

# Identification of two putative novel deltapartitiviruses and an enamovirus in coriander transcriptomes

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## Short Report

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## Abstract

Coriander is a herbaceous spice and condiment crop also known for its medicinal properties. The present study identified two putative novel deltapartitiviruses and an enamovirus tentatively named as *Coriandrum sativum* deltapartitivirus 1, 2 (CsDPV1, 2) and *Coriandrum sativum* enamovirus (CsEV) in the publicly available transcriptome-assembled contigs derived from coriander grown in India. CsDPV1 and 2 contained tripartite and bipartite genomes, respectively with each genome segment encoding for a single ORF. CsEV contained five ORFs encoding for proteins P0, P1, P1–2, P3 and P3–5. Phylogenetic analysis revealed three distinct subgroups of deltapartitiviruses wherein CsDPV1 and 2 grouped in subgroup 3 and 1, respectively while CsEV formed a distinct sub-clade within enamoviruses. Further, presence of CsDPV2 in fruit samples of one of the cultivars from where the virus was identified was validated through RT-PCR assay and Sanger sequencing. The study highlights the need for further studies on understanding the importance and biological properties of identified novel viruses.

## Full Text

*Coriandrum sativum* L., commonly known as coriander, is an economically important herbaceous spice crop belonging to the family Apiaceae. Though native to the Mediterranean and Middle East, the crop is widely cultivated in different regions of the world. Leaves of coriander are generally used as flavouring agents in continental preparations like curries and soups while its fruits are used as condiments in pickle and curry powder preparation. Besides culinary uses, coriander fruits are valued for their medicinal properties in Ayurvedic medicine. Products like oleoresins and volatile oil derived from coriander fruits have huge demand in the global market (Sharma and Sharma, 2012, Choudhary et al., 2019). India is among the leading producer and exporter of coriander in the world (Sharma and Sharma, 2012).

In recent times, novel plant viral sequences are being increasingly discovered by probing the plant transcriptome-assembled contigs available in Transcriptome Shotgun Assembly (TSA) database of National Centre for Biotechnology Information (Sidharthan et al., 2022a, b, c, Sidharthan et al., 2023, Bejerman et al., 2021, Bejerman et al., 2022, Bejerman and Debat, 2022). In the present study, we mined the transcriptome-assembled contigs derived from leaves and fruits of three coriander cvs. AgCr-1, CO-2 and Pant haritma grown in India, and deposited in the NCBI under the Bioproject PRJNA472685 for putative novel viral sequences. Contigs were retrieved from NCBI, imported into Galaxy Australia sever (Community, 2022) and subjected to BLASTN search (evaluate cutoff: 1e-5) against the reference viral genomes downloaded at <https://ftp.ncbi.nlm.nih.gov/refseq/release/viral/>. Contigs longer than or equal to 500 nt and sharing similarity with known plant viruses were only regarded as plant viral contigs. The longest contig of an identified putative novel virus across libraries was regarded as genome/genome segment of that virus. Open reading frame (ORF) prediction in viral genomes, motif, transmembrane helix (TMH) prediction in and molecular weight estimation of viral genome-encoded proteins were performed as described in Sidharthan and Baranwal, 2021 while – 1 ribosomal frameshift site was predicted as described in Sidharthan et al., 2022a. Phylogenetic trees were constructed after MUSCLE alignment of viral protein sequences in MEGA7 v 7.0.26 (Kumar et al., 2016) using neighbourhood-joining (NJ) method and Poisson model with 1000 bootstrap replicates. Total RNA was isolated from fruits samples of coriander cvs. CO-2 and Pant haritma derived from the same lots that were originally used for transcriptome sequencing using Spectrum™ Plant Total RNA kit (Sigma, USA) following manufacturer's protocol. cDNAs were synthesized from the isolated 500 ng RNA using 2µM of each random hexamers and oligo-dT primers using FIREScript RT cDNA synthesis kit (Solis Biodyne, Estonia) following manufacturer's protocol. To detect *Coriandrum sativum* deltapartitivirus 2 (CsDPV2), reverse transcriptase polymerase chain reactions (RT-PCR) were performed in 25µL final reaction volume containing 50 ng of cDNA, 0.4 mM of each forward (5'-ATCCACCGTTTCATCACA-3') and reverse (5'-TGCTCTGTAAGCCGAAATC-3') primers (designed from the recovered CsDPV2 RNA1 sequence) and 1X Dream Taq PCR master mix polymerase (Thermo Scientific) with the following cycling conditions: initial denaturation at 95°C for 3 min, 35 cycles of 95°C for 30 s, 58°C for 45 s, 72°C for 45 s followed by a final denaturation step at 72°C for 10 s in a thermalcycler (Eppendorf Mastercycler nexus GX2 PCR system). PCR amplicons were visualized in 1.8% agarose gel, eluted, cloned and sequenced as described in Damini et al., 2023.

