A novel virus is associated with the ringspot disease in Common oak (*Quercus robur*)

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Introduction

Virus-suspected symptoms like mottle, chlorotic spots and ringspots on oaks were already observed in the 1970s [1] but no causal agent was found so far. Using modern High-throughput sequencing methods a previously unknown virus was identified in an oak tree with this observed disorder. Sequence analysis revealed closest relationship to emaraviruses, a genus of segmented negative stranded RNA viruses [2] (Fig. 1). Specific RT-PCRs were established to study the association of the virus with observed symptoms and to confirm the four identified viral RNAs in diseased oaks.

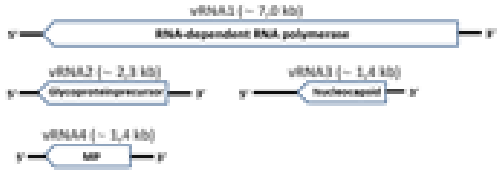


Fig. 1: Genomic organization of the novel putative emaravirus. Viral segments with fragment length and encoding proteins are shown. MP = movement protein

Sample collection

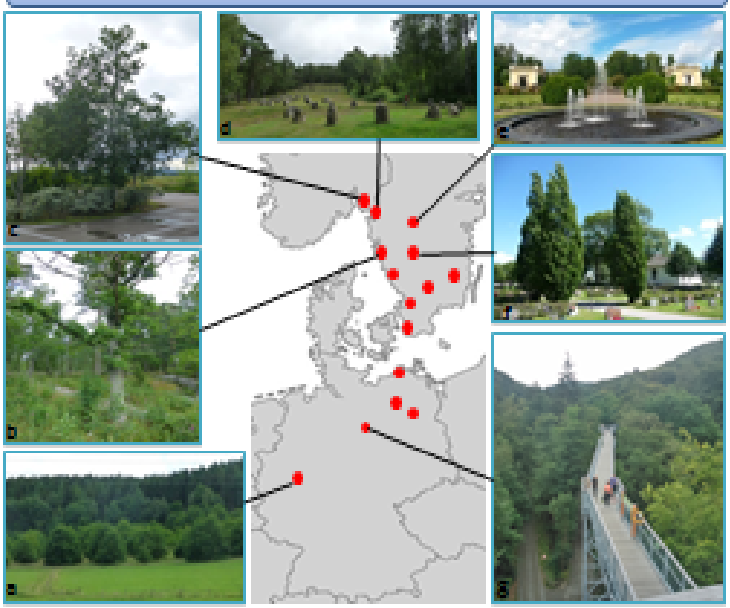
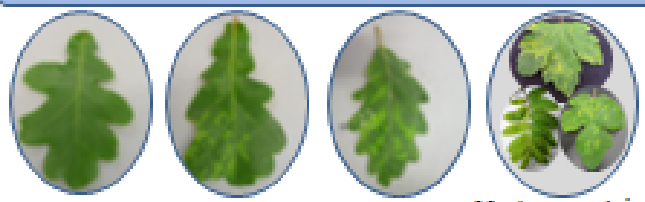


Fig. 2: Sampling sites with diseased oaks
Examples of sampling sites are illustrated, including a seed collection stand (a), parking lot (c), churchyard (f), park trees (e) and forest trees (b, d and g), in different European countries

Results



RNA-Extraction

Detection of viral genome segments via RT-PCR

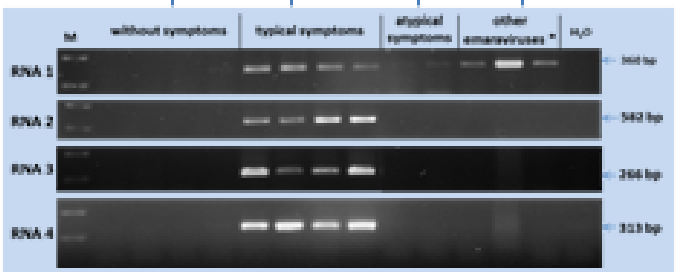


Fig. 3: Establishment of virus-specific PCR as shown by analysis of PCR products after agarose gelelectrophoresis. Detection of emaraviruses by generic RT-PCR targeting viral RNA1, according to Elbeaino et al. 2015. Virus-specific detection by RT-PCR targeting the viral RNA2, RNA3 and RNA4.
*included *Fig mosaic virus*, *European mountain ash ringspot-associated virus* and Emaravirus in maple

Table 1: Detection of emaravirus in sampled oaks by virus-specific RT-PCR targeting viral RNA3 and RNA4

Samples (n)	Location*	RNA 3 detectable	RNA 4 detectable
Symptomatic (129)	D (Fe, Ha, Pr) S (Asp, Gu, Fo, Sä, Vi) N (Hu, Ås)	81/ 83	123/ 129
Asymptomatic (17)	D (Fe) S (Asp, Gu, Sä, Vi) N	0/17	0/17
Atypical (16)	D (Da, Ha, Hvl, Pf)	0/17	0/17

*D = Germany (Da = Darß, Fe = Fellinghausen, Ha = Harz, Hvl = Havelland, Pf = Pfaueninsel, Pr = Prerow)
N = Norway (Ås = Ås, Hu = Hunn)
S = Sweden (Asp = Aspeberget, Fo = Fossum, Gu = Gustavsberg, Sä = Säffle, Vi = Vittsyke)

CONCLUSIONS

Detection of the novel emaravirus in oak is closely correlated with chlorotic ringspot symptoms while it was neither detectable in leaf material collected from trees without virus-like symptoms nor in *Quercus* spp. showing atypical symptoms like regular chlorotic patterns or partial chloroses of leaves (Fig. 3, Table 1). The putative new emaravirus is widespread in oak populations confirmed to occurr in Germany, southern Sweden and Norway (Fig. 2, n = 162).

References
[1] Nienhaus (1975) Z Pflkrankh Pflschutz 82, 738-749; [2] Mielke-Ehret, Mühlbach (2012) Viruses, 1535-1558; [3] Elbeaino, Whitfield, Sharma, Digiaro (2015) Journal of Virological Methods, 57–60

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