



**HAL**  
open science

## **Amoebae: Hiding in Plain Sight: Unappreciated Hosts for the Very Large Viruses**

Victória Fulgêncio Queiroz, Rodrigo Araújo Lima Rodrigues, Paulo Victor De  
Miranda Boratto, Bernard La Scola, Julien Andreani, Jônatas Santos

► **To cite this version:**

Victória Fulgêncio Queiroz, Rodrigo Araújo Lima Rodrigues, Paulo Victor De Miranda Boratto, Bernard La Scola, Julien Andreani, et al.. Amoebae: Hiding in Plain Sight: Unappreciated Hosts for the Very Large Viruses. *Annual Review of Virology*, 2022, 9 (1), pp.79-98. 10.1146/annurev-virology-100520- . hal-03948777

**HAL Id: hal-03948777**

**<https://amu.hal.science/hal-03948777>**

Submitted on 20 Jan 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

*Annual Review of Virology*

# Amoebae: Hiding in Plain Sight: Unappreciated Hosts for the Very Large Viruses

Victória Fulgêncio Queiroz,<sup>1</sup>  
Rodrigo Araújo Lima Rodrigues,<sup>1</sup>  
Paulo Victor de Miranda Boratto,<sup>1</sup> Bernard La Scola,<sup>2,3</sup>  
Julien Andreani,<sup>2,3,4</sup> and Jônatas Santos Abrahão<sup>1</sup>

<sup>1</sup>Laboratório de Vírus, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil; email: jonatas.abrahao@gmail.com

<sup>2</sup>Department of Microbes, Evolution, Phylogeny and Infection, Institut de Recherche pour le Développement, Assistance Publique-Hôpitaux de Marseille, Aix-Marseille Université, Marseille, France; email: bernard.la-scola@univ-amu.fr

<sup>3</sup>Institut Hospitalo-Universitaire Méditerranée Infection, Marseille, France

<sup>4</sup>Laboratoire de Virologie, Centre Hospitalier Universitaire Grenoble-Alpes, Grenoble, France

ANNUAL  
REVIEWS **CONNECT**

[www.annualreviews.org](http://www.annualreviews.org)

- Download figures
- Navigate cited references
- Keyword search
- Explore related articles
- Share via email or social media

Annu. Rev. Virol. 2022. 9:79–98

First published as a Review in Advance on June 2, 2022

The *Annual Review of Virology* is online at [virology.annualreviews.org](http://virology.annualreviews.org)

<https://doi.org/10.1146/annurev-virology-100520-125832>

Copyright © 2022 by Annual Reviews. This work is licensed under a Creative Commons Attribution 4.0 International License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. See credit lines of images or other third-party material in this article for license information.

## Keywords

amoebae, giant virus, NCLDV, *Nucleocytoviricota*, viral diversity, genome complexity

## Abstract

For decades, viruses have been isolated primarily from humans and other organisms. Interestingly, one of the most complex sides of the virosphere was discovered using free-living amoebae as hosts. The discovery of giant viruses in the early twenty-first century opened a new chapter in the field of virology. Giant viruses are included in the phylum *Nucleocytoviricota* and harbor large and complex DNA genomes (up to 2.7 Mb) encoding genes never before seen in the virosphere and presenting gigantic particles (up to 1.5 μm). Different amoebae have been used to isolate and characterize a plethora of new viruses with exciting details about novel viral biology. Through distinct isolation techniques and metagenomics, the diversity and complexity of giant viruses have astonished the scientific community. Here, we discuss the latest findings on amoeba viruses and how using these single-celled organisms as hosts has revealed entities that have remained hidden in plain sight for ages.



## INTRODUCTION

### Importance

Viruses are the most abundant biological entities on Earth (1). As intracellular parasites of organisms from the three domains of life, they exert important top-down selective pressures. They are agents of evolution and play fundamental roles in ecosystems (1–3). Viral studies are important for medicine, economics, and biotechnology (4). Therefore, it is not surprising that the known virosphere is extremely anthropocentric (5). Nonetheless, most viruses are not associated with humans, and their study can unravel an unprecedented part of the world. The discovery of *Acanthamoeba polyphaga* mimivirus (APMV) is a notable example (6).

The description of a virus with genome and particles of such complexity opened the door to an innovative area of study. Virologists revisited the concepts that defined viruses and even led to the discovery of satellite-like associated viruses, i.e., virophages (7). Therefore, it is not surprising that the search for other viruses that infect amoebae has recently intensified. With advances in molecular techniques, studies of metagenomics have detected these ubiquitous and abundant viruses in different parts of the world (8, 9). These prospective studies and viral isolation have made it possible to make an in-depth characterization of some of these viruses to help shed light on viral diversity, evolution, structure, genomics, replication cycle, interactions with hosts, and what role these complex organisms play in nature.

### Discovery of Giant Viruses of Amoebae

A pneumonia outbreak in 1992 in Bradford, England, led researchers to seek to identify the causative agent of the disease. Water samples were collected from cooling towers, and different microorganisms were isolated in association with amoebae including an apparent Gram-positive bacteria described at the time as the Bradford coccus (6). Several molecular attempts to identify the new microorganism failed. The mystery began to be solved when transmission electron microscopy analysis was performed at Aix-Marseille University, France. This revealed gigantic icosahedral virus-like particles surrounded by extensive fibril structures (6). The new species was then named *Acanthamoeba polyphaga* mimivirus in reference to its host organism and for mimicking another microorganism. The newly discovered mimivirus had a particle size of approximately 700 nm in diameter that could not be filtered in standard 0.2- $\mu$ m membranes. The viral particle was nonenveloped and had an  $\sim$ 1.2-Mb genome that was larger than some cellular organisms (6, 10). The mimivirus was associated with a group of nucleocytoplasmic large DNA viruses (NCLDV), thus establishing the new family *Mimiviridae*.

The search for new giant viruses intensified after the discovery of APMV. New mimivirus isolates, as well as metagenomic analyses, revealed the ubiquity and diversity of these viruses on the planet (11–14). In 2009, six years after the discovery of mimiviruses, a new *Acanthamoeba*-infecting virus was discovered from water samples collected from cooling towers in Paris and was named marseillevirus (15). The novel virus was an icosahedral particle with an approximate size of 250 nm and a circular genome of 370 kb characterized by genomic mosaicism (15). This was the first representative of the new family *Marseilleviridae*, which expanded over the years to include different marseillevirus lineages (16–19). Since then, several groups of giant amoeba viruses have been discovered, pushing the limits once imagined for viral particle size and genomic complexity as evidenced by pandoraviruses, pithoviruses, and tupanviruses, thus expanding the diversity of putative members of the phylum *Nucleocytoviricota* (20–23).

## Advances in Virus Isolation Techniques

Rowbotham (24, 25) pioneered the use of *Acanthamoeba* coculture techniques. At that time, Pontiac fever was a recently solved mystery, and he proposed an enrichment step for the isolation of *Legionella* spp. directly from patient samples. This technique involved culturing *Acanthamoeba* spp. in xenic culture with UV-irradiated *Klebsiella aerogenes*. This technique allowed the characterization of many intracellular bacteria (26, 27).

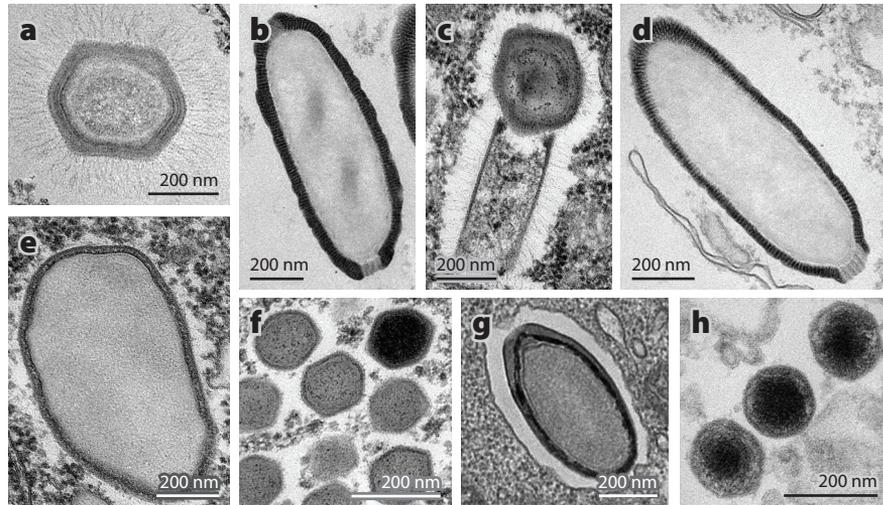
During the first decade after the description of APMV, the coculture strategy for amoebae was mainly based on the use of *Acanthamoeba polyphaga* (Linc-AP1) and *Acanthamoeba castellanii*. A total of 7,000 samples were inoculated to discover the A, B, and C lineages of *Mimiviridae* and *Marseilleviridae* using different cocktails of antimicrobials and using Page's amoebal saline medium to avoid microbial proliferation (28). Some viruses remained unexplored because they were considered endosymbionts (29). This high-throughput procedure paved the way for new viral isolates even though many modifications and adaptations were subsequently made (30). The ingenuity and creativity deployed allowed one to explore and revealed new viruses notably by adapting amoebae as for the isolation of pandoravirus (20) or by a pre-enrichment step using rice grains and incubation of samples in the dark for almost a month, thus resulting in the isolation of megavirus chilensis and samba virus with its virophage (11, 31). Finally, a high-throughput strategy was developed for amoebae by coupling coculture with flow cytometry and electron microscopy (32). In parallel, new cell types such as *Vermamoeba vermiformis* have been explored in an appropriate medium called TS (starvation buffer) (33, 34).

This past decade has shown a rapid expansion of viral isolates on different platforms including nonamoebal hosts (35–38). Ultimately, a high-throughput system was developed to detect nonlytic viruses. This system could be coupled with different image techniques including optic and electron microscopy as well as fluorescence staining for the rapid detection of giant viruses directly from culture (39). Moreover, the use of a new support is always a challenge, such as the use of the xenic amoeba *Stenamoeba* sp. to isolate virus with a virophage named “Sissivirus” (under characterization) (35). Another strategy is based on the separation of amoebae by capturing the infected amoeba via double viral infections as for faustovirus and clandestinovirus via a single microaspiration technique (40). More recently, myriad new viruses have been identified, causing our perception of the virosphere to change regularly.

## Unexpected Diversity of Amoebae Viruses

The family *Mimiviridae* is the most diverse among the groups of giant viruses known to date. Mimiviruses ushered in a new era of virology and expanded the limits of the virosphere in terms of both structure and genomic complexity (41). These viruses have pseudoicosahedral particles approximately 700 nm in diameter with long fibrils immersed in a layer of peptidoglycan-like component, thus justifying the erroneous identification as Gram-positive cocci in the early 1990s (42) (**Figure 1**). This family contains one of the most complex amoebae viruses isolated to date: the tupanviruses (22).

