Complete genome sequence of a novel mitovirus isolated from Paris polyphylla var. yunnanensis

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Research Article

Keywords: parmv1, genome, sequence, mitoviruses, paris, mitovirus, structure

DOI: https://doi.org/10.21203/rs.3.rs-890311/v1

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Abstract

Paris mitovirus 1 (ParMV1) is a positive-sense RNA virus isolated from diseased *Paris polyphylla* var. *yunnanensis* plants in Wenshan, Yunnan. The complete genome sequence of ParMV1 consists of 2,751 nucleotides with a genome structure typical of the mitoviruses. ParMV1 genome has a single open reading frame (ORF: 358-2,637 nt) that encodes RNA-dependent RNA polymerase (RdRp) with a molecular mass of 86.42 kDa. ParMV1 contains six conserved motifs (I-VI) that are unique to mitoviruses. In addition, the 5’ and 3’ terminals of the genome have a stable secondary structure, and the reverse complementary sequence forms a panhandle structure. Comparative genome analysis revealed that ParMV1 shares 23.1–40.6% amino acid (aa) and 32.3–45.7% nucleotide (nt) sequence identities with the RdRp of other mitoviruses. The phylogenetic tree inferred from RdRp aa sequence showed that ParMV1 clusters with mitoviruses, and hence should be considered as a new member of the genus *Mitovirus* in the family *Motiviridae*. This is the first report of a novel mitovirus infecting *Paris polyphylla* var. *yunnanensis*.

Introduction

*Paris polyphylla* var. *yunnanensis* is a perennial herb, which has important medicinal and economical values with a huge market [1,2]. With the rapid development of the Chinese traditional medicine industry, the demand for *P. polyphylla* var. *yunnanensis* as a raw material increased greatly. With the aim of meeting market demand, the practice of single planting structure at large-scale artificial planting bases in Qujing, Wenshan, Pu’er and other places in Yunnan has led to the emergence of various viral diseases in *P. polyphylla* var. *yunnanensis*. Currently, Paris mosaic necrosis virus (PMNV), Paris polyphylla virus X (PPVX), pepper mild mottle virus (PMMoV), Paris virus 1 (ParV1), Yunnan paris negative-stranded virus (YPNSV), and Paris virus 2 (ParV2) [3–8] have been reported to infect *P. polyphylla* var. *yunnanensis*.

The genus *Mitovirus* were formerly members of the family *Narnaviridae*, but have now been assigned to the new family *Motiviridae* (https://talk.ictvonline.org/taxonomy/). Which currently contains only one genus. Most of these viruses are tentative species. Five species of the genus *Mitovirus* that have been classified include *Cryphonectria mitovirus 1* (CMV1), *Ophiostoma mitovirus 3a* (OMV3a), *Ophiostoma mitovirus 4* (OMV4), *Ophiostoma mitovirus 5* (OMV5), and *Ophiostoma mitovirus 6* (OMV6) [9–13]. Mitoviruses have small non-segmented positive-sense RNA genomes (generally 2.1 to 4.4 kb) that contain a single ORF encoding RdRp [14–18]. The mitovirus RdRp is recognized to define a conserved protein domain family (pfam05919) and contains six conserved motifs, that may play a role in virus replication and synthesis. In addition, the 5’ and 3’ terminal untranslated regions (UTRs) of mitovirus genomes have variable lengths, and their residues are used as the stem-loop structure for RdRp recognition and initiation of replication [19–21].

Mitoviruses have been reported mostly in phytopathogenic fungal genera, such as *Sclerotinia*, *Ophiostoma*, *Puccinia*, *Rhizoctonia*, *Cryphonectria*, and *Alternaria* and *Botrytis*, with a few from *Thielaviopsis*, *Thanatephorus*, *Helicobasidium*, *Gremmeniella*, arbuscular mycorrhizae glomus and

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ectomycorrhizal fungi.[19–22] Plant mitoviruses, like fungal ones, lack coat protein (CP) and virions. They are usually considered to exist in the host mitochondria in the form of intracellular ribonucleoprotein complexes (RNP), which are transmitted during cell division and fusion without being exposed to the extracellular environment[15,23–24]. This means that they cannot be spread by mechanical inoculation and grafting, but can only rely on seeds to be transmitted vertically[25–26].

