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Review

Characteristics of virophages and giant viruses

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Five years after being discovered in 2003, some giant viruses were demonstrated to play a role of the hosts for virophages, their parasites, setting out a novel and yet unknown regulatory mechanism of the giant viruses presence in an aqueous. So far, 20 virophages have been registered and 13 of them have been described as a metagenomic material, which indirectly impacts the number of single- and multi-cell organisms, the environment where giant viruses replicate.

Key words: virophages, giant viruses, MIMIVIRE, Sputnik

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Abbreviations: ACMV, acanthamoeba castellanii mamavirus; ALM, ace lake mavirus; APMV, acanthamoeba polyphaga mimivirus; CroV, cafateria roenbergensis virus; DNA, deoxyribonucleic acid; dsDNA, double stranded DNA; DSLV, Dishui Lake virophage; dsRNA, double stranded RNA; IEM, independent entry mode; IRG1, immune responsive gene 1; NCLDV, nucleocytoplasmic large DNA viruses; OLV, organic lake virophage; ORF, open reading frames; QLV, Qinghai Lake virophage; PEM, paired entry mode; PCR, polymerase chain reaction; PGV, phaeocystis globosa virophage; RNA, ribonucleic acid; RNV, Rio Negro virophage; RVP, rumen virophage; SMBV, Samba virus; ssDNA, single-stranded DNA; ssRNA, single-stranded RNA; tRNA, transfer RNA; YSLV, Yellowstone Lake virophage

VIROPHAGES

Among the 20 virophages described so far, 13 are in the form of a metagenomic material, and the hosts were revealed for 16 virophages (Table 1). Out of the group of the 16 hosts, 8 giant viruses (items 1–6, 12 and 13 in Table 1) were characterized, with the other 8 hosts identified as the 'probable' giant viruses (items 7–11 and 18–20 in Table 1). Currently, the virophages are classified to belong to the *Lavidaviridae* family (Krupovic *et al*., 2016). Although they differ significantly between each other, they are considered to be satellite- or satellite-like viruses (Table 2).

Sputnik was the first virophage to be identified in 2008 in a *Mamavirus* – ACMV (*Acanthamoeba castellanii mamavirus*), a giant virus (Table 1) of the *Mimivirus* genus of Mimiviridae family (Table 3). The virus was found inside the protozoan *Acanthamoeba (A.) castellanii,* in a Paris water-cooling tower (Table 1). Research on ACMV *Mamavirus* revealed an eclipse phase, called Sputnik (from a Russian word meaning 'a companion in a journey'), to celebrate the first artificial satellite of the Earth (Taylor *et al*., 2014). Given the analogy with the term bacteriophages, it is also referred to as a virophage, which stands for a 'virus eater' (La Scola *et al*., 2008). Later, Sputnik virophage was demonstrated to infect the giant virus APMV (*Acanthamoeba polyphaga*) *Mimivirus*, that was identified in 2003 (Table 1) and belongs to the *Mimivirus* genus, *Mimiviridae* family (Table 3). It was found in the protozoan *A. polyphaga* in a water-cooling tower in Bradford (Table 1). Sputnik has a spherical dsDNA genome closed in a capsid with icosahedral symmetry, 50–74 nm in size, inside which there is a lipid membrane made of phosphatidylserine, which probably protects the genetic material of the virophage (Claverie *et al*., 2009; Desnues *et al*., 2012). Sputnik's genome has 18343 base pairs with 21 ORFs that encode proteins of 88 to 779 amino acids. They compose the capsids and are responsible for N-terminal acetylation of amino acids and transposases (Claverie *et al*., 2009; Desnues *et al*., 2012; Tokarz-Deptula *et al*., 2015). Sputnik's genome does not have an RNA-dependent DNA polymerase. Hence, in infection, Sputnik uses *Mamavirus* – ACMV or *Minivirus* – APMV synthesized polymerase (La Scola *et al*., 2008; Desnues *et al*., 2012).

Mavirus (Table 1) is a virophage that was identified in 2011 and infects a giant virus *Cafateria roenbergensis* (CroV) of the genus *Cafateriavirus*, family *Mimiviridae* (Table 3). Mavirus was isolated for the first time from the flagellate *Cafateria roenbergensis* that populates the coastal waters of Gulf of Mexico in Texas (Table 1). This virophage also has a spherical dsDNA genome, which probably encodes 20 proteins (Fischer *et al*., 2011; Sliwa-Dominiak *et al*., 2016), and a capsid with icosahedral symmetry that is similar to that identified in Sputnik (Fischer *et al*., 2011). The Mavirus virophage's genome is homologous to eukaryotic DNA transposons, which suggests that the virophages could indeed have been involved in their origin (Fischer *et al*., 2011; Desnues *et al*., 2012). Interestingly, virophages play an important role in the ecology of the protists' natural populations (Fischer *et al*., 2011; Desnues *et al*., 2012; Sliwa-Dominiak *et al*., 2016; Krupovic *et al*., 2016; Fischer *et al*., 2016).

The third discovered virophage was isolated in 2011 in the salty waters of Antarctica. It was OLV (*Organic lake virophage*), which preyed on an algae- infecting giant virus (no genus given), of a *Phycodnaviridae* family (Table 1). OLV, like the Sputnik, also has a double stranded DNA genome that is circular in shape and 26421 bp in size and encodes 24 proteins which are 27–42% identical with the Sputnik proteins (Yau *et al*., 2011; Beklitz *et al*., 2016). The OLVs' effect on giant viruses infecting algae impacts their count and regulates organic matter in their aqueous environment (Yau *et al*., 2011).

Sputnik 2, which was discovered in 2012, has an 18338 bp, circular, double stranded DNA genome (Gaia *et al*., 2013; Beklitz *et al*., 2016), with a capsid that has icosahedral symmetry (Beklitz *et al*., 2016). It infects *Lentille virus*, a giant virus, genus *Mimivirus*, family *Mimiviridae* (Table 3), that was found in *A. polyphaga* eukaryote harvested from a contact lens fluid (Desnues *et al*., 2012).

