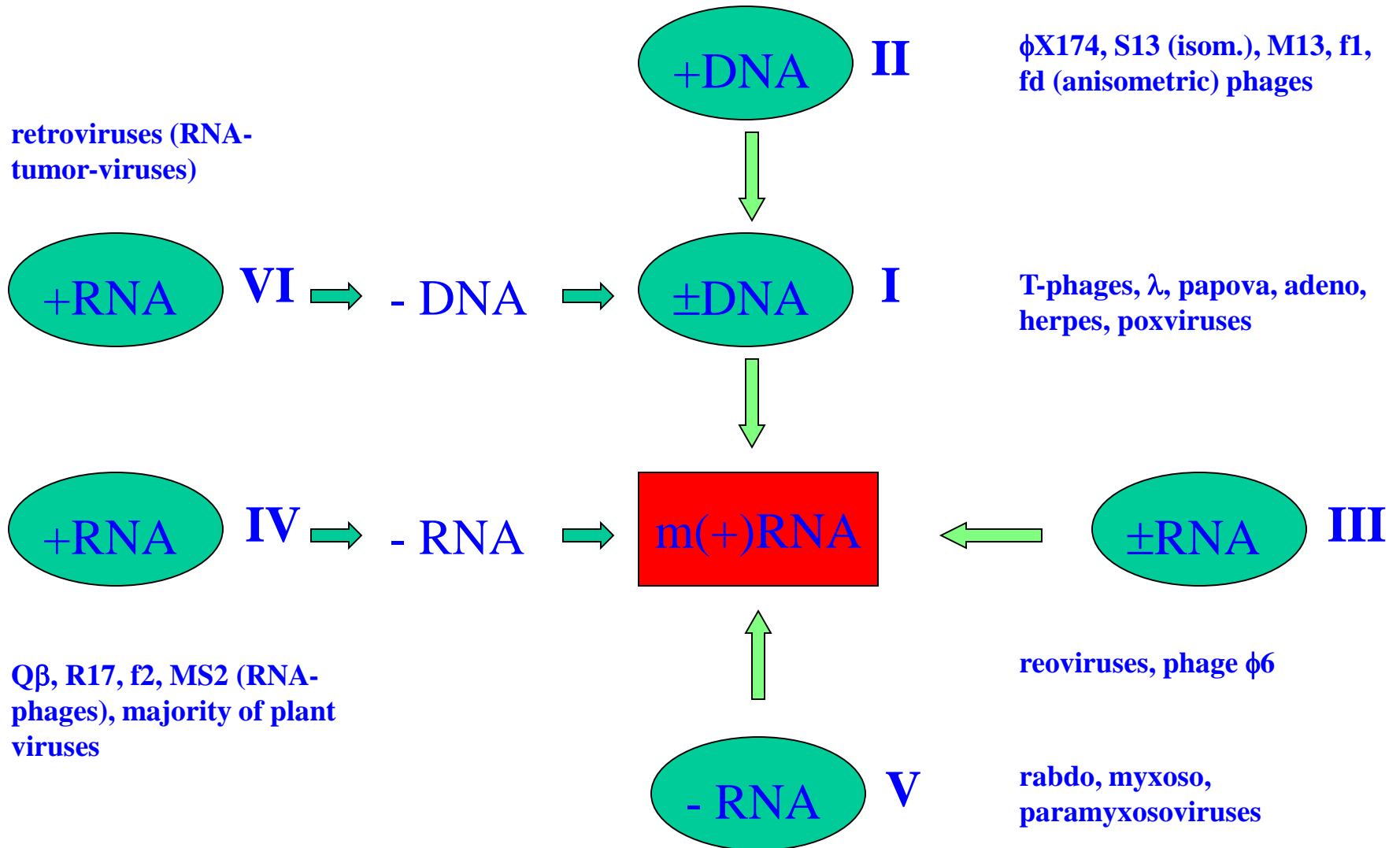
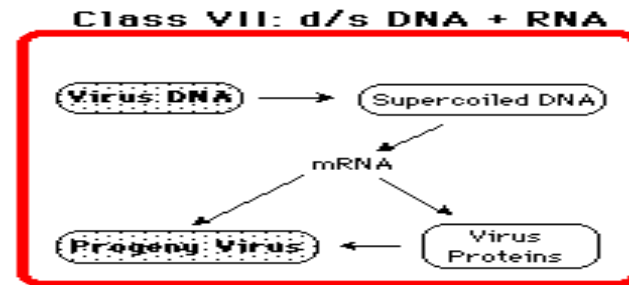
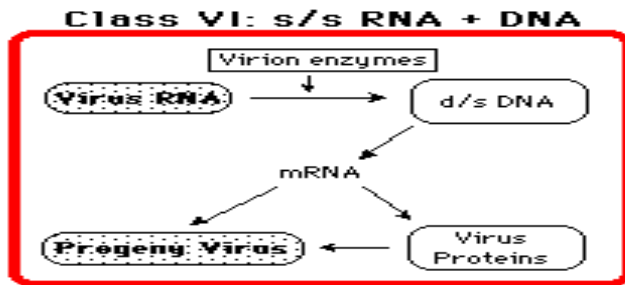
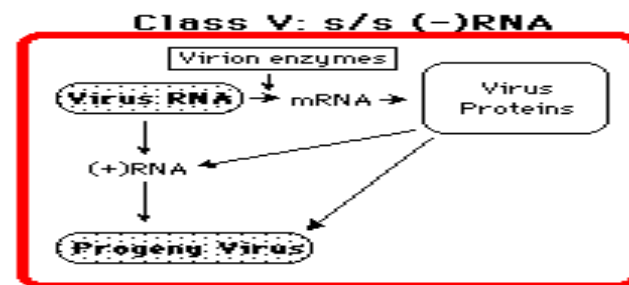
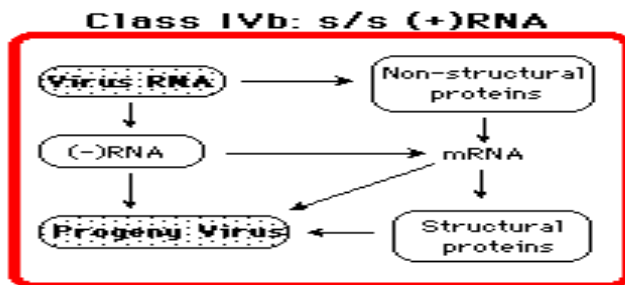
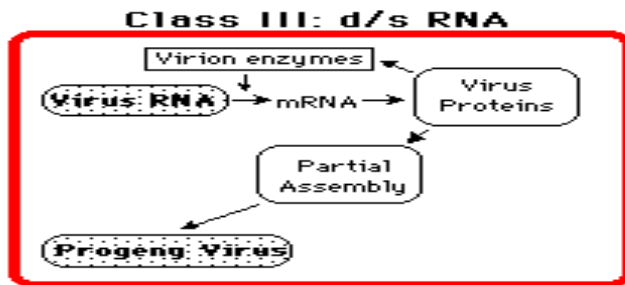
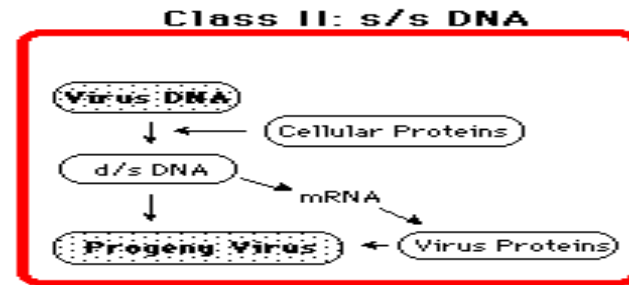
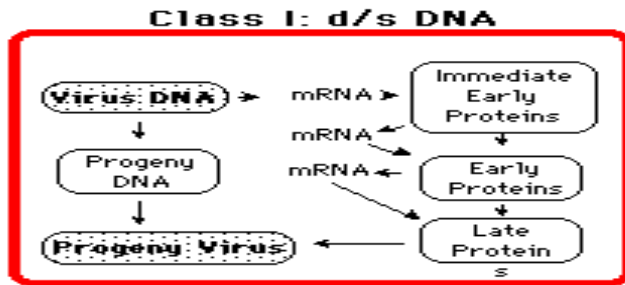


Overview of the virus genomes and replication strategies



- Replication and expression of viral genomes



Replication strategy – the way virus genome is organized and the information therein is expressed with the aim of producing viable virus progeny.

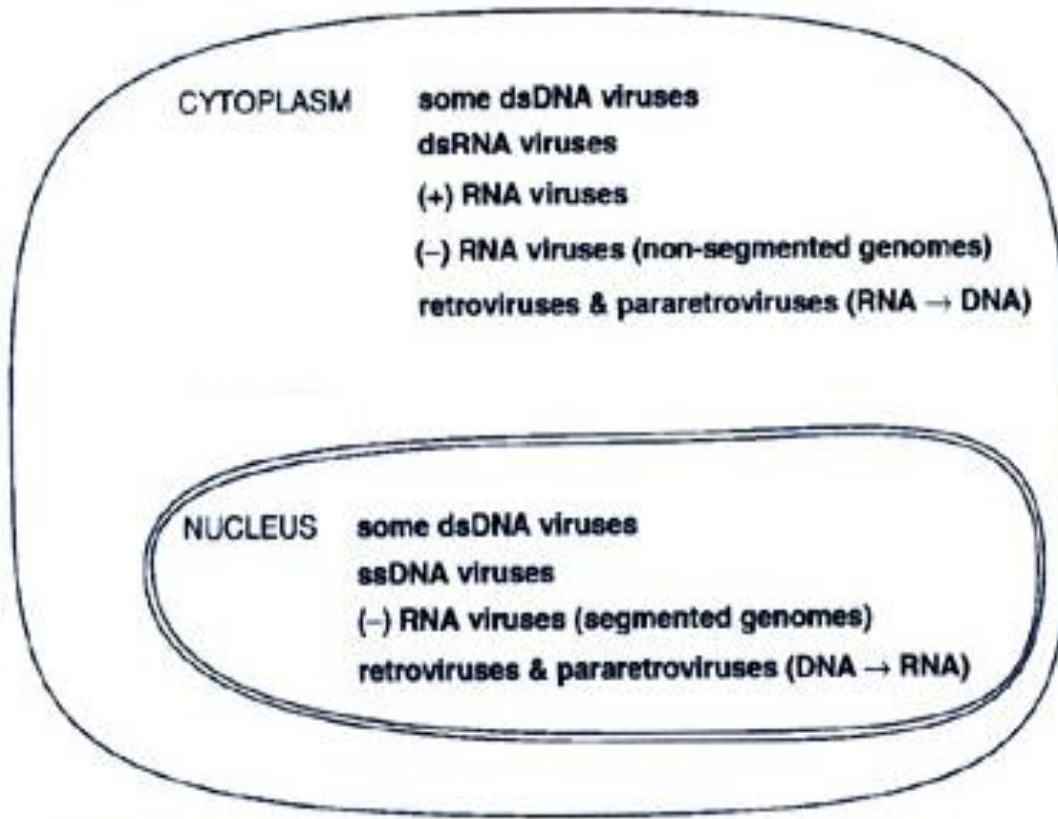
Importance - understanding of viral cycle, pathogenesis, epidemiology, treatments, virus classification.

