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Virophages question the existence of satellites

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In a recent Comment (Virophages or satellite viruses? *Nature Rev. Microbiol.* **9**, 762–763 (2011))[1], Mart Krupovic and Virginija Cvirkaite-Krupovic argued that the recently described virophages, Sputnik and Mavirus, should be classified as satellite viruses. In a response[2], to which Krupovic and Cvirkaite-Krupovic replied[3], Matthias Fisher presented two points supporting the concept of the virophage: first, Sputnik and Mavirus are not defective particles but fully functional viruses, and second, they have been shown (at least *in silico*) to divert the machinery of the host virus, rather than that of the host cell, for genome replication and transcription.

Here, we want to go beyond semantics and look at the problem another way. We propose a remodelled classification based on our previously suggested bipartite system of capsid-encoding organisms (CEOs) and ribosome-encoding organisms (REOs)[4], which, in addition to the existing viruses of Bacteria (bacteriophages), Eukarya and Archaea, would incorporate a new group called 'viruses of Viruses', encompassing virophages and traditional satellite RNA viruses.

What is a virophage?

A virophage is a viral parasite of giant viruses, thriving by exploiting the machinery of the viral host for genome replication and transcription, impairing production of the viral host and causing the generation of abnormal and degenerated forms of the viral host[5].

A virophage is a virus. Sputnik (the first virophage described)[5] and the recently discovered Mavirus[6] are particles of about 50 nm diameter, with double-stranded DNA genomes of 18,343 bp and 19,063 bp coding for 21 and 20 ORFs, respectively. Both possess structural (for example, major capsid protein (MCP)) and non structural (for example, polymerase and genome-packaging ATPase) proteins. As the Sputnik and Mavirus genomes harbour their own capsid-encoding genes, they are consistent with the definition of viruses as CEOs[4,7]. The absence of significant similarities between the genome sequences of Sputnik, Mavirus and Organic Lake virophage (OLV; a virophage sequence identified from metagenomic data)[8] and genome sequences of other known viruses indicates that the virophages probably belong to a new viral family. This is further supported by structural analysis of Sputnik, which showed that MCP probably adopts a double-jelly-roll fold, although there is no sequence similarity between the virophage MCPs and those of other members of the bacteriophage PRD1–adenovirus lineage[9]. With the size of the Sputnik particle, the nature and complexity of its genome, and the presence of a ready-to-use set of viral RNAs in the virion[10], Sputnik challenges the current definition of satellite viruses, which relies on the idea of a defective particle or subviral agent.

A virophage is a parasite of another virus. To illustrate the similarity between the dependence of a satellite virus on the helper virus and the dependence of a virophage on its viral host, Krupovic and Cvirkaite-Krupovic used the example of the satellite tobacco necrosis virus (STNV), the single-stranded RNA genome of which is replicated by the RNA-dependent RNA polymerase (RdRp) of the helper virus, TNV[1]. We believe that this is equivalent to comparing cows and apples. As the single-stranded RNA genomes serve directly as mRNA, the replication cycle of the STNV–TNV couple does not have a transcription stage. In addition, the RdRp is a typical viral enzyme that is not found in eukaryotic, bacterial or archaeal genomes, so to replicate, STNV has no choice but to rely on this enzyme. As an alternative (although still imperfect) comparison, a parallel could have been made with the adeno-associated virus (AAV)–adenovirus system, as both are DNA viruses. Following the AAV example, and as is the case for DNA viruses of comparable size, the Sputnik genome would be expected to transit into the nucleus, where it would be replicated and transcribed by the host cell machinery. However, recent *in situ* hybridization experiments have shown that the Sputnik infection cycle has no nuclear phase and that genome replication takes place inside the viral factory, with a maximum of activity at 6–7 hours post-infection (C.D. and D.R., unpublished observations). In addition, the previously described polyadenylation signals and the AT-rich conserved promoter motifs located in front of 12 out of 21 Sputnik coding sequences and all 20 *Cafeteria roenbergensis* virus coding sequences (both promoters being associated with the late expression of genes) imply that virophage gene expression is governed by the transcription machinery of the host virus during the late stages of infection.

Another point concerns the effect of the virophage on the host virus. It has been argued that the effect of Sputnik or Mavirus on the host is similar to that observed for STNV and its helper virus[1]. However, in some cases the infectivity of TNV is greater when inoculated along with STNV (or its nucleic acid) than when inoculated alone, suggesting that STNV makes cells more susceptible to TNV[11]. Such an effect has never been observed for Sputnik or Mavirus, for which a negative effect on the host virus is always obtained. In addition, a satellite virus has never been shown to produce a 'diseased' form of its helper virus or (even though rare with Sputnik) to be fully encapsidated within it.