Here, we report a new plant mitovirus infecting P. polyphylla var. yunnanensis. This new mitovirus is tentatively named as “Paris mitovirus 1 (ParMV1)”. This study enhances our knowledge on the evolution and classification of plant mitoviruses, and further research on P. polyphylla var. yunnanensis viral disease.

Provenance Of The Virus Material

In July 2019, diseased P. polyphylla var. yunnanensis plants with foliar chlorotic were observed during a survey. To determine the virus species responsible for the infection, samples of the diseased plants were collected in Wenshan, Yunnan and sent to OE biotech (Shanghai, China) for high throughput sequencing (HTS) on the Illumina HiSeq-2500 platform. The raw reads obtained by HTS were filtered and reassembled into contigs using Trinity algorithm. The assembled contigs were then used to performed online BLASTx (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to determine the virus sequence. To confirm the presence of the virus, total RNA was extracted from the symptomatic and asymptomatic leaves using Plant RNA Kit (Omega Bio-tek, USA). The ParMV1-specific primers (ParMV1-1F/R; ParMV1-2F/R; ParMV1-3F/R; ParMV1-4F/R; ParMV1-5F/R; ParMV1-6F/R; ParMV1-7F/R) (Table S1) designed from the virus sequence. Prime ScriptTM II 1st Strand cDNA Synthesis Kit (TaKaRa Bioengineering, Dalian) and 2× Taq PCR Master Mix (BioTeke, Beijing) were used to perform RT-PCR and amplify the complete genome sequence of the virus. The ORF of the virus genome and molecular weight of the protein were predicted by using the NCBI ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder/) and EditSeq program of DNASTAR 7.1. The stem-loop structures in the 3’ and 5’ terminal of the virus genome were predicted using the Mfold Web server (http://unafold.rna.albany.edu/?q=mfold)[9,19]. Multiple sequence alignments and determination of conserved motifs were performed using the ClustalW tool in BioEdit 7.0. Phylogenetic trees were constructed with neighboring joining (NJ) method and 1,000 bootstrap replicates using MEGA 7.0.

Sequence Properties

The 24,886,212 raw reads obtained by HTS were processed and reassembled de novo into transcripts using the paired-end splicing method in Trinity to obtain 1,224 contigs. BLASTx analysis revealed that a contig consisting of 2,751 nt had the highest amino acid sequence identity (44.41%) with Cannabis sativa mitovirus 1 (Accession no. BK010438/ DAB41756). The presence ParMV1 in P. polyphylla var. yunnanensis symptomatic leaves was confirmed by RT-PCR (Fig. 1C).
The complete genome sequence of ParMV1 (Accession no. MT269666) consist of 2,751 nt with an A+U content of 56.05%. ParMV1 contains a single ORF (nt:358-2,637) which is predicted to encode an RNA-dependent RNA polymerase (RdRp) with a molecular mass of about 86.42 kDa, and 5’ and 3’ untranslated regions (UTRs) of 357 bp and 114 bp respectively (Fig. 1A). The complete genome sequence and the size of the 5’- and 3’-UTR fragments are consistent with previously reported plant mitoviruses. In addition, the BLASTx analysis showed that ParMV1 has high sequence similarity with plant mitoviruses (Score 507–599, Query Cover 76–79% and E-value of 0.0). These results indicate that ParMV1 has a typical mitovirus genome structure.

Six highly conserved motifs (I to VI) of mitovirus were identified in ParMV1 by multiple sequence alignments of RdRp amino acid sequences of ParMV1 and other mitovirus, based on the criteria of Bruenn et al. [15,16,27–30]. These motifs include motif-I (307FGKLACAIEGGKKRIFAIGNYVKQRLLRPYHDWSTVLGRIPNDGTYNQL357), Motif-II (368LLYSFGLKSAIDRWP382), Motif-III (427FVTGQPLGYHCSWPLFALSHHWVWWMAA454), Motif-IV (464FQNYALGDIDVADSSVAAQYSIL489), Motif-V (492LGVEISYQKSLVS504), Motif-VI (510EFAKR514) (Fig. 2A). Two potential stem-loop structures with initial ΔG values of -27.4 kcal/mol and -34.5 kcal/mol respectively were predicted in the 5’ and 3’ UTRs of ParMV1 (Fig. 1B). A potential panhandle structure was also predicted with a ΔG value of -13.2 kcal/mol because of the inverted complementarity of the 5’ and 3’ UTRs. These motifs and terminal sequence features (stem-loop and panhandle structures) typical of mitoviruses confirm that ParMV1 is typical of mitovirus. Comparative sequence alignment analysis showed that the RdRp of ParMV1 shared 23.1–40.6% amino acid and 32.3–45.7% nucleotide sequence identities with the species of Mitovirus (Table S2, S3). Cannabis sativa mitovirus 1 isolate (Accession no. BK010438/ DAB41756) had the highest sequence identities (45.7% aa and 40.6% nt) with ParMV1, inferring that ParMV1 is a plant mitovirus.

