



# Complete nucleotide sequence of a new virus, peach chlorotic leaf spot virus, isolated from flat peach in China

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## Abstract

Contigs with sequence homologies to apple chlorotic leaf spot virus (ACLSV) were identified by high-throughput sequencing analysis in three peach samples. Complete genomic sequences of RP19-1 and RP19-2 of the virus consisted of 7,466 and 7,465 nucleotides (nts), respectively, excluding the poly (A) tails. They shared the highest identity with Ta Tao 5, but lower than 70% of sequence similarity with other ACLSV isolates. Furthermore, phylogenetic analysis revealed that these two isolates clustered with Ta Tao 5, which is distinct from other ACLSV isolates. According to the criteria for species demarcation within the genus *Trichovirus*, these two isolates as well as Ta Tao 5 should be recognized as a new virus species, tentatively named “*Peach chlorotic leaf spot virus*”.

Viruses classified taxonomically as *Apple chlorotic leaf spot virus*, the type species of the genus *Trichovirus* within the family *Betaflexiviridae*, possess a positive-strand RNA genome of approximately 7.5 kb [1]. Apple chlorotic leaf spot virus (ACLSV) viruses have high genetic diversity [2–5]. Sequence similarity between ACLSV isolate Ta Tao 5 and other ACLSV isolates for which complete genome sequences are available is 72–75.6% [6], which is less than the standard percentage assigned for different species in the genus *Trichovirus* [1]. In addition, the ‘Ta Tao 5’ group is

distinguishable from others in a phylogenetic tree of ACLSV [5, 7]. Based on this observation we should be able to assign Ta Tao 5 to a new virus species within the genus *Trichovirus*. On the other hand, the C-terminal part of the CP of Ta Tao 5 has only one aa mutation compared to other ACLSV isolates, and Ta Tao 5 could be detected by ACLSV primers targeting the polymerase [6]. Thus, this question is still open, and the identification of more ACLSV isolates like Ta Tao 5 should be helpful to resolve this question. Here, we identified two new isolates and found that they clustered into the ‘Ta Tao 5’ group.

Three leaf samples, with yellowing and mosaic disease, from flat peaches (cv. ‘Rui Pan 19’) were collected in Beijing in June 2017. They were mixed and RNA sequencing was performed using the Illumina HiSeq 4000 platform (Novogene, Tianjin, China). The obtained reads were assembled to produce contigs using Trinity [8]. BLAST analysis identified two contigs of sizes 7,439 nt and 4,584 nt which were related to ACLSV Ta Tao 5 isolate, with 82% and 81% nucleotide sequence similarity.

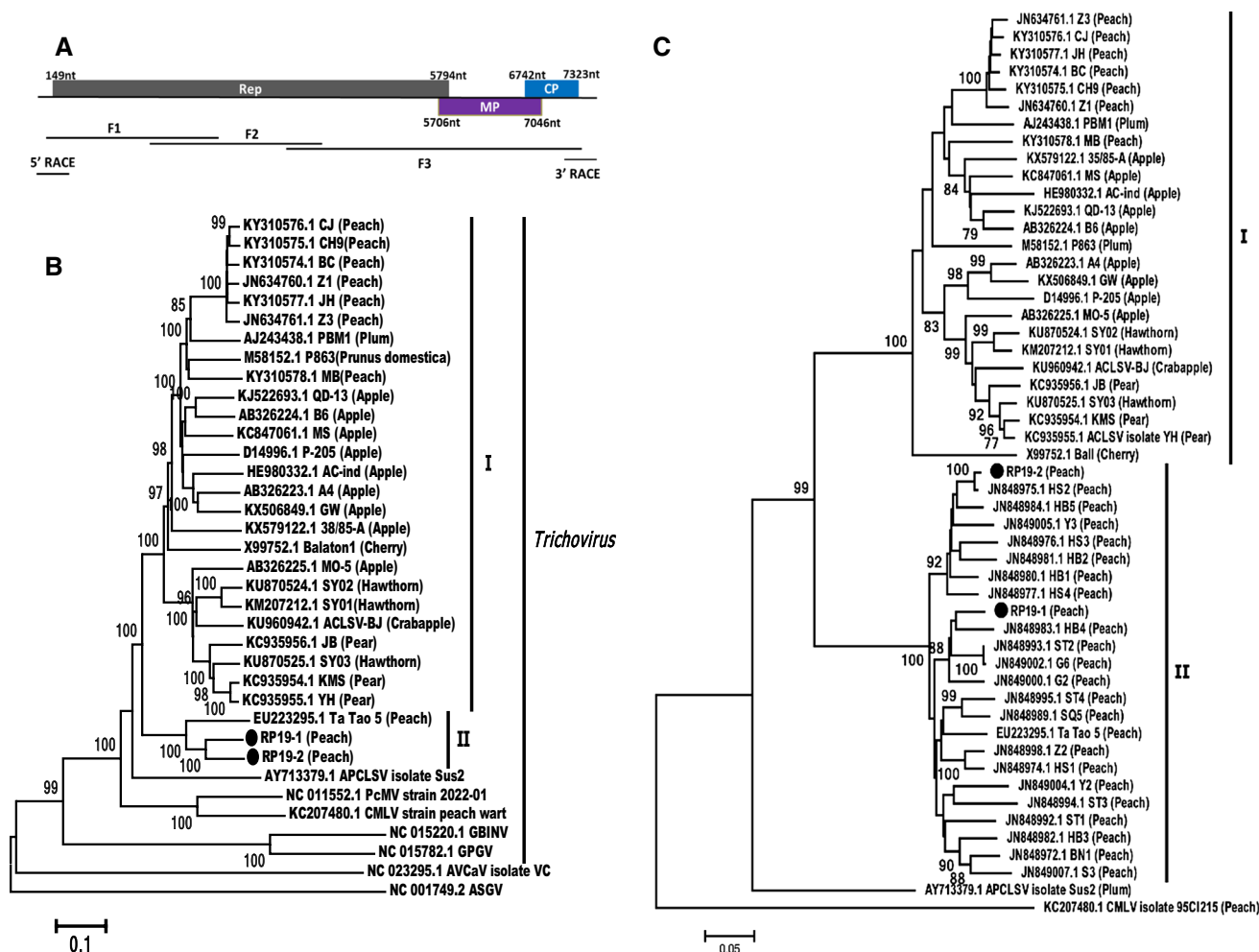
To determine the complete genomic RNA sequence of these ACLSV-like isolates, RT-PCR was performed using the primer sets designed in this study (Fig. 1A, and Table S1). The 5’ and 3’ terminal sequences of genome were determined using a SMARTer® RACE 5’/3’ Kit (Clontech, Dalian, China). Sequencing these fragments, and assembling the contiguous sequence was performed as described previously [7]. Phylogenetic analysis was performed using MEGA 6.06 [9].

