Template for Taxonomic Proposal to the ICTV Executive Committee
Creating Species in an existing genus

Code† 2005.007P.04 To designate the following as species in the genus: ***Nepovirus***

belonging to the family° : ***Comoviridae***

Grapevine deformation virus
Grapevine deformation virus (GDefV) AY291208
Grapevine Anatolian ringspot virus
Grapevine Anatolian ringspot virus (GARSV) AY291207

† Assigned by ICTV officers
° leave blank if inappropriate or in the case of an unassigned genus

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Old Taxonomic Order

Order: None
Family: *Comoviridae*
Genus: *Nepovirus*
Type Species: *Tobacco ringspot virus*
Species in the Genus: 8 (subgroup A); 10 (subgroup B); 15 (subgroup C)
Tentative Species in the Genus: None
Unassigned Species in the family: None

New Taxonomic Order

Order: None
Family: *Comoviridae*
Genus: *Nepovirus*
Type Species: *Tobacco ringspot virus*
Species in the Genus: 9 (subgroup A); 11 (subgroup B); 15 (subgroup C)
Tentative Species in the Genus: None
Unassigned Species in the family: none

ICTV-EC comments and response of the SG

Species demarcation criteria in the genus

The criteria demarcating species in the genus are:

- CP aa sequence less than 75% homologous,
- Polymerase aa sequence less than 75% homologous,
- No pseudo-recombination between components possible,
- Differences in antigenic reactions,
- Different vector species.

Argumentation to justify the designation of new species in the genus

To give a taxonomic allocation to two recently described grapevine viruses with isometric particles, based on biological, physico-chemical, serological, ultrastructural, and molecular evidence, as detailed in the annex

List of created Species in the genus

Grapevine deformation virus
Grapevine deformation virus (GDefV)
Grapevine Anatolian ringspot virus
Grapevine Anatolian ringspot virus (GARSV)

References

Cigsar I., Digiaro M., Gokalp K., Abou Ghanem-Sabanadzovic N., De Stradis A., Boscia D., Martelli G.P., 2003.Grapevine deformation virus, a novel nepovirus from Turkey. *Journal of Plant Pathology*. **85**: 183-191.

Gokalp K., Digiaro M., Cigsar I., Abou Ghanem-Sabanadzovic N., De Stradis A., Boscia D., Martelli G.P., 2003. Properties of a previously undescribed nepovirus from south-east Anatolia. *Journal of Plant Pathology*. **85**: 35-41.

Abou Ghanem-Sabandzovic N:, Sabandzovic S:, Digiaro M. Martelli G.P., 2005. Complete nucleotide sequence of RNA-2 of Grapevine deformation and Grapevine ringspot viruses. *Virus Genes* **30**: 333-338.

ANNEX

Grapevine deformation virus (GDefV)

GDefV, a virus with isometric particles about 30 nm in diameter and angular contour, was recovered by mechanical transmission from grapevines with fanleaf-like symptoms growing in Cappadocia (Central Turkey).

Biological properties. GDefV infected a restricted range of herbaceous hosts. In *Chenopodium amaranticolor,* it induced systemic symptoms very similar to those given by *Grapevine fanleaf virus* (GFLV) and *Arabis mosaic virus* (ArMV) but, unlike them, did not elicit symptom in *Gomphrena globosa* and most of the the *Nicotiana* species tested. The virus had a field incidence of 3.4% and was not transmitted through grapevine seeds. Search for possible nematode vectors was unsuccessful.

