Taxonomic Proposals from the ICTV Dicistrovirus Study Group

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Christian, P. (Chairperson), Carstens, E., Domier, L., Johnson, J., Johnson, K., Nakashima, N., Scotti, P. and van der Wilk, F.

Proposal 1. 2003.089F
Assign Aphid lethal paralysis virus to the Genus *Cripavirus* in the family *Dicistroviridae*.

1.1 Purpose

Since the last revision of the family ratified at the Paris meeting last year, the full genomic sequence of Aphid lethal paralysis virus (ALPV) has been published (van Munster *et al*., 2002). The known properties of this virus are very similar to those of the viruses currently recognised as comprising the *Dicistroviridae*. In particular ALPV shows the following defining characteristics of the *Dicistroviridae*:

- positive sense ssRNA of around 10kb, polyadenylated at the 3' end
- 3 major capsid proteins (generally between 28 and 37 kDa)
- icosahedral/spherical particles (about 30nm in diameter)
- a genome comprising two distinct ORFs separated by an untranslated region and no known sub-genomic RNAs
- genome organised with the ORF encoding the non-structural proteins towards the 5' of the genome (ORF 1)and the structural proteins encoded by the ORF at the 3' end of the genome (ORF2).
- coding sequence for the capsid proteins (from 5' to 3') are organised VP2, VP3 and VP1. The terminology for these proteins is derived by homology with the structural proteins of picornaviruses and as exemplified by *Cricket paralysis virus* – for which the 3D structure is known.
- the deduced amino acid sequences of ORF1 contain core motifs for the RNA helicase, cysteine protease and the RNA polymerase.

ALPV shows all of the above characters as do all other current members of the *Dicistroviridae*.

Two other polythetic characters are also helpful in defining the *Dicistroviridae,* namely:

- presence of a small (<10kDa) minor protein in the capsid that is analogous to the VP4 of picornaviruses and is located on the internal surface of the 5-fold axis below VP1. This protein (VP4) is derived from a precursor (usually termed VP0) - that is sometimes also present as a minor capsid component – comprising VP4+VP3.
- presence of a covalently linked protein at the 5' end of the genome (VPg)

There is no evidence that ALPV exhibits either of these characters - although ALPV does show a minor capsid component that may correspond to VP0 and a consensus cleavage site between the putative VP4 and VP3.

1.2 Taxonomic Implications

The recognised members of the Genus will now comprise the species as shown below:

Official virus species names are in italics. Tentative virus species names , alternative names( ), strains, or serotypes are not italicized. Virus names, genome sequence accession numbers [ ], and assigned abbreviations ( ) are:

Cricket paralysis virus	[AF218039]	(CrPV)
Aphid lethal paralysis virus	[AF536531]	(ALPV)
Black queen cell virus	[AF183905]	(BQCV)
Drosophila C virus	[AF014388]	(DCV)
Himetobi P virus	[AB017037]	(HiPV)
Plautia stali intestine virus	[AB006531]	(PSIV)
Rhopalosiphum paid virus	[AF022937]	(RhPV)
Taura syndrome virus	[AF277675]	(TSV)
Triatoma virus	[AF178440]	(TrV)
Tentative Species in the genus		
Acute bee paralysis virus	[AF150629]	(ABPV)

1.3 Derivation of proposed names

Names and abbreviations follow those widely recognised and in current use with workers in the field.

1.4 References

van Munster,M., Dullemans,A.M., Verbeek,M., van den Heuvel,J.F., Clerivet,A. and van der Wilk,F. (2002) Sequence analysis and genomic organization of Aphid lethal paralysis virus: a new member of the family *Dicistroviridae.* J. Gen. Virol. **83**; 3131-3138.

Proposal 2. 2003.090F Move *Taura syndrome virus* (TSV) from the Genus *Cripavirus* and move *Acute bee paralysis virus* (ABPV) from being a tentative member of the genus *Cripavirus* to both become Unassigned Species in the Family Dicistroviridae.

2.1 Purpose

Taura syndrome virus (TSV) was assigned to the genus *Cripavirus* in the last revision of the family that was ratified at the Paris meeting last year. Prior to this, *Acute bee paralysis virus* (ABPV) had been assigned as a tentative member of the genus *Cripavirus* – the reason for it not then being included in *Cripavirus* was that it showed some features that indicated it may represent a distinct lineage from the other dicistroviruses. Since the acceptance of the two proposals above further analysis has been carried out on ABPV and TSV that show that while both viruses are clearly dicistroviruses they are very distinct from all other members of the family i.e. the species that comprise *Cripavirus*.

The main lines of evidence indicating the TSV and ABPV are more distantly aligned with the dicistroviruses placed in the genus *Cripavirus* are:

1) The predicted tertiary structure of the IRES from the intergenic region of these two viruses is quite different from that of all the viruses currently assigned to *Cripavirus* (Figure 1).
2) Phenetic analysis of the coding regions of the three major capsid proteins (VP2, VP3 and VP1) all indicate that

Predicted Tertiary Structure of the Intergenic IRES element.

