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Phylogenetic justification for splitting the *Rymovirus* genus of the taxonomic family *Potyviridae*

Brief Report

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Summary. The *Potyviridae* family has been divided into four genera on the basis of vector transmission, as follows: *Potyvirus* genus (aphid), *Rymovirus* genus (mite), *Bymovirus* genus (fungus) and *Ipomovirus* genus (whitefly). However recent sequence comparisons of the coat protein and 3′ NCR regions of the potyviruses have demonstrated that the rymoviruses appear to be a group of two unrelated clusters namely Ryegrass Mosaic Virus (RGMV), Agropyron Mosaic Virus (AgMV) and Hordeum Mosaic Virus (HoMV) in one group and Wheat Streak Mosaic Virus (WSMV) and Brome Streak Mosaic Virus (BrSMV) in the second group. We therefore propose that RGMV, AgMV and HoMV remain in the genus *Rymovirus* and WSMV and BrSMV form a separate genus, possibly the *Whestrevirus* genus.

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Viruses of the taxonomic family *Potyviridae* are numerous and diverse, infecting many plant species, both wild and cultivated, often having devastating effects [1]. Members of the group as it is currently constituted are described by Brunt [2]. The *Potyviridae* family has been divided into four genera, largely on the basis of vector transmission, as follows: *Potyvirus* genus (aphid-transmission), *Rymovirus* genus (mite-transmission), *Bymovirus* genus (fungal-transmission) and *Ipomovirus* genus (whitefly-transmission).

Potyviridae taxonomy has partially been clarified by recent systematic application of serological and physico-chemical techniques, and 3′ terminus coat protein and NTR sequence comparisons [1, 10, 14]. Genome sequencing has confirmed that accepted members of the *Potyvirus* genus share a common genomic organisation and that the genetic distances between their coat proteins are generally in agreement with previouxly observed properties, such as cross-protection and serological relationships [16, 17].

Although viruses belonging to the genera *Rymovirus*, *Bymovirus* and *Ipomovirus* share the described morphological and physico-chemical characteristics of the *Potyviridae*, there has been little sequence data for any of the other genera, and assignment of a virus to a genus has generally been by vector-transmission only. However, recently sequence data have become available for

Table 1. Virus nucleotide sequence sources and accession numbers

Virus	Source	Accession no.
1. Plum pox virus (PPV-NAT)	GenBank	M92280
2. Ornithogalum mosaic virus (OMV)	GenBank	D00615
3. Turnip mosaic virus (TuMV-Hu)	GenBank	L12396
4. Potato virus Y (PVY-N)	GenBank	M95491
5. Tobacco etch virus (TEV-HAT)	GenBank	M11458
6. Johnsongrass mosaic virus (JGMV-JG)	GenBank	L31350
7. Ryegrass mosaic virus (RGMV-SA)	GenBank	U27383
8. Agropyron mosaic virus (AgMV)	GenBank	U30616
9. Hordeum mosaic virus (HoMV)	GenBank	U30615
10. Brome streak mosaic virus	GenBank	Z48506
11. Wheat streak mosaic virus (WSMV)	7	
12. Barley yellow mosaic virus (BaYMV-GE)	5; 6	
13. Barley mild mosaic virus (BaMMV-GE)	GenBank	X74095
14. Sweet potato mild mottle virus (SPMMV)	3	

several members of the *Rymovirus* genus [3, 4, 7, 11, 13] and preliminary alignments between the *Potyviruses* and putative members of the *Rymovirus* genus have indicated that some rymoviruses share more sequence similarity with *Potyviruses* than with other rymoviruses [3].

In this article we present the alignment of the coat protein and 3′-terminal non-coding region (3′NCR) nucleotide and amino acid sequences of several putative rymoviruses with other *Potyviridae* in order to determine the phylogenetic relationships between members and to ascertain whether division of this family into the current genera is justifiable.

