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A novel deltacryptic virus identified in *Allium cepa* from Brazil

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Summary. – This work describes a novel partitivirus genome assembled from RNA-seq data generated from onion tissue from fields in Brazil. A new partitivirus genome composed of three dsRNAs, which was closely related to arhar cryptic virus 1, was assembled from *Allium cepa* samples from Brazil. The genomic sequences were also identified from available transcriptomic datasets of onion samples from China, Czech Republic, India, South Korea and USA. According to the species demarcation in the *Partitiviridae* family, the new virus was classified into the genus *Deltapartitivirus* with the suggested name of allium deltapartitivirus. This is the first report of the occurrence of a cryptic virus in plants of the genus *Allium*, and therefore, this work contributes to the understanding of the genetic diversity of partitiviruses that infect the genus *Allium*.

Keywords: Allium sp.; high-throughput sequencing; partitiviruses

Currently, the *Partitiviridae* family consists of 45 species distributed into five genera, and 15 species not yet classified (ICTV, 2022). Members of the genera *Alphapartitivirus* and *Betapartitivirus* infect plants or fungi, those of the genus *Gammapartitivirus* infect only fungi, of the *Deltapartitivirus* only plants, and there is only one approved species of the genus *Cryspovirus* that infects protozoa (Nibert *et al*., 2014). Genomes consist of two or three genomic segments of double-stranded RNA packaged separately in isometric particles (30–42 nm) that are not enveloped (Pan *et al*., 2009; Vainio *et al*., 2018; Byrne *et al*., 2021). The RNA-dependent RNA polymerase (RdRp) is coded on dsRNA-1, and the coat protein (CP) on dsRNA-2 (Nibert *et al*., 2014). Occasionally partitiviruses are associated with satellite RNAs or defective RNAs (Chiba *et al*., 2013).

Infections caused by partitiviruses in their respective hosts are persistent, and natural vectors are unknown or do not exist (Boccardo *et al*., 1987; Nibert *et al*., 2009; Vainio and Hantula, 2016; Cross *et al*., 2020). Fungal partitiviruses are transmitted through hyphal anastomosis and sporogenesis but can be also transmitted through contacts between hyphae (Ihrmark *et al*., 2002; Sasaki *et al*., 2006; Bhatti *et al*., 2011; Liu *et al*., 2012; Chiba *et al*., 2013; Xiao *et al*., 2014), while plant partitiviruses are transmitted through ovule and by pollen to the seed embryo (Valverde and Gutierrez, 2008; Roossinck, 2010). There are no reports of graft transmission and apparently no cell-to-cell movement and transmission occurs strictly vertically via meiosis (Boccardo *et al*., 1987; Ghabrial *et al*., 2008; Nibert *et al*., 2014). Members of the genus *Deltapartitivirus* have two or more nucleic acid segments (Sabanadzovic and Valverde, 2011) and are found at very low concentration in the host plant (Yang *et al*., 2022). Other additional RNAs have been found in deltapartitiviruses, which also encode for another full-length CP with an unknown function

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Abbreviations: ADp = allium deltapartitivirus; ArCV-1 = arhar cryptic virus 1; CP = coat protein; ICTV = International Committee on Taxonomy of Viruses; ORF(s) = open reading frame(s); SDT = sequence demarcation tool; TSA = transcriptome shotgun assembly

(Sabanadzovic and Valverde, 2011; Kim *et al*., 2018; Kumar *et al*., 2017).

