

Characterization of an unusual tobacco rattle virus isolate and a novel phenuivirid in the Jerusalem sage

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Research Article

Keywords: bunyaviricetes, coguvirus, entovirus, phenuiviridae, phlomis, tobnavirus, TRV, virgaviridae

Posted Date: May 9th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-6578422/v1>

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Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Virology Journal on August 6th, 2025. See the published version at <https://doi.org/10.1186/s12985-025-02896-3>.

Abstract

Two RNA viruses were identified by high-throughput sequencing analysis of leaf samples of Jerusalem sages (*Phlomis fruticosa*) in Switzerland. The first virus, representing a novel isolate of tobacco rattle virus (TRV) named "Phlo", was identified in symptomatic plants but not in healthy ones. Phlo is distinguished by its exceptionally long RNA2 that harbours two ORFs preceding the CP ORF. This peculiar genetic make-up is shared by a set of closely-related European TRV RNA2s, and it could be associated with host-specific systemic infection ability. The second virus, detected both in symptomatic and asymptomatic sages, is a novel member of the family *Phenuiviridae* named "Phlomis phenuivirus 1" (PPV1). PPV1 exhibits a "cogu-like" architecture with a probable bi-segmented, ambisense RNA genome encoding a replicase, nucleocapsid, and putative movement protein (MP). PPV1 is related to Muscari virus A, a virus identified in an ornamental in Australia, and together they likely constitute a new genus for which the name "Maladivirus" is proposed. This taxon represents a sister clade of the genus *Entovirus*, whose members have been identified in a fungus and diverse environmental samples. Intriguingly, the putative MPs of maladiviruses and entoviruses cluster with those of lentinuviruses (i.e. cogu-like mycoviruses) and with putative endogenous viral elements from a mycorrhizal fungus, suggesting a role in fungal hosts.

Introduction

Advances in high-throughput sequencing (HTS) technologies have revolutionized plant virology. HTS allows for the unbiased analysis of nucleic acids, which is particularly relevant in the cases of untransmissible and/or latent phytoviruses that would be otherwise omitted by traditional diagnostics methods. HTS has therefore significantly expanded the known plant virome (1), and becomes increasingly popular in routine phytosanitary analyses as costs decrease (2). Here, we used HTS to investigate the etiology of diseased plants of the Jerusalem sage (*Phlomis fruticosa*), an ornamental in the family *Lamiaceae*. Native to the eastern Mediterranean regions, this evergreen shrub is widely grown across Europe for its attractive yellow flowers. This species can readily propagate over several years in temperate areas, which favors a potential role as pathogen reservoir. So far, the sole virus identified in Jerusalem sage is Phlomis mottle virus (PhMV), a trichovirus discovered in Italy (3). Our HTS analysis of symptomatic Jerusalem sages revealed the mixed infection of an atypical isolate of tobacco rattle virus (TRV, *Tobravirus tabaci*) and a novel virus belonging to the family *Phenuiviridae*.

TRV is a member of the genus *Tobravirus* (family *Virgaviridae*; order *Martellivirales*) which also accommodates pea early-browning virus (PEBV, *Tobravirus pisi*) and pepper ringspot virus (PRV, *Tobravirus capsica*) (4). The history of TRV dates back to the late 1890s in Germany, when the "Mauche" disease was first reported on tobacco (5). Nowadays, although TRV is best known as a versatile biotechnology tool (6), it still remains problematic on ornamentals and crops such as potato. TRV is ubiquitous in many agricultural soils worldwide, inducing latent to strongly symptomatic infections (7). Its host range is exceptionally high, encompassing over 400 plant species from 50 families (7). The virus is transmitted by nematodes from the genera *Paratrichodorus* and *Trichodorus*, and actively attracts

these vectors through modulation of plant root volatiles (8). Transmission can also occur via seeds (9). TRV genome consists of two positive sense, single-stranded RNAs (RNA1 and 2) encapsidated in rod-shaped virions (10). Both RNAs are 5'-capped and end by a tRNA structure (11). RNA1 encodes the RNA-dependant RNA polymerase (RdRp) subunits, a movement protein (MP) and a cysteine-rich protein (CRP). Both the MP and CRP act as viral suppressors of RNA silencing (12, 13), and a recent study has also highlighted a role in virion formation for the CRP (14).

While TRV RNA1 is highly stable among isolates (6), RNA2 is prone to reassortment, recombination, insertion and deletion (15). Two main architectures have been described for this RNA. The most common architecture consists of the capsid protein (CP) ORF located in 5' and followed by the ORFs encoding 2b and 2c, two proteins involved in nematode transmission (16). Some isolates exhibiting this architecture have shortened versions of 2b and/or 2c, and a few isolates have additional small ORFs in the 3' region (17). The second RNA2 architecture, previously framed "rule-breaking" architecture, consists of the CP ORF preceded by one or several ORFs. It was first described for the "Spinach Yellow Mottle" (SYM) isolate in England in 1979, for which RNA2 harbours three CP-preceding ORFs encoding hypothetical proteins (HPs) (18, 19). In 2014, the Polish isolate Mlo7 identified in potato was also predicted to encode a small CP-preceding HP closely related to one of SYM HPs (20). A similar architecture was found in 2015 for the German isolates Da and Db, both retrieved from strongly-symptomatic potato tubers, and encoding a CP-preceding HP of 35 kDa (35K) unrelated to SYM HPs (21). Last, the RNA2 of the isolate IFA65, first identified in Italy on pepper in the 1970s (22), has been recently sequenced, revealing a long CP-preceding ORF related to SYM HPs (22). The biological role of all these aforementioned CP-preceding ORFs remains unknown.

