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:	***2012.001a-fP***	(to be completed by ICTV officers)

Short title: New genus *Velarivirus* in the family *Closteroviridae* and new species *Cordyline Virus1* to be assigned to the new genus

(e.g. 6 new species in the genus *Zetavirus*)

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

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 study group

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

Responses to queries:

(i) **Which method was used to build the tree?** Answer: Neighbor Joining MEGA 5 (see References in the proposal) for all trees.

(ii) **Why is the HSP70h protein used for the tree?** Answer: HSP70h is a gene unique to the family *Closteroviridae* and the one that has been and still is the most widely used to infer molecular divergences/ relationships among individual species and strains within a species. The literature is loaded with papers grouping sequenced isolates of individual closteroviral species into "lineages" or the like. These intraspecific groups are most commonly identified using HSP70h sequences. We have constructed new trees using the amino acid sequences of the polymerase and CP sequences. As you can see, they do not differ from the one built with HSP70h sequences, confirming that velariviruses are phylogenetically closer to criniviruses than closteroviruses and ampeloviruses, but are separate from criniviruses. A comparable allocation of velariviruses was found in a number of additional phylotrees made by Dr. S. Sabanadzovic using different systems.

Other comments:

One could express reservations on the taxonomic validity of the proposal based on the position of Velarivirus in the phylotrees (two separate clades in the same branch). In addition,

one could reason that one of the major traits that discriminates genera within the family *Closteroviridae* is the diversity of the vectors. Isolates of the three species in the proposed new genus do not have known vector. If by any chance Cordyline virus 1 (CoV-1) and any of the three putative new species denoted CoV-2, -3 and -4, which are being described, will prove to be vectored by whiteflies, this would be a strong argument in favour of the designation of velariviruses as a subgroup in the genus *Crinivirus.* However, even if CoV isolates were shown to have a whitefly vector (it is most unlikely that this would happen with LChV-1 and GLRaV-7), the undivided genome would still be determinant in retaining Velarivirus as a *bona fide* separate genus. Just think of the biological implications of a fractionated genome, e.g the possibility of giving rise to pseudorecombinants.

We hope that the new version of the Velarivirus proposal satisfies the EC and that it will be re-discussed (and hopefully approved) after a year of "probation".

Date first submitted to ICTV:	May 2012
Date of this revision (if different to above):	November 2012

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	***2012.001aP***	(assigned by ICTV officers)

To create 1 new species within:

Genus:	***Velarivirus*** (new)
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:**
Cordyline virus 1	HM588723

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

Cordyline virus 1 (CoV-1) was discovered in *Cordyline fruticosa* plants from Hawaii containing high molecular weight dsRNAs (16-18 kbp) (Mezer *et al*., 2011). The virus is not mechanically transmissible and has no known vector. Its completely sequenced genome is 16,833 nts in size and, as shown in Fig. 1, comprises 9 ORFs encoding, in the order: the replication-associated proteins (ORF1A and 1B); a small (4.4 kDa) hydrophobic protein (ORF2); the 62.5 kDa HSP70h protein (ORF3); another small-sized polypeptide (5.7 kDa) which does not share sequence similarity with any known protein in databases (ORF4); a 61 kDa protein homologous to the comparable product coded for by all known closteroviruses (ORF5); the 35.6 kDa coat protein (ORF6); the 70.3 kDa minor coat protein (ORF7); a 26 kDa protein (ORF8) and a 29 kDa protein (ORF9), neither of which shows similarity with known proteins in databases. CoV-1 genome structure resembles that of Little cherry virus 1 (LChV-1) and Grapevine leafroll-associated virus 7 (GLRaV-7) (Jelkmann *et al*., 1997; Al Rwahnih *et al.*, 2012, Jelkmann *et al*., 2012) both of which are members of the newly proposed genus Velarivirus (see Module 3). With these viruses CoV-1 shares also the lack of transmissibility by sap inoculation and of a recognized vector. However, the three viruses in question show a divergence in the amino acid sequence of the polymerase, HSP70h and coat protein far exceeding the 25% threshold set as demarcation criterion for species identification in the family *Closteroviridae* (Martelli *et al.*, 2011) (Table 1), i.e. a clear indication that they can be retained as separate species. As to the relationships with members of the three extant genera of the family *Closteroviridae (Closterovirus, Ampelovirus* and *Crinivirus)* in phylogenetic trees constructed with the complete nucleotide sequence of the HSP70h gene, CoV-1, GLRaV-7 and LChV-1 form a clade distinct from those of these genera (Fig. 2), supporting the notion that they may be classified in separate genus.

MODULE 3: **NEW GENUS**

creating a new genus

Ideally, a genus should be placed within a higher taxon.