Site directed mutagenesis and *in vitro*-translation studies with several members of the family *Dicistroviridae* i.e. *Cricket paralysis virus* (CrPV), *Plautia stali intestine virus* (PSIV) and *Rhopalosiphum padi virus* (RhPV)

have shown that certain sequences in the 3' end of the intergenic region (IGR) of the viral genome are necessary for these sequences to function as internal ribosome entry sites (IRES).Tertiary structure models of these intergenic region sequences reveal the presence of a pseudoknot structure that incorporates the essential bases identified in the mutational analyses. The triplet immediately upstream of the codon directing the first amino acid of the structural polyprotein has an essential role in forming the pseudoknot structure and in determining where protein synthesis begins.
Most of the dicistroviruses characterised to date appear to have the pseudoknot structure within the IGR conserved, and empirical data for several viruses have shown that the structural polyprotein is indeed initiated with non-methionine residues. In the case of all the current members of *Cripavirus* the predicted structure of the IGR-IRES is highly conserved (as exemplified by Figure 1 A and B). However, in the case of TSV and ABPV the "bulge" between the predicted helices of Stem-loop III is replaced with a more ordered structure (shown in yellow in Figure 1 C and D) and there is also an additional side stem-loop in the 3' proximal stem (Stem-loop VI - shown in purple in Figure 1 C and D). These differences in structure form a major distinction between the viruses in *Cripavirus* and ABPV/TSV.

Phenetic analysis of Capsid Protein Coding Regions
Phenetic analysis of the capsid proteins of the current members of *Dicistroviridae* and the proposed new member (ALPV) shows that ABPV and TSV are the most divergent members of the family. Using VP2 – the most conserved of the three capsid proteins the relationships shown in Figure 2 are revealed. Bootstrap analysis indicates that the clade comprising CrPV, DCV, RhPV and ALPV is well supported by the data as that comprising HiPV, BQCV, PSIV and TrV. The data also supports the sister-group relationship of the above clades. These are the taxa that currently comprise the genus *Cripavirus* (the placement of ALPV in this genus as outlined in Proposal 1). However, the data show no clear relationship between ABPV and TSV and demonstrates that they basally diverge from the genus *Cripavirus*.

Similar analyses conducted with VP3 and VP1 show similar results – although the less conserved nature of these proteins provides less convincing support for the groupings outlined above. Nevertheless, all three analyses show that::

1) CrPV, DCV, RhPV and ALPV form a consistent clade
2) HiPV, BQCV, PSIV and TrV form a consistent clade – which is a sister group to the above clade
3) ABPV and TSV are the most divergent members of the family and show no clear relationship to each other

Summary
Based on the analyses of the predicted structure of the IGR-IRES and the phenetic relationships between family members the genus *Cripavirus* should comprise CrPV, DCV, RhPV, ALPV HiPV, BQCV, PSIV and TrV. ABPV and TSV are clearly members of *Dicistroviridae* on the basis of their biophysical properties and genomic arrangement and fulfil the criteria for inclusion as full species:

- Natural host range: species can be differentiated on the basis of their natural host range and their relative ability to replicate in a range of cultured insect cells.
- Serology: all species are serologically distinct.
- Sequence identity between the capsid proteins of isolates and strains of a species is above 90%.

While there is evidence to indicate that new genera could be established for these taxa there are some data that indicate that this would be premature. For instance, analysis of the non-structural polyprotein shows that this region of the genome shares affinities with CrPV and DCV (van Munster *et al.,* 2002). Therefore, the most parsimonious treatment of ABPV and TSV at this point in time is to include them as Unaligned Members of the Family until such time as further evidence becomes available to make a convincing case for their placement in the genus *Cripavirus* or the establishment of a new genus (genera) to accommodate them.

2.2 Taxonomic Implications

The structure of the family will be as shown below:

Official virus species names are in italics. Tentative virus species names , alternative names( ), strains, or serotypes are not italicized. Virus names, genome sequence accession numbers [ ], and assigned abbreviations ( ) are:

Genus: *Cripavirus*
Type Species: *Cricket Paralysis Virus*

Cricket paralysis virus	[AF218039]	(CrPV)
Aphid lethal paralysis virus	[AF536531]	(ALPV)
Black queen cell virus	[AF183905]	(BQCV)
Drosophila C virus	[AF014388]	(DCV)
Himetobi P virus	[AB017037]	(HiPV)
Plautia stali intestine virus	[AB006531]	(PSIV)
Rhopalosiphum padi virus	[AF022937]	(RhPV)
Triatoma virus	[AF178440]	(TrV)

Unassigned Species in the Family

Acute bee paralysis virus	[AF150629]	(ABPV)
Taura syndrome virus	[AF277675]	(TSV)

2.3 Derivation of proposed names

Names and abbreviations follow those widely recognised and in current use with workers in the field.

2.4 References

Kanamori,Y. and Nakashima,N. (2001) A tertiary structure model of the internal ribosome entry site (IRES) for methionine-independent initiation of translation. RNA. **7**; 266-274.

van Munster,M., Dullemans,A.M., Verbeek,M., van den Heuvel,J.F., Clerivet,A. and van der Wilk,F. (2002) Sequence analysis and genomic organization of Aphid lethal paralysis virus: a new member of the family *Dicistroviridae.* J. Gen. Virol. **83**; 3131-3138.