The family *Phenuiviridae* (order *Hareavirales*; class *Bunyaviricetes*) accommodates viruses with negative and ambisense RNA infecting eukaryotes including humans (23–26). These viruses have segmented genomes encoding at least an RdRp and a nucleocapsid (N). While many phenuivirids produce glycoproteins (G) and are protected in an envelope, others are "naked" and appear as flexuous filaments consisting of genomic RNA associated with N proteins. This is the case for plant phenuivirids such as members of the genera *Tenuivirus*, *Mechlorovirus*, *Coguvirus* and *Rubodvirus* (27–31). In recent years, *Phenuiviridae* has greatly expanded through the accumulation of HTS data, which has led the International Committee on Taxonomy of Viruses (ICTV) to recognize several new "cogu-like" genera: *Entovirus*, *Laulavirus*, *Lentinivirus* and *Bocivirus*. These viruses are presumably naked and encode an RdRp, N and putative MP that is structurally similar to proteins from the tobacco mosaic virus 30 kDa (30K) family (32, 33). Cogu-like viruses have been retrieved from various eukaryotes (plants, fungi, arthropods) and environmental samples (34–39). Remarkably, a recent study has shown that the laulavirus "Valsa mali negative-strand RNA virus 1" (VmNSRV1) can be found in natural populations of fungi and plants, leading to its description as the first "phyto-mycovirus" (40).

Material and method

Plant material

Leaves of healthy and symptomatic plants of Jerusalem sage were collected in a private garden in Lausanne (Switzerland), in proximity of the so-called “Maladière” area in August 2022. Symptomatic leaves were used for the mechanical inoculation of several indicator plant species. Briefly, a sap inoculum was produced by grinding leaves in cold phosphate buffer (1 mM diethyldithiocarbamate, 20 mM Na₂HPO₄, pH 7.6) in a mortar using a pestle. The resulting sap was rubbed on the first true leaves of indicator species using carborundum (400-mesh silicon carbide, Aldrich) as abrasive. Inoculated plants were maintained in greenhouses (24°C, 14/10 h photoperiod) for one month with daily inspections.

Virion observation and inoculation

For the visualisation of virions, a simplified protocol dedicated for the semi-purification of TRV particle was followed (41). Briefly, 10 g of symptomatic sage leaves were first ground in liquid nitrogen in a mortar using a pestle. The resulting powder was mixed with 20 ml of CP buffer (0.18 M McIlvaine buffer, pH 7) supplemented with 0.2% thioglycolic acid, and squeezed through a nylon filter cloth. One vol of diethylether: carbon tetrachloride was added, followed by slow mixing for 5 min. The phases were then separated by low-speed centrifugation (20 min, 4,000 rpm) and the upper phase was used for high-speed centrifugation (1 h, 40,000 rpm). The resulting pellet was suspended in 1 mL CP buffer. The low and high-speed centrifugation steps were repeated, and the final pellet was resuspended in 0.1 mL CP buffer. Particles were observed by transmission electron microscopy (TEM) using the Tecnai G2 Spirit electron microscope as previously described (42). Semi-purified particles were also used for plant inoculation as described in the previous section.

RNA extraction and RT-PCR

RNA extraction and RT-PCR

Total RNA was extracted from 0.5 g of leaves using a 3% CTAB protocol, which was followed by DNase treatment as previously-described (43). RNA concentration was estimated with the Qubit 3 Fluorometer (Invitrogen). RT-PCR were conducted using the M-MLV reverse transcriptase and the Go Taq polymerase (Promega) as described before (43). The primer pair Katu/Katd was used to assess the presence of PhMV (44). TRV RNA1 was amplified using the primer pair TRV_F/R (45), while the new primer pair TRV2JS_F/R (TCGGGGTTTACTTGTTCCG/CTCCTAACGCATTGTTGGCG) was designed to amplify RNA2. The segments L and S of the phenuivirid were amplified using the pairs PPV1_F2/R2 (GCTAGGGACGATGCAGAGGG/GCACCTGAGCTTTGTAGACGC) and PPV2_F1/R1 (CCGCCAGAGTGAGTGAGAGG/AGACAGAGCAAGGAGGCGAG). PCR amplicons were analysed by agarose gel electrophoresis.

Genome sequencing

The DNase-treated RNA extracted from the leaves of two symptomatic Jerusalem sages was sent to Macrogen (South Korea) for an Illumina sequencing as previously documented (46). A total of 95,985,190 reads were generated, of which 93.6% exhibited high quality scores (> Q30) and were further used for trimming using Trimmomatic (47) and *de novo* assembly with Spades (48). Contigs longer than 500 bp were then submitted to BlastX searches against a local virus database. Contigs with hits E-value < 0.001 were further examined by online BlastX and BlastN searches.