Code	***2012.001bP***	(assigned by ICTV officers)

To create a new genus within:

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 family is specified, enter "**unassigned**" in the family box

naming a new genus

Code	***2012.001cP***	(assigned by ICTV officers)

To name the new genus: *Velarivirus*

Assigning the type species and other species to a new genus

Code	***2012.001dP***	(assigned by ICTV officers)

To designate the following as the type species of the new genus

Grapevine leafroll-associated virus 7

Every genus must have a type species. This should be a well characterized species although not necessarily the first to be discovered

The new genus will also contain any other new species created and assigned to it (Module 2) and any that are being moved from elsewhere (Module 7b). **Please enter here the TOTAL number of species (including the type species) that the genus will contain:**

3

Reasons to justify the creation of a new genus:

Additional material in support of this proposal may be presented in the Appendix, Module 9

GLRaV-7 was originally found in an unidentified apparently symptomless white-berried grapevine cultivar from Albania (accession AA42) which, however, induced leafroll symptoms onto grafted cv. Cabernet sauvignon indicators, thus justifying the name given to the virus (Choueiri *et al*., 1996). The geographical distribution of GLRaV-7 is rather wide, as it comprises European (Albania, Armenia, Greece, Hungary, Italy, Switzerland), Near East (Egypt, Palestine, Turkey), North (USA) and South (Chile) American countries and China (Martelli, 2009).

GLRaV-7 has very flexuous filamentous particles with a most frequent length of 1500-1700 nm (with a predominance of 1500 nm long particles), exhibiting the cross banding and open structure of typical closterovirid virions. Its identification as a different species was largely based on the lack of reaction of an antiserum raised to its coat protein with any of the six grapevine-infecting closteroviruses known at that time (Choueiri *et al*., 1996). However, partial sequencing of the viral genome (Turturo *et al*., 2000) disclosed differences with members of the two genera of the family *Closteroviridae* with a monopartite genome (*Closterovirus* and *Ampelovirus*) suggesting GLRaV-7 be classified as an unassigned putative species to the family, a position that it shares with Little cherry virus 1 (LChV-1) (Martelli *et al*., 2011a).

Contrary to all approved species of the genus *Ampelovirus*, GLRaV-7 and LChV-1 do not have a known vector.

Two GLRaV-7 isolates have been sequenced originating, respectively, from the Albanian accession AA42 (Mikona *et al*., 2009; Jelkmann *et al.,* 2012) and a Swiss selection of cv. Pinot Noir [accession FPS PN-23 (Al Rwahnih *et al.* (2012)].

The viral genome consists of 16,496 nucleotides (nt) arranged in 10 open reading frames (ORFs) (Fig. 1). As reported (Mikona *et al.*, 2009; Jelkmann *et al*., 2012; Al Rwahnih *et al.*, 2012), the genome encodes in the 5'→ 3' direction: (i) a polyprotein 267 kDa in size comprising the protease, methyltransferase, and helicase domains (ORF1a) and the 60 kDa RNA-dependent RNA polymerase (ORF 1b); (ii) a 8 kDa putative protein (ORF2) that overlaps ORF1b. This protein has predicted transmembrane helices and resembles small membrane proteins encoded by other closteroviruses, such as *Beet yellows virus* (BYV), where it is expressed by a subgenomic messenger RNA (Al Rwahnih *et al*., 2012); (iii) a 4 kDa hydrophobic protein with a putative transmembrane domain (ORF3); (iv) the 62 kDa HSP70h protein (ORF4); (v) a 10 kDa protein showing homology with the small-sized proteins (p4 to p10) coded for by RNA-2 of some criniviruses at the same genomic position (ORF5); (vi) a 61 kDa protein matching the comparable product, referred to as "p60", encoded by all members of the family *Closteroviridae* (ORF6); (vii) the coat protein (CP) 34 kDa in size (ORF6) and (viii) the minor coat protein (CPm) 69 kDa in size (ORF7). ORF9 and ORF10 putatively code for a 25 kDa and a 27 kDa protein, respectively, neither of which shares similarities with other viral proteins in the current database. It is to be noted that the AUG initiation codons of ORFs 2, 3 and 5 are not in an optimal context for expression (Lutcke *et al.*, 1987). The 5' and 3' untranslated regions (UTRs) are 47 and 283 nt in size, respectively, and have no apparent similarity with those of other closteroviruses. By analogy with other members of the family *Closteroviridae*, the genome expression strategy is thought to encompass direct translation and proteolytic processing of the polyprotein encoded by ORF1, a +1 ribosomal frameshift for the expression of the RdRp domain encoded by ORF1b and the expression of downstream ORFs by 3' co-terminal subgenomic RNAs (Martelli *et al*., 2011a).

