

[Click here to view linked References](#)

A novel deltapartitivirus from red clover

Paul Telengech¹, Sabitree Shahi¹, Hideki Kondo¹, and Nobuhiro Suzuki¹

1. Institute of Plant Science and Resources, Okayama University, Kurashiki, 710-0046, Japan.

Corresponding author:

Nobuhiro Suzuki

e-mail: nsuzuki@okayama.u-ac.jp

Tel. +81(086) 434-1230

Fax. +81(086) 434-1232

MS information: Abstract, 133 words; Main text, 1160 words excluding references; Figure, 1; Supplemental figures, 3; **Supplementary table, 1.**

Keywords: deltapartitivirus, dsRNA virus, bi-segmented RNA virus, red clover

Sequence data availability. The complete nucleotide genomic sequences of red clover cryptic virus 3 were deposited in the GenBank/ENA/DDBJ database under accession numbers LC652484 and LC652485.

Abstract

The family *Partitiviridae* has five genera among which is the genus *Deltapartitivirus*. We report here the complete genomic sequence of a deltapartitivirus from red clover, termed red clover cryptic virus 3 (RCCV3). RCCV3 has a bi-segmented double-stranded (ds) RNA genome. DsRNA1 and dsRNA2 are 1580 and 1589 nucleotides (nts) in length and would encode RNA-directed RNA polymerase (RdRP) and capsid protein (CP), respectively. RCCV3 RdRP shares the highest sequence identity with a previously reported deltapartitivirus, *Medicago sativa* deltapartitivirus 1 (MsDPV1) (76.5%), while RCCV3 CP show 50% sequence identity to MsDPV1 CP. RdRP- and CP-based phylogenetic trees place RCCV3 into a clade of deltapartitiviruses. The sequence and phylogenetic analyses clearly indicate that RCCV3 represents a new species within the genus *Deltapartitivirus*. RCCV3 was detectable in all three tested cultivars of red clover.

Members of the family *Partitiviridae* are omnipresent in fungi and plants. The family has five established genera: *Alphapartitivirus*, *Betapartitivirus*, *Gammapartitivirus*, *Deltapartitivirus* and *Cryspovirus* [13, 20]. The first two genera accommodate both plant and fungal viruses, while the genera *Gammapartitivirus* and *Deltapartitivirus* exclusively encompass fungal or plant viruses, respectively. Cryspoviruses infect protozoans. A few other genera, such as “Epsilonpartitivirus” and “Zetapartitivirus”, were recently proposed to accommodate fungal and insect viruses [5, 12]. Members of these genera or proposed genera are differentiated by phylogenetic relation, molecular attributes and sequence identities in addition to host organisms. Partitiviruses commonly have a bi-segmented, bi-particulate double-stranded (ds) RNA genome which is encapsidated by a 120-subunit $T=1$ capsid of 25-54 nm in diameter [2, 14].

The genus *Deltapartitivirus* has a relatively small number of members, the genomes of which range from 3.1 kbp to 3.2 kbp [13]. Deltapartitiviruses have been reported in herbaceous and woody plants such as pepper, beet, watermelon, crimson glory vine, and Japanese pear [1, 4, 11, 16, 17, 21]. Deltapartitiviruses show biological properties similar to other plant partitiviruses that include high rates of seed transmission, symptomless nature, very low virus content in infected cells, lack of mechanical transmissibility and cell-to-cell movement. A noteworthy difference between deltapartitiviruses and other partitiviruses is a relatively long size range for their 5' untranslated regions (UTR). During the study of the characterization of diverse fungal and plant partitiviruses initiated in 2008, we discovered a deltapartitivirus from red clover (*Trifolium pratense* L.) designated as red clover cryptic virus 3 (RCCV3). In this study, we report on its molecular characterization and prevalence in red clover variety.