Bioinformatics analyses

Viral genomes were analysed on Jalview (49) and Ugene (50). Functional annotation was performed using the MOTIF webserver (<https://www.genome.jp/tools/motif/>). Accession numbers for the proteins used in this study are available in Table S2 and S3. Protein alignments were obtained using Muscle (51). For phylogenetic analyses, alignments were first loaded into ModelFinder (52) in order to find the best aa substitution model. Maximum-likelihood (ML) phylogenetic trees were then built with IQ-Tree (53) in combination with ultrafast bootstrap (54). The ML trees were manually curated on iTol (55). RNA secondary structures were predicted using the RNAfold webserver (56). Homologs of viral proteins in fungal genomes were further screened on the MycoCosm portal (57).

Results

A new TRV isolate

In the summer 2022, large chlorotic rings and irregular patches were noted on leaves of Jerusalem sages in a private garden in Lausanne (Fig. 1A). Total RNA samples extracted from these plants tested negative for PhMV by RT-PCR. Sap inoculations of several indicator species were unsuccessful, and examination of crude extracts by TEM did not reveal viral particle. Furthermore, a cellulose-based extraction protocol failed to evidence any dsRNA element in the leaves (data not shown). Altogether, these results prompted an HTS analysis of total RNA. Following *de novo* assembly and Blast analyses, two viral contigs (6,752 and 4,590 nt) were identified, corresponding to the RNA1 and 2 of a new TRV isolate. Both contigs were assembled with high coverages (1,547x and 691x, respectively) and exhibited partial TRV termini (data not shown), indicating near-complete sequencing. This new TRV isolate is hereafter dubbed “Phlo”, and its RNA1 and RNA2 sequences are available in NCBI Genbank under accession numbers PV297904 and PV297905, respectively.

RT-PCR analyses confirmed the presence of Phlo RNA1 and 2 in symptomatic Jerusalem sages, while there was no detection in samples from healthy sages collected at the same location (Fig. S1). Rod-shaped particles *ca.* 50–150 nm in length were observed in semi-purified extracts from sage leaves (Fig. 1B). These particles exhibited a clear central canal, in line with previous characterization of TRV virions (10). Mechanical inoculation using semi-purified virions was successful on plants of sugar beet (*Beta vulgaris*), *Chenopodium* (*C. quinoa* and *C. amaranticolor*), tobacco (*Nicotiana tabacum* and *N.*

benthamiana) and periwinkle (*Catharanthus roseus*). Mild chlorosis appeared at 4 days post inoculation (dpi) on leaves of both *Nicotiana* species, but systemic symptoms were only noted on plants of *N. tabacum*, on which ringspots and severe mosaic developed locally at 7 dpi, and later spread on upper tissues as well (Fig. 1C). Periwinkles did not exhibit local symptoms but showed chlorotic spots on upper leaves after one week. Conversely, plants of sugar beet developed local chlorotic spots at 7 dpi, but no systemic symptom was noted. Chlorotic and necrotic spots appeared 3 dpi on the inoculated leaves of the two *Chenopodium* species. Inoculated leaves of *C. quinoa* eventually became completely necrotic and collapsed. After one week, chlorosis, necrotic spots and deformations were noted on the upper leaves of these species. This last result is quite remarkable as long-distance movement in *Chenopodium* has only been reported for SYM among TRV isolates (18).

Analysis of Phlo genome

Phlo RNA1 is highly similar to other TRV RNA1s, showing a classic architecture, with the closest sequence being ppk20 RNA1 (AF406990.1, 100% cover, 99.5% nt id.). In contrast, Phlo RNA2 is rather unusual, as it is longer than other reported TRV RNA2 and harbours five long (> 200 nt) ORFs dubbed “ORF1-5” (Fig. 2A). BlastP searches on these ORFs yielded intriguing results (Table 1). Both ORF1 and 2 show homology to the CP-preceding HP of IFA65, but also to the HPs of SYM and Mlo7. ORF3 corresponds to the CP, while ORF4 encodes a distant homolog of 2b from TRV 11r21, an isolate collected at the same location of Mlo7 (20). ORF5 hypothetically encodes a small protein of 9 kDa (9K) that is also present on IFA65 RNA2, but does not show homology to any other sequence in the NCBI databases. Consistent with these results, a BlastN search on Phlo RNA2 yielded IFA65 RNA2 as the closest sequence (ON156783.1, 77% cover, 98% nt id.). Both RNAs are almost identical except for the 2b-encoding region lacking in IFA65 (Fig. 2A).

Table 1
Description of the best BlastP hits for the ORFs of TRV Phlo RNA2.

ORF	Size (aa)	Best hit	Cover (%)	ID (%)	Size (aa)	Acc. number
1	314	IFA65 RNA2 ORF1	100	98	527	WCJ12589.1
2	200	IFA65 RNA2 ORF1	100	97	527	WCJ12589.1
3	221	IFA65 RNA2 CP	100	99	221	WCJ12590.1
4	371	11r21 RNA2 2b	99	47	369	AHG52767.1
5	70	IFA65 RNA2 ORF3	100	99	70	WCJ12591.1

A phylogeny of the *Tobravirus* CPs, which is the only conserved gene in RNA2, reveals that most isolates fall in four main lineages: “alpha”, “beta”, “gamma”, and “delta” (Fig. 2B), consistent with a previous study (21). The isolates harboring CP-preceding ORFs belong to two groups within the alpha lineage, with Da and Db forming the “alpha 1” subclade, while Phlo and the remaining isolates forming the “alpha 2” subclade (Fig. 2C). A third group, the subclade “alpha 3” accommodates isolates with a classic

architecture shared with other TRV RNA2s. Hypothetically, three gene acquisitions occurred among members of the alpha 1 and 2 subclades, and several deletion events also occurred. 2b is truncated in SYM and totally lost in Mlo7 and IFA65. The CP-preceding HPs also have experienced deletions, with total lost in 11r21. An alignment of these HPs shows that no isolate seem to harbor a full-length ancestral version (Fig. S2).