Contigs of 12 viruses, including three putative novel viruses tentatively named as *Coriandrum sativum* deltapartitivirus 1 (CsDPV1), CsDPV2 and *Coriandrum sativum* enamovirus (CsEV), were identified across libraries. Library SRR7212291 housed the maximum number of viruses (8) while SRR7212212 library had the least number of viruses (2) (Table 1).

CsDPV1 possessed tripartite genome designated as RNA1–3, each containing a single large ORF. CsDPV1 RNA1 (1699 nt) ORF encodes for an RNA-dependent RNA polymerase (RdRp) (PF00680) motif-containing 475 aa (54.91 kDa) protein that shared a maximum of 72.36% aa identity [at 99% query coverage(qcov)] with RdRp sequences of Camellia cryptic virus 1 (CCV1) (UZZ64748, UZZ64751, QED42868). CsDPV1 RNA2 (1422 nt) ORF encodes for a 340 aa (37.83 kDa) protein that shared 33.63% aa identity (at 100% qcov) with CCV1 coat protein (CP) 1 sequence (UZZ64765) while CsDPV1 RNA3 (1416 nt) ORF encodes for a 352 aa (38.99 kDa) protein that shared 43.30% aa identity (at 99% qcov) with CCV1 coat protein (CP) 2 sequence (UZZ64791). No motif was predicted in the encoded CP sequences when searched against Pfam database.

CsDPV2 possessed bipartite genome designated as RNA1 and 2, each containing a single large ORF. ORF predicted in CsDPV2 RNA1 (1594 nt) encodes for an RdRp (PF00680) motif-containing 479 aa (54.54 kDa) protein that shared 73.74% aa identity (99% qcov) with RdRp sequence of Panax cryptic virus 1 (PCV1) (QED42879). ORF predicted in CsDPV2 RNA2 (1413 nt) encodes for a 407 aa (46.25 kDa) protein that shared 44.95% aa identity (100% qcov) with PCV1 CP sequence (QED42881). No Pfam motif was predicted in the CsDPV2 CP sequence. Phylogenetic analysis based on RdRp and CP sequences revealed three subgroups of deltapartitiviruses namely subgroup 1–3. Subgroup 1 contained bipartite viruses except beet cryptic virus 2 while subgroup 3 contained tripartite viruses. Subgroup 2 that included fig cryptic virus and spinach deltapartitivirus 1 with bipartite genomes was related to subgroup 3 viruses than subgroup 1 viruses. CsDPV1 with tripartite genome grouped with subgroup 3 deltapartitiviruses while CsDPV2 with bipartite genome grouped with subgroup 1 deltapartitiviruses (Fig. 1). Based on the species demarcation criteria of the genus *Deltapartitivirus* ( $\leq 90\%$  RdRp and  $\leq 80\%$  CP aa sequence identities with known members) (Vainio et al., 2018), CsDPV1 and CsDPV2 can be regarded as putative novel deltapartitivirus members.

Proteins encoded by five predicted ORFs in CsEV genome (5415 nt) are designated as P0, P1, P2, P3 and P5. P0 (328 aa; 37.55 kDa) that shared no significant similarity with any of the existing sequences in GenBank contained no predicted Pfam motif. P1 (818 aa; 91.90 kDa) contained peptidase S39 motif (PF02122), and shared 26.49% aa identity (96% qcov) with P1 sequence of Celmisia lyallii enamovirus (CIEV) (DAZ87600). P1–P2 fusion protein (1245 aa; 140.12 kDa), expressed via a -1 ribosomal frameshifting at 2127 nt due to the presence of slippery sequence (2121-TTTAAA-2127), contained peptidase S39 (PF02122) and RdRp (PF02123) motifs, and shared 38.62% aa identity (98% qcov) with P1–P2 sequence of CIEV (DAZ87601). Similar to CIEV (Sidharthan et al., 2022a), five TMHs were predicted in the N-terminal region of P1 and P1–P2 protein sequences. P3 (191 aa; 21.28 kDa) contained luteovirus CP motif (PF00894), and shared 36.08% aa identity (96% qcov) with grapevine enamovirus (GEV-1) P3 sequence (UBK09883). P3–P5 (414 aa; 46.68 kDa), possibly produced by translational read-through of stop codon of ORF3, contained luteovirus CP (PF00894) and potato leaf roll virus read-through protein (PF01690) motifs, and shared 35.00% aa identity (98% qcov) with GEV-1 P3–P5 sequence (UBK09882). Phylogenetic analysis based on the CP sequences placed CsEV in a distinct sub-clade among the enamoviruses (Fig. 2). Based on the species demarcation criteria for the genus *Enamovirus* ( $> 10\%$  amino acid sequence divergence of any of the encoded proteins with known members) (Sõmera et al., 2021), CsEV that shared  $> 10\%$  amino acid sequence divergence of all the encoded proteins with known members can be regarded as a putative novel enamovirus.