Red clover cultivar “Hokuseki” was grown and maintained in the experimental field of the Institute of Plant Science and Resources, Okayama University. This is part of the virome study of biota associated with the experimental field [7]. Three root samples were collected from three symptomless plants and used for total RNA isolation using TaKaRa RNAiso Plus Reagent (TaKaRa Biotech. Co., Shiga, Japan) as described by the manufacturer. The red clover RNA samples were pooled together with that of three carrot root samples prepared by the same method and subjected to an RNA-seq analysis as a part of root virome analyses in this field. The pooled RNA (18.3 µg, RIN 6.8) was sent to Macrogen Inc. (Tokyo, Japan) for cDNA library construction (TruSeq Stranded Total RNA LT Sample Prep Kit-Plant, Illumina) and pair-end high-throughput sequencing (HTS) (100 bp pair-end reads) using the Illumina HiSeq 2500 platforms (Illumina). After HTS, *de novo* assembly of 48 M pair-end raw reads was performed using the CLC Genomics Workbench (version 11, CLC Bio-Qiagen, Aarhus, Denmark). The assembled sequence contigs (total 97,222) derived from the pooled sample were subjected to local Blast searches against the viral reference sequence (RefSeq) dataset of NCBI. Virus-like contigs related to five plant partitiviruses (so-called cryptoviruses) and two plant RNA viruses (alfalfa mosaic virus and red clover nepovirus A) together

with some mycovirus-like sequences were detected. Among them, a putative deltapartitivirus (RCCV3) was characterized for molecular properties in this study, and other remaining virus-like sequences will be published elsewhere after their thorough characterization.

The contigs 540 (1566 nt, 9457 reads) and 791 (1568 nt, 8267 reads) corresponded to dsRNA1 and dsRNA2 of RCCV3. The contig sequences were confirmed by Sanger sequencing of RT-PCR clones obtained using **contig-specific** primer sets ([Supplementary Table 1](#)). The terminal sequences of the genomic segments were determined by RLM-RACE (RNA ligase mediated rapid amplification of complementary DNA ends) [19]. The complete sequences of both genomic segments are available from the GenBank/ENA/DDBJ databank with their accession numbers LC652484 and LC652485. The genome organization is shown in [Fig. 1A](#). RCCV3 dsRNA1 of 1580 bp encodes a putative **RNA-directed RNA polymerase (RdRP)** of 477 amino acids, while RCCV3 dsRNA2 is 1589 bp in length with a single large ORF encoding a putative CP of 430 amino acids. Phylogenetic analysis with RCCV3 **deduced** RdRP ([Fig. 1B](#)) and CP ([Supplementary Fig. S1](#)) **sequences and their respective protein homologs** showed that the virus is placed in the genus *Deltapartitivirus* with the highest **phylogenetic** affinity to *Medicago sativa* deltapartitivirus 1 (MsDPV1) [6, 10]. Other closely related **putative** deltapartitiviruses, revealed by Blastp search, **included** *Pyrus pyrifolia* cryptic virus [15], *Vitis* cryptic virus [11], and pepper cryptic virus 1 [17]. The sizes of the RCCV3 genome (3.2 kb), encoded proteins (RdRP, 477 amino acids; CP, 430 amino acids), 5'-UTR (95 nts for dsRNA1; 83 nt for dsRNA2) and **3'-UTR (51 nts for dsRNA1; 213 nt for dsRNA2)** fall well within a range expected for a deltapartitivirus. Generally, RdRP-encoding dsRNA1 is larger than CP-encoding dsRNA2 in partitiviruses, while there are a few exceptions reported [3]. RCCV3 has a relatively long 5'-UTR and shares the hepta-nucleotide (5'-AGAAUUU....) in the extreme terminal sequences of both segments ([Supplementary Fig. S2A](#)) and in many other deltapartitiviruses ([Supplementary Fig. S2B](#)). Among five other partitiviral candidates (two alpha-, one beta- and one delta partitiviruses), the betapartitivirus has been confirmed to be present in the red clover samples (data not shown). The contig 19527 (2,415 nt, 533 reads) has potentially derived from the dsRNA1 segment of this virus and has 99.6% sequence identity to crimson clover cryptic virus 2 (CCCV2) dsRNA1 [9]. The putative dsRNA2 sequence could be identified only as partial fragments (~354 nts with low row reads) from the sequence pool.