A novel phenuivirid

In addition to Phlo, a second RNA virus was identified in the HTS dataset. Indeed, two contigs (5,832 and 3,372 nt) were found harbouring ORFs related to proteins from phenuivirids (BlastX hits with E-value = 0). These contigs were thus assigned to the long (L) and short (S) segments of a novel virus tentatively named “Phlomis phenuivirus 1” (PPV1, accession numbers PV370485 and PV370486). The sequencing coverages of PPV1 segments (71x and 141x, respectively) were significantly lower than that of Phlo, indicative of a lower viral titre. Interestingly, RT-PCR analyses showed the presence of PPV1 not only in symptomatic but also in healthy Jerusalem sages (Fig. S1), suggesting that the virus might be latent on this species. While TRV was detected by RT-PCR in the mechanically-inoculated symptomatic plants, there was no signal for PPV1 RNAs (data not shown). A RACE protocol was followed to obtain the sequences of the RNA termini, without success.

A representation of the genome of PPV1 is provided on Fig. 3A. PPV1 segment L encodes a large (partial) protein of 1,919 aa with the *Bunyavirus* RdRp domain pfam04196 at positions 615–1286. PPV1 segment S is ambisense, harboring two ORFs in opposite frames. The 425 nt-long intergenic region separating these ORFs is highly rich in AT, and it is predicted to fold into a stable secondary structure (Fig. 3B), which are features described in many phenuivirids. The first ORF of PPV1 segment S encodes a protein of 359 aa containing the *Tenuivirus/Phlebovirus* N domain pfam05733 (positions 47–307), and the second ORF encodes a HP of 448 aa with no known domain. BlastP search on this HP revealed distant homology (< 30% aa id.) with the putative MPs associated with several other cogu-like viruses.

Phylogenetic analyses of PPV1

ML phylogenetic trees were built for PPV1 proteins and their homologs from representative phenuivirids (Table S2). In the RdRp tree (Fig. 3C, left panel), PPV1 is placed in a strongly-supported clade with Muscari virus A (MvA), a virus identified by HTS in grape hyacinth (*Muscari neglectum*) in Western Australia (58). Both viruses arguably form a new genus of cogu-like viruses as their RdRp share very low level of aa identities (< 37%) with the closest sequences. We propose therefore the novel genus “Maladivirus” (from the location of the sampled sages in the “Maladière” area) to accommodate PPV1 and MvA.

Maladiviruses represent a sister clade of the genus *Entovirus* that consists of the mycovirus “Entoleuca phenui-like virus 1” (EnPLV1) and sequences retrieved from metaviromics studies of soils and sediments in China (59, 60). The genera “Maladivirus” and *Entovirus* share a common bi-segmented genome

architecture with other cogu-like viruses, with some exceptions found among laulaviruses and bociviruses for which the N and MP ORFs are located on distinct segments (Fig. 3C, right panel).

In the phylogenetic tree of N proteins (Fig. 4A), which is not available for MvA, PPV1 appears again most closely related to entoviruses. In contrast to the RdRp and N that are shared with other phenuivirids, the MPs of cogu-like are unique in the family *Phenuiviridae* and show distant homology to the MPs of ophioviruses (family *Aspiviridae*), which are negative-strand RNA viruses infecting a great diversity of plants (28, 32). The MP tree was therefore built using ophioviruses' MPs as outgroup (Fig. 4B). In this tree, cogu-like viruses form two distinct clades, with maladiviruses again closely related to several members of the genus *Entoviruses*. The MPs of maladiviruses appear also related to the MPs of lentinuviruses, which have been exclusively associated with fungi (36, 61). Interestingly, in both N and MP trees, a few phylogenetic incongruences appear among coguviruses and entoviruses, suggesting possible events of recombination/reassortment.

Table 2
Endogenous viral elements homolog to PPV1 MP in *G. morchelliformis* GMNE.BST.

EVE	Scaffold	CDS positions	Strand	CDS accessions	Size (aa)
G	401	67,301 – 68,401	+	KAF8490304.1	366
G'	158	206,301-206,667 and 206,884 – 207,395	+	KAF8510981.1	292
G''	153	207,359 – 207,644 and 207,720 – 207,760	-	KAF8511336.1	108