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**Fig. 1** Amplification of overlapping fragments of the new isolates and an unrooted phylogenetic tree reconstructed for the genus *Trichovirus*. **A**: Schematic representation of the new isolates' genome organization (drawn to scale) and the regions that were cloned and sequenced; **B**: Neighbor-joining phylogenetic trees based on the complete genome (B) and CP (C) sequences of representative members of the genus *Trichovirus*. Bootstrap values were obtained from 1,000 replications, and bootstrap values < 75% were collapsed. The isolates available in GenBank are followed by their hosts and accession numbers.

The complete genomes of the two isolates were obtained individually and were 7,466 nt for isolate RP19-1 and 7,465 nt for isolate RP19-2, excluding the poly (A) tails (accession numbers MH084695 and MH084696). They contained three overlapping open reading frames (ORFs 1, 2 and 3). ORF 1, ORF 2 and ORF 3 extend from nt 149 to 5794, 5706 to 7046, and 6742 to 7323, respectively. They have 86.5% nucleotide sequence identity with each other and share 79.6% and 79.5% nt identity with Ta Tao 5 (Table 1). However, they have only 66.9%–68.8% and 66.8%–68.7% nucleotide identity with other ACLSV isolates (Tables S2 and S3). The aa sequence of Rep, MP, and CP of RP19-1 and RP19-2 share 87.0–87.3%, 85.2–85.7%, and 95.3–97.4%

identity with Ta Tao 5, but only share 70.8–74.6%, 57.0–61.3%, and 71.5–76.7 identity with other isolates (Table S2 and S3). According to the species demarcation criteria for the genus *Trichovirus*, family *Betaflexiviridae* (less than 72% nt identity or 80% amino acid identity between respective CP or replicase genes) [1], the current species of *Apple chlorotic leaf spot virus* can be split into two, with the new isolates as well as Ta Tao 5 being recognized as representative of a new virus species.

To further reveal the relationship between RP19-1, RP19-2 with ACLSV, a phylogenetic tree of 27 completely sequenced isolates and these two isolates was generated. Phylogenetic analysis showed that these isolates were

**Table 1** Comparison of the complete genome sequence and different genomic regions of RP19-1 (a) and RP19-2 (b) with Ta Tao 5

Isolate	Genome		5' UTR		Rep <sup>a</sup>		MP <sup>a</sup>		CP <sup>a</sup>		3' UTR	
	nt <sup>b</sup>	nt% <sup>c</sup>	nt	nt%	nt	nt% (aa% <sup>bc</sup> )	nt	nt% (aa%)	nt	nt% (aa%)	nt	nt%
(a)												
RP19-1	7466	-	148	-	5646	-	1341	-	582	-	143	-
RP19-2	7465	86.5	148	92.8	5646	85.4 (92.6)	1341	88.9 (89.8)	582	90.4 (95.9)	142	95.5
Ta_Tao_5	7474	79.6	159	80.7	5643	77.8 (87.3)	1341	83.6 (85.2)	582	89.5 (97.4)	143	96.9
(b)												
RP19-2	7465	-	148	-	5646	-	1341	-	582	-	142	-
RP19-1	7466	86.5	148	92.8	5646	85.4 (92.6)	1341	88.9 (89.8)	582	90.4 (95.9)	143	95.5
Ta_Tao_5	7474	79.5	159	80.1	5643	77.7 (87.0)	1341	84.2 (85.7)	582	89.5 (95.3)	143	95.1

a: Rep, replication-associated protein; MP, movement protein; CP, coat protein

b: nt, nucleotide; aa, amino acid

c: nt%, aa%, the nucleotide and amino acid identities between RP19-1 or RP19-2 and other isolates

divided into two distinct clades I and II (Table S4; Fig. 1B, 1C and S1). Clade I included most of the isolates from peach, plum, cherry, apple, pear, and hawthorn. Clade II ('Ta Tao 5' group) only contained RP19-1, RP19-2, and Ta Tao 5. In line with previous reports [5, 7], the 'Ta Tao 5' group is distinguished from others in the phylogenetic tree of ACLSV. Based on evidence of the new isolates and those already published, RP19-1, RP19-2, and Ta Tao 5 should be considered as members of a new species in the genus *Trichovirus*. Due to all isolates of this 'Ta Tao 5' group being isolated from peach, we tentatively name the new virus "*Peach chlorotic leaf spot virus*".

A previous study revealed that many CP gene sequences of ACLSV from peach in China were also clustered into the 'Ta Tao 5' group [7], suggesting that peach chlorotic leaf spot viruses are widespread in China. These results will underpin further understanding of the molecular diversity of this virus and will provide a new insight into its evolution.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that we 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.

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