New Disease Reports

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First Detection of a Phytoplasma From the 16SrV-C (Elm yellows) Group in *Alnus glutinosa* in the Netherlands

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In September 2024, a phytoplasma was detected in six mature black alder (*Alnus glutinosa*) trees growing naturally in the municipality of Wageningen, the Netherlands. The trees displayed no phytoplasma- or virus-like symptoms. The samples were collected as part of a project, which aimed to enhance knowledge of viruses and other non-culturable organisms in forestry tree species. Black alder was included in this study because it has been reported as a host for the EU quarantine pest grapevine flavescence dorée phytoplasma (GFDP) (Malembic-Maher et al. 2020). GFDP is regulated due to its role in causing 'flavescence dorée' (FD), a grapevine disease characterised by severe symptoms and decline. GFDP was previously considered as '*Candidatus* Phytoplasma vitis'; however, as its 16S rRNA gene sequences share over 98.65% identity with those of other phytoplasmas (Bertaccini et al. 2022), it is not formally classified as a distinct '*Candidatus* Phytoplasma' species. This genetic similarity complicates its classification and regulatory status.

Leaves were collected from different branches of each tree. DNA was extracted (DNeasy Plant Mini Kit; Qiagen, the Netherlands) from the midribs and secondary veins. Phytoplasma was detected in all six trees by generic real-time PCR primers JH-F1/JH-F all/JH-R and probe JH-P (Hodgetts et al. 2009). One sample was further tested by nested PCR using the P1/P7 and R16F2n/R16R2 primers following EPPO Standard PM7/133 (European and Mediterranean Plant Protection Organization 2018). The PCR amplicon obtained through conventional PCR was purified (QIAquick PCR Purification Kit; Qiagen, the Netherlands) and sequenced using Illumina technology. According to the *i*PhyClassifier (Zhao et al. 2013), the resulting 16S rRNA sequence (GenBank Accession No. PV565616) belonged to the 16SrV-C subgroup. A BLASTn search showed 99.92% identity with several 16SrV phytoplasmas including GFDP (AF176319), Alder yellows phytoplasma (PP097681) and '*Candidatus* phytoplasma ulmi' (MH279539). To differentiate between these 16SrV phytoplasmas, the *secY-map* sequence was amplified from the same sample by nested PCR with primer pairs FD9f5/MAPr1 and FD9f6/MAPr2 (Arnaud et al. 2007). The PCR amplicon was purified and Sanger sequenced (Macrogen Europe, the Netherlands). The *secY-map* sequence (PV563858) was compared with known map genotypes in CLC Genomics Workbench (version 24, Qiagen, Slovenia) with BLAST tool and matched genotype M44 (Figure 1). Genotype M44 is not linked to epidemic clusters Map-FD1, Map-FD2 or Map-FD3, which include genotypes associated with *Scaphoideus titanus* transmission and FD outbreaks. Instead, the M44 genotype has previously been reported in black alder in France, Germany and Italy, and in the vector *Oncopsis alni* in France and Germany (Malembic-Maher et al. 2020).

To our knowledge, this is the first report of phytoplasma from the 16SrV-C (Elm yellows) group detected in the Netherlands. Following previous reports that 16SrV phytoplasmas do not always cause symptoms in black alder (Malembic-Maher et al. 2020),

P.P.M. de Koning and R. Schoen contributed equally to this work.