Table 1. Virophages and their "host" – giant viruses

No.	The name of the Virophages and the year of finding	Species, type or family of giant viruses	Host and place of occurance of giant viruses	Reference
1.	Sputnik (2008)	Mamavirus (ACMV)	Amoeba *Acanthamoeba (A.) castellanii* – water of cooling tower in Paris (France)	La Scola *et al*., 2008
		Mimivirus (APMV)	Amoeba *A. polyphaga* – water of cooling tower in Bradford (England).	
2.	Mavirus (2011)	Cafateria roenbergensis virus (CroV)	Flagellate *Cafateria roenbergensis* – sea water in Texas coast (USA)	Fischer & Suttle, 2011
3.	OLV (Organic Lake Virophage) (2011)	Phycodnaviridae	Algae (no name) – saline waters of Antarctica Lake	Yau *et al*., 2011
4.	Sputnik 2 (2012)	Lentille virus	Amoeba *A. polyphaga* – contact lenses liquid (France)	Desnues *et al.,* 2012
5.	Sputnik 3 (2013)	*Mimiviridae*, probably Mamavirus	Amoeba *A. polyphaga* – soil samples (France)	Gaia *et al*., 2013
6.	PGV (*Phaeocystis globosa* virophage) – metagenomic material (2013)	*Phaeocystis globose virus* (PgV-16T)	Algae *Phaeocystis* type – water of the North Sea samples (coast of the Netherlands)	Santini *et al*., 2013
7.	YSLV 1 (Yellowstone Lake Virophage 1) – metagenomic material (2013)	Probably *Phycodnaviridae* or Mimivirus	*Algae – water of the Yellowstone Lake (USA**)**	Zhou *et al*., 2013
8.	YSLV 2 (Yellowstone Lake Virophage 2) – metagenomic material (2013)			
9.	YSLV 3 (Yellowstone Lake Virophage 3) – metagenomic material (2013)			
10.	YSLV 4 (Yellowstone Lake Virophage 4) – metagenomic material (2013)			
11.	ALM (Ace Lake Mavirus) – metagenomic material (2013)	Probably Mimiviridae	*Protozoa (no name) – water of Antarctica Lake	Zhou *et al*., 2013
12.	RNV (Rio Negro virophage) (2014)	Samba wirus (SMBV)	Amoeba *A. castellanii* – water of the Negro River (Brazil)	Campos *et al*., 2014
13.	Zamilon virophage (2014)	Mont1 wirus	Amoeba *A. polyphaga* – soil samples (Tunisia)	Gaia *et al*., 2014
14.	YSLV 5 (Yellowstone Lake Virophage 5) – metagenomic material (2015)	Not described	No "host" for the virus-lake water in Yellowstone Park (USA)	Zhou *et al*., 2015
15.	YSLV 6 (Yellowstone Lake Virophage 6) – metagenomic material (2015)			
16.	YSLV 7 (Yellowstone Lake Virophage 7) – metagenomic material (2015)			
17.	Zamilon 2 – metagenomic material (2015)	Not described	Probably amoeba *Acanthamoeba* sp. – water in a poplar wood bioreactor (USA))	Beklitz *et al*., 2015
18.	RVP (Rumen virophage) – metagenomic material (2015)	Probably Mimiviridae	* Protozoa (no name) – sheep's rumen (USA)	Yutin *et al*., 2015
19.	DSLV (Dishui Lake virophage) – metagenomic material (2016)	Probably Phycodnaviridae	*Algae (no name) – water of the Dishui Lake (China)	Gong *et al*., 2016
20.	QLV (Qinghai Lake virophage) – metagenomic material (2016)	Probably Phycodnaviridae	*Algae (no name) – water of the Qinghai Lake (Tibet)	Oh *et al*., 2016

*supposed „host" for giant viruses

Table 2. Selected feature of virophages and satellite viruses (satellite-like)

No.	Feature	Virophages	Satellite viruses (satellite- like)	Reference
1.	Host	Giant viruses	Mammals, plants in presence of helper viruses	Fischer *et al*., 2011; Taylor *et al*., 2014
2.	Impact on giant viruses or viruses (helper viruses)	Negatively impact on giant viruses	No negatively impact on helper viruses	Taylor *et al*., 2014
3.	Impact of virophages on host of giant viruses (protozoa, algae) and impact of satellite viruses on host of viruses (mammals and plants)	No interactions	Negatively or no interactions	Taylor *et al*., 2014; Tokarz-Deptuła *et al*., 2013
4.	Place of replication	„Factories of giant viruses"	Cell nucleus of eukaryotic organisms	Colson *et al*., 2010; Abergel *et al*., 2015
5.	Type of genetic material	dsDNA	ssRNA, dsRNA and ssDNA	Krupovic *et al*., 2016
6.	Genome size	18,0 -19,0 thousand bp	11,0 thousand nt	Krupovic *et al*., 2016
7.	Differentiation in genome building	High degree of diversity, because they have sequences from eukaryotes, including algae and giant viruses	Typical diversity - only viral sequences exist	Fischer *et al*., 2011; Desnues *et al*., 2012; Krupovic *et al*., 2016
8.	Size of capsid	50 – 70 nm	about 20 nm	Krupovic *et al*., 2016
9.	Capsid organization	Not typical because for example Sputnik virophage have a double-layered lipid film under capsid	Typical	Yutin *et al*., 2015
10.	Origin	Unclear, but probably from eukaryotes, bacteria and archaea	Unclear	Fischer *et al*., 2011; Desnues *et al*., 2012; Krupovic *et al*., 2016

Another virophage, called Sputnik 3, was identified in 2013. It has an 18338 bp, circular, double stranded DNA genome (Gaia *et al*., 2013; Beklitz *et al*., 2016), with a capsid that also has an icosahedral symmetry (Beklitz *et al*., 2016). Virophage Sputnik 2 was isolated for the first time from a soil sample taken outside Marseilles in France and containing *A. polyphaga* amoeba (Table 1). To extract Sputnik 3 from its host giant virus, a co-culture of 20 giant virus strains of *Mimiviridae* family was used as a reporter. Sputnik 3 was then found in a soil sample filtrate added to the culture (giant viruses + amoebae being their hosts), using PCR (Gaia *et al*., 2013). Thus, it was assumed that the virophage replicates only in *Mamavirus* – ACMV co-culture, genus *Mimivirus*, family *Mimiviridae* (Table 3). Now, Sputnik, Sputnik 2 and 3 are known to replicate in so-called 'giant virus replication factories' (Table 1). Although they colonize different giant viruses, all three Sputniks share as much as 99% of their DNA (Table 1).