Genome size – the hindrance in achieving specific genome replication, time and space control of its expression within the host cell.

Fundamental differences also exist in the control of genome replication and expression of eukaryotic and prokaryotic cells and their viruses.

• Replication of viral genomes

in eukaryotic cell

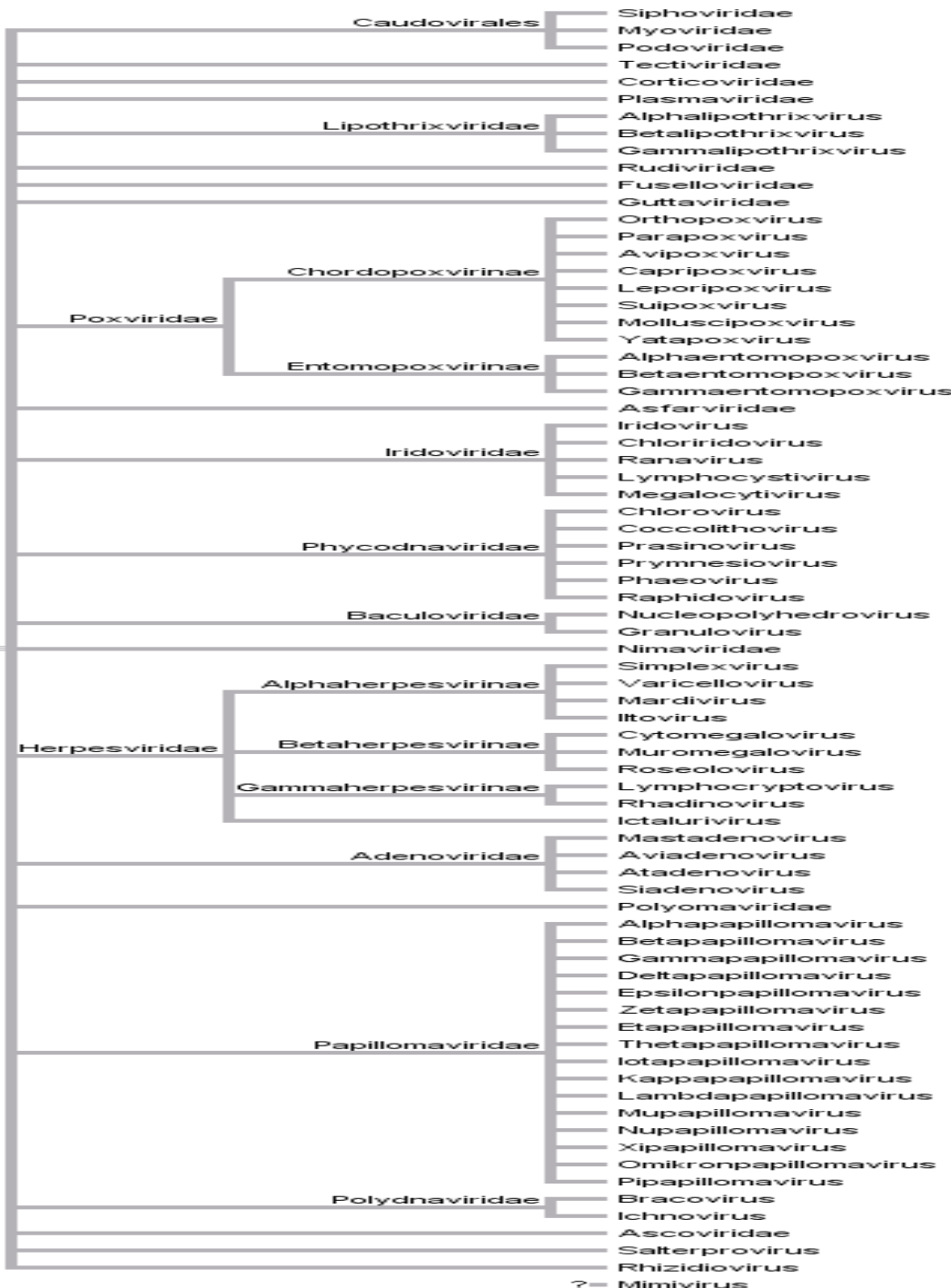


Different types and complexity of viral genomes – viruses with dsDNA-genomes are the most similar to their hosts.

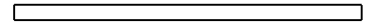
Primers



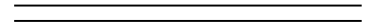
Carter & Saunders, 2007.



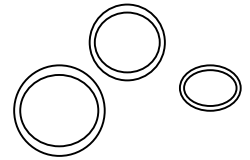
Poxv.



Herpesv.



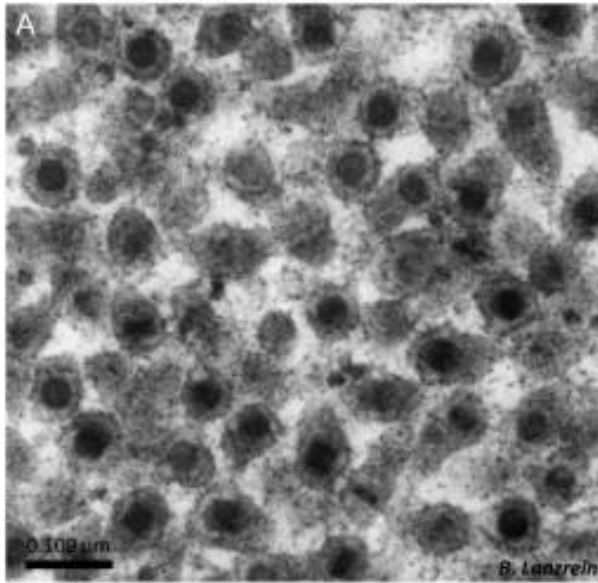
Bracov.



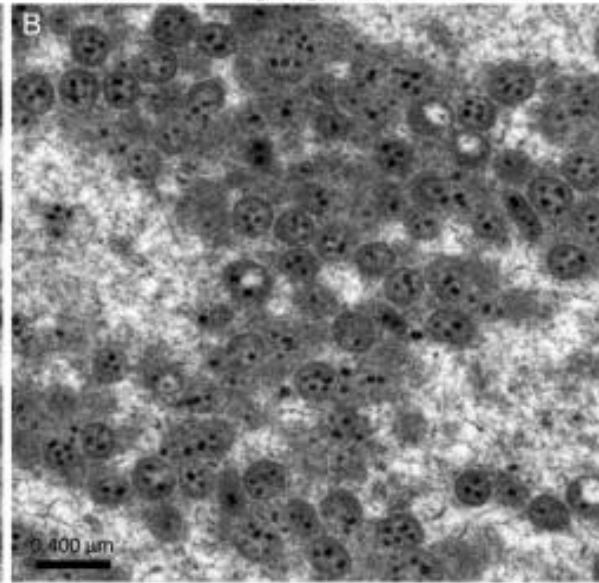
Mimivirus?

dsDNA

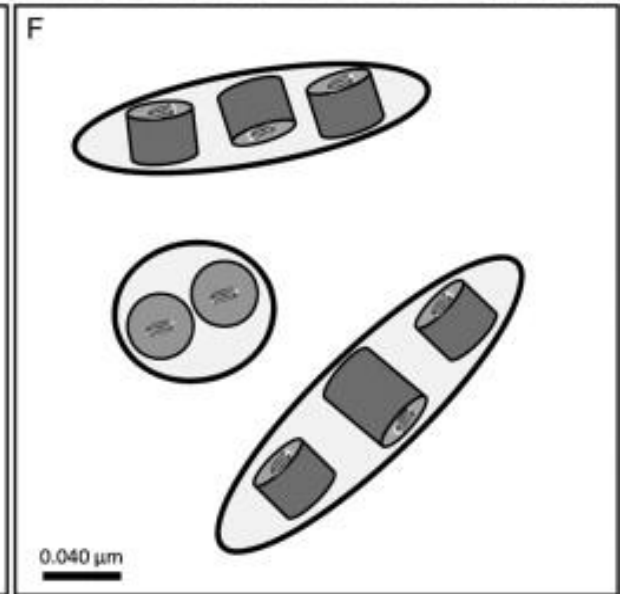
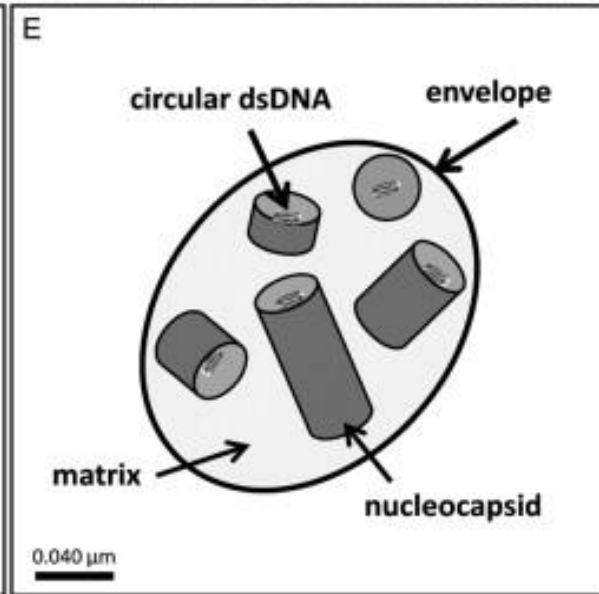
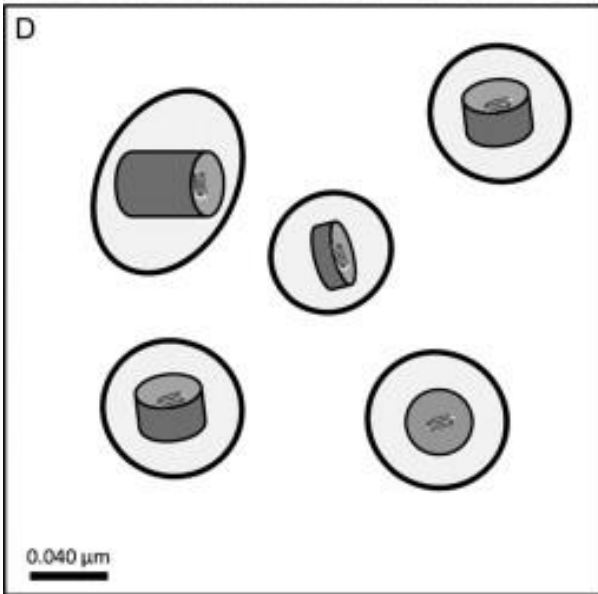
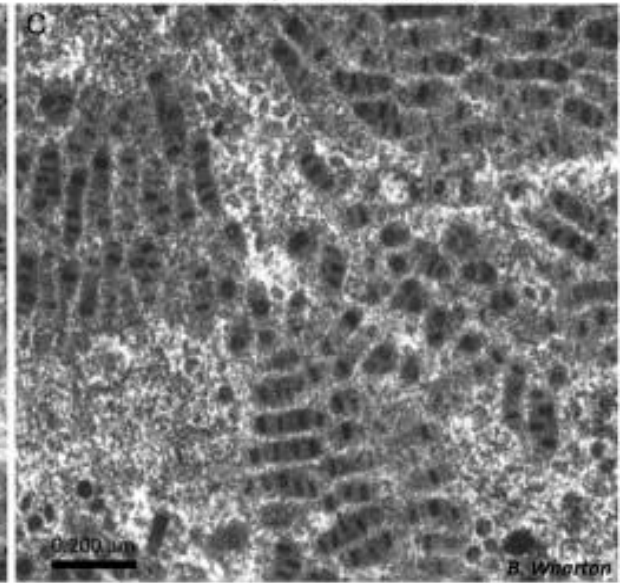
Chelonus inanitus bracovirus