Curiously, an online BlastP search revealed a distant homolog of PPV1 MP (KAF8490304.1, 366 aa, 64% cover, 27% id.) in the genome of the mycorrhizal fungus *Gautieria morchelliformis* strain GMNE.BST (assembly “Gaumor1_1”, acc. number: GCA_015179125.1 (62)). The associated ORF likely represents an “endogenous viral element” (dubbed “EVE-Gm”) which presumably results from the reverse-transcription and integration of a viral sequence into its host genome (63). A previous study already evidenced an EVE related to lentinuviral N in the genome of the powdery mildew pathogen *Erysiphe cichoracearum* (36). EVE-Gm is specifically related to the MPs of maladiviruses, entoviruses and lentinuviruses (Fig. 4B). Additional BlastN searches revealed two other similar EVEs (EVE-Gm' and EVE-Gm'', Table 2) on other scaffolds of the same assembly. These sequences are truncated and likely correspond to pseudogenized EVEs. In terms of genomic neighbourhood, EVE-Gm and EVE-Gm'' are preceded by an ORF harboring the domain COG3378 (Fig. 4C), corresponding to a DNA primase/helicase associated with mobile genetic elements (MGE). BlastP search on this ORF yielded significant hits in other fungal genomes (data not shown), suggesting that it may represent a fungus-specific MGE. In the case of EVE-Gm', the MP-related ORF is followed by a HP with no known domain.

Discussion

Our HTS analysis revealed the mixed infection of two RNA viruses in symptomatic samples of Jerusalem sages. Genome analysis for these viruses provides interesting evolutionary insights for distant taxa within the *Riboviria* realm.

A near-complete genome was reconstructed for Phlo, which corresponds to a new TRV isolate most likely responsible for the chlorotic patches and ring patterns observed on the sages. These symptoms are indeed consistent with those previously described on other plant species upon TRV infection (7). Of particular interest, Phlo RNA2 is remarkably long, and is closely related to the RNA2s of the European isolates IFA65, SYM, Mlo7, and 11r21. All these RNA2s presumably derive from a common ancestor that acquired one (or multiple) CP-preceding ORF, and experienced various deletion events. Similar deletions have been previously reported for other TRV RNA2s upon repeated mechanical inoculations or extended *in planta* infection (21, 64). As for the CP-preceding HPs, their function remain uncertain. Reverse genetics studies on SYM mutants lacking HPs have not revealed changes in viral titre or spread in *N. benthamiana* (19). Nevertheless, early studies on this isolate have linked its RNA2 with long-distance movement in *C. quinoa*, an ability that is shared by Phlo but absent in isolates with a common architecture (18, 65). Hence, the CP-preceding HPs might be involved in systemic infection in a host-specific manner. This could be easily experimentally tested using infectious clones in future research. It is important to highlight that most TRV sequences available to date have been obtained from potato (Table S1). Consequently, further characterization of TRV isolates from alternative host species is warranted to improve our understanding of the genomic diversity of this virus.

The second RNA virus identified in the sages is PPV1, which represents a novel phenuivirid likely latent on sages. Together with MvA, PPV1 belongs to the proposed genus “Maladivir” within the large group of cogu-like viruses. A fascinating aspect of these viruses lies in their host diversity (Fig. 3C). Some genera have been associated with one host kingdom. This is the case for coguviruses that infect plants and lentinuviruses that are mycoviruses. All members of *Bocivirus* have been described as mycoviruses, except Sanya phenuivirus 1 (SPV1) that has been detected in a horse fly. The recently-described laulavirus VmNSRV1 has been described both in plant and fungus, and other laulaviruses have been identified in plants, fungi and even arthropods. The case of *Entovirus* is unclear as the host status has only been clearly established for the mycovirus EnPLV1, whereas other members of this genus have been obtained from soil and sediment metaviromics studies. As far as maladiviruses are concerned, both MvA and PPV1 were detected in plant samples. However, clear demonstration of plant infection is still needed, as it is possible that these viruses actually infect plant-associated organisms (e.g. fungal endophytes) rather than plants themselves. Altogether, the host diversity of cogu-like viruses remind the case of members the family *Partitiviridae*, which are dsRNA viruses detected in multiple eukaryotic kingdoms. A recent study has demonstrated replication of several partitivirids in plants, fungi and animals (66), and this triple cross-kingdom infection capacity is worth testing for cogu-like viruses.

Another intriguing aspect of cogu-like viruses concerns their putative MPs. These proteins are arguably involved in the intercellular movement of coguviruses and plant-infecting cogu-like viruses, which is supported by the localization of VmNSRV1 MP in plasmodesmata (40). However, the presence of such

homologs in cogu-like viruses not associated with plants is puzzling. Either these proteins might be required during a hypothetical plant stage, or they could serve another function in non-plant hosts. Interestingly, the MPs of maladiviruses are particularly close to homologs found in mycoviruses as well as putative EVEs from the fungus *G. morchelliformis*. These findings further question the true host of PPV1 and suggest a function in fungal hosts for these putative MPs.

Declarations

Acknowledgements

We would like to thank Sabine Bonnard, Stefan Kellenberger, Marc Passerat and Larissa Grosu for taking care of the plants. We are also thankful to Jacques Mahillon and Raphaël Groux for critical review of the manuscript.

Funding

This research received no external funding.

Availability of data and materials

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author Contribution

MM performed the plant sampling. ND and MM performed virus Purification and TEM. JB, IK and MM produced the HTS and conducted subsequent bioinformatics and molecular analyses. JB and MM performed biological assays. MM wrote the initial draft. AB and OS critically reviewed the manuscript. All authors have read the manuscript and agree for publication.

