

Identification of a novel waikavirus infecting *Pittosporum tobira* in China

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Research Article

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Abstract

A new waikavirus, tentatively named *pittosporum tobira waikavirus* (PtWV), was identified in *Pittosporum tobira* plants with symptoms of leaf abnormality and ringspot in Yunnan, China. The complete genome sequence was determined using high-throughput sequencing and rapid amplification of cDNA ends. The RNA genome of PtWV is 12,709 nt in length and contains a large open reading frame (ORF) encoding a polyprotein of 3,669 aa. Furthermore, it includes a smaller ORF that encodes for a 13.2 kDa BVCV-like protein. Phylogenetic analysis and sequence alignment consistently demonstrated that PtWV shares the closest evolutionary relationship and the highest amino acid sequence of the Pro-Pol region identity with *Actinidia yellowing virus 1* (AcYV1). This study represents the first report of a novel waikavirus identified in *P. tobira*.

Full Text

Waikaviruses are monopartite genome-containing secovirids with an approximate genome size of 12 kb that contains at least two well-conserved open reading frames (ORFs) [1]. In general, the genomic RNAs of waikaviruses contain a large ORF (designated as ORF1) encoding for a polyprotein, which is cleaved into seven mature proteins in the order-hypothetical protein (P1), three coat proteins (CP1, CP2, CP3), nucleoside triphosphate-binding protein with helicase motif (HEL), 3C-Pro, and RNA-dependent RNA polymerase (RdRp) [1-2]. The genus *Waikavirus* is widely recognized to have 12 members, of which five have been registered and extensively annotated with coding protein information in the NCBI GenBank database [1-7]. The small overlapping ORF predicted in the +1 frame of ORF1 (designated as ORFX) in waikaviral genomes encodes a 9–14 kDa protein of unknown function. In the genus *Waikavirus*, only *Camellia virus A* has been proven to encode a VPg protein that can be linked to the 5' end [1, 2, 8]. The presence of VPg protein is also predicted in the remaining virus genus members. Furthermore, waikaviruses are usually spread by insects like aphids and leafhoppers [9].

Pittosporum tobira is an ornamental plant with great ecological and medicinal value [10]. Five viruses infecting *P. tobira* have been documented in Asia, Europe, and North America [10-12]. These viruses are identified as *Pittosporum tobira parvo-like virus*, *Pittosporum tobira picorna-like virus*, *Eggplant mottled dwarf virus*, *Pittosporum tobira CRESS virus*, and *Pittosporum tobira opiovirus*. In 2018, *P. tobira* plants exhibiting yellow ringspot and mosaic symptoms (Fig. 1A) were found in Mengzi and Kunming, Yunnan, China, with about 10-30% incidence. Total RNA was extracted from symptomatic *P. tobira* leaves (~100 mg) using an RNAprep Pure Plant Kit (Tiangen, BJ, CN) and subjected to transcriptome sequencing at BioMarker, Inc., China, on an Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA) to identify the potential viral pathogen. A total of 69,055,238 clean reads with a Q30 of 91.56% were obtained after trimming adapter sequences and filtering low-quality sequences using Trimmomatic software [13]. The virus-associated clean reads were assembled de novo into 111,204 contigs using the rnaviralSPAdes method in the SPAdes v3.15.3 software (<https://github.com/ablab/spades>) [14]. Further BLAST analysis showed that three were contigs with lengths of 12,709 nt that shared 36.0% nucleotide sequence identity with the complete genome of

Actinidia yellowing virus 1 (AcYV1) (GenBank no. MN180070). Therefore, the PtWV isolate associated with *P. tobira* yellow ringspot and mosaic disease that was collected in Yunnan province, China, during this study was named 22YN-PiTi.

To verify the presence of PtWV in the sample identified by HTS and determine its complete genome sequence, specific detection primers were designed based on the obtained contigs (Supplementary Table S1). Single-stranded cDNA synthesis and PCR reactions were performed using ABScript III RT Master Mix for qPCR (ABclonal, WH, CN) and 2× M5 HiPer plus Taq HiFi PCR mix (Mei5, BJ, CN), respectively. The 5'- and 3'-terminal sequences of the putative waikavirus were determined by the RACE method using a SMARTer™ RACE cDNA Amplification Kit (Clontech, PA, USA) according to the manufacturer's instructions. The amplified products were ligated into the pTOPO-TA vector (Aidlab, BJ, CN) and sequenced by Sangon Biotech, Inc. (SH, CN). Finally, the viral genome sequence obtained by combining the entire sequence resulting from RNA-Seq and the terminal sequence obtained using the RACE method was assembled using Vector NTI and submitted to the GenBank database with the accession number OR659471. Genomic ORFs and conserved protein domains were predicted using ORF Finder (<https://www.ncbi.nlm.nih.gov/orffinder/>) and the Conserved Domain Database (CDD, <https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>), respectively. Homologous proteins were identified by local BLASTp analysis against viral databases. The genome sequences of other waikaviruses available in the GenBank database were downloaded, and the nucleotide and amino acid sequence identity values were calculated using the Clustal Omega algorithm (<https://www.ebi.ac.uk/Tools/msa/clustalo/>).

