First Report of Cucurbit Aphid-Borne Yellows Virus Causing Yellowing Disease on Pumpkin (*Cucurbita pepo*) and Cucumber (*Cucumis sativus*) in Bulgaria

V. Radeva-Ivanova,^{1,†} G. Pasev,^{1,†} R. Lyall,² M. Angelov,³
A. N. Nankar,⁴ and D. Kostova-Protochristova⁴

¹ Virus Immunity Laboratory, Maritsa Vegetable Crops Research Institute, 4000 Plovdiv, Bulgaria

² Bioinformatics Department, Center of Plant Systems Biology and Biotechnology, 4000 Plovdiv, Bulgaria

³ Molecular Stress Physiology, Department Center of Plant Systems Biology and Biotechnology, 4000 Plovdiv, Bulgaria

⁴ Vegetable Breeding Department, Center of Plant Systems Biology and Biotechnology, 4000 Plovdiv, Bulgaria

V. Radeva-Ivanova and G. Pasev contributed equally to this work.

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Cucurbit aphid-borne yellows virus (CABYV) was first reported in France in 1992 but has since been observed worldwide (Buzkan et al. 2017; Choi and Choi 2016; Khanal and Ali 2018; Lecoq et al. 1992; Vidal et al. 2018; Zindovic et al. 2017). This virus has caused severe losses to different crops, especially to members of the family Cucurbitaceae, and yield losses can reach up to 40 to 50% if infection occurs at early stages (Lecoq et al. 1992). In July 2017, leaf samples showing virus-like symptoms were collected from five pumpkin (*Cucurbita pepo* L. var. *Clypeata Alefield*) and two cucumber (*Cucumis sativus* L. cv. *Azuma matsunari*) plants in a field near Sadovo, Bulgaria. Nearly all plants in the field were affected and displayed green or yellow mosaic, interveinal yellowing, blisters, and leaf deformation. The collected samples were all symptomatic and were subjected to a double antibody sandwich or triple antibody sandwich enzyme-linked immunosorbent assay to determine the viral agent(s). Specific monoclonal antibodies (Leibniz Institute DSMZ, Germany) raised against cucumber leaf spot virus, cucurbit chlorotic yellows virus, cucurbit yellow stunting disorder virus, cucumber mosaic virus (CMV), melon necrotic spot virus, beet western yellows virus (BWYV), CABYV, watermelon mosaic virus (WMV), zucchini yellow mosaic virus (ZYMV), and cucumber green mottle mosaic virus were

used. The total number of tested samples was seven ($n = 5$ from pumpkin and $n = 2$ from cucumber). All of them displayed positive signals for CABYV and BWYV, both belonging to genus *Polerovirus*, family *Luteoviridae*. In addition, ZYMV and/or WMV were detected in pumpkins while CMV and/or WMV were detected in cucumber samples. To confirm the presence of CABYV and/or BWYV, total RNA was isolated from all seven samples by TRI Reagent (Sigma, St. Louis, USA) and converted to cDNA with a First Strand cDNA Synthesis Kit (Thermo Scientific, Cat. #K1612). Reverse transcription (RT)-PCR was performed using two pairs of primers (CABYV1FW: 5'-TTATCAGGGGACTATGTTTA-3' and CABYV14REV: 5'-GAGGGGATTTTAACTGACTG-3', and BWYV1FW: 5'-AGTAAGTCCTCCCCAACTGA-3' and BWYV2REV: 5'-CTACCCACGACCGTATTTCAT-3'), specifically designed to detect CABYV and BWYV, respectively. Amplicons with expected sizes of 1,930 bp were obtained only with CABYV primers for all samples while no fragments were amplified with BWYV primers. The obtained products from two samples (pumpkin and cucumber) were purified and sent to Macrogen (South Korea) for direct sequencing in both directions. High-quality nucleotide sequences were submitted to GenBank with accession numbers MK671010 (656 bp) and MK671014 (712 bp). These sequences contained ORFs encoding CABYV P1-P2 fusion proteins as determined by BLASTp analysis (<https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins>). A phylogenetic tree constructed by the neighbor-joining method using 18 CABYV accessions and Potato leafroll virus as an outlier showed that the closest accessions to MK671010 and MK671014 were NC003688 (France) and EU636992 (China), with respective nucleotide identities of 98% and 99%. In 2019, another outbreak was observed in the same field near Sadovo and in a field near Plovdiv planted with pumpkins. Nearly 30% of the plants showed leaf yellowing typical of *Polerovirus* infection. Screening of collected samples ($n = 17$) by RT-PCR confirmed CABYV presence in 15 samples. To our knowledge, this is the first report of CABYV in Bulgaria.

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[†]Indicates the corresponding authors.

V. Radeva-Ivanova; vesela_radeva@izk-maritsa.org,
and G. Pasev; gipasev@aol.com