Tupanviruses have a capsid similar to mimiviruses but have a long cylindrical tail attached, thus leading to an average size of 1.2  $\mu\text{m}$  of viral particles (**Figure 1**). In 2017, metagenomic analysis from samples collected at a wastewater treatment plant in Klosterneuburg, Austria, revealed the existence of klosneuviruses (43). These viruses have a unique genomic complexity with several genes related to protein synthesis in an amount and diversity never seen before (second only to tupanviruses) (22, 43). Recently, other members of the possible subfamily “Klosneuvirinae” were identified by metagenomics of soil samples, thus increasing the diversity of this group (44).



**Figure 1**

Diversity of amoebae-infecting viruses. Electron microscopy images evidence the structural diversity and complexity of viruses isolated from different amoebae hosts: (a) mimivirus, (b) cedratvirus, (c) tupanvirus, (d) pithovirus, (e) orpheovirus, (f) marseillevirus, (g) pandoravirus, and (h) kaumoebavirus.

Over the past 15 years, diverse groups of amoeba viruses have been isolated and characterized. Viruses with widely varied structures and genomes were uncovered using *Acanthamoeba* as an isolation platform. Marseilleviruses have icosahedral particles of approximately 250 nm (**Figure 1**) and circular genomes, thus constituting a diverse family of viruses with at least five recognized lineages (A–E) (15–19). Pithoviruses and cedratviruses have ovoid particles that can reach 1.5  $\mu\text{m}$  in length and present one and two cork structures, respectively, in apical positions of the viral particles, i.e., regions where their circular genomes are released during the replication cycle (21, 45) (**Figure 1**). There are currently only a few isolated pithoviruses, but several others have been identified by metagenomics, thus forming the putative family “Pithoviridae” (46, 47). Pandoraviruses are 1.0- $\mu\text{m}$  ovoid particles (**Figure 1**), and linear genomes reach up to 2.7 Mb. These are the largest viral genomes known to date (20, 48). These viruses have already been isolated on different continents, thus revealing distinct lineages and an enormous genetic diversity (48–50). Pandoraviruses are phylogenetically close to molliviruses—another group of *Acanthamoeba*-infecting viruses with only two known isolates (51, 52). Pacmanvirus and medusavirus are other viruses isolated in *A. castellanii*, with two and one known isolates to date, respectively (53–55).

Other groups also infect *Vermamoeba* sp.: Faustoviruses were the first to be isolated in this system with circular genomes and 250-nm icosahedral particles (34). These viruses are phylogenetically close to the kaumoebaviruses—a newly discovered group of viruses with only two isolates to date (56–58) (**Figure 1**). Clandestinovirus was identified in a mixed coculture with a faustovirus and has similar characteristics to medusavirus, possibly constituting a new family called “Medusaviridae” (59). Finally, viruses with very large genomes and particles were also identified and infect *Vermamoeba*. Yasminevirus was the first amoeba virus of “Klosneuvirinae” to be isolated. It has a large set of translation-related genes (60). Orpheovirus is currently the largest virus isolated using *Vermamoeba* and is part of the putative family “Pithoviridae” (61). This virus has ovoid particles nearly 1.1  $\mu\text{m}$  in diameter (**Figure 1**) and a circular genome of 1.4 Mb, in contrast to pithoviruses and cedratviruses with genomes up to 610 kb.

Finally, a recent analysis by global metagenomics highlighted the enormous diversity of giant viruses. There are more than 2,000 new viral genomes, thus increasing the phylogenetic diversity of NCLDVs by eleven-fold in relation to what was previously known (8). This reveals the importance of this type of analysis and expands our knowledge about viral diversity. It is also an important limitation because it highlights fundamental questions about the biology of these viruses including the difficulty of identifying rare viruses in the environment. This was evidenced with the discovery of yaravirus—a small amoeba virus with 90% of the gene content unknown and undetectable in a set of more than 8,500 available metagenomes (62).

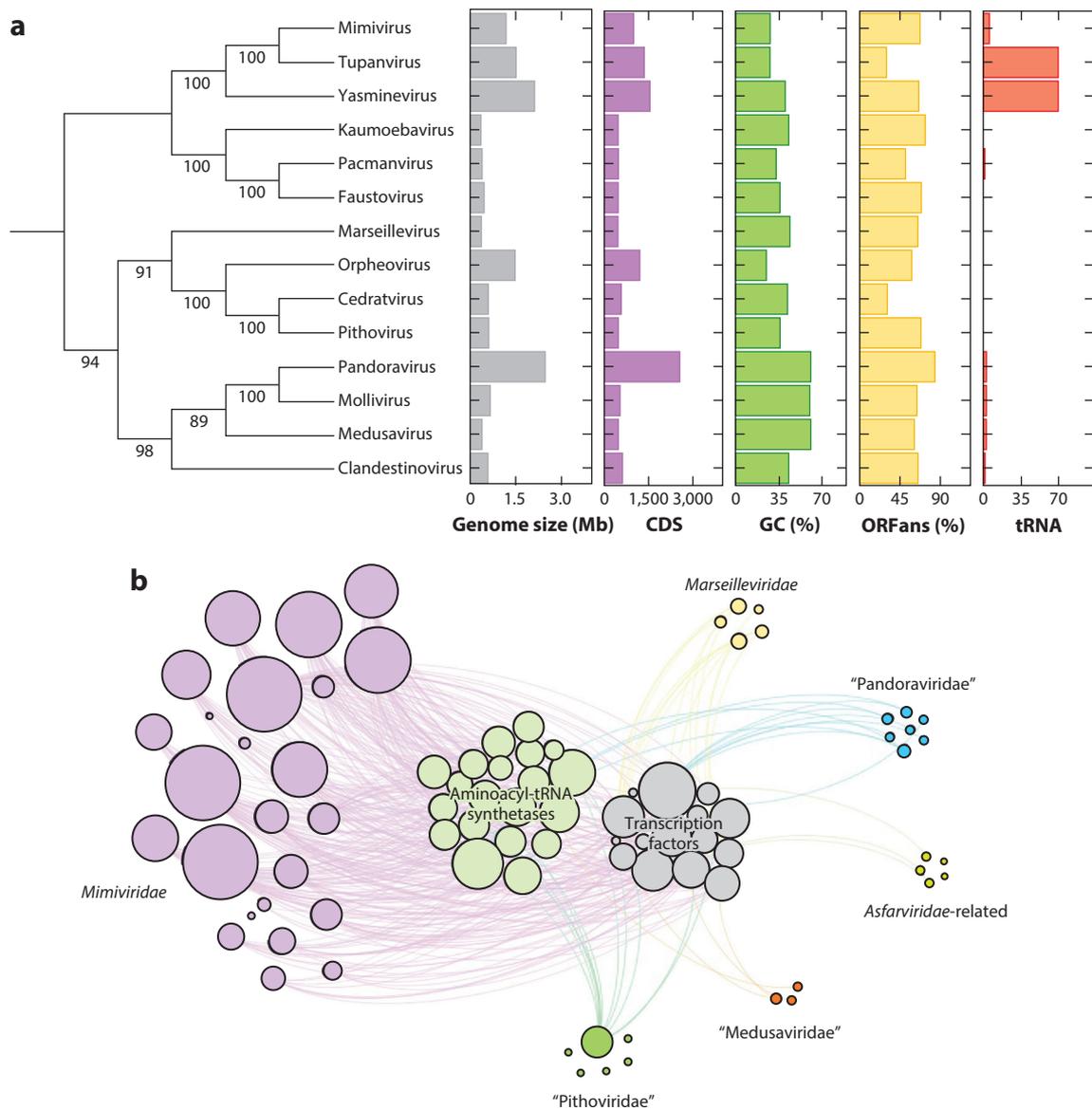
## BASIC VIROLOGY

### The Complex Genomes of Amoebae Viruses

One of the most striking features of viruses is the presence of short genomes encoding only a few genes due to their intracellular parasitic nature. However, some viruses break this pattern and encode hundreds of genes such as large DNA viruses such as poxviruses, chloroviruses, and jumbophages (63–65). The discovery of mimiviruses crossed this limit—these viruses have genomes over 1.0 Mb that encode more than 1,000 genes, thus exhibiting a genomic complexity never conceived of before in the virosphere (66) (**Figure 2a**). Mimiviruses have dozens of genes related to metabolic processes with an emphasis on elements related to protein translation—something hitherto exclusive to the cellular world (10, 66). Proteins such as IF4A, eF-TU, and eRF1 are involved in initiation, elongation, and release of the polypeptide chain, respectively. These are found in mimivirus genomes and a diverse set of aminoacyl-transfer RNA (tRNA) synthetases (aaRSs)—this is unprecedented in the virosphere (10). The discovery of klosneuviruses and later tupanviruses dramatically increased the genomic complexity of *Mimiviridae*. These viruses have 20 aaRSs covering all amino acids and up to 70 tRNAs covering all cognate amino acids found in the tupanvirus genome (22, 43) (**Figure 2a,b**). Other giant viruses such as pandoraviruses and orpheovirus also have genes related to protein synthesis, but in much less abundance and diversity than *Mimiviridae* (20, 61) (**Figure 2b**).

In addition to the translational machinery, other features stand out in the genome of several groups of giant viruses. Marseilleviruses, medusavirus, and clandestinovirus have genes that encode histones. These proteins are homologous to those found in eukaryotes and are involved in the formation of nucleosomes and DNA organization (54, 59, 67, 68). Mimiviruses have genes related to post-translational modification processes and are involved in different sugar pathways such as UDP-D-viosamine and UDP-L-rhamnose, thus having elements that form their own glycosylation machinery (69, 70). Pandoraviruses have a set of enzymes involved in the tricarboxylic acid cycle for energy production. These genes are expressed during the viral replication cycle, and at least one of the encoded proteins was verified to have biological activity based on isocitrate dehydrogenase—a key enzyme in the process (71). Furthermore, some giant viruses have their own set of mobile genetic elements (named as mobiloma) such as mimiviruses, marseilleviruses, and faustoviruses that have genes containing introns [e.g., RNA polymerase and major capsid protein (MCP)] and the presence of linear transposable elements, or transpovirons, detected in all three mimivirus lineages (72–75). Furthermore, mimiviruses have satellite-like viruses that depend on their enzymatic machinery for replication, called virophages, which can also integrate into the genomes of their hosts, thus forming proviropages (7, 75).

