Template for Taxonomic Proposal to the ICTV Executive Committee To create a new Family

$\operatorname{Code}^{\dagger}$	2007.036P	To create a new family* in	nassigned			
$\operatorname{Code}^{\dagger}$	2007.037P	Γο name the new family* <i>Virgaviridae</i>				
$\operatorname{Code}^{\dagger}$	2007.038P	To designate the following genera as part of the new family*:				
		Furovirus Hordeivirus Pecluvirus Pomovirus Tobamovirus Tobravirus				

[†]Assigned by ICTV officers

^o Leave blank is not appropriate
* repeat these lines and the corresponding arguments for each genus created in the family

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Old Taxonomic Order

Order	Unassigned
Family	Unassigned
Genus	Furovirus, Hordeivirus, Pecluvirus, Pomovirus, Tobamovirus, Tobravirus

New Taxonomic Order

Order	Unassigned
Family	Virgaviridae
Genus	Furovirus, Hordeivirus, Pecluvirus, Pomovirus, Tobamovirus, Tobravirus

ICTV-EC comments and response of the SG

Argumentation to create a new family:

The new family groups together 6 currently unassigned genera of ssRNA plant viruses that are characterised by:

- 1. Single-stranded RNA + sense genomes with a 3'- t-RNA like structure and no polyA tail
- 2. Rod-shaped virions 20-25nm in diameter with a central "canal"
- 3. Alpha-like replication proteins that form a distinct phylogenetic "family" (Koonin & Dolja, 1993)
- 4. Coat proteins of 19-24kDa
- 5. Movement proteins that are either a single protein of the '30K' superfamily (Melcher, 2000) or from a distinct subset of TGBs (Morozov & Solvyev, 2003)

This includes all the rod-shaped plant viruses except those of the genus *Benyvirus* that are much more distantly related in phylogenetic analyses. The genera themselves are justified largely on the basis of genome organization (Fig. 1) and phylogenetic analysis and their major properties are summarized in Table 1.

Table 1. Major properties of the genera proposed for inclusion in the new family *Virgaviridae* (See also Fig. 1)

Genus	RNAs	RdRP ^a	MP ^b	CP ^c	3' structure ^d	Transmission
Furovirus	2	RT	'30K'	19K+RT	t-RNA ^{Val}	"fungus"
Hordeivirus	3	Separate	TGB	22K	t-RNA ^{Tyr}	seed
Pecluvirus	2	RT	TGB	23K	t-RNA ^{Val}	"fungus" + seed
Pomovirus	3	RT	TGB	20K+RT	t-RNA ^{Val}	"fungus"
Tobamovirus	1	RT	'30K'	17-18K	t-RNA ^{His}	mechanical
Tobravirus	2	RT	'30K'	22-24K	t-RNA ⁻	nematode

^a relation of RdRp to the replication protein (Methyltransferase, Helicase); RT, in a readthrough domain at the C-terminus

^b MP, movement protein either of the '30K' superfamily (Melcher, 2000) or a Triple gene block (TGB; Morozov & Solovyev, 2003)

^c CP, coat protein size (with indication of RT, a readthrough domain at the C-terminus if present) ^d t-RNA^{Val/Tyr/His/-}, t-RNA like structure accepting Valine, Tyrosine, Histidine or not aminoacylated respectively

The only genus with rod-shaped virions excluded from this list is *Benyvirus* because this is much more distantly related in phylogenetic analyses of the polymerase (see below) and because (unlike the others) its members have a polyadenylated genome, and the polymerase is processed by autocatalytic protease activity.

1. The replication protein

More than 10 years ago, Koonin & Dolja (1993) included all these viruses within RdRp Supergroup 3, which they sub-divided into 3 lineages that they suggested might correspond to Orders: Tymo (including tymoviruses and the genera that we now have in the family *Flexiviridae*), Rubi (including benyviruses, alphaviruses and others) and Tobamo (including the viruses in the 6 genera considered here together with *Closteroviridae*, *Bromoviridae* and *Idaeovirus*). Phylogenetic analysis of the RdRp domain, of the whole replication protein or of the fused Met-Hel-RdRp domains continues to support this grouping (see Fig. 2).