In this work, we used RNA-seq data of *Allium cepa* cultivated in Brazil to assemble a putatively new genomic sequence of a deltapartitivirus closely related to arhar cryptic virus 1 (ArCV-1). Three genomic sequences were assembled, molecularly characterized, and were also found in TSA datasets of onions from different countries around the world. Therefore, we first collected symptomatic tissue of onion leaves (*Allium cepa* L.) from producing fields in the city of Petrolândia, Pernambuco, Brazil. Total RNA was extracted using the Quick-RNA™ Plant Miniprep Kit (Zymo Research, CA, USA) according to the manufacturer's instructions and sent for High-Throughput Sequencing to Macrogen (Seoul, South Korea). Two files RB-1_1.fastq.gz (1.2 Gb), and RB-1_2.fastq.gz (1.3 Gb) were generated with a total of 5,270,386,040 reads (GC content of 43.65%, AT of 56.35%, the proportion of bases with Phred quality score higher than 20 (Q20%) was 98.72% and 30 (Q30%) was 95.85%). Also, seven transcriptome datasets (TSA prefixes GAAO, GBJZ, GBRO, GBRQ, GEOY, GETF, and GHMJ) of *Allium cepa* were retrieved from NCBI and used to assemble complete or partial partitiviral sequences of dsRNA-1, dsRNA-2 and dsRNA-3. The bioinformatics pipeline for *de novo* assembly of viral genomes was performed according to Blawid *et al.* (2017). RdRp amino acid sequences of partitiviruses were used in multiple sequence alignments using MAFFT v.7 (Katoh and Standley, 2013). The best model of protein evolution was evaluated with ProtTest 3.4.2 (Darriba *et al*., 2011). Based on the AIC and BIC criteria, the LG substitution matrix with I, G and F distributions were chosen, with a confidence interval of 100%. Phylogenetic analyses were then performed with the predetermined parameters of PhyML 3.0 (Guindon *et al*., 2010, http://www.atgc-montpellier.fr/phyml/execution.php). The starting tree was obtained by BioNJ and optimized by both branch length and tree topology. Branch support values (%) were estimated by the approximate likelihood ratio test (aLRT) with SH-like criteria. Phylogenetic tree was visualized and edited with iTOL v4 (https://itol.embl.de/tree/) (Letunic and Bork, 2021). The Sequence Demarcation Tool (SDTv.1.2) (Muhire *et al*., 2014) was used to analyze multiple alignments at the amino acid level of the RdRp (dsRNA-1, 502 aa) and the CPs (dsRNA-2, 345 aa; dsRNA-3, 338 aa) of partitiviruses. GenBank Acc. Nos. of the partitivirus sequences used for analysis in this publication are in Supplementary Table S1.

As a result, three segments (dsRNA1- OP313027, dsRNA2- OP313028, dsRNA3- OP313029) were assembled from onion samples from Brazil, which were closely related to deltapartitiviruses. According to ICTV, the binomial name allium deltapartitivirus (ADp) was suggested. dsRNA-1 consists of 1734 bp and has an ORF of 1509 bp (nucleotide position 103 to 1611) that putatively encodes a 502 aa RdRp (MW 59.41 kDa). Blast analyses revealed the highest amino acid sequence identity of 77.80% (94% coverage) with the unclassified RdRp sequence of ArCV-1 (NC_024014) followed by 74% with the RdRp aa sequence of rosa multiflora cryptic virus (RMCV, ABV89762). SDT analyses using the complete ADp RdRp amino acid sequence revealed the highest percentage identities of 42.8–77.3 with unclassified RdRp deltapartitivirus sequences (ArCV-1, NC_024014; fragaria chiloensis cryptic virus, FClCV, NC_009519; RMCV; rose cryptic virus 1, RoCV1, NC_010346 and others) followed by 37 to 39.3% identities with deltapartitivirus sequences (beet cryptic virus 2, NC_038846; fig cryptic virus, NC_015494; pepper cryptic virus 1, NC_037095; pepper cryptic virus 2, NC_034159) (Supplementary Fig. S1). Currently the *Partitiviridae* family is divided into five genera corresponding to the clustering of RdRp sequences (Nibert *et al*., 2009; Nibert *et al.,* 2014). Based on partitiviral RdRp-like sequences phylogenetic analysis, unclassified deltapartitiviruses and sorted deltapartitiviruses form a monophyletic group (Liu *et al*. 2012; Nibert *et al*., 2014; Kumar *et al*., 2017). Indeed, our RdRp phylogenetic analysis using RdRp amino acid sequences of sorted and unclassified partitiviruses also placed ADp closely related to unclassified deltapartitiviruses (Fig. 1).

