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Phylogenetic Relationships Within the Family *Potyviridae*: Wheat Streak Mosaic Virus and Brome Streak Mosaic Virus Are Not Members of the Genus *Rymovirus*

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ABSTRACT

Stenger, D. C., Hall, J. S., Choi, I.-R., and French, R. 1998. Phylogenetic relationships within the family *Potyviridae*: Wheat streak mosaic virus and brome streak mosaic virus are not members of the genus *Rymovirus*. *Phytopathology* 88:782-787.

The complete nucleotide sequence of wheat streak mosaic virus (WSMV) has been determined based on complementary DNA clones derived from the 9,384-nucleotide (nt) RNA of the virus. The genome of WSMV has a 130-nt 5' leader and 149-nt 3'-untranslated region and is polyadenylated at the 3' end. WSMV RNA encodes a single polyprotein of 3,035 amino acid residues and has a deduced genome organization typical for a member of the family *Potyviridae* (5'-P1/HC-Pro/P3/6K1/CI/6K2/VPg-NIa/NIb/CP-3'). Because WSMV shares with ryegrass mosaic virus (RGMV) the biological property of transmission by eriophyid mites, WSMV has been assigned to

the genus *Rymovirus*, of which RGMV is the type species. Phylogenetic analyses were conducted with complete polyprotein or NIb protein sequences of 11 members of the family *Potyviridae*, including viruses of monocots or dicots and viruses transmitted by aphids, whiteflies, and mites. WSMV and the monocot-infecting, mite-transmitted brome streak mosaic virus (BrSMV) are sister taxa and share a most recent common ancestor with the whitefly-transmitted sweet potato mild mottle virus, the type species of the proposed genus "*Ipomovirus*." In contrast, RGMV shares a most recent common ancestor with aphid-transmitted species of the genus *Potyvirus*. These results indicate that WSMV and BrSMV should be classified within a new genus of the family *Potyviridae* and should not be considered species of the genus *Rymovirus*.

Additional keywords: proposed genus "*Tritimovirus*."

Wheat streak mosaic virus (WSMV) is an important pathogen of wheat (*Triticum aestivum* L.) that is transmitted by the eriophyid mite, *Aceria tosichella* Keifer (7). WSMV is currently classified as a member of the genus *Rymovirus* in the plant virus family *Potyviridae* (33,36). Viruses of the family *Potyviridae* have been classified into genera that coincide with vector taxa (33). The numerous and economically important aphid-transmitted viruses belong to the genus *Potyvirus* (the type species is potato virus Y) and are the most thoroughly characterized viruses of the family due to the numerous virus species for which complete sequences are available. During the past decade, it has become apparent that fungus-transmitted, bipartite viruses, such as barley yellow mosaic virus (BaYMV), share an evolutionary relationship with the aphid-transmitted potyviruses (20,23) and are members of the genus *Bymovirus* (the type species is BaYMV) within the family *Potyviridae*. The whitefly-transmitted sweet potato mild mottle virus (SPMMV) is clearly a member of the family *Potyviridae*, and the genus "*Ipomovirus*" has been proposed, with SPMMV as the sole species proposed (12). Thus, for these three genera of the family *Potyviridae*, sequence comparisons have validated species affiliations within genera that group virus species transmitted by the same vector taxa.

It is the eriophyid mite-transmitted viruses that have been problematic with respect to defining phylogenetic relationships within the family *Potyviridae*. Mite-transmitted species of the family *Potyviridae* include ryegrass mosaic virus (RGMV [29]), wheat streak mosaic virus (11,22), brome streak mosaic virus (BrSMV [17]), and Agropyron mosaic virus (AgMV [26]). The genus *Rymovirus* (the type species is RGMV) currently includes these four mite-transmitted species plus Hordeum mosaic virus (HoMV [26]), for which a vector has yet to be identified, and several other virus species that have been partially characterized (19,36). Recently, based on analysis of coat protein (CP) and, in some cases, partial NIb sequences (6,16,27,29), it has been suggested that the genus *Rymovirus* is not monophyletic and should be split into at least two genera in which RGMV, HoMV, and AgMV remain within the genus *Rymovirus*, whereas WSMV and BrSMV constitute a new genus within the family *Potyviridae* (27). However, in previous efforts, <25% of the viral genomes were analyzed. Thus, the relationships among members of the genus *Rymovirus* remain uncertain. In this paper, we report the complete nucleotide sequence of the Sidney 81 isolate of WSMV and extend the phylogenetic analysis of the genus *Rymovirus*, using as data sets complete polyprotein and mature NIb sequences for three species of the genus *Rymovirus* and eight additional species of the family *Potyviridae*.

