This form should be used for all taxonomic proposals. Please complete all those modules that are applicable (and then delete the unwanted sections). For guidance, see the notes written in blue and the separate document "Help with completing a taxonomic proposal"

Please try to keep related proposals within a single document; you can copy the modules to create more than one genus within a new family, for example.

MODULE 1: **TITLE, AUTHORS, etc**

| | | |
|---|---|---|
| **Code assigned:** | ***2011.014aV*** | (to be completed by ICTV officers) |

**Short title:** Create species *Murid herpesvirus 8* in the genus *Muromegalovirus*, subfamily *Betaherpesvirinae*, family *Herpesviridae*, order *Herpesvirales*
(e.g. 6 new species in the genus *Zetavirus*)

| | | | | | |
|---|---|---|---|---|---|
| **Modules attached** (modules 1 and 9 are required) | **1** ☒ | **2** ☒ | **3** ☐ | **4** ☐ | **5** ☐ |
| | **6** ☐ | **7** ☐ | **8** ☐ | **9** ☒ | |

**Author(s) with e-mail address(es) of the proposer:**

Sebastian Voigt (voigts@rki.de) & Gary S Hayward (ghayward@jhmi.edu)

**List the ICTV study group(s) that have seen this proposal:**

| | |
|---|---|
| A list of study groups and contacts is provided at http://www.ictvonline.org/subcommittees.asp . If in doubt, contact the appropriate subcommittee chair (fungal, invertebrate, plant, prokaryote or vertebrate viruses) | None |

**ICTV-EC or Study Group comments and response of the proposer:**

After a discussion period, the proposal was approved by the Herpesvirales Study Group without dissent.

| | |
|---|---|
| Date first submitted to ICTV: | Feb. 15, 2011 |
| Date of this revision (if different to above): | June 17, 2011<br>October 12, 2011 (minor corrections) |

MODULE 2: **NEW SPECIES**

creating and naming one or more new species.

If more than one, they should be a group of related species belonging to the same genus. All new species must be placed in a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family. Wherever possible, provide sequence accession number(s) for one isolate of each new species proposed.

| Code | ***2011.014aV*** | (assigned by ICTV officers) |
|---|---|---|

**To create 1 new species within:**

| | |
|---|---|
| Genus: | ***Muromegalovirus*** |
| Subfamily: | ***Betaherpesvirinae*** |
| Family: | ***Herpesviridae*** |
| Order: | ***Herpesvirales*** |

Fill in all that apply.

- If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
- If no genus is specified, enter "**unassigned**" in the genus box.

| **And name the new species:** *Murid herpesvirus 8* | **GenBank sequence accession number(s) of reference isolate:** |
|---|---|
| | Sequences are available for six genomic segments encompassing 26,795 bp: AF302184, FJ477243, EU267790, GU018179, U62396, and AY166871. |

**Reasons to justify the creation and assignment of the new species:**

- Explain how the proposed species differ(s) from all existing species.
  - If species demarcation criteria (see module 3) have previously been defined for the genus, **explain how the new species meet these criteria**.
  - If criteria for demarcating species need to be defined (because there will now be more than one species in the genus), please state the proposed criteria.
- Further material in support of this proposal may be presented in the Appendix, Module 9

Two isolates of rat cytomegalovirus (RCMV), the Maastricht and the English (RCMV-M and RCMV-E, respectively), have been reported and RCMV-M has been classified as *Murid herpesvirus 2*, genus *Muromegalovirus*, subfamily *Betaherpesvirinae*, family *Herpesviridae*, order *Herpesvirales*. RCMV-M was first described by Bruggeman et al. (1982), and, in the same year, Priscott and Tyrrell reported on the existence of another rat CMV that was later termed the "English" isolate (Priscott and Tyrrell, 1982; Burns et al., 1988). Both viruses had been isolated from *Rattus norvegicus*.

