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NEW DISEASE REPORT

First report of a putative new badnavirus in association with wisteria mosaic disease in Italy

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KEYWORDS

Badnavirus wisteriae, Chinese wisteria, Wisteria vein mosaic virus

In 2022, severe symptoms of wisteria mosaic disease (WMD), such as irregular leaf spots, interveinal patterns and chlorotic patches (Figure 1), were observed on a Chinese wisteria (*Wisteria sinensis*) growing in an urban garden (Bari, Southern Italy). Preliminary RT-PCR investigations led to the detection of Wisteria vein mosaic virus (WVMV, *Potyvirus wisteriae*, genus *Potyvirus*) (D'Attoma et al., 2023). Further characterisation, based on Illumina® high-throughput sequencing of a ds-RNA library isolated in the following season, confirmed the presence of WVMV and revealed the potential occurrence of badnavirus contigs (Morelli et al., 2024).

To validate the HTS findings, total DNA extracted from a pooled sample of ten leaves, randomly collected from the same diseased plant, was tested using the BadnaFP/RP PCR universal primer set, designed within the core of RT/RNase H domain (Yang et al., 2003). All amplifications returned an amplicon of 576 bp, which was cloned into the pSC-A-amp/kan vector (Agilent Technologies, USA) and sequenced (GenBank Accession No. PQ878522). A pairwise MAFFT alignment with the corresponding amino acid sequence of 68 assigned species in the genus *Badnavirus* revealed the highest sequence identity (nt 74.7%, aa 84.4%) with Wisteria badnavirus 1 (WBV-1, *Badnavirus wisteriae*) (Figures 2–3). Based on the species demarcation criterion for the genus *Badnavirus*, a nucleotide identity value <80% in the RT/RNase H coding region suggests that the isolate could be classified as a new virus, for which the tentative name Wisteria badnavirus 2 (WBV-2) is proposed. The tentative phylogenetic relationships of the WBV-2 Bari isolate were elucidated by running the PhyML workflow available at NGPhylogeny.fr and based on the LG substitution model. TreeClus clustering placed WBV-2 and WBV-1 in a subclade of a broader lineage that included as closest neighbour, pagoda yellow mosaic associated virus isolated from *Styphnolobium japonicum*, another ornamental plant of Asian origin (Figure 4).



FIGURE 1 Symptoms of wisteria mosaic disease observed on Chinese wisteria (*Wisteria sinensis* cv. Sweet) leaves, co-infected by Wisteria vein mosaic virus and a badnavirus provisionally named Wisteria badnavirus 2. The compound leaves showed interveinal mottling, chlorotic patches and ringspots, in some cases evolving to blotches, necrotic flecks and slight distortions.

A survey conducted in the immediate proximity of the original infected wisteria plant identified eleven trees with the same symptoms. When a second set of specific primers, designed for the detection of WBV-1 (Mehrvar et al., 2022) and spanning the C-terminal region of the coat protein, was used, nine of the eleven plants tested positive for WBV-2. The amplicon (727 bp) was sequenced and deposited in GenBank (PQ878523). In addition, two asymptomatic plants were found to be positive, while three further asymptomatic plants were negative. All plants which tested positive for WBV-2 were found to be co-infected with WVMV when tested by RT-PCR. Only one of

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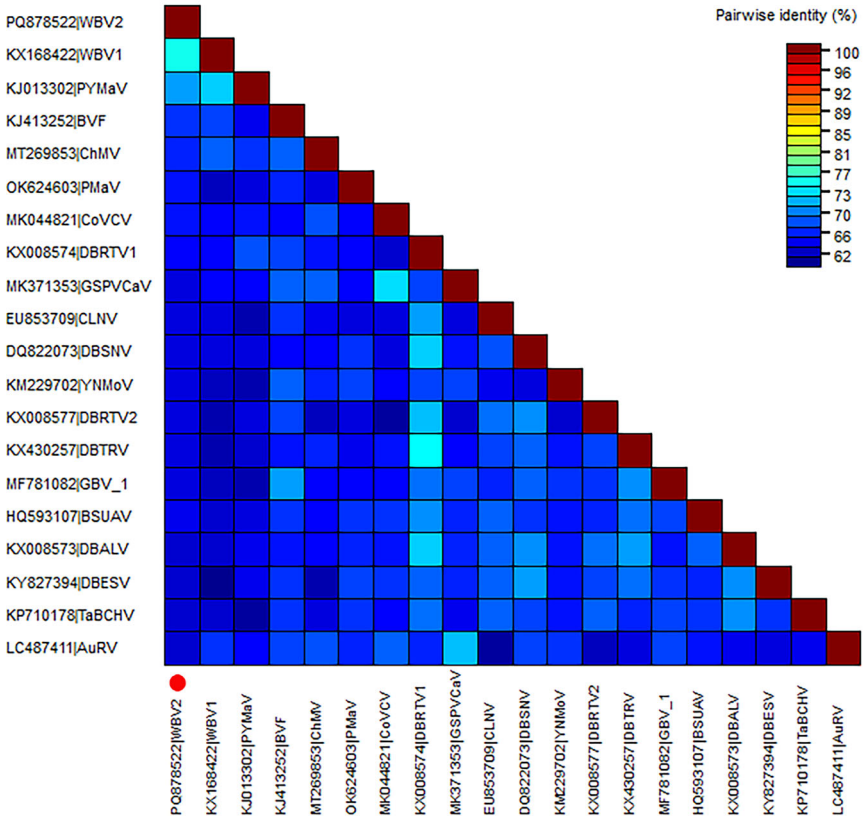