Finally, a surprising amount of ORFans in all known amoeba viruses reached approximately 90% in pandoraviruses (20) (**Figure 2a**). This reflects the enormous diversity and genetic novelty in these viruses and highlights the importance of isolating and characterizing new amoeba viruses. Furthermore, pan-genome studies performed with different groups of amoeba viruses such as



**Figure 2**

The genomic complexity of giant viruses. (a) Phylogenetic tree based on concatenate alignment of the three most conserved genes among *Nucleocytoviricota* (DNA polymerase B family, D5-primase-helicase, and viral late transcription factor 3) including representatives of different groups of giant viruses of amoebae. Simple bar charts indicate general genomic features of these viruses. (b) Network graph connecting representatives of distinct groups of giant viruses of amoebae to translation-related elements. Aminoacyl-tRNA synthetases and translation factors (initiation, elongation, and release) are represented with light green and gray colors, respectively. Different groups of viruses are indicated with distinct colors. The node sizes are proportional to the degree of connections. Abbreviations: CDS, coding DNA sequence; GC (%), percentage of guanine and cytosine content in the nucleic acid; ORFans (%), percentage of protein-coding regions that have no recognizable homolog; tRNA, transfer RNA.

mimivirus, marseillevirus, faustovirus, and pandoravirus revealed an open pan-genome, showing that the genomic diversity and complexity of amoeba viruses is far from reaching stability (18, 50, 56, 76).

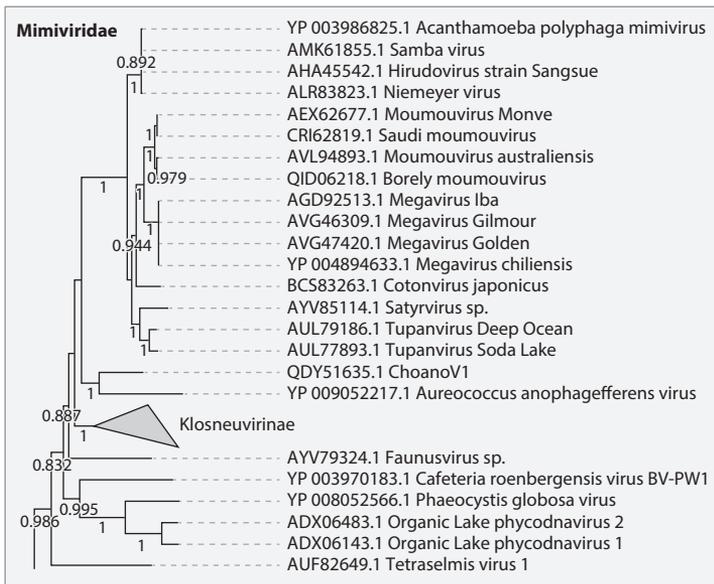
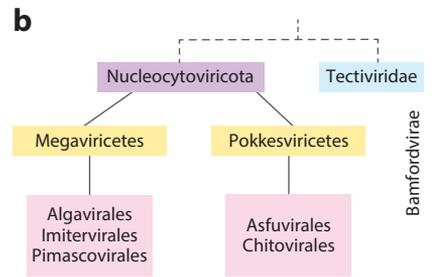
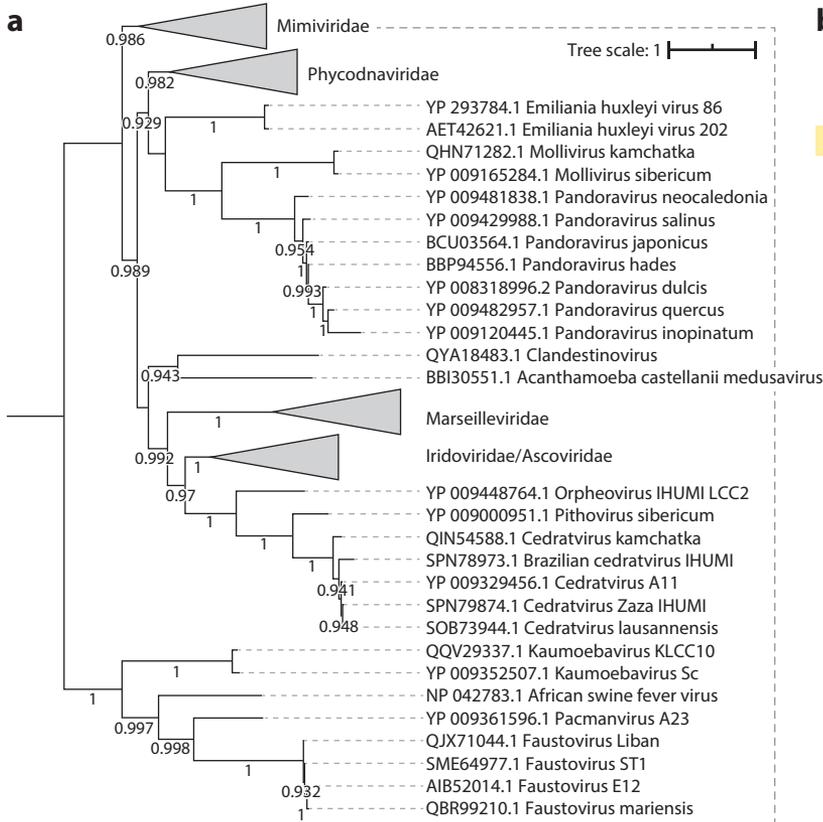
## Evolution and Taxonomy of Giant Viruses

After the discovery of mimiviruses and other amoeba viruses, they were grouped together with NCLDV [now officially the phylum *Nucleocytoviricota*, kingdom *Bamfordvirae* (23)]. Some genes are conserved in most known viruses in this group, e.g., DNA polymerase B family, which allows for reconstruction of the evolutionary history of the group, thus evidencing the phylogenetic relationship between the different amoeba viruses (**Figure 3a**). Mimiviruses are phylogenetically close to phycodnaviruses including some viruses formerly included in the family *Phycodnaviridae* that should be reclassified and will be part of the *Mimiviridae* family. It is now clear that both families group together viruses that infect microalgae found either in freshwater or in the marine environment (77, 78). These families are currently included in the orders *Algavirales* and *Imitervirales*, respectively. Both are included in the class *Megaviricetes* (**Figure 3b**). Pandoraviruses and molliviruses are not yet officially classified into any group, but they have a close relationship to viruses of the *Phycodnaviridae* family—especially the coccolithoviruses (**Figure 2a**), thus suggesting their inclusion in the order *Algavirales*. Marseilleviruses and pithoviruses, as well as related viruses (cedratviruses and orpheovirus), are phylogenetically close to iridoviruses and ascoviruses; they are classified in the order *Pimascovirales* (**Figure 3**). Other large amoeba viruses such as faustoviruses, pacmanviruses, and kaumobaviruses are phylogenetically related to the African swine fever virus, family *Asfarviridae*, thus suggesting that they belong to the order *Asfuvirales*, class *Pokkesviricetes* (**Figure 3b**). Other evidence points to a common origin among these viruses as regulatory elements of gene transcription (79), although new and more robust analyses must be conducted to better elucidate the relationship between these amoeba viruses and the asfarviruses.

Other evidence suggests a common origin of these giant viruses where they would have shared a common ancestor with smaller viruses—possibly belonging to the group of bacteriophages of the family *Tectiviridae* (80, 81) (**Figure 3b**). Molecular and morphological aspects support this hypothesis (82). Phylogenetic analyses based on different genes conserved within *Nucleocytoviricota* point to a common origin of these viruses that would have occurred before the emergence of modern eukaryotes from smaller viruses, thus emphasizing how ancient giant viruses are (83). Furthermore, analyses based on genes associated with the protein translation process point to a similar pathway in which the giant amoeba viruses would have acquired several genes through horizontal gene transfer events, thus substantially increasing the size and genomic complexity of these viruses (43, 81). However, analyses based on different proteins of these viruses point to an opposite pathway in which giant viruses coexist with cellular ancestors as they were a separate group of complex organisms that evolved mostly due to a loss of genes and adaptation to a parasitic lifestyle (84, 85). Given this scenario, further studies must be carried out to settle this important question about giant amoeba viruses leading to important information about the origin and evolution of one of the most complex groups in the virosphere.

## Structure of Giant Viruses

Giant viruses of amoeba have complex particles that come in a wide variety of shapes and sizes (15, 21, 22, 51, 59–62, 86–91). Isolates with smaller dimensions have particles of icosahedral symmetry (15, 34, 53, 54, 57, 59, 60, 62, 87). Some (faustoviruses, kaumobaviruses, and yaraviruses) seem to present two-capsid protein shells (62, 86). Different shapes start to appear in particles with 400 nm or more. Molliviruses have spherical particles ranging from 500 to 600 nm (51, 52).



(Caption appears on following page)

**Figure 3** (Figure appears on preceding page)

Evolution and classification of giant amoeba viruses. (a) Maximum likelihood phylogenetic tree of *Nucleocytoviricota* based on the DNA polymerase B family gene. Sequences in amino acid were aligned using Multiple Sequence Comparison by Log-Expectation (MUSCLE) software. The tree was built using FastTree with the Jones-Taylor-Thornton (JTT) model and 1,000 bootstrap replicates and then visualized in Interactive Tree Of Life (iTOL). The *Mimiviridae* clade is shown in the light gray box. (b) Schematic representation of the classification and evolution of giant viruses based on the International Committee on Taxonomy of Viruses–approved megataxonomy. A putative evolutionary relationship between *Nucleocytoviricota* and *Tectiviridae* is represented by dotted lines.

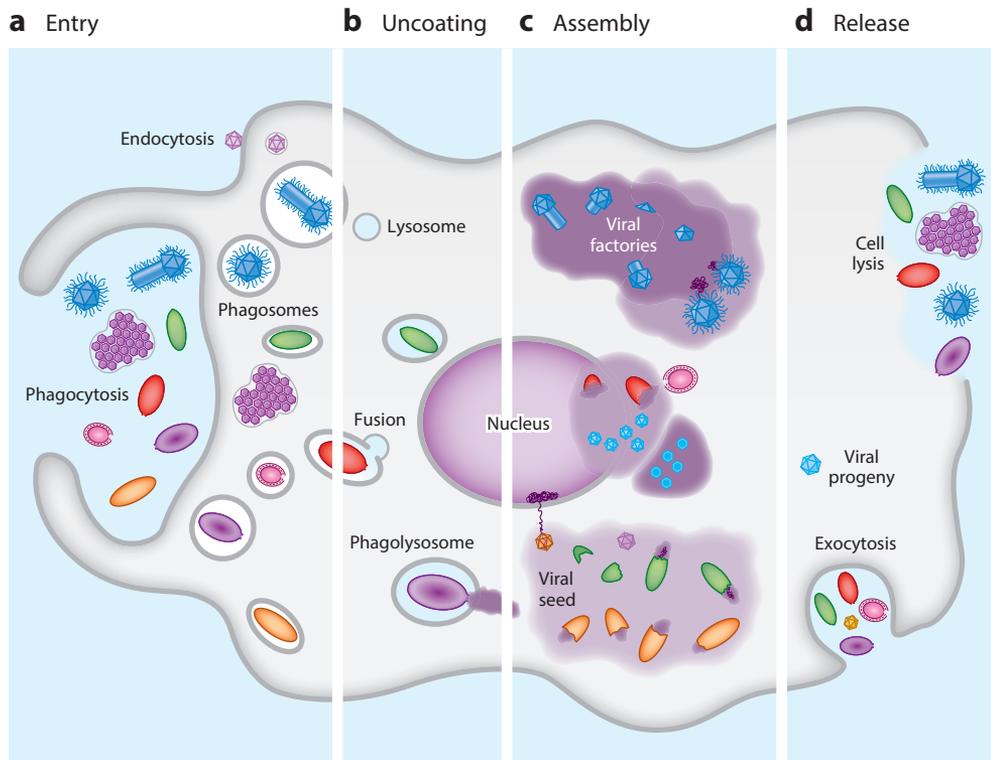
Pandoraviruses, orpheovirus, pithoviruses, and cedratviruses have ovoidal shaped particles that frequently present over 1,000 nm in length (20, 21, 45, 61). Different formats of particles can be observed among representatives of the family *Mimiviridae*. Their capsids have a pseudo/quasi-icosahedral symmetry and are often covered by fibrils, ranging from 450 to 527 nm in representatives of the genus *Mimivirus*, making the total particle size range between 700 and 834 nm in diameter (88). Although tupanviruses' capsids are similar to mimiviruses', these viruses' particles have a complex symmetry in which the capsid is followed by a cylindrical tail that varies in size in the viral progeny (22).