We tested two red clover cultivars other than “Hokuseki”, **used in the above experiments, to evaluate RCCV3 infection by one-step** RT-PCR with dsRNA1-specific primers 5'-GTCGGAGAAGTTACAGACCTTG-3' (Forward) and 5'-ACTTGGTCCATGTCCGGTGATA-3' (Reverse). As shown in [Supplementary Fig. S3](#), three red clover cultivars, “Taisetsu,” “Natsuyu,” and “Hokuseki” tested positive for RCCV3, whereas white clover (*Trifolium repens* L.) and carrot (*Daucus carota*) were RCCV3-negative.

In this study, we characterized the genome of RCCV3. A few partitiviruses have been reported from red clover, such as red clover cryptic virus 1 (RCCV1) and red clover cryptic virus 2 (RCCV2) [8, 9]. These previously identified partitiviruses, RCCV1 and RCCV2, belong to the genera *Alphapartitivirus* and *Betapartitivirus*, respectively. Partitivirus species demarcation criteria set by ICTV are: $\leq 90\%$ amino acids sequence identity in the RdRP, and $\leq 80\%$ amino acid sequence identity in the CP (Vainio et al., 2018). The closest relative of RCCV3 is MsDPV1, which shares 76.5% and 50% amino acid sequence identities to RCCV3 RdRP and CP, respectively, that are far below the threshold identity scores. Therefore, RCCV3 appears to represent a new species in the genus *Deltapartitivirus*.

Supplementary Information

The online version contains supplementary material available at ----.

Acknowledgements

SS is a JSPS fellow. The authors are grateful to Tokachi Station, National Livestock Breeding Center (NLBC) for generous gift of the three red clover cultivars (Hokuseki, Natsuyu, Taisetsu). PT is thankful to the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan for MEXT scholarship.

Author contributions Conceptualization: N. S., Investigation; P. T., S. S., H. K. Writing - original draft: N. S., P. T. Writing – reviewing & editing: P. T., H. K. Funding acquisition: N. S.

Funding

This study was supported in part by Yomogi Inc., the Ohara Foundation for Agriculture Research (to NS), Grants- in-Aid for Scientific Research on Innovative Areas from the Japanese Ministry of Education, Culture, Sports, Science and Technology (KAKENHI 21H05035, 21K18222, 16H06436, 16H06429 and 16K21723 to NS and HK). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Compliance with ethical standards

Conflicts of interest

The authors declare that there are no conflicts of interest.

