

Template for Taxonomic Proposal to the ICTV Executive Committee To create a new Genus in an existing Family

Code [†]	2004.008P.04	To create a new genus in the family*	<i>Bromoviridae</i>
Code [†]	2004.009P.04	To name the new genus*	<i>Anulavirus</i>
Code [†]	2004.010P.04	To designate the species As the type species of the new genus*	<i>Pelargonium zonate spot virus</i>
Code [†]	2004.011P.04	To designate the following as species of the new genus*:	<i>Pelargonium zonate spot virus</i>
Code [†]		To designate the following as tentative species in the new genus*:	None

[†] Assigned by ICTV officers

* repeat these lines and the corresponding arguments for each genus created in the family

Author(s) with email address(es) of the Taxonomic Proposal

Giovanni P. Martelli: martelli@agr.uniba.it
 Donato Gallitelli: gallitel@agr.uniba.it
 Mariella Finetti-Sialer: mariellafinetti@hotmail.it

Old Taxonomic Order

Order
 Family *Bromoviridae*
 Genus
 Type Species
 Species in the Genus
 Tentative Species in the Genus
 Unassigned Species in the family

New Taxonomic Order

Order
 Family *Bromoviridae*
 Genus *Anulavirus*
 Type Species *Pelargonium zonate spot virus*
 Species in the Genus *Pelargonium zonate spot virus*
 Tentative Species in the Genus

Unassigned Species in the family
ICTV-EC comments and response of the SG

Accepted to go forward but needs to substantiate the proposal and provide references.

Argumentation to choose the type species in the genus

PZSV is thoroughly characterized. In any case, the genus is monotypic, thus the choice was unavoidable

Species demarcation criteria in the genus

Not applicable for the genus is monotypic

List of Species in the created genus

Pelargonium zonate spot virus (PZSV)

List of Tentative Species in the created genus

None

Argumentation to create a new genus:

Accommodating PZSV in a new genus plant viruses with quasi-spherical particles 25-35 nm in diameter and a tripartite ssRNA genome, with the properties described in the annex
Distinguishing this genus from other genera of plant viruses with a tripartite genome, in recognition of differences in particle morphology, physicochemical, molecular, and biological properties (host range and epidemiology), type and size of encapsidated RNA.
The assignment of the genus *Anulavirus* to the family *Bromoviridae* is justified by the possession of a tripartite genome and by similarities in genome organization, strategy of replication, and phylogenetic relationships (i.e. similarities in the sequence of all genome products with those of one or another of the current genera in the family).

Origin of the proposed genus name

From *anular*, Latin for concentric, because of the type of symptoms (concentric bands or rings) shown by major natural hosts (pelargonium, tomato)

References

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- Castellano M. A., Martelli G.P., 1981. Electron microscopy of Pelargonium zonate spot virus in host tissues. *Phytopathologia Mediterranea*, **20**: 64-71.
- Finetti-Sialer M., Gallitelli D. 2003. Complete nucleotide sequence of Pelargonium zonate spot virus and its relationship with the family *Bromoviridae*. *Journal of General Virology*, **84**:3143-3151.
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- Vovlas C., Gallitelli D, Conti M., 1989. Preliminary evidence for an unusual mode of transmission in the ecology of Pelargonium zonate spot virus (PZSV). *4th Plant Virus Epidemiology Workshop, Montpellier 1989*: 302-305.

Annex:

Pelargonium zonate spot virus (PZSV) is a mechanically transmissible virus first isolated in southern Italy from *Pelargonium zonale* plants showing concentric chrome-yellow bands in the leaves (Quacquarelli and Gallitelli, 1979; Gallitelli *et al.*, 1983) and characterized by Gallitelli (1982). This virus was later found infecting vegetables (tomato, artichoke) and weeds (*Capsella bursa-pastoris*, *Chrysanthemum segetum*, *Diplotaxis erucoides*, *Picreis echioides*, and *Sonchus oleraceus*) and was recorded also from France and Spain (see Finetti-Sialer and Gallitelli, 2003). Like some ilarviruses, PZSV is seed-borne in *D. erucoides* and is transmitted from this weed to tomato in association with pollen grains carried on the body of thrips that feed on its flowers (Vovlas *et al.*, 1989).

Virus particles are non-enveloped, quasi-spherical, with a diameter ranging from 25 to 35 nm, and have a poorly resolved surface structure. Virions sediment as three components in sucrose density gradient and analytical centrifugation but have the same buoyant density at equilibrium in CsCl.

