

# First detection of *European mountain ash ringspot-associated virus* in *Sorbus aria* and *Sorbus intermedia*



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So far, *Sorbus aucuparia* was the only known host of EMARaV. Here, we present evidence for infection of two additional tree species with EMARaV in Sweden.

## Introduction

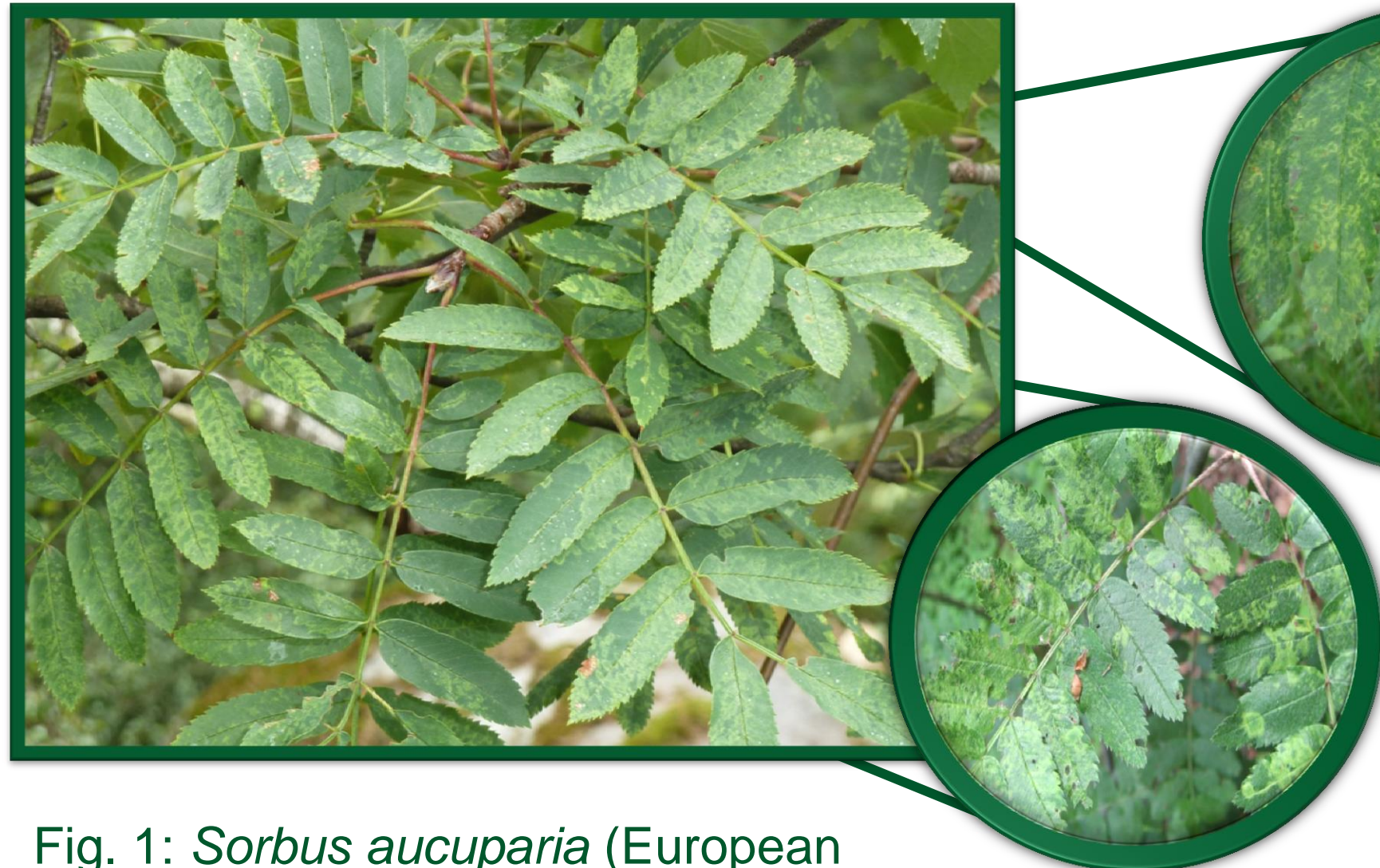


Fig. 1: *Sorbus aucuparia* (European mountain ash) with mottle and chlorotic ringspots.

