Evidence for a novel partitivirus isolated from the entomopathogenic nematode *Steinernema ceratophorum*

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Abstract

Nematodes are abundant, but little is known about their viruses. In this study, we report a novel partitivirus isolated from the entomopathogenic nematode species *Steinernema ceratophorum*, named "Steinernema ceratophorum partitivirus 1" (ScPV-1). The complete genome of ScPV-1 comprises two dsRNA segments, dsRNA1 (2352 bp) and dsRNA2 (2196 bp). Each dsRNA contains a single open reading frame (ORF), encoding a putative RNA-dependent RNA polymerase (RdRp) and a coat protein (CP), respectively. The sequences of the RdRp and CP showed the highest similarity (47% and 33% identity, respectively) to *Plasmodiophora viticola* associated partitivirus 7 (PvAP-7). A multiple sequence alignment and phylogenetic analysis of the RdRp of ScPV-1 and other selected viruses indicated that ScPV-1 is a new member of the genus *Betapartitivirus* in the family *Partitiviridae*.

Nematodes are a group of organisms that were previously thought to be immune to viral infections and therefore have not been adequately tested for the presence of infectious viruses. Recent advances in genomic approaches have led to the rapid identification of unknown viruses from recalcitrant environments and organisms [2, 3]. The first nematode viruses were identified in *Caenorhabditis elegans* and *Caenorhabditis briggsae* [4, 5]. Orsay virus, the first known infectious nematode virus, was isolated from *C. elegans* [4]. Santeuil virus and Le Blanc virus were isolated from *C. briggsae* [5]. These newly discovered viruses cause abnormal intestinal morphology without an obvious effect on longevity or brood size. Orsay virus particles have been observed intracellularly in both intestinal cells and in somatic gonads [4]. Sequence analysis of the three nematode viruses isolated thus far revealed that they all belong to the family *Nodaviridae* [4, 5]. Subsequently, plant-parasitic nematode viruses were reported in the soybean cyst nematode (SCN) *Heterodera glycines* [6, 7]. The identification of viruses from natural nematode populations supports the notion that nematode viruses might be common but have been overlooked [4]. Entomopathogenic parasitic nematodes (EPNs) are known to control a wide range of insect pests and are safe alternatives to chemical insecticides, which can be hazardous to the environment and human health [1].

The evidence discussed above indicates that EPNs can be infected with viruses, and here we report the identification of a dsRNA virus named "Steinernema ceratophorum partitivirus 1" (ScPV-1), which was isolated from *S. ceratophorum* strain D43. ScPV-1 belongs to the family *Partitiviridae*, which consists of five genera: *Alphapartitivirus*, *Betapartitivirus*, *Cryspovirus*, *Deltapartitivirus*, and *Gammapartitivirus* [8]. Recently, the creation of two new genera in this family, "Epsilonpartitivirus" and "Zetapartitivirus", has been proposed [9, 10]. Phylogenetic analysis of the putative RNA-dependent RNA polymerase (RdRp) of ScPV-1 and the RdRps of other related viruses confirmed that ScPV-1 is a new member of the genus *Betapartitivirus*.

Provenance of the virus material

Strain D43 of *S. ceratophorum*, confirmed by the method described by Bekal et al. [6], was stored at 4°C and grown in *Galleria mellonella* (wax moth) larvae at 23°C for 28 h.
Nematodes from wax moth cadavers were collected in dishes stored in the dark for 5-7 days at 4°C. Total nematode RNA was extracted using a TransZol RNA Extraction Kit according to the manufacturer’s instructions. Extracted RNA was stored at -80°C for constructing cDNA libraries.

dsRNA was extracted using CF-11 cellulose (Sigma) chromatography [11]. Contaminating DNA and rRNA was removed by digestion with DNase I and S1 nuclease, respectively (Takara, Dalian, China), and purified dsRNAs were fractioned by electrophoresis in a 1% (w/v) agarose gel. Strain D43 was found to harbor several dsRNA segments. A cDNA library was constructed using the tagged random primer dN6 (5’-GACGTCAGATCGCGAATTCNNNNNN-3’) and reverse transcriptase [12]. The resulting cDNAs were amplified using a specific primer set (forward primer, 5’-CCAGGTCCATTAGGTTGCTGA-3’; reverse primer, 5’-GCAGGTTCCTATGGGTGG-3’) and PrimeSTAR HS DNA Polymerase (TaKaRa, Dalian, China). The 5’- and 3’-terminal sequences of ScPV-1 were cloned using RNA-ligase-mediated rapid amplification of cDNA ends (RLM-RACE). All PCR amplicons were cloned into the PMD™19-T vector (Takara, Dalian, China), recombinants were sequenced using the Sanger method, and sequences were analyzed using the DNAMAN program and the BLASTx program on the NCBI website.