PGV (*Phaeocystis globosa* virophage) was identified in 2013 (Table 1) in *Phaeocystis globosa* giant virus (PgV-16T), genus *Prymneovirus*, family *Phycodnaviridae* (Table 3), which infected *Phaeocystis* algae in Dutch coastal waters of the North Sea (Table 1). The virophage has a circular double stranded DNA of 19527 bp, closed in a capsid of an undefined symmetry. It encodes 16 proteins, some of which have homologs in *Mavirus* or OLV (Santini *et al*., 2013). Since no genes encoding capsid proteins have been found in PGV's genome, it has been suggested that it replicates as a linear plasmid in PgV-16T particles or is integrated in its host virus genome as a provirophage (Santini *et al*., 2013). PgV virophage replicates in PgV-165 giant virus particle factories like the Sputniks (Santini *et al*., 2013).

Five more new metagenomic sequences were identified in 2013. They were defined as ALM and YSLV1-4 virophages (Table 1). They all have circular double stranded DNA and icosahedral symmetry of the capsid (Beklitz *et al*., 2016; Yutin *et al*., 2015). One of the sequences called ALM (*Ace Lake Mavirus*) is 17767 bp long and encodes 22 ORFs, 14 of which are homologous to those found in Mavirus virophage (Zhou *et al*., 2013; Beklitz *et al*., 2016). ALM probably infects *Mimiviridae* giant viruses found in (unspecified) eukaryotes in Antarctica lakes (Table 1).

Four successive metagenomic sequences, defined as YSLV 1–4 virophages, were found in the water samples from the Yellowstone Lake (USA) (Table 1). They were homologous to OLV with replication mechanism similar to that of YSLV 1–4 in the algae infecting *Phycodnaviridae* giant virus hosts. Giant viruses, genus *Mimivirus*, family *Mimiviridae*, were also suggested as their eukaryotic hosts (Tables 1 and 3).

Table 3. Giant viruses – host of virophages

No.	Family of giant viruses	Type of giant viruses	Species of viruses and place of occurance	References
1.	Mimiviridae*	Mimivirus	Mimivirus *Acanthamoeba polyphaga mimivirus* (APMV) – 2003, amoeba - Acanthamoeba (A.) *polyphaga*	La Scola *et al*., 2003
			Mamavirus *Acanthamoeba castellani mimivirus* (ACMV) – 2008, amoeba – *A. castellani*	La Scola *et al*., 2008
			Lentille virus – 2012, amoeba – *A. polyphaga*	Desnues *et al.,* 2012
			Samba virus (SMBV) – 2014, amoeba – *A. castellani*	Campos *et al*., 2014
			Mont1 – 2014, amoeba – *A. polyphaga*	Gaia *et al*., 2014
		Cafateriavirus	*Cafateria roenbergensis virus (CroV) – 2010,* flagellate *Cafateria roenbergensis*	Fischer *et al*., 2011
2.	Phycodnaviridae**	Prymneovirus	*Phaeocystis globosa virus* (PgV-16T) – 2013, algae *Phaeocystis globosa*	Santini *et al*., 2013; Wilson *et al*., 2009
		No data	No species – 2011, sea algae (no name)	Yau *et al*., 2011

*In this family could be included giant viruses (not described), which colud be the host of virophages Sputnik 3 and probably megaviruses (without specifying the family and genus), the "hosts" of the virophages ALM and RVP and giant viruses (Mimivirus), the "hosts" of virophages YSLV1, YSLV2, YSLV3, YSLV4 (Table 1). **This family should probably include megaviruses, without specifying the family and type that are the "hosts" of the virophage YSLV1, YSLV2, YSLV3, YSLV4 and DSLV and QLV (Table 1).

RNV (*Rio Negro* virophage) was identified in 2014 in the Negro River in the Amazon rainforest in Brazil. It was found in *A. castellanii* infected with Samba, a SMBV giant virus, of the genus *Mimivirus*, family *Mimiviridae* (Table 1 and 3). It has double stranded DNA. No data is available, though, on whether it has a circular or linear shape. RNV's capsid was demonstrated to have icosahedral symmetry with a diameter of approximately 35 nm (Campos *et al*., 2014; Beklitz *et al*., 2016). Through the infection of Samba giant virus replicating in *A. castellanii* hosts, RNV causes abnormal shape of Samba's capsid and reduces its standard concentration in amoebas by over 80% (Yau *et al*., 2011; Krupovic *et al*., 2016). RNV is also responsible for the defective capsid shape of APMV giant virus infecting *A. castellanii* hosts (Campos *et al*., 2014).

Zamilon virophage was isolated in 2014 from the soil samples from Tunisia (Table 1). It infected a *Mont1* giant virus, genus *Mimivirus*, family *Mimiviridae* (Table 3) in its *A. polyphaga* host. It contains a double stranded spherical DNA genome of 17276 bp with 20 ORFs (Gaia *et al*., 2014; Beklitz *et al*., 2016), some of which encode proteins that are homologous to other known virophage proteins, ATPases, helicases and transposases (Gaia *et al*., 2014). According to Gaia and others (Gaia *et al*., 2014), Zamilon has a 70–76% genetic identity with Sputnik, Sputnik 2, Sputnik 3 and *Megavirus chilensis* giant virus. Interestingly, it is the only virophage that infects lineage C of *Mimiviridae* giant viruses (Santini *et al*., 2013, Campos *et al*., 2014; La Scola *et al*., 2008; Gaia *et al*., 2013; Yau *et al*., 2011; Fischer *et al*., 2011; Zhou *et al*., 2013; Sliwa-Dominiak *et al*., 2016; Desnues *et al*., 2012). All the other virophages characterized so far (Table 1) infect lineage A giant viruses of *Mimiviridae* family as well (Campos *et al*., 2014; La Scola *et al*., 2008; Gaia *et al*., 2013; Desnues *et al*., 2012). Zamilon has a 50–60 nm icosahedral capsid. Zamilon causes abnormal capsid shape in the infected *Mont1* giant viruses. However, it does not affect neither their replication, nor the lytic ability (Gaia *et al*., 2014).

