



Taxonomic update for giant viruses in the order *Imitervirales* (phylum *Nucleocytoviricota*)

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Abstract

Large DNA viruses in the phylum *Nucleocytoviricota*, sometimes referred to as “giant viruses” owing to their large genomes and virions, have been the subject of burgeoning interest over the last decade. Here, we describe recently adopted taxonomic updates for giant viruses within the order *Imitervirales*. The families *Allomimiviridae*, *Mesomimiviridae*, and *Schizomimiviridae* have been created to accommodate the increasing diversity of mimivirus relatives that have sometimes been referred to in the literature as “extended *Mimiviridae*”. In addition, the subfamilies *Aliimimivirinae*, *Megamimivirinae*, and *Klosneuvirinae* have been established to refer to subgroups of the *Mimiviridae*. Binomial names have also been adopted for all recognized species in the order. For example, *Acanthamoeba polyphaga* mimivirus is now classified in the species *Mimivirus bradfordmassiliense*.

Introduction

Large dsDNA viruses belonging to the phylum *Nucleocytoviricota*, often referred to as “giant viruses”, include the largest viruses that have yet been characterized, both in terms of virion size and genome length [1]. Of specific interest are viruses in the order *Imitervirales*, which are particularly abundant and widespread in ecosystems around the

globe [2–5]. Despite their importance, until recently, only two members of the *Imitervirales* were classified by the International Committee on Taxonomy of Viruses (ICTV): *Acanthamoeba polyphaga* mimivirus (APMV) and *Cafeteria roenbergensis* virus (CroV). Both of these viruses belong to the family *Mimiviridae*, but a large number of recently sequenced viruses that are only distantly related to either APMV or CroV, sometimes referred to as “extended *Mimiviridae*” or “extended family *Mimiviridae*” [6, 7], have recently been reported but not classified. Hence, there has

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been an urgent need to update the taxonomy of the order *Imitervirales* to reflect current knowledge on these viruses by classifying those with recently sequenced genomes and organizing them in a hierarchical order by establishing new families, subfamilies, and genera, as needed. Here, we describe taxonomic updates for giant viruses in the order *Imitervirales* that have recently been adopted by the ICTV.

Phylogenomic analysis

We analyzed 43 genomes of viruses that are, or are related to, current members of the order *Imitervirales*. We used a concatenated phylogenetic approach (Fig. 1) and also examined pairwise average nucleotide identity (ANI). The concatenated tree is based on seven marker genes: DNA polymerase family B (PolB), RNA polymerase large subunit (RNAPL), A32 packaging enzyme (A32), topoisomerase family II (TopoII), virus late transcription factor 3 (VLTF3), transcription factor IIB (TFIIB), and a superfamily II helicase (SFII). These marker genes have previously been benchmarked and shown to provide high-fidelity phylogenetic trees for viruses belonging to the phylum *Nucleocyotoviricota* [8]. Families, subfamilies, and genera are demarcated such that they have approximately equivalent phylogenetic breadths (i.e., distance from the root). We only describe classification of viral isolates here, but for our concatenated phylogenetic approach, we also included metagenome-derived viral genome sequences. This was done to improve phylogenetic reconstruction by providing additional evolutionary context and to avoid long branches that arise when only isolates are used. For this purpose, a manually selected set of metagenome-derived genome sequences were obtained from the Giant Virus Database (<https://faylward.github.io/GVDB/>).

