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VIROLOGY DIVISION NEWS

Virgaviridae: a new family of rod-shaped plant viruses

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Abstract The new plant virus family *Virgaviridae* is described. The family is named because its members have rod-shaped virions (from the Latin *virga* = rod), and it includes the genera *Furovirus, Hordeivirus, Pecluvirus, Pomovirus, Tobamovirus* and *Tobravirus*. The chief characteristics of members of the family are presented with phylogenetic analyses of selected genes to support the creation of the family. Species demarcation criteria within the genera are examined and discussed.

The International Committee on Taxonomy of Viruses (ICTV) has recently approved a proposal to create a plant virus family *Virgaviridae*. The family is named because its members have rod-shaped virions (from the Latin *virga* = rod), and it includes the genera *Furovirus, Hordeivirus, Pecluvirus, Pomovirus, Tobamovirus* and *Tobravirus*. The chief characteristics of members of the family are:

1. Alpha-like replication proteins that form a distinct phylogenetic "family" [5].
2. Single-stranded RNA + sense genomes with a 3′-t-RNA-like structure and no polyA tail.
3. Rod-shaped virions 20–25 nm in diameter with a central "canal".
4. Coat proteins of 19–24 kDa.

It contains some viruses in which there is a single cell-to-cell movement protein (MP) of the '30K' superfamily [7] and others that encode a triple gene block (TGB) [8]. There are also differences in the number of genomic RNAs (1, 2 or 3 depending on the genus), but sequence analysis of the polymerase and other genes suggests that the viruses form a coherent taxonomic unit (see below). Some properties of the six genera included in the family are summarized in Table 1, and their genome organization is shown in Fig. 1. Biologically, the viruses are fairly diverse. They have been reported from a wide range of herbaceous and mono- and dicotyledonous plant species, but the host range of individual members is usually limited. All members can be transmitted experimentally by mechanical inoculation, and for those in the genus *Tobamovirus*, this is the only known means of transmission. In some genera, transmission is by soil-borne vectors, while members of the genus *Hordeivirus* are transmitted through pollen and seed. 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 a polymerase that is processed by autocatalytic protease activity.

On the basis of their analysis of the RNA-dependent RNA polymerase (RdRp) gene from a wide range of viruses, Koonin and Dolja [5] included viruses from the six genera described in this paper within RdRp Supergroup 3, which they sub-divided into three lineages that they suggested might correspond to orders. One of these lineages, which they named Tobamo, included the six genera

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Table 1 Major properties of the genera included in the new family *Virgaviridae*

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 [7] or a Triple gene block (TGB [8])

[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

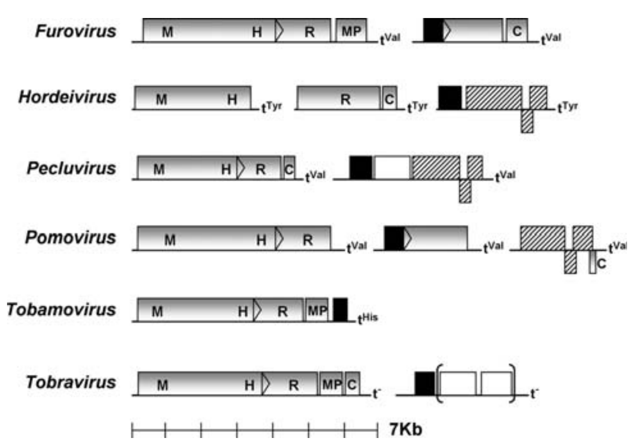

Fig. 1 *Diagram* showing the genome organization of the six genera included in the family *Virgaviridae*. *Domains* marked in the replication proteins are Methyltransferase (M), Helicase (H) and RNA-dependent RNA polymerase (R). Triple gene block proteins (TGB) are cross-hatched, and coat proteins are in *black*. MP, movement protein of the '30K' superfamily; C, cysteine-rich protein. Positions of "leaky" stop codons are shown by *triangles* (*filled triangles*). 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

