

Uncovering the worldwide diversity of little cherry virus 1 and 2 and the associated viruses in *Prunus* spp.

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Introduction

The transnational consortium and project EUPHRESKO aimed at creating an extensive network of research partners that work on Little cherry disease (LChD) to gain information on the distribution of both little cherry virus (LChV) pathogens (LChV-1&2) in the European Union (EU). To further unravel the LChD complex, high-throughput sequencing (HTS) techniques were used to gain a better insight in the pathogenesis and global distribution in sweet and sour cherries, as well as in ornamental *Prunus* spp.

Virus	Country	# complete sequences	# partial sequences
Little cherry virus 1 (LChV-1)	Austria	2	-
	Bosnia	-	1
	Canada	8*	1
	France	1	-
	Germany	3	4
	Italy	-	4
	Slovenia	-	1
	Spain	1	-
	Switzerland	-	1
	The Netherlands	3	-
The United Kingdom (UK)	1	-	
Little cherry virus 2 (LChV-2)	Belgium	1	-
	Canada	5*	2
	Japan	3	6
	Switzerland	2	2
	The Netherlands	1	-

*In one sample, two isolates were reconstructed.

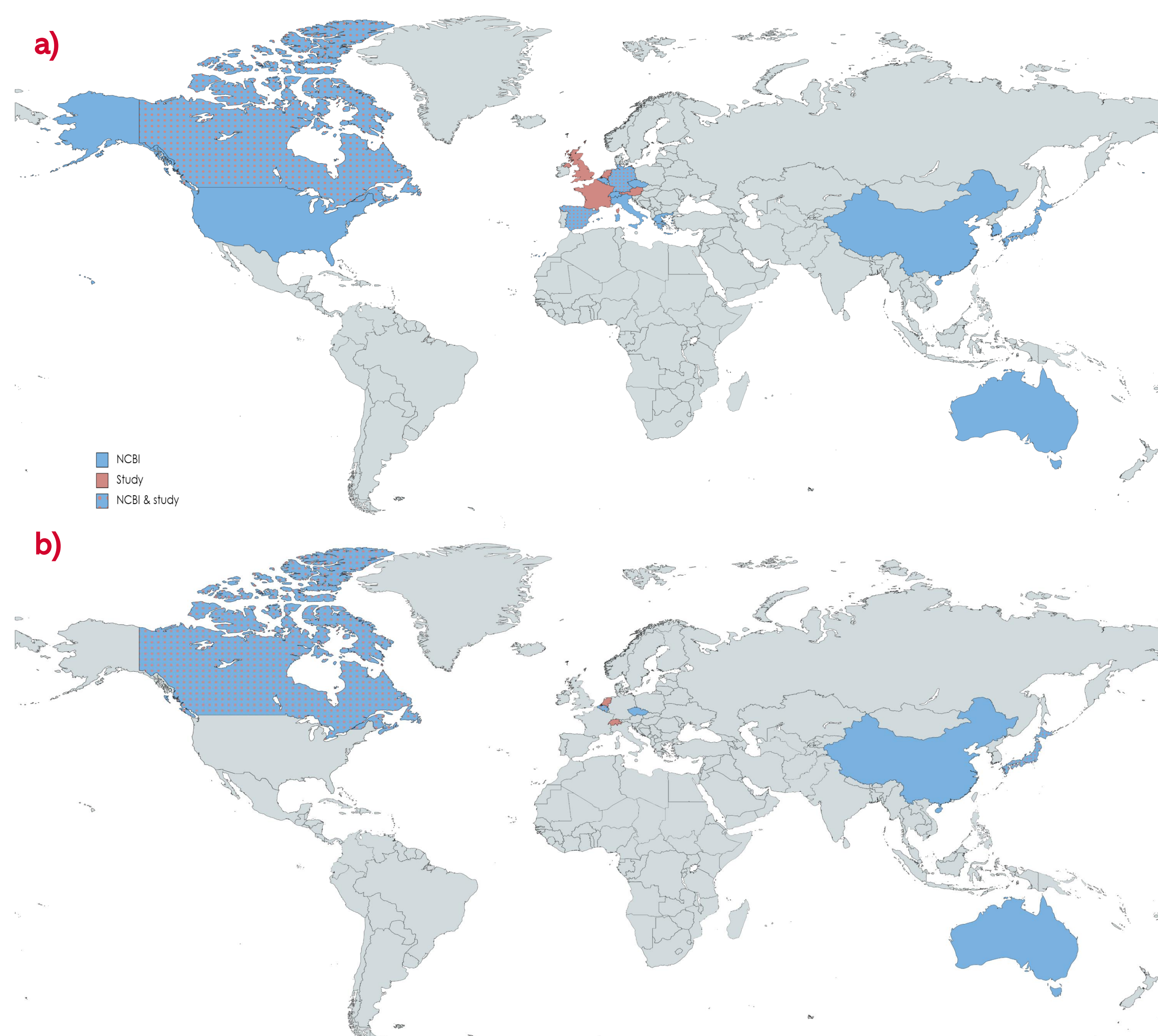


Figure 1. Map of the countries of origin of the reconstructed complete sequences (without UTRs) of (a) LChV-1 and (b) LChV-2 analysed in this study (light pink) and the complete genomes from NCBI (blue). The countries where both NCBI and reconstructed genomes are highlighted in a blue background with light pink dots. The map was created with mapchart.net.