Guidelines used for binomial species names

Newly adopted family names have the suffix “-mimiviridae” to denote evolutionary relatedness to the *Mimiviridae* (*Allomimiviridae*, *Mesomimiviridae*, *Schizomimiviridae*) and to acknowledge the original name of this group of viruses proposed in 2008 [9]. New subfamilies are either named by officially adopting names that are commonly used in the literature (*Megamimivirinae*, *Klosneuvirinae*) or using a simple Latin prefix (*Aliimimivirinae*; alii- Latin for “other”). Subfamilies were created only for the family *Mimiviridae* because the most viral isolates are available for this family. For species, we adopted binomial names. Genus names refer to the names of Titans or gods in Greek mythology (*Biavirus*, *Kratosvirus*, *Heliosvirus*, *Oceanusvirus*, *Rheavirus*, *Tethysvirus*, and *Theiavirus*) unless another genus name

was already in common usage in the literature (*Megavirus*, *Moumouvirus*, *Fadolivirus*, *Yasminevirus*, *Cotonvirus*, and *Tupanvirus*). The second name in the binomial refers to features or geography where viruses were sampled or isolated (i.e., *sinusmexicani* denoting isolation from the Gulf of Mexico). For consistent use of binomial names, we also renamed the currently recognized species *Acanthamoeba polyphaga mimivirus* and *Cafeteria roenbergensis virus* to *Mimivirus bradfordmassiliense* and *Rheavirus sinusmexicani*, respectively, to fit the new Linnaean binomial format. A full list of all official binomial names can be found in Table 1.

Proposed families

Family *Allomimiviridae*

“Allo-” from Greek allos “other, different”. This family contains the species *Heliosvirus raunefjordense* and *Oceanusvirus kaneohense*. Members of both species are marine viruses that infect green algae (*Pyramimonas orientalis* and *Tetrasetmis* sp., respectively). The name denotes the broad phylogenetic relationship of this family to the family *Mimiviridae*.

Family *Schizomimiviridae*

“Schizo-” from Greek schizo, “split”. This family contains *Biavirus raunefjordense* and *Kratosvirus quantuckense* to classify marine viruses that infect haptophyte and heterokont hosts, respectively. The name refers to the splitting of these viruses from others that have commonly been referred to “extended *Mimiviridae*”.

Family *Mesomimiviridae*

“Meso-” from Greek mesos, “middle”. This family contains *Tethysvirus hollandense*, *T. ontarioense*, and *T. raunefjordense*, all of which include aquatic viruses that infect haptophytes. These viruses form a highly supported monophyletic clade in our concatenated phylogeny, and we therefore propose that they should be classified in the same genus. The name refers to the previous description of these viruses as “extended *Mimiviridae*” and reflects their medium-sized genome lengths, as compared to other *Imitervirales* members. This lineage was previously suggested to represent a subfamily within the family *Mimiviridae* with the name *Mesomimivirinae* [6], but analysis of the concatenated phylogeny demonstrates that this group can be clearly distinguished from the *Mimiviridae* and that a new, family-level rank is appropriate (Fig. 1).