Reverse transcription polymerase chain reaction (RT-PCR) amplification of identified putative novel viral sequences in RNA isolated from fruit samples of cvs. CO-2 and Pant haritma obtained from the same lots that were used for transcriptome sequencing yielded a desired amplicon of 583 bp in CO-2 sample for CsDPV2 RNA1. Amplified sequence (accession number: OQ506517) shared 98.1% nt sequence identity with the recovered CsDPV2 RNA1 sequence. Other identified novel viral sequences could not be amplified from the RNA isolations. This might be because of the long-term storage of fruits or lesser proportion of infected fruits in the lot. Based on the consensus statement report by Simmonds et al. (2017), the identified putative novel viruses in the current study can be regarded as *bona fide* ones. Further studies on understanding the importance and biological properties of identified putative novel viruses are needed.

## Declarations

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## Author contributions

V. Kavi Sidharthan: conceptualization, methodology, formal analysis and investigation, writing- original draft preparation; Damini Diksha: formal analysis and investigation; Ravindra Singh: resources, writing- review and editing; Sharda Choudhary: resources, writing- review and editing; Mahantesha B. N. Naika: resources, writing- review and editing; V. K. Baranwal: conceptualization, resources, supervision, writing- review and editing.

## Data availability

The viral genome sequences described in the study are submitted to NCBI Third Party Annotation database, and are provided in the ESM\_1.

## Compliance with ethical standards

## Conflict of interest

The authors declare that they have no conflicts of interest.

## Research involving human participants and/or animals

This work does not contain any animal or human participants.

## Informed consent

This work does not contain any animal or human participants.

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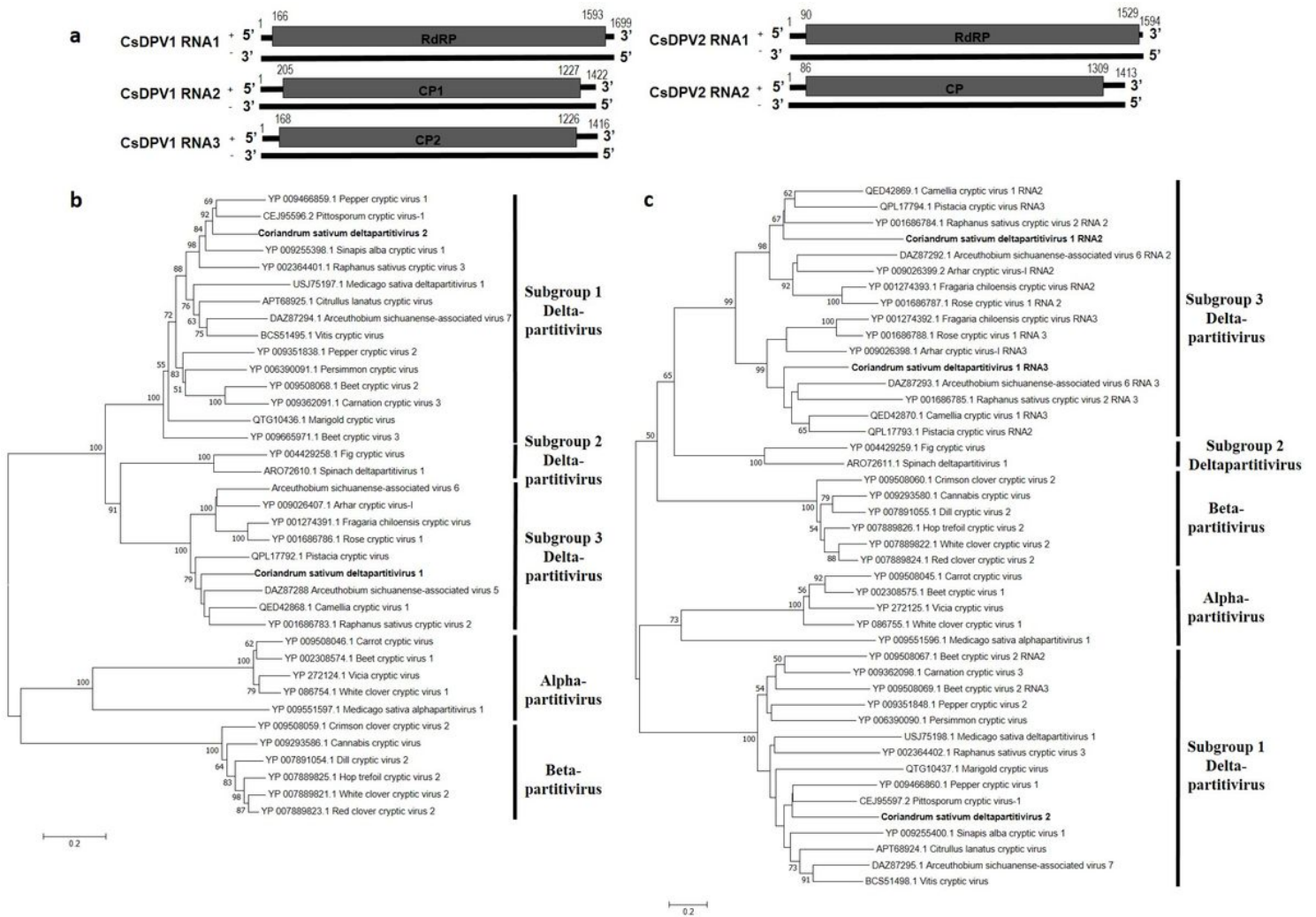
## Table