considered here, together with the families *Closteroviridae* and *Bromoviridae* and the genus *Idaeovirus*. Phylogenetic analysis (using several different methods) of the RdRp domain, of the whole replication protein or of the fused Methyl transferase–Helicase–RdRp domains continues to support this grouping and shows that the genus *Benyvirus* is much too distantly related to be grouped in this family (see Fig. 2). The inclusion within the branch of the families *Closteroviridae* and *Bromoviridae* also justifies the inclusion of all six genera within the single family *Virgaviridae.* 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. For example, the TGB proteins of the genera *Hordeivirus*, *Pecluvirus* and *Pomovirus* are clearly related and form a distinct group separate from those of the genus *Benyvirus* and the filamentous viruses in the family *Flexiviridae* (recently split into two families). A tree for TGBp1 sequences is provided in Fig. 3, and more details supporting this classification of the TGB proteins are provided by Morozov and Solovyev [8]. 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. 4). There are also close relationships between the small cysteine-rich proteins of *Furovirus*, *Hordeivirus*, *Pecluvirus* and *Tobravirus*, although those of *Pomovirus* do not align well with them (data not shown).

The taxonomic structure of the new family and the species currently included are listed in Table 2.

Sequence differences between and within the existing species in the family were examined and compared with molecular criteria for species discrimination provided by the relevant study groups in the 8th ICTV report [2]. Individual pairwise comparisons were therefore made using the nt and aa sequences of each fully sequenced gene from every available accession in the family *Virgaviridae* contained in the international databases. Comparisons used the GCG [1] program GAP (with a gap creation penalty of 50 and a gap extension penalty of 3 for nt comparisons and values of 8 and 2, respectively, for aa comparisons). This program aligns the two sequences selected and calculates the percentage identity and similarity between them. To assist with the large numbers of comparisons, software was written (Antoniw, unpublished) to generate batch files that were run in GCG and also to extract and summarize data from the output files. Some of the chief features of the data for the replication protein, the RdRp and the coat protein

