



# The complete nucleotide sequence and genome organization of a novel virus of the order *Tymovirales* isolated from *Prunus davidiana* (Carr.) Franch. in Liaoning, China

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Received: 19 January 2019 / Accepted: 22 February 2019 / Published online: 28 March 2019  
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## Abstract

In September 2017, a yellow spot leaf disease was noted on the leaves of *Prunus davidiana* (Carr.) Franch. plants in Liaoning, China, and spherical virions (approx. 30 nm in diameter) were later observed in preparations of symptomatic leaves. Subsequent deep sequencing of small RNA revealed the presence of a virus in these symptomatic leaves. The complete genome of this viral isolate consists of 6,072 nucleotides, excluding the poly(A) tail. The virus showed the closest genetic relationship to grapevine-associated tymo-like virus, reported in Colmar, France (GaTLV, MH383239), which is the sole member of the newly proposed genus “*Gratylivirus*” within the order *Tymovirales*, which is currently unassigned to a particular family. The virus clustered closely with GaTLV in a phylogenetic tree constructed based on complete genomic sequences. On the basis of the nucleotide and amino acid sequences of the replicase and coat protein genes, this virus shares the highest (although still relatively low) sequence similarity with those of GaTLV (41.6%–60.8% identity), indicating that the virus is a distinct member of the order *Tymovirales*, for which the name “prunus yellow spot-associated virus” (PYSaV) is proposed. To our knowledge, this is the first report of a virus naturally infecting *P. davidiana*.

The order *Tymovirales* contains viruses that are characterized by a single molecule of positive sense ssRNA that is between 5.9 and 9.0 kb in length. *Tymovirales* is divided into five families, namely *Alphaflexiviridae*, *Betaflexiviridae*, *Deltaflexiviridae*, *Gammaflexiviridae*, and *Tymoviridae* (<https://talk.ictvonline.org/>). Of these, only viruses in the family *Tymoviridae* have typical icosahedral virions of

approximately 30 nm in diameter, whereas members of the other four families have flexuous filaments, which are typically 12–13 nm in diameter and approximately 470 to 1000 nm in length [1]. Recently, grapevine-associated tymo-like virus (GaTLV, MH383239), isolated from grapevine in France, was proposed to be a member of a tentatively named new genus, “*Gratylivirus*”, that is currently unassigned to a particular family, within the order *Tymovirales* [2]; however, no information on the particle morphology of this virus has been published. In recent studies, many *Tymovirales* members have been characterized from plants, fungi, or insects worldwide [3–7]; however, to date, no *Tymovirales* members have been reported to infect the plants of *Prunus davidiana* (Carr.) Franch. (subgenus *Amygdalus*, Rosaceae). In China, *P. davidiana* is an excellent ornamental tree that is widely planted for the greening of urban landscapes because of its efficient water and soil conservation properties [8]. Although fungal and bacterial diseases had previously been identified in *P. davidiana*, prior to 2017, no virus-like diseases had been reported [8]. However, in September 2017, a yellow spot leaf disease was observed on leaves of *P. davidiana* plants growing in Shenyang, Liaoning Province, China (Fig. 1a). Here, we describe the complete genome sequence

Handling Editor: F. Murilo Zerbini.

