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:	***2012.002a,bP***	(to be completed by ICTV officers)

Short title: Revision of the genus *Ampelovirus* in the family *Closteroviridae*
(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:

G.P. Martelli (martelli@agr.uniba.it) on behalf of the *Closteroviridae* Study Group

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)	*Closteroviridae* SG

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

Date first submitted to ICTV: May 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	***2012.002aP***	(assigned by ICTV officers)

To create 1 new species within:

Genus:	***Ampelovirus***
Subfamily:	
Family:	***Closteroviridae***
Order:	

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:	**GenBank sequence accession number(s) of reference isolate:**
Grapevine leafroll-associated virus 4	**FJ467503**

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

Please see supporting case in Appendix, section 9

MODULE 7: **REMOVE and MOVE**

Use this module whenever an existing taxon needs to be removed:

- *Either* to abolish a taxon entirely (when only part (a) needs to be completed)
- *Or* to move a taxon and re-assign it e.g. when a species is moved from one genus to another (when BOTH parts (a) and (b) should be completed)

Part (a) taxon/taxa to be removed or moved

Code	***2012.002bP***	(assigned by ICTV officers)

To remove the following taxon (or taxa) from their present position:

Grapevine leafroll-associated virus 5

The present taxonomic position of these taxon/taxa:

Genus:	***Ampelovirus***	Fill in all that apply.
Subfamily:		
Family:	***Closteroviridae***	
Order:		

If the taxon/taxa are to be abolished (i.e. not reassigned to another taxon) write "yes" in the box on the right	**YES**

Reasons to justify the removal:
Explain why the taxon (or taxa) should be removed

Please see supporting case in Appendix, section 9

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

Summary of the proposal: Following a reevaluation of species demarcation criteria in the genus, the study group proposes to create the species *Grapevine leafroll-associated virus 4* (GLRaV-4) that regroups several related virus isolates and to demote *Grapevine leafroll-associated virus 5* (GLRaV-5) from species to isolate of the new *Grapevine leafroll associated virus-4* species. The name *Grapevine leafroll-associated virus 4* is chosen for the amalgamated species because (1) GLRaV-4 was the first virus of this group of isolates for which the entire sequence became available and (2) this group of virus isolates is already widely referred to as GLRaV-4-like viruses in the literature.

Main text of the proposal: The membership of the genus *Ampelovirus*, family *Closteroviridae*, has been determined by the discriminating criteria for the identification of virus species approved by the ICTV (Martelli *et al.* 2005) and enforced up to 2011:

(i) Particle size
(ii) Size of CP, as determined by deduced amino acid sequence data
(iii) Serological specificity using discriminatory monoclonal or polyclonal antibodies
(iv) Genome structure and organization (number and relative location of the ORFs)
(v) Amino acid sequence of relevant gene products (CP, CPm, HSP70h) differing by more than 10%
(vi) Vector species and specificity

(vii) Magnitude and specificity of natural and experimental host range
(vii) Cytopathological features (aspect of inclusion bodies and origin of cytoplasmic vesicles).

The fully or partially sequenced closteroviruses identified in leafroll-diseased vines (Table 1) were found to possess extensive variation in the size and structure of their genomes. Due to the ease of obtaining molecular data in comparison to biological data and controversial serological data caused by the inconsistent reactivity of some of the available reagents, the molecular parameters became the single most important criterion for the recognition of new species. When the narrow 10% boundary in the sequence identity of taxonomic relevant genes was broken, novel putative species were identified, thus favouring the growth of the population of leafroll-associated agents to the unprecedented current size.

The increase of the discriminating threshold from 10% to 25% for three taxonomically relevant genes (polymerase, HSP70h and CP) recently approved by the ICTV (Martelli *et al*., 2011a) and the production of new sets of monoclonal antibodies to GLRaVs (Gugerli, 2009), now allows to re-consider the taxonomic structure of the genus *Ampelovirus* and the validity of its grapevine-infecting members.

The identification of GLRaV-4, -5, -6 and -9 as distinct ampelovirus species was dictated primarily by the apparent lack of serological relationship among them (Boscia *et al*., 1995; Alkowni *et al*, 2004). However, when molecular data emerged (Good and Monis, 2001; Abou Ghanem-Sabanadzovic *et al*., 2003, 2006; Dovas and Katis, 2003; Saldarelli *et al*., 2006) it became evident that these four viruses formed a coherent phylogenetic cluster, that grew to seven members when the sequences of GLRaV-Pr, GLRaV-De, and GLRaV-Car became available (Maliogka *et al*., 2008, 2009; Abou Ghanem-Sabanadzovic *et al*., 2010). GLRaV-De has recently been identified as a variant of GLRaV-6 (Abou Ghanem-Sabanadzovic *et al*., 2012).

