南方科技大学
SOUTHERN UNIVERSITY OF SCIENCE AND TECHNOLOGY



The seasonality and dynamics of marine giant viruses in a decadal metagenomic time-series

Shengwei Hou

2025/07/01

Presenting author: Shengwei Hou
Email: hougw@sustech.edu.cn

海洋科学与工程系
Department of Ocean Science and Engineering



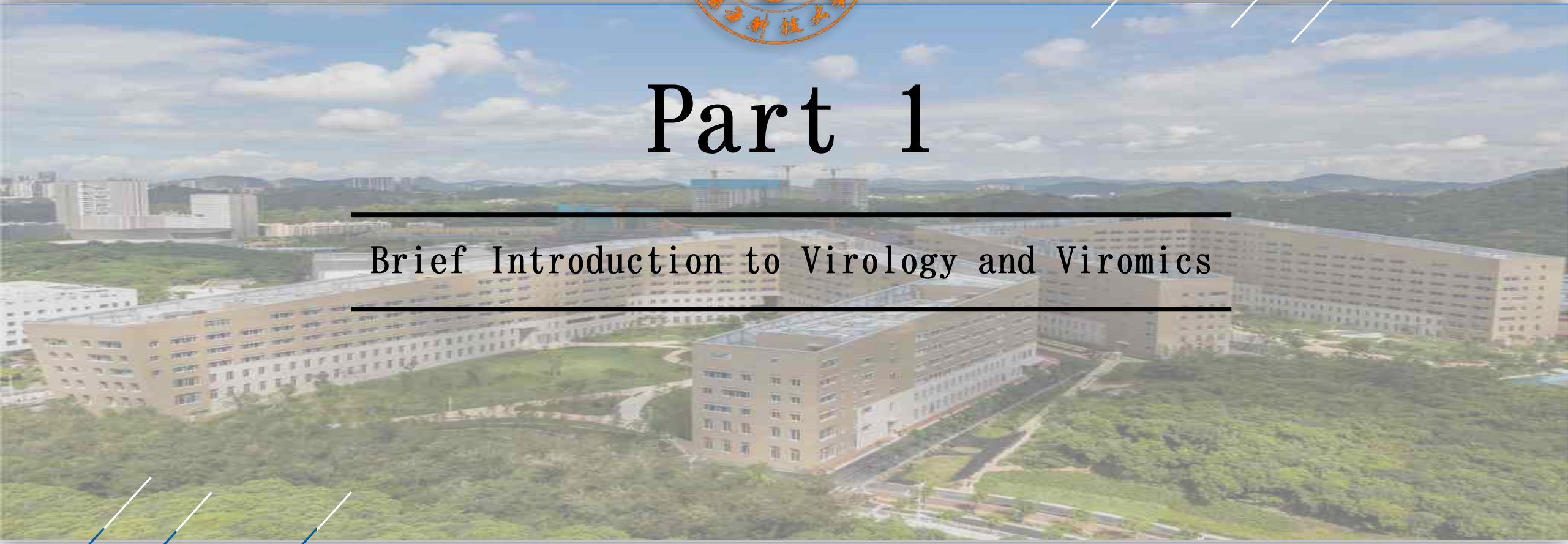
Content

01： Brief Introduction to Virology and Viromics

02： Brief Introduction to Giant Viruses

03： Seasonality of Giant Viruses at SPOT

04： Imitervirales under Global Warming



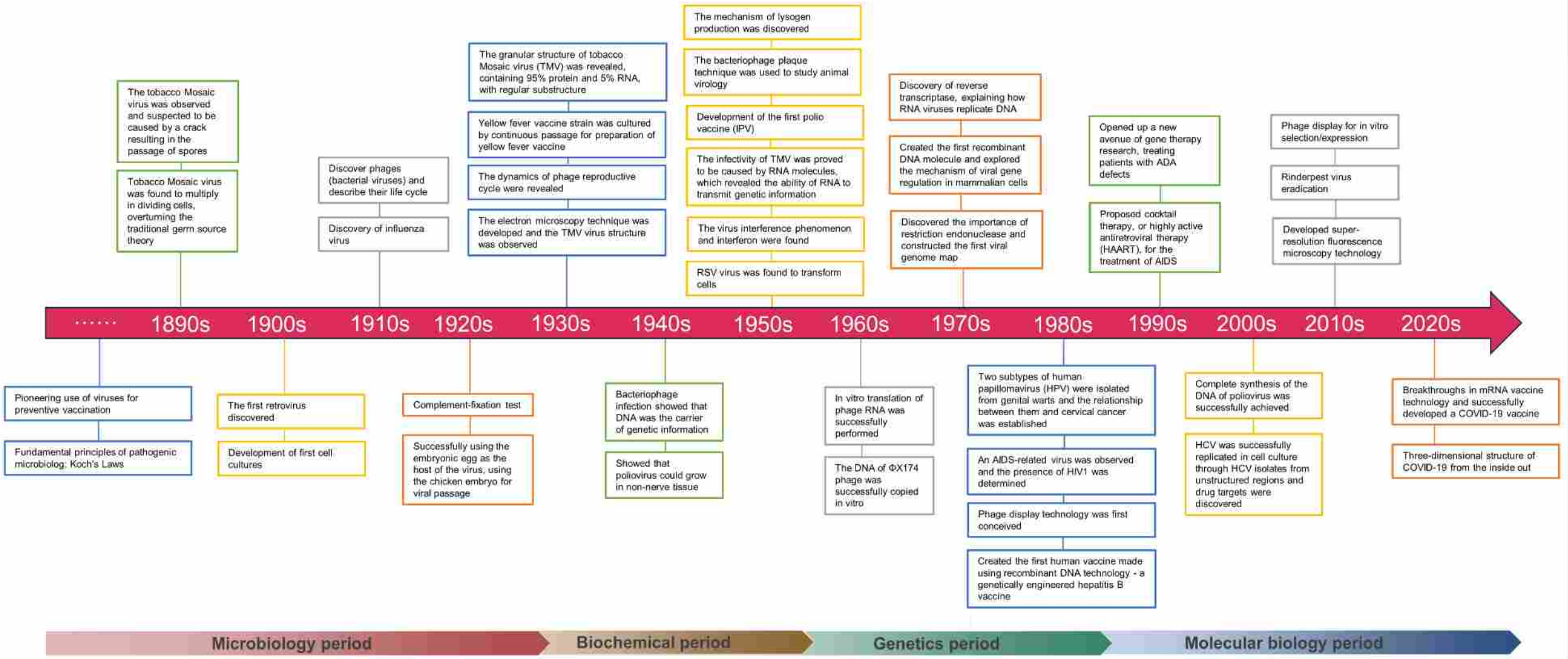
Part 1

Brief Introduction to Virology and Viromics

Key events in the timeline of virus discoveries

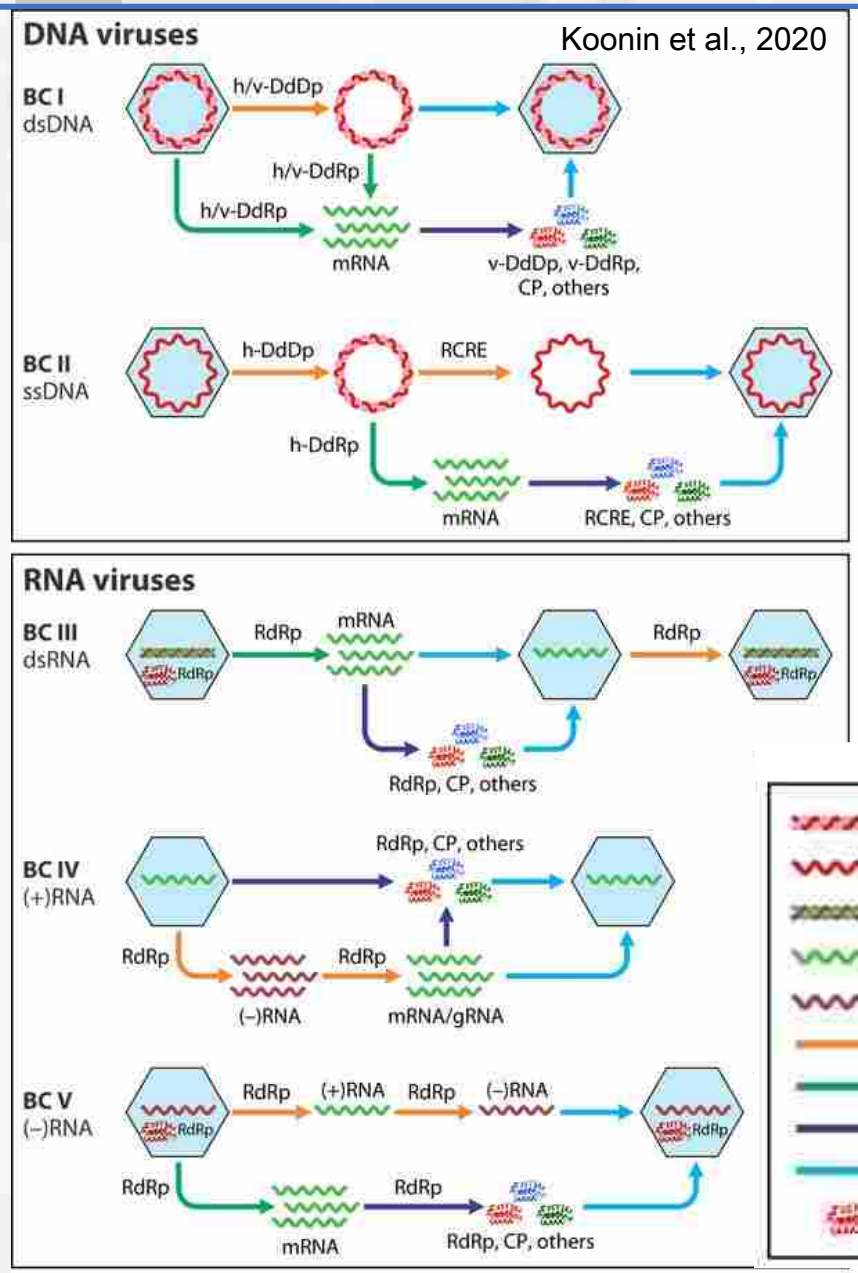
Evolution of Virology: Science History through Milestones and Technological Advancements

Zuo et al., 2024



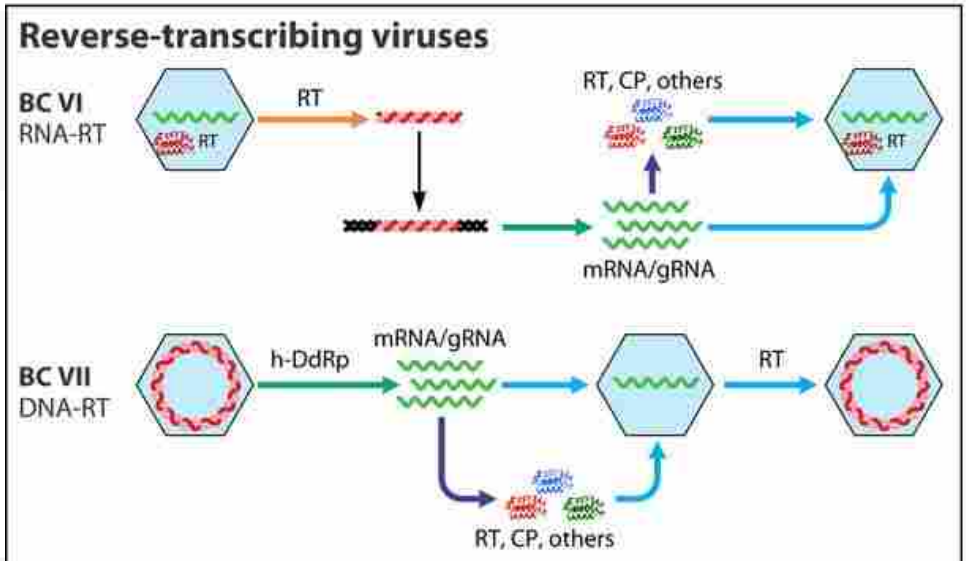
Baltimore classification of viruses

海洋科学与工程系
Department of Ocean Science and Engineering



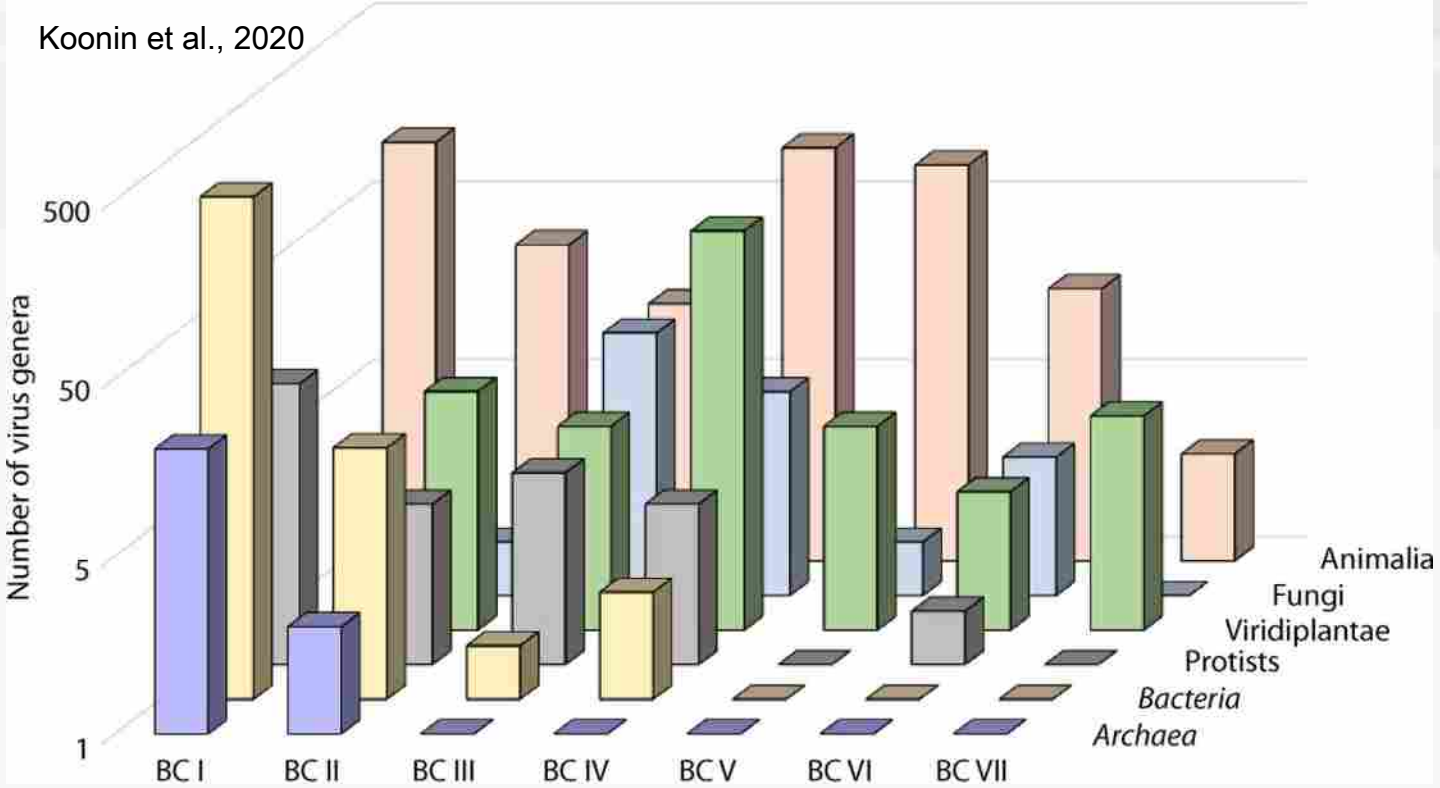
Baltimore classification: **I**. Double-stranded DNA (dsDNA) viruses share the same replication-expression strategy as in cellular life forms; **II**. Single-stranded DNA (ssDNA) viruses that replicate mostly via a rolling-circle mechanism; **III**. dsRNA viruses; **IV**. Positive-sense RNA [(+)RNA] viruses have ssRNA genomes with the same polarity as the virus mRNA(s); **V**. Negative-sense RNA [(−)RNA] viruses have ssRNA genomes complementary to the virus mRNA(s); **VI**. RNA reverse-transcribing viruses that have (+)RNA genomes replicate via DNA intermediates synthesized by reverse transcription; **VII**. DNA reverse-transcribing viruses replicate via reverse transcription, but incorporate a dsDNA or an RNA-DNA form of the virus genome into virions.

CP, capsid protein; DdDp, DNA-directed DNA polymerase; DdRp, DNA-directed RNA polymerase; gRNA, genomic RNA; RdRp, RNA-directed RNA polymerase; RT, reverse transcriptase; RCRE, rolling-circle replication (initiation) endonuclease.



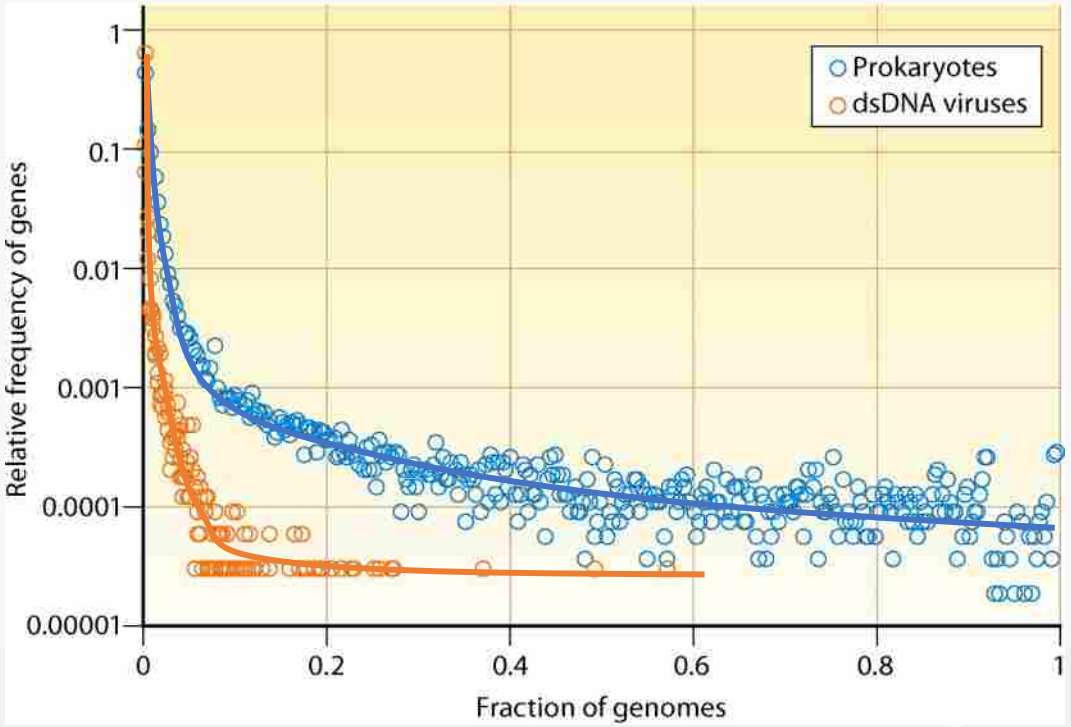
Baltimore classification of viruses

Koonin et al., 2020

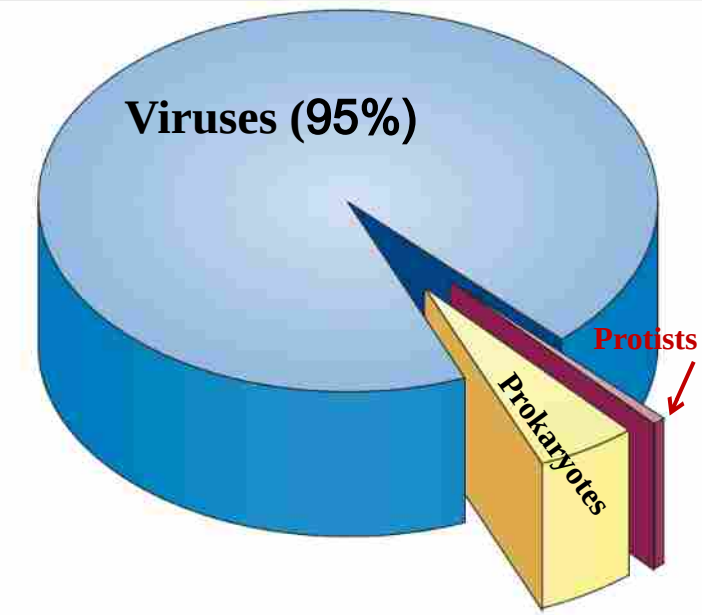


- Viruses across Baltimore Classifications (BC) vary widely in abundance, diversity, and distribution among cellular hosts.
- The BC system categorizes viruses based on mRNA synthesis, which is determined by their genome type and replication strategy.
- BC I (dsDNA) viruses are abundant and diverse in prokaryotes, while BC IV (positive-strand RNA) viruses dominate eukaryotic viromes.

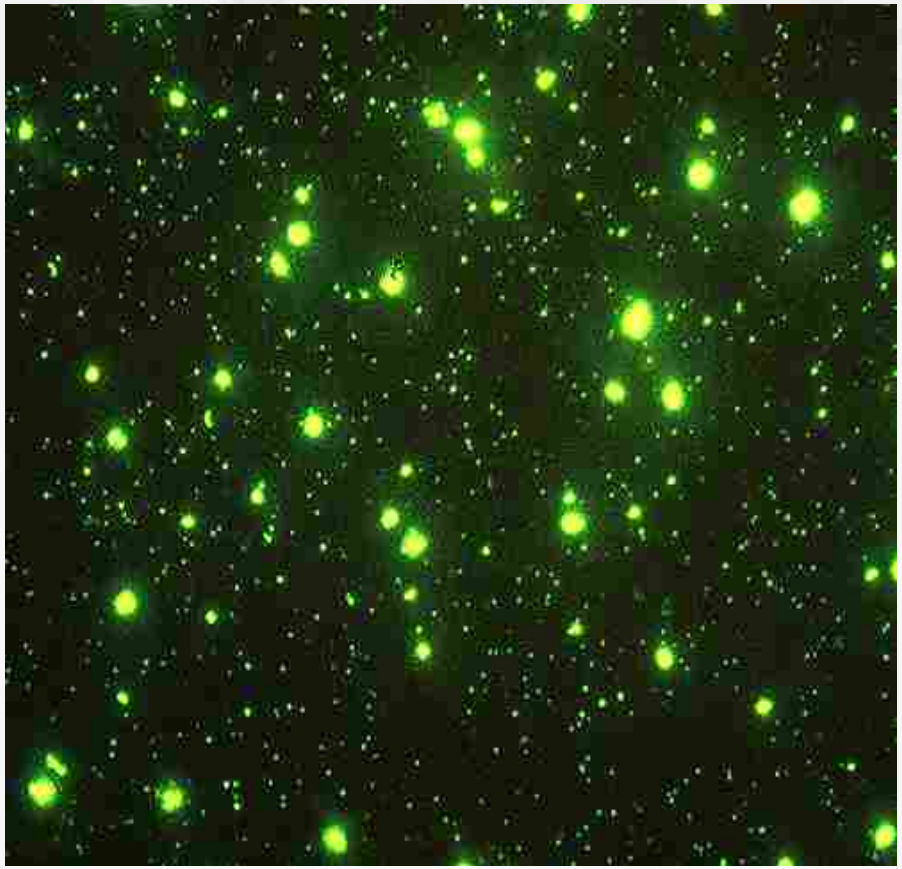
- The gene distribution for dsDNA virus genomes is similar but differs quantitatively, with more viral genes in the poorly conserved/unique cloud and a tiny core of conserved genes. These core genes, along with the (nearly) universal genes in other BC viruses, are known as virus hallmark genes.