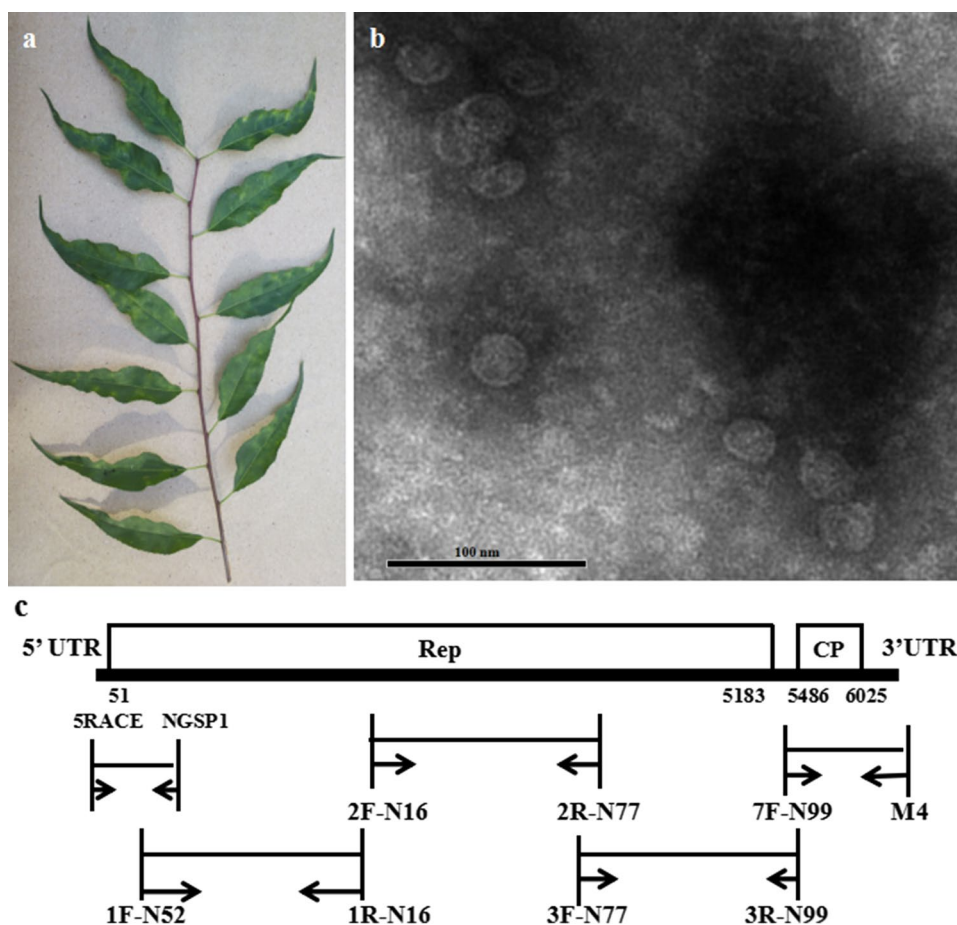
**Electronic supplementary material** The online version of this article (<https://doi.org/10.1007/s00705-019-04220-1>) contains supplementary material, which is available to authorized users.

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**Fig. 1** Diseased leaves with the yellow-spot symptom (a) in *Prunus davidiana* (Carr.) Franch. plants, viral particles in a diseased leaf (b), and genomic organization and PCR amplification strategy for PYSaV (c)



of a new member of the order *Tymovirales* (isolate TS) isolated from *P. davidiana*.

In order to identify the causal agent of the disease, samples of symptomatic leaves collected from infected *P. davidiana* plants were prepared for transmission electron microscopy, and in these crude preparations we observed spherical viral particles of approximately 30 nm in diameter (Fig. 1b). For species-level identification, we constructed a small RNA (sRNA) library using an NEBNext Multiplex Small RNA Library Prep Set (New England Biolabs). sRNAs of 18–30 nt in length were extracted from one diseased sample and sequenced using the BGISEQ-500RS platform (Huada Gene, Shenzhen, China). From the sequences generated, we obtained a total of 31,623,739 clean reads, from which the adaptor sequences were trimmed, followed by contig assembly using Velvet 0.7.3 [9]. The 16,473 contigs with lengths equal to or greater than 50 nt were used for BLASTN and BLASTX searches for similar sequences in the GenBank database (<http://www.ncbi.nlm.nih.gov/>). Fifteen contigs were found to show similarities to genome fragments of grapevine-associated tymo-like virus, turnip yellow mosaic virus, eggplant mosaic virus, alfalfa virus F, Ek Balam virus, grapevine fleck virus, Culex-originated Tymoviridae-like virus, maize rayado fino virus, and Medicago sativa

marafivirus 1 of the family *Tymoviridae* (order *Tymovirales*). On this basis of the detected similarities, we used the grapevine-associated tymo-like virus (GaTLV, MH383239) genome sequence as a reference, and contig sequences were used to design specific primers to amplify the viral genome of the new isolate (Fig. 1c; Supplementary Table 1). Total RNA was extracted using an RNASimple Total RNA Kit and was then used for first-strand cDNA synthesis using the primer M4-T(15) and a TIANScript RT Kit (Tiangen, Beijing, China). We accordingly amplified five amplicons from samples of symptomatic leaves, whereas no products were obtained from the leaves of healthy plants (Supplementary Fig. 1). The 5'-terminal sequences of the amplicons were determined using the specific primers GSP1 and NGSP1 in conjunction with a SMARTer® RACE 5'/3' Kit (Clontech, USA). Using primer GSP1, we initially obtained a 0.45-kb amplicon, which was subsequently used as a template for nested PCR using the primer NGSP1, following instructions provided in the kit manual, and accordingly obtained a 0.36-kb amplicon (Supplementary Fig. 1; Supplementary Table 1). All of the PCR products were then cloned into the pMD20T vector (TaKaRa, Dalian, China) and sequenced. Sequence data were assembled and analyzed using DNA-MAN 9 (Lynnon, Quebec, Canada). The complete sequence