Furthermore, it is possible to observe the delivery portal in those larger particles—a differentiated area of the capsid through which the internal content of the particle is released. The delivery portal in molliviruses is a circular depression 160–200 nm in diameter (51). For pandoraviruses and orpheovirus, the internal content is released through an ostiole-like structure located on the apex of the particle (49, 92). In pithoviruses and cedratviruses, the delivery portal is sealed by a structure named cork. While pithoviruses present a single cork on the apex of the particle (21), cedratviruses present two corks—one at each end of the particle—but the internal content is delivered just from one (91). Capsids within family *Mimiviridae* members present a fivefold vertex called stargate that is sealed by a proteinaceous complex, the starfish, which collectively prevents the capsid from opening (93). The processes that trigger the opening of the capsids are described in the section titled Replication Cycle. The delivery portal is usually not covered by fibrils, whether they are short (molliviruses and orpheovirus) or long (family *Mimiviridae*) (51, 92, 94).

Proteomic analysis of the purified viral particles showed that the majority of amoebal viruses' particles are composed of a large number of proteins ranging from 26 to over 200 proteins, thus forming a mature particle of different groups (62, 95). Studies have shown that a smaller number of proteins are detected in the mature viral particle when compared to the diversity of proteins present in the viral factory. Moreover, the number of proteins is not associated with the particle or genome size (21, 22, 95). In contrast to icosahedral viruses, the MCP of marseilleviruses is not the most abundant protein in the mature viral particle (95). Even so, proteomic analyses reveal that most of the proteins that form amoebal viruses' particles still have no known function or orthologs (54, 62, 95, 96). Nevertheless, other methodologies have helped to elucidate some components of viral particles. A recent study demonstrated that the *Pandoravirus massiliensis* capsid is composed of a peripheral layer of polysaccharide nature that resembles cellulose (97). Interestingly, *P. massiliensis* particles also present membrane potential (proton gradient). This result, in association with bioinformatics data, suggests that the *P. massiliensis* genome has distant homolog genes predicted to encode tricarboxylic acid elements. This raises questions about energy generation associated with pandoravirus particles during replication (71).

## Replication Cycle

Many viruses within the family *Mimiviridae* present fibrils on the surface of the capsid (11, 22, 94, 98). In APMV, fibrils play a role in particle attachment on the amoeba's surfaces (99). But the fibrils do not seem to be essential for viral replication, since the virus loses many fibrils when passaged



**Figure 4**

Representative scheme of the amoeba viruses' replication cycle. (a) Giant virus entry can occur through the phagocytic pathway or endocytic pathway. (b) The drop in pH due to fusion with the lysosome triggers the release of the viral seed. (c) Establishment of the viral factories where the assembly of the viral progeny takes place. (d) Viral release can occur via exocytosis, cell lysis, or both.

multiple times in cell culture (100). Other giant viruses also have fibrils and, although shorter, they can also play a similar role in viral particle adhesion: the  $\sim 12$ -nm fibrils of marseilleviruses, the 50- to 100-nm fibrils of molliviruses, the  $\sim 50$ -nm fibrils of orpheovirus, and amoeba klosneuvirus fibrils (15, 51, 60, 87, 92). Aside from fibrils, medusavirus presents flexible spherical-headed spikes of  $\sim 14$  nm that also might be involved with viral attachment and entry (54).

The size of the particle is a determinant element for the initial steps of the replication cycle. It was experimentally demonstrated that latex beads over 500 nm trigger phagocytosis in *Acanthamoeba* cells (101). Many giant viruses have dimensions greater than 500 nm, and it has been frequently hypothesized that they are consequently phagocytosed (61, 92, 102) (**Figure 4a**). In fact, being able to explore the phagocytic pathway as an entry route seems so advantageous that strategies to bypass the minimum size requirement were selected in the smaller giant viruses. This is the case of 250-nm viruses within the *Marseilleviridae* family. By the end of their replication cycle, the viral progeny can be grouped together, thus forming viral aggregates inside or outside vesicles that can reach more than 1,000 nm and are successfully phagocytosed. In addition to the phagocytic pathways, individual particles can enter the host cell through endocytosis (103) (**Figure 4a**). The endocytic pathway might be a route for other amoebal viruses that do not fulfill the size requirement as suggested for yaravirus (62). Another entry route for these viruses can be macropinocytosis (103).

Once inside the host cell, the particle uncoating generally occurs during the endosomal or phagosomal escape. Some redox proteins have been described as components of amoebal virus

virions and may interact with oxidative elements inside amoebal endosomes and phagosomes. An inner membrane has been described inside most giant virus capsid/tegument shells. This membrane is essential during virus uncoating because it fuses with the cell compartment membrane (endosome/phagosome). Such fusion occurs through the delivery portal whose opening is triggered by a drop in pH. This is likely due to fusion with lysosomes during the natural course of infection (94). Low pH alone was insufficient to fully open samba virus and tupanvirus capsids in *in vitro* studies, thus indicating that other interactions are required for the complete release of the internal contents (93) (**Figure 4b**).

After the internal content/viral seed is released inside the host cell, an intense rearrangement occurs until the formation of a mature viral factory. In most giant viruses, this formation occurs near to the amoeba's nucleus, but the nucleus is no longer observed after the formation of the mature viral factory of pandoraviruses, molliviruses, yaraviruses, and clandestinovirus (51, 59, 62, 89). Some viral factories have specialized areas, which are important for particle morphogenesis. The periphery of mimiviruses' electron-dense viral factory is electron lucent and has an apparent fibrillar nature. When the newly formed capsids pass through this region, they acquire long polysaccharidic fibrils. Therefore, this part of the viral factory was named the fibril acquisition area (94). Furthermore, the electron-lucent viral factory of yaravirus presents an electron-dense periphery. When the newly formed empty capsids migrate to this part of the viral factory, they are filled with their internal content (62) (**Figure 4b,c**).

For most amoebal viruses, DNA replication occurs inside the viral factory, except for medusaviruses, whose DNA replicates in the periphery of the host nucleus, and the particle morphogenesis occurs in the cytoplasmic viral factory (54). Along with medusavirus and the viruses that form their viral factories in the cell nucleus, other viruses, such as marseilleviruses, also depend on host nuclear functions for their replication. Marseilleviruses replicate in the cytoplasm, but they transiently recruit the nuclear transcription machinery to their cytoplasmic viral factory to generate their earliest transcripts (95) (**Figure 4c**).

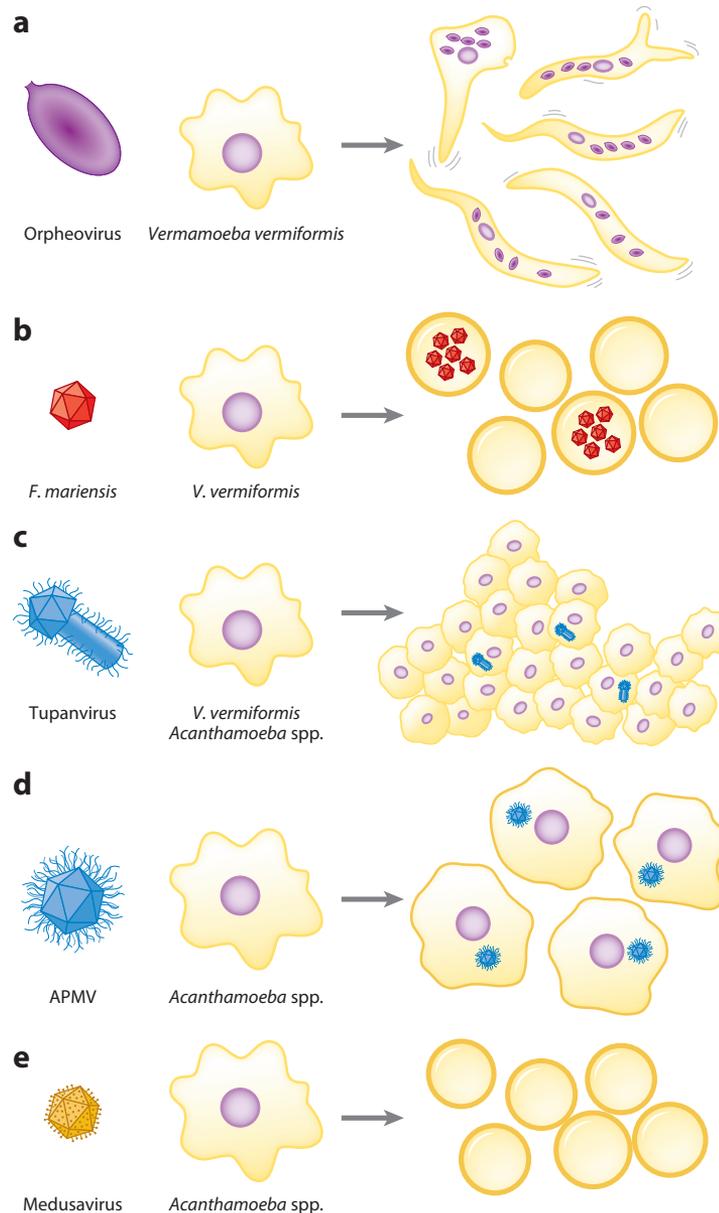
The particle morphogenesis and the internal content of marseilleviruses, molliviruses, pandoraviruses, and pithoviruses were described to occur simultaneously (15, 21, 51, 89), but these can also occur separately. Most giant viruses begin particle morphogenesis with the formation of semicircular structures, named crescents. These are usually filled by the internal content as they expand (62, 91, 92, 94, 104). For mimiviruses, the capsid assembly starts with the formation of the stargate, and genome acquisition then occurs through the opposite vertex concomitantly with fibril acquisition (94) (**Figure 4c**).