Cotesia congregata bracovirus



Mirax sp. bracovirus



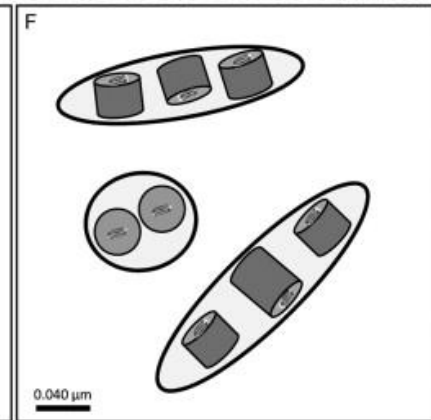
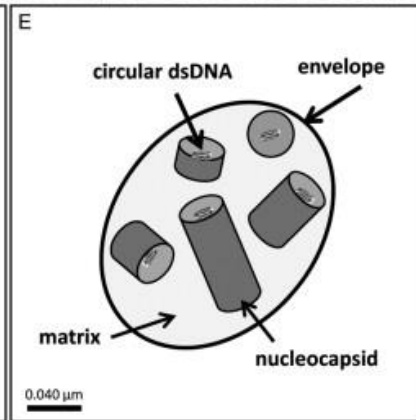
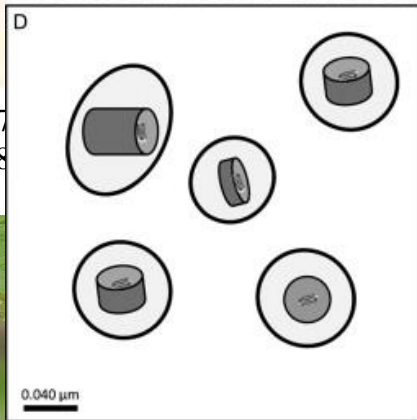
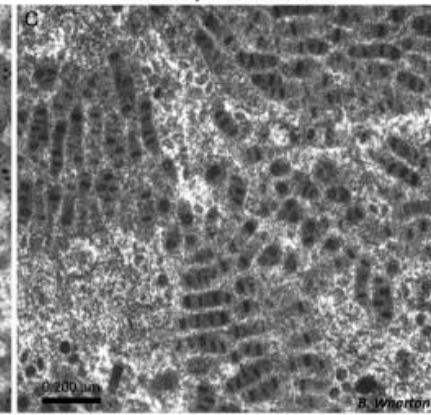
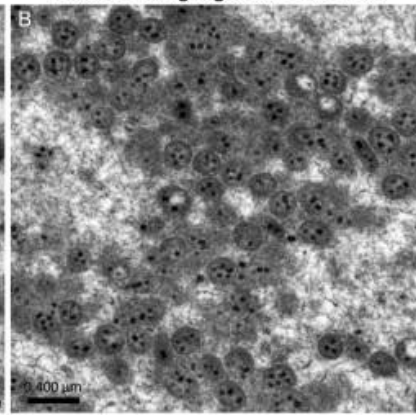
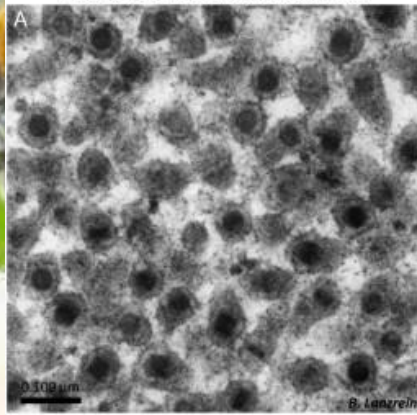


PDV persists in wasp somatic and germ cells as a provirus

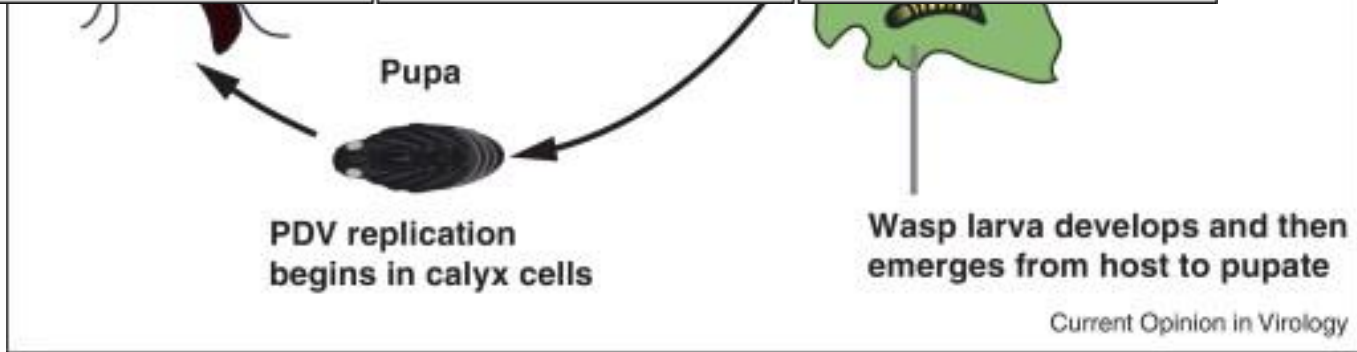
Chelonus inanitus bracovirus

Cotesia congregata bracovirus

Mirax sp. bracovirus



Burke GR, 2016, PLoS ONE 11(7)
DOI: 10.1371/journal.pone.01588



Viruses with dsDNA genomes (1):

linear

Poxviridae (130-375 kbp, the only dsDNA viruses with genome replication in cytoplasm, polyA-tail discovered in vaccinia virus)

Herpesviridae (genome circularizes, 105-235 kbp)

Adenoviridae (30-38 kbp, the discovery of introns – alternative splicing)

What are NCLDV?

Nucleocytoplasmic Large DNA Viruses

NCLDV – giant virions:

Mimiviridae - 1,2 Mbp (*Amoeba* and ?)

Poxviridae - (insects, birds, mammals)

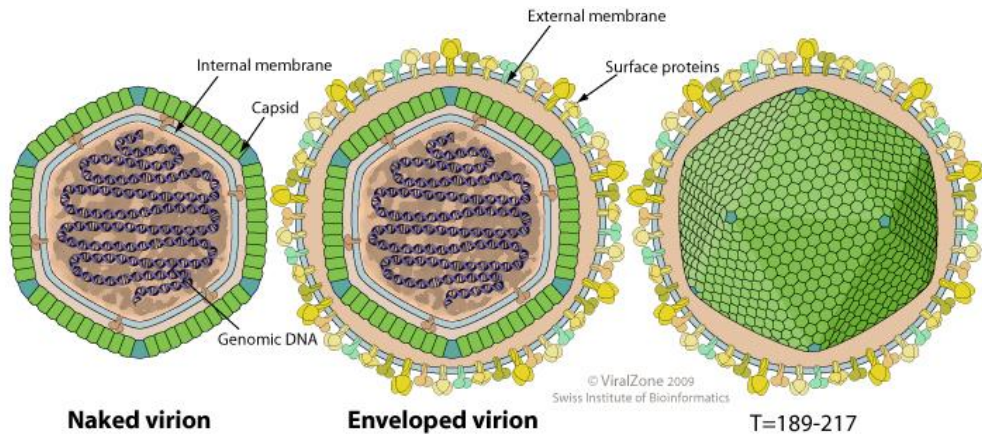
Phycodnaviridae - > 300 kbp (*Paramecium bursaria* *Chlorella virus*),

Iridoviridae - up to 280 kbp (insects, fishes, amphibians)



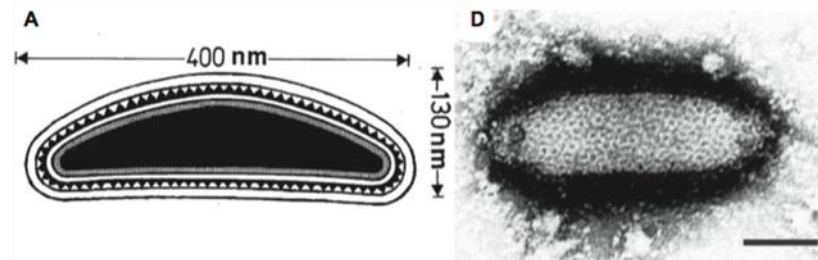
www.microbiologybites.com

Costelytra zealandica



Asfarviridae about 170 kbp (*African swine fever v.*)

Ascoviridae - cdsDNA about 169kbp – invertebrate pathogens (lepidopteran larvae, originated from iridoviruses, similar cycle).



Mimivirus – obligate intracellular parasite of *Amoeba*.