Mitoviruses were previously thought to infect only fungi, but the detection of complete genome sequences (2.7 to 3.0 kb) of nearly 20 plant mitoviruses in beet, hemp and petunia in the past years indicates that mtoviruses can also infect plants. A number of mitovirus sequences have been identified in the transcriptomes of a large collection of invertebrates [31], but the origin of these viruses still remains unclear. It has been proposed that the mitoviruses either arose from plant genetic elements or by horizontal transfer from fungal mitochondria to plant mitochondria [32–35]. Subsequent studies have suggested that plant mitoviruses are formed by the integration of fungal mitovirus cDNA fragments in the mitochondrial DNA of vascular plants [36]. Systematic analysis of mitoviruses in 10 plant species revealed that plant mitoviruses did not originate from pathogenic fungi [26].

In the diseased P. polyphylla var. yunnanensis leaves from which ParMV1 was detected and isolated, no fungal infection was detected. These diseased plants only exhibited viral-like symptoms. Besides, if ParMV1 had evolved from endophytic fungi, ParMV1 should have clustered with fungal mitoviruses in the clade containing fungal mitoviruses, but not with plant mitoviruses as seen in the RdRp-inferred
phylogenetic tree (Fig. 2B). These results therefore indicate that ParMV1 is a plant mitovirus rather than a fungal mitovirus.

The presence of this novel mitovirus was confirmed by HTS and RT-PCR, combined with complete genome structure analysis, molecular phylogeny and comparative genome sequence analysis. Although studies have shown that fungal mitoviruses can affect the growth and virulence of fungi, leading to morphological abnormalities in mitochondria and growth defects, it is uncertain whether or not mitoviruses have a direct impact on plant health \[^{34,37-38}\]. As a new plant mitovirus, it is uncertain whether ParMV1 directly caused the observed viral disease symptoms in the host plant. Since plant mitoviruses rely on hosts mitochondrial for replication, it is plausible that these viruses may have potential effects on plant hosts mitochondria that may be detrimental to the plant. Further studies are therefore required to ascertain the effects of mitoviruses on their plant host.

**Declarations**

**Compliance with ethical standards**

**Funding**

This study was funded by the National Natural Science Foundation of China (81860774).

**Conflict of interest**

The authors declare that they have no conflict of interest.

**Ethical approval**

This article does not contain any studies with human participants or animals performed by any of the authors.

**Data availability**

The dataset generated during the current study is available in the GenBank database. (Accession no. MT269666)

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**Figures**
Figure 1

(A) Schematic representation of the genomic organization of ParMV1. The open reading frame (ORF) and the untranslated regions (UTRs) are indicated by blue bar and single lines, respectively. The nucleotide positions of the initiation and termination codons are shown above the ORFs. (B) Predicted secondary structures of the 5’ (left) and 3’ termini (middle) of ParMV1. A putative panhandle structure (right) formed by the inverted complementarity at the terminal sequences is also shown. (C) 1% agarose gel
electrophoresis of RT-PCR detected ParMV1. M: DNA molecular weight marker (2000bp); 1F/R-7F/R: specific primers 1-7 amplicons of the complete genome of ParMV1 isolated from P. polyphylla var. yunnanensis diseased leaf samples.

Figure 2

(A) Multiple amino acid sequence alignment of the RNA-dependent RNA polymerase (RdRp) domains of ParMV1 and those of other members in the genus Mitovirus. Asterisks (*) indicate highly similar amino
acid residues. (B) Phylogenetic analysis of the RdRp domains of ParMV1 and other related viruses of the genera Mitovirus and Narnavirus. The scale bar represents a genetic distance of 0.1, values at the branches refer to bootstrap values (%). Individual accession numbers precede each mitovirus name. The blue dots indicate plant mitoviruses, and the red triangle indicates the position of ParMV1. Viral genome sequences were downloaded from NCBI viral genome database (http://www.ncbi.nlm.nih.gov/genome/viruses/).

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