The genome structure of GLRaV-7 resembles that of LChV-1 and of Cordyline virus 1 (CoV-1), a novel closterovirus-like virus infecting ti plants (*Cordyline fruticosa*) in Hawaii (see Module 2) (Melzer *et al*., 2011), except for the apparent lack in LChV-1 of ORF2 and ORF5, and of ORF2 in CoV-1 (Fig. 1).

In phylogenetic trees built with the HSP70h, polymerase and coat protein sequences, GLRaV-7, LChV-1 and CoV-1 group together in a clade related to that comprising members of the genus *Crinivirus* (Fig. 2, 3, 4 and 5). All trees were constructed on alignments generated with MUSCLE (Edgar, 2004) using the Neighbor Joining method (Saitou and Nei, 1987) implemented in MEGA 5.05 (Tamura *et al*., 2011).

Furthermore, from a comparative analysis of three taxonomic relevant genes (polymerase, HSP70h and CP) of the three viruses (including both GLRaV-7 isolates) with comparable genes of members of the genera *Closterovirus*, *Ampelovirus* and *Crinivirus*, it appears that the highest identity at the amino acid level is shared with criniviruses (Table 1).

As discussed by Jelkmann *et al.* (2012), differences of GLRaV-7, LChV-1 and CoV-1 with members of the three extant genera of the family *Closteroviridae* reside in:

Ampeloviruses: (i) genome size and structure [number of genes intermediate between that of the largest (Subgroup I) and the smallest (Subgroup II) members of the genus]; (ii) biological traits, i.e. lack of a recognized vector, transmission through dodder to herbaceous hosts [ascertained for GLRaV-7 and LChV-1 (Jelkmann *et al.*, 2009; Mikona and Jelkman, 2010)]; (iii) distant phylogenetic relationships (RdRp, HSP70h and CP protein identity at the amino acid level always lower than 30% for any gene).

Closteroviruses: (i) genome size and structure (lower number of genes, CPm preceding CP);

(ii) lack of a recognized vector; (iii) distant phylogenetic relationships, RdRp, HSP70h and CP protein identity at the amino acid level always lower than 30% for any gene.

Criniviruses: Genome structure (monopartite versus bipartite/tripartite, diverse gene arrangement); (ii) lack of a recognized vector; (iii) phylogenetic relationships closer than that with closteroviruses and ampeloviruses but still distant (RdRp, HSP70h and CP protein identity at the amino acid level slightly exceeding 50% only for the polymerase gene).

Overall, these differences seem to be relevant enough to support the suggestion (Al Rwahnih *et al*., 2012; Jelkmann *et al.*, 2012; Martelli *et al.*, 2012) that a fourth genus comprising GLRaV-7, LChV-1 and CoV-1 be created within the family *Closteroviridae* provisionally denoted *Velarivirus* (Al Rwahnih *et al*., 2012).

Origin of the new genus name:

Fron Latin "*Velari*" (veiled, cryptic) because naturally infected vines may not show symptoms

Reasons to justify the choice of type species:

GLRaV-7 is well characterized and was described earlier than the two other species in the genus

Species demarcation criteria in the new genus:

If there will be more than one species in the new genus, list the criteria being used for species demarcation and explain how the proposed members meet these criteria.

Species demarcation criteria are the same as those reported for the other genera of the family Closteroviridae (Martelli *et al*., 2011a):

(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 (polymerase, CP, HSP70h) differing by more than 25%
(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).

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	***2012.001eP***	(assigned by ICTV officers)

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

Grapevine leafroll-associated virus 7
Little cherry virus 1

The present taxonomic position of these taxon/taxa:

Genus:	
Subfamily:	
Family:	***Closteroviridae,*** unassigned species
Order:	

Fill in all that apply.

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

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

To become the type species (GLRaV-7) and an approved species (LChV-1) of a new genus

Part (b) re-assign to a higher taxon

Code	***2012.001fP***	(assigned by ICTV officers)

To re-assign the taxon (or taxa) listed in Part (a) as follows:

Genus:	***Velarivirus*** (new)
Subfamily:	
Family:	***Closteroviridae***
Order:	

Fill in all that apply.
- If the higher taxon has yet to be created write "**(new)**" after its proposed name and complete relevant module to create it.

If no genus is specified, enter "**unassigned**" in the genus box.

Reasons to justify the re-assignment:
- If it is proposed to re-assign species to an existing genus, please 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.
- Provide accession numbers for genomic sequences
- Further material in support of this proposal may be presented in the Appendix, Module 9

See Module 3 and appendix for justification of the new genus

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

Al Rwahnih M., Dolja V.V., Daubert S., Koonin E.V., Rowhani A., 2012. Genomic and biological analyses of a virus from a symptomless grapevine support a new genus within the family *Closteroviridae*. *Virus Research* **163**: 302-309.