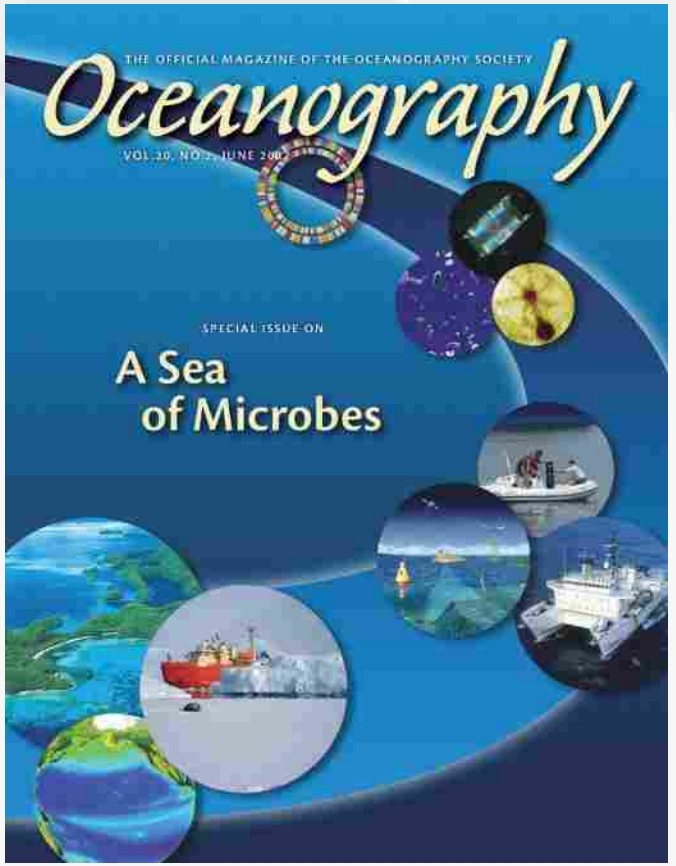


Viruses are the most abundant entities on earth



Relative abundance of Marine microbes

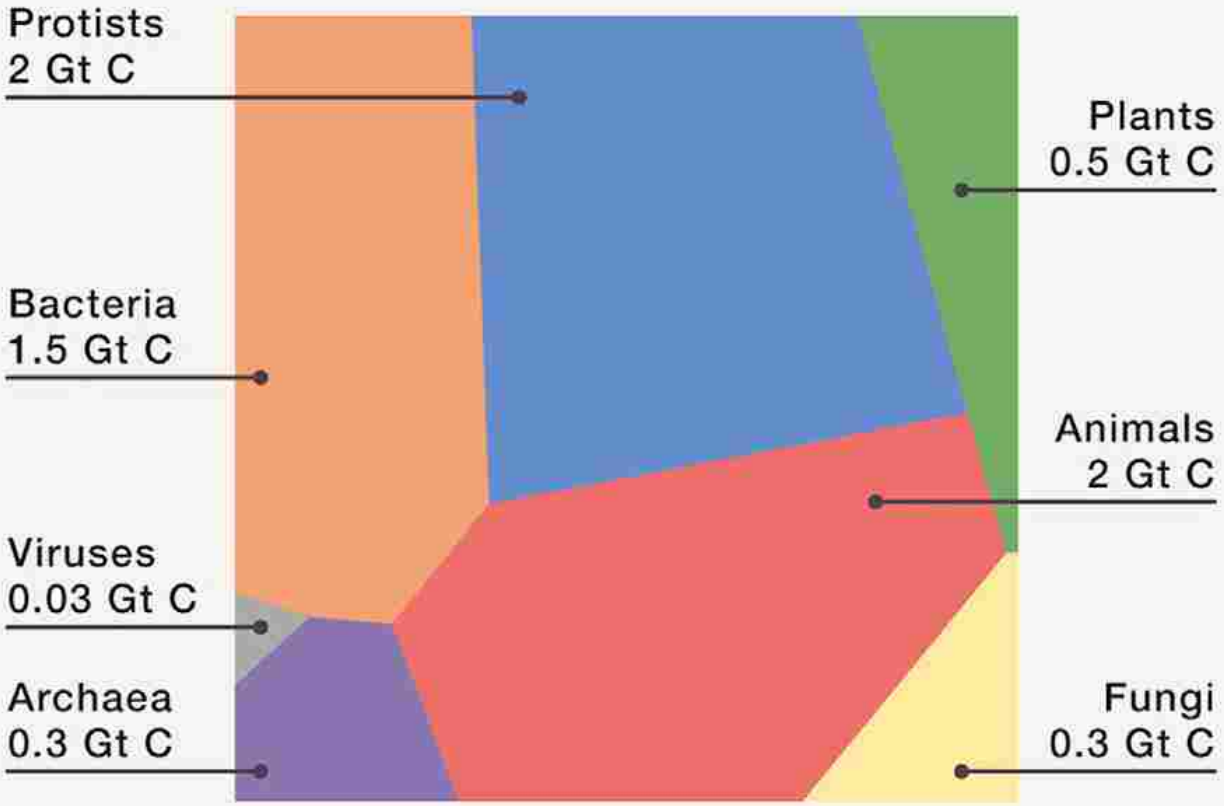


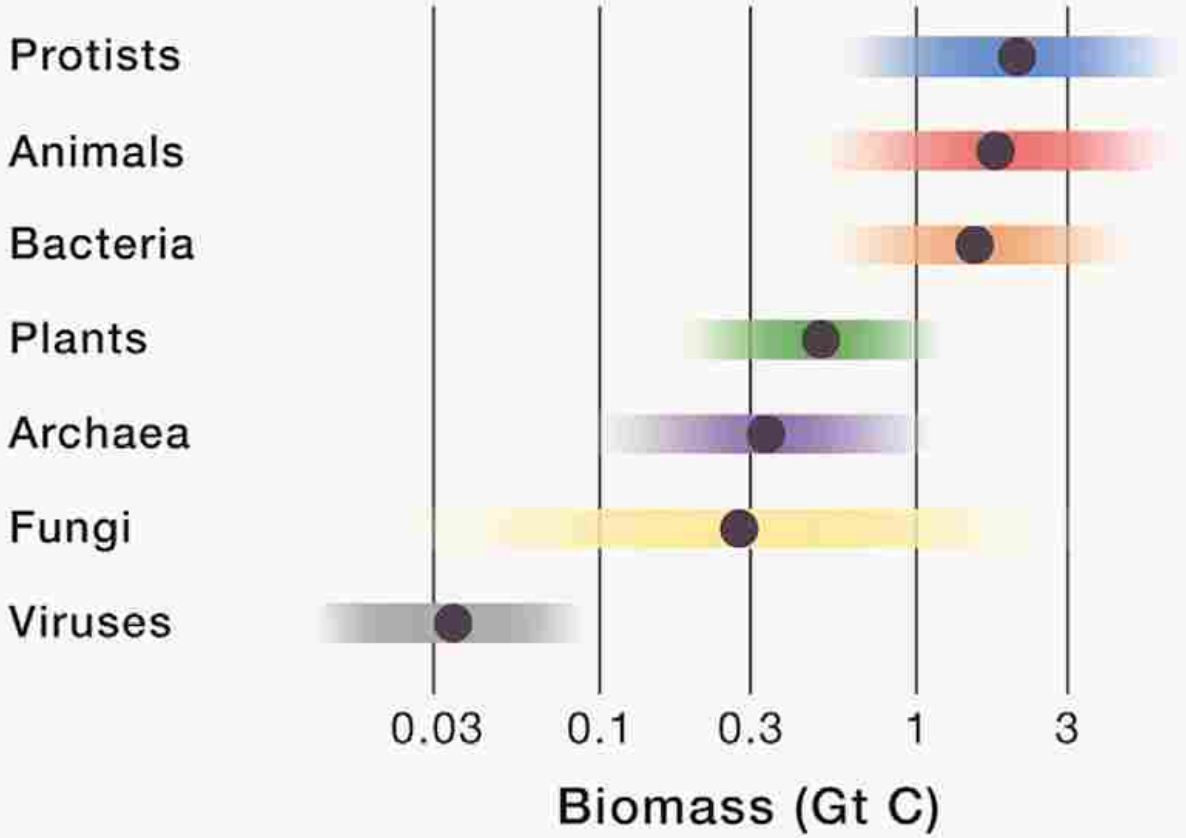


1 ml of seawater: 5-10 million viruses (10^7) ; The global ocean: 10^{30} viruses

Suttle, Nature Reviews Microbiology, 2007

Global marine viral biomass



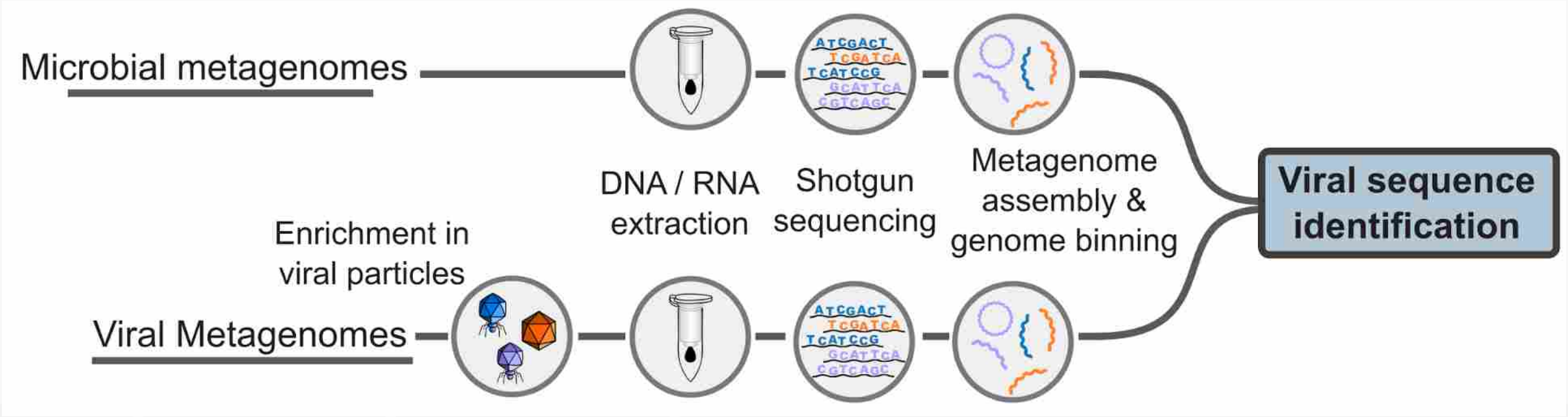


Bar-On & Milo, 2019, Cell

Until ~ 2000 - most virus work based on lab cultures

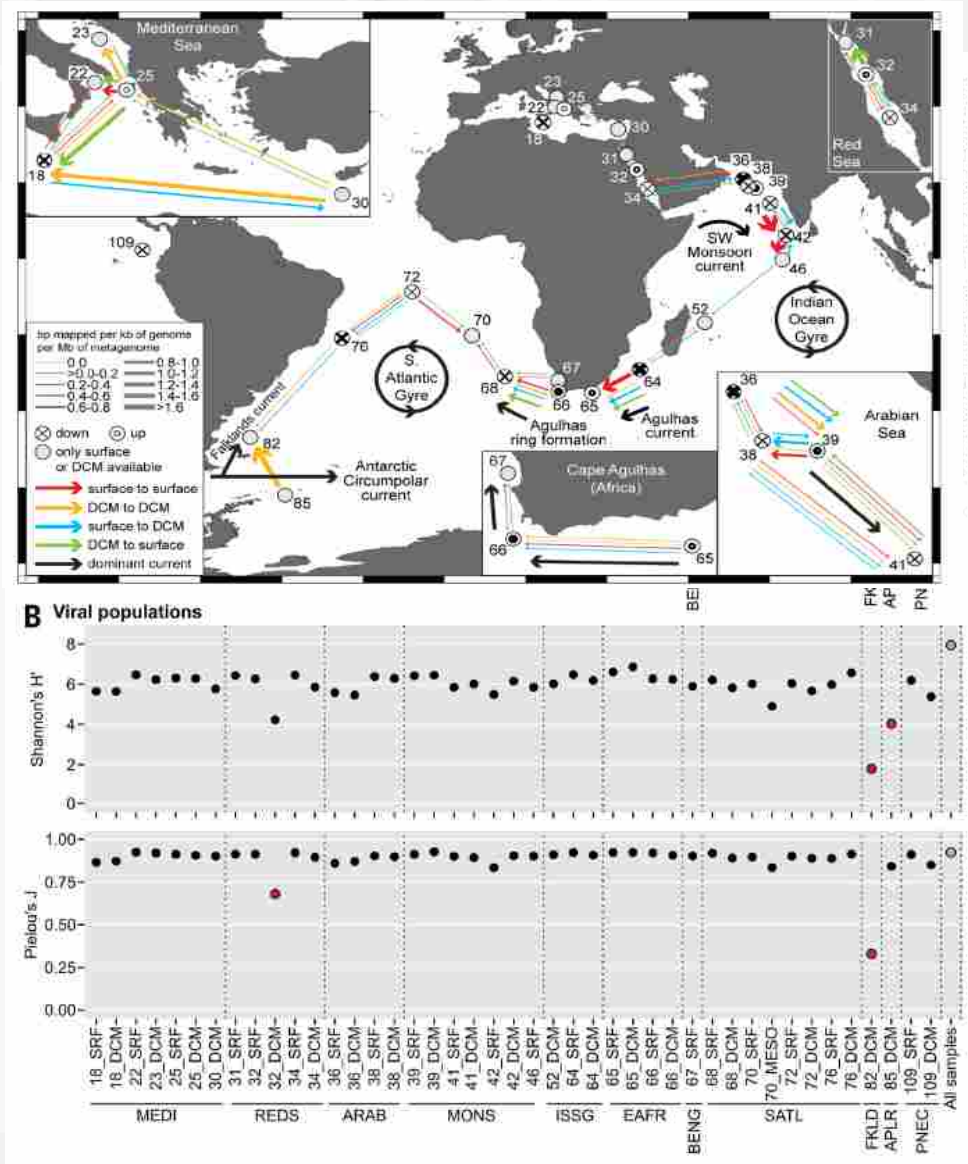
- No viruses for uncultivated hosts (except global counts)
- For cultivated hosts: needs to identify conditions for virus propagation

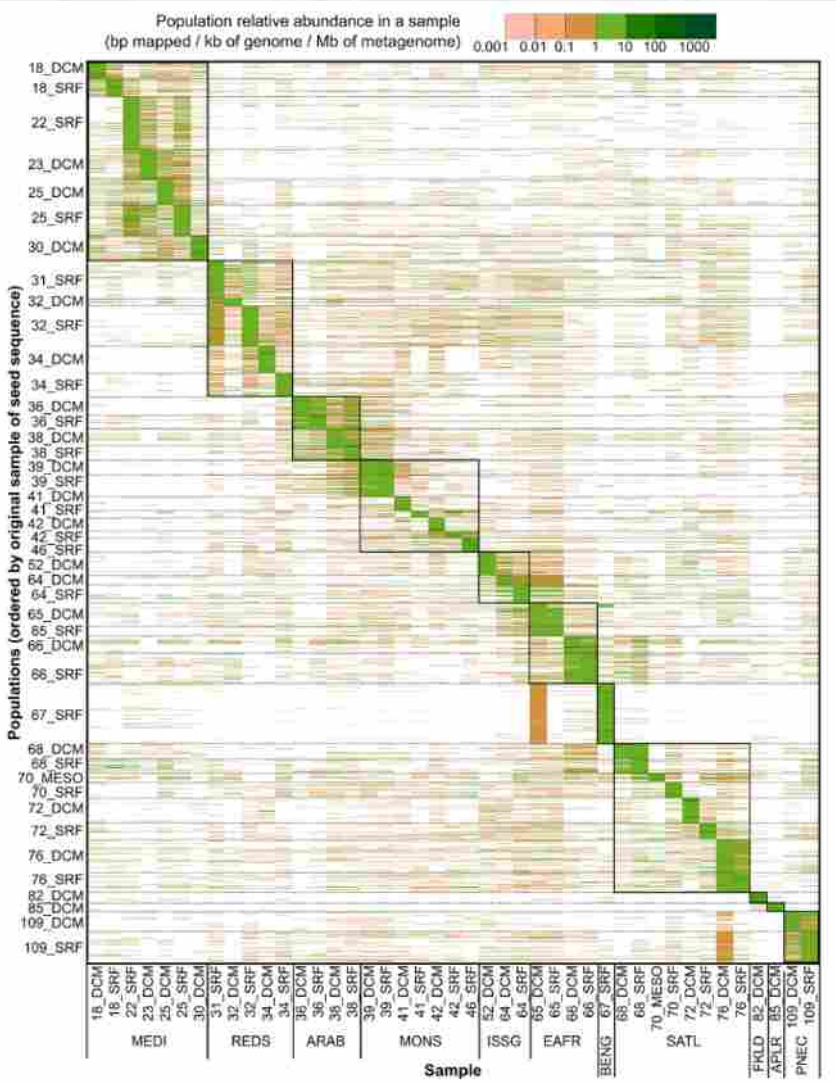
Metagenomics bypasses requirement for cultivation



Adapted from Matthew Sullivan, 2019

Global marine viral diversity





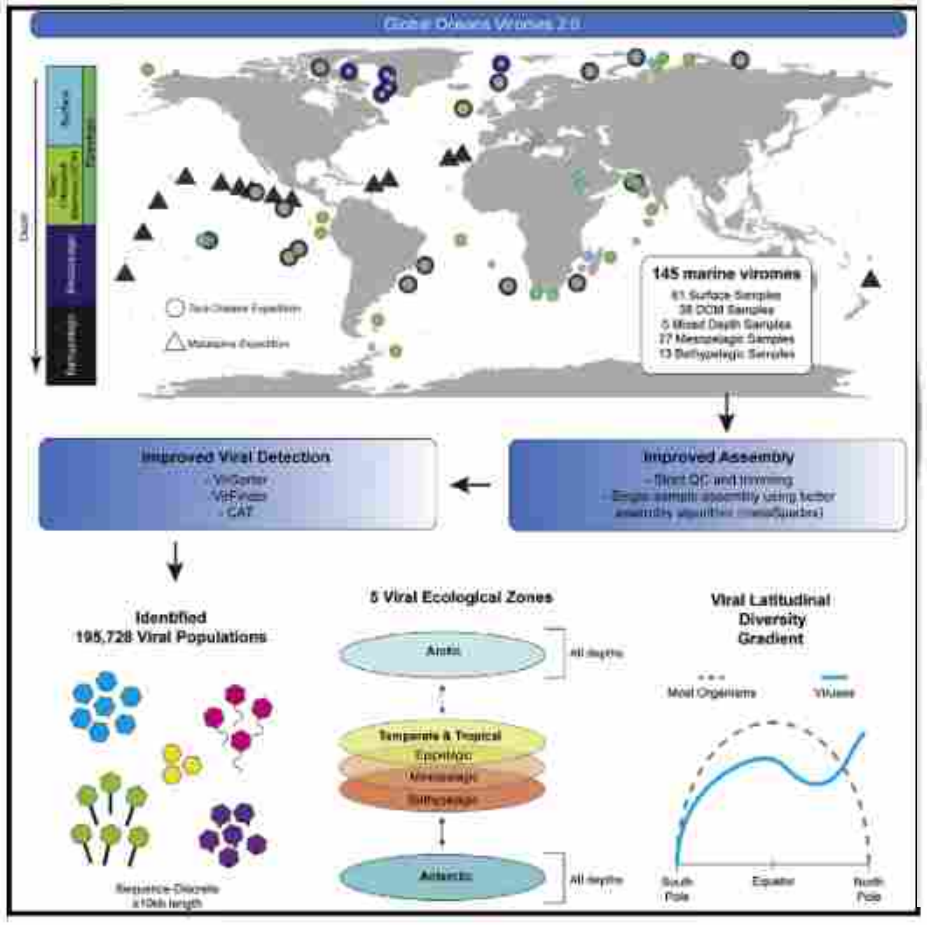
- Global marine viruses represent biogeographic patterns
- Viral diversity and community structure are influenced by local environmental conditions and passive dispersal with oceanic currents
- Viral diversity: local > global
- The seed-bank hypothesis

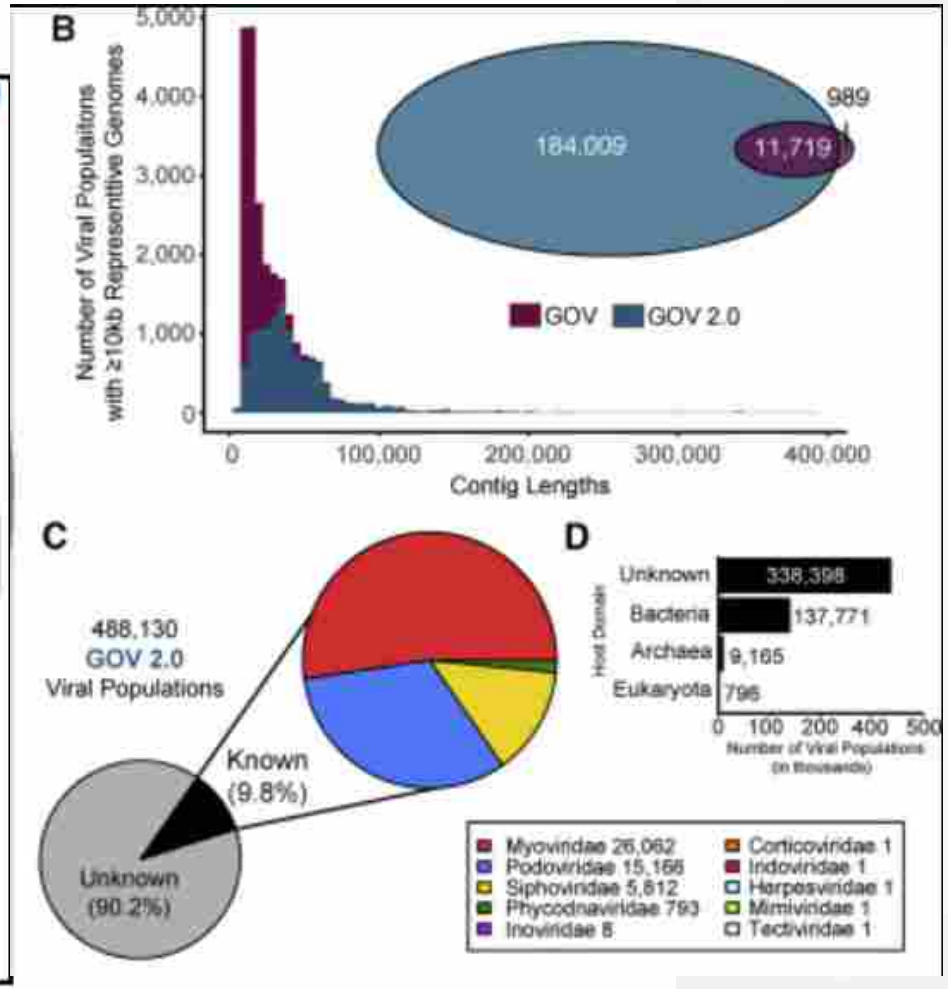
Brum， et al., 2015, Science

Cell

Marine DNA Viral Macro- and Microdiversity from Pole to Pole

Graphical Abstract

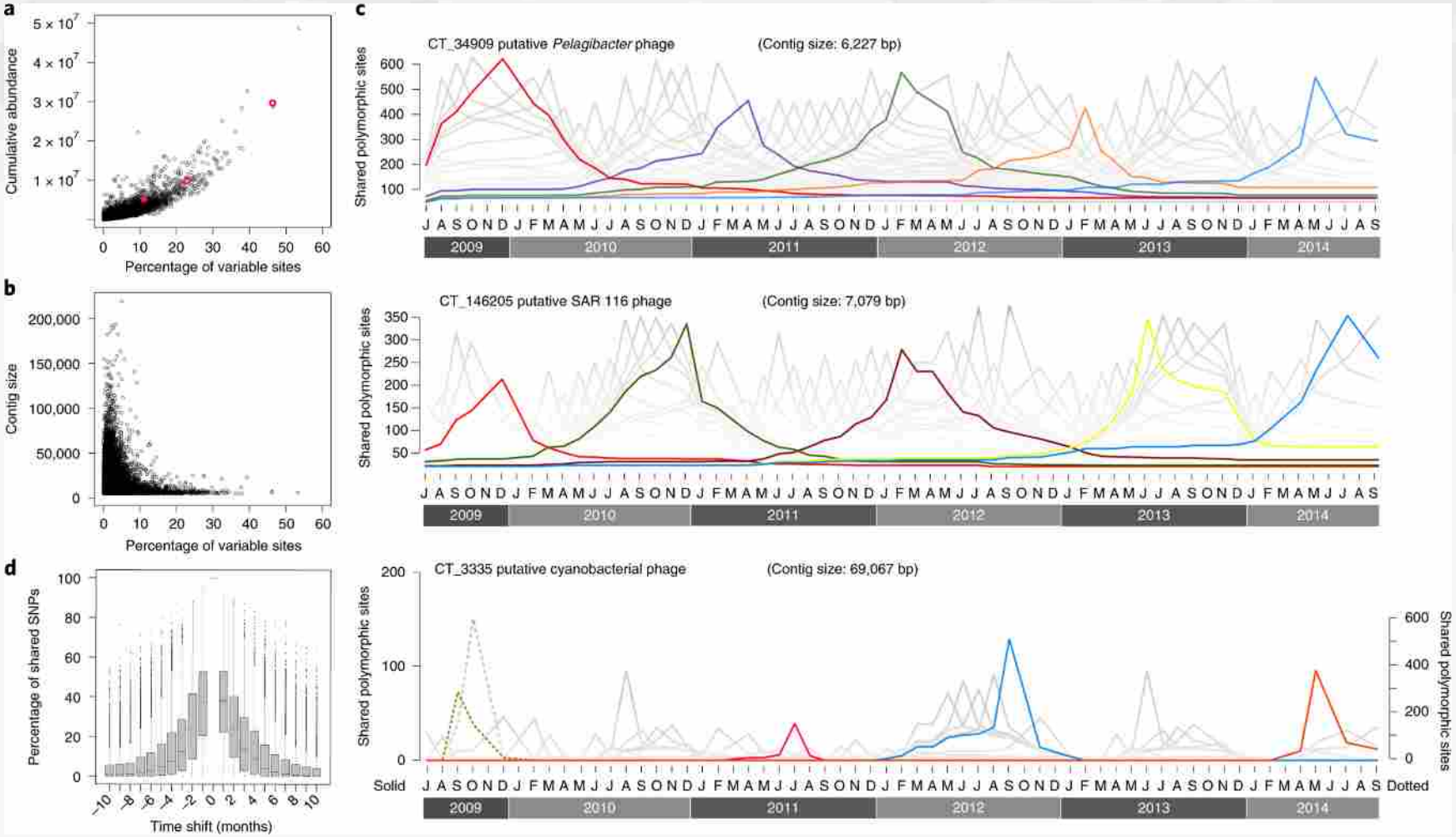




- 195,728 virus populations were identified in 145 viromes.
- The global marine DNA viral diversity can be divided into five ecological zones, of which the Arctic is a biodiversity hotspot.
- Viral diversity (micro and macro) didn't represent a significant latitude gradient.