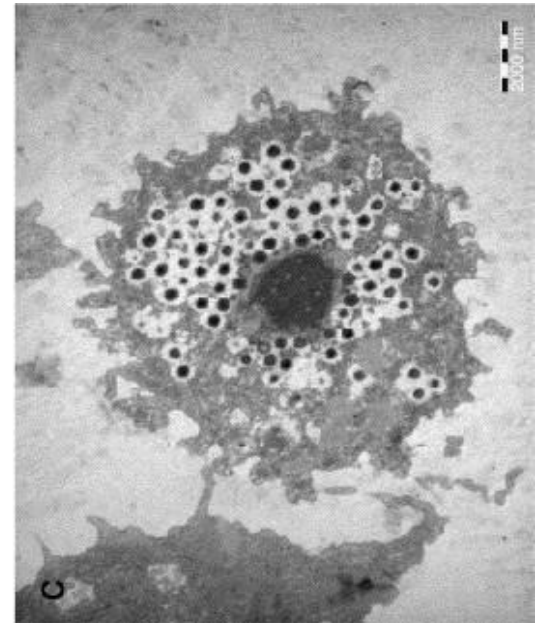
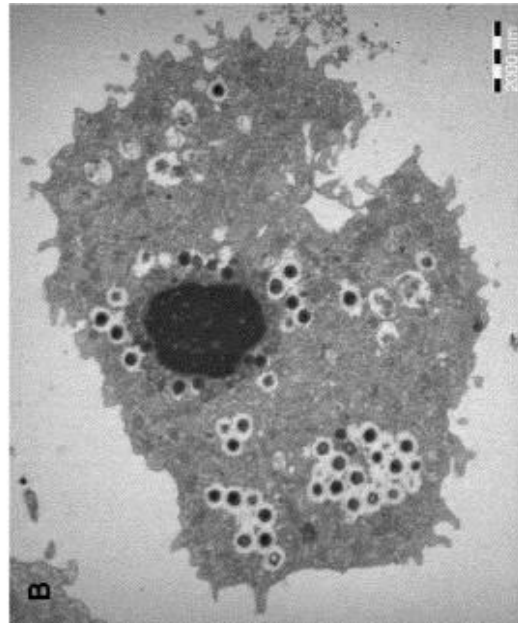
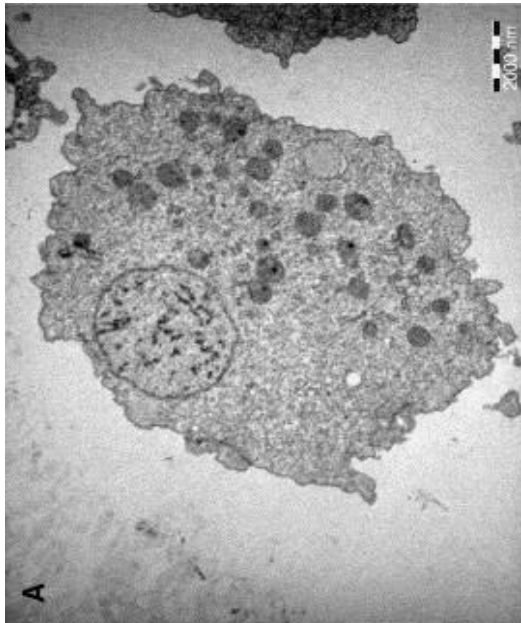
Acanthamoeba polyphaga,

A. castellanii

A. mauritaniensis, etc.

It has a lytic cycle.

LLAP – Legionella-like Amoebal Pathogens.



Taxon Mimivirus

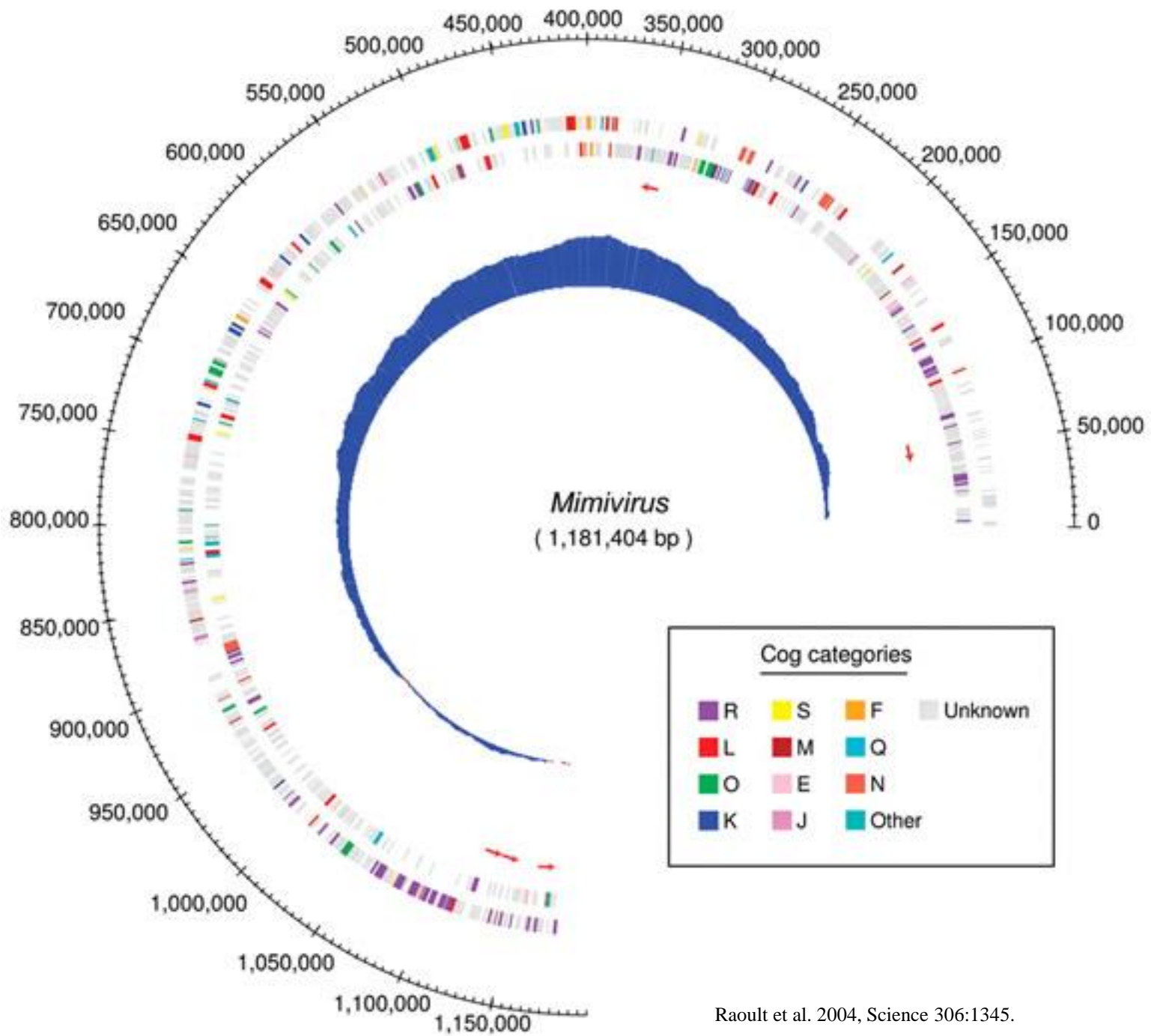
Discovery in 2003 - Amoeba from cooling tower in Bradford, UK.

Microbe **mimicking** virus

Acanthamoeba polyfaga mimivirus (APMV)

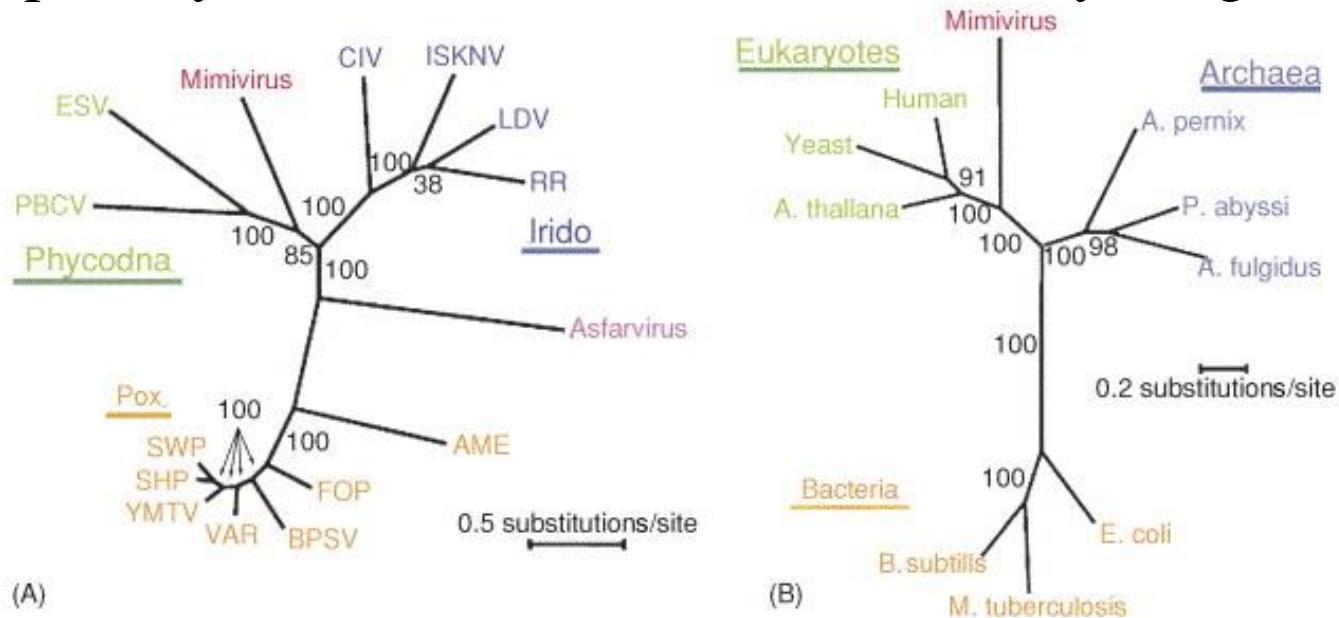
The first giant virus discovered (diameter ~ 650 nm). Huge and very complex genome, unusual features.

Four genes for translation (aa-tRNA synthetases, translation elongation factor EF-Tu, 6 tRNA genes), some genes for sugar, lipid and protein metabolism, DNA-repair, protein folding.