Physico-chemical and molecular properties. In sucrose density gradient centrifugation the virus sedimented as tree components, T (empty shells), M, and B, both consisting of apparently intact particles. Virus preparations contained two RNA species with mol. wt 2.6 x 10^6 Da (RNA-1) and 1.3 x 10^6 Da (RNA-2). The coat protein (CP) subunits were of a single type with M_r of *c.* 53,000. Viral RNA-2 was totally sequenced and shown to consist of 3753 nucleotides excluding the poly(A) tail (a size compatible with that of viral species in subgroup A of the genus *Nepovirus*). It contained a single open reading frame encoding a polyprotein of 122 kDa. Full-length nucleotide sequence comparison disclosed 71-73% homology of GDefV RNA-2 with RNA-2 of GFLV and ArMV, respectively. The CP cistron had 69% identity at the amino acid level with the CP of ArMV and 58% identity with the CP of GFLV. In a phylogenetic tree constructed with nepoviral CP sequences, GDefV clustered with ArMV and other subgroup A species.

Serology. A virus-specific polyclonal antiserum (titre 1:1024) did not react with healthy plant antigens and gave a single precipitin line in gel double diffusion plates. GDefV was serologically unrelated to 16 different nepoviruses, including all those known to infect grapevines. A distant positive reaction was obtained only with ArMV in immunodiffusion (serological differentiation index = 4) and immuno-electron microscopy tests, and when leaf extracts from naturally infected grapevines were tested in ELISA with commercial antisera to ArMV.

Cytopathology. The most striking ultrastructural feature of infected *C. amaranticolor* mesophyll cells was the presence of inclusion bodies which were usually located next to the nuclei and had an overall aspect somewhat differing from that observed in cells infected by other nepoviruses. Virus particles were either scattered in the cytoplasm or, more often, arranged in rows or in microcrystals, or were close to or within plasmodesmata. Tubule-containing particles were not seen.

Diagnosis. PCR primers were designed on the CP sequence and used successfully for virus detection in grapevine crude sap extract. An ELISA kit produced with the GDefV antiserum was used in a field survey conducted in Turkey.

Grapevine Anatolian ringspot virus (GARSV)

GARSV a virus with isometric particles about 30 nm in diameter and angular contour, was recovered by mechanical transmission from grapevines with mild fanleaf-like symptoms growing in Anatolia (Turkey).

Biological properties. GARSV infected a restricted range of herbaceous hosts inducing reactions comparable to those induced by nepoviruses. It had a field incidence of about 3%. Search for possible nematode vectors was unsuccessful.

Physico-chemical and molecular properties. In sucrose density gradient centrifugation the virus sedimented as tree components, T (empty shells), M, and B, both of which consisted of apparently intact particles. Virus preparations contained two RNA species with mol. wt 2.2 x 10^6 Da (RNA-1) and 1.4 x 10^6 Da (RNA-2). The coat protein (CP) subunits were of a single type with M_r of *c.* 55,500. The totally sequenced viral RNA-2 consisted of 4607 nucleotides excluding the poly(A) tail (a size compatible with that of nepovirus species in subgroup B), and contained a single open reading frame encoding a polyprotein of 150 kDa. Full-length nucleotide sequence comparison disclosed a 62-64% homology of GARSV RNA-2 with RNA-2 of Grapevine chrome mosaic virus (GCMV) and Tomato black ring virus (TBRV), respectively. The CP cistron had 62% identity at the amino acid level with the CP of GCMV and 49% identity with the CP of both TBRV and Artichoke Italian latent virus (AILV). In a phylogenetic tree constructed with nepoviral CP sequences, GARSV clustered with GCMV and other species of subgroup B.