Human and animal rights

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

References

1. Accotto GP, Boccardo G (1986) The coat proteins and nucleic acids of two beet cryptic viruses. *Journal of General Virology* 67:363-366
2. Byrne M, Kashyap A, Esquirol L, Ranson N, Sainsbury F (2021) The structure of a plant-specific partitivirus capsid reveals a unique coat protein domain architecture with an intrinsically disordered protrusion. *Commun Biol* 4:1155
3. Chiba S, Lin YH, Kondo H, Kanematsu S, Suzuki N (2016) A novel betapartitivirus RnPV6 from *Rosellinia necatrix* tolerates host RNA silencing but is interfered by its defective RNAs. *Virus Res* 219:62-72
4. Fuji S, Mochizuki T, Okuda M, Tsuda S, Kagiwada S, Sekine KT, Ugaki M, Natsuaki KT, Isogai M, Maoka T, Takeshita M, Yoshikawa N, Mise K, Sasaya T, Kondo H, Kubota K, Yamaji Y, Iwanami T, Ohshima K, Kobayashi K, Hataya T, Sano T, Suzuki N (2022) Plant viruses and viroids in Japan. *J Gen Plant Pathol*:in press
5. Jiang Y, Wang J, Yang B, Wang Q, Zhou J, Yu W (2019) Molecular characterization of a debilitation-associated partitivirus Infecting the pathogenic fungus *Aspergillus flavus*. *Front Microbiol* 10:626
6. Kim H, Park D, Hahn Y (2018) Identification of novel RNA viruses in alfalfa (*Medicago sativa*): an Alphapartitivirus, a Deltapartitivirus, and a Marafivirus. *Gene* 638:7-12
7. Kondo H, Fujita M, Hisano H, Hyodo K, Andika IB, Suzuki N (2020) Virome analysis of aphid populations that Infest the barley field: the discovery of two novel groups of nege/kita-like viruses and other novel RNA viruses. *Front Microbiol* 11:ARTN 509. doi: 510.3389/fmicb.2020.00509
8. Lesker T, Maiss E (2013) In planta protein interactions of three alphacryptoviruses and three betacryptoviruses from white clover, red clover and dill by bimolecular fluorescence complementation analysis. *Viruses* 5:2512-2530
9. Lesker T, Rabenstein F, Maiss E (2013) Molecular characterization of five betacryptoviruses infecting four clover species and dill. *Arch Virol* 158:1943-1952
10. Li J, Gu H, Liu Y, Wei S, Hu G, Wang X, McNeill MR, Ban L (2021) RNA-seq reveals plant virus composition and diversity in alfalfa, thrips, and aphids in Beijing, China. *Arch Virol* 166:1711-1722
11. Nabeshima T, Abe J (2021) High-throughput sequencing indicates novel varicosavirus, emaravirus, and deltapartitivirus infections in *Vitis coignetiae*. *Viruses* 13:827
12. Nerva L, Silvestri A, Ciuffo M, Palmano S, Varese GC, Turina M (2017) Transmission of *Penicillium aurantiogriseum* partiti-like virus 1 to a new fungal host (*Cryphonectria parasitica*) confers higher resistance to salinity and reveals adaptive genomic changes. *Environ Microbiol* 19:4480-4492
13. Nibert ML, Ghabrial SA, Maiss E, Lesker T, Vainio EJ, Jiang D, Suzuki N (2014) Taxonomic reorganization of family *Partitiviridae* and other recent progress in partitivirus research. *Virus Res* 188C:128-141
14. Ochoa WF, Havens WM, Sinkovits RS, Nibert ML, Ghabrial SA, Baker TS (2008) Partitivirus structure reveals a 120-subunit, helix-rich capsid with distinctive surface arches formed by quasisymmetric coat-protein dimers. *Structure* 16:776-786

15. Osaki H, Kudo A, Ohtsu Y (1998) Nucleotide sequence of seed- and pollen-transmitted double-stranded RNA, which encodes a putative RNA-dependent RNA polymerase, detected from Japanese pear. *Biosci Biotechnol Biochem* 62:2101-2106
16. Osaki H, Sasaki A, Nakazono-Nagaoka E, Ota N, Nakaune R (2017) Genome segments encoding capsid protein-like variants of *Pyrus pyrifolia* cryptic virus. *Virus Res* 240:64-68
17. Sabanadzovic S, Valverde RA (2011) Properties and detection of two cryptoviruses from pepper (*Capsicum annuum*). *Virus Genes* 43:307-312
18. Sato Y, Shamsi W, Jamal A, Bhatti MF, Kondo H, Suzuki N (2020) Hadaka virus 1: a capsidless eleven-segmented positive-sense single-stranded RNA virus from a phytopathogenic fungus, *Fusarium oxysporum*. *mBio* 11:e00450-00420. DOI: 00410.01128/mBio.00450-00420
19. Suzuki N, Supyani S, Maruyama K, Hillman BI (2004) Complete genome sequence of Mycoreovirus-1/Cp9B21, a member of a novel genus within the family *Reoviridae*, isolated from the chestnut blight fungus *Cryphonectria parasitica*. *J Gen Virol* 85:3437-3448
20. Vainio EJ, Chiba S, Ghabrial SA, Maiss E, Roossinck M, Sabanadzovic S, Suzuki N, Xie J, Nibert M, Ictv Report C (2018) ICTV Virus Taxonomy Profile: *Partitiviridae*. *J Gen Virol* 99:17-18
21. Xin M, Cao M, Liu W, Ren Y, Lu C, Wang X (2017) The genomic and biological characterization of *Citrullus lanatus* cryptic virus infecting watermelon in China. *Virus Res* 232:106-112

