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First Report of Plum Pox Virus Strains M, D, and Rec Infecting *Prunus* spp. in the Republic of North Macedonia

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Sharka, caused by plum pox virus (PPV, genus *Potyvirus*), is one of the most important *Prunus* diseases (Scholthof et al. 2011). Its worldwide presence includes almost all Eastern and Central European countries (Rimbaud et al. 2015).

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Caption

Strawberry plant showing leaf blight caused by *Pantoea ananatis* FANS-1 (Bajpai et al.). Photo credit: B. Prithiviraj. *Plasmopara velutina* causing downy mildew on *Impatiens balsamina* (R. M. Silva et al.). Photo credit: R. W. Barreto.

Metrics

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Surprisingly, there is still no official report of PPV presence in the Republic of North Macedonia (previously: former Yugoslav Republic of Macedonia), despite the mention of sharka on plum trees in old (**Josifović 1964**) and local (**Mitrev et al. 2012**) documents and its official presence in the surrounding countries (i.e., Albania, Serbia, Bulgaria, and Greece [**EPPO 2019**]). In June 2018, we carried out a countrywide survey targeting cultivated (commercial orchards and private gardens), subspontaneous, and wild *Prunus* trees, including *P. persica*, *P. armeniaca*, *P. domestica,* and *P. cerasifera*. Leaf samples were collected from 173 trees exhibiting typical (chlorotic rings and vein yellowing; 115 samples), atypical (necrotic spots; 29), or no (29) sharka symptoms. In the absence of typical symptoms, PPV detection was performed by double antibody sandwich enzyme-linked immunosorbent assay (Agdia, Elkhart, IN) followed, for all positive samples, by immunocapture reverse transcription polymerase chain reaction (IC-RT-PCR) with the PPV-polyvalent P10/P20 primers (**Olmos et al. 2002**). The samples with typical sharka symptoms were directly subjected to IC-RT-PCR. Strains were typed using strain-specific primers (CIP-M/MR and CIP-D/DR, in combination with P3M/P4 and P3D/P4), allowing the amplification of either PPV-M or PPV-D genomic fragments in the (Cter)P3-6K1-(Nter)CI and (Cter)NIb-(Nter)CP regions, respectively (**Candresse et al. 1998**; **Kamenova et al. 2011**). Successful PCR amplifications with CIP-D/DR and P3M/P4 primer pairs suggested the presence of recombinant PPV-Rec. The mD5/mM3 primers spanning the PPV-Rec recombination breakpoint (**Šubr et al. 2004**) were used in a second step to identify PPV-Rec isolates and mixed-strain infections. PPV was detected in 69% of the samples (110, 8, and 1 samples with typical, atypical, and no sharka symptoms, respectively), among which 88 were typed as PPV-M (74%), 10 as PPV-D (8%), and 21 as PPV-Rec (18%). Mixed

infection (M+D) was detected in only one sample. To confirm the strain typing, the genomic region consisting of the (Cter)NIb, full CP, and 3'UTR (1,252 nt) was sequenced for nine isolates typed as PPV-M (MK41, MK112, and MK175), -D (MK52, MK61, and MK169), and -Rec (MK121, MK137, and MK158). Each sequence was assembled from two overlapping PCR fragments amplified (and directly sequenced) using primer pairs P3M or P3D/P1, and P2 (**Wetzel et al. 1991**)/PolyT9 (5'-T_{21}GTCTCTTGC-3'), respectively. The Macedonian isolates typed as PPV-M, -Rec, and -D showed the highest nucleotide identities with PPV-M (98.7 to 99.3% with either M92280 or AJ243957), PPV-Rec (99.3 to 99.4% with HG964685), and PPV-D (98.2 to 99% with either HQ452356 or LT600780) strains, respectively. Phylogenetic reconstruction confirmed (with high support values) strain assignment. The corresponding sequences were deposited in GenBank (accession nos. MK562730 to MK562738). This is the first official report of PPV in the Republic of North Macedonia, where all sampled peach and plum orchards were infected. This work bridges a knowledge gap in the distribution of this quarantine pathogen, which induces up to 100% yield loss for the most susceptible local plum cultivars (e.g., Požegača) (**Scholthof et al. 2011**).

The author(s) declare no conflict of interest.

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