Although it had been pointed out that these viruses could represent genetic variants of a single species (Martelli, 2009; Elbeaino *et al*., 2009), no action for a taxonomic revision could be taken until the complete genomic sequences of GLRaV-4, -5 and -6 were obtained, as this represented the third obstacle standing on the way, the others being the above recalled

References:

Abou Ghanem-Sabanadzovic N., Sabanadzovic S., Roy G., Rowhani A., 2003. Partial molecular characterization of Grapevine leafroll-associated virus 4. *Extended Abstracts 14th Meeting of ICVG, Locorotondo (Bari), Italy*: 42.

Abou Ghanem-Sabanadzovic N., Sabanadzovic S., Gugerli P., Rowhani A., 2006. Molecular characterization of grapevine leafroll associated viruses 4 and 6. *Extended Abstracts 14th Meeting of ICVG, Stellenbosch, South Africa:* 30-31.

Abou Ghanem-Sabanadzovic N., Sabanadzovic S., Uyemoto J.K., Golino D., Rowhani A., 2010. A putative new ampelovirus associated with grapevine leafroll disease. *Archives of Virology* **155**: 1871-1876.

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Alkowni R., Rowhani A., Daubert S., Golino D., 2004. Partial characterization of a new ampelovirus associated with grapevine leafroll disease. *Journal of Plant Pathology* **86**: 123-133.

References:

Boscia D., Greif C., Gugerli P., Martelli G.P., Walter B., Gonsalves D., 1995. Nomenclature of leafroll-associated putative closteroviruses. *Vitis* **34**: 171-175.

Dolja V.V., Kreuze J.F., Valkonen J.P.T., 2006. Comparative and functional genomics of closteroviruses. *Virus Research* **117**: 38-51.

Dovas C.I., Katis N.I., 2003. Application of spot multiplex nested RT-PCR for the simultaneous detection of viruses associated to rugose wood and leafroll grapevine diseases. *Extended Abstracts 14th Meeting of ICVG, Locorotondo (Bari), Italy*: 192.

Elbeaino T., Numic F., Digiaro M., Sabanadzovic S., Martelli G.P., 2009. Partial characterization of a grapevine leafroll-associated virus from an infected Cypriot vine of cv. Mavro. *Journal of Plant Pathology* **91**: 479-484.

Good X., Monis J., 2001. Partial genome organization, identification of the coat protein gene, and detection of Grapevine leafroll-associated virus 5. *Phytopathology* **91**: 274-281.

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Le Maguet J., Beuve M., Herrbach E,, Lemaire O., 2012. Transmission of six ampeloviruses and two vitiviruses by *Phenacoccus aceris. Phytopathology* **102** (in press).

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Martelli G.P., 2009. Grapevine virology highlights 2006-2009. *Extended Abstract 16th Meeting of ICVG, Dijon, France*: 15-23.

Martelli G.P., Agranovsky A.A., Bar-Joseph M., Boscia D., Candresse T., Coutts R.H.A., Dolja V.V., Hu J.S., Jelkmann W., Karasev A.V., Martin R.R., Minafra A., Namba S., Vetten H.J., 2011a. Family *Closteroviridae.* In: King A., Adams M.J., Carstens E.B., Lefkowitz E. (eds). Virus Taxonomy. Ninth Report of the International Committee on Taxonomy of Viruses, pp. 987-1001. Elsevier-Academic Press, Amsterdam, The Netherlands.

Saldarelli P., Cornuet P., Vigne E., Talas F., Bronnebkant I., Dridi A.M., Andret-Link P., Boscia D., Gugerli P., Fuchs M., Martelli G.P., 2006. Partial characterization of two divergent variants of grapevine leafroll-associaed virus 4. *Journal of Plant Pathology* **88**: 204-214.

Sim S.T., Rowhani A., Alkowni R., Golino D.A., 2003. Experimental transmission of Grapevine associated virus 5 and 9 by longtailed mealybugs. *Extended Abstract 14th Meeting of ICVG, Locorotondo, Italy*: 211-212.

References:

Thompson J.R., Fuchs M., Perry K., 2012. Genomic analysis of *Grapevine leafroll-associated virus 5* and related viruses. *Virus Research* **163**: 19-27.