Three new virophages, YSLV5, YSLV6 and YSLV7, were identified in 2015 as a metagenetic material (Zhou *et al*., 2015) in the Yellowstone Lake (US). They showed genetic homology to Zamilon. Their DNA was double stranded and spherical and their capsids were probably icosahedral (La Scola *et al*., 2008; Gaia *et al*., 2013). Their genomes were 22000–29000 bp in size and contained 26 to 32 ORFs (Zhou *et al*., 2015; Beklitz *et al*., 2016). No giant viruses or organisms were identified to be the hosts to YSLV5, YSLV6 and YSLV7 (Table 1). The YSLV5-7 virophages show a significant homology to YSLV1, YSLV2, YSLV3 and YSLV4, which were isolated in the same waters of Yellowstone Lake back in 2013 (Table 1).

A homologous to Zamilon strain of dsDNA discovered in 2015 was named Zamilon 2 (Table 1). Although no giant virus was implicated, a probable host of Zamilon 2 is *Acanthamoeba sp.* giant virus (Table 1), first found in a bioreactor in the state of New York (US). Zamilon 2 virophage has a capsid that is probably icosahedral (Beklitz *et al*., 2016; Yutin *et al*., 2015). Its genome is only 6616 bp in size, and 392 base pairs are identical with Zamilon genome (Beklitz *et al*., 2015).

RVP (*Rumen* virophage) was identified in a metagenetic material in 2015. It probably infects *Mimiviridae* giant viruses that replicate in (unspecified) eukaryotic hosts in the sheep rumen (Table 1). RVP probably has an icosahedral capsid (Beklitz *et al*., 2016; Yutin *et al*., 2015). Its linear genome is different from the genomes of the other virophages and owing to this it is referred to as a 'hybrid virophage' – a combination of a virophage and

Table 4. Selected feature of giant viruses and viruses ("classic" viruses)

No.	Feature	Giant viruses	Viruses („classical" viruses)	References
1.	Genetic material	Doubled-strended DNA	DNA or RNA, single or double-stranded, circular	La Scola *et al*., 2003; Fischer *et al*., 2011
2.	Size of genome	1.181 Mb	0.035 Mb	La Scola *et al*., 2003; Campos *et al*., 2014; Monti *et al*., 2008; Gaia *et al*., 2014; Fischer *et al*., 2011; Wilson *et al*., 2009; Desnues *et al*., 2012; Abergel *et al*., 2015
3.	The content of the genome	Genes of viral, prokaryotic, archaeonic and eukaryotic origin	Typical for viruses	Raoult *et al*., 2004; Suzan-Monti *et al*., 2007; Claverie *et al*., 2009; Cortines *et al*., 2015; Abergel *et al*., 2015
4.	DNA repair genes, transcription factors, genes responsible for protein buffering and modification, mRNA synthesis genes, genes encoding tRNA polysaccharide synthesis genes and mobile genetic elements	They have them, which determines the mosaicism of their genome, gives it instability and can expand their infectious spectrum	Absent	Suzan-Monti *et al*., 2006; Suzan--Monti *et al*., 2007; Claverie *et al*., 2009; Cortines *et al*., 2015; Abergel *et al*., 2015
5.	Presence of atypical elements	Presence for example transposons, inteins, introns, rope plasmids	Absent	Sharma *et al*., 2016; Suzan-Monti *et al*., 2006; Xiao *et al*., 2009; Raoult *et al*., 2004; Colson *et al*., 2010; Santini *et al*., 2013; Claverie *et al*., 2016
6.	Replication	„Factories of giant viruses"	In cell nucleus, but also in cytoplasm of macro organism	Colson *et al*., 2010; Abergel *et al*., 2015
7.	Size of capsid	200–1000 nm	~17–200 nm	La Scola *et al*., 2003; Colson *et al*., 2010
8.	Capsid organization	Capsid coverd with 150 nm of peptydoglycan-based fibers, glycosylation glycoproteins	Typical for "classic" viruses"	La Scola *et al*., 2003; Suzan-Monti *et al*., 2006; Raoult *et al*., 2004; Cortines *et al*., 2015; Abergel *et al*., 2015
9.	Resistance system	MIMIVIRE similar to CRISP-Cas mechanism commonly present in bacteria and archaea	Absent	Levasseuer *et al*., 2016
10.	Host – place of „living"	Water – protozoa (amoeba, flagellate), algae, sponge, coral, mollusc, insects. Soil (desert, prairies, tundra) – amoeba.Mammals – human and animals (sheep, cattle)	Eukaryotes (including mammals), prokaryotes and archea	La Scola *et al*., 2003; Santini *et al*., 2013; Campos *et al*., 2014; Monti *et al*., 2008; Gaia *et al*., 2014; Fischer *et al*., 2011; Wilson *et al*., 2009; Desnues *et al*., 2012; Abergel *et al*., 2015

large polinton, DNA transposon, i.e. giant virus transpoviron DNA (Yutin *et al*., 2015).

New metagenetic material, defined later as two novel virophages, was isolated in Asia (Table 1). The first was DSLV (*Dishui Lake* virophage) with a circular double stranded DNA genome, 28788 bp in size, that contained 28 ORFs (Gong *et al*., 2016; Beklitz *et al*., 2016), and showed a significant homology to all the virophages identified in Yellowstone Lake (YSLV 1-7) (Table 1). It was particularly homologous to YSLV3 and OLV (Gong *et al*., 2016; Beklitz *et al*., 2016). DSLV was extracted from Dishui Lake in Shanghai, China. Although it was assigned no giant virus host, the probable candidate may be *Phycodnaviridae* virus that infects (unspecified) algae (Table 1). DSLV's genome has 23379 bp and contains 25 ORFs (Oh *et al*., 2016, Beklitz *et al*., 2016).