Figure legends

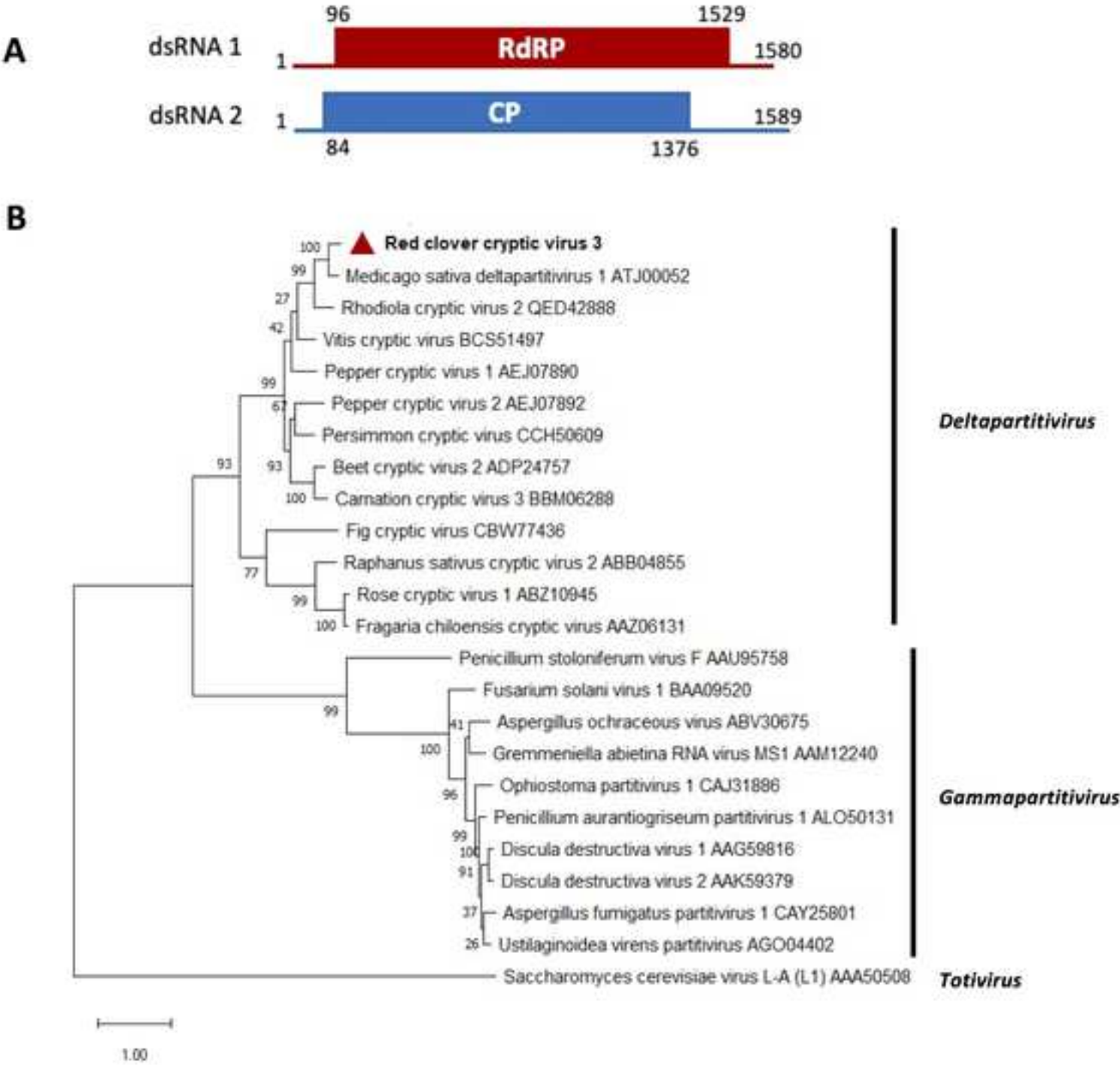
Figure 1. (A) A schematic genome organization of a novel deltapartitivirus, red clover cryptic virus 3 (RCCV3). (B) Phylogenetic relationship of RCCV3 with members of family *Partitiviridae*. Maximum-likelihood phylogenetic tree (a best-fit model LG+G+I) based on an RdRP sequence alignment were generated by MEGA11. The values next to the branches indicate the bootstrap probability for 1000 replicates. GenBank accession numbers follow virus names. The RdRP of a totivirus (*Saccharomyces cerevisiae* virus L-A) was included as an outgroup. RCCV3 is highlighted by a red triangle. The scale bar represents a genetic distance of 1.00 amino acid substitutions per site.