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MATERIALS AND METHODS

Construction of WSMV cDNA clones. Virions of the Sidney 81 isolate of WSMV (8) were partially purified from wheat cv. Centurk by the minipurification method of Lane (21), except that the extraction buffer was 0.1 M K₂H/KH₂PO₄, pH 8.4, containing 1.4 M NaCl, 0.1% 2-mercaptoethanol, and 0.5% CHCl₃. WSMV RNA was recovered from virions by digestion with proteinase K in the presence of sodium dodecyl sulfate, followed by phenol/

chloroform extraction and ethanol precipitation. Polyadenylated viral RNA was purified further, using a PolyA Tract I mRNA isolation kit (Promega, Madison, WI). Complementary DNA (cDNA) was synthesized, using a Pharmacia Biotech (Piscataway, NJ) cDNA synthesis kit, with cloned moloney murine leukemia virus reverse transcriptase in the first-strand reaction and oligo dT or random hexamers as primers. Second-strand synthesis was accomplished with the Klenow fragment of DNA polymerase I. *EcoRI*/*NotI* adapters were ligated to the dsDNA products of the second-strand reaction and cloned into the *EcoRI* site of pACT2 (Clontech Laboratories, Palo Alto, CA). The cloned cDNA inserts initially were characterized by sequencing the termini, and selected inserts were subcloned into pGEM5Zf+ as *NotI* fragments. To obtain WSMV cDNA clones containing the 5' end of the viral RNA, cDNA was prepared with the 5'/3'-RACE kit (Boehringer Mannheim, Indianapolis, IN), and the amplified polymerase chain reaction product was cloned into the pGEM-T vector (Promega).

Sequencing of the WSMV genome. Four overlapping cDNA clones encompassing the complete genome of WSMV (Fig. 1) were selected and used to construct nested deletions, using the Erase-a-Base kit supplied by Promega. Nested deletions were obtained in both directions for each clone, and the deletion derivatives served as sequencing templates. The complete nucleotide sequence of WSMV RNA was obtained for both strands, with an average redundancy of 4.2 determinations/nucleotide (nt). The complete WSMV sequence was compiled by the University of Wisconsin-Madison GCG program.

Similarity and phylogenetic analyses. Pairwise alignments of complete polyprotein sequences of selected potyviral genomes were conducted by the PILEUP function of GCG, with alignment parameters set at default values of gap weight = 12 and gap length weight = 4. The distribution of amino acid (aa) residue identity of each virus relative to WSMV or RGMV was determined by pairwise alignments, using the Sequence Similarity Presenter program (15) with a window size of 10 aa residues and a window shift of 2 aa residues. Multiple alignments of complete polyprotein or NIb protein sequences of 11 viruses of the family *Potyviridae* (listed in Table 1) were obtained using the PILEUP function of GCG, with alignment parameters set at default values. Parsimony and neighbor-joining analyses of amino acid sequence data were performed by PAUP 4.0.0d60, provided by D. Swofford (Smithsonian Institution, Washington, DC). Inferred gaps in the multiple alignments were treated as missing data. The heuristic search option was used to find and retain all most parsimonious trees for bootstrap-re-

sampled data sets based on 1,000 replications. A distance matrix of amino acid sequences was generated and used to infer neighbor-joining trees for nonresampled data sets or bootstrap-resampled data sets (1,000 replications). For both types of phylogenetic analyses, RNA 1 of BaYMV was defined as the outgroup.

RESULTS

Genome organization of WSMV. The complete nucleotide sequence of WSMV RNA is 9,384 nt, excluding the variable-length polyadenylated tail, and has been deposited in GenBank as accession AF057533. The genome of WSMV has the features of a typical potyvirus and consists of a single large open reading frame encoding a polyprotein of 3,035 aa residues flanked at the termini by a 130-nt 5' leader and a 149-nt 3'-untranslated region (Fig. 1). The 5'-leader sequence is A-T rich and devoid of G residues at the extreme 5' end, as is common for other potyviruses for which 5'-leader sequences are known.

