Identification and Complete Genome Sequence of Mulberry Cryptic Virus (Genus *Deltapartitivirus* Family *Partitiviridae*)

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Research Article

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Abstract

A cryptic virus, named *Mulberry cryptic virus* (MuCV), was identified in mulberry (*Morus alba*) transcriptome dataset and RACE methods. The genome sequence of MuCV composed of two double-stranded RNAs (dsRNA) of 1605 bp and 1627 bp in size, encoding an RNA-dependent RNA polymerase (RdRp) and a coat protein (CP), respectively. The 5'-AGAAUUAU-3' sequence in the 5'-UTR was conserved in the two dsRNAs. The sequence similarity and phylogenetic analyses based on the RdRp and CP both indicated that MuCV is a deltapartitivirus (Family *Partitiviridae*).

Main Text

The *Partitiviridae* is a family of viruses that are composed of two dsRNA segments which encode an RNA-dependent RNA polymerase (RdRp) and a coat protein (CP), respectively. Both types of dsRNA molecules are separately encapsidated in non-enveloped isometric particles measuring 29–37 nm in diameter [1,2]. The *Partitiviridae* is presently classified into five genera by host and genome length [3]. The genome length of genus *Deltapartitivirus* are 3.1–3.2 kbps and its hosts are mainly plants. There is only one seed transmission mode for genus *Deltapartitivirus*, which led to the accidental detection of these viruses [4]. The first obtained complete genome sequence was White clover cryptic virus 1 in 2005, which was significantly later than other plant viruses [5]. Now, new partitiviruses of plants, especially deltapartitiviruses, have been found by next-generation sequencing (NGS), and more than 30 complete genome sequences had been found by 2019.

Mulberry (*Morus alba*) is an economically important woody plant grown widely in China, Brazil, India, and several other countries across the globe [6]. Mulberry leaves are the sole feed for silkworm. The yield and quality of mulberry leaves could be seriously affected by the diseases, and then affect the development of sericultural industry. In this study, mulberry leaves with yellow vein symptoms (Fig.1a) were collected from the mulberry garden in Ankang City, Shaanxi Province, China. The total RNA was extracted with TRIzol reagent according to the manufacturer's instructions. Then, the cDNA library was constructed and subjected to NGS. The raw reads were processed by adapter trimming, quality and length trimming, and the clean reads were then used in further analyses.

As a result, some sequences in the clean data of Huaiyin No.4 mulberry sample were found to have 99% identity with RdRp of Hubei partiti-like virus 58 (GenBank accession no. APG78223.1) [7], and the CP gene of Mulberry cryptic virus 1 (GenBank accession no. AWN09390.1) [8]. Then, the primers was designed according to the obtained sequence (Table S1), and the 5'-terminus and the 3'-terminus were amplified using the 5'-RACE and 3'-RACE techniques. The two complete nucleotide sequences of viral genome were spliced by BioEdit 7.2.6, with 1 605 bp and 1 627 bp in size, encoding an RdRp and a CP (GenBank No. MW029480, MW029479), and named Mulberry cryptic virus (MuCV).

To investigate MuCV in different mulberries, leaves from 80 asymptomatic mulberry plants were collected and RNA were extracted using total RNA isolation reagent (Promega). RT-PCR amplifications with specific

primers (Table S1) were performed. The agarose gel electrophoresis and sequencing of PCR products confirmed the presence of MuCV contigs in some asymptomatic Huaiyin No.4 (Fig.1b).

Then, the conserved sequences and secondary structures in the untranslated regions (UTRs) of the 5'-terminus of MuCV RdRp and CP were analyzed. A sequence of 5'-AGAAUUAU-3' was found by BlastN algorithms in the RdRp and CP of MuCV and partial deltapartitiviruses (Fig.2a). Meanwhile, another conserved sequence of 5'-ATAATGATC-3' was found existing in some other members of genus *Deltapartitivirus* (Fig.2b). A stable secondary structure could be formed in the 5'-UTR of MuCV RdRp and cp (Fig.2c,d), and the last four or five bases of conserved region are involved in the structure. The secondary structure was found existing widely in the 5'-UTR of genus *Deltapartitivirus*, which are generally thought to play important roles in the replication process, RdRp recognition, or CP packaging [9].