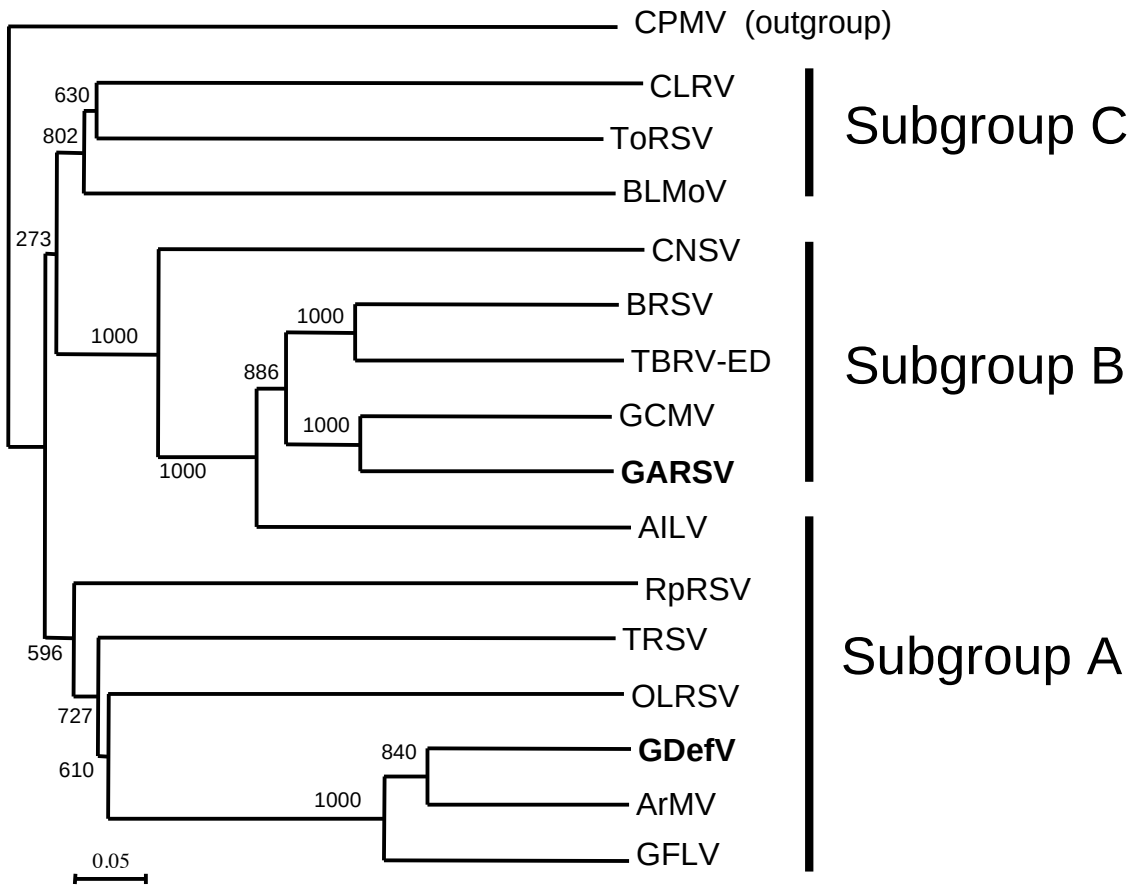
Serology. A virus-specific polyclonal antiserum (titre 1:256) did not react with healthy plant antigens and gave a single precipitin line in gel double diffusion plates. GARSV was serologically unrelated to 17 different nepoviruses, including all those recorded from grapevines.

Cytopathology. Infected *Nicotiana benthamiana* cells had a cytopathology comparable with that elicited by most nepoviruses. Inclusion bodies resembled the vesiculate-vacuolate cytopathological structures associated with nepovirus infections. Virus particles were scattered in the cytoplasm or were within tubular structures associated with plasmodesmata.

Diagnosis. PCR primers were designed on the CP sequence and used successfully for virus detection in grapevine crude sap extract. An ELISA kit produced with the antiserum to the virus was used in a field survey conducted in Turkey.

Concluding remarks

Although the level of homology of polymerase aa sequence of GDefV/GARSV and those of other nepoviruses was not determined, nor pseudo-recombination experiments were conducted, the biological, serological, physico-chemical, cytopathological, and the available molecular properties of these viruses are such so as to strongly suggest that they can be regarded as *bona fide* members of the subgroup A (GDefV) and B (GARSV) of the genus *Nepovirus,* the taxonomic allocation that we propose.



Phylogenetic tree generated from the alignment of the overall coat protein amino acid sequences of members of the *Nepovirus* genus

From: Abou Ghanem-Sabanadzovic *et al*., 2005. *Virus Genes* **30**:333-338