Tsai C.-W., Rowhani A., Golino D.A., Daane K.M., Almeida R.P.P., 2010. Mealybug transmission of grapevine leafroll viruses: An analysis of virus-vector specificity. *Phytopathology* **100**: 830-834.

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. Current classification and some properties of Grapevine leafroll-associated ampeloviruses.

Virus	Genus	Coat protein (kDa)	Genome size (nts) (GenBank accession No.)	ORFs (No.)	Vectors	First record *fide* Boscia *et al*. (1995) and this paper
GLRaV-1	*Ampelovirus*	34	18,659 (JQ023131)	9	Mealybugs, soft scale isects	Gugerli *et al*. (1984)
GLRaV-3	*Ampelovirus*	35	18,498 (EU259806)	12	Mealybugs, soft scale and scale insects	Zee *et al*. (1987)
GLRaV-4	*Ampelovirus*	35	13,830 (FJ467503)	6	Mealybugs	Hu *et al*. (1990)
GLRaV-5	*Ampelovirus*	35	13,384[a] (FR822696)	6	Mealybugs	Zimmermann *et al*. (1990); Walter and Zimmermann (1991)
GLRaV-6	*Ampelovirus*	35	13,807 (FJ467504)	6	Mealybugs	Gugerli and Ramel (1993); Gugerli *et al*. (1997)
GLRaV-8[b]	*Ampelovirus*	37	ND	ND	Unknown	Monis (2000)
GLRaV-9	*Ampelovirus*	35	12,588[a] (AY29781)	6	Mealybugs	Alkowni *et al*. (2004)
GLRaV-Pr	*Ampelovirus*	30	13,696 (AM182328)	6	Mealybugs	Maliogka *et al*. (2009);
GLRaV-Car	*Ampelovirus*	29	13,626 (FJ907331)	6	Unknown	Abou Ghanem-Sabanadzovic *et al*. (2010)

[a] Nearly complete sequence; [b] Cancelled from the 9th ICTV Report (Martelli *et al*., 2011a); ND, not determined.

Table 2. RNA-dependent RNA polymerase amino acid sequence identity (%) of members of the "GLRaV-4 cluster"

	GLRaV-9	GLRaV-5	GLRaV-6	GLRaV-4	GLRaV-Pr	GLRaV-Car	Mean divergence (%)
GLRaV-9	100	90	89	85	83	77	15
GLRaV-5	90	100	89	86	82	76	15
GLRaV-6	89	89	100	87	85	78	14
GLRaV-4	85	86	87	100	82	74	17
GLRaV-Pr	83	82	85	82	100	77	18
GLRaV-Car	77	76	78	74	77	100	24

Table 3. Heat shock protein 70 homologue amino acid sequence identity (%) of members of the "GLRaV-4 cluster"

	GLRaV-De	GLRaV-6	GLRaV-9	GLRaV-5	GLRaV-4	GLRaV-Pr	GLRaV-Car	Mean divergence (%)
GLRaV-De	100	92	85	86	81	79	67	18
GLRaV-6	92	100	84	85	78	78	66	20
GLRaV-9	85	84	100	90	82	80	67	19
GLRaV-5	86	85	90	100	82	80	68	18
GLRaV-4	81	78	82	82	100	78	67	22
GLRaV-Pr	79	78	80	80	78	100	69	23
GRLaV-Car	67	66	67	68	67	69	100	**33**

Table 4. Coat protein amino acid sequence identity (%) of members of the "GLRaV-4 cluster"

	GLRaV-De	GLRaV-6	GLRaV-5	GLRaV-9	GLRaV-4	GLRaV-Car	GLRaV-Pr	Mean divergence (%)
GLRaV-De	100	91	86	86	81	76	76	17
GLRaV-6	91	100	85	84	81	79	76	17
GLRaV-5	86	85	100	87	84	79	78	17
GLRaV-9	86	84	87	100	82	79	78	17
GLRaV-4	81	81	84	82	100	79	77	19
GLRaV-Car	76	79	79	79	79	100	77	22
GLRaV-Pr	76	76	78	78	77	77	100	23

Table 5. Proposed new taxonomic configuration of the genus *Ampelovirus*

Approved species	**Vectors**
Subgroup I	Mealybugs, soft scale and scale insect
Grapevine leafroll-associated virus 1 (GLRaV-1)	