Gregory et al., 2019

Viral microdiversity through time

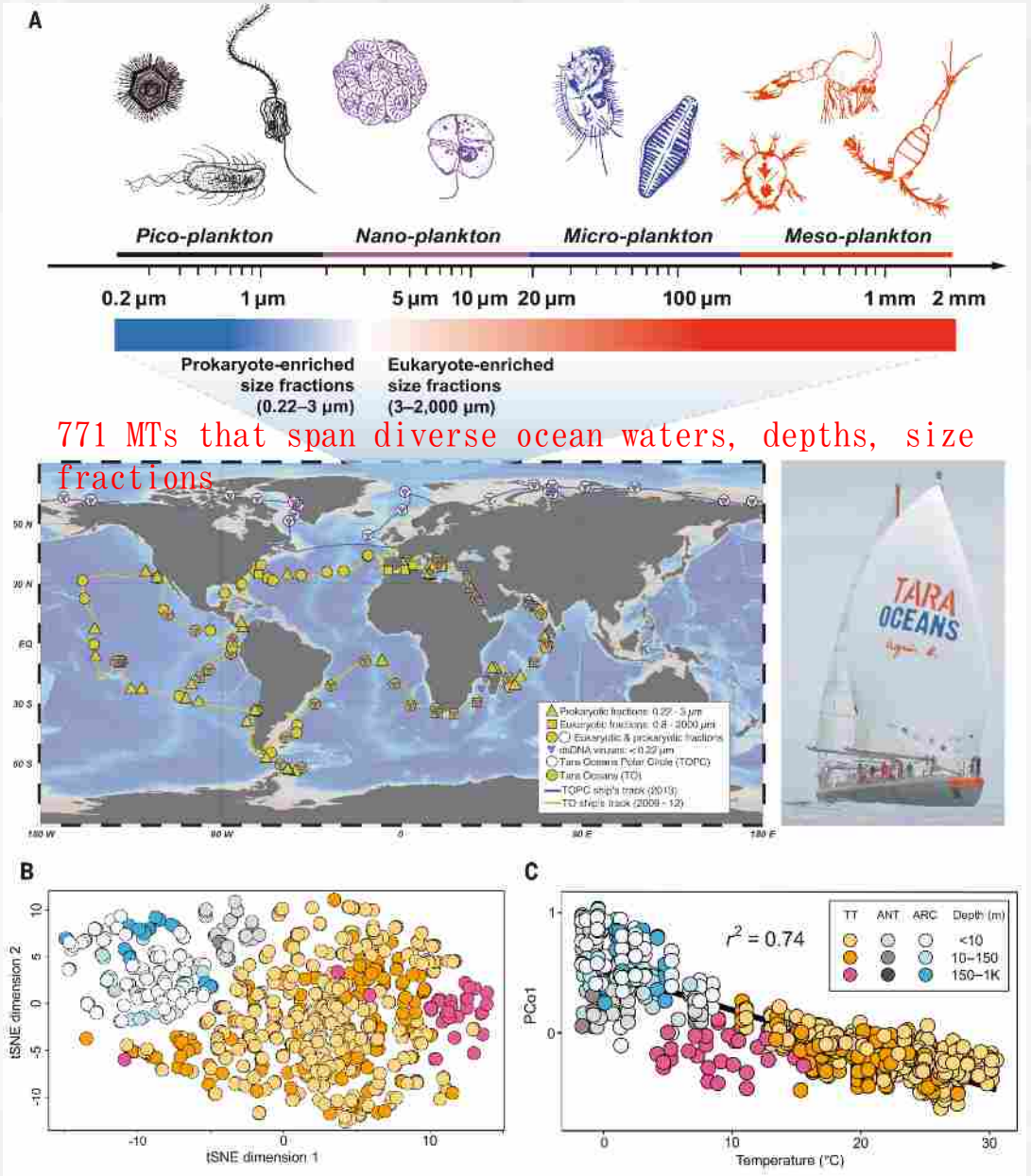


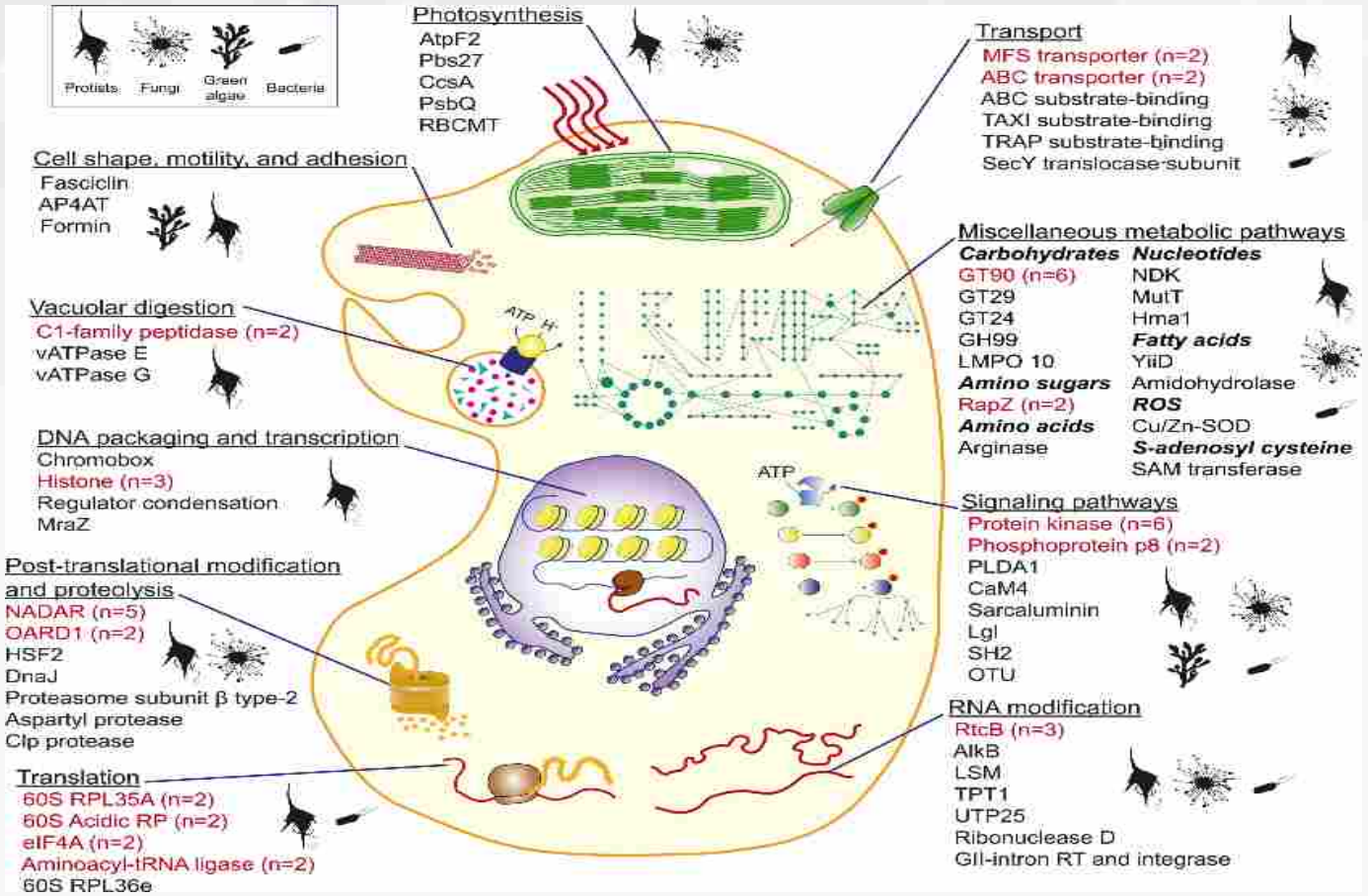
- Of 19,907 assembled viral contigs (>5 kb, mean 15 kb), 97% were found in each sample to have relative abundances that ranged over seven orders of magnitude, with limited temporal reordering of rank abundances.
- Seasonal variations in viral community composition were superimposed on the overall stability.
- transient variations in single-nucleotide polymorphisms (SNPs) and constant turnover of minor population variants, each rising and falling over a few months, reminiscent of Red Queen dynamics

- The rise and fall of variants within populations, is consistent with the hypothesis that fluctuating selection acts on a microdiverse cloud of strains, and this succession is associated with ever-shifting virus–host defences and counterdefences.

Ignacio Espinoza et al., 2020

Global marine RNA viral diversity





- The global marine RNA viral diversity can be divided into four ecological zones, characterized by distinct latitudinal and depth diversity patterns.
- RNA viruses cause reprogramming of diverse host metabolisms, including photosynthesis and carbon cycling, and RNA virus abundances predict ocean carbon export.

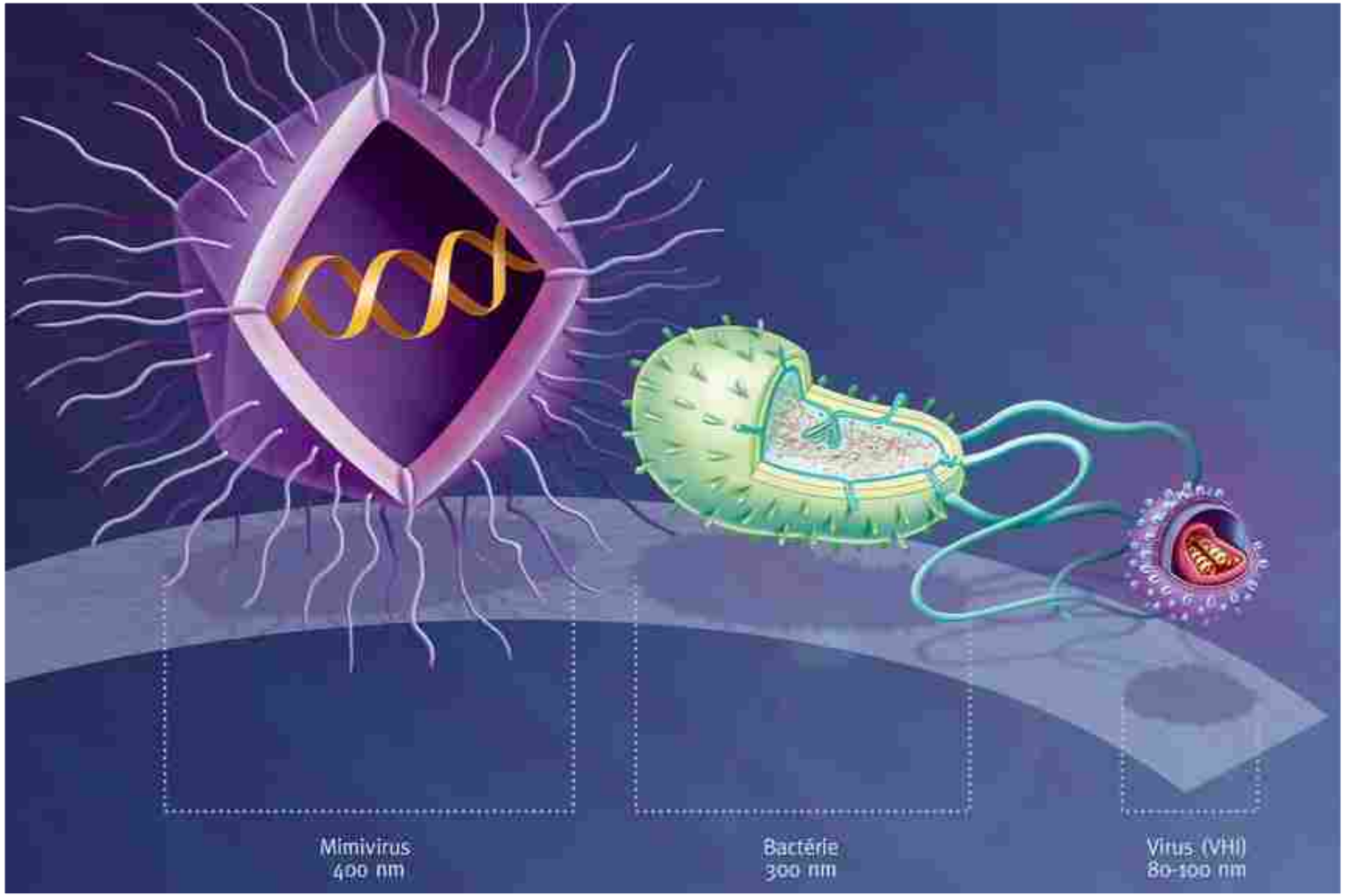
Dominguez-Huerta et al., 2022



Part 2

Brief Introduction to Giant Viruses

Giant Viruses – introduction



Comparison of the size of a Mimivirus (400 nm) with that of a bacterium *Escherichia coli* (300 nm), and of a virus HIV (100 nm).
Photo: BSIP via Getty Images

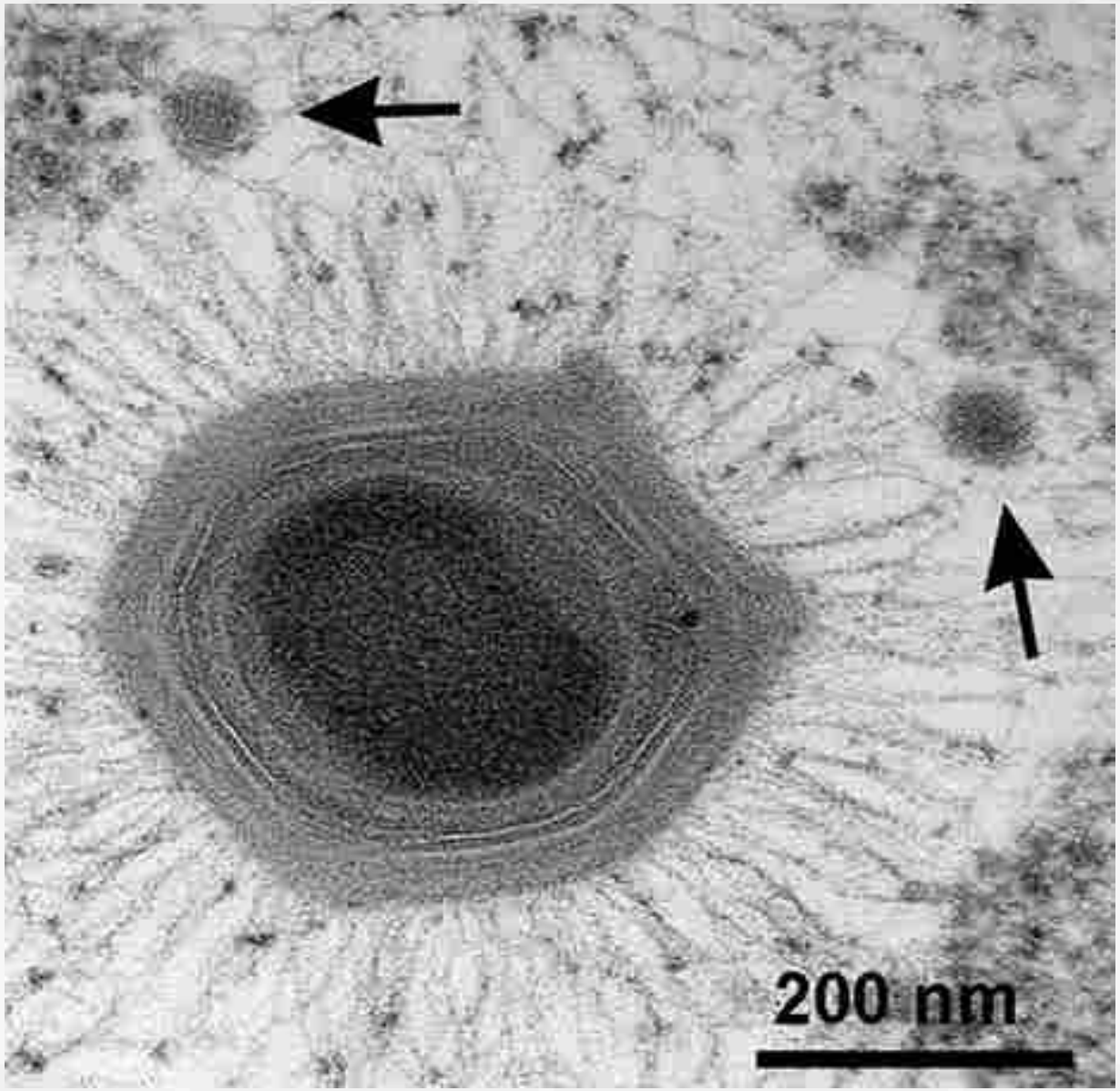
https://www.syfy.com/syfy-wire/are-giant-arctic-viruses-combatting-climate-change



July-August 2011
Volume 99, Number 4
Page 304

https://www.americanscientist.org/article/giant-viruses

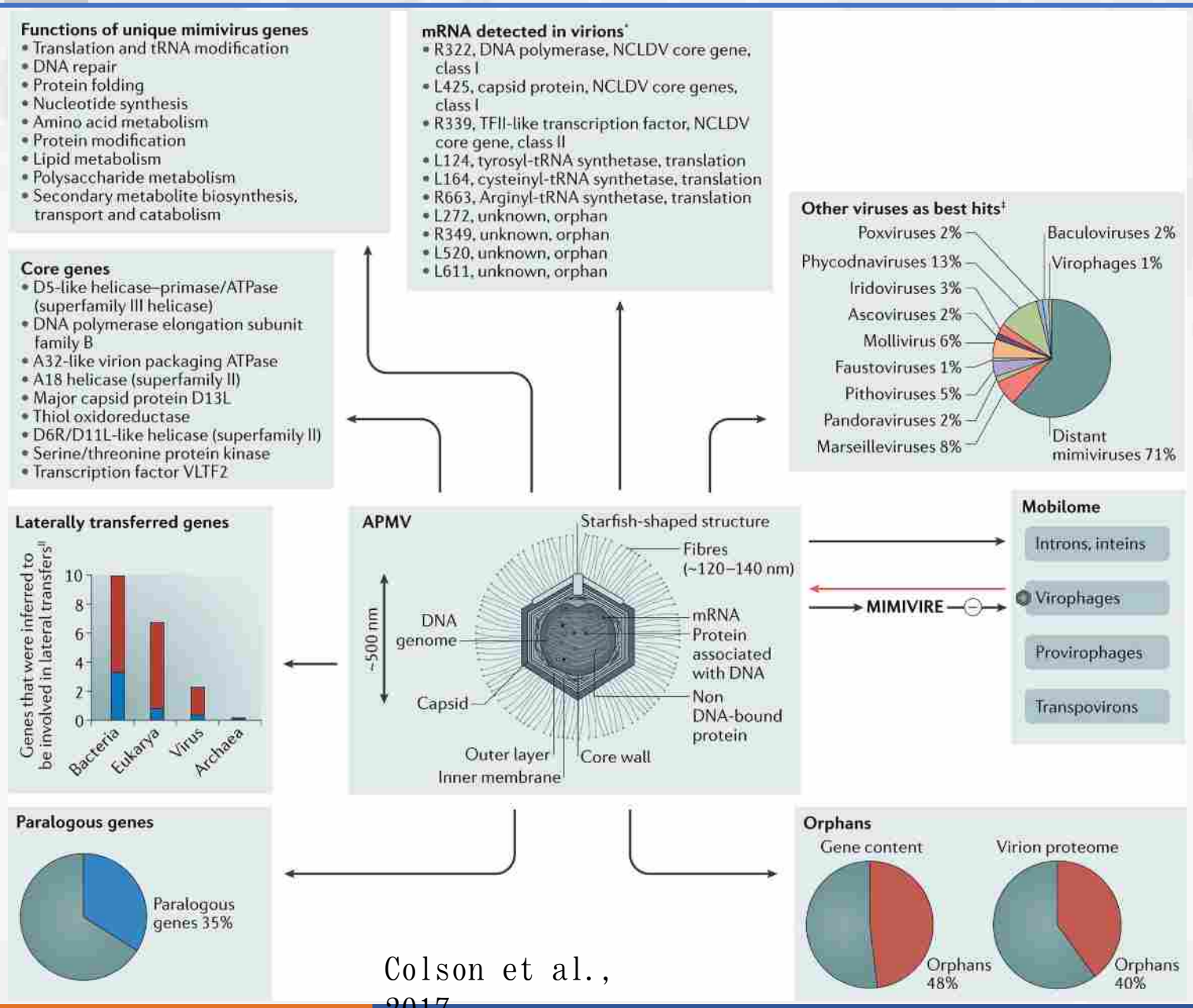
Giant Viruses – introduction



https://en.wikipedia.org/wiki/Mimivirus

- APMV was discovered accidentally in 1992 in *Acanthamoeba polyphaga* during legionellosis research by scientists from Marseille and Leeds. It was seen in a Gram staining and mistakenly identified as a Gram-positive bacterium, leading to its name Bradfordcoccus, after Bradford, England, where the amoeba originated.
- In 2003, researchers from the Université de la Méditerranée in Marseille, identified the microorganism as a virus. They named it "mimivirus" (meaning "mimicking microbe") because it resembles a bacterium in Gram staining.
- The same team that discovered the mimivirus later identified a slightly larger virus called the mamavirus, along with the Sputnik virophage that infects it.

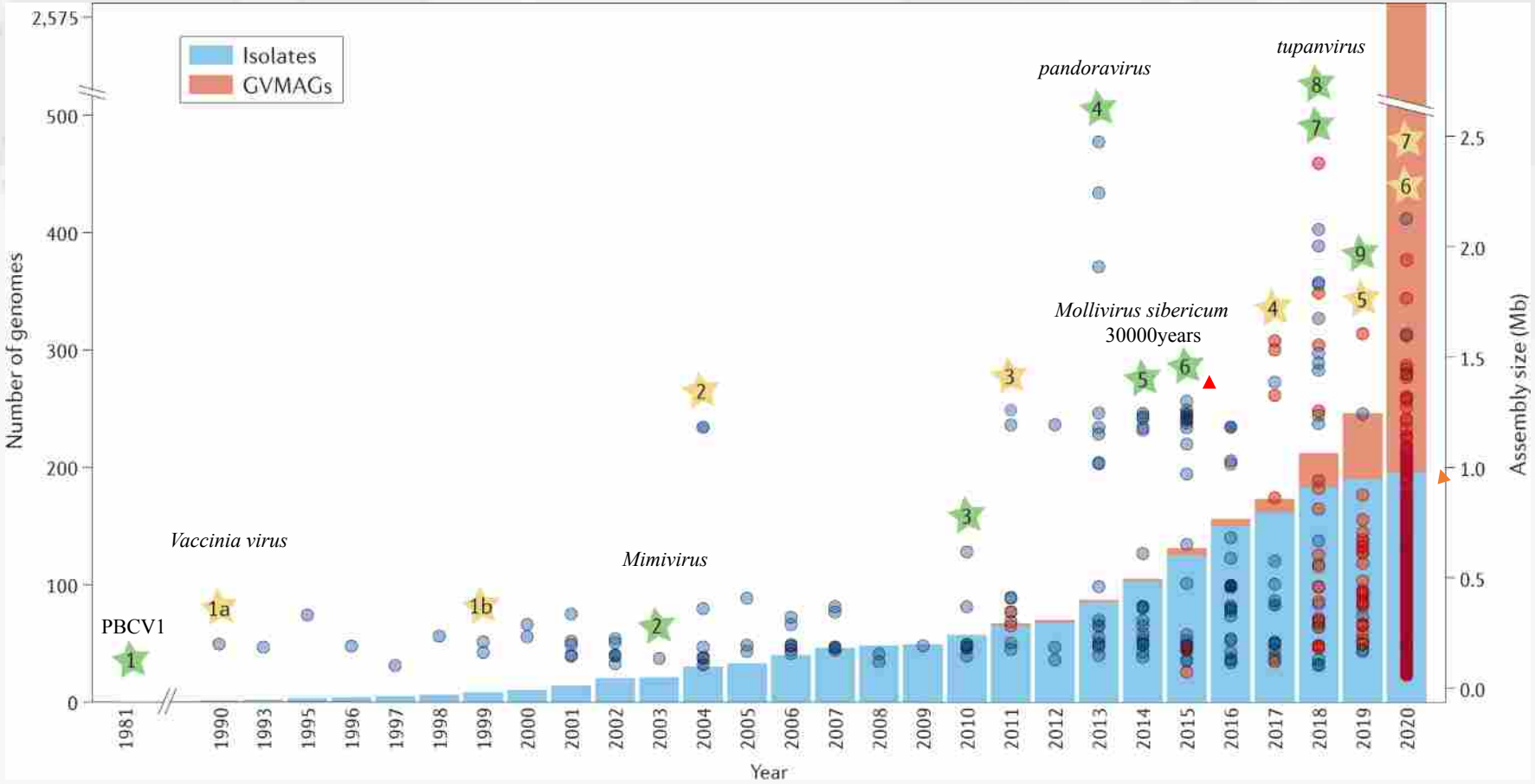
Giant Viruses – introduction



Colson et al., 2017

- The APMV capsid is ~500 nm in size and is covered by fibrils that are ~120–140 nm long and 1.4 nm thick.
- The capsid proteins have a double jelly-roll fold and are icosahedral, except for the starfish-shaped vertex (termed 'stargate'), which is devoid of fibers.
- APMV has a double-stranded DNA genome that is 1.2 Mb in length and contains 979 genes, only 12% of which encode known proteins.
- The mRNAs are expressed as polyadenylated transcripts that often end with short palindromes that form hairpin-like structures.

