



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:	2012.004aF	(to be completed by ICTV officers)			
Short title: Five new species in the genus <i>Chrysovirus</i> , family <i>Chrysoviridae</i> (e.g. 6 new species in the genus <i>Zetavirus</i>)					
Modules attached (modules 1 and 9 are required)	1 <input checked="" type="checkbox"/> 6 <input type="checkbox"/>	2 <input checked="" type="checkbox"/> 7 <input type="checkbox"/>	3 <input type="checkbox"/> 8 <input type="checkbox"/>	4 <input type="checkbox"/> 9 <input checked="" type="checkbox"/>	5 <input type="checkbox"/>

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

Said Ghabrial (chair of the <i>Chrysoviridae</i> study group): saghab00@email.uky.edu

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

<i>Chrysoviridae</i> SG

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

Date first submitted to ICTV: July 2, 2012

Date of this revision (if different to above):

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	2012.004aF	(assigned by ICTV officers)
To create 5 new species within:		
Genus:	<i>Chrysovirus</i>	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.
Subfamily:		
Family:	<i>Chrysoviridae</i>	
Order:		
And name the new species:		GenBank sequence accession number(s) of reference isolate (RNA1-RNA4:
<i>Amasya cherry disease associated chrysovirus</i>		(Cherry chlorotic rusty spot associated chrysovirus; synonym of Amasya cherry disease associated chrysovirus) [AJ781163 - AJ781166] [AJ781397 - AJ781400]
<i>Aspergillus fumigatus chrysovirus</i>		[FN178512 -FN178515]
<i>Cryphonectria nitschkei chrysovirus</i>		[GQ290649, GQ290645, HM013825, HM013826]
<i>Fusarium oxysporum chrysovirus</i>		[EF152346 - EF152348]
<i>Verticillium dahliae chrysovirus</i>		[HM004067-HM004070]

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

Criteria to differentiate species in the genus *Chrysovirus* (as listed in the Ninth Report of ICTV):

• Most species in which the viruses naturally occur; chrysoviruses lack natural vectors and they are confined to the fungal host species from which they were first isolated.

• Size of dsRNA segments.

• Length of 3' UTR.

• Phylogenetic relationships.

The five newly proposed species meet the outlined criteria for species demarcation (see below)

Virions of members of the genus *chrysovirus* contain four unrelated segments of dsRNA, dsRNA1 encodes the RNA-dependent RNA polymerase (RdRp), dsRNA2 codes for the capsid protein (CP), dsRNAs 3 and 4 code for proteins of unknown function. The four segments are encapsidated separately. The genomes of at least 7 members and probable members of the genus *Chrysovirus* have been sequenced (Table 1). The genomic structure of *Penicillium chrysogenum virus* (PcV), the prototype of the genus *Chrysovirus*, is schematically represented in Fig. 1). Except for ACDACV (and CCRSACV) dsRNAs, the 5' UTRs of chrysovirus dsRNAs are relatively long, between 140 and 400 nucleotides in length. In addition to the strictly conserved 5'- and 3'-termini, a 40–75 nt region with high sequence identity is present at the 5' UTR of all four dsRNAs (box 1; Fig. 1). A second region of strong sequence similarity is present immediately downstream from 'box 1' (Fig. 1). This consists of a stretch of 30–50 nt containing a reiteration of the sequence 'CAA'. The (CAA)_n repeats are similar to the enhancer elements present at the 5' UTRs of tobamoviruses.

BLAST searches of PcV RdRp amino acid sequence showed that it shared significant sequence identity with AfCV RdRp (70%) followed by the RdRps of CnCV1, VdCv1, HvV145S, FoCV1 and ACDACV, (51%, 48%, 39%, 39% and 38%, respectively). High similarities (BLAST hits of 1e–09 or lower) were also found with the RdRps of several members of the family *Totiviridae*. Interestingly, no significant hits were evident with any of the viruses in the family *Partitiviridae*, another validation for the removal of chrysovirus from the family *Partitiviridae* and their placement in the recently created family *Chrysoviridae*. A Neighbor-joining phylogenetic tree constructed based on full-length amino acid sequences of RdRps of members and probable members of the family *Chrysoviridae*, as well as selected members of the family *Totiviridae*, is shown in Fig. 2 and is consistent with the sequence identity inferences made above. Interestingly, the phylogenetic tree also defines two clusters of chrysovirus, those in which segment 3 codes for P3 (PcV, AfCV, CnCV1 and VdCV1, upper cluster) and those in which segment 3 codes for P4 (HvV145S, ACDACV and FoCV1, lower cluster).