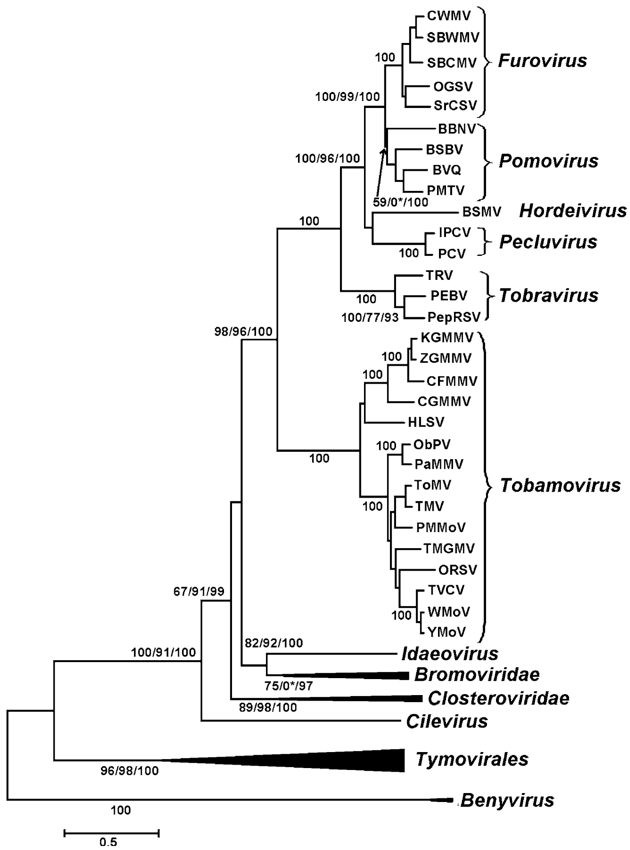

Fig. 2 Phylogenetic tree of the amino acid sequences of the fused Met–Hel–RdRp domains of the members of the six genera included in the family *Virgaviridae* together with some other related viruses. Distantly related genera and families that formed well-supported monophyletic clades were collapsed into a triangle, the length of which corresponds to the variation found within the clade. The recently established order *Tymovirales* includes the families *Tymoviridae* and *Flexiviridae* (which has also been divided). The neighbour-joining (NJ) tree is shown, but nearly identical trees were produced from the alignment using Maximum Composite Likelihood (ML) and Bayesian tree building algorithms. Percentage bootstrap support (out of 1,000 replications) for NJ and ML trees and posterior probability for the Bayesian tree are, respectively, indicated on the corresponding *branches* separated by *slashes* if they differed from each other. Values are only indicated on the *major branches* when >60%, and when values were identical, only one number is indicated (*asterisk*). The consensus tree generated by ML did not support the inclusion of BBNV into a *Pomovirus* clade and grouped the genus *Idaeovirus* within the *Bromoviridae* clade. The scale indicates JTT amino acid distances. Alignments were made from translated nucleotide sequences using the ClustalW algorithm in the Alignment Explorer module of MEGA4 [9] as described previously [6]. A total of 500 amino acid positions corresponding to 1,500 nt positions were used for the alignment. NJ and ML trees were generated using standard settings for these algorithms in MEGA4 [9] from protein and back-translated nucleotide alignments, respectively. The Bayesian tree was generated from back-translated nucleotide alignment using MrBayes v3.1.2 [4], employing the general time reversible model with gamma-shaped rate variation with a proportion of invariable sites; 1,000,000 generations of MCMC analysis were the point at which the average standard generation of split frequency between two parallel runs had reached 0.009565

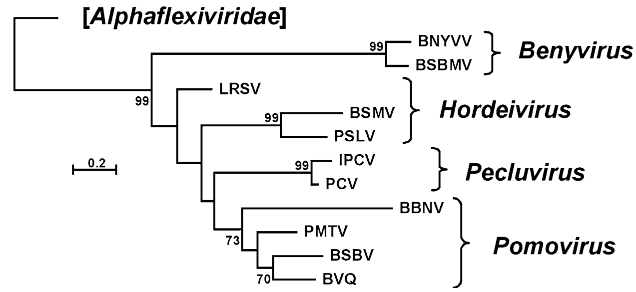

Fig. 3 Phylogenetic (neighbor-joining) tree of the amino acid sequences of the TGBp1 proteins of members of the genera included in the family *Virgaviridae* together with other TGB-containing viruses. *Numbers on branches* indicate percentage of bootstrap support out of 1,000 bootstrap replications (when >60%). The *scale* indicates JTT amino acid distances. Tree produced in MEGA4 [9]. A tree of similar typology was obtained by maximum-likelihood analysis (PROML in PHYLIP [3])

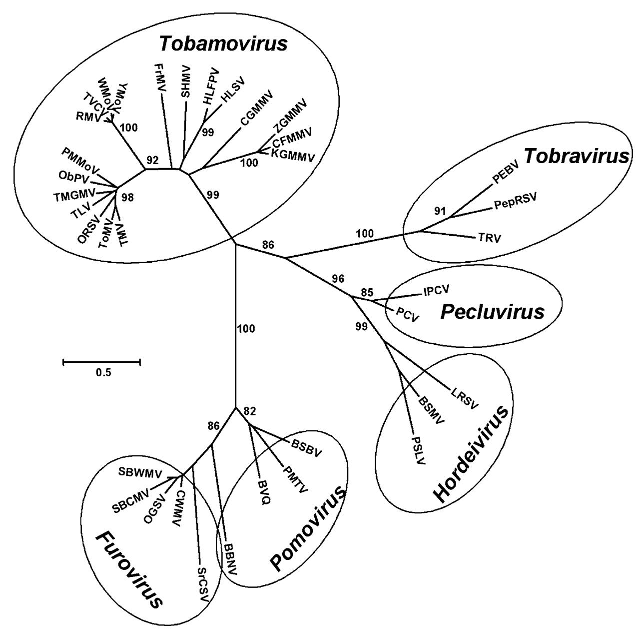

Fig. 4 Phylogenetic (neighbor-joining) tree of the amino acid sequences of the coat proteins of members of the genera included in the family *Virgaviridae*. *Numbers* on major branches indicate percentage of bootstrap support out of 1,000 bootstrap replications (when >60%). The *scale* indicates JTT amino acid distances. Tree produced in MEGA4 [9]. A tree of similar typology was obtained by maximum-likelihood analysis (PROML in PHYLIP [3])

genes are summarized in Table 3. Within some genera, there are rather few species and sequences, but some conclusions may nevertheless be reached. For the genus *Tobravirus*, it is already known that coat protein sequences (from RNA2) are of little taxonomic value [2], and this appears also to be the case for the genus *Pecluvirus*. Within the replication protein and RdRp, isolates of the same species usually had >90% nt or aa sequence identity. Comparisons between genera show that some existing