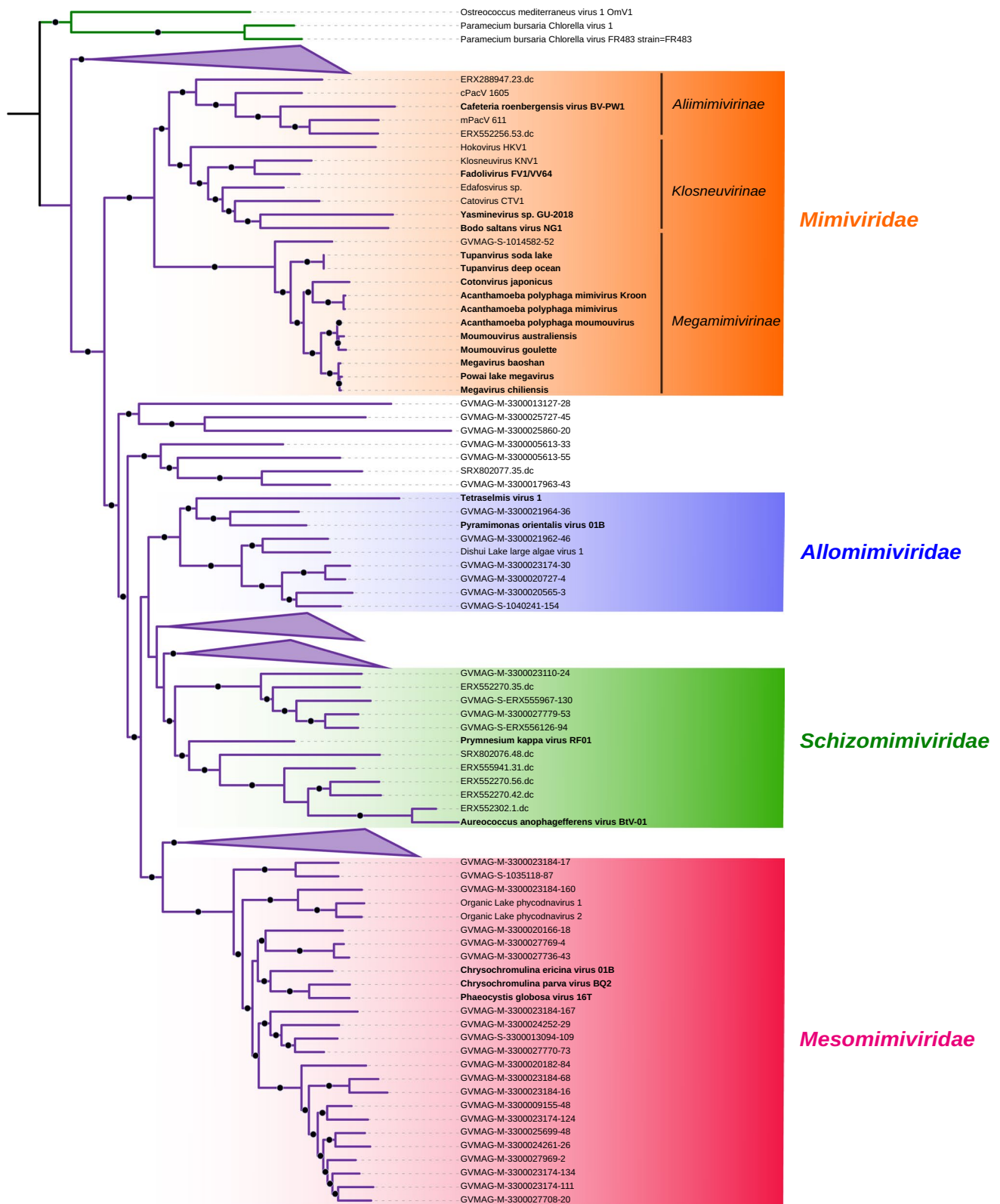


Fig. 1 Phylogeny of the members of the order *Imitervirales*, based on an alignment of seven concatenated marker genes (see main text for details). Viruses classified in new or renamed species are shown in bold. Reference genome sequences were obtained from the Giant

Virus Database (<https://faylward.github.io/GVDB/>). The tree was constructed using the ncldv_markersearch workflow (https://github.com/faylward/ncldv_markersearch), which has been described previously [8]