The online blastp comparison of MuCV RdRp and CP was launched, respectively. The results showed that RdRp of MuCV had 74% identity with that of *Carnation cryptic virus* 3 (CarCV3), 72% with *Beet cryptic virus* 2 (BCV2). CP of MuCV had 54% identity with that of *Carnation cryptic virus* 3 (CarCV3), and 44%~47% identity with *Panax cryptic virus* 2 (PCV2), *Persimmon cryptic virus* (PerCV), and *Beet cryptic virus* 2 (BCV2). Moreover, The multiple sequence alignment (MSA) for RdRp were conducted by T-coffee [10]. RdRp contained several conserved motifs such as “GWS/ARSY/FY” and “PDVGYTRTQL” [11], which is similar to other deltapartitiviruses.

To illustrate the phylogenetic relationship of the MuCV with other deltapartitiviruses, the genome of other 28 members of genus *Deltapartitivirus* were downloaded from GenBank. Phylogenetic trees based on RdRp and CP were separately drawn using Mega X [12]. The MuCV in both trees were adjacent to CarCV3 and BCV2 (Fig.3), which support the taxonomic result of MuCV belonging to genus *Deltapartitivirus*. In addition, the two trees showed a certain consistency, both are divided into two clades, similar to the two types of conserved sequences in 5'-terminus. The site mutation rates of RdRp and CP calculated by the ML method and the JTT model were 0.8134 and 2.3321, respectively. Therefore, RdRp were often used in unraveling the genealogical history of many viruses owing to its low evolution rate [13]. The reasons for the rapid evolution of CP proteins may be due to their structural role, which often needs to adapt to the influence of host [14].

Regarding the pathology of MuCV, further studies are needed to ensure whether MuCV itself can cause any clinical signs, and the effect of MuCV on symptoms in coinfection mulberries.

Declarations

Acknowledgements

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Conflict of interest

All authors declare that they have no conflict of interest.

Ethical statement

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

Consent to participate and publication

All authors confirm that they have read the manuscript and participated in the study.

