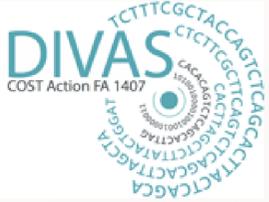


# TWO NOVEL VIRUS-LIKE SEQUENCES FROM GRAPEVINE

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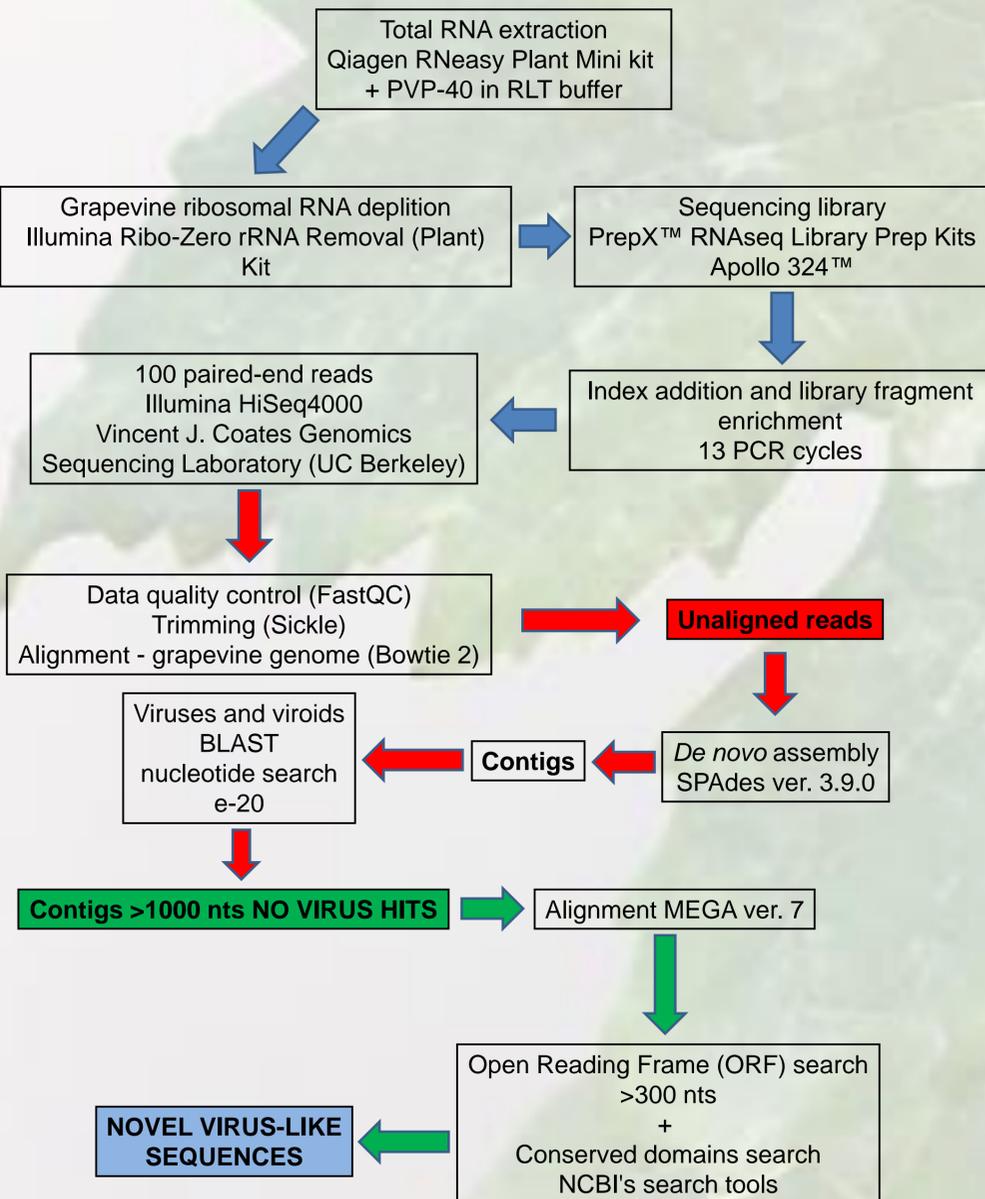
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Since the discovery of *Grapevine fanleaf virus* as the first virus-infecting grapevines over 50 years ago, about 70 different viruses have now been identified from this plant host. Next generation sequencing (NGS, high throughput sequencing, or deep sequencing) has become an important diagnostic tool to detect known, but also novel viruses.