Nucleotide sequence data were obtained from the EMBL or GenBank databases at the NCBI or from the literature. Accession numbers and literature references of the viruses used in this study are given in Table 1. Predicted amino acid sequences were compiled using Genepro version 4 software (Riverside Scientific) or the GCG set of programs (Genetics Computer Group). Multiple alignments of the amino acid sequence data using pairwise distance measurements were carried out using Clustal W Multiple Sequence Alignments [14]. Gaps were introduced for maximum alignment and weighting in protein matrices was by default. Neighbour-joining trees [12] were produced from Clustal W treefile output using TreeView version 1.2 [8], with BaYMV and BaMMV as outgroups. Runs were performed with gaps taken into account, and ignored.

Phylogenetic analysis of the rymovirus sequences aligned with generic *Rymo-*, *Bymo-*, *Ipomo-* and *Potyvirus* sequences and construction of a tree (Fig. 1) revealed that rymoviruses ryegrass mosaic virus strains (RGMV-SA and RGMV-DK), *Agropyron* mosaic virus (AgMV) and *Hordeum* mosaic virus (HoMV) cluster separate from a well defined cluster of generic *Potyviruses*. Rymoviruses wheat streak mosaic virus (WSMV) and brome streak mosaic virus (BrSMV) however cluster together on a branch basal to the monophyletic cluster incorporating the RGMV and *Potyvirus* clusters. Bymoviruses barley yellow mosaic virus (BaYMV) and barley mild mosaic virus (BaMMV) cluster together separate from both the generic *Rymo-* and *Potyviruses* and *Ipomovirus* sweet potato mild mottle virus (SPMMV) forms a separate branch altogether. The RGMV/AgMV/HoMV cluster are as distinct from one another as are distinct members of the genus *Potyvirus*; the *Rymo-* and *Potyvirus* groupings are also not obviously basal to one another and are obviously more closely related than any are to the WSMV/BrSMV cluster or to generic bymoviruses. It is worth noting that sequence distances between poty- and RGMV-like viruses are smaller than between these viruses and the WSMV group or the bymoviruses, or even than between WSMV and BrSMV within the WSMV cluster, or BaMMV and BaYMV in the bymoviruses. The fact that the newly sequenced putative "Ipomovirus" SPMMV clusters more strongly with the rymoviruses WSMV and BrSMV than these do with the RGMV/AgMV/HoMV cluster (83% of the time in bootstrap analyses) and that SPMMV branches between the WSMV and BrSMV clusters lends even more weight to the need for taxonomic revision of the *Rymovirus* genus, emphasizing the probable dual origins of the genus.