QLV (*Qinghai Lake* virophage) was the second virophage to be found in the region (Table 1). It is most closely related to OLV and YSLV (Gong *et al*., 2016; Beklitz *et al*., 2016). It was isolated from Qinghai Lake in Tibetan mountains. Like DSLV, QLV probably infects *Phycodnaviridae* giant viruses found in unspecified algae (Tables 1 and 3).

GIANT VIRUSES, WHICH CAN BE VIROPHAGES' HOSTS

The studies on giant viruses – megaviruses, including *Mimiviridae* and *Phycodnaviridae* families that act as hosts for virophages, showed that they are abundant in the natural environment and have properties that (classic) viruses do not display (Table 4). Giant viruses are also called nucleocytoplasmic large DNA viruses (NCLDVs). Prior to isolation of *Mimiviridae* viruses that act as virophage hosts, several other viruses were classified as giant viruses, including PgV-16T viruses of family *Phy-*

codnaviridae, genus *Prymneovirus* that host virophages but replicate in algae (Table 3), the viruses that infect vertebrates from family *Asfarviridae*, the viruses that infect vertebrates and insects from family *Poxviridae* and the viruses from family *Iridoviridae* that infect eukaryotes found in aqueous environment (La Scola *et al*., 2003).

Six species of giant viruses, including APMV, ACMV, Lentilevirus, SMBV, Mont1, CroV, and eight other unspecified viruses were extracted from *Mimiviridae* family (Table 1). They replicate in eukaryotes (amoebae and flagellates) and belong to *Mimivirus* and *Cafateriavirus* genera (Table 3). They have linear or circular double stranded DNA (La Scola *et al*., 2003; Campos *et al*., 2014; La Scola *et al*., 2008; Gaia *et al*., 2014; Yau *et al*., 2011; Boughalmi *et al*., 2013) and a large genome ranging from 0.6 to over 1 Mb (La Scola *et al*., 2003; Campos *et al*., 2014; La Scola *et al*., 2008; Gaia *et al*., 2014; Wilson *et al*., 2009; Desnues *et al*., 2012; Abergel *et al*., 2015). The viruses were demonstrated to have MIMIVIRE – genes regulating immunity system against virophages. They are the common genes found in (classical) viruses, giant virus particles, including transpovirons, polintons (Tokarz-Deptula *et al*., 2015), genes typical of bacteria, archaea and eukarya, i.e. transposons, inteins, introns and linear plasmids. The giant viruses that host virophages have a mosaic-like genome (Sharma *et al*., 2016; Xiao *et al*., 2009; Suzan-Monti *et al*., 2006; Raoult *et al*., 2004; Colson *et al*., 2010; Santini *et al*., 2013; Claverie *et al*., 2016). Abergel and others (Abergel *et al*., 2015), meaning that the genome contains approximately 21% of genes that originate from the eukaryotic, prokaryotic and archaeal organisms.

The first giant virus of Mimiviridae family is *Acanthamoeba castellanii* Mamavirus (a strain of giant ACMV, genus *Mimivirus*), from which Sputnik was for the first time isolated in 2008 (La Scola *et al*., 2008) (Table 1). The Mamavirus was discovered in 2003 and called mimivirus ("mimicking microbe") in the amoeba *Acanthamoeba polyphaga* residing in a Bradford water-cooling tower (England) (Table 1).

APMV was at first called *Bradfordcoccus* owing to its resembling of the Gram-positive cocci. It was identified in 1992 and genetic analysis was not available at that time (La Scola *et al*., 2003). Later on, the electron microscopy methods (La Scola *et al*., 2003) showed that it has properties similar to those of a virus. A new family of *Mimiviridae* (Table 2) was identified as a part of the NCLDV superfamily (La Scola *et al*., 2003). The mimivirus has icosahedral capsid, approximately 440 nm long. It does not seem to have an outer envelope. The virus replicates in amoeba's cytoplasm creating so-called 'viral factories' (Colson *et al*., 2010; Abergel *et al*., 2015). On its surface, it has the fibrilis (collagen) protrusions (Suzan-Monti *et al*., 2006; Raoult *et al*., 2004), that are covered with 150 nm fibers made of peptidoglycan, an element common to bacteria (Abergel *et al*., 2015). This layer is probably responsible for the virus's adhesion to amoeba cells during the infection (Rodriggues *et al*., 2015). It also regulates virophage adhesion to the virus during their common entry into amoeba cells (Taylor *et al*., 2014). Like all *Mimiviridae* family, mimivirus genome consists of the linear dsDNA and is up to 1181 Mbp long, carrying 1262 potential genes. It contains the capsid genes, infection-inducing genes and, never observed in (classic) viruses, the DNA repair genes, transcription factors, mRNA synthesis genes (including genes encoding tRNA), genes of mobile genetic elements, polysaccharide synthesis genes that also include peptidoglycan, and 911 protein-coding genes, including protein folding and protein modification genes (Raoult *et al*., 2004; Suzan-Monti *et al*., 2007; Claverie *et al*., 2009; Rodriggues *et al*., 2015; Tokarz-Deptula *et al*., 2013; Abergel *et al*., 2015). All these elements of APMV result in the mosaic character of its genome and make the genome unstable, which may broaden the virus's spectrum of infection (Raoult *et al*., 2004; Suzan-Monti *et al*., 2007; Claverie *et al*., 2009; Rodriggues *et al*., 2015; Tokarz-Deptula *et al*., 2013; Abergel *et al*., 2015). APMV infects *Acanthamoeba polyphaga* usually through phagocytosis. However, the mechanism of the virus's replication inside amoebae has not been explained yet (Suzan-Monti *et al*., 2006).