In this study we selected four grapevine samples from the Croatian region of Kaštela, in Central Dalmatia: VB-108 (variety Babica), VD-102 (var. Dobričić), VLJ-178 (var. Ljutun) and VVL-101 (var. Vlaška). Kaštela region has a long viticultural tradition and numerous grapevine varieties locally grown that are considered to be autochthonous. In addition, previous studies suggested deteriorated sanitary status of varieties from mentioned region with common mixed infections with different viruses.

## Materials and Methods



## Results and Discussion

*De novo* assembly of data from grapevine accessions VB-108, VD-102, VLJ-178, and VVL-101 generated 436, 467, 459, and 251 contigs longer than 1000 nucleotides, respectively. Detailed analysis of these contigs identified two novel grapevine-associated viruses, tentatively named Grapevine virus G and Grapevine badnavirus-1.

## Acknowledgments

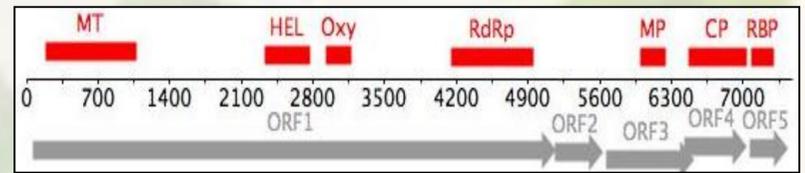
The research leading to these results received funding from the European Union Seventh Framework Programme (FP7 2007-2013) under grant agreement n° 291823 Marie Curie FP7-PEOPLE-2011-COFUND (The new International Fellowship Mobility Programme for Experienced Researchers in Croatia - NEWFELPRO). This article was prepared as a part of a project "Ecology of an emerging grapevine virus in Croatia and California (EcoGVCC)" which received funding through NEWFELPRO under grant agreement n°51. The work used the Vincent J. Coates Genomics Sequencing Laboratory at UC Berkeley, supported by NIH S10 OD018174 Instrumentation Grant.

## Grapevine virus G (GVG)

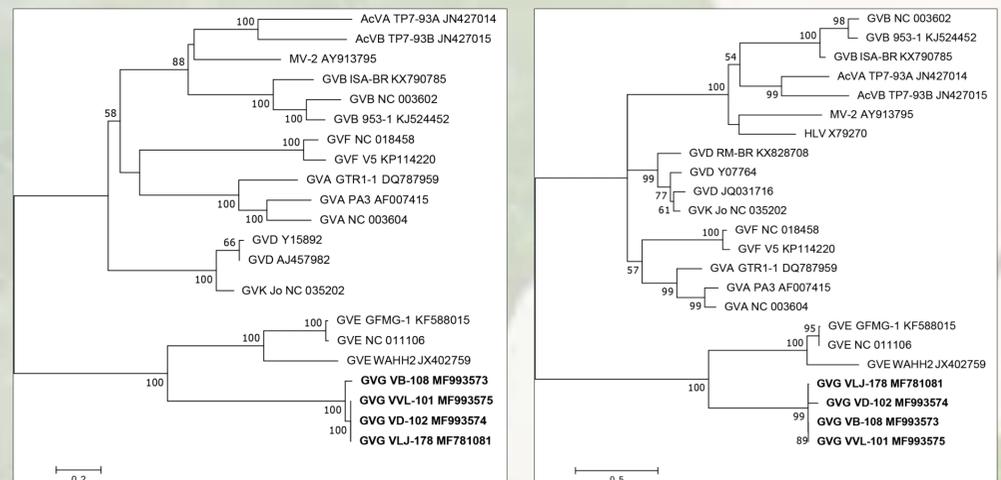
- in all grapevine accessions

Genome: linear RNA, 5 ORFs, 7475 nts + poly(A) tail (Fig. 1).

- distinct species in the genus *Vitivirus* (Fig. 2).



