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High genetic diversity of *Plum pox virus* in subspontaneous trees in North Macedonia sheds new light into its evolutionary history

S. Dallot¹, M. Brevet¹, D. Filloux², E. Fernandez², R. Rusevski³, B. Kuzmanovska³ and G.Thébaud¹

¹PHIM Plant Health Institute, INRAE, Univ Montpellier, CIRAD, Institut Agro, IRD, Montpellier, France, ²PHIM, CIRAD, Montpellier, France, ³Department of Plant Pathology, Ss. Cyril and Methodius University, Skopje, North Macedonia. (sylvie.dallot@inrae.fr)

Understanding plant virus spread and evolution at the agro-ecological interface is crucial to design appropriate disease management strategies and prevent variant emergence. Perennial plants are prone to harbor a high viral genetic diversity due to repeated and long-lasting infections and may thus provide a conducive environment to the emergence of new virus genotypes. Sharka, a serious disease of stone fruits (Prunus) worldwide, is caused by the plum pox virus (PPV, genus Potyvirus). Ten strains have been described so far, among which only three are widely distributed across Europe. In this study, we assessed the role of the wild compartment as a potential reservoir of PPV genetic diversity. We carried out a countrywide survey in North Macedonia, targeting cultivated trees, but also subspontaneous myrobalan trees (P. cerasifera) and wild blackthorn (P. spinosa) bushes. For PPV diagnosis and strain assignment, we designed new or used previously published polyvalent and strain-specific RT-PCR tests followed by partial sequencing. In P. cerasifera, we identified the three strains that are widespread in Europe (i.e., PPV-M, -D, -Rec), the geographically restricted PPV-T recombinant as well as PPV-An, one of the putative parents of the epidemic PPV-M strain and previously detected only once (in Albania). Furthermore, a PPV isolate distant from all known strains was detected and fully sequenced using Nanopore and Sanger technologies. Based on genetic distance, this isolate belongs to a new PPV strain, that we called PPV-P. Its aphid transmissibility and its ability to infect cultivated Prunus species were assessed experimentally, evidencing that this new strain has the potential to be epidemic in stone fruits. In contrast to P. cerasifera, cultivated species harbored only one (peach), two (apricot) or four (plum) PPV strains. These results suggest that P. cerasifera in the Balkans may be involved in the emergence of the PPV strains spreading in Europe.