Table 1  
Summary of plant viruses identified in transcriptome-assembled contigs of coriander

Bioproject ID	TSA accession from where the viral contigs were identified	SRA ID	Biosample ID	Cultivar	Tissue	Viruses identified	Novel viral genome/ genome segment recovered <sup>b</sup>
PRJNA472685	GGPO01000000	SRR7212291	SAMN09240492	Pant haritma	Fruit	CsDPV1 <sup>a</sup> , CsEV <sup>a</sup> , CsVLRA, VDMV, PVX, PVS, CCYV, CMV	Nil
	GGPG01000000	SRR7212212	SAMN09240486	Pant haritma	Leaf	CsVLRA, VDMV	Nil
	GGPN01000000	SRR7212214	SAMN09240491	CO-2	Fruit	CsDPV1 <sup>a</sup> , 2 <sup>a</sup> , CsEV <sup>a</sup> , CsVLRA, VDMV, PVX, M, LMV	CsDPV1 RNA1 (GGPN01005130), RNA3 (GGPN01000159), CsDPV2 RNA1 (GGPN01021718), CsEV (GGPN01011425),
	GGPM01000000	SRR7212211	SAMN09240485	CO-2	Leaf	CsDPV2 <sup>a</sup> , CsVLRA, VDMV, LMV	Nil
	GGPF01000000	SRR7212185	SAMN09240482	AgCr-1	Leaf	CsDPV1 <sup>a</sup> , 2 <sup>a</sup> , SSCV, LMV, PVS	CsDPV1 RNA2 (GGPF01002528), CsDPV2 RNA2 (GGPF01001296)