Examination of the WSMV polyprotein revealed potential viral-encoded proteinase cleavage sites (Table 2; Fig. 1), similar to those characterized for tobacco etch virus (TEV [9,10,14,31]), that would result in production of mature proteins (P1, HC-Pro, P3, 6K1, CI, 6K2, VPg-NIa, NIb, and CP) that also are encoded by other species of the genera *Rymovirus*, *Potyvirus*, and "*Ipomovirus*". Two motifs that are conserved among all potyviruses, the polymerase motif of the NIb protein and the nucleotide binding motif of the CI protein, occur in the WSMV polyprotein sequence at the expected map positions (Fig. 2). In addition, the WSMV protein sequence contains a conserved 'GYCYM' pentapeptide motif beginning at amino acid position 623 within the carboxy-terminal third of HC-Pro. A tyrosine residue at position 1843 of the peptide sequence occurs in the motif 'YGFDP,' matching exactly the context of the tyrosine, which forms the phosphoester linkage of TEV VPg to the 5' end of the viral RNA (28).

Similarity of potyviral polyproteins. Comparisons of polyprotein amino acid residue percent identity of WSMV and four other

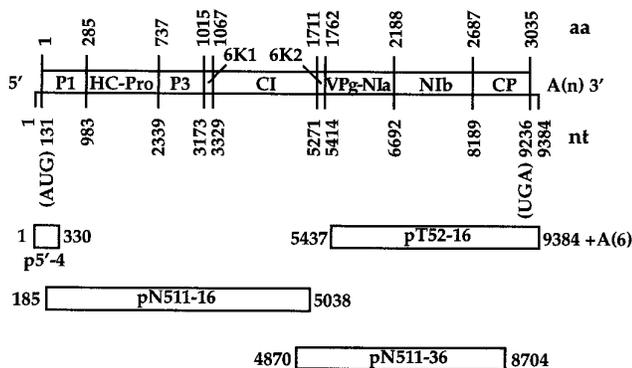


Fig. 1. Genome organization of wheat streak mosaic virus (WSMV). The map of the WSMV genome depicts the locations of polyprotein open reading frame start and stop codons, the amino acid (aa) and nucleotide (nt) coordinates of predicted proteinase cleavage sites delineating mature potyviral proteins (P1, HC-Pro, P3, 6K1, CI, 6K2, VPg-NIa, NIb, and CP), and the relative locations and the nucleotide coordinates of complementary DNA (cDNA) clones used to determine the complete nucleotide sequence of WSMV. pT52-16 was obtained by oligo dT priming of the first-strand cDNA reaction; pN511-16 and pN511-36 were obtained by random hexamer priming of first-strand synthesis; and p5'-4 was obtained by the 5'/3'-RACE procedure.

TABLE 1. Potyvirus sequences used in similarity and phylogenetic analyses

Virus species	Genus ^a	GenBank Accession	Reference
Brome streak mosaic virus	<i>Rymovirus</i>	Z48506	17
Barley yellow mosaic virus			
RNA 1	<i>Bymovirus</i>	X69757	23
Johnsongrass mosaic virus	<i>Potyvirus</i>	Z26920	18
Plum pox virus	<i>Potyvirus</i>	X16415	30
Potato virus Y	<i>Potyvirus</i>	X12456	24
Ryegrass mosaic virus	<i>Rymovirus</i>	Y09854	29
Sweet potato mild mottle virus	" <i>Ipomovirus</i> "	Z73124	12
Tobacco etch virus	<i>Potyvirus</i>	M15239	2
Tobacco vein mottling virus	<i>Potyvirus</i>	X04083	13
Wheat streak mosaic virus	<i>Rymovirus</i>	AF057533	This paper
Zucchini yellow mosaic virus	<i>Potyvirus</i>	L31350	34

^a Current taxonomic status. Quotes indicate proposed genus.

TABLE 2. Predicted proteinase cleavage sites in wheat streak mosaic virus polyprotein

Proteinase	Peptide junction	Amino acid sequence ^a
P1	P1/HC-Pro	GFITTY_S
HC-Pro	HC-Pro/P3	KDYKIG_G
NIa	P3/6K1	ELVEYQ_G ^b
NIa	6K1/CI	FNCEYQ_G ^b
NIa	CI/6K2	SHVSYQ_A ^b
NIa	6K2/VPg-NIa	RSVKFE_G ^b
NIa	VPg-NIa/NIb	DLVSYQ_S ^b
NIa	NIb/CP	QYCVYE_S ^b

^a Underlined gap denotes peptide bond cleavage position.