The replication proteins constitute the majority of the genomes of these viruses and provide the best phylogenetic trees but there are also indications of relatedness amongst the other genes (see below).

2. The movement proteins

(a) The '30K' proteins of *Furovirus*, *Tobamovirus* and *Tobravirus* are related but do not form a coherent subgroup of MPs as those of *Furovirus* are more closely related to those of *Dianthovirus* and *Bromoviridae* than to the other rod-shaped genera (Melcher, 2000) (Fig. 3).

(b) The Triple gene block (TGB) proteins of the genera *Hordeivirus*, *Pecluvirus* and *Pomovirus* are clearly related, while those from the genus *Benyvirus* are more distantly related. A tree for TGBp1 sequences is provided in Fig. 4. The only other viruses with a TGB module are from the filamentous viruses in the family *Flexiviridae*. The TGBs of the rod shaped viruses differ from these in having a larger TGBp1 and TGBp3 (the latter having two, rather than one, transmembrane domains) and form a distinct group in phylogenetic anlayses. For more details supporting this classification of the TGB proteins, see Morozov & Solovyev (2003).

3. The coat proteins

The small size of the coat protein and its inherent variability make it less suitable for phylogenetic analysis. Nevertheless, significant groupings of genera occur (Furo- with Pomo-; Peclu- with Hordei- and Tobra- a bit more distant) which correspond with those found within the RdRp (Fig. 5).

4. The small cysteine-rich proteins

There are close relationships between the small cysteine-rich proteins of *Furovirus*, *Hordeivirus*, *Pecluvirus* and *Tobravirus* (Fig. 6) although those of *Pomovirus* do not align well with them.

Origin of the proposed family name

From *virga* (Latin = rod) from the morphology of the virions.

References

Koonin, E.V. & Dolja, V.V. (1993). Evolution and taxonomy of positive-strand RNA viruses: implications of comparative analysis of amino acid sequences. *Crit Rev Biochem Mol Biol.*, 28, 375-430.

Melcher, U. (2000). The '30K' superfamily of viral movement proteins. J Gen Virol., 81, 257-266.
Morozov, S.Y. & Solovyev, A.G. (2003). Triple gene block: modular design of a multifunctional machine for plant virus movement. J Gen Virol., 84, 1351-1366.

Annex:



Figure 1. Diagram showing the genome organisation of the 6 genera proposed for inclusion in the family *Virgaviridae*. Replication proteins are shown in blue with the methyltransferase (M), helicase (H) and RdRp (R) domains. Coat proteins are in red, '30K'-type movement proteins in green, triple gene block movement protein modules in yellow and cysteine-rich proteins in grey. Other ORFs are shown in white and positions of "leaky" stop codons by triangles (\triangleright). t^{Val/Tyr/His/-}: t-RNA like structure accepting Valine, Tyrosine, Histidine or not aminoacylated respectively. Brackets indicate ORFs that are missing from some strains.



Figure 2. Phylogenetic (Neighbor-joining) tree of the amino acid sequences of the fused Met-Hel-RdRp domains of the members of the 6 genera proposed for inclusion in the family *Virgaviridae* (pale blue) together with other related viruses. Distantly related genera and families which formed well-supported monophyletic clades were collapsed into a triangle, the length of which corresponds to the variation found within the clade. Numbers on branches indicate percentage of bootstrap support out of 1000 bootstrap replications. Scales indicate JTT amino acid distances.



Figure 3. Phylogenetic (Neighbor-joining) tree of the amino acid sequences of the '30K' movement proteins of members of the genera (circled) proposed for inclusion in the family *Virgaviridae* together with other related viruses.



Figure 4. Phylogenetic (Neighbor-joining) tree of the amino acid sequences of the TGBp1 proteins of members of the genera proposed for inclusion in the family *Virgaviridae* together with other related viruses.



Figure 5. Phylogenetic (Neighbor-joining) tree of the amino acid sequences of the coat proteins of members of the genera proposed for inclusion in the family *Virgaviridae* together with other related viruses.



Figure 6. Phylogenetic (Neighbor-joining) tree of the amino acid sequences of the cysteine-rich proteins of members of four genera proposed for inclusion in the family *Virgaviridae*.