The complete genome sequences of both MuHV-2 (RCMV-M) and MuHV-1 (Smith strain; classical mouse CMV often called MCMV) have been published (Vink et al., 2000; NC_002512; 230,138 bp and Rawlinson et al., 1996; NC_004065; 230,278 bp) as well as those of four more very closely related isolates of MuHV-1 (Smith et al., 2008). The complete genome of RCMV-E has been sequenced (Voigt et al.) but has been published only partially. Initially, MuHV-2 (RCMV-M) and RCMV-E proved to have totally different restriction enzyme cleavage patterns suggesting that they represent different betaherpesviruses species rather than different strains of the same virus (Beisser et al. 1998, Voigt et al. 2005). From both physical analysis and the genome sequencing data, it is evident that the RCMV-E genome is considerably smaller (about 203 kb) and thus differs significantly in size from both MuHV-1 and MuHV-2 (Burns et al., 1988 and Voigt, unpublished). The following sequence data totalling 26,795-bp encompassing six selected interesting genomic segments of RCMV-E have been published: (1) a genomic fragment comprising a spliced C-type lectin-like gene located at the left terminus (AF302184, 4306 bp; Voigt et al., 2001); (2) the anti-apoptotic protein e41.1 homologue (FJ477243; 168 bp; Cam et al., 2010); (3) the e38.5 homologue (EU267790; 435 bp; Jurak et al., 2008); (4) RCMV-E glycoprotein B and DNA polymerase (GU018179; 5786 bp; Teterina et al., 2009); (5) the RCMV-E immediate early 1 and 2 genes including the promoter region (U62396; 5936 bp; Sandford et al., 1993; Sandford and Burns, 1996); and (6) the region upstream of the major immediate-early (MIE) region encoding a CD200

and a beta chemokine orthologue (AY166871; 12164 bp; Voigt et al., 2005). Together with the other published RCMV-E sequences, data for the RCMV-E MIE region especially substantiates the claim that RCMV-E is a type of CMV that lies within the *Muromegalovirus* genus (Sandford et al. 1993, Sandford et al. 1996, Voigt et al. 2005). Observations on the biology of RCMV-E in terms of locations and patterns of persistent and latency also closely resemble those expected of a muromegalovirus (Sandford et al., 2010).

The protein sequences of the majority of RCMV-E genes, as well as the gene order and arrangement resemble that found in MuHV-1 and MuHV-2 genomes, but at both the nucleotide and protein level most RCMV genes although nearly equally diverged from both MuHV-1 (MCMV) and MuHV-2 (RCMV-M) are somewhat more closely related to MuHV-1 (Voigt, unpublished). However, RCMV-E encodes several additional genes with immunomodulatory potential, including a C-type lectin-like gene and a CD200 orthologue (Voigt et al. 2001; Voigt et al., 2005) that are not found in either of the other two viruses. Within individual coding regions, amino acid sequence identity ranges from 10 to 87% between MuHV-1 and RCMV-E and from 10 to 84% between MuHV-2 and RCMV-E (Voigt, unpublished). A published phylogenetic analysis reveals that RCMV-E is only distantly related to MuHV-1 and MuHV-2 (Teterina et al., 2009). Based on the phylogenetic trees and comparison of levels of DNA identity for eight conserved genes (see module 9 below), RCMV-E is far more highly diverged (15% to 45% different) from both MuHV-1 and MuHV-2 than are human CMV (HHV5 Merlin or HHV5 AD169) and rhesus CMV (RhCMV) from each other at all eight of these loci.

In conclusion, we believe that RCMV-E meets the criteria to be classified as a new species and suggest the name *Murid herpesvirus 8*.

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

## References:


Beisser PS, Kaptein SJ, Beuken E, Bruggeman CA, Vink C. The Maastricht strain and England strain of rat cytomegalovirus represent different betaherpesvirus species rather than strains. Virology. 1998; 246:341-351.

Bruggeman CA, Meijer H, Dormans PH, Debie WM, Grauls GE, van Boven CP. Isolation of a cytomegalovirus-like agent from wild rats. Arch Virol. 1982; 73:231-241.

Burns WH, Barbour GM, Sandford GR. Molecular cloning and mapping of rat cytomegalovirus DNA. Virology. 1988; 166:140-148.

Cam M, Handke W, Picard-Maureau M, Brune W. Cytomegaloviruses inhibit Bak- and Bax-mediated apoptosis with two separate viral proteins. Cell Death Differ. 2010; 17:655-665.

