

## The family *Closteroviridae* revised

**G.P. Martelli (Chair)<sup>1</sup>, A. A. Agranovsky<sup>2</sup>, M. Bar-Joseph<sup>3</sup>, D. Boscia<sup>4</sup>,  
T. Candresse<sup>5</sup>, R. H. A. Coutts<sup>6</sup>, V. V. Dolja<sup>7</sup>, B. W. Falk<sup>8</sup>, D. Gonsalves<sup>9</sup>,  
W. Jelkmann<sup>10</sup>, A.V. Karasev<sup>11</sup>, A. Minafra<sup>12</sup>, S. Namba<sup>13</sup>, H. J. Vetten<sup>14</sup>,  
G. C. Wisler<sup>15</sup>, N. Yoshikawa<sup>16</sup>**

**(ICTV Study group on closteroviruses and allied viruses)**

<sup>1</sup>Dipartimento Protezione Piante, University of Bari, Italy; <sup>2</sup>Laboratory of Physico-Chemical Biology, Moscow State University, Moscow, Russia; <sup>3</sup>Volcani Agricultural Research Center, Bet Dagan, Israel; <sup>4</sup>Istituto Virologia Vegetale CNR, Sezione Bari, Italy; <sup>5</sup>Station de Pathologie Végétale, INRA, Villenave d'Ornon, France; <sup>6</sup>Imperial College, London, U.K.; <sup>7</sup>Department of Botany and Plant Pathology, Oregon State University, Corvallis, U.S.A.; <sup>8</sup>Department of Plant Pathology, University of California, Davis, U.S.A.; <sup>9</sup>Pacific Basin Agricultural Research Center, USDA, Hilo, Hawaii, U.S.A.; <sup>10</sup>Institut für Pflanzenschutz im Obstbau, Dossenheim, Germany; <sup>11</sup>Department of Microbiology and Immunology, Thomas Jefferson University, Doylestown, U.S.A.; <sup>12</sup>Istituto Virologia Vegetale CNR, Sezione Bari, Italy; <sup>13</sup>Graduate School of Agricultural and Life Sciences, University of Tokyo, Japan; <sup>14</sup>Biologische Bundesanstalt, Braunschweig, Germany; <sup>15</sup>Department of Plant Pathology, University of Florida, Gainesville, U.S.A.; <sup>16</sup>Iwate University, Morioka, Japan

**Summary.** Recently obtained molecular and biological information has prompted the revision of the taxonomic structure of the family *Closteroviridae*. In particular, mealybug-transmitted species have been separated from the genus *Closterovirus* and accommodated in a new genus named *Ampelovirus* (from *ampelos*, Greek for grapevine). Thus, the family now comprises three genera. Their major properties are (i) *Closterovirus*: type species *Beet yellows virus*, genome monopartite, 15.5–19.3 kb in size, a 22–25 kDa major coat protein (CP), the gene encoding the divergent CP analogue (CPd) upstream of the CP cistron, transmission by aphids, a membership of 8 definitive and 4 tentative species; (ii) *Ampelovirus*: type species *Grapevine leafroll virus 3*, genome monopartite 16.9–19.5 kb in size, a 35–37 kDa major CP, a CPd cistron generally located downstream of the CP gene, transmission by pseudococcid and coccid mealybugs, a membership of 6 definitive and 5 tentative species; (iii) *Crinivirus*: type species *Lettuce infectious yellows virus*, genome essentially bipartite 15.3–19 kb in size, a 28–33 kDa CP, a CPd cistron downstream of the CP gene, transmission by whiteflies (*Bemisia*, *Trialeurodes*), a membership of 7 definitive and 3 tentative species. There are five unassigned species in the family.