Almost every infection culminates in cellular lysis at the end of the replication cycle, but yaravirus, pandoraviruses, orpheovirus, and cedratviruses start releasing their particles before the cell lysis by exocytosis (62, 89, 91, 92). Only clandestinovirus and molliviruses seem to not cause significant cell lysis (51, 59) (**Figure 4d**).

## VIRUS-HOST INTERACTION

### Virus-Amoeba Interaction

To provide a good understanding of the relationships surrounding a virus and its host, studies generally focus on key elements regarding the contagium of that cell. During infection by giant viruses, a series of structural and morphological changes occur in the amoebal cells as part of the natural outcome of most viral infections (**Figure 5**). These changes, also called cytopathic effects (CPEs), are generally the consequence of an efficient viral replication and have been extremely important to help identify novel isolates of giant viruses (105). Each cell may develop different CPEs depending on which virus is currently replicating. For most infections involving amoebae and giant



**Figure 5**

Representative scheme of the CPEs. (a) Orpheovirus causes cells to shift to a stretched fusiform shape during the early stages of infection. (b) *Faustovirus mariensis*-infected trophozoites release a soluble factor that promotes the encystment of infected and uninfected cells. (c) Tupanvirus's infected cells form bunches with uninfected cells. (d) APMV decreases the levels of serine-proteinase transcription and translation of infected cells, thus preventing encystation. (e) Medusavirus stimulates an encystment-like process. Abbreviations: APMV, *Acanthamoeba* polyphaga mimivirus; CPE, cytopathic effect.

viruses, the standard observed CPE involves progressive cell rounding and loss of the trophozoite motility and lysis with subsequent release of the viral progeny from inside the cell (106). Amoebal viruses found in the above-described CPEs are mimiviruses, marseilleviruses, pandoraviruses, cedratviruses, faustoviruses, yasminevirus, and yaravirus (19, 34, 49, 60, 62, 91, 106, 107).

Different sets of CPEs may be observed in a different context of infection by other groups of amoebal viruses (**Figure 5**). For example, in addition to promoting rounding of *A. castellanii* cells, infection by medusaviruses was described as stimulating an encystment-like process of a subpopulation of amoebal cells as early as 2 days post infection (54) (**Figure 5e**). A similar effect was also observed for *Vermamoeba vermiformis* cells when infected by the giant *Faustovirus mariensis* (104). A detailed description of the replication cycle of orpheovirus showed the occurrence of different steps of CPEs before amoebal cells lyse (92). During the Souza et al. study (92), the authors found that *V. vermiformis* amoebae shift to a stretched fusiform shape as early as 3 hpi. The effect becomes more obvious as the time of infection progresses. At 12 hpi, the morphology evolves to a branched fusiform shape. Later, around 24 hpi, amoebae start to emerge as rounded cells until they are lysed by the virus (92) (**Figure 5a**).

In another study, an interesting biological feature that links the successful replicative cycle of tupanvirus to the CPEs that are established during infection of *A. castellanii* cells was described (108). When amoebae are infected by tupanviruses, they progressively develop some of the standard effects described above for most of the infections with giant viruses. First, the cell becomes round. Then the massive viral production forces lead to amoebal lysis. However, when these amoebal cells are just beginning to become round, several neighboring uninfected cells are induced to aggregate to them and form bunches with other cells that were already infected (108). This behavior was very important during infection, thus establishing an advantage for viral dissemination. Such behavior has also been linked to relevant changes in the expression of mannose-binding protein genes by tupanvirus (108) (**Figure 5c**). Interestingly, a similar CPE has also been described for viruses belonging to a marseillevirus lineage (109).

As known hosts of giant viruses, amoebae of the *Acanthamoeba* and *Vermamoeba* genera may also play important roles in many of the biological and evolutionary aspects of the giant viruses mentioned above. Both amoebae are considered to be free-living organisms and are encountered in a diversity of environments with a worldwide distribution (110, 111). Amoebae's life cycle proceeds through two stages: The first is called the trophozoite and is a form that is generally observed under favorable growth conditions (abundant food supply, appropriate temperature, neutral pH) (110, 111). During this stage, amoebae are motile and can perform most of their life tasks including feeding and reproducing (110, 111). The second stage, best known as cysts, is a natural form of resistance against adverse/stressful conditions. In contrast to trophozoites, the cysts are static (110–112).

Since the discovery of the first amoebal giant viruses, only a few studies have investigated aspects of viral infection in a context that considers both cellular stages. One of the first studies regarding this subject explored some specific kinds of interactions among the APMV and amoebae of the *Acanthamoeba* spp. in the framework of the encystment stimulation commonly observed in the natural environment. *Acanthamoeba* cysts are resistant to APMV infection (113). Interestingly, this resistance was maintained as long as the stimulus for cell encystment was triggered before the viral infection, even if the amoebae had not yet suffered massive turnover of the cellular components necessary to become a cyst (113). However, the protection is severely nullified when the mimivirus gets to the cell before the stimulus of encystation. The virus can then infect the amoeba (113). Strategies for viral evasion of this antiviral mechanism were also uncovered. Prior work found a serine proteinase that is a crucial component in mediating *A. castellanii* cells to encyst (114). Mimivirus decreases the levels of amoebal serine-proteinase transcription and translation,

thus preventing *Acanthamoeba* encystations (113) (**Figure 5d**). Finally, a novel antiviral mechanism involving *F. mariensis* and its amoebal host, *Vermamoeba vermiformis*, was described (104).

Giant viruses invade amoebal trophozoites and use their metabolic machinery to produce the viral progeny and induce cell lysis to disseminate the progeny. However, upon *F. mariensis* infection, *V. vermiformis* trophozoites release a soluble factor (likely containing  $Mg^{2+}$ ). This soluble factor induces encystment of neighboring cells to enclose faustovirus progeny inside the newly formed cysts. These cysts are no longer viable and therefore reduce the viral progeny in the supernatant (104) (**Figure 5b**).

## Virophages

The discovery in 2008 of satellite-like viruses, named Sputnik virophages, was exciting in the biology of giant amoeba virus. Virophages are large-viruses dependent or associated viruses classified in the family *Lavidaviridae*. They have 50- to 70-nm icosahedral capsids and double-stranded DNA genomes ranging from 17 to 30 kb, and they are dependent on giant viruses to complete their replication in host cells. The fundamental basis of the virophage/giant virus relationship still requires further study, but virophages might exploit the giant viruses' transcriptional machinery especially during the late phase of giant virus replication. Consequently, virophages might impact, to different degrees, the production of giant viruses' progeny. Virophages have a structural module (MCP with a double-jelly roll structure), thus suggesting a common origin among those viral entities (7, 115–118). Although virophages were first described in association with amoebal mimiviruses, a few years later, a novel virophage named mavirus was described in association with Cafeteria roenbergensis virus (CrOV) infecting the flagellate *Cafeteria roenbergensis*. Later metagenomics studies revealed that virophages are widespread especially in aquatic environments (116–119).

Comprehensive studies have demonstrated that the virophage-giant virus-protist tripartite relationship is complex and ancient. Remarkably, virophages seem to integrate into either giant viruses or protist genomes (provirophages). In one notable example, the virophage mavirus could integrate into the chromosome of the flagellate *C. roenbergensis* (120). Virophage genes are expressed upon CrOV infection, thus resulting in production of mavirus virions. Such newly formed virophage progeny promote the overall protection of the flagellate *C. roenbergensis* population. In contrast, giant viruses also developed mechanisms to mitigate virophage impacts on their replication. A defense system against a specific group of virophages (group named Zamilon) has been described in mimiviruses lineage A. The mechanisms of the so-called mimivirus virophage resistance element system need further investigation, but it was hypothesized that it acts analogously to the CRISPR-Cas system and is guided by the *R349* gene containing 15- or 28-nucleotide Zamilon-like repeats (121). Therefore, the discovery of virophages has fueled debates regarding the nature of nested parasitism and the definition of satellite viruses.

## FINAL CONSIDERATIONS

Throughout history, the study of viruses has provided unique insights to understand complex events of nature. This has helped to develop biology and anthropology (4). The discovery of giant viruses broke the mold of classical virology and demonstrated that even the most accepted definitions within basic science are not immutable. These new findings open the field to even more exciting discoveries in the future (6). New advances in viral prospecting and isolation are needed to explore this new field of study. In addition to changes in isolation techniques, the use of other amoebae as prospecting platforms has contributed to the discovery of new viruses. This has led to a broader view of the virosphere (34). Even with the diversity of amoeba viruses already isolated,

some viral groups still have only one representative isolated (59, 61, 62). Metagenomic studies have indicated that giant viruses are diverse and ubiquitous on Earth (8). Indeed, different representatives of giant viruses have been isolated among the most diverse samples worldwide, but some of these viruses have such singular genomes that isolation and characterization studies are critical to identify these organisms. It could not be done solely by metagenomics (62). Therefore, further prospecting and characterization studies are essential to understand the virosphere.

Although APMV was found in 1992, giant viruses that infect amoebae remained hidden in plain sight for more than 10 years without being identified as viruses (6). One of the main reasons why this organism was not considered to be a virus was its large size, which contradicted the definition of viruses at the time (6). In fact, giant viruses' particles are so unique and diverse that some reported finding a noncultivable endocytobiont even after the description of APMV as a virus (29, 122, 123).

A universe of viral diversity was revealed through prospective and characterization studies of giant viruses. This work significantly contributed to our biological understanding of these unique viruses and the virosphere. Nevertheless, much remains to be elucidated about giant viruses, and studies regarding these viruses remain an important field of research to understand possible ecological roles, the host spectrum and evolutionary aspects of this group, and the biological diversity of viruses.

## DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

## ACKNOWLEDGMENTS

We are thankful for our colleagues from GEPViG and the Laboratório de Vírus, Universidade Federal de Minas Gerais, for their support over the last 11 years. We also thank Pro-Reitoria de Pesquisa and Pós-Graduação da UFMG, CAPES, CNPq, and FAPEMIG for scholarships and other financial supports. J.S.A. is a CNPq researcher. J.S.A. and B.L. are thankful to the Editorial Committee for inviting our teams to write this review in collaboration. The data presented here were partially or fully registered at SISGEN (AA3B21E, A702EB8, A25764F, AC31840, A473BD3, A3DAB3E, AC3045D, A96431C, ABF23CC, A2F8816, A580BBD, and AEC3EAA). Collection authorization SISBIO numbers are 33326, 34293, and 80252.