FIGURE 2 Distance matrix of pairwise MAFFT-based comparison of the RT/RNase H region (576 nt) in the gene coding region for the ORF3 polyprotein of species in the genus *Badnavirus*. Only sequences sharing the highest aa identity with Wisteria badnavirus 2 (WBV-2) isolate Bari (indicated by the red dot) are shown. Sequences are identified by their accession numbers and virus acronyms.

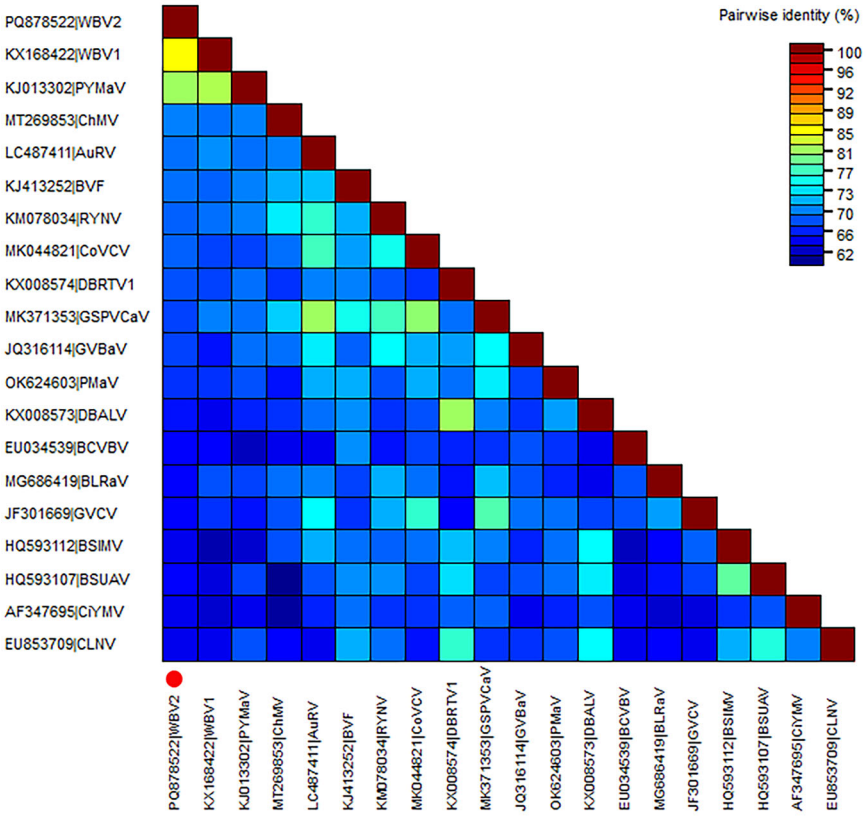


FIGURE 3 Distance matrix of pairwise MAFFT-based comparison of the RT/RNase H region (192 aa) in the ORF3 polyprotein of species in the genus *Badnavirus*. Only sequences sharing the highest aa identity with Wisteria badnavirus 2 (WBV-2) isolate Bari (indicated by the red dot) are shown. Sequences are identified by their accession numbers and virus acronyms.