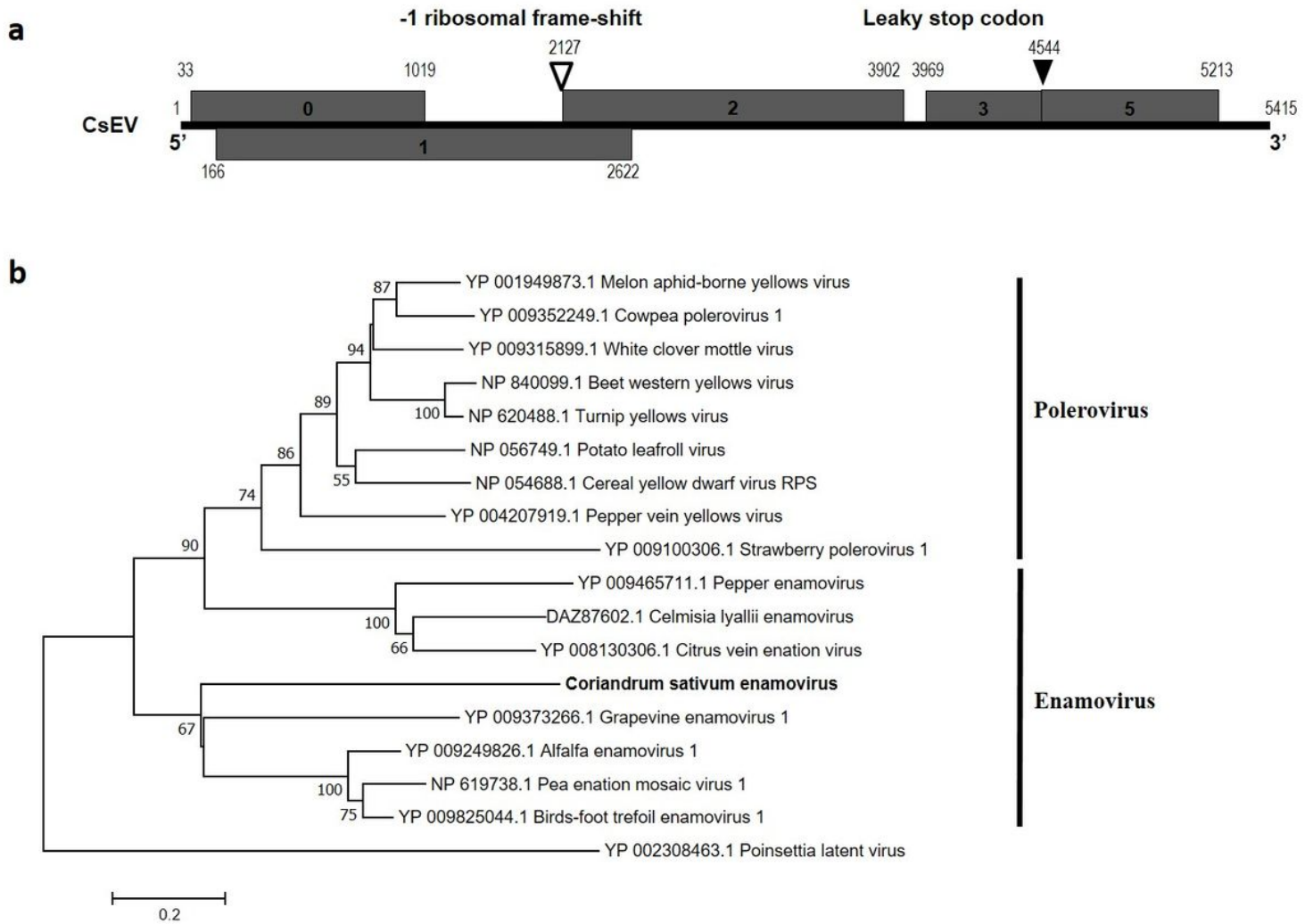
<sup>a</sup>Putative novel viruses; <sup>b</sup>TSA accession numbers of viral contigs are mentioned in brackets; *Coriandrum sativum* deltapartitivirus 1, 2 (CsDPV1, 2), *Coriandrum sativum* enamovirus (CsEV), *Coriandrum sativum* virus-like RNA assemblies (CsVLRA), cucumber mosaic virus (CMV), cucurbit chlorotic yellows virus (CCYV), lettuce mosaic virus (LMV), potato virus M (PVM), potato virus S (PVS), potato virus X (PVX), *Sedum sarmentosum* crinivirus (SSCV), vanilla distortion mosaic virus (VDMV).

## Figures



**Figure 1**

Genome organization of *Coriandrum sativum* deltapartitivirus 1 and 2 (CsDPV 1 and 2) identified in the current study (**a**). Phylogenetic relationship of deltapartitiviruses identified in the current study with other partitiviruses based on RdRp (**b**) and CP sequences (**c**). Bootstrap values >50% are only indicated. CsDPV 1 and 2 are shown in bold.



**Figure 2**

Genome organization of *Coriandrum sativum* enamovirus (CsEV) identified in the current study (**a**). Phylogenetic relationship of CsEV with other enamo- and poleroviruses based on P3 sequence (**b**). Bootstrap values >50% are only indicated. CsEV is shown in bold. Poinsettia latent virus was used as outgroup.

## Supplementary Files

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- [ESM1.txt](#)