of the *P. davidiana* isolate (designated “prunus yellow spot-associated virus”, PYSaV) (MK404133) was used for a BLASTn search of homologous sequences, which were then used for sequence comparisons and construction of a phylogenetic tree. Pairwise percentage identities of nucleotide and amino acid sequences were calculated using MegAlign based on the Clustal W method in DNASTAR 6.0 (DNASTAR; WI, USA; Supplementary Table 2). Multiple sequence alignments based on the full genome sequences were performed using the MUSCLE (Codon) program of MEGA7 [10]. Phylogenetic analysis was performed using the maximum-likelihood method implemented in IQ-TREE [11] based on the GTR+F+R5 nucleotide substitution model, which was chosen by ModelFinder [12]. Branch support for the inferred maximum-likelihood tree was assessed using the SH-aLRT test [13] and ultrafast bootstrapping (UFboot) with 5000 replicates [14].

The complete sequence of PYSaV consists of 6,072 nucleotides, excluding the poly(A) tail. The 5′ and 3′ untranslated regions (UTRs) were 50 and 47 nt in length, respectively. The genomic organization is similar to that of GaTLV, encoding a large replicase polyprotein (Rep: 51–5183 nt, 1710 amino acids, 192.27 kDa) and a coat protein (CP: 5486–6025 nt, 179 amino acids, 18.724 kDa) (Fig. 1c) [2]. The presence of conserved domains was determined using the NCBI Conserved Domain Search program, which revealed that the Rep protein contains methyltransferase (MTR), RNA helicase (Hel), and RNA-dependent RNA polymerase (RdRp) domains. MTR, which is involved in capping, which enhances mRNA stability, has been identified in a wide range of ssRNA viruses [1, 15, 16]. The Hel domain plays multiple roles at different stages of viral RNA replication, as has been demonstrated by mutational analysis [17–19].

Sequence comparisons revealed that PYSaV shares the highest nucleotide sequence similarity with the unclassified virus GaTLV (59.7% identity in the complete genome sequence, 12% in the 5′-UTR, and 57.4% in the 3′-UTR). PYSaV shows the highest nucleotide and amino acid sequence similarity with GaTLV with respect to the Rep protein (60.8% and 55.1% identity, respectively), and the coat protein (53.3% and 41.6% identity, respectively). In contrast, PYSaV shares less than 30.0% identity in queries for all the other assessed viruses (Supplementary Table 2). A phylogenetic tree constructed based on viruses in the order *Tymovirales* with spherical particle morphology revealed that viruses in the family *Tymoviridae* grouped into three major clades according to genus. PYSaV was found to most closely related to GaTLV, with which it clustered in the unique “*Gratylinivirus*” group (Fig. 2).

Considering the observed particle morphology, the results of phylogenetic analysis, and the relatively low homology to GaTLV, the virus isolated from *P. davidiana* appears to

be the second member of the newly proposed viral genus “*Gratylinivirus*” within the order *Tymovirales*, which to date, has yet to be assigned to a particular family [1, 2]. Since viruses of the order *Tymovirales* have a wide range of hosts [3–7], viral investigation will be conducted not only on plants but also on fungi and insects to better elucidate the origin and epidemiological characteristics of PYSaV.

**Acknowledgments** This work was supported by funds from the Natural Science Foundation of Liaoning Province (20180550863, 2015020806) and the Fundamental Research Funds for the Central Universities (XDJK2018AA002). We thank Zhenguo Du and Fangluan Gao of Fujian Agriculture and Forestry University for useful discussions and help in constructing the phylogenetic trees, respectively.