These symptoms are wide spread in European mountain ash from North and Central Europe (Robel *et al.*, 2013) and were associated with the *European mountain ash ringspot-associated virus* (EMARaV) (Mielke *et al.*, 2007), the type-species of the newly established genus *Emaravirus* (Mühlbach & Mielke-Ehret 2011). The genome of EMARaV consists of four single stranded RNAs (RNA1-RNA4) of negative polarity. Each segment encodes a single protein (P1-P4) translated from the complementary strand (figure 4).

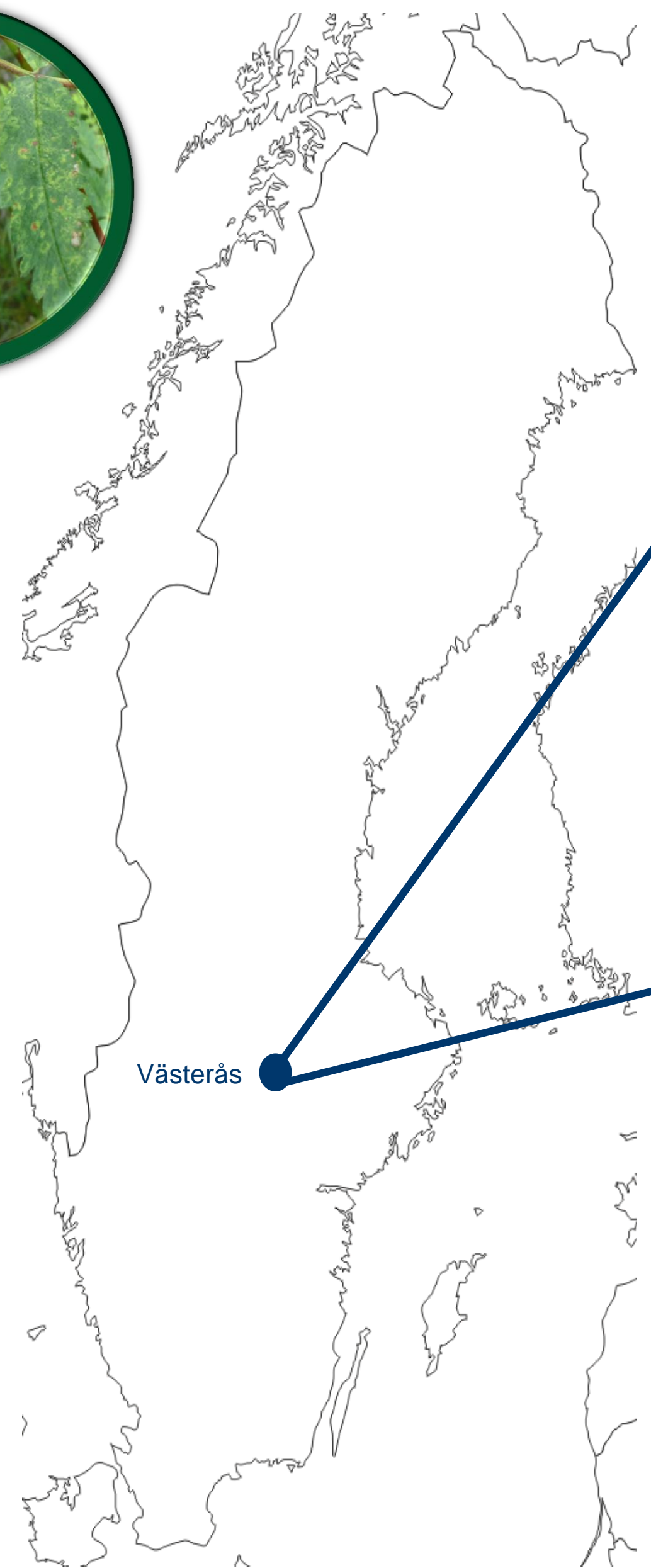


Fig. 2: *Sorbus intermedia* (Swedish whitebeam) with mottle and chlorotic ringspots.

