First report of *European mountain ash ringspot-associated virus* in *Sorbus aucuparia* in Norway

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In July 2012, leaf mottle and intensive chlorotic ringspots were observed on urban, forest or roadside mountain ash trees (*Sorbus aucuparia* L., rowan) of different ages in Norway during visual inspection of native broad-leaf forest tree species (supplemental fig. 1). Symptoms resembled those caused by *European mountain ash ringspot-associated virus* (EMARaV), the type-member of the newly established genus *Emaravirus*, containing segmented ss(-)RNA and infecting woody host species (1). Leaves of nine out of 30 assessed rowan trees exhibiting characteristic symptoms were sampled in the counties of Nordland and Nord-Trøndelag (between 63.511806° and 66.304680° northern latitude). Three of them were infested by the potential vector the eriophyid gall mite *Phytoptus pyri*. EMARaV was detected from total RNA extracts of leaves by reverse transcription-PCR using virus-specific primers amplifying 300 bp of RNA2 and 204 bp of RNA3, respectively (2). PCR fragments were directly sequenced from both ends and submitted to the EMBL database (accession numbers HG428680-HG428697). Sequenced fragments comprising the partial gene encoding the glycoprotein-precursor (261 nucleotides of RNA2 omitting primer sequences) obtained from the nine sampled trees showed identities of 97-98 % to the sequence of the reference strain of EMARaV from Hamburg, Germany (database accession AY563041). Comparison of 159 nucleotides of the 3´ untranslated region (3´ UTR) of viral RNA3 of the nine investigated

rowans in Norway exhibited higher sequence diversity on nucleotide level (up to 50 nucleotide exchanges, or 31 %) as previously reported from EMARaV variants from other European countries (3). When subjected to BLASTN search through the GenBank, only 3 partial RNA3 sequences generated in this study showed sequence identities of 96 % to the reference isolate (accession DQ831831). The other 6 sequences revealed only 68-73% identity to RNA3 sequences of EMARaV variants from the GenBank database. This led to formation of a separate cluster in phylogenetic analysis of partial RNA3 sequences of the six EMARaV variants from Norway when compared to previously characterized strains from the Czech Republic (n=2), Finland (n=17), Germany (n=1), Great Britain (n=5), Russia (n=3), and Sweden (n=10) (supplemental fig. 2). From three Norwegian samples clustering separately in the tree based on the partial 3´ UTR of RNA3, the partial vRNA1 was amplified by RT-PCR using a generic primer set Motif-A-sense/Motif-C-antisense (4). Sequence analyses of these PCR fragments confirmed the viruses as members of the *Emaravirus* genus which were most closely related to EMARaV (data not shown). This is the first report of EMARaV in Norway infecting *Sorbus aucuparia*, a valuable native plant of northern Europe. The data obtained suggest a higher genetic variability of the EMARaV population in mountain ash trees in Norway than in other locations in Central and Northern Europe. However, whether the EMARaV variants identified in this study represent new strains of the virus have to be investigated in the future.

References: (1) N. Mielke-Ehret. and H. P. Mühlbach. Viruses 4: 1515-1536, 2012. (2) N. Mielke et al. For. Path. 38: 371-380, 2008. (3) S. von Bargen et al. For. Path. 43: 429-423, 2013. (4) T. Elbeaino et al. J. Virol. Meth. 188: 37-40, 2013.

