First Report of *Coguvirus eburi* Infecting Pear (*Pyrus communis*) in South Africa

K. Bougard,¹ H. J. Maree,^{1,2} G. Pietersen,¹ J. Meitz-Hopkins,³ and R. Bester^{1,2,†}

¹ Department of Genetics, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa

² Citrus Research International, PO Box 2201, Matieland, 7602, South Africa

³ Department of Plant Pathology, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa

Funding: The project was funded by Hortgro (PO-20-USGEN-PM01). Plant Dis. 106:772, 2022; published online as <https://doi.org/10.1094/PDIS-08-21-1630-PDN>. Accepted for publication 26 August 2021.

Coguvirus eburi is a member of the genus *Coguvirus* in the family *Phenuviridae* (Kuhn et al. 2020). The species *Coguvirus eburi* was established to include citrus virus A (CiVA), which is a negative-sense, single-stranded RNA virus that was first found infecting sweet orange in southern Italy via high-throughput sequencing (HTS) (Navarro et al. 2018). This virus was also found to infect pome fruits in France, such as pear (Svanella-Dumas et al. 2019). More recently, CiVA infections have been associated with impietratura disease in citrus (Beris et al. 2021). In the summer of 2021, leaf samples were collected from a pear tree (*Pyrus communis* cv. Bosc, B175) in the Koue Bokkeveld, South Africa, as part of a virus survey. Sample B175 displayed no visual disease symptoms. One gram of leaf petioles was used for total RNA extraction, using a modified CTAB extraction protocol (Ruiz-García et al. 2019). Ribo-depleted RNA was prepared (Ribo-Zero Plant kit) and a sequencing library constructed (Illumina TruSeq Stranded Total RNA). The RNA library was paired-end (2 × 100 bp) sequenced on an Illumina HiSeqX instrument (Macrogen, South Korea). A total of 47,750,152 reads were obtained. Raw data were trimmed for quality with Trimmomatic (SLIDINGWINDOW:3:20, MINLEN:20) (Bolger et al. 2014). De novo assembly performed with CLC Genomics Workbench 11.0.1 (Qiagen) (default parameters) using high quality reads yielded 75,250 contigs. BLASTn analysis identified two viral contigs with high nucleotide (nt) identity to apple stem pitting virus (ASPV) and CiVA. The CiVA contig was 9,400 nt and on closer

examination, a concatamer of CiVA RNA1 and RNA2. The concatenation occurred due to the characteristic near-identical nucleotides shared at the 5' and 3' ends of RNA1 and RNA2 of these negative-stranded RNA viruses (Navarro et al. 2018). After splitting and curation, the RNA1 contig was 6,664 nt and the RNA2 contig 2,686 nt. A total of 51,397 and 34,820 reads were used to construct these contigs, resulting in an average depth of coverage of 761 and 1,281 for RNA1 and RNA2, respectively. The contigs had the highest nt identity to the complete CiVA GenBank accessions MT720885.1 (95.53%) and MW148460.1 (96.03%), spanning 99.6% and 98.1% of the genomes of RNA1 and RNA2, respectively. These contigs were submitted as partial genomes to GenBank as accessions MZ463039 and MZ463040. RT-PCR was used to validate the presence of CiVA in sample B175. Two RT-PCR assays, directed at RNA1 and RNA2, respectively (Bester et al. 2021), were used to generate amplicons. Amplicon sequences were confirmed with bidirectional Sanger sequencing. Twenty-one additional samples from the same orchard as B175 as well as other samples from the Koue Bokkeveld and Elgin areas, including cultivars Abate (10 samples), Forelle (10 samples), Early Bon Chretien (3 samples), Packham's Triumph (12 samples), and Rosemarie (3 samples), were all surveyed for CiVA using the same RT-PCR assays as mentioned above. Thirty-six of the 59 samples tested were positive for CiVA, which further confirms the presence and widespread distribution of this virus in the limited survey conducted in pears in South Africa. However, no association with any disease symptoms or specific cultivar were identified. This is the first report of CiVA infecting pear in South Africa. This study therefore contributed to investigating the distribution of this virus and will assist the South African plant material certification scheme to assess the incidence of CiVA in South Africa.

References:

- Beris, D., et al. 2021. Phytopathology 111:1782.
- Bester, R., et al. 2021. J. Citrus Pathol. 8. <https://doi.org/10.5070/C481049000>.
- Bolger, A. M., et al. 2014. Bioinformatics 30:2114.
- Kuhn, J. H., et al. 2020. Arch. Virol. 165:3023.
- Navarro, B., et al. 2018. Front. Microbiol. 9:2340.
- Ruiz-García, A. B., et al. 2019. Page 163 in: Citrus Tristeza Virus. Methods in Molecular Biology. Humana, New York.
- Svanella-Dumas, L., et al. 2019. Plant Dis. 103:2703.

The author(s) declare no conflict of interest.

Keywords: CiVA, citrus virus A, viruses and viroids, tree fruits

[†]Indicates the corresponding author.
R. Bester; rachelle@sun.ac.za