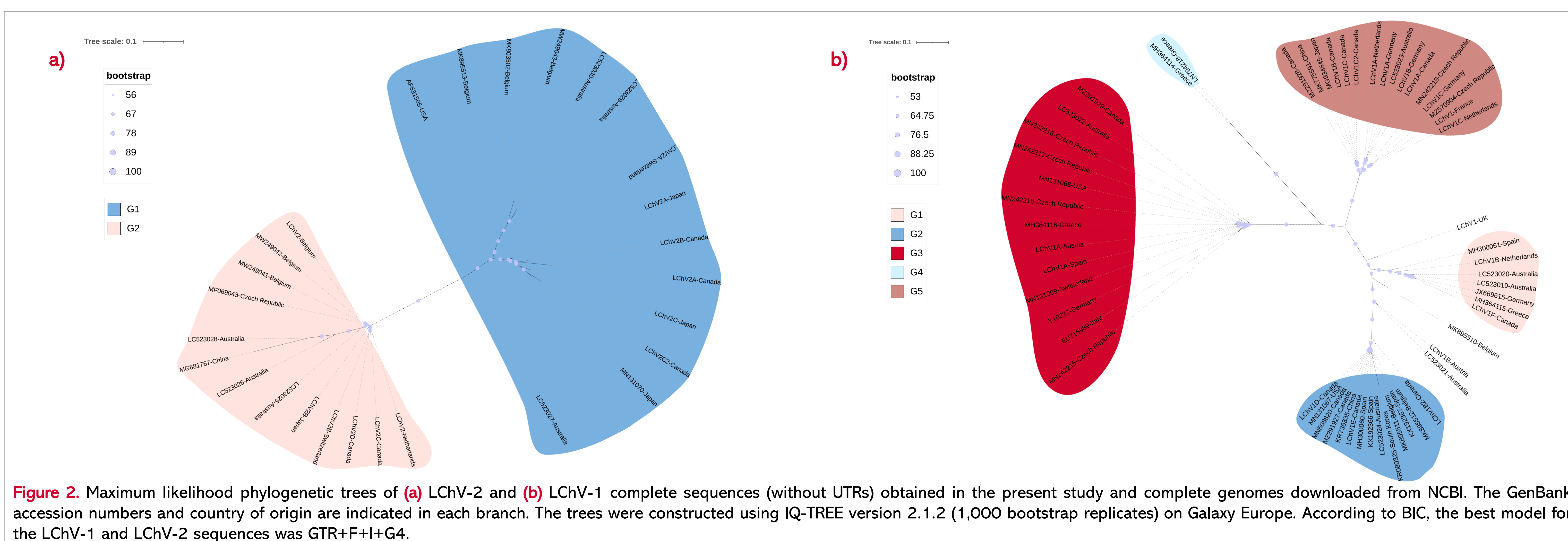


Figure 2. Maximum likelihood phylogenetic trees of (a) LChV-2 and (b) LChV-1 complete sequences (without UTRs) obtained in the present study and complete genomes downloaded from NCBI. The GenBank accession numbers and country of origin are indicated in each branch. The trees were constructed using IQ-TREE version 2.1.2 (1,000 bootstrap replicates) on Galaxy Europe. According to BIC, the best model for the LChV-1 and LChV-2 sequences was GTR+F+I+G4.

Diversity of *Prunus* spp. infecting viruses

The most prevalent viruses identified in the samples were cherry virus A (CVA), prune dwarf virus (PDV), apricot pseudo-chlorotic leaf spot virus (APCLSV), and american plum line pattern virus (APLPV). Thanks to genome reconstructions, this study almost doubled the number of genomes available for LChV1 (19 new genomes compared to 37 already available), LChV2 (12 new genomes compared to 15 available), PDV (14 new genomes compared to 30 RNA 1, 26 RNA 2, and 19 RNA 3 sequences) and APLPV (8 new genomes compared to 3).

Acknowledgments

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Discussion and conclusion

This work has resulted in an increase of the genomic information and will be available in public datasets in the foreseeable future. In the phylogenetic analysis of the reconstructed LChV-1 genomes, the four known groups were recovered (Figure 2b), although there are some isolates in between them. Interestingly, the two groups obtained in the phylogenetic analysis of LChV-2 genomes have the characteristic difference that group 1 (G1) has a nucleotide (nt) sequence of 1,386 nt that encodes the protein p53, while genomes in group 2 (G2) the protein p53 is encoded by a sequence of 1,383 nt. The addition of these newly reconstructed genomes provides a good baseline to further design better specific diagnostic tests for LChV1&2 and to help optimize the existing ones.