APMV-genome (*Mimiviridae*) analysis:

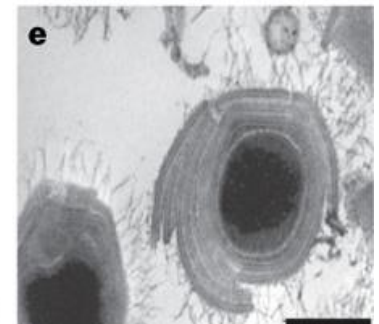
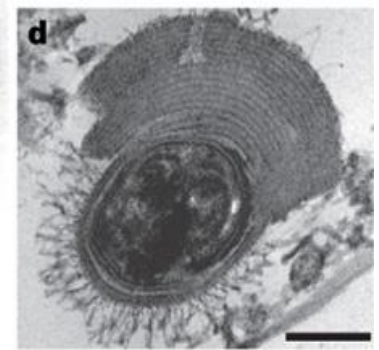
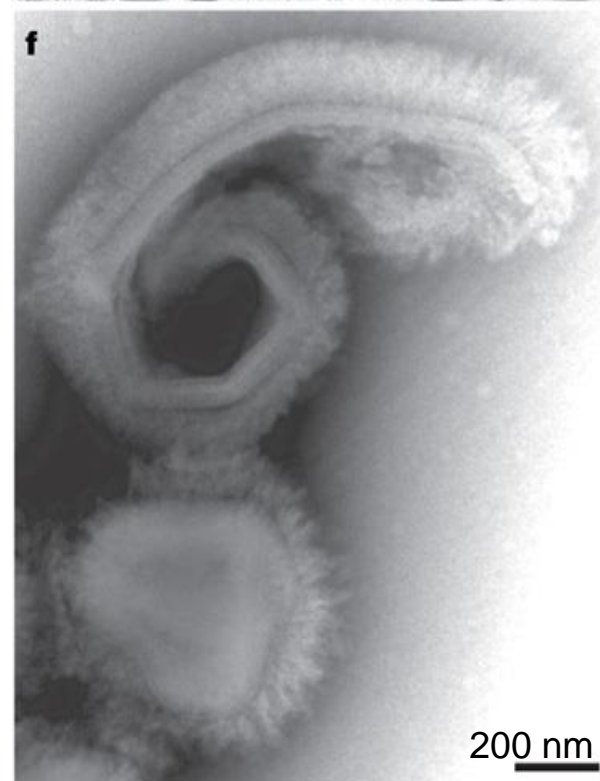
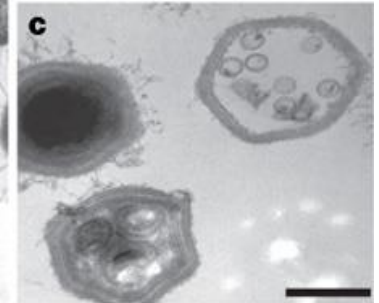
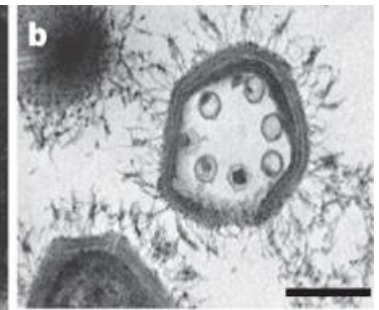
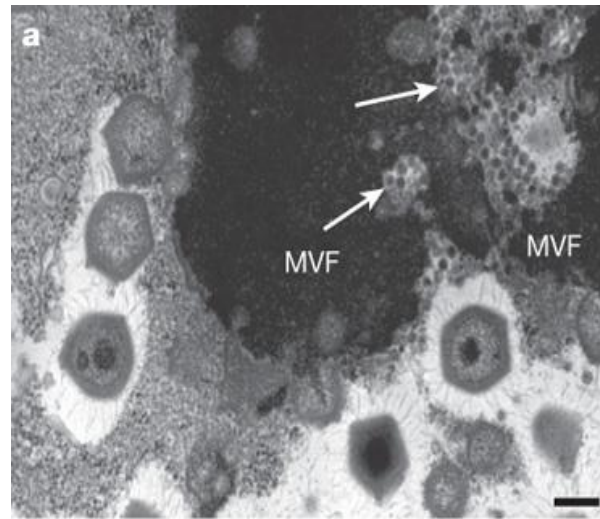
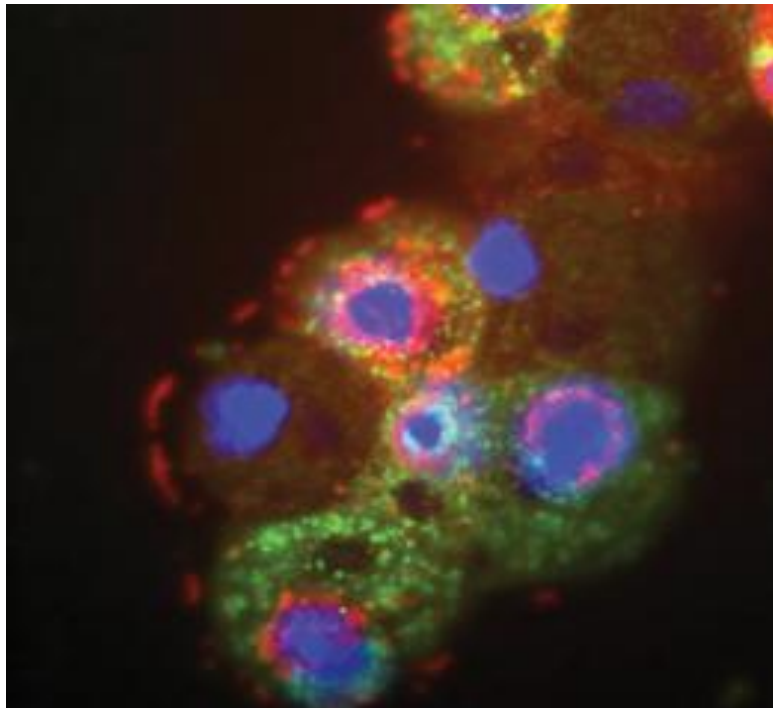
- linear dsDNA, 1.2 Mb (GenBank AY653733) - circularization of the genome ends is possible (repetitive sequences).
- 1262 genes (298 functional), 26 out of 31 NCLDV-homologous genes
- identified genes belong to unknown virus taxa, homologous to NCLDV, prokaryotic (Archea, Bacteria) and eukaryotic genes.



New APMV strain discovered in a cooling tower in Paris, 2008.

Mamavirus – bigger than Mimivirus (ACMV),

harbors “virophage” Sputnik (SNV, 50 nm).



Sputnik cannot replicate by itself in amoeba. It has to coinfect it with a Mamavirus. Otherwise, it has a typical viral cycle.

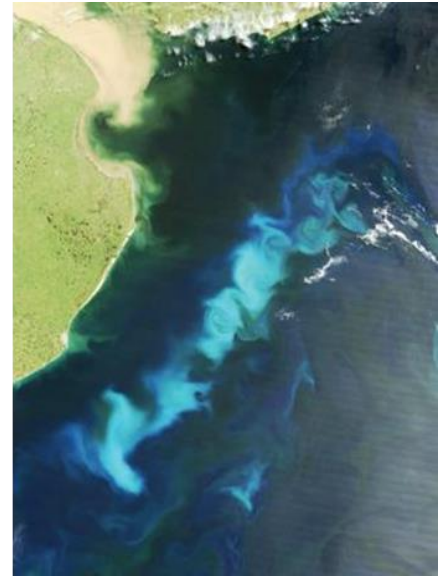
Occasionally, encapsidated in the APMV virus particles.

It also uses MVFs (Mamavirus Virus Factories) for synthesizing its virion.

Functional analogy with phages – harmful for APMV – named virophage.

This was just the first example of a virophage...there are more discovered afterwards.

Sputnik – the first representative of a new virus family.



“ORFan genes” – viral genes with no homologues in known databases. In Sputnik, 13/21 are ORFans! The homologues from the GOS (Global Ocean Sampling Expedition, JCV Institute).

Other genes homologous to – archeal viruses (or plasmids), Mimi/Mamavirus, putative new virus family linked with NCLDV.

Marseillevirus

Discovered in 2009 – giant virus (250 nm) of amoeba but smaller than Mimivirus.

DNA genome, 368000bp, different origins of genes, contains also RNAs (chimeric genome) in the virion.

The role of amoeba (and other phagocytic protists) in the constitution of new gene sets - potential new cell parasites.

New viral family?

Boyer M., Raoult D. et al. **Giant Marseillevirus highlights the role of amoebae as a melting pot in emergence of chimeric microorganisms.** *Proceedings of the National Academy of Sciences*, 2009; 106 (51): 21848. DOI: 10.1073/pnas.0911354106

In 2011, the biggest virus that far discovered from an ocean sample (Las Cruces, Chile) - *Megavirus chilensis* (MCHV, 1.26 Mbp genome).

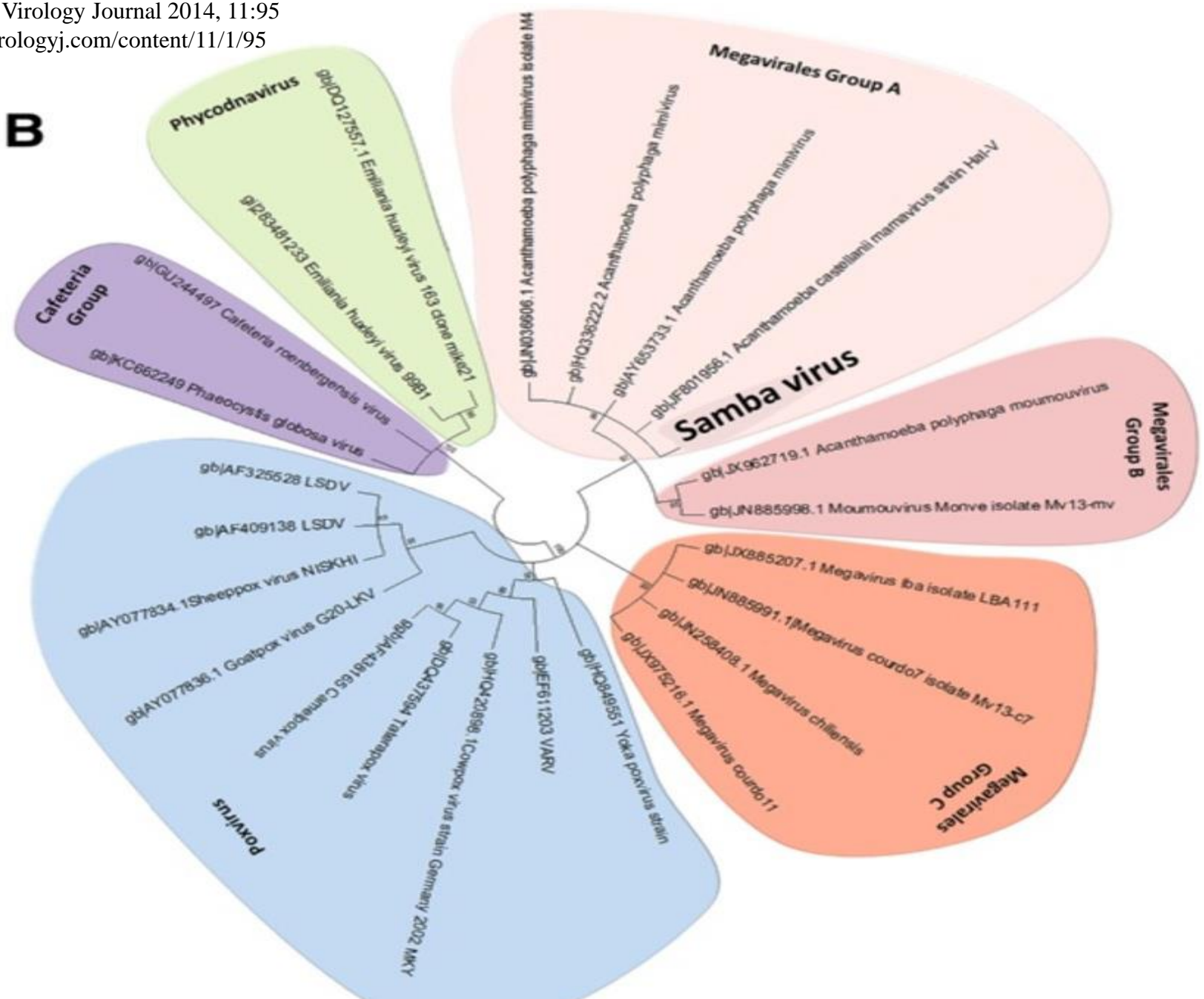
Grows in laboratory cultures of *Amoeba castellani*, *A. poyphaga*, *A. griffini*.