^b Amino acid residue 3 is hydrophobic, amino acid residue 5 is aromatic, and amino acid residue 6 is Q or E.

members of the family *Potyviridae* (Fig. 2A) clearly indicate that WSMV is a distinct virus species. The WSMV polyprotein was most similar to that of BrSMV, although extensive regions of the polyprotein have diverged even between these two mite-transmitted species. Interestingly, the WSMV polyprotein is no more similar to RGMV than it is to TEV. The polyprotein of RGMV is more similar to the polyprotein of TEV than to other potyviral polyproteins (Fig. 2B).

Based on the percent identity plots presented in Figure 2, it is apparent that certain regions of the potyviral polyprotein have diverged preferentially. Nib represents the most conserved region of the viral polyprotein, whereas the P1 region is the most divergent. Both observations agree with literature comparisons of potyviral sequence identities (1,17,32,35). Among more closely related taxa (e.g., WSMV and BrSMV or RGMV and TEV), discontinuous clusters of high sequence identity (<70%) occur in various regions of the polyprotein outside the Nib domain. In particular, WSMV and BrSMV share clusters of high sequence identity within the CI protein, the carboxy-terminal portion of HC-Pro, and the amino-terminal portion of P3 (Fig. 2A). RGMV and TEV also

share clusters of high sequence identity within the CI, VPg-NIa, and CP regions (Fig. 2B). In contrast, RGMV and TEV share only three short stretches of high sequence identity in HC-Pro and none in P3.

The SPMMV polyprotein sequence is ≈28% identical to that of WSMV overall, with higher identities in the 6K1, CI, 6K2, VPg-NIa, Nib, and CP regions and lower identities in P1, HC-Pro, and P3. However, residues 47 to 129 of WSMV P1 are 27 and 32% identical to P1 of SPMMV and BrSMV, respectively, and show no similarity to other potyviral polyproteins. Other notable sequence identities also are found in the amino-terminal portion of HC-Pro. The first 82 aa residues of the WSMV HC-Pro, corresponding to the region required for the aphid-transmission function of HC-Pro in the genus *Potyvirus* (3,4), share no similarity with TEV or RGMV but are 37 and 32% identical to the corresponding HC-Pro regions of SPMMV and BrSMV, respectively.

Phylogenetic analyses of the family *Potyviridae*. Trees depicting the phylogenetic relationships among species of the family *Potyviridae* based on complete polyprotein (Fig. 3) or Nib protein (Fig. 4) sequences, using neighbor-joining or parsimony methods, consistently placed WSMV and BrSMV as sister taxa. For all