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Figures

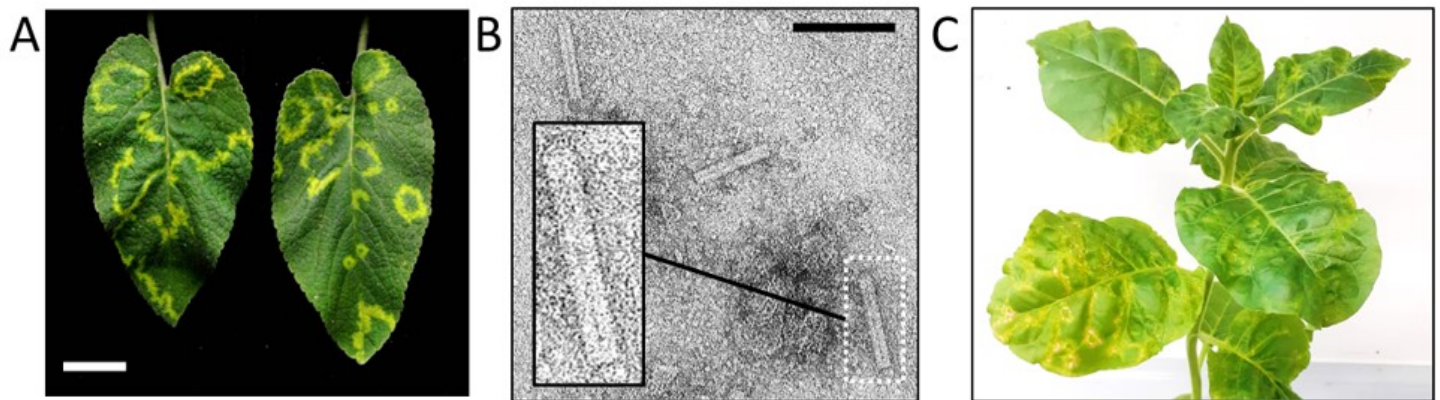


Figure 1

Symptoms and particles associated with TRV Phlo. **A.** Leaves of Jerusalem sages with virus-like symptoms that were used for the HTS analysis. The white bar represents 1 cm. **B.** Electron micrograph of three TRV Phlo virions. Particles were stained with phosphotungstic acid. The black bar represents 100 nm. The inner panel gives a closer look at the central canal. **C.** Mosaic and ringspot symptoms on upper leaves of *N. tabacum* cv. Xanthii, 30 days post inoculation with semi-purified TRV Phlo particles.