Girus – a term for giant virus taxon?

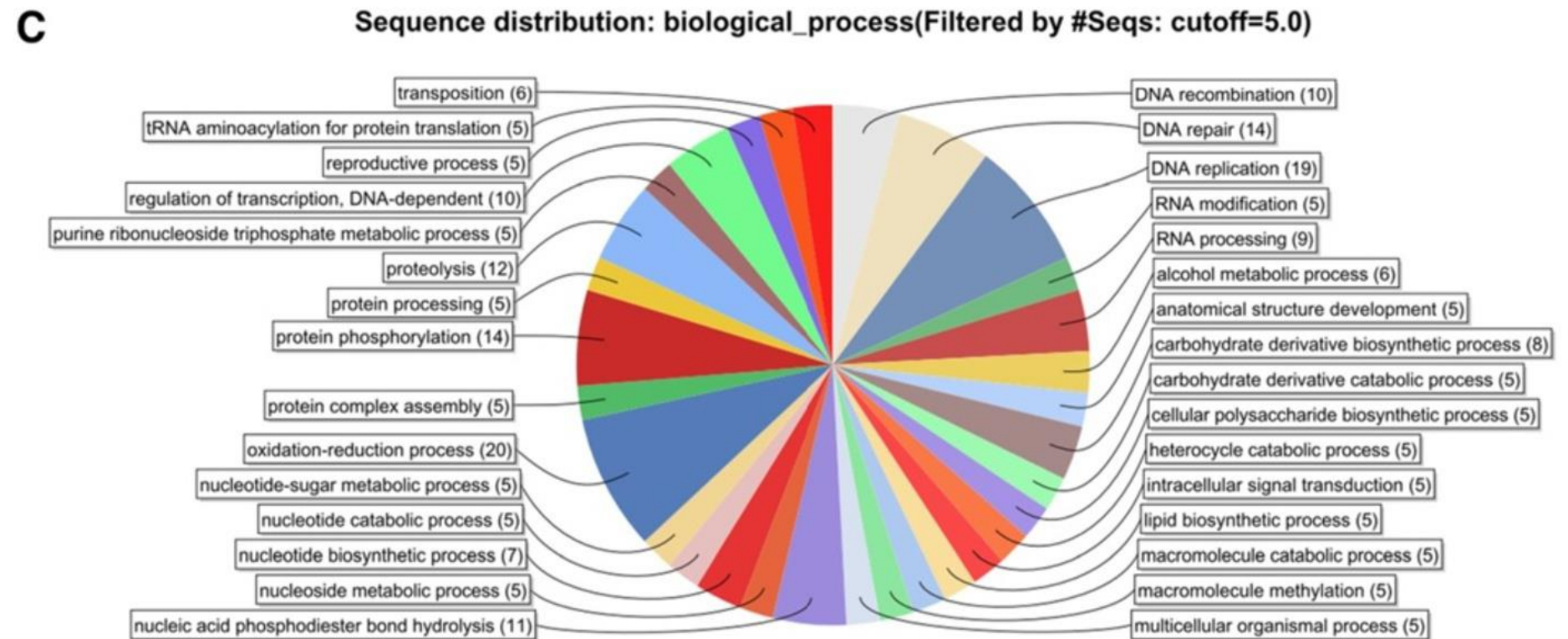
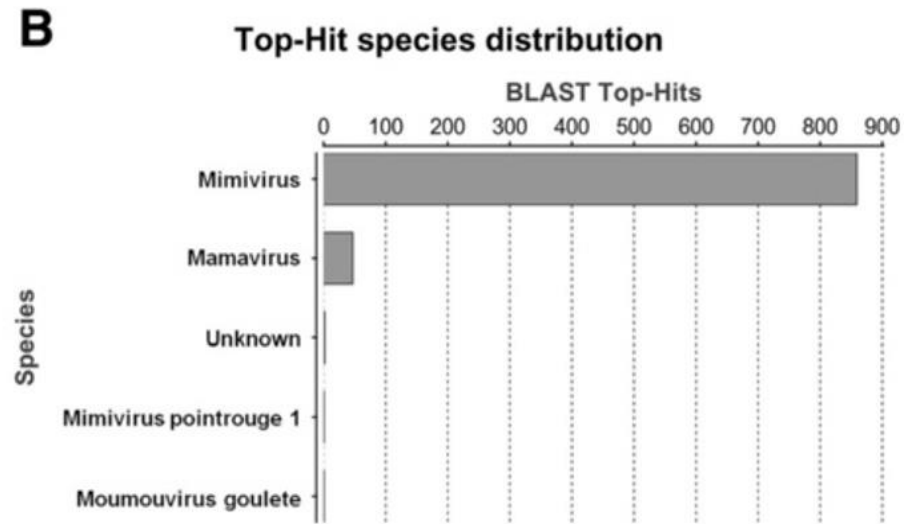
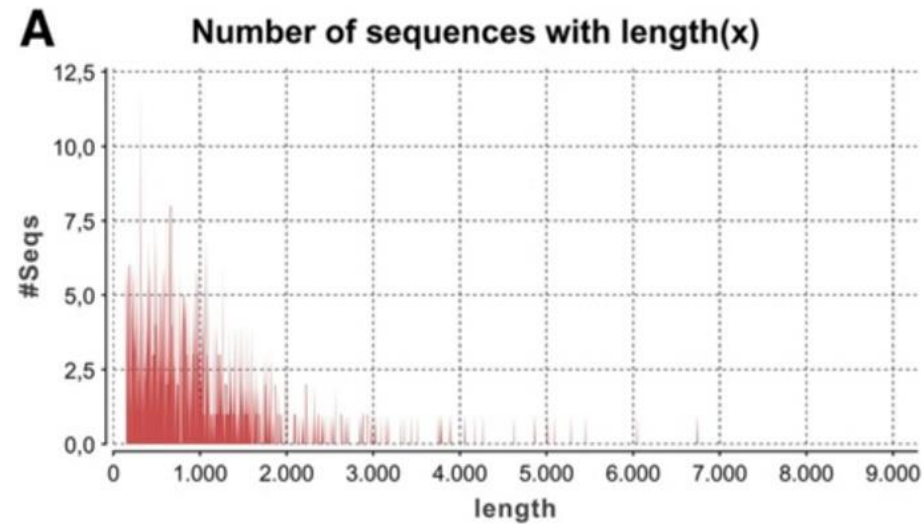
Maybe viral families?

Evolved from an ancestral genome by reductive evolution?

B



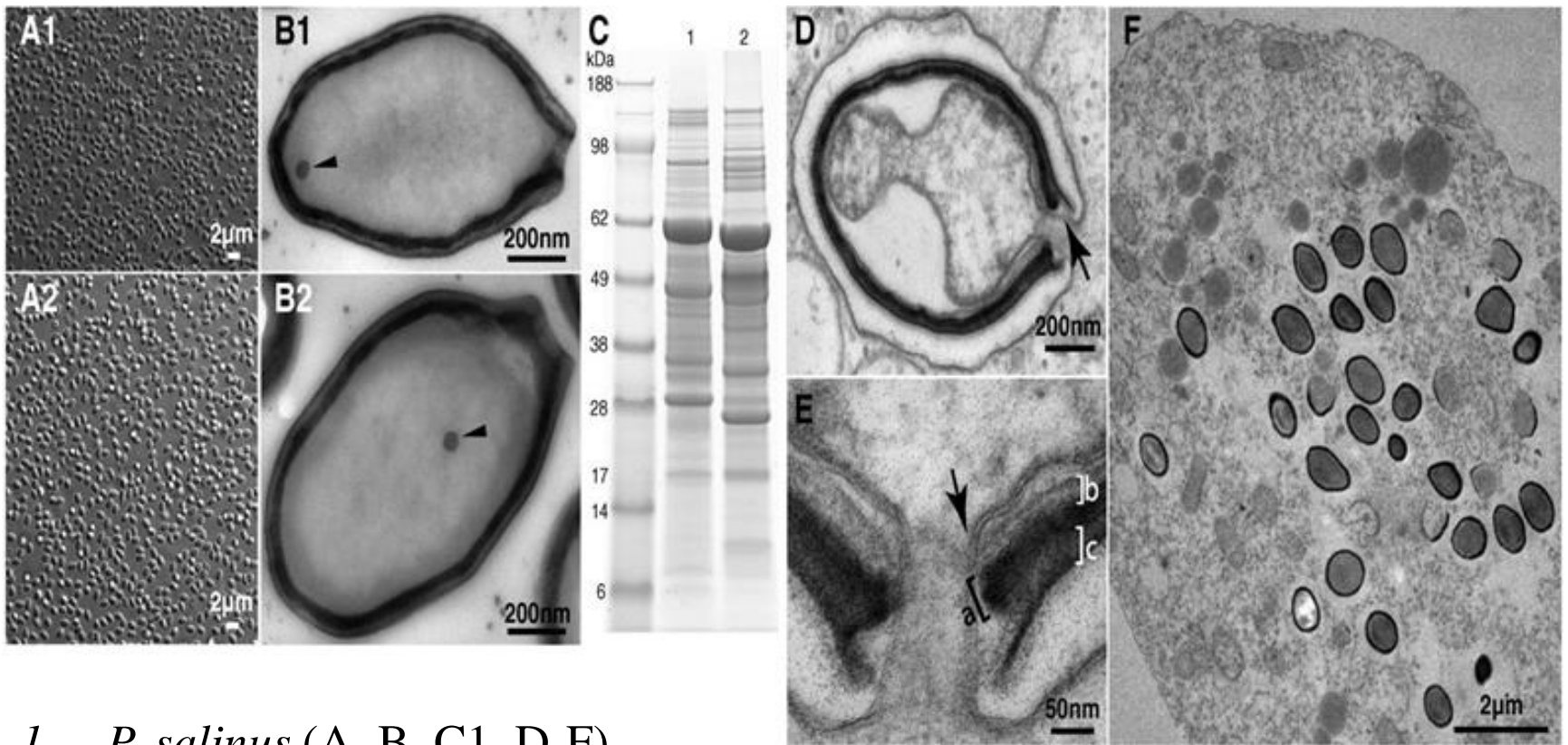
SMBV (Sambavirus)– 1.2 Mbp circular genome!