Giant Viruses – introduction



1981: First isolation

1990: First whole genome

2011: First GVMAGs

2013: *Pandoravirus* (2.5Mb)

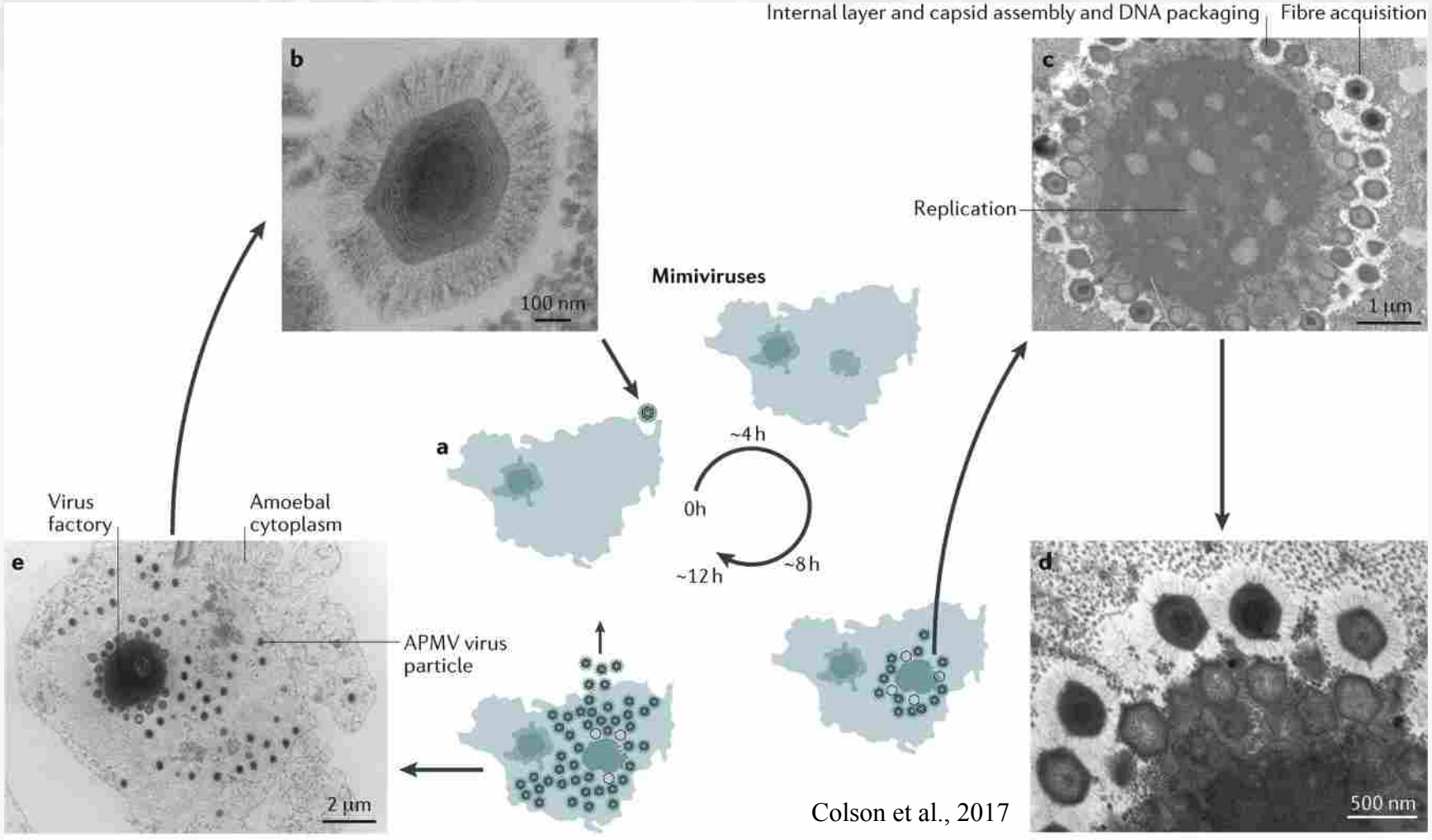
2019: Single-cell genomics

2020: 2074 GVMAGs

- Giant viruses, belonging to the phylum Nucleocytoviricota, are a monophyletic group of nucleocytoplasmic large DNA viruses (NCLDVs).
- They possess double-stranded DNA genomes ranging from 50 Kb to 2.5 Mb, with viral particles reaching up to 2 μm in length, infecting a variety of eukaryotic hosts, including protists and animals.
- **Traditional co-cultivation methods have yielded only ~200 viral isolates.**
- Metagenomics has been demonstrated to be a powerful tool for discovering novel lineages of giant viruses.

Schulz et al. 2022, Nature reviews microbiology

Replication cycle of giant viruses

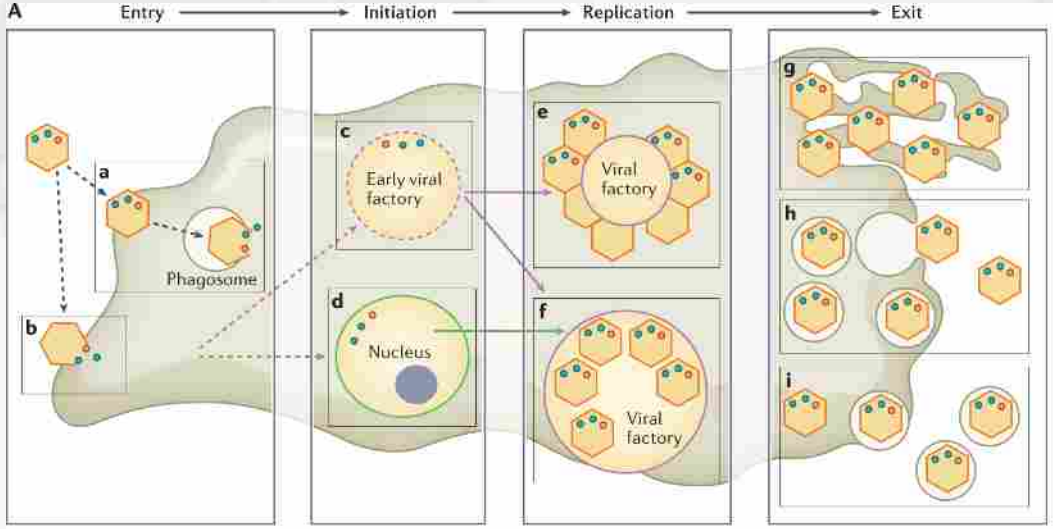


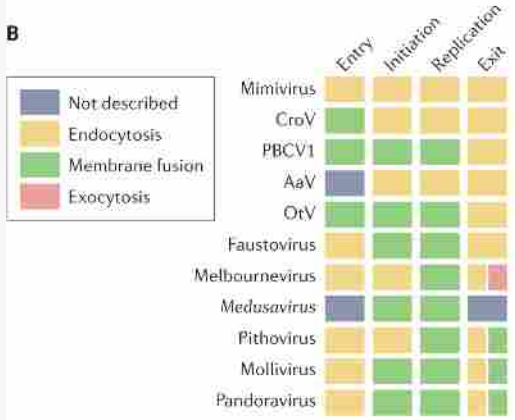
Colson et al., 2017

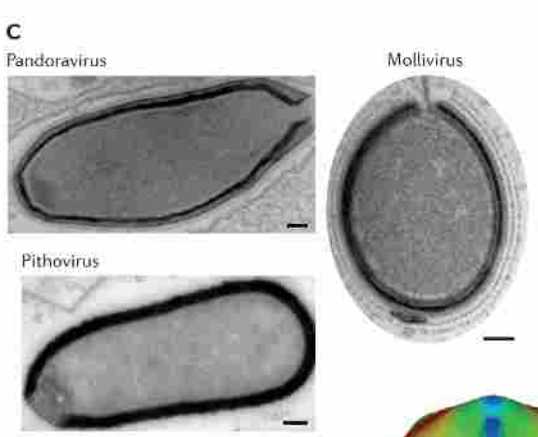
- Virus particles are visible on the *Acanthamoeba* sp. surface at the first cycle stage.
- After virus entry via phagocytosis, the APMV genome is released into the amoebal cytoplasm, initiating the formation of the virus factory, which is located away from the nucleus.
- From ~approximately 8 hours post-infection, virus particles assemble at the periphery of the factory.
- Amoebal lysis occurs approximately 12 hours post-infection.

- The mimivirus replication cycle in Amoeba. The replicative cycle of APMV lasts about 12 h and occurs in the amoebal cytoplasm. The size of the amoebal nucleus decreases approximately twofold during APMV replication.

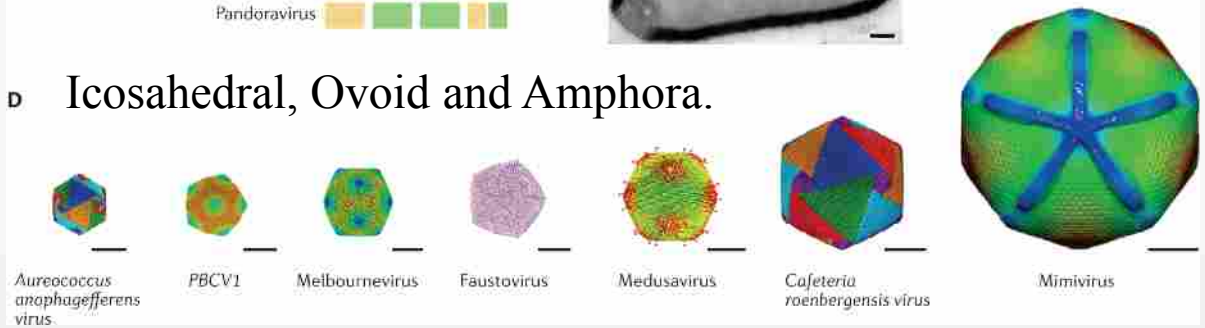
Infection and replication



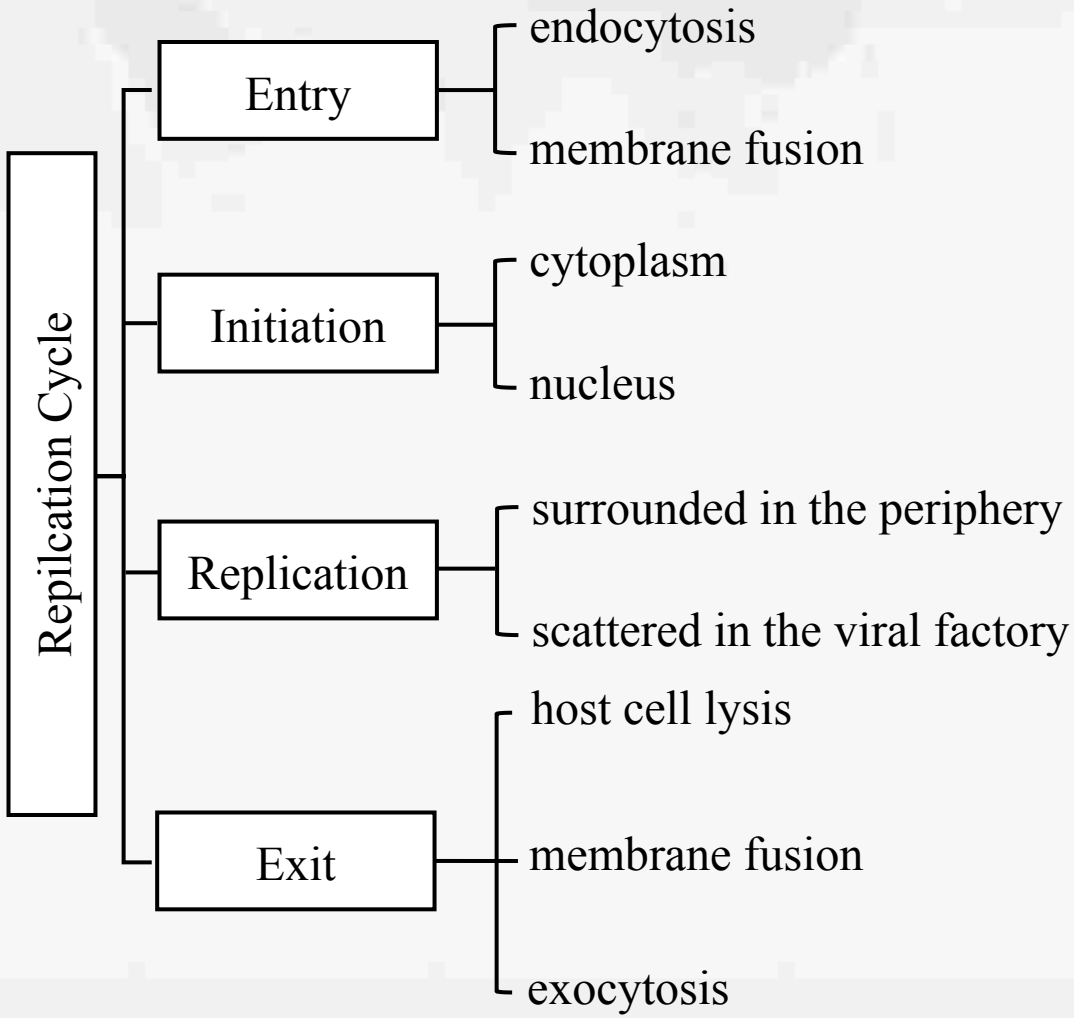




D Icosahedral, Ovoid and Amphora.

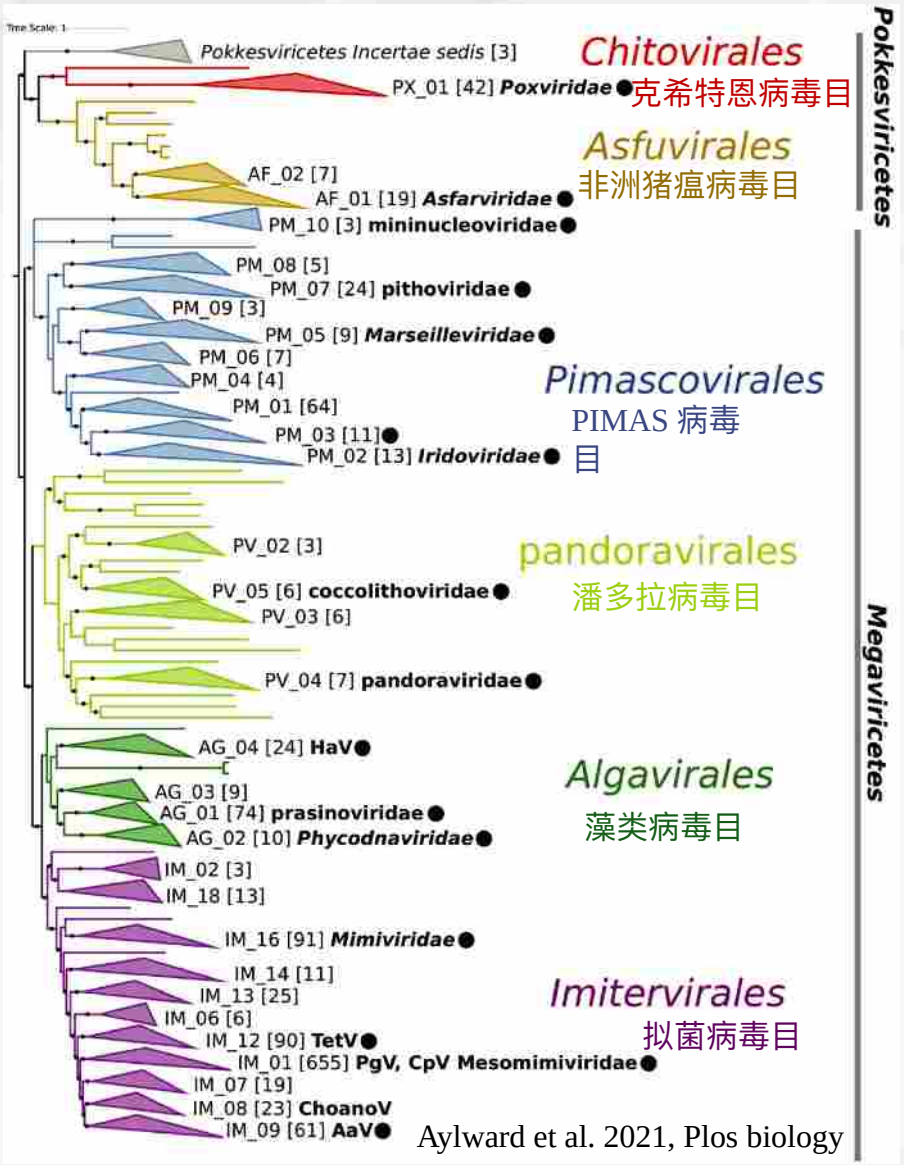


NCLDVs typically establish viral factories either in the host cell cytoplasm or nucleus to complete their replication and assembly processes.



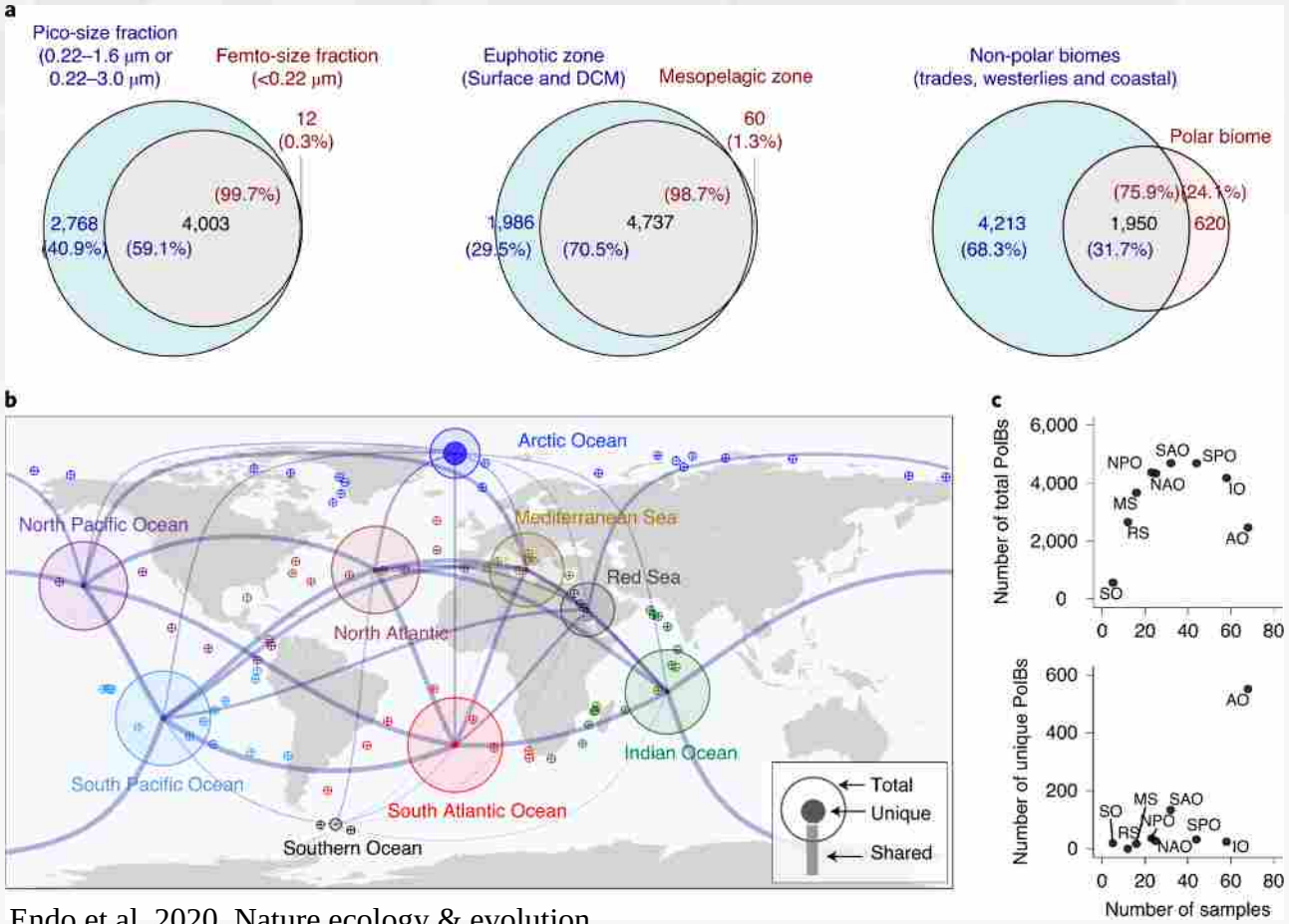
Schulz et al. 2022, Nature reviews microbiology

Diversity and distribution of giant viruses



Aylward et al. 2021, Plos biology

NCLDVs have been classified into 6 orders and 32 families

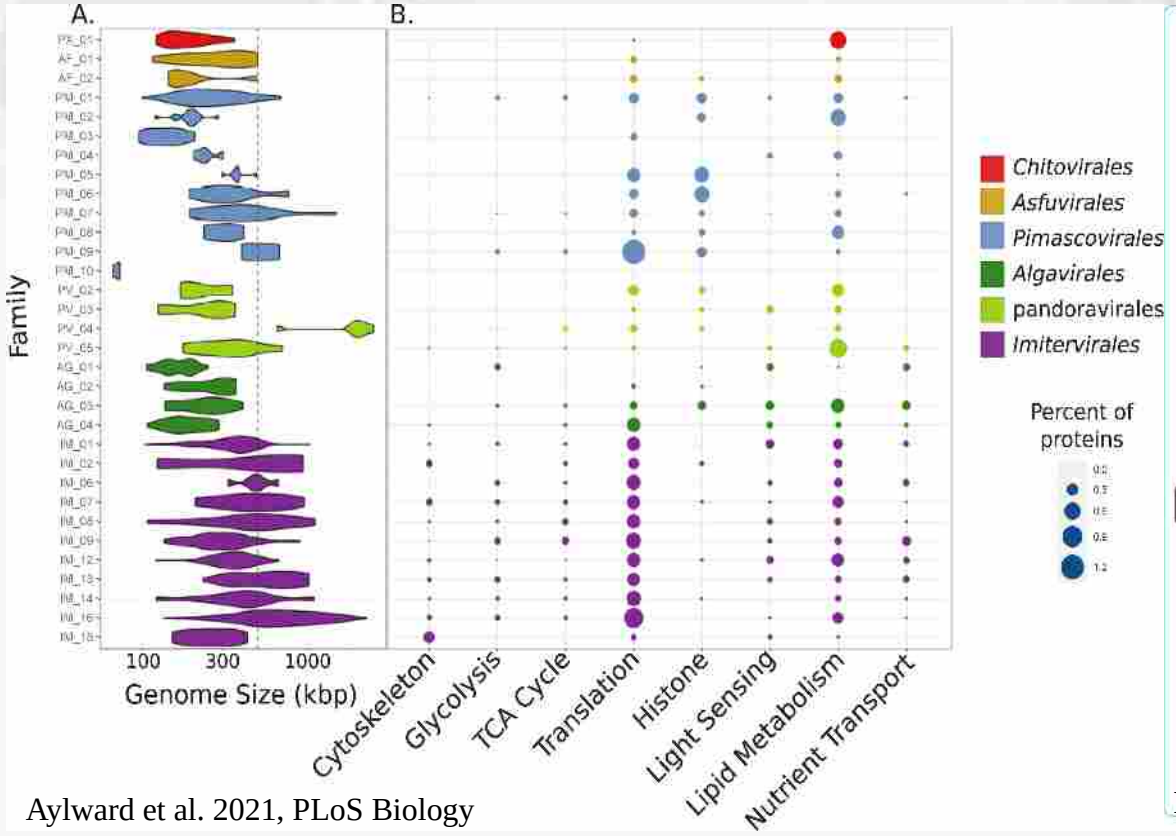


Endo et al. 2020, Nature ecology & evolution

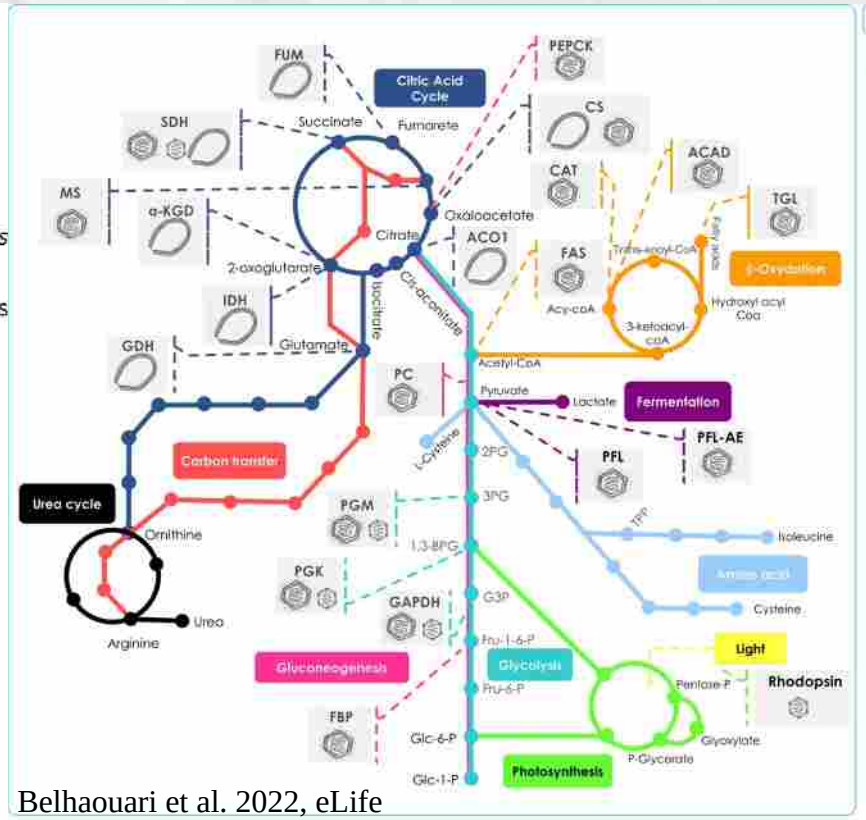
- **Numerous novel lineages await discovery to expand the current phylogenetic framework.**
- Giant viruses are ubiquitously found in diverse aquatic systems, from freshwater to ocean habitats.