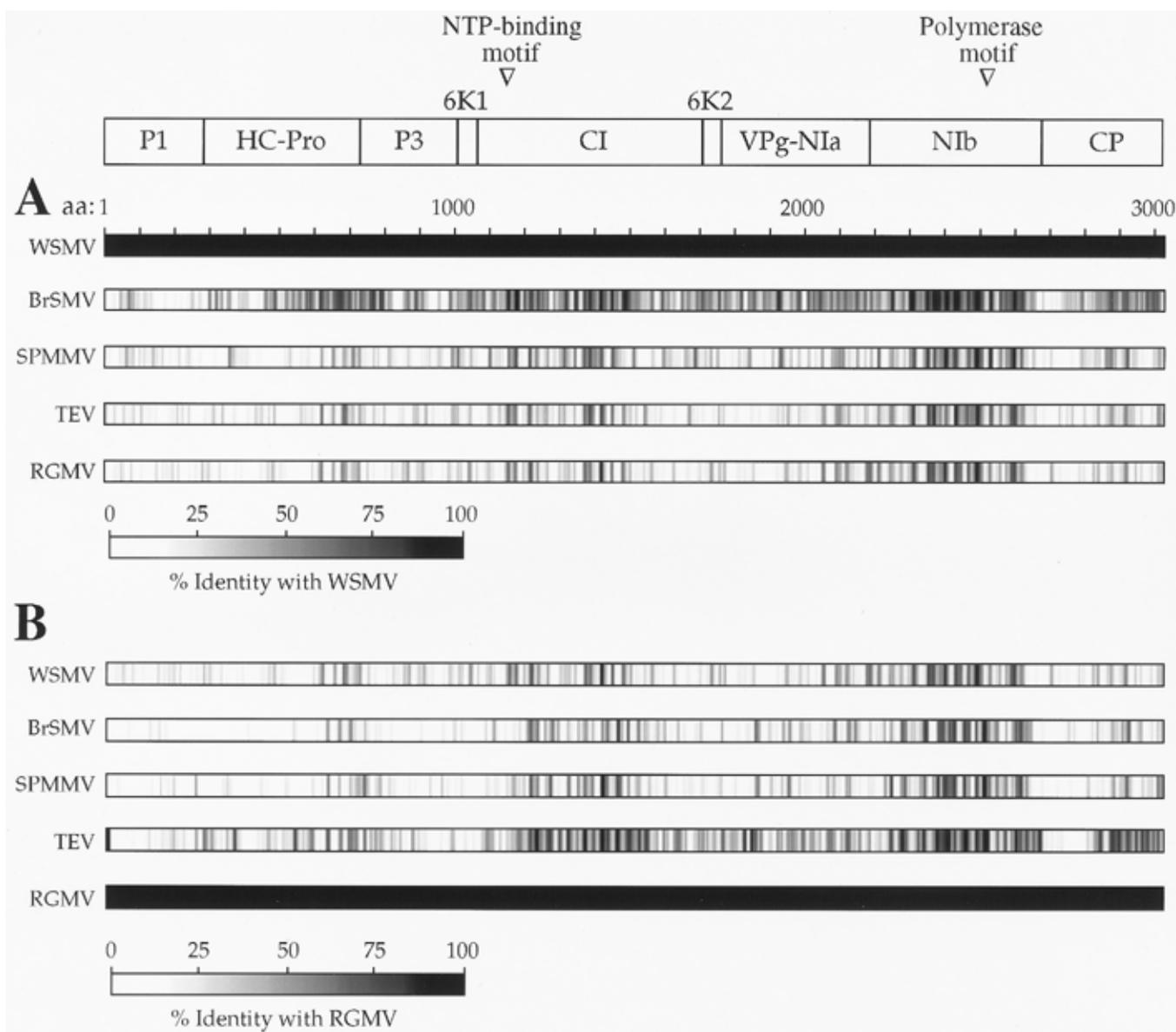


Fig. 2. Similarity of potyviral polyproteins. The plots depict the distribution of amino acid (aa) percent identity of selected potyviral polyproteins with **A**, wheat streak mosaic virus (WSMV), or **B**, ryegrass mosaic virus (RGMV), as determined based on the Sequence Similarity Presenter software program (15), with a window size of 10 aa residues, a window shift of 2 aa residues, and a minimum identity threshold value of 15%. The locations of mature potyviral proteins predicted after proteinase cleavage are indicated at the top, as are the locations of two motifs conserved in all species of the family *Potyviridae* for which nucleotide sequences are available. BrSMV = brome streak mosaic virus; SPMMV = sweet potato mild mottle virus; and TEV = tobacco etch virus.

trees, regardless of the type of analysis performed, RGMV shared a most recent common ancestor with a clade containing all aphid-transmitted species of the genus *Potyvirus*. The specific arrangement of taxa within a clade containing all dicot-infecting species of the genus *Potyvirus* varied depending on the specific data set and analytical method. Nonetheless, all of the dicot-infecting species of the genus *Potyvirus* cluster in a single clade that shared a most recent common ancestor with the monocot-infecting species Johnsongrass mosaic virus.

In all but one of the trees, the separate clades containing RGMV or only WSMV and BrSMV did not share a common node above the base of the trees. In the lone exception, the bootstrap-resampled neighbor-joining tree based on NIB protein sequences (Fig. 4) resulted in a tree topology that grouped the WSMV-BrSMV clade with the clade containing RGMV at the first node above the

base of the tree. However, support for this node was weak (51% of the trees examined by bootstrapping) and barely above the threshold to appear in a 50% majority-rule consensus tree. If this node were collapsed to yield a polytomy, the basal topology of the neighbor-joining bootstrap-resampled NIB tree would be identical to that obtained by parsimony methods performed on the same data set.

