



First report of European mountain ash ringspot-associated virus in *Karpatisorbus × hybrida* in Finland

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We report the first detection of European mountain ash ringspot-associated virus (EMARaV) in *Karpatisorbus × hybrida* in Finland. The host species varies in morphology, containing primary diploid hybrids of *Sorbus aria* and *Sorbus torminalis* as well as stable apomictic and vegetative propagating forms (Sennikov & Kurttio, 2017). Due to the decorative flowers, berries and leaf colouration in the autumn, the hybrid is planted in urban areas as a woody ornamental in the same way as other *Sorbus* species.

We sampled leaf material from six trees of *Karpatisorbus × hybrida* cultivated in a public park in the city of Helsinki, Finland, showing chlorotic ringspots, mottle, line patterns, sometimes accompanied by leaf deformation and decline (Figs. 1-4, Table 1). The observed disease resembled symptoms caused by EMARaV in *Sorbus* spp. (von Bargen *et al.*, 2019) and related hybrid species (Grimová *et al.*, 2015). Additionally, we collected samples from a rowan tree (*S. aucuparia*) with chlorotic ringspots on leaves growing adjacently. The rowan was infested by the pear blister gall mite, *Phytoptus pyri*, which is considered to be the vector of EMARaV.

To confirm the presence of EMARaV, we performed RT-PCR from extracted total RNA. We could demonstrate that six *Karpatisorbus × hybrida* and the *S. aucuparia* were affected by an emaravirus by amplification of a 360 bp fragment from the sampled leaf material using generic primers targeting RNA1 (Elbeaino *et al.*, 2013). Additionally, EMARaV-specific RT-PCRs (von Bargen *et al.*, 2019) detected all tested genome segments (RNA2-RNA4 and RNA6) of the virus in the corresponding samples, while none of the genome segments were detectable in a sample taken from a tree of *Karpatisorbus × hybrida* without leaf symptoms. By sequencing PCR products amplified from viral RNA1 and RNA4 we could confirm that all seven sampled trees with leaf symptoms were infected by EMARaV. We compared the nucleotide sequences of the partial RNA1 (348 bp) and the complete coding region of RNA4 (699 bp) with reference sequences from GenBank (Table 1). The minimum nucleotide identity was 97.4% (RNA1) and 98.1% (RNA4), respectively, (Table 1) confirming the virus as EMARaV according to the current species demarcation criteria for the genus (Elbeaino *et al.*, 2018). Sequences have been deposited in the European Nucleotide Archive (ENA) and are available under the accession numbers LR811990-LR812003.

This is the first record of EMARaV affecting *Karpatisorbus × hybrida* in Finland. The rowan tree growing adjacently was infected by the virus, with the putative vector *P. pyri* also being found on the tree. Kallinen *et al.* (2009) confirmed the virus to be widespread in rowan in Finland and Grimová *et al.* (2015) demonstrated that EMARaV is graft-transmissible within species of the *Rosaceae*. It is therefore possible that the virus was

transmitted from the rowan to the *Karpatisorbus × hybrida* population by root grafting. However, how the trees in the park in Helsinki acquired the virus remains unknown as their origin and history could not be determined.

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Figure 1



Figure 2



Figure 3



Figure 4

Table 1. Overview of samples and results of European mountain ash ringspot-associated virus (EMARaV) detection by generic segment-specific RT-PCR (EMARV-RNA1; 148 bp) and RNA4 (699 bp) specific fragments from symptomatic and asymptomatic reference sequences (RNA1, AY508402; RNA4, LR818811). Nucleotide sequence identities to the respective reference sequences are indicated (%)

Sample	Species	Symptoms	EMARaV identified by RT-PCR				
			RNA1	RNA2	RNA3	RNA4	RNA6
E14675	<i>Karpatisorbus × hybrida</i>	chlorotic ringspot & spots, chlorotic mottles, decline	98.1	+	+	98.1	+
E14677	<i>Karpatisorbus × hybrida</i>	chlorotic ringspot, mottle	98.1	+	+	98.1	+
E14678	<i>Karpatisorbus × hybrida</i>	chlorotic ringspot, mottle, decline	98.1	+	+	98.1	+
E14679	<i>Karpatisorbus × hybrida</i>	mottle, line pattern	98.1	+	+	98.2	+
E14681	<i>Karpatisorbus × hybrida</i>	chlorotic ringspot, mottle, line pattern	98.1	+	+	98.2	+
E14682	<i>Karpatisorbus × hybrida</i>	chlorotic ringspot, mottle, line pattern	97.4	+	+	99.4	+
E14683	<i>Sorbus aucuparia</i>	chlorotic ringspot, mottle, galls	98.1	+	+	98.4	+
E14684	<i>Karpatisorbus × hybrida</i>	no symptoms	-	-	-	-	-

+ = positive RT-PCR, - = negative RT-PCR

Figure 5

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