Supplementary Figure S1. Capsid protein-based phylogenetic tree. See the legend to Fig. 1 for a CP amino acid sequence alignment and phylogenetic tree generation.

Supplementary Figure S2. Conserved 5'-terminal sequences of deltapartitiviruses. The 5'-terminal sequences of dsRNA1 and dsRNA2 of red clover cryptic virus 3 (A) and selected members of the genera *Deltapartitivirus* (B). Strictly conserved nucleotides are shaded.

Supplementary Figure S3. Detection of the deltapartitivirus red clover cryptic virus 3, RCCV3. Total RNA was prepared from carrot, white clover and red clover for one-step RT-PCR [18]. PCR products were electrophoresed through a 1% agarose gel in 1X TAE. Lane numbers 1–3 refer to the three red clover cultivars, Taisetsu, Natsuyu, and Hokuseki, respectively. M indicates GeneRuler 1-kb DNA ladder (Thermo Fisher Scientific., Inc., Waltham, MA, USA). The arrow on the right points to the RT-PCR product of RCCV3 (576 bp).

Figure 1



Supplementary Data for
A novel deltapartitivirus from red clover

Paul Telengech¹, Sabitree Shahi¹, Hideki Kondo¹, and Nobuhiro Suzuki¹

1. Institute of Plant Science and Resources, Okayama University, Kurashiki, 710-0046, Japan.

Corresponding author:

Nobuhiro Suzuki

e-mail: nsuzuki@okayama.u-ac.jp

Tel. +81(086) 434-1230

Fax. +81(086) 434-1232

Supplementary figures, S1 to S3

Supplementary table, 1

Legends to Supplementary Figures

Supplementary Figure S1. Capsid protein-based phylogenetic tree. See the legend to Fig. 1 for a CP amino acid sequence alignment and phylogenetic tree generation.