Table 2 List of species recognised within the genera belonging to the new family *Virgaviridae* with accession numbers for complete genome nucleotide sequences

Species	Abbreviation	Isolate genome sequence(s)
Genus *Furovirus*		
Chinese wheat mosaic virus	CWMV	AJ012005 + AJ012006 (NC_002359 + NC_002356); AJ271838 + AJ271839; AB299271 + AB299272
Oat golden stripe virus	OGSV	AJ132578 + AJ132579 (NC_002358 + NC_002357)
Soil-borne cereal mosaic virus	SBCMV	AJ132576 + AJ132577 (NC_002351 + NC_002330); AF146278 + AF146282; AJ252151 + AJ252152
Soil-borne wheat mosaic virus[e]	SBWMV	L07937 + L07938 (NC_002041 + NC_002042); AB033689 + AB033690[a]
Sorghum chlorotic spot virus	SrCSV	AB033691 + AB033692 (NC_004014 + NC_004015)
Genus *Hordeivirus*		
Anthoxanthum latent blanching virus	ALBV	No sequences available
Barley stripe mosaic virus[e]	BSMV	J04342 + X03854 + M16576 (NC_003469 + NC_003481 + NC_003478); U35768 + U35772 + U13918; U35766 + U35769 + U13916; U35767 + U35770 + U13917; AY789693 + AY789694 + AY787207
Lychnis ringspot virus	LRSV	No complete genome sequences available
Poa semilatent virus	PSLV	No complete genome sequences available
Genus *Pecluvirus*		
Indian peanut clump virus	IPCV	X99149 + AF447397 (NC_004729 + NC_004730)
Peanut clump virus[e]	PCV	L07269 + Z97873 (NC_003668 + NC_003520)
Genus *Pomovirus*		
Beet soil-borne virus	BSBV	Z97873 + U64512 + Z66493 (NC_003520 + NC_003518 + NC_003519); EF545138 + EF545140 + EF545142; EF545139 + EF545141 + EF545143; FJ971717 + FJ971718 + FJ971719
Beet virus Q	BVQ	AJ223596 + AJ223597 + AJ223598 (NC_003510 + NC_003511 + NC_003512)
Broad bean necrosis virus	BBNV	D86636 + D86637 + D86638 (NC_004423 + NC_004424 + NC_004425)
Potato mop-top virus[e]	PMTV	AJ238607 + AJ243719 + AJ277556 (NC_003723 + NC_003724 + NC_003725)
Genus *Tobamovirus*		
Brugmansia mild mottle virus	BruMMV	AM398436 (NC_010944)
Cucumber fruit mottle mosaic virus	CFMMV	AF321057 (NC_002633)
Cucumber green mottle mosaic virus	CGMMV	D12505 (NC_001801); AB015146; AF417242; AF417243; EF611826; AB369274; EU352259
Frangipani mosaic virus	FrMV	No complete genome sequences available
Hibiscus latent Fort Pierce virus	HLFPV	No complete sequence but FJ196834,AY596456 and AY250831 provide the coding sequences]
Hibiscus latent Singapore virus	HLSV	AF395898 (NC_008310)
Kyuri green mottle mosaic virus	KGMMV	AJ295948 (NC_003610); AB015145; AB162006
Obuda pepper virus	ObPV	D13438 (NC_003852); L11665
Odontoglossum ringspot virus	ORSV	X82130 (NC_001728); U34586; U89894; S83257; AY571290; DQ139262
Paprika mild mottle virus	PaMMV	AB089381 (NC_004106)
Pepper mild mottle virus	PMMoV	M81413 (NC_003630); AB000709; AJ308228; AB069853; AY859497; AB126003; AB113116; AB113117; AB254821; AB276030
Rehmannia mosaic virus	ReMV	EF375551 (NC_009041)
Ribgrass mosaic virus	RMV	No complete genome sequences available
Sammons's Opuntia virus	SOV	No sequences available
Streptocarpus flower break virus	SFBV	AM040955 (NC_008365)
Sunn-hemp mosaic virus	SHMV	An almost complete sequence is provided from a combination of U47034 and J02413
Tobacco latent virus	TLV	No complete genome sequences available
Tobacco mild green mosaic virus	TMGMV	M34077 (NC_001556); AB078435; DQ821941; EF469769
Tobacco mosaic virus[e]	TMV	V01408 (NC_001367); V01409; X68110; AF165190; AJ011933; D63809; AF273221; AF395127; AF395128; AF395129; AB369275; AB369276
Tomato mosaic virus	ToMV	AF332868 (NC_002692); AF155507; AJ243571; Z92909; X02144; AJ132845; AJ417701; AB083196; DQ873692
Turnip vein-clearing virus	TVCV	U03387 (NC_001873); Z29370