### Introduction

When established in 1998 [5], the family *Closteroviridae* comprised two genera, *Closterovirus* and *Crinivirus*, whose major differentiating trait was the possession of monopartite and bipartite genomes, respectively. As new molecular and biological information became

available, revision of the taxonomic structure of the family became desirable. Thus, Karasev [3] argued that closteroviruses be classified by type of insect vector rather than by the number of genomic RNAs. He proposed the establishment of a genus named “*Vinivirus*” (from *Vitis vinifera* the natural host of the type species) having *Grapevine leafroll-associated virus 3* (GLRaV-3) as type species, to accommodate mealybug-transmitted virus species. The reasons underlying this proposal were biological, i.e., type of vector (mealybugs versus aphids or whiteflies) and molecular, i.e. reverse order of the structural protein genes (setting a difference from closteroviruses); possession of a monopartite genome and a duplication of the leader protease (setting a difference from criniviruses); presence of a 600–1100 nt long intergenic untranslated region downstream of the RdRp-encoding ORF1b.

This proposal was reviewed by the ICTV Study Group on Closteroviruses and Allied Viruses, which, recognizing that the name “*Vinivirus*” was very like that of genus *Vitivirus*, a quite different extant genus [4] and could thus generate confusion, changed it to *Ampelovirus* (from *ampelos*, Greek for grapevine), and analyzed the position of individual species of the whole family. The revised version of the family *Closteroviridae* was approved by ICTV in July 2002.

### Taxonomic structure of the family

Family *Closteroviridae*

Genus *Closterovirus* Type species: *Beet yellows virus* (BYV)

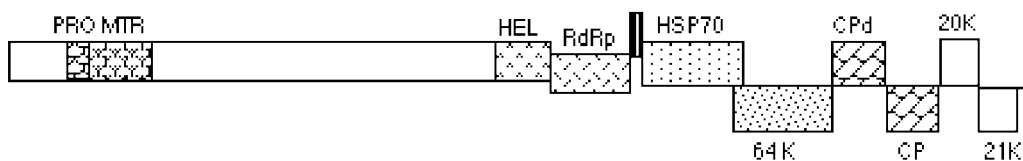
Genus *Ampelovirus* Type species: *Grapevine leafroll-associated virus 3* (GLRaV-3)

Genus *Crinivirus* Type species: *Lettuce infectious yellows virus* (LYIV)

The common characterizing traits of the family are the same as described in the 7<sup>th</sup> Report of ICTV [5]. A concise description of the genera follows.

#### Genus *Closterovirus*

Virions are of one size (> 1000 nm, usually 1250–2200 nm in length) and contain a single molecule of linear, positive sense single-stranded RNA 15.5–19.3 kb in size. The major coat protein (CP) subunit (22–25 kDa) coats most of the virion length, and CPd, the divergent CP analogue (24–27 kDa) coats a short segment (*c.* 75 nm) at one end of the particle. There are three types of genome structure in the genus typified by *Beet yellows virus* (BYV) (Fig. 1), *Citrus tristeza virus* (CTV), and *Beet yellow stunt virus* (BYSV). The organization of BYSV genome is intermediate between that of BYV and CTV, suggesting that these three



**Fig. 1.** Genome structure of *Beet yellows virus* (BYV) the type species of the genus *Closterovirus*, showing the relative position of the ORFs and their expression products. *PRO*, papain-like protease; *MTR*, methyltransferase; *HEL*, helicase; *RdRp*, RNA polymerase; *HSP70*, heat-shock-related protein; *CPd*, divergent coat protein analogue; *CP*, coat protein

viruses might represent three distinct stages in closterovirus evolution. In contrast to the other two genera in the family (*Ampelovirus* and *Crinivirus*), the closterovirus gene encoding the CPd is upstream of the CP gene. The dsRNA-containing cytoplasmic vesicles that accumulate in the cytoplasm of infected cells, arise primarily by proliferation of the endoplasmic reticulum. The genus contains viruses that are transmitted only by aphids in a semi-persistent manner and that primarily infect dicotyledonous hosts. Several of the members are transmissible by sap inoculation, though with some difficulty.