References:

Covelli, L., Coutts, R.H.A., Di Serio, F., Citir, A., Acikgoz, S., Hernandez, C., Ragozzino., A. and Flores, R. (2004). Cherry chlorotic rusty spot and Amasya cherry diseases are associated with a complex pattern of mycoviral-like double-stranded RNAs. I. Characterization of a new species in the genus *Chrysovirus*. *J. Gen. Virol.*, **85**, 3389–3397.

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Ghabrial, S.A., Soldevila, A.I. and Havens, W.M. (2002). Molecular genetics of the viruses infecting the plant pathogenic fungus *Helminthosporium victoriae*. In S. Tavantzis (Ed.), *Molecular Biology of Double-stranded RNA: Concepts and Applications in Agriculture, Forestry and Medicine* (pp. 213–236). Boca Raton, FL: CRC Press.

Jiang, D. and Ghabrial, S.A. (2004). Molecular characterization of *Penicillium chrysogenum* virus: reconsideration of the taxonomy of the genus *Chrysovirus*. *J. Gen. Virol.*, **85**, 2111–2121.

Kim, J.-M., Kim, J.-A., Park, S.-M, Cha, B.-J., Yang, M.-S., Kim, D.-H. (2010). Nucleotide sequences of four segments of chrysovirus in Korean *Cryphonectria nitschkei* BS122 strain. *Virus Genes* **41**, 292–294.

Liu, Y-C., Dynek, J.N., Hillman, B.I. and Milgroom, M.G. (2007). Diversity of viruses in *Cryphonectria parasitica* and *C.nitschkei* in Japan and China, and partial characterization of a new chrysovirus species. *Mycol. Res.*, **111**, 433–442.

Luque, D., González, J.M., Garriga, D., Ghabrial, S.A., Havens, W.M., Trus, B., Verdaguier, N., Carrascosa, J.L. and Castón, J.R. (2010). The T=1 capsid protein of *Penicillium chrysogenum* virus is formed by a repeated helix-rich core indicative of gene duplication. *J. Virol.*, **84**, 7256–7266.

Jamal, A., Bignell, E. M., and Coutts, R. H. A. (2010). Complete nucleotide sequences of four dsRNAs associated with a new chrysovirus infecting *Aspergillus fumigatus*. *Virus Res.* **153**, 64-70.

Cao, Y-F, Zhu, X-W, Xiang, Y., Li, D-Q., Yang, J-R. Mao, Q-Z., and Chen, J-S. (2011). Genomic characterization of a novel dsRNA virus detected in the phytopathogenic fungus *Verticillium dahliae* Kleb. *Virus Res.* **159**, 73-78.

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.

Table 1. List of members and probable members (and synonyms) of the family *Chrysoviridae*, dsRNA segment number, genome size, encoded protein and size and GenBank accession number

Virus (synonym)	Abbreviation	dsRNA segment number, length in bp, encoded protein, molecular mass in k	GenBank accession number
<i>Aspergillus fumigatus</i> chrysovirus	AfCV	1 (3560, RdRp, 128) 2 (3159, CP, 107) 3 (3006, P3, 99) 4 (2863, P4, 95)	FN178512 FN178513 FN178514 FN178515
<i>Amasya cherry disease associated chrysovirus</i> *	ACDACV	1 (3399; RdRp, 124) 2 (3128; CP, 112) 3 (2833; P4, 98) 4 (2498; P3, 77)	AJ781166 AJ781165 AJ781164 AJ781163
(<i>Cherry chlorotic rusty spot associated chrysovirus</i>) ^a	CCRSACV	1 (3399; RdRp, 129) 2 (3125; CP, 112) 3 (2833; P4, 98) 4 (2499; P3, 77)	AJ781397 AJ781398 AJ781399 AJ781400
<i>Cryphonectria nitschkei</i> chrysovirus 1	CnCV1	1 (2978; RdRp, 110) 2 (2980; CP, 99) 3 (2960; P3, 81) 4 (2552; P4, 91)	GQ290649 GQ290645 HM013826 HM013825
<i>Fusarium oxysporum</i> chrysovirus 1 ^b	FoCV1	1 (2574; RdRp, 99)	EF152346
<i>Helminthosporium victoriae</i> virus 145S*	HvV145S	1 (3612; RdRP, 125) 2 (3134; CP, 100) 3 (2972; P4, 93) 4 (2763; P3, 81)	NC_005978 NC_005979 NC_005980 NC_005981
<i>Penicillium chrysogenum</i> virus	PcV	1 (3562; RdRP, 129) 2 (3200; CP, 109) 3 (2976; P3, 101) 4 (2902; P4, 95)	NC_007539 NC_007540 NC_007541 NC_007542
<i>Verticillium dahliae</i> chrysovirus 1	VdCV1	1 (3594; RdRP, 127) 2 (3313; CP, 113) 3 (2983; P3, 84) 4 (2931; P4, 90)	HM004067 HM004068 HM004069 HM004070

^a Synonym of ACDACV.