Furthermore, the 5' ends of ADp dsRNAs nucleotide sequences have the conserved sequence 5'-GAUAAU GAUC-3', also found in some unclassified deltapartitiviruses. This consensus follows the described consensus 5'-GAWWWUNMYC-3' (Nibert *et al.,* 2014) found for all deltapartitiviruses so far. Finally, several structural RdRp (A-G) motifs (Bruenn, 1993; Xie *et al*., 1993; Bartholomäus *et al*., 2016; Jia and Gong, 2019) were found: Motifs A (DX_4D), B ($P/A/SGX_3TX_4SX_2N$), the C box (GDD), D (KS, KC, KT or KL), E ($LX_{22}P/L/TE/K/R$), F ($PX_6R\,X_2IX_7KXR$) and G (AG, AE, VE. AD or TG) (Supplementary Fig. S2).

ADp dsRNA-2 consists of 1474 bp and has a 1038 bp ORF-2 (345 aa, MW ~38.29 kDa) that putatively codes for a coat protein (CP1). Blastp analysis of the CP1 amino acid sequence showed the highest amino acid sequence identity of 45.99% with the ArCV-1 coat protein (NC_024011, cover 97%). dsRNA-3 is 1413 bp long and has an ORF consisting of 1017 bp that also putatively codes for another CP (CP2, 338 aa, MW ~37.9 kDa). The highest amino acid identity of 47.9% (97% coverage, Blastp) was found with the hypothetical protein LR48_Vigan07g013900 from *Vigna angulares* (KOM46435) followed by 50.2% (86% coverage) with the CP2 of ArCV-1 (NC_024010). SDT matches using CP1 and CP2 sequences at the amino acid level revealed higher identities to unclassified partitiviruses (Supplementary Fig. S3), in which the CP1 aa sequence shared 45.7% identity with the CP1 sequence of ArCV-1 (NC_024011), and

43.8% with the FClCV sequence (NC_009520). The CP2 of ADp shares 47.6% identities with the CP2 sequence of ArCV-1 (NC_024010), and 45.2% with the CP2 of RoCV1 (NC_010348) and RMCV (EU024676). When CP1 and CP2 aa sequences were compared with other partitivirus sequences, the highest identity between 19.0% to 43.3% were observed (Supplementary Fig. S3). The CP1/CP2 length of dsRNA-2 and dsRNA-3 are in the range of other deltapartitiviruses, which varies from 1415–1598 bp (337–430 aa). We also performed a phylogenetic analysis using CP1 and CP2 amino acid sequences of partitiviruses (Supplementary Fig. S4) besides identifying the low identities among partitiviruses. ADp CPs grouped with unclassified partitiviruses sequences (ArCV-1, FClCV, RoCV1, RMCV), showing a close phylogenetic relationship with unclassified partitivirids. The CPs sequences of ADp grouped into two clades formed with CP1 and CP2 sequences, respectively. The closest relationship of ADp CP1/CP2 was found with the CP1/CP2 of ArCV-1.

Concerning the poly (A) uninterrupted stretches at their 3'-terminal ends, deltapartitiviruses are known to have 12–19 A residues in the last 50 nt of the 3'-terminal of dsRNA-1 and 9–18 of dsRNA-2 sequences (Nibert *et al.*, 2014; An *et al.*, 2017). ADp dsRNA-1, dsRNA-2 and dsRNA-3 have 11, 14 and 11 A residues at its last 50 nt, respectively.