## Material & Methods

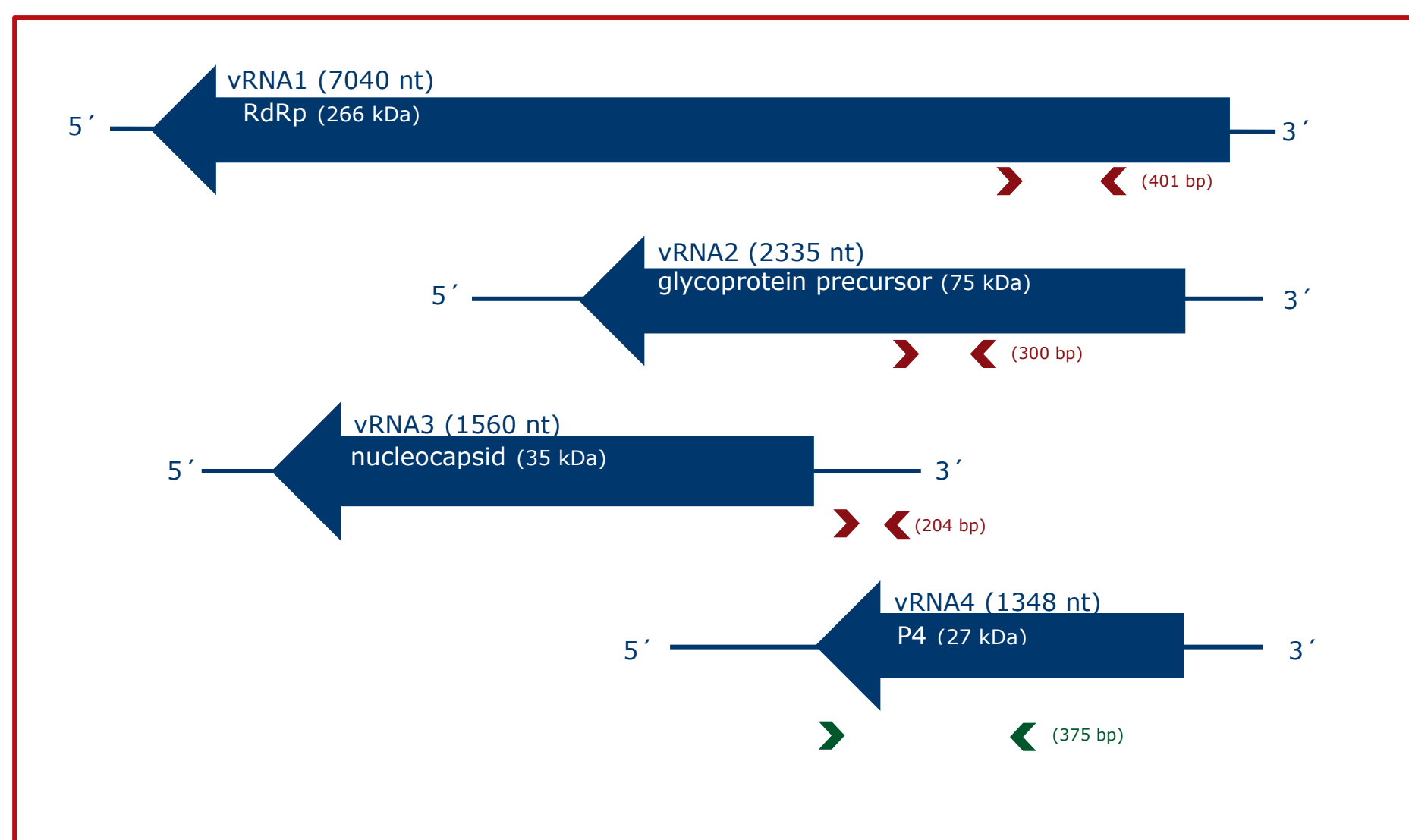


Fig. 4: Genome organization of EMARaV with locations of primers used for amplification of specific fragments from all four genome segments.

Total RNA from symptomatic leaves (figure 2 and 3) of affected common whitebeam and Swedish whitebeam sampled in 2012 in Västerås (Sweden) was isolated according to the protocol by Mielke and Muehlbach (2007).

Fragments of all four RNAs comprising the viral genome were amplified by RT-PCR and directly sequenced. Amplification of RNA1, RNA2 and RNA3 fragments (red arrow heads) was performed according to Mielke *et al.* (2008). A 375 base pair fragment of RNA4 was amplified with the primer pairs p4-for and p4-375-rev (green arrow heads).