Jurak I, Schumacher U, Simic H, Voigt S, Brune W. Murine cytomegalovirus m38.5 protein inhibits Bax-mediated cell death. J Virol. 2008; 82:4812-4822.

Priscott PK, Tyrrell DA. The isolation and partial characterisation of a cytomegalovirus from the brown rat, Rattus norvegicus. Arch Virol. 1982; 73:145-160.

Sandford GR, Burns WH. Rat cytomegalovirus has a unique immediate early gene enhancer. Virology. 1996; 222:310-317.

Sandford GR, Ho K, Burns WH. Characterization of the major locus of immediate-early genes of rat cytomegalovirus. J Virol. 1993; 67:4093-4103.

Sandford GR, Schumacher U, Ettinger J, Brune W, Hayward GS, Burns WH, Voigt S. Deletion of the rat cytomegalovirus immediate-early 1 gene results in a virus capable of establishing latency, but with lower levels of acute virus replication and latency that compromise reactivation efficiency. J Gen Virol. 2010; 91:616-621.

Smith LM, McWhorter AR, Masters LL, Shellam GR, Redwood AJ. Laboratory strains of murine cytomegalovirus are genetically similar to but phenotypically distinct from wild strains of virus. J Virol. 2008; 82:6689-6696.

Teterina A, Richter D, Matuschka FR, Ehlers B, Voigt S. Identification of a novel betaherpesvirus in Mus musculus. Virol J. 2009; 6:225.

Vink C, Beuken E, Bruggeman CA. Complete DNA sequence of the rat cytomegalovirus genome. J Virol. 2000; 74:7656-7665.

Voigt S, Sandford GR, Ding L, Burns WH. Identification and characterization of a spliced C-type lectin-like gene encoded by rat cytomegalovirus. J Virol. 2001; 75:603-611.

Voigt S, Sandford GR, Hayward GS, Burns WH. The English strain of rat cytomegalovirus (CMV) contains a novel captured CD200 (vOX2) gene and a spliced CC chemokine upstream from the major immediate-early region: further evidence for a separate evolutionary lineage from that of rat CMV Maastricht. J Gen Virol. 2005; 86:263-274.

Figure 1. Phylogenetic trees. Amino acid sequences were aligned with Mega5. Trees were generated using Maximum analysis (JTT+F+G+I model). Branch lengths are proportional to evolutionary distance (scale bar). Bootstrap scores are indicated

Figure 1A. Phylogenetic tree using DNA polymerase (E54/UL54) sequences of indicated viruses.