Interestingly, we also were able to assemble RdRp, CP1 and CP2 sequences of ADp from TSA datasets of onions from China, Czech Republic, India, South Korea and USA,

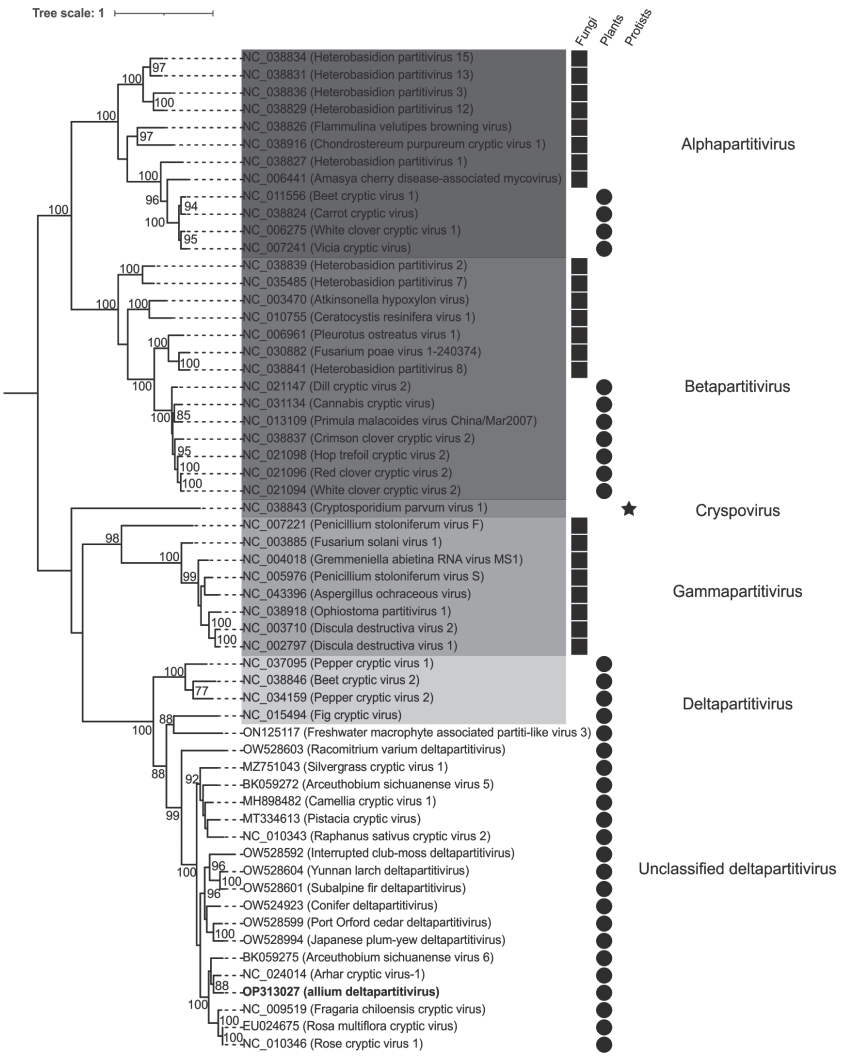

Fig. 1

Phylogenetic relationships between members of the *Partitiviridae* family and unclassified partitiviruses based on the amino acid sequences of the RdRp region

The newly assembled sequence of *Allium* deltapartitivirus is shown in bold. Nodes display branch support values (%).

which shared aa identities of 84-100% with the corresponding sequences (Supplementary Table S2). Pairwise multiple sequence comparisons at the amino acid level showed identities as high as 99.7% and 99.4% in the CP1 and CP2 regions, respectively, with other sequences derived from transcriptomic data. This suggests that ADp is widespread in *A. cepa* indicating that ADp might have co-evolved with the host over a long time.

So far, unclassified partitiviruses have been detected in asymptomatic and symptomatic plants, as found for RoCV1 (James *et al.*, 2015), and some authors have suggested that partitivirus possibly plays a role in disease-associated virus complex (Martin and Tzanetakis, 2008). Although we found ADp from symptomatic onion leaves, future studies should be performed to clarify whether partitivirus infection plays a role in disease expression in onions. This work contributes to the understanding of the diversity of partitiviruses in onions and enunciates the first genome of a partitivirus infecting plants of *Allium cepa*.

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Supplementary information is available in the online version of the paper.

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