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Figures

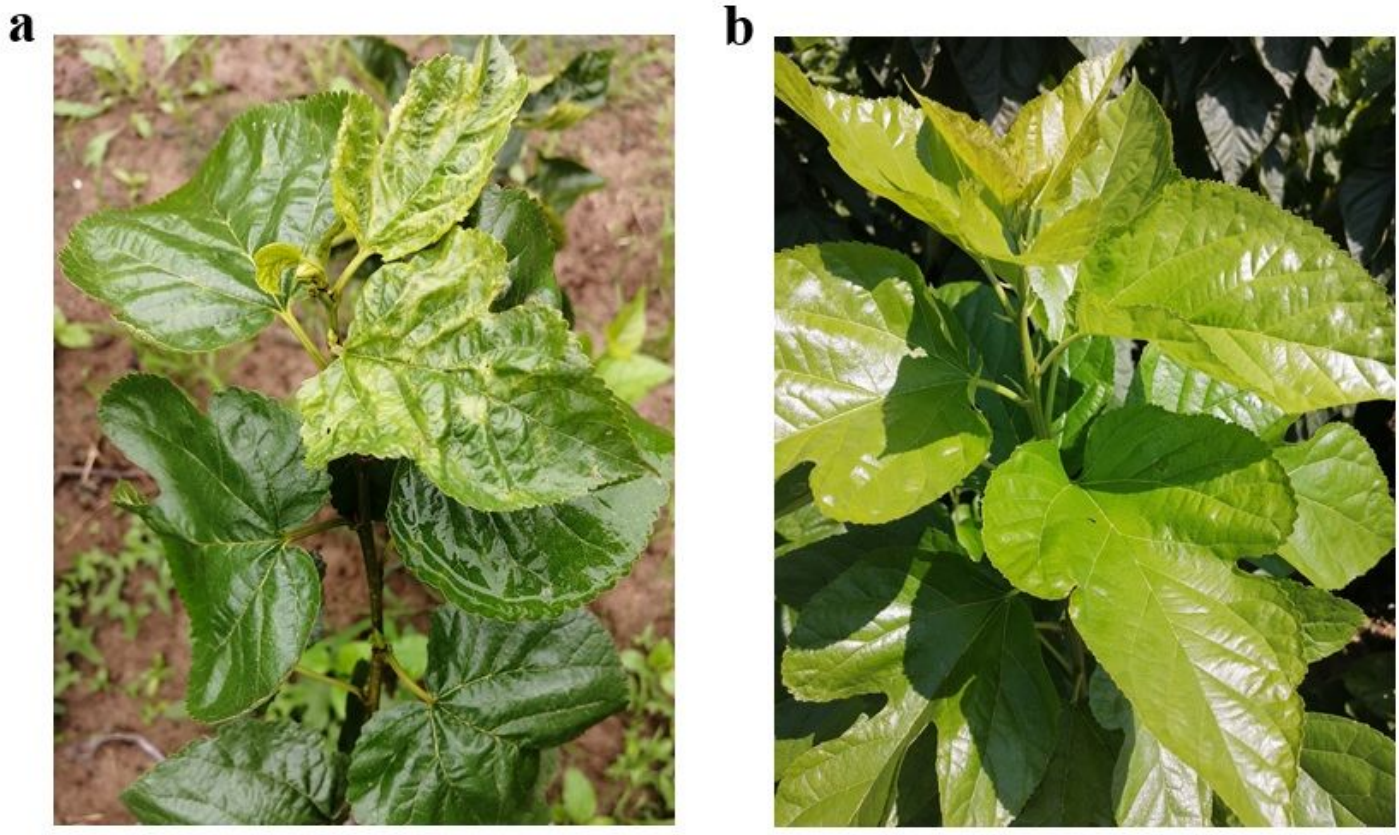

Figure 1

(a) Mulberry with yellow vein symptoms; (b) Mulberry of asymptomatic.

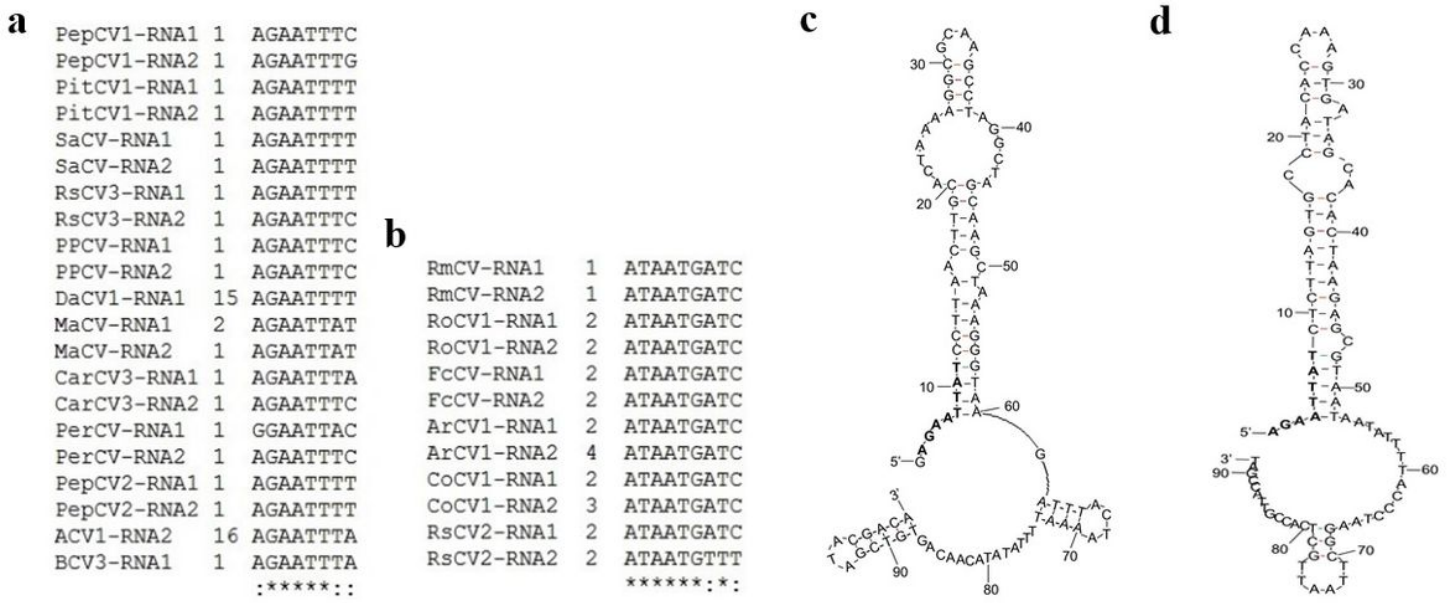

Figure 2

(a and b) The conserved sequences in the 5'-terminus of deltapartitiviruses RNA1 and RNA2; (c and d) The secondary structure in 5'-UTR of MuCV RpRd (c) and cp (d). The bold nucleotides were the conserved sequences.