Table 1 Taxonomic update for the order *Imitervirales*

Family	Subfamily	Genus	Species	Virus isolate	NCBI accession no.
<i>Allomimiviridae</i>	–	<i>Heliosvirus</i>	<i>Heliosvirus raunefjordenense</i>	Pyramimonas orientalis virus 01B	MT663534:MT663543
	–	<i>Oceanusvirus</i>	<i>Oceanusvirus kaneohense</i>	Tetraselmis virus 1	KY322437
<i>Mesomimiviridae</i>	–	<i>Tethysvirus</i>	<i>Tethysvirus hollandense</i>	Phaeocystis globosa virus PgV-16T	KC662249
				Phaeocystis globosa virus PgV-12T	HQ634147
				Phaeocystis globosa virus PgV-14T	HQ634144
			<i>Tethysvirus ontarioense</i>	Chrysochromulina parva virus BQ2	MH918795
			<i>Tethysvirus raunefjordenense</i>	Chrysochromulina ericina virus CeV-01B	KT820662
<i>Mimiviridae</i>	<i>Aliimimivirinae</i>	<i>Rheavirus</i>	<i>Rheavirus sinismexicani</i>	Cafeteria roenbergensis virus BV-PW1	GU244497
	<i>Klosneuvirinae</i>	<i>Fadolivirus</i>	<i>Fadolivirus algeromassiliense</i>	Fadolivirus FV1/VV64	MT418680
		<i>Theiavirus</i>	<i>Theiavirus salishense</i>	Bodo saltans virus strain NG1	MF782455
		<i>Yasminevirus</i>	<i>Yasminevirus sauidmassiliense</i>	Yasminevirus sp. GU-2018	UPSH01000001
	<i>Megamimivirinae</i>	<i>Cotonvirus</i>	<i>Cotonvirus japonicum</i>	Cotonvirus japonicus	AP024483
		<i>Megavirus</i>	<i>Megavirus baoshanense</i>	Megavirus baoshan strain SH	MH046811
			<i>Megavirus chilense</i>	Megavirus chilensis	JN258408
				Megavirus lba isolate LBA111	JX885207
				Megavirus terra1 genome	KF527229
				Megavirus courdo11	JX975216
				Acanthamoeba polyphaga mimivirus ASM381511v1	MG602508
				Acanthamoeba polyphaga mimivirus ASM381513v1	MG602507
				Megavirus vitis isolate vigne	MG807319
			<i>Megavirus powaiense</i>	Powai lake megavirus isolate 1	KU877344
		<i>Mimivirus</i>	<i>Mimivirus bradfordmassiliense</i>	Acanthamoeba polyphaga mimivirus ViralProj60053	HQ336222
				Mimivirus terra2 genome	KF527228
				Acanthamoeba castellanii mamavirus Hal-V	JF801956
				Acanthamoeba castellanii mimivirus kasaii	AP017644
				Acanthamoeba castellanii mimivirus shirakomae	AP017645
				Acanthamoeba polyphaga mimivirus M4	JN036606
				Acanthamoeba polyphaga mimivirus Oyster	KM982401
				Acanthamoeba polyphaga mimivirus	AY653733
				Hirudovirus strain Sangsue	KF493731

Table 1 (continued)

Family	Subfamily	Genus	Species	Virus isolate	NCBI accession no.
				Mimivirus Bombay isolate 1	KU761889
				Niemeyer virus	KT599914
				Samba virus	KF959826
				Acanthamoeba polyphaga mimivirus strain Amazonia	KM982403
			<i>Mimivirus lagoaense</i>	Acanthamoeba polyphaga mimivirus Kroon	KM982402
		<i>Moumouvirus</i>	<i>Moumouvirus australiense</i>	Moumouvirus australiensis isolate 10A	MG807320
			<i>Moumouvirus goulettemassiliense</i>	Moumouvirus goulette	KC008572
			<i>Moumouvirus moumou</i>	Acanthamoeba polyphaga moumouvirus	JX962719
				Saudi moumouvirus	KY110734
		<i>Tupanvirus</i>	<i>Tupanvirus altamarinense</i>	Tupanvirus deep ocean	MF405918
			<i>Tupanvirus salinum</i>	Tupanvirus soda lake	KY523104
<i>Schizomimiviridae</i>	–	<i>Biavirus</i>	<i>Biavirus raunefjordense</i>	Prymnesium kappa virus RF01	HG999358
	–	<i>Kratovirus</i>	<i>Kratovirus quantuckense</i>	Aureococcus anophagefferens virus isolate BtV-01	KJ645900

Proposed subfamilies

Three new subfamilies have been created within the family *Mimiviridae*, all of which form highly supported clades of approximately the same phylogenetic breadth. The literature often refers to the subfamilies *Megamimivirinae* and *Klosneuvirinae*, and we therefore felt it is appropriate to formalize these names and also to introduce the subfamily *Aliimimivirinae* such that consistent reference can be made to all subclades within this family.