Giant Viruses – metabolism

海洋科学与工程系
Department of Ocean Science and Engineering



Aylward et al. 2021, PLoS Biology

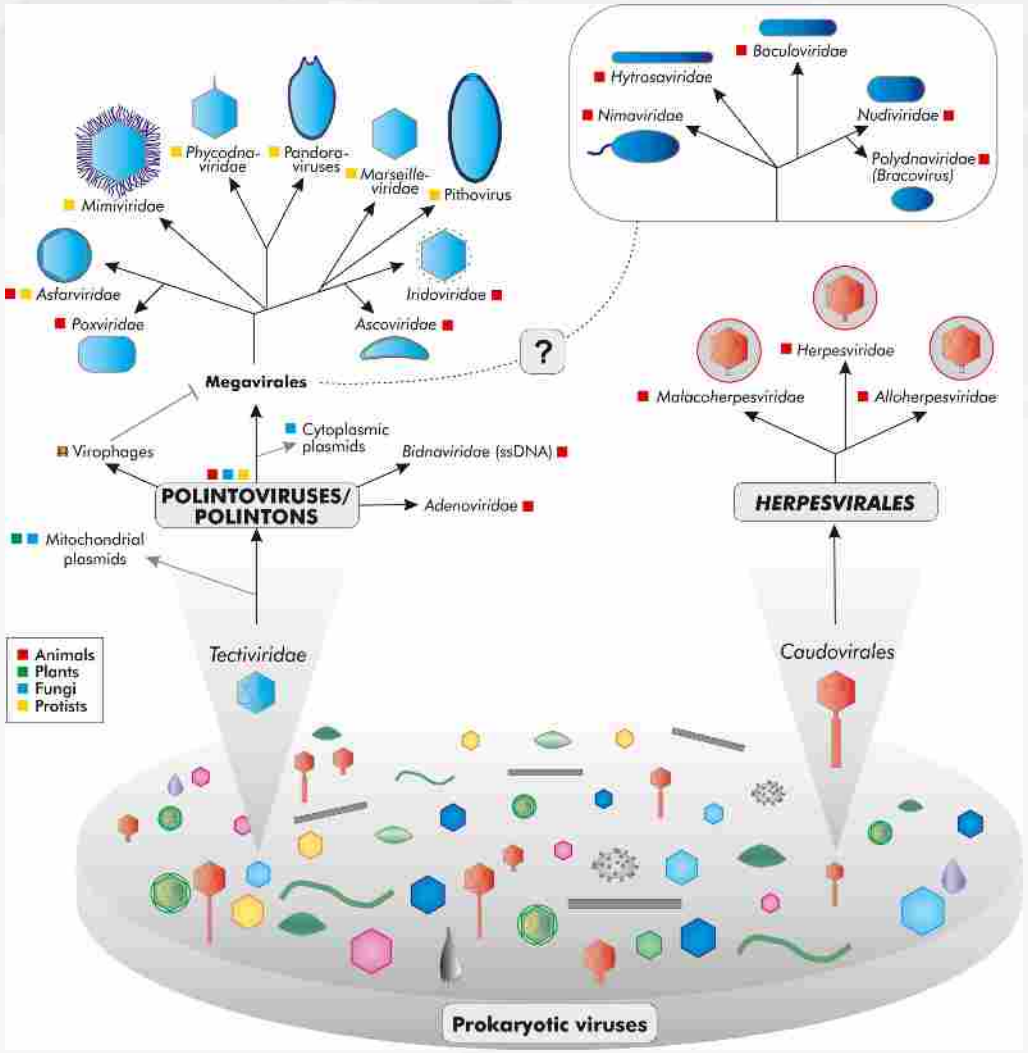


Belhaouari et al. 2022, eLife

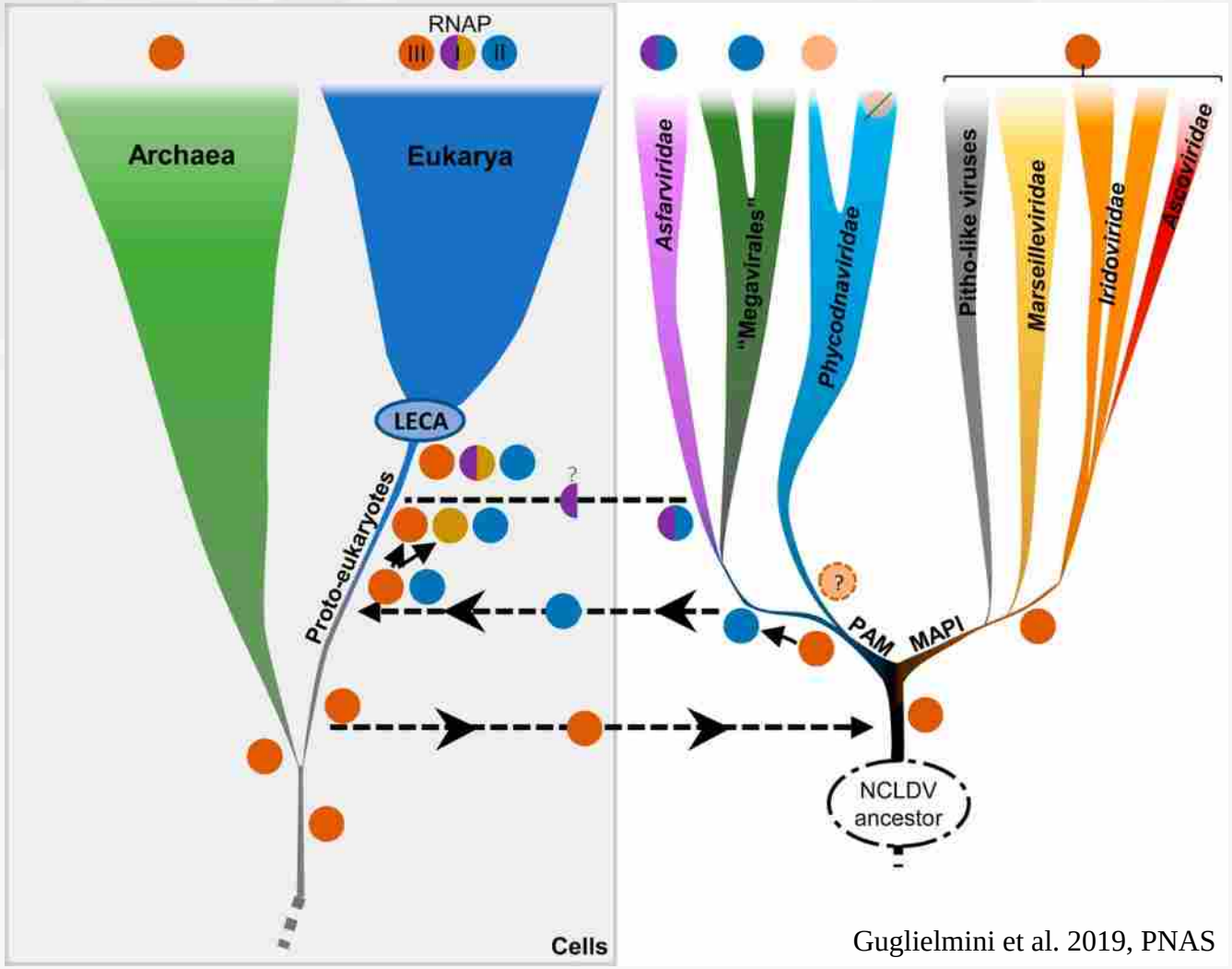
- Genome sizes are highly variable (i.e., pandoravirus);
- Most giant viruses encode components of the translation machinery and lipid metabolism;
- Imitevirales (mimivirus) encode diverse metabolic genes involved in energy metabolism and transport.

- Schematic figure showing the identified cellular enzymes and putative substrates that encoded in NCLDV genomes, represented by Mimiviridae, Phycodnaviridae, and Pandoraviridae;
- TCA cycle feeding into the Urea cycle; Carbon transfer; Gluconeogenesis; Glycolysis; Photosynthesis; Amino acid metabolism; Fermentation; and lipid β-oxidation.

Evolution of giant viruses

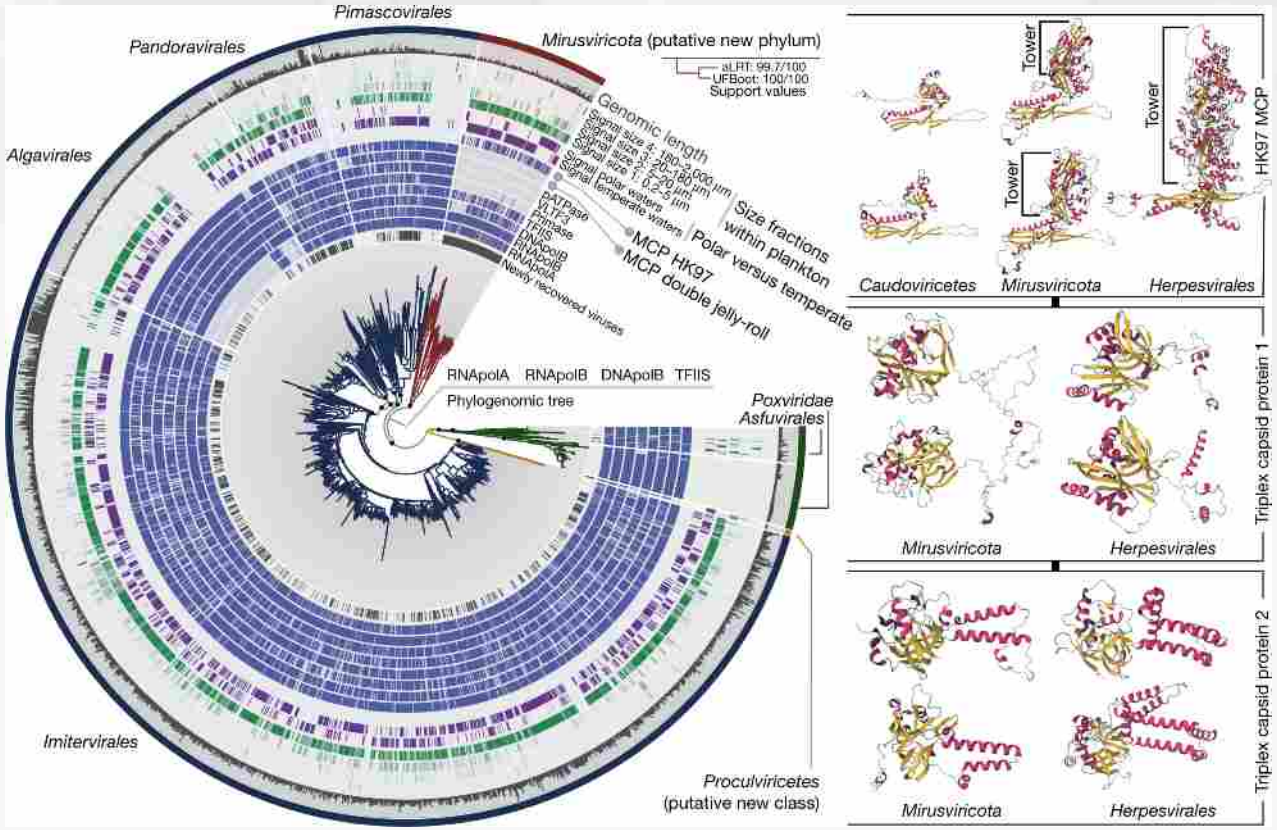


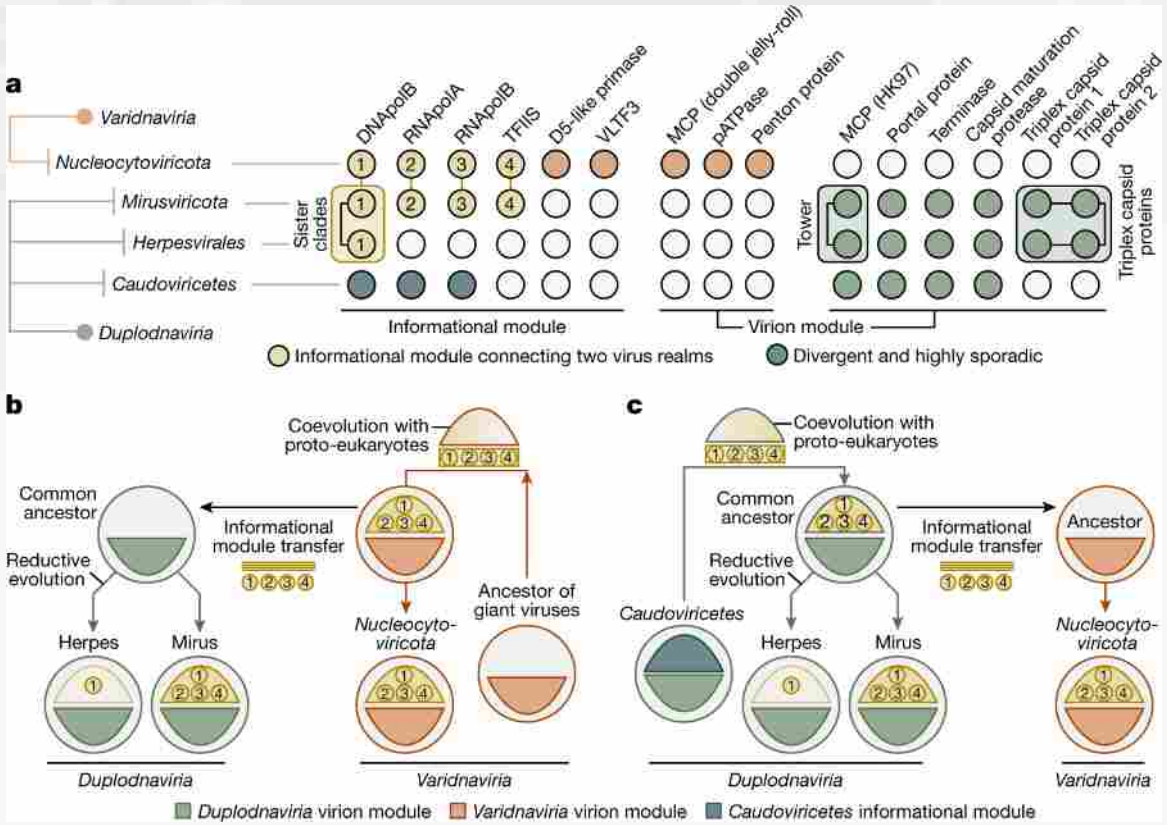
Koonin et al. 2015, The ultimate modularity



Guglielmini et al. 2019, PNAS

- **Giant viruses originated from smaller DNA viruses-Tectiviridae and predate the last eukaryotic common ancestor, representing an ancient viral lineage.**

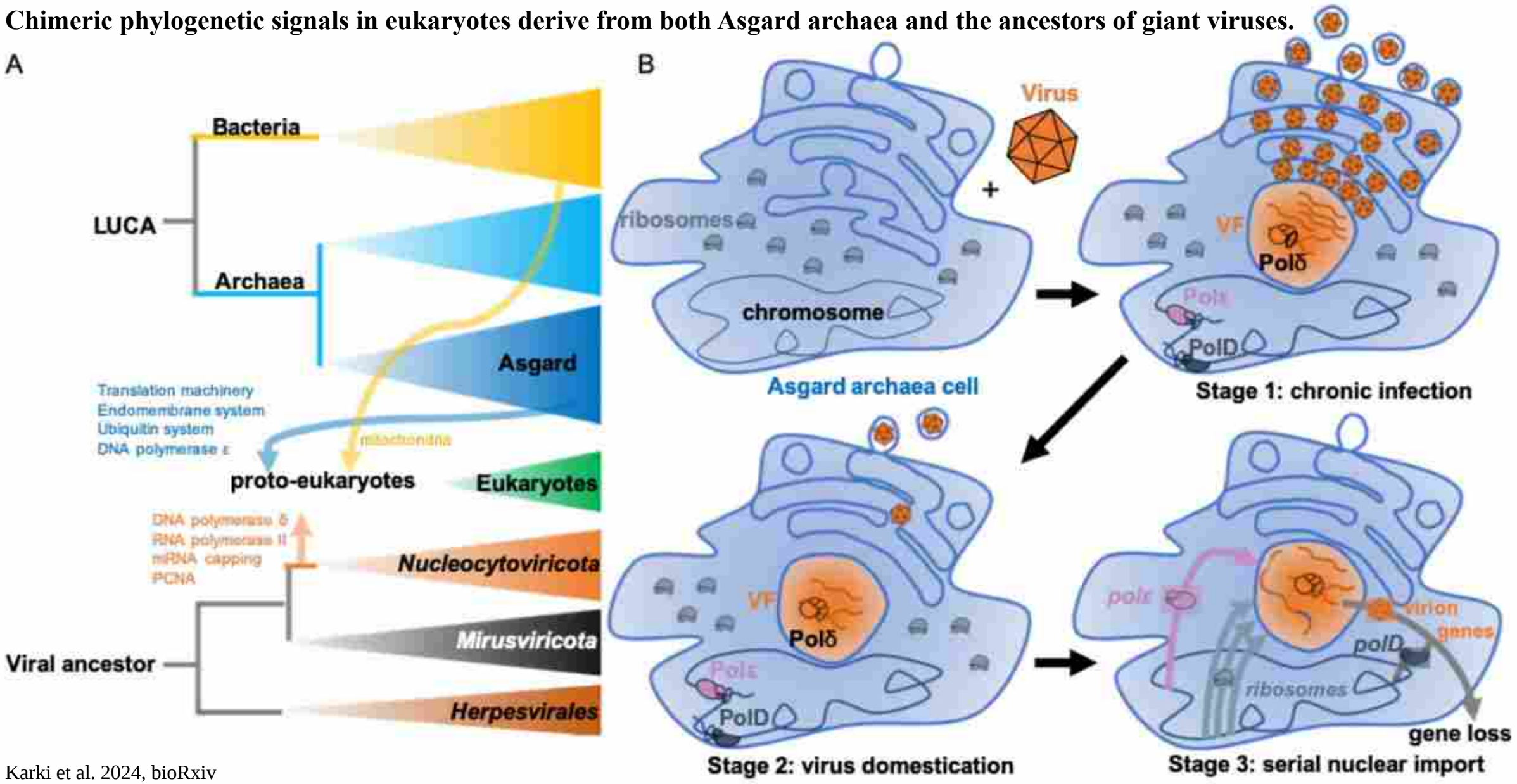




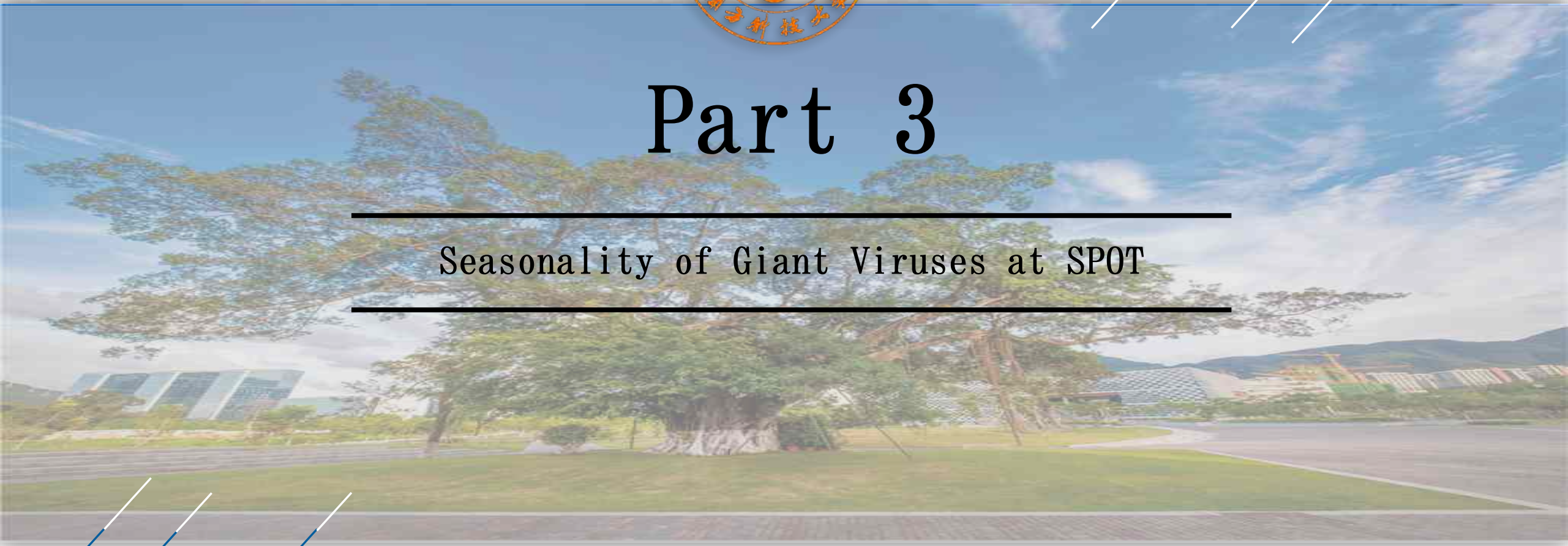
- The newly established phylum **Mirusviricota** exhibits a chimeric evolutionary architecture: its structural modules (e.g., the HK97-fold MCP) show homology with Herpesvirales (within Duplodnaviria), while its informational and functional modules align more closely with NCLDVs (Varidnaviria), bridging a critical gap between these two viral domains.
- **Only over 100 mirusvirus MAGs have been obtained to date, with no isolated viruses yet cultivated, and their eukaryotic host range remains largely unknown.**

Gaïa et al. 2023, Nature

Chimeric phylogenetic signals in eukaryotes derive from both Asgard archaea and the ancestors of giant viruses.