Mamavirus – ACMV (*Acanthamoeba castellanii* mamavirus) is a strain of APMV virus that has a nucleoid which is 99% identical with that of APMV (La Scola *et al*., 2008). As mentioned above, the Sputnik virophage was isolated from it (Table 1). The new giant virus called *A. castellanii* mamavirus (ACMV) was isolated in 2008 from *Acanthamoeba castellanii* found in a cooling water tower, and also in many pulmonary infections in patients from a Paris hospital (Table 1). Like *Mimivirus* – APMV, the ACMV mamavirus has an icosahedral capsid (Zhou *et al*., 2015; Raoult *et al*., 2010) and replicates in the viral factories. Its genome is 1191 Mbp long linear double stranded DNA. ACMV is therefore 10000 bp longer than APMV, although at the end of the 5th section it has approximately 13000 bp which were not found in AMPV (Zhou *et al*., 2015).

Apart from the Mimivirus – APMV and Mamavirus – ACMV (Table 1), the other giant viruses that act as virophage hosts include a Lentille virus which hosts Sputnik 2, a probable Mamavirus – ACMV which hosts Sputnik 3, a Samba virus which hosts Rio Negro virophage, a Mont1 virus which hosts Zamilon (Table 1), a virophage of genus *Mimivirus*, family *Mimiviridae* (Table 3) which, like giant viruses (APMV and ACMV), replicate in *A. polyphaga* and *A. catellanii* amoebae. The Sputnik 2, Sputnik 3, Rio Negro and Zamilon virophages replicate in virus replication factories in amoeba's cytoplasm and were found parasitizing 4 species of giant viruses, including Lentille, Mamavirus – ACMV, Samba and Mont1 (Table 1). All the virophages have a linear double stranded DNA genome closed in an icosahedral capsid (La Scola *et al*., 2003; Sharma *et al*., 2016; Xiao *et al*., 2009; Raoult *et al*., 2004; Campos *et al*., 2014; Suzan-Monti *et al*., 2007; Saadi *et al*., 2013a; Saadi *et al*., 2013b; La Scola *et al*., 2008; Gaia *et al*., 2014; Tokarz-Deptula *et al*., 2013; La Scola *et al*., 2005).

CroV (*Cafateria roenbergensis virus*) is another representative of the giant viruses. It comes from a family of *Mimiviridae,* genus *Cafateriavirus,* that hosts Mavirus virophage, found in flagellate *Cafateria roenbergensis* (Tables 1 and 3). Crov has a linear double stranded DNA genome of 0.78 Mb, closed in an icosahedral capsid. Like other giant viruses of the genus *Mimivirus*, family *Mimiviridae* that were discussed above, CroV replicates in viral factories in *Cafateria roenbergensis* cytoplasm. *Mimiviridae* family of viruses includes unspecified giant viruses that host ALM, RVP, YSLV1-YSLV4 virophages (Tables 1 and 3). The latter may also be hosted by *Phycodnaviridae* giant viruses (Tables 1 and 3).

PgV-16T (*Phaeocystis globosa virus*) from the genus *Prymneovirus* of the *Pycodnaviridae* family (Table 3) infects algae and is an obligate host of the virophages. PgV-16T acts as host for PGV (*Phaeocystis globosa virophage*) (Table 1). Similar to other *Mimiviridae,* this giant virus has a linear double stranded DNA genome and replicates in the algae cytoplasm. Its icosahedral capsid is smaller (by up to 220 nm) than that of the *Mimiviridae* viruses. PgV-16T

genome is 470 000 bp long and contains a duplication of the two types of virus core genes packing ATPases and RNA polymerases (Santini *et al*., 2013; Wilson *et al*., 2009; Baudoux *et al*., 2005). PgV-16T is similar to APMV and CroV from the *Mimiviridae* family, which host virophages and contain DNA sequences common for bacteria, archaea and eukaryotes (Santini *et al*., 2013). Giant viruses of the *Phycodnaviridae* family, (no genus available) are reported to host OLV (*Organic lake virophage*), DSLV (*Dishui lake virophage*), QLV (*Qinghai lake virophage*) and YSLV1-4 virophages, likewise the *Mimiviridae* viruses.

To sum up the data on giant viruses that act as hosts or probable hosts for 16 out of the total of 20 virophages identified to date (Table 1), the 5 species of giant viruses were found in amoebae, 1 species in flagellate, 7 probable (unknown) *Mimiviridae* giant viruses in eukaryotes and 8 types of *Phycodnaviridae* viruses in algae (Tables 1 and 3). The giant viruses from *Mimiviridae* family (Table 3) were found not only in amoebae and flagellates (Table 1), but they can also infect sponges, coral, sheep, cattle and people (Yutin *et al*., 2015; Saadi *et al*., 2013a; Saadi *et al*., 2013b; La Scola *et al*., 2005; Almeida *et al*., 2017; LaScola *et al*., 2014; Raoult *et al*., 2010; Kutikhin *et al*., 2014). They were demonstrated to constitute a part of the microbiome of the human respiratory system, as they were identified in bronchoscopic samples of the healthy people as well as in the samples taken from patients diagnosed with pneumonia (Saadi *et al*., 2013a; Saadi *et al*., 2013b; LaScola *et al*., 2005; Almeida *et al*., 2017; Raoult *et al*., 2010; Kutikhin *et al*., 2014). They were secondarily identified in the blood of the patients suffering from respiratory diseases. That would explain the pneumonia cases in the patients from Bradford and Paris, where the first *Mimiviridae* viruses: *Mimivirus* – APMV and *Mamavirus* – ACMV, were discovered (Saadi *et al*., 2013a; Saadi *et al*., 2013b; LaScola *et al*., 2005; Almeida *et al*., 2017; Raoult *et al*., 2010; Kutikhin *et al*., 2014). Currently (Almeida *et al*., 2017), the APMV *Mimivirus* was demonstrated to trigger a novel type of immune response in the human body, regulated by the activity of interferons (IFNs), and IFN-β in particular. The infection with APMV was shown to facilitate IFN-β activity and induce immune responsive gene 1 (IRG1) in macrophages, resulting in itaconic acid release which activated antiviral and antibacterial immunity and metabolic processes (Almeida *et al*., 2017). *Marseillieviridae* giant viruses were isolated from the human blood, macrophages and lymphoid tissue as well as from the *Limnoperna fortunei* bivalvia and *Eristalis tenax* larva (Almeida *et al*., 2017; Dos Santos *et al*., 2016; Boughalmi *et al*., 2013), which shows that giant viruses are quite common risk factors in the environment.