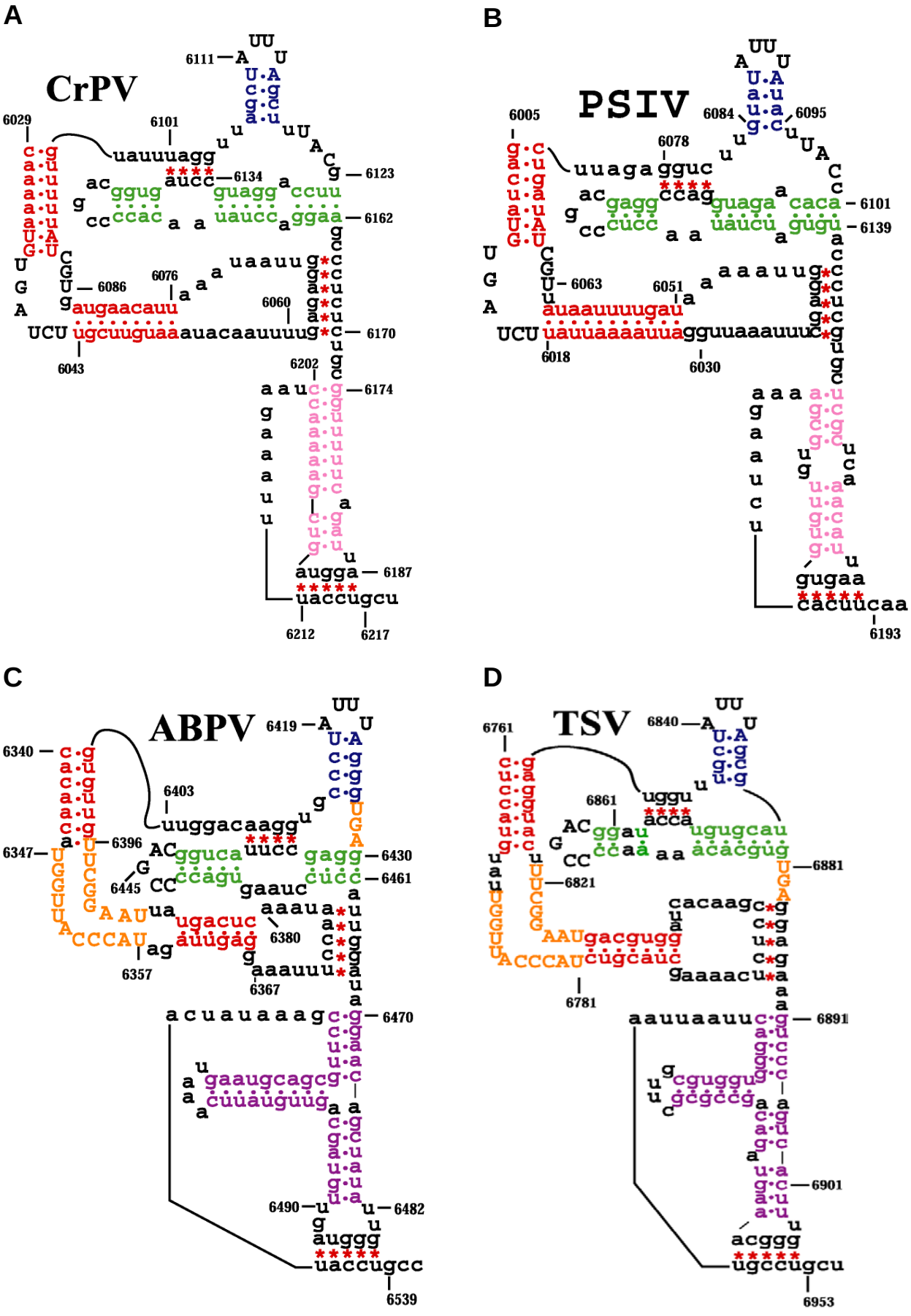

Figure 1.

Figure1. Predicted structures of the intergenic region (IGR) IRES elements of CrPV (A), PSIV (B), APBV (C) and TSV (D). The nomenclature for the stem-loops are as proposed by Kanamori and Nakashima (2001) (Red = Stem Loop III; Blue = Stem-Loop IV; Green = Stem-loop V; Pink = Stem-Loop VI in CrPV and PSIV and Purple = Stem-loop VI in ABPV and TSV). Red asterisks show base-pair interactions in putative pseudoknots and dots represent interactions in putative helical structures of the stem-loops

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Figure 2. Phenogram showing the relationships between current and proposed (ALPV – see Proposal 1) members of the *Dicistroviridae*. The phenogram was generated using amino-acid identities between the coding regions of the VP2 capsid protein and used the Neighbour-joining algorithm of the MEGA software package. The phenogram was generated using Infectious flacherie virus (IFV, Accession number AB000906) as an outgroup. Brach lengths are proportional to distances between sequences.