Choueiri E., Boscia D., Digiaro M., Castellano M.A., Martelli G.P., 1996. Some properties of a hitherto undescribed filamentous virus of the grapevine. *Vitis* **35:** 91-93.

Edgar R.C., 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* **5**: 113.

Jeklmann W., Fechtner B., Agranovsky A.A. 1997. Complete genome structure and phylogenetic analysis of little cherry virus, a mealybug transmissible closterovirus. *Journal of General Virology* **78**: 2067-2071.

Jelkmann W., Hergenhahn F., Berwarth C., 2009. Transmission of Little cherry virus 1 (LChV-1) by Cuscuta europea to herbaceous host plants. In: Julius-Kühn-Archiv, Neustadt, Germany. Julius Kühn-Institut: 272-274.

Jelkmann W., Mikona C., Turturo C., Navarro B., Rott M.E., Menzel W, Saldarelli P., Minafra A., Martelli G.P., 2012. Molecular characterization and taxonomy of Grapevine leafroll-associated virus 7. *Archives of Virology* **157**: 359-362.

Lutcke H.A., Chowl K.C., Mickell F.S., Moss K.A. Kern H.F., Scheelel G.A., 1987. Selection of AUG initiation codons differs in plants and animals. *The EMBO Journal* **6**: 43-48.

Martelli G.P., 2009. Grapevine virology highlights 2006-2009. *Extended Abstracts 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.

Martelli G.P., Abou Ghanem-Sababadzovic N., Agranovsky A.A., Al Rwahnih M., Dolja V.V., Dovas C.I., Fuchs M., Gugerli P., Hu J.S., Jelkmann W., Katis N.I., Maliogka V.I., Melzer M.J., Menzel W., Minafra A., Rott M.E., Rowhani A., Sabanadzovic S., Saldarelli P., 2012. Taxonomic revision of the family Closteroviridiae with special referene to the grapevine leafroll-associated members of the genus Ampelovirus and the putative species unassigned to the family. *Journal of Plant Pathology* **94:** 7-19.

Melzer M.J., Sehter D.M., Borth W.B., Mersino E.F., Hu J.S., 2011. An assemblage of closteroviruses infects Hawaiian ti (*Cordyline fruticosa* L.). *Virus Genes* **42**: 254-260.

References:

Mikona C., Turturo C., Navarro B., Menzel W., Minafra A., Rott M.E., Martelli G.P., Jelkmann W., 2009. Taxonomy, complete nucleotide sequence and genome organization of Grapevine leafroll-associated virus 7. *Extended Abstracts 16th Meeting of ICVG, Dijon, France*: 275.

Mikona C., Jelkmann W., 2010. Replication of Grapevine leafroll-associated virus 7 (GLRaV-7) by Cuscuta species and its transmission to herbaceous plants. *Plant Disease* **94**: 471-476.

Saitou N., Nei M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**: 406-425

Tamura K., Peterson D., Peterson N., Stecher G., Nei M., Kumar S., 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods**.** *Molecular Biology and Evolution* **28:** 2731-2739

Turturo C., Rott M.E., Minafra A., Saldarelli P., Jelkmann W., Martelli G.P., 2000. Partial molecular characterization and RT-PCR detection of Grapevine leafroll-associated virus 7. *Extended Abstracts 13th Meeting of ICVG, Adelaide, Australia:* 17-18.

Woodham Krake, 1983. Invstigations on transmission of grapevine leafroll, yellow speckle and fleck diseases by dodder. *Phytopathologische Zeitschrift* **106**: 193-198.

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. Amino acid sequence identity/divergence (%) in the RNA-dependent RNA polymerase, HSP70h and coat protein of CoV-1, GLRaV-7 and LChV-1.

RNA-dependent RNA polymerase			
Identity/divergence	GLRaV-7	LChV-1	CoV-1
GLRaV-7	---	52.2/47.8	51.6/48.4
LChV-1	52.2/47.4	---	48.1/51.9
CoV-1	51.6/48.4	48.1/51.9	---

HSP70h			
Identity/divergence	GLRaV-7	LChV-1	CoV-1
GLRaV-7	---	37.2/62.8	40.5/59.5
LChV-1	37.2/62.8	---	37.3/62.7

CoV-1	40.5/59.5	37.3/62.7	---

Coat protein			
Identity/divergence	GLRaV-7	LChV-1	CoV-1
GLRaV-7	---	243.3/75.7	29.0/71.0
LChV-1	24.3/75.7	---	23.5/76.5
CoV-1	29.0/71/0	23.5/76.5	---

Table 2. Amino acid identity (%) of complete sequences of three taxonomically relevant genes [RNA-dependent RNA polymerase (polymerase), heat shock protein 70 homologue (HSP70h), and coat protein (CP)] of GLRaV-7-Alb (numerator) and GLRaV-7-Swi (denominator) with the comparable genes of Little cherry virus 1 (LChV-1) and Cordyline virus 1 (CoV-1), and of all definitive members of the three current genera of the family *Closteroviridae.*