*Species in the genus*

1. Aphid-transmitted:

<i>Beet yellows virus</i> (13)	[X73476, AF056575]	(BYV)
<i>Beet yellow stunt virus</i> (207)	[U51931]	(BYSV)
<i>Burdock yellows virus</i>		(BuYV)
<i>Carnation necrotic fleck virus</i> (136)		(CNFV)
<i>Carrot yellow leaf virus</i>		(CYLV)
<i>Citrus tristeza virus</i> (33,353)	[U56902; U16304]	(CTV)
<i>Wheat yellow leaf virus</i> (157)		(WYLV)

2. Vector unknown:

<i>Grapevine leafroll-associated virus 2</i>	[Y14131, AF039202]	(GLRaV-2)
--	--------------------	-----------

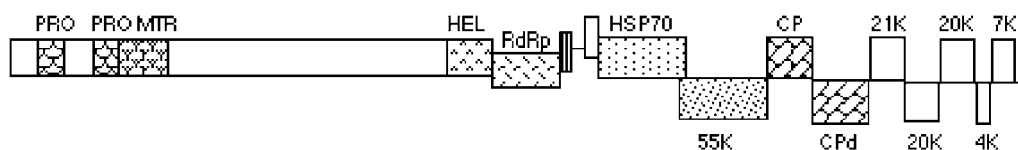
*Tentative species in the genus*

1. Aphid-transmitted:

Clover yellows virus	(CYV)
Dendrobium vein necrosis virus	(DVNV)
Heracleum virus 6	(HV-6)
Festuca necrosis virus	(FNV)

Genus *Ampelovirus*

Virions are of one size (> 1000 nm, usually 1400–2200 nm) in length and contain a single molecule of linear, positive sense, ssRNA 16.9–19.5 kb in size. CP subunits have a high molecular mass (35–39 kDa). There are two types of genome structure in the genus, typified by GLRaV-3 (Fig. 2) and *Little cherry virus 2* (LChV-2). With GLRaV-3 and several other sequenced members of the genus, the CPd gene follows the CP gene, whereas with LChV-2, the CPd gene is five ORFs upstream of the CP cistron [6, and personal communication]. *Grapevine leafroll-associated virus 1* (GLRaV-1) shows a further peculiarity in that its CPd gene is duplicated [2]. The dsRNA-containing vesicles that accumulate in the cytoplasm of



**Fig. 2.** Genome structure of *Grapevine leafroll-associated virus 3* (GLRaV-3) the type species of the genus *Ampelovirus*, showing the relative position of the ORFs and their expression products. *PRO*, papain-like protease; *MTR*, methyltransferase; *HEL*, helicase; *RdRp*, RNA polymerase; *HSP70*, heat-shock-related protein; *CP*, coat protein; *CPd*, divergent coat protein analogue

infected cells may arise either by proliferation of the endoplasmic reticulum or from vesiculation and fragmentation of mitochondria [1]. The genus contains viruses that infect only dicotyledonous hosts, and that are transmitted semi-persistently by coccid (*Parthenolecanium*, *Pulvinaria*, *Neopulvinaria*) or pseudococcid (*Pseudococcus*, *Planococcus*, *Saccharicoccus*, *Dysmiococcus*, *Phenacoccus*, *Heliococcus*) mealybugs. None of the members is transmissible by sap inoculation.

#### Species in the genus

<i>Grapevine leafroll-associated virus 1</i>	[AF 195822]	(GLRaV-1)
<i>Grapevine leafroll-associated virus 3</i>	[U82937, Y1589]	(GLRaV-3)
<i>Grapevine leafroll-associated virus 5</i>	[AF039552]	(GLRaV-5)
<i>Pineapple mealybug wilt-associated virus 1</i>	[AF414119]	(PMWaV-1)
<i>Pineapple mealybug wilt-associated virus 2</i>	[AF283103]	(PMWaV-2)
<i>Little cherry virus 2</i>	[AF295922, AF333237]	(LChV-2)

#### Tentative species in the genus

##### 1. Mealybug-transmitted:

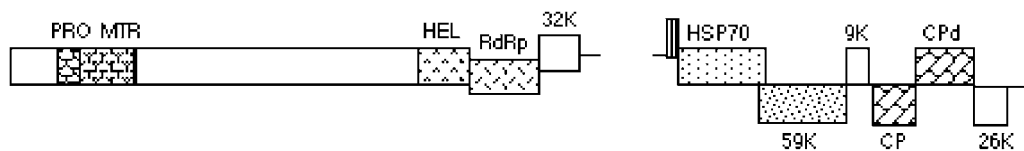
Sugarcane mild mosaic virus (SMMV)