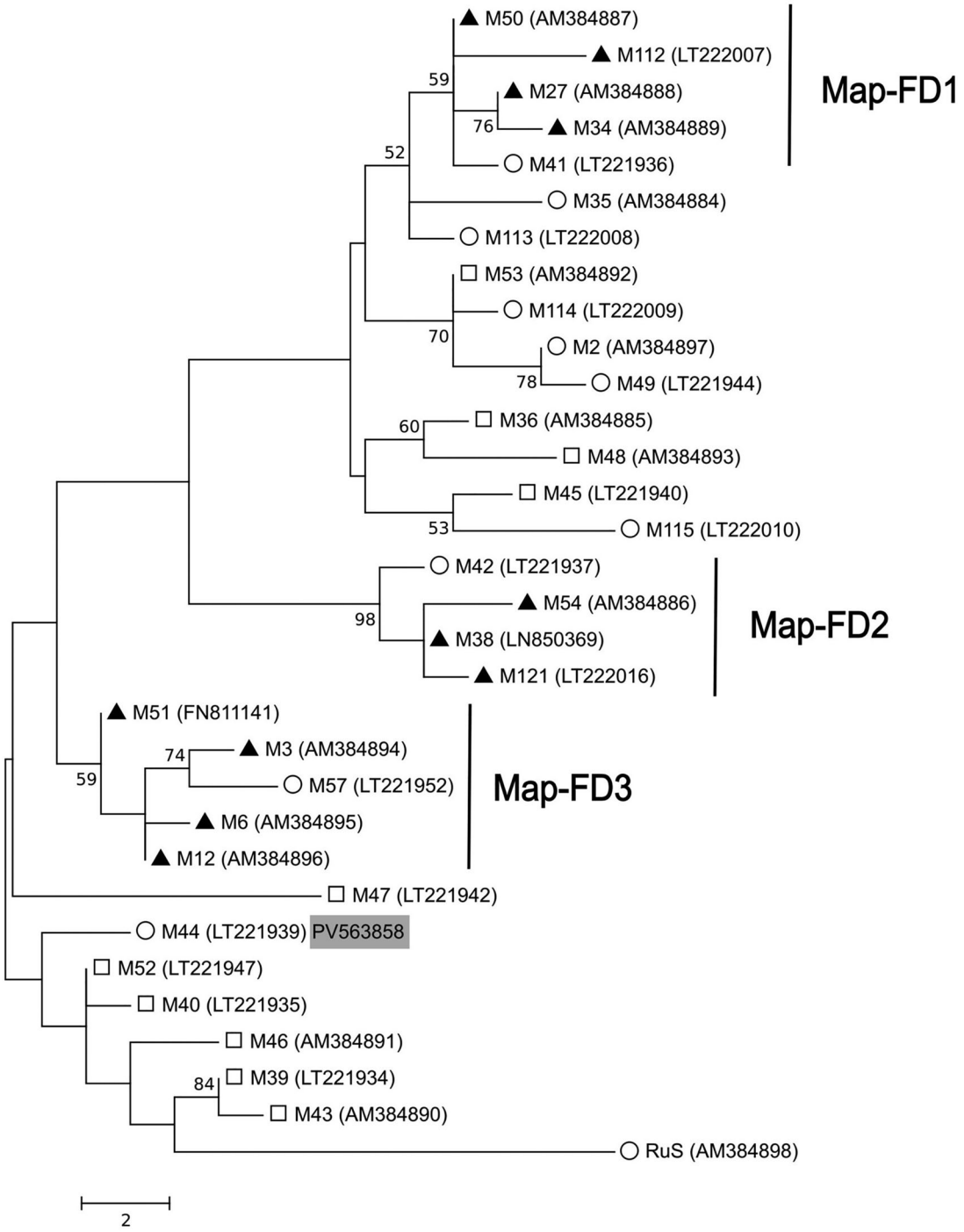

FIGURE 1 | Unrooted maximum parsimony phylogenetic tree of the *secY-map* sequence of the M44 isolate from *Alnus glutinosa* in the Netherlands (marked in grey) and reference sequences of 16SrV phytoplasmas. GFDP isolates are marked with filled triangles, grapevine non-epidemic isolates with squares and 16SrV isolates from other plants with circles; RuS—'*Candidatus* Phytoplasma rubi'; the tree was constructed in MEGA7 with 500 bootstrap replicates (bootstrap values indicated on branches when ≥50) based on a ClustalW alignment of 674 bp sequences; the scale bar represents the number of nucleotide changes per site.

the sampled trees in this study appeared healthy. The identified phytoplasma was not considered a quarantine pest, and therefore, no phytosanitary measures were taken. This finding highlights the value of baseline studies, including asymptomatic sampling, to identify reservoir hosts and gain insight in phytoplasma distribution, diversity and ecology. Such studies are essential for risk assessment, regulation refinement and improving early detection strategies. Moreover, this example underlines the complexity of phytoplasma taxonomy and the challenges in diagnosing regulated phytoplasma species.

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Data Availability Statement

The nucleotide sequences from this study can be found in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) with GenBank accession no. PV565616 and PV563858.

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