	Polymerase	HSP70h	CP
Little cherry virus 1 (LChV-1)	54/54	42/43	32/31
Cordyline virus 1 (CoV-1)	54/54	40/39	25/29
Crinivirus	47-53/48-49	34-40/37-39	15-21/18-22
Ampelovirus	23-29/25-28	21-27/25-26	8-14/10-14
Closterovirus	29-35/27-29	24-25/24-26	10-12/12-13

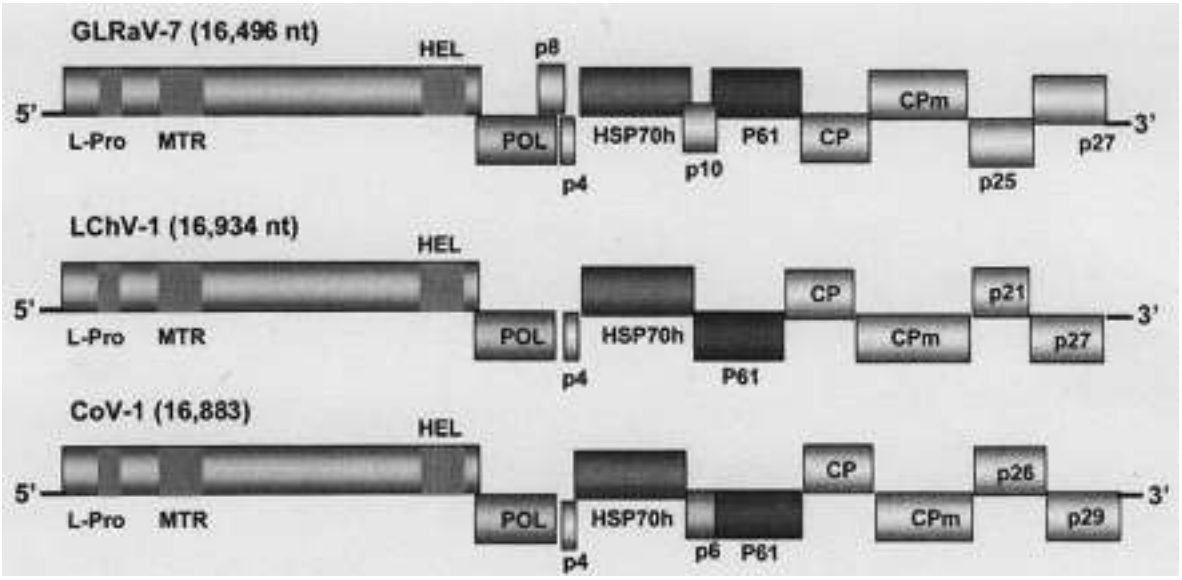

Fig. 1. Schematic representation of the genome structure of the members of the novel genus *Velarivirus*.