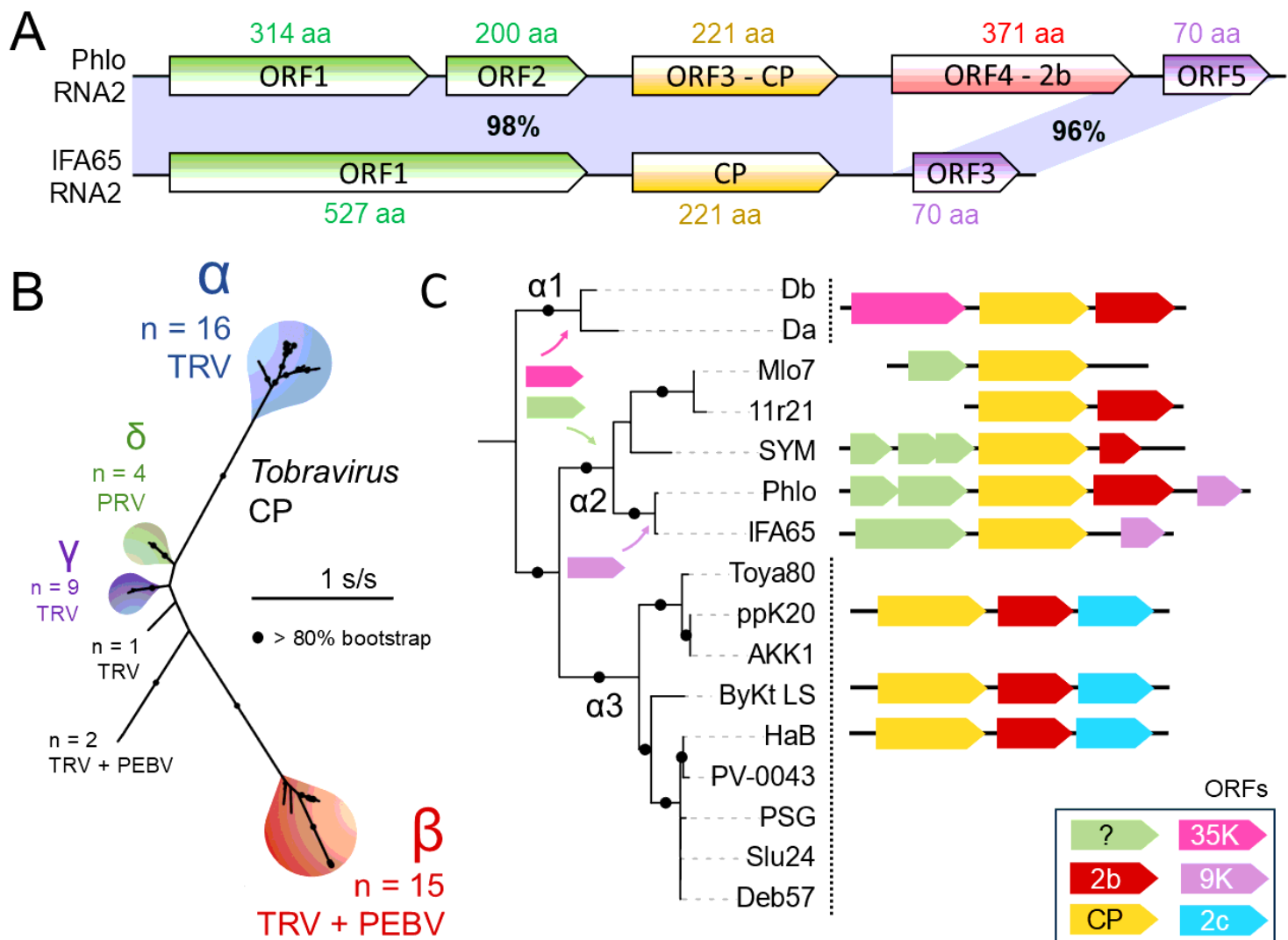


Figure 2

Genomic and phylogenetic analyses of TRV Phlo RNA2. A. Comparison of Phlo and IFA65 RNA2. Colored arrows represent ORFs. The shaded areas indicate nt identities (in percent). **B.** Unrooted ML phylogenetic tree of the *Tobravirus*CPs (see Table S1 for accession numbers). The tree was built using the model JTT+G4. Black circles on branch indicate > 80 % bootstrap support. “n” indicates the number of isolates in each clade. **C.** Details on the CP lineage alpha. For each isolate, the corresponding RNA2 architecture is represented when available. Description of the ORFs are given in the lower right panel.