Grapevine leafroll-associated virus 3 (GLRaV-3)	
Little cherry virus 2 (LChV-2)	
Pineapple mealybug wilt-associated virus 2 (PMWaV-2)	
Subgroup II	Mealybugs
Grapevine leafroll-associated virus 4 (GLRaV-4)	
Pineapple mealybug wilt-associated virus 1 (PMWaV-1)	
Pineapple mealybug wilt-associated virus 3 (PMWaV-3)	
Prunus bark necrosis stem pitting-associated virus (PBNSPaV)	

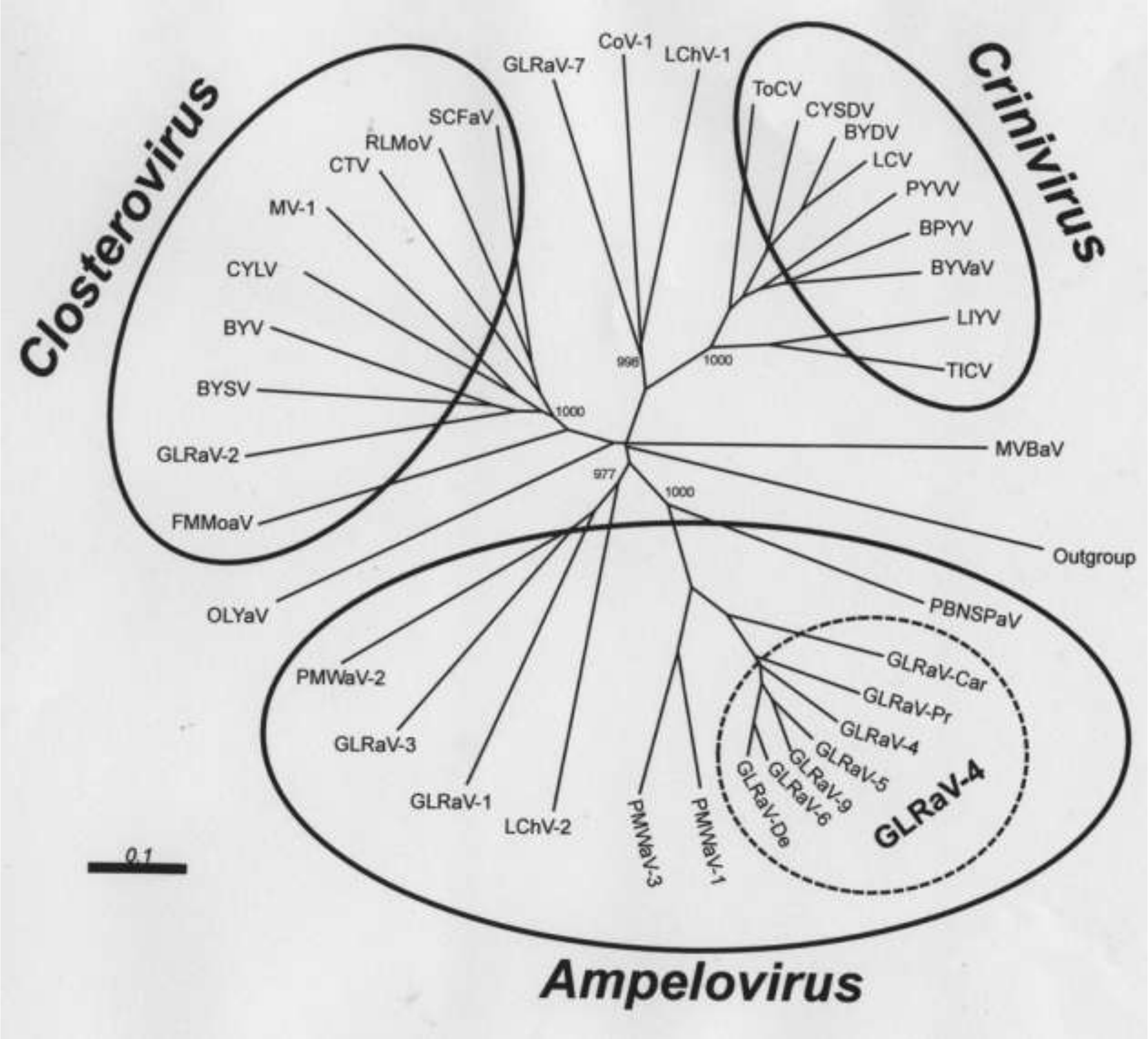

Fig. 1. Phylogenetic tree constructed with complete amino acid sequences of the HSP70h gene of members of family *Closteroviridae*. Distances are proportional to branch lengths. Bootstrap values are indicated at the main branch nodes. The bar represents 0.1 amino acid change per site. Viruses used in the tree, their abbreviations and accession numbers are: genus *Ampelovirus*: *Grapevine leafroll-associated virus 1* (GLRaV-1, AAF22740); *Grapevine leafroll-associated virus 3* (GLRaV-3, NP_813799); Grapevine leafroll-associated virus 4 (GLRaV-4, FJ467503); Grapevine leafroll-associated virus 5 (GLRaV-5, NC_016081); Grapevine leafroll-associated virus 6 (GLRaV-6, FJ467504); Grapevine leafroll-associated virus 9 (GLRaV-9, AAL63810); Grapevine leafroll-associated virus Car (GRLaV-Car; ACT67478); Grapevine leafroll-associated virus Pr (GLRaV-Pr, YP_002364305); Grapevine leafroll-associated virus De (GLRaV-De, AM494395); *Little cherry virus 2* (LChV-2; AF531505); *Pineapple mealybug wilt-associated*