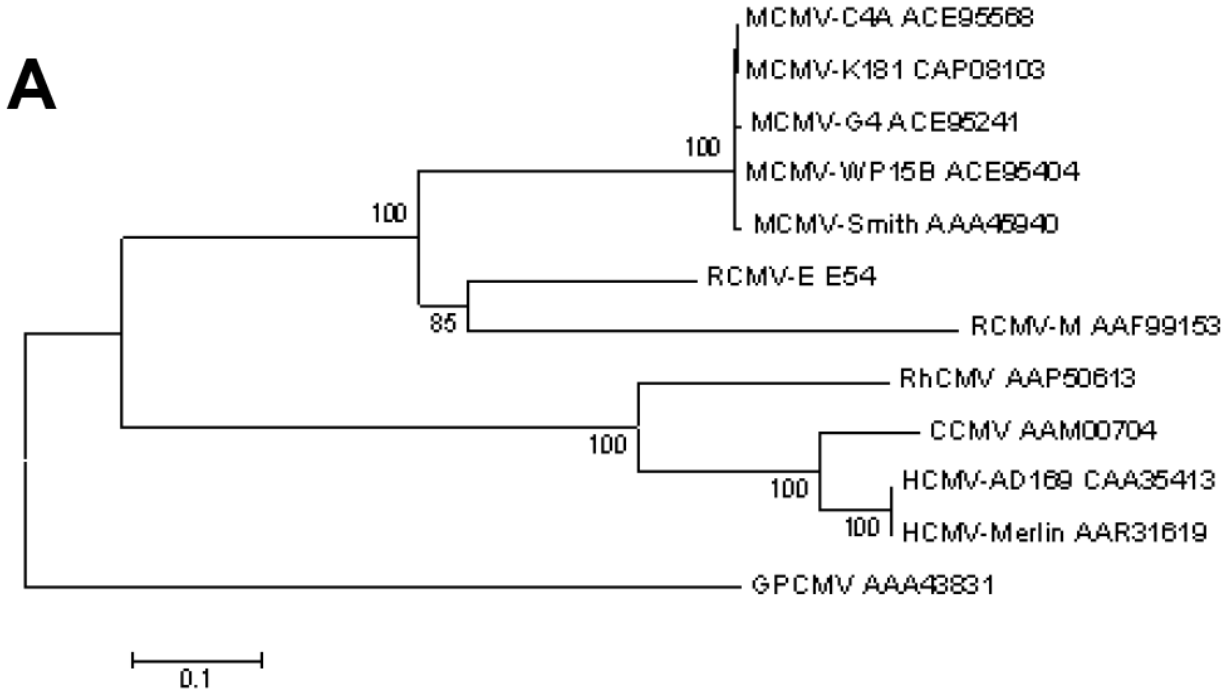


Figure 1B. Phylogenetic tree using glycoprotein B (E55/UL55) sequences of indicated viruses.

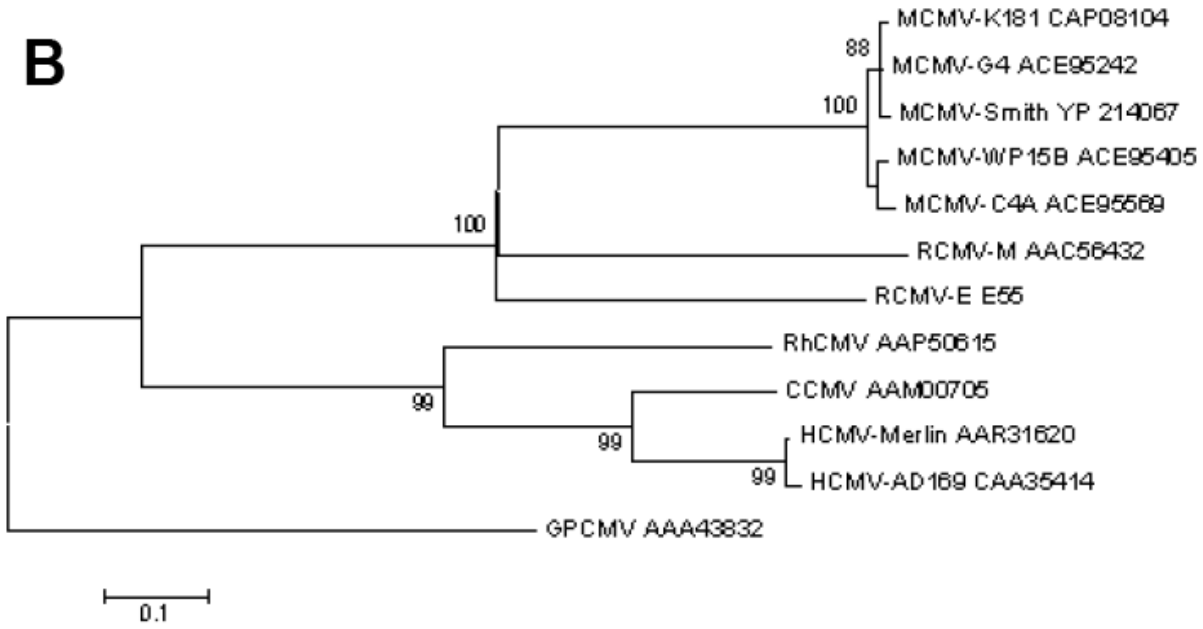

Figure 1C. Phylogenetic tree using terminase subunit (E56/UL56) sequences of indicated viruses.