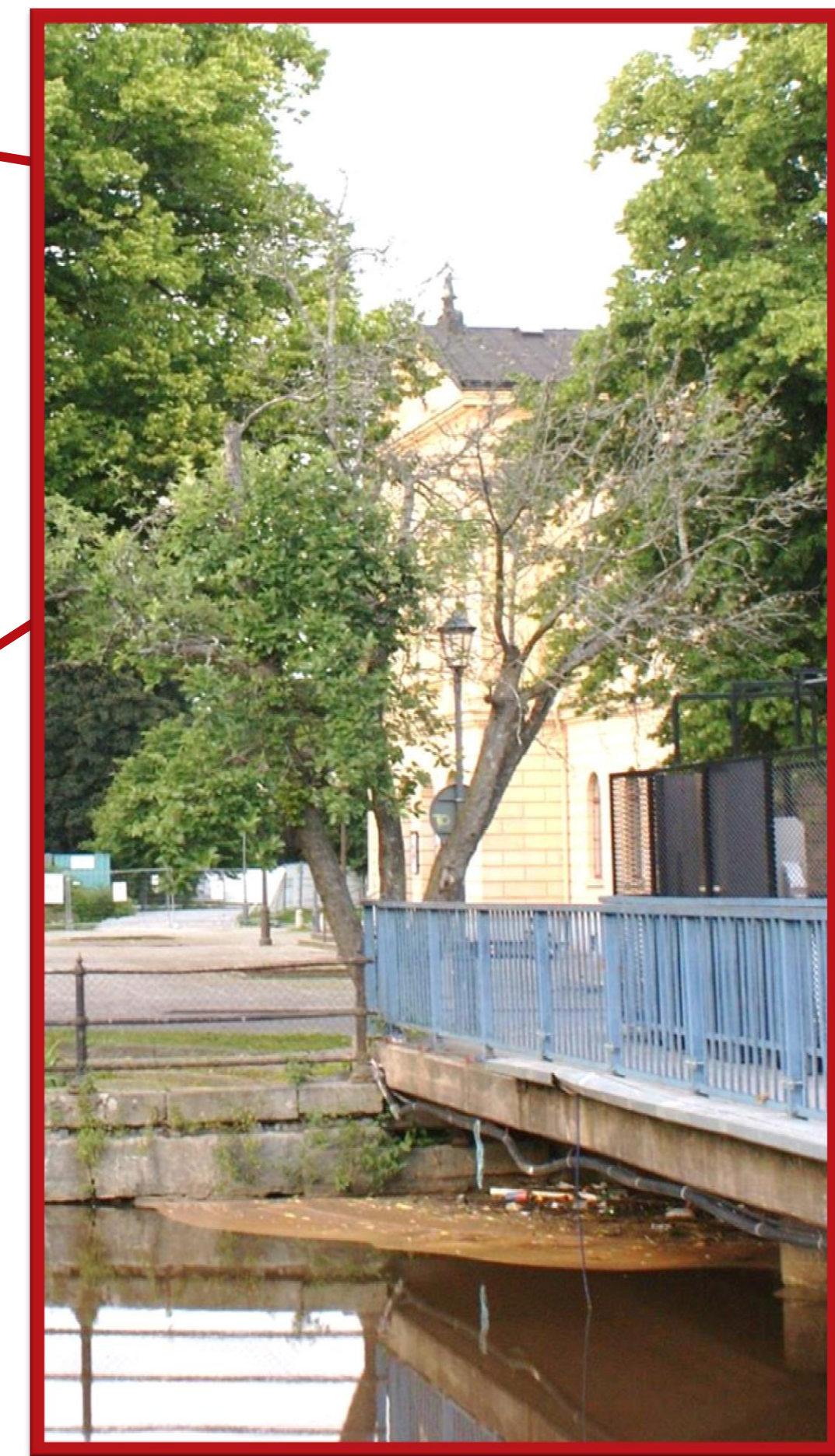


Fig. 3: *Sorbus aria* (Common whitebeam) with chlorotic line pattern on leaves of a degenerating tree.