Karki et al. 2024, bioRxiv



Part 3

Seasonality of Giant Viruses at SPOT

1) STATION LOCATION and DEPTHS - monthly samples

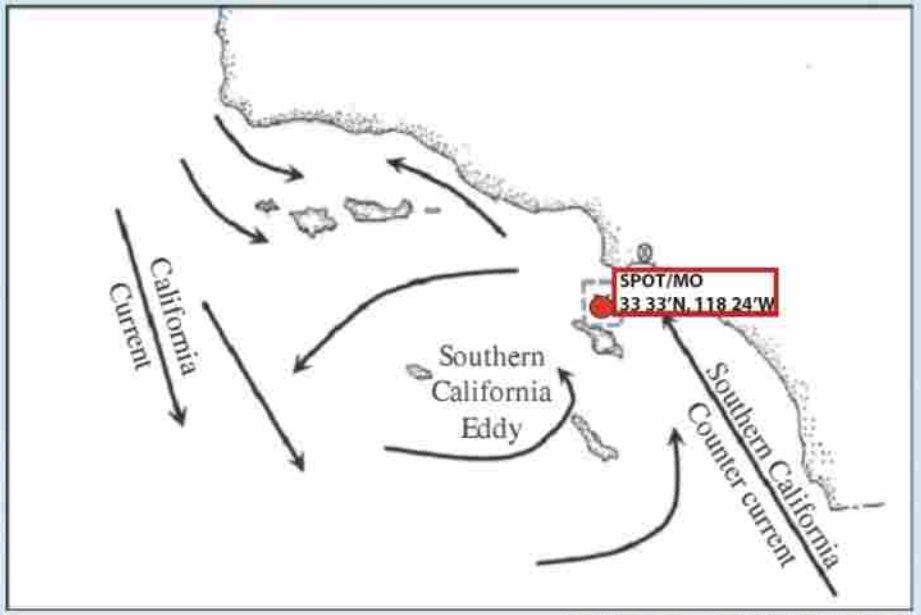
1998 - current : SPOT program (physical, chemical): 0, 10, 20, 30, 40, 50, 60, 100, 250, 500, 750, 885 m

2000 - current : MO program (diversity, biomass, rates): 5 m, DCM, 150 m, 500 m, 885 m





Coastal temperate system
Oligotrophic to mesotrophic
San Pedro Basin (sill depth ~740 m)
Maximum depth ~890 m



Collins et al. (2011), *DSR-I*

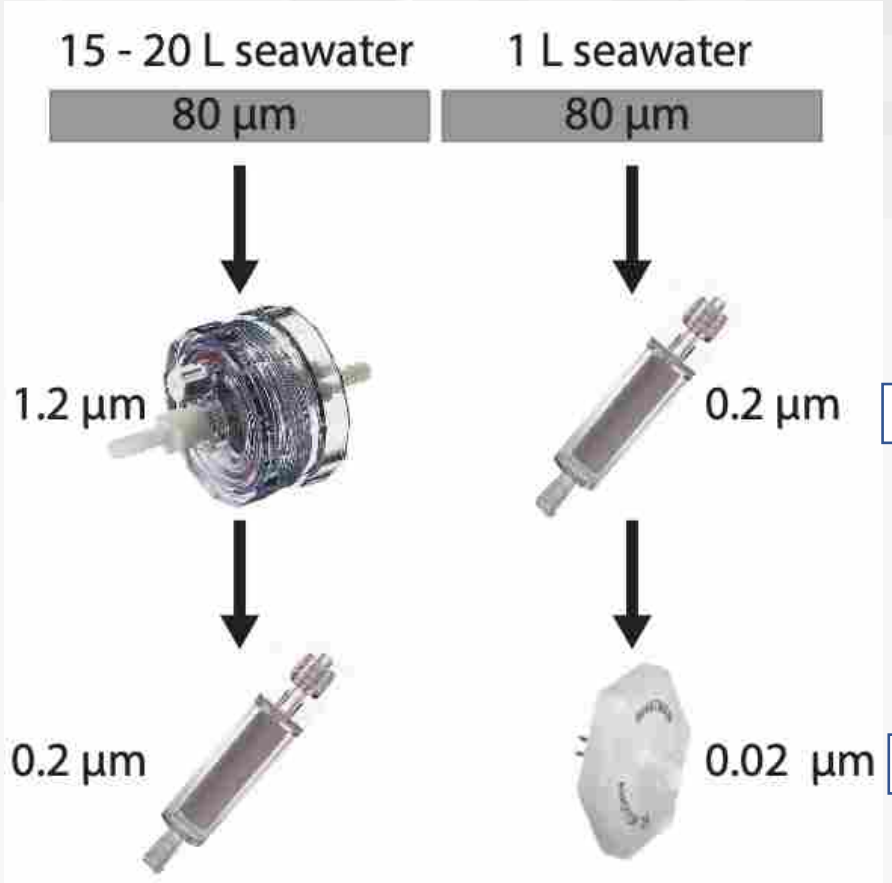
San Pedro Ocean Time-series (SPOT)

>20 years of monthly microbial

Presented by Diane Kim at the International Ocean Carbon Coordination Project (IOCCP) and the Ocean Carbon and Biochemistry Program (OCB) International Time-series Workshop (http://www.whoi.edu/website/TS-workshop/home), November 28-30, 2012

SPOT Microbial Observation

海洋科学与工程系
Department of Ocean Science and Engineering



AE filter, 1.2 - 80 µm：
Eukaryotes + particle-associated prokaryotes

Sterivex, 0.2 - 80 µm：
Prokaryotes and eukaryotes

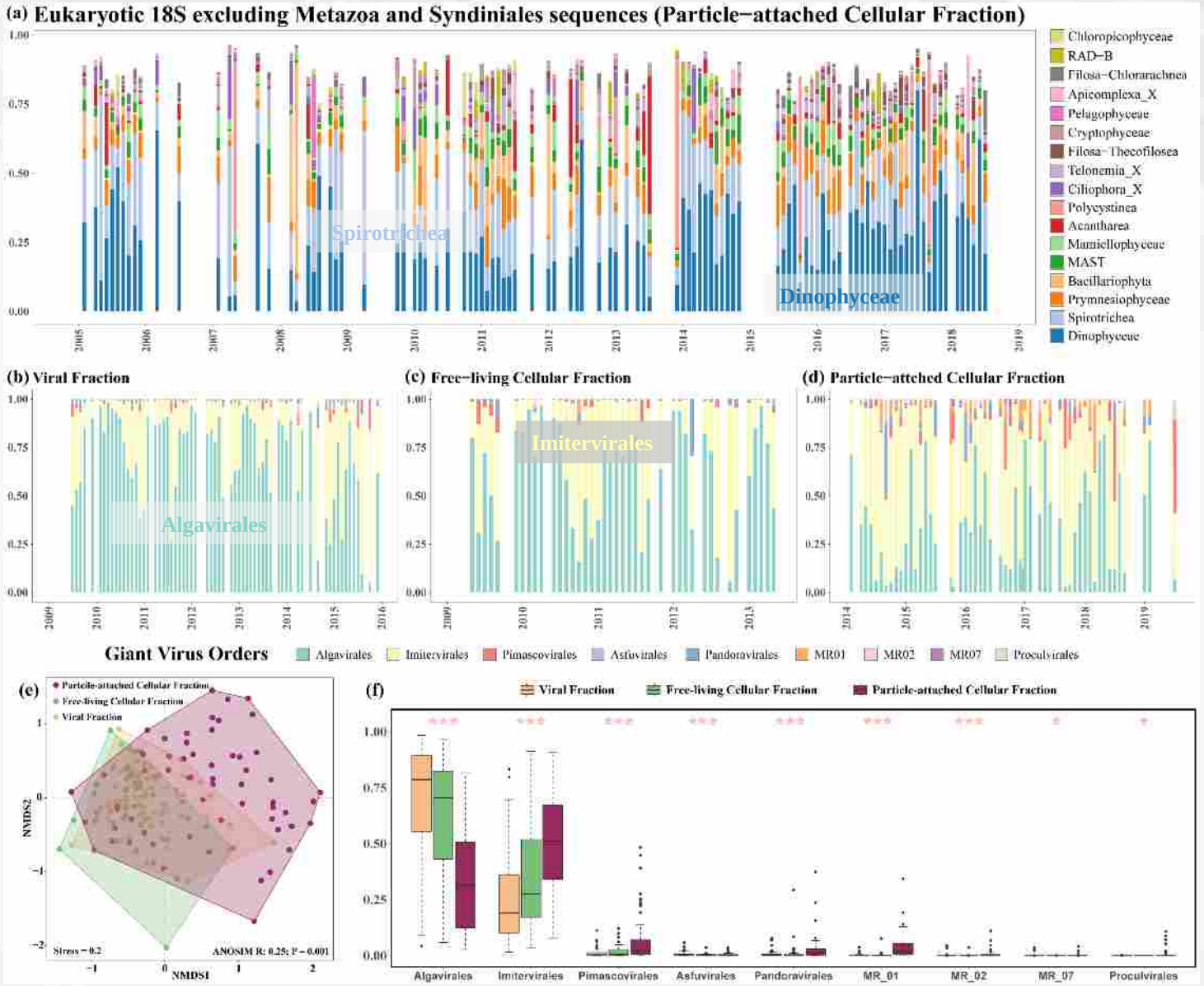
Sterivex, 0.2 - 1.2 µm：
Free-living prokaryotes and picoeukaryotes

Anotop, 0.02 - 0.2 µm：
Viruses and lysed cell fragments

- **Metagenomes**:
  - 2009 – 2013: 0.22 - 1 µm size fraction surface (5m)
  - 2014 – 2019: 1 - 80 µm size fraction surface (5m)
- **Viromes:**
  - 2009 – 2016: 0.02 – 0.2 µm size fraction surface (5m)
- **V4-V5 Amplicons:**
  - 2005 – 2019: monthly, variable depths and size fractions

Eukaryotic and giant virus communities

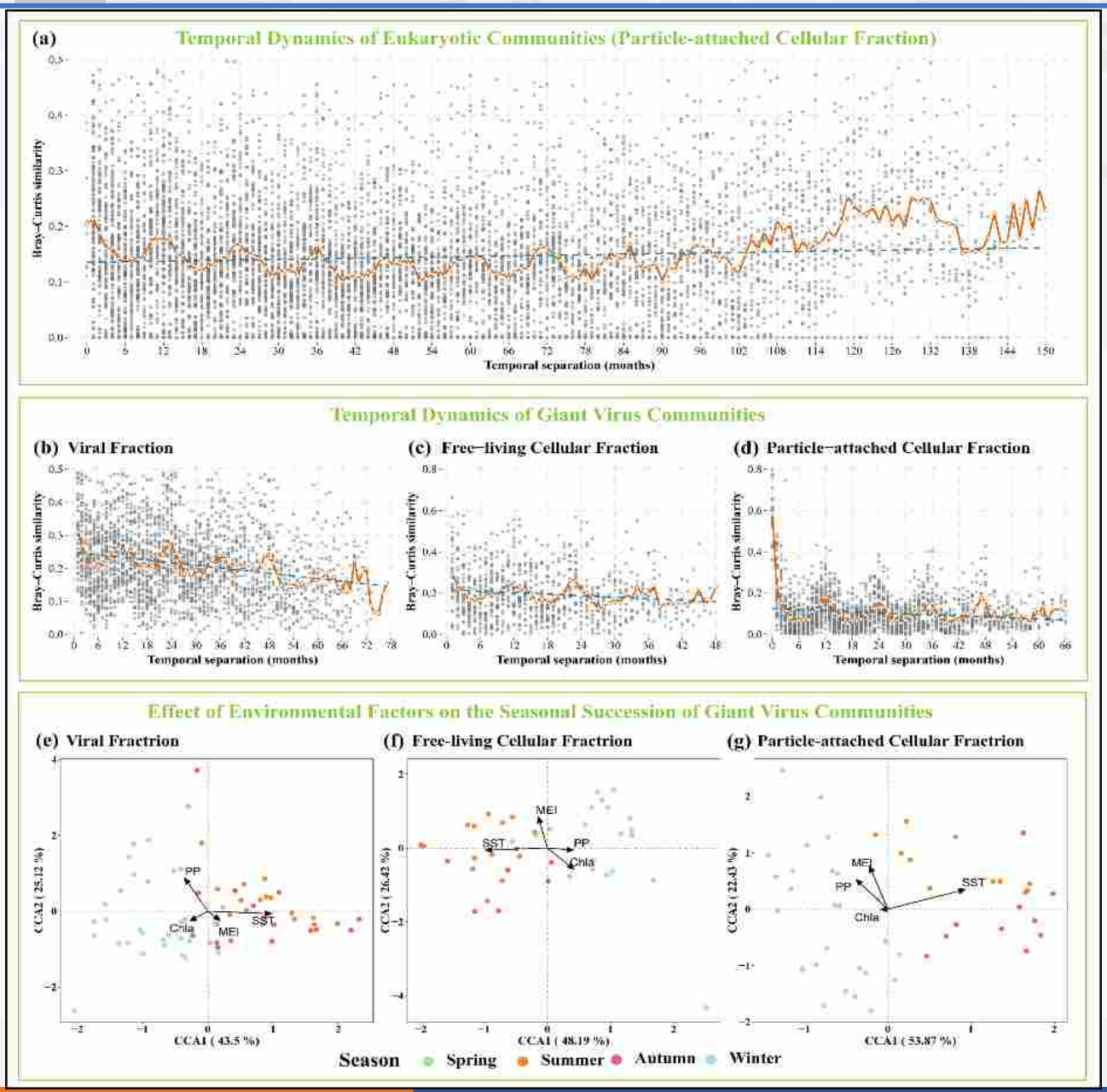
海洋科学与工程系
Department of Ocean Science and Engineering



- Dinophyceae (including photosynthetic and heterotrophic dinoflagellates; 28.5%), Spirotrichea (18.5%), Prymnesiophyceae, and Bacillariophyta are the main eukaryotic groups.
- **Dinophyceae peak in summer**, while **Spirotrichea peak during the spring algal bloom**.
- Algavirales and Imitervirales are the domainat giant viruses. The relative abundance of **Algavirales peaked in spring and winter**, while **Imitervirales reached its peak during summer and autumn**.
- **With increasing particle size**, the relative abundance of **Algavirales gradually decreased**, whereas that of **Imitervirales increased**.
- The particle-attached cellular fraction exhibited a significant increase in giant virus diversity. Notably, Pimascovirales showed a marked rise in relative abundance during July and October. Additionally, the relative abundance of MR_01 increased significantly in spring.

Feng et al., unpublished

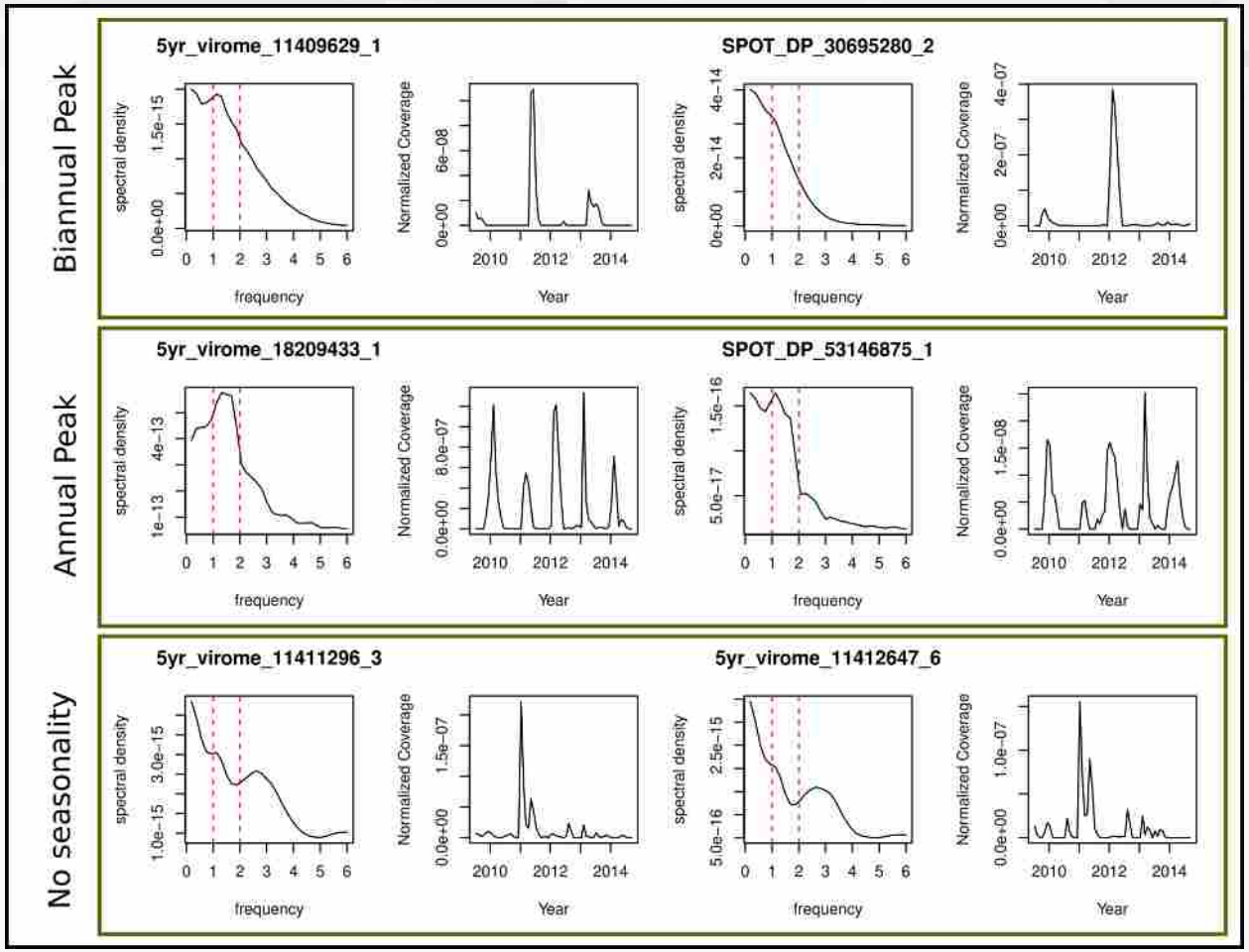
Temperature drives the seasonality of giant viruses

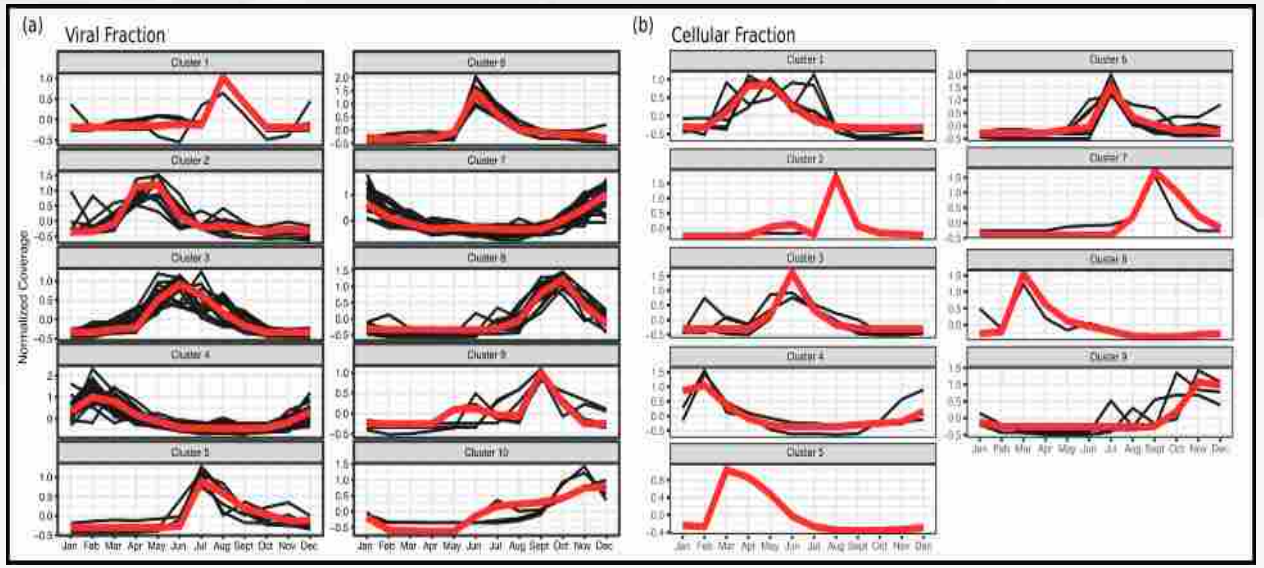


- Overall, the SPOT **18S eukaryotic community** exhibits a **seasonal variation** with one peak community similarity (Bray-Curtis) at 1-year intervals;
- Likewise, the **giant virus community** exhibited **seasonally-predictable changes** in composition, with peak community similarity occurring at 1-year intervals;
- From the CCA analysis results, it is evident that the seasonal dynamics of giant viruses can be explained by seasonally recurring patterns, such as **temperature** fluctuations (dominant environmental driver) and spring phytoplankton blooms.

Feng et al., unpublished

Seasonality of SPOT giant viruses





- The NCLDVs with an annual seasonality showed abundance patterns peaked in different months;
- 10 clusters in the viral fraction and 9 clusters in the cellular metagenomic fraction were found.

- Different NCLDV phylotypes showed seasonal variations with bi-annual and annual peaks, respectively;
- Non-seasonal giant viruses showed stochastic variations.

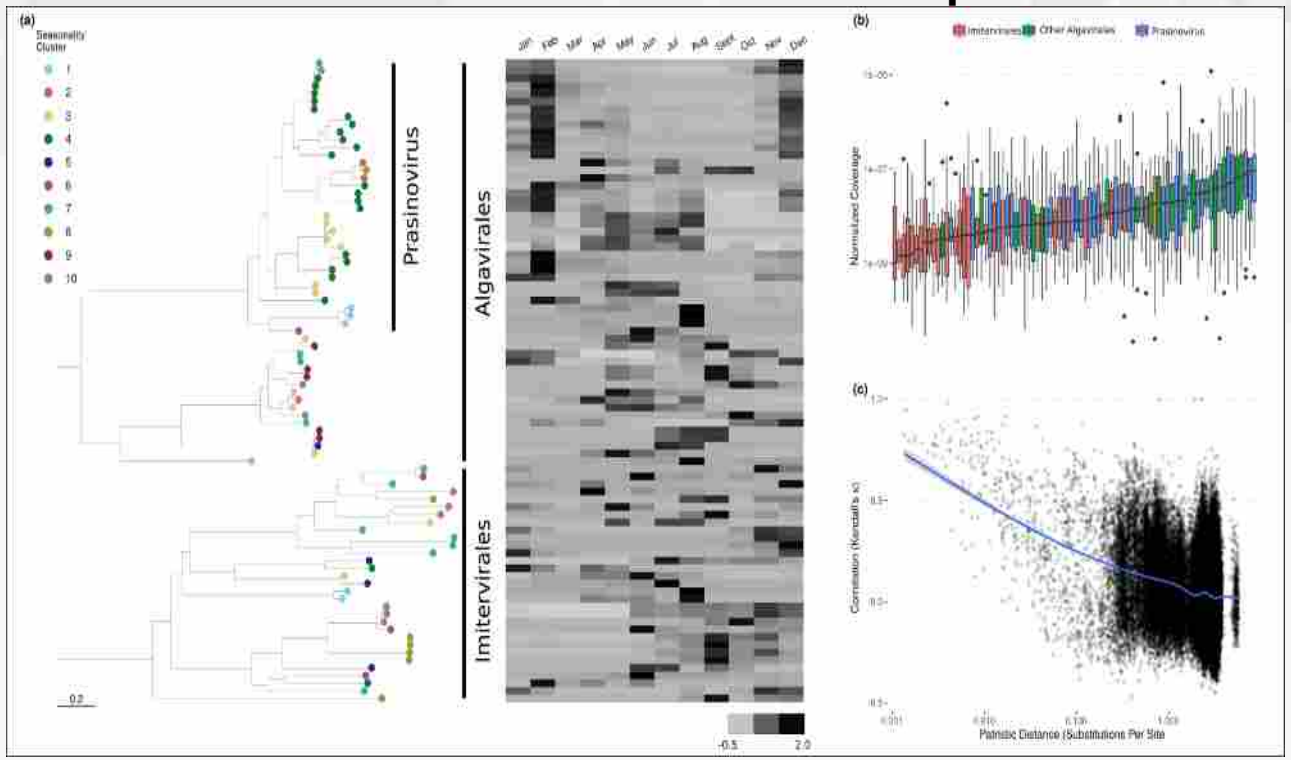
Laperriere SM#, Minch B#, Weissman JL, **Hou S***, Yeh YC, Ignacio-Espinoza JC, Ahlgren NA, Moniruzzaman M*, Fuhrman JA*. Phylogenetic proximity drives temporal succession of marine giant viruses in a five-year metagenomic time-series. [2024 preprint version on bioRxiv]

Laperriere et al., under review

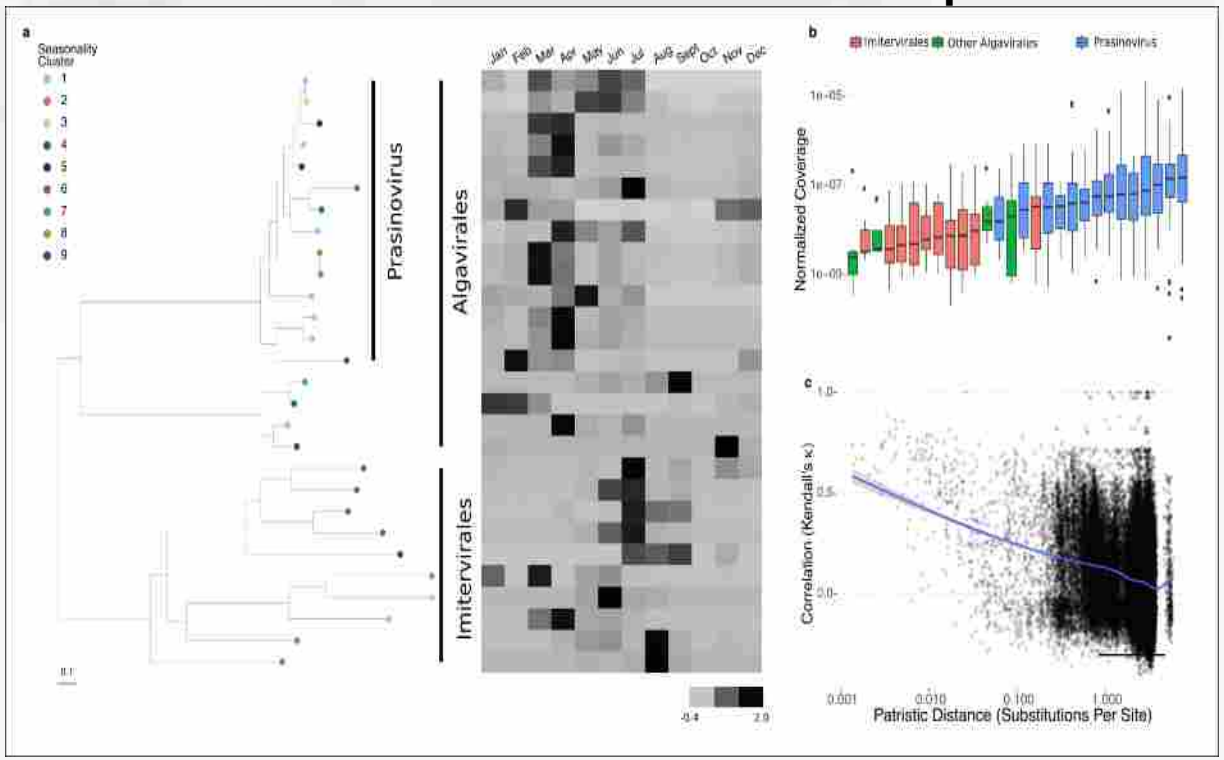
Phylogenetic proximity also drives temporal succession

海洋科学与工程系
Department of Ocean Science and Engineering

viral size fraction : 0.02 - 0.2 μm



cellular size fraction : 0.2 - 1.2 μm



- Giant viruses with closer phylogenetic distances have consistent seasonal succession patterns;
- When the seasonality patterns of closely related viruses are inconsistent, it is usually coupled with a switch in the host being infected;
- Algavirales mainly form clusters in spring and early winter, while Imitervirales mainly form clusters in late summer and autumn.