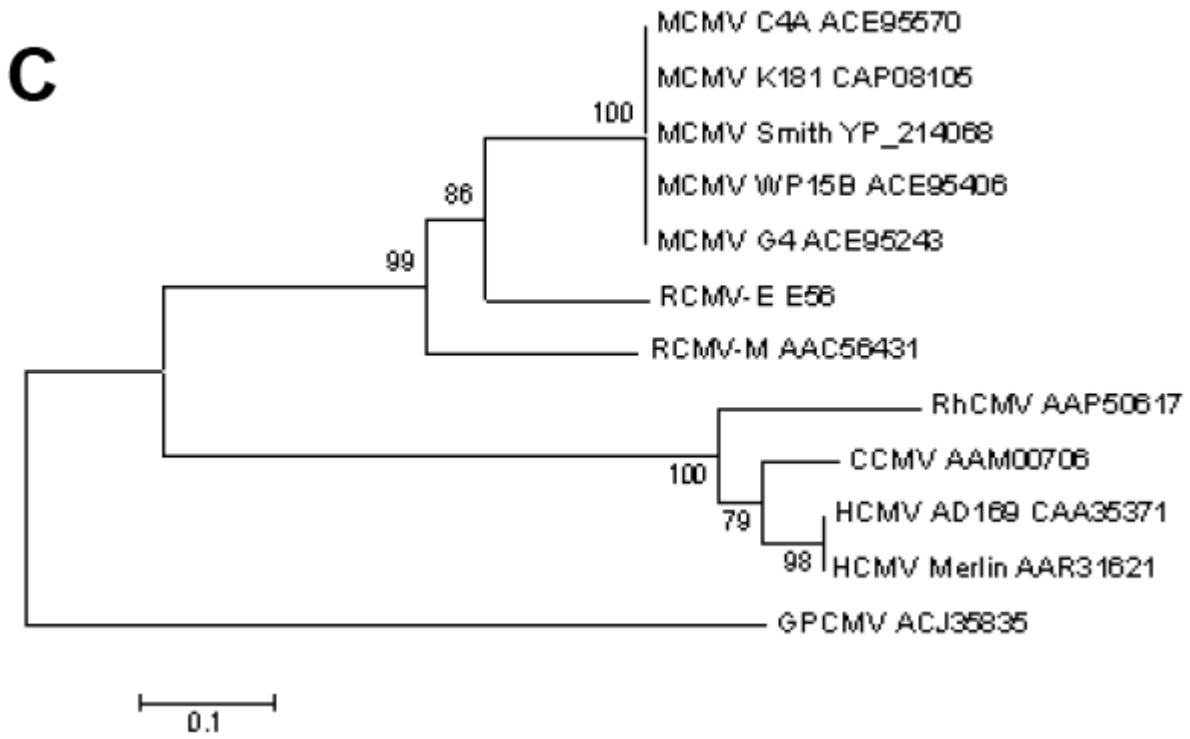


Figure 1D. Phylogenetic tree using major DNA binding protein (E57/UL57) sequences of indicated viruses.