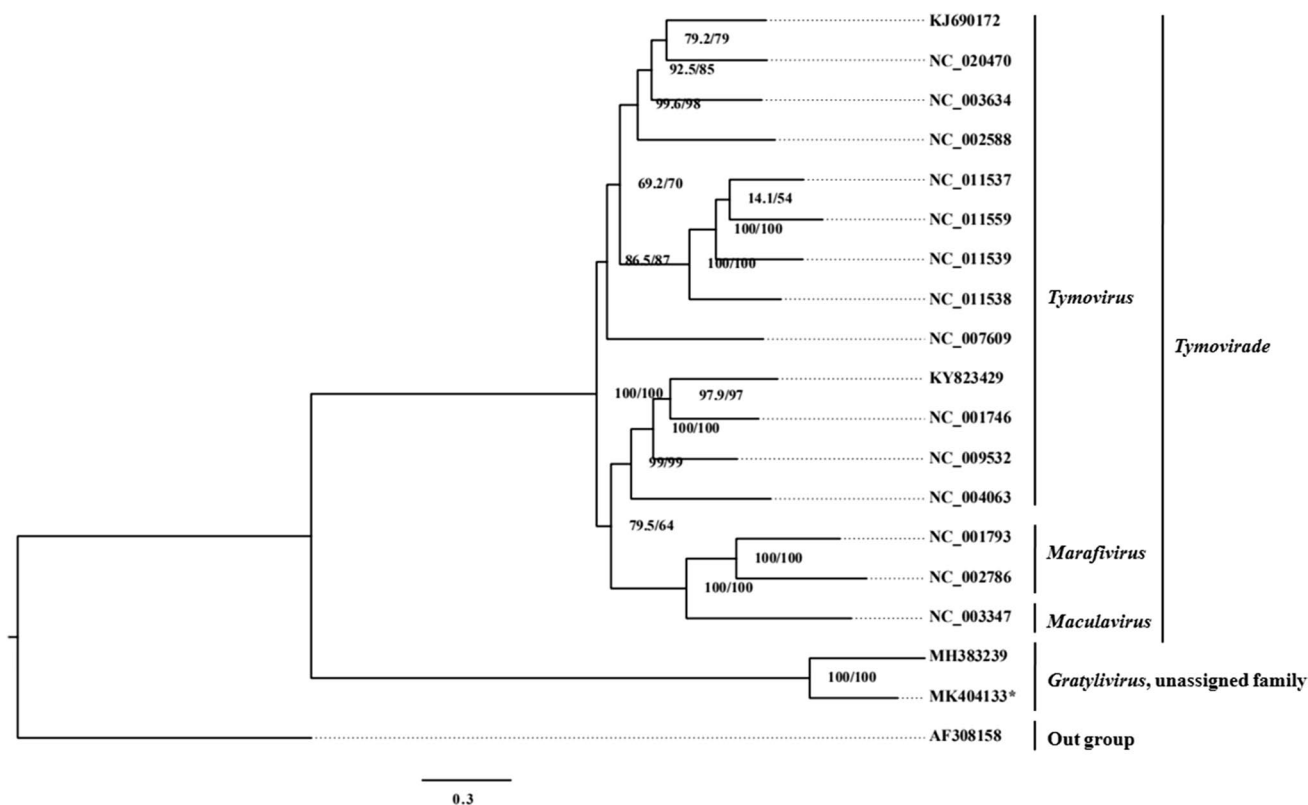
## Compliance with ethical standards

**Conflict of interest** The authors declare no competing interests.

**Ethical approval** This article does not contain any experiments involving humans or animals that have been performed by any of the authors.

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**Fig. 2** Midpoint-rooted phylogenetic tree based on the complete nucleotide sequences of PYSaV (MK404133) from *P. davidiana* and representative members of the family Tymoviridae. For each node, only values >75% for SH-aLRT and 95% for UFboot are shown above the branches. The viruses included eggplant mosaic virus (EMV, KJ690172), passion fruit yellow mosaic virus (PFYMV, KY823429), grapevine associated tymo-like virus (GaTLV, MH383239), kennedy yellow mosaic virus (KYMV, NC\_001746), oat blue dwarf virus (OBDV, NC\_001793), chayote mosaic virus (ChMV, NC\_002588), maize rayado fino virus (MRFV, NC\_002786),

grapevine fleck virus (GFV, NC\_003347), physalis mottle virus (PhMV, NC\_003634), turnip yellow mosaic virus (TYMV, NC\_004063), dulcamara mottle virus (DMV, NC\_007609), okra mosaic virus (OMV, NC\_009532), scrophularia mottle virus (SMV, NC\_011537), nemesia ring necrosis virus (NRNV, NC\_011538), plantago mottle virus (PIMV, NC\_011539), anagryis vein yellowing virus (AVYV, NC\_011559), and Andean potato latent virus (APLV, NC\_020470). Cactus virus X (AF308158, genus *Potexvirus*, family *Alphaflexiviridae*) was used as an outgroup

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