## Results

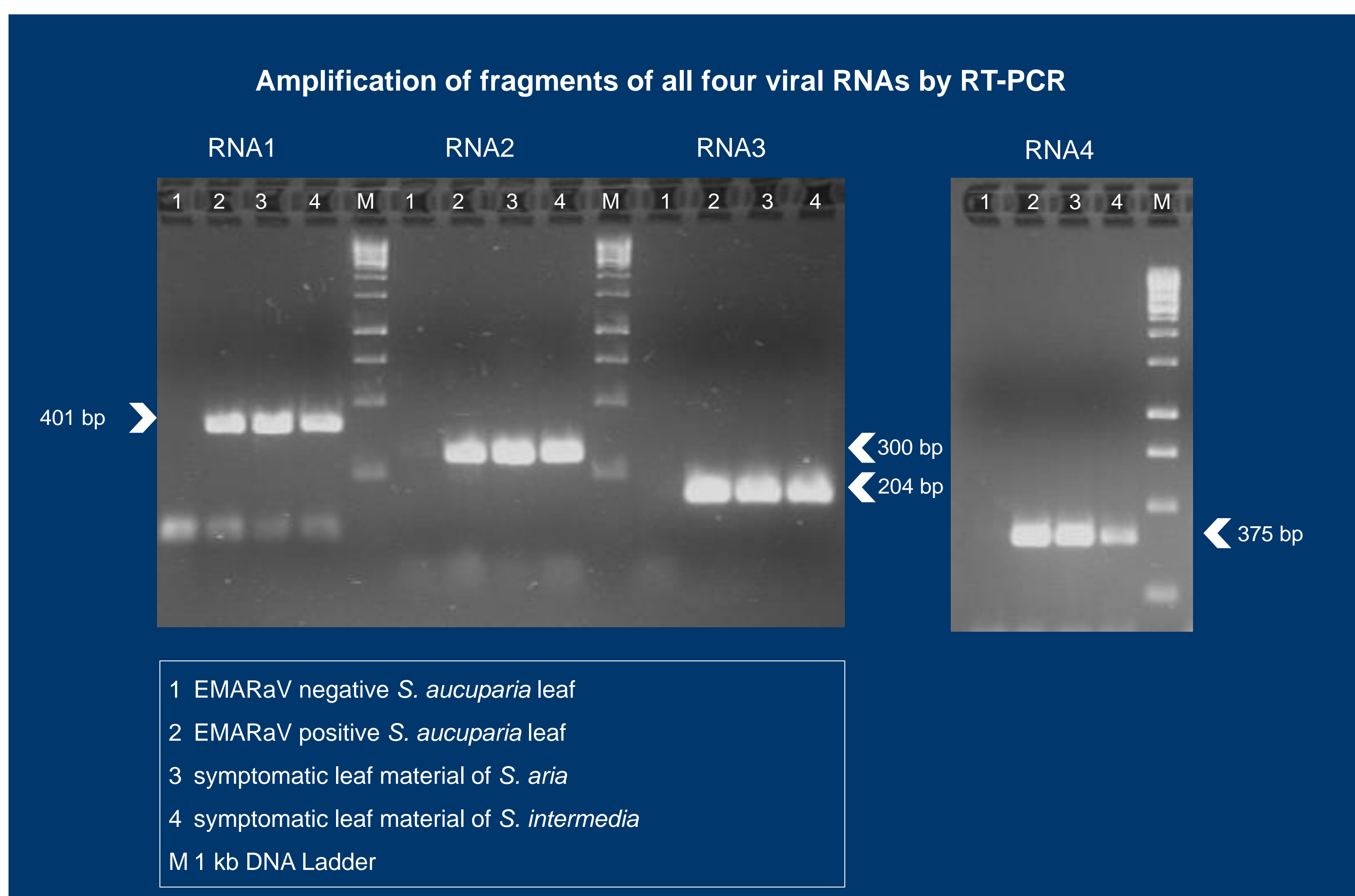


Fig. 5: Gel electrophoretic separation of RT-PCR products.

### *Sorbus intermedia*:

- The 3' UTR fragment with 99 % identity to other EMARaV variants from Sweden, Finland, and Germany
- RNA4 fragment showed 100 % identity to the German EMARaV RNA4 sequence (DQ831828)

➤ EMARaV has different hosts and infects other *Sorbus* species besides *Sorbus aucuparia*.

➤ In a neighbour-joining phylogenetic tree generated from the 3' UTR fragment, the sequence variants separates in two different clusters (figure 6).

- All four EMARaV RNAs were detected in Common whitebeam (*Sorbus aria*) and Swedish whitebeam (*Sorbus intermedia*) from Västerås (figure 5).