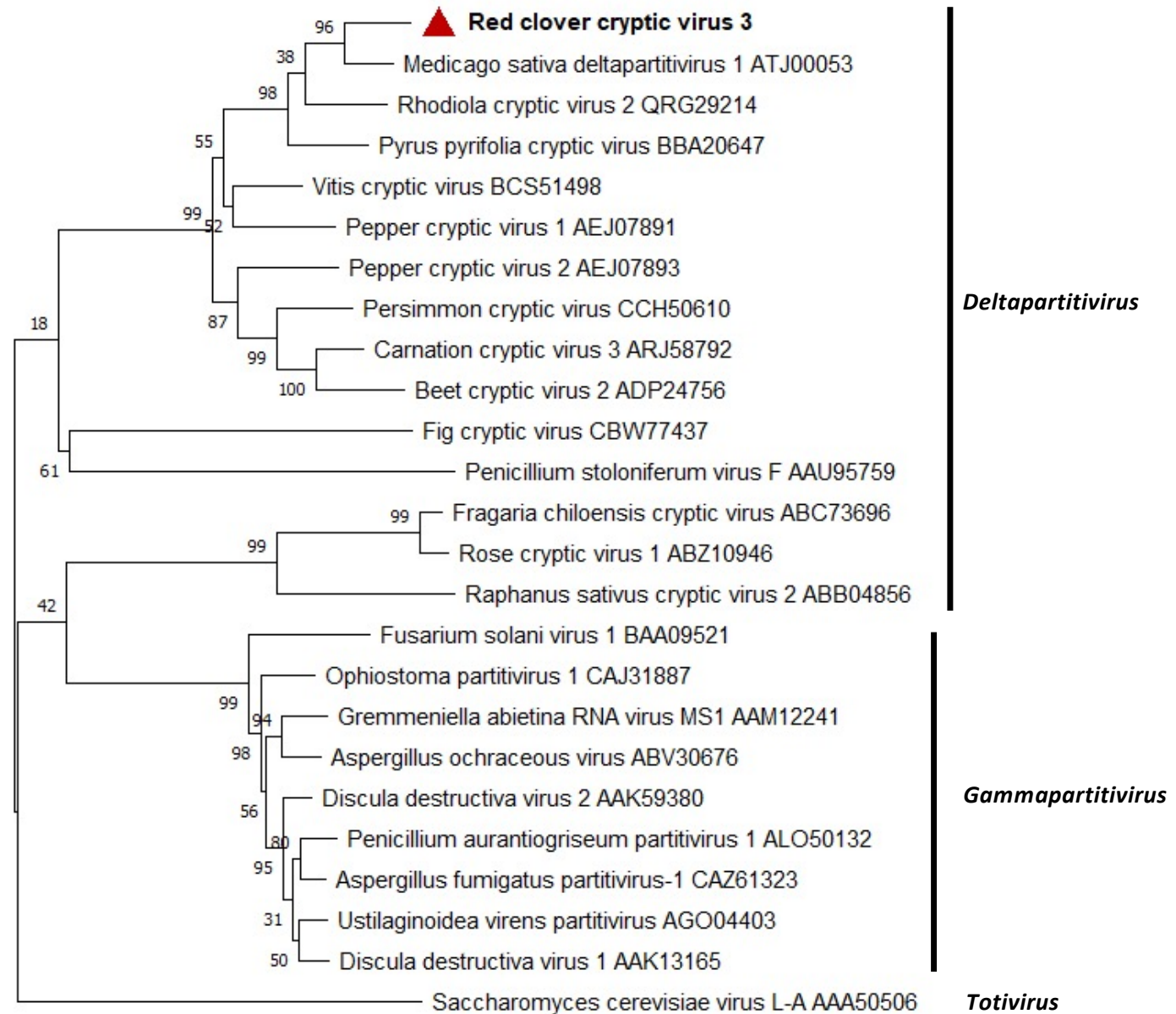
Supplementary Figure S2. Conserved 5'-terminal sequences of deltapartitiviruses. The 5'-terminal sequences of dsRNA1 and dsRNA2 of red clover cryptic virus 3 (A) and selected members of the genera *Deltapartitivirus* (B). Strictly conserved nucleotides are shaded.

Supplementary Figure S3. Detection of the deltapartitivirus red clover cryptic virus 3, RCCV3. Total RNA was prepared from carrot, white clover and red clover for one-step RT-PCR [1]. PCR products were electrophoresed through a 1% agarose gel in 1X TAE. Lane numbers 1–3 refer to the three red clover cultivars, Taisetsu, Natsuyu, and Hokuseki, respectively. M indicates GeneRuler 1-kb DNA ladder (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The arrow on the right points to the RT-PCR product of RCCV3 (576 bp).

1. Sato Y, Shamsi W, Jamal A, Bhatti MF, Kondo H, Suzuki N (2020) Hadaka virus 1: a capsidless eleven-segmented positive-sense single-stranded RNA virus from a phytopathogenic fungus, *Fusarium oxysporum*. mBio 11:e00450-00420. DOI: 00410.01128/mBio.00450-00420