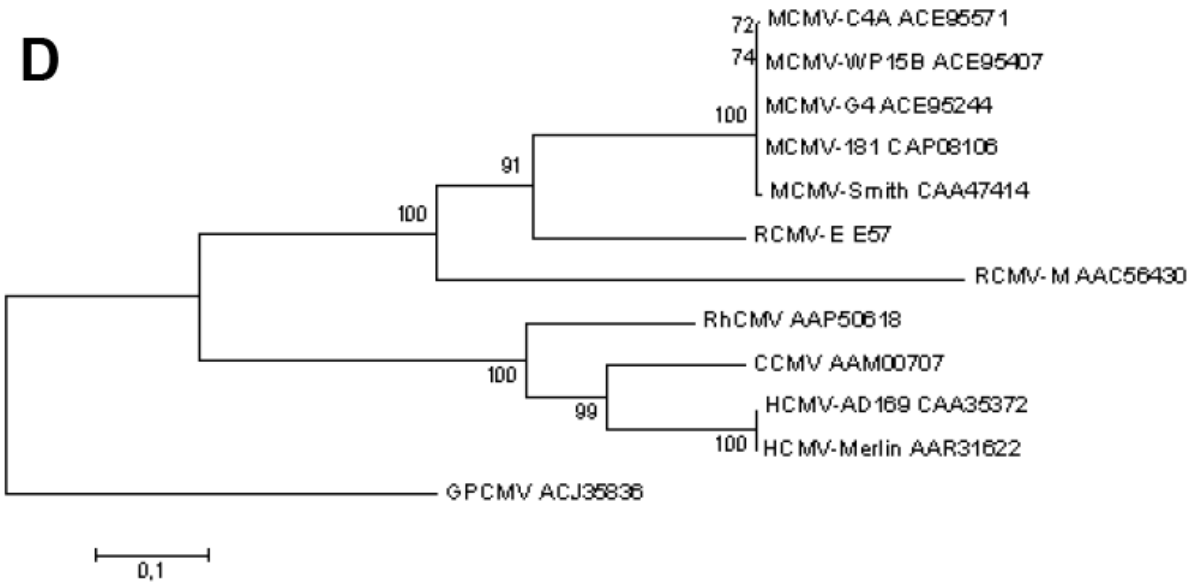

Figure 1E. Phylogenetic tree using major capsid protein (e86/UL86) sequences of indicated viruses.

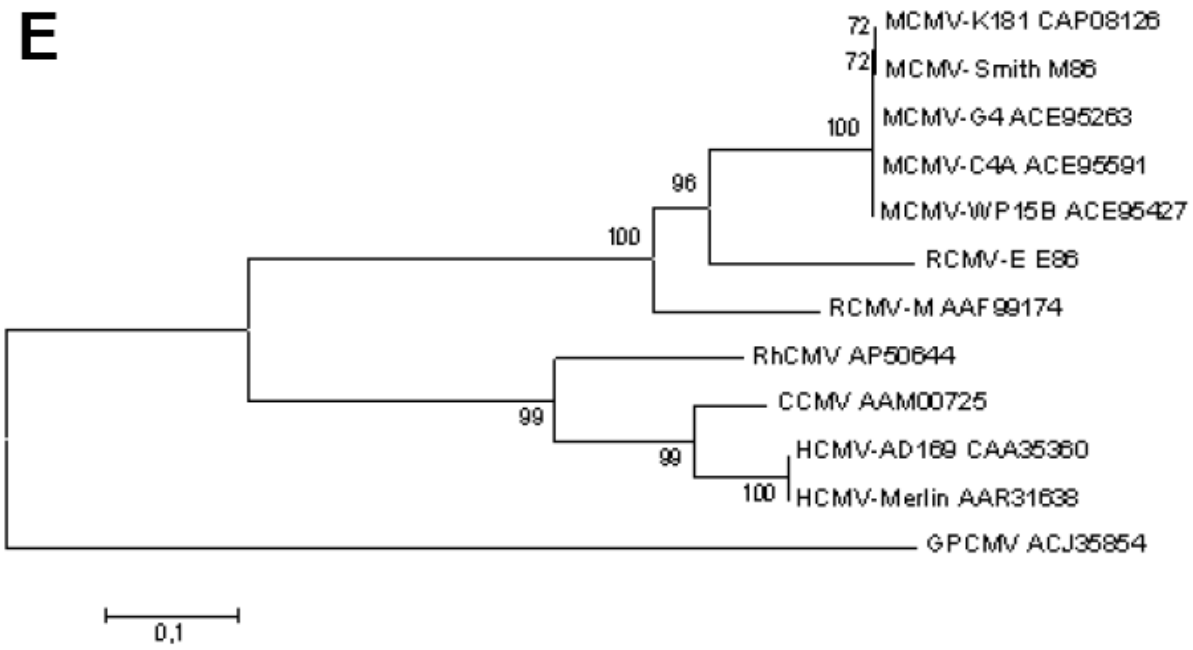


Figure 1F. Phylogenetic tree using DNA packaging protein (E89/UL89) sequences of indicated viruses.