**Figure 1.** Grapevine virus G genome. Viral ssRNA - black horizontal line; ORFs/genes - grey; conserved domains - red: MT – methyltransferase, Oxy – oxygenase, HEL – helicase, RdRp – RNA dependent RNA polymerase, MP – movement protein, CP – coat protein, RBP – putative RNA binding protein. ORF2 - hypothetical protein 17-kDa.



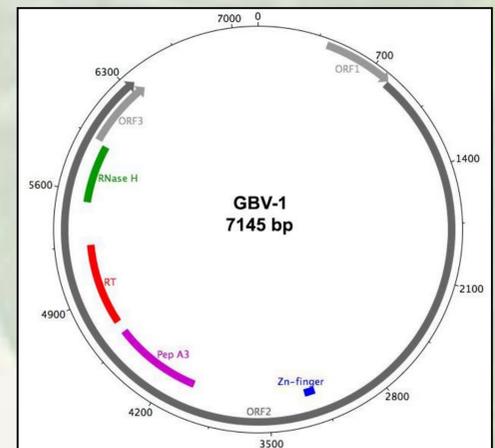
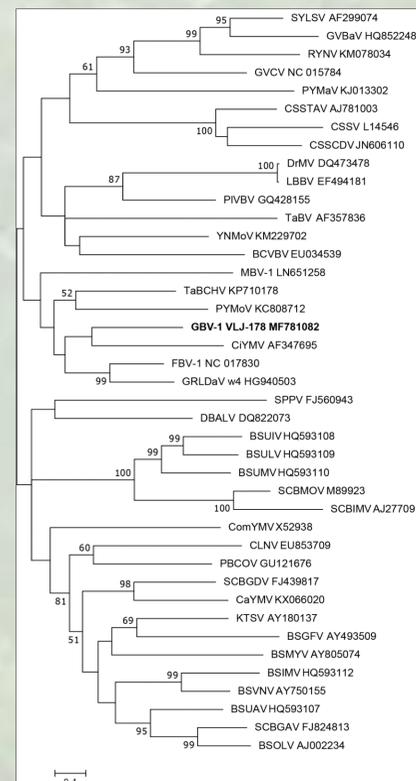
**Figure 2.** Maximum-likelihood phylogenetic trees of the genus *Vitivirus* constructed using codon-aligned nucleotide sequences of replicase (left) and coat protein (right) genes of representative isolates, with respective GenBank accession numbers included. Bootstrap values (1,000 replicates) are shown on branches with >50% support. Isolates of novel virus, tentatively named Grapevine virus G (GVG), are in bold. Abbreviations: AcVB – *Actinidia virus A*, ACVB – *Actinidia virus B*, MV-2 - *Mint virus 2*, GVA – *Grapevine virus A*, GVB – *Grapevine virus B*, GVD – *Grapevine virus D*, GVE – *Grapevine virus E*, GVF – *Grapevine virus F*, GVK - *Grapevine virus K*.

## Grapevine badnavirus 1 (GBV-1)

- grapevine accessions VLJ-178 and VVL-101 (identical sequence)

Genome: circular DNA, three ORFs, 7145 nts (Fig. 3).

- distinct species in the genus *Badnavirus* (Fig. 4).



**Figure 3.** Grapevine badnavirus 1 genome. Viral DNA - thick black circular line; three open reading - grey; conserved domains in the ORF2 polyprotein - color. Abbreviations: RNase H – ribonuclease H, RT – reverse transcriptase, Pep A3 – *Caulliflower mosaic virus* peptidase (A3), Zn-finger – Zinc knuckle. ORF1 and ORF3 - hypothetical proteins of 33- and 36-kDa, respectively.

**Figure 4.** Maximum-likelihood phylogenetic tree from codon aligned nucleotide sequences of the RT/RNase H region from members of the genus *Badnavirus*, with respective GenBank accession numbers. Bootstrap values of 1000 replicates are shown on branches when >50% support. The position of the novel badnavirus, tentatively named Grapevine badnavirus 1 (GBV-1), is in bold.

## Conclusion

We report the *de novo* assembly of two novel virus-like sequences from total host plant RNA as a template. It is clear that NGS data will lead to the discovery of new plant viruses, as shown here with only four samples. However, it is important to stress the fact that biological information about most grapevine viruses is very limited or non-existent, and the ability to detect a large number of putative pathogens is of limited applied value if there are no studies describing their respective roles, or how to reduce their impact to a crop of economic importance.