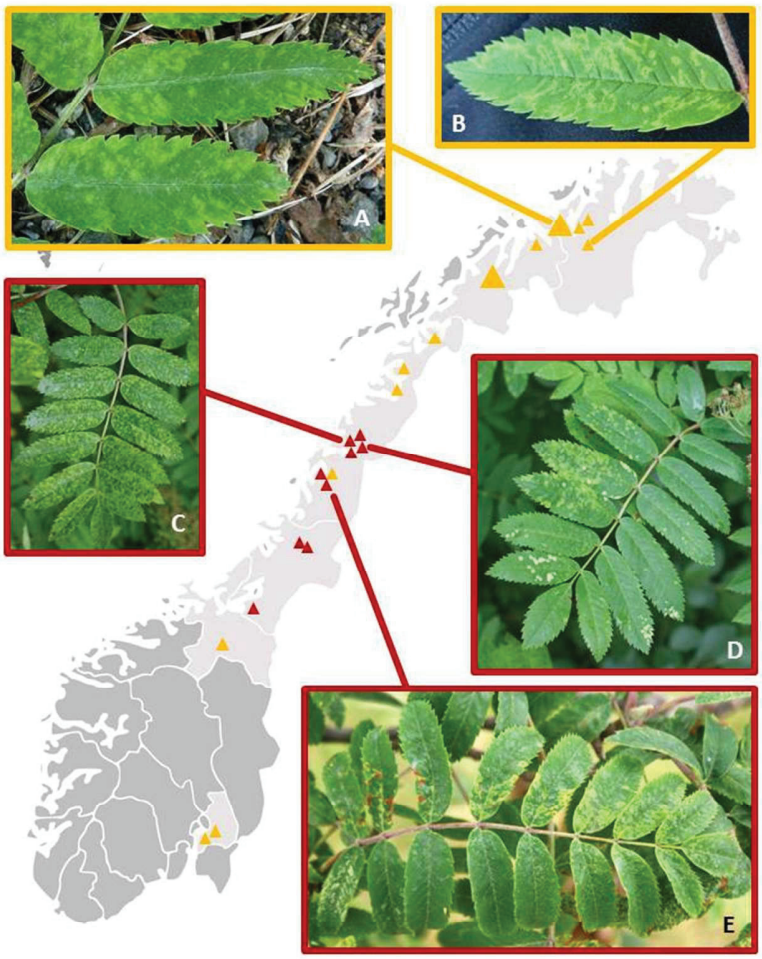

Fig. 1: Symptomatic rowans (yellow triangles) and detection of *European mountain ash ringspot-associated virus* (red triangles) in trees in different locations. Small triangles indicate individual trees, large triangles represent 5 trees. Chlorotic ringspots (A, E), line pattern (B), mottle (C) in leaflets. Leaf with irregular distributed chlorotic ringspots and galls indicative of an infestation with *Phytoptus pyri* (D).

190x275mm (96 x 96 DPI)

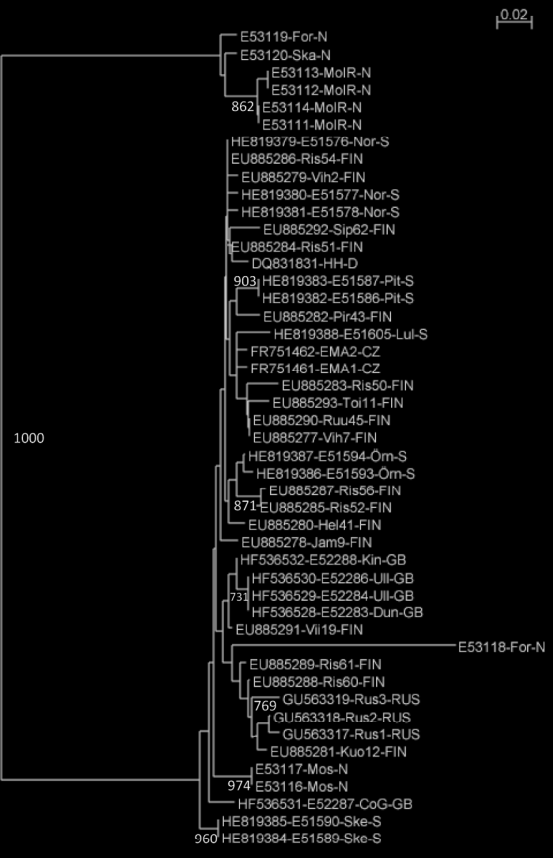

Fig 2: Neighbour-joining phylogenetic tree inferred from 159 nucleotides of the partial 3′ untranslated region of EMARaV-RNA3 using ClustalX 2.0 (bootstrap values above 700 are indicated). The scale bar represents 2 exchanges per 100 nucleotides. Sequences obtained from the database are indicated by their accession number.

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