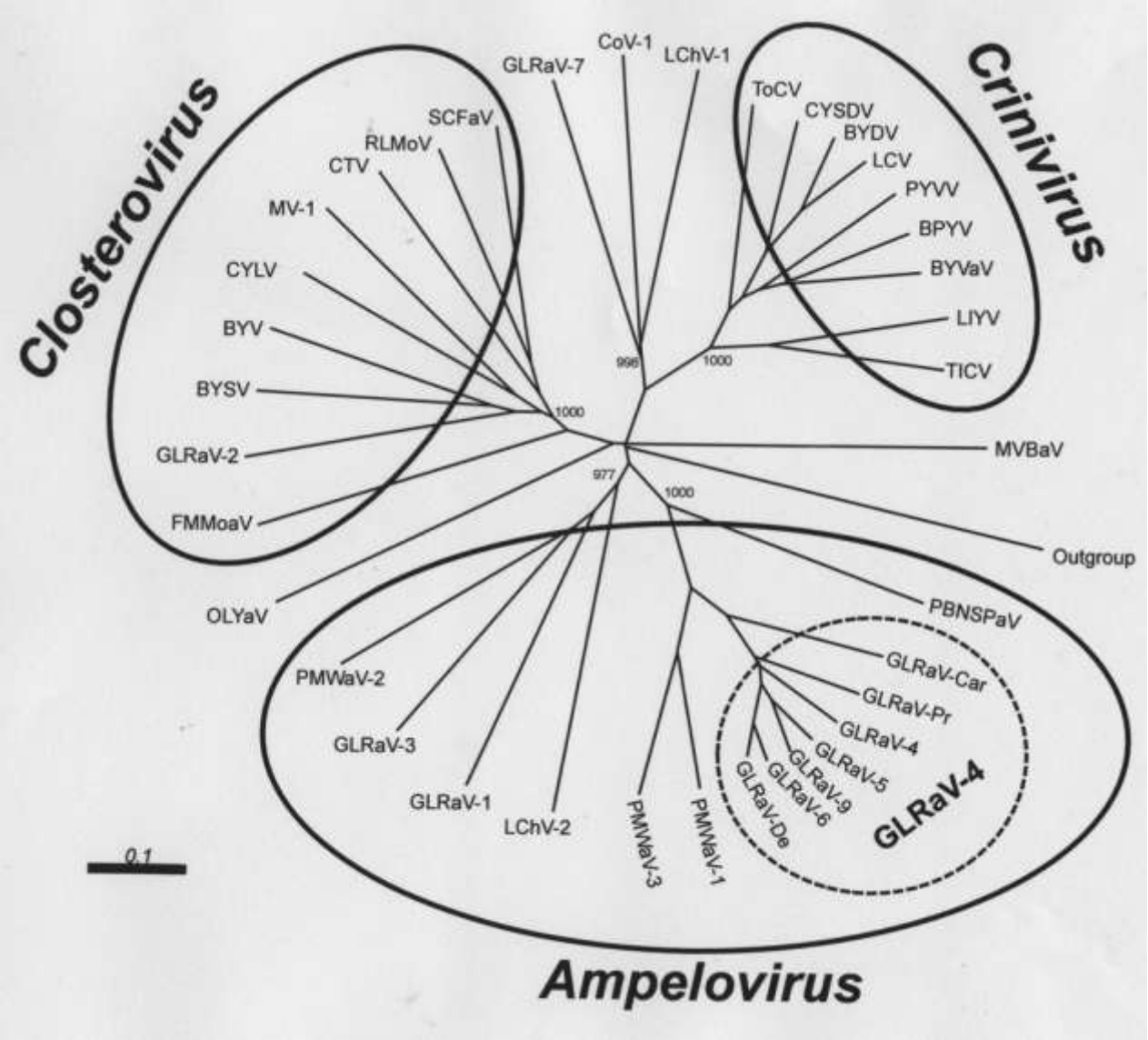

Fig. 2. Phylogenetic tree (Neighbor Joining MEGA 5.05) constructed with complete amino acid sequences of the HSP70h gene of members of family *Closteroviridae*. Distances are proportional to branch lengths. Bootstrap values are indicated at the main branch nodes. The bar represents 0.1 amino acid change 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), *Lettuce chlorosis virus* (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-associad 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. All phylotrees shown in Fig. 2 to 5 wre construced with

