Template for Taxonomic Proposal to the ICTV Executive Committee
To create a new Family

Code† **2005.004F.04** To create a new family* °

Code† **2005.005F.04** To name the new family* ***Mimiviridae***

Code† To accommodate the new genus created:

Mimivirus

† Assigned by ICTV officers
° Leave blank is not appropriate
* repeat these lines and the corresponding arguments for each genus created in the family

Author(s) with email address(es) of the Taxonomic Proposal

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Old Taxonomic Order

Order	
Family	
Genus	***Mimivirus***
Type Species	*Acanthamoeba polyphaga mimivirus*
Species in the Genus	*Acanthamoeba polyphaga mimivirus*
Tentative Species in the Genus	
Unassigned Species in the family	

New Taxonomic Order

Order	
Family	***Mimiviridae***
Genus	***Mimivirus***
Type Species	*Acanthamoeba polyphaga mimivirus*
Species in the Genus	*Acanthamoeba polyphaga mimivirus*
Tentative Species in the Genus	
Unassigned Species in the family	

ICTV-EC comments and response of the SG

Argumentation to create a new family:

Acanthamoeba polyphaga mimivirus (AY653733) is an emerging virus characterized in 2002-2004. It is a nucleocytoplasmic large DNA virus (NCLDV) (virions are 600 nm in diameter) the complete genome of which has been sequenced (Raoult et al., 2005, Science *306(5700):1344-50)*. The genome size is 1.2 Mb and contains 1261 ORFs (greater than 300 nt), including 911 very likely protein coding genes. The virus exhibits many original features such as numerous translation apparatus-related genes (e.g. 4 aminoacyltRNA synthetases, 4 translation factors, enzymes for all DNA repair mechanism, and 3 types of topoisomerase 1a, 1b, and 2). Phylogenetic trees derived from a concatenation of the sequences of 7 NCLDV conserved gene sequences indicate that it occupies an intermediate position between *Iridoviridae,* and *Phycodnaviridae.*

Origin of the proposed family name

Mimiviridae, mimi-for "microbe mimicking" because of its particle size that makes it visible under the light microscope, ressembling to a small Gram-positive coccus on Gram staining (mimicking microbe).

References

La Scola, B., Audic S., Robert, C., Jungang, L., de Lamballerie, X., Drancourt, M., Birtles, R., Claverie, L.M., and Raoult, D. (2003). A giant virus in amoebae. Science **299**, 2033.

Raoult D, Audic S, Robert C, Abergel C, Renesto P, Ogata H, La Scola B, Susan M, Claverie JM. The 1.2-Mb Genome Sequence of Mimivirus. *Science. 2004*, *306:1344-50*.

Annexes:

A Giant Virus in Amoebae

Bernard La Scola,[1] Stéphane Audic,[2] Catherine Robert,[1] Liang Jungang,[1] Xavier de Lamballerie,[3] Michel Drancourt,[1] Richard Birtles,[1] Jean-Michel Claverie,[2]* Didier Raoult[1]*

During a study following a pneumonia outbreak in 1992, a microorganism growing in amoebae and resembling a small Gram-positive coccus (Fig. 1A) was isolated from the water of a cooling tower in Bradford, England. Despite attempts with various extraction protocols and low-stringency polymerase chain reaction, no amplification product was obtained with universal 16*S* rDNA bacterial primers (*1*).

Study of this microorganism within *Acanthamoeba polyphaga* (*2*) revealed a characteristic viral morphology with mature particles of 400 nm in diameter and surrounded by an icosahedral capsid. This structure is consistent with the finding that Mimivirus is not filterable through 0.2-μm pore size filters. No envelope was observed, but 80-nm fibrils attached to the capsid were visible (fig. S1). A typical virus developmental cycle, including an eclipse phase, was observed (fig. S2). As it resembles a bacterium on Gram staining, it was named Mimivirus (for Mimicking microbe) (Fig. 1A). DNA digestion by Sal I and Sac II treatment of purified particles (*2*), followed by pulsed-field gel electrophoresis, demonstrated that Mimivirus has a double-stranded DNA circular genome of about 800 kilobase pairs (kbp). Its genome is thus larger than the sequenced genomes of several bacteria, including *Mycoplasma genitalium* (580 kbp), *Ureaplasma urealyticum* (752 kbp), *Buchnera* sp. (641 kbp), and *Wigglesworthia brevipalpis* (698 kbp) (*3*). Consistent with this large genome, Mimivirus particles have a size comparable to that of small bacteria such as *U. urealyticum* (Fig. 1B). The viruses with the largest genomes previously described are a Phycodnavirus infecting *Pyramimonas* algae (560 kbp) and phage D of *Bacillus megaterium* (670 kbp) (*4*, *5*).