virus 1 (PMWaV-1; AAL66711); *Pineapple mealybug wilt- associated virus 2* (PMWaV-2; AAG13941); *Pineapple mealybug wilt-associated virus 3* (PMWaV-3; ABD62350); *Plum bark necrosis stem pitting-associated virus* (PBNSPaV; YP_001552326). Genus *Closterovirus: Beet yellow stunt virus* (BYSV, AAC55662); *Beet yellows virus* (BYV, AAF14302), *Citrus tristeza virus* (CTV; NP_042864); *Carrot yellow leaf virus* (CYLV; YP_003075968); *Grapevine leafroll-associated virus 2* (GLRaV-2, AAC40858); *Mint virus 1* (MV-1, YP_224093); *Raspberry leaf mottle virus* (RLMoV, ABO15357), *Strawberry chlorotic fleck-associated virus* (SCFaV, ABI23185); Fig mild mottle-associated virus (FMMoaV, ACU57193). Genus *Crinivirus: Beet pseudoyellows virus* (BPYV, NP_940788); *Blackberry yellow vein-associated virus* (BYVaV, AAW67738); *Cucurbit yellow stunting disorder virus* (CYSDV, CAA11494); *Lettuce infectious yellows virus* (LIYV, NP_619695), *Lettice chlorosis viru*s (LCV, ACQ82510); *Potato yellow vein virus* (PYVV, YP_054417); *Tomato infectious chlorosis virus* (TICV, ACN88745); *Tomato chlorosis virus* (ToCV, AAD01790); *Bean yellow disorder virus* (BYDV, ABY66965). Unassigned or unclassified viruses: Little cherry virus 1 (LChV-1, NP045004), Mint vein banding-associead virus (MVBaV, AAS57941); Olive leaf yellowing-associated virus (OLYaV, CAD29309); Grapevine leafroll-associated virus 7 (GLRaV-7, HE588185); Cordyline virus 1 (CoV-1; HM588723), Heat shock 70 protein from *Arabidopsis thaliana* (NP_187864) was used as outgroup.