The coat protein (CP) is made up of a single type of subunits with Mr of c. 23 kDa, which have no effect on the infectivity of RNA preparations. Nucleic acid is a positive sense, single-stranded RNA accounting for c. 18% of the particle weight, originally reported to consist of two separately encapsidated functional species with mol. wts of 0.95×10^6 and 1.25×10^6 , respectively (Gallitelli, 1982; Gallitelli *et al.*, 1983).

Cytopathological alterations are severe. Virus particles are plentiful in infected cells accumulating in the nucleus and cytoplasm in large non-crystalline aggregates or in single rows within tubular structures (Castellano and Martelli, 1981).

Thus, PZSV closely resembles members of the genus *Iilarvirus* in particle morphology, some physicochemical properties, and epidemiology, but appear to differ significantly from ilarviruses in having only two RNA species, no need of coat protein for RNA infectivity, and for eliciting a distinctively diverse cytopathology: For these reasons and because no molecular information was available, PZSV remained unclassified and was included among unassigned viruses in the 7th ICTV Report (Calisher *et al.*, 2000).

Recent studies have shown that, contrary to previous findings (Gallitelli *et al.*, 1983), PZSV has three positive strand RNA species which have been completely sequenced and proven to possess the organization of members of the family *Bromoviridae* (Finetti-Sialer and Gallitelli, 2003).

The whole viral genome comprises 8477 nt distributed in three RNA species encoding four proteins. In particular:-

- (i) The 3383 nt long RNA-1 consists of a single ORF (ORF 1) encoding a polypeptide with Mr of 108,419 Da (protein 1a), containing the conserved motifs of methyltransferase and helicase.
- (ii) RNA-2 is 2435 nt in size, has a single ORF (ORF 2) which encodes a polypeptide of Mr 78,944 da (protein 2a), containing the conserved motifs of RdRp
- (iii) The bicistronic RNA-3 is 2569 nt in size (i.e. slightly larger than RNA-2, a feature that, among bromovirids, is unique to this virus). ORF 3 codes for a 34K protein with similarities to movement proteins of the 30K superfamily, whereas ORF 4 is the CP cistron, encoding a 23K product. CP is expressed via a subgenomic RNA 1118 nt in size.

None of the PZSV proteins has a level of similarity higher than 78% with any of the comparable proteins coded for by viruses of other genera in the family *Bromoviridae* (Table 1 in Finetti-Sialer and Gallitelli, 2003). In fact, pairwise comparisons of amino acid sequence similarities between methyltransferase (MET), helicase (HEL), RdRp, putative movement protein (MP), and CP of PZSV and sequenced bromovirids, gave values ranging between 58.7 and 73.6% (MET); 59.1 and 78.4% (RdRp); 52.3 and 65.9% (HEL); 35.7 and 48.3% (MP); 35.6 and 49.2% (CP).

In phylogenetic tree constructed with CPs sequences (trees in Finetti-Sialer and Gallitelli, 2003), PZSV stands on its own, being clearly separated from all other bromovirids. By contrast: (i) in the MP tree, PZSV clusters with *Olive latent virus 2* (the type species of the monotypic genus *Oleavirus*) in a separate subclade; (ii) in the RdRp tree, PZSV is in a clade comprising members of the genera *Cucumovirus* and *Bromovirus*; (iii) in the HEL tree, PZSV clusters with members of the genera *Oleavirus*, *Bromovirus*, *Cucumovirus*, and *Ideovirus*; (iv) in the MET tree, PZSV clusters with members of the genera *Oleavirus* and *Cucumovirus* (Finetti-Sialer and Gallitelli, 2003).

The overall biological and genomic characteristics of PZSV show affinities in diverging directions with one or the other of the viruses in the family *Bromoviridae*. While phylogenetic trees establish a clear separation between PZSV CP and CPs of other genera in this family, the remaining PZSV gene products seem less similar to those of alfamoviruses and ilarviruses than those of members of the genera *Bromovirus*, *Cucumovirus*, *Oleavirus* and *Idaeovirus*. This suggests that PZSV may have originated from ancestors common to viruses in these latter genera, but acquired the CP gene from another source.

From a consideration of all of the above, it seems plausible to conclude that PZSV is the representative of a novel monotypic genus in the family *Bromoviridae*, for which the name of *Anulavirus* is proposed.