##### 2. Vector unknown:

<i>Grapevine leafroll-associated virus 4</i>	[AF039553]	(GLRaV-4)
<i>Grapevine leafroll-associated virus 6</i>		(GLRaV-6)
<i>Grapevine leafroll-associated virus 8</i>		(GLRaV-8)
Plum bark necrosis and stem pitting-associated virus	[AF195501]	(PBNSPaV)

#### Genus *Crinivirus*

Virions are shorter than 1000 nm and have two modal lengths (650–850 and 700–900 nm). The genome is a linear, positive sense, ssRNA 15.3–19 kb in size, divided into two molecules that are both needed for infectivity and are separately encapsidated (Fig. 3). CP size of known members is rather uniform ranging from 28 to 33 kDa, but the size of CPd can be as large as *c.* 80 kDa. RNA-1 of LIYV is a bicistronic molecule that encodes replication-related proteins (ORF1) and a *trans* enhancer for RNA-2 accumulation (ORF2) [7]. RNA-2 has seven ORFs. It contains the five-gene module typical of the family, which, however, differs from that of the *Closterovirus* and *Ampelovirus* genera because of the insertion of an extra small gene (ORF4) upstream of the CP gene. In all members of the genus, the CPd



**Fig. 3.** The bipartite genome structure of *Lettuce infectious yellows virus* (LIYV), the type species of the genus *Crinivirus*, showing the relative position of the ORFs and their expression products. *PRO*, papain-like protease; *MTR*, methyltransferase; *HEL*, helicase; *RdRp*, RNA polymerase; *HSP70*, heat-shock-related protein; *CP*, coat protein; *CPd*, divergent coat protein analogue

ORF is downstream of the CP gene. The dsRNA-containing vesicles that accumulate in the cytoplasm of infected cells may arise by proliferation of the endoplasmic reticulum. None of the members can be transmitted by sap inoculation. Natural vectors are whiteflies (*Bemisia*, *Trialeurodes*) which transmit in a semipersistent manner. A few *Trialeurodes*-transmitted viruses with a seemingly undivided genome and an arrangement of genes similar to that of bipartite viral species, have been assigned to this genus as tentative species on the grounds that the type of vector transmission is a key biological character that needs to be recognized as a major classification feature of closteroviruses [3].

*Species in the genus*

<i>Abutilon yellows virus</i>		(AbYV)
<i>Cucurbit yellow stunting disorder virus</i>	[U67448, AJ439690]	(CYSDV)
<i>Lettuce chlorosis virus</i>		(LCV)
<i>Lettuce infectious yellows virus</i>	[U15440, U15441]	(LIYV)
<i>Sweet potato chlorotic stunt virus</i> (Sweet potato sunken vein virus)	[X80995, AJ010914]	(SPCSV)
<i>Tomato chlorosis virus</i>	[AF024630]	(ToCV)
<i>Tomato infectious chlorosis virus</i>	[U67449]	(TICV)

*Tentative species in the genus*

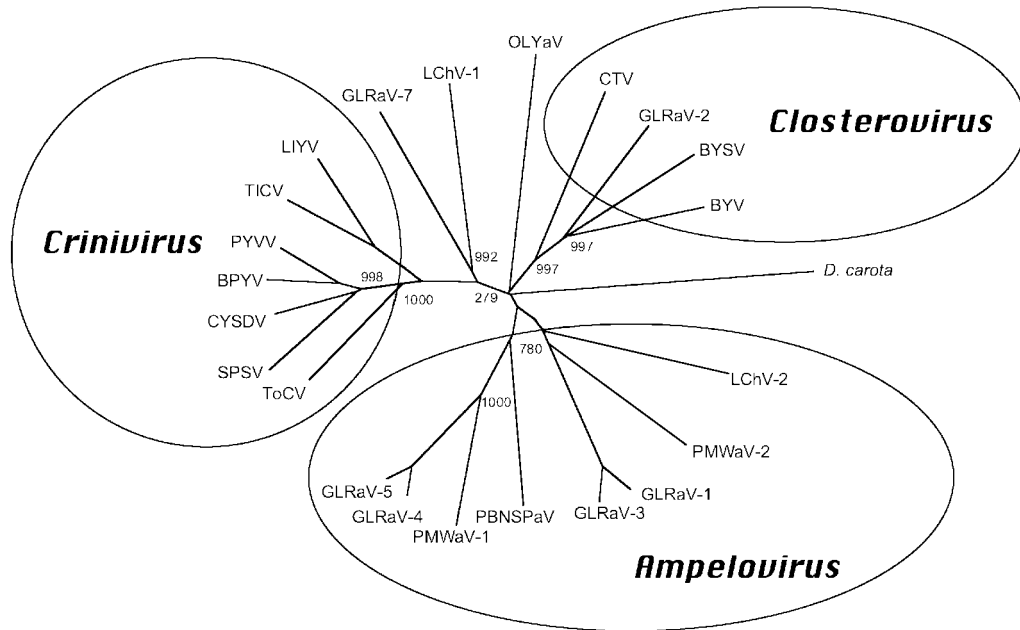
Potato yellow vein virus	[AF150984]	(PYVV)
Beet pseudoyellows virus (Cucumber chlorotic spot virus)	[Y15568, U67447]	(BPYV)
Diodea vein chlorosis virus		(DVCV)