Neighbor-joining trees based on nonresampled data sets (Figs. 3 and 4), in which mean character distances among taxa were depicted by horizontal branch lengths, indicated considerable divergence among species of the family *Potyviridae*. Thus, the extent of divergence between the sister taxa BrSMV and WSMV was not unusual and was similar to that observed in the neighbor-joining analyses of nonresampled data sets for TEV and tobacco vein mottling virus, which clustered as sister taxa within the genus

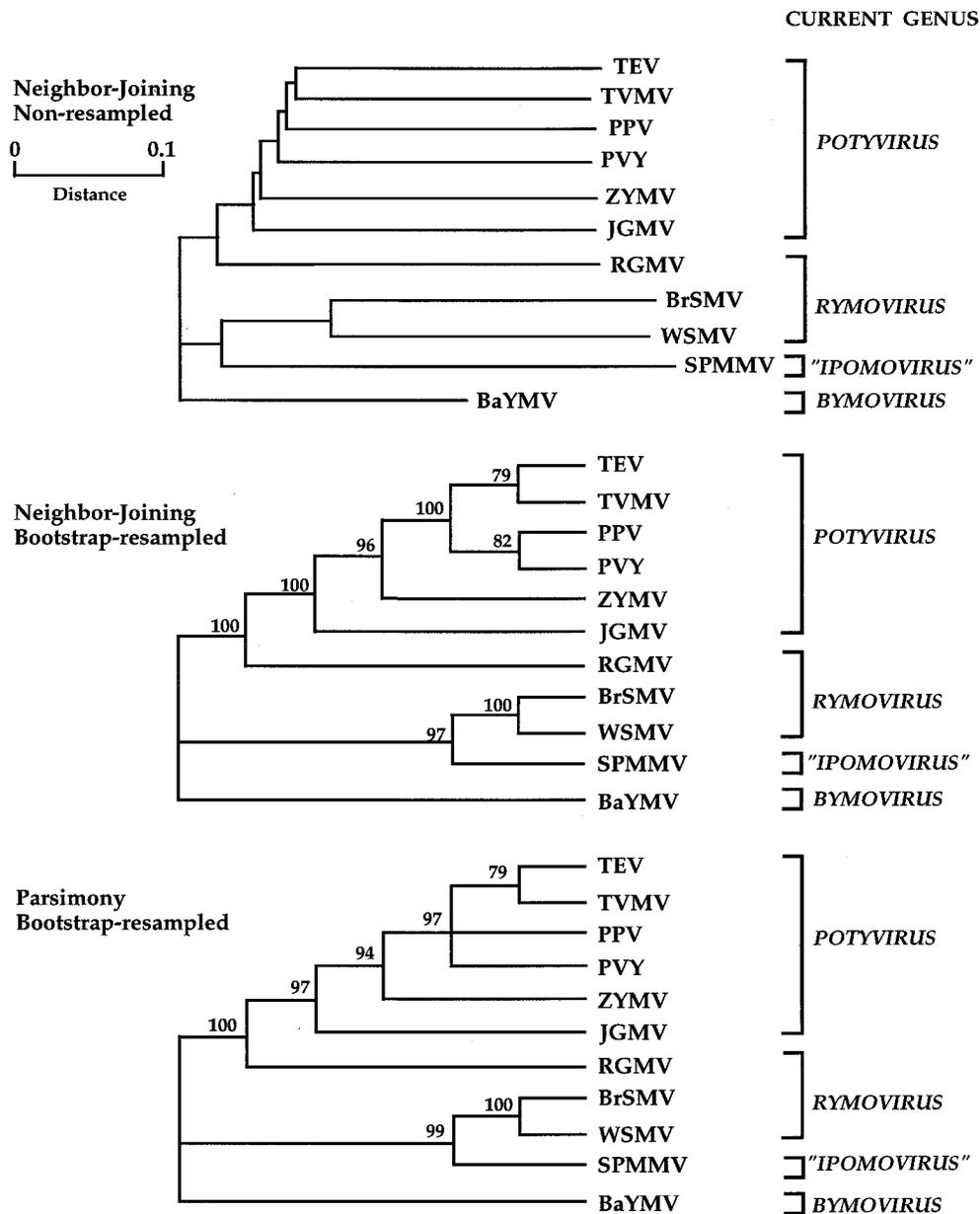


Fig. 3. Phylogenetic relationships among 11 species of the family *Potyviridae*, based on multiple alignment of complete polyprotein sequences. Trees were obtained by the neighbor-joining or parsimony methods, with barley yellow mosaic virus (BaYMV) RNA 1 defined as the outgroup. Horizontal branch lengths for the neighbor-joining tree, derived from the nonresampled data set, correspond to mean character distances between taxa; vertical lengths are arbitrary. Trees derived from bootstrap-resampled data sets (1,000 replications) are based on a 50% majority-rule consensus, in which only tree topology is relevant; both vertical and horizontal branch lengths are arbitrary. Bootstrap percentages of clades are shown (when >50%) along internal branches of trees derived from bootstrap-resampled data sets. TEV = tobacco etch virus; TVMV = tobacco vein mottling virus; PPV = plum pox virus; PVY = potato virus Y; ZYMV = zucchini yellow mosaic virus; JGMV = Johnsongrass mosaic virus; RGMV = ryegrass mosaic virus; BrSMV = brome streak mosaic virus; WSMV = wheat streak mosaic virus; and SPMMV = sweet potato mild mottle virus.