INTERACTIONS BETWEEN VIROPHAGES AND GIANT VIRUSES

To examine the interactions between virophages and their *Mimiviridae* and *Phycodnaviridae* giant viruses hosts, it is important to understand the way virophages enter viruses and the way giant viruses infected with virophages enter their specific hosts, i.e. protozoa (amoebae and flagellates), algae and mammals. Because virophages can only replicate in the "viral factories" of the giant viruses, the mechanism of their co-infections is important to know.

Giant virus infection mechanisms were elucidated by Taylor and coworkers (Taylor *et al*., 2014), who used a mathematical model to show the two probable ways in which virophage – infected giant viruses enter amoebae and flagellates (Fig. 1). The first way is called IEM (*independent entry mode*) and is common for Mavirus virophage and its giant virus – CroV. They both independently enter a protozoan where they later both replicate (Fig. 1a). The other way, called PEM (*paired entry mode*), is thought to be used by Sputnik virophage and its giant virus – *Mimivirus* (APMV). In this way, the co-infection occurs when the giant virus and virophage are entangled and together enter the host organism – *A. polyphaga*. This way consists of two phases. First, Sputnik adheres to Mimivirus (APMV) and this complex successively enters the amoeba *via* phagocytosis. This entry stage was confirmed with electron microscope photos showing *Mamavirus* (ACMC) giant virus and its virophage – Sputnik occurring in the phagocytic vacuole (Desnues *et al*., 2010). Forming of a "complex" of the virophage and giant virus, is also enabled by the long collagen fibers appearing on the surface of *Mamavirus* (ACMC). The complex (entanglement) is easily absorbed by amoeba – *A. castellanii* (Xiao *et al*., 2009; Taylor *et al*., 2014). This hypothesis was confirmed by the study of Boyer and others (Boyer *et al*., 2011), which showed that virophages (Sputnik), were not able to penetrate and replicate when co-cultured with the giant viruses (*Mamavirus*) without fibers. This suggests the important role of these fibers in the formation of giant virus – virophage complex and in their penetration into the host *via* PEM (Desnues *et al*., 2010; Taylor *et al*., 2014). In the second phase of PEM pathway, the Mimivirus (APMV) sheds its capsid and the genome of the entangled virophage enters the so-called "viral replication factories", where the virophages are replicated (Taylor *et al*., 2014) (Fig. 1b).

Replication of the virophages starts 3–6 hours after the entry of a giant virus to the host cell, i.e. when its eclipse phase has completed (Desnues *et al*., 2010; Marie *et al*., 2016). The replication of a giant virus involves laying and creating the offspring virions, which remain in eukaryotic (amoebae and flagellates) cells' cytoplasm until their lysis (Desnues *et al*., 2010; Marie *et al*., 2016). Currently (Taylor *et al*., 2014), it has been suggested that the infection of protozoans with a virophage and a giant virus relies on the PEM pathway. Another proposed way of the eukaryotic infection is when a giant virus and a virophage replicate independently in the environment. When the protozoa come into contact, the infection is passed over to the other organisms (Yau *et al*., 2011). Taylor and coworkers (Taylor *et al*., 2014) pointed out that both IEM and PEM entry pathways of the virophage-infected giant viruses to amoebae and flagellates depend equally on the same three elements – a host (amoeba), a giant virus and a virophage. However, studies by Yau and coworkers (Yau *et al*., 2011), showed the association of a giant virus and a virophage can be present in a predator-prey context (predator-prey system), where the increase in the number of virophages can be, theoretically, independent of the final host (Fig. 1c). However, it should be remembered, that the virophages require the presence of both – the host and the giant virus for their own replication. Therefore, such a theory is debatable, because no replication of virophages, without co-infection with the giant virus, has ever been observed in nature, in any eukaryotic host (La Scola *et al*., 2008).

However, regardless of the way of entry or co-infection of eukaryotes (amoebae and flagellates) with giant viruses and virophages, the infection reduces the number of the hosts. This decrease was demonstrated to be greater when infection was caused only by a giant virus