*Unassigned species in the family*

Little cherry virus 1	[Y10237]	(LChV-1)
Grapevine leafroll-associated virus 7	[Y15897]	(GLRaV-7)
Alligatorweed stunting virus		(AWSV)
Megakepasma mosaic virus		(MegMV)
Olive leaf yellowing-associated virus	[AJ440010]	(OLYaV)

The above species could not be comfortably allocated to any of the genera either because the available information is scanty and does not provide any solid taxonomic rationale (MegMV and AWSV), or because more molecular (OLYaV, GLRaV-7) or biological (LChV-1) data are needed for the unequivocal definition of their phylogenetic relationships.

## Phylogenetic relationships within the family



**Fig. 4.** Phylogenetic tree showing the relationships between the species and genera of the family *Closteroviridae* based on the sequence of the HSP70 gene. The neighbour-joining tree was produced and bootstrapped using CLUSTAL W. Branch lengths are proportional to sequence distances. *GLRaV-7*, *LChV-1*, and *OLYaV* are unassigned species in the family

## References

1. Faoro F (1997) Cytopathology of closteroviruses infecting grapevines. In: Monette PL (ed) Filamentous viruses of woody plants. Research Signpost, Trivandrum, pp 29–47
2. Fazeli FC, Rezaian MA (2000) Nucleotide sequence and organization of ten open reading frames of the grapevine leafroll-associated virus 1 genome and identification of three subgenomic RNAs. *J Gen Virol* 81: 605–615
3. Karasev AV (2000) Genetic diversity and evolution of closteroviruses. *Annu Rev Phytopathol* 38: 293–324
4. Martelli GP, Minafra A, Saldarelli P (1997) *Vitivirus*, a new genus of plant viruses. *Arch Virol* 142: 1930–1932
5. Martelli GP, Agranovsky AA, Bar-Joseph M, Boscica D, Candresse T, Coutts RHA, Dolja VV, Falk BW, Gonsalves D, Jelkmann W, Karasev AV, Minafra A, Namba S, Vetten HJ, Wisler GC, Yoshikawa N (2000) Family *Closteroviridae*. In: Van Regenmortel MHV, Fauquet CL, Bishop DHL, Carstens E, Estes MK, Lemon S, Maniloff J, Mayo MA, McGeoch D, Pringle CR, Wickner RB (eds) *Virus taxonomy*. Seventh Report of the International Committee on Taxonomy of Viruses. Academic Press, San Diego, pp 943–952
6. Rott ME, Jelkmann W (2001) Detection and partial characterization of a second closterovirus associated with little cherry disease, Little cherry virus-2. *Phytopathology* 91: 261–267
7. Yeh HH, Tian T, Rubio L, Crawford B, Falk BW (2000) Asynchronous accumulation of *Lettuce infectious yellows virus* RNAs 1 and 2 and identification of an RNA 1 *trans* enhancer of RNA 2 accumulation. *J Virology* 74: 5762–5768

Authors' address: Dr. G. P. Martelli, Dipartimento di Protezione dell Pianta, Via Giovanni Amendola 165A, 70126 Bari, Italy.