Subfamily *Megamimivirinae*

This subfamily includes members of the genera *Tupanvirus*, *Cotonvirus*, *Mimivirus*, *Megavirus*, and *Moumouvirus*, all of which infect amoebae. The name is already used frequently in the literature (e.g., [6]) and reflects their genome lengths in the megabase-pair range.

Subfamily *Klosneuvirinae*

This subfamily includes the viruses Fadolivirus FV1/VV64 (species *Fadolivirus algeromassiliense*), Yasminevirus sp. GU-2018 (species *Yasminevirus saudimassiliense*), and Bodo saltans virus (species *Theiavirus salishense*). The first two of these infect amoeba hosts, while the third infects a kinetoplastid phagotrophic protozoan (*Bodo saltans*). The name is already used frequently in the literature (e.g., [10]).

Subfamily *Aliimimivirinae*

“Alii-”, Latin for “other”. This subfamily contains a single species, *Rheavirus sinusmexicani*, to classify Cafeteria roenbergensis virus (CroV), which infects a flagellate protozoan (*Cafeteria roenbergensis*).

Demarcation criteria

Members of the same species have pairwise ANI > 95% for >75% of the predicted genes in each genome. The species *Mimivirus bradfordmassiliense*, *Moumouvirus 22moumou*, *Megavirus chilense*, and *Tethysvirus hollandense* include multiple viral isolates that fit these criteria (Table 1).

Families and genera are defined as monophyletic clades in the concatenated tree that have high bootstrap support (>80%) and approximately equivalent phylogenetic breadth (Fig. 1). Our family-level demarcations are consistent with a phylogenomic framework that has been proposed recently [8].

This phylogenetic methodology should be adequate for adding new taxonomic levels in the future. Although it relies on seven marker genes (PolB, A32, SFII, VLTF3, RNAPS, TopoII, and TFIIB), genome sequences can be included even if they encode only a subset of these genes. For example, prasinoviruses that lack RNA polymerase subunits were still included using this phylogenetic approach, and they had robust phylogenetic placement [8]. Therefore, novel viral lineages that are discovered in the future could still be included using these methods even if their genomic composition differed slightly from the viruses examined here. We propose that new families should be created once the first complete genome sequence is available for a viral lineage that represents a phylogenetic breadth consistent with the existing families. Most of these complete genome sequences will likely be derived from isolated viruses, but in the future it may be possible for high-quality genome sequences that are suitable for classification to be obtained using culture-independent methods, which is in full agreement with recently adopted ICTV standards and recommendations for development of a universal sequence-based virus taxonomy [11, 12].

Conclusions

Recent studies have shown that members of the family *Mimiviridae* and other viruses in the order *Imitervirales* are abundant in a wide range of ecosystems across the planet [2, 4, 5, 13, 14]. These viruses infect diverse protists from across the eukaryotic tree of life, including amoebae, green algae, euglenoids, stramenopiles, and haptophytes [15–19]. Furthermore, members of the order *Imitervirales* typically have large genomes that encode proteins with a wide range of complex functions, including rhodopsins, cytoskeletal structural proteins, and predicted components of the TCA cycle and glycolysis [4, 20–24]. As the number of known viruses within the order *Imitervirales* has grown, it has

become clear that the establishment of several new families is needed to classify the burgeoning diversity within this group. For example, a recent study using primarily metagenome-derived giant virus genome sequences suggested that the order *Imitervirales* contains at least 11 families [8]. The newly adopted families *Allomimiviridae*, *Mesomimiviridae*, and *Schizomimiviridae* are therefore likely to be the first of several new families that will be demarcated within the order *Imitervirales* as new viruses are isolated and complete genome sequences become available.

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Declarations

Conflict of interest The authors have not disclosed any competing interests.

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