The complete genome of PtWV is 12,709 nt in size, excluding the poly (A) tail (Fig. 1B). The 5' untranslated region (UTR) has a length of 962 nt, followed by a long ORF1 (nt 963–11,972), which potentially encodes a large polyprotein (3,669 aa, 403.6 kDa). CDD analysis of the polyprotein revealed seven conserved domains (e-value <0.01): chromosome-anchoring protein RacA (P1) (acc. cl26654; aa 620–711), waikavirus capsid protein 1 (CP1) (cl19643; aa 774–942), Picornavirus capsid protein domain_like (CP2) (cl13999; aa 1000–1,157), Picornavirus capsid protein domain_like (CP3) (cl13999; aa 1,208–1,308), RNA helicase (pfam00910; aa 1,885–1,988), Tungro spherical virus-type peptidase (Pro) (cl13774; aa 2,828–3,033), and RNA-dependent RNA polymerase (RdRp) (cd23169; aa 3,256–3,568). This polyprotein may be proteolytically cleaved by Pro into seven protein products: hypothetical protein P1, three distinct CPs (CP1, CP2, CP3), NTB, 3C-like Pro, and RdRp. Dipeptide cleavage sites for Pro were predicted in the unstructured regions by sequence comparison with other waikaviruses (Fig. 2). Based on BLASTp analysis, it was shown that there are a small waikavirus-associated proteins (ORF2, nt 1,186–1,545) that overlaps with ORF1 in PtWV. The 3' UTR is 763 nt in length.

To further examine the taxonomic position of PtWV, the amino acid sequences of Pro-Pol and the CPs of PtWV and other waikaviruses were aligned using the ClustalW algorithm in MEGA11, and maximum-likelihood (ML) phylogenetic trees were constructed with 1000 bootstrap replicates using MEGA11 under the substitution model LG+G+I, which was chosen as the best-fit substitution model using the 'Find Best DNA/Protein Models' program in MEGA11. A currently classified AcYV was identified

as the closest relative of PtWV. The results revealed that PtWV clusters with AcYV in the same branch, indicating a close phylogenetic relationship (Fig. 2). The demarcation criteria for new species in the family *Secoviridae* are less than 75% amino acid sequence identity in the CPs (two or three) and less than 80% in the Pro-Pol region [15]. The sequence identity of PtWV to other waikaviruses in the CPs (23.4%-43.7%) and the Pro-Pol region (36.8%-50.7%) is less than the threshold values (Supplementary Table S2), suggesting that PtWV is a member of a new species in the genus *Waikavirus*, family *Secoviridae*.

In conclusion, the genomic organization and phylogenetic analysis confirm that PtWV represents a novel species within the genus *Waikavirus*. The discovery of PtWV enriches the understanding of the virome associated with *P. tobira* and underscores the importance of continuous surveillance for emerging plant pathogens."

Declarations

Declarations

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

This article does not contain any experiments involving humans or animals, and no ethical approval was required.

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Data availability

All data included in this study are available upon request by contact with the corresponding author.