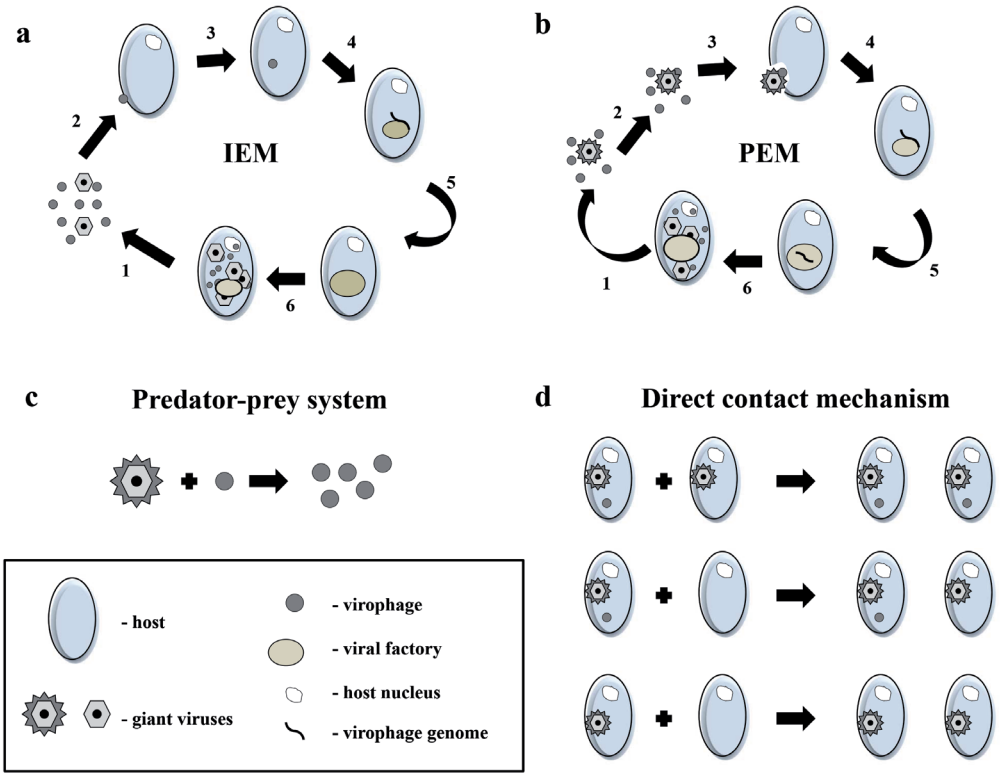

Figure 1. Virophage and giant virus co-infection lifecycle.
(**A**) Independent entry mode – IEM (Taylor *et al.,* 2014). Step 1: A free virophage and a giant virus following a host's lysis. Step 2: A free virophage enters the host. Step 3: A free giant virus enters the host – amoeba. Step 4: The viral particles lose capsids. Step 5: The virophage genome enters the viral factory (viral factory expands). Step 6: The virophages leave the viral factory and wait for the lysis (by host). (**B**) Paired entry mode – PEM (Taylor *et al.,* 2014). Step 1: A free virophage and a giant virus following a host's lysis. Step 2: A virophage and a giant virus entangle. Step 3: The entanglement enters the host (co-infection). Step 4: The viral particles lose capsids. Step 5: The virophage genome enters the viral factory (viral factory expands). Step 6: The virophages leave the viral factory and wait for the lysis (by host). (**C**) Predator-prey system (Yau *et al.,* 2011). A virophage replicates via the infection and lysis of a giant virus, in the absence of a host. (**D**) Direct contact mechanism (Wodarz, 2013). Replication of a virophage and a giant virus, where the free viral particles are not released into the environment.

compared to when infection was caused by a virus and a virophage. This finding shows that virophages are infection factors, protecting amoebae and flagellates against giant viruses. Infection of a giant virus with a virophage was shown to reduce the mortality of the infected amoebae and flagellates and to cause abnormal shape of the infected giant viruses (Campos *et al*., 2014; Gaia *et al*., 2014). This was reported in a study of Zamilon virophage that infects the Mont1 virus (Gaia *et al*., 2014). In a study of the Mont1 giant virus, a sequence was isolated that was not found in (classic) viruses. It was called MIMIvirus VIrophage Resistant Element (MIMIVIRE) and it the virus against Zamilon infection (Lavasseuer *et al*., 2016). The system is also suggested (Lavasseuer *et al*., 2016) to be present in other Mimiviridae giant viruses. It is similar to CRISPR/Cas mechanism, which is widespread in bacteria and archaea (Lavasseuer *et al*., 2016) and based on the short palindromic repeats created after RNA transcription, which are then used as a guide for enzymatic proteins, including helicases and nucleases, for cleaving of the foreign nucleic acids. After cleavage, the foreign DNA with palindromic sequences is included in-between the repeats. In the next infection of bacteria and archaea with a similar factor, they can act directly against the foreign DNA, e.g. DNA of bacteriophages. A study on MIMIVIRE system in the Mont1 virus showed 28 nucleoid sequence repeats that did not contain open reading frames (ORFs) (Lavasseuer *et al*., 2016). Although the MIMIVIRE system, defined as a model of the giant virus immunity against virophage infection, can follow a different mechanism (Claverie *et al*., 2016), the authors of the study gave no further details.

Regardless of whether the elements of immunity against virophages exist in the giant viruses, the infection of giant viruses with virophages lower their number, which ultimately protects their hosts – protozoa (Taylor *et al*., 2014). The presence of Sputnik virophage infection of the ACMV *Mamavirus* was demonstrated to reduce the count of *A. polyphaga* amoebae by 13% less then when infected with ACMV *Mamavirus* only (Taylor *et al*., 2014). A similar picture was observed in a culture of *Cafateria roenbergensis* flagellate infected with Crov and Mavirus virophages (Fischer *et al*., 2011). Therefore, the virophages were called the friends of the giant virus hosts (eukaryotes and algae), which differentiates them from typical satellite- or satellite-like viruses, which they are often compared to (Table 2). Through destruction of the giant viruses, the virophages participate in a biological loop. It was recorded in Antarctica lakes that they affect the growth of blooming algae (Santini *et al*., 2013; Yau *et al*., 2011). Another model of the giant viruses and virophages spread, presented by Wodarz (Wodarz, 2013) is in opposition to that presented by Taylor and coworkers (Taylor *et al*., 2014) and describes this phenomenon

through a direct contact of a virophage and a giant virus, which does not include the presence of a "free" giant virus or virophage in environment (Fig. 1d).

The virophages were shown to have a positive effect on bacteria (Slimani *et al*., 2013). Superinfection of eukaryotes with a giant virus and BABL1 bacteria increases the count of BABL1 and virophages and reduces the number of giant viruses. This finding suggests that through their effect on giant viruses, the virophages affect the count of BABL1 bacteria, probably due to bacteria and giant viruses competing for the host (Slimani *et al*., 2013). Since the effect of the virophages on the giant viruses is that the count of the latter is reduced, it provides better conditions for bacteria to thrive (Slimani *et al*., 2013). The number of virophages in aqueous environment depends on water temperature and its chemical composition, just like in case of the bacteriophages. This correlation was demonstrated in Yellowstone Lake water, where the number of virophage metagenomes correlated with water temperature and sun exposure (Zhou *et al*., 2015). Yet more experimental data on the co-infection and dynamics of virophage presence in the giant viruses is needed to accurately describe the process of virophages replication, as well as the mechanism of entry and interaction with a giant virus and their host.

SUMMARY

The discovery and isolation of the virophages and their hosts – giant viruses has brought some novel facts into virology. The analysis of available data on virophages and giant viruses, evokes a question if the current taxonomic division into three domains (bacteria, archaea and eukaryotes) is indeed a right one. The properties of virophages and giant viruses, that have not been previously identified in the infection factors, may suggest that this division lacks precision. The data concerning virophages, giant viruses and their interactions, including a novel mechanism of the giant viruses' defense systems, constitute some of the new discoveries of biology of the 21st century and reveals the imperfections of the current three domains division of living organisms.

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