Laperriere SM*, Winch B*, Weissman JL, **Hou S**, Yeh YC, Ignacio-Espinoza JC, Ahlgren NA, Moniruzzaman M, Fuhrman JA. Phylogenetic proximity drives temporal succession of marine giant viruses in a five-year metagenomic time-series. [2024 preprint version on bioRxiv]

Laperriere et al., under review



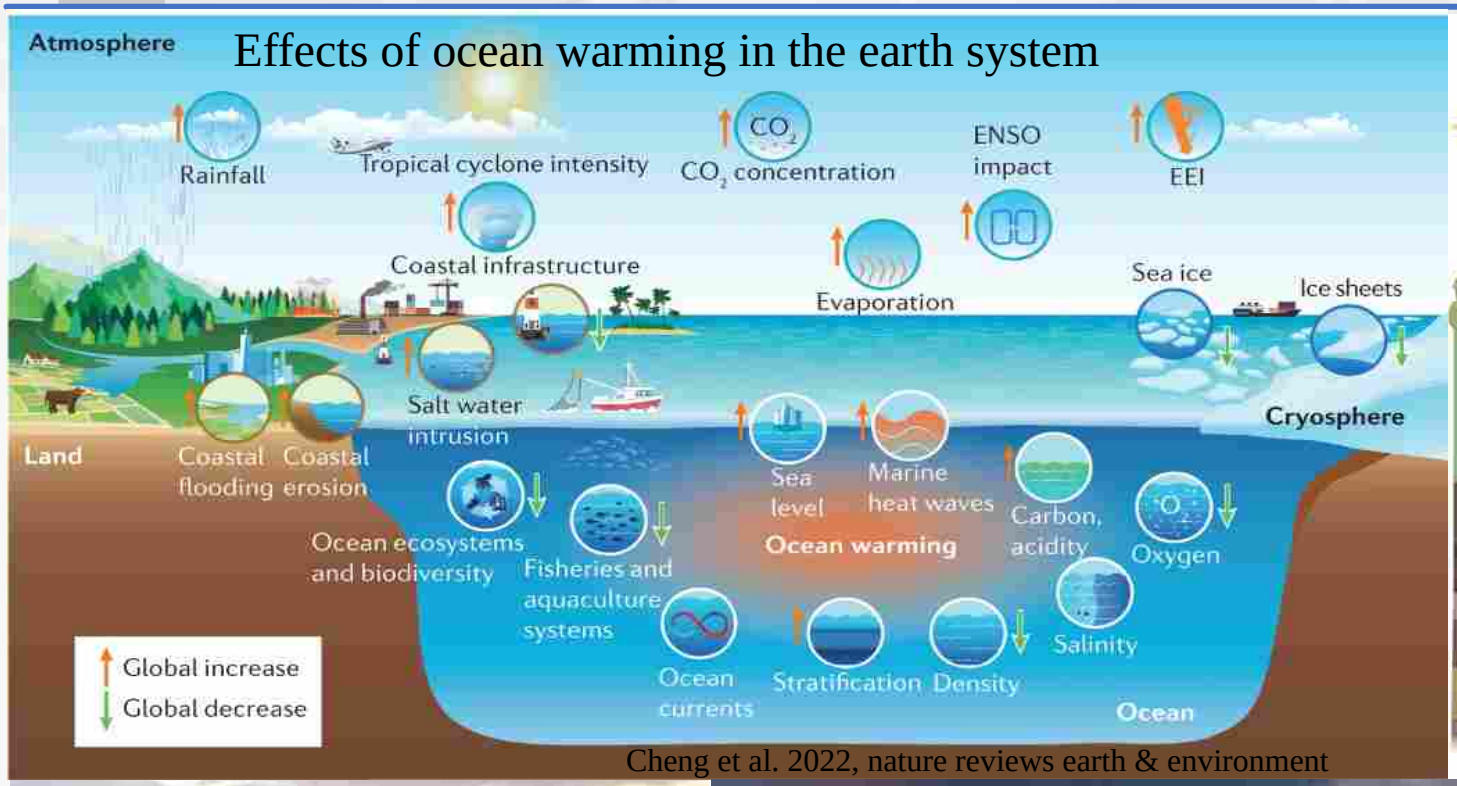
Part 4

Imitervirales under Global Warming

Ocean warming and giant viruses

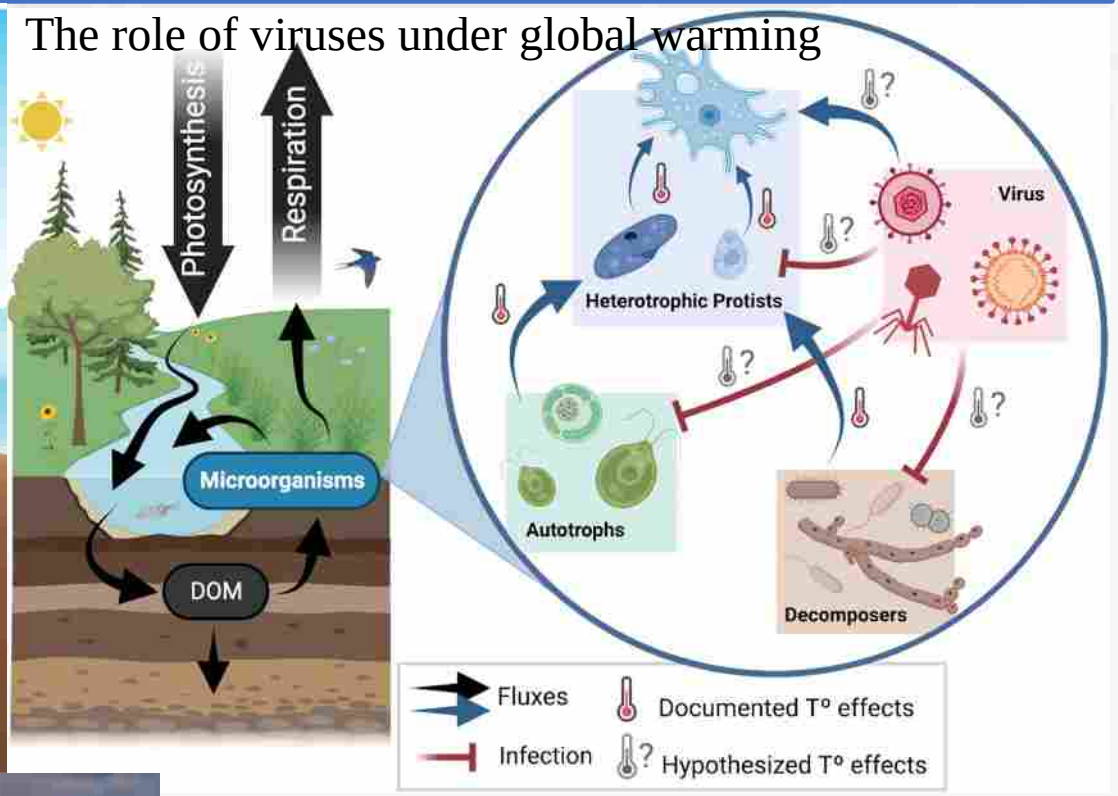
海洋科学与工程系
Department of Ocean Science and Engineering

Effects of ocean warming in the earth system



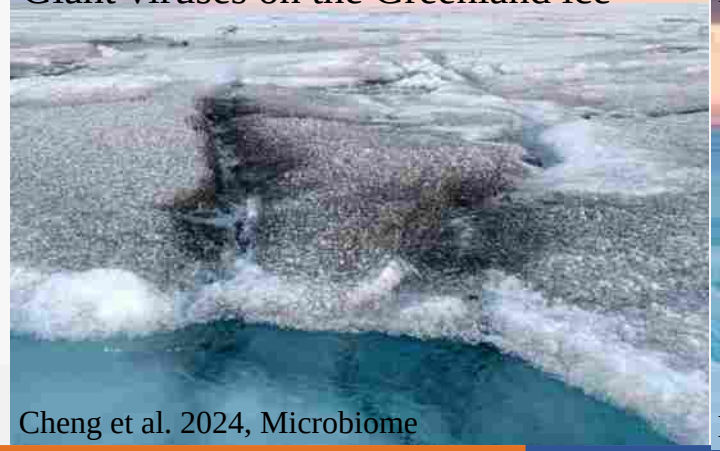
Cheng et al. 2022, nature reviews earth & environment

The role of viruses under global warming



Wieczynski et al. 2023, FEMS Microbiology Ecology

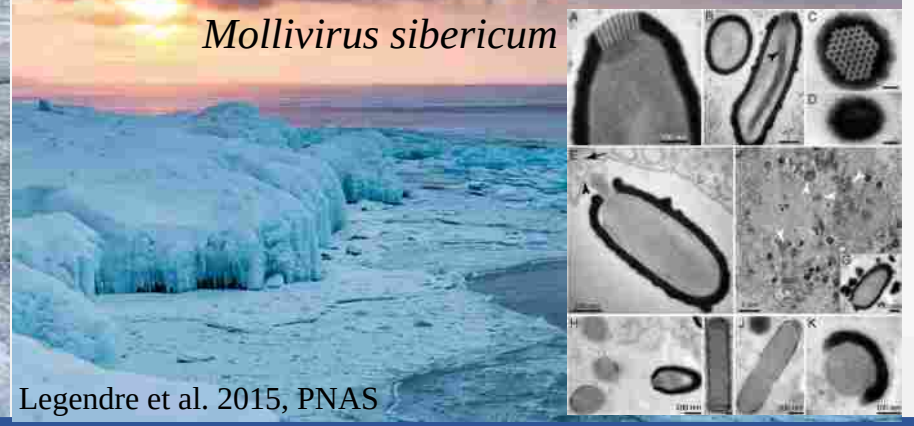
Giant viruses on the Greenland ice



Cheng et al. 2024, Microbiome

Thirty-thousand-year-old giant virus from permafrost

Mollivirus sibericum

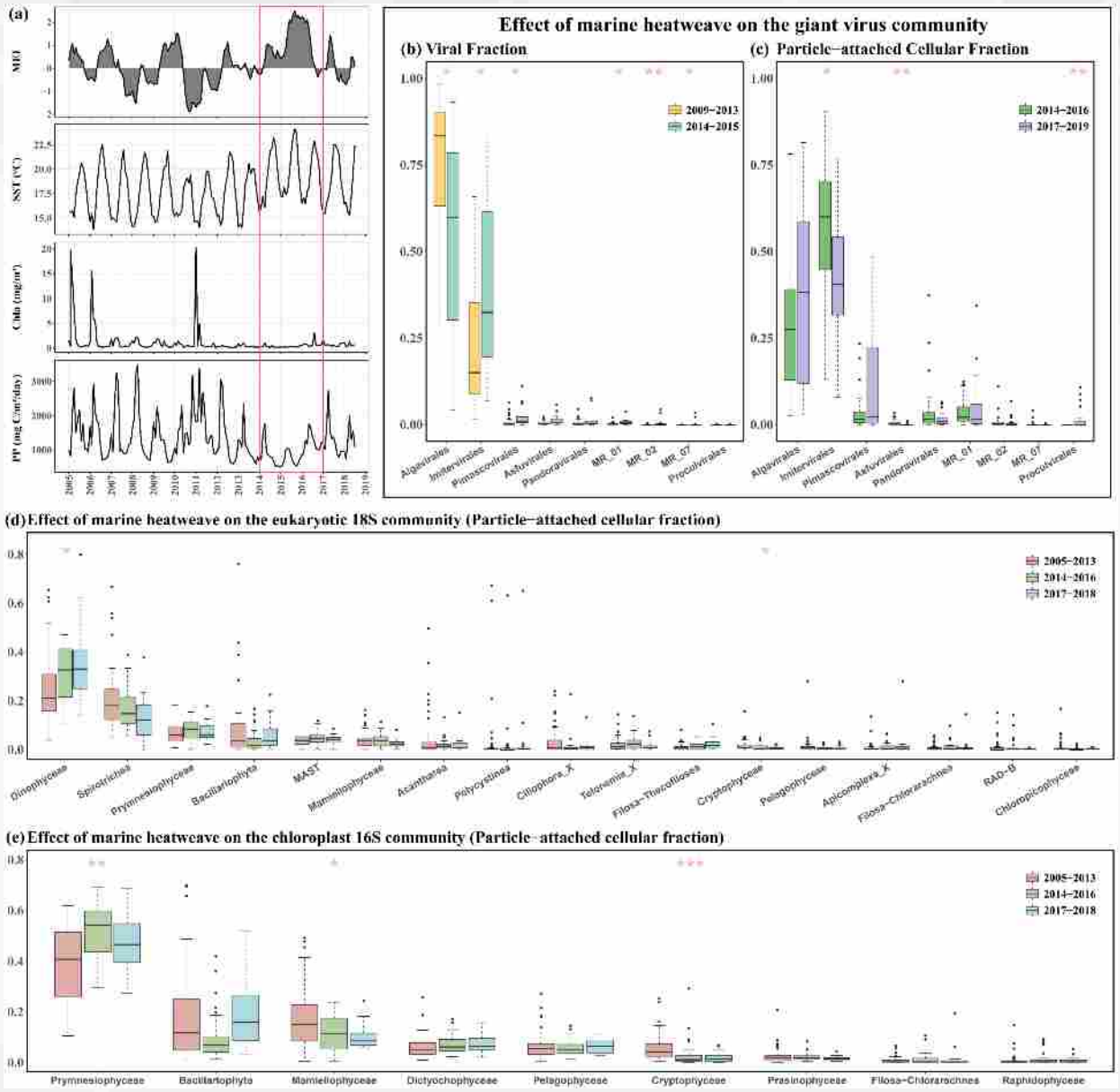


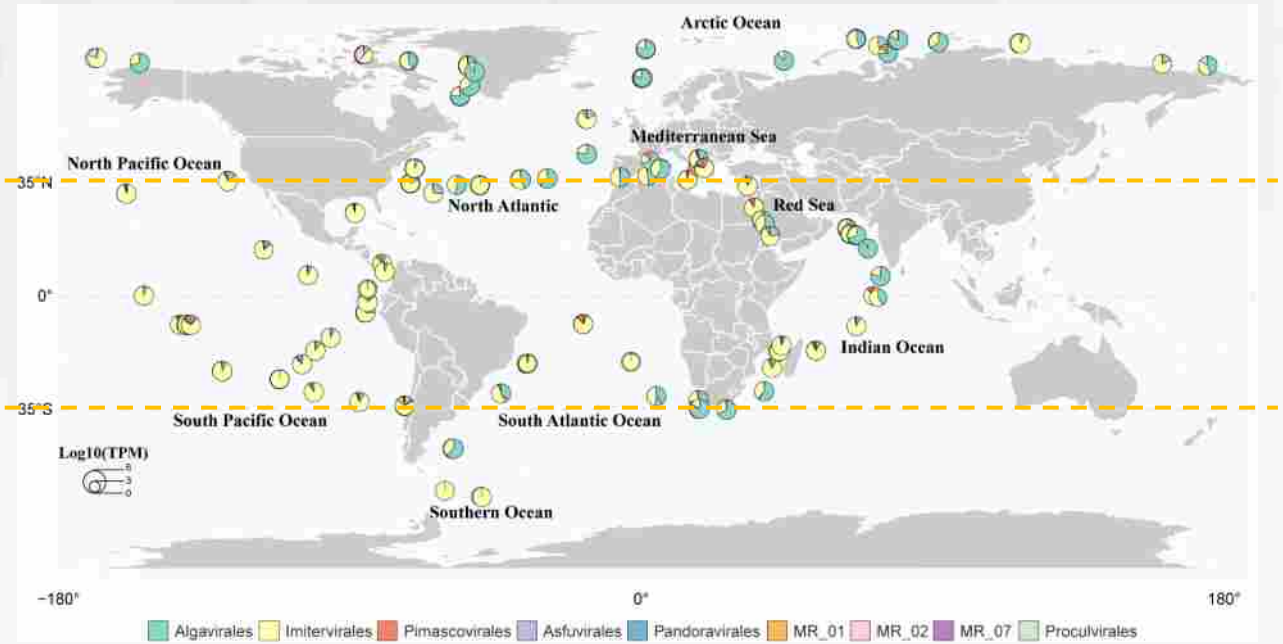
Legendre et al. 2015, PNAS

- Under global warming, how do giant virus communities evolve to regulate marine primary productivity?
- **Large-scale time-series metagenomic dataset may help answer this question.**

Effect of marine heatwave on the giant viruses

海洋科学与工程系
Department of Ocean Science and Engineering



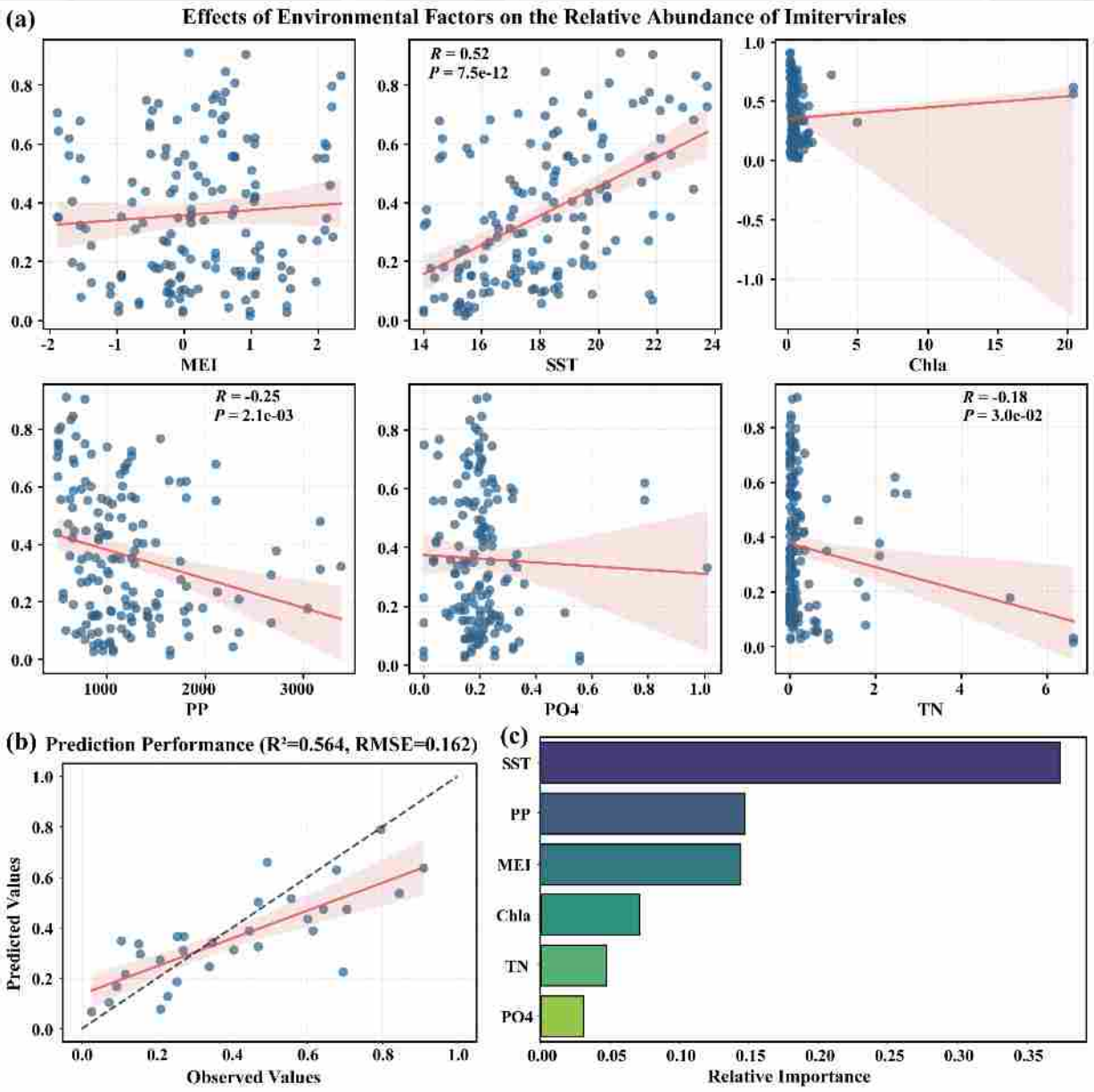


- The relative abundance of **Imitervirales increased significantly during marine heatwave** followed by rapid decline, while Algavirales exhibited an opposite pattern with significant decreases during heatwaves followed by subsequent recovery;
- **This trend persisted in the global ocean** with Imitervirales dominating in low-latitude (within 35°N-35°S) seas, while Algavirales prevailed in high-latitude or highly productive tropical ocean regions.

Feng et al., unpublished

Correlations between Imitervirales and environmental factors

海洋科学与工程系
Department of Ocean Science and Engineering

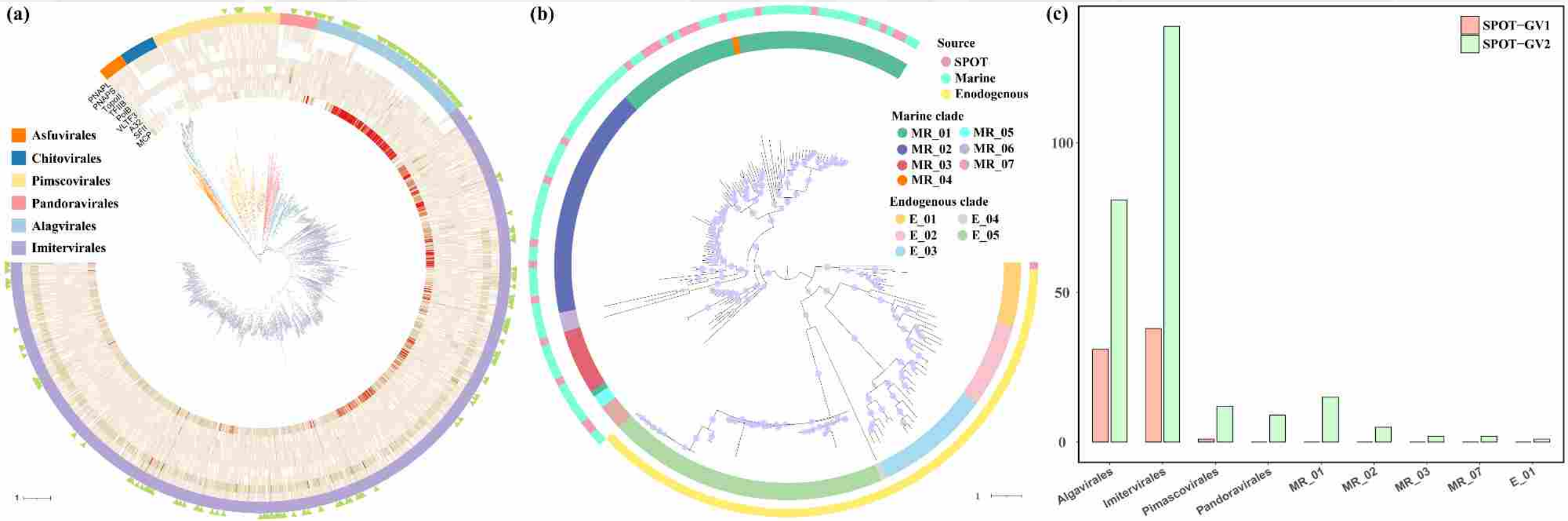


- The relative abundance of **Imitervirales** exhibited a significantly **positive linear correlation with temperature**, while showing negative correlations with both primary productivity and total nitrogen.
- We randomly split the dataset into an 8:2 ratio, allocating 80% as the training set and 20% as the test set, and employed a **gradient boosting regression model** (best model) to predict changes in Imitervirales abundance in response to environmental factors.
- The future distribution of Imitervirales will be predicted under global ocean warming by integrating projected marine environmental parameters spanning 2025-2100 from CMIP6 into our models.