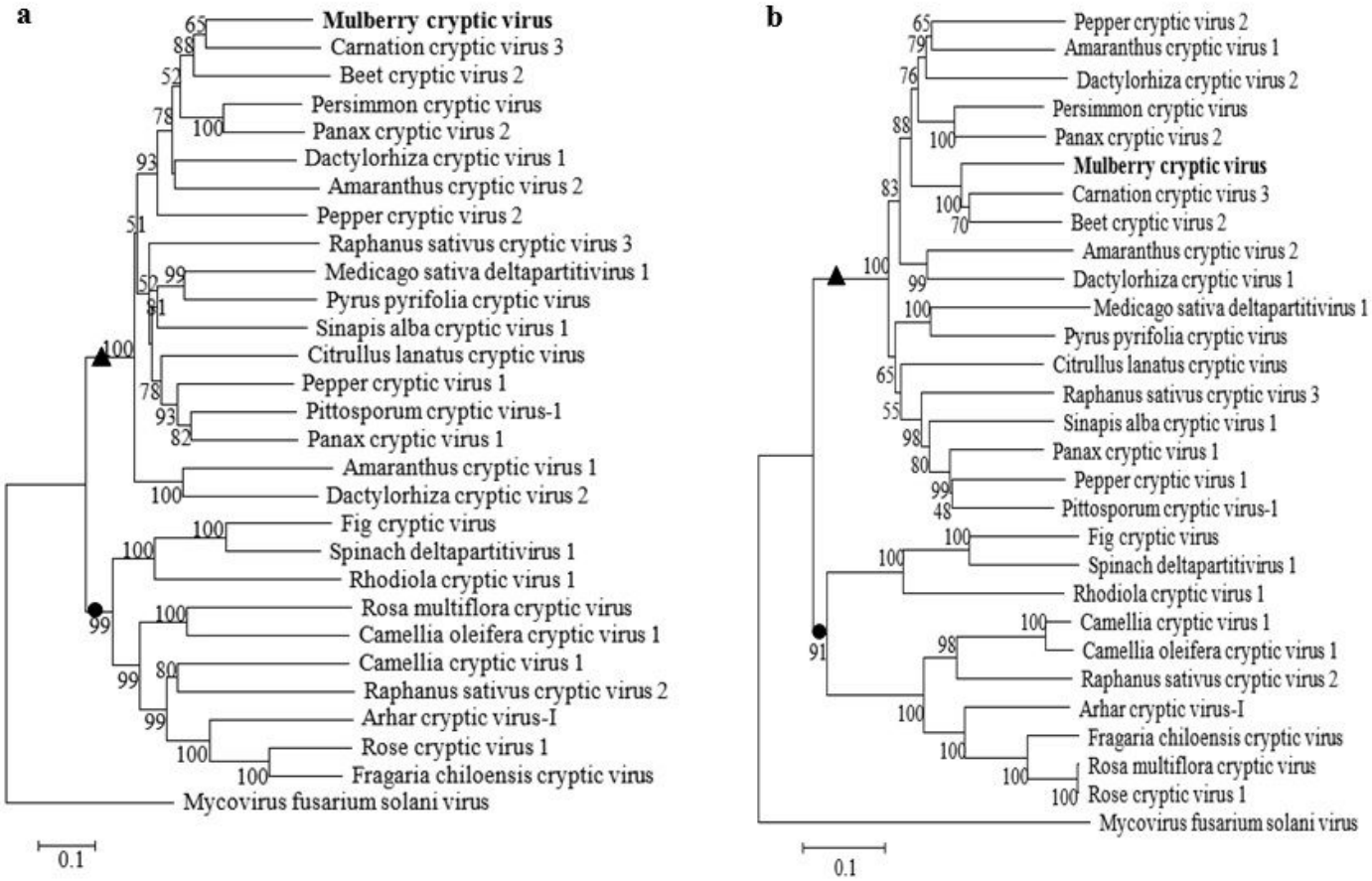

Figure 3

Phylogenetic analysis based on RdRp (A) and CP (B) sequence of deltapartitiviruses. The phylogenetic trees were generated using Maximum Likelihood method by MEGA X with a 1000 bootstrap replications. The Mycovirus fusarium solani virus (MFusoV) genome was used as outgroup. The black triangle and black dot were the two clades in the two trees.

Supplementary Files

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

- Submission2385320.txt
- TableS1.xlsx
- renamed1ca4d.rar