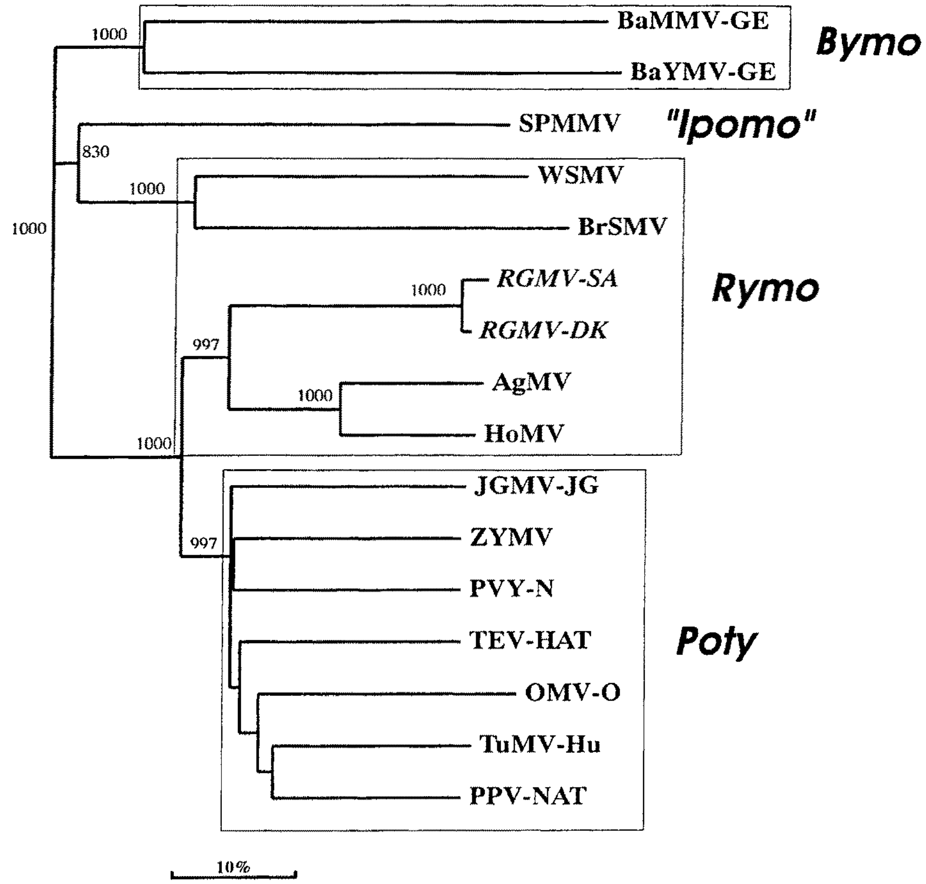

Fig. 1. Neighbour-joining relationship dendrogram for partial polyproteins of selected *Potyviridae.* The predicted partial polyprotein sequence of RGMV-SA (part of the putative NIb and the complete coat protein) was aligned with the cognate sequences of other *Potyviridae* using Clustal W Vers. 1.5, then a relationship dendrogram was calculated with bootstrapping (1000 replicates) using the neighbour-joining option in Clustal W with alignment gaps ignored for comparison purposes. The dendrogram was rooted using the *Bymovirus* sequences BaYMV and BaMMV. All horizontal distances are proportional to sequence differences (see inset scale); vertical distances are arbitrary. Numbers adjacent to nodes are bootstrap scores (out of 1000 replicates). Blocks indicate presently-accepted taxons: "*Ipomo*" is the proposed generic name for SPMMV and related viruses [3]

Review of the available data on the rymoviruses and *Potyviruses* reveals several interesting points. Firstly, RGMV and AgMV are transmitted by the mite *Abacarus hystrix,* while WSMV and BrSMV are transmitted by the mite *Aceria tulipae.* Secondly, while the RGMV cluster all exhibit a number of conserved motifs typical of the *Potyviruses* such as the MVWCIENG, LAPYAF and QKMAAAI motifs [1], these motifs are absent from the WSMV cluster. Thirdly, WSMV and BrSMV share 50% sequence similarity with each other but only 20–25% with other rymoviruses and *Potyviruses,* while AgMV and HoMV share nearly 70% sequence similarity with each other and about 40% similarity with RGMV strains. Lastly, serological studies have shown that RGMV, AgMV and HoMV all exhibit cross-reactivity with antisera to a number of generic *Potyviruses* such as TuMV [9] while BrSMV does not appear to be serologically related to other *Potyviruses* [9, 13] although WSMV has been shown to be related serologically to the potyvirus Johnsongrass mosaic virus (JGMV) [15].

The rymoviruses thus appear to be a group of two essentially unrelated clusters of viruses, sharing only the mode of mite transmission, which in itself is an inadequate classification criterion as the vectors of several putative rymoviruses such as oat necrotic mottle virus, HoMV and spartina mottle virus are as yet unknown and in addition, within the *Rymovirus* genus, two distinct mite genera are involved, as mentioned previously. We therefore propose that the classification be reviewed and make two suggestions. Firstly, RGMV, AgMV and HoMV could be classified as a mite-transmitted subgenus of the *Potyvirus* genus with aphid-transmitted *Potyviruses* forming a second subgenus and with WSMV and BrSMV retaining their status as generic rymoviruses. Alternatively a new genus could be formed to include RGMV, AgMV and HoMV, retaining the status of *Rymoviruses*, with WSMV and BrSMV forming a second genus, possibly the *Whestreviruses*. The latter solution is supported by sequence, serological and transmission evidence which seems to indicate that the RGMV cluster of viruses appear to be intermediates between the *Potyviruses* and the true rymoviruses. Additional sequence and transmission information on the other putative rymoviruses would be critical in deciding the future status of the rymoviruses.

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