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Figures

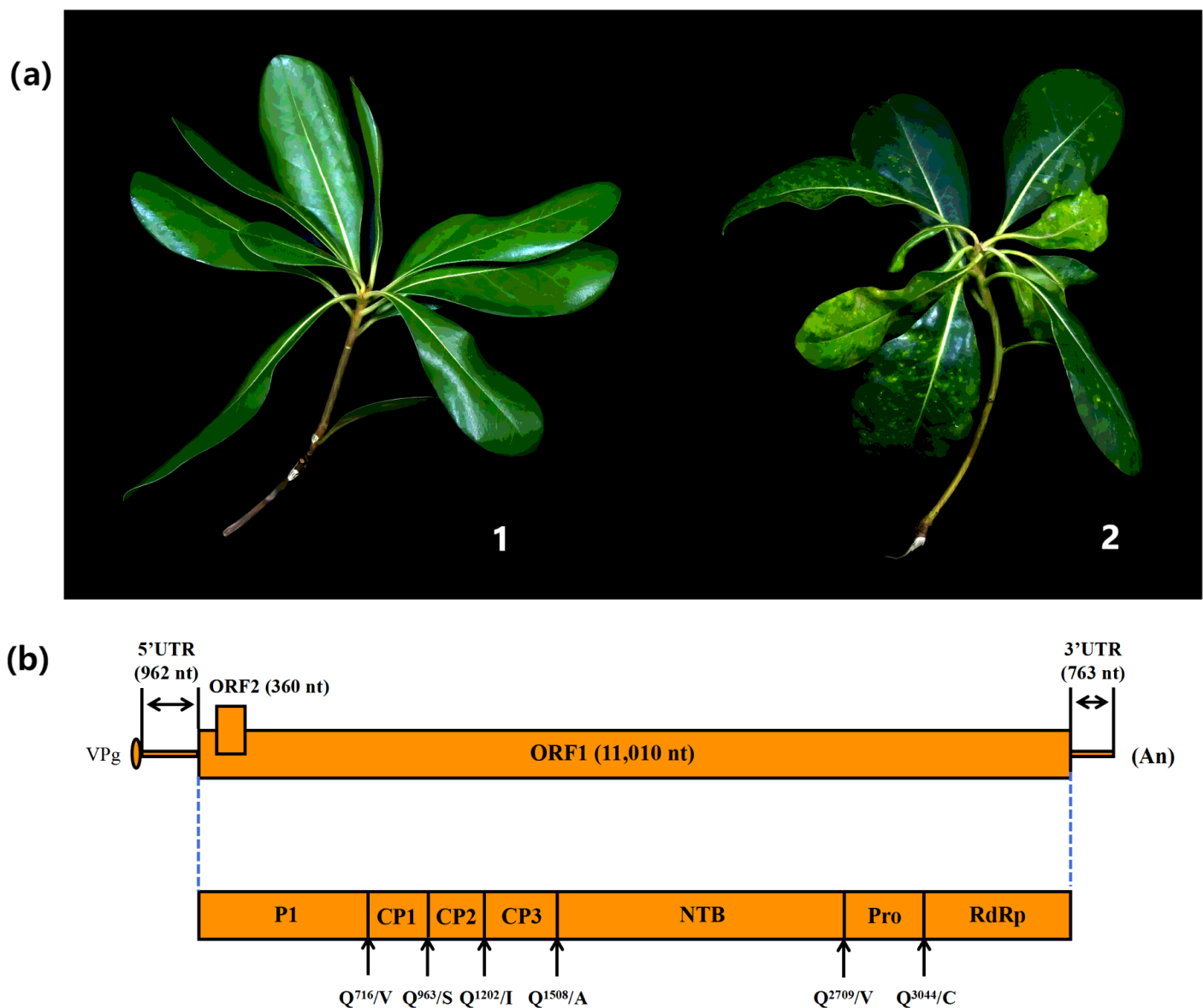


Figure 1

(a1) Samples of *Pittosporum tobira* in healthy condition; (a2) Symptoms on the leaf of a *P. tobira* infected with pittosporum tobira waikavirus (PtWV). (b) Schematic representation of the pittosporum tobira waikavirus genome. The genome contains two ORFs. ORF1 (11,010 nt) encodes a polyprotein (3,669 aa) precursor that is processed into several mature proteins: the P1 protein, three coat proteins (CP1, CP2, CP3), an NTP-binding protein, a 3C-like proteinase (Pro), and an RNA-dependent RNA polymerase (RdRp). The aa residues and positions of the putative cleavage sites are indicated by vertical lines. The seven mature proteins are indicated with their respective predicted sizes. An, poly (A) tail.; UTRs are represented by lines.

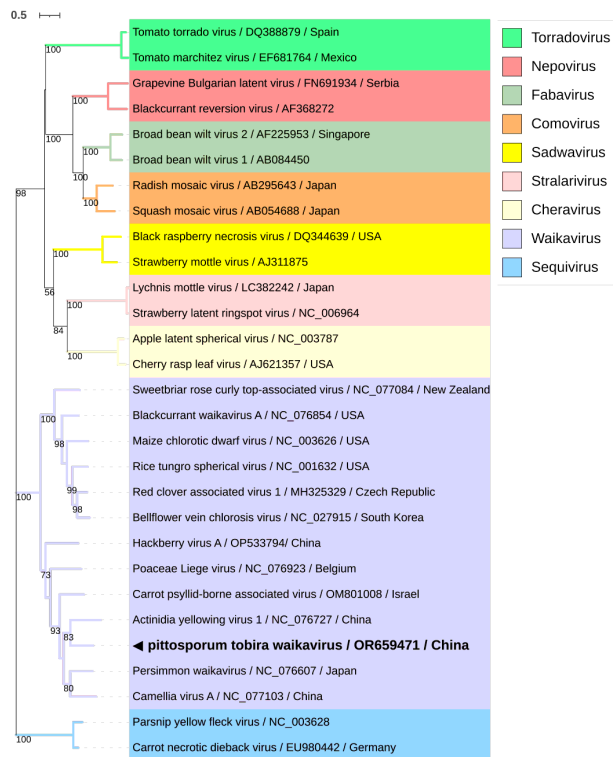


Figure 2

Phylogenetic relationships for *pittosporum tobira waikavirus* (PtWV) and other representative members of the family Secoviridae resulting from analysis of the “Pro-RdRp” amino acid sequences. For phylogenetic trees, the maximum-likelihood (ML) method with the recommended substitution models LG + G + I (“Pro-RdRp”) was applied. Viruses are indicated with different colors for differentiation of genera; bootstrap values (%) are shown at the corresponding nodes; and PtWV is indicated by a red triangle.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Pittosporumtobirawaikavirus.txt](#)
- [Supplementarymaterials.docx](#)