## LITERATURE CITED

1. Suttle CA. 2005. Viruses in the sea. *Nature* 437(7057):356–61
2. Suttle CA. 2007. Marine viruses—major players in the global ecosystem. *Nat. Rev. Microbiol.* 5(10):801–12
3. Prangishvili D, Bamford DH, Forterre P, Iranzo J, Koonin EV, Krupovic M. 2017. The enigmatic archaeal virosphere. *Nat. Rev. Microbiol.* 15(12):724–39
4. Flint J, Racaniello VR, Rall GF, Hatzioannou T, Skalka AM. 2020. *Principles of Virology*. Washington, DC: ASM. 5th ed.
5. Rodrigues RA, dos Santos Pereira Andrade AC, Boratto PV, Trindade GD, Kroon EG, Abrahão JS. 2017. An anthropocentric view of the virosphere-host relationship. *Front. Microbiol.* 8:1673
6. La Scola B, Audic S, Robert C, Jungang L, de Lamballerie X, et al. 2003. A giant virus in amoebae. *Science* 299(5615):2033
7. La Scola B, Desnues C, Pagnier I, Robert C, Barrassi L, et al. 2008. The virophage as a unique parasite of the giant mimivirus. *Nature* 455(7209):100–4

8. Schulz F, Roux S, Paez-Espino D, Jungbluth S, Walsh DA, et al. 2020. Giant virus diversity and host interactions through global metagenomics. *Nature* 578:432–36
9. Endo H, Blanc-Mathieu R, Li Y, Salazar G, Henry N, et al. 2020. Biogeography of marine giant viruses reveals their interplay with eukaryotes and ecological functions. *Nat. Ecol. Evol.* 4(12):1639–49
10. Raoult D, Audic S, Robert C, Abergel C, Renesto P, et al. 2004. The 1.2-megabase genome sequence of Mimivirus. *Science* 306:1344–50
11. Arslan D, Legendre M, Seltzer V, Abergel C, Claverie J-M. 2011. Distant Mimivirus relative with a larger genome highlights the fundamental features of Megaviridae. *PNAS* 108(42):17486–91
12. Yoosuf N, Yutin N, Colson P, Shabalina SA, Pagnier I, et al. 2012. Related giant viruses in distant locations and different habitats: *Acanthamoeba polyphaga moutouvirus* represents a third lineage of the *Mimiviridae* that is close to the *Megavirus* lineage. *Genome Biol. Evol.* 4(12):1324–30
13. Ghedin E, Claverie JM. 2005. Mimivirus relatives in the Sargasso sea. *Virol. J.* 2:62
14. Mihara T, Koyano H, Hingamp P, Grimsley N, Goto S, Ogata H. 2018. Taxon richness of “Megaviridae” exceeds those of bacteria and archaea in the ocean. *Microbes Environ.* 33(2):162–71
15. Boyer M, Yutin N, Pagnier I, Barrassi L, Fournous G, et al. 2009. Giant Marseillevirus highlights the role of amoebae as a melting pot in emergence of chimeric microorganisms. *PNAS* 106(51):21848–53
16. Thomas V, Bertelli C, Collyn F, Casson N, Telenti A, et al. 2011. Lausannevirus, a giant amoebal virus encoding histone doublets. *Environ. Microbiol.* 13(6):1454–66
17. Aherfi S, Boughalmi M, Pagnier I, Fournous G, La Scola B, et al. 2014. Complete genome sequence of Tunisivirus, a new member of the proposed family *Marseilleviridae*. *Arch. Virol.* 159(9):2349–58
18. Dornas FP, Assis FL, Aherfi S, Arantes T, Abrahão JS, et al. 2016. A Brazilian Marseillevirus is the founding member of a lineage in family *Marseilleviridae*. *Viruses* 8(3):76
19. dos Santos RN, Campos FS, Medeiros de Albuquerque NR, Finoketti F, Côrrea RA, et al. 2016. A new marseillevirus isolated in Southern Brazil from *Limnoperna fortunei*. *Sci. Rep.* 6:35237
20. Philippe N, Legendre M, Doutre G, Couté Y, Poirot O, et al. 2013. Pandoraviruses: amoeba viruses with genomes up to 2.5 Mb reaching that of parasitic eukaryotes. *Science* 341(6143):281–86
21. Legendre M, Bartoli J, Shmakova L, Jeudy S, Labadie K, et al. 2014. Thirty-thousand-year-old distant relative of giant icosahedral DNA viruses with a pandoravirus morphology. *PNAS* 111(11):201320670
22. Abrahão J, Silva L, Silva LS, Khalil JYB, Rodrigues R, et al. 2018. Tailed giant Tupanvirus possesses the most complete translational apparatus of the known virosphere. *Nat. Commun.* 9(1):749
23. Koonin EV, Dolja VV, Krupovic M, Varsani A, Wolf YI, et al. 2020. Global organization and proposed megataxonomy of the virus world. *Microbiol. Mol. Biol. Rev.* 84:e00061-19
24. Rowbotham TJ. 1980. Preliminary report on the pathogenicity of *Legionella pneumophila* for freshwater and soil amoebae. *J. Clin. Pathol.* 33(12):1179–83
25. Rowbotham TJ. 1983. Isolation of *Legionella pneumophila* from clinical specimens via amoebae, and the interaction of those and other isolates with amoebae. *J. Clin. Pathol.* 36(9):978–86
26. Birtles RJ, Rowbotham TJ, Michel R, Pitcher DG, La Scola B, Raoult D. 2000. ‘*Candidatus* Odysseella thessalonicensis’ gen. nov., sp. nov., an obligate intracellular parasite of *Acanthamoeba* species. *Int. J. Syst. Evol. Microbiol.* 50:63–72
27. La Scola B, Birtles RJ, Greub G, Harrison TJ, Ratcliff RM, Raoult D. 2004. *Legionella drancourtii* sp. nov., a strictly intracellular amoebal pathogen. *Int. J. Syst. Evol. Microbiol.* 54:699–703
28. Pagnier I, Reteno DG, Saadi H, Boughalmi M, Gaia M, et al. 2013. A decade of improvements in *Mimiviridae* and *Marseilleviridae* isolation from amoebae. *Intervirology* 56(6):354–63
29. Scheid P, Zöller L, Pressmar S, Richard G, Michel R. 2008. An extraordinary endocytobiont in *Acanthamoeba* sp. isolated from a patient with keratitis. *Parasitol. Res.* 102(5):945–50
30. Khalil JYB, Andreani J, La Scola B. 2016. Updating strategies for isolating and discovering giant viruses. *Curr. Opin. Microbiol.* 31:80–87
31. Campos RK, Boratto PV, Assis FL, Aguiar ER, Silva LCF, et al. 2014. Samba virus: a novel mimivirus from a giant rain forest, the Brazilian Amazon. *Virol. J.* 11(1):95
32. Khalil JYB, Robert S, Reteno DG, Andreani J, Raoult D, La Scola B. 2016. High-throughput isolation of giant viruses in liquid medium using automated flow cytometry and fluorescence staining. *Front. Microbiol.* 7:26

33. Dornas FP, Khalil JYB, Pagnier I, Raoult D, Abrahão J, La Scola B. 2015. Isolation of new Brazilian giant viruses from environmental samples using a panel of protozoa. *Front. Microbiol.* 6:1086
34. Reteno DG, Benamar S, Khalil JB, Andreani J, Armstrong N, et al. 2015. Faustovirus, an asfarvirus-related new lineage of giant viruses infecting amoebae. *J. Virol.* 89(13):6585–94
35. Rolland C, Andreani J, Louazani AC, Aherfi S, Francis R, et al. 2019. Discovery and further studies on giant viruses at the IHU Mediterranean infection that modified the perception of the virosphere. *Viruses* 11(4):312
36. Fischer MG, Allen MJ, Wilson WH, Suttle CA. 2010. Giant virus with a remarkable complement of genes infects marine zooplankton. *PNAS* 107(45):19508–13
37. Santini S, Jeudy S, Bartoli J, Poirot O, Lescot M, et al. 2013. Genome of *Phaeocystis globosa* virus PgV-16T highlights the common ancestry of the largest known DNA viruses infecting eukaryotes. *PNAS* 110(26):10800–5
38. Deeg CM, Chow C-ET, Suttle CA. 2018. The kinetoplastid-infecting Bodo saltans virus (BsV), a window into the most abundant giant viruses in the sea. *eLife* 7:e33014
39. Francis R, Ominami Y, Khalil JYB, La Scola B. 2019. High-throughput isolation of giant viruses using high-content screening. *Commun. Biol.* 2:216
40. Sahmi-Bounsiar D, de Miranda Boratto PV, Oliveira GP, Khalil JY, La Scola B, Andreani J. 2019. Single cell micro-aspiration as an alternative strategy to fluorescence-activated cell sorting for giant virus mixture separation. *J. Vis. Exp.* 152:e60148
41. Colson P, La Scola B, Levasseur A, Caetano-Anollés G, Raoult D. 2017. Mimivirus: leading the way in the discovery of giant viruses of amoebae. *Nat. Rev. Microbiol.* 15(4):243–54
42. Xiao C, Kuznetso YG, Sun S, Hafenstein SL, Kostyuchenko VA, et al. 2009. Structural studies of the giant Mimivirus. *PLOS Biol.* 7(4):e1000092
43. Schulz F, Yutin N, Ivanova NN, Ortega DR, Lee TK, et al. 2017. Giant viruses with an expanded complement of translation system components. *Science* 356(6333):82–85
44. Schulz F, Alteio L, Goudeau D, Blanchard J, Woyke T, et al. 2018. Hidden diversity of soil giant viruses. *Nat. Commun.* 9:4881
45. Andreani J, Aherfi S, Khalil JYB, Di Pinto F, Bitam I, et al. 2016. Cedratvirus, a double-cork structured giant virus, is a distant relative of pithoviruses. *Viruses* 8(11):300
46. Levasseur A, Andreani J, Delerce J, Bou Khalil J, Robert C, et al. 2016. Comparison of a modern and fossil *Pithovirus* reveals its genetic conservation and evolution. *Genome Biol. Evol.* 8(8):2333–39
47. Bäckström D, Yutin N, Jørgensen SL, Dharamshi J, Homa F, et al. 2019. Virus genomes from deep sea sediments expand the ocean megavirome and support independent origins of viral gigantism. *mBio* 10(2):e02497-18
48. Legendre M, Fabre E, Poirot O, Jeudy S, Lartigue A, et al. 2018. Diversity and evolution of the emerging *Pandoraviridae* family. *Nat. Commun.* 9(1):2285
49. dos Santos Pereira Andrade AC, de Miranda Boratto PV, Rodrigues RAL, Bastos TM, Azevedo BL, et al. 2019. New isolates of pandoraviruses: contribution to the study of replication cycle steps. *J. Virol.* 93(5):e01942-18
50. Aherfi S, Andreani J, Baptiste E, Oumessoum A, Dornas FP, et al. 2018. A large open pangenome and a small core genome for giant pandoraviruses. *Front. Microbiol.* 9:1486
51. Legendre M, Lartigue A, Bertaux L, Jeudy S, Bartoli J, et al. 2015. In-depth study of *Mollivirus sibericum*, a new 30,000-year-old giant virus infecting *Acanthamoeba*. *PNAS* 112(38):5327–35
52. Christo-Foroux E, Alempic J-M, Lartigue A, Santini S, Labadie K, et al. 2020. Characterization of *Mollivirus kamchatka*, the first modern representative of the proposed *Molliviridae* family of giant viruses. *J. Virol.* 94(8):e01997-19
53. Andreani J, Khalil JYB, Sevvana M, Benamar S, Di Pinto F, et al. 2017. Pacmanvirus, a new giant icosahedral virus at the crossroads between *Asfarviridae* and faustoviruses. *J. Virol.* 91(14):e00212-17
54. Yoshikawa G, Blanc-Mathieu R, Song C, Kayama Y, Mochizuki T, et al. 2019. Medusavirus, a novel large DNA virus discovered from hot spring water. *J. Virol.* 93(8):e02130-18
55. Yoshida K, Zhang R, Garcia KG, Endo H, Gotoh Y, et al. 2021. Draft genome sequence of *Medusavirus stheno*, isolated from the Tatakai river of Uji, Japan. *Microbiol. Resour. Announc.* 10(1):e01323-20