Mimivirus is a nucleocytoplasmic large DNA virus (NCLDV). This group of viruses includes four other families, including the enveloped *Poxviridae*, which infect vertebrates (*Chordopoxvirinae*) and insects (*Entomopoxvirinae*). The three others are also icosahedral. *Iridoviridae* and *Phycodnaviridae* are aquatic viruses, and *Asfarviridae* infect vertebrates (*6*). Whole genome shotgun sequencing is under way. Two libraries (5-kb and 9-kb inserts obtained by mechanic shearing, cloned in pcdna 2.1 with Bst XI adaptators) were constructed. Plasmid inserts were sequenced from both ends with flanking vector sequences and dye terminator primers. The preliminary assembly [using the Phred/Phrap software (*7*)] of 6X coverage shotgun data confirmed that the Mimivirus genome is about 800 kbp (734 kbp of preliminary sequence data with phrap score >20 is available in the WGS section of GenBank, accession # AABV01000000). More than 900 open reading frames (ORFs) longer than 100 amino acids were identified, representing ~82.4% of the available genome, a coding fraction comparable to other NCLDVs. Comparisons to DNA and protein sequence databases (GenBank, Swissprot, and TrEMBL) did not reveal any sign of amoebal or other contamination.

Following Iyer *et al.* (*8*), we compared Mimivirus ORFs with viral proteins only, allowing greater sensitivity in relating it to one of the established families of large eukaryotic DNA viruses (*2*). We identified 21 Mimivirus proteins with known functional attributes and clear homologs in at least one of these virus families, as follows: nine in *Phycodnaviridae*, six in *Poxviridae*, five in *Iridoviridae*, and one in *Baculoviridae*. Some of the genes also exhibited lower similarity to *Baculoviridae* or *Asfarviridae* homologs. These results suggest that Mimivirus occupies an intermediary position between *Poxviridae*, *Iridoviridae*, and *Phycodnaviridae*, with which Mimivirus appears to share the Vp54 capsid protein and a glucosamine synthetase unique to the *Paramecium bursaria Chlorella* virus. Mimivirus appears as a deep branch in the phylogenetic tree (Fig. 1C), suggesting early divergence from other virus families.

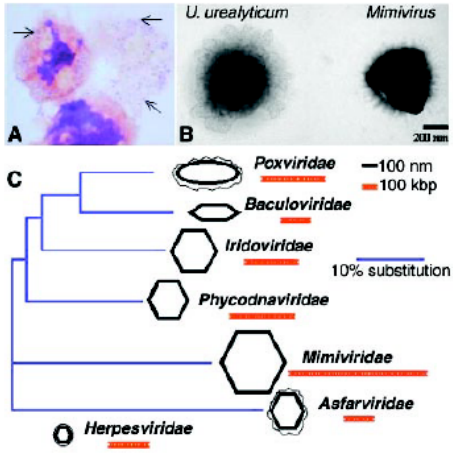

Fig. 1. (**A**) Mimivirus (arrows) in cytocentrifuged *A. polyphaga* as Gram-positive particles. (**B**) Electronic microscopy of Mimivirus and *U. urealyticum*. (**C**) Phylogenetic tree from alignment of ribonucleotide reductase small subunit sequences (*9*). Similar trees are obtained with ribonucleotide reductase large subunits and topoisomerase 2.

Although further characterization is needed, Mimivirus's icosahedral ultrastructure and the typical eclipse phase in its life cycle support its viral nature. Furthermore, Mimivirus lacks universal bacterial genes, such as those encoding ribosomal RNA or proteins, as well as other ubiquitous bacterial proteins involved in protein translation. The high fraction (80%, P value $< 10^{-6}$) of ORFs without significant similarity to other organisms is also typical of viruses. Finally, the Mimivirus genome has 21 genes encoding homologs to proteins highly conserved in most NCLDVs (*8*). We propose that Mimivirus is a member of a new family of giant virus, the *Mimiviridae*, that represents a divergent taxon within the NCLDV group.

References and Notes

1. R. Birtles *et al.*, *Lancet* **349**, 925 (1997).
2. Materials and Methods are on *Science* Online.
3. See www.ncbi.nlm.nih.gov/PMGifs/Genomes/eub_g.html
4. R. A. Sandaa *et al.*, *Virology* **290**, 272 (2001).
5. M. S. Hutson *et al.*, *Biopolymers* **35**, 297 (1995).
6. M. H. V. Van Regenmortel *et al.*, *Virus Taxonomy: 7th Report of the International Committee on Taxonomy of Viruses* (Academic Press, San Diego, CA, 2000).
7. B. Ewing *et al.*, *Genome Res.* **8**, 175 (1998).
8. L. M. Iyer *et al.*, *J. Virol.* **75**, 11720 (2001).
9. Multiple alignment was done with T-COFFEE software (igs-server.cnrs-mrs.fr) and the tree computed on the EBI server (www.ebi.ac.uk/clustalw/) with the default options, ignoring gaps, correcting distances and phylip tree. Accession numbers: Mimivirus, AF529888; Cowpox virus, NP_619839; *Lymantria dispar* nucleopolyhedrovirus, NP_047757; *Paramecium bursia chlorella* virus 1, NP_048832; infectious spleen and kidney necrosis virus, NP_612246; and African swine fever virus, NP_042738.
10. We thank T. Rowbotham for providing the isolate now identified as Mimivirus; W. F. Denis for helpful discussion; and B. Guimelli, A. Carlioz, and L. Barrassi for technical help. Supported by a grant from the Ministère Français de l'Aménagement du Territoire et de l'Environnement (convention EN00C13).

Supporting Online Material
www.sciencemag.org/cgi/content/full/299/5615/2033/DC1
Materials and Methods
Figs. S1 and S2

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