Feng et al., unpublished

Phylogenetic tree of giant viruses and mirusviruses

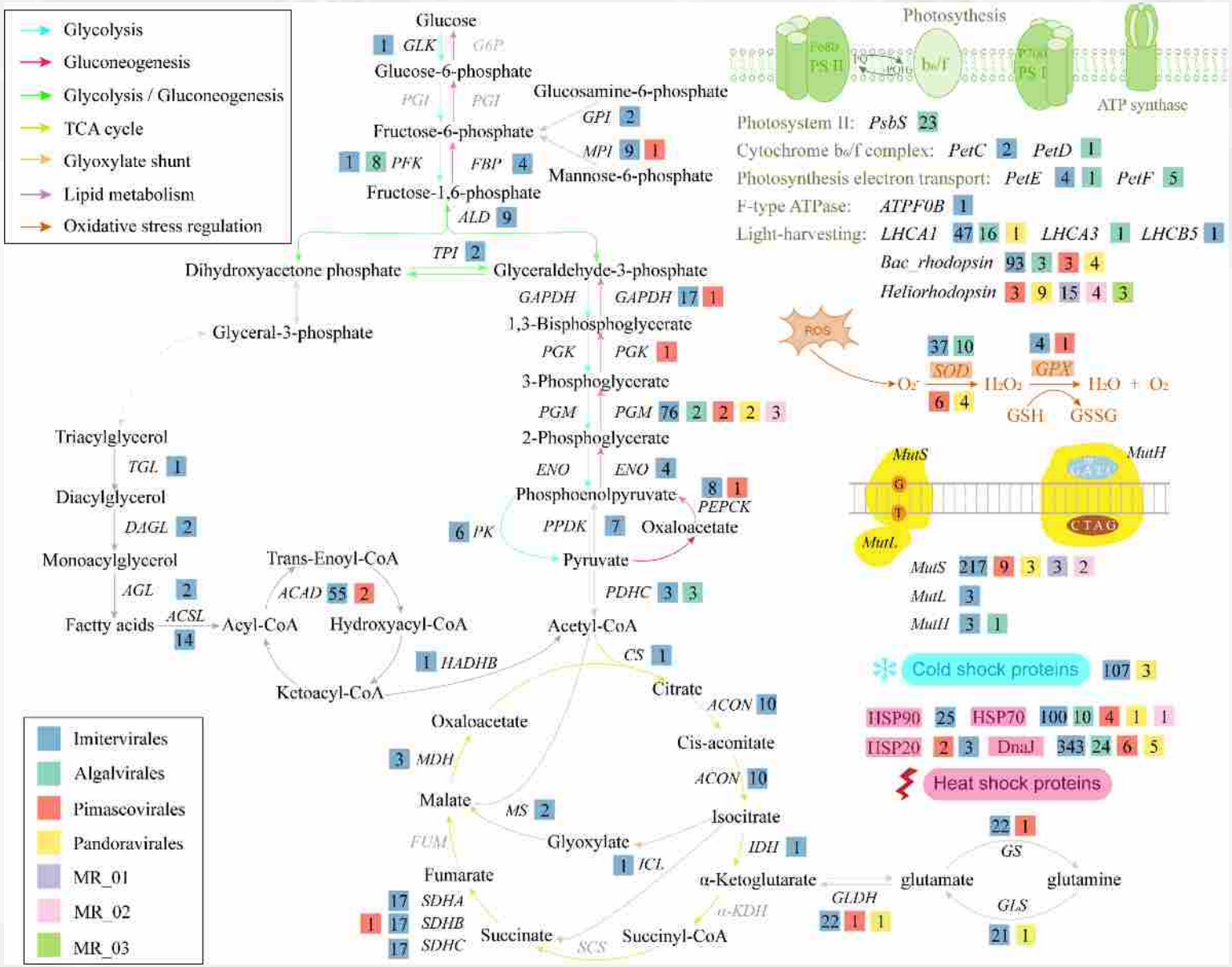
海洋科学与工程系
Department of Ocean Science and Engineering



- We recovered 241 high-quality, non-redundant giant virus metagenome-assembled genomes (MAGs), representing four taxonomic orders: Imitervirales (n=139), Algavirales (n=81), Pimascovirales (n=12), and Pandoravirales (n=9);
- Additionally, we recovered 25 non-redundant Mirusvirus MAGs, spanning the MR_01 (15), MR_02 (5), MR_03 (2), MR_07 (2), and E_01 (1) lineages, consistent with the findings from the TARA Oceans Mirusvirus study, where MR_01 was the most abundant and MR_07 the least.
- The known diversity of SPOT giant viruses at SPOT was increased by over threefold.

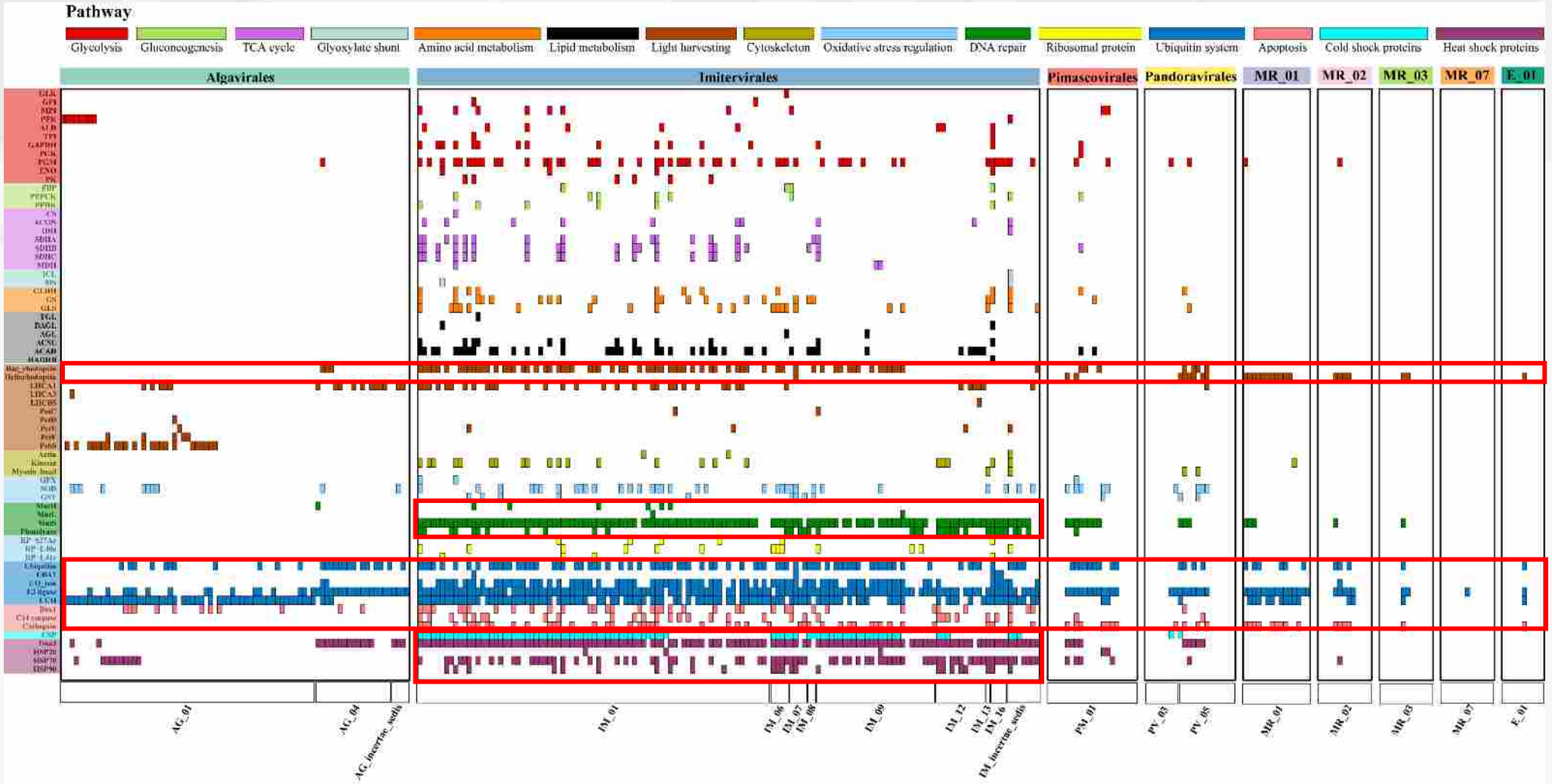
Feng et al., unpublished

Imitervirales show adaptation to warming



- SPOT giant viruses (primarily Imitervirales) **encode all enzymes of glycolysis except for glucose-6-phosphate isomerase**. However, they compensate for the production of fructose-6-phosphate by utilizing mannose-6-phosphate isomerase and glucosamine-6-phosphate isomerase;
- They could produce energy through the glyoxylate cycle, **bypassing the decarboxylation step of isocitrate to α-ketoglutarate and subsequently to succinate** in the TCA cycle, suggesting adaptation to oligotrophic environments.
- Imitervirales and Algavirales are two major orders of giant viruses that primarily infect algae. They encode a variety of photosynthesis-related proteins. Algavirales encodes photosystem proteins, among which PsbS is exclusively found in Prasinviridae (AG_01). In contrast, Imitervirales encodes **multiple light-harvesting-related proteins**.
- **Imitervirales** encode multiple **DNA mistake repair, heat shock proteins, and ROS-scavenging enzymes**, conferring enhanced adaptability to global ocean warming.

Auxiliary metabolic genes

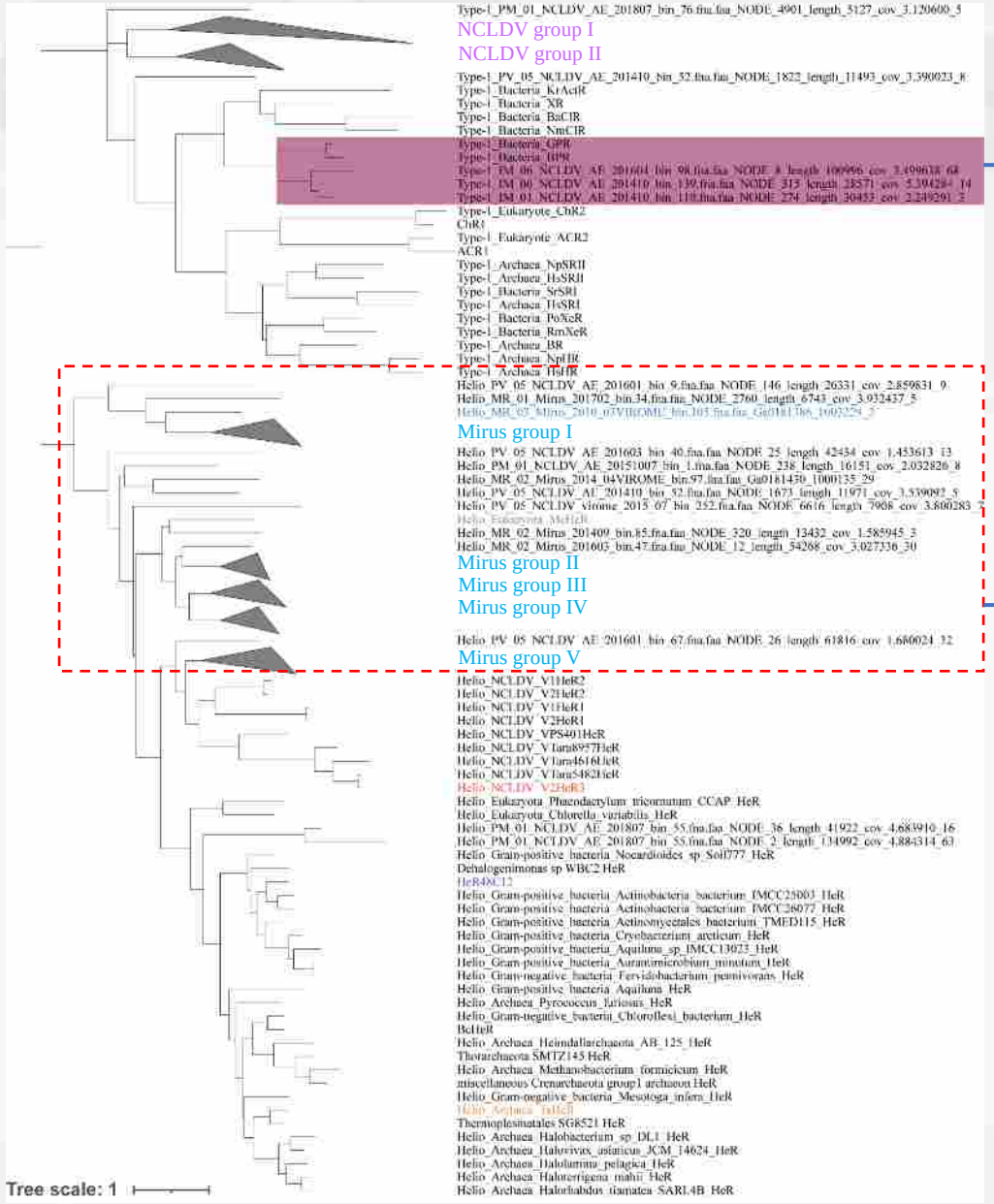


- SPOT NCLDVs and mirusviruses ubiquitously encode genes involved in the ubiquitin system, deubiquitination, and apoptosis processes;
- Bac_rhodopsin is ubiquitous in Imitervirales infecting photosynthetic hosts, whereas heliorhodopsin is prevalent in mirusviruses and NCLDVs primarily infecting non-photosynthetic hosts.

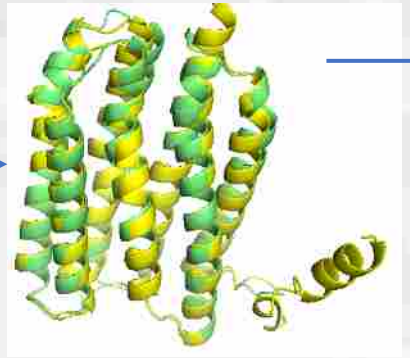
Feng et al., unpublished

Functional diversity of rhodopsins

海洋科学与工程系
Department of Ocean Science and Engineering

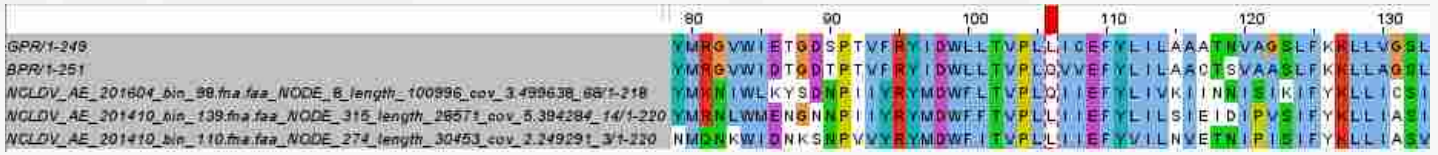


PR

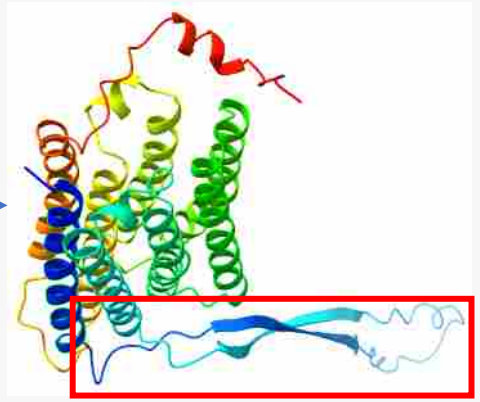


RMSD = 0.919 (1082 to 1082 atoms) < 1A°

106L: Green light-absorbing Proteorhodopsin
106Q: Blue light-absorbing Proteorhodopsin



Three rhodopsins from giant viruses share identical structures with GPR and BPR.

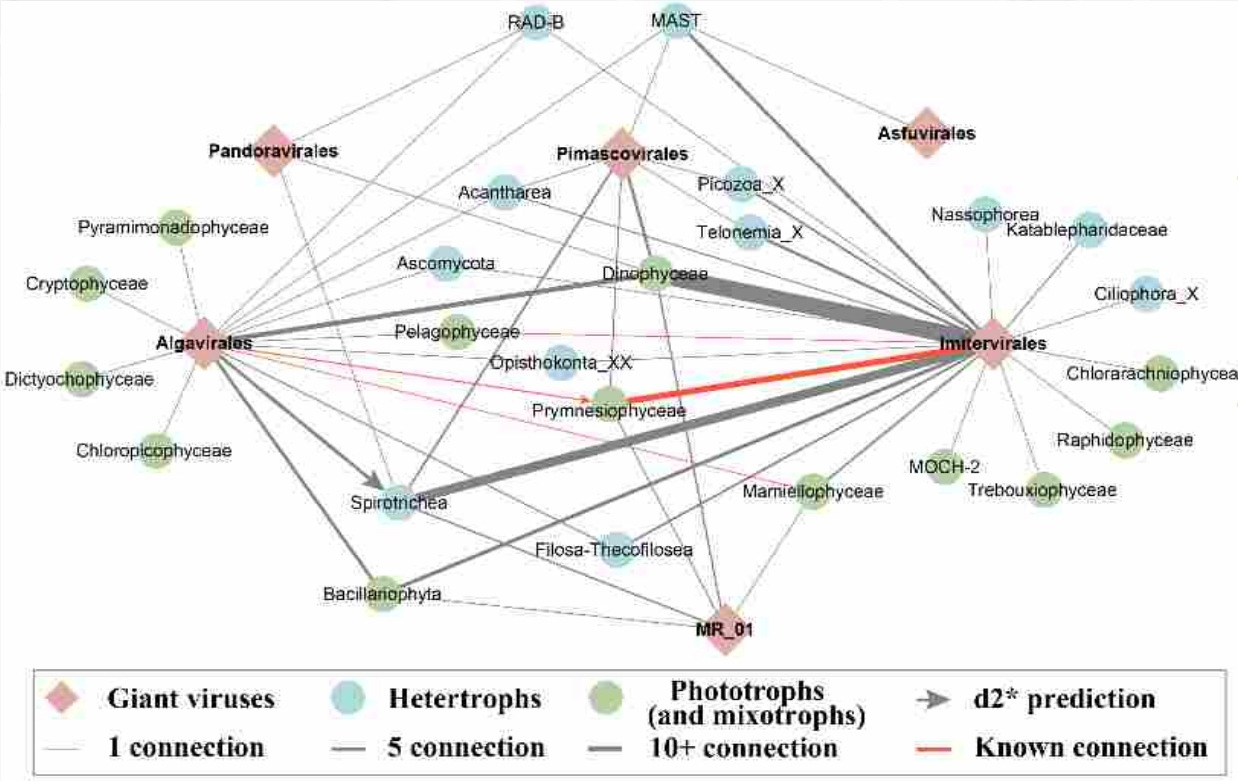


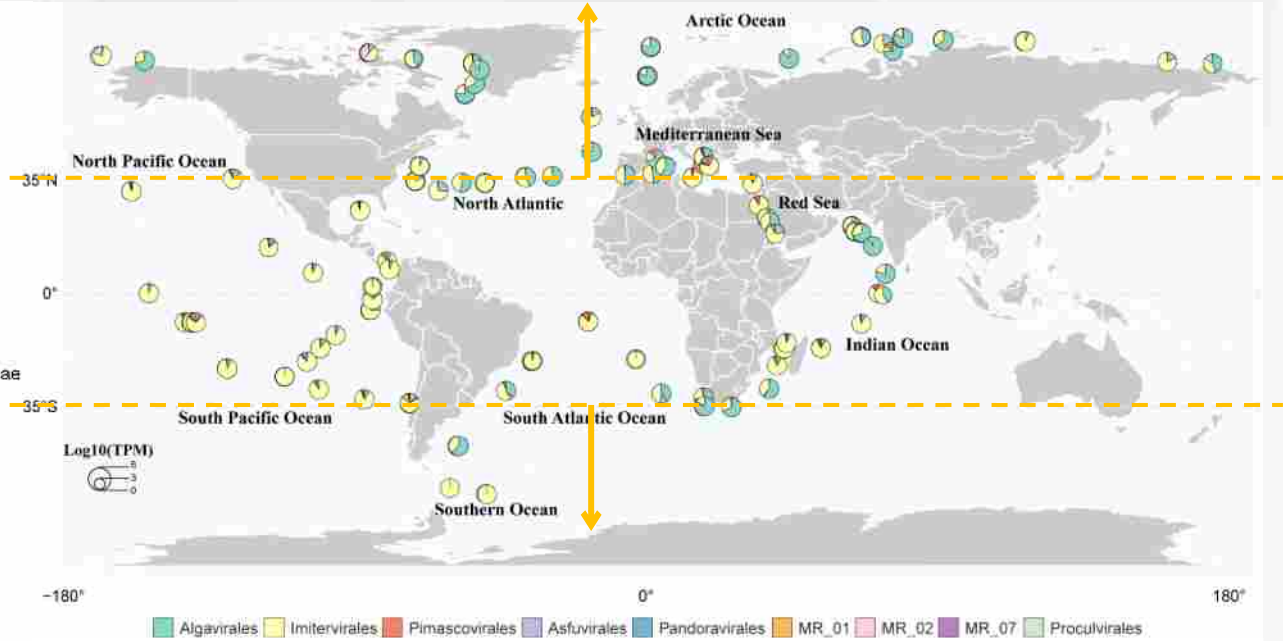
Potential enzymatic protein from mirusviruses

- Rhodopsins of giant viruses may play unrecognized biological roles.

Feng et al., unpublished

Host of giant viruses





- The co-occurrence network analysis indicates that **dinophyceae** are the primary hosts of **Imitervirales**.
- We predict future ocean warming will increase the abundance of Imitervirales infecting dinophyceae, while decreasing the abundance of Algavirales infecting pico-sized algae.

Feng et al., unpublished

海洋科学与工程系
Department of Ocean Science and Engineering

Main conclusions:

- The diversity of SPOT NCLDVs is high and exhibits seasonal patterns.
- Temperature is the primary driver of seasonal fluctuations in giant virus communities.
- The genetic diversity of Imitervirales underpins their adaptability to ocean warming.
- Giant viruses can serve as predictors for global ocean warming.

Future perspectives:

- The phylogeny, metabolic potential, and hosts of unclassified NCLDVs.
- The origin and ecological significance of metabolic genes of giant viruses.
- High-throughput identification methods for hosts of NCLDVs and mirusviruses.

Acknowledgments

海洋科学与工程系
Department of Ocean Science and Engineering

The Fuhrman Lab：

- Jed A. Fuhrman
- Andrew M. Long
- Jesse C. McNichol
- Jake L. Weissman
- Fuhrmanlab members



The Sun Lab：

- Fengzhu Sun
- Tianqi Tang
- Siliangyu Cheng
- Other members







The Hou Lab：

- Jianyi Feng
- Xiaomeng Wang
- Shuaishuai Xu
- Others



南方科技大学
SOUTHERN UNIVERSITY OF SCIENCE AND TECHNOLOGY

SIMONS FOUNDATION
GORDON AND BETTY MOORE FOUNDATION
NIH
NSF

NSFC
National Natural Science Foundation of China

cbiomes
Simons Collaboration on Computational Biogeochemical Modeling of Marine Ecosystems

USC Wrigley Institute
San Pedro Ocean Time-series

南方科技大学
SOUTHERN UNIVERSITY OF SCIENCE AND TECHNOLOGY



Thank you for your attention!

Presenting author: Shengwei Hou
Email: houqw@sustech.edu.cn

海洋科学与工程系
Department of Ocean Science and Engineering

Files for omics practice

海洋科学与工程系
Department of Ocean Science and Engineering

文件 / HKUST_Summer_school_HOU URL to online drive： https://pan.quark.cn/s/132729f16038



Scan to download