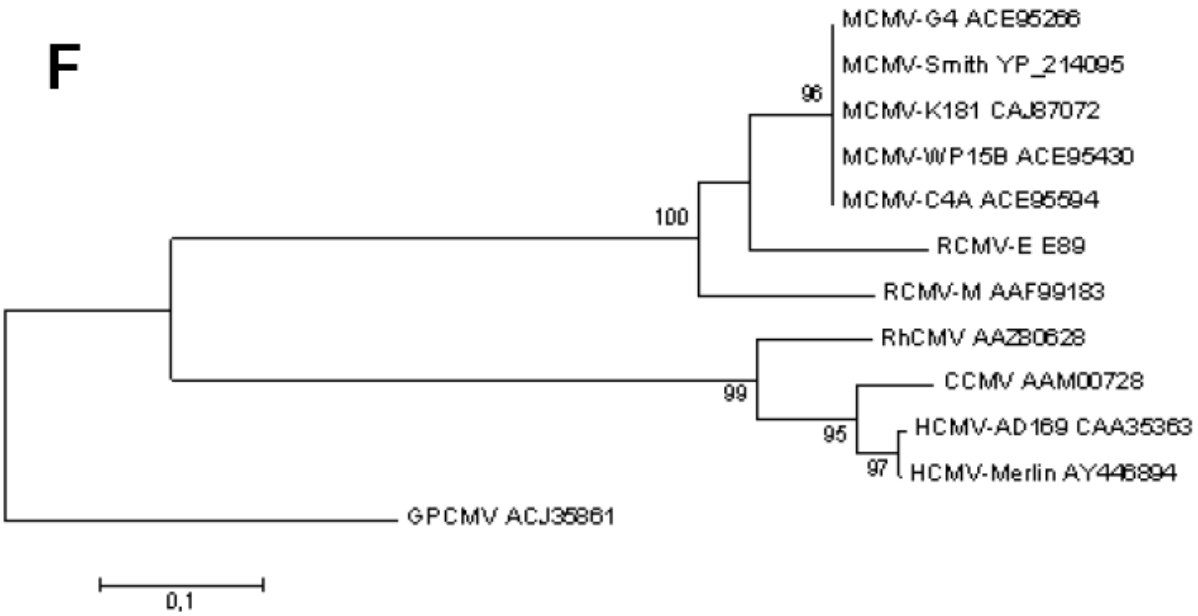

Figure 1G. Phylogenetic tree using DNA helicase protein (e105/UL105) sequences of indicated viruses.

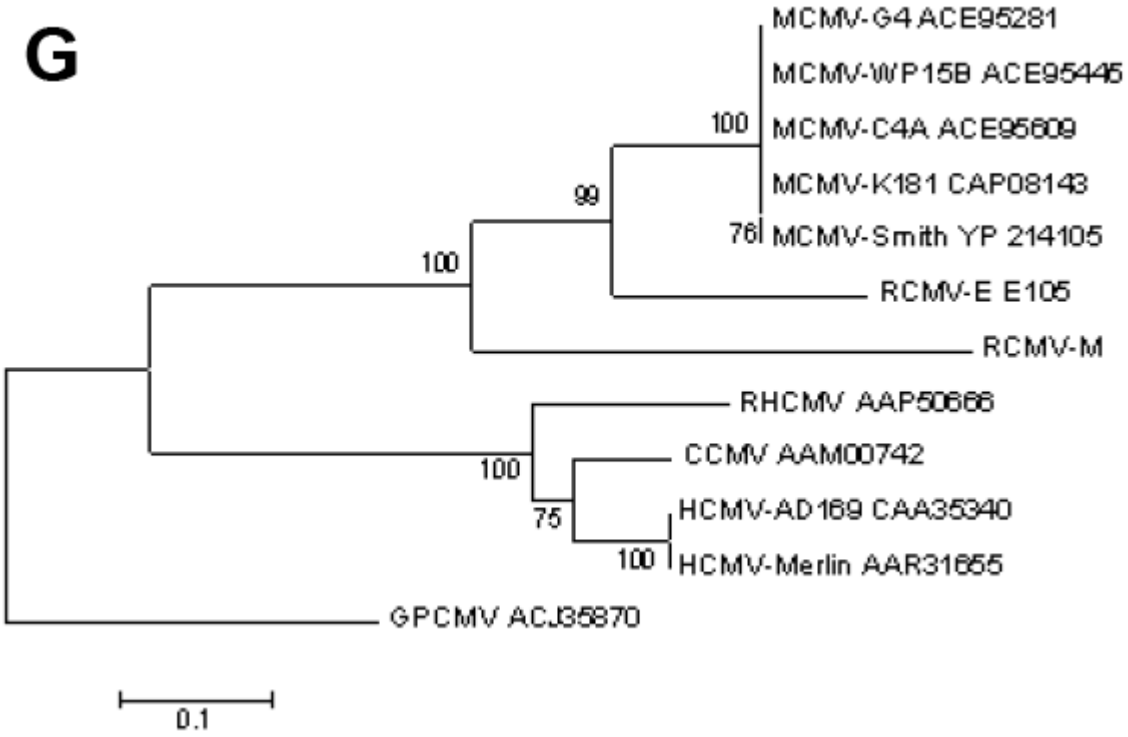


Figure 1H. Phylogenetic tree using sequences concatenated from the sequences used in panels A through G.