56. Benamar S, Reteno DGI, Bandaly V, Labas N, Raoult D, La Scola B. 2016. Faustoviruses: comparative genomics of new megavirales family members. *Front. Microbiol.* 7:3
57. Bajrai LH, Benamar S, Azhar EI, Robert C, Levasseur A, et al. 2016. Kaumobavirus, a new virus that clusters with Faustoviruses and *Asfarviridae*. *Viruses* 8(11):278
58. Geballa-Koukoulas K, Andreani J, La Scola B, Blanc G. 2021. The kaumobavirus LCC10 genome reveals a unique gene strand bias among “extended *Asfarviridae*.” *Viruses* 13(2):148
59. Rolland C, Andreani J, Sahmi-Bounsiar D, Krupovic M, La Scola B, Levasseur A. 2021. Clandestinovirus: a giant virus with chromatin proteins and a potential to manipulate the cell cycle of its host *Vermamoeba vermiformis*. *Front. Microbiol.* 12:715608
60. Bajrai LH, Andreani J, Baptiste E, Delerce J, Raoult D, et al. 2020. Isolation of Yasminevirus, the first member of *Klosneuvirinae* isolated in coculture with *Vermamoeba vermiformis*. *J. Virol.* 94(1):e01534-19
61. Andreani J, Khalil JYB, Baptiste E, Hasni I, Michelle C, et al. 2018. Orpheovirus IHUMI-LCC2: a new virus among the giant viruses. *Front. Microbiol.* 8:2643
62. de Miranda Boratto PV, Oliveira GP, Machado TB, dos Santos Pereira Andrade AC, Baudoin JP, et al. 2020. Yaravirus: a novel 80-nm virus infecting *Acanthamoeba castellanii*. *PNAS* 117(28):16579–86
63. Moss B. 2014. *Poxviridae*. In *Fields Virology*, ed. D Knipe, P Howley, p. 2129. Philadelphia: Lippincott, Williams & Wilkins. 6th ed.
64. Van Etten JL, Agarkova IV, Dunigan DD. 2019. Chloroviruses. *Viruses* 12(1):20
65. Yuan Y, Gao M. 2017. Jumbo bacteriophages: an overview. *Front. Microbiol.* 8:403
66. Legendre M, Santini S, Rico A, Abergel C, Claverie J-M. 2011. Breaking the 1000-gene barrier for Mimivirus using ultra-deep genome and transcriptome sequencing. *Virol. J.* 8(1):99
67. Valencia-Sánchez MI, Abini-Agbomson S, Wang M, Lee R, Vasilyev N, et al. 2021. The structure of a virus-encoded nucleosome. *Nat. Struct. Mol. Biol.* 28(5):413–17
68. Liu Y, Bisio H, Toner CM, Jeudy S, Philippe N, et al. 2021. Virus-encoded histone doublets are essential and form nucleosome-like structures. *Cell* 184(16):4237–50.e19
69. Piacente F, Marin M, Molinaro A, De Castro C, Seltzer V, et al. 2012. Giant DNA virus mimivirus encodes pathway for biosynthesis of unusual sugar 4-amino-4,6-dideoxy-D-glucose (viosamine). *J. Biol. Chem.* 287(5):3009–18
70. Piacente F, De Castro C, Jeudy S, Molinaro A, Salis A, et al. 2014. Giant virus *Megavirus chilensis* encodes the biosynthetic pathway for uncommon acetamide sugars. *J. Biol. Chem.* 289(35):24428–39
71. Aherfi S, Belhaouari DB, Pinault L, Baudoin JP, Decloquement P, et al. 2021. Incomplete tricarboxylic acid cycle and proton gradient in *Pandoravirus massiliensis*: Is it still a virus? *ISME J.* 16(3):695–704
72. Azza S, Cambillau C, Raoult D, Suzan-Monti M. 2009. Revised Mimivirus major capsid protein sequence reveals intron-containing gene structure and extra domain. *BMC Mol. Biol.* 10:39
73. Louazani AC, Baptiste E, Levasseur A, Colson P, La Scola B. 2018. Faustovirus E12 transcriptome analysis reveals complex splicing in capsid gene. *Front. Microbiol.* 9:2534
74. Rodrigues RAL, Louazani AC, Picorelli A, Oliveira GP, Lobo FP, et al. 2020. Analysis of a Marseillevirus transcriptome reveals temporal gene expression profile and host transcriptional shift. *Front. Microbiol.* 11:651
75. Desnues C, La Scola B, Yutin N, Fournous G, Robert C, et al. 2012. Provirophages and transpovirons as the diverse mobilome of giant viruses. *PNAS* 109(44):18078–83
76. Assis FL, Bajrai L, Abrahão JS, Kroon EG, Dornas FP, et al. 2015. Pan-genome analysis of Brazilian lineage A amoebal mimiviruses. *Viruses* 7(7):3483–99
77. Claverie J-M, Abergel C. 2018. *Mimiviridae*: an expanding family of highly diverse large dsDNA viruses infecting a wide phylogenetic range of aquatic eukaryotes. *Viruses* 10(9):506
78. Gallot-Lavallée L, Blanc G, Claverie J-M. 2017. Comparative genomics of *Chrysochromulina ericina* virus and other microalga-infecting large DNA viruses highlights their intricate evolutionary relationship with the established *Mimiviridae* family. *J. Virol.* 91(14):e00230-17
79. Oliveira GP, de Aquino ILM, Luiz APMF, Abrahão JS. 2018. Putative promoter motif analyses reinforce the evolutionary relationships among Faustoviruses, Kaumobavirus, and Asfarvirus. *Front. Microbiol.* 9:1041
80. Koonin EV, Krupovic M, Yutin N. 2015. Evolution of double-stranded DNA viruses of eukaryotes: from bacteriophages to transposons to giant viruses. *Ann. N.Y. Acad. Sci.* 1341(1):10–24

81. Yutin N, Wolf YI, Koonin EV. 2014. Origin of giant viruses from smaller DNA viruses not from a fourth domain of cellular life. *Virology* 466–467:38–52
82. Rodrigues RAL, de Souza FG, de Azevedo BL, da Silva LCF, Abrahão JS. 2021. The morphogenesis of different giant viruses as additional evidence for a common origin of *Nucleocytoviricota*. *Curr. Opin. Virol.* 49:102–10
83. Guglielmini J, Woo A, Krupovic M, Forterre P, Gaia M. 2019. Diversification of giant and large eukaryotic dsDNA viruses predated the origin of modern eukaryotes. *PNAS* 116(39):19585–92
84. Nasir A, Kim KM, Caetano-Anolles G. 2012. Giant viruses coexisted with the cellular ancestors and represent a distinct supergroup along with superkingdoms Archaea, Bacteria and Eukarya. *BMC Evol. Biol.* 12(1):156
85. Colson P, Levasseur A, La Scola B, Sharma V, Nasir A, et al. 2018. Ancestrality and mosaicism of giant viruses supporting the definition of the fourth TRUC of microbes. *Front. Microbiol.* 9:2668
86. Klose T, Reteno DG, Benamar S, Hollerbach A, Colson P, et al. 2016. Structure of faustovirus, a large dsDNA virus. *PNAS* 113(22):6206–11
87. Andreani J, Schulz F, Di Pinto F, Levasseur A, Woyke T, La Scola B. 2021. Morphological and genomic features of the new *Klosneuvirinae* isolate fadolivirus IHUMI-VV54. *Front. Microbiol.* 12:719703
88. Schrad JR, Young EJ, Abrahão JS, Cortines JR, Parent KN. 2017. Microscopic characterization of the Brazilian giant samba virus. *Viruses* 9(2):30
89. dos Santos Pereira Andrade AC, Arantes TS, Rodrigues RAL, Machado TB, Dornas FP, et al. 2018. Ubiquitous giants: a plethora of giant viruses found in Brazil and Antarctica. *Virol. J.* 15(1):22
90. Andreani J, Khalil JYB, Sevvana M, Benamar S, Di Pinto F, et al. 2017. Pacmanvirus, a new giant icosahedral virus at the crossroads between *Asfarviridae* and Faustoviruses. *J. Virol.* 91(14):e00212–17
91. dos Santos Silva LK, dos Santos Pereira Andrade AC, Dornas FP, Rodrigues RAL, Arantes T, et al. 2018. *Cedratovirus getuliensis* replication cycle: an in-depth morphological analysis. *Sci. Rep.* 8(1):4000
92. Souza F, Rodrigues R, Reis E, Lima M, La Scola B, Abrahão J. 2019. In-depth analysis of the replication cycle of Orpheovirus. *Virol. J.* 16(1):158
93. Schrad JR, Abrahão JS, Cortines JR, Parent KN. 2020. Structural and proteomic characterization of the initiation of giant virus infection. *Cell* 181(5):1046–61.e6
94. dos Santos Pereira Andrade AC, Rodrigues RAL, Oliveira GP, Andrade KR, Bonjardim CA, et al. 2017. Filling knowledge gaps for mimivirus entry, uncoating, and morphogenesis. *J. Virol.* 91(22):e01335–17
95. Fabre E, Jeudy S, Legendre M, Trauchessec M, Claverie J, Abergel C. 2017. Noumeavirus replication relies on a transient remote control of the host nucleus. *Nat. Commun.* 8:15087
96. de Miranda Boratto PV, dos Santos Pereira Andrade AC, Rodrigues RAL, La Scola B, Abrahão JS. 2019. The multiple origins of proteins present in tupanvirus particles. *Curr. Opin. Virol.* 36:25–31
97. Belhouari DB, Baudoin J-P, Gnankou F, Di Pinto F, Colson P, et al. 2019. Evidence of a cellulosic layer in *Pandoravirus massiliensis* tegument and the mystery of the genetic support of its biosynthesis. *Front. Microbiol.* 10:2932
98. Bajrai LH, de Assis FL, Azhar EI, Jardot P, Robert C, et al. 2016. Saudi Mouvovirus, the first group B mimivirus isolated from Asia. *Front. Microbiol.* 7:2029
99. Rodrigues RAL, dos Santos Silva LK, Dornas FP, de Oliveira DB, Magalhães TFF, et al. 2015. Mimivirus fibrils are important for viral attachment to the microbial world by a diverse glycoside interaction repertoire. *J. Virol.* 89(23):11812–19
100. Boyer M, Azza S, Barrassi L, Klose T, Campocasso A, et al. 2011. Mimivirus shows dramatic genome reduction after intraamoebal culture. *PNAS* 108(25):10296–301
101. Korn ED, Weisman RA. 1967. Phagocytosis of latex beads by *Acanthamoeba*. II. Electron microscopic study of the initial events. *J. Cell Biol.* 34(1):219–27
102. Silva LCF, Rodrigues RAL, Oliveira GP, Dornas FP, La Scola B, et al. 2019. Microscopic analysis of the *Tupanvirus* cycle in *Vermamoeba vermiformis*. *Front. Microbiol.* 10:671
103. Arantes TS, Rodrigues RAL, dos Santos Silva LK, Oliveira GP, de Souza HL, et al. 2016. The large marseillevirus explores different entry pathways by forming giant infectious vesicles. *J. Virol.* 90(11):5246–55
104. Borges I, Rodrigues RAL, Dornas FP, Almeida G, Aquino I, et al. 2019. Trapping the enemy: *Vermamoeba vermiformis* circumvents faustovirus mariensis dissemination by enclosing viral progeny inside cysts. *J. Virol.* 93(14):e00312–19

