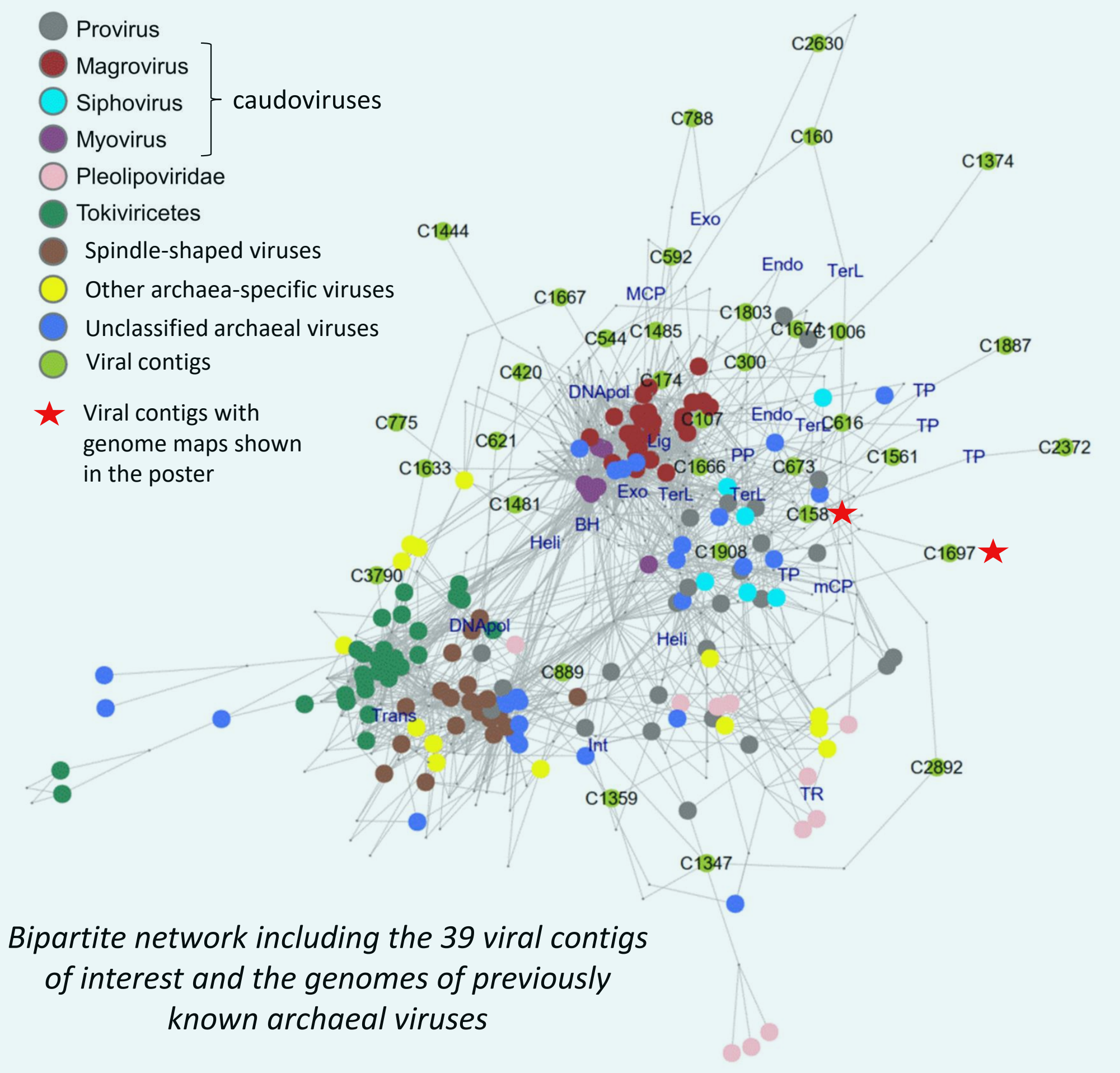
Virus-like particle (VLP) diversity



Transmission Electron Microscopy images

→ A great diversity of morphotypes was observed in the microcosms, including head-tailed, rod-shaped, spherical and spindle-shaped ones. Spindle-shaped viruses are specific to Archaea.

Bipartite network of 39 viral contigs predicted to have an archaeal host



Bipartite network including the 39 viral contigs of interest and the genomes of previously known archaeal viruses

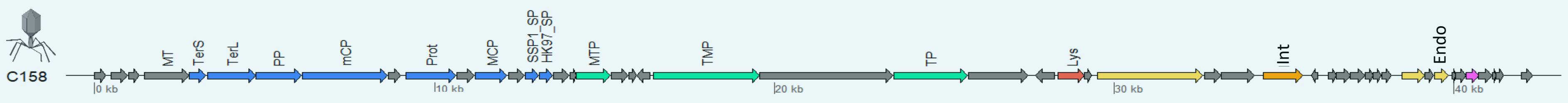
→ Most of the 39 viral contigs share genes with archaeal caudoviruses (e.g. C158)

→ Several of them share only few genes with previously known archaeal viruses (e.g. C1697)

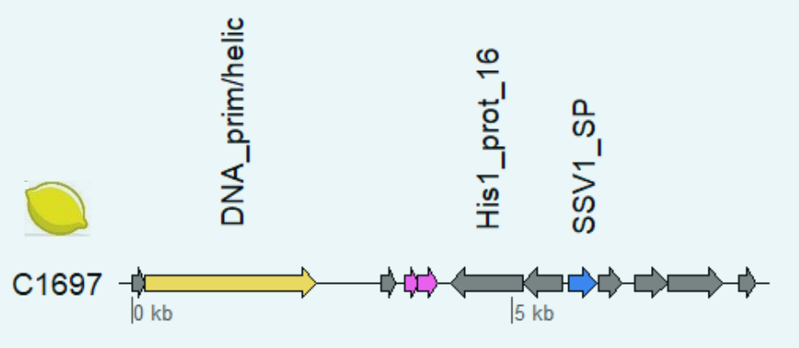
→ Several of them share genes with viruses belonging to viral families specific to Archaea

Examples of viral contigs associated to ^{13}C-enriched/unenriched methanogens

RPKM = reads per kilobase of contig per million reads mapped



Caudovirus, complete genome, predicted host = ^{13}C-enriched *Methanobacterium*, based on gene content and k-mer composition - new family proposed: *Speroviridae* (abundance: RPKM ≈ 263 at d17)



Spindle-shaped virus, partial genome, host = unenriched *Methanosarcina*, based on gene content and k-mer composition - likely represents a new family (abundance: RPKM ≈ 9 at d17)

SSV1_SP: similar to the main structural protein of SSV1, a spindle-shaped virus (Fuselloviridae) indecting Sulfolobus solfataricus, an acidothemophilic archaeon
His1_prot_16: similar to prot_16 of His1, a spindle-shaped virus (Halspiviridae) infecting Haloarcula hispanica, a halophilic archaeon

Conclusions

- ✓ Significantly expands the knowledge on the diversity of viruses of methanogens (one new caudovirus family, two putative new families of spindle-shaped viruses)
- ✓ Reinforces the notion of wide environmental and phylogenetic distribution of spindle-shaped archaeal viruses
- ✓ Work recently published: Ngo *et al*, *Environmental microbiology*, 2022, 1– 16

Perspectives

- ☐ Original approach that can be applied to virtually any type of microbial community
- ☐ and coupled with other experimental methods for host identification (e.g. PhageFISH, epicPCR, …).

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