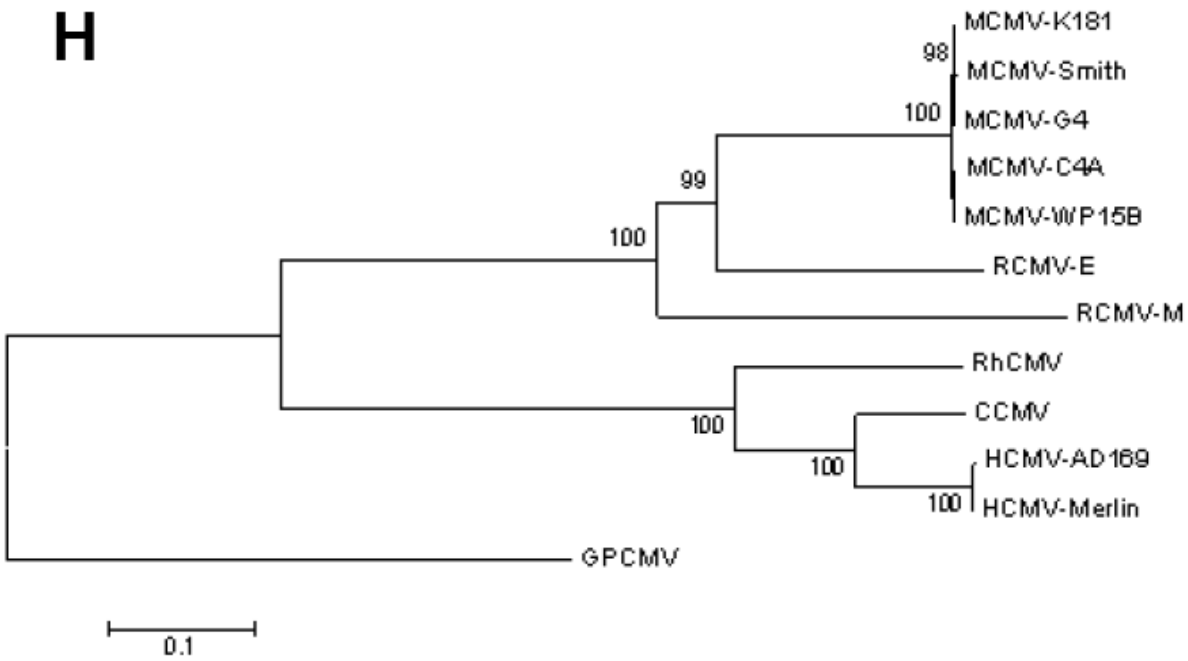

Table 1. Comparison of protein identities between well conserved, CMV-specific proteins. DNA POL: DNA polymerase; gB: glycoprotein B; term subunit: terminase subunit; MDBP: major DNA binding protein; MCP: major capsid protein; DPP: DNA packaging protein. The higher % identity is highlighted in green.

| | Protein Identity (%) | |
|---|---|---|
| | **RCMV-E** | |
| | RCMV-M | MCMV |
| Protein | | |
| DNA POL (E54) | 58.4 | **66.4** |
| gB (E55) | **54.6** | 54.3 |
| term subunit (E56) | 63.4 | **77.3** |
| MDBP (E57) | 51.7 | **68.3** |
| MCP (e86) | 75.9 | **77.4** |
| DPP (E89) | 83.7 | **86.4** |
| Helicase (e105) | 57.4 | **69.8** |

## Annex:

Include as much information as necessary to support the proposal, including diagrams comparing the old and new taxonomic orders. The use of Figures and Tables is strongly recommended but direct pasting of content from publications will require permission from the copyright holder together with appropriate acknowledgement as this proposal will be placed on a public web site. For phylogenetic analysis, try to provide a tree where branch length is related to genetic distance.