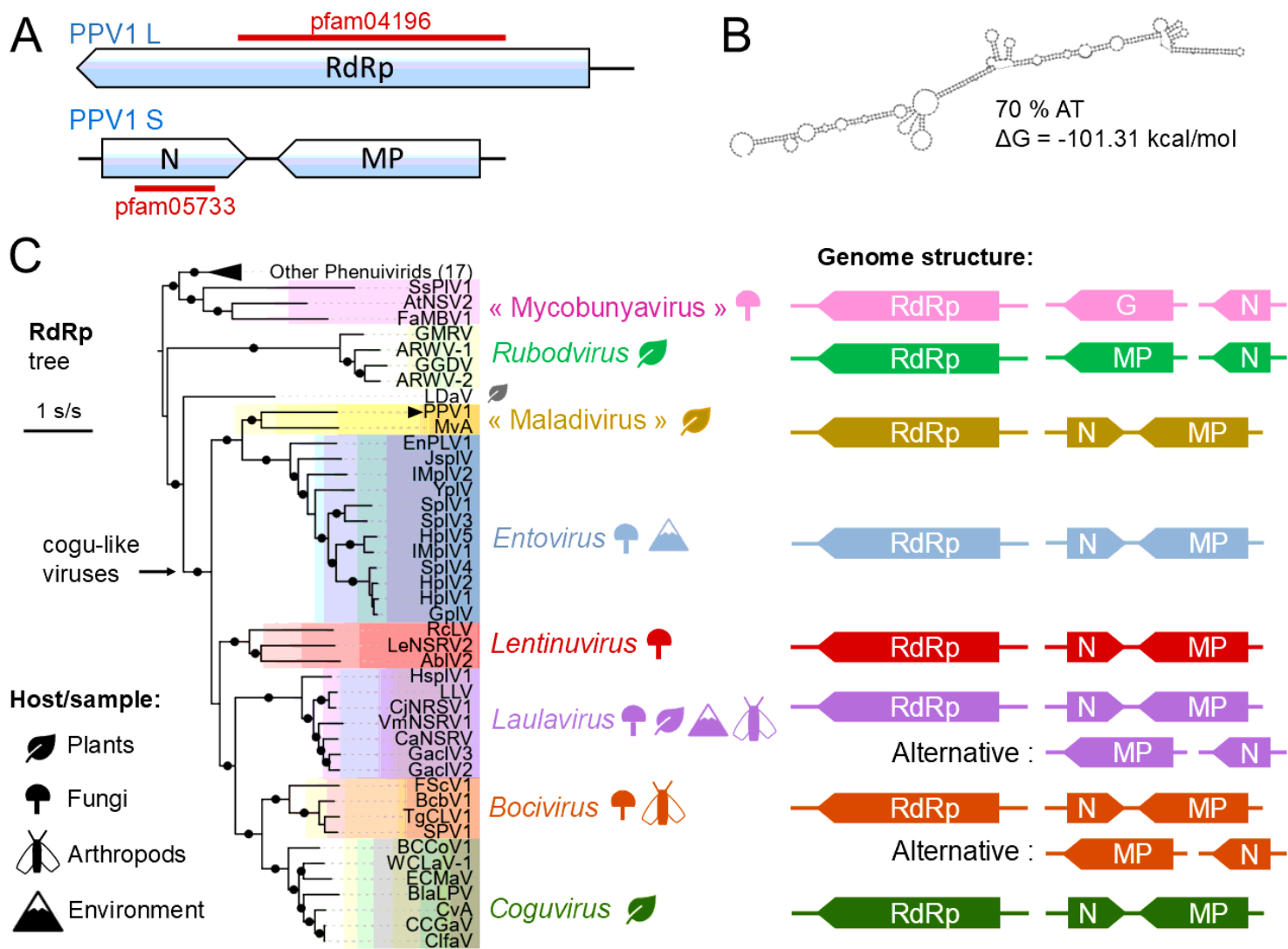


Figure 3

Genomic and phylogenetic analyses of PPV1. A. Schematical representation of PPV1 genomic segments L and S. Colored arrows represent ORFs. B. Predicted secondary structure for PPV1 segment S intergenic region. The percentage of AT and minimal energy are indicated. C. Left: ML phylogenetic tree for the RdRp of PPV1 and related phenuivirids. The tree was built using the substitution model rtREV + I + G4. Black circles on branch indicate > 80 % bootstrap support. The position of PPV1 is indicated by a black arrow. Right: Genome architectures. RdRp: RNA-dependant RNA replicase; G: glycoproteins; N: nucleocapsid; MP: movement protein.

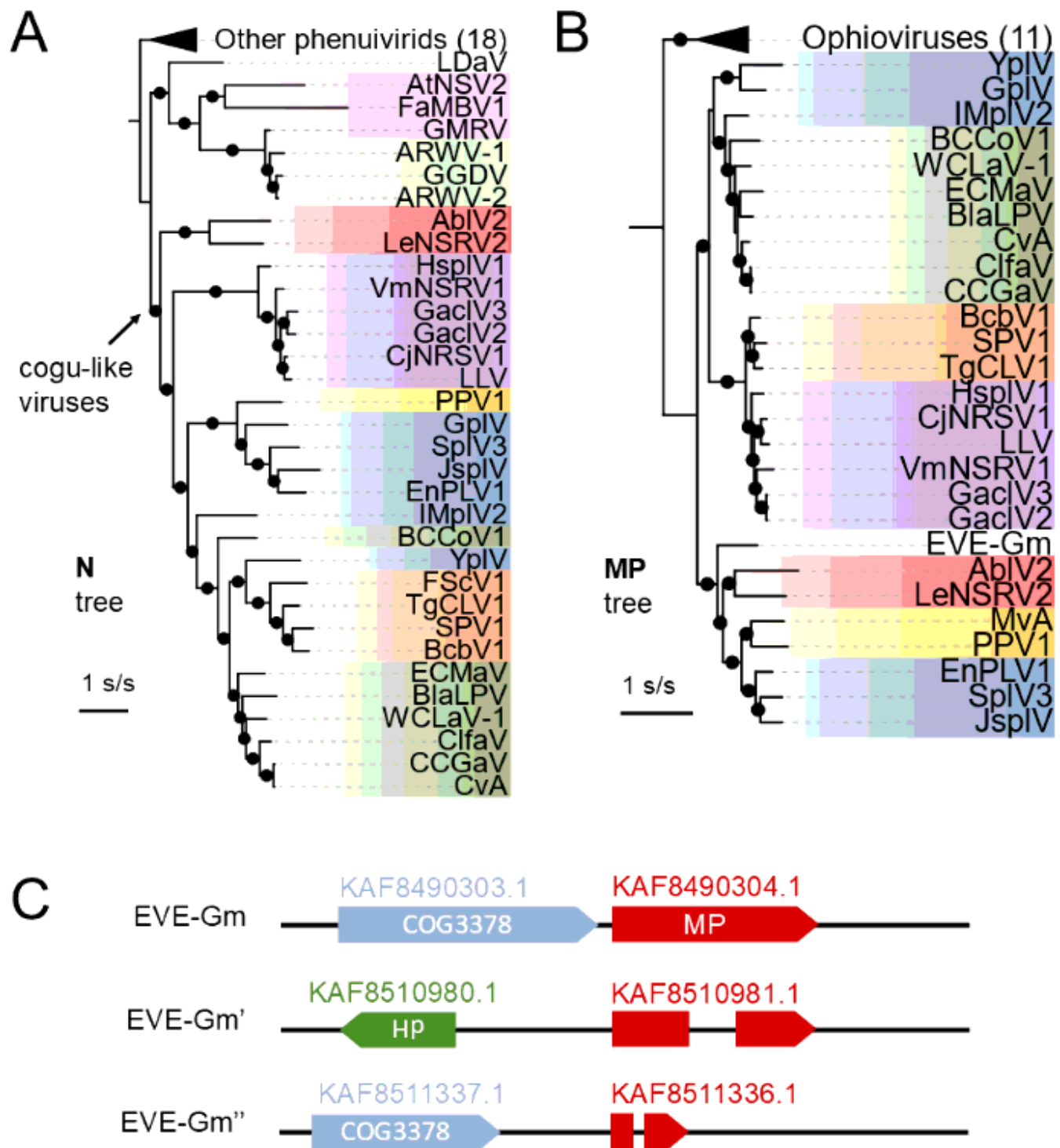


Figure 4

ML phylogenetic trees for the proteins N (A) and MP (B) of PPV1 and related phenuivirids. The trees were built using the substitution models LG+F+G4 and rtREV+F+I+G4, respectively. Black circles on branch indicate > 50 % bootstrap support. Colored boxes represent genera as indicated on Figure 3C. C. Schematical representation of the three endogenous viral elements (EVE) detected in the genome of *G. morchelliformis* GMNE.BST.

Supplementary Files

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