^b Only partial sequences are available of segments 2 (accession # EF152347), which encodes partial sequence of CP, and segment 3 (accession # EF152348) that encodes partial sequence of P4.

* For viruses marked with asterisks, segment #3 codes for P4 and segment # 4 codes for P3.

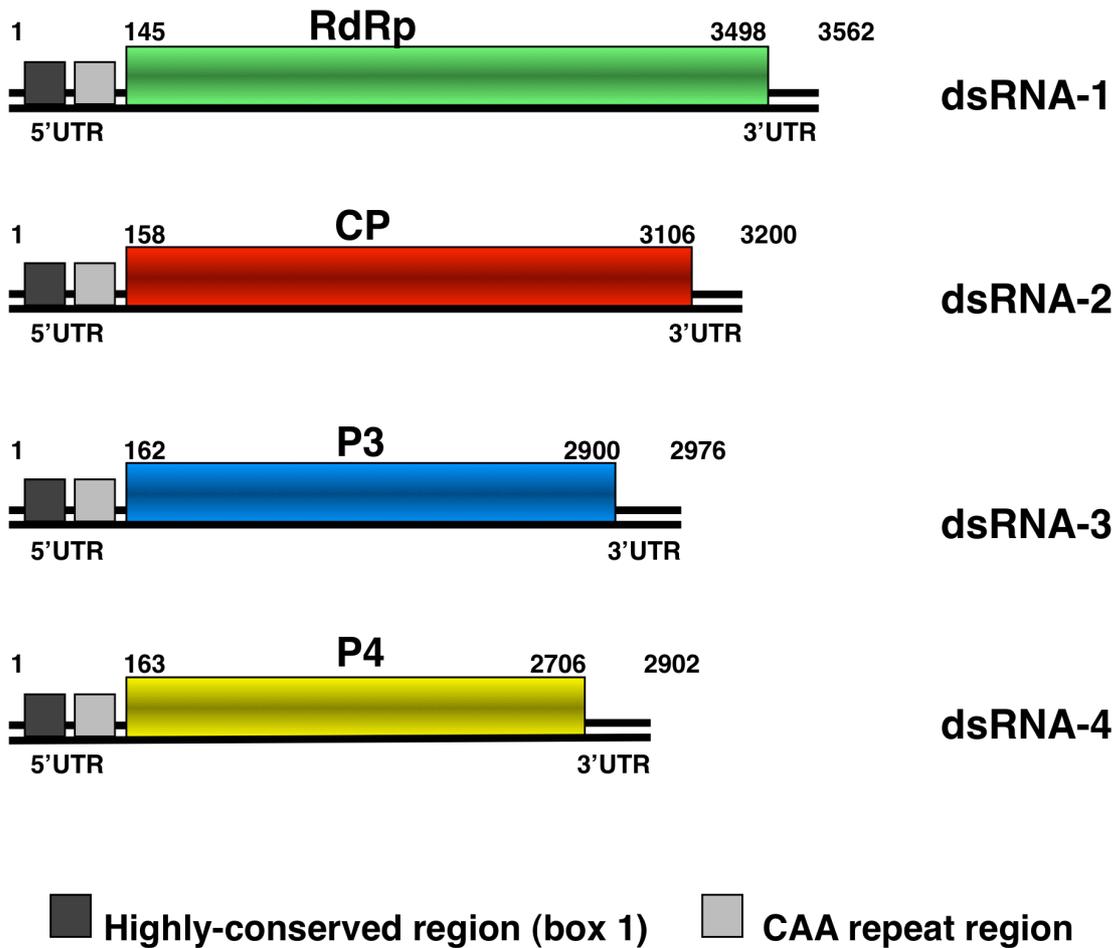


Figure 1. Genome organization of *Penicillium chrysogenum* virus (PcV). The genome consists of four dsRNA segments; each is monocistronic. The RdRp ORF (nt positions 145 to 3,498 on dsRNA-1), the CP ORF (nt positions 158 to 3,106 on dsRNA-2), the P3 ORF (nt positions 162 to 2900 on dsRNA-3) and the P4 ORF (nt positions 163 to 2706 on dsRNA-4) are represented by rectangular boxes. Although the functions of P3 and P4 are unknown, the N-terminal region of P3 shares high sequence identity with the corresponding N-terminal region of RdRp (RNA-binding region?) and P4 is a putative cysteine protease.