Potyvirus. Comparison of branch lengths obtained for SPMMV, using the neighbor-joining analyses (nonresampled data sets) of complete polyprotein or NIB protein sequences, indicated that SPMMV is no more similar to WSMV and BrSMV than RGMV is to any member of the genus *Potyvirus*.

DISCUSSION

Previous phylogenetic analyses (6,16,27,29) of the genus *Rymovirus* used CP or partial NIB sequences to reconstruct evolutionary history. In these studies, AgMV and HoMV formed a clade with RGMV, whereas WSMV and BrSMV clustered in a separate clade. Based on phylogenetic analyses of full-length polyprotein or NIB protein sequences presented in the current study, it is clear that the genus *Rymovirus*, as presently defined, is not monophyletic. Although the neighbor-joining and parsimony methods utilize differ-

ent assumptions to estimate phylogenetic relationships among taxa, both methods produced trees that place WSMV and BrSMV as sister taxa that do not share a most recent common ancestor with the type species of the genus *Rymovirus* (RGMV). Thus, evolution of the mite-transmission trait within the family *Potyviridae* is paraphyletic. How the capacity for mite-transmission arose within two lineages of the family *Potyviridae* remains an unanswered question, but we noted that transmission by eriophyid mites is not unique to potyviruses; several potex- and carla-like viruses are vectored by eriophyid mites (25). A similar situation exists within the aphid-transmitted viruses of the family *Potyviridae*. Recently, analysis of 3'-terminal sequences of narcissus latent and Maclura mosaic viruses indicate that these two aphid-transmitted viruses are more closely related to members of the genus *Bymovirus* than they are to members of the genus *Potyvirus*, as a result the genus "*Macluravirus*" has been proposed (5).