105. Albrecht T, Fons M, Boldogh I, Rabson AS, Baron S. 1996. Effects on cells. In *Medical Microbiology*, ed. S Baron, ch. 44. Galveston, TX: University of Texas Medical Branch at Galveston. 4th ed.
106. Fukaya S, Takemura M. 2021. Kinetic analysis of *Acanthamoeba castellanii* infected with giant viruses quantitatively revealed process of morphological and behavioral changes in host cells. *Microbiol. Spectr.* 9(1):e00368-21
107. Ben Yaakov L, Mutsa Y, Porat Z, Dadosh T, Minsky A. 2019. Kinetics of *Mimivirus* infection stages quantified using image flow cytometry. *Cytom. A* 95(5):534–48
108. Oliveira G, Silva L, Leão T, Mougari S, da Fonseca FG, et al. 2019. Tupanvirus-infected amoebas are induced to aggregate with uninfected cells promoting viral dissemination. *Sci. Rep.* 9(1):183
109. Aoki K, Fukaya S, Takahashi H, Kobayashi M, Sasaki K, Takemura M. 2021. *Marseilleviridae* lineage B diversity and bunch formation inhibited by galactose. *Microbes Environ.* 36(1):ME20139
110. Marciano-Cabral F, Cabral G. 2003. *Acanthamoeba* spp. as agents of disease in humans. *Clin. Microbiol. Rev.* 16(2):273–307
111. Siddiqui R, Makhlof Z, Khan NA. 2021. The increasing importance of *Vermamoeba vermiformis*. *J. Eukaryot. Microbiol.* 68(5):e12857
112. dos Santos Silva LK, de Miranda Boratto PV, La Scola B, Bonjardim CA, Abrahão JS. 2016. Acanthamoeba and mimivirus interactions: the role of amoebal encystment and the expansion of the “Cheshire Cat” theory. *Curr. Opin. Microbiol.* 31:9–15
113. Boratto P, Albarnaz JD, de Freitas Almeida GM, Botelho L, Fontes ACL, et al. 2015. *Acanthamoeba polyphaga* mimivirus prevents amoebal encystment-mediating serine proteinase expression and circumvents cell encystment. *J. Virol.* 89(5):2962–65
114. Moon E-K, Chung D-I, Hong Y-C, Kong H-H. 2008. Characterization of a serine proteinase mediating encystation of *Acanthamoeba*. *Eukaryot. Cell* 7(9):1513–17
115. Sun S, La Scola B, Bowman VD, Ryan CM, Whitelegge JP, et al. 2010. Structural studies of the Sputnik virophage. *J. Virol.* 84(2):894–97
116. Fischer MG, Suttle CA. 2011. A virophage at the origin of large DNA transposons. *Science* 332(6026):231–34
117. Mougari S, Sahmi-Bounsiar D, Lévassieur A, Colson P, La Scola B. 2019. Virophages of giant viruses: an update at eleven. *Viruses* 11(8):733
118. Gaia M, Benamar S, Boughalmi M, Pagnier I, Croce O, et al. 2014. Zamilon, a novel virophage with *Mimiviridae* host specificity. *PLOS ONE* 9(4):e94923
119. Blanc G, Gallot-Lavallée L, Maumus F. 2015. Provirophages in the Bigelowiella genome bear testimony to past encounters with giant viruses. *PNAS* 112(38):E5318–26
120. Fischer MG, Hackl T. 2016. Host genome integration and giant virus-induced reactivation of the virophage mavirus. *Nature* 540(7632):288–91
121. Lévassieur A, Bekliz M, Chabrière E, Pontarotti P, La Scola B, Raoult D. 2016. MIMIVIRE is a defence system in mimivirus that confers resistance to virophage. *Nature* 531(7593):249–52
122. Michel R, Schmid EN, Hoffmann R, Müller KD. 2003. Endoparasite KC5/2 encloses large areas of sol-like cytoplasm within *Acanthamoebae*. Normal behavior or aberration? *Parasitol. Res.* 91(4):265–66
123. Scheid P, Hauröder B, Michel R. 2010. Investigations of an extraordinary endocytobiont in *Acanthamoeba* sp.: development and replication. *Parasitol. Res.* 106(6):1371–77



# Contents

## History

- Opportunities, Technology, and the Joy of Discovery  
*Patricia G. Spear* ..... 1
- The Ups and Downs of an Out-of-the-Box Scientist with  
a Curious Mind  
*Marilyn J. Roossinck* ..... 19
- Tobacco Mosaic Virus and the History of Molecular Biology  
*Angela N.H. Creager* ..... 39

## Ecology and Evolution

- Understanding the Impacts of Bacteriophage Viruses:  
From Laboratory Evolution to Natural Ecosystems  
*Britt Koskella, Catherine A. Hernandez, and Rachel M. Wheatley* ..... 57
- Amoebae: Hiding in Plain Sight: Unappreciated Hosts for the Very  
Large Viruses  
*Victória Fulgêncio Queiroz, Rodrigo Araújo Lima Rodrigues,  
Paulo Victor de Miranda Boratto, Bernard La Scola, Julien Andreani,  
and Jônatas Santos Abrahão* ..... 79
- Viruses in Subsurface Environments  
*Jennifer Wirth and Mark Young* ..... 99
- Natural Selection, Intracellular Bottlenecks of Virus Populations,  
and Viral Superinfection Exclusion  
*Camila Perdoncini Carvalho, Ruifan Ren, Junping Han, and Feng Qu* ..... 121
- A Trait-Based Approach to Predicting Viral Host-Range Evolvability  
*Hannah M. Strobel, Elizabeth C. Stuart, and Justin R. Meyer* ..... 139
- The Case for Studying New Viruses of New Hosts  
*Mark D. Stenglein* ..... 157
- The Ecology of Viral Emergence  
*Edward C. Holmes* ..... 173

## Genome Replication, Regulation of Gene Expression, and Biosynthesis

- Crowning Touches in Positive-Strand RNA Virus Genome Replication  
Complex Structure and Function  
*Masaki Nishikiori, Joban A. den Boon, Nuruddin Unchwaniwala,  
and Paul Ablquist* ..... 193
- The Role of Viral RNA Degrading Factors in Shutoff  
of Host Gene Expression  
*Léa Gaucherand and Marta Maria Gaglia* ..... 213
- The Life Cycle of the Vaccinia Virus Genome  
*Matthew D. Greseth and Paula Traktman* ..... 239
- Nuclear Capsid Uncoating and Reverse Transcription of HIV-1  
*Thorsten G. Müller, Vojtech Zila, Barbara Müller, and Hans-Georg Kräusslich* ..... 261

## Virus Cell Biology

- Liquid Phase Partitioning in Virus Replication: Observations  
and Opportunities  
*Chao Wu, Alex S. Holehouse, Daisy W. Leung, Gaya K. Amarasinghe,  
and Rebecca Ellis Dutch* ..... 285
- Replication Compartments of Eukaryotic and Bacterial DNA Viruses:  
Common Themes Between Different Domains of Host Cells  
*David M. Knipe, Amy Prichard, Surendra Sharma, and Joe Pogliano* ..... 307
- Viral G Protein–Coupled Receptors Encoded by  $\beta$ - and  
 $\gamma$ -Herpesviruses  
*Mette M. Rosenkilde, Naotaka Tsutsumi, Julius M. Knerr, Dagmar F. Kildedal,  
and K. Christopher Garcia* ..... 329
- The Role of Viruses in Identifying and Analyzing RNA Silencing  
*David C. Baulcombe* ..... 353
- APOBEC3: Friend or Foe in Human Papillomavirus Infection  
and Oncogenesis?  
*Cody J. Warren, Mario L. Santiago, and Dobun Pyeon* ..... 375
- Systems Biology of Virus-Host Protein Interactions: From Hypothesis  
Generation to Mechanisms of Replication and Pathogenesis  
*Priya S. Shah, Nitin S. Beesabathuni, Adam T. Fishburn, Matthew W. Kenaston,  
Shiaki A. Minami, Oanh H. Pham, and Inglis Tucker* ..... 397

## Pathogenesis

- Citrus Tristeza Virus*: From Pathogen to Panacea  
*Svetlana Y. Folimonova and Yong-Duo Sun* ..... 417

Advances in Understanding Neuropathogenesis of Rift Valley Fever Virus <i>Kaleigh A. Connors and Amy L. Hartman</i> .....	437
--	-----

## Immunity

Cyclic Nucleotide Signaling in Phage Defense and Counter-Defense <i>Januka S. Athukoralage and Malcolm F. White</i> .....	451
Shifting the Immune Memory Paradigm: Trained Immunity in Viral Infections <i>Esther J.M. Taks, Simone J.C.F.M. Moorlag, Mibai G. Netea, and Jos W.M. van der Meer</i> .....	469

## Vaccines

Lessons from Acquired Natural Immunity and Clinical Trials to Inform Next-Generation Human Cytomegalovirus Vaccine Development <i>Xintao Hu, Hsuan-Yuan Wang, Claire E. Otero, Jennifer A. Jenks, and Sallie R. Permar</i> .....	491
RNA-Based Vaccination of Plants for Control of Viruses <i>Andreas E. Voloudakis, Athanasios Kaldis, and Basavaprabhu L. Patil</i> .....	521

## Errata

An online log of corrections to *Annual Review of Virology* articles may be found at <http://www.annualreviews.org/errata/virology>