### *Sorbus aria*:

- Nucleotide BLAST of 159 bp of the 3' untranslated region (3' UTR) of RNA3 showed highest identities (98 %) to EMARaV variants from Sweden, Czech Republic, and Finland
- Sequence fragments of RNA4 showed 99 % identity to EMARaV sequences from the database

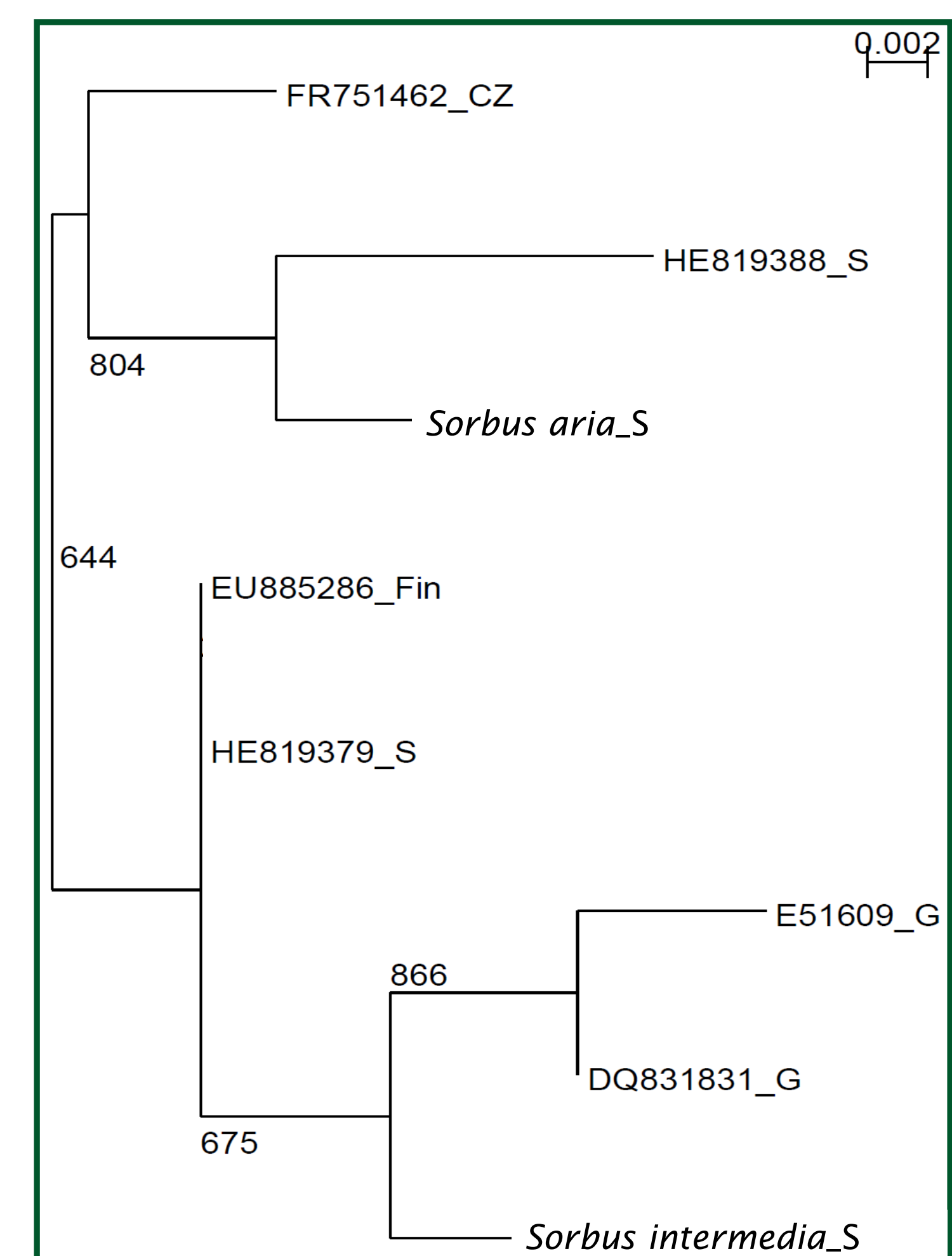


Fig. 6: Neighbour-joining phylogenetic tree using ClustalX2.0 generated from the partial 3' UTR.

## Outlook

- Examination of more whitebeam and Swedish whitebeam trees to verify the two species as a host plant of EMARaV.
- Investigation of additional plant species (e.g. other members of family Rosaceae) to find other putative host plants.
- Study of modes of transmission of the virus. Currently, the gall mite *Phytoptus pyri* is the only known putative vector of EMARaV.

## References

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 Mühlbach H P, Mielke-Ehret N. 2011. Emaravirus. Elsevier Academic Press, San Diego/USA: 767-770.  
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## Acknowledgement

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