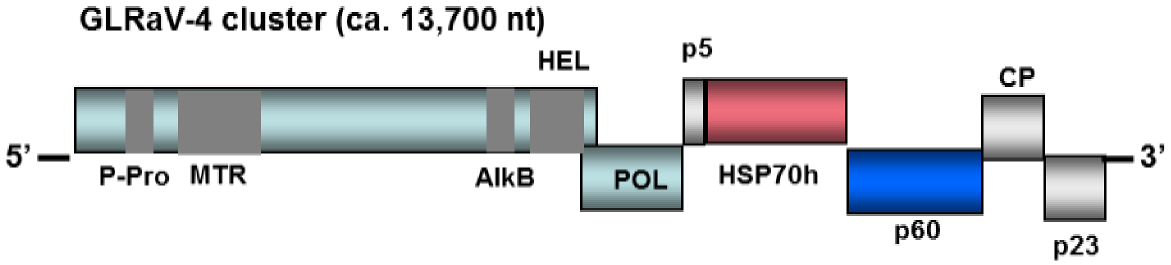

Fig. 2 Genomic organization of the GLRaV-4 species

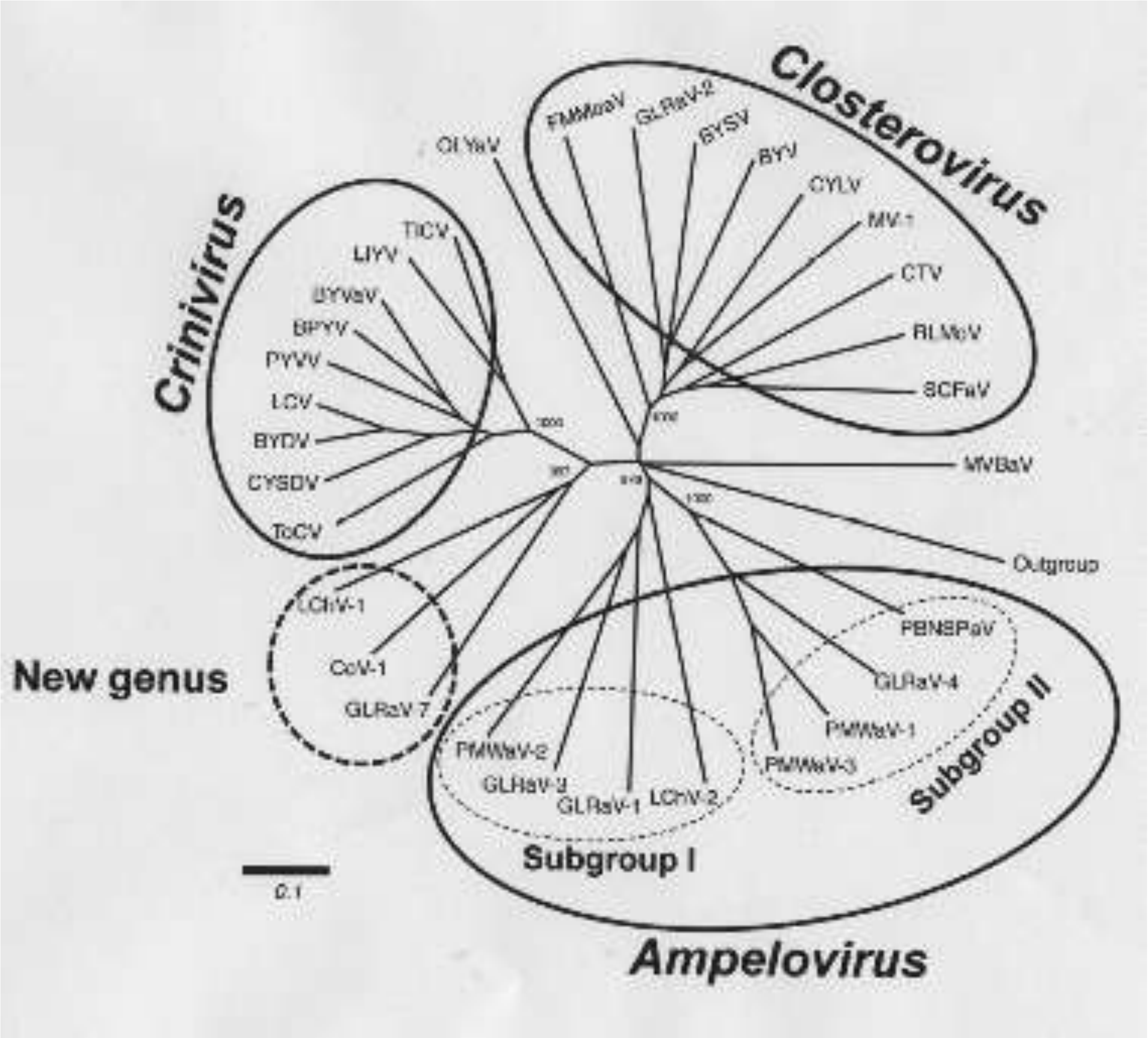

Fig. 3. Phylogenetic tree constructed with complete amino acid sequences of the HSP70h gene of members of family *Closteroviridae*. Distances are proportional to branch lengths. Bootstrap values are indicated at the main branch nodes. The bar represents 0.1 amino acid per site. Viruses used in the tree, their abbreviations and accession numbers are the same as in Fig. 2. The tree shows: (i) the suggested splitting of the genus *Ampelovirus* into two coherent subgroups including viral species with a large (in excess of 17,000 nts) and complex (9 to 12 ORFs) genome (Subgroup I) and with a smaller (approximately 13,000-14,000 nts) and simpler (6 ORFs) genome (Subgroup II); (ii) the allocation of the three virus species (GLRaV-7, LChV-1 and CoV-1) included in the novel genus *Velarivirus,* in a branch of the tree next to that comprising members of the genus *Crinivirus*.