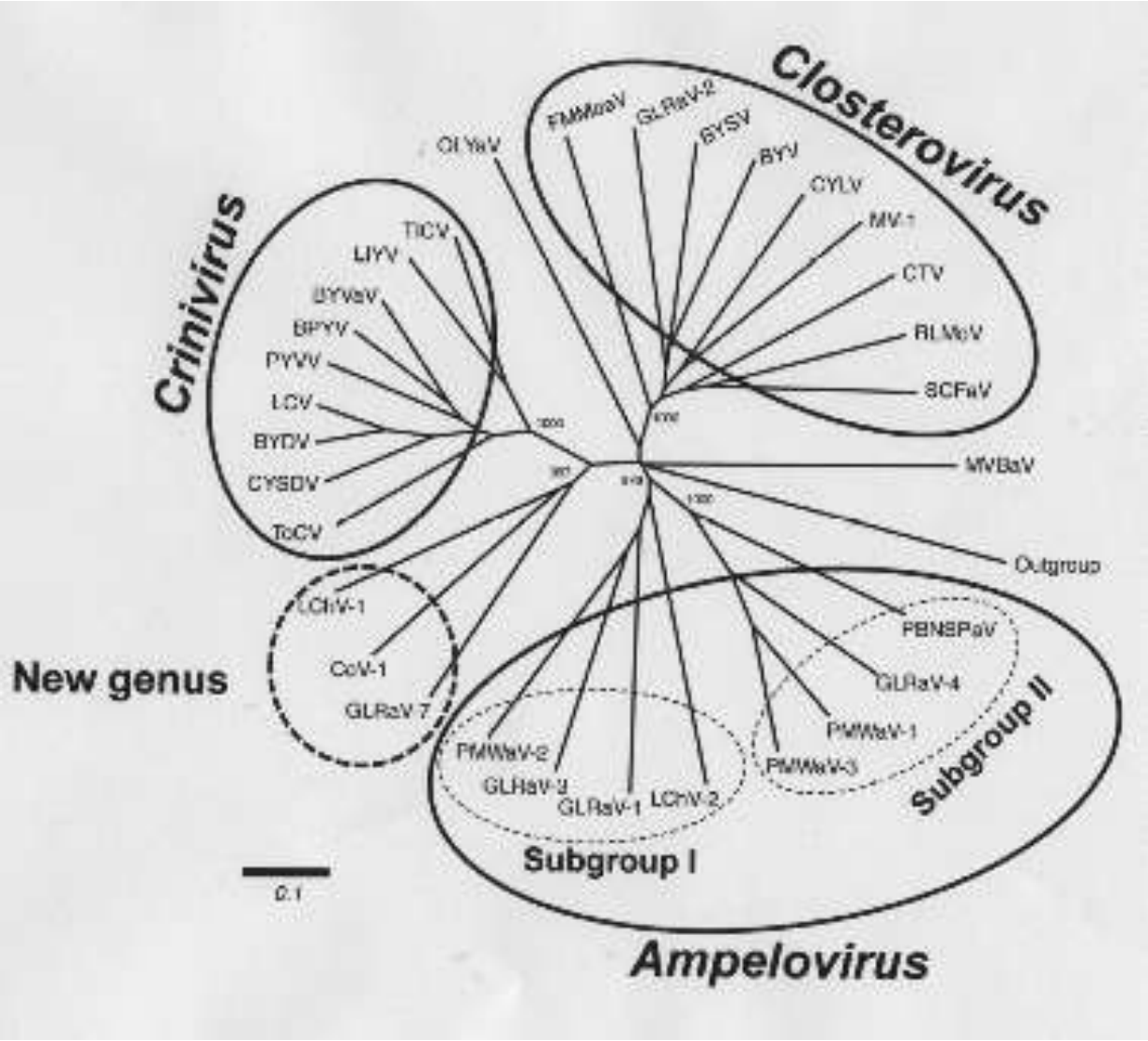

Fig. 3. Phylogenetic tree constructed with complete amino acid sequences of the **HSP70h gene** of members of the family *Closteroviridae*. Distances are proportional to branch lengths. Bootstrap values are indicated at the main branch nodes. The bar represents 0.1 amino acid per site. Viruses used in 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 (aproximately 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*.

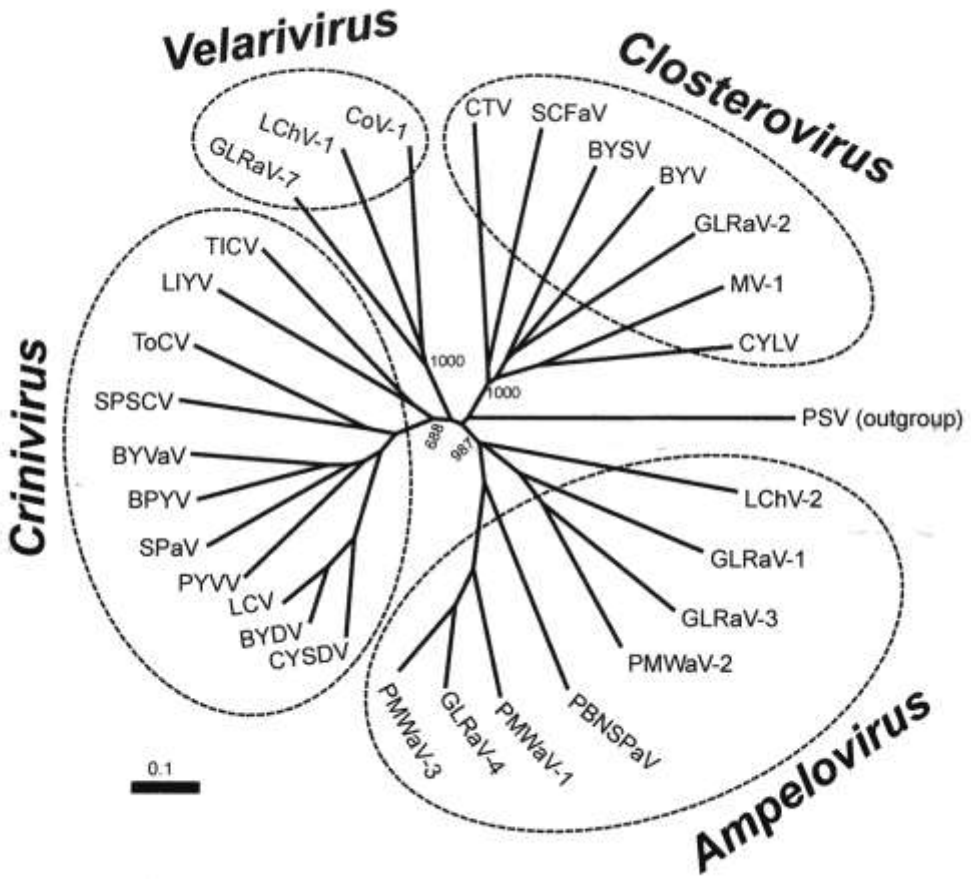

Fig. 4. Phylogenetic tree constructed with complete amino acid sequences of the **coat protein** gene of members of the family *Closteroviridae* availabe in database. Distances are proportional to branch lengths. Bootstrap values are indicated at the main branch nodes. The bar represents 0.1 amino acid per site. Viruses used in the tree, their abbreviations and accession numbers are the same as in Fig. 2. Like in the HSP70h tree, the virus species (GLRaV-7, LChV-1 and CoV-1) included in the novel genus *Velarivirus,* are allocated in a branch of the tree next to that comprising members of the genus *Crinivirus*. The CP gene of *Peanut stunt virus* (PSV) was used as outgroup.