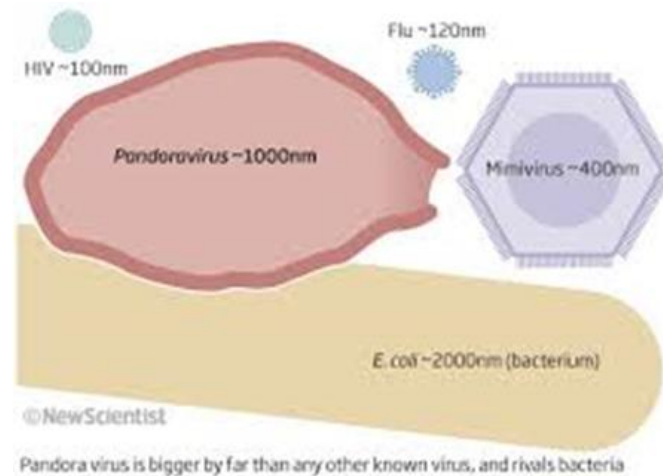
Bigger than the biggest but quite different... *Pandoravirus* (~ 1 μm)
Pandoravirus salinus (2,5 Mbp) coastal seawater of the central Chile,
P. dulcis (1,9 Mbp) a lake near Melbourne, Australia.



Philippe *et al.* (2013) *Science* 341: 281-6.
<http://en.wikipedia.org/wiki/Pandoravirus>

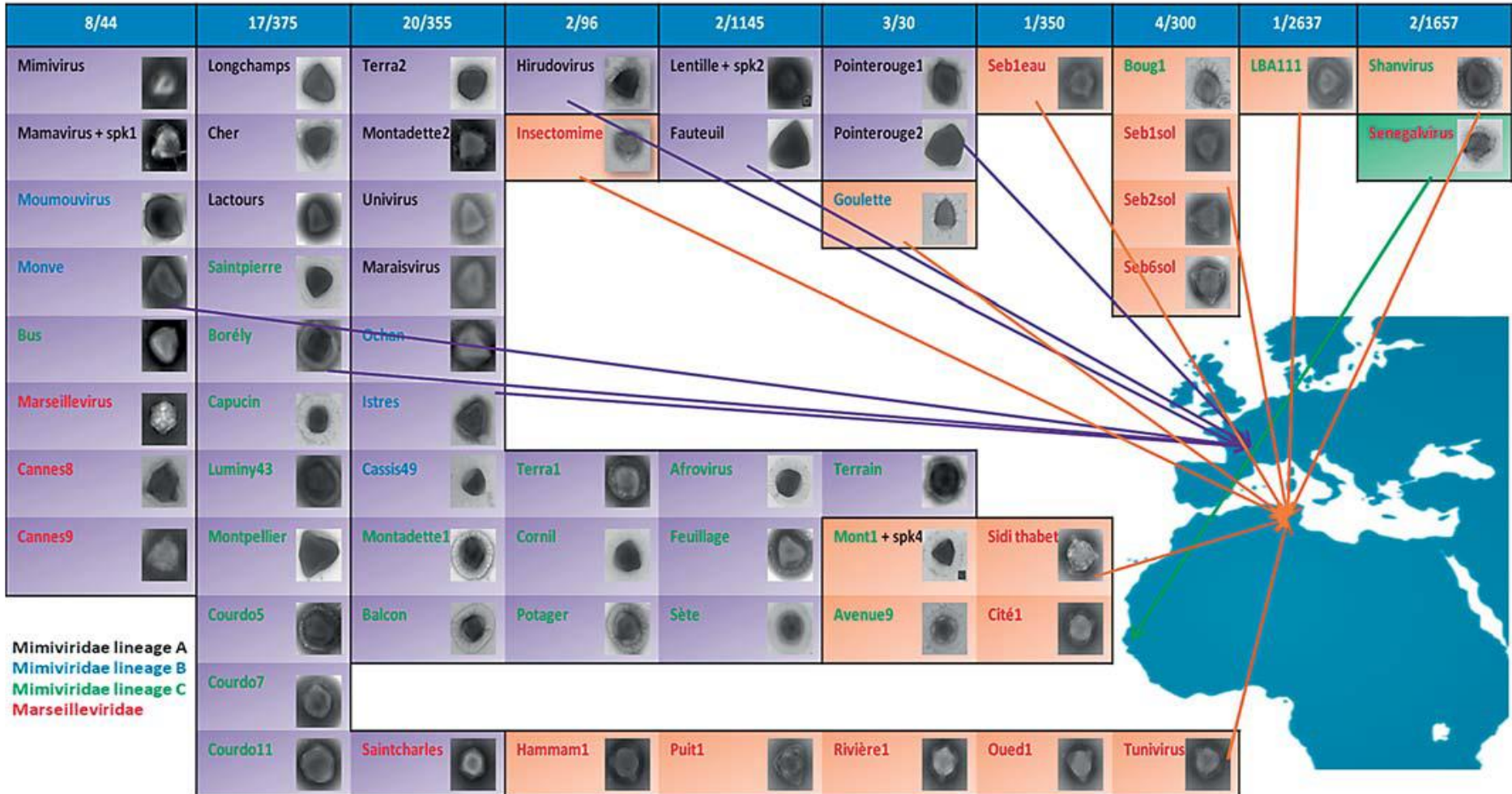


1. *P. salinus* (A, B, C1, D-F)
2. *P. dulcis*

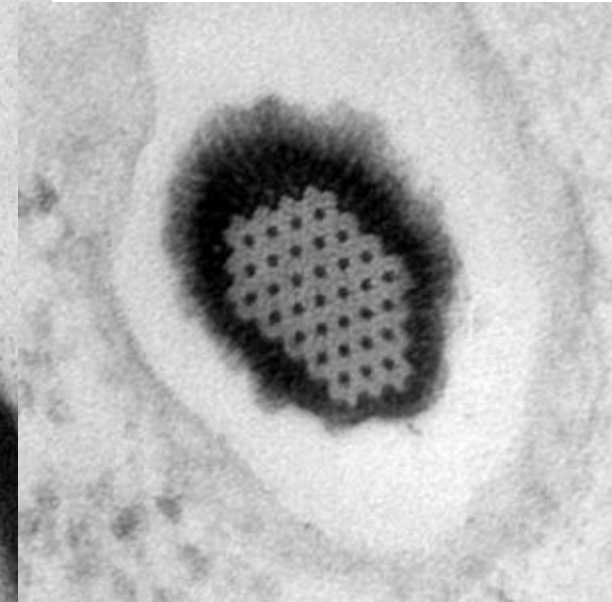
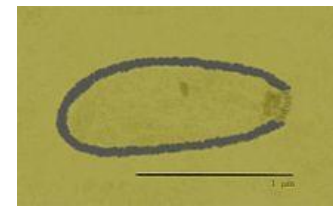


Philippe *et al.* (2013) Science 341: 281-6.
<http://en.wikipedia.org/wiki/Pandoravirus>

Giant viruses are isolated from diverse ecological niches:



Girus group grows further...*Phitovirus sibericum*



Pleistocene sediment of Siberian permafrost, 30 m depth.

(Legendre, M.; Bartoli, J.; Shmakova, L.; Jeudy, S.; Labadie, K.; Adrait, A.; Lescot, M.; Poirot, O.; Bertaux, L.; Bruley, C.; Coute, Y.; Rivkina, E.; Abergel, C.; Claverie, J.-M. (2014). "Thirty-thousand-year-old distant relative of giant icosahedral DNA viruses with a pandoravirus morphology". *Proc. Natl. Acad. Sci. U.S.A.* (11): 4274–9.

Twice as large ($1,5 \times 0,5 \mu\text{m}$) as *Pandoravirus* members, but smaller and less dense genome (about 610000 bp, 467 genes).

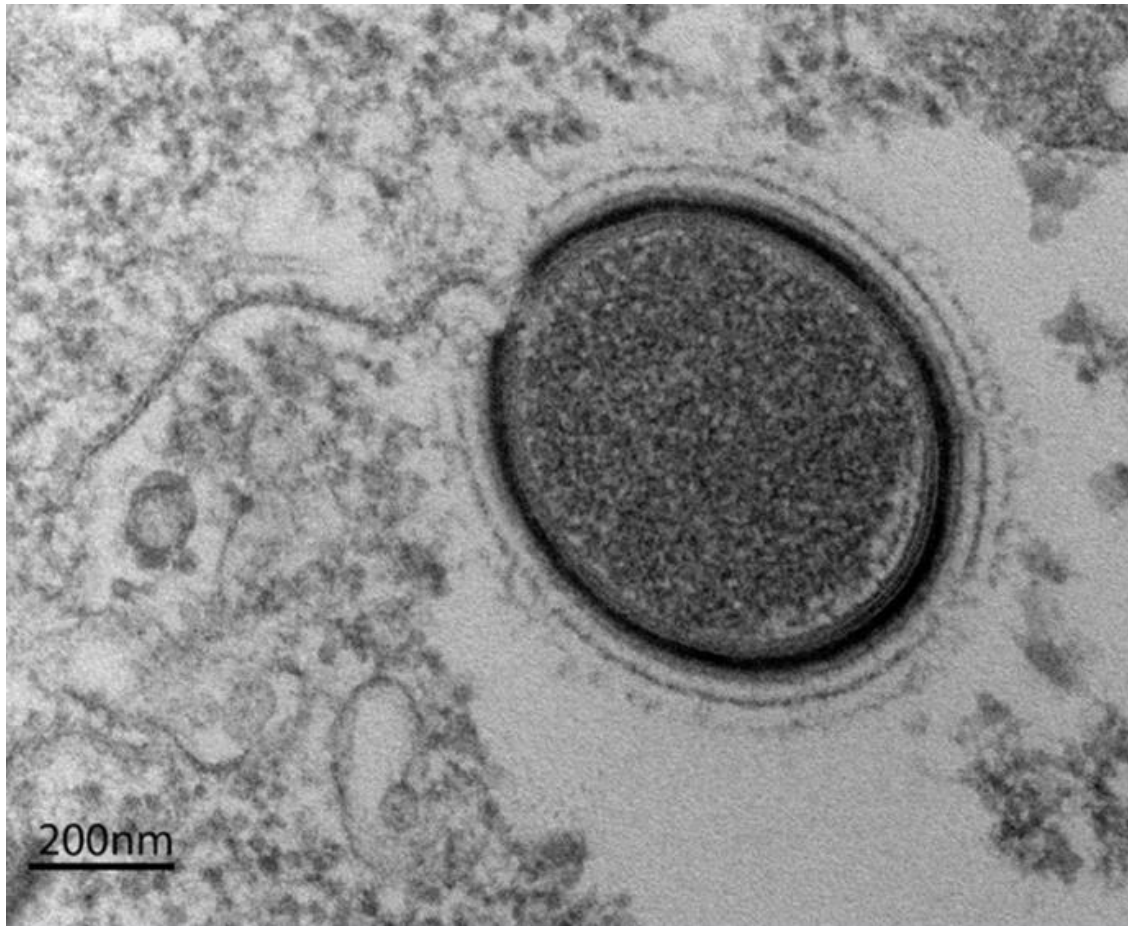
Science 25 September 2015:
Vol. 349 no. 6255 pp. 1501-1502
DOI: 10.1126/science.349.6255.1501-e

A recent fascinating development in basic virology has been the discovery of “giant” viruses that are visible by light microscopy.