Putative ORFs were predicted using the ORF Finder program at NCBI (https://www.ncbi.nlm.nih.gov/orffinder/). Conserved domain and homology searches were performed using the NCBI Conserved Domain Database (CDD) and the BLASTp program. Multiple sequence alignment of ScPV-1 with other partitiviruses was performed using the MAFFT program (https://www.ebi.ac.uk/Tools/msa/mafft/). The complete nucleotide (nt) sequences of both dsRNAs were deposited in the GenBank database under the accession numbers MZ964623 for dsRNA1 and MZ964624 for dsRNA2. A phylogenetic tree was constructed using the maximum-likelihood (ML) method with 1000 bootstrap samples, using MEGA 7.0 software [13].

Fig. 1 (A) Schematic representation of the ScPV-1 genomic structure. ORF1 encodes an RdRp, and ORF2 encodes a CP. The black lines indicate the 5’ and 3’ UTRs. (B) Comparison of the 5’- and 3’-terminal sequences of ScPV-1 dsRNA1 and dsRNA2. The conserved parts of the 5’-UTR and 3’-UTR sequences of ScPV-1 dsRNA1 and dsRNA2 are shaded in black and grey.
**Sequence properties**

The complete genome of ScPV-1 is composed of two segments of dsRNA1 and dsRNA2, which are 2352 bp and 2196 bp in length, respectively, with poly(A) tails at their 3' termini. The 5' untranslated region (UTR) and 3'-UTR of dsRNA1 are 84 nt and 210 nt long, respectively (Fig. 1A), whereas the 5'- and 3'-UTRs of dsRNA2 are 58 nt and 245 nt long, respectively (Fig. 1A). Alignment of the 5'- and 3'-UTR sequences of both ScPV-1 dsRNAs revealed significant similarities between them (Fig. 1B). Sequence analysis revealed that each dsRNA contains a single ORF: ORF1 and ORF2, respectively.

ORF1 extends from nt 85 to nt 2226 and is predicted to encode a 713-amino-acid (aa) protein with a predicted molecular mass (M_r) of 83 kDa (Fig. 1A). BLASTp searches

![Fig. 2](image-url)
indicated that the dsRNA1 product has the highest similarity to the RdRps of Partitiviridae family members, particularly with Plasmopara viticola associated partitivirus (PvAP-7; GenBank accession number QHD64796.1; E value = 2e-142, 98% query coverage and 47% percent identity). A CDD search showed that RdRp contains a conserved viral RdRp domain (RT-like superfamily, cl02808). A multiple sequence alignment of RdRps based on aa sequences of ScPV-1 and other related viruses indicated the presence of six conserved motifs (III-VIII) (Fig. 2A). ORF2 extends from nt 59 to nt 1951, encoding a 630-aa protein with a calculated Mr of 71 kDa (Fig. 1A). A BLASTp search indicated that this protein was most closely related to the PvAP-7 CP, with 33% aa sequence identity (E value, 1e-109; 96% query coverage). Additionally, a multiple sequence alignment based on the CPs of ScPV-1 and other closely related members of the family Partitiviridae also showed high conservation of some regions (Supplementary Fig. S1A).

Phylogenetic analysis based on RdRp (Fig. 2B) and CP (Supplementary Fig. S1B) sequences showed that ScPV-1 grouped with members of the genus Betapartitivirus. Selected members of the family Partitiviridae were divided into four recognized genera (Alphapartitivirus, Betapartitivirus, Gammapartitivirus, and Deltapartitivirus) and two proposed genera (“Epsilonpartitivirus” and “Zetapartitivirus”); (Fig. 2B) (Supplementary Fig. S1B). Therefore, ScPV-1 can be considered a new member of the genus Betapartitivirus.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00705-021-05314-5.

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Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval This article does not contain any studies involving human participants or animals.

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