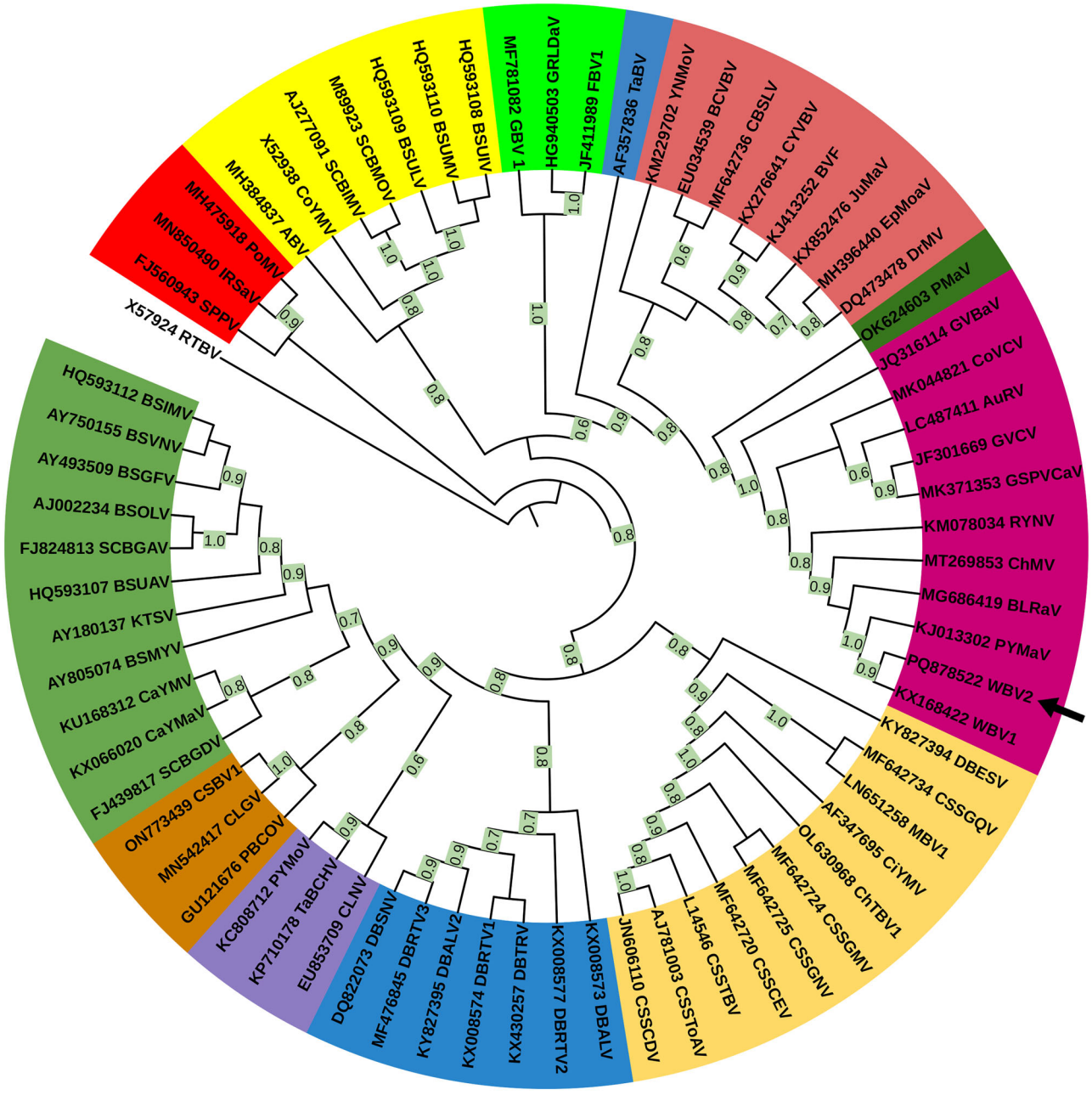


FIGURE 4 Radial cladogram based on the RT/RNase H region (192 aa) of the ORF3 polyprotein of WBV-2 isolate Bari and species in the genus *Badnavirus*. Phylogenetic reconstruction was performed with the PhyML workflow available at the NGPhylogeny web service. Computation was based on the LG substitution model and branch support was evaluated with the Shimodaira-Hasegawa (SH)-like likelihood ratio test (values >0.5 are shown above the branches). The tree was rooted using rice tungro bacilliform virus (RTBV) as an outgroup. The branch length is unscaled. The cluster analysis was performed with the TreeClus algorithm with a dissimilarity threshold >50%. Distinct clades are shaded in different colours. Sequences are identified by their accession numbers and virus acronyms.

the three asymptomatic plants negative for WBV-2 was positive for WVMV.

To date, WBV-1 is the only badnavirus reported in wisteria, having been identified in China (Li et al., 2017) and Iran (Mehrvar et al., 2022) in *W. sinensis* showing leaf crinkling and mosaic symptoms, and in co-infection with WVMV. To our knowledge, this is the first finding of a badnavirus in WMD-affected wisteria in Italy. However, it should be noted that the genome of badnaviruses can become integrated, in whole or part, into the the genome of their hosts. Further work is required to demonstrate the presence of replicating virus, e.g. electron microscopy or selective detection of the episomal genomic DNA. The correlation between WVMV and WBV-2 infection means that it is not possible to draw conclusions on the pathogenicity of this putative new virus.

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