Table 2 continued

Species	Abbreviation	Isolate genome sequence(s)
Ullucus mild mottle virus	UMMV	No sequences available
Wasabi mottle virus	WMoV	AB017503 (NC_003355)[c]; AB017504
Youcai mosaic virus	YMoV	U30944 (NC_004422); AF254924 (NC_002792)[d]; D38444; AY318866; DQ223770; AB261175; EU571218
Zucchini green mottle mosaic virus	ZGMMV	AJ295949 (NC_003878); AJ252189
Genus *Tobravirus*		
Pea early browning virus	PEBV	X14006 + X51828 (NC_002036 + NC_001368)
Pepper ringspot virus	PepRSV	L23972 + X03241 (NC_003669 + NC_003670)
Tobacco rattle virus[e]	TRV	AF166084 + Z36974 (NC_003805 + NC_003811); AF034622 + AF034621

[a] Probably a different species

[b] There are sequences annotated as *Ribgrass mosaic virus*, but the definition of this species appears uncertain

[c] Annotated as crucifer tobamovirus wasabi strain

[d] Annotated as *Ribgrass mosaic virus* but seems to belong here while the definition of RMV appears uncertain

[e] Denotes type species

Table 3 Values from pairwise sequence comparisons for three genome regions amongst viruses in the family *Virgaviridae*

	Most distantly related isolates of same species		Most closely related species		Most distantly related species	
	aa	nt	aa	nt	aa	nt
Replication protein						
Furovirus	95.1	94.3	82.5	72.7	52.7	56.5
Hordeivirus	96.5	94.7	NA	NA	NA	NA
Pecluvirus	NA	NA	88.6	78.0	88.6	78.0
Pomovirus	99.7	99.6	54.9	59.2	45.3	54.9
Tobamovirus	94.4	86.8	93.8	83.6	39.3	49.4
Tobravirus	98.8	99.2	63.6	63.0	52.9	57.8
RdRp						
Furovirus	96.7	95.9	90.4	78.9	72.4	68.0
Hordeivirus	98.1	98.4	NA	NA	NA	NA
Pecluvirus	NA	NA	95.1	79.4	95.1	79.4
Pomovirus	99.2	99.4	76.6	70.3	65.0	64.2
Tobamovirus	95.4	87.2	96.2	86.0	52.8	57.1
Tobravirus	99.2	99.0	79.8	71.6	75.5	68.7
Coat protein						
Furovirus	92.0	86.2	95.5	94.2	43.2	49.1
Hordeivirus	98.0	97.7	55.6	60.1	40.8	48.1
Pecluvirus	40.6	50.1	66.5	64.8	36.5	45.8
Pomovirus	97.7	98.7	53.0	58.8	29.1	42.4
Tobamovirus	87.7	88.5	93.0	90.9	26.7	38.9
Tobravirus	38.6	45.2	89.2	77.5	36.2	48.6

Amino acid (aa) and nucleotide (nt) identities are provided for each genus, showing the most distantly related isolates of the same species and the minimum and maximum values for comparisons between different species. Criteria for species discrimination listed in the in 8th ICTV report [2] are also shown

Furovirus: less than about 75 or 80% nt identity on RNAs 1 and 2, respectively

Hordeivirus: no criteria provided

Pecluvirus: no molecular criteria provided

Pomovirus: less than about 80% identical over the whole sequence; less than about 90% identical in CP amino acid sequence

Tobamovirus: less than 10% overall nt sequence difference is considered to characterize strains of the same species

Tobravirus: nucleotide sequences of RNA-1 show <75% identity; RNA-2 sequences are of limited value species in the genus *Tobamovirus* are rather closely related and there may be merit in re-examining the species demarcation criteria within this genus.

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