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### First Report of Grapevine Enamovirus 1 in *Vitis vinifera* Cultivar Meunier in France

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*Grapevine enamovirus 1* (GEV-1) is a member of the genus *Enamovirus* in the family *Solemoviridae*. GEV-1 was first described in 2017 in a few grapevine cultivars in Brazil (Silva et al. 2017) and subsequently in China (Ren et al. 2021). We first identified GEV-1 using high-throughput sequencing (HTS) (Illumina, NOVASeq SP, TruSeq mRNA stranded 2 × 150 bp of ribosomal RNA depleted total RNA extracts using an RNeasy Plant mini kit) (Qiagen) from a *Vitis vinifera* 'Meunier' leaf sample collected in a more than 20-year-old commercial vineyard in the Champagne region of France in 2019. Analyses of the 47,573,330 total reads were performed using CLC Genomics Workbench 12.0 software (Qiagen) as previously described (Hily et al. 2018). The GEV-1 genome, determined only from the HTS data (isolate GEV-1-Fr; GenBank accession no. MW760844), is 6,262 nucleotides (nt) long and fully covered with 5,706 reads (mapping parameters of 0.5 in length and 0.7 in similarity fractions using CLC). Compared with the previously determined sequences (NC\_034836 and KX645875) from Brazil, the GEV-1-Fr sequence contained a few indels, including a deletion of 9 nt in the 5'

untranslated region (UTR), an insertion of 3 nt located in the overlapping region of the open reading frame (ORF)1 and ORF2, and a single nt insertion in the noncoding region between ORF2 and ORF3. These indels also exist within the sequence of isolate SD-CG from China (MT536978). However, GEV-1-Fr contains a unique 45-nt insertion in the 3'-UTR, although this needs to be verified using standard assays. Overall, GEV-1-Fr exhibits 88.7, 89.1, and 93.3% identity at the nt level with isolates from Brazil (NC\_034836, KX645875) and China (MT536978), respectively. The GEV-1-infected Meunier grapevine showed symptoms of light chlorotic patterns on the leaves that were probably due to the presence of other coinfecting viruses, including grapevine fanleaf virus, grapevine Pinot gris virus, grapevine rupestris stem pitting-associated virus, and grapevine fleck virus. The detection of GEV-1 was further confirmed in the Meunier grapevine via RT-PCR using newly designed primer pairs Fwd\_GEV\_5600 (GCAAGGAGCA GCCCTATAATGCT) and Rev\_GEV\_6075 (CTAGTCGATACGATCTATA GCGGAGG) that amplified a 474-bp fragment of ORF5. We also designed a TaqMan assay in ORF5 with the following primers and probe: Fwd\_GEV\_5662 (ACAAGTGCCYGTTCATAG), Probe\_GEV\_5724-FAM (TTTA CCGAGGACTATGACGCCGC), and Rev\_GEV\_5772 (CACCGGCTCCAT AACCAT). Among all the samples from different grapevine cultivars and geographic regions in France that were tested with the TaqMan assay ( $n = 188$ ), only the original Meunier plant from Champagne was positive for GEV-1. To our knowledge, this is the first report of GEV-1 in France and in European vineyards in general. Although many aspects of the virus biology are yet to be elucidated, our results expand its geographical range. New GEV-1 detection primers can be developed, considering its genetic diversity, to facilitate its detection and further define its evolutionary history.

#### References:

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The author(s) declare no conflict of interest.

**Keywords:** grapevine, grapevine enamovirus 1, complete sequence, high-throughput sequencing

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