Legendre *et al.* now report a **fourth type of giant virus** called *Mollivirus sibericum*.

Like its cousin *Pithovirus sibericum*, it can still infect acanthamoeba (a common soil protozoan) after being found in 30,000-year-old Siberian permafrost. Its diameter spans 0.6 μm , with a 623-kb genome, but it differs from other giant viruses in how it replicates, how its genome is organized, and in the proteins it encodes. Nearly 65% of the proteins encoded by *Mollivirus* have no known homologs.

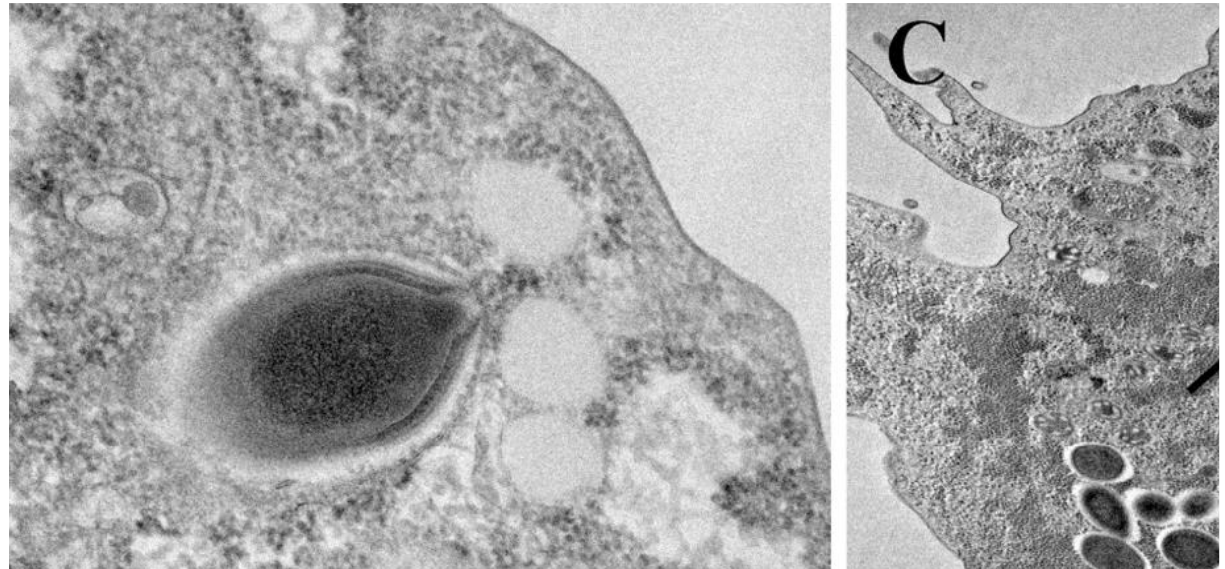
Proc. Natl. Acad. Sci. U.S.A. 10.1073/pnas.1510795112 (2015).



Transmission electron micrograph of a *Mollivirus* particle

"PHOTO: M. LEGENDRE ET AL., PNAS PLUS (8 SEPTEMBER 2015) © PNAS"

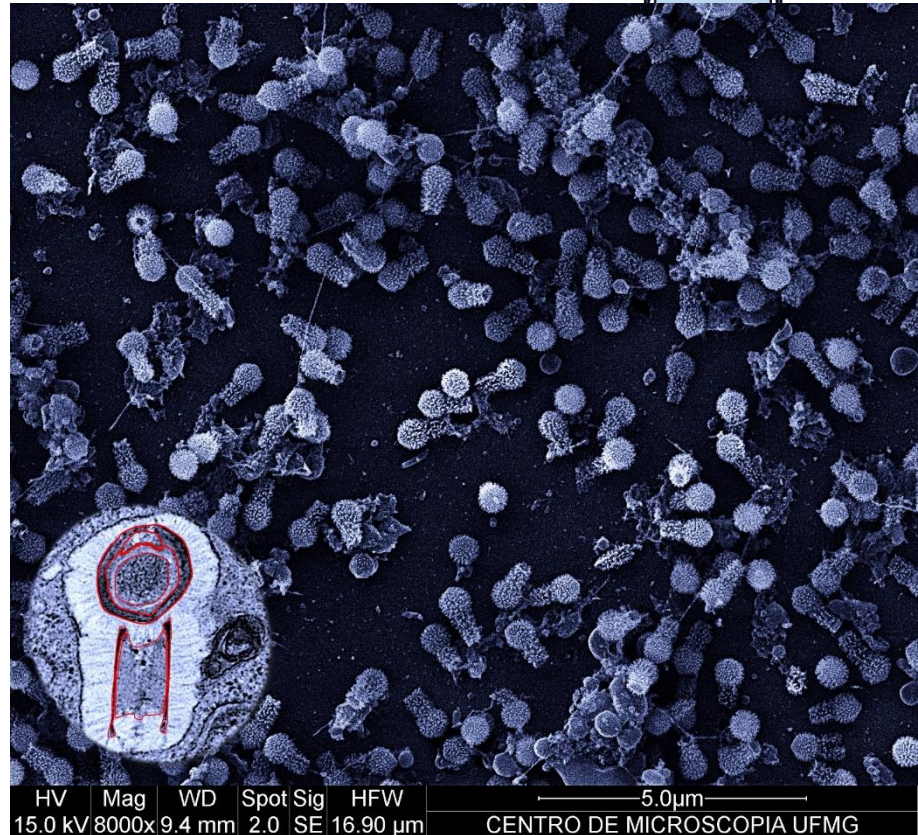
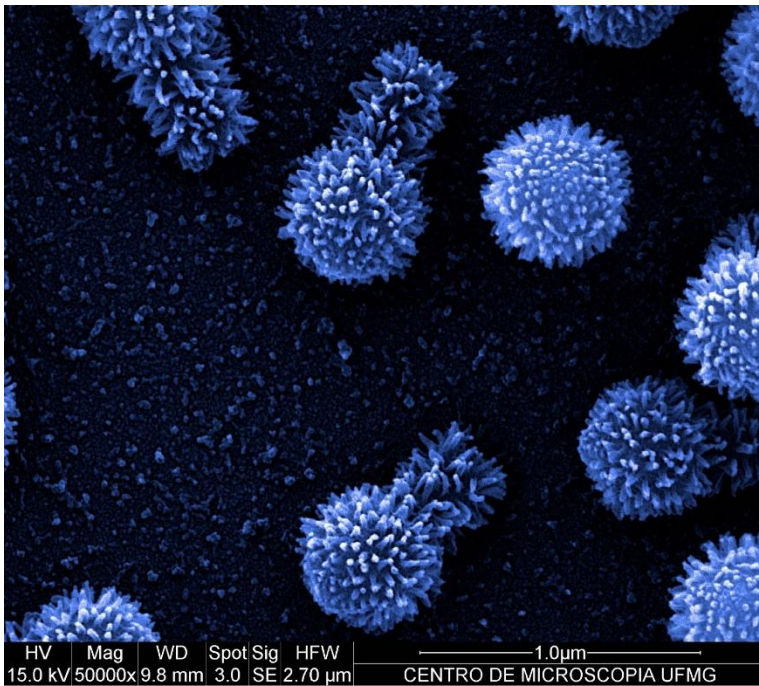
Orpheovirus – HUMI-LCC2, cdsDNA, 1,4 Mbp, 900-1300 nm,
from *Vermamoeba vermiformis*, wastewaters, rat's feces
Phitoviridae



Andreani J, Khalil JYB, Baptiste E, Hasni I, Michelle C, Raoult D,
Levasseur A and La Scola B (2018) Orpheovirus IHUMI-LCC2: A New Virus among
the Giant Viruses. *Front. Microbiol.* 8:2643.
doi: 10.3389/fmicb.2017.02643

„Viruses from Brazil

<https://naturemicrobiologycommunity.nature.com/users/83804-jonatas-abrahamo/posts/30723-precious-giants-from-extreme-environments-brazil>



Tupanvirus – Guarani tribe’s deity Tupan, Tupã
Pantanal of Nhecolandia – soda lakes (extreme salinity and pH),
3000 m deep Atlantic ocean sediments.
Amoebal hosts, 1,2 μm long, the „head” diameter is 450 nm.

Tailed giant Tupanvirus possesses the most complete translational apparatus of the known virosphere.

Abrahão J, Silva L, Silva LS, Khalil JYB, Rodrigues R, Arantes T, Assis F, Boratto P, Andrade M, Kroon EG, Ribeiro B, Bergier I, Seligmann H, Ghigo E, Colson P, Levasseur A, Kroemer G, Raoult D, La Scola B. Nat Commun. 2018 Feb 27;9(1):749. doi: 10.1038/s41467-018-03168-1

Abstract

Here we report the discovery of two Tupanvirus strains, the longest tailed Mimiviridae members isolated in amoebae. Their genomes are **1.44-1.51 Mb linear double-strand DNA** coding for **1276-1425 predicted proteins**. Tupanviruses share the same ancestors with mimivirus lineages and these giant viruses present the **largest translational apparatus within the known virosphere, with up to 70 tRNA, 20 aaRS, 11 factors for all translation steps, and factors related to tRNA/mRNA maturation and ribosome protein modification**. Moreover, two sequences with significant similarity to intronic regions of 18 S rRNA genes are encoded by the tupanviruses and highly expressed. In this translation-associated gene set, **only the ribosome is lacking**. At high multiplicity of infections, tupanvirus is also cytotoxic and causes a severe shutdown of ribosomal RNA and a progressive degradation of the nucleus in host and non-host cells. The analysis of tupanviruses constitutes a new step toward understanding the evolution of giant viruses.

References:

La Scola et al. 2003. A giant virus in amoeba. *Science* 299:2033.

Raoult et al. The 1.2-megabase genome sequence of Mimivirus. 2004. *Science* 306: 1344-1350.

Claverie et al. 2006. Mimivirus and the emerging concept of "giant" virus. *Virus Res.* 117:133-44.

Suzan-Monti et al. 2006. Genomic and evolutionary aspects of Mimivirus. *Virus Res.* 117:145-55

Raoult et al. 2007. The discovery and characterization of Mimivirus, the largest known virus and putative pneumonia agent. *Clin Infect Dis.* 45:95-102.

Moreira, Brochier-Armanet. 2008. Giant viruses, giant chimeras: the multiple evolutionary histories of Mimivirus genes. *BMC Evol Biol.* 8:12.

Raoult, Forterre. 2008. Redefining viruses: lessons from Mimivirus. *Nat Rev Microbiol.* 6:315-319.

La Scola et al. 2008. The virophage as a parasite of a giant mimivirus. *Nature* 455:100-104.

Claverie et al. 2009. Mimivirus and Mimiviridae: giant viruses with an increasing number of potential hosts including corals and sponges. *J. Invert. Pathol.* 101: 171-180.