Supplementary Fig. S1

CP



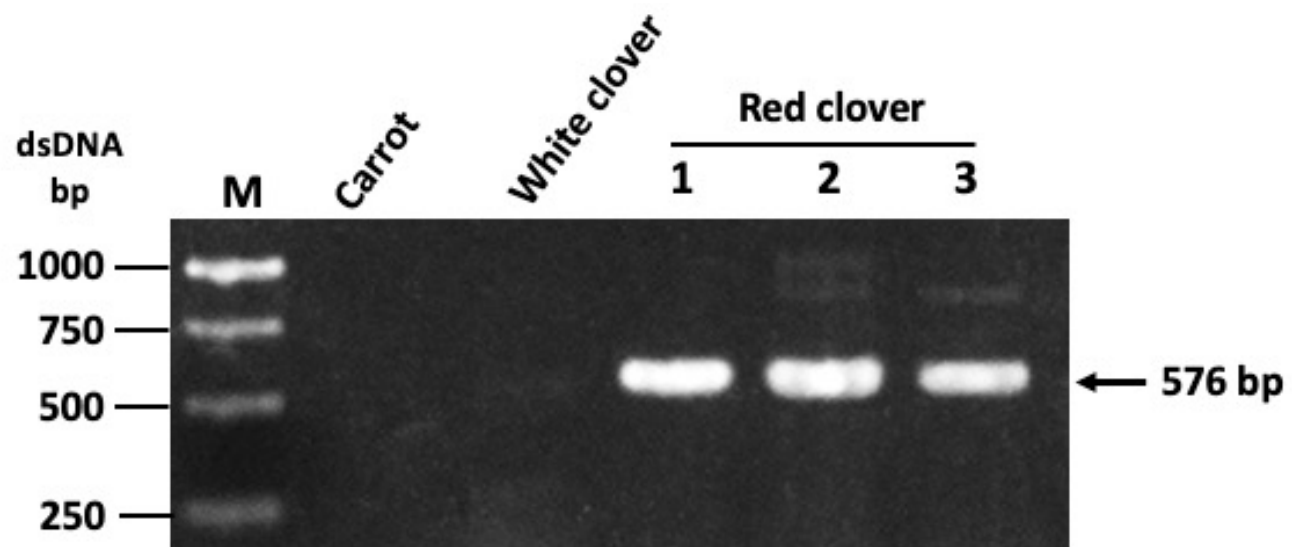
Supplementary Fig. S2

A dsRNA1 5' -AGAAUUUAAACCAGUCGUAAAC--
dsRNA2 5' -AGAAUUUCUCGCAACUACGUAU--

B

Red clover cryptic virus 3 dsRNA1	AGAAUUUA
Red clover cryptic virus 3 dsRNA2	AGAAUUUC
Pepper cryptic virus 1 dsRNA1	AGAAUUUC
Pepper cryptic virus 1 dsRNA2	AGAAUUUG
Pepper cryptic virus 2 dsRNA1	AGAAUUUU
Pepper cryptic virus 2 dsRNA2	AGAAUUUU
Beet cryptic virus 2 dsRNA1	AGAAUUAU
Beet cryptic virus 2 dsRNA2a	AGAAUUAU
Beet cryptic virus 2 dsRNA2b	AGAAUUUC
Persimmon cryptic virus dsRNA1	GGAAUUAC
Persimmon cryptic virus dsRNA2	AGAAUUUC
Carnation cryptic virus 3 dsRNA1	AGAAUUUA
Carnation cryptic virus 3 dsRNA2	AGAAUUUC
Rhodiola cryptic virus 2 dsRNA2	AGAAUUUA

Supplementary Fig. S3



Supplementary Table S1. List of primers used in this study

No.	Primer name	Primer sequences (5'- 3')	Purpose	Target virus
1	Ca Delta Confirm F	GTCGGAGAAGTTACAGACCTTG	RT-PCR	RCCV3
2	Ca Delta Confirm R	ACTTGGTCCATGTCCGGTGATA	RT-PCR	RCCV3
3	RC delta CP F	GCCGCTATTAATTCTGGTATCCG	RT-PCR	RCCV3
4	RC delta CP R	GCTGTTGTGTGTTGAGCAGTGAA	RT-PCR	RCCV3
5	Car Delta RdRp 5T	CGCACTGATAACCATAGCCAG	RLM-RACE	RCCV3
6	Car Delta RdRp 3T	CGACACATTCATCCCACCAGA	RLM-RACE	RCCV3
7	Car Delta Cp 5T	ATGTGAGTGGGGCGGATAC	RLM-RACE	RCCV3
8	Car Delta Cp 3T	GACATTCTGCCCCTGAAGGT	RLM-RACE	RCCV3
9	RC/carrot beta F	ACCTCTGTTGATTCCGACCCAAAT	RT-PCR	CCCV2
10	RC/carrot beta R	ATGTGGTTGCGGGTGAACAGTAA	RT-PCR	CCCV2