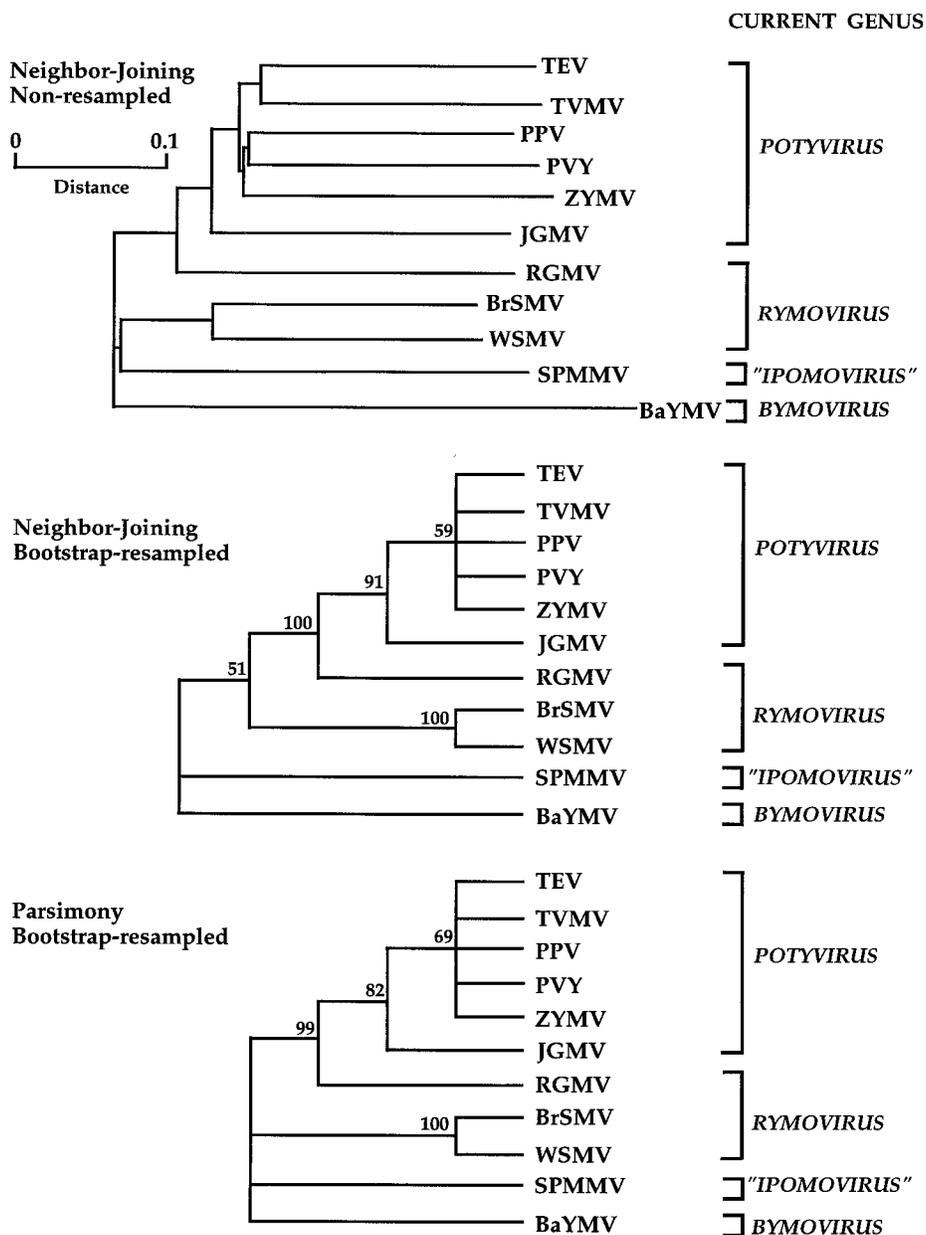


Fig. 4. Phylogenetic relationships among 11 species of the family *Potyviridae*, based on multiple alignment of NIB protein sequences. Trees were obtained by neighbor-joining or parsimony methods, with barley yellow mosaic virus (BaYMV) RNA 1 defined as the outgroup. Horizontal branch lengths for the neighbor-joining tree, derived from the nonresampled data set, correspond to mean character distances between taxa; vertical lengths are arbitrary. Trees derived from bootstrap-resampled data sets (1,000 replications) are based on a 50% majority-rule consensus, in which only tree topology is relevant; both vertical and horizontal branch lengths are arbitrary. Bootstrap percentages of clades are shown (when >50%) along internal branches of trees derived from bootstrap-resampled data sets. TEV = tobacco etch virus; TVMV = tobacco vein mottling virus; PPV = plum pox virus; PVY = potato virus Y; ZYMV = zucchini yellow mosaic virus; JGMV = Johnsongrass mosaic virus; RGMV = ryegrass mosaic virus; BrSMV = brome streak mosaic virus; WSMV = wheat streak mosaic virus; and SPMMV = sweet potato mild mottle virus.

Sufficient data are available to split the current genus *Rymovirus* into two distinct genera. Because the partial nucleotide sequence data available for AgMV and HoMV indicate these potyviral genomes are closely related to RGMV (26,27), the genus *Rymovirus* should retain these three viruses as member species. WSMV and BrSMV, however, should be classified as something other than species of the genus *Rymovirus*. One possibility would be to place WSMV and BrSMV into the same genus as SPMMV, the potyviral genome with which WSMV and BrSMV share a most recent common ancestor. However, given the substantial sequence divergence between SPMMV and WSMV or BrSMV and the fact that SPMMV is transmitted by whiteflies, a separate genus for SPMMV is justified.

The International Committee on Taxonomy of Viruses (ICTV) currently is in the process of revising the taxonomy and nomenclature of the family *Potyviridae*. The ICTV Executive Committee recently approved the establishment of the genus "*Tritimovirus*," which contains WSMV (type species) and BrSMV as members (P. Berger, *personal communication*), and it is expected that this revision will appear in the next report from the ICTV. In addition, the recently discovered sugarcane streak mosaic virus (19) from Pakistan appears to be a close relative of WSMV and BrSMV (J. S. Hall, *unpublished data*) that may represent the third member species of the genus "*Tritimovirus*."

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