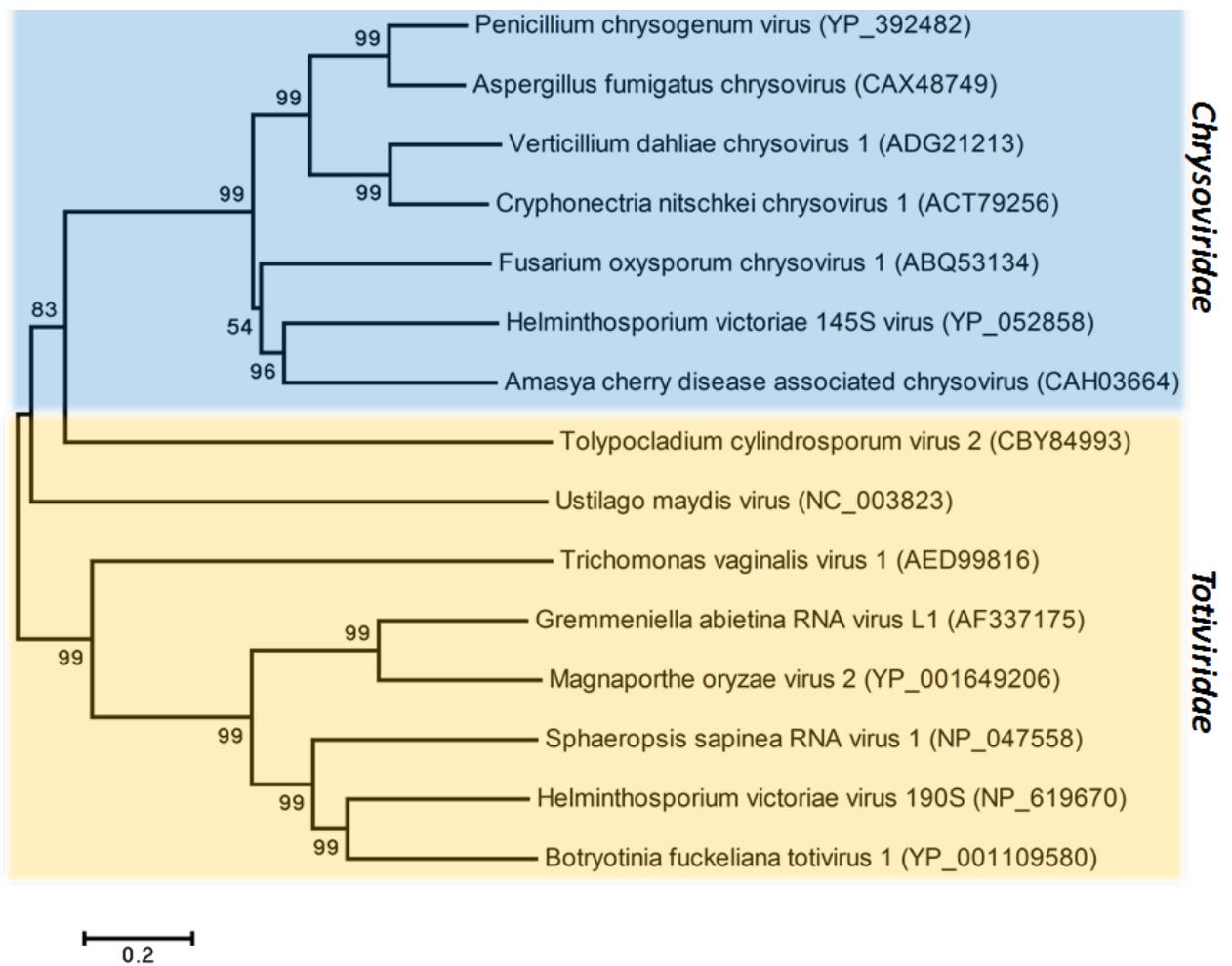


Figure 2. Neighbor-joining phylogenetic tree constructed based on the complete amino acid sequences of RdRps of members and probable members of the family *Chrysoviridae* and selected viruses from the family *Totiviridae*. The amino acid sequences were aligned using the program CLUSTAL X. The phylogenetic tree was generated using the MEGA 5 phylogenetic package. Bootstrap values as a percent of 2,000 replicates are indicated at each node.