First Report of *Ranunculus* White Mottle Ophiovirus in Slovenia in Pepper with Yellow Leaf Curling Symptom and in Tomato

M. P. S. Rivarez,^{1,2} Z. Kogej,^{1,2} N. Jakš,¹ A. Pecman,^{1,2} G. Seljak,¹ A. Vučković,¹ M. Ravnkar,¹ N. Mehle,^{1,3} and D. Kutnjak^{1,†}

¹ Department of Biotechnology and Systems Biology, National Institute of Biology, 1000 Ljubljana, Slovenia

² Jožef Stefan International Postgraduate School, 1000 Ljubljana, Slovenia

³ School for Viticulture and Enology, University of Nova Gorica, 5000 Nova Gorica, Slovenia

M. P. S. Rivarez and Z. Kogej contributed equally to this work.

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Pepper (*Capsicum annuum*) and tomato (*Solanum lycopersicum*) plants showing virus-like disease symptoms were collected in 2017, 2019, and 2020 in different parts of Slovenia. Total RNA was extracted from the leaf tissue of individual samples using an RNeasy Plant Mini kit (Qiagen) and pooled in four composite samples as follows: two pepper plants from 2017 (D2017), five pepper and four tomato plants from 2019 (D2019_P1), seven tomato plants (D2020_P1), and two pepper and four tomato plants (D2020_P3) from 2020. The pooled RNA samples were sequenced using Illumina platforms (details of the sequencing experiments are in the supplementary material). Reads were analyzed using CLC Genomics Workbench (v. 20.0, Qiagen) following the pipeline for plant virus discovery (Pecman et al. 2017). Reads and contigs mapping to *Ranunculus* white mottle ophiovirus (RWMV) (GenBank accession no. AY542957 or NC_043389) were detected in all pools. The longest contig (1,255 bp) was obtained from the 2019 composite sample, which mapped to the coat protein-coding RNA 3 segment of the RWMV genome (accession no. AY542957). Details of mapping, genome coverage, and other viruses detected in the pools are summarized in the supplementary material. To identify individual RWMV-infected plants from the pools, PCR primers were designed to target the coat protein gene. Two pepper samples from two

different farms collected in 2017 and 2019 in southwest Slovenia and four tomato samples from two different farms collected in 2020 in central Slovenia tested positive for RWMV. To assess the diversity of RWMV isolates, amplicons were purified using QIAquick PCR purification kit (Qiagen) and sent for Sanger sequencing. Based on maximum likelihood phylogenetic analysis, Italian and Slovenian RWMV isolates form a monophyletic clade within the genus. Pairwise nucleotide identities of the Slovenian isolates (accession nos. MZ507604 to MZ507609) relative to the original Italian isolate coat protein (accession no. AY542957) range from 92 to 97%, indicating a moderate level of diversity among isolates. Since only RWMV, bell pepper alphaendornavirus (BPEV), and pepper cryptic virus 2 (PepCV2) were present in a pepper sample from 2017, and BPEV and PepCV2 infection in pepper are not known to be associated with any disease symptoms (Okada et al. 2011; Saritha et al. 2016), the symptoms observed on this plant might be associated with RWMV infection. We observed mottling with interveinal chlorosis or yellowing, slight to full curling of leaves from lamina inward, as well as necrotic and aborted flowers on this plant. We cannot easily associate observed symptoms with RWMV for RWMV-positive tomatoes, since several viruses were detected in the pools containing these samples. Nevertheless, the prominent symptoms in tomato plants were mottling with interveinal chlorosis and leaf curling, similar to those observed in pepper plants. RWMV was discovered and characterized in buttercups (*Ranunculus asiaticus*) and detected in anemones (*Anemone coronaria*) from Italy (Vaira et al. 1996, 1997, 2000, 2003). It was recently detected in pepper plants from Australia showing vein yellowing (Gambley et al. 2019). Here, we detected RWMV for the first time in Slovenia and reported its first detection in tomato and pepper plants from Europe. These findings call for further studies on the effects of RWMV infection on tomato and pepper production and its monitoring in neighboring European countries.

References:

- Gambley, C., et al. 2019. New Dis. Rep. 40:13.
- Okada, R., et al. 2011. J. Gen. Virol. 92:2664.
- Pecman, A., et al. 2017. Front. Microbiol. 8:1998.
- Saritha, R. K., et al. 2016. Virusedisease. 27:327.
- Vaira, A. M., et al. 1996. Acta Hort. 432:36.
- Vaira, A. M., et al. 1997. Arch. Virol. 142:2131.
- Vaira, A. M., et al. 2000. Plant Dis. 84:1046.
- Vaira, A. M., et al. 2003. Arch. Virol. 148:1037.

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[†]Indicates the corresponding author.
D. Kutnjak; denis.kutnjak@nib.si