A virophage has ecological and evolutionary significance. By predating on giant viruses such as members of the families *Mimiviridae* or *Phycodnaviridae*, virophages regulate viral population dynamics and probably influence the whole microbial food web. In a recent study, Yau *et al.* used a Lotka–Volterra simulation to model the effect of a virophage on the phycodnavirus–green alga interaction[8]. The addition of a virophage to this system led to an increase in the survival of the host algal cell population and a deviation of the microbial loop towards secondary production.

The presence of genes from different viral origins in the Sputnik genome has stressed the role of Sputnik as a probable vehicle for gene transfer among viruses[5]. In addition, on the basis of the similarities between the Mavirus genome and transposable elements of the Maverick–Polinton family, it has even been suggested that large DNA transposons found in eukaryotic genomes emerged from a virophage ancestor[6].

The presence of numerous giant virus and virophage signatures in environmental metagenomic data sets suggests that, as for bacteriophages in the ocean[12,13], virophages are common in the environment and play an unrecognized part in regulating virus–host interactions and contributing to global gene flow in the virosphere.

What is a satellite virus?

The International Committee on Taxonomy of Viruses (ICTV) describes satellite viruses as "subviral agents lacking genes that could encode functions needed for replication and

depending on the co-infection of a host cell with a helper virus for their multiplication" (REFS 14,15). Unlike satellite nucleic acids, satellite viruses also encode a structural protein that encapsidates their genome. Until the release of *Virus Taxonomy. Ninth Report of the International Committee on Taxonomy of Viruses* (on 8 November 2011)[15], the satellite viruses were classified in a unique category, itself subdivided into two subgroups: subgroup 1, with the chronic bee-paralysis virus-associated satellite virus as representative, and subgroup 2, with satellites that resemble STNV. Thus, when we described Sputnik (which means satellite in Russian) the category of satellite viruses included only small particles of 15 to 17 nm diameter encapsidating a single-stranded RNA molecule of 796 to 1,239 bp and displaying only one to two ORFs.

The AAVs, which are commonly called satellite viruses in the literature and were cited as a representative of satellite viruses by Krupovic and Cvirkaite-Krupovic[1], were previously not included in the satellite virus category of the ICTV but instead were classified with the single-stranded DNA viruses and belong to the *Parvoviridae* family (Dependovirus genus). The ninth report of the ICTV now also includes AAVs in the satellite section and specifies that "this group of satellites is anomalous, having been placed in a genus Dependovirus within the family *Parvoviridae*". AAVs are thus now found in two different sections of the ICTV. More confusingly, the ICTV specifies in section 3.38 ('Rules about sub-viral agents') that "satellites and prions are not classified as viruses but are assigned an arbitrary classification as seems useful to workers in the particular fields" (REF. 15).

This provides a useful illustration of the complexities of classifying viruses and the fact that, sometimes, new findings do not fit into pre-existing boxes.

The new 'viruses of Viruses' domain

As Karl Popper said, "The logical probability of a statement is complementary to its degree of falsifiability: it increases with decreasing degree of falsifiability." (REF. 16.) We believe that a non-recognized satellite category — in which all 'elements associated with viruses' are brought together and the definition is adapted to accommodate more and more diversity — no longer has a basis in reality. We thus propose to add the new domain of 'viruses of Viruses' to our previously suggested classification of CEOs (FIG. 1). This domain would include the virophages, AAVs and traditional satellite viruses. Finally, the 'other' category, also called 'orphan replicons', would contain mobile genetic elements such as plasmids, transposons and satellite nucleic acids.

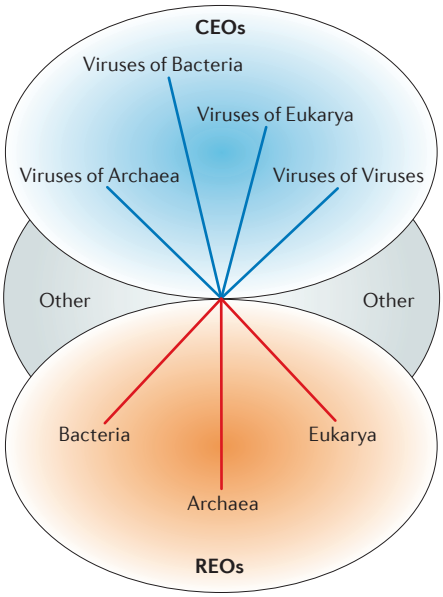

Figure 1 | **The capsid-encoding organisms (CEOs), including the traditional viruses of Bacteria, viruses of Archaea and viruses of Eukarya plus the new 'viruses of Viruses' domain.** The capsid-encoding organisms (CEOs) rely on ribosome-encoding organisms (REOs) for the synthesis of proteins and the production of energy. Figure is modified, with permission, from REF. 4 © (2008) Macmillan Publishers Ltd. All rights reserved.

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Competing interests statement
The authors declare no competing financial interests.

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