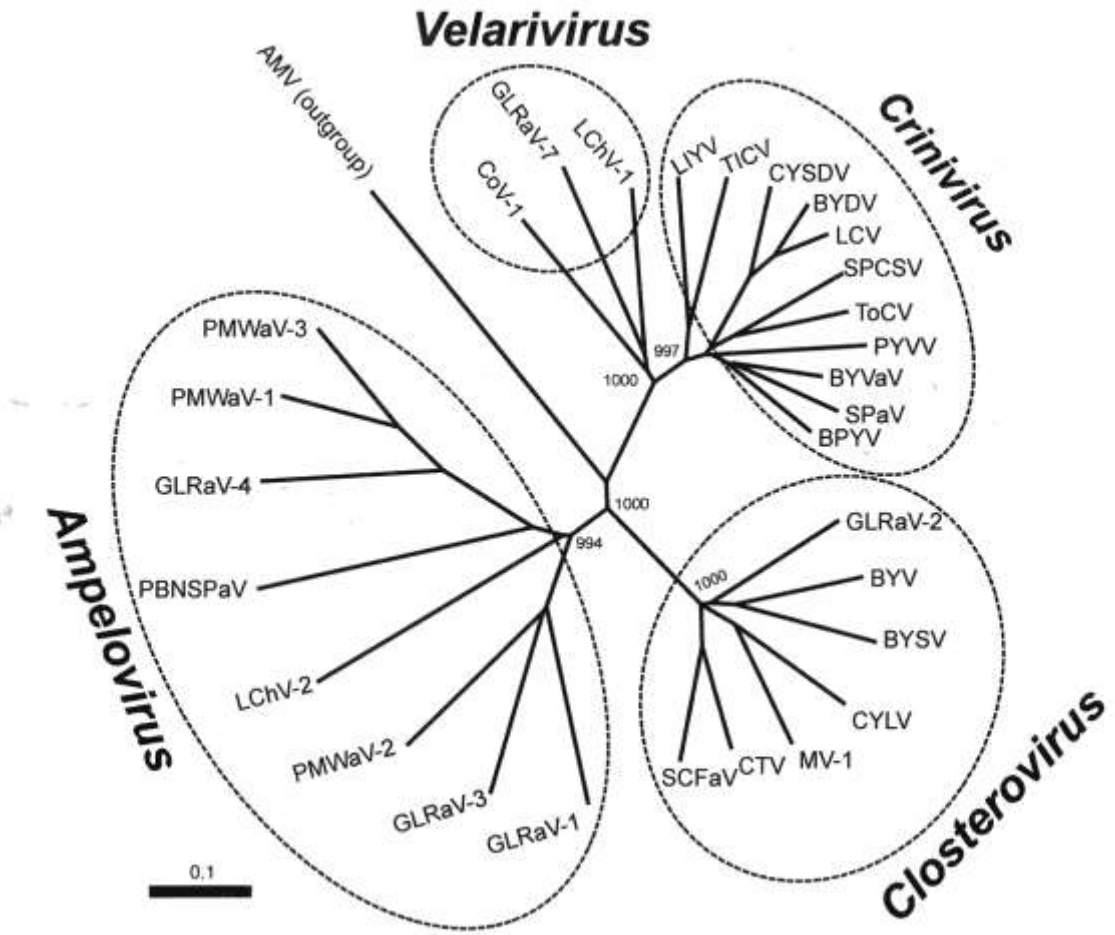

Fig. 5. Phylogenetic tree constructed with complete amino acid sequences of the **polymerase** gene of members of the family *Closteroviridae* available in database. Distances are proportional to branch lengths. Bootstrap values are indicated at the main branch nodes. The bar represents 0.1 amino acid per site. Viruses used in the tree, their abbreviations and accession numbers are the same as in Fig. 2. Like in the HSP70h, and CP trees, the virus species (GLRaV-7, LChV-1 and CoV-1) included in the novel genus *Velarivirus,* are allocated in a branch of the tree next to that comprising members of the genus *Crinivirus*